DETERMINATION OF DIGESTION PARAMETERS TO DEVELOP AND EVALUATE FORAGE MIXTURES FOR PASTURE-FED RUMINANTS

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ABSTRACT

Animal production can be improved by lessening the dependence on ryegrass-based pastures as the sole source of nutrients for production. Ryegrass varies in quality and availability and supplementation with appropriate forages will maintain or improve production. This thesis defines the nutritive characteristics of a range of forages, including temperate and tropical grasses, legumes with and without condensed tannins, herbs and silages, in terms of chemical composition, products of degradation and rates of digestion using *in sacco* and *in vitro* methods. The forages assessed varied in crude protein concentration (CP; 7.6 – 29.9 % of dry matter; DM) and neutral detergent fibre (NDF; 22.4 – 57.8% DM), with commensurate net appearance of plant N as ammonia (0 to 49%) and *in sacco* DM, CP and NDF degradation rates (%/h) from 3 – 26, 3 – 19 and 4 – 28, respectively. The Cornell Net Carbohydrate Protein System (CNCPS) was used to evaluate the ability of forages to meet the energy and protein requirements of dairy cows. Data suggested sulla (*Hedysarum coronarium*), lucerne (*Medicago sativa*), red clover (*Trifolium pratense*) and white clover (*Trifolium repens*) as potential forages for feeding with medium to low quality pasture.

Lambs were fed pasture, white clover, lucerne and sulla alone or in mixtures and production, rumen digestion parameters and estimates of protein synthesis were measured. Lambs fed white clover, sulla, lucerne:sulla and white clover:sulla had the highest daily intakes (1.47 – 1.54 kg DM) and liveweight gains (281 – 308 g) compared to lambs fed pasture (1.10 kg DM; 116 g). Sulla had potential for feeding with pasture and lucerne, but energy limited production. Protein synthesis between lambs fed lucerne, sulla and lucerne:sulla were similar (162 – 180 g/day) and greater than pasture (93 g/day). In a trial with dairy cows fed pasture (P), supplementation with maize silage (M) or sulla (S) did affect *in sacco* degradation and the maize silage lowered *in sacco* DM degradation rates (P, 7; M, 4; S, 16; P:M; 5; P:S, 11 and P:M:S, 6 %/h).

The work presented provides a foundation for formulating mixed forage rations to meet cow nutrient requirements and improve productivity in ryegrass-based pasture systems. Animal trials demonstrated synergistic effects of dietary components on both animal production and rumen microbial function.

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LIST OF ABBREVIATIONS

#	number
β	beta
μ	micro
μmol	micromole
^{35}S	labelled sulphur
^{3}H	labelled hydrogen
A	soluble fraction
A:P	acetate:propionate ratio
(A+B):P	acetate + butyrate:propionate ratio
AA	amino acids
ADF	acid detergent fibre
ADL	acid detergent lignin
B	degradable insoluble fraction
Bq	becquerel
c.	about
CP	crude protein
CT	condensed tannin
CV	coefficient of variation
CW	carcass weight
DM	dry matter
dpm	disintegrations per minute
ED	effective degradability
EDTA	ethylene diamine tetra acetic acid
EMA	eye muscle area
FDG	freeze-dried and ground
FSR	fractional synthesis rate
FV	feeding value
g	gram
x *g*	gravitational field
GR	fat depth 11 cm from the mid line of the 11th and 12th ribs
GT	grazing time
h	hour

I	intake
i.u	international units
i.d	internal diameter
IB	herbage intake per bite
ILR	irreversible loss rate
k	fractional disappearance rate per hour
kBq	kilobecquerel (10^3)
kg	kilogram
kJ	kilojoule
L	litre
LS means	least-square means
LW	liveweight
M	maize silage
MBq	megabecquerel (10^6)
ME	metabolisable energy
mg	milligram
min(s)	minute(s)
MJ	megajoule
mL	millilitre
mm	millimetre
mmol	millimole
N	nitrogen
n	number
NAN	non ammonia nitrogen
NDF	neutral detergent fibre
NH_3	ammonia
NH_3-N	ammonia nitrogen
NIRS	Near InfraRed Spectroscopy
nmol	nanomole
NV	nutritive value
o.d	outside diameter
°C	degree celcius
OM	organic matter
OMD	organic matter digestibility
P	pasture

PD	potential degradability
P:M	pasture:maize silage
P:M:S	pasture:maize silage:sulla
P:S	pasture:sulla
Pr	probability
r	correlation coefficient
RB	rate of biting
REML	residual maximum likelihood
RFC	rapidly fermentable carbohydrates
S	sulla
SC	soluble carbohydrate
SD	standard deviation
SDS	sodium dodecyl sulphate
SEM	standard error of the means
SO_4	sulphate
Sp(p)	species
SRA	specific radioactivity
SS	sulla silage
t DM/ha/yr	tonnes dry matter per hectare per year
t	incubation time
TBq	Terabecquerel (10^{12})
TMR	total mixed ration
TQ	transfer quotient
UDP	undegraded protein
US	United States
VFA	volatile fatty acids
VFI	voluntary feed intake
vs.	versus
w/v	weight to volume
WBPS	whole body protein synthesis
WC	white clover
x M	maintenance feeding

LIST OF PUBLICATIONS

Publications produced from this thesis.

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CHAPTER 1

GENERAL INTRODUCTION

CHAPTER 1: GENERAL INTRODUCTION

1.1 INTRODUCTION

Pasture is the predominant source of feed offered to livestock in New Zealand. The temperate climate and fertile soils enable high annual production from pasture which can be grazed year round. These characteristics make New Zealand a low cost producer of milk, meat and wool and provide an economic advantage over many other developed countries. Research has improved pasture production through soil fertility, drainage and grazing management, and has provided highly productive pasture species suitable for contrasting environments within New Zealand. However, our reliance on low cost pasture presents major constraints to maximising livestock production because nutrient intakes do not meet requirements of animals with genetic potential for high production.

Our system is sensitive to climatic changes affecting total pasture production, seasonal production and pasture quality, which do not always match animal nutrient requirements. The quality and quantity of nutrients available to grazing animals are extremely variable throughout a season and between seasons because of weather conditions that affect growth, growth rate, onset of grass maturation, sward composition and feeding management practices. Ryegrass-based pasture can have low dry matter and high fibre concentrations which may restrict feed intake so cow nutrient requirements are often not met. Ryegrass-based pastures are limited by metabolisable energy and have rapidly degradable protein that is excess to requirements which could impose an energetic cost to the animal (Kolver and Muller, 1998; Waghorn, 2002). Therefore, strategies need to be implemented to avoid the inadequacies of our pasture base. These must maintain our low cost advantage and be incorporated into grazing systems without too much disruption. One option is to take advantage of our temperate climate to grow and use high quality forages (eg. legumes, herbs or cereal crops fed fresh or ensiled) for feeding with pasture and provide sufficient quantities of appropriate nutrients for different classes of ruminants. Researchers have investigated a range of forages that have a higher feeding value than perennial ryegrass *(Lolium perenne)*, including other grasses (eg. Italian ryegrass), legumes (eg. lucerne, red clover), cereal crops (eg. wheat silage, maize silage), brassicas (eg. turnips) and herbs (eg. chicory). Recent options have included legumes containing condensed tannins (CT), eg. *Lotus corniculatus, Lotus pedunculatus* and sulla. However, most studies have focussed on forages fed as sole diets rather than as mixtures or with pasture.

1.2 OBJECTIVES

A series of trials were undertaken to identify potential forages that would be complementary to each other and to ryegrass-based pasture to meet animal nutrient requirements and maximise production, particularly in dairy cows. The objectives of this research include:

1. To describe contrasting forages in terms of their chemical composition, degradation and fermentation kinetics using *in sacco* and *in vitro* techniques (Chapter 3).

2. To identify forages with digestion kinetics that have potential for feeding with medium quality pasture using modelling techniques (Chapter 3).

3. To measure responses of animals fed forage mixtures in terms of liveweight gain (Chapters 4 and 5) and milk production (Chapter 6), and to use degradation and fermentation kinetics (Chapter 3, 4 and 6), rumen and blood parameters (Chapter 4 and 6), radioactive isotopes (Chapter 5) and modelling (Chapters 3, 4 and 6) to explain nutrient utilisation and animal production differences.

1.3 FORMAT OF THE THESIS

The thesis is presented in seven chapters. This introduction is followed by a review of literature (Chapter 2), in which the limitations of the pasture-based system relative to animal requirements are defined and compared. Forages with good potential for ruminant nutrition are briefly summarised and the laboratory techniques (*in sacco and in vitro*) used in this thesis to define the degradation and fermentation kinetics are described. Chapter 3 reports the results of *in sacco* and *in vitro* techniques to define the degradation and fermentation kinetics of a range of contrasting forages (grasses, legumes, herbs, silages and hays). The information presented in Chapter 3 formed the basis for identifying potential forages for feeding with medium quality pasture and some of these were evaluated in animal experiments in Chapters 4, 5 and 6. The legume, sulla (*Hedysarum coronarium*) which contains CT, was identified as having excellent potential in both yield and feeding value. Chapter 4 compares the production of lambs fed sulla with lambs fed pasture, lucerne, white clover and three mixtures containing 50% sulla, in terms of liveweight (LW) gain and rumen parameters. Chapter 5 reports protein synthesis in the whole body and tissues of lambs fed four of the diets evaluated in Chapter 4. The last experiment (Chapter 6) investigated the milk production of dairy cows fed two-thirds pasture with sulla and/or maize silage and uses the *in sacco* and techniques to define the digestion kinetics of mixed forage diets and the effect diet offered can have on digestion kinetics. Chapter 7 summarises the results of this thesis, identifies future research and discusses the practical application of feeding mixed forage diets in the pasture-based grazing system.

CHAPTER 2

REVIEW OF LITERATURE

CHAPTER 2: REVIEW OF LITERATURE

2.1 INTRODUCTION

New Zealand livestock production systems are based on feeding temperate pasture that is made up of about 75 – 80% perennial ryegrass and 15 – 20% white clover. Animal production on these pastures is restricted and this review will address the characteristics of pasture and why pasture is not suitable to meet the nutrient requirements of high producing ruminants and discuss other forages that may be suitable for feeding with pasture. Techniques to measure the degradation and fermentation characteristics of feeds in the rumen will be discussed.

2.2 NUTRITION OF THE GRAZING RUMINANT

The ruminant in New Zealand grazes predominantly on perennial ryegrass and white clover pasture that can produce relatively high dry matter (DM) yields (12 – 16 t DM/ha/year), because of the temperate climate, and has the ability to withstand intense grazing in most environments. The production of animals on this pasture-based system is dependent on nutrient availability, ingestion and the utilisation of ingested nutrients. For a grazing ruminant to be efficient at producing milk, meat or fibre, the demand for nutrients must be matched by the supply of nutrients. Ryegrass-based pastures are dynamic with changing quantity and quality during a season and are unable to supply enough and/or an ideal balance of nutrients for maximum animal production. The imbalances of nutrients supplied by ryegrass-based pasture result in sub-optimal animal production. These imbalances will be discussed in this review.

Feeding value (FV) is often used to describe and compare the nutritive characteristics of herbages. It is defined as the animal production response to total consumption of a specific feed and is a function of voluntary feed intake (VFI) and nutritive value (NV) per unit of intake (Ulyatt, 1973). Feeding value is typically measured as liveweight (LW) gain for growing animals or milk production for lactating animals.

$$FV = VFI \times NV / \text{unit of DM intake}$$

VFI is the amount of feed an animal will consume when given free access to the feed (Ulyatt, 1981). VFI has been estimated to account for 50 – 70% of the variation between pastures in their capacity to sustain animal production while the remaining variation is a result of digestibility differences (Ulyatt, 1984). Hodgson (1990) defined the daily amount of herbage eaten for the grazing animal as a product of the time spent grazing (GT) and the rate of herbage intake during grazing (rate of biting and intake per bite). Herbage mass and sward height are the two major components that influence grazing behaviour of temperate pastures (Hodgson, 1985; Poppi *et al.*, 1987).

$$I = GT \times RB \times IB$$

I = daily intake of herbage of a grazing animal (mg OM/kg LW/day)
RB = rate of biting during the grazing period (bites/min)
IB = herbage intake per bite or bite size (mg OM/kg LW)
GT = grazing time (min/day)

Nutritive value is defined as the concentration of nutrients in a feed, or animal response per unit of intake. The NV of a diet is thus dependent on the proportion of nutrients digested (apparent digestibility), and the efficiency with which these digested nutrients are absorbed and utilised by the animal's tissues (Ulyatt, 1981).

Several studies have compared the feeding value of forage species many of which have values higher than that of perennial ryegrass (Ulyatt, 1981; Stevens *et al.*, 1992; Stevens *et al.*, 1993; Fraser and Rowarth, 1996; Johnson and Thomson, 1996). For example, growth rates of lambs fed ryegrass-based pasture are typically between 98 to 136 g/day (Brown, 1990; Fraser and Rowarth, 1996), while lambs grazing white clover have grown twice as fast as ryegrass-fed lambs, and studies have clearly shown the benefits of white clover for milk production (Rogers *et al.*, 1982; Rogers and Robinson, 1984; Thomson, 1984). White clover has a higher feeding value than ryegrass due to lower levels of structural carbohydrate, higher digestible protein, faster breakdown in the rumen and a faster rate of passage through the rumen (Ulyatt, 1981), which results in higher voluntary feed intakes (Rogers *et al.*, 1982). Under white clover grazing, bacterial populations in the rumen are larger and protein and amino acids are used more efficiently compared to ryegrass grazing (Thomson, 1984). Other forages that have higher feeding values than ryegrass are due to all or some of the same reasons why white clover has a higher feeding value than ryegrass. Table 2.1 summarises the nutrient composition of a range of forage species and their feeding values relative to medium quality ryegrass.

The New Zealand dairy cow grazing ryegrass-based pasture on average produces about 3,800 kg of milk and 179 kg milkfat and 136 kg milk protein (315 kg milksolids) over a lactation period of 270 days (LIC, 2002/2003). Cows in the United States (US) characteristically produce more milk, have longer lactation lengths and are larger animals, but their longevity is often shortened. The New Zealand dairy cow is capable of producing more milk when concentrates are included in the diet compared to feeding ryegrass-based pasture (Peterson, 1991; Kolver *et al.*, 2002). Recently Kolver *et al.* (2002) fed New Zealand and US dairy cows either ryegrass-based pasture or a total mixed ration (TMR) that has been specifically formulated to meet cow requirements. TMR are widely fed to Northern Hemisphere dairy cows and are made up of conserved forage and concentrates. They generally contain less structural fibre and degradable protein and more soluble carbohydrate than high quality pasture, dry matter content is generally higher than pasture and the particle size of TMR are smaller (Waghorn, 2002). Table 2.2 illustrates that the New Zealand cow can produce more milk if fed a diet that is balanced to meet her nutrient requirements, compared to cows grazing ryegrass-based pastures. Kolver and Muller (1998) showed that 61% of the lower milk production (29.6 vs. 44.1 kg/day) in cows grazing high quality pasture (the DM contain 43% NDF, 25% CP; 19% non-structural carbohydrates and was 77% digestible) compared to cows fed a TMR (the DM contained 31% NDF, 19% CP, 29% non-structural carbohydrates and was 76% digestible) was due to the lower DM intake of pasture compared with cows fed the TMR. A TMR system is generally not economically viable in the New Zealand grazing system, therefore more economical feeds need to be incorporated into

TABLE 2.1. Comparative feeding value in terms of sheep liveweight gain, forage dry matter (DM) content, composition (% of DM), and metabolisable energy concentration for fresh species (Burke *et al.*, 2002b).

Forage	Feeding value[1]	DM (%)	Soluble Carbohydrates	Crude Protein	ADF[2]	NDF[3]	ADL[4]	CT[6]	ME[7]
White clover	100	15	12	27	19	26	5.9	-	11.5
Chicory	95	14	11	19	21	24	7.0	-	12.5
Birdsfoot trefoil	87	16	13	22	20	28	7.0[5]	0.2 – 4	11.0
Lotus major	84	16	12	22	22	33	17.0[5]	5 – 9	12.0
Grasslands Tama	83	15	16	21	16	37	2.9	-	12.7
Lucerne	82	24	9	30	21	30	6.1	-	10.9
Sulla[8]	81	12	18	23	18	22	8.5[5]	5 – 8	12.7
Perennial ryegrass	52	19	9	16	26	49	2.9	-	11.0

[1] All values relative to white clover (Waghorn and Barry, 1987)

[2] Acid detergent fibre (cellulose + lignin)

[3] Neutral detergent fibre (cellulose + hemicellulose + lignin)

[4] Acid detergent lignin

[5] Values elevated due to condensed tannin and other phenolic compounds associated with lignin

[6] Condensed tannins (phenolic compound that reduce rumen proteolysis)

[7] Metabolisable energy (MJME/kg DM)

[8] Mean of liveweight gain for lambs fed sulla during autumn, spring and winter (Terrill *et al.*, 1992).

the diet of grazing dairy cows in New Zealand to improve milk production. Forages with unique nutritive characteristics have the potential to be economically grown in New Zealand and fed with ryegrass-based pasture diets. However, more information is needed on how these forages are digested and utilised by the ruminant and the production of ruminants on these forages alone and in mixtures. Forages that have potential will be discussed in section 2.8 of this review.

TABLE 2.2. Mean annual milk production, liveweight and body condition score of New Zealand and overseas Holstein-Friesians[1] grazing pasture (Grass) or fed total mixed ration (TMR) during the 2000/2001 season (Kolver *et al.*, 2002).

	New Zealand		Overseas	
	Grass	TMR	Grass	TMR
Days in milk	300	300	298	298
Milk yield (kg/cow)	5300	7300	5900	10100
Milkfat (kg/cow)	267	335	253	365
Milk protein (kg/cow)	198	267	206	355
Milksolids[2] (kg/cow)	465	602	459	720
Efficiency (kg MS/kg $LW^{0.75}$)	4.42	5.26	3.97	5.72
Liveweight at end of season	532	624	561	684
Condition score at end of season	5.0	7.6	4.6	6.1

[1] Genetics obtained from North America and the Netherlands

[2] Milksolids = milkfat + milk protein yield

2.3 CHEMICAL COMPOSITION OF PASTURE

2.3.1 Carbohydrates

Carbohydrates are the major components of plant tissues accounting for 50 – 80% of the forage DM (Moore and Hatfield, 1994; Van Soest, 1994), and are the primary source of energy in ruminant diets (Moore and Hatfield, 1994; Mertens, 1996). Carbohydrates serving as storage and energy reserves in plants are rapidly digested and are categorized as non-structural carbohydrates (Moore and Hatfield, 1994). They may be water-soluble and are sometimes termed as soluble carbohydrates. However, pectins are also rapidly digested but are categorised as structural carbohydrates (Smith, 1973). Non-fibrous carbohydrate is also a term used by nutritionists and includes the non-structural carbohydrates plus pectin, while the fibrous carbohydrates are the hemicellulose, cellulose and lignin components of a feed (NRC, 2001).

2.3.1.1 Structural carbohydrates (cell wall)

Structural carbohydrates make up plant cell walls and are composed of hemicellulose, cellulose and pectin, linked by lignin, and a low concentration of nitrogen (Wilson, 1994; Mertens, 1996). Structural carbohydrates are insoluble and provide structural support and protection to ensure plant survival. Their rate of digestion is wide ranging and slow rates of digestion are due in part to cross-linkages with lignin and phenolic acids (ferrulic and p-coumaric acids). When digestion of structural carbohydrates is slow feed intake is limited (Buxton *et al.*, 1996; Mertens, 1996). Crude fibre, acid detergent fibre (ADF) and neutral detergent fibre (NDF) are the most common measures of fibre used for routine feed analysis, but none of these fractions are chemically uniform. Fibre content is determined by the detergent system (Goering and Van Soest, 1970). Neutral detergents and acid detergents are used to determine the NDF (cellulose + hemicellulose + lignin) and ADF (cellulose + lignin) concentrations of feeds, respectively, and 72% sulphuric acid can be used to separate cellulose and lignin from the ADF residue.

Fibre is important for rumen function because it stimulates rumination and production of saliva, and the cation exchange properties of fibre facilitate rumen buffering (Van Soest *et al*., 1991). This is particularly important in TMR where fibre intake is less than that of pasture-based diets. It has been suggested that fibre in forage-based diets, particularly spring pasture, may not be effective for salivation which aids in creating an environment in the rumen for optimum digestion (Waghorn, 2002). However, Kolver *et al*. (1998a) estimated that 40% of the NDF in high quality pasture was effective based on comparisons with the effective NDF of other feeds and the association between 23 effective fibre and passage rate. With this estimate of the percentage of fibre that is effective, a NDF content of 34 to 40% in high quality spring pasture was related to a pH of 6.0 to 6.1, which are values suitable for optimum digestibility (De Veth and Kolver, 2001a). If the NDF content of spring pasture is less than 34%, which is rare in NZ ryegrass-based pastures (Table 2.4), ruminal pH is likely to be lower than 6.0, and if the NDF content of spring is greater than 34%, the fibre content is likely to be more than adequate to maintain pH and high levels of digestibility (De Veth and Kolver, 2001a).

Impact of cell walls on nutritive value

The cell wall content of forages varies between species, plant components, plant maturity and environment (Wilson, 1994). Plant morphology and tissue changes explain many differences in digestibility (Akin, 1989) and particle breakdown (Wilson, 1991) for contrasting forages. Plant tissues are colonized and degraded at different rates by rumen microbes (Akin, 1989). For example, mesophyll and phloem cells are degraded more rapidly and before the outer bundle sheath and epidermal cells. Bacteria do not attach to sclerenchyma and vascular bundle cells; hence these cells are slowly and incompletely degraded (Akin, 1989). The varying proportion of different cell types in plants correlates to the digestibility and nutritive value differences of plants (Wilson and Hattersley, 1989).

Differences in the forage cell wall composition between legumes and grasses

The cell wall concentration (on a DM basis) of legumes is typically less (about 10 %) than that of grasses and consequently legumes are more rapidly digested (Van Soest, 1994). The composition of grass and legume cell walls is different and generally legumes have a higher concentration of pectin (Buxton *et al.*, 1987; Hatfield, 1989; Van Soest, 1994), sucrose and other soluble sugars (Moore and Hatfield, 1994) than grasses (Table 2.3).

Legumes require less rumination and chewing than grasses because they easily break into small cuboidal particles (Kelly and Sinclair, 1989; Buxton *et al.*, 1996), compared with grasses of all types which degrade into long vascular and sclerenchyma strands. The long strands have no obvious weak points for natural breakage, whereas legume leaves have a reticular venation with angular vein junctions that are the natural points of breakage (Buxton *et al.*, 1996). Consequently, grass particles require extensive rumination to break across the many walls within the strand to reduce fibre length. The vascular bundles of legumes are also relatively weaker than grasses because they are not surrounded or capped by layers of sclerenchyma cells as in grasses (Wilson, 1994). These differences contribute to faster degradation of DM and fibre (NDF), higher intakes and higher production of ruminants fed legumes compared to perennial ryegrass. *In sacco* studies have shown that the DM and NDF (41 to 50 % of DM) of vegetative perennial ryegrasses degraded between 6 to 10%/h and 2 to 9 %/h, respectively, while the degradation rate of legume DM and NDF (26 to 31% of DM) was faster and ranged between 15 to 20 %/h and 5 to 12 %/h, respectively. Selecting ryegrass with a low concentration of sclerenchyma tissue has resulted in faster rates of particle breakdown, shorter rumination times and higher daily intakes (Inoue *et al.*, 1989), but effects on animal production have been inconsistent (Inoue *et al.*, 1994).

Tropical grasses generally have a higher structural carbohydrate concentration than temperate grasses (Moore and Hatfield, 1994; Table 2.3) and their anatomical characteristics and leaf structure also influences digestibility. Tropical grasses have a higher proportion of thick-walled tissues (eg. vascular bundles, sclerenchyma tissues and a specialised sheath surrounding each bundle) in their leaves and leaf sheath compared to temperate pasture species (Akin, 1989; Wilson *et al.*, 1983). There are few differences in stem anatomy between tropical and temperate grasses (Buxton *et al.*, 1996), but stems are less digestible than leaves. Therefore, the low forage quality of tropical grasses may be due to a greater proportion of stem compared to temperate grasses (Twidell *et al.*, 1988). Total plant herbage of temperate grasses contains less NDF and less lignin than tropical grasses (Jung and Vogel, 1986).

TABLE 2.3. Typical concentrations of carbohydrates (g/kg DM) in temperate legumes, and temperate and tropical grasses (Moore and Hatfield, 1994)

Category	Temperate Legumes	Temperate grasses	Tropical grasses
Non-structural carbohydrates			
Soluble sugars	20 – 50	30 – 60	10 – 50
Starch	10 – 110	0 – 20	10 – 50
Fructans	-	30 – 100	-
Structural carbohydrates			
Cellulose	200 – 350	150 – 450	220 – 400
Hemicelluloses	40 – 170	120 – 270	250 – 400
Pectin	40 – 120	10 – 20	10 – 20

Cell wall digestion

Both lignin and cuticular waxes affect digestion by restricting microbial and enzyme access to digestible components (Wilson and Kennedy, 1996). Degradation of lignified tissues takes place from the inside of the cell because microbes cannot penetrate through the lignified middle lamella and primary wall, therefore rumen microbes must enter the interior of plant cells through the stomata, fractures in the cuticle or through cut or sheared surfaces (Wilson, 1993). The tough epidermis of leaves is an effective barrier to digestion (Monson *et al.*, 1972) and digestion is made faster by increasing the number of cut surfaces and rupturing the cuticle (Monson and Burton, 1972).

Rumen bacteria, fungi and protozoa are all responsible for the degradation of fibre to varying degrees, but effectiveness requires penetration of barriers. Orpin and Ho (1991) reported that anaerobic fungi preferentially colonize lignified tissues, particularly sclerenchyma, and are capable of degrading lignified secondary walls, but their rate of digestion appears slow and poorly defined. Bacteria are primarily responsible for cell wall digestion in ruminants. Varga and Kolver (1997) reported generation times of 6 – 9 hours for fungi compared to 0.5 – 3.0 hours for bacteria.

Fibre digestion begins with a physical association between bacteria and feed particles and successive colonization within the adherent population, enabling digestion and nutrient release (Cheng *et al.*, 1991). Although physical attachment to particles does take place, the degree of colonization and mode of action is specific to each microbial species (Kudo *et al.*, 1990). Weimer (1993) reported 10 – 50% of cells within strains of cellulolytic bacteria did not adhere to feed particles, despite prolonged incubation times. If most microbes must attach to the surface of the wall for effective digestion, the surface area for colonization relative to the volume of the wall to be digested is limited in thick-walled cells. As the cell wall thickness increases the surface area:cell wall volume ratio is expected to decrease (Wilson, 1993), consequently limiting microbial degradation of cell walls.

2.3.1.2 Non-structural carbohydrates (NSC)

Sugars, starches, organic acids and other carbohydrate reserves (eg. fructans) make up the NSC fraction associated with storage, energy transfer and metabolism in plants (Smith, 1973). The NSC, relative to cell walls, make up a small component of the total carbohydrate concentration of pastures and legumes, whereas NSC of cereals or root crops can account for 50 – 90% of the total carbohydrate content. The NSC in maize silage, grains and seed by-products (eg. soybean meal, cottonseeds) is mainly starch, while fructans and sucrose are the major components of NSC in grasses (Moore and Hatfield, 1994; NRC, 2001). Legumes contain higher concentrations of sucrose and other soluble sugars than grasses, and concentrations of rapidly digestible pectin are higher in legumes than in grasses (Table 2.3; Moore and Hatfield, 1994). Small quantities of soluble sugars in plant cell walls are only released when the cell wall is broken, but degradation of cellulose and hemicellulose may yield soluble sugars.

Fructans

Fructans are composed almost entirely of 5 carbon fructose units with 2,1 or 2,6 linear linkages or highly branched combinations. The inulin fructans (linear β2,1-linked) are the reserve carbohydrates found in the roots of some plants, for example chicory, while the levan fructans (linear β2,6-linked) are found in the leaves and stems of grasses. Branched fructans containing both β2,1 and β2,6 glycosidic linkages are present in the leaves of cereals, wheat, rye, barley and oats (Van Soest, 1994). Levans are more soluble than inulin and account for 3 – 10% of the DM in temperate grasses (Van Soest, 1994), but may account for one-third of grass stem DM. Soluble sugars (sucrose, glucose, fructose and maltose and others) are generally present in higher concentrations in legumes than grasses and are usually higher in temperate grasses than tropical grasses (Moore and Hatfield, 1994). A wide variety of other organic substances occur in low concentrations, and all NSC are rapidly degraded by rumen micro-organisms (Lassiter and Edwards, 1982).

Starch

Starch is the most important storage carbohydrate in cereal grains, seeds and tubers and is made up of two configurations of D-glucose units, amylose and amylopectin. Amylose consists of linear chains of D-glucose units linked by α-1,4 bonds, usually in a helical form with six glucose units per turn. Amylopectin molecules are highly branched and the α-1,4 linkages form short chains with α-1,6 linkages at about every 20th glucose unit (Moore and Hatfield, 1994; Van Soest, 1994). Chain lengths appear to vary with maturity (Lassiter and Edwards, 1982).

Starch makes up a minor component (< 1% of DM) of grass or legume leaves and stems. A small amount of starch is stored in the seeds of temperate grasses (0 – 2% DM), whereas in most legume species the starch accumulates in the roots (1 – 11% DM), although in white clover the starch is stored in the stolons (Moore and Hatfield, 1994). Amylopectin comprise about 75% of the total starch concentration in grasses (McIlroy, 1967), whereas amylose is the predominant form in maize and sorghum stems (Bailey, 1973).

Starch accounts for 50 – 100% of the NSC in most grains and seeds and can have negative effects on ruminant production if the NSC concentration exceeds 30 – 40% dietary DM (Nocek, 1997). The starch (90%) in most cereal grains (oats, barley or wheat) is rapidly fermented in the rumen (Orskov, 1986; Nocek and Tamminga, 1991), but up to 40% of the starch in maize or sorghum can escape fermentation in the rumen and is fermented post-ruminally (Owens *et al.*, 1986).

2.3.2 Protein

Plant proteins enable photosynthesis and utilisation of energy for growth and reproduction. Nitrogen (N) in fresh forages is 70 – 90% true protein and 10 – 30% non-protein nitrogen (nucleic acids, amino acids, peptides and nitrate), but the proportion of true protein and non-protein nitrogen (NPN) and the rumen degradability of the true protein varies with feed type. Silages and immature pasture have a higher proportion of NPN (Leng and Nolan, 1984), especially free amino acids, NH_3, and amines, with lower concentrations of peptides and nitrates than forages. The NPN concentrations of most grasses are less than 12% of the total nitrogen (Van Soest, 1994).

Forage proteins comprise three main fractions, with chloroplasts containing about 75% of the total leaf protein, of which about half is Fraction 1 protein and is identical to the photosynthetic enzyme, Rubisco (ribulose-1, 5-biphosphate carboxylase). Fraction 2 proteins constitute about 25% of total leaf protein and are derived from the cytoplasm and chloroplasts. The third fraction (membrane fraction) makes up about 40% of plant protein and is a mixture of protein from chloroplasts and nuclear and mitochondrial membranes of plants (Mangan, 1982). Fraction 1 is rapidly degraded in the rumen (Nugent and Mangan, 1981), while the other two fractions are degraded more slowly (McNabb *et al.*, 1996; Min *et al.*, 2000).

The different proportions of NPN and protein fractions in plants affect the rates of ruminal degradation of plant protein, with protein from fresh forage being more rapidly degraded than protein in dried plant material (Beever *et al.*, 1976; Minson, 1990). Drying forages and processing cereal grains reduce protein solubility and increase proportions of protein that are undegradable in the rumen. Protein degradation rates have been measured using *in sacco* and *in vitro* techniques similar to those discussed in section 2.7 of this review (Orskov and MacDonald, 1979; Hoffman *et al.*, 1993; Stern *et al.*, 1994). Leafy legume protein is 45 – 50% soluble and degraded at 15 – 20 %/h compared to protein in leafy grasses which is 35 – 45 % soluble and is degraded at 6 – 11 %/h (Hoffman *et al*., 1993). Maturation reduces protein solubility and degradation rates, but values for legumes are always higher than for pasture. Application of nitrogen fertiliser increases protein solubility and degradation rate (Van Vurren *et al*., 1990). Degradation rates of protein in fresh forages using the *in sacco* technique are comparable to data obtained from *in vivo* experiments when fresh forages were fed (Beever *et al*., 1986; Van Vuuren *et al*., 1990, 1991, 1992).

2.3.3 Lipids

Lipids make up a small energy dense component of forages, and ruminant diets generally contain between 2 to 5% total lipids, of which half are fatty acids (Tamminga and Doreau, 1991). Surface lipids are mainly indigestible waxes and fatty acid components of glycolipids and phospholipids present in the mitochondria, endoplasmic reticulum and plasma membranes (McDonald *et al*.1995). Storage lipids in fruits and seeds are predominantly triglycerides and the diversity of lipids affects their availability through digestion and absorption. The presence of fat in the diet reduces the ability of rumen bacteria to digest dietary fibre and when the dietary concentrations exceed 5 – 7% of the DM fibre digestion is reduced (NRC, 2001).

Of the total amount of lipid present in forage (grass and clover) leaves (about 5% DM is ether extract) fatty acids contribute about 43%, and non-fatty acids (cuticular waxes, pigments eg. chlorophyll and other unsaponifiable substances) make up the rest (Palmquist and Jenkins, 1980). Fatty acid chains in plants are predominantly 16 – 18 carbon atoms long and may contain two or three double bonds (polyunsaturated fatty acids, PUFA). Fatty acid chains in forages generally contain 18 carbon atoms. Linolenic acid (C18:3) accounts for 60 – 75% of fatty acids in pasture (Palmquist, 1988), linoleic acid (C18:2), a component of triglycerides in cereal grains and oil seeds, accounts for 5 – 19% of fatty acids in pasture, and palmitic acid (C16:0) accounts for 6 – 16% of fatty acids in pasture (Palmquist, 1988). In TMR where cereal grains, oilseeds (eg. barley, oats, wheat, cottonseed, soybean, sunflower) and maize are major ingredients of the diet, linoleic acid and palmitic acid are dominant (Kay *et al*., 2002). On average TMR contain more lipids, by using oilseeds and grains in the diet, than forage-based diets, and can be more easily manipulated to include the maximum amount of lipid that a ruminant should consume.

2.3.4 Condensed Tannins

Tannins are naturally occurring plant polyphenols which are contained in intracellular vacuoles and combine with protein and other polymers such as cellulose and hemicellulose to form stable complexes when cells are ruptured (Mangan, 1988). There are two types of tannins, hydrolysable tannins (HT), which are present in leaves of trees and browse shrubs, and condensed tannins (CT) present in forage plants.

Condensed tannins are more common in dicotyledon species than grasses (Waghorn *et al.*, 1997), especially in seed coats or hulls (eg. lucerne, faba beans, cotton seed) and sometimes in flower petals (eg. white clover) but less often in the foliage. Some temperate forages express CT in the foliage (eg. *Lotus spp*) but CT are more common in forage plants originating from warmer climates (eg. sainfoin and sulla) and are widespread in tropical trees, shrubs and herbaceous plants. CT are oligomers of flavanols which can account for up to 30% of plant DM, but rarely exceed 10% in forage species (Waghorn *et al.*, 1997).

CT may have both beneficial and anti-nutritional qualities. Dietary CT reduces microbial proteolysis in the rumen and increase the supply of amino acids to the small intestine. Providing intestinal absorption is not compromised, the increased flux of amino acids to the small intestines may result in improved LW gain, wool growth, milk production, reduced bloat and reduced impacts of parasitism in ruminants (Waghorn, 1996). However, the concentration, structure and type of the CT in some forages (Foo *et al.* 1997) can have detrimental effects on animal production (Waghorn *et al.*, 1997, 1998). When the concentrations of CT are greater than 4 – 6% of DM in temperate forages they are likely to be detrimental to production, depending on astringency (Mangan, 1988).

2.4 PASTURE AS A NUTRIENT SOURCE

2.4.1 Limitations to pasture

The sheep and cattle industries in New Zealand have evolved around the seasonal pattern of pasture production with lambing and calving coinciding with spring pasture growth. However, pasture at some times of the year has limitations that restrict animal production. The main limitations of the pasture based diet include:

1. Moderate energy concentrations and limited digestibility.
2. Low DM content and excessive fibre (measured as neutral detergent fibre; NDF) in ryegrass which at times is slow to digest, which will restrict feed intake.
3. Low concentrations of rapidly fermentable carbohydrates (soluble sugars, organic acids and pectin) relative to crude protein (CP) and structural carbohydrate concentrations.
4. High CP and insufficient undegradable protein (UDP) concentrations, which require excess ammonia (NH_3) to be removed at a metabolic cost.
5. Quantities and proportions of volatile fatty acids (VFA) arising from fermentation which may not be optimal for rapid growth or milk production.

Mineral element deficiencies, excess potassium, and the incidence of endophyte and other toxins may also limit animal production, but are not discussed in this review.

2.4.2 Seasonal changes in pasture

The seasonal changes in pasture digestibility and nutrient composition can further exacerbate the limitations of pasture. Some studies and surveys have attempted to identify the seasonal changes in pasture composition (Moller *et al.*, 1996; Stevenson *et al.*, 2003; Corson, unpublished; Prewer, unpublished). Table 2.4 illustrates the changes in the nutrient composition of ryegrass-based pasture throughout the year. The nutrient composition of pasture is extremely variable with large variation within a year and between years.

Spring pasture is predominantly green leaf, with a relatively low concentration of fibre (40 – 45% NDF), high digestibility (80 – 85%) and high metabolisable energy concentration (ME; 11.5 – 12.0 MJME/kg DM). The concentration of soluble carbohydrate is low (10 – 15% DM) relative to CP (25 – 30% DM) and consequently there is often not enough energy in the diet for efficient microbial synthesis. High quantities of CP coupled with high degradability in the rumen (70 – 80%; Ulyatt and Waghorn, 1993) result in a high concentration of NH_3 absorbed into the bloodstream. Animals divert energy from production to remove excess NH_3 as urea (Danfaer *et al.*, 1980; Oldham, 1984) and Beever (1993) suggested hepatic NH_3 removal might further deplete amino acid availability for production in forage fed animals.

Spring pastures contain 12 – 16% DM, so large quantities need to be consumed to meet animal energy requirements. Energy is often the first limiting nutrient in a pasture-based system and coupled with the low DM content and high concentration of slowly degrading fibre in ryegrass compared to legumes, an animal is unable to consume sufficient feed to meet nutrient requirements for high production (Kolver and Muller, 1998). The soft flexible leaf requires little chewing or salivation to swallow, but the fibre needs to be broken into small pieces by chewing in order to pass from the rumen. Maturation of ryegrass during late spring reduces feed and nutrient intake and exacerbates the rapid decline in milk production that is characteristic of pastoral feeding.

In contrast, summer pasture is mature and the proportion of seed head, stem and dead matter increases relative to leaf (Waghorn and Barry, 1987). Increased concentration of NDF (45 – 55% DM), lower concentration of CP (< 20% DM) and lower digestibility (< 70%) and ME (< 10.5 MJME/kg DM) (Wilson and Moller, 1993; Moller *et al.*, 1996) are the most obvious pasture quality changes. Chaves *et al.* (2002a) has described the relationships between ryegrass and maturity. As ryegrass matures NDF concentration increases and organic matter digestibility decreases (OMD vs. NDF concentration, $r^2 = 0.89$), CP concentration decreases with increasing maturity (CP concentration vs. harvesting age, $r^2 = 0.94$) with commensurate reductions in NH_3 concentration during *in vitro* fermentation (CP concentration vs. *in vitro* NH_3 concentration, $r^2 = 0.85$). The slow digestion of fibre and low CP content of mature pasture will limit intakes of energy and protein, so animal production will be sustained by substitution of mature pasture with feeds containing energy and adequate concentration of protein (eg. rapidly digested forages and concentrate feeds). Maturing ryegrass coincides with a time of the year when pasture availability is limited, therefore when a supplement is fed less substitution of the pasture for the supplement is likely to occur compared to feeding a supplement when pasture was in plentiful supply and fed *ad libitum*.

Pastures in autumn are of similar quality to spring pasture with high CP concentration relative to soluble carbohydrates. Fibre concentrations in autumn pasture are generally higher than spring pastures (Wilson and Moller, 1993; Moller *et al*., 1996) and feed supply insufficient, so supplementation is crucial to sustain productivity.

In this thesis ryegrass-based pastures are considered to be high, medium or low quality if they have a ME, CP and/or NDF concentration and digestibility within the ranges described by the following criteria:

	Pasture quality with regard to:			
Pasture quality	Metabolisable energy (MJME/kg DM)	Crude protein (% DM)	Neutral detergent fibre (% DM)	Digestibility (%)
High	> 11.0	> 25	< 45	> 80
Medium	10.0 – 11.0	15 – 25	45 – 50	70 – 80
Low	< 10.0	< 15	> 50	< 70

TABLE 2.4. Average (and standard deviation) nutrient composition of pasture from dairy farms through New Zealand.

Nutrient	Stage of lactation				
	Early (August to October)	Mid (November to January)	Late (February to April)	Early Dry (May)	Late Dry (June)
Source: Stevenson *et al.* (2003)[1]					
Number of samples	18	22	16	5	8
Dry matter (g/kg DM)	186 (24)	225 (39)	218 (21)	167 (22)	198 (41)
Metabolisable energy (MJ/kg DM)	10.8 (0.4)	10.5 (0.5)	10.8 (0.5)	11.3 (0.4)	11.3 (0.3)
Non-structural carbohydrate (g/kg DM)	219 (42)	243 (40)	235 (29)	252 (23)	229 (64)
Crude protein (g/kg DM)	229 (28)	179 (29)	225 (24)	261 (33)	252 (33)
Neutral detergent fibre (g/kg DM)	454 (43)	481 (45)	443 (35)	390 (23)	422 (40)
Source: Prewer (unpublished)[1]					
Number of samples	40	40	27	10	18
Dry matter (g/kg DM)	163 (24)	180 (23)	186 (49)	168 (24)	149 (22)
Metabolisable energy (MJ/kg DM)	12.3 (0.4)	11.8 (0.4)	11.6 (0.5)	12.1 (0.5)	12.2 (0.5)
Non-structural carbohydrate (g/kg DM)	104 (27)	100 (23)	81 (25)	105 (30)	119 (73)
Crude protein (g/kg DM)	241 (38)	204 (31)	206 (36)	208 (36)	227 (41)
Neutral detergent fibre (g/kg DM)	460 (27)	495 (44)	500 (52)	444 (44)	452 (41)
Source: Corson (unpublished)[2]					
Number of samples	415	365	53	28	101
Metabolisable energy (MJ/kg DM)	11.8 (0.7)	10.7 (1.2)	9.4 (1.2)	10.2 (1.2)	11.2 (1.3)
Non-structural carbohydrate (g/kg DM)	97 (33)	87 (33)	68 (22)	70 (36)	79 (30)
Crude protein (g/kg DM)	269 (47)	217 (61)	217 (56)	236 (62)	275 (52)
Neutral detergent fibre (g/kg DM)	408 (48)	451 (71)	475 (67)	436 (71)	428 (65)

[1] Samples collected during 1996/97 season.
[2] Samples collected during 2000/01 season.

2.4.3 Dry matter content of pasture

Pasture is an extremely moist feed with water content varying from 90% in early spring to 70 – 80% in mid summer. It is thought that water content is a major factor limiting intake of forage nutrients (John and Ulyatt, 1987). Verite and Journet (1970) found that the threshold at which intracellular water content of fresh forages limited intake was between 15 – 18% DM, and for every 1% increase in water content in forages, with a water content above 81.9%, voluntary intake of lactating dairy cows decreased by 0.34 kg DM. Together the higher water content and bulk density of fresh forage limits feed intake by increasing the volume of material that a ruminant has to physically consume to eat the same amount of DM as a ruminant fed a diet that was dried, chopped or ground and pelleted (Ulyatt and Waghorn, 1993). Data summarised by Waghorn (2002) illustrate that the low DM content of ryegrass-based pastures is one factor affecting feed intake and production of cows fed ryegrass-based pastures. Compared to TMR that are 50% concentrate and 50% forage, ryegrass-based pastures have a lower DM content (13 – 24 vs. 50 – 60% DM), cows physically consume 2 – 4 times more wet material (72 – 121 vs. 36 – 40 kg wet material/cow/day), and DM intakes of cows are 10 to 40% lower (14.0 – 17.6 vs. 19.8 – 22.2 kg DM/cow/day) (Holmes *et al.,* 1987; Moller *et al.*, 1996; Kolver *et al.*, 1998b; Kolver *et al.*, 2000; NRC, 2001; Waghorn, 2002)

2.4.4 Particle size and fibre content of pasture

The upper limit of particles able to pass from the rumen in sheep and cattle are particles able to pass through sieves with aperture sizes of about 1 and 2 mm, respectively. Larger particles need to be regurgitated and reduced in size by rumination (Poppi *et al.*, 1980, 1981; Ulyatt, 1983; Ulyatt *et al.*, 1986) to enable clearance from the rumen.

The fibre component of pasture (measured as NDF), comprising hemicellulose, cellulose and lignin, has the greatest impact on particle breakdown, the rate particles pass out of the rumen and feed intake. Fibrous particles are slowly digested and fermented resulting in longer rumen retention times and consequently reduced feed intake (Buxton and Redfearn, 1997).

Fibre content and particle size differences between forages (ryegrass vs. legumes) and diets (ryegrass-based pasture vs. TMR) affect particle breakdown, passage rate and feed intake (feeding value) (Waghorn, 2002). Comparing legumes with temperate grasses, legumes have a relatively low concentration of structural carbohydrates, especially in leaves, and chewing is more effective at reducing particle size than pasture, with associated effects on feed intake and animal production. In a comparison between ryegrass and lucerne fed to cows, chewing during eating reduced 61% of lucerne DM and only 46% of ryegrass DM to a size able to pass a 2 mm sieve (Waghorn *et al.*, 1989). Increasing fibre content with maturity is exacerbated in pastoral systems because mature pasture makes up most of a ruminants diet over the summer months. Consequently feed intake and animal production is limited.

Comparing diets, TMR have a different nutrient composition and particle size to that of high quality pasture (Waghorn, 2002). TMR contain much less structural fibre (NDF = 26 – 34 vs. 42 – 58% DM), especially hemicellulose (8 – 11 vs. 20 – 24% DM), more rapidly fermentable carbohydrate (35 – 40 vs. 5 – 24% DM) and less rumen degradable protein (RDP = 60 – 65 vs. 70 – 75% CP) which affects the rate particles are degraded and fermented in the rumen (Waghorn, 2002). At feeding, the particle sizes of TMR are shorter than pasture because about 50% of it is roughage – either hay or silage that has been chopped to lengths of 1 – 2 cm. The remaining 50% are concentrates that are made up of grains that are small and often processed and the protein supplements are small grains and/or powders. Consequently, nutrient composition, particle size and DM content differences between cows fed ryegrass-based pasture and TMR combine to affect digestion, fermentation, passage rate and feed intake with concomitant effects on cow production.

2.5 DIGESTION AND FERMENTATION OF NUTRIENTS

Feeds and their components are exposed to microbial (primarily bacterial) digestion and degradation in the rumen before residues pass to the small intestine. The dietary constituents (eg. ryegrass, maize silage, white clover etc.) and their components (cell walls, NSC, CP, CT, lipid) are degraded by the rumen microflora, with changes in structure and degradation affecting the net yield of metabolites absorbed from the rumen and intestine to provide energy and nutrients required for growth and production. A brief summary is given to indicate the fate of products of digestion of plant components.

2.5.1 Carbohydrates

Structural and non-structural carbohydrates are digested to produce VFA which account for the majority of the energy disappearing from the rumen and between 50 – 70% of the digestible energy intake (Sutton, 1972, 1979; Thomas and Clapperton, 1972). Structural carbohydrates are a more important source of energy than NSC and pectins for ruminants fed forages, but are fermented slowly relative to NSC. A greater proportion of maize starch is soluble (20 – 50% NSC); Nocek and Tamminga, 1991; Offner *et al.,* 2003) and degradation rates range between 4 to 30%/h (Nocek and Tamminga, 1991; Offner *et al.,* 2003) compared to NDF in perennial forages which was less soluble (0 to 30% NDF; Hoffman *et al.*, 1993) and degraded between 2 to 15%/h (Hoffman *et al.*, 1993). Carbohydrates must be degraded into simple sugars (glucose, fructan, uronic acids and pentoses) by microbial extracellular enzymes prior to absorption and metabolism by rumen micro-organisms to yield VFA, lactate and energy (ATP). The energy is used for maintenance and growth of the microbial population. Sucrose and fructans are fermented most rapidly, followed by starch, then cell wall carbohydrates (Lassiter and Edwards, 1982). Digestion of cell walls is dependent upon physical rupture, allowing bacterial access to digestible constituents, especially in lignified tissues.

Acetate, propionate and butyrate are the main VFA produced from carbohydrate fermentation. Rumen contents of animals fed pasture and forage diets typically contain 60 – 72% acetate, 15 – 23% propionate and 12 – 18% butyric acid with total concentration of VFA in rumen liquor varying between 50 – 150 mmol/L (Bergman, 1990; Holmes *et al.*, 2002), but diet composition and microbial population affects the products of fermentation. Forage maturation increases acetate production at the expense of propionate, whereas a high proportion of NSC results in a relatively higher molar proportion of propionic and lactic acids (Murphy *et al.*, 1982). Butyrate production may be increased when some concentrates are fed due to high protozoa populations that occur with some concentrate diets, but the most common effects of concentrates are to increase the proportions of propionic (up to 50%) and lactic acids (Bergman, 1990; Van Houtert, 1993).

Starch is degraded by α-amylase to dextrins (Moore and Hatfield, 1994) and further fermentation yields glucose. Small grains can easily escape the rumen without digestion, which is wasteful, so processing (flaking, crushing) ensures high utilisation of grain crops. However, the low rumen pH associated with these feeds due to the rapid fermentation and large amounts of lactic acid that are produced can reduce the number of cellulolytic and fibre digesting bacteria, resulting in a low acetate:propionate ratio and acidosis from lactic acid accumulation. When high concentrate diets are fed, propionate is predominantly produced via the lactate and acrylate pathway, whereas propionate is produced through the succinate pathway from forages (Van Houtert, 1993; McDonald *et al.*, 1995).

2.5.2 Protein

When fresh forages are fed, 65 – 75% of true protein is degraded to peptides, amino acids and ammonia (NH_3) by rumen microbes (MacRae and Ulyatt, 1974), while the undegraded protein passes to the small intestine for digestion and absorption. Rumen microbes also utilise NH_3, peptides and amino acids to synthesise microbial protein, which passes into the abomasum and small intestine for digestion and absorption by the animal. The amount of dietary N leaving the rumen is determined principally by the total N in the diet, the extent to which it is fermented in the rumen and the rumen retention time (Minson, 1990). When degradation of protein to NH_3 exceeds NH_3 utilisation for synthesis of microbial protein, substantial absorption occurs (often 25 – 40% of nitrogen intake; Leng and Nolan, 1984). Urea synthesised in the liver may be recycled as saliva but most is excreted as urine (Leng and Nolan, 1984). Urea synthesis from excess NH_3 can be a costly process for the dairy cow (Danfaer, 1980; Oldman, 1984; Beever, 1993; Lobley *et al.*, 1995) in terms of both energy and amino acids. Baldwin (1995) estimated ammonia production in excess of bacterial utilisation is converted to urea at a net metabolic cost of about 12 kJ/g NH_3-N. Danfaer (1980) found that a 25% CP pasture may depress milk production by 2 litres/day and a 30% CP pasture could decrease production by a further 2 to 3 litres per day. Modelling with the

Cornell Net Energy and Protein model (CNCPS) suggested that the energetic cost of detoxification of NH_3 to urea if the CP content of pasture was 20% CP would be 4.1 MJ/day and at 30% CP the cost would increase to 12.2 MJ/day. Therefore assuming that one litre of milk requires 5 MJME (Holmes *et al.*, 2002) the cost of increasing the CP content from 20% to 30%, assuming no other nutrient component changes, equates to about 1.6 litres of milk which was similar to the difference of 1.8 litres of milk from the model. Lean *et al.* (1996) also used the CNCPS model and found that the cost of excess ammonia by converting it to urea, in combination with the negative effect of low availability of carbohydrates associated with high CP pastures, may decrease potential milk production by 11 litres per cow per day, if the CP of ryegrass increased from 20 to 35%. Cohen (1991) estimated that the energy costs associated with the removal of excess N, if animals were fed white clover of varying maturity, was equivalent to the energy needed for the production of 0.5 to 2.0 kg of milk per day, and in late lactation some of this energy may also be at the expense of liveweight gain. Infusions of ammonia (as ammonium bicarbonate) given to lactating ewes resulted in a 15% drop in milk production (Malik *et al.*, 1999).

Non-ammonia nitrogen (NAN) absorbed from the intestine is composed of undegraded plant protein, microbial CP and endogenous NAN (McNabb *et al.*, 1996). Approximately 80% of the NAN entering the small intestine is true protein and the remainder is nucleic acids. Any undigested CP that enters the large intestine may be fermented to VFA, or is excreted in the faeces.

Diets can be manipulated to include more bypass protein and less rapidly degraded protein. These changes are relatively simple when TMR are formulated using forages and grains, but more difficult with forages. One method of reducing ruminal proteolysis in forage-based diets is to incorporate CT into the diet, but care must be taken to ensure the protected protein can be absorbed from the small intestine.

2.5.3 Lipids

Dietary lipids are a small part of an animals diet. They are hydrolysed by bacterial and protozoal lipases. But rumen microbes are unable to metabolise the fatty acids, although rumen microbes can synthesise odd chain (15 – 17 carbons) and branched chain fatty acids which are incorporated into cell membrane phospholipids (Van Soest, 1994).

The glycerol and galactose portions of plant lipids are fermented by rumen microbes to VFA, whilst unsaturated linoleic (18:2) and linolenic (18:3) fatty acids are hydrogenated to stearic acid (C18:0) (Drackley, 2000). Most lipids entering the small intestine are saturated fatty acids which adsorb to the surface of small feed particles and are emulsified by bile acids to enable absorption. Microbial lipid accounts for 15% of the lipids reaching the small intestine. Meat and milk contain relatively more saturated fatty acids than the diet they consume because of the hydrogenation process.

2.5.4 Condensed tannins and the effect on ruminants

Condensed tannins are able to reduce protein degradation during digestion in the rumen. The CT-protein complexes resulting from cell rupture are stable and insoluble in the pH range of 3.5 to 7.0, but are unstable and release protein at a pH less than 3 and over 8 (Jones and Mangan, 1977). Reactions between CT and protein are highly specific for sources of tannin and protein (Asquith and Butler, 1986). The CT-protein bond reduces protein degradation in the rumen with 30 – 70% lower concentration of NH_3 in rumen liquor, less rumen digestion of protein, and increased flow of dietary N to the intestine compared to a similar diet without CT (Waghorn *et al.*, 1997). Barry and Manley (1984) found that increasing the dietary concentration of CT linearly increased duodenal NAN flow per unit total N intake. However, the increased flow of dietary protein to the small intestine does not always equate to an increase in the absorption of amino acids in the small intestine (Waghorn *et al.*, 1997). Condensed tannins are able to bind with and precipitate protein when fed with non-CT forages (Waghorn and Jones, 1989) and mixtures of *Lotus spp.* and ryegrass have demonstrated CT in *Lotus pedunculatus* to have a greater astringency than CT in *Lotus corniculatus* (Waghorn and Shelton, 1995; 1997).

Reduced rumen proteolysis has equated to improved animal production when *Lotus corniculatus* and sulla have been fed. The effects of CT on nutritional performance is determined by giving polyethylene glycol (PEG) which preferentially binds with CT to remove the effect of the CT. Therefore, daily administration of PEG to animals results in a CT-free diet (Jones and Mangan, 1977). Compared to sheep given PEG, CT in *Lotus corniculatus* and/or sulla increased wool production (Wang *et al.*, 1996a), increased milk production in sheep (Wang *et al.*, 1996b) and dairy cows (Woodward *et al.*, 1999), and increased LW gain in deer (Hoskin, 1998) and lambs (Barry, 1989; Douglas *et al.*, 1995; Wang *et al.*, 1996b). Condensed tannins in sulla *and Lotus pedunculatus* also provide protection to grazing sheep against the deleterious effects of intestinal nematodes (Robertson *et al.*, 1995).

2.6 ENERGY AND OTHER NUTRIENT REQUIREMENTS OF DAIRY COWS

Energy is considered the first limiting nutrient of diets based on fresh pasture (Kolver and Muller, 1998; Kolver, 2000), but a deficiency of other nutrients at particular times can cause sub-optimal production of ruminants, compared with diets that are balanced to meet nutrient requirements (Table 2.5). Energy for ruminants is supplied by the breakdown of VFA that have been mostly derived from carbohydrate fermentation, but amino acids and lipid degradation in the rumen can also contribute to energy supply. However, the efficiency with which amino acids and lipids are used for energy is less than that of VFA. However, the catabolism of the major VFA acetate is relatively inefficient in supplying energy. For example, if the digestion of fresh pasture yields approximately 68% acetate, 18% propionate and 14% butyrate in the rumen, only about 50% of the energy supplied to the cow comes from acetate, 22% from propionate and 28% from butyrate (Holmes *et al.*, 2002).

TABLE 2.5. Nutrient requirements for a 400 kg dairy cow compared to nutrients supplied in a ryegrass-based pasture and total mixed ration (TMR) (Adapted from Moller *et al*., 1996; Kolver, 2000; NRC, 2001; Waghorn, 2002).

Dietary nutrient	Requirements for a 400 kg cow		Spring pasture	Summer pasture	TMR
DM	Stage of lactation	kg DM/cow/day	kg DM/ha/day		
	Early[1]	15 – 16	60 – 80	10 – 30	-
	Late[2]	12 – 14			
Energy		MJME/cow	MJME/kg DM		
	Early	160 – 180	10 – 11	8 – 9	11 – 12
	Late	110 – 130			
Soluble carbohydrate (% DM)	36-44		10 - 15	5 – 10	35 – 40
Protein (% DM)	Early	18 – 20	25 - 30	< 20	16 – 18
	Mid[3]	16 – 18			
	Late	14 – 16			
ADF (% DM)	15 – 25		20 – 25	25 – 35	18 – 23
NDF (% DM)	25 – 33		35 – 45	45 – 55	26 – 34

[1] Cow producing 23 litres of milk with 4.7% mikfat, 3.3% milk protein and gaining no liveweight.

[2] Cow producing 15 litres of milk with 4.7% milkfat, 3.3% milk protein and gaining 0.25 kg/day of liveweight.

[3] Cow producing 19 litres of milk with 4.7% milkfat, 3.3% milk protein and gaining no liveweight.

Glucose is the main source of energy, but ruminants absorb very little glucose from the digestive tract; therefore it must be synthesised in the liver from glucogenic precursors, particularly propionate, but also glycerol and certain amino acids. Gluconeogenesis from amino acids is marginally less efficient resulting in approximately 29% energy capture, compared to 36% from propionate (Waghorn and Barry, 1987; Holmes *et al*., 2002). Table 2.6 summarises the efficiency of energy capture (as high energy phosphate bonds) from a range of substrates. The process of using glucogenic amino acids to meet glucose requirements (eg. for lactation) is marginally inefficient, but also wastes amino acids that could be used for protein synthesis (Beever, 1993), especially when protein is not in excess. An additional cost results from the removal of NH_3 as urea (Danfaer *et al*., 1980; Baldwin, 1995). This illustrates the importance of feeding diets able to provide sufficient nutrients to meet animal needs and to minimise transformation and wastage of nutrients.

TABLE 2.6. Efficiency of energy capture (as high-energy phosphate bonds) from a range of substrates in support of maintenance processes (Waghorn and Barry, 1987; Baldwin, 1995; Holmes *et al*. 2002).

Substrate	Heat of combustion (kJ/mole)	Net yield of ~P[1]/mole	Heat of combustion/~P produced (kJ/mole)	Heat loss relative to glucose	Efficiency of conservation[2]
Glucose	2813	38	74	100	41
Stearate	11336	146	77	104	39
Acetate	873	10	87	118	35
Propionate	1534	18	85	115	36
Butyrate	2190	27	81	109	38
Casein[3]	2461	23.2	106	143	29

[1] High energy phosphate bond

[2] Based on free energy of hydrolysis of ATP to ADP (30.5 kJ/mole) x yield ~P/mole substrate ÷ Δ of substrate.

[3] ΔH for 115g casein (equivalent to 1 mole AA) is corrected for ΔH of urea resulting from AA catabolism. An additional 4~P are required to synthesise each mole of urea from ammonia.

Energy intakes can fluctuate in the grazing ruminant due to changes in pasture quality and supply, therefore the adipose tissue becomes an important source of energy if energy intake is restricted. Mobilisation of adipose tissue involves the breakdown of triglycerides into glycerol and fatty acids. Glycerol may contribute to gluconeogenesis, whilst fatty acids undergo ß-oxidation to acetate and ß-hydroxy butyrate for production of ATP.

Amino acids cannot be synthesised (essential AA) so requirements must be met via nutrient supply or catabolism of tissue protein. An ideal diet would provide the amino acids required for production and maintenance, in the correct ratios. It is important that imbalances in AA supply are avoided, and no AA is limiting for animal requirements, because this would necessitate substantial catabolism of those AA in excess of requirements. Rumen modifications of dietary protein make provisions for an optimal balance of AA difficult, but reduced proteolysis (eg. with CT) and minimal losses to NH_3 (eg. increasing the supply of rapidly fermentable carbohydrates) can lower the costs of providing amino acids to the animal. Research has shown that increasing the proportion of rapidly fermentable carbohydrates and decreasing the proportion of structural carbohydrates of a pasture-based diet can improve the utilisation of dietary N and increase microbial protein synthesis in lactating dairy cows (Carruthers *et al.*, 1996; Carruthers and Neil, 1997; Kolver and Muller, 1998).

Long chain fatty acids derived from feed, microbes and mobilisation of body fat are metabolised by the liver and utilised by adipose and mammary gland tissues or catabolised by tissues to provide energy, depending on the physiological status of the animal. Lipids are synthesised from acetate, butyrate and circulating long chain fatty acids. The cost of synthesising triglycerides using fully-formed fatty acids from circulating lipids is more efficient than from fatty acids synthesized from VFA (Holmes *et al.*, 2002).

In lactating ruminants, the mammary gland is the major site of triglyceride synthesis. Ruminant milkfat is composed of short chain fatty acids (SCFA; 50% of fatty acids), as well as medium and long chain fatty acids, in contrast to adipose tissue which is predominantly made up of long chain fatty acids (LCFA). Acetate and butyrate are precursors for SCFA and medium chain fatty acid synthesis while the circulating blood lipids provide LCFA. The concentration and composition of milkfat can be altered by the diet. Milk from cows fed pasture has higher fat content compared to milk from cows fed TMR or diets containing a high amount of concentrates (Kelly *et al.*, 1998; Auldist *et al.*, 2002; Kolver *et al.*, 2002).

The composition of the diet can indicate the feeding value, in so far as the amount and type of fibre will influence voluntary intake, and the proportions or concentrations of protein, lipid and NSC will indicate the rate and type of nutrients available for absorption and metabolism. To some extent dietary composition can indicate the ME and protein supply, but it is the utilisation and quantity of absorbed nutrients that determines animal production. The efficiency with which energy and other nutrients are utilised is indicated in feed tables as net energy (NE) values of diets (NRC, 2001) or k or q parameters that predict energy retention (AFRC, 1993) for specific purposes, but is dependent on many dietary and animal factors. One of the challenges addressed in this thesis will be to use degradation kinetics from *in sacco* techniques (section 2.7.1), and fermentation products (NH_3 and VFA yields and proportions) from *in vitro* techniques (section 2.7.2) to predict the supply of nutrients to the animal and how these nutrients will be utilised.

2.7 TECHNIQUES TO MEASURE THE DIGESTION AND FERMENTATION OF FEEDS

Manipulation of dietary components to maximise animal production requires accurate data that describes the quality of feeds being fed to ruminants and the effects these feeds will have on animal production. Feed composition, digestibility, the rate at which feed components (eg. DM, protein, fibre, soluble carbohydrates) are broken down and passed out of the rumen, pool sizes of feed components, products of digestion, and absorption and utilisation of nutrients are all components that affect the feed value of feeds. The ability of forages to supply nutrients can be assessed using animals by measuring production and measuring the losses of feed components across the whole digestive tract. However, obtaining this information from animal trials is often time consuming, expensive, requires large amounts of test forage and animals, and is therefore not an appropriate method for routine evaluations of feeds (Weiss, 1994). Consequently other indirect methods (*in sacco* and *in vitro* methods) are being used to evaluate feed characteristics.

In vivo balance experiments involve collecting faeces and urine from animals eating measured amounts of feed (usually close to maintenance requirements) over a 7 – 10 day feeding period (McDonald *et al.*, 1995) to calculate digestibility coefficients of feed components. Data collected has created a reference library of forage digestibilities which are used to calibrate and validate indirect methods for predicting digestibility. A range of *in vitro* laboratory procedures have been developed to simulate ruminant digestion and predict DM digestibility, extent and rate of protein degradation, yield and proportions of VFA, and rate at which feeds and their components are degraded. However an *in vitro* system is only valid if it correlates with *in vivo* digestion (McDonald *et al*, 1995). *In sacco* methods have also been developed to measure the rate that feed and its components (DM, protein, soluble carbohydrates, fibre) are broken down in the rumen over time. This method does not measure products of fermentation, but allows pool sizes of the feed and the rate at which these pools are degraded to be determined.

Digestibility measured *in vivo* does not permit differentiation between feed degradation in the rumen and postruminal degradation; however about 60 – 65% of digestion does occur in the rumen and this influences the digesta presented to the intestines. Hence, rumen digestion is pivotal to feed utilisation, and procedures for predicting *in sacco* and *in vitro* rumen digestion will provide information about the feeding value (nutritive value and intake) of diets and their constituents.

In this thesis, *in sacco* methods have been used to measure degradation kinctics, and products of fermentation have been determined by an *in vitro* technique. The size of the soluble component (eg. DM, protein and fibre), the slowly degradable fraction, and the undegradable fraction, and the rate that the slowly degradable fraction is broken down over a time period (eg. 72 hours) is determined by the *in sacco* method, while the *in vitro* method measures NH_3 and VFA production from feeds over time (eg. 24 hours). This information will allow a greater understanding about the rate and extent feeds and their components are degraded and fermented in order to predict feeding value and animal production, without conducting animal trials.

2.7.1 *In sacco* digestibility

The *in sacco* or *in situ* method measures the rate and extent feeds are degraded in the rumen by placing a small amount of feed in an undegradable porous bag suspended in the rumen of the animal (Huntington and Givens, 1995). This technique is used to characterise rumen digestibility of DM and nutrient fractions, such as N (AFRC, 1992) and fibre (Aerts *et al.*, 1977; Navaratre *et al.*, 1990). Digestibility values for forages determined by *in sacco* techniques have been shown to be highly correlated with *in vivo* digestibility of forages in the rumen (Table 2.7; Huntington and Givens, 1995; Kitessa *et al.*, 1999; Carro *et al.*, 2002) and ranking of forages in terms of *in sacco* dry matter disappearance (after either 48 or 72 hours of incubation) and *in vivo* DM digestibility are similar (Judkins *et al.*, 1990; Messman *et al.*, 1991; Flachowsky and Schneider, 1992).

However, because the *in sacco* technique varies between researchers and the purpose of the research, *in sacco* degradability values within specific feed types can be different. Therefore, some researchers have attempted to standardise the *in sacco* method (Huntington and Givens, 1995; Orskov, 2000). Bag characteristics, sample composition, preparation and amount of sample incubated, dietary and animal effects, placement of bags in the rumen, length of time sample is incubated, post-rumen processing, effects of microbial contamination, data analysis and modelling systems can influence prediction of rumen degradability values (Nocek, 1988; Weiss, 1994; Huntington and Givens, 1995). A brief overview of these sources of variation is presented in this review.

TABLE 2.7. Correlations (r) between *in sacco* DM degradability and *in vivo* digestibility.

Feed type	*In vivo* parameter	*In sacco* parameter	r	Reference
By-product of fruit and vegetable canning industry	OMD[1]	DM disappearance after 48 hour incubation	0.97	Gasa *et al*. (1989)
Hay	DMD[2]	Potential DM digestibility[4] Effective DM digestibility[5]	0.89 0.98	Carro *et al*. (1994)
Hay	DMD[2]	Potential DM disappearance[6]	0.95	Khazaal *et al*. (1993)
Hay	DMD[2]	Potential DM disappearance[6]	0.83	Khazaal *et al*. (1995)
Maize Silage	OMD[1]	Potential DM disappearance[6]	0.86	Ferret *et al*. (1997)
Grass Silage	OMD[1]	Potential DM disappearance[6]	0.82	Barber *et al*. (1990)
Hay (Legume and grass)	IVDMD[3]	Potential DM digestibility[4] Effective DM digestibility[5]	0.81 0.98	Carro *et al*. (2002)
Grass Hay	IVDMD[3]	Potential DM digestibility[4] Effective DM digestibility[5]	0.95 0.98	Carro *et al*. (2002)

[1] Organic matter digestibility

[2] Dry matter digestibility

[3] *In vivo* dry matter digestibility

[4] Potential dry matter digestibility = degradable fraction (a + b)

[5] Effective dry matter digestibility = degradable fraction that accounts for passage rate of feed out of the rumen

[6] Calculated from *in sacco* degradation in the rumen alone using equation $P = a + b\,(1 - e^{kt})$ where P = potential DM disappearance at time t; a + b = total potentially degradable DM where a = soluble fraction; b = degradable but insoluble fraction; k = rate of degradation.

2.7.1.1 Bag characteristics

Fabric type

Fabric type, size and uniformity of pores, and bag size can affect *in sacco* results (Weiss, 1994). Bags are made out of synthetic fabrics such as nylon, polyester or dacron (Huntington and Givens, 1995). Of greater importance than the fabric type is the weave structure and pore size.

The SCA (1990) recommended that the preferred material used to make *in sacco* bags should be polyester, but states a monofilamentous weave structure should be used and not a multifilamentous one. Cloth that has a monofilamentous structure is precision woven and is heat treated after weaving which results in the formation of permanent corrugations where the filaments cross, resulting in pores with a precisely defined size which will not distort. Cloth that has a multifilamentous structure has an irregular weave and pore size and a greater propensity to deform causing a greater loss of undegraded particles from the bag (Huntington and Givens, 1995).

Pore size

In theory, the porosity of the bag must permit the influx of digesting agents and buffers, and limit the influx of rumen contents not associated with the test feed, but prevent the efflux of undegraded sample whilst allowing the removal of degradation end products (Nocek, 1988; Huntington and Givens, 1995). However, the ideal bag does not exist. Pore size has significant effects on *in sacco* degradation with DM disappearance generally increasing as pore size increases above 10 µm (Lindberg and Varvikko, 1982; Uden and Van Soest, 1984; Nocek, 1985), but also the loss of particles through washout increases with increasing bag pore size (Lindberg and Knutsson, 1981). Most studies use bags with pore sizes between 35 and 54 µm (Huntington and Givens, 1995).

Bag size and sample size

Although bag size per se is unimportant, initial sample size and the ratio of sample dry weight to bag surface area have significant impacts on kinetics of digestion, and often the ratio is governed by the amount of sample needed post incubation for accurate weight determination and analysis. It is important not to fill bags too full as this may result in delayed bacterial attachment, increased lag times and an underestimation of degradability. Alternatively, too little sample may lead to an overestimation of sample degradability (Nocek, 1985). As sample size increases relative to surface area of the bag, the rate and extent of disappearance decreases (Mehrez and Orskov, 1977). Published data relate mainly to the evaluation of dried material placed in bags and although ruminal wetting will increase the volume of material it will always be lower than an equivalent amount of chewed forage DM. Huntington and Givens (1995) and Nocek (1988) have suggested 10 – 20 mg DM/cm^2 of bag surface area to be an optimal fill for bags with 40 – 60 μm pore size. However, fresh forages contain a high proportion of water, therefore in order to get a large enough sample for post-incubation analyses *in sacco* bags may need to be overfilled compared to if freeze-dried and ground material was used. If bags contain too much sample, which may occur when fresh forages are incubated, the rate at which bacteria enter the bag and colonise the material may be affected.

2.7.1.2 Sample preparation

Sample preparation does affect *in sacco* and *in vitro* digestion (Barrell *et al.*, 2000; Cohen and Doyle, 2001) and when evaluating feeds, samples should be prepared in a manner similar to material swallowed by the ruminant (eg. fresh, ensiled or dried), including effects of chewing and masticating. Feeds used for incubation should be homogenous and not altered by the preparation procedure. Most studies use feeds which have been dried to reduce changes in composition through respiration and to facilitate grinding to a consistent particle size (Huntington and Givens, 1995). Oven drying (60°C), freeze-drying or no drying had little effect on *in sacco* disappearance of oesophageal samples (Playne *et al.* 1978), but drying samples above 60°C has been shown to alter the chemical composition of samples and is not recommended (Weiss, 1994). Whilst drying grains may be acceptable, drying and grinding does not replicate chewing of fresh or ensiled forage (Barrell *et al.*, 2000).

There is considerable debate as to whether prepared material should mimic fed or masticated material, but there is agreement that sample preparation should be similar to the resultant particle distribution post mastication and rumination (Weiss, 1994; Orskov, 2000). Bags incubated in the rumen or *in vitro* systems are not subjected to these processes, and microbial fermentation and ruminal muscular activity are the only means by which particle size may be reduced sufficiently to escape the bag (Huntington and Givens, 1995). The literature is inconclusive as to the degree particle size influences digestion. Generally, longer and coarser material is associated with slower rates of digestion and greater variation between replicates. However, finely ground material is subject to greater mechanical losses from *in sacco* bags (sometimes resulting in unrealistically rapid rates of digestion) but variation between replicates is lower (Nocek, 1988). The particle size distribution of the feed should be identified when determining digestion to obtain a relative comparison with other researchers.

In systems where fresh forages are grazed, the extent of particle size reduction is dependent on the type of forage and the amount of chewing during eating and rumination. Very little investigation has been conducted on appropriate methods for forage preparation of fresh material. Some researchers have chopped fresh plant material in lengths of 5 – 10 mm (Howarth *et al*., 1982; Van Vuuren *et al*., 1991; Goplen *et al*., 1993; Kolver *et al*., 1998b) whereas others (Waghorn and Caradus, 1994; McNabb *et al*., 1996) have minced forages to achieve a particle size distribution similar to chewed material of sheep and cows. Chopping is probably not an appropriate preparation method because forages have a tough epidermis that restricts bacteria penetration and bacteria would only be able to digest the cut ends of the leafy material and have limited access to internal structures via stomata (Wilson, 1991). Cell wall digestion is limited by lignified structures, so bacteria access to cells is only possible following cell rupture.

The particle size distribution of DM in boli swallowed by sheep eating fresh forages had 49 – 51% retained on a sieve with 1 mm aperture and 32 – 38% was soluble in water (Ulyatt *et al*., 1986), and in cows fed fresh forages 42 – 47% was over 1 mm and 17 – 22% was soluble. Therefore mincing fresh forage may replicate chewing and masticating of fresh forage better than chopping (Waghorn and Caradus, 1994; Barrell *et al*., 2000). Waghorn and Caradus (1994) developed a method of mincing fresh forages that had a particle size distribution similar to material chewed and swallowed by grazing sheep and cattle (Table 2.8). Initially mincing involved the use of hand-operated mincers, requiring the same operator to avoid effects of technique (cutting vs. squeezing through the sieve plates) but improved understanding enabled a mechanised mincer to be used (to avoid operator effects).

Barrell *et al*. (2000) compared freeze-drying and grinding (FDG), chopping or mincing of ryegrass, white clover and *Lotus corniculatus* and found that the preparation affected the ranking of the forages, in terms of digestion losses (*in sacco*), NH_3 and VFA production (*in vitro*). They suggested mincing was an ideal preparation for fresh forages because particle size distribution was similar to chewed forage of cows and sheep and there were no lag periods during incubations, in contrast to chopped and FDG preparations of the same forage. McNabb *et al*. (1996) compared freshly minced and conventional FDG *Lotus pedunculatus in sacco* and *in vitro*. Mincing fresh *Lotus pedunculatus* was more representative of chewed *Lotus pedunculatus* and rumen contents than the FDG *Lotus pedunculatus*, and was more suitable for the evaluation of protein solubilization and degradation of fresh forages.

TABLE 2.8. Particle size distribution of DM in boli (chewed during eating) or rumen contents of sheep[1, 2, 3, 5] and cows[4] compared to average particle size of minced grasses, legumes and minced forage[5]. Data are % DM retained on sieves with aperture sizes (sides of square hole) as indicated.

			Sieve aperture size			
	Forage	Source	> 4 mm	1 + 2 mm	0.5 + 0.25 mm	< 0.25 mm[a]
1	White clover	Chewed boli	19	11	13	56
	Red clover	Chewed boli	14	8	14	64
2	White clover	Boli	33	18	9	40
	White clover	Rumen digesta	11	4	14	72
	Young ryegrass	Boli	42	11	7	39
	Young ryegrass	Rumen digesta	14	3	15	68
	Immature ryegrass	Boli	23	22	18	36
	Immature ryegrass	Rumen digesta	9	10	39	41
3[b]	Ryegrass	Boli	36.6	14.8	4.5	48.6
	Red clover	Boli	26.8	21.6	5.4	37.6
	Lucerne	Boli	33.8	20.8	4.7	31.9
	Lucerne hay	Boli	19.2	40.1	7.6	22.9

4	Forage	Source	4 – 2 mm	1 mm	0.5 + 0.25 mm	< 0.25 mm[a]
	Red clover	Rumen digesta	19.5	5.2	21.0	54.3
	Lucerne	Rumen digesta	24.9	11.2	24.2	39.7
	Lucerne hay	Rumen digesta	25.5	15.7	23.3	35.5

5[c]		4 mm	2 mm	1 mm	0.5 mm	0.25 mm	Residue	Soluble
	Chewed	15	6	5	6	8	20	40
	Minced	13	10	14	8	6	3	46

[1] Waghorn and Shelton, 1988; [2] Dellow *et al*., unpublished; [3] Ulyatt *et al*., 1986; [4] Waghorn, 1986; [5] Barrell *et al*., 2000.

[a] Residues + solubles.

[b] Material passing the 0.5 mm sieve (7.3 – 10.3% of total) was divided equally between the 0.5 + 025 mm and < 0.25 mm pools.

[c] Particle size distribution (expressed on a DM% basis) of chewed fresh forage and rumen contents of sheep fed fresh ryegrass and white clover as separate diets (chewed) and that obtained by mincing ryegrass, white clover and *Lotus corniculatus* through a sieve plate with 12 mm holes (minced).

2.7.1.3 Diet effects

The diet fed to the recipient animal will affect the rate and extent of degradation of the *in sacco* sample because the diet influences the rumen microflora (Nocek, 1988; Weiss, 1994; Weimer *et al*., 1999), but results have been inconsistent. Generally when a relative ranking of forages is required, the type of forage fed to the recipient animal is probably not important, but best results are probably obtained when the recipient animal is fed a similar diet to the forage being tested (SCA, 1990; AFRC, 1992; Weiss, 1994; Weimer *et al*., 1999).

The basal diet of the recipient animal should meet the nitrogen and energy requirements of the microflora and include long fibre to abrade the bag and maintain a flux between the rumen contents and the bag interior (Huntington and Givens, 1995). This is particularly important when maize is a major component of the basal diet because the pores of the bags can be blocked by bacterial slime. The abrasive action between the bag surface and fibrous material of high forage diets could help prevent blockage and improve rumen fluid flux across the bag (Weakley *et al*. 1983).

2.7.1.4 Animal effects

The *in sacco* digestion technique has been utilised mainly in cattle and sheep. Differences between species of mature ruminants appear to be small, although rates of degradation tend to be higher in sheep than cattle, possibly due to shorter rumen retention time and outflow rates (Poppi, 1980, 1981; Rees and Little, 1980; Ha and Kennelly, 1984; Prigge *et al*., 1984), even though *in sacco* digestion is a function of the microflora and not influenced by chewing. As well as reported effects of sex and physiological state on *in sacco* degradability (Huntington and Givens, 1995), there are substantial differences between individual animals when given the same diet (Waghorn and Caradus, 1994; Weimer *et al*., 1999). Between animal variation is often masked by using several animals and presenting results as group means. It is important to use animals at a similar physiological state for *in sacco* incubations and to use a basal diet that is similar to the feed being tested.

2.7.1.5 Rumen techniques for incubation

Bags should be placed in the rumen so they are squeezed during muscular contractions facilitating fluid exchange between the internal environment of the bag and the rumen (Strizler *et al*., 1990). Generally bags are weighted and attached to the cannula with about 60 cm of cord (Strizler *et al*., 1990; Huntington and Givens, 1995). Weighting *in sacco* bags is essential to prevent flotation, but the bag is likely to be caught in the raft of solid material for some of the time.

Digestion rates can also be influenced by bag removal sequence. Most often all bags are placed in the rumen at the same time, and removed at various times over 72 hours. Alternatively, bags can be placed into the rumen at various times and all bags removed at the same time (Weiss, 1994). The procedure is primarily a matter of individual choice, often affected by post-removal processing. Some researchers freeze bags upon removal for washing as a single group at a later date. Paine *et al*. (1982) found no difference between methods for rate or extent of *in sacco* DM disappearance of forages.

When feeding is intermittent as in New Zealand dairying systems, conditions in the rumen show a definite diurnal pattern which may affect the rate of digestion. Therefore, under grazing conditions it may be best to place bags in the rumen at the time of feeding so the diurnal pattern exerts its effects on all bags. When bags are placed into the rumen of grazing cows at different times, the diurnal pattern may exert its effects differently on different bags resulting in an inaccurate pattern of degradation. The effect of the diurnal pattern is reduced when animals are fed more frequently which is characteristic of cows fed TMR in the feedlot situation.

2.7.1.6 Post incubation techniques

Post incubation rinsing is required to remove all rumen digesta that has entered the bag leaving residues of the incubated plant material. (Huntington and Givens, 1995). However, the washing technique can be a source of variation that affects the quantity of residue remaining after incubation (Mehrez and Orskov, 1977; Weakley *et al*., 1983). For example, unsatisfactory washing will elevate the quantity of residue remaining and affect assessment of forage degradability. Washing methods include rinsing bags in running warm water until the solution is clear (Cherney *et al.*, 1990; Huntington and Givens, 1995), or using a household washing machine. The first method is subjective (Weiss, 1994), but Cherney *et al.* (1990) found that machine rinsing with cold water for five minutes resulted in excessive DM losses and a two-minute wash was a more appropriate alternative to hand rinsing. However, differences between washing techniques were small, but there is a need for a standardised post incubation washing procedure. Some researchers have indicated a need to halt microbial degradation by placing bags in iced water (Aerts *et al.*, 1977; Ehle *et al.*, 1982), ethanol (Lusk *et al.*, 1962; McManus *et al.*, 1972) or acid-pepsin (Cottyn *et al*, 1986) but exposure to aerobic conditions and either refrigeration or drying at 60°C will also halt fermentation.

2.7.1.7 Microbial contamination

Bacterial attachment to particles within bags can inflate the amount of DM and other components (eg. N) and underestimate the extent and rate of digestion. The degree of contamination is affected by substrates with high fibre forages having greater microbial contamination than concentrates (Nocek and Grant, 1987). Microbial matter can account for 10 – 20% of the DM found in bags (Olubokokun *et al.*, 1990). There is confusion as to whether the extent of microbial contamination increases linearly with time of incubation (Mathers and Aitchison, 1981), or increases at a decreasing rate with time of incubation (Nocek and Grant, 1987). Nocek and Grant (1987) suggested that bacteria attach to the forage particles, but as the incubation proceeds particles get smaller resulting in less sites for bacteria to attach to. Despite not knowing how microbial contamination occurs during an *in sacco* incubation, researchers must be aware that it may be significantly contributing to the extent and rate of digestion of a feed, particularly a roughage. Therefore, researchers may need to use appropriate microbial markers (not discussed in this review) to evaluate the extent of microbial contamination. However, using inappropriate markers may create more error than not correcting for microbial contamination (Broderick and Merchen, 1992).

2.7.1.8 Statistical analyses

Variation among bags, animals and time, as well as differences between procedures used for *in sacco* incubations affect the amount and types of replication needed.

However, researchers place different emphasis on the source of variation. Mehrez and Orskov (1977) using barley and Michalet-Doreau and Ould-Bah (1992) using dehydrated lucerne as substrates have shown that the variation between *in sacco* degradation kinetics was greatest between animals, followed by between-day variation, with between-bag variation being the least important source of variation. However, Van der Koelen *et al.* (1992) using TMR as the substrate, reported that between bag variation was most important and among-animal variation was least important. Incubations should be replicated over animals, time and within animals and time to account for all sources of variation (Weiss, 1994). Most researchers remove between two and four bags for each treatment at each measurement time, but the number of replications is dependent on the total number of bags being incubated. The use of standard feeds with known and consistent disappearance has been suggested as a means of reducing variation among animals and time periods (Ayres, 1991).

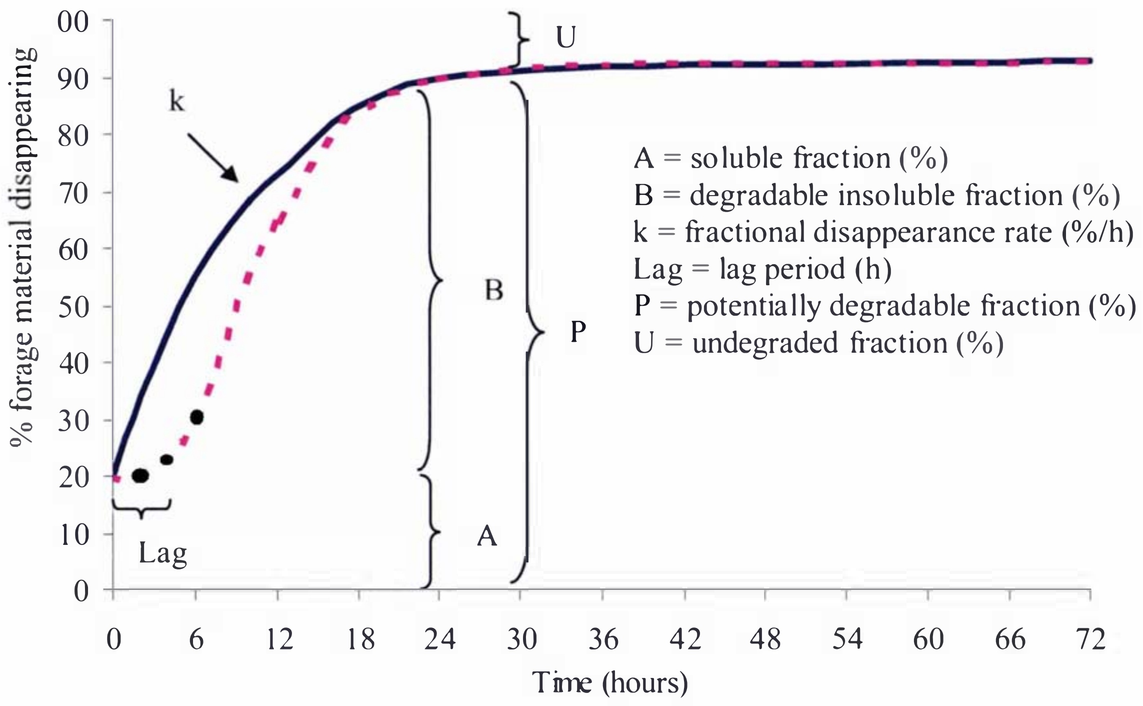
When designing experiments, it is critical that the appropriate number of time points are used to detect an observable lag time which is the period when either no digestion occurs, or digestion occurs at a greatly reduced rate (McDonald, 1981); to detect rate of degradation; and to detect an end point of digestion. For most concentrate substrates, 48 hours of incubation is appropriate to detect a ruminal digestion end-point but forages require 72 to 96 hours (Nocek, 1988).

2.7.1.9 Model fitting

Data obtained from *in sacco* incubations can be plotted against time and mathematical models can be fitted to the curves to explain the data (Figure 2.1). Several mathematical models have been used to describe the digestion kinetics of feed fractions (Lopez *et al.*, 1999). Typically digestion kinetics are explained by first order kinetics which includes a fraction that is immediately soluble (and degradable) in the rumen (A), a fraction that is slowly degradable (B), and a third fraction that is insoluble and not degradable (U) (Equation 2.1, Figure 2.1). The rate that the slowly degradable fraction is degraded can also be predicted (k). Together this information can predict the potential degradability of the feed (P). Orskov and McDonald (1979) recommend that the effect of rumen outflow rate be taken into consideration when calculating degradability and this is known as effective degradability (ED) of the feed sample

(Equation 2.2). A lag parameter may also need to be fitted into the model and is the period during which either no digestion occurs, or digestion occurs at a greatly reduced rate (McDonald, 1981). The lag period is substrate specific and is dependent upon the accurate determination of losses of nutrients from the bag at zero time, and sufficient sample times at the start of the incubation (Equation 2.3, Figure 2.1). McDonald (1981) and Sinclair *et al*. (1993) used equations that include a lag phase to predict ED for feed components, but for the purposes of this thesis ED has been estimated without including the effect of lag. NRC (2001) did not include a lag phase when estimating ED and because cows are continuously eating it is assumed that the rumen is always in a state of digestibility that a lag phase does not exist.

FIGURE 2.1. Stylised disappearance curve for dry matter, protein or the fibre component of feed that does not have a lag period (solid curve) and feed that has a lag period (dotted curve).



Equation 2.1.

Potential degradation of dry matter, crude protein, fibre

$$P_{\mathrm{DM;\ CP;\ Fibre}} = A + B\,(1\text{-}e^{-k\,(t)})$$

Where;

$P_{\mathrm{DM;\ CP;\ Fibre}}$ = potential degradation of the nutrient at time t (%)

A = soluble DM (%DM),

B = degradable insoluble (%DM),

k = fractional degradation rate per hour of the B fraction (%/h),

t = incubation time (h)

(Orskov and McDonald, 1979)

Equation 2.2. Includes passage rate

$$\text{Effective degradability} = A + [(B*k)/(k + C)]\,(1\text{-}e^{-(k+c)t})$$

Where; C = passage rate out of the rumen

(Sinclair *et al*., 1993)

Equation 2.3. Includes a lag phase:

$$P_{DM;\,CP;\,Fibre} = A + B\,(1 - e^{-k\,(t - Lag)})$$

Where; Lag = lag time (h)

(Kolver *et al.*, 1998b)

2.7.2 *In vitro* incubations

Weiss (1994) summarised early *in vitro* techniques to measure DM disappearance of a feed by incubation in the presence of ruminal contents and a buffer solution under anaerobic conditions, but the principles can also be applied to measure other nutrient (eg. protein) digestion and fermentation. Development of a suitable buffer (McDougall, 1948) based on the mineral composition of sheep saliva allowed long term *in vitro* incubations to become possible. Several different *in vitro* systems have been developed (Johnson, 1963), but all must have the following criteria for evaluation (Warner, 1956):

1. The maintenance of a normal microbiological population.
2. The maintenance of normal digestion rates.
3. The ability to predict *in vivo* results.

In this thesis *in vitro* techniques have been used to measure the products of digestion (VFA and NH_3) over time. The principles of the following techniques can be applied when measuring DM digestibility, protein or fibre degradation, and products of fermentation (NH_3, VFA, microbial growth).

The Tilley and Terry method

Tilley and Terry (1963) developed an incubation procedure that gave a high correlation between *in vivo* and *in vitro* DM. Their two stage method combines rumen and enzyme protein digestion by firstly incubating a finely ground sample for 48 hours with buffered rumen liquor in a screw cap tube under anaerobic conditions, followed by 48 hours of digestion with pepsin in acid solution. The insoluble residue is filtered, dried and combusted to give an estimate of digestible DM. This method agrees with *in vivo* values for DM digestibility (Van Soest, 1994), but the traditional Tilley and Terry method does not provide information on the kinetics of forage digestion. However, the method can be modified to determine the rate of digestion by using replicates sampled at different time intervals (Getachew *et al*., 1998)

Enzymatic methods

Enzymatic digestion techniques have been developed to overcome the problems associated with biological *in vitro* assays (eg. the requirement for cannulated animals and variation in the activity of rumen fluid; Weiss, 1994). The assays use commercial enzymes in place of micro-organisms (Jones and Hayward, 1973, 1975; McQueen and Van Soest, 1975; De Boever *et al*., 1986, 1988) to estimate the digestibility of forages. Enzymes are routinely used to determine end-point digestibility, but do not provide kinetic information. As with sources of rumen inoculum the source of cellulase can influence the digestibility data, but values can be obtained which are highly correlated with *in vivo* digestibility (Weiss, 1994).

Actual digestibility values obtained using enzymes are often much lower than *in vitro* procedures using rumen fluid and *in vivo* digestion, so regression equations are used to predict digestibility from enzymatic disappearance. The regression coefficients are affected by forage species (Dowman and Collins, 1982; De Boever *et al*., 1988), method of pre-treatment (Davis *et al*., 1990), and source of enzyme (Clark and Beard, 1977; Dowman and Collins, 1982). Pre-treatment of forages with pepsin-HCl or neutral detergent solution prior to cellulase treatment increases DM disappearance probably allowing the enzyme to have greater access to cell wall components (Weiss, 1994).

Gas production methods

The gas production technique can predict the digestibility and energy value of a wide range of feeds because of a high correlation between *in vitro* gas production and *in vivo* apparent digestibility. The gas production method determines the kinetics of degradation based on the amount of gas released from a buffered rumen fluid during fermentation of a feedstuff over time (Menke *et al*., 1979). When a feedstuff is incubated with buffered rumen fluid, carbon dioxide is produced from the direct fermentation of the carbohydrates to VFA (acetate, propionate and butyrate), and through acidification of the buffer (Beuvink and Spoelstra, 1992). Gas production from protein fermentation is relatively small compared to carbohydrate fermentation (Wolin, 1960), and there is negligible production from fat (Getachew *et al*., 1998). The amount of gas produced during fermentation of carbohydrate is related to the proportion of the carbohydrate going through each of the fermentation pathways described in Table 2.9 for glucose. Depending on the fermentation pathway, 1 g of glucose results in 267 mL of gas when only propionic acid is produced, 400 mL of gas when only butyric acid is produced, and 533 mL gas when only acetic acid is formed (Cone *et al.,* 1994). Diets high in rapidly fermentable carbohydrates (RFC; grain based) yield relatively more propionate relative to acetate compared with high fibre diets that yield more acetate relative to propionate (Orskov and Ryle, 1990), therefore there would be less gas produced from fermentation of grain-based feeds and more from the fermentation of high fibre diets. Therefore, the amount of gas produced can reflect the composition of the VFA produced, but VFA analysis still needs to occur at the completion of or during the incubation which is a disadvantage of the gas production method.

TABLE 2.9. Direct and indirect gas production from glucose fermented to different acidic endpoints.

Gas production from substrate

$$\text{Glucose} + 2H_2O \longrightarrow 2\text{ acetic acid} + 2\,CO_2 + 8H$$
$$\text{Glucose} + 8H \longrightarrow 2\text{ propionic acid} + 2H_2O$$
$$\text{Glucose} \longrightarrow 1\text{ butyric acid} + 2CO_2 + 4H$$
$$8H + CO_2 \longrightarrow CH_4 + 2H_20$$

Gas production from acidification of buffer

$$H+ + HCO_3- \longrightarrow H_2CO_3 \longrightarrow CO_2 + H_2O$$

The advantage of gas production over the Tilley and Terry (1963) *in vitro* method is it provides information on the digestion kinetics of the substrate being fermented. Kinetics can only be obtained from Tilley and Terry digestion by using replicates sampled at different time intervals.

Gas measurement focuses on the appearance of fermentation products, whereas other *in vitro* methods are based on gravimetric measurements which follow disappearance of the substrate (components which may or may not contribute to fermentation; Getachew *et al*., 1998). Gas is measured either at atmospheric pressure and its volume determined directly, or accumulated in a fixed volume container, so that the volume is calculated from pressure changes.

The available gas measuring techniques are:

a. Hohenheim gas method or Menke's method (Menke *et al*., 1979);
This *in vitro* gas measuring technique is conducted in large (100 mL) calibrated glass syringes containing the feedstuff (~0.2 g DM) and a buffered rumen fluid (Menke *et al*., 1979).

b. Liquid displacement system (Beuvink *et al*., 1992);
Feed is incubated with buffered rumen fluid in fermentation bottles and connected to a water displacement system. The amount of liquid displaced by the gas is collected and weighed to determine gas production.

c. Manometric method (Waghorn and Stafford, 1993);
This technique measures gas production from buffered feed samples and rumen liquor in a manometric measuring device which enables gas volumes to be measured at atmospheric pressure.

d. Pressure transducer systems: manual (Thedorou *et al*., 1994), computerised (Pell and Schofield, 1993) or a combination of pressure transducer and gas released systems (Cone *et al*, 1994, 1996).

Continuous culture systems (steady state systems)

Continuous culture systems have been developed as a means of studying rumen microbial metabolism under conditions which more closely approximate *in vivo* fermentation than closed vessel incubations. The system (Hoover *et al*. (1976a, b; Hannah *et al*., 1986) was designed to simulate differential flows of liquids and solids, and environmental conditions (eg. pH and temperature) to examine rumen fermentation of various substrates. They provide a reasonable estimate of rumen fermentation without the cost and labour of *in vivo* experiments (Hannah *et al.*, 1986; Mansfield *et al*, 1995; De Veth and Kolver, 2001a, b). A typical continuous culture system consists of a glass vessel with inputs of feed and buffer and one output orifice through which flows a homogenous mixture of solids, liquids, fermentation products and micro-organisms (Hoover *et al.*, 1976a) with variations to the basic structure, liquid and solid retention times, pH, temperature, mixing, and others variables, depending on the research being conducted. However, these systems are expensive.

Protein degradation methods

In vitro techniques have been used to measure protein degradation of a feed by measuring the amount of NH_3 produced when the feed is incubated under *in vitro* conditions with rumen fluid (Little *et al.*, 1963; Broderick, 1978; Raab *et al.*, 1983). Non-ammonia nitrogen is the primary end-product of rumen proteolysis, therefore the concentration of NH_3 present in the *in vitro* apparatus can be used to calculate rate and extent of protein degradation of the feed (Broderick, 1978; Michalet-Doreau and Ould-Bah, 1992). Numerous parameters can influence the production of NH_3 from a feed, such as the nature of the substrate, the incubation time, and the addition of a buffer.

The concentration of NH_3 is a product of both protein degradation and utilisation of NH_3-N by the rumen microbes, which may be as much as 10% of white clover N (Barrell *et al.*, 2000). The concentration and type of carbohydrate can affect microbial NH_3-N utilisation (Broderick, 1978). Feeds with high concentrations of RFC and low CP concentration (eg. maize silage) have low or negative rates of NH_3 release and protein degradation because more of the NH_3-N is utilised by rumen microbes than is being released (Burke *et al.*, 2000). Attempts have been made to suppress the microbial uptake of released NH_3 by inhibiting microbial synthesis (Broderick, 1978), or controls have been used to take into account the NH_3 taken up by microbes (Raab *et al.*, 1983).

The amount of NH_3 produced depends on the length of the *in vitro* incubation. Microbial activity rapidly decreases due to the difficulty in maintaining anaerobic conditions for long periods, the accumulation of end-products of degradation or the decline of pH. Buffer solutions have been added to help maintain *in vitro* pH, as discussed below.

2.7.2.1 Preparation of feed sample

Guidelines for preparing forages for *in vitro* digestion are similar to those for *in sacco* digestion. Freeze-dried and ground (FDG) preparations are a common method, but mincing appears to be more appropriate when fresh forage samples are being tested. Proteolysis and VFA production of chopped fresh forages (ryegrass, white clover and *Lotus corniculatus*) was reduced relative to the same forages FDG and the same forages minced (Barrell *et al*., 2000). McNabb *et al*. (1996) also showed differences between FDG *Lotus corniculatus* and minced *Lotus corniculatus* that were incubated *in vitro*.

2.7.2.2 Buffering systems

A range of buffers have been used to maintain pH near neutrality and include artificial saliva (McDougall, 1948), complete buffers with microelements and tripticase (Goering and Van Soest, 1970; USDA buffer) and citrate buffers (Grant and Mertens, 1992a, b). The buffer should maintain pH similar to *in vivo* conditions. The buffer that has been warmed and gassed is added to the substrate (also warmed and gassed) often with a reducing agent and rumen inoculum. The rumen inoculum can be prepared by collecting rumen digesta from a fistulated ruminant, homogenising the solid rumen digesta, squeezing through cheesecloth and chilling (Furchtenicht and Broderick, 1987). Homogenising ensures that the rumen inoculum contains high numbers of fibre-associated bacteria to maximise NDF and protein degradation (Craig *et al*. 1984), but some researchers do not homogenise (Waghorn and Caradus, 1994) or chill rumen inoculum (Waghorn and Caradus, 1994; Grant and Mertens, 1992a, b, c) used for *in vitro* incubations. An important difference between the methods of Furchtenicht and Broderick (1987) and Grant and Mertens (1992a, b, c) and that of Waghorn and Caradus (1994), is that the cheese cloth used by the former researchers has much smaller pores and was folded into eight layers to restrict passage of very large particles, whereas that used by Waghorn and Caradus (1994) had large pores, was double thickness and small particles were able to pass into the rumen liquor. Therefore, Waghorn and Caradus (1994) did not homogenise the rumen contents because plenty of fibre-digesting bacteria were able to pass through the cheesecloth attached to the small fibre particles.

2.7.2.3 Effect of gassing and reducing agents

Grant and Mertens (1992a) recommended that *in vitro* systems used to measure digestion kinetics should be gassed continuously with carbon dioxide to maintain an anaerobic environment, maximise rates of NDF digestion and reduce lag. However many systems rely on about 60 minutes of gassing the buffer and purging incubation flasks with carbon dioxide. Addition of a cysteine sulphide reducing agent to incubations reduced variation between replicates, reduced lag and maximised rates of fermentation and degradation (Grant and Mertens, 1992a).

2.7.2.4 Effect of pH

Ideally pH should be maintained at 6.5 to 6.8 for rumen cellulolytic microbes to maximise fibre digestion (Stewart 1977; Russell *et al.*, 1979) but production of VFA reduces buffering capacity. Typical mean rumen pH of cows fed diets of high quality pasture range between pH 5.8 – 6.4 (Van Vuuren *et al.*, 1992; Carruthers *et al.*, 1997; Mackle *et al.*, 1996; Kolver *et al.*, 1998a), which may be sub-optimal for fibre digestion. The Cornell Net Carbohydrate and Protein System (CNCPS) regards pH values of less than 6.2 to impair fibre digestion (Pitt *et al.*, 1996), but De Veth and Kolver (2001a) found that pH values less than 5.8 were detrimental to fibre digestion of high quality pasture (NDF concentration of 37.9% of the DM) in continuous culture. It is important to use correct amounts of substrate, so the pH is not reduced to unrealistically low levels with prolonged fermentation. A reduction in pH may have negative consequences on celluloytic bacteria activity (Therion *et al.*, 1982), especially if the pH goes below the critical pH value which impairs fibre digestion. For pasture this is less than 5.8, and for conserved forage and concentrate diets this less is less than 6.0 to 6.2 (Pitt et al., 1996).

2.7.2.5 Effect of rumen inoculum

Rumen bacteria, protozoa and fungi are responsible for fermenting the feed into products that are utilised by the animal for production. The interactions between the diet, animal and rumen microbes are complicated and lead to significant differences between bacterial populations of individual animals (Weimer *et al.*, 1999). Horton *et al.* (1980) reported that rumen fluid collected from sheep produced different *in vitro* DM digestibility values than rumen fluid collected from cattle, and Waghorn and Stafford (1993) reported differences between sheep and deer. For research purposes, the donor animal should be the same species as the target animal.

Populations and activities of microbes vary and are related to time of feeding and diet. Fasting will lower microbial activity and transfer of digesta to incubation vessels reduces the proportion of protozoa in the microflora (Weiss, 1994). Therefore, it is recommended that the same animal should be used as an inoculum donor for comparison and ranking of diets. It should be fed a common diet and at the same time relative to sampling to minimise differences in inoculum activity.

2.8 THE FEEDING VALUE OF FORAGES

Several forages grown in the New Zealand environment have higher feeding values than ryegrass and can be fed with ryegrass-based pasture at strategic times of the year to improve diet quality. However, most growth is during spring, summer and autumn, therefore some will have to be harvested and conserved as a silage or hay for feeding at other times of the year.

The ideal forage mixture for high performing ruminants needs to have less structural fibre than a sole diet of medium quality pasture (as previously defined in section 2.4.2) provides, and the fibre needs to be rapidly digested so as not to limit intake. The degradability of protein should be sufficient to maximise rumen microbial protein synthesis but not be in excess, so that loss of NH_3-N across the rumen is minimised. The diet should also provide sufficient microbial and undegraded dietary protein to meet the animal's metabolisable protein requirements. The concentration of RFC, either as soluble sugars and starch or rapidly degradable fibre, should be sufficient to provide energy for efficient microbial fermentation and enable the capture of a high proportion of NH_3 produced during proteolysis for microbial protein synthesis. Pasture will form the basis of mixed forage diets but fresh or conserved forages may be used to increase the dietary supply of metabolisable energy and/or protein.

Legume and herb species have characteristics that are ideal for feeding with pasture because they have low structural carbohydrate to soluble carbohydrate ratios, rapid breakdown in the rumen and passage rate out of the rumen. This should facilitate higher feed intakes and improved animal production. When ryegrass and white clover mixtures were fed, maximum milk production was obtained when white clover made up 55 – 65% of the ryegrass-based diet (Harris *et al*., 1997a, b). Other feeding trials have shown the advantages in intake and animal production from feeding forages with low concentrations of structural fibre with pasture or as sole diets (eg. sulla, chicory, white clover, turnips, red clover and *Lotus corniculatus*; Table 2.10).

Legumes that contain CT (eg. *Lotus corniculatus, Lotus pedunculatus*, sulla) may have greater potential in pasture-based systems because the CT can reduce protein degradation in the rumen and allow a greater passage of undegraded protein to the small intestine for absorption. This may be an excellent way of reducing the rumen degradation of protein in spring pasture, which is high in degradable protein, reduce the excretion of excess protein as urea through the urine and improve the efficiency of protein utilisation by the animal. Cows *fed Lotus corniculatus* have produced 51% more milk than pasture-fed cows, with 46% of the response due to the effect of CT (Woodward *et al*., 1999). CT in combination with low structural fibre may encourage higher intakes and better utilisation of dietary protein. Forages that contain CT concentrations that are detrimental to animal production may be mixed with a non-CT forage to dilute the CT effect.

Maize silage is used to supplement pasture in spring because of the high grain (40 – 50% DM) and low crude protein concentration (7 – 8% DM) in the DM. When maize silage makes up one-third of the DM and pasture two-thirds, starch from the maize silage provides rapidly fermentable energy for the microbes to capture NH_3 for growth, and the low protein content dilutes the high protein concentration of spring pasture (Kolver *et al*., 2001). The other advantage of maize silage is the high DM yields (16 – 25 t DM/ha) that can be achieved, potentially making it a more economical crop to grow and incorporate into the pasture-based system than other crops that are able to be grown in New Zealand. Achieving economical yields may be the problem with some of the other forages that have potential to provide high quality feed. For example, yields of *Lotus pedunculatus* have been reported to be between 7 – 13 t DM/ha, compared to

Lotus corniculatus and sulla that have achieved yields of 10 – 15 t DM/ha and 12 – 25 t DM/ha, respectively (Waghorn *et al.*, 1998).

In most feeding trials, the type and amount of supplement that was fed was based on filling feed deficits, but the results have been variable (Waghorn, 2002). Table 2.10 illustrates some of the responses from supplements with pasture and feeding high quality legumes to cows in the second half of lactation. In the NZ dairy system, a high energy intake is important for early lactation, and little research has focussed on feeding forages other than pasture, at this time of the year, but grain feeding benefits milk production in early lactation (Penno *et al.*, 1998). To date, most research has focused on feeding pasture supplemented with one other forage, but optimum animal production may require a mixture of several forages that have the nutrient composition and digestion characteristics that optimise the supply of metabolisable energy and/or protein and ensure efficient utilisation of consumed nutrients. Feeding trials are expensive and time consuming, therefore information obtained from *in sacco* and *in vitro* incubations will provide a scientific means to identify forage mixtures that optimise animal production using complex ration balancing models.

TABLE 2.10. Lactation responses of New Zealand cows in the second half of lactation when fed supplements with medium to low quality pasture or fed high quality legumes.

Diet	DMI (kg)	ME (MJ ME/kg diet DM)	CP (%)	NDF (%)	Milk yield (kg/day)	Milksolids yield (kg/day)	Response (g MS/ kg DM fed)	Marginal response
[1] Ryegrass + 20%WC	10.9	10.3	21.5	49.8	8.5	0.80	74	-
Ryegrass + 50% WC	12.1	10.5	22.6	45.6	10.0	0.93	77	100
Ryegrass + 80% WC	12.0	10.6	23.8	41.2	9.8	0.93	77	111
[2] Ryegrass	12.1	9.5	14.3	61.8	10.2	0.96	79	-
Ryegrass + 25% WC	13.1	10.1	16.4	52.6	12.5	1.17	90	216
Ryegrass + 50% WC	14.8	10.5	18.4	47.7	13.6	1.24	84	102
Ryegrass + 75% WC	15.8	10.7	21.9	40.4	13.7	1.26	82	90
[3] Pasture	-	9.7	24.3	48.5	10.4	0.87	-	-
Pasture + turnips	-	9.8	22.6	43.2	11.3	0.99	34	30
Pasture + chicory	-	9.9	24.0	42.8	10.8	0.93	32	15
[4] Pasture	9.6	10	19.7	55.9	8.6	0.73	76	-
Pasture + turnips	12.1	10.7	17.1	44.8	10.6	0.93	82	80
Pasture + sorghum	11.0	9.7	17.4	62.1	9.2	0.82	80	64
[5] Pasture	12.4	10.4	11.6	53.2	12.4	0.93	75	-
Grass + 75% WC	15.0	11.3	19.1	36.8	16.6	1.26	84	146
Grass + 75% *Lotus C.*	13.8	11.8	20.8	25.0	18.3	1.38	100	348
[6] Ryegrass	14.2	10.6	18.2	52.9	10.0	0.83	59	-
Lotus C. – CT	16.7	11.4	25.6	30.4	13.8	1.13	68	120
Lotus C.+ CT	16.8	11.4	25.6	30.4	16.5	1.40	83	219
[7] Pasture (restricted)	12.5	10.0	17.4	47.5	13.2	1.00	80	-
Pasture (full)	18.5	10.1	18	45.3	17.0	1.11	70	48
Pasture + PS	17.0	10.3	16.9	46.8	14.3	1.11	65	24
Pasture + M	16.6	10.1	14.4	44.5	13.7	1.12	68	29
Pasture + *Lotus* CT.	17.2	10.3	19.1	35.5	13.7	1.29	75	62
Pasture + SS	15.7	10.0	16.7	50.8	13.7	1.10	70	31
[8] Pasture (rest)	10.4	10.6	21.5	44.1	13.2	0.99	95	-
Pasture (full)	15.7	10.7	21.7	43.9	17.2	1.30	83	95
Pasture + M + SS (60:25:15)	14.6	10.5	16.5	45.1	14.3	1.02	70	7
Pasture + SS + M (60:25:15)	14.4	10.6	18	45.8	13.7	1.00	70	2
Pasture + SS (60:40)	13.9	10.6	18.8	47.9	13.7	0.98	71	12
Pasture + M (60:40)	14.3	10.6	15.4	43.0	13.7	1.01	70	3
Mean							**74**	**85**
Range							**32 – 100**	**3 – 348**

Abbreviations: DMI, DM intake; ME, metabolisable energy, CP, crude protein concentration (% of DM); NDF, neutral detergent fibre concentration (% of DM); MS, milksolids yield includes fat and protein yield; WC, white clover; *Lotus C*, *Lotus corniculatus*; M, maize silage; SS, sulla silage; *Lotus* CT, *Lotus corniculatus* silage; CT, condensed tannin (– CT indicates inactivation by daily administration of polyethylene glycol).

[1] Harris *et al.* (1997a): *ad lib* feeding indoors; [2] Harris *et al.* (1997b): *ad lib* grazing; [3] Waugh *et al.* (1998): 4 – 8 kg DM supplement + 25 kg DM pasture offered/cow; [4] Clark *et al.* (1997): 4 kg supplement + 25 kg DM pasture offered/cow; [5] Harris *et al.* (1998): *ad lib grazing*; [6] Woodward *et al.* (1999): indoor feeding; [7] Woodward *et al.* (2002): 5kg DM supplement + 25 kg DM pasture allowance/cow; [8] Chaves *et al.* (2002c): 18 kg DM pasture allowance.

2.9 SIMULATION MODELLING

Simulation models can be used to predict requirements and feed utilisation by ruminants and should integrate knowledge of feed, intake, digestion and passage rates in relation to feed composition, energy concentration, digestion and escape of dietary protein and microbial growth (Fox and Barry, 1994). Models have been developed for ration balancing (eg. Camdairy) that are empirical rather than causative models, and are often not interactive or user friendly. In empirical models, relationships between components are described by functions that best fit the data. They may be algebraic or statistical (eg. regression). In contrast, relationships in mechanistic models are described by functions representing the actual biophysical processes. For example, in mechanistic models, milk production is a function of metabolites delivered to the mammary gland, rather than a function of DM intake (Wastney *et al.* 2002). Models used for ruminal studies, including MOLLY (Baldwin, 1995) and Dijkstra's rumen model (Dijkstra *et al.*, 1992), are mechanistic, more complex, difficult to use and are not designed for interpreting cow production in relation to feed characteristics. The Dexcel whole farm system model (WFM) has attempted to link multiple aspects (animals, pastures or crops, soil nutrients, climate, management and economics) into a farm systems model (Sherlock *et al.*, 1997). The WFM was developed as a research tool to relate pasture management (McCall pasture model, McCall, 1994) together with cow metabolism (MOLLY; Baldwin, 1995), contain the latest research information, and represent any realistic farm management scenario (Wastney *et al.*, 2002).

The prediction of ruminal fermentation of feeds with different characteristics is much better understood than the metabolism of nutrients in many models (SCA, 1990; AFRC, 1993). The Cornell Net Carbohydrate and Protein System (CNCPS) was developed to evaluate the diets of cattle by predicting nutrient requirements and supply with wide variations in animal, feed and environmental conditions (Fox *et al.*, 1995). It uses mechanistic and empirical relationships to predicts the metabolisable energy (ME) and protein (MP) requirements of cattle and the supply of these components from the diet using the ruminal digestion kinetics of diets (Kolver *et al.*, 1998a). The equations and validation for the CNCPS model have been published by Russell *et al.* (1992), Sniffen *et al.* (1992), Fox *et al.* (1992), O'Connor *et al.* (1993), Pitt *et al.* (1996), Tylutki and

Fox (1997) and Kolver *et al*., (1998a). Diets based on pasture have been evaluated using the CNCPS and it was concluded that the model can be used in a grazing system to make realistic predictions of production (Kolver *et al*., 1998a). However, the CNCPS is generally designed to predict nutrient supply from a given feed intake and it does not satisfactorily estimate intake (Kolver *et al*., 1998a) or the partitioning of energy and protein between the competing demands for milk production and liveweight change (Chaves *et al*., 2003). St-Pierre and Thraen (1999) highlighted that the CNCPS is a requirement system, not a response system. It calculates the nutrients required to support a given level of milk production and composition. Milk production is an input and is used to estimate DM intake, but constraints of digesta clearance from the rumen and the ability of cows to convert body reserves into milk may account for the poor DM intake predictions.

The CNCPS model offers good potential for predicting responses (eg. milk yield) to forage mixtures, but its inability to account for partitioning of nutrients between milk yield and liveweight gain and its inability to estimate potential intake of the feed is a major limitation of the model. Despite these limitations the CNCPS V5.0 (Fox *et al*., 2003) will be used in this thesis with the digestion kinetic data collected from *in sacco* and *in vitro* incubations to evaluate and identify forages that could be combined to improve nutrient supply and milk production at a given intake.

2.10 CONCLUSIONS

Dairy cow production is affected by feed quality and availability. The nutrient composition of ryegrass-based pastures vary considerably within a year and between years and at certain times of the year will not meet cow nutrient requirements. There are alternative forages with characteristics that when combined with ryegrass-based pastures will increase and balance the supply of metabolisable energy and protein to the animal, but very little is understood about the digestion and fermentation and likely production response when some of these forages are combined and fed. The agronomic characteristics and production response of some of the potential forages have been examined as individual diets but not as mixtures, and it might be as mixtures that they have there greatest benefit. Rather than conduct several individual animal experiments to determine the effect of the infinite number of forages and mixtures that could have potential *in sacco* and *in vitro* methods and modelling will be used. These methods will identify possible forages for feeding with ryegrass-based pastures and animal experiments, together with *in sacco* and *in vitro* studies and modelling will be conducted to support the use of forages in a ryegrass-based pasture system.

CHAPTER 3

DIGESTION KINETICS OF CONTRASTING FORAGE SPECIES

CHAPTER 3: DIGESTION KINETICS OF CONTRASTING FORAGE SPECIES

3.1 ABSTRACT

The work described here is the first step in formulating mixed forage rations for dairy cows, based on chemical composition and digestion kinetics derived from *in sacco* and *in vitro* incubations with interpretations using the CNCPS mechanistic nutrition model. Kinetic information for dry matter (DM), crude protein (CP) and fibre was obtained from 23 contrasting fresh and conserved forages species, including temperate and tropical grass species, legumes (including species containing condensed tannins), herbs, silages, lucerne hay and maize grain. All forages were minced to a particle size similar to chewed material. *In sacco* incubations were used to determine the degradation parameters of contrasting forages in terms of soluble (A) and degradable (B) fractions, degradation rates (k) and lag periods of forage components. All components of legumes and herbs had faster degradation rates (%/h) and shorter lag periods compared to temperate and tropical grasses. Estimates of DM degradation ranged from 4.2 to 33.2 %/h, with rates for plantain, white clover, perennial ryegrass, kikuyu and maize silage of 33.2, 21.1, 10.6, 7.1 and 4.2 %/h, respectively. Protein and fibre degradation rates were of a similar magnitude for each of these forages but the presence of CT reduced the losses of protein to ammonia, relative to comparable legumes. A major effect of feed type was the extent of the lag period associated with DM degradation from about 1.5 hours for chicory, plantain, white clover, lucerne, red clover, *Lotus corniculatus* and sulla to between 4.0 – 5.0 hours for perennial ryegrass, cocksfoot, yorkshire fog, and tama ryegrass and 7.8 hours for kikuyu. Products of fermentation (ammonia and volatile fatty acids) were measured by *in vitro* incubations. After 6 h of *in vitro* incubation there was a five-fold difference between forages in net ammonia (NH_3) release, and by 24 hours the difference had increased further. For low protein forages (eg. maize silage, maize grain, kikuyu and paspalum) all NH_3 released had been utilised after 6 hours, but concentration for legumes had increased to more than 40% of forage-nitrogen after 24 hours. There were 2–3 fold differences in volatile fatty acid (VFA) yield and a two-fold difference in the acetate:propionate ratio after 24 hours of

incubation across forage types. The data were entered into the CNCPS as individual forages and as mixtures, mostly based on pasture with other forages. The model indicated whether metabolisable energy or protein were first limiting for milk production in cows at defined levels of intake. The information provided a nutritional basis for mixed forage ration formulations to optimise the nutrient supply for high producing dairy cows, especially to complement perennial ryegrass pasture on the basis of their digestion and fermentation characteristics.

3.2 INTRODUCTION

The New Zealand dairy industry is a low-cost pasture-based system, but the ryegrass-dominant diet has nutritional constraints which limit animal production. Kolver and Moller (1998) have demonstrated that 61% of the difference in milk production of cows grazing high quality spring pasture (with a composition (% of DM) of 25% CP, 43% NDF and 19.3 % total non structural carbohydrates) compared to TMR, was due to the lower DM intake of pasture. These authors used the NRC (2000) feeding standards and the Cornell Net Carbohydrate and Protein System (CNCPS) to show that energy intake was first limiting for production and the lower proportion of dietary N appearing as milk N incurred a greater metabolic cost for urea synthesis compared to cows fed TMR.

There are alternative fresh and conserved forages able to be grown in New Zealand which have nutritive characteristics that are superior to many ryegrass pastures. This has been demonstrated in cattle and sheep fed legume-based diets, such as lucerne (*Medicago sativa*), white clover (*Trifolium repens*), *Lotus corniculatus* (birdsfoot trefoil), *Lotus pedunculatus* (lotus) and *Hedysarum coronarium* (sulla) in comparison with ryegrass-based pastures (Ulyatt, 1981; Brown, 1990; Terrill *et al*., 1992a; Woodward *et al*., 1999). Legumes and other forages may be fed with ryegrass to improve animal production.

In North America, where concentrates are relatively inexpensive, nutrient balancing is used to formulate total mixed rations (TMR) for high producing dairy cows. The same approach could be adopted in New Zealand using combinations of available forages with diverse chemical and structural characteristics. However, there is insufficient information concerning the digestion kinetics of fresh forages to formulate mixed forage-based rations.

Several methods are available for predicting nutritive value, each with their own advantages and disadvantages. Conventional feed evaluation to obtain estimates of digestibility, together with animal production, is expensive and labour intensive. Chemical analyses of feeds determine nutrient composition (eg. Corson *et al.*, 1999), but do not predict animal production, therefore alternative procedures for estimating nutritive value and digestion kinetics (Section 2.7) have been used. These techniques include *in sacco* (placing minced fresh forages in porous bags in the rumen) and *in vitro* (incubating minced fresh forages with rumen inoculum) methods. These two procedures enable digestion to be evaluated in terms of both rates of disappearance *in sacco* and products of digestion *in vitro* (ammonia, NH_3; and volatile fatty acids, VFA) to provide information about the kinetics of digestion, fermentation products and the nutritive value of feeds. Together this information could be used to formulate forage mixtures that supply optimum concentrations of nutrients to the animal.

The objective of this study was to define the digestion and fermentation kinetics of a diverse range of fresh and conserved forages to formulate mixed forage rations best able to meet the nutritional requirements of high producing dairy cows in New Zealand.

3.3 METHOD

3.3.1 Experimental procedure

Digestion kinetics using *in sacco* and *in vitro* laboratory techniques were measured on 23 contrasting fresh and conserved forages comprising of eight species of grasses, five legume species (including *Lotus spp.*), two species of herbs, five types of silages, lucerne hay and maize grain (Table 3.2). Species evaluated in this study are identified by botanical and common names in Table 3.2 of this chapter, and thereafter are referred to by their common name, except *Lotus corniculatus and Lotus pedunculatus* which will be referred to by their botanical name.

3.3.2 Forage collection and processing

Fresh forages were collected (about April to May 1999) by harvesting the top leafy horizon of each sward in a vegetative state, and for conserved forages care was taken to obtain representative samples from the stack or bale. All material was frozen at –20°C immediately following collection and maintained frozen until used in incubations.

Two to three days prior to evaluation, frozen material was chopped to about 30 mm to facilitate mincing (whilst frozen) in a Kreft Compact meat mincer R70 fitted with a sieve plate with 12 mm holes (Kreft, Gmbh; Photograph 3.1). Frozen and chopped forage was passed through the mincer once and then refrozen. Mincing was found to be the most appropriate method for preparing fresh forages for *in sacco* and *in vitro* incubations (Barrell *et al*., 2000) because it resulted in a particle size distribution of the dry matter (DM) similar to chewed material (Waghorn, 1986; Waghorn *et al*., 1989), and had significantly reduced lag periods compared to freeze-dried and ground (FDG) or chopped preparations (Barrell *et al*., 2000; Cohen and Doyle, 2001). The frozen minced material was either sealed into dacron bags for *in sacco* incubation, or weighed into bottles for *in vitro* incubation. Samples of minced forage were dried at 60°C and chemical composition determined by Near InfraRed Reflectance Spectroscopy (NIRS). Lignin concentration (acid detergent lignin; ADL) of forages was determined by sequential extraction described by Chaves *et al*. (2002b). Dry matter content of the forages was determined by drying about 50 g of wet minced forage at 95°C for 24 hours and enabled the correct amount of wet minced forage to be placed into bags and bottles. Further sub-samples (100 g wet weight) of minced forage were retained to determine particle size distribution by wet sieving (Waghorn, 1986).

3.3.3 Particle size distribution

The particle size distribution of minced forage was determined using the method described by Waghorn (1986; Appendix 3.1). A wet sieving apparatus (Turner and Newall Ltd) with sieve sizes (length of side of square holes) of 4, 2, 1, 0.5 and 0.25 mm was used. Minced forage samples (30 – 50 g wet weight) were sieved by washing with 1300 mL water recirculated through the sieves at a flow rate of 4 litres/minute for 5 minutes. Material retained on the sieves was transferred by gentle washing to tared filter papers and dried at 95°C for 24 hours to determine particle dry weight.

Material that passed through all sieves was mixed, a 1-litre aliquot was taken and centrifuged at 2,000 x *g* for 20 minutes. The pellet was transferred to filter papers and dried as above. This fraction was termed 'residues'. The quantity of material not retained on sieves or as residues can be calculated 'by difference' from the sample wet weight and DM % and from the sum of recovered particulate DM fractions. This fraction was termed 'solubles'.

3.3.4 *In sacco* and *in vitro* digestion

The procedures for *in sacco* and *in vitro* incubations considered most appropriate for fresh forages fed to dairy cows in New Zealand were based on methods discussed in 2.7. *In sacco* and *in vitro* incubations (Appendix 3.2) were carried out simultaneously, with three forages evaluated during each incubation. Eight incubations were conducted over a two-month period. Previous experience has shown substantial variation between cows when measuring digestion kinetics (eg. Waghorn and Caradus, 1994; Weimer *et al*., 1999); therefore one ruminally cannulated non-lactating Holstein-Friesian cow was fed lucerne hay at maintenance for all incubations. This enabled comparison between forages without the effect of the cow or diet.

3.3.4.1 *In sacco* digestion

Approximately 5 – 6 g DM (20 – 40 g wet forage) was weighed into each 100 x 100 mm dacron bag (mean pore size 35 μm; Photograph 3.2). Ten bags of each forage were placed into the rumen and duplicate bags removed at 2, 6, 12, 24 and 72 hours. Removal of bags at each time was facilitated by putting two bags of each forage into lingerie bags (6 dacron bags/lingerie bag). Immediately after removal from the rumen, bags were hand-rinsed with cold water until no further colour appeared. Bags representing 0 hours were not placed in the rumen but were washed and handled in the same manner as the bags placed in the rumen. Bags were dried at 60°C for 48 hours, weighed and residues removed for analysis. Residues and forages were analysed by

NIRS to estimate crude protein (CP) and fibre (neutral detergent fibre, NDF; and acid detergent fibre, ADF) contents. Residues from 60 *in sacco* bags were also analysed by wet chemistry to determine CP concentration, and from 68 *in sacco* bags to determine ADF and NDF content, enabling calibration of the residue composition with the NIRS. The R^2 and standard error of the calibration equation are given in Table 3.1 and Appendix 3.3 to illustrate the relationship between wet chemistry determinants and NIR predictions of CP, ADF and NDF concentrations in dacron bag residues.

Procedures for chemical analyses were; CP by instrumental combustion using the Carlo Erba Nitrogen Analyzer (Carlo Erba Strumentazion, Milan Cable Erbadas); NDF by the method of Goering and Van Soest (1970), involving sequential extraction in neutral detergent, acid detergent and acid digestion in 72% sulphuric acid; and ADF by the AOAC (1990) method (No. 973.18). Residues following the respective extractions comprise NDF (hemicellulose, cellulose, lignin and ash), ADF (cellulose, lignin and ash) and lignin with ash. When CT was present, they were analysed by sequential extraction using butanol-HCl (Terrill *et al.*, 1992b). The CT resides in the lignin fraction following detergent and acid digestion.

TABLE 3.1. Statistics to support the accuracy of Near InfraRed Reflectance Spectroscopy (NIRS) calibration equation to estimate the composition of *in sacco* residues (% of DM).

Constituents (% of DM)	No	R^2 of the calibration equation	SE of calibration equation[1] (% of DM)	R^2 of cross validation	SE of cross validation (% of DM)[2]
Protein	60	0.99	0.54	0.91	3.00
ADF	68	0.95	1.55	0.92	1.89
NDF	68	0.97	2.54	0.99	3.00

Abbreviations: ADF, acid detergent fibre; NDF, neutral detergent fibre; SE, standard error.

[1] SE of the calibration equation is the SE between actual and predicted *in sacco* residue compositions.

[2] SE of cross validation is the SE between the actual and predicted composition of a random 10% sample of *in sacco* residues.

Two empty bags were placed into the rumen during two incubations as blanks and removed after 72 hours to measure contamination by inflow of rumen DM into bags. There was no net accumulation of DM in the bags, so this process was discontinued.

Relativity between incubation runs was determined by incubating two bags of each of the 23 feeds for 12 hours in the same cow fed lucerne hay on a separate occasion to the eight incubations. Dry matter disappearance over this time demonstrated relative losses to digestion for each forage and was compared to the DM loss of each forage incubated in the eight separate incubations. Coefficient of variation (CV; standard deviation divided by the average) between runs was calculated and values greater than 5% indicated poor repeatability.

3.3.4.2 *In vitro* digestion

About 2.5 g of freshly minced forage (approximately 0.5g DM) was weighed into 50 mL bottles (Photograph 3.3) and warmed to 39°C with 12 mL of McDougal's buffer (artificial saliva), 0.5 mL of reducing agent (cysteine sulphide) and 3 mL of strained rumen liquor (Appendix 3.2). Bottles were placed in a shaking incubator (90 oscillations/minute) for the duration of the incubation (Photograph 3.4). Triplicate bottles of each forage were removed from the incubator after 0, 2, 4, 6, 8, 10, 12 and 24 hours of incubation, pH was measured and sub-samples taken for determination of NH_3. Samples taken at 0, 6, 12 and 24 hours were analysed for VFA concentrations. pH of rumen liquor used for each incubation was measured immediately after collection and sub-samples taken to measure NH_3 and VFA concentrations (Appendix 3.4). Freeze-dried and ground (FDG) lucerne was used as a standard and included in each incubation run to monitor variation between incubations. Triplicate bottles of FDG lucerne were removed after 2 and 8 h of incubation. pH of the *in vitro* media was measured and sub-samples taken to determine NH_3 and VFA concentrations.

3.3.4.3 pH, ammonia and VFA analyses

The pH of strained rumen liquor and *in vitro* incubation media was measured using a MeterLab® (PHM210, Radiometer Pacific Limited, Copenhagen). The meter was recalibrated immediately prior to each set of measurements (Appendix 3.4).

For NH_3 determination, sub-samples (1 mL) were taken from each *in vitro* bottle at each time and from strained rumen liquor, acidified with 15 μL of concentrated hydrochloric acid (HCl), mixed and centrifuged (14,000 x *g*; 15 minutes; HermleZ160M). The supernatant was transferred and frozen for analysis of NH_3 concentration by the colourimetric method described by Chaney and Marbach (1962) (Appendix 3.5). Ammonia concentrations from *in vitro* incubations were corrected for concentrations in rumen inocula and have been expressed in terms of forage nitrogen (N) incubated. Absolute concentrations of NH_3 (corrected for NH_3 concentration in rumen inocula, mmol/L) are presented in Appendix 3.10.

For VFA determination, two sub-samples (1.5 mL) of strained rumen liquor and incubation media were combined from triplicate *in vitro* bottles and centrifuged (14,000 x *g* for 15 mins; HermleZ160M), with the supernatant transferred and frozen. VFA concentrations were determined by gas liquid chromatography described by Attwood *et al*. (1998) (Appendix 3.6). VFA concentrations (mg) have been corrected for VFA concentrations of rumen inocula and are expressed in terms of forage DM incubated and proportions and ratios of non-glucogenic:glucogenic VFA are reported.

PHOTOGRAPH 3.1. Kreft compact mincer used to mince fresh forage

PHOTOGRAPH 3.2. Dacron bags that were used for *in sacco* incubations

PHOTOGRAPH 3.3. 50 mL bottles used for *in vitro* incubations

PHOTOGRAPH 3.4. Incubator used for *in vitro* incubations

3.3.5 Statistics

Kinetic parameters of DM, CP, NDF and ADF disappearance over time from *in sacco* bags were predicted by fitting data from bag residues to a non-linear equation (Ørskov and McDonald, 1979) using the NLIN procedure and Marquardt method within SAS (1996) for each forage (Appendix 6.2). Fitting non-linear models within SAS to estimate parameters of the equation is an iterative process. For each forage, initial values of each parameter were estimated based on actual curves and the NLIN procedure adjusted estimates to optimise the fit and achieve convergence. The Marquardt algorithm was used to calculate the iterations and the optimal fit minimised the residual sums of squares. The Marquardt algorithm first uses the steepest descent method in initial iterations after which the algorithm gradually switches to the Gauss-Newton method to minimise the residual sum of squares (Motulsky and Ransnas, 1987). The non-lag and lag equations (Equations 3.1 and 3.2) were fitted to the DM, CP, ADF and NDF data. Residual mean squares and degrees of freedom from fitting both models were recorded and the best fit (least residual error). The R^2 (square of the correlation coefficient) of the non-linear regression equation was calculated from the residual sum of squares and the total corrected sum of squares [1 – (Residual sum of squares / Total corrected sum of squares)] and reported.

It was necessary to choose either the non-lag (Equation 3.1) or lag (Equation 3.2) model because parameters cannot be compared from a mixture of both models. Although the best fit was obtained with the non-lag model in many instances there were a number of data sets that could not be explained by the non-lag model. In order to compare all data sets, the lag model was used to define degradation kinetics for all components of all forages.

EQUATION 3.1: $P_x (\%) = A + B (1 - e^{-kt})$ (Orskov and McDonald, 1979)
EQUATION 3.2: $P_x (\%) = A + B (1 - e^{-k (t-Lag)})$ (Kolver *et al.*, 1998b)

Where:
PD_x = potential degradation of x at time t,
x = DM, CP, ADF or NDF (%)

A = soluble fraction of x (% DM)
B = degradable insoluble fraction of x (% DM)
k = fractional disappearance rate of x per hour (%/h)
t = incubation time (h)
Lag = lag time (h). This is the period that takes into account the lag phase which is the period during which either no digestion occurs, or digestion occurs at a greatly reduced rate (McDonald, 1981).

Effective degradability (ED) of forages was calculated according to Sinclair *et al.* (1993) using Equation 3.3.

EQUATION 3.3: $ED_x (\%) = A + [(B\,k)/(k + c)]\,(1 - e^{-(k+c)t})$

Where:
ED_x = effective degradability of x,
c = rumen outflow rate of each constituent assumed to be at 6 %/h (Kolver *et al.*, 1998b),
Other abbreviations are defined above.

Although passage rate will not be constant for all forages, few comparable data (with fresh forages) are available and a 6 %/h passage rate has been used in several studies (Hoffman *et al.*, 1993; Kolver *et al.*, 1998b; Elizadale *et al.*, 1999) to calculate ED of fresh forages. AFRC (1992) has reported outflow rates of 2 %/h for dairy cows fed a maintenance diet; 5 to 6 %/h for cows producing up to 15 kg milk/day (< 2 x maintenance); and 8 %/h for high producing dairy cows (> 15 kg milk/day and high DMI of > 2 x maintenance). The work undertaken here was designed to feed cows producing an average of 15 kg milk/day or less, so 6 %/h was used across all comparisons.

Effective rumen degradability of crude protein (ERDP; AFRC, 1993) was calculated for all forages and is a measure of the total N supply that is available for utilisation by the rumen microbes for growth and synthetic purposes. The ERDP (g/kg DM) content of forages is defined in Equation 3.4 and is compared with the CP and NDF content of forages (g/kg DM) and the ED of forage DM (% DM).

EQUATION 3.4: $\text{ERDP (g/kg DM)} = \text{CP}\,[(0.8\,\text{A}) + ((\text{B}\,k)/(k + c))]$.

Where:
ERDP = effective rumen degradability of crude protein,
Other abbreviations are defined above.

Multiple regression analyses were conducted to determine the effect of diet composition (% DM) on the k, ED and lag values for DM, CP, NDF and ADF of all forages. The forward model-selection method of the stepwise procedure of SAS (1996) was used. In order to prevent the selection of too many variables in the multiple regression models, the level of significance was set at $Pr < 0.10$. The aim of the multiple regression analysis was to identify the variables most likely responsible for variation of DM, CP, NDF and ADF kinetic parameters. Nutrient variables included in the multiple regression models were based on correlation coefficients determined by correlation analyses and an understanding of nutritive characteristics. Soluble carbohydrate (SC), CP, NDF, ADF and lignin content of the diet were included to explain the variation.

Degradation and fermentation parameters of individual forages are presented in tables of this chapter, and figures provide a graphical comparison of the mean degradation and fermentation products of forage types over the period of the incubations. Forages have been grouped as:

C3 grasses:	perennial ryegrass, cocksfoot, tall fescue, yorkshire fog, tama ryegrass, prairie grass.
C4 grasses:	kikuyu, paspalum.
Legumes:	white clover, lucerne, red clover.
Legumes with CT:	*Lotus corniculatus, Lotus pedunculatus*, sulla.
Herbs:	chicory, plantain.
Grass silages:	pasture silage, oat silage.
Legume silages:	lucerne silage, sulla silage.
Maize silage.	
Maize grain.	
Lucerne hay.	

Data are presented mainly as means, often derived from curve fitting equations and also in terms of yield (eg. net NH_3 or VFA) per unit DM incubated for defined incubation times. Data have not been used to statistically compare forages or forage types because the objective of this work was to define factors affecting nutritive value, rather than to make statistical comparisons. However, an evaluation between incubation variability was made on the basis of *in sacco* DM loss (Section 3.3.4.1) and incubation of FDG lucerne *in vitro* (Section 3.3.4.2). For *in sacco* data a coefficient of variation less than 5% indicated small variation between incubation runs. Data for rumen inoculum (pH, NH_3 and VFA yields and molar percentages) used for each incubation and *in vitro* pH and NH_3 concentration of FDG lucerne at 2 and 8 hours are presented. The GLM procedure within SAS was used to determine the effect of incubation run on the NH_3 concentration of rumen inoculum, and the effect of incubation run, time during incubation and the run x time interaction were determined for *in vitro* pH and NH_3 concentration of FDG lucerne incubated *in vitro*.

3.4 RESULTS

3.4.1 Chemical composition

The chemical composition of the forages is summarised in Table 3.2. Dry matter of fresh forages ranged from 11.6% to 30.9%. Composition of the DM was wide ranging with the CP content ranging from 7.6% to 29.9%, soluble carbohydrate from 3.1% to 41.7% and NDF contents from 22.4% to 57.8%. Of the eight grasses evaluated, paspalum had the lowest soluble carbohydrate (4.2% DM) and CP (13.5% DM), and highest NDF (57.8% DM) and consequently had the lowest predicted organic matter digestibility (64.9%). Legumes and herbs (chicory and plantain) had low fibre concentrations compared with most of the grasses and silages, and high CP contents ranging from 19.3% DM to 29.9% DM. Silages generally had low soluble carbohydrate contents ranging from 3.1% DM to 8.0% DM, indicating the conversion of sugars to lactic acid during the fermentation process when ensiled.

TABLE 3.2. Forage dry matter (DM) content and composition (% of DM) and predicted organic matter digestibility (OMD) determined by Near InfraRed Reflectance Spectroscopy for fresh and conserved species.

Forage	DM (%)	Soluble Carbohydrate	Crude Protein	Acid detergent fibre	Neutral detergent fibre	Acid detergent lignin	OMD (%)
Fresh							
Lolium perenne (Perennial ryegrass)	18.8	9.1	15.5	25.5	48.7	3.4	77.3
Dactylis glomerata (Cocksfoot)	26.8	7.4	23.7	23.6	47.5	5.1	74.7
Festuca arundinacea (Tall fescue)	25.3	11.9	16.4	23.8	41.6	3.8	75.6
Holcus lanatus (Yorkshire fog)	16.3	12.3	23.7	19.3	39.9	3.1	85[1]
Bromus willdenowii (Prairie grass)	19.1	9.9	19.9	23.1	44.8	3.8	75.2
Lolium multiflorum (Grasslands Tama)	15.2	16.4	21.3	16.2	36.5	2.9	85[1]
Pennisetum clandestinum (Kikuyu)	17.2	6.7	16.4	29.5	47.7	3.8	65.9
Paspalum dilatatum (Paspalum)	30.9	4.2	13.5	33.7	57.8	6.9	64.9
Trifolium repens (White clover)	15.0	12.1	26.9	19.0	25.6	5.9	82.1
Medicago sativa (Lucerne)	23.9	8.6	29.9	21.4	29.5	6.1	73.0
Trifolium pratense (Red Clover)	14.8	8.5	27.4	26.6	33.6	6.2	85[1]
Lotus corniculatus (Birdsfoot trefoil)[3]	16.2	13.0	22.2	19.6	28.2	7.2	76.9
Lotus pedunculatus (Lotus)[3]	16.3	12.2	21.5	22.2	33.1	16.9	80.3
Hedysarum coronarium (Sulla)[3]	11.6	17.8	23.0	17.7	22.4	8.5	85[1]
Cichorium intybus (Chicory)	14.3	11.4	19.3	21.2	23.8	7.0	83.9
Plantago lanceolata (Plantain)	13.0	14.0	24.7	24.3	28.3	21.1	85[1]
Conserved							
Pasture silage	40.8	3.7	17.2	33.5	50.3	4.3	-[2]
Oat silage	40.0	3.1	17.8	32.6	53.2	4.3	-[2]
Lucerne silage	57.4	8.0	23.3	23.1	30.5	8.0	-[2]
Sulla silage[3]	22.6	4.1	21.2	29.5	36.2	4.3	-[2]
Maize silage	34.7	41.7	7.6	24.5	40.5	4.4	-[2]
Lucerne hay`	89.9	4.9	24.2	32.5	39.1	8.5	65.6
Zea maize (Maize grain)	87.1	71.0	10.2	4.11	10.9	2.0	

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[1] Very high estimated digestibility, constrained to 85%

[2] OMD not predicted for silages by NIRS.

[3] Condensed tannin concentration in DM: *Lotus corniculatus* = 3.1%; *Lotus pedunculatus* = 5.1%; Sulla = 5.3%; Sulla silage = 1.1%.

3.4.2 Particle size distribution

Mincing forages resulted in particle size distribution (Table 3.3; Appendix 3.7) that was representative of fresh forages that had been chewed by cows and sheep (Table 2.9; Appendix 3.8). The differences in chemical composition of forages, especially fibre content, were supported by the particle size distribution of minced material. On average temperate grasses and legumes had a similar proportion of DM present on sieves with an aperture size 1 mm and greater (34.9 and 34.7% DM, respectively), but very fibrous tropical grass (kikuyu and paspalum) had 43.7% DM present on the sieves greater than 1 mm. The different chemical composition of temperate grasses and legumes affected the distribution of particles on the 4 mm and 1 + 2 mm sieves. The average percentage of material present on the 4 mm sieve for temperate grasses was greater (11.2% DM) than that of legumes (5.3% DM) with less on the 1 + 2 mm sieves for temperate grasses (23.7% DM) compared to legumes (29.7 %DM). Tropical grasses with high fibre content had less soluble and residual material (25 – 33%), than legumes and temperate grasses which had 41.8% DM and 47.5% DM, respectively.

TABLE 3.3. Particle size distribution of minced forages used for *in sacco* and *in vitro* incubations. Data are % of dry matter (DM) retained on sieves with aperture sizes (sides of square hole; mm) indicated, or passing through a sieve with 0.25 mm aperture size (soluble and residues).

Forage	> 4	1 + 2	0.25 + 0.50	< 0.25[1]
Fresh				
Perennial ryegrass	14.0	24.8	15.5	45.7
Cocksfoot	6.2	27.0	31.0	35.8
Tall fescue	9.3	24.9	17.4	48.4
Yorkshire fog	12.7	24.1	13.4	49.8
Prairie grass	8.0	22.1	18.8	51.1
Grasslands Tama	16.8	19.1	9.7	53.9
Kikuyu	14.1	27.3	25.6	33.0
Paspalum	10.7	35.3	28.5	25.5
White clover	8.4	33.1	20.7	37.7
Lucerne	11.1	27.1	17.6	44.3
Red clover	8.3	36.3	24.3	31.1
Lotus corniculatus	3.1	28.9	18.4	49.6
Lotus pedunculatus	2.3	25.0	29.8	42.9
Sulla	3.3	27.2	23.4	46.2
Chicory	4.1	31.8	20.0	44.2
Plantain	1.9	25.7	34.0	38.4
Conserved				
Pasture silage	13.6	25.4	17.6	43.4
Oat silage	20.2	22.1	12.6	45.1
Lucerne silage	25.3	29.5	15.5	29.8
Sulla silage	4.1	27.0	22.4	46.5
Maize silage	9.6	23.6	26.0	40.9
Lucerne hay	16.4	39.9	17.5	26.3
Maize grain	1.4	66.3	14.7	17.6

[1] Soluble + residue DM

3.4.3 Variation between incubations

Incubation run did not affect DM loss at 12 h for forages incubated in the eight separate incubations. The DM loss for all forages incubated in the eight incubations ranged from 45.2 to 92.5 % DM and averaged 67.0 ± 3.18 % DM which was similar to the 12 h DM loss when all forages were incubated at once with DM losses ranging from 44.9 to 86.3 % DM and averaged 69.0 ± 2.99 % DM. The CV ranged from 1.6 to 6.8 with an average of 3.9.

Table 3.4 shows the rumen inoculum used for most incubations were similar, except for the third run where rumen pH was lower (6.16) and VFA yield higher (165 mmol/L) than the other runs. Rumen pH ranged from 6.33 to 6.54, except for the third incubation. VFA concentrations and molar percentages for all runs except the third were similar ranging from 107 to 145 mmol/L. Rumen NH_3 concentration for the first and last runs were significantly lower than other runs at 17.2 and 13.4 mmol/L, respectively, and run 3 had the significantly highest rumen NH_3 concentration at 30.8 mmol/L, and the remaining five runs ranged from 21.0 to 26.5 mmol/L.

TABLE 3.4. Rumen pH (n = 1), rumen ammonia (NH_3; n = 4) and volatile fatty acid (VFA) concentrations (n = 1) and molar percentages of inocula[1] used for eight *in sacco* incubation runs.

	Incubation run							
	1	2	3	4	5	6	7	8
<u>Rumen parameters</u>								
Rumen pH	6.47	6.41	6.16	6.53	6.57	6.33	6.54	6.40
Rumen NH_3 (mmol/L)[2]	17.2^{b}	24.7^{de}	25.3^{e}	23.9^{ad}	30.8^{f}	25.3^{de}	21.0^{c}	13.4^{a}
Total Rumen VFA (mmol/L)	132.3	144.5	163.5	111.2	125.2	106.7	130.9	118.7
Acetate (%)	65.2	65.9	64.6	67.5	70.6	67.0	69.9	68.6
Propionate (%)	19.4	17.7	18.1	17.3	18.5	20.2	17.7	20.0
Butyrate (%)	10.5	11.0	12.2	9.8	6.6	9.3	7.6	6.7
Minor (%)	4.8	5.4	5.2	5.4	4.3	3.5	4.7	4.7

[1] Fresh rumen inocula was collected for each incubation from the same cow fed lucerne hay at maintenance.

[2] Statistical differences between incubation runs were only carried out for rumen NH_3 concentration. Number of samples per incubation run were 4; standard error of the mean = 0.49; Pr < 0.05.

[a, b] LS means within columns with a common superscript letter do not differ significantly (Pr < 0.05).

In vitro pH and NH_3 concentration when FDG lucerne was incubated were similar. Incubations 1 and 8 resulted in a slower rate of digestion than the rest of the runs, indicated by a significantly lower net release of lucerne N to NH_3-N at 2 and 8 hours (Table 3.5). This corresponded with lower NH_3 concentration of rumen inocula. *In vitro* NH_3 yield of run 3 was not different to the majority of data, despite the low pH and high concentration of VFA of rumen inocula (Table 3.4).

TABLE 3.5. *In vitro* pH and NH_3 of freeze-dried and ground lucerne at 2 and 8 hours when incubated in eight separate *in vitro* incubation runs. *In vitro* NH_3 and pH data are the least-square (LS) means of triplicate samples at each time in each incubation run (n = 3). LS means and associated standard error of the means (SEM) are presented.

Incubation Run	*In vitro* pH		*In vitro* NH_3 concentration (mmol/L) derived from degradation of forage[1]		*In vitro* NH_3 concentration (% forage nitrogen recovered as NH_3)[1]	
	2 hours	8 hours	2 hours	8 hours	2 hours	8 hours
1	6.93	6.71^{bc}	5.0^{a}	14.6^{a}	7.8^{ab}	23.5^{a}
2	6.86	6.69^{bc}	6.6^{abc}	22.9^{cd}	11.0^{bc}	39.2^{d}
3	6.87	6.48^{a}	7.1^{bc}	21.4^{c}	11.7^{c}	32.5^{bc}
4	6.83	6.73^{c}	6.2^{ab}	20.5^{bc}	10.0^{abc}	31.9^{bc}
5	6.89	6.59^{abc}	8.2^{c}	22.3^{c}	13.2^{c}	35.3^{c}
6	6.80	6.52^{a}	7.4^{bc}	22.6^{c}	11.4^{c}	35.1^{c}
7	6.86	6.63^{abc}	7.1^{bc}	24.6^{d}	11.8^{c}	40.3^{d}
8	6.85	6.56^{ab}	4.8^{a}	19.3^{b}	7.1^{a}	29.9^{b}
SEM	0.029		0.38		0.63	
Run effect (Pr)	< 0.05		< 0.05		< 0.05	
Time effect (Pr)	< 0.05		< 0.05		< 0.05	
Run x Time (Pr)	< 0.05		< 0.05		< 0.05	

[1] Corrected for NH_3 concentration in rumen liquor (Table 3.4).

[a, b] LS means within rows with a common superscript letter do not differ significantly ($Pr < 0.05$).

3.4.4 *In sacco* digestion

The *in sacco* technique used in this study determined degradation kinetics for DM, CP, NDF and ADF for the 23 forages. The distribution of DM, CP or fibre fractions between pools, potential and effective degradability and rates of degradation with lag times are presented in Tables 3.6 to 3.9 and Figures 3.1, 3.3, 3.4 and 3.5. The effect of contamination by the inflow of material from the rumen was minimal. When empty *in sacco* bags were placed in the rumen for 72 hours they increased in weight by only 30 to 40 mg.

3.4.4.1 Dry matter digestion kinetics

The proportion of DM released into the soluble (A) pool is a function of plant structure and mincing. This fraction was higher in temperate grasses (41 to 56% DM) than in tropical grasses (26 to 31%) and values for legumes and herbs ranged from 22% (red clover) to 52% of DM with sulla (Table 3.6). White clover, *Lotus pedunculatus*, chicory and plantain had a soluble DM fraction of about 40% DM. For most silages the soluble fraction was between 42 to 49% DM, except for maize silage where only 28% DM was soluble.

Fractional DM degradation rates of the degradable (B) fraction ranged from 6.4 %/h (paspalum) to 33.2 %/h (plantain); and in the case of silages, from 4.2 %/h (maize) to 16.2 %/h (lucerne; Table 3.6). Figure 3.1 illustrates the DM degradation curves for contrasting forage types evaluated in this study. The herbs and white clover with less than 30% NDF were degraded more rapidly than any of the other legumes and temperate (C3) grasses. As expected tropical grasses (C4) had the slowest degradation rates, as did grass silages including maize silage (4.2 %/h), which is often used as a supplement to pasture systems.

The period during which either no digestion occurs, or digestion occurs at a greatly reduced rate, is generally referred to as the lag phase (McDonald, 1981). In this study the duration of the lag phase ranged from 0 hours (sulla silage and lucerne hay) to 9.0

hours (tall fescue). The lag period was less than 1.5 hours for legumes and ranged between 4 to 5 hours for temperate grasses and 8 to 9 hours for tropical grasses.

Effective DM degradability was lowest for tropical grasses at 59%, greatest for rapidly digested legumes and herbs at 81 to 84% and about 76% for temperate grasses. Red clover and the annual tetraploid Tama ryegrass had ED values of 66% and 82%, which differed from values for each forage type (legumes and temperate grasses). Conservation of lucerne lowered ED by 2% units as silage, but substantially more as hay (24% units), and ED of sulla silage was 69% compared to 81% for fresh sulla. Maize silage DM ED was only 50%.

FIGURE 3.1. Dry matter (DM) degradation curves for eight forage types (Section 3.3.5) evaluated *in sacco*. Means ± standard error of the mean at each time are presented.

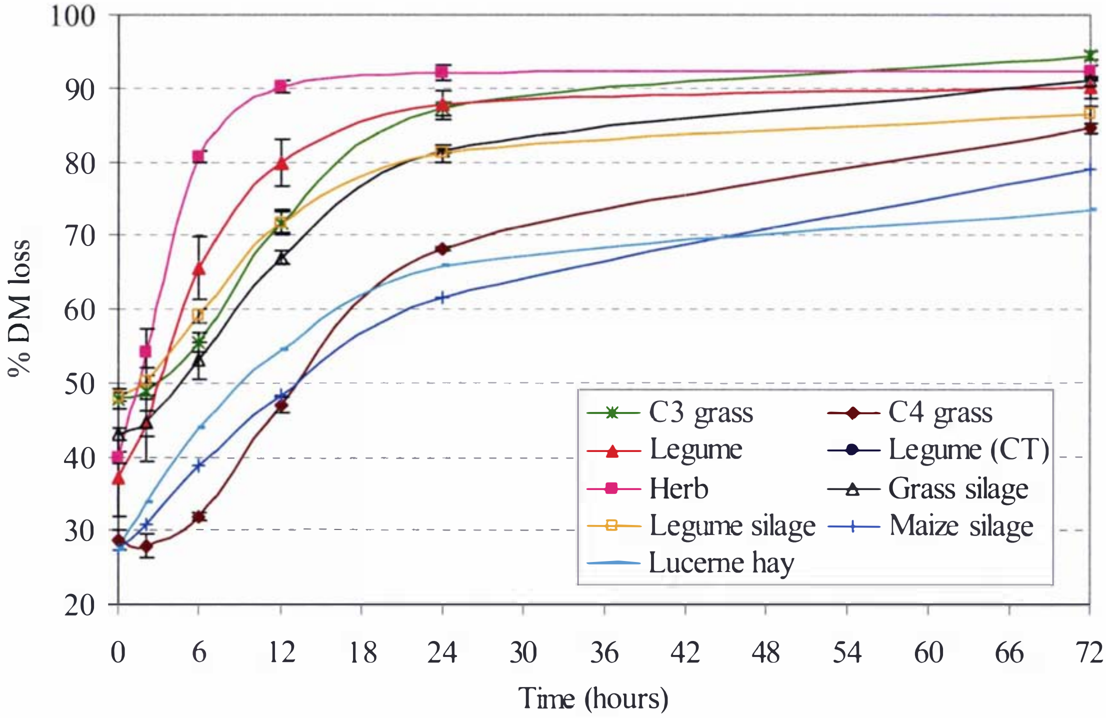


TABLE 3.6. *In sacco* forage dry matter (DM) degradation characteristics (% of DM) as defined by soluble (A) and degradable insoluble (B) fractions, potential degradability (PD), fractional degradation rate (k, %/h), lag time (hours) and effective degradability (ED) which takes into account the effect of passage from the rumen. The R^2 value (square of the correlation coefficient) of the non-linear regression equation is presented to illustrate the goodness of fit. (Standard errors for A, B, k and Lag parameters are presented in Appendix 3.9).

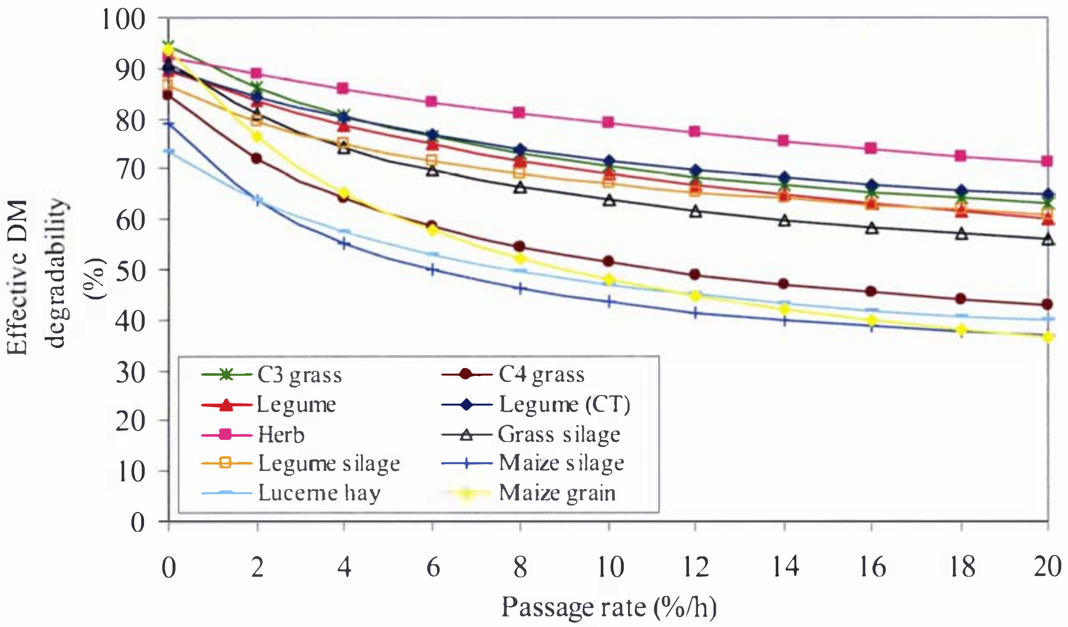
Forage	A	B	PD	k	Lag	ED[1]	R^2
Fresh							
Perennial ryegrass	44.1	50.8	94.9	10.6	4.2	76.2	1.00
Cocksfoot	49.3	43.7	93.0	11.6	4.9	78.1	0.93
Tall fescue	48.6	45.3	93.9	7.9	9.0	74.3	0.98
Yorkshire fog	48.5	46.7	95.2	8.7	4.0	76.1	0.99
Prairie grass	41.4	50.4	91.8	9.2	0.7	71.9	1.00
Grasslands Tama	55.5	41.5	97.0	10.1	4.7	81.5	0.99
Kikuyu	31.0	55.3	86.3	7.1	7.8	61.0	0.99
Paspalum	26.3	57.8	84.1	6.4	4.2	56.1	0.98
White clover	39.9	54.1	94.0	21.1	1.2	82.0	1.00
Lucerne	49.8	38.6	88.4	13.5	0.7	76.5	0.99
Red clover	21.8	64.1	85.9	13.4	1.1	66.1	0.94
Lotus corniculatus	50.5	39.4	89.9	15.0	1.1	78.6	0.99
Lotus pedunculatus	43.7	40.9	84.6	11.4	4.8	70.5	1.00
Sulla	51.7	43.6	95.3	12.3	0.2	81.0	0.97
Chicory	41.6	52.4	94.0	26.0	0.4	84.2	1.00
Plantain	39.1	51.3	90.4	33.2	1.4	82.6	0.97
Conserved							
Pasture silage	41.6	49.5	91.1	8.4	4.2	70.5	0.98
Oat silage	44.5	47.1	91.6	6.5	1.0	69.0	0.99
Lucerne silage	47.0	37.5	84.5	16.2	4.0	74.4	0.99
Sulla silage	49.1	39.8	88.9	5.9	0	68.8	0.96
Maize silage	27.5	54.4	81.9	4.2	0.4	49.9	0.96
Lucerne hay	27.4	46.5	73.9	7.3	0	52.9	0.98
Maize grain	17.5	76.9	94.4	6.6	0.4	57.8	0.99

[1] Calculated using an assumed fractional passage rate of 6 %/h.

3.4.4.2 Dry matter effective degradability

In this study, ED of forages has been reported and compared at an outflow rate of 6 %/h (Tables 3.6, 3.7, 3.8 and 3.9), but the passage rate of material out of the rumen is affected by the type of forage consumed and level of intake. Figure 3.2 illustrates the sensitivity of ED for different forage types when rumen passage rates range between 0 to 20%/h. Effective degradability of low fibre herbs and legumes decreased as outflow rate increased from 0 to 20%, but to a lesser extent than slowly degraded tropical species, maize silage, maize grain and lucerne hay (Figure 3.2).

FIGURE 3.2. Effective dry matter (DM) degradability of forage types when DM outflow rate varies from 0 to 20 %/h.



3.4.4.3 Crude protein digestion kinetics

The soluble fraction of CP had a greater range across forages (29% to 73%; Table 3.7) than the soluble fraction of DM (27% to 55%). On average 54% of the CP in temperate grasses was soluble while for legumes and herbs the soluble CP component ranged between 29 and 52%. Oat and pasture silage had a soluble CP content of 73% whilst values for lucerne and sulla silage (53 and 55% of CP) were similar to fresh lucerne (52%) and sulla (50%; Table 3.7; Figure 3.3).

Degradation rates for degradable CP ranged from 3.4 %/h for maize silage to 34.9 %/h for plantain, and CP degradation rates of forages had similar rankings to their DM degradation rates (Table 3.7; Figure 3.3). Chicory and plantain had the most rapid degradation of protein with very small lag periods. The legumes, white clover, *Lotus corniculatus*, lucerne, red clover and sulla, all had lag periods less than 1 hour and degradation rates between 11 to 19 %/h, but *Lotus pedunculatus* had a considerably longer lag period and slower degradation rate. Temperate grasses had longer lag periods than legumes, but once digestion commenced, degradation was between 13 and 17 %/h. Kikuyu and paspalum had long lag periods (11 and 12 h) and degradation rates of 7.8 and 12.5 %/h, but 50% of CP in paspalum was undegradable.

Condensed tannins in legumes appeared to decrease the soluble CP fraction by about 6% units and degradation rate of the insoluble CP fraction was slower (12.7 %/h) than those legumes not containing CT (14.9 %/h).

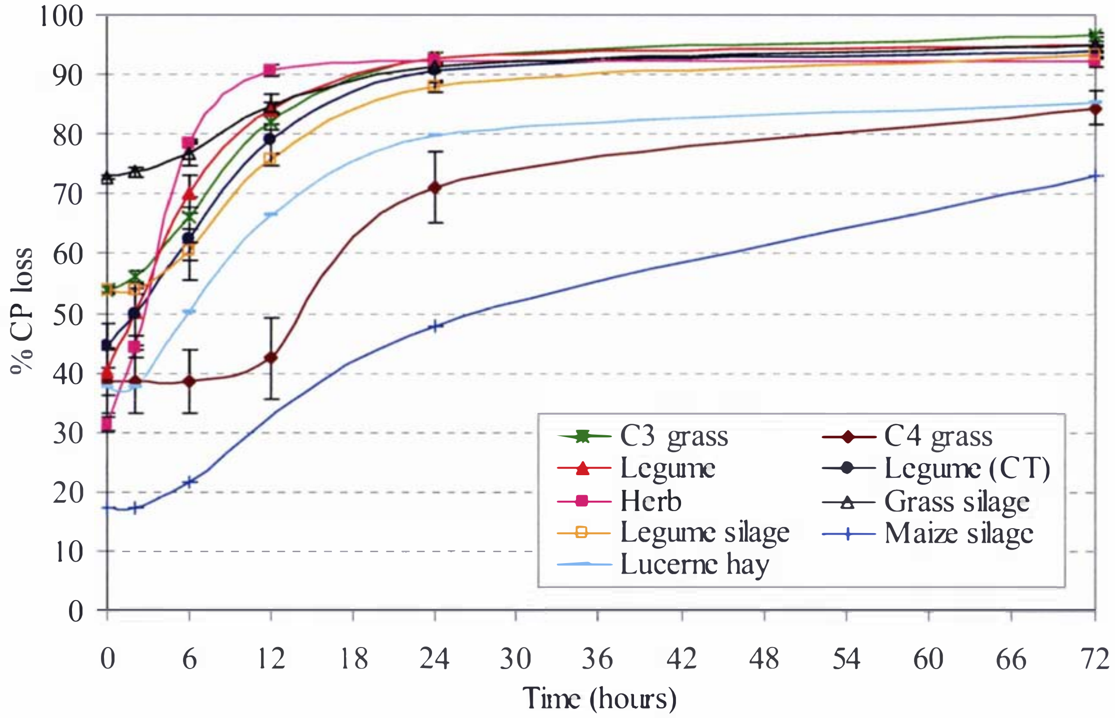
The ED of CP in fresh C3 grasses and legumes were similar at about 80% with the exception of tall fescue (77%), red clover (72%) and *Lotus pedunculatus* (71%) within their respective forage types. Low protein C4 grasses, paspalum and kikuyu, had low ED of 41% and 76%, respectively, and maize silage with an even lower CP content had an ED of 36% CP.

TABLE 3.7. Crude protein (CP) concentration in the DM and *in sacco* degradation characteristics (% of CP) of forages as defined by soluble (A) and degradable insoluble (B) fractions, potential degradability (PD), fractional degradation rate (k, %/h), lag time (hours) and effective degradability (ED) which takes into account the effect of passage from the rumen. The R^2 value (square of the correlation coefficient) of the non-linear regression equation is presented to illustrate the goodness of fit. (Standard errors for A, B, k and Lag parameters are presented in Appendix 3.10).

Forage	CP	A	B	PD	k	Lag	ED[1]	R^2
Fresh								
Perennial ryegrass	15.5	52.2	43.5	95.7	15.0	4.6	83.3	1.00
Cocksfoot	23.7	55.0	41.1	96.1	15.9	5.0	84.8	0.95
Tall fescue	16.4	53.0	43.1	96.1	7.5	3.2	76.9	0.97
Yorkshire fog	23.7	54.8	43.6	98.4	8.7	1.0	80.6	0.99
Prairie grass	19.9	52.4	43.0	95.4	17.1	0.9	84.2	1.00
Grasslands Tama	21.3	56.4	41.7	98.1	13.9	4.3	85.5	1.00
Kikuyu	16.4	47.9	41.5	89.4	12.5	10.7	75.9	1.00
Paspalum	13.5	29.1	20.9	50.0	7.8	11.6	40.9	0.91
White clover	26.9	38.4	58.0	96.4	19.1	1.3	82.5	1.00
Lucerne	29.9	52.0	41.5	93.5	15.3	0	81.8	0.98
Red Clover	27.4	30.1	65.4	95.5	10.4	0	71.6	0.94
Lotus corniculatus	22.2	51.1	44.3	95.4	15.0	1.0	82.7	0.99
Lotus pedunculatus	21.5	33.0	58.0	91.0	11.4	4.8	71.0	0.99
Sulla	23.0	49.5	46.4	95.9	11.7	0	80.2	0.98
Chicory	19.3	29.3	65.4	94.7	27.3	0.8	82.9	1.00
Plantain	24.7	33.3	57.1	90.4	34.9	1.6	82.0	0.92
Conserved								
Pasture silage	17.2	72.1	22.8	94.9	10.4	5.5	86.6	0.98
Oat silage	17.8	73.3	21.7	95.0	7.8	0.9	85.6	0.99
Lucerne silage	23.3	52.5	40.0	92.5	14.1	5.0	80.6	1.00
Sulla silage	21.2	55.4	39.4	94.8	7.4	3.2	77.2	0.96
Maize silage	7.6	17.4	62.0	79.4	3.4	3.8	39.8	0.99
Lucerne hay	24.2	37.6	48.0	85.6	10.2	3.0	67.8	0.99
Maize grain	10.2	0	90.2	90.2	7.8	9.5	51.0	0.98

[1] Calculated using an assumed fractional passage rate of 6 %/h.

FIGURE 3.3. Crude protein (CP) degradation curves for eight forage types (Section 3.3.5) evaluated *in sacco*. Means ± standard error of the mean at each time are presented.



3.4.4.4 Fibre digestion kinetics

Mincing did release "soluble" fibre, which ranged from 0 to 40% of NDF and 0 to 25% of ADF (Tables 3.8 and 3.9). Most values for soluble NDF were less than 25% with less than 10% soluble ADF in most instances and 70 to 90% of NDF was potentially degradable for nearly all forages.

Degradation rate of NDF in plantain, white clover and chicory ranged between 24.1 and 33.9 %/h, while lucerne and sulla degraded at 17.0 and 16.4 %/h, respectively, and *Lotus corniculatus*, lucerne silage and red clover NDF degraded more slowly (11.2 to 13.0 %/h). These forages also had short lag periods of less than 1 hour, except for red clover and lucerne where the lag periods were 2 and 4 hours, respectively. Concentration of CT in sulla and *Lotus pedunculatus* were similar (5.3 vs. 5.1%), but degradation rate was faster and lag period shorter (0.8 and 4.6 h) in sulla compared to *Lotus pedunculatus* (k = 16.4 vs. 8.1 %/h; l = 0.8 and 4.6 h). The degradable NDF fractions of temperate and tropical grasses and silages were degraded more slowly than legumes, with rates between 5 and 10 %/h. Maize silage, lucerne hay and pasture silage NDF degraded at less than 5 %/h (Figure 3.4)

ADF is a component of NDF and the ranking of ADF and NDF were similar for the forages and silages evaluated here (Table 3.9; Figure 3.5). Herbs and white clover had the most rapid ADF degradation (24.1 to 39.0 %/h) with lag periods less than 1 hour (Table 3.9). Other legumes had small lag periods of less than 2 hours, but degradation rate was slower, between 9.0 %/h and 14.6 %/h. *Lotus pedunculatus* had a lag period of 5.3 hours. Temperate and tropical grasses had degradation rates between 6.2 and 11.1 %/h, and lag periods were between 4 and 9 hours. Slowest degradation occurred for hay and silages (except for lucerne silage) averaging 4.5 %/h with only 3 hours or less of lag.

The potential degradability of NDF (Table 3.8) in temperate grasses exceeded 90% in most instances, averaged 80% for tropical grasses and ranged from 73 to 84% for legumes. Degradability of silage NDF was variable (61 – 88%). However when outflow rates from the rumen were set at 6 %/h, NDF ED for temperate grasses ranged between 54 to 71% NDF, for kikuyu and paspalum was 31 and 49% NDF and for legumes ranged between 41 to 73% NDF. Effective degradability for silage and hay NDF were very low between 21 to 59%, but would be higher if fractional outflow rates were lower. Values for both potential degradability and ED for ADF followed a similar pattern to NDF, but values were often substantially lower (Table 3.9).

TABLE 3.8. Neutral detergent fibre (NDF) concentration in the DM and *in sacco* degradation characteristics (% of NDF) of forages as defined by soluble (A) and degradable insoluble (B) fractions, potential degradability (PD), fractional degradation rate (k, %/h), lag time (hours) and effective degradability (ED)[1]. The R^2 value (square of the correlation coefficient) of the non-linear regression equation is presented to illustrate the goodness of fit. (Standard errors for A, B, k and Lag parameters are presented in Appendix 3.11).

Forage	NDF	A	B	PD	k	Lag	ED[1]	R^2
Fresh								
Perennial ryegrass	48.7	20.7	71.6	92.3	9.3	3.9	64.2	0.99
Cocksfoot	47.5	40.0	49.5	89.5	9.9	4.0	70.8	0.99
Tall fescue	41.6	15.6	74.8	90.4	6.4	9.1	54.2	0.98
Yorkshire fog	39.9	22.9	68.6	91.5	7.7	3.9	61.5	0.99
Prairie grass	44.8	18.3	68.4	86.7	7.1	0	55.4	0.99
Grasslands Tama	36.5	36.2	58.7	94.9	7.8	5.1	69.4	1.00
Kikuyu	47.7	0	79.6	79.6	5.8	5.6	39.1	0.98
Paspalum	57.8	8.3	72.2	80.5	7.8	3.9	49.1	0.98
White clover	25.6	21.2	62.3	83.5	28.3	1.0	72.6	1.00
Lucerne	29.5	39.9	33.2	73.1	17.0	4.2	64.4	0.96
Red Clover	33.6	6.0	68.0	74.0	13.0	2.0	52.5	0.95
Lotus corniculatus	28.2	0	73.1	73.1	12.3	1.0	46.1	0.99
Lotus pedunculatus	33.1	1.7	67.6	69.3	8.1	4.6	40.5	0.99
Sulla	22.4	0	84.5	84.5	16.4	0.8	61.9	0.95
Chicory	23.8	0	82.1	82.1	33.9	0	69.8	0.98
Plantain	28.3	12.1	75.4	87.5	24.1	0	72.5	0.98
Conserved								
Pasture silage	50.3	11.9	76.6	88.5	4.7	0.9	45.5	0.99
Oat silage	53.2	24.4	62.4	86.8	7.3	3.6	58.7	0.99
Lucerne silage	30.5	5.3	56.2	61.5	11.2	0.5	41.9	0.98
Sulla silage	36.2	29.4	43.2	72.9	6.3	0	51.5	0.94
Maize silage	40.5	0	63.7	63.7	4.1	0.4	25.8	0.94
Lucerne hay	39.1	0	52.8	52.8	4.1	0	21.4	0.94
Maize grain	10.9	0	69.0	69.0	7.8	7.8	39.0	0.98

[1] Calculated using an assumed fractional passage rate of 6 %/h.

TABLE 3.9. Acid detergent fibre (ADF) concentration in the DM and *in sacco* degradation characteristics (% of ADF) of forages as defined by soluble (A) and degradable insoluble (B) fractions, potential degradability (PD), fractional degradation rate (k, %/h), lag time (hours) and effective degradability (ED) which takes into account the effect of passage from the rumen. The R^2 value (square of the correlation coefficient) of the non-linear regression equation is presented to illustrate the goodness of fit. (Standard errors for A, B, k and Lag parameters are presented in Appendix 3.12).

Forage	ADF	A	B	PD	k	Lag	ED[1]	R^2
Fresh								
Perennial ryegrass	25.5	10.3	79.7	90.0	10.2	4.1	60.5	0.99
Cocksfoot	23.6	5.0	81.8	86.8	10.0	4.2	56.1	0.98
Tall fescue	23.8	9.1	80.0	89.1	6.7	9.5	51.3	0.98
Yorkshire fog	19.3	5.7	83.9	89.6	8.1	4.4	53.9	0.99
Prairie grass	23.1	0	81.0	81.0	10.1	3.8	50.8	0.99
Grasslands Tama	16.2	5.6	85.6	91.2	11.1	7.8	61.2	1.00
Kikuyu	29.5	0	79.7	79.7	6.2	4.9	40.5	1.00
Paspalum	33.7	0	79.8	79.8	10.6	7.9	51.0	0.94
White clover	19.0	0	81.6	81.6	24.1	1.5	65.3	0.99
Lucerne	21.4	24.6	45.6	70.2	12.3	1.8	55.3	0.99
Red Clover	26.6	0	72.1	72.1	9.0	1.8	43.3	0.84
Lotus corniculatus	19.6	3.0	70.1	73.1	11.2	1.4	48.7	0.99
Lotus pedunculatus	22.2	0	58.8	58.8	10.4	5.3	37.3	0.98
Sulla	17.7	0	84.1	84.1	14.6	1.3	59.6	0.96
Chicory	21.2	0	83.2	83.2	26.3	0	57.7	0.98
Plantain	24.3	24.6	61.4	86.0	39.0	1.9	77.8	0.99
Conserved								
Pasture silage	33.5	10.0	78.0	88.0	5.1	0.9	45.8	1.00
Oat silage	32.6	19.9	66.4	86.3	7.2	3.1	56.1	0.99
Lucerne silage	23.1	10.5	51.2	61.7	15.5	4.4	47.4	0.99
Sulla silage	29.5	23.3	54.9	78.2	4.6	0	47.1	0.96
Maize silage	24.5	0	59.9	59.9	5.1	0	27.5	0.97
Lucerne hay	32.5	0	59.9	59.9	3.4	0	21.6	0.95
Maize grain	4.1	0	43.3	43.3	7.2	13.0	23.6	0.98

[1] Calculated using an assumed fractional passage rate of 6 %/h.

FIGURE 3.4. Neutral detergent fibre (NDF) degradation curves for eight forage types (Section 3.3.5) evaluated *in sacco*. Means ± standard error of the mean at each time are presented.

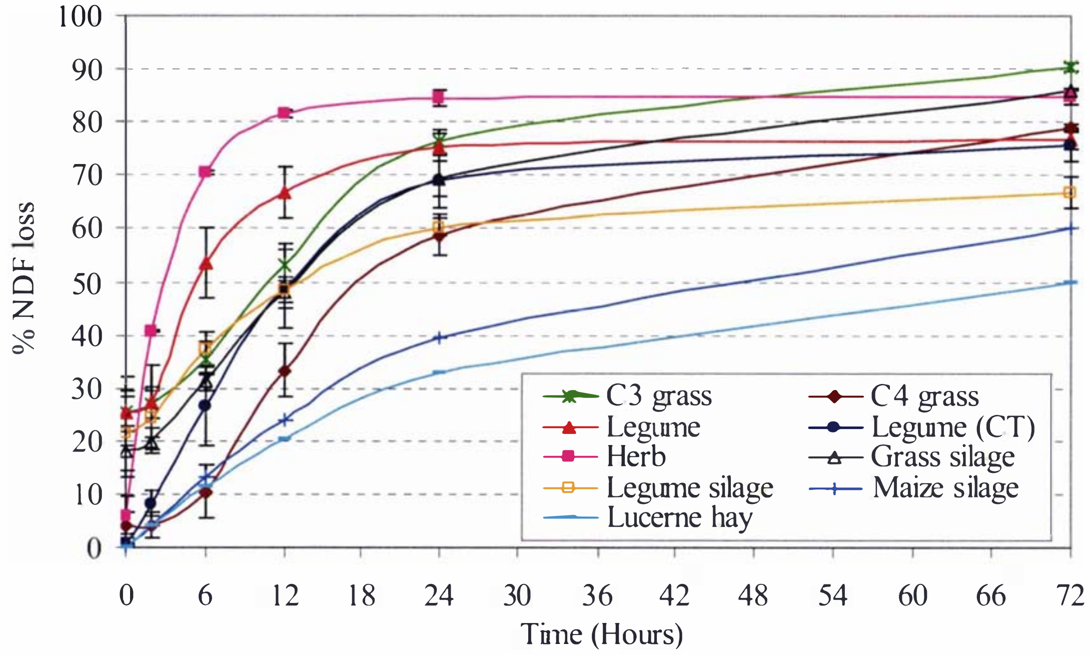
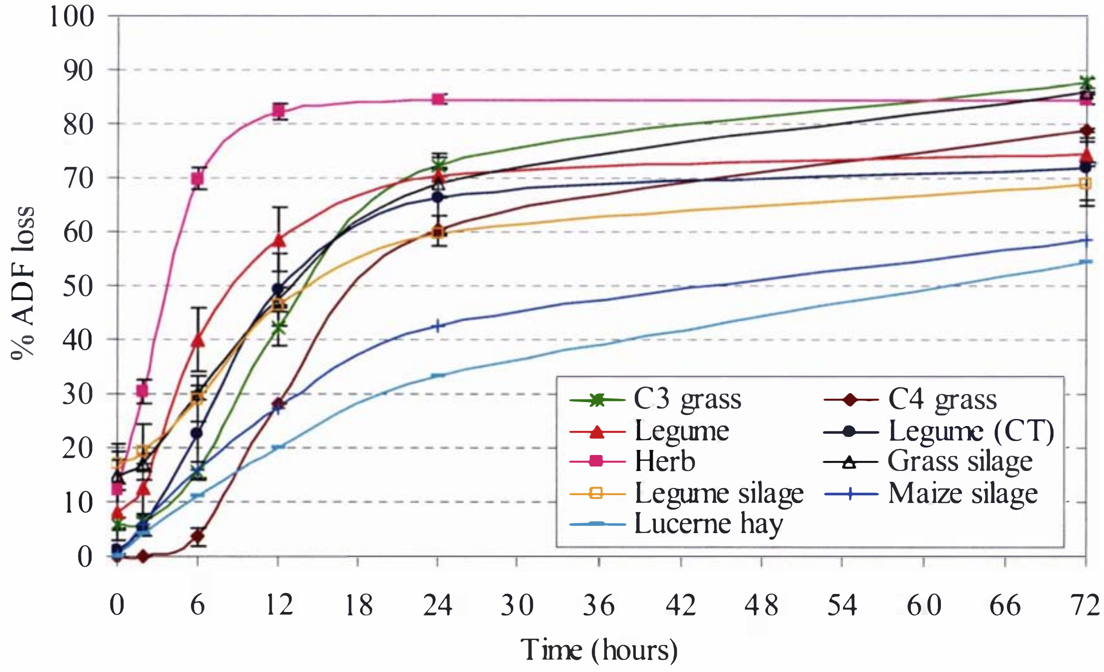


FIGURE 3.5. Acid detergent fibre (ADF) degradation curves for eight forage types (Section 3.3.5) evaluated *in sacco*. Means ± standard error of the mean at each time are presented.



3.4.4.5 Relationships between fractions

There was a positive relationship between CP content (g/kg DM) and ERDP (g/kg DM) across forages, which accounted for 92.5% of the variability (Figure 3.6). There were also significant ($Pr < 0.05$) positive relationships between ERDP of forages with ED of forage DM (% DM; R^2 = 53%; Figure 3.7) and NDF content (g/kg DM; R2 = 51%; Figure 3.8).

FIGURE 3.6. Relationship between the crude protein content (CP, g/kg DM) and effective rumen degradability of CP (ERDP, g/kg DM) of forages at a rumen outflow rate of 6 %/h.

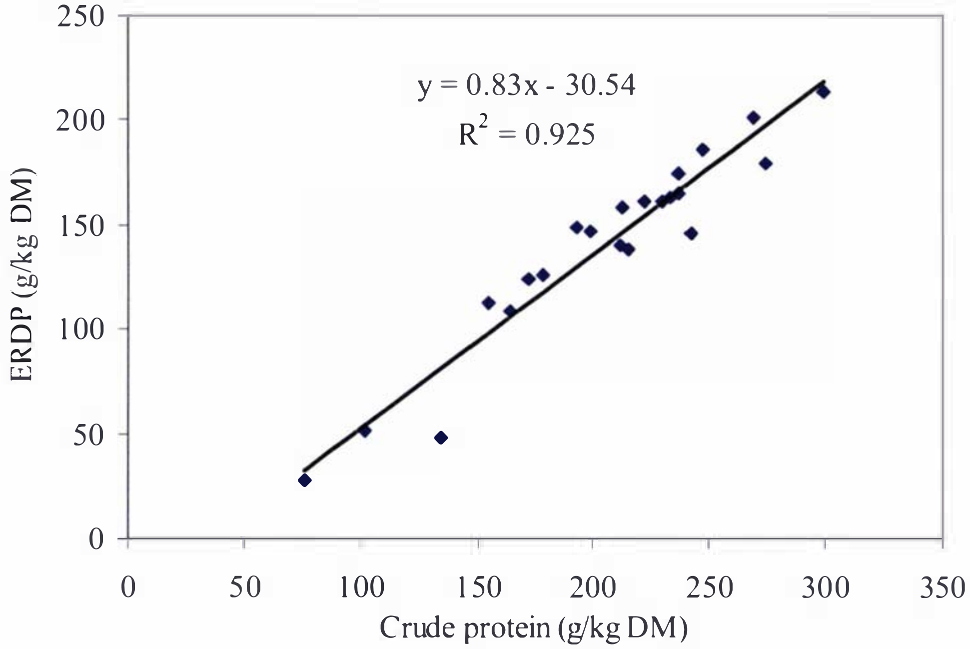


FIGURE 3.7. Relationship between the effective degradability of DM (ED_{DM}, % DM) and effective rumen degradability of crude protein (ERDP, g/kg DM) of forages at a rumen outflow rate of 6 %/h.

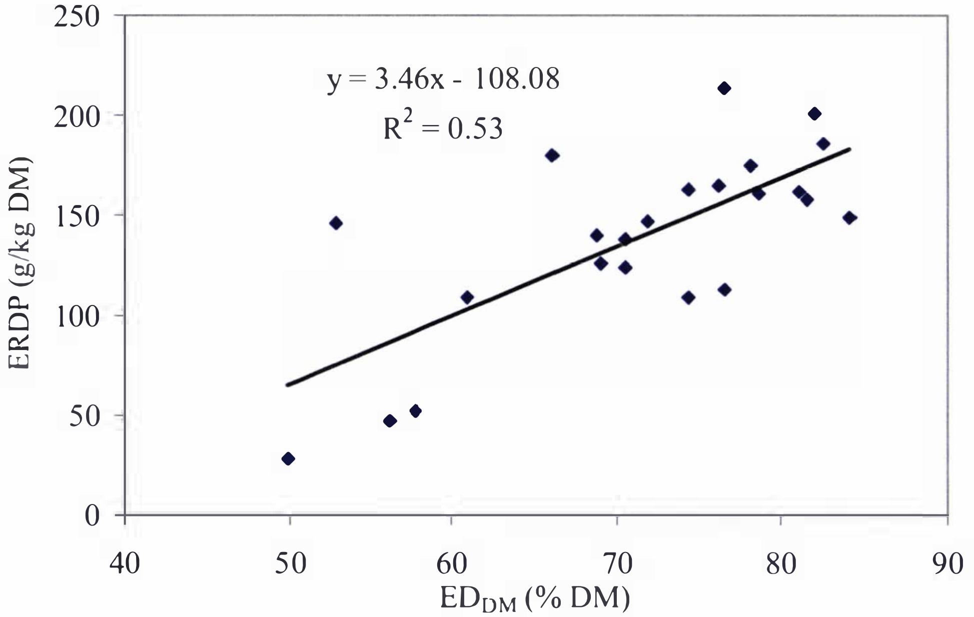
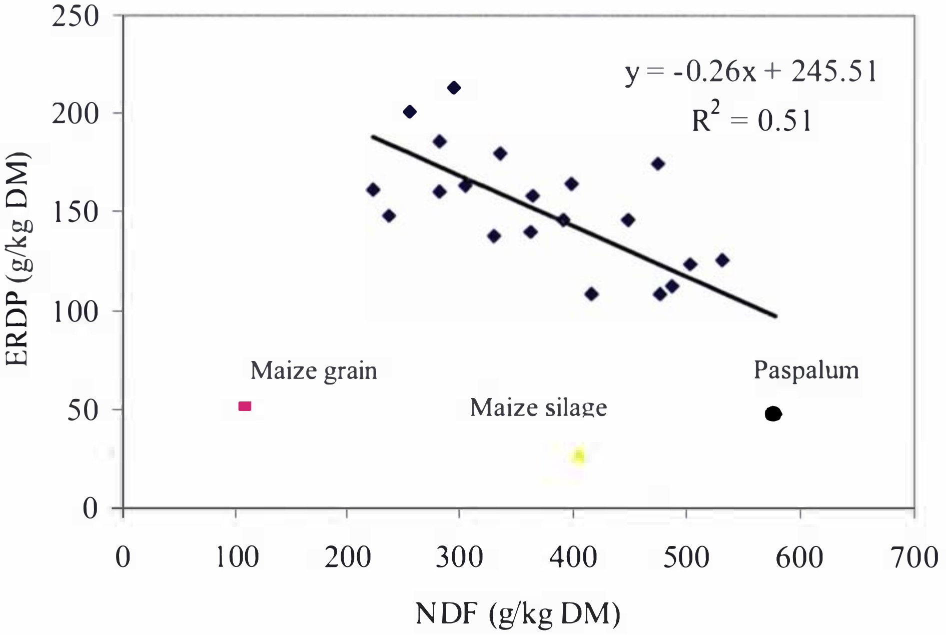


FIGURE 3.8. Relationship between the neutral detergent fibre (NDF, g/kg DM) and effective rumen degradability of crude protein (ERDP, g/kg DM) of forages at a rumen outflow rate of 6 %/h. Maize silage and maize grain were not included in the relationship.



Degradation rates of forage DM and NDF decreased as NDF content increased up to a concentration of 400 g/kg DM, after which degradation of DM and NDF was between 5 and 10 %/h and there was no relationship. A power equation explained 51% and 55% of the variation for NDF content compared to DM and NDF degradation rate, respectively (maize grain was not included; Figures 3.9 a and b).

FIGURE 3.9a. Relationship between neutral detergent fibre content (NDF, g/kg DM) and dry matter degradation rate (%/h) of forages. (Maize grain was not included in the equation).

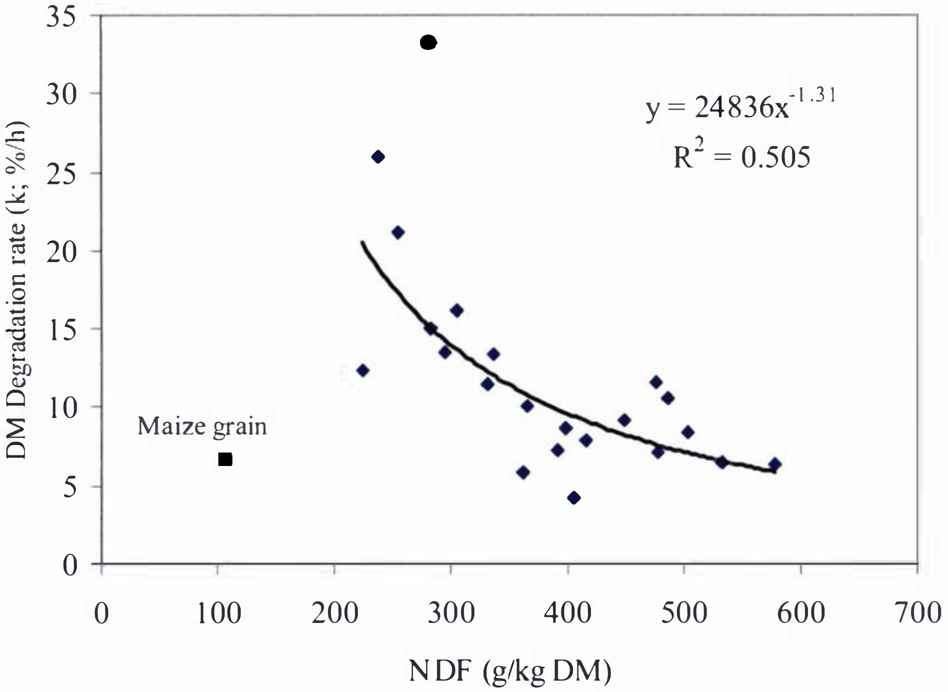
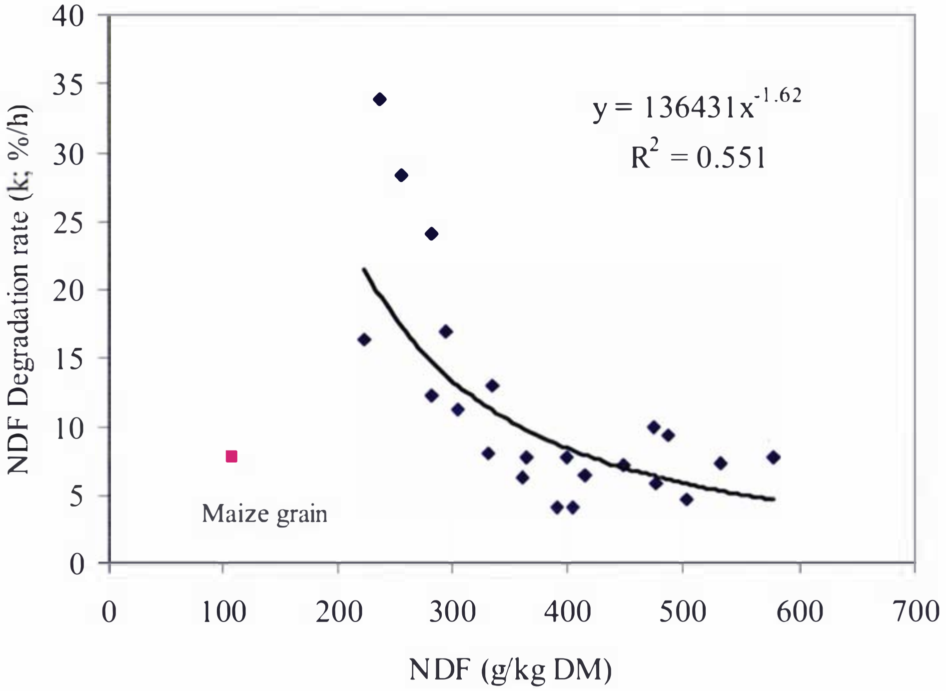


FIGURE 3.9b. Relationship between neutral detergent fibre content (NDF, g/kg DM) and NDF degradation rate (%/h) of forages. (Maize grain was not included in the equation).



Multiple regression analysis

Table 3.10 reports the results of multiple linear regression analysis on the k (%/h), ED (%) and lag values (h) for DM, CP, NDF and ADF degradation. Multiple regression analysis was not good at explaining the variation ($R^2 < 0.50$) of the k value of CP, NDF and ADF; the lag period of DM, CP, NDF and ADF degradation; and ED of CP, NDF and ADF. Degradation rate (k) of CP, NDF and ADF had 32%, 48% and 49% (respectively) of the variation explained while DM degradation rate had 61% of the variation explained. Soluble carbohydrate (SC) and NDF concentration explained NDF degradation, ADF and lignin concentration explained ADF degradation, and SC, NDF and lignin concentration explained DM degradation. Effective degradability of DM had 74% of the variation explained by the SC, CP and ADF content of the forages.

TABLE 3.10. Multiple regression analysis to indicate dietary components best able to explain the variation in dry matter (DM), crude protein (CP), acid detergent fibre (ADF) and neutral detergent fibre (NDF) kinetic parameters.

Nutrient		Equation	R^2
DM			
k	=	23.2 – 0.18 SC – 0.36 NDF + 0.68 lignin	0.61
ED	=	132.4 – 0.88 SC – 0.23 CP – 1.88 ADF	0.74
Lag	=	0.073 – 0.20 ADF + 0.20 NDF	0.32
CP			
k	=	7.6 + 0.88 lignin	0.32
ED	=	44.2 + 1.54 CP	0.38
Lag	=	11.1 – 0.37 CP	0.37
NDF			
k	=	36.6 – 0.28 SC – 0.58 NDF	0.48
ED	=	29.4 + 1.18 CP	0.20
Lag	=	No solution	0.27
ADF			
k	=	11.9 – 0.35 ADF + 1.23 lignin	0.49
ED	=	27.0 + 1.01 CP	0.15
Lag	=	2.22 + 0.10 SC	0.20

Abbreviations: k = degradation rate (%/h); ED = effective degradability (%); SC = soluble carbohydrate.

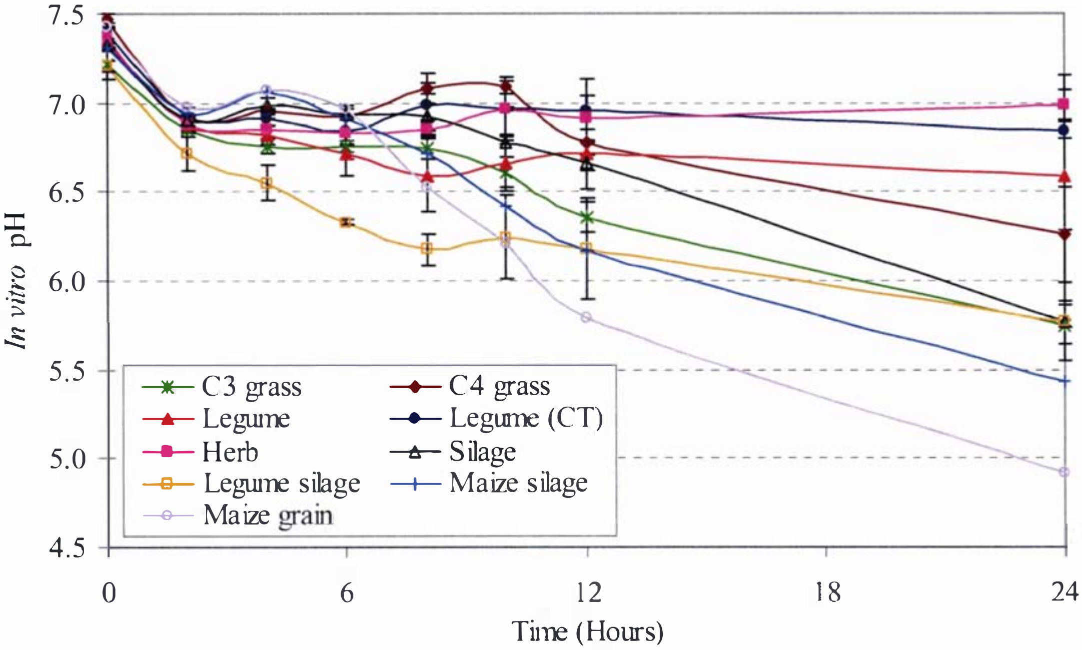
3.4.5 *In vitro* digestion

In vitro incubation enabled NH_3 and VFA to be determined over a 24 hour period to indicate rates and products of microbial fermentation.

3.4.5.1 *In vitro* pH

In vitro pH was used to monitor incubations especially to indicate the extent to which they were representing *in vivo* digestion. In most situations the rumen digesta of forage fed ruminants remained above a mean pH of 5.8 and any mean values of 5.6 or below were considered unrepresentative of normal digestion. Figure 3.10 illustrates the change in *in vitro* pH of the eight forage types and Appendix 3.9 gives the individual *in vitro* values over the 24 hour incubation. *In vitro* pH at 0 hours for all feeds ranged between 7.0 and 7.4 and after 2 hours declined to between 6.6 and 7.0. After 2 hours of incubation the pH of all forage types remained above 6.0 for a further 10 hours, except for maize grain which declined to 5.7 after 12 hours of incubation. The majority of information was derived from the first 12 hours of *in vitro* incubations, but values after 24 hours of incubation showed tall fescue, cocksfoot, perennial ryegrass, pasture silage, lucerne silage and maize silage had an average *in vitro* pH below 5.6 and maize grain had an *in vitro* pH of 4.9 (Figure 3.10). Total concentration of VFA and *in vitro* pH at 12 and 24 hours were not significantly correlated (r = -0.14 and -0.21, respectively).

FIGURE 3.10. *In vitro* pH when eight forage types were incubated for 24 hours. Data are the average pH of all forages in each forage type at each time point with associated standard error bars.



3.4.5.2 Ammonia yield

Changes in NH_3 concentration in incubation media, after correction for input from rumen inoculum (Table 3.4) and utilisation for bacterial growth gives an indication of proteolysis. Values given here always underestimate true proteolysis because the net incorporation into bacteria was not measured, but after 6 hours there was a five-fold difference between forages in the percentage of plant N released to NH_3-N (paspalum, 4.0 vs. pasture silage, 20.7; Table 3.11). Interpretation of the data is complicated by contrasting concentrations of N in forage DM, ranging from 7.6% with maize silage to nearly 30% in fresh lucerne but they do indicate a wide range in the extent of protein degradation.

The net yield of NH_3 accounted for 43% of plant-N in legumes after 24 hours with 49% from white clover, 46% from red clover and 35% from lucerne (Table 3.11). Forages containing CT (*Lotus spp*. and sulla) had very little increase in NH_3 concentration after 6 hours of incubation (Table 3.11) with only 4 to 25% of plant N appearing as NH_3-N at

24 hours (Figure 3.11), despite a relatively high N concentration in the DM (21.5 – 23.0%). Net release rate of NH_3 over 24 hours was rapid and linear for legumes without CT, but NH_3 release over the first 12 hours was most rapid for grass silages (Table 3.12).

Temperate grasses showed a net release of N in the first 6 hours of incubation equivalent to 11 to 23% of plant N, after which net release rates declined. Forages with a low N content (eg. Tropical (C4) species) showed a maximum net yield of NH_3 after 6 hours, but bacterial utilisation exceeded N release after 6 hours (Figure 3.11). Kikuyu had a greater net release rate of NH_3 than paspalum during the first 6 hours of incubation (Table 3.12). Maize silage and maize grain had low N and high starch concentrations, and N utilisation exceeded release after the first 6 hours of incubation. Net losses suggest N incorporation into microbial biomass exceeded N release from plant protein degradation.

Ensiled sulla and pasture had NH_3 concentrations nearly three-fold greater than fresh sulla and pasture after 6 hours, and rate of net release was rapid for the first 12 hours of incubation for all silages with slower values between 12 and 24 hours (Tables 3.11 and 3.12).

TABLE 3.11. Net ammonia production[1] (% forage nitrogen released as NH_3) of forages evaluated in *in vitro* for 24 hours (h). (Standard errors are presented in Appendix 3.14).

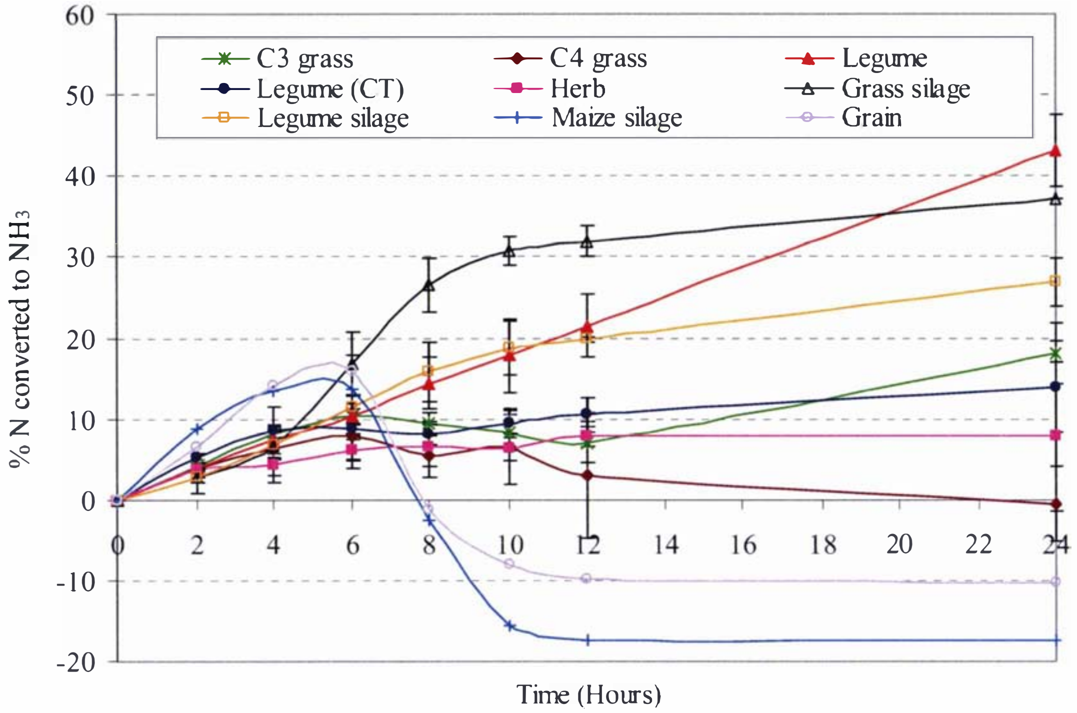
	Time of incubation						
Forage	2 h	4 h	6 h	8 h	10 h	12 h	24 h
Fresh							
Perennial ryegrass	4.3	6.8	6.8	4.7	-0.9	-3.3	3.3
Cocksfoot	3.7	7.6	10.1	9.7	10.7	10.2	16.5
Tall fescue	4.5	9.6	10.2	9.2	5.9	6.2	16.2
Yorkshire fog	5.3	11.4	13.7	15.3	15.3	16.7	30.9
Prairie grass	3.3	5.8	9.2	8.2	9.8	5.5	18.7
Grasslands Tama	4.4	8.0	12.0	9.1	9.4	8.5	23.1
Kikuyu	4.3	7.8	11.9	8.1	11.0	10.8	4.1
Paspalum	3.5	5.1	4.0	2.8	2.0	-4.7	-5.1
White clover	4.3	7.7	13.7	15.7	21.7	21.0	49.2
Lucerne	2.0	4.4	5.5	8.4	8.7	15.0	34.6
Red Clover	5.5	10.4	12.2	19.1	23.0	28.6	45.5
Lotus corniculatus	4.7	8.1	9.9	10.9	12.9	14.7	25.2
Lotus pedunculatus	6.0	8.8	9.0	8.1	8.5	8.9	9.2
Sulla	5.3	9.0	7.6	5.6	7.1	8.1	7.6
Chicory	3.6	3.1	4.7	4.2	4.7	6.6	17.0
Plantain	3.9	5.7	7.5	9.2	8.1	9.0	-1.4
Conserved							
Pasture silage	3.7	7.7	20.7	29.8	32.6	33.9	37.1
Oat silage	2.2	5.3	12.9	23.3	29.1	30.1	37.2
Lucerne silage	0.9	2.1	5.1	12.2	15.5	19.5	23.9
Sulla silage	4.6	11.4	17.9	19.5	22.0	20.1	29.9
Maize silage	8.8	13.5	13.7	-2.5	-15.5	-17.4	-17.4
Lucerne hay	4.2	8.3	11.9	20.4	24.7	27.2	38.0
Maize grain	6.5	14.0	16.0	-1.2	2.3	-9.8	-10.2

[1] Net ammonia production = ammonia concentration at each time – ammonia concentration in rumen inocula used for each incubation. The concentration of rumen ammonia for each incubation ranged between 13.4 and 30.8 mmol/L as listed in Table 3.4.

TABLE 3.12. Net ammonia produced (mmol NH_3/mmol forage nitrogen) for each forage incubated between 0 – 6, 6 – 12, 12 – 24 and 0 – 24 hours (h). Data are the average of triplicate bottles of each forage and negative values indicate a net utilisation.

Forage	0 – 6 h	6 – 12 h	12 – 24 h	0 – 24 h
Fresh				
Perennial ryegrass	11.3	-16.8	5.5	0.14
Cocksfoot	16.8	0.1	5.3	0.69
Tall fescue	17.0	-6.7	8.3	0.67
Yorkshire fog	22.9	3.2	12.7	1.29
Prairie grass	15.3	-6.0	11.0	0.78
Grasslands Tama	20.0	-5.9	12.2	0.96
Kikuyu	19.9	-1.8	-5.6	0.17
Paspalum	6.7	-1.5	-0.4	-0.21
White clover	22.9	12.1	24.7	2.05
Lucerne	9.2	15.9	16.3	1.44
Red Clover	20.3	27.4	14.1	1.90
Lotus corniculatus	16.5	8.0	8.8	1.05
Lotus pedunculatus	15.0	-0.2	0.3	0.38
Sulla	12.6	0.8	-0.4	0.32
Chicory	7.9	3.2	8.6	0.71
Plantain	12.5	2.6	-8.7	-0.06
Conserved				
Pasture silage	34.6	21.9	2.7	1.55
Oat silage	21.5	28.6	6.0	1.55
Lucerne silage	8.6	24.0	3.7	1.00
Sulla silage	29.8	3.7	8.2	1.25
Maize silage	22.7	-51.8	0.04	-0.72
Lucerne hay	19.9	25.5	9.0	1.58
Maize grain	26.6	-42.9	-0.3	-0.42

FIGURE 3.11. Net ammonia (NH_3) yield for nine forage types evaluated *in vitro*.



3.4.5.3 VFA yield

Yields of VFA showed a two-fold range across forages at 6, 12 and 24 hours of *in vitro* incubations (Figure 3.12) and there were substantial differences between forages in the rates of VFA production over 24 hours (Tables 3.13 to 3.15). Figure 3.12 shows that on average legumes had the highest yield of VFA throughout the 24 hours and maize grain, maize silage and tropical (C4) grass species produced low quantities of VFA throughout. Surprisingly, the herb plantain produced the smallest amount of VFA (172 mg/g DM) after 24 hours and the yield was half that of chicory (344 mg/g DM). After 24 hours the VFA yield was equivalent to 36 to 40% of DM in white clover, red clover or lucerne, with similar values for several grasses (Table 3.15). The yield of VFA produced from legumes containing CT was less than legumes not containing CT (Figure 3.12).

Figures 3.13 to 3.16 illustrate the changes in acetate, propionate, butyrate and minor VFA concentrations throughout the 24 hours of *in vitro* incubation for forage types and Table 3.16 gives the rates per hour of total VFA production at 6, 12 and 24 hours of the incubation, expressed in terms of DM incubated. After 24 hours of incubation *Lotus pedunculatus* had produced the highest proportion of acetate but most forages resulted in about 45 to 55% acetate. The acetate:propionate (A:P) ratio ranged from a high of 3.6 with *Lotus pedunculatus* to a low of 1.8 with sulla. The A:P ratio showed a substantial difference between forage types over the incubation. After 6 hours of incubation maize silage and plantain had the highest A:P ratio of 4.8 and 4.1 (Table 3.13), although the ratio declined to 2.0 and 2.6 for the respective forages after 24 hours (Table 3.15). In contrast, tama ryegrass, perennial ryegrass, white clover and sulla had the lowest A:P ratios after 6 hours (1.4, 1.7, 1.7, 1.8; respectively) and the A:P ratios of sulla and WC were similar for the entire 24 hours. Sulla had the lowest A:P ratio for the entire 24 hours (1.8).

Within the legume forage type, red clover differed from the others with more acetate relative to propionate being produced compared to either white clover or lucerne after 24 hours. Within the CT legume forage type, *Lotus pedunculatus* produced more acetate relative to propionate compared to *Lotus corniculatus* or sulla after 24 hours (Table 3.15).

FIGURE 3.12. Net yield of VFA (mg/g DM) produced when forages were evaluated *in vitro*. Standard error bars for C3 and C4 grasses, legumes with and without CT, grass silage and legume silages.

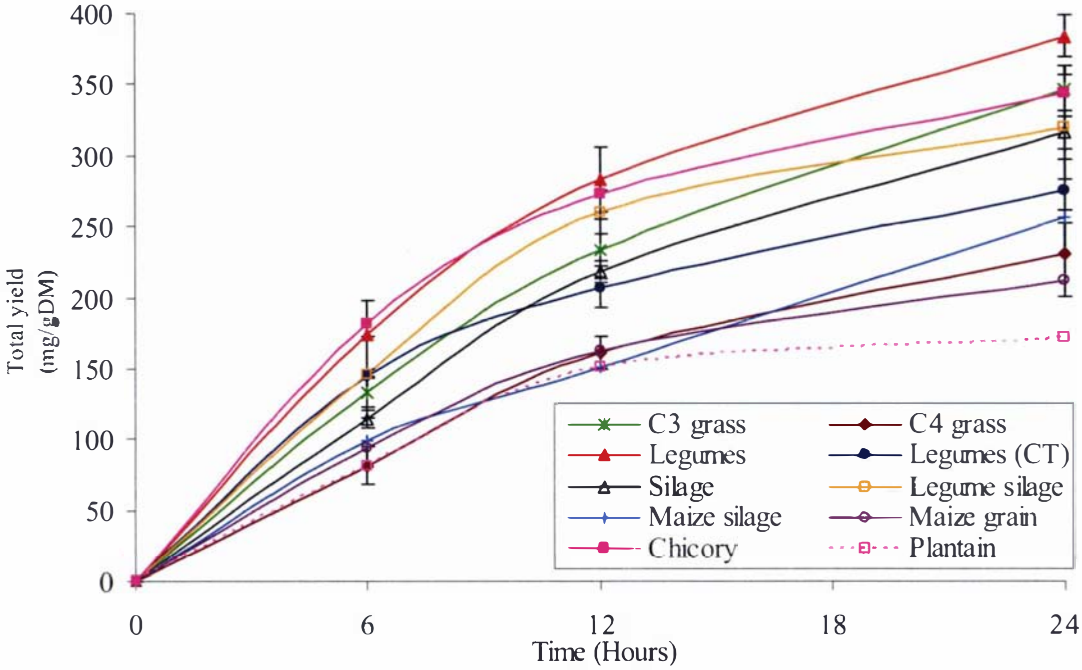


FIGURE 3.13. Net yield of acetate produced (mg/g DM) when forages were evaluated *in vitro*. Standard error bars for C3 and C4 grasses, legumes with and without CT, grass silage and legume silages.

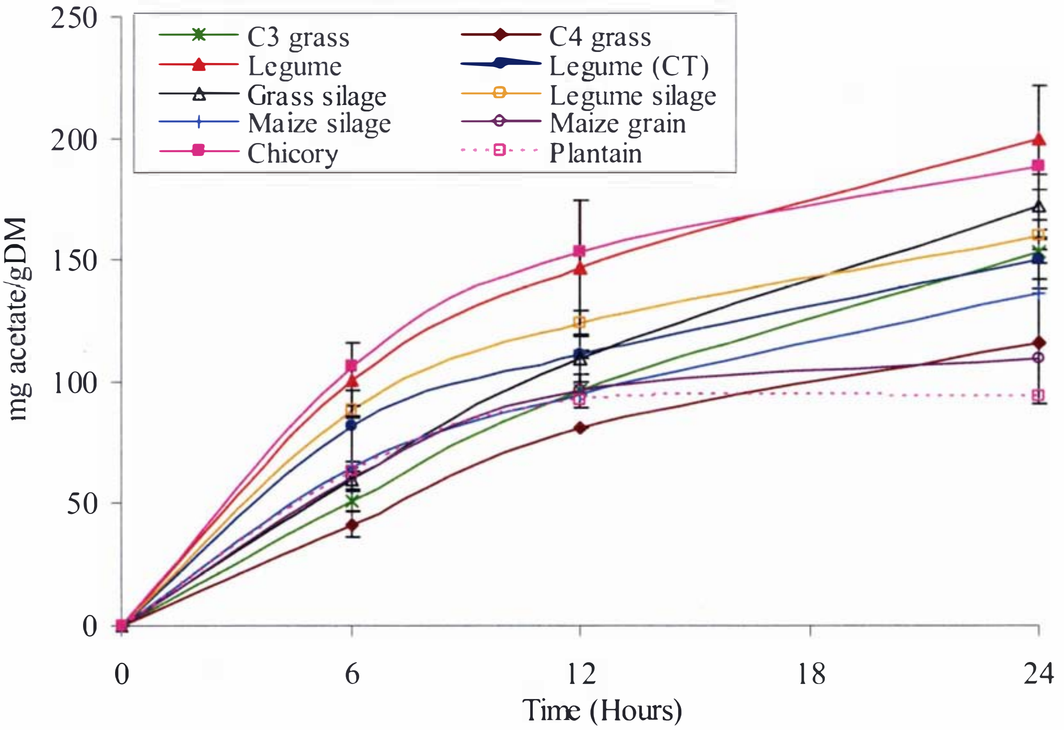


FIGURE 3.14. Net yield of propionate produced (mg/g DM) when forages were evaluated *in vitro*. Standard error bars for C3 and C4 grasses, legumes with and without CT, grass silage and legume silages.

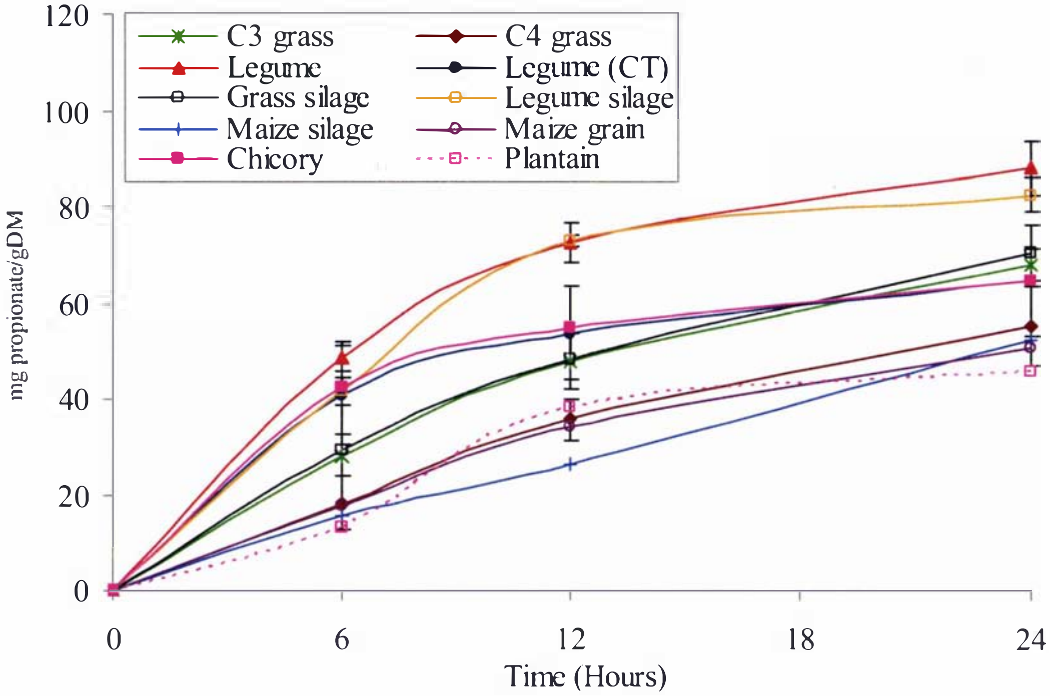


FIGURE 3.15. Net yield of butyrate produced (mg/g DM) when forages were evaluated *in vitro*. Standard error bars for C3 and C4 grasses, legumes with and without CT, grass silage and legume silages.

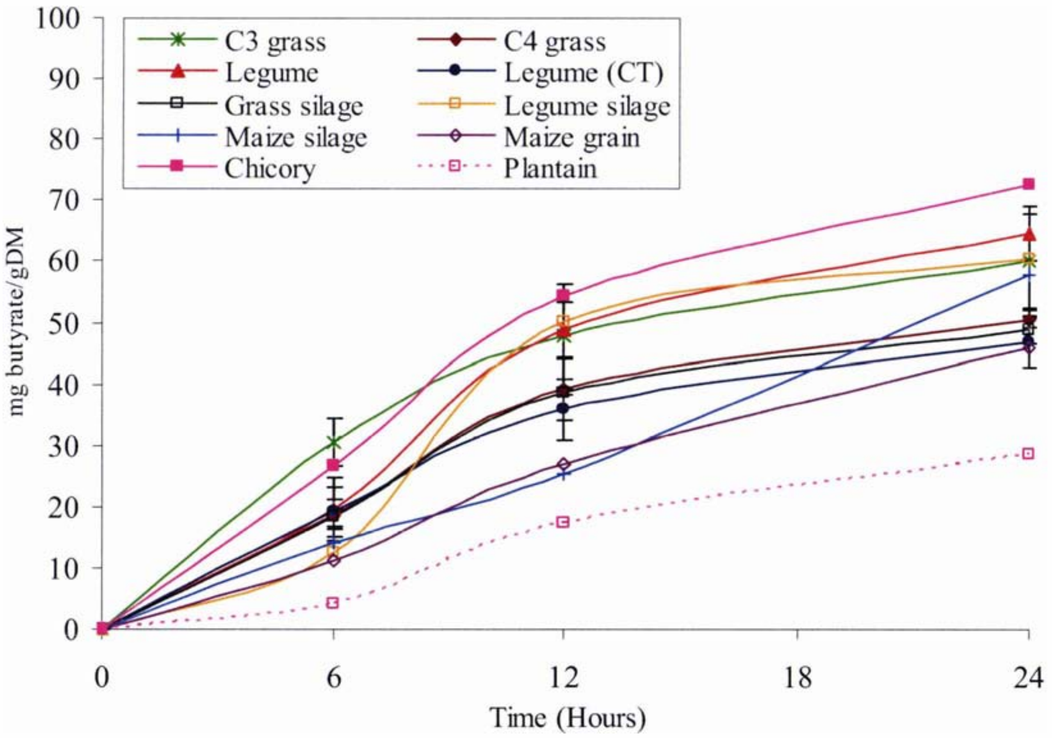


FIGURE 3.16. Net yield of minor VFA produced (mg/g DM) when forages were evaluated *in vitro*. Standard error bars for C3 and C4 grasses, legumes with and without CT, grass silage and legume silages.

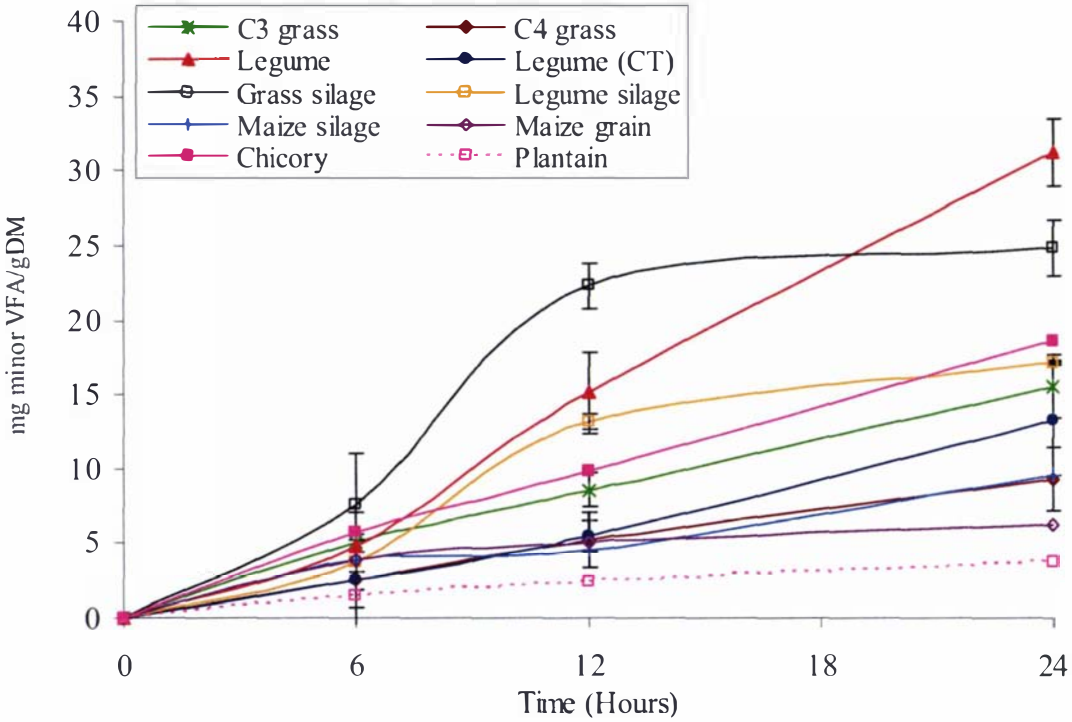


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TABLE 3.13. *In vitro* yield of VFA (mg/g DM) after 6 hours (h) of incubation with molar percentages and molar ratios of acetate (A), propionate (P) and butyrate (B).

Forage	6 h (mg/g DM)	A (%)	P (%)	B (%)	Minor[1] (%)	A:P	(A+B):P
Fresh							
Perennial ryegrass	130.0	49.0	29.1	19.0	2.9	1.7	2.3
Cocksfoot	115.8	49.1	20.7	24.4	5.7	2.4	3.5
Tall fescue	157.8	43.1	22.8	29.4	4.7	1.9	3.2
Yorkshire fog	108.8	41.0	21.1	33.0	4.9	1.9	3.5
Prairie grass	117.0	46.8	21.8	27.1	4.2	2.1	3.4
Grasslands Tama	173.9	40.0	28.9	26.9	4.3	1.4	2.3
Kikuyu	94.7	49.4	25.3	22.3	3.0	2.0	2.8
Paspalum	68.0	54.1	18.7	24.2	3.1	2.9	4.2
White clover	169.5	53.8	30.8	12.7	2.8	1.7	2.2
Lucerne	133.8	59.5	31.8	7.6	1.1	1.9	2.1
Red Clover	217.5	59.9	23.8	12.6	3.7	2.5	3.1
Lotus corniculatus	108.2	56.8	30.1	11.4	1.6	1.9	2.3
Lotus pedunculatus	124.7	59.9	23.1	15.2	1.8	2.6	3.3
Sulla	200.9	54.7	30.5	13.1	1.7	1.8	2.2
Chicory	181.5	58.8	23.3	14.8	3.2	2.5	3.2
Plantain	81.4	77.2	16.1	5.0	1.7	4.8	2.1
Conserved							
Pasture silage	108.0	51.7	27.5	15.5	5.2	1.9	2.4
Oat silage	121.1	52.5	23.6	16.2	7.7	2.2	2.9
Lucerne silage	142.9	63.2	27.1	7.8	1.9	2.3	2.6
Sulla silage	149.8	57.6	29.7	9.4	3.2	1.9	2.3
Maize silage	84.0	55.2	19.1	22.0	3.7	4.1	5.0
Lucerne hay	96.1	72.4	20.6	5.6	1.5	3.5	3.8
Maize grain	93.6	65.1	18.8	12.0	4.1	3.5	4.1

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[1] Minor VFA include iso-butyrate, iso-valerate and n-valerate.

TABLE 3.14. *In vitro* yield of VFA (mg/g DM) after 12 hours (h) of incubation with molar percentages and molar ratios of acetate (A), propionate (P) and butyrate (B).

Forage	12 h (mg/g DM)	A (%)	P (%)	B (%)	Minor[1] (%)	A:P	(A+B):P
Fresh							
Perennial ryegrass	212.1	51.7	28.4	17.2	2.8	1.8	2.4
Cocksfoot	166.4	52.2	23.0	19.8	4.9	2.3	3.1
Tall fescue	228.5	48.2	22.1	25.4	4.4	2.2	3.3
Yorkshire fog	220.0	47.8	22.5	24.2	5.5	2.1	3.2
Prairie grass	255.2	49.3	21.5	25.3	3.9	2.3	3.5
Grasslands Tama	320.4	42.6	25.3	27.8	4.3	1.7	2.8
Kikuyu	172.7	47.1	23.3	25.6	4.1	2.0	3.1
Paspalum	150.4	54.2	20.9	22.6	2.2	2.6	3.7
White clover	250.8	45.1	30.6	19.3	5.0	1.5	2.1
Lucerne	271.4	46.3	28.2	21.0	4.6	1.6	2.4
Red Clover	327.3	61.5	19.7	12.5	6.3	3.1	3.8
Lotus corniculatus	212.2	51.3	30.2	15.4	3.0	1.7	2.2
Lotus pedunculatus	177.7	62.3	19.4	16.3	1.9	3.2	4.0
Sulla	232.7	49.7	27.4	20.0	2.9	1.8	2.5
Chicory	272.6	56.3	20.2	19.9	3.6	2.8	3.8
Plantain	151.9	61.5	25.5	11.4	1.6	2.4	2.9
Conserved							
Pasture silage	210.2	47.5	24.0	18.6	9.9	2.0	2.8
Oat silage	226.4	52.3	20.2	17.0	10.5	2.6	3.4
Lucerne silage	244.5	52.9	30.3	11.2	5.6	1.7	2.1
Sulla silage	276.1	43.0	26.0	26.5	4.6	1.7	2.7
Maize silage	196.3	62.8	17.4	16.8	3.0	3.6	4.6
Lucerne hay	198.8	65.6	19.8	9.0	5.7	3.3	3.8
Maize grain	162.9	59.3	21.0	16.5	3.1	2.8	3.6

[1] Minor VFA include iso-butyrate, iso-valerate and n-valerate.

TABLE 3.15. *In vitro* yield of VFA (mg/g DM) after 24 hours (h) of incubation with molar percentages and molar ratios of acetate (A), propionate (P) and butyrate (B).

Forage	24 h (mg/g DM)	A (%)	P (%)	B (%)	Minor[1] (%)	A:P	(A+B):P
Fresh							
Perennial ryegrass	286.5	55.5	25.4	16.0	3.1	2.2	2.8
Cocksfoot	328.4	53.1	24.6	16.9	5.4	2.2	2.8
Tall fescue	352.7	53.4	21.5	20.3	4.8	2.5	3.4
Yorkshire fog	339.2	52.3	21.9	18.7	7.1	2.4	3.2
Prairie grass	375.6	50.7	20.8	23.6	5.0	2.4	3.6
Grasslands Tama	398.0	46.5	23.8	24.1	5.6	2.0	3.0
Kikuyu	200.8	45.1	23.3	25.9	5.7	1.9	3.0
Paspalum	262.1	54.2	24.2	18.8	2.7	2.2	3.0
White clover	356.4	48.5	25.5	18.3	7.7	1.9	2.6
Lucerne	388.4	47.6	24.8	18.5	9.1	1.9	2.7
Red Clover	407.2	59.5	19.0	13.9	7.6	3.1	3.9
Lotus corniculatus	316.9	53.8	25.7	16.6	3.9	2.1	2.7
Lotus pedunculatus	240.4	63.0	17.7	16.2	3.0	3.6	4.5
Sulla	268.1	47.9	26.4	18.3	7.5	1.8	2.5
Chicory	344.3	54.7	18.8	21.1	5.4	2.9	4.0
Plantain	171.8	54.6	26.7	16.6	2.1	2.0	2.7
Conserved							
Pasture silage	305.4	52.1	23.5	16.8	7.5	2.2	2.9
Oat silage	328.1	56.5	21.1	14.3	8.1	2.7	3.4
Lucerne silage	283.6	54.4	28.0	11.6	6.0	1.9	2.4
Sulla silage	357.3	46.5	24.1	24.6	4.8	1.9	3.0
Maize silage	256.9	53.2	20.5	22.6	3.7	2.6	3.7
Lucerne hay	276.8	59.7	19.6	12.8	7.9	3.0	3.7
Maize grain	212.1	51.5	23.9	21.6	3.0	2.2	3.1

[1] Minor VFA include iso-butyrate, iso-valerate and n-valerate.

TABLE 3.16. Net VFA yields (mg/g DM/h) after 6, 12 and 24 hours (h) and rates of production (mg/g DM/h) from 0 – 6 h, 6 – 12 h, 12 – 24 h and 0 – 24 h.

Forage	Net VFA yield (mg/g DM)			Rates of production (mg/g DM/h)			
	6 h	12 h	24 h	0 – 6	6 – 12	12 – 24	0 – 24
Fresh							
Perennial ryegrass	130.0	212.1	286.5	21.7	13.7	6.2	11.9
Cocksfoot	115.8	166.4	328.4	19.3	8.4	13.5	13.7
Tall fescue	157.8	228.5	352.7	26.3	11.8	10.4	14.7
Yorkshire fog	108.8	220.0	339.2	18.1	18.5	9.9	14.1
Prairie grass	117.0	255.2	375.6	19.5	23.0	10.0	15.7
Grasslands Tama	173.9	320.4	398.0	29.0	24.4	6.5	16.6
Kikuyu	94.7	172.7	200.8	18.8	13.0	2.3	8.4
Paspalum	68.0	150.4	262.1	14.0	18.7	7.2	10.9
White clover	169.5	250.8	356.4	28.3	13.6	8.8	14.9
Lucerne	133.8	271.4	388.4	22.3	22.9	9.8	16.2
Red Clover	217.5	327.3	407.2	36.3	18.3	6.7	17.0
Lotus corniculatus	108.2	212.2	316.9	18.0	17.3	8.7	13.2
Lotus pedunculatus	124.7	177.7	240.4	20.8	8.8	5.2	10.0
Sulla	200.9	232.7	268.1	33.6	4.5	3.3	11.2
Chicory	181.5	272.6	344.3	30.2	15.2	6.0	14.3
Plantain	81.4	151.9	171.8	13.7	11.4	1.8	7.2
Conserved							
Pasture silage	108.0	210.2	305.4	18.0	17.0	7.9	12.7
Oat silage	121.1	226.4	328.1	20.2	17.6	8.5	13.7
Lucerne silage	142.9	244.5	283.6	23.8	16.9	3.3	11.8
Sulla silage	149.8	276.1	357.3	25.0	21.1	6.8	14.9
Maize silage	84.0	196.3	256.9	16.5	8.6	8.9	10.7
Lucerne hay	96.1	198.8	276.8	16.0	17.1	6.5	11.5
Maize grain	93.6	162.9	212.1	15.6	11.5	4.1	8.8

3.5 DISCUSSION

This study has produced a comprehensive set of degradation data for forages prepared in a manner similar to those consumed by ruminants. The combined use of *in sacco* and *in vitro* techniques indicate actual losses through digestion in the rumen (*in sacco*), and the net yield of metabolites from *in vitro* fermentation available for absorption and metabolism.

An important component of these data was the use of fresh, minced forages to replicate chewed material characteristic of ruminant diets in New Zealand. Although incubations have been undertaken with fresh, chopped perennial ryegrass, cocksfoot, white clover and *Lotus corniculatus* (Van Vuuren *et al*., 1991; Goplen *et al*., 1993; Hoffman *et al*., 1993; Kolver *et al*., 1998b; Barrell *et al*., 2000) most evaluations have been based on FDG preparations. The type of preparation has very significant effects on degradation kinetics, in terms of DM disappearance, proteolysis, VFA production and microbial growth (McNabb *et al*., 1996; Barrell *et al*., 2000; Cohen and Doyle, 2001; Lee *et al*., 2002). Barrell *et al*. (2000) showed no lag during *in sacco* degradation and greater *in vitro* NH_3 production at 2 hours for minced forage compared to chopped and FDG preparations, and McNabb *et al*. (1996) and Cohen and Doyle (2001) showed more rapid release of the soluble fraction of protein when fresh forage was minced compared to chopped, FDG or oven-dried preparations. Together these studies indicate that mincing reflected *in vivo* digestion more closely than other preparations.

Mincing of fresh forages produced a particle size distribution similar to that of chewed boli and rumen contents of sheep and cows (Table 2.9 in Chapter 2). Typical particle size distribution of DM in boli swallowed by sheep eating fresh forages comprise 49 – 51% larger than 1 mm and 32 – 38% soluble (Ulyatt *et al*., 1986), whilst rumen contents of cows fed lucerne and ryegrass comprise 42 – 47% over 1 mm and 17 – 22% soluble DM (Waghorn *et al*., 1989). Rumen contents of cows fed low fibre diets have smaller proportions of DM greater than 1 mm compared with fibrous diets. Waghorn (1986) reported that the rumen contents of cows fed red clover (37% NDF) had as much as 26% of DM retained on sieves greater than 1 mm and 24% soluble compared to the rumen contents of cows fed lucerne hay (59% NDF) where 38% was greater than 1 mm and 17% was soluble. The particle size distribution of rumen contents of cows fed different types of forages will depend on rate of eating, fibre content and plant anatomy but values reported in these studies were similar to that of minced forage (Table 2.9 in Chapter 2). This technique replicated the chewing during eating and rumination of forages when eaten by cows.

The extent to which particles are degraded is dependent on forage type and the amount of chewing that occurs during eating and rumination (Ulyatt *et al*., 1986). During eating about 60% of cell contents are released (Reid *et al*., 1962; Waghorn *et al*., 1989). The extensive cell rupture and greater amount of protein in intracellular contents corresponds to the higher proportion of soluble (A) fraction for protein compared to DM and to fibre which comprises plant cell walls.

3.5.1 *In sacco* digestion kinetics

The kinetic data of forages in this study corresponds to the rapid degradation and higher intakes of legumes and some grasses with a high nutritive value compared to the poorer quality tropical forages and some silages that have slower degradation and lower intakes (Weston, 1985; Ulyatt *et al*., 1986). Particle size distribution was similar for all minced preparations and was comparable to particle size distribution of swallowed boli, although swallowed boli of immature grass or legumes may have a higher proportion of larger particles than mature grasses which require more chewing for bolus formation. Substantial cell rupture occurs with extensive chewing of grasses but succulent forages undergo rapid cell rupture in the rumen (Waghorn, 1986; Kelly and Sinclair, 1989; Waghorn, *et al*., 1989). DM, CP and fibre degradation kinetics for succulent herbs and legumes were faster and degradation began earlier compared to fibrous grasses, especially tropical species, which had slower degradation rates and longer lag periods. Mincing temperate forages released about 41% DM into the soluble pool and this would be rapidly degraded. The insoluble degradable (B) fraction averaged 47% DM and variation in digestion of this fraction will affect nutrient supply and rumen clearance. The kinetics presented here represented bacterial activity without further structural breakdown associated with rumination, but the variation between forages agrees with differences in animal production (Ulyatt, 1981; Brown, 1990; Stevens *et al.*, 1992; Stevens *et al.*, 1993; Fraser and Rowarth, 1996; Johnson and Thomson, 1996; Burke *et al.*, 2002a; Chapter 2; Chapter 4). For example, the k value for perennial ryegrass DM (10.6 %/h) was much lower than that for white clover DM (21.1 %/h) and the initial degradation of perennial ryegrass was slow (Lag = 4.2 h) relative to white clover (Lag = 1.2 h). These differences are indicated by the effective degradability (ED; Table 3.7) of the two feeds and are supported by the relatively lower production of ruminants fed medium quality perennial ryegrass relative to high quality white clover (Ulyatt, 1981; Harris *et al.* 1997a, b; Chapter 4). Most data were derived from leafy and immature plant material that contained no stem, dead matter or seed head, and differences would be greater with more mature forage typical of summer growing conditions (Clark and Brougham, 1979; Van Vuuren *et al.*, 1991; Hoffman *et al.*, 1993; Elizadale *et al.*, 1999; Cohen, 2001; Chaves *et al.*, 2002a) where rumen fill (Weston, 1982, 1985, 1996) and voluntary intake are likely to further limit animal production. Perennial ryegrass in this study was lush, leafy and appeared to be of high quality at the time of collection, but NIR analyses showed a NDF concentration of 48.7% of DM and only 15.5% CP in the DM. The ryegrass studied here was typical of pasture during late spring, summer and early autumn (Table 2.4 in Chapter 2; Moller *et al.*, 1996; Stevenson *et al.*, 2003; Corson, unpublished; Prewer, unpublished). Ryegrass grown in late winter/early spring (June to September) has a lower NDF concentration (Table 2.4 in Chapter 2) and a higher feeding value than that studied here.

The 23 forages evaluated in this study include examples of slow degrading forages such as kikuyu and paspalum where 26 and 31% of DM were released into the soluble pool and rates of DM loss were slow at 7.1 %/h and 6.4 %/h, respectively with substantial lag times (7.8 and 4.2 h, respectively). In contrast chicory and plantain were rapidly degraded with DM losses of 26.0 %/h and 33.2 %/h, respectively with short lag times (0.4 h and 1.4 h, respectively). These data demonstrate some of the reasons why animal production can be very poor on some diets. Forages with slow degradation have high NDF content, provide limited energy (VFA) to the animal, less availability of N for microbial growth (ERDP) and generally rumen clearance is slower which can only be countered by extensive chewing. Physical factors appear to limit feed intakes of dairy cows in New Zealand (Waghorn, 2002) despite very large rumen digesta pool sizes. Diets which are slowly digested may require benefit from substitution with rapidly degraded forages to achieve improved productivity, and data presented here will enable diet formulation based on kinetic information to achieve a balance between nutrient release from digestion and limitations to intake associated with breakdown of structural fibre.

Low-fibre legumes and herbs were more easily minced than temperate grasses which were more easily minced than tropical species. During forage preparation for this trial the mincer required four to five times more current to mince grasses compared to white clover, twice as much current was needed to mince chicory compared to white clover, and tama ryegrass used 17% less current than white clover (Waghorn, unpublished). Rupture of herbage during chewing is a function of both tensile and shear strength (Wilson, 1965; MacKinnon *et al.*, 1988) and both tensile and shear strength have been used to indicate the resistance of herbage to breakdown, and brittleness and fragmentation of herbage during chewing and rumination (Easton, 1989; Stone, 1994). Research by Evans (1964, 1967a, b) reported that ryegrass leaves with high concentrations of cellulose and sclerenchyma tissues had high leaf tensile strength due to the high tensile stiffness of sclerenchyma tissues (Vincent, 1982). Leaves with high tensile and shear strength required more chewing and rumination, were digested more slowly, and sheep had smaller feed intakes than ryegrass leaves with low tensile strength and low concentrations of cellulose and sclerenchyma tissues (John *et al.*, 1989; Inoue *et al.*, 1989, 1994). Weston (1985) estimated that the energy required to reduce clover to < 1 mm would be less than that of poorly digested grass with high cell wall contents (Table 3.17). As ryegrass matures fibre content increases and cross linkages between lignin and other constituents occur (Hatfield *et al.*, 1999) affecting the structural resilience of fibre. These linkages are not indicated by either lignin or NDF concentration, but they appear to account for differences in rates of physical breakdown which affect nutrient release and clearance from the rumen.

TABLE 3.17. Mean data on the composition and digestion by sheep of 20 herbages grouped on the basis of dry matter (DM) digestibility (Weston, 1985).

	Clover	Grass		
Digestibility	High	High	Medium	Low
Cell wall contents (g/kg DM)	360	430	560	670
Comminution energy[1] (kJ/kg DM)	24	31	64	117
DM digestibility (%)	78	81	72	56
Voluntary DM intake (g/day/lwt[2])	85	75	65	51
Voluntary DE[3] intake (MJ/day/lwt[2])	1.28	1.15	0.89	0.55
Time spent eating	2.8	3.6	5.2	6.8
Time spent ruminating	3.7	5.1	8.4	11.1

[1] Energy to reduce particles that pass through a 1 mm aperture screen
[2] Liveweight (kg $^{0.75}$)
[3] Digestible energy

Effective degradability estimates the amount of forage likely to be digested *in vivo* and is calculated (Equation 3.3) using predetermined outflow rates, however the type of forage and level of intake will affect retention time, rumen degradation and ED. For example, ruminants fed low fibre forages (eg. white clover and chicory) have shorter DM retention times and consequently faster outflow rates compared to medium quality perennial ryegrass fed to ruminants (Thornton and Minson, 1973; Kusmartono *et al*., 1996; Kusmartono and Barry, 1997). Dairy cows fed at maintenance have slower outflow rates compared to cows fed at 3 x maintenance (AFRC, 1992), so ED enables comparative measurements of rumen parameters that are affected by feed and physiological characteristics. Slow degradation rates will limit feed intake because of constraints associated with rumen fill, although lactation may override this regulation to a limited extent (Weston, 1985, 1996).

3.5.2 Forage characteristics and animal production

Tall fescue and red clover had degradation parameters for DM, CP and fibre components that were not typical of perennial temperate grasses or legumes, respectively, which may explain production by animals on these forages. Tall fescue can produce more DM in summer-dry regions compared to perennial ryegrass, but milk production has not been improved (Thomson *et al.*, 1988; McCallum *et al.*, 1992; Rollo *et al.*, 1998). The tall fescue used for incubations had adequate CP, but there was a substantial lag in DM and NDF degradation. The slower DM and NDF degradation of tall fescue resulted in DM ED of 74% and NDF ED of 54% for tall fescue compared to 76 to 81% DM and 64 to 71% NDF for other temperate grasses. Ammonia and VFA yields suggested an adequate digestion but clearance of fibre may limit production. In field trials animal production benefited from tall fescue pastures, but this was mainly due to the greater clover content in tall fescue swards (Davies and Morgan, 1982; Milne *et al*, 1997; Hyslop *et al.*, 2000). Tall fescue can grow rapidly during late spring and summer and digestibility can fall rapidly as it approaches reproductive maturity (Minson *et al.*, 1964), therefore intensive grazing management is necessary to maintain the sward in a leafy state and prevent deterioration of feed quality (Rollo *et al.*, 1998). Compared to maturing tall fescue, the decline in feed quality of other maturing temperate grass species is not as early and severe (Minson *et al.*, 1964).

The unusual degradation of red clover was reported by Hoffman *et al.* (1993) where the *in sacco* degradation of NDF was slower than other legumes, and Broderick and Albrecht (1997) suggested an *in vitro* protein degradability that was comparable with forages containing low levels of tannins. Liveweight gain has been improved by feeding red clover compared to ryegrass and white clover swards (Niezen *et al.*, 1993; Semiadi *et al.*, 1993; Soetrisno *et al.*, 1994) and by feeding red clover silage compared to lucerne silage (Broderick *et al.*, 2000) and by feeding red clover compared to chicory (Niezen *et al.*, 1993). Milk production of dairy cows was greater when fed red clover swards in autumn compared to pasture-based swards (Brookes and Wilson, 1983).

Studies comparing perennial ryegrass with annual or short-rotation ryegrasses (eg. Grasslands Tama) have demonstrated the better production of animals fed annual ryegrass species compared to perennial ryegrass (Ulyatt, 1971). The lower fibre and higher soluble carbohydrate content of Grasslands Tama ryegrass compared to perennial ryegrass and other temperate grass species resulted in a faster breakdown and passage (Ulyatt, 1971). Calculated ED for DM, CP and NDF, and rates of NH_3 and VFA production from *in vitro* incubations complement measurements of animal production (Ulyatt, 1981).

Forages containing CT released a similar proportion of soluble N as other legumes but NH_3 concentrations in the media were lower. These findings complement *in vivo* studies in sheep fed *Lotus corniculatus* (Waghorn *et al*., 1987a, b; Wang *et al*., 1996a, b), sulla (Stienezen *et al*., 1996; Douglas *et al*., 1999) and *Lotus pedunculatus* (Waghorn *et al*., 1994b) where CT lowered the concentration of NH_3. Waghorn *et al*. (1994a) also demonstrated lower rumen DM turnover (rumen clearance) attributable to CT in *Lotus pedunculatus* fed to sheep. The effect of CT in reducing protein solubility, degradation and microbial activity are further documented by Waghorn and Jones (1989), McNabb *et al*. (1996) and Min *et al*. (2000). In order to determine the effect of CT on *in sacco* digestion in this study, bags of CT and non-CT forages should have been incubated in a cow fed a CT containing diet and the same diet with daily administration of polyethylene glycol (PEG) to remove effects of CT. Min *et al*. (2000) showed CT in *Lotus corniculatus* slowed the rates of solubilization of Rubisco and that CP from white clover was degraded more rapidly than that from *Lotus corniculatus* with or without CT. There were no differences in *in sacco* losses of Rubisco from minced *Lotus pedunculatus* incubated in sheep fed *Lotus pedunculatus* with and without an intraruminal infusion of PEG (McNabb *et al*., 1996). Effects of CT on *in sacco* digestion appear to differ with feed type.

The slow initial degradation of the insoluble DM, CP and fibre components of *Lotus pedunculatus* and low ED values compared to *Lotus corniculatus* and sulla are most likely due to the type and concentration of CT in *Lotus pedunculatus* (Waghorn and Shelton, 1995; 1997) which had a similar chemical composition to other CT legumes but very different degradation kinetics. Animal studies support reduced digestibility and low DM intakes when *Lotus pedunculatus* with high CT concentration has been fed even when the CT content has been diluted by feeding with ryegrass (Waghorn and Shelton, 1995).

3.5.3 The *in sacco* procedure

The method used in this study enabled comparison and ranking of forages on the basis of their DM, CP and fibre digestion kinetics which were similar to those observed in field studies. Some aspects of the procedure recommended by Broderick and Cochran (1999) differed to those used in this study, particularly the use of one cow fed a single diet. However, the purpose here was ranking, in which case the use of a single cow was adequate (Orskov, 2000). The variation between cows in *in sacco* digestion kinetics is well known (Chapter 2; Weimer *et al*., 1999) and the effects of diet on degradation rate (Mertens *et al*., 1998; Weimer *et al*., 1999; Chapter 2) are also important and have been addressed in Chapter 6. However, of primary importance for this study was the need for a consistent ruminal environment to achieve a defensible data set and lucerne hay was chosen over ryegrass pasture which has rapid and significant changes in composition over the growing season (Wilson and Moller, 1993; Moller *et al*., 1996).

The ruminal environment was monitored at each incubation (pH, NH_3, VFA) and a single incubation was used to determine relativity between runs. However future trials of this type would benefit from inclusion of an appropriate forage standard. Perennial ryegrass has demonstrated inconsistent behaviour, especially in *in vitro* incubations (Waghorn and Burke, unpublished). Use of vegetative lucerne may provide an optimal standard as it comprises rapidly degraded leaf as well as fibrous stem, and bags also need to be removed more frequently than the single (12 h) time point used here.

In this study forage samples were collected, frozen and held frozen until incubating *in sacco* and *in vitro*. MacRae (1970), MacRae *et al*. (1975) and Kohn and Allen (1992) showed that freezing and thawing fresh and ensiled forage changed the chemical composition of the forage, but Huntington and Givens (1997) showed freezing forage prior to *in sacco* incubation had small effects on DM degradability and the difference between fresh and frozen material was of limited practical significance. We are confident that harvesting and freezing fresh forage samples for later use is a satisfactory method to store forage for analysis. The benefits of this system far outweighed the difficulties associated with harvest and incubation within a limited time period, especially in view of diurnal (Fulkerson and Trevaskis, 1997; Ciavarella *et al*., 2000;

Trevaskis *et al.*, 2001) and seasonal (Wilson and Moller, 1993; Moller *et al.*, 1996) effects on forage composition.

Crude protein degradation kinetics in this study were not corrected for microbial contamination (adhesion to fibre). The extent of microbial colonization (Nocek and Grant, 1987; Messman *et al.*, 1996) on degradation curves of low protein forages were shown as a net increase in forage N content and an underestimation of degradation in the first 6 hours (similar to CP disappearance for maize silage in Appendix of 6.6). Adjusting for microbial adhesion may reduce the lag period and estimates of rate and extent of degradation may increase (Olubobokun *et al.*, 1990; Wanderley *et al.*, 1993), but this would have only affected maize grain, maize silage, kikuyu and paspalum. Comparison of estimates using the lag and non-lag equations did not shift the relative ranking of forages.

3.5.2 *In vitro* products of fermentation

The measurement of *in vitro* pH was used as a check on the buffering of the closed system to ensure the *in vitro* system did represent the *in vivo* situation. Dairy cows grazing pasture have significant changes in rumen pH often declining from about 6.8 pre-feeding to about 5.6 (Holden *et al.*, 1994; Mackle *et al.*, 1996; Carruthers and Neil, 1997; Carruthers *et al.*, 1997). Rumen pH does not fall below 5.6 under pastoral grazing (Holden *et al.*, 1994; Mackle *et al.*, 1996; Carruthers and Neil, 1997; Carruthers *et al.*, 1997) so instances of very low *in vitro* pH (eg. below 5.0) were considered atypical and sub-optimal and the data were not used in evaluations. In this study five feeds had an *in vitro* pH less than 5.6 at 24 hours of the incubation: maize silage, maize grain, perennial ryegrass, cocksfoot and tall fescue. Low pH has been reported in cattle when maize silage and maize grain were fed (Bergman, 1990; Nocek, 1997; NRC, 2001) and the rapid pH decline *in vitro* matches the *in vivo* situation. The rapid decline in *in vitro* pH when ryegrass was incubated has also been observed *in vivo* (Holden *et al.*, 1994; Mackle *et al.*, 1996; Carruthers and Neil. 1997; Carruthers *et al.*, 1997) and may contribute to slow degradation of fibre in cows fed pasture. De Veth and Kolver (2001a) reported a rumen pH value of less than 5.8 and the length of time that the rumen pH was less than 5.8 (De Veth and Kolver, 2001b) will impair microbial protein synthesis and fibre digestibility of high quality pasture diets.

The NH_3 concentration *in vitro* is a consequence of both protein degradation and NH_3-N utilisation by rumen microbes and can indicate the extent of wasteful protein degradation that occurs during digestion. Excess NH_3 production incurs a metabolic cost for disposal and has been implicated in relatively poor production of ruminants fed fresh forages (Beever, 1993), although the true impact of excess NH_3 on metabolism is not well understood (Fitch *et al*., 1989; Beever, 1993; Lobley *et al*., 1995; Greaney *et al*., 1996; Cohen, 2001). This study has shown that forages with a high concentration of CP and a low fibre concentration (eg. white clover, red clover, lucerne) have rapid DM, CP and fibre degradation. The excess NH_3 produced is not utilised by microbes and has to be excreted as urea at a net metabolic cost to the animal (Beever, 1993). In contrast, forages with insufficient degradable protein (eg. paspalum and maize silage) have low NH_3 production and high utilisation which may limit microbial growth and fibre degradation, but VFA production did not appear to be closely related to NH_3 supply. Utilisation of NH_3-N is also affected by the amount and nature of the carbohydrate fermented. For example, in the case of maize silage and maize grain the high concentration of fermentable carbohydrate (grain component) enabled all of the NH_3-N to be utilised and bacterial growth was likely to be limited by N availability (Broderick, 1982).

Condensed tannin reduced degradation of DM, CP and fibre and reduced proteolysis (McNabb *et al*. 1996; Broderick and Albrecht, 1997; Min *et al*. 2000). Differences in net release of NH_3 between legumes containing CT and legumes not containing CT appeared after 6 hours of incubation and were evident over 24 hours (Figure 3.11). Forages with CT may complement forages releasing excess NH_3 because CT is able to bind protein from other plants (Waghorn and Jones, 1989) and result in a more efficient protein and energy capture from a mixed diet than either fed alone. For example, forages with CT, particularly sulla and *Lotus corniculatus* may have characteristics that could complement high protein and low fibre white clover. In contrast, maize silage contains insufficient N for maximum microbial growth (Satter and Slyter, 1974) and feeding with rapidly digested legumes or grasses should improve animal production (Beever, 1993). Provision of available energy can increase microbial growth and capture of NH_3 (Dellow *et al.*, 1988; Obara *et al.*, 1991), and trials have optimised the ratio of N and rapidly fermentable carbohydrate in an attempt to improve microbial protein synthesis and animal production. Those trials have generally reported reduced rumen NH_3-N concentration and improved microbial protein synthesis, but effects on milk production were inconsistent (Van Vuuren *et al.*, 1990; Sinclair *et al.*, 1993; Trevaskis *et al.*, 2001). An attempt to synchronise rapidly fermentable carbohydrate with pasture N has appeared to improve the capture of ruminal N, based on changes in ruminal ammonia concentrations, but effects were transient and did not change the N status or production of dairy cows (Kolver *et al.*, 1998b).

VFA represent about 65 – 75% of energy absorbed by ruminants and both rates of production and proportions of individual VFA affect lactose synthesis and milk production. Acetate and butyrate typically account for 75% or more of the VFA produced from degradation of pasture by cows (Church, 1976; Bergman, 1990; Mackle *et al.*, 1996), but diets that produce relatively more propionate to acetate and butyrate improve energy capture and ensure adequate precursors for glucogenesis without catabolism of glucogenic amino acids (Van Soest, 1994). Sulla and *Lotus corniculatus* were two examples of forages that had relatively low A:P and (A+B):P ratios compared to diets incorporating substantial amounts of grain or root crops which can result in substantial lactic acid production from fermentation, lower rumen pH, inhibition of fibre degradation and in some cases milk fat depression (Van Soest, 1994; Holmes *et al.*, 2001). This study did not measure *in vitro* lactate concentration, but the incubations did enable an effective and rapid evaluation of several forages to indicate products of fermentation and ranking in terms of (A+B):P ratios.

Total VFA concentration of forages incubated *in vitro* support differences observed in animal studies. Ruminants fed white clover have higher total rumen VFA concentration than those fed perennial ryegrass (Ulyatt, 1971; Beever *et al.* 1986) which were similar to relationships from *in vitro* incubations. Condensed tannin reduced the molar proportion of minor VFA and proteolysis in sheep fed *Lotus corniculatus* (Wang *et al.*, 1996a, b) and sulla (Terrill *et al.*, 1992a). Similar differences between legumes with and without CT were evident when incubated *in vitro*.

In vitro incubations have produced some interesting insights into feed value. For example, degradation and fermentation kinetics of red clover and sulla in this study suggest they could be given more prominence in diets. Red clover lost favour with producers because of its association with bloat and the presence of oestrogenic compounds in some cultivars that lowered sheep fertility, but bloat can be controlled by routine drenching and new cultivars have low concentrations of oestrogenic compounds. In contrast, plantain digestion was rapid *in sacco*, but minimal *in vitro*, suggesting anti-microbial compounds might be present and could impact on the microflora and animal production. Mixed results have been reported when plantain swards have been fed to sheep resulting in both poor liveweight gain relative to chicory, ryegrass and white clover (Robertson *et al*., 1995; Fraser and Rowarth, 1996), improved liveweight gain relative to pasture (Moorhead *et al*., 2002) and no improvements on milk production from cows (Nicolas, unpublished). *In sacco* and *in vitro* fermentation data support the poor production of animals fed *Lotus pedunculatus* (Barry and Duncan, 1984; Barry and Manley, 1984; Barry, *et al*., 1986) compared to other legumes containing CT (*Lotus corniculatus* and sulla).

3.5.3 CNCPS diet evaluation

The CNCPS is a process driven model based on the NRC (2001) feeding requirements for dairy cows. It has been designed and validated for dairy cattle fed forage and concentrate diets in confinement (Fox et al., 1992; Russell et al., 1992; Sniffen et al., 1992) and modified to accommodate pasture-based diets (Kolver *et al*., 1998a). The model predicts nutrient supply from digestion and absorption, nutrient requirements for metabolism and production, and nutrient excretion. It also attempts to predict supply and requirements for amino acids (AA) by dairy cows. Degradation rates of pasture fibre and protein, the value given to effective fibre and the lignin content significantly affected estimates of ME and metabolisable protein (MP) supply as well as the profile of amino acids for milk production (Kolver *et al*., 1998a). Rates of protein and fibre degradation and distribution of components between the A, B and C fractions for forages from this experiment were used in the CNCPS model to evaluate the capability of individual forages and forage mixtures for meeting nutrient requirements of cows.

Inputs to the model were based on industry milk production data (Livestock Improvement Corporation; Dexcel Limited; Holmes *et al*., 2002) for a 500 kg Friesian cow in early lactation (90 days since calving) producing 22.7 kg milk with 4.7% milkfat and 3.3% protein (1.82 kg milksolids/day) and late lactation (210 days since calving and 120 days pregnant) producing 15.0 kg milk with 4.7% milkfat and 3.3% protein (1.2 kg milksolids/day). CNCPS predicted that the defined cow would consume 17.1 kg DM/day in early lactation and 14.6 kg DM/day in late lactation and this intake was used to evaluate all forages. Data collected from this experiment provided feed composition (Table 3.2), the amount of protein that was soluble (Table 3.7) and degradation rates for the slowly degradable (B_2) NDF (Table 3.8) and protein (Table 3.7) fractions of forages, and the CNCPS feed library provided additional values required for the model. Feed composition and degradation rates for the carbohydrate and protein fractions used for the CNCPS evaluation are presented in Tables 3.18 to 3.20. For the defined cow eating 17.1 and 14.6 kg DM/day in early and late lactation the model predicted milk production and liveweight change and estimated microbial growth and other parameters to indicate feed digestion and utilisation of nutrients for all forages examined in this experiment.

The model is particularly sensitive to the concentration of effective fibre in each forage and because these values are poorly defined for grazed forages, simulations were initially carried out using both 40% and 60% NDF values for effective fibre for all forages (except paspalum, kikuyu, silages and hays) to evaluate the impact of effective fibre concentrations on model predictions. Paspalum and kikuyu were only given values of 60% NDF and library values from Chaves (unpublished) were used for silages and hays. Tables illustrating the CNCPS outputs for forages at both 40% and 60% NDF of effective fibre are presented in Appendix 3.17.

Predictions of cow production when fed individual forages were based on a value of 60% NDF, except for white clover, chicory, plantain and sulla which had rapid degradation rates and an effective fibre concentration of 40% NDF was used (Appendix 3.17). Comparisons of individual forages were also made with high quality perennial ryegrass derived from the CNCPS library. The high quality ryegrass from the CNCPS library contained 43.4% NDF and 26.3% CP in the DM and the amount of effective fibre was 40% NDF for over-grazed (OG) and 60% NDF for well-managed (WM) ryegrass pasture, compared with the perennial ryegrass evaluated in this chapter (Table 3.2) with an NDF and CP concentration of 48.7 and 15.5% of the DM, respectively. Several forage mixtures were also evaluated using the same procedure as for individual forages.

TABLE 3.18. Composition of forages evaluated using the CNCPS model.

Forages	DM (%)	NDF (% DM)	Lignin (% NDF)	Fat (% DM)	Ash (% DM)	Starch (% DM)
Perennial ryegrass	18.8	48.7	6.98	4.0	10.7	48.0
Cocksfoot	26.8	47.5	10.73	6.4	9.7	46.0
Tall fescue	25.3	41.6	9.10	6.4	9.7	46.0
Yorkshire fog	16.3	39.9	7.80	6.4	9.7	46.0
Prairie grass	19.1	44.8	8.50	6.4	9.7	46.0
Grasslands Tama	15.2	36.5	8.00	4.0	10.7	48.0
Kikuyu	17.2	47.7	8.00	6.4	9.7	46.0
Paspalum	30.9	57.8	11.90	6.4	9.7	46.0
White clover	15.0	25.6	23.10	3.9	10.7	60.0
Lucerne	23.9	29.5	20.70	6.4	9.7	60.0
Red clover	14.8	33.6	18.50	3.9	10.7	60.0
Lotus corniculatus	16.2	28.2	25.50	3.9	10.7	60.0
Lotus pedunculatus	16.3	33.1	30.00	3.9	10.7	60.0
Sulla	11.6	22.4	38.00	3.9	10.7	60.0
Chicory	14.3	23.8	29.40	3.9	10.7	60.0
Plantain	13.0	28.3	30.00	3.9	10.7	60.0
Pasture silage	40.8	50.3	8.55	2.6	7.5	59.0
Oat silage	40.0	53.2	8.08	3.1	10.1	53.0
Lucerne silage	57.4	30.5	26.20	3.2	9.0	89.0
Sulla silage	22.6	36.2	11.90	5.2	10.1	64.0
Maize silage	34.7	40.5	10.90	3.1	4.0	80.0
Lucerne hay	89.9	39.1	21.70	2.5	9.0	64.0
Maize grain	88.0	10.0	2.22	4.3	1.6	97.5
Pasture-Ryegrass – OG[1]	25.0	43.4	7.83	7.3	11.4	48.0
Pasture-Ryegrass – WM[1]	25.0	43.4	7.83	7.3	11.4	48.0

[1] High quality pasture ryegrass from the CNCPS library, either defined as over-grazed (OG) or well-managed (WM). The only difference between the two ryegrasses is the effective fibre concentration. The effective fibre values for OG ryegrass was 40% NDF and for WM ryegrass was 60%.

Abbreviations: Dry matter, dry matter; NDF, neutral detergent fibre.

TABLE 3.19. Energy and protein values for forages evaluated by the CNCPS model.

Forages	CP (% DM)	UIP (% DM)	Sol-P (% CP)	NPN (% Sol- P)	NDFIP (% CP)	ADFIP (% CP)
Perennial ryegrass	15.5	22.4	52.2	2.44	4.54	1.65
Cocksfoot	23.7	27.7	55.0	2.44	9.00	1.70
Tall fescue	16.4	27.7	53.0	2.44	9.00	1.70
Yorkshire fog	23.7	27.7	54.8	2.44	9.00	1.70
Prairie grass	19.9	27.7	52.4	2.44	9.00	1.70
Grasslands Tama	21.3	22.4	56.4	2.44	4.54	1.65
Kikuyu	16.4	27.7	47.9	4.76	15.89	4.70
Paspalum	13.5	27.7	29.1	4.76	15.89	4.70
White clover	26.9	29.1	38.4	2.17	4.30	2.14
Lucerne	29.9	27.7	52.0	4.76	15.89	4.70
Red clover	27.4	29.1	30.1	2.17	4.30	2.14
Lotus corniculatus	22.2	29.1	51.1	2.17	4.30	2.14
Lotus pedunculatus	21.5	29.1	33.0	2.17	4.30	2.14
Sulla	23.0	29.1	49.5	2.17	4.30	2.14
Chicory	19.3	29.1	29.3	2.17	4.30	2.14
Plantain	24.7	29.1	33.3	2.17	4.30	2.14
Pasture silage	17.2	26.5	72.1	100.00	31.00	8.00
Oat silage	17.8	34.2	73.3	100.00	30.00	10.00
Lucerne silage	23.3	30.1	52.5	70.00	27.00	15.00
Sulla silage	21.2	38.4	55.4	28.00	15.00	10.00
Maize silage	7.6	22.0	17.4	100.00	16.00	7.00
Lucerne hay	24.2	33.1	37.6	70.00	18.00	11.00
Maize grain	9.8	51.9	12.0	73.00	15.00	5.00
Pasture-Ryegrass – OG[1]	26.3	22.4	43.0	4.76	9.10	3.04
Pasture-Ryegrass – WM[1]	26.3	22.4	43.0	4.76	9.10	3.04

[1] High quality pasture ryegrass from the CNCPS library, either defined as over-grazed (OG) or well-managed (WM). The only difference between the two ryegrasses is the effective fibre concentration. The effective fibre values for OG ryegrass was 40% NDF and for WM ryegrass was 60%.

Abbreviations: CP, crude protein; UIP, undegraded intake protein; Sol-P, soluble protein; NPN, non-protein nitrogen; NDFIP, neutral detergent insoluble protein; ADFIP, acid detergent insoluble protein.

TABLE 3.20. Degradation rates for forages evaluated by the CNCPS model.

Forages	Degradation rates (%/h)					
	Carbohydrate			Protein		
	A	B_1	B_2	B_1	B_2	B_3
Perennial ryegrass	85	21.5	9.3	200	15.0	2.00
Cocksfoot	85	31.3	9.9	200	15.9	2.00
Tall fescue	85	31.3	6.4	200	7.5	2.00
Yorkshire fog	85	31.3	7.7	200	8.7	2.00
Prairie grass	85	31.3	7.1	200	17.1	2.00
Grasslands Tama	85	21.5	7.8	200	13.9	2.00
Kikuyu	85	25.0	5.8	200	12.5	2.00
Paspalum	85	25.0	7.8	200	7.8	2.00
White clover	34	32.9	28.3	150	19.1	1.25
Lucerne	34	32.9	17.0	150	15.3	2.00
Red clover	34	32.9	13.0	150	10.4	1.25
Lotus corniculatus	34	32.9	12.3	150	15.0	1.25
Lotus pedunculatus	34	32.9	8.1	150	11.4	1.25
Sulla	34	32.9	16.4	150	11.7	1.25
Chicory	34	32.9	33.9	150	27.3	1.25
Plantain	34	32.9	24.1	150	34.9	1.25
Pasture silage	10	25.0	4.7	200	10.4	1.75
Oat silage	10	50.0	7.3	300	7.8	0.20
Lucerne silage	10	25.0	11.2	150	14.1	1.75
Sulla silage	10	25.0	6.3	150	7.4	1.75
Maize silage	10	35.0	4.1	300	3.4	0.25
Lucerne hay	10	30.0	4.1	150	10.2	1.25
Maize grain	200	10.0	4.0	135	6.0	0.10
Pasture-Ryegrass – OG[1]	85.3	19.2	14.0	200	12.0	2.00
Pasture-Ryegrass – WM[1]	85.3	19.2	14.0	200	12.0	2.00

[1] High quality pasture ryegrass from the CNCPS library, either defined as over-grazed (OG) or well-managed (WM). The only difference between the two ryegrasses is the effective fibre concentration. The effective fibre values for OG ryegrass was 40% NDF and for WM ryegrass was 60%.

Abbreviations for digestion rates: Carbohydrate: A, sugar; B_1, starch and pectic; B_2, available neutral detergent fibre. Protein: B_1, rapidly degraded protein; B_2, intermediately degraded protein; B_3, slowly degraded protein.

3.5.3.1 Evaluation of individual forages

Evaluation of individual forages were carried out in early lactation with grasses, legumes, conserved forages and differently managed ryegrass from the CNCPS library (Table 3.21), and in late lactation with the same forage types (Tables 3.22).

In both early and late lactation the first limiting nutrient for milk production was ME for white clover, sulla, red clover, lucerne, *Lotus pedunculatus* and *Lotus corniculatus*, cocksfoot, yorkshire fog, tama ryegrass, WM and OG ryegrass and lucerne hay. Metabolisable protein was the first limiting nutrient for milk production when perennial ryegrass, tall fescue, prairie grass, kikuyu, paspalum, chicory, plantain and silages made from pasture, sulla, maize and oats were evaluated. Tama ryegrass, *Lotus corniculatus* and plantain were the only forages where the effective fibre concentration altered the nutrient that was limiting milk production and this applied for both early and late lactation. Metabolisable protein was first limiting for Tama ryegrass, *Lotus corniculatus* and plantain when 40% of NDF was effective, whereas ME limited milk production if 60% NDF was used for effective fibre. However, differences in predicted milk production were small for *Lotus corniculatus* and plantain.

In early lactation predicted milk production from ME ranged between 14.5 (Lucerne hay) to 24.4 kg milk/day (WM ryegrass) and predicted milk production from MP ranged between 8.6 (pasture silage) to 34.7 (red clover) kg milk/day. The difference between predicted milk production from ME and MP supply ranged between 0.3 (tama ryegrass) to 14.6 kg milk/day (red clover).

Milk production from ME in late lactation ranged between 10.6 (lucerne hay) and 19.0 (WM ryegrass) and from MP ranged between 7.2 (pasture silage) to 27.8 (red clover) kg milk/day. Differences between predicted milk production supplied by ME and MP in late lactation were of similar magnitude to early lactation ranging from 0.3 (plantain) to 12.6 (red clover) kg milk/day.

Potential milk production for all grasses ranged between 16 kg/day (paspalum) to 24.4 kg/day (WM ryegrass) in early lactation and 12.9 to 18.6 kg/day for the same grasses in late lactation. For legumes the range was from 16.2 (*Lotus pedunculatus*) to 23.1

kg/day (lucerne) in early lactation and in late lactation from 11.9 (*Lotus pedunculatus*) to 17.7 kg/day (lucerne). With the conserved feeds, potential milk production ranged from 8.6 to 17.1 kg/day in early lactation and 7.2 to 13.7 kg/day in late lactation for pasture silage and sulla silage, respectively.

The supply of ME and MP are given in Tables 3.21 and 3.22. Total MP ranged between 1550 to 2000 g/day for grasses, 1727 to 2405 g/day for legumes and 1476 to 1547 g/day for herbs in early lactation. Comparable values (g/day) in late lactation were 1309 to 1668 (pastures), 1431 to 1987 (legumes) and 1229 to 1268 (herbs). The CNCPS model evaluates microbial protein production and undegraded feed protein values, based on the integration of feed carbohydrate and protein fraction pool sizes, microbial growth on fibre and non-fibre fractions, digestion and passage rates. The proportion of MP that is supplied by microbes is given in the tables. Microbial protein supply ranged between 34% (red clover) to 65% (pasture silage) in early lactation with similar percentages in late lactation. White clover, lucerne, red clover, sulla, *Lotus pedunculatus* and plantain supplied between 34 to 43% of microbial protein, whereas microbial protein supply was 51% for *Lotus corniculatus* and 52% for chicory. Microbial protein supplied from most grasses ranged between 44 to 57% of total MP with the exception of the OG and WM ryegrasses (high quality ryegrass) at 35 – 38% and ryegrass used in this experiment (medium quality ryegrass) at 65%. Silages supplied between 51 to 65% of MP as microbial protein.

The supply of ME in early lactation ranged between 142 (lucerne hay) to 220 (maize grain) MJME/day and for late lactation ranged between 123 and 192 MJME/day. The supply of MP (g/day) per MJME has been calculated and forages supplied between 6.8 to 13.8 g MP/MJME/day in early lactation and 6.6 to 13.3 g MP/MJME/day in late lactation and the ratio of predicted milk from MP supply and ME supply ($\mathrm{MP{:}ME_{milk\ ratio}}$) ranged between 0.47 to 1.73 and 0.51 to 1.83 for early and late lactation, respectively. Regression analysis of the predicted MP:ME milk ratio against the MP/ME supplied by the diet (g MP/MJME) in early lactation gave an equation of: $\mathrm{MP{:}ME_{milk\ ratio}}$ 0.156 x (diet MP/ME; g MP/MJME) – 0.579 ($R^2 = 0.86$). Therefore if the diet provided a balance of energy and protein to produce the same amount of milk (predicted milk $\mathrm{MP{:}ME_{milk\ ratio}} = 1$) 10.1 g MP/MJME would need to be supplied, while in late lactation 9.4 g MP/MJME would be required to supply a balanced supply of MP and ME [$MP:ME_{\text{milk ratio}} = 0.191 \times$ (diet MP/ME; g MP/MJME) – 0.806; $R^2 = 0.91$] for milk production.

The urea cost estimated by CNCPS is the energetic cost of excreting excess absorbed nitrogen as urea. Forages with higher CP concentrations (white clover, red clover and lucerne) resulted in higher costs of urea excretion than forages with lower CP concentrations (kikuyu, paspalum and maize silage). The cost of urea excretion for high protein legumes ranged between 10.6 to 12 MJ/day (about 6.5 to 7.3 % ME consumed in early lactation) compared to lower values for legumes containing CT (5.5 to 7.9 MJ/day or 3.7 to 4.7% of ME intake). The rapidly digested chicory and plantain had urea costs of 5.9 (3.4% ME intake) and 10.2 (6.2% ME intake) MJ/day. The cost of urea excretion for temperate grasses was between 1.5 (perennial ryegrass) and 9.6 MJ/day (OG ryegrass) (0.8 to 4.9% ME intake) in early lactation with very low values for paspalum and kikuyu (0 to 1.4% ME intake). The urea costs were slightly less during late lactation with similar relativity between forages.

In this experiment forages that are balanced had equal predicted milk production from the supply of ME and MP when modelled using CNCPS. High quality OG and WM ryegrasses are "well balanced" for ME and MP in early lactation (MP:ME milk ratio = 1.01) and have surplus MP in late lactation (MP:ME milk ratio = 1.34). This in contrast to the ryegrass used in this experiment which was deficient in MP in both early (MP:ME milk ratio = 0.85) and late (MP:ME milk ratio = 0.89) lactation.

Forages identified as suitable for feeding with medium quality ryegrass used in this experiment were those which could provide the limiting nutrient (MP), maximise milk production and/or reduce the cost of excreting excess N. The CNCPS model identified lucerne, red clover *Lotus corniculatus*, *Lotus pedunculatus*, sulla and sulla silage for feeding with medium quality pasture. The feeding value of legumes were limited by ME and would be ideal for feeding with medium quality pasture that was limited by MP. As an individual forage, lucerne had excellent potential for milk production and exceeded target milk production in early (23.1 vs. 22.7 kg/day) and late lactation (17.7 vs. 15.0 kg/day). Lucerne grows predominantly during spring to autumn, can achieve high yields up to about 18 tDM/ha and has potential to be fed with pasture. The high MP for red clover, relative to ME (sufficient for 34.7 and 20.1 kg/day in early lactation) suggested excellent potential for feeding with a forage where ME was high and MP limiting. Sulla and *Lotus corniculatus* had lower urea costs and predicted milk production from ME and MP differed by only about 1 kg/day and should be suitable as sole diets for milk production. The potential of sulla silage was apparent with ME and MP milk production in early lactation of 21.6 and 17.1 kg/day, respectively, which was 2.5 kg better than the next best conserved forage, lucerne silage, making sulla silage an excellent forage for feeding with MP limiting forages, eg. legumes.

TABLE 3.21. Early lactation metabolisable energy (ME; MJ/day) and metabolisable protein (MP; g/day) supplied by forages and predicted milk production of dairy cows expected to produce 22.7 litres milk/day and consume 17.1 kgDM/day using CNCPS. When the ratio between predicted milk from MP and ME (MP:ME milk ratio[1]) is greater than 1, ME is limited and when the ratio is less than 1, MP is limiting.

Forage	ME supplied by diet (MJ/day)	Urea cost (MJ/day)	MP supplied by diet (g/day)	MP from microbial protein (%)	MP/ME supplied by diet (gMP/MJME)	Predicted milk production (kg/day) based on supply of:		MP:ME milk ratio[1]
						ME	MP	
Red Clover	174	10.6	2405	34	13.8	20.1	34.7	1.73
Lotus pedunculatus	149	5.5	1892	42	12.7	16.2	22.3	1.38
Lucerne	192	12.0	2056	38	10.7	23.1	28.5	1.23
White clover	180	10.8	1863	36	10.4	21.0	24.6	1.17
Yorkshire fog	192	7.5	2000	45	10.4	23.9	27.3	1.14
Lucerne hay	142	7.8	1593	41	11.2	14.5	16.0	1.10
Sulla	169	7.9	1731	39	10.2	19.7	21.0	1.07
Cocksfoot	180	7.3	1828	44	10.2	21.6	22.8	1.06
Lotus corniculatus	171	6.6	1727	51	10.1	20.3	21.1	1.04
OG ryegrass[2]	195	9.6	2200	35	11.3	24.1	24.4	1.01
WM ryegrass[2]	195	9.4	2203	38	11.3	31.5	31.7	1.01
Grasslands Tama	181	5.5	1759	56	9.7	22.4	22.7	1.01
Tall fescue	180	1.5	1736	57	9.6	23.0	21.1	0.92
Plantain	164	10.2	1547	43	9.4	18.3	16.9	0.92
Paspalum	152	0	1611	51	10.6	17.9	16.0	0.89
Prairie grass	180	5.1	1646	54	9.1	22.3	19.3	0.87
Perennial ryegrass	171	1.5	1577	65	9.2	21.2	18.1	0.85
Lucerne silage	157	6.2	1457	58	9.3	17.8	14.5	0.81
Kikuyu	171	2.3	1550	47	9.1	21.0	16.5	0.79
Sulla silage	178	5.7	1523	51	8.6	21.6	17.1	0.79
Chicory	174	5.9	1476	52	8.5	20.9	16.4	0.78
Maize grain	220	0	1489	52	6.8	31.0	19.6	0.63
Maize silage	166	0	1344	53	8.1	20.5	12.3	0.60
Oat silage	159	4.4	1272	63	8.0	18.5	11.1	0.60
Pasture silage	161	5	1155	65	7.2	18.4	8.6	0.47

[2] High quality pasture ryegrass from the CNCPS library, either defined as over-grazed (OG) or well-managed (WM). The only difference between the two ryegrasses is the effective fibre concentration. The effective fibre values for OG ryegrass was 40% NDF and for WM ryegrass was 60%.

TABLE 3.22. Late lactation metabolisable energy (ME; MJ/day) and metabolisable protein (MP; g/day) supplied by forages and predicted milk production of dairy cows expected to produce 15 litres milk/day and consume 14.6 kgDM/day using CNCPS. When the ratio between predicted milk from MP and ME supply (MP:ME milk ratio[1]) is greater than 1, ME is limited and when the ratio is less than 1, MP is limiting.

Forage	ME supplied by diet (MJ/day)	Urea cost (MJ/day)	MP supplied by diet (g/day)	MP from microbial protein (%)	MP/ME supplied by diet (gMP/MJME)	Predicted milk production (kg/day) based on supply of:		MP:ME milk ratio[1]
						ME	MP	
Red Clover	149	10.1	1987	46	13.3	15.2	27.8	1.83
Lotus pedunculatus	127	5.6	1572	44	12.4	11.9	17.5	1.47
WM ryegrass[2]	168	9.1	1823	53	10.9	19	25.4	1.34
OG ryegrass[2]	168	9.2	1820	53	10.8	18.8	25.2	1.34
Lucerne	164	11.2	1698	40	10.4	17.7	22.6	1.28
White clover	154	10.3	1529	38	9.9	16	19.2	1.20
Lucerne hay	123	6.8	1333	43	10.8	10.6	12.5	1.18
Yorkshire fog	165	7.4	1668	47	10.1	18.6	22	1.18
Sulla	145	7.4	1431	41	9.9	14.8	16.4	1.11
Cocksfoot	154	7.3	1515	47	9.8	16.6	18	1.08
Lotus corniculatus	148	6.3	1437	53	9.7	15.4	16.7	1.08
Grasslands Tama	157	5.6	1472	58	9.4	17.3	18.2	1.05
Tall fescue	155	1.9	1468	59	9.5	17.9	17.2	0.96
Paspalum	132	0	1362	53	10.3	13.7	12.9	0.94
Plantain	140	9	1268	46	9.1	13.7	12.8	0.93
Perennial ryegrass	148	1.3	1333	67	9.0	16.5	14.7	0.89
Prairie grass	155	4.6	1377	57	8.9	17.4	15.4	0.89
Lucerne silage	136	5.3	1238	60	9.1	13.4	11.7	0.87
Sulla silage	152	5	1282	53	8.4	16.7	13.7	0.82
Kikuyu	148	2	1309	59	8.8	16.4	13.3	0.81
Chicory	149	5.2	1229	54	8.2	16	12.7	0.79
Oat silage	139	3.6	1107	64	8.0	14.2	9.4	0.66
Maize grain	192	0.3	1269	55	6.6	25.2	16.4	0.65
Maize silage	142	0	1146	64	8.1	15.7	9.9	0.63
Pasture silage	139	4.2	1001	66	7.2	14.2	7.2	0.51

[2] High quality pasture ryegrass from the CNCPS library, either defined as over-grazed (OG) or well-managed (WM). The only difference between the two ryegrasses is the effective fibre concentration. The effective fibre values for OG ryegrass was 40% NDF and for WM ryegrass was 60%.

3.5.3.2 Evaluation of mixed forage rations

Using the results from *in vitro* incubations and CNCPS evaluations of individual forages, mixtures of forages that are able to best supply the nutrient requirements to maximise animal production were evaluated. The data suggest forages containing CT have potential value because of lower protein degradation, lower cost to remove excess nitrogen and small differences between predicted ME and MP milk production. Ryegrass-based pasture was part of most forage mixtures evaluated. Pasture comprised 80% ryegrass and 20% white clover. The milk production and rumen characteristics of forage mixtures fed to early and late lactating dairy cows are illustrated in Tables 3.23 and 3.24, respectively, and Appendix 3.18.

Forage mixtures included 80:20 ryegrass:white clover (pasture), 50:50 ryegrass: white clover, 20:80 ryegrass:white clover and 50:50 mixtures of pasture with lucerne, sulla, *Lotus corniculatus*, *Lotus pedunculatus*, plantain, chicory, red clover, lucerne silage, sulla silage and oat silage. In addition 50% pasture was modelled with 25% lucerne and maize silage; red cover and maize grain; and 67% pasture with 33% maize silage, 33% sulla and with 17% maize silage and 17% sulla. Other mixtures included 50:50 white clover:sulla, lucerne:sulla, lucerne:chicory, lucerne:plantain and lucerne:sulla silage.

Increasing the proportion of white clover in a medium quality ryegrass-based diet increased the ME and MP predicted milk production due to an increase in the supply of ME and MP (Table 3.23). When white clover is 50% or more of the diet ME is the limiting nutrient (MP:ME milk ratio > 1), while MP was the limiting nutrient when white clover made up only 20% of the diet (MP:ME milk ratio < 1). Increasing white clover reduced MP from bacteria and substantially increased supply from undegraded feed and increased the cost of excess N disposal from 2.9 to 8.6 MJ/day (Table 3.23).

Mixing lucerne with pasture, chicory, plantain, sulla and sulla silage (Table 3.23) in early lactation suggested the highest milk production from the lucerne:chicory mixture (22.7 kg/day) followed by lucerne:sulla silage (22.5 kg/day), pasture:lucerne (22.3 kg/day), lucerne:sulla (21.4 kg/day) and lucerne:plantain (21.2 kg/day). In late lactation (Table 3.24), ranking was similar with 17.3 kg milk/day produced from lucerne:chicory and lucerne:sulla silage, 17.1 kg milk/day from pasture:lucerne, 16.3 kg/day from lucerne:sulla and 16.1 kg/day from lucerne:plantain. ME was the limiting nutrient in all mixtures, but for lucerne:chicory and lucerne:sulla silage ME and MP milk production were very similar in early lactation (Table 3.23) and a 1 kg/day difference in late lactation (Table 3.24). Sulla was also mixed with pasture and white clover. For the 50:50 mixtures of pasture:sulla, white clover:sulla and lucerne:sulla, ME was limiting for all mixtures and milk production from the limiting nutrient was highest for the lucerne:sulla mixture, and lowest for white clover:sulla. MP from metabolisable protein was less than 40% and the urea cost was about 6% of ME intake (about 9.0 MJ/day) in early and late lactation for lucerne:sulla and white clover:sulla compared with 48% MP from microbial protein and a urea cost of about 3% ME intake (about 5 MJ/day) for the pasture:sulla mixture.

Adding 25% maize silage and 25% lucerne to a diet containing 50% of medium quality pasture (40% ryegrass and 10% white clover) resulted in a more balanced diet than the 50:50 pasture:lucerne mixture. This was most evident in early lactation where pasture:lucerne:maize silage resulted in ME and MP milk production of 22.3 and 22 kg/day.

The CNCPS model was used to identify forages that could be fed with red clover which has high MP for milk production but ME was limiting. No forage or mixture of forages fed with red clover enabled ME and MP for milk to be balanced, however when pasture, red clover and maize grain were combined in early lactation at a ratio of 50:25:25 ME and MP milk production was 23.8 kg/day and 23.4 kg/day, respectively. In contrast a diet of 50:50 pasture and red clover resulted in ME and MP milk production of 20.8 and 27.5 kg/day, respectively.

A common practice on farm has been to feed two-thirds pasture with one-third supplement, generally to fill a feed deficit. A diet containing two-thirds medium quality pasture and the remaining one-third being maize silage and sulla together was a well balanced diet for late lactation with ME and MP milk production of 16.5 and 16.6 kg/day, respectively, but this diet was not so ideal for early lactation with a 1 kg/day difference and MP was the limiting nutrient. In early lactation, a diet containing two-thirds medium quality pasture and one-third sulla reduced the difference in predicted milk production to only 0.5 kg milk/day and ME was the limiting nutrient.

A major constraint to predictions made here are voluntary intake of forages and mixtures which are not considered by CNCPS. Intake is the primary determinant of production and trials should be undertaken to evaluate the merit of CNCPS predictions of forage mixtures.

TABLE 3.23. Early lactation metabolisable energy (ME; MJ/day) and metabolisable protein (MP; g/day) supplied by forage mxitures and predicted milk production of dairy cows expected to produce 22.7 litres milk/day and consume 17.1 kgDM/day using CNCPS. When the ratio between predicted milk from MP and ME (MP:ME milk ratio[1]) is greater than 1, ME is limited and when the ratio is less than 1, MP is limiting.

Forage mixture	ME supplied by diet (MJ/day)	Urea cost (MJ/ day)	MP supplied by diet (g/day)	MP from microbial protein (%)	MP/ME supplied by diet (gMP/ MJME)	Predicted milk production (kg/day) based on the supply of:		ME: MP milk ratio[1]
						ME	MP	
Pasture:red clover (50:50)	174	6.5	2038	45	11.7	20.8	27.5	1.32
Lucerne:sulla (50:50)	180	9.7	1890	39	10.5	21.4	24.7	1.15
Ryegrass:white clover (20:80)	183	8.6	1880	44	10.3	22.2	25.3	1.14
Pasture:*Lotus P* (50:50)	161	4.2	1784	51	11.1	18.8	21.3	1.13
White clover:sulla (50:50)	174	9.2	1787	37	10.3	20.3	22.5	1.11
Pasture:lucerne (50:50)	183	7.1	1878	49	10.3	22.3	24.6	1.10
Lucerne:plantain (50:50)	180	10.4	1820	40	10.1	21.2	23.2	1.09
Ryegrass:white clover (50:50)	178	5.3	1821	54	10.2	21.8	23.6	1.08
Pasture:sulla (50:50)	171	5	1745	48	10.2	20.6	21.5	1.04
Pasture:*Lotus C* (50:50)	173	4.6	1727	56	10.0	20.8	21.2	1.02
Lucerne:chicory (50:50)	185	8.1	1789	45	9.7	22.7	23.1	1.02
Lucerne:sulla silage (50:50)	185	8.1	1801	44	9.7	22.5	23.0	1.02
Pasture:sulla (66:34)	171	3.8	1738	56	10.2	20.8	21.3	1.02
Pasture:lucerne: maize silage (50:25:25)	178	2.6	1764	57	9.9	22.3	22.0	0.99
Pasture:plantain (50:50)	169	6.1	1658	54	9.8	20.2	19.6	0.97
Pasture:red clover:grain (50:25:25)	185	2.9	1797	54	9.7	23.8	23.1	0.97
Pasture:chicory (50:50)	176	3.7	1654	59	9.4	22.7	21.7	0.96
Ryegrass:white clover (80:20)	173	2.9	1676	60	9.7	21.4	20.3	0.95
Pasture:maize silage:sulla (66:17:17)	173	1.8	1696	60	9.8	21.4	20.4	0.95
Pasture:maize silage (66:34)	173	0	1664	64	9.6	21.9	19.7	0.90
Pasture:lucerne silage (50:50)	*166*	4.7	1551	60	9.3	19.6	17.1	0.87
Pasture:sulla silage	176	4.3	1599	56	9.1	21.5	18.7	0.87
Pasture:oat silage (50:50)	166	3.6	1470	64	8.9	19.9	15.6	0.78

TABLE 3.24. Late lactation metabolisable energy (ME; MJ/day) and metabolisable protein (MP; g/day) supplied by forage mxitures and predicted milk production expected to produce 15.0 litres milk/day and consume 14.6 kgDM/day using CNCPS. When the ratio between predicted milk from MP and ME (MP:ME milk ratio[1]) is greater than 1, ME is limited and when the ratio is less than 1, MP is limiting.

Forage mixture	ME supplied by diet (MJ/day)	Urea cost (MJ/day)	MP supplied by diet (g/day)	MP from microbial protein (%)	MP/ME supplied by diet (gMP/ MJME)	Predicted milk production (kg/day) based on the supply of		ME: MP milk ratio[1]
						ME	MP	
Pasture:red clover (50:50)	149	6.5	1696	47	11.38	15.9	22.0	1.38
Pasture:*Lotus P* (50:50)	139	4.5	1491	53	10.73	14.2	17.0	1.20
Lucerne:sulla (50:50)	155	9.3	1561	41	10.07	16.3	19.4	1.19
Ryegrass:white clover (20:80)	158	8.3	1552	47	9.82	17.0	20.0	1.18
Pasture: lucerne (50:50)	157	7.0	1564	51	9.96	17.1	19.7	1.15
White clover:sulla (50:50)	149	8.8	1470	40	9.87	15.3	17.5	1.14
Ryegrass:white clover (50:50)	154	5.5	1522	56	9.88	16.7	18.9	1.13
Lucerne:plantain (50:50)	154	9.9	1498	43	9.73	16.1	18.1	1.12
Pasture:sulla (50:50)	148	5.0	1456	54	9.84	15.7	17.1	1.09
Pasture:sulla (66:34)	148	3.9	1456	59	9.84	15.9	17.1	1.08
Pasture:*Lotus C* (50:50)	148	4.5	1445	59	9.76	16.0	16.9	1.06
Lucerne:sulla silage (50:50)	158	7.9	1500	46	9.49	17.3	18.3	1.06
Lucerne:chicory (50:50)	159	7.9	1483	47	9.33	17.3	18.2	1.05
Pasture: lucerne: maize silage (50:25:25)	154	3.0	1489	59	9.67	17.2	17.9	1.04
Pasture:red clover: grain (50:25:25)	161	3.4	1502	58	9.33	18.7	19.0	1.02
Pasture:plantain (50:50)	146	4.3	1379	57	9.45	15.4	15.5	1.01
Pasture:maize silage:sulla (66:17:17)	149	2.0	1432	62	9.61	16.5	16.6	1.01
Ryegrass:white clover (80:20)	151	2.9	1409	63	9.33	16.6	16.4	0.99
Pasture:chicory (50:50)	152	3.65.5	1387	62	9.13	16.7	16.1	0.96
Pasture:maize silage (66:34)	149	0.3	1414	66	9.49	17.0	16.2	0.95
Pasture:lucerne silage (50:50)	143	4	1312	62	9.17	15	13.8	0.92
Pasture:sulla silage	151	3.8	1345	58	8.91	16.7	15.0	0.90
Pasture:oat silage (50:50)	143	3.1	1258	65	8.80	15.4	12.9	0.84

3.6 CONCLUSIONS

The data presented here provided a ranking of grasses, legumes, herbs and silages in terms of their solubility, rates of degradation and fermentation and effective degradability of DM, protein and fibre (ADF and NDF). Differences between the degradation and fermentation characteristics of forages are supported by differences obtained when forages were evaluated in animal studies. The CNCPS model was used to evaluate individual forages in terms of ME and MP available for milk production, but the data of individual forages also needs be used in simulation models that balance nutrient yields with dairy cow requirements and include predictions of voluntary feed intakes. The information derived from *in sacco* and *in vitro* incubations and CNCPS evaluations provides a scientific basis for formulating forage-based rations. Forages used in formulations should complement each other in terms of their digestion and fermentation characteristics and thereby optimising the nutrient (ME and MP) supply for high-producing dairy cows in New Zealand.

Forages able to balance the supply of nutrients and increase intake were identified from the methods used in this chapter. High quality legumes, for example lucerne and white clover, have potential to increase the supply of MP to diets in which MP is limiting (eg. medium quality ryegrass), but also legumes containing CT (eg. sulla, *Lotus spp*.) have the potential to reduce the energy losses associated with excess dietary N breakdown in the rumen. Another determinant of which forages have potential in the pasture-based system are those that are capable of achieving high agronomic yields (eg. lucerne and sulla).

CHAPTER 4

AN EVALUATION OF SULLA (*HEDYSARUM CORONARIUM*) WITH PASTURE, WHITE CLOVER AND LUCERNE FOR LAMBS

CHAPTER 4: AN EVALUATION OF SULLA (*HEDYSARUM CORONARIUM*) WITH PASTURE, WHITE CLOVER AND LUCERNE FOR LAMBS

4.1 ABSTRACT

Pasture-based diets restrict animal production, due to limitations in voluntary feed intake and insufficient release of nutrients during digestion to meet nutrient requirements for production purposes. Opportunities for improving the efficiency of pasture utilisation include the addition of a soluble carbohydrate source to enable improved capture of ammonia by rumen bacteria, or the inclusion of condensed tannins (CT) to reduce protein degradation. A feed evaluation trial was conducted at AgResearch Grasslands to investigate the ability of sulla (*Hedysarum coronarium*), a forage containing high concentrations of soluble carbohydrate and CT, to be fed with pasture and legume species. Fifty-six weaned ram lambs were allocated to seven contrasting diets: pasture (80% ryegrass and 20% white clover), white clover, lucerne, sulla, or 50:50 mixtures (DM basis) of pasture:sulla, white clover:sulla and lucerne:sulla. Measurements included feed composition, intakes, daily gain, wool growth, carcass characterisitcs, rumen ammonia (NH_3) and volatile fatty acids (VFA) and blood glucose concentration. Lambs fed sulla, white clover, white clover:sulla and lucerne:sulla had the most rapid daily gains (281 – 308 g/day) while lambs fed pasture gained 116 g/day. Clean wool yield of lambs fed pasture and pasture:sulla were lower than for lambs fed white clover:sulla, sulla and lucerne:sulla. Sulla added to pasture, white clover and lucerne diets significantly reduced rumen ammonia concentrations and acetate:propionate ratios in lambs. Sulla improved lamb production when fed with pasture and lucerne, but not white clover. Production was strongly correlated with ME intake. Protein, and effects of CT, did not appear to influence liveweight gain, wool growth or carcass characteristics.

4.2 INTRODUCTION

New Zealand's livestock industries are reliant on ryegrass/white clover dominant pastures, but quality and quantity is extremely variable within and between years. Ryegrass pasture has a relatively high concentration of fibre from about October to April (Table 2.4 in Chapter 2; Wilson and Moller, 1993; Moller *et al*., 1996; Stevenson *et al*., 2003; Corson, unpublished; Prewer, unpublished) which can limit feed intake, a low soluble carbohydrate concentration all year and a variable (often excessive) concentration of rapidly degradable crude protein (CP) requiring substantial energy expenditure for excretion as urea. Several studies have shown significant improvements in animal production by feeding forages other than perennial-ryegrass-based pasture (Ulyatt, 1981; Stevens et al., 1992; Stevens et al., 1993; Fraser and Rowarth, 1996; Johnson and Thomson, 1996). Relative feeding values, based on animal trials have shown white clover (*Trifolium repens*) to be twice that of perennial ryegrass (*Lolium perenne*), and lucerne (*Medicago sativa*), *Lotus pedunculatus*, sainfoin (*Onobrychis viciifolia*) and Italian ryegrass (*Lolium multiflorum*) have substantially higher feeding values than perennial ryegrass for lamb (Ulyatt, 1981). Similar effects of higher feeding value forages on liveweight gains have been demonstrated in steers and deer. Daily liveweight gains of steers from 8- to 30-months-of-age were 13% greater when grazing high quality pastures (annual ryegrass during winter and spring and red clover and lotus in summer and autumn) compared to grazing a ryegrass-white clover based pasture (Cosgrove *et al*., 1996). Hoskin et al. (1999) demonstrated the potential of sulla (*Hedysarum coronarium*) over ryegrass-white clover pastures for deer growing from 3- to 12-months of age with 34% and 13% greater LW gains over autumn and winter, respectively. Trials with lactating cows have also demonstrated significant advantages of white clover when fed with ryegrass (Harris *et al*., 1997) or maize silage (Stockdale, 1995) for milk production. These benefits are a function of feed intake and nutrient supply.

In sacco and *in vitro* studies (Chapter 3) showed legumes, including those containing CT, have a high nutritive value and could complement ryegrass pasture. Degradation rates of legumes were more rapid and total volatile fatty acid production was greater than for grasses. Legumes containing CT had reduced protein solubility and degradation relative to legumes without CT and *in vitro* incubations showed that only 8% of dietary N in sulla was converted to NH_3 after 24 hours compared to 49% of white clover N. Sulla also has a relatively low concentration of neutral detergent fibre (NDF; c. 18 – 22% DM), a high concentration of soluble carbohydrate (18 – 25% DM) and the CT increases the flow of amino acids (AA) to the small intestine for absorption (Waghorn *et al.*, 1998). CT from one plant species is able to bind with and precipitate protein from other plants (Waghorn and Jones, 1989; Min *et al.*, 2000), so the CT in sulla may reduce rumen degradation of dietary protein when fed with pasture and legume species. The higher concentration of rapidly digested soluble carbohydrates will supply immediate energy to the animal which was evident from *in vitro* incubations (Chapter 3) where the production of volatile fatty acids from sulla was 20 to 50% higher than white clover and lucerne after 6 hours of incubation.

The *in sacco* and *in vitro* studies, in conjunction with CNCPS evaluation of data have enabled forages to be ranked in terms of degradation rate, proteolysis, VFA production and predicted milk production by cows at defined levels of intake. The CNCPS predictions (Chapter 3) showed ME to be first limiting for most legumes and that high quality ryegrass could promote high levels of production when CP concentrations were adequate (Tables 3.21 to 3.24). The similarity of CNCPS predictions for milk production by dairy cows fed temperate grasses and legumes in early lactation (Table 3.21) is in marked contrast to responses of growing lambs where actual production varied widely (Ulyatt 1981; Brown 1990) and suggests that intake was dominating lamb production or that dairy cows respond to diet quality quite differently to lambs.

Cow trials where ryegrass pasture has been supplemented with white clover or *Lotus corniculatus* (Harris *et al.* 1997a, b; 1998) have resulted in 30 – 60% increases in milk production, in association with the change in diet composition and increased intakes. These trials (Harris *et al.* 1997 a, b) also showed that 55 – 65% of white clover with ryegrass maximised milk production in dairy cows, so ryegrass with 50% white clover or other legumes were possible choices for sheep feeding trials.

As well as providing crude protein, adding legumes to grasses should dilute dietary fibre and improve feed intakes. White clover, lucerne and sulla were three forages identified from *in sacco* and *in vitro* incubations, from modelling with the Cornell Net

Carbohydrate Protein System (CNCPS), and from potential annual DM yield, as having potential to improve the production of pasture-fed animals. Predicted responses determined in Chapter 3 showed combinations of ryegrass (MP limiting) and white clover, lucerne or sulla (ME limiting) in a 50:50 mixture provided an increased supply of both ME and MP, although there was now a small surplus of MP over requirements (Tables 3.23 and 3.24).

The chemical composition of sulla, especially the high soluble carbohydrate, presence of CT, and low NDF made it a forage of choice for evaluation, especially as relatively little nutritional information is available and because of potentially high DM yields (Waghorn *et al.*, 1998). Preliminary *in sacco* studies (Appendix 4.1) of forage mixtures showed sulla increased the DM, CP and NDF degradation rates of lucerne:sulla relative to lucerne alone. White clover:sulla mixtures had slower DM, CP and NDF degradation rates than white clover alone. *In vitro* incubations (Appendix 4.1) have shown reduced *in vitro* ammonia (NH_3) production when sulla was incubated with either ryegrass or white clover.

The objective of this study was to determine the effect of supplementing ryegrass/white clover pasture and legumes with sulla on animal production. Lamb liveweight gain, wool growth and carcass characteristics were complemented by rumen and metabolic parameters including measurement of whole body and tissue protein synthesis (in four treatment groups; Chapter 5).

4.3 MATERIALS AND METHODS

4.3.1 Experimental design

An experiment was conducted with lambs fed pasture, white clover, lucerne, sulla (Photograph 4.1) and mixtures of pasture:sulla, white clover:sulla and lucerne:sulla over an eight week period. Measurements included LW gain, wool growth, carcass composition (eg. eye-muscle area, GR and back fat-depths measured by ultrasound), carcass weight (CW), feed intakes, concentration of blood glucose, rumen NH_3 and volatile fatty acids (VFA) and rumen pH. All procedures were reviewed and approved by the Crown Research Institute Animal Ethics Committee in Palmerston North, New Zealand according to the Animals Protection Act (1960) and Animal Protection Regulations (1987) and amendments. Table 4.1 illustrates the timetable of events throughout the trial. Forages used in this trial were also used for *in sacco* and *in vitro* incubations to determine products and rates of digestion according to the methods described in Chapter 3 (Results in Appendix 4.5).

4.3.2 Animals and diets

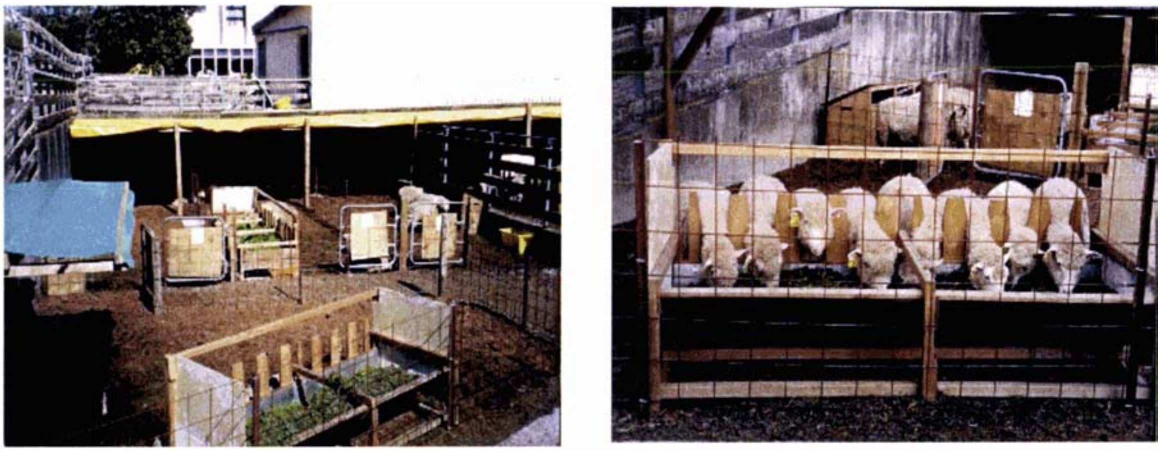
Fifty-six weaned ram lambs (aged 12 weeks) were fed seven contrasting forage diets *ad libitum* for eight weeks from 19 October to 18 December 2000. The pasture comprised 80% ryegrass and 20% white clover (ryegrass and white clover weighed out separately to ensure an 80:20 ratio was fed), and the sulla mixtures were on a 50:50 DM basis. All forages were grown and harvested daily at Aorangi Research Farm, Manawatu and transported to the feeding facility at Grasslands Research Centre, Palmerston North by 1000 hours. The ryegrass, lucerne and sulla were chopped to 3 – 6 cm lengths using a JF Forage chopper (Model FC80) immediately after harvesting to facilitate easy and accurate mixing for feeding.

All lambs were drenched with Selenium Cydectin (Fort Dodge New Zealand Ltd) two weeks before the start of the trial, Leviben at the start of the trial and Leviben with Selenium (Novartis New Zealand Ltd) at week four of the trial.

Each group of eight lambs were held on sawdust feed pads (10 m x 3 m), fed *ad libitum* from troughs and provided with shelter and water (Photographs 4.1 and 4.2). Each group was given their daily allowance in the morning at about 1100 hours and refusals were about 15 – 25% of feed offered. Refusals were removed and weighed prior to feeding. Average lamb DM intakes and intakes of DM constituents over the entire experiment were calculated from feed offered less feed refused (mean DM intake). Actual lamb intakes (alkane intake/lamb; Table 4.7) were determined during week six of the trial using alkane markers (Dove and Mayes, 1991). Tablets containing 32.5 mg of C32 alkane (n-Dotriacontane) were administered twice daily from day 35, and faecal samples were collected twice daily for five days from day 39 to day 44 from all lambs (Appendix 4.2).

PHOTOGRAPH 4.1. Sulla (*Hedysarum coronarium*)

PHOTOGRAPH 4.2. Feeding of lambs on feed pads



Sub-groups of three lambs fed pasture, sulla, lucerne and lucerne:sulla were taken from the feed pads and placed indoors in metabolism crates from days 35 to 44, and further groups of three lambs from those treatments were placed indoors from days 44 to 53 to enable methane production to be measured for a separate trial (not reported here). Indoor facilities and timing meant that indoor measurements could only be conducted on four treatments, therefore these four dietary treatments were considered to be the most contrasting in terms of animal production. Alkane recovery was also determined for those 24 lambs to determine if either C31 or C33 herbage marker should be used for estimating intakes. The second group of twelve lambs had intakes measured by alkanes both indoors and outdoors enabling all indoor data to be adjusted to the feed pad situation.

Sixteen lambs from the pasture, lucerne, sulla and lucerne:sulla treatments (including some used to measure methane) were also removed from the feed pads and placed indoors and fed in metabolism crates to measure whole body and fractional protein synthesis rates of tissues (Chapter 5). The first eight lambs were placed indoors on day 46 of the trial and were slaughtered in two groups of four on day 55 and 56. A further eight lambs from the same treatments were brought indoors on day 56 and slaughtered on days 62 and 63 of the trial (Chapter 5).

Feed samples were taken daily, and refusals taken every two days and held at –20°C until being freeze-dried and analysed. Faecal samples from lambs on the feed pads and 10% aliquots from those 24 lambs held indoors were frozen and dried at 60°C and alkane concentrations of the feed and faeces were determined by gas chromatography (Dove, 1992). Individual intakes were calculated according to the equation of Dove and Mayes, (1991):

$$\text{kg DM intake/day} = \left(\frac{F_i}{F_j} \times D_j\right) \div \left(H_i \times \frac{F_i}{F_j} \times H_j\right)$$

Where F_i = concentration of C31 or C33 alkane in faeces

F_j = concentration of C32 alkane in faeces

H_i = concentration of C31 or C33 alkane in herbage

H_j = concentration of C32 alkane in herbage

D_j = daily dose of C32 alkane in tablet

The C31 alkane was used to predict DM intake of lambs fed white clover, lucerne, sulla and white clover:sulla, and the C33 alkane was used to predict DM intake of lambs fed pasture, lucerne:sulla and pasture:sulla. Choice of alkane to predict DM intake was based on concentration of each alkane in the forages (Method described in Appendix 4.2).

Fresh forage samples and refusals collected throughout the trial were analysed by Near InfraRed Spectroscopy (NIRS) to determine chemical composition, including CT concentration. In addition to conventional measures of feed composition the NIRS (FeedTECH; AgResearch Grasslands) had been calibrated to determine total, unbound, protein bound and fibre bound CT in sulla. Lignin concentration (Acid detergent lignin, ADL) of forages was determined by sequential extraction described by Chaves *et al.* (2002b). Chemical composition of the mixed diets was calculated from the proportion of each forage used in the mixtures. Group DM and nutrient intake were calculated from DM and nutrients offered less DM and nutrients refused. The forage components (leaf or stem) the lambs consumed or refused from individual diets and mixtures were noted during the day and when refusals were weighed. DM content was determined daily by drying representative forage samples for 24 h at 95°C.

All forages were maintained in a vegetative state. Changes in composition were monitored throughout the trial and efforts were made to maintain forages in a vegetative state and consistent quality. Procedures included raising the cutting height to minimise the harvest of stems from ryegrass, lucerne and sulla and harvesting vegetative regrowth. Ryegrass quality was difficult to maintain and deterioration in quality by day 39 resulted in harvest from a second paddock which did not contain reproductive material. White clover was cut from several plots to maintain a consistent cutting height.

4.3.3 Lamb production

Lambs were weighed weekly prior to feeding, at about 0900 hours throughout the trial (fed LW), and fasted LW were taken on days 8 and 59. Average daily gains were calculated between day 6 and 58 of the trial as well as from fasted LW. On days 6, 30 and 54 lambs were weighed and subjected to ultrasound scans (Portable ultrasound) to measure eye muscle area (EMA), fat depth about 11 cm from the mid line in the region of the 11th and 12th ribs (GR) and depth of fat on the back (at the point of greatest muscle depth). Eye muscle area (mm^2) of the 12th rib was calculated by multiplying the width (A; mm) and depth (B; mm) of the muscle by 0.77. Fed LW taken on day 58 of the trial and CW at slaughter was used to calculate dressing-out % (CW/fed LW x 100). However, 16 lambs on the pasture, lucerne, sulla and lucerne:sulla treatments used to determine absolute whole body protein synthesis and fractional protein synthesis rates (Chapter 5) were not fasted; therefore their fasted weights were predicted based on the average difference in LW between fed lambs on day 58 and fasted lambs on day 59 of the remaining lambs.

Forty lambs were slaughtered and dressed in a commercial abattoir (Lakeview Farm Fresh Ltd, Levin) and 16 lambs used for whole body and fractional protein synthesis rates (Chapter 5) were slaughtered and dressed at Grasslands Research Centre. Carcass weight and fat depths over the 11th and 12th ribs (GR) were recorded from all lambs at slaughter.

Wool was shorn from both sides of the lambs at the commencement of the trial and wool growth was determined from 10 cm x 10 cm patches shorn on days 54 or 55. Samples were washed in a four-bowl aqueous mini scour to obtain yield and clean weights (Kenyon *et al.*, 1999).

4.3.4 Rumen measurements

Rumen contents were obtained by lavage (stomach tube) on days 9, 14, 21, 29, 35 and 42 of the experiment, 2 – 4 hours after feeding, to measure pH, NH_3 and VFA. On day 14 lavage samples were taken from all lambs 2 hours prior to feeding and 2, 5 and 8 hours after feeding to determine the diurnal pattern of pH and NH_3. Rumen digesta pH was measured at the time of sampling using a MeterLab® (PHM210, Radiometer Pacific Limited, Copenhagen) which was recalibrated immediately prior to each set of measurements. Ten mL of rumen digesta were centrifuged (28,000 x *g*; 10 minutes) to obtain supernatant for analyses (Appendix 3.4). One aliquot (1.5 mL) was frozen for VFA analysis by gas-liquid chromatography (Attwood *et al.*, 1998; Appendix 3.6). A further 1 mL of supernatant was acidified (15 μL of concentrated HCl), mixed, micro-centrifuged (14,000 x *g*; 15 minutes) and the supernatant frozen for NH_3 determination. Ammonia concentration was determined by the enzymatic method of Bergemeyer and Beutler (1985) using a commercial kit (Cat. # 171-C; Sigma Chemicals) and measured on a Cobas Fara II (Hoffman LaRoche, Basel; Appendix 6.2).

4.3.5 Blood measurements

Two hours after feeding, blood samples (10 mL) were collected from the jugular vein of all lambs into heparinized vacutainers on days 16, 27 and 41. Samples were mixed gently, held on ice for no more than 30 minutes and an aliquot taken to measure blood glucose and lactate concentrations using a Blood Gas Analyser (ABL 615, Radiometer Pacific Limited, Copenhagen) calibrated to measure blood glucose and lactate.

4.3.6 Statistical Analysis

Effects of diet on DM intakes (calculated from feed offered and feed refused), LW gain, wool growth, CW and carcass GR fat-depth at slaughter, rumen VFA and individual DM intakes measured by alkanes were determined using the GLM procedure of SAS (1996). Repeated-measures analysis using the MIXED procedure of SAS (1996) was used to determine the effects of diet, day of measurement and the interaction of diet x day of measurement on lamb LW on each day of weighing, rumen pH and rumen NH_3 concentration, blood glucose and lactate concentrations, and EMA, GR and back fat-depth measured by ultrasound scanning on live animals throughout the experiment. Lamb LW on the day of ultrasound scanning was also used as a covariate in the analysis of EMA, GR and back fat depth and CW was used as a covariate for back fat-depth of carcasses at slaughter. Liveweight and CW as covariates were significant for the variables described and results of these analyses are presented in Appendix 4.4.

Contrasts were used to determine whether the DM intake, LW gain and rumen NH_3 concentrations of lambs fed the mixed diets (pasture:sulla, lucerne:sulla and lucerne:sulla) were significantly different from the expected LW gain and NH_3 concentration using data from feeding the sole forages to lambs.

All data were checked for normality and homogeneity. Blood lactate concentration was $\log_{10}$ transformed in order for the data to satisfy ANOVA assumptions (normality and homogeneity) and the effects of diet determined on both absolute and transformed concentrations.

Results were expressed as least-squares (LS) means ± standard errors of the mean (SEM) and the LSD test was used to compare treatments. Statistical differences between treatments were indicated when probabilities (Pr) were less than or equal to 0.05.

Separate multiple regression analyses were conducted on mean LW gain and mean CW for each dietary treatment using composition (% DM) and intake of nutrients eaten (kg). The forward model-selection method of the stepwise procedure of SAS (1996) was used. In order to prevent the selection of too many variables in the multiple regression models, the level of significance was set at $Pr < 0.10$. The aim of the multiple regression analyses was to identify the variables most likely responsible for variation in LW gain and CW. Nutrient variables included in the multiple regression models were based on correlation coefficients determined by correlation analyses (Table 4.11) and an understanding of nutritive characteristics. In the first model, the content (% of DM) of soluble carbohydrate (SC), crude protein (CP), neutral detergent fibre (NDF) and predicted digestibility (dig) of diet were included, while in the second model the intake (kg) of soluble carbohydrate (SC), crude protein (CP), NDF and predicted digestibility (dig) of diet were included to explain the variation. A separate multiple regression was used to determine the nutrients (% of DM) responsible for the variation in ME content of the diet (MJME/kg DM). Soluble carbohydrate, CP, ADF and NDF as a % of the DM were included in the model.

TABLE 4.1. Schedule of events for lambs fed seven contrasting diets for eight weeks.

Day	Event
Pre-Trial	All lambs weighed and randomised into groups of similar average weight. Right and left side of lambs shorn to skin prior to treatment allocation.
1	Lambs allocated to treatments. Drenched with Leviben.
6	Ultrasound scan and liveweight.
7	Lambs removed off feed at 1930 hours.
8	Fasted liveweight.
9	Lavage all lambs.
14	Diurnal lavage from lambs allocated to pasture, white clover, lucerne and sulla treatments.
15	Diurnal lavage of lambs allocated to pasture:sulla, white clover:sulla and lucerne:sulla treatments.
16	Blood sampling.
21	Lavage all lambs.
22	Liveweight.
23	Preventative treatment for foot rot and drenched with Cydectin
27	Liveweight and blood sampling.
29	Lavage all lambs.
30	Ultrasound scan and liveweight.
34	Liveweight.
35	Lavage all lambs. Permeation tubes[1] given to 12 group A[2] lambs for methane measurement.
36	Alkane tablets given to all lambs (including group A lambs) until day 44.
39	12 lambs placed indoors[3] for methane measurement (group A).
40	First faecal sampling of all lambs to estimate individual intakes.
41	Liveweight and blood sampling of all lambs.
42	Lavage all lambs. Permeation tubes given to 12 group B[2] lambs for methane measurement.
44	Last faecal sampling of all lambs for intake estimation.
45	Alkane tablets given to lambs in group B until day 51.
46	Group A lambs outdoors. 12 lambs indoors for methane measurement (group B).
47	Liveweight of all lambs. Eight lambs[4] indoors to measure protein metabolism.

52	Group B lambs outdoors.
54	Ultrasound scan and liveweight. Mid-side wool patches (10 cm x 10 cm) trimmed.
55	Protein: Four lambs slaughtered (Group A); another four lambs brought indoors (Group B).
56	Protein: Four lambs slaughtered (Group A); another four lambs brought indoors (Group B).
58	Liveweight. All lambs outdoors removed off feed at 1930 hours.
59	Fasted liveweight of all lambs outdoors.
61	All lambs outdoors slaughtered.
62	Protein: Four lambs slaughtered (Group B).
63	Protein: Four lambs slaughtered (Group B).

[1] Permeation tubes weigh about 25 g and are about 30 mm x 17 mm in size and release an inert gas (SF_6) at about 1 mg/day to enable measurement of methane production.

[2] Groups A and B comprised three lambs selected at random from pasture, lucerne, sulla and lucerne:sulla treatments on days 35 (Group A) and 42 (Group B) for measurement of methane, alkane recovery and digestibility.

[3] Indoors refers to confinement in metabolism crates in a well ventilated building for 7 day periods.

[4] Each group of eight lambs comprised two lambs from pasture, lucerne, sulla and lucerne:sulla treatments.

4.4 RESULTS

4.4.1 Feed Composition

Table 4.2 illustrates the contrasting nature of the seven forage diets offered. The dietary DM content ranged from 13.8% (sulla) to 24.0% (pasture composed of 86% ryegrass and 14% white clover) and the CP content (% of DM) ranged from 15.5 (pasture) to 27.9 (white clover). Pasture had a high neutral detergent fibre (NDF) concentration, averaging 48% of DM, compared to other diets, whilst sulla contained only 21.5% NDF in the DM. Sulla and diets containing sulla had a higher soluble:structural carbohydrate ratio than pasture, white clover or lucerne. The mean concentration of soluble carbohydrate for sulla was 21.8% of DM compared to only 12.1% DM in pasture. Predicted digestibility and metabolisable energy (ME) content of diets offered was greatest for sulla, white clover:sulla and white clover diets at 83.3 to 84.2% and 11.8 to 12.2 MJME/kg DM and lowest for pasture and lucerne diets at 70.0 and 70.2%, and 10.1 and 10.0 MJME/kg DM, respectively. Combining sulla with pasture and lucerne increased the predicted digestibility and ME of these mixed diets to 76.7 and 77.1%, and 11.1 MJME/kg DM. The concentration of total CT in sulla averaged 5.6% of the DM and ranged from 4.5 to 6.7% DM throughout the eight week trial period. Sixty-two percent of sulla CT was unbound (and therefore able to interact with other proteins), 34% bound to protein and 4% bound to fibre. The CT concentration in pasture:sulla, white clover:sulla and lucerne:sulla ranged from 2.7 to 3.0% DM.

The concentration of soluble carbohydrate, protein, fibre (acid detergent fibre, ADF; and NDF), predicted digestibility and ME content of ryegrass, white clover, lucerne and sulla offered to lambs over the eight week period are illustrated in Figure 4.1a – f.

White clover had a consistent composition over the eight week period, although soluble carbohydrate concentration decreased from 16% to about 14% DM at day 40 with corresponding increases in CP concentration from 26 to 29% DM.

Lucerne composition changes were mostly evident for fibre concentration as the plants matured (Figures 4.1c, d) with increasing proportions of stem. Overall changes were minor but use of regrowth lucerne on day 32 caused a decrease in NDF concentration from 33 to 29% of DM. Regrowth lucerne had a higher soluble carbohydrate concentration than mature material, ranging from 12 to 15% of DM. The soluble carbohydrate concentration in regrowth lucerne declined as it matured but the increase in protein content in regrowth lucerne (26 vs. 24% of DM) was sustained. The combined changes resulted in a constant ME and predicted digestibility for lucerne over the entire trial.

Sulla maintained a high concentration of soluble carbohydrate throughout the trial, but the protein concentration declined from about 22 to 17% of DM by day 28, then increased to about 20% of DM at the end of the trial. Small changes in fibre combined with changes in soluble carbohydrate and protein concentrations in the second half of the trial resulted in a small decline in ME content, but very little change in digestibility.

Ryegrass quality deteriorated over the first 40 days of the trial with increasing fibre (NDF increased from 50.7 to 54.1%; ADF increased from 30.1 to 33.2%) and decreasing CP concentrations (decreased from 13.6 to 10.1%). On day 39 a new stand of ryegrass was harvested for the remainder of the trial, which had a lower fibre concentration (NDF = 48.2 – 51.3%; ADF = 27.9 – 30.5%) and higher crude protein concentration (15.9 – 18.4%) compared to the ryegrass used for the first 39 days. The ME content and digestibility of ryegrass tended to decline over the duration of the trial and this affected the quality of mixed diets containing pasture. Pasture comprised 86% ryegrass and 14% white clover.

Lambs consumed 77 to 94% of feed offered (Table 4.3). Stems made up a large proportion of the refused DM of the sulla, lucerne, lucerne:sulla and white clover:sulla diets. The composition of feed consumed for white clover and white clover:sulla diets were similar to the composition of feed offered (Tables 4.2 and 4.3). Lambs fed pasture, lucerne, sulla, pasture:sulla and lucerne:sulla consumed a higher proportion of protein, soluble carbohydrate and ME and less fibre (ADF and NDF) relative to the diet offered. The CT concentration was higher in sulla DM eaten relative to feed offered.

TABLE 4.2. Average dry matter content (DM), percentage of sulla fed, and composition (% of DM) of the seven diets offered to lambs over eight weeks. Means ± standard errors are presented for each diet (8 feed samples for each diet).

Nutrient	DM %	Sulla fed (%)	Soluble Carbohydrate	Crude Protein	ADF[1]	NDF[2]	Lipid	Condensed Tannin	ME[3] (MJ/kg DM)	OMD[4] (%)
Pasture[5]	24.0 ± 0.45	-	12.1 ± 0.27	15.5 ± 1.08	29.5 ± 0.52	48.0 ± 0.69	2.8 ± 0.11	0	10.1 ± 0.15	70.0 ± 1.09
White Clover	14.6 ± 0.25	-	14.5 ± 0.36	27.9 ± 0.44	21.4 ± 0.23	26.4 ± 0.35	3.1 ± 0.05	0	11.8 ± 0.04	83.3 ± 0.28
Lucerne	21.2 ± 0.43	-	12.3 ± 0.53	24.4 ± 0.39	27.8 ± 0.33	32.3 ± 0.53	2.8 ± 0.03	0	10.0 ± 0.07	70.2 ± 0.53
Sulla	13.8 ± 0.16	100	21.8 ± 0.50	19.2 ± 0.72	22.2 ± 0.70	21.5 ± 0.89	2.2 ± 0.06	5.6 ± 0.27	12.2 ± 0.12	84.2 ± 0.41
Pasture[5]:Sulla	19.2 ± 0.32	48 ± 0.96	16.7 ± 0.37	17.3 ± 0.71	26.0 ± 0.35	35.3 ± 0.65	2.5 ± 0.07	2.7 ± 0.14	11.1 ± 0.13	76.7 ± 0.74
White Clover:Sulla	14.2 ± 0.18	53 ± 0.48	18.5 ± 0.37	23.0 ± 0.43	21.8 ± 0.39	23.9 ± 0.50	2.6 ± 0.03	3.0 ± 0.15	12.0 ± 0.07	83.7 ± 0.26
Lucerne:Sulla	17.6 ± 0.28	50 ± 0.88	17.0 ± 0.42	21.8 ± 0.48	25.1 ± 0.37	26.9 ± 0.54	2.5 ± 0.03	2.8 ± 0.17	11.1 ± 0.07	77.1 ± 0.37

[1] ADF, Acid detergent fibre (cellulose and lignin).
[2] NDF, Neutral detergent fibre (hemicellulose, cellulose and lignin).
[3] ME, Metabolisable energy.
[4] OMD, Predicted organic matter digestibility.
[5] Pasture is composed of 86% ryegrass and 14% white clover.

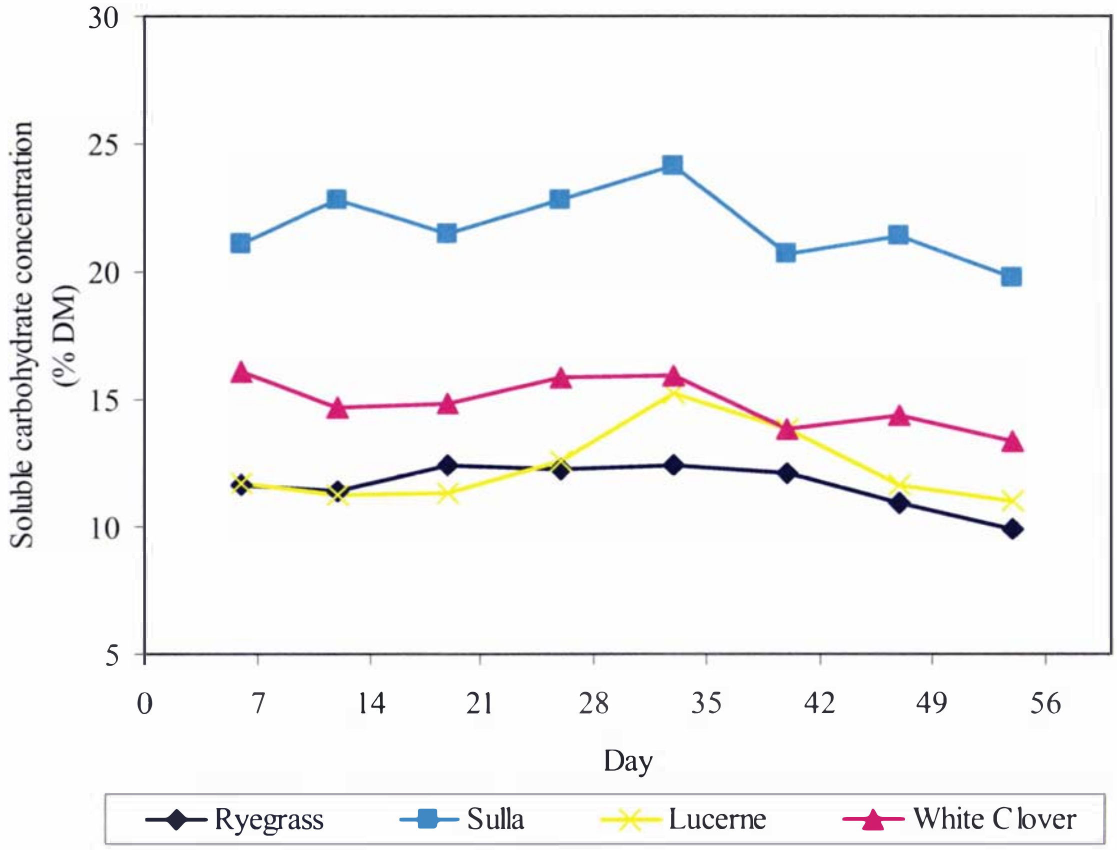
TABLE 4.3. Average composition[1] (% DM) and percentage of feed eaten for the seven diets fed to lambs over eight weeks. Means ± standard errors are presented for each diet (8 feed samples for each diet).

Nutrient	Soluble Carbohydrate	Crude Protein	ADF[2]	NDF[3]	Lipid	Condensed Tannin	ME[4] (MJ/kg DM)	OMD[5]	Intake as % DM offered
Pasture	13.2 ± 0.25	16.3 ± 1.43	28.6 ± 0.80	45.8 ± 1.19	2.8 ± 0.14	0	10.3 ± 0.12	71.9 ± 0.94	77 ± 1.1
White Clover	15.3 ± 0.35	27.5 ± 0.49	21.0 ± 0.25	26.1 ± 0.39	3.0 ± 0.06	0	11.8 ± 0.04	83.6 ± 0.31	94 ± 0.7
Lucerne	14.3 ± 0.62	25.6 ± 0.73	25.6 ± 0.80	28.7 ± 1.21	2.9 ± 0.06	0	10.3 ± 0.12	72.5 ± 0.99	82 ± 1.5
Sulla	23.9 ± 0.61	20.1 ± 0.81	19.6 ± 0.88	17.0 ± 1.37	2.3 ± 0.09	6.4	12.7 ± 0.15	87.5 ± 1.12	83 ± 1.5
Pasture:Sulla	17.9 ± 0.39	17.9 ± 0.82	25.0 ± 0.38	32.7 ± 0.75	2.4 ± 0.09	2.9	11.4 ± 0.11	78.5 ± 0.61	86 ± 1.1
White Clover:Sulla	19.2 ± 0.41	23.6 ± 0.45	20.8 ± 0.39	22.4 ± 0.55	2.6 ± 0.04	3.0	12.2 ± 0.08	84.9 ± 0.40	92 ± 0.7
Lucerne:Sulla	18.8 ± 0.61	23.0 ± 0.70	22.7 ± 0.38	22.8 ± 1.46	2.6 ± 0.06	3.1	11.5 ± 0.15	80.1 ± 1.26	86 ± 1.7

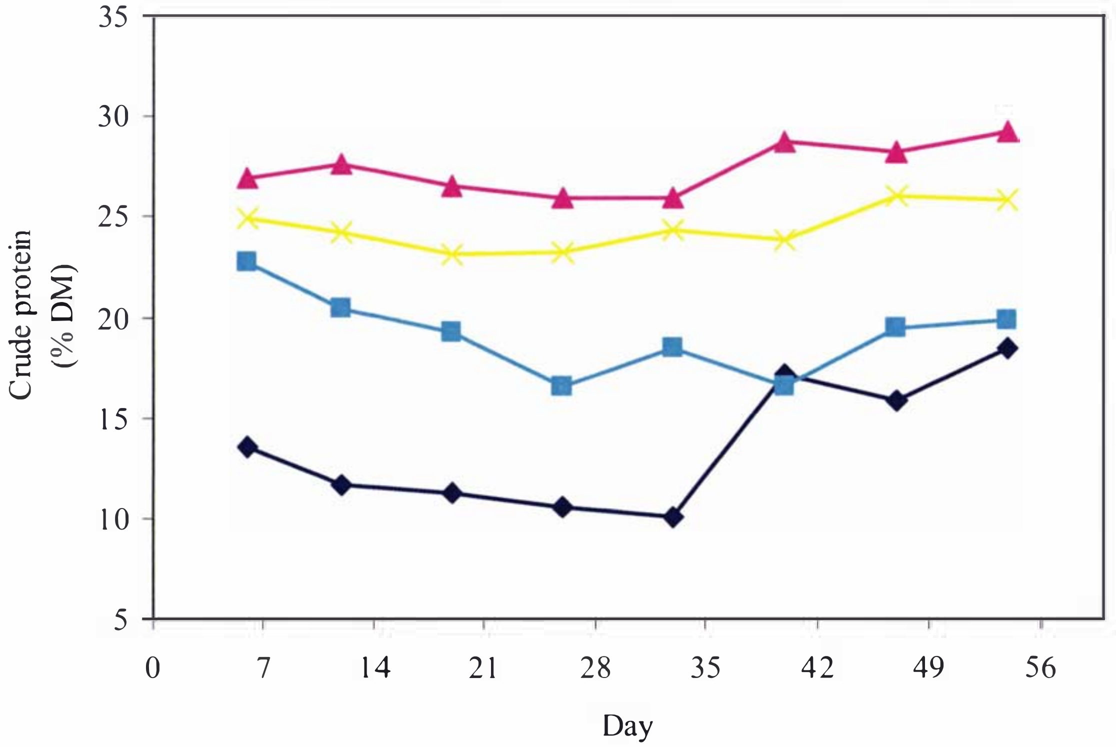
[1] Intake calculated from amount of feed offered and refused and composition of the offered and refused diets.
[2] ADF, Acid detergent fibre (cellulose and lignin).
[3] NDF, Neutral detergent fibre (hemicellulose, cellulose and lignin).
[4] ME, Metabolisable energy.
[5] OMD, Predicted organic matter digestibility.

FIGURE 4.1. Chemical composition of ryegrass, white clover, lucerne and sulla offered to lambs over the eight week experimental period.

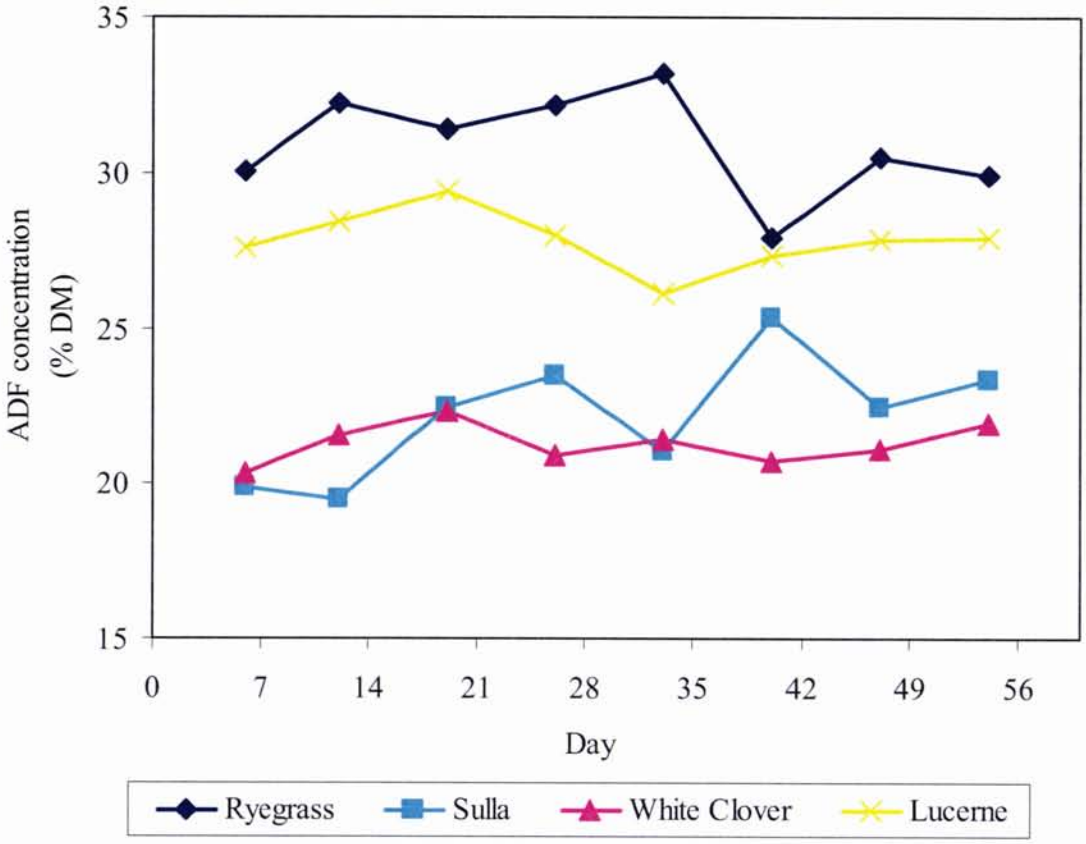
4.1a. Soluble carbohydrate.



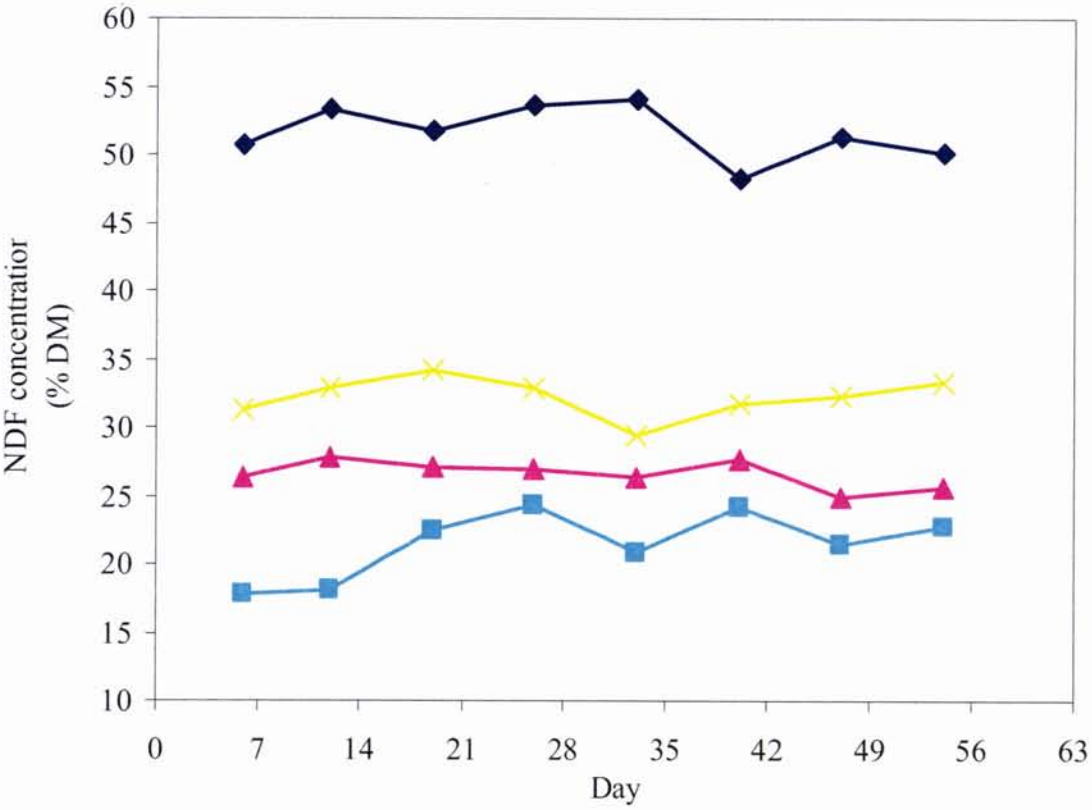
4.1b. Crude protein.



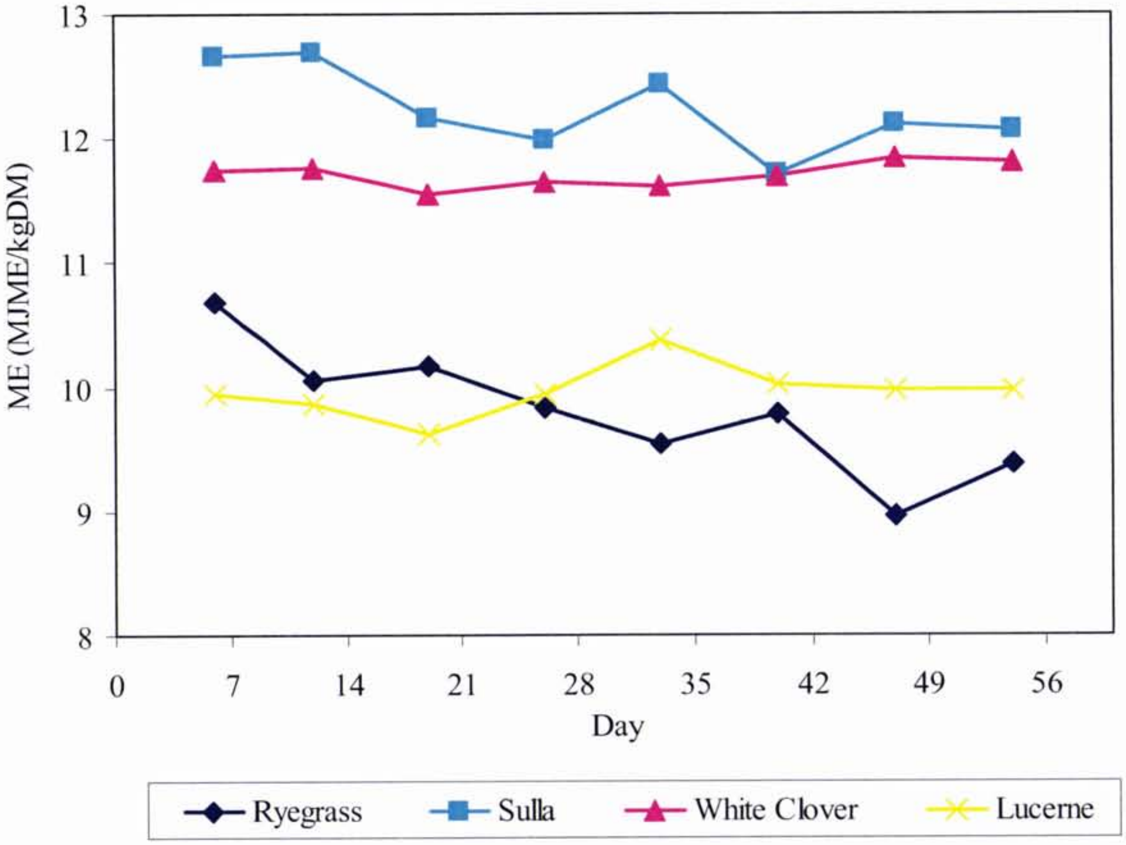
4.1c. Acid detergent fibre (ADF).



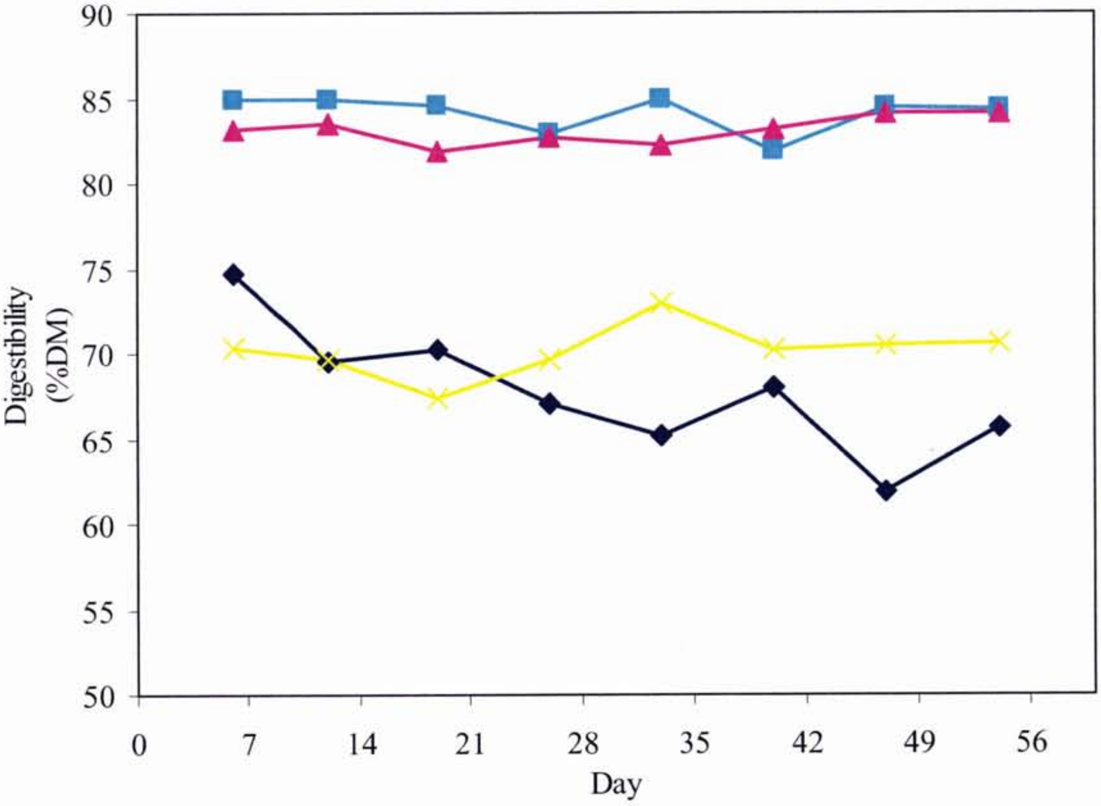
4.1d. Neutral detergent fibre (NDF).



4.1e. Metabolisable energy (ME).



4.1f. Predicted digestibility.



4.4.2 Lamb production

4.4.2.1 Liveweight gain

Liveweights of lambs (at 0900 h) on day 1 and 6 were 28.6 ± 0.76 kg and 28.3 ± 0.76 kg, respectively, and differences between treatment daily gains were evident after two weeks of feeding (Figure 4.2). Between day 6 and the end of the trial pasture-fed lambs grew at 116 g/day over the duration of the trial and achieved a final LW of 34.5 ± 0.79 kg which was significantly lower than all other treatments (Table 4.4; Figure 4.2). Lambs fed sulla had the most rapid daily gain (308 g/day) but did not grow significantly faster than lambs fed white clover, white clover:sulla or lucerne:sulla (281g/day). Lambs fed white clover, sulla, white clover:sulla and lucerne:sulla achieved a final LW of 43.1 to 43.5 kg, whilst those fed lucerne and pasture:sulla had daily gains of 207 and 190 g/day and their respective final LW of 39.5 and 37.9 kg, were significantly less than other treatments (Table 4.4). Contrasts showed that the actual LW gains of lambs fed pasture:sulla (190 g/day), white clover:sulla (281 g/day) and lucerne:sulla (281 g/day) diets were not significantly different ($Pr < 0.05$) from the expected LW gains (pasture:sulla, 212 g/day; white clover:sulla, 295 g/day; lucerne:sulla, 258 g/day) which were based on the LW gains of lambs fed the individual forage diets.

FIGURE 4.2. Liveweight profile of lambs fed pasture, white clover, lucerne, sulla and 50:50 mixtures of pasture:sulla, white clover:sulla and lucerne:sulla over eight weeks. Least-square (LS) means ± standard errors of the mean (SEM) are presented.

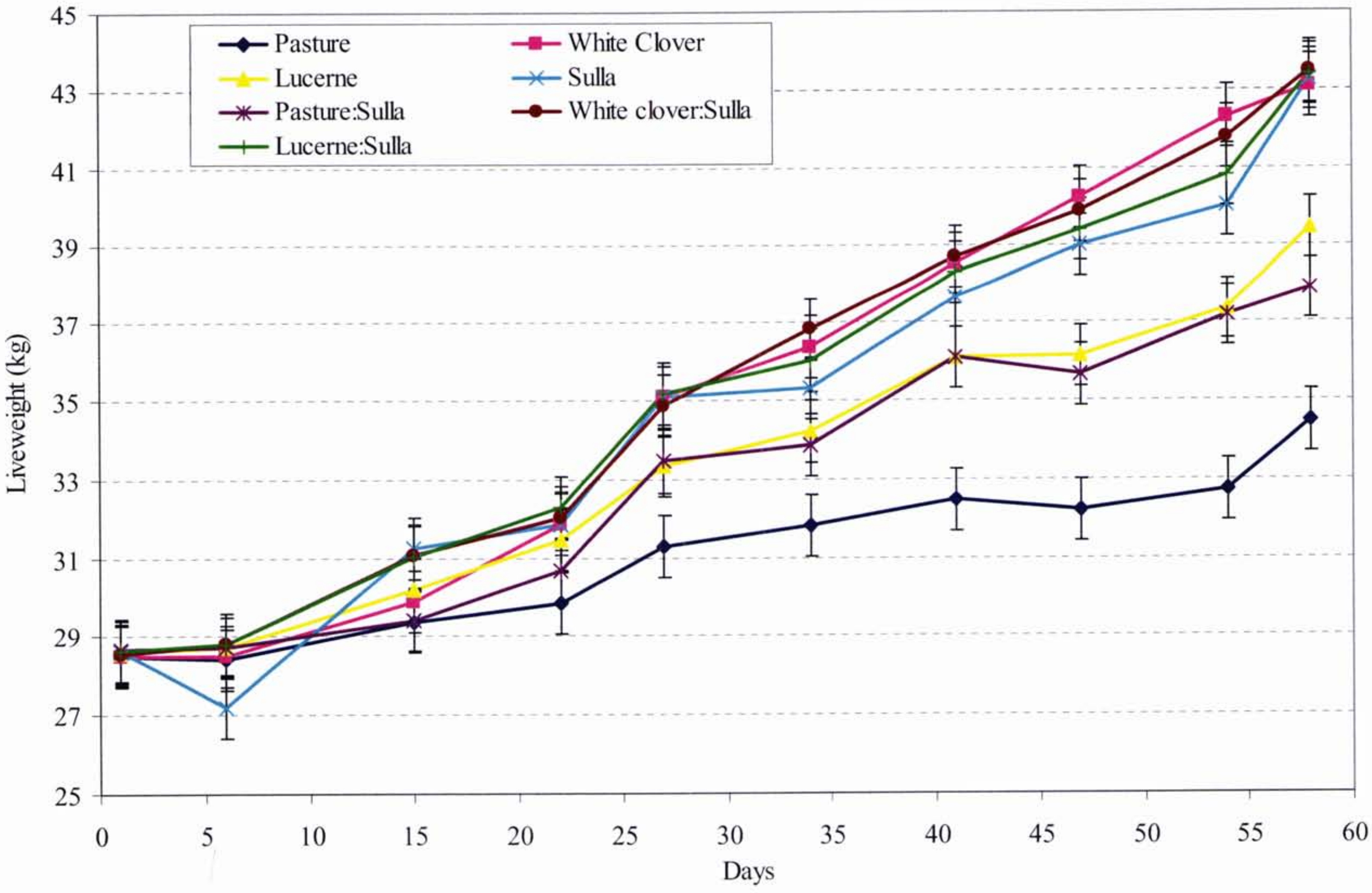


TABLE 4.4. Daily dry matter (DM) intake, liveweight (LW) gain, clean wool yield of lambs fed seven forage diets over eight weeks, carcass weight (CW) and dressing-out % of lambs at slaughter and efficiency of lamb production in terms of LW gain per kg DM eaten, metabolisable energy (ME) eaten and metabolisble protein (MP) supplied. Least-square (LS) means and standard error of the means (SEM) are presented.

Diet	During trial			At slaughter		Efficiency[2]		
	DM intake/lamb[1] (kg DM/day)	LW gain (g/day)	Wool yield (mg/100 cm^2 per day)	CW (kg)	Dressing-out %	g LW gain/ kg DM	g LW gain/ MJME eaten	g LW gain/ MP supplied
Number	51	8	16	8	8	-	-	-
Pasture	1.10^{a}	116^{a}	47^{a}	13.0^{a}	40.7^{a}	105	10.3	1.10
White Clover	1.48^{d}	281^{d}	71^{d}	19.0^{d}	42.2^{d}	190	16.1	1.58
Lucerne	1.37^{c}	207^{c}	59^{cb}	16.1^{b}	41.3^{bc}	151	14.7	1.28
Sulla	1.47^{d}	308^{d}	68^{d}	18.3^{cd}	41.0^{cd}	210	16.5	1.85
Pasture:Sulla	1.21^{b}	190^{b}	47^{a}	15^{b}	41.5^{b}	157	13.8	1.60
White Clover:Sulla	1.49^{d}	281^{d}	55^{ab}	18.5^{cd}	41.1^{cd}	189	15.5	1.53
Lucerne:Sulla	1.54^{d}	281^{d}	66^{cd}	17.7^{c}	40.0^{bc}	182	15.9	1.63
SEM	0.031	13.0	3.1	0.44	0.63	-	-	-
Diet effect (Pr)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	-	-	-

[1] DM intake/lamb/day is calculated from total feed offered less total feed refused per day divided by the number of lambs in each treatment group/day.

[2] Efficiency values for g LW gain per kg DM, MJME eaten and MP supplied were calculated from means of each parameter and no statistical analyses were conducted because individual lamb intakes were not measured.

[a, b] LS means within columns with a common superscript letter do not differ significantly (Pr < 0.05).

4.4.2.2 Fed versus fasted liveweight gain

This trial was designed to monitor the production of lambs fed contrasting diets, so routine weighing was an essential component but it was considered inappropriate to fast lambs every week to obtain empty (or fasted) LW. Most calculations have been based on weekly measurements of fully fed lambs, but a comparison was made between fed and fasted LW taken at the beginning and end of the trial (Table 4.5).

Fed liveweights of lambs at the start and end of the trial were greater than fasted LW and differences associated with fasting were affected by the diet ($Pr < 0.01$). However, effects of fasting at the beginning and end of the trial were inconsistent. At the start of the trial the difference between fed and fasted LW ranged between 0 kg (sulla) to 2.3 kg (white clover:sulla) while at the end of the trial differences ranged between 0.9 kg (pasture:sulla) and 3.0 kg (lucerne:sulla; Table 4.5).

Daily gains based on fed or fasted LW were similar for lambs fed lucerne but values were higher for fasted weights with lambs fed white clover, sulla and lucerne:sulla and lower in lambs fed pasture, pasture:sulla and white clover:sulla. These effects illustrate the importance of feed type on fasting weight loss and inconsistencies between diets (Table 4.5). The data confirm the importance of multiple weighing of fully fed lambs to determine daily gain.

4.4.2.3 Carcass characteristics

Differences in LW gain were correlated with carcass weight (CW; Table 4.4). The heaviest carcasses were from lambs that had the highest rate of gain (CW vs. fed LW gain, $r = 0.85$, $Pr < 0.01$; CW vs. fasted LW gain, $r = 0.84$; $Pr < 0.001$; Figure 4.3). Carcasses of lambs fed white clover, sulla and white clover:sulla were heavier (18.3 – 19.0 kg) than other treatments ($Pr < 0.05$), but CW of lambs fed sulla and white clover:sulla did not differ significantly from lucerne:sulla-fed lambs (17.7 kg). Lambs fed lucerne:sulla had the same daily gains (281 g/day) as white clover-fed lambs, but their carcasses were significantly lighter than lambs fed white clover ($Pr < 0.05$).

Dressing-out % was significantly lowest in pasture-fed lambs (37.7%) and highest in lambs fed white clover (43.9%). Dressing-out % of lambs fed white clover:sulla (42.3%) and sulla (42.2%), lucerne:sulla (40.7%) and lucerne (40.7%) were not significantly different (Table 4.4). The diet effects on dressing-out % were removed when dressing-out % was adjusted to a common carcass weight.

FIGURE 4.3. The relationship between carcass weight (kg) and fasted liveweight gain (g/day) for lambs fed seven forage-based diets over eight weeks.

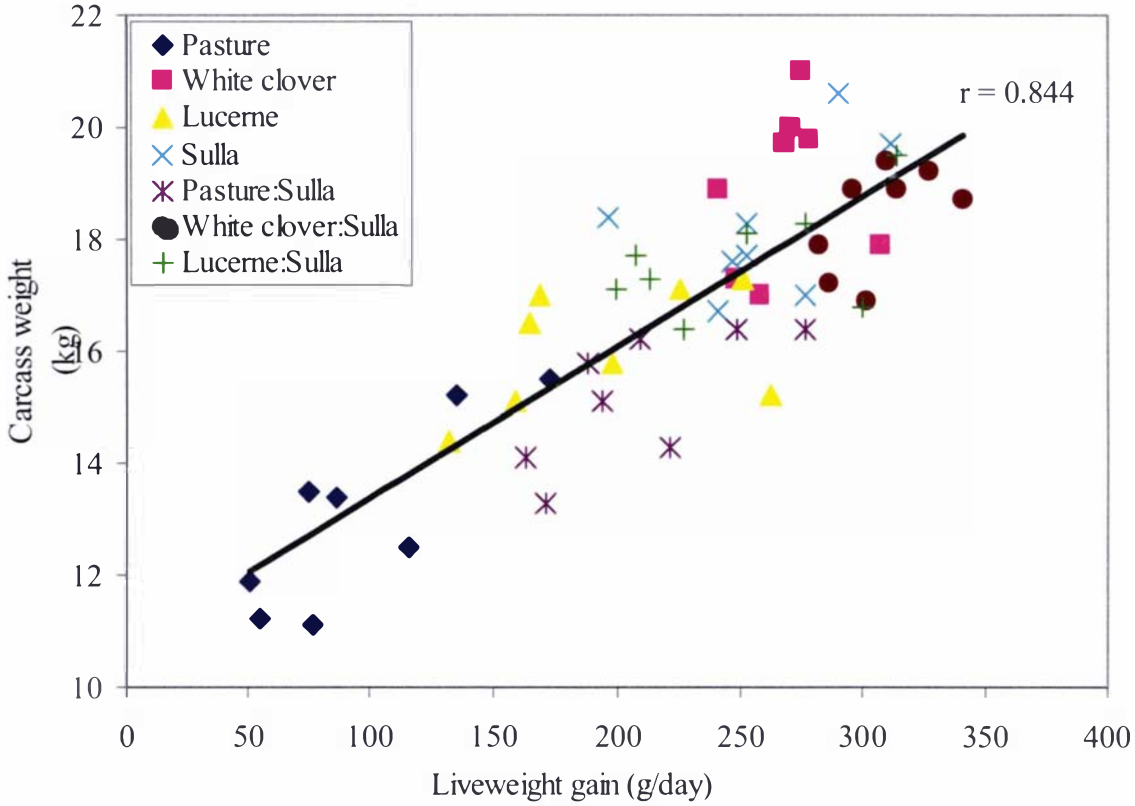


TABLE 4.5. Fed and fasted liveweights (LW) of lambs at the start and end of the trial, difference between fed and fasted LW at the start (day 6 and 8) and end (day 58 and 59) of the trial and LW gains of fed and fasted lambs fed seven forage diets over the eight weeks. Least-square (LS) means and standard error of the mean (SEM) are presented.

Diet	Day 1	Day 6	Day 8	Day 6 – 8 LW	Day 58	Day 59	Day 58 – 59 LW	LW gain	
	LW (kg)	Fed LW (kg)	Fasted LW (kg)	Difference (kg)	Fed LW (kg)	Fasted LW (kg)	Difference (kg)	Fed (g/lamb/day)	Fasted (g/lamb/day)
Pasture^{1}	28.5	28.4	28.0	0.4^{ab}	34.5^{a}	33.4 ± 0.98$^{1\ a}$	1.2 ± 0.29$^{1\ ab}$	116^{a}	127 ± 16.6$^{1\ a}$
White Clover	28.5	28.5	27.9	0.6^{ab}	43.1^{c}	41.6 ± 0.76^{c}	1.5 ± 0.21^{ab}	281^{c}	269 ± 11.7^{c}
Lucerne^{1}	28.7	28.7	27.4	1.3^{bc}	39.5^{b}	37.1 ± 0.98$^{1\ b}$	2.1 ± 0.29$^{1\ bc}$	207^{b}	208 ± 16.6^{b}
Sulla^{1}	28.6	27.2	27.2	0^{a}	43.3^{c}	41.5 ± 0.98$^{1\ c}$	2.6 ± 0.29$^{1\ c}$	308^{c}	281 ± 16.6$^{1\ c}$
Pasture:Sulla	28.7	28.0	26.3	1.7^{bc}	37.9^{b}	37.0 ± 0.76^{b}	0.9 ± 0.21^{a}	190^{b}	209 ± 11.7^{b}
White Clover:Sulla	28.5	28.8	26.6	2.3^{c}	43.5^{c}	42.3 ± 0.76^{c}	1.2 ± 0.21^{ab}	281^{c}	307 ± 11.7^{c}
Lucerne:Sulla^{1}	28.6	28.8	27.7	1.1^{bc}	43.7^{c}	40.7 ± 0.98$^{1\ c}$	3.0 ± 0.29$^{1\ c}$	281^{c}	250 ± 16.6$^{1\ c}$
SEM	0.79	0.79	0.79	0.30	0.79	-	0.29	13.0	-
Diet (Pr)	NS	NS	NS	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

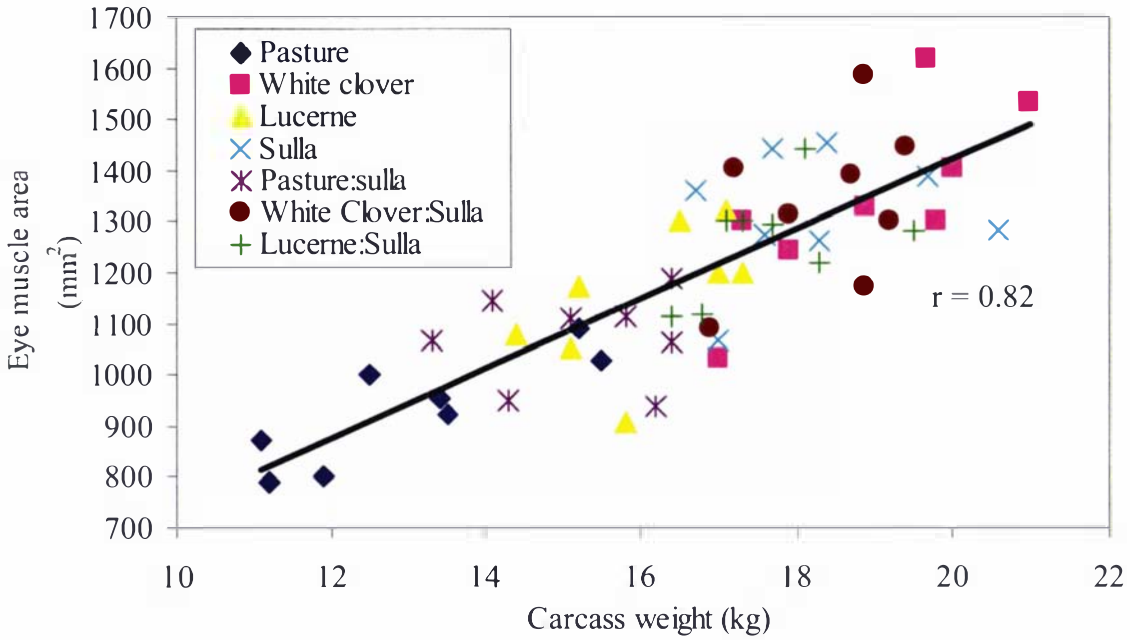
[1] Fasted liveweight and fasted liveweight gain based on 4 lambs only in pasture, lucerne, sulla and lucerne:sulla treatments. The other 4 lambs were used in the protein synthesis study and were not fasted. LS means ± SEM for each treatment group are presented.

[a, b] LS means within columns with a common superscript letter or with no superscript letter do not differ significantly ($Pr < 0.05$).

Diet significantly affected all measurements taken on live animals using the ultrasound (GR fat-depth, back fat-depth and EMA; Table 4.6). On day 6 of the experiment all lambs had the same EMA, GR and back fat-depths and by day 30 differences between dietary treatments were evident. By day 54 lambs fed pasture had the least amount of fat (back fat and GR) and muscle (EMA) compared to lambs fed other diets. EMA and fat depths were greatest in lambs fed sulla, white clover, white clover:sulla and lucerne:sulla. From day 6 to day 54 of the experiment changes in fat-depths and EMA were small or non-existent for slow growing lambs fed pasture and pasture:sulla, compared to fast growing lambs fed white clover, white clover:sulla, lucerne:sulla, sulla where changes occurred throughout the experiment. Differences between diets seen on live animals were also evident at slaughter with the carcasses of lambs fed pasture having the smallest GR fat depths and lambs fed white clover, sulla, white clover:sulla and lucerne:sulla the greatest. However, GR fat depths measured by ultrasound were much lower on day 54 (mean GR across treatments = 2.6 mm) compared to a mean value of 7.8 mm measured seven days later at slaughter. There was a strong correlation between CW and EMA of lambs on day 54 of the trial ($r = 0.82$; Figure 4.4), and the correlation between EMA and LW increased from day 6 to 54 of the trial (day 6, $r = 0.41$; day 30, $r = 0.68$; day 54, $r = 0.75$).

The LW covariate had a significant effect on all ultrasound measurements taken on live animals and the CW covariate had a significant effect on GR fat-depths of carcasses at slaughter (Appendix 4.4). After adjusting for LW and CW there was no effect of diet on GR fat-depths at slaughter and no effect of diet or diet x day of measurement on GR fat-depths measured by ultrasound. Diet and diet x day of measurement interaction affected EMA measured by ultrasound ($Pr < 0.10$) and only diet affected back fat-depths ($Pr < 0.05$). By day 54 of the experiment lambs fed pasture and pasture:sulla had smaller EMA than lambs fed any other diet, and on average over the entire trial, lambs fed pasture and pasture:sulla had smaller back fat-depths than lambs fed white clover, sulla and lucerne:sulla. On average lambs fed white clover:sulla tended to have less back fat than lambs fed white clover over the entire trial ($Pr < 0.10$), whereas the opposite was the case when sulla was added to lucerne with lucerne:sulla-fed lambs tending to have greater back fat-depths than lambs fed lucerne on average over the entire trial ($Pr < 0.10$).

FIGURE 4.4. The relationship between carcass weight (kg) and eye muscle area (mm2) on day 54 of the experiment for lambs fed seven forage-based diets over eight weeks.



4.4.2.4 Wool production

Pasture and pasture:sulla-fed lambs had significantly lower clean wool yields (47 mg/100 cm^2/day) than lambs fed white clover:sulla, sulla and lucerne:sulla (71, 68 and 66 mg/100 cm^2/day). Effects of supplementing lambs with sulla were mixed (Table 4.4) with a reduction in wool growth over eight weeks in lambs fed white clover:sulla vs. white clover (55 vs. 71 mg/100 cm^2/day; $Pr < 0.05$), no change when pasture:sulla was fed (47 g/100 cm^2) and improved yield when lucerne:sulla was fed compared to lucerne (66 vs. 59 mg/100 cm^2/day; $Pr < 0.05$). Clean wool weights were 70 – 90% of greasy weights.

TABLE 4.6. Fat depth at the 11th and 12th rib (GR; mm), back-fat depth (mm) and eye muscle area[1] (mm^2) estimated by ultrasound on days 6, 30 and 54 for lambs fed on seven forage diets over eight weeks, and GR fat-depth of lamb carcasses at slaughter (day 61). Least-square (LS) means and standard error of the mean (SEM) are presented.

Diet	GR			Carcass GR	Back fat-depth			Eye muscle area[1]		
Day	6	30	54	61	6	30	54	6	30	54
Pasture	2.3^{a}	2.3^{abc}	2.1^{a}	3.9^{a}	1.6^{a}	1.6^{a}	2.0^{a}	958^{a}	972^{a}	932^{a}
White Clover	2.5^{a}	2.5^{ab}	3.5^{b}	10.0^{d}	2.0^{a}	2.5^{bc}	3.1^{b}	1017^{a}	1189^{bcd}	1345^{b}
Lucerne	2.1^{a}	2.0^{ac}	2.8^{c}	6.9^{c}	1.8^{a}	2.0^{ac}	2.3^{ac}	933^{a}	1080^{ad}	1155^{cd}
Sulla	2.5^{a}	2.8^{b}	3.0^{bc}	8.8^{bd}	2.0^{a}	2.6^{bd}	2.9^{bd}	960^{a}	1241^{b}	1316^{b}
Pasture:Sulla	2.5^{a}	1.8^{c}	2.9^{c}	7.3^{cb}	1.6^{a}	1.8^{a}	2.4^{acd}	959^{a}	1092^{ac}	1072^{d}
White Clover:Sulla	2.5^{a}	2.1^{ac}	3.5^{b}	8.6^{bd}	1.8^{a}	2.1^{ad}	2.6^{bc}	950^{a}	1184^{bc}	1338^{b}
Lucerne:Sulla	2.4^{a}	2.0^{ac}	3.1^{bc}	8.9^{bd}	2.0^{a}	2.6^{bd}	3.1^{b}	955^{a}	1144^{bc}	1259^{bc}
SEM		0.24		0.62		0.21			43.8	
Diet effect		< 0.01		< 0.01		< 0.01			< 0.01	
Day effect		< 0.01		-		< 0.01			< 0.01	
Diet x day effect		< 0.05		-		NS			< 0.01	

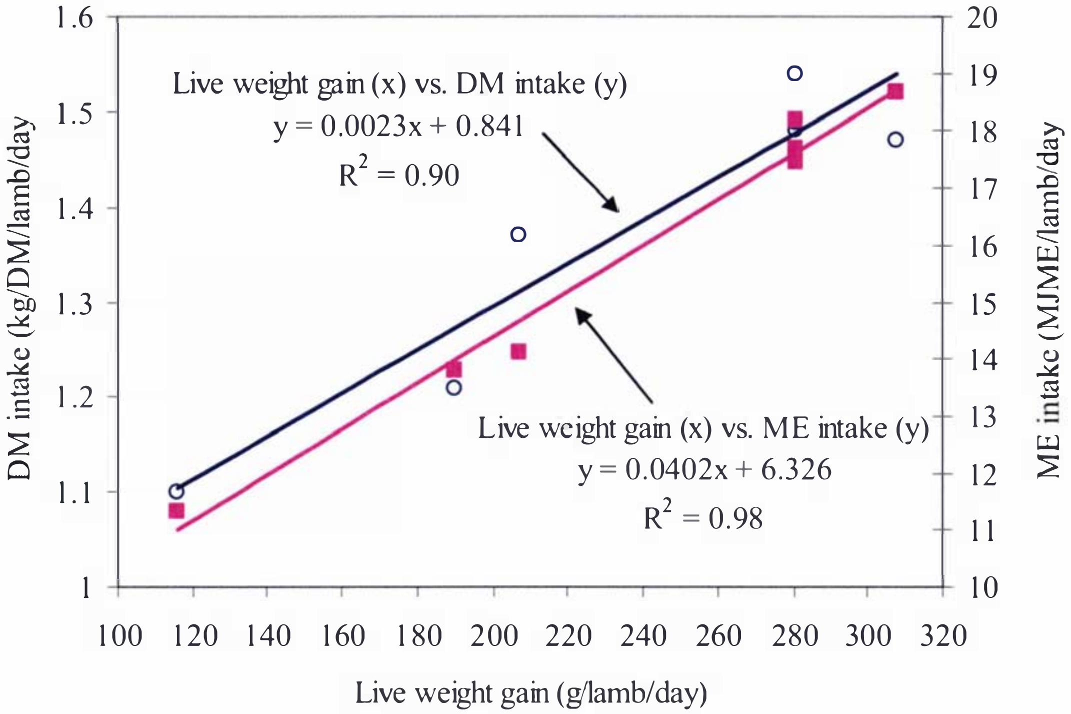
[1] Eye muscle area calculated as width (A; mm) x depth (B; mm) x 0.77.

[a, b] LS means within columns with a common superscript letter do not differ significantly (Pr < 0.05).

4.4.2.5 Feed intakes

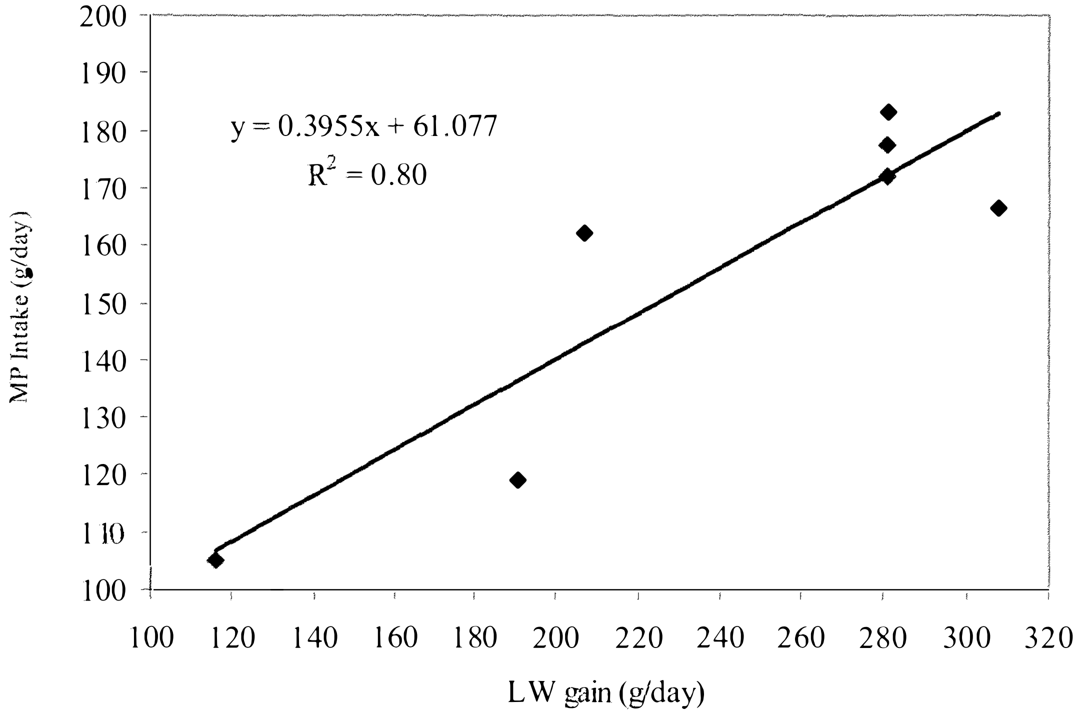
DM intakes calculated from total daily feed intake of treatment groups averaged over the 60 days of the trial were highest for lambs fed white clover, white clover:sulla, sulla and lucerne:sulla (1.47 to 1.54 kg DM/lamb/day), than 1.10 to 1.37 kg DM/lamb/day for lambs fed pasture, pasture:sulla and lucerne (Table 4.4). Intakes increased over the duration of the trial for all treatments. Average intake of all lambs increased from 1.18 kg DM/lamb/day in week one to 1.57 kg DM/lamb/day in week eight of the experiment with a smaller increase for lambs fed pasture, pasture:sulla and lucerne. Analysis using contrasts showed that the actual DM intake of lambs fed lucerne:sulla (1.54 kg DM/day) was significantly greater ($Pr < 0.05$) than the expected intakes (1.42 kg DM/day), but for lambs fed pasture:sulla actual DM intakes tended to be less than the expected intakes (1.21 vs. 129 kg DM/day). Mean DM intakes for the treatment groups (Table 4.4) were strongly correlated with mean LW gain from day 6 to 58 (Table 4.5) of the trial ($r = 0.95$; Figure 4.5), and the correlation between LW gain and mean ME intake was 0.99 (Figure 4.5). In order for 25 to 50 kg lambs to gain an extra 100 g/day, daily DM and ME intake would need to increase 0.23 kg and 40.2 MJME, respectively (Figure 4.5). Actual intakes of individual lambs within dietary treatments were determined during week 4 and 5 when alkane markers were given to individual lambs (Appendix 4.2).

FIGURE 4.5. Average liveweight (LW) gain of lambs from day 6 to 58 of the trial compared to dry matter (DM) intake and metabolisable energy (ME) intake of lambs fed seven forage diets.



Metabolisable protein (MP) supply was calculated using AFRC (1992) equations (Appendix 4.6) for the seven diets fed to lambs. Calculations were based on CP, soluble (A) and slowly degradable (B) fractions and degradation rate (k) for each diet, measured by the *in sacco* method (Appendix 4.5). Metabolisable protein intake was strongly correlated with LW gain (r = 0.90; Figure 4.6), although three diets supplied a similar amount of MP (172 to 183 g/day) with daily gains of 281 g. Metabolisable protein supplied by pasture, pasture:sulla, lucerne, sulla, lucerne:sulla, white clover and white clover:sulla was 105, 119, 162, 166, 172, 178 and 183 g/day, respectively. Regression analysis showed that for 25 to 50 kg lambs an extra 39.6 g MP/day would increase LW gain by 100 g/day (Figure 4.6).

FIGURE 4.6. Average metabolisable protein (MP) intake and average liveweight (LW) gain of lambs from day 6 to 58 of the trial when fed seven forage diets.



In this study alkane predictions were of limited value as the predicted intakes did not appear to be correct, and were not similar to DM intakes of lambs on the feed pads (Table 4.7). Correlations between alkane predicted DM intakes of the 56 lambs in this study and individual gains from day 6 to 60 were poor and probably incorrect (Table 4.7). Correlations between LW gain and alkane predicted DM intakes were 0.07 ($Pr > 0.10$; Figure 4.7) and with predicted ME intake were 0.31 ($Pr < 0.05$; Figure 4.8). Predictions were especially poor with the lucerne diet, where intakes were over-predicted. Omission of data for lambs fed lucerne improved correlations between LW gain and DM intake ($r = 0.24$; $Pr > 0.10$; Figure 4.7), and ME intake ($r = 0.44$; $Pr < 0.01$; Figure 4.8).

When effects of over- or under-prediction of group intakes were removed by adjusting individual lamb intakes to equal the total (measured) group intakes (for day 26 to 38 of the trial), relationships with production improved. Adjusted individual intakes of lambs (Group DM intake; Table 4.7) were calculated by calculating the relative intake of each lamb using predicted alkane intakes. These proportions were multiplied by the total amount of feed actually consumed by lambs on the feed pads to give intake per lamb during week 4 and 5 of the trial. Figure 4.9 shows a significant (Pr < 0.01) relationship between LW gain from day 6 to 60 and DM intake (r = 0.58) and as expected there was a very strong relationship between ME intake and LW gain (r = 0.68).

The unsatisfactory predictions of individual intakes based on the alkane procedure resulted in all comparisons being made on the basis of average intakes over the entire trial (Table 4.7).

FIGURE 4.7. The relationship between dry matter (DM) intake (predicted by alkanes) and liveweight gain of all lambs on seven forage diets, and when data from lambs fed lucerne were not included.

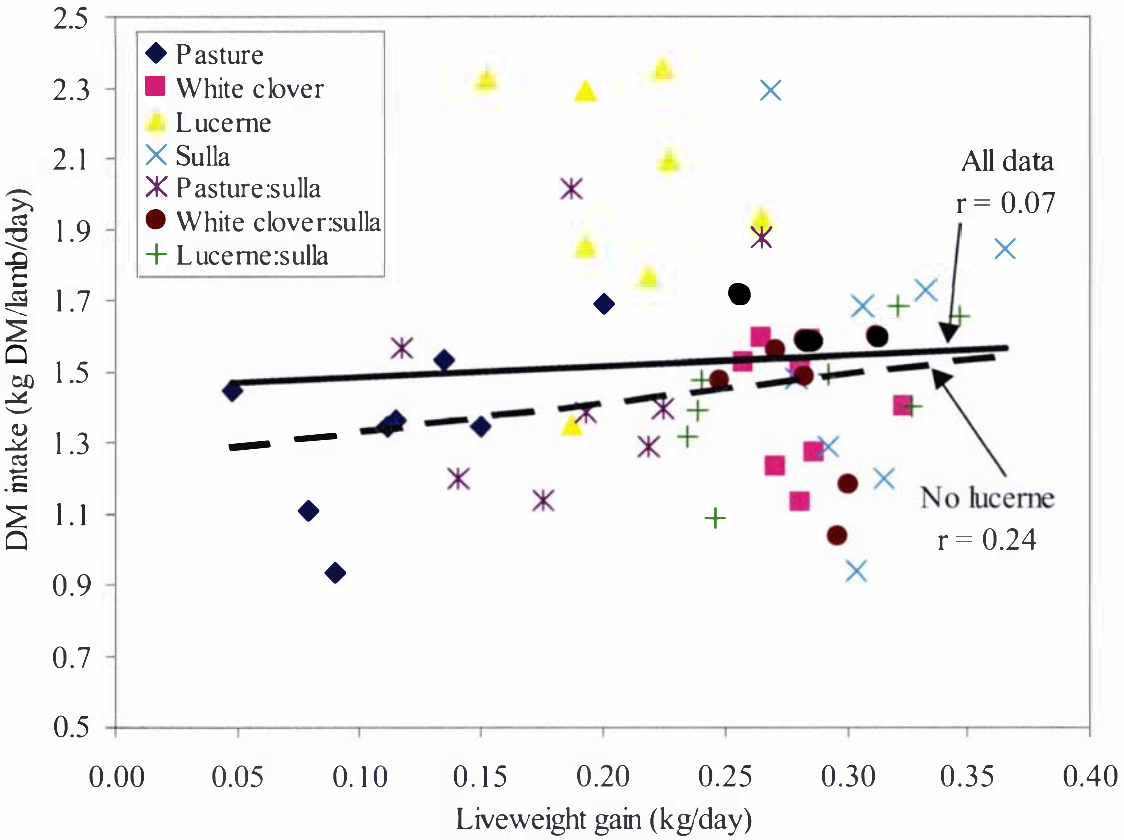


FIGURE 4.8. The relationship between metabolisable energy (ME) intake (predicted by alkanes) and liveweight gain of all lambs on seven forage diets and when data from lambs fed lucerne were not included.

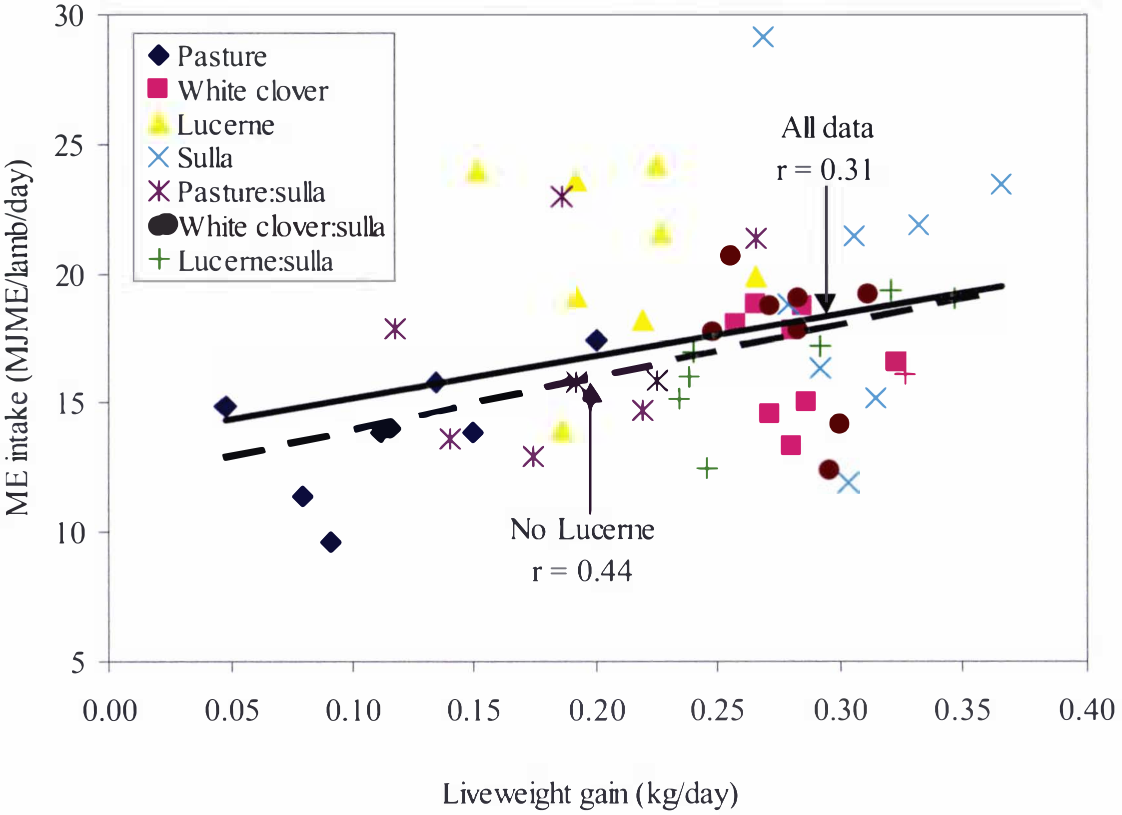


FIGURE 4.9. The relationship between dry matter (DM) intake (predicted by alkanes) and liveweight gain adjusted for over- and under-prediction of group intakes

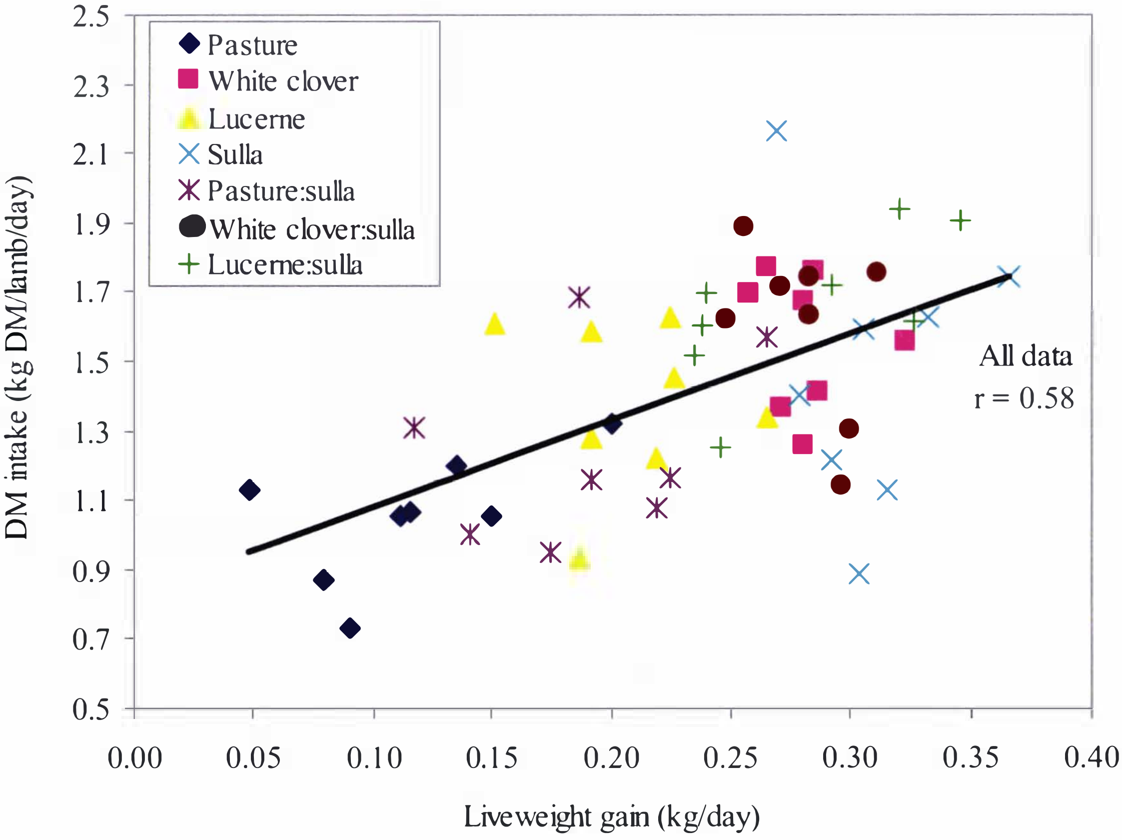


TABLE 4.7. Dry matter (DM) intake (kg DM/lamb/day) of individual lambs predicted with alkane markers[1] and calculated group DM intake during weeks 4 and 5 of the trial[2], and average DM intakes[3] and liveweight (LW) gain over the eight week duration of the trial. Least-square (LS) means and standard error of the mean (SEM) are presented.

Diet	Mean DM intake per lamb using alkanes[1]	Group average intake per lamb[2]	Predicted DM intake – group DM intake[4]	Intake and LW gain over eight weeks of trial	
				Mean DM intake per lamb[3]	LW gain (g/day)
Pasture	1.35^{a}	1.05^{a}	+0.30	1.10^{a}	116^{a}
White Clover	1.41^{a}	1.60^{bc}	-0.29	1.48^{d}	281^{c}
Lucerne	2.00^{b}	1.38^{bc}	+0.62	1.37^{c}	207^{b}
Sulla	1.56^{a}	1.47^{bc}	+0.09	1.47^{d}	308^{c}
Pasture:Sulla	1.48^{a}	1.24^{ab}	+0.24	1.21^{b}	190^{b}
White clover:Sulla	1.46^{a}	1.56^{bc}	-0.10	1.49^{d}	281^{c}
Lucerne:Sulla	1.44^{a}	1.66^{c}	-0.22	1.54^{d}	281^{c}
SEM	0.101	0.092	-	0.031	13.0
Diet effect	< 0.01	< 0.01	-	< 0.01	< 0.01

[4] No statistical analysis was conducted because difference is based on mean intake measured with alkanes and mean group intake of lambs.

[a, b] LS means within columns with a common superscript letter do not differ significantly ($Pr < 0.05$).

4.4.2.6 Efficiency of DM and ME utilisation

The efficiency of LW gain/DM intake (g/kg) calculated from mean LW gain and mean DM intake of each diet ranged from 105 to 210. Lambs fed sulla had a higher value (210 g/kg DM intake) than lambs fed white clover, white clover:sulla and lucerne:sulla (190, 189 and 182 g LW/kg DM) or pasture:sulla, lucerne and pasture (157, 151 and 105 g LW/kg DM; Table 4.4).

Efficiency of LW gain from ME intake calculated from the mean LW gain and mean ME intake of each diet ranged between 10.3 g LW gain/MJME for pasture-fed lambs to 16.5 for lambs fed sulla. The ranking of treatment groups for utilisation of ME differed from DM, with the lucerne-fed lambs ranking better than lambs fed pasture:sulla (Table 4.4).

Energy and protein requirements of the lambs fed the seven diets were calculated from mean LW and LW gain using SCA (1990) and AFRC (1992) equations, respectively (Appendix 4.6). Figure 4.10 illustrates that the ME requirements were similar to the ME consumed by lambs, but MP requirements were 80% of the MP intake (Figure 4.11). Calculated NE retention was strongly correlated with ME intake above maintenance (0.99; Figure 4.12) but less than half of the MP consumption above maintenance was retained and the correlation was $r = 0.88$ (Figure 4.13).

The utilisation of ME above maintenance for LW gain (Appendix 4.6) was calculated from the mean LW gain and mean ME used above maintenance and ranged between 36.5 to 49.3 MJME/kg LW gain for lambs fed sulla and lucerne, respectively (Table 4.8). Adding sulla to pasture, white clover and lucerne reduced the amount of ME utilised for LW gain relative to the individual diets (Table 4.8).

FIGURE 4.10. Relationship between metabolisable energy (ME) requirements and ME intake for lambs fed seven forage diets.

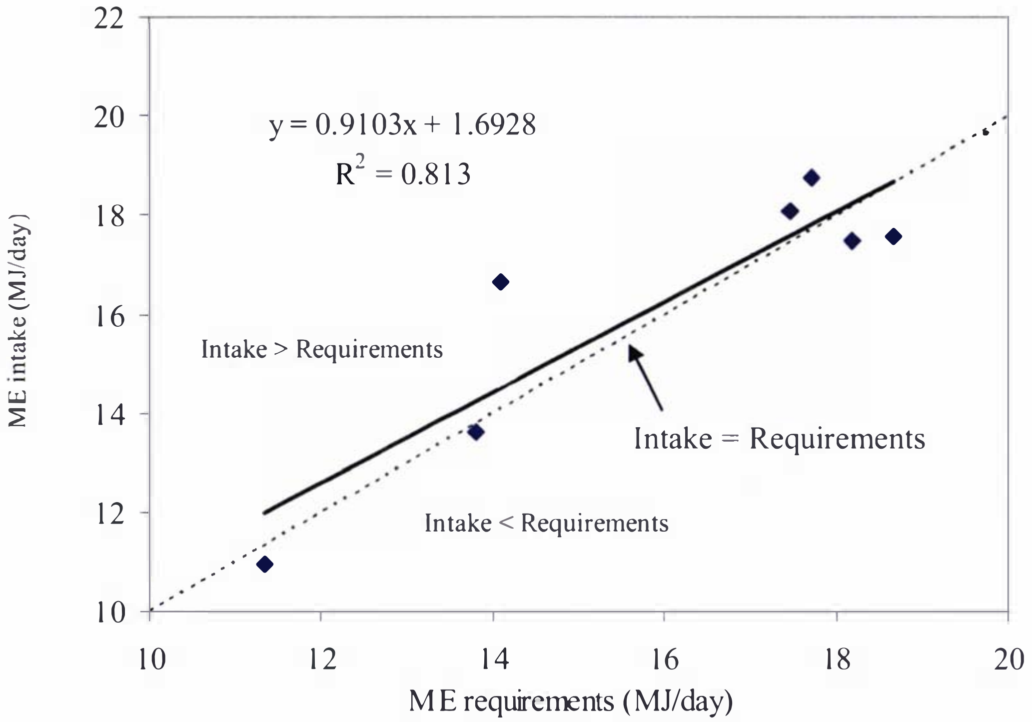


FIGURE 4.11. Relationship between metabolisable protein (MP) requirements and MP intake for lambs fed seven forage diets.

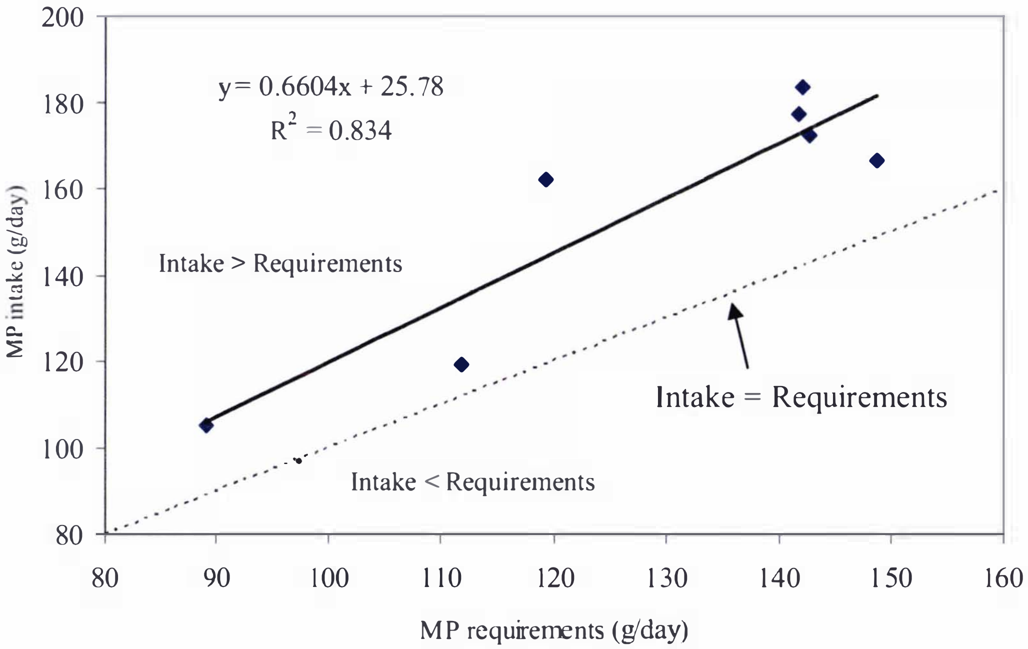


FIGURE 4.12. Relationship between metabolisable energy (ME) eaten above maintenance and net energy (NE) retained as liveweight gain.

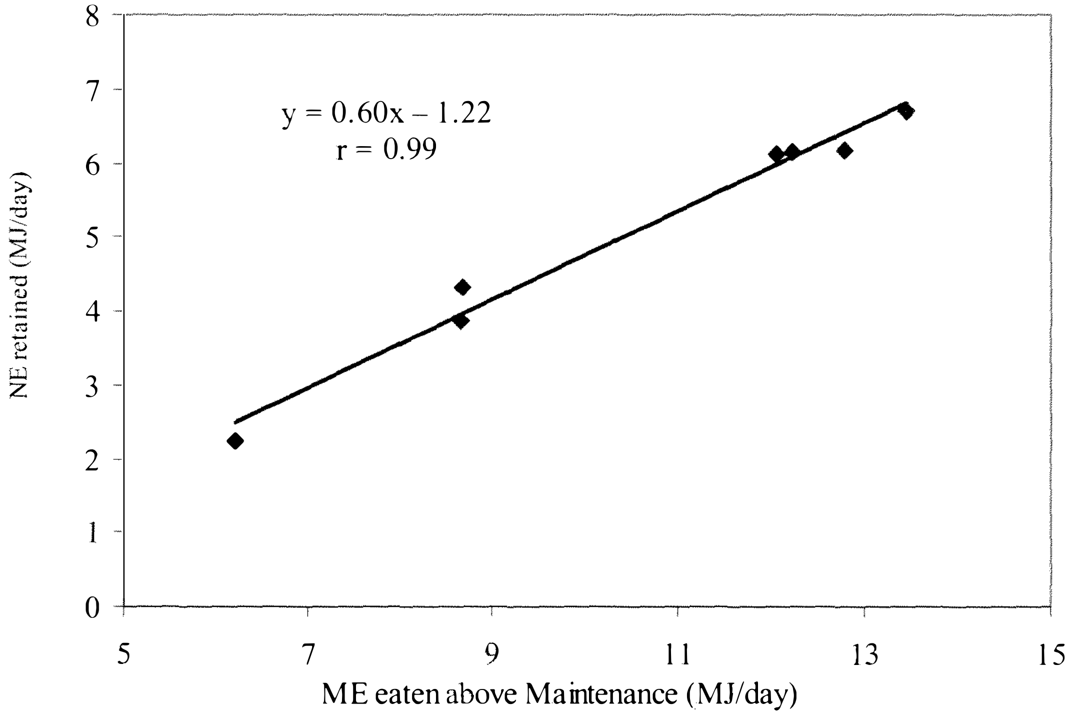


FIGURE 4.13. Relationship between metabolisable protein (MP) eaten above maintenance and net protein (NP) retained as liveweight gain and wool.

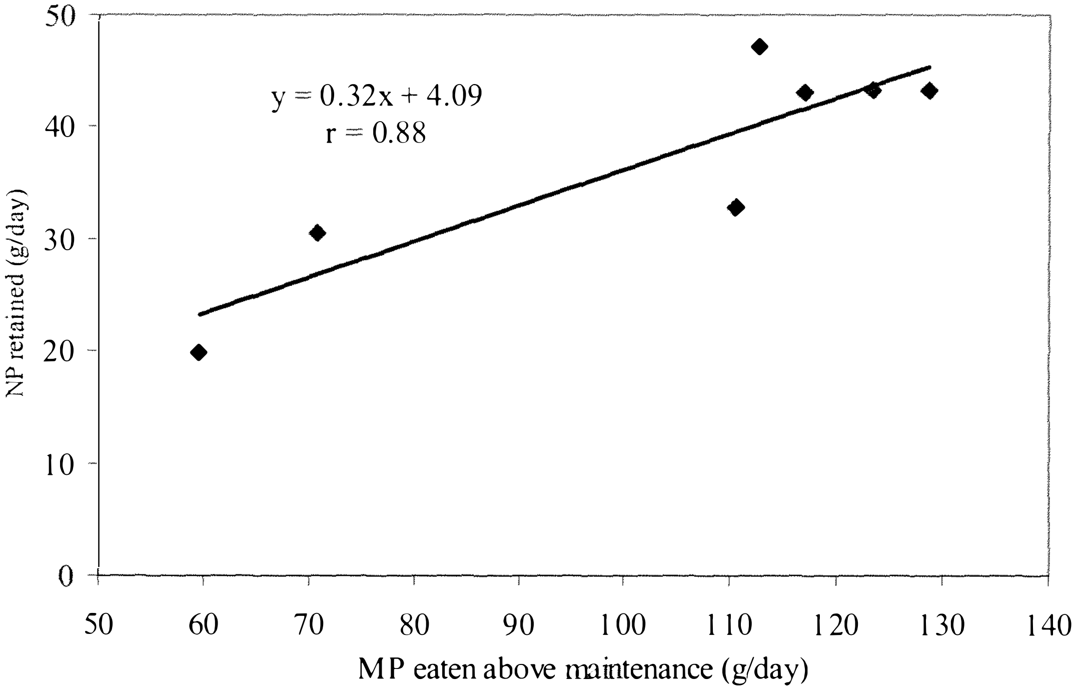


TABLE 4.8. Metabolisable energy (ME) requirement[1] for maintenance and liveweight (LW) gain and the efficiency which ME used above maintenance was converted into LW gain.

Diet	ME required for			LW gain (g/day)	Efficiency of ME utilisation per kg LW gain
	Maintenance	LW gain	Total[2]		
Pasture	5.1	5.3	11.0	116	45.8
White clover	5.4	11.3	18.1	281	41.0
Lucerne	5.4	10.2	16.7	207	49.3
Sulla	5.2	11.2	17.6	308	36.5
Pasture:Sulla	5.1	7.7	13.6	190	40.5
White clover:Sulla	5.4	11.0	17.5	281	39.2
Lucerne:Sulla	5.5	12.1	18.8	281	43.0

[1] Calculated using SCA (1990) equations in Appendix 4.6.

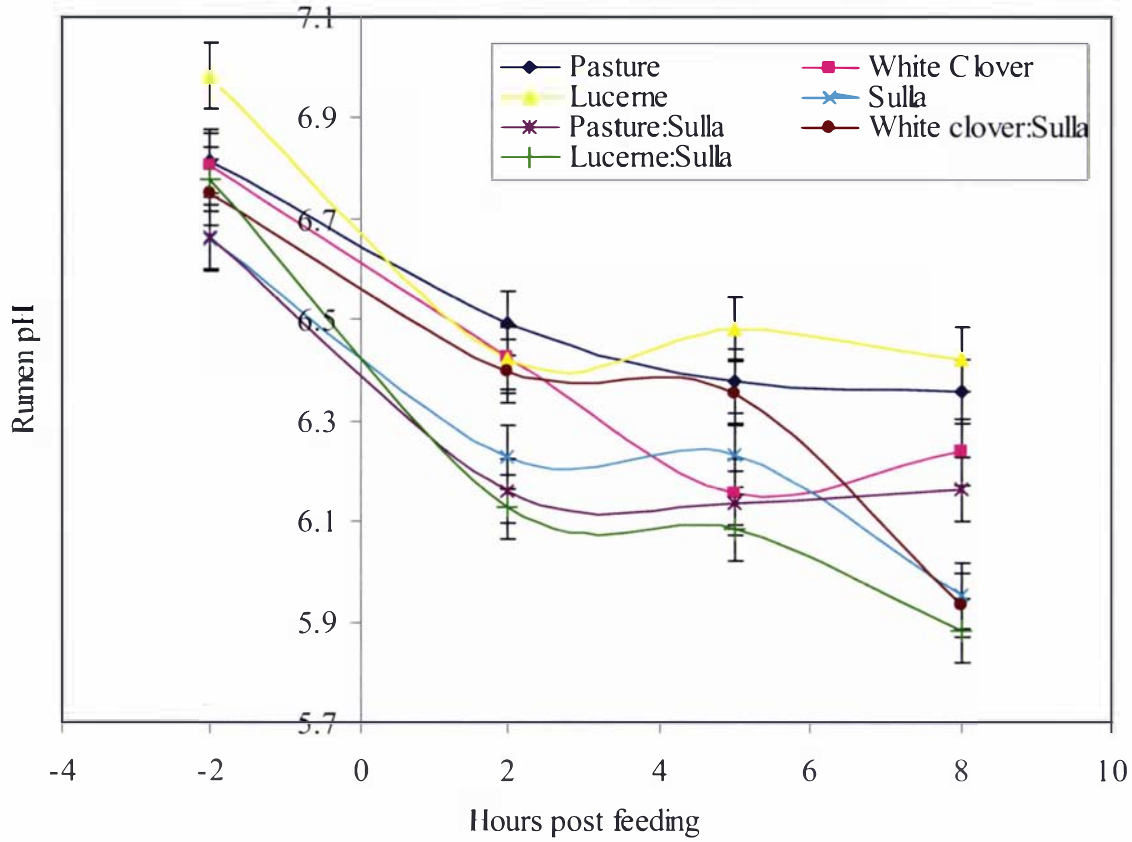
[2] Includes a value for support (10% of ME used for LW gain)

4.4.3 Rumen pH, ammonia and volatile fatty acids

4.4.3.1 Rumen pH

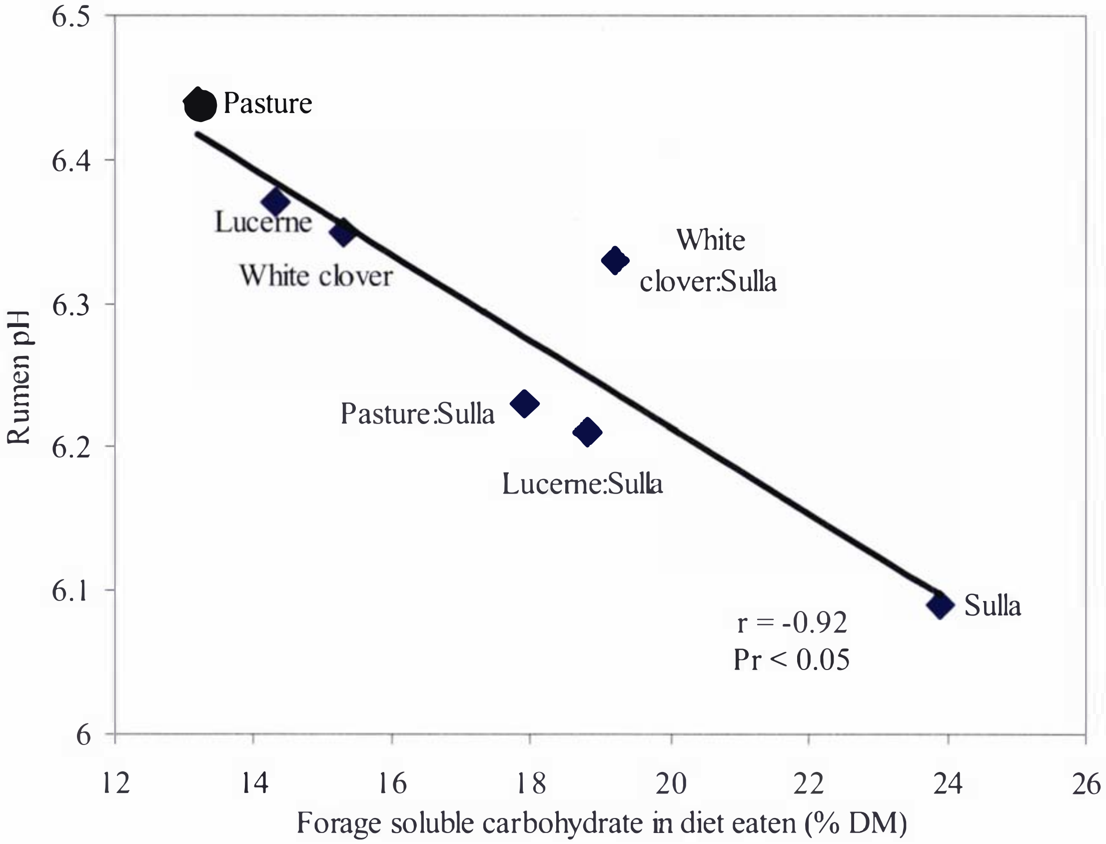
Figure 4.14 illustrates rumen pH changes from pre-feeding to 8 hours after feed was offered on one day of the experiment. Pre-feeding pH ranged from a high of 6.98 for lambs fed lucerne to 6.66 for lambs fed either pasture:sulla or sulla. Feeding resulted in a rapid decline to values between 6.12 and 6.49 (lucerne:sulla- and pasture-fed lambs, respectively) after which pH remained essentially constant for lucerne, pasture and pasture:sulla, but continued to decline in lambs fed white clover:sulla, white clover, sulla and lucerne:sulla. The lucerne:sulla diet was associated with the lowest pH at 2, 5 and 8 hours post-feeding whereas the lucerne diet resulted in the highest pH at 5 to 8 hours post-feeding (Figure 4.14). Sulla also had low pH pre-feeding and 8 hours after feeding, but after 2 and 5 hours rumen pH was mid-way between lambs fed lucerne, pasture, and white clover:sulla and lambs fed lucerne:sulla, pasture:sulla and white clover.

FIGURE 4.14. Changes in mean rumen pH from two hours pre-feeding to eight hours post-feeding for lambs fed the seven forage diets on one day of the experiment. Least-square means ± standard errors of the mean at each time of measurement are presented.



Mean pH from all lambs measured 2 – 4 hours post-feeding on six occasions during the experiment (Table 4.9) showed rumen pH to be lowest in lambs fed sulla (6.09) and the addition of sulla to pasture and lucerne also reduced rumen pH (pasture vs. pasture:sulla = 6.44 vs. 6.23; lucerne vs. lucerne:sulla = 6.37 vs. 6.21). Rumen pH was negatively correlated with soluble carbohydrate intake (Figure 4.15; r = -0.92; Pr < 0.05). The relationships between rumen pH and NDF and ADF concentrations in DM eaten were not significantly correlated (NDF, r = 0.71, Pr < 0.10; ADF, r = 0.63, Pr > 0.10).

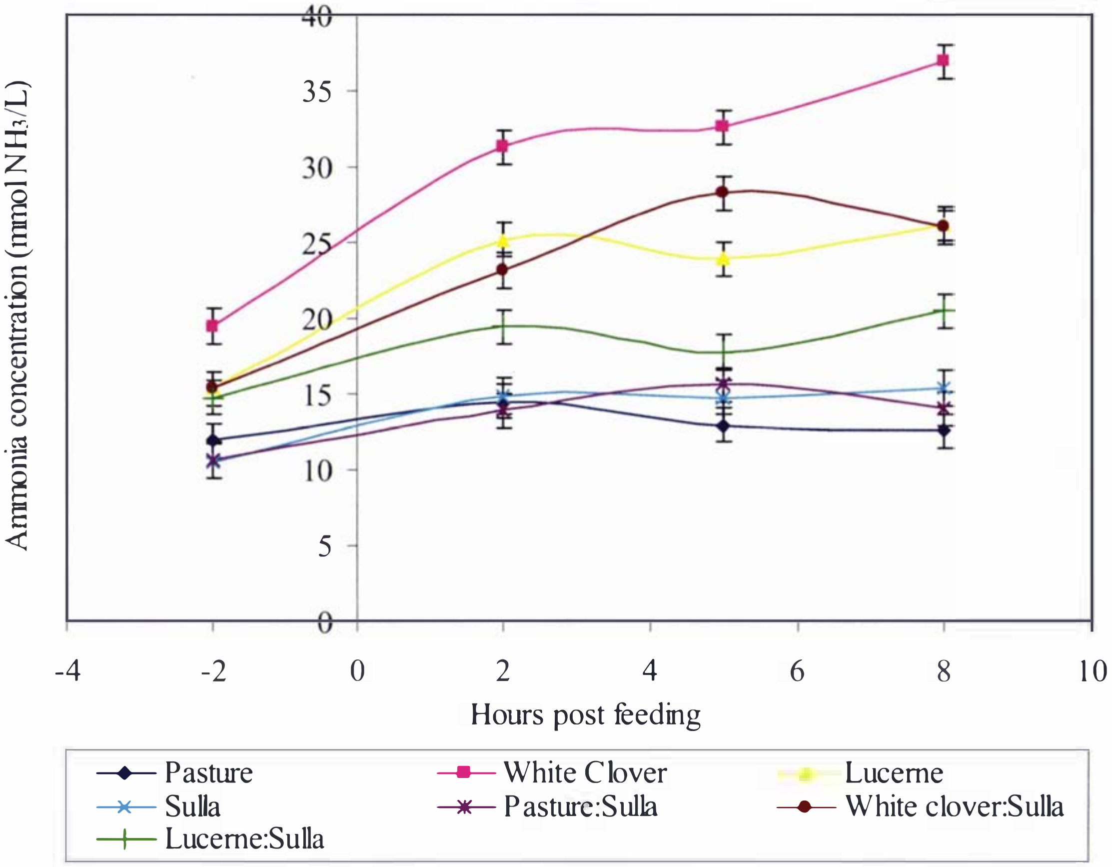
FIGURE 4.15. The relationship between mean rumen pH measured 2 – 4 hours after eating on six occasions during the experiment and mean soluble carbohydrate content (% dry matter; DM) of forages fed to lambs over eight weeks of the experiment.



4.4.3.2 Rumen ammonia

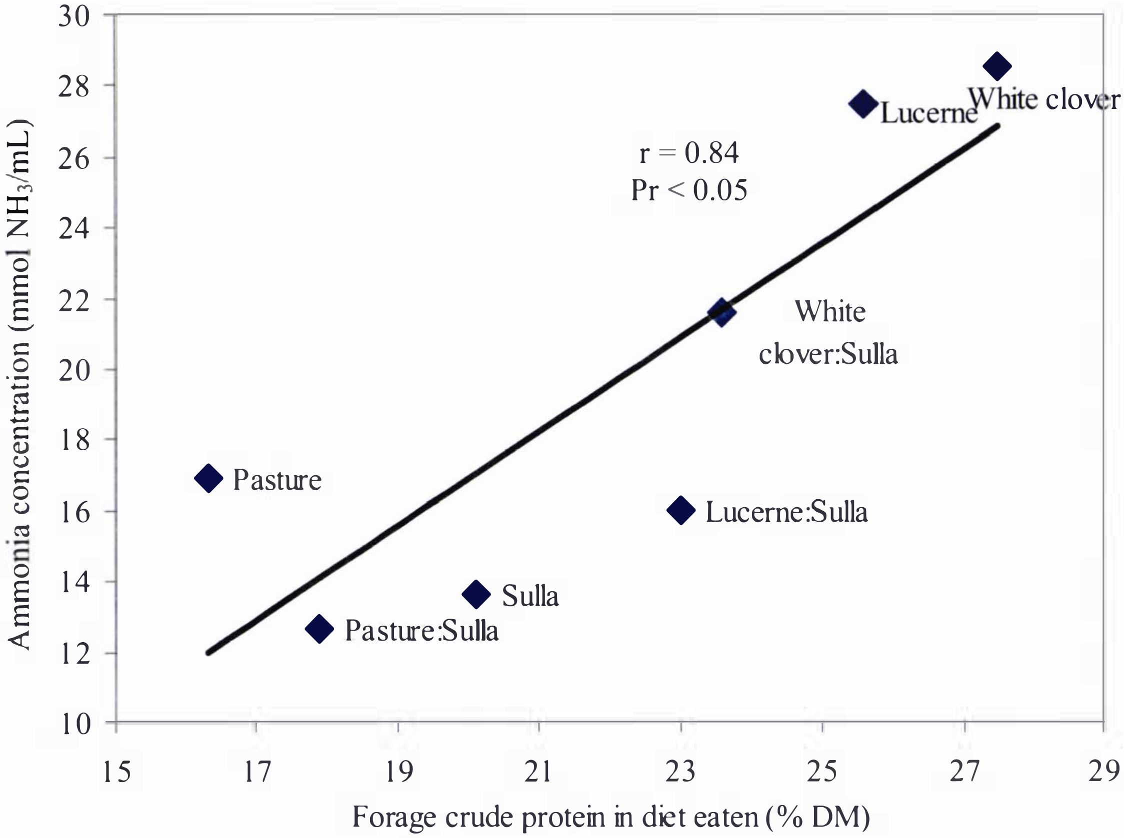
Changes in the rumen NH_3 concentration from pre-feeding until 8 hours after feed was offered on one day of the experiment (Figure 4.16) showed significant increases with the white clover diet from 19 to 37 mmol/L, minimal changes with sulla, pasture:sulla and pasture, but moderate increases with white clover:sulla and lucerne. Addition of sulla to lucerne and white clover reduced the extent of change relative to lucerne and white clover fed alone. The rumen NH_3 concentration of lambs fed white clover and white clover:sulla continued to increase up to 5 hours post feeding, whereas the rumen NH_3 concentration of lambs fed on other diets tended to peak 2 hours after feeding and remained at this concentration for all samplings (up to 8 h post feeding).

FIGURE 4.16. Changes in mean rumen ammonia (NH_3) from two hours pre-feeding to eight hours post-feeding for lambs fed the seven forage diets on one day of the experiment. Least-square means ± standard errors of the mean at each time of measurement are presented.



The mean rumen NH_3 concentration measured 2 – 4 hours after feeding on six occasions over the eight week trial (mmol/L; Table 4.9) was highest in lambs fed diets containing a high concentration of CP (white clover, 28.5 mmol/L and lucerne, 27.5 mmol/L) relative to lambs fed pasture (17.0 mmol/L) and sulla (13.6 mmol/L). On average adding sulla to pasture, lucerne and white clover significantly reduced mean NH_3 concentration compared to values from lambs fed diets without sulla (Table 4.9). Rumen NH_3 concentration in lambs fed pasture:sulla and lucerne:sulla were significantly lower than the concentration predicted from values for each diet fed alone. There was a strong correlation between rumen NH_3 concentration and concentration of CP in the DM eaten (Figure 4.17; $r = 0.84$; $Pr < 0.05$). When pasture data were removed the correlation increased to $r = 0.94$ ($Pr < 0.01$) suggesting a higher proportion of pasture CP appeared as NH_3 compared to the other six diets.

FIGURE 4.17. The relationship between mean rumen ammonia (NH_3) concentration measured 2 – 4 hours after eating on six occasions during the experiment and concentration of crude protein in the diet eaten (% dry matter; DM) for seven forage diets fed to lambs and averaged over eight weeks of the experiment.



4.4.3.3 Volatile fatty acids

Lambs fed lucerne, white clover, white clover:sulla and lucerne:sulla produced the higher concentrations of rumen VFA (90 – 101 mmol/L) relative to pasture, sulla and pasture:sulla (79 – 83 mmol/L; Table 4.10). Rumen contents of sulla-fed lambs had a higher proportion of propionate (23.7%) and butyrate (12.0%) and less acetate (62.1%) compared to lambs fed other diets, particularly when compared to pasture (propionate = 16.9%, butyrate = 9.1% and acetate = 69.6%). When sulla was fed with pasture and lucerne more propionate and butyrate were produced relative to pasture and lucerne diets. The ratio of acetate:propionate (A:P) and (acetate + butyrate):propionate ((A+B):P) indicate relative glucogenic values for VFA and were lowest (most glucogenic) when lambs were fed sulla compared to other diets. Relative to pasture and lucerne diets, the A:P ratio was reduced when sulla was fed with pasture (4.1 to 3.3) and lucerne (3.4 to 3.0). The proportion of minor VFA (iso-butyrate, iso-valerate and n-valerate) was significantly less in lambs fed sulla compared to all other diets, and was lower in white clover:sulla-, pasture:sulla- and lucerne:sulla-fed lambs compared to lambs fed white clover, pasture and lucerne, indicating a reduction in ruminal AA degradation.

There were significant correlations between the mean soluble carbohydrate, ADF and NDF concentrations (% DM) eaten by lambs and the mean proportion of acetate or propionate present in rumen contents. There were strong relationships between dietary concentration of soluble carbohydrate and proportions of rumen propionate ($r = 0.90$, $Pr < 0.01$) and acetate ($r = -0.82$, $Pr < 0.05$). Diets with high concentrations of NDF and ADF had positive relationships with the proportion of acetate (NDF, $r = 0.91$, $Pr < 0.01$; ADF, $r = 0.94$, $Pr < 0.05$) and negative relationships with the proportion of propionate (NDF, $r = -0.87$, $Pr < 0.05$; ADF, $r = -0.75$, $Pr < 0.05$) in the rumen contents.

4.4.4 Blood glucose and lactate

Blood glucose concentration was significantly higher for lambs fed white clover, sulla, white clover:sulla and lucerne:sulla compared to lambs fed pasture, lucerne and pasture:sulla (Table 4.9). The addition of sulla to white clover and pasture diets did not affect the blood glucose concentration relative to pasture and white clover diets, but the addition of sulla to lucerne increased the blood glucose concentration compared to lambs fed lucerne.

There was a significant correlation between the concentration of blood glucose and A:P ratio (Figure 4.18; $r = -0.57$; $Pr < 0.01$), A+B:P ratio ($r = -0.531$; $Pr < 0.01$) and the proportion of propionate in rumen liquor ($r = 0.48$; $Pr < 0.01$). Despite the significant correlation between dietary soluble carbohydrate concentration and proportion of propionate in rumen contents, the relationship between blood glucose concentration and soluble carbohydrate concentration (0.56; $Pr > 0.10$) and soluble carbohydrate intake (0.71; $Pr > 0.05$) was weak. There was no effect of diet on blood lactate concentration.

FIGURE 4.18. The relationship between mean blood glucose concentration and acetate:propionate ratio (A:P) of individual lambs fed seven forage diets.

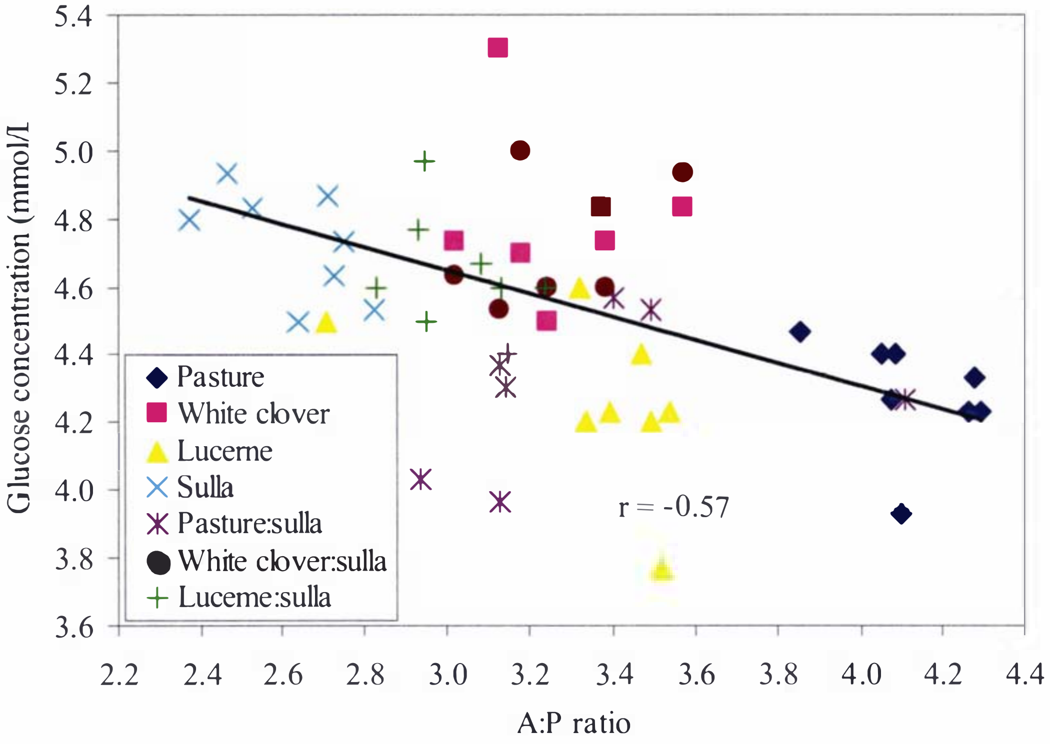


TABLE 4.9. Mean rumen pH and ammonia concentration measured on six occasions during the experiment, predicted rumen ammonia concentration, nitrogen (N) intake and blood glucose and blood lactate concentrations measured on three occassions during the experiment in lambs fed seven forage diets over eight weeks of the experiment. Least-square (LS) means and associated standard error of the means (SEM) are presented.

Diet	pH	Ammonia (mmol/L)	Predicted Ammonia[1] (mmol/L)	Nitrogen intake[2] (g N/lamb/day)	Blood Glucose (mmol/L)	Log$_{10}$ Blood Lactate[3] (mmol/L)
Number	48	48	-	-	24	24
Pasture	6.44^{d}	17.0^{b}	-	27.3	4.3^{a}	0.40 (1.7)
White Clover	6.35^{cd}	28.5^{d}	-	66.1	4.8^{b}	0.33 (1.6
Lucerne	6.37^{cd}	27.5^{d}	-	53.5	4.3^{a}	0.32 (1.5)
Sulla	6.09^{a}	13.6^{a}	-	45.2	4.7^{b}	0.54 (1.9)
Pasture:Sulla	6.23^{b}	12.6^{a}	15.4	33.5	4.4^{a}	0.51 (1.8)
White Clover:Sulla	6.33^{c}	21.6^{c}	20.6	54.8	4.7^{b}	0.38 (1.5)
Lucerne:Sulla	6.21^{b}	16.0^{b}	20.6	53.7	4.6^{b}	0.53 (1.9)
SEM	0.033	0.72	-	-	0.06	0.116
Diet effect (Pr)	< 0.01	< 0.01	-	-	< 0.01	NS

[1] Expected ammonia concentration calculated from proportion of forage in mixed diet and actual rumen ammonia concentration for individual diets; contrasts were conducted to measure statistical differences between expected and actual ammonia concentrations. The only statistical differences were between the pasture:sulla and lucerne:sulla diets.

[2] Nitrogen intake = mean nitrogen content of diet x mean group intake. No statistical analysis conducted.

[3] Data are Log$_{10}$ transformed (enabling treatment comparisons) with actual values in parenthesis.

[a, b] LS means within columns with a common superscript letter do not differ significantly ($Pr < 0.05$).

TABLE 4.10. Volatile fatty acid concentration in rumen digesta, proportion of acetate, propionate, butyrate and minor (iso-butyrate, valerate and iso-valerate) fatty acids, and ratio of acetate:propionate (A:P) and acetate + butyrate:propionate ((A+B):P) from lambs fed seven forage diets over eight weeks[1]. Least-square (LS) means and associated standard error of the means (SEM) are presented.

Diet	Total concentration (mmol/L)	Percentage of				Ratios	
		Acetate	Propionate	Butyrate	Minor	A:P	(A+B):P
Pasture	79.2^{a}	69.6^{c}	16.9^{a}	9.1^{b}	4.4^{d}	4.1^{d}	4.7^{d}
White clover	96.2^{bc}	64.1^{b}	19.6^{b}	10.5^{c}	5.8^{e}	3.3^{c}	3.8^{c}
Lucerne	101.3^{c}	67.8^{d}	19.7^{b}	8.0^{a}	4.5^{d}	3.4^{c}	3.8^{c}
Sulla	82.2^{a}	62.1^{a}	23.7^{d}	12.0^{d}	2.2^{a}	2.6^{a}	3.1^{a}
Pasture:Sulla	82.9^{a}	66.7^{d}	20.2^{bc}	10.3^{c}	2.8^{b}	3.3^{c}	3.8^{c}
White clover:Sulla	91.1^{b}	65.4^{c}	19.7^{b}	10.4^{c}	4.6^{d}	3.3^{c}	3.9^{c}
Lucerne:Sulla	90.3^{b}	64.2^{b}	21.2^{c}	11.0^{c}	3.6^{c}	3.0^{b}	3.6^{b}
SEM	2.38	0.39	0.38	0.30	0.22	0.08	0.09
Diet effect (Pr)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

[1] Six rumen samples were pooled within individuals over the duration of the trial so values are means for individual lambs (n = 8/treatment).

[a, b] LS means within columns with a common superscript letter do not differ significantly ($Pr < 0.05$).

4.4.5 Correlation and multiple regression analyses

Table 4.11 shows the coefficients of correlation (r) between mean LW gain and CW of dietary treatments with the chemical composition (% DM) and ME (MJME/kg DM) of diet eaten and intake of nutrients (kg/day) and total ME eaten (MJME/day). The only correlations that were significant for both LW gain and CW at $Pr < 0.05$ were ADF, NDF, ME and DM digestibility. As expected the relationship between ADF and NDF composition and intake with LW gain and CW were negative, while all other variables were positive. The correlation between soluble carbohydrate content and LW gain (0.72), and between crude protein content and CW (0.73) were significant at $Pr < 0.10$, However, the same nutrients with CW (0.56) and LW gain (0.59), respectively, were not significant. In contrast, soluble carbohydrate and crude protein eaten were significantly correlated with both LW gain and CW (0.76 – 0.89). The correlation of CT concentration and intake with LW gain and CW were low and not significant (0.40 – 0.62).

Multiple regression analysis was used to explain the relationships between lamb production and diet. These analyses excluded ME because factors contributing to ME (dietary constituents and digestibility), were used in the regression. Furthermore NDF content includes ADF, so only NDF was used in the regression, as it composed a higher proportion of the forage than ADF (which omitted hemicellulose). Condensed tannins were poorly correlated with LW gain and CW and were present in only four of the seven diets, so they were omitted from the regression.

The variables included in the model for both DM concentration (% DM) and intake were soluble carbohydrate (SC), crude protein (CP), NDF and DM digestibility (DM dig) of diet.

Multiple regression analyses were also conducted to determine the relationship between nutrient composition (soluble carbohydrate, crude protein, ADF and NDF content as a % DM) and ME content (MJME/kg DM) and between DM intake and nutrient composition (soluble carbohydrate, crude protein and NDF content as a % DM and DM digestibility).

TABLE 4.11. Correlation coefficients between mean liveweight gain (g/day) or carcass weight (kg) and composition of the diet eaten (% DM) and intake of nutrients (kg).

Variable	Liveweight gain (g/day)	Carcass weight (kg)
Nutrient in feed eaten[1]		
Soluble carbohydrate (% DM)	0.72†	0.56
Crude protein (% DM)	0.59	0.73†
ADF (% DM)	-0.97*	-0.95*
NDF (% DM)	-0.97*	-0.91*
CT (% DM)	0.59	0.40
ME (MJME/kg DM)	0.84*	0.78*
DM digestibility (%)	0.88*	0.84*
Total intake of nutrients[2]		
Soluble carbohydrate (kg/day)	0.89*	0.76*
Crude Protein (kg/day)	0.76*	0.86*
ADF (kg/day)	-0.13	-0.09
NDF (kg/day)	-0.89*	-0.78*
CT (kg/day)	0.62	0.44
ME (MJME/day)	0.99*	0.96*

[1] Nutrient composition (% DM), MJME/kg DM or digestibility % of diets eaten (Table 4.3) were calculated from nutrient composition of diet offered and refused.

[2] Intake of nutrient = nutrient concentration in the diet eaten (% DM or MJME/kg DM) x mean DM intake (kg/lamb/day)

* Correlations are significant Pr < 0.05
† Correlations are significant at Pr < 0.10

4.4.5.1 Liveweight gain

The regression model which best explained the relationship between diet composition and LW gain comprised only one variable. The % NDF explained 93% of the total variation and the regression model is given as Equation 4.1 (± standard error; SEM):

(4.1) LW gain = 437.4 (± 24.86) – 7.15 (± 0.85) NDF (% DM);
$R^2 = 0.93$; $Pr < 0.001$.

The relationship between LW gain and nutrient intake of lambs composed three variables. NDF intake (kg/day) explained 79% of the total variation and inclusion of CP intake (kg/day) and DM dig increased the variation explained to 94% and 98%, respectively. The multiple regression model that best explained LW gain was Equation 4.2 (± SEM):

(4.2) LW gain = -70.4 (± 162.3) – 0.35 (± 0.122) NDF (g/day) + 0.35 (± 0.070) CP (g/day) + 4.17 (± 1.540) DM dig (%);
$R^2 = 0.98$; $Pr < 0.01$.

However, Figure 4.5 illustrated the strong correlation of ME intake with LW gain. If ME intake was used as a variable in the multiple regression analysis the relationship between LW gain and nutrient intake is solely due to ME intake with 98% of the total variation being explained (Equation 4.3):

(4.3) LW gain = -150.5 (± 22.84) + 24.4 (± 1.42) ME intake (MJME/day);
$R^2 = 0.98$; $Pr < 0.0001$.

4.4.5.2 Carcass weight

The relationship between CW and nutrient composition of the diet was explained by the same variable as LW gain. The inclusion of NDF (% DM) explained 82% of the total variation and the relationship is indicated by Equation 4.4 (± SEM):

(4.4) $CW = 22.8\ (\pm 1.30) - 0.21\ (\pm 0.045)\ NDF\ (\%\ DM);\ R^2 = 0.82,\ Pr < 0.01.$

Three variables were selected to explain the variation between nutrient intake and CW. Crude protein intake (kg/day) explained 74% of the total variation and this was increased to 99.7% by including DM digestibility (%). Inclusion of soluble carbohydrate and NDF allowed 100% of the variation to be explained. The multiple regression model that explained the relationship between CW and consumption of nutrients is indicated in Equation 4.5 (± SEM):

(4.5) $CW = -4.13\ (\pm 2.384) + 15.13\ (\pm 2.355)\ CP\ (g) + 0.20\ (\pm 0.036)\ DM\ dig\ (\%);$
$R^2 = 0.72,\ Pr < 0.0001.$

If ME intake was used as a variable instead of DM digestibility the relationship between CW and nutrient intake is explained by ME and crude protein intake with 92 and 98% of the variation being explained (Equation 4.6).

(4.6) $CW = 5.1\ (\pm 0.86) + 0.55\ (\pm 0.077)\ ME\ (MJME) + 0.009\ (\pm 0.003)\ CP\ (g);$
$R^2 = 0.72,\ Pr < 0.0001.$

4.4.5.3 ME content vs. nutrient composition

Multiple regression analysis found ADF and crude protein concentration to be the two variables that explained variation between nutrient composition (% DM) and ME content (MJME/kg DM). ADF concentration (% DM) explained 86% of the total variation and this increased to 99% when crude protein was included in the multiple regression model (Equation 4.7; ± SEM).

(4.7) $ME\ (MJME/kg\ DM) = 20.9\ (\pm 0.62) - 0.32\ (\pm 0.018)\ ADF\ (\%\ DM) - 0.091\ (\pm 0.014)\ CP\ (\%\ DM);$
$R^2 = 0.99,\ Pr < 0.0001.$

4.4.5.4 DM intake vs. nutrient composition

The concentration of NDF in the DM was the only variable that was associated with DM intake and the relationship was negative. The inclusion of NDF explained 83% of the total variation between DM intake and nutrient composition and is illustrated below in Equation 4.8 (± SEM).

(4.8) DM intake (kg DM/lamb/day) = 1.83 (± 0.01) – 0.016 (± 0.003) NDF (% DM);
$R^2 = 0.83$, $Pr < 0.01$.

4.4.6 Predicting DM and ME intake and LW gain

The regression equations similar to those in section 4.4.5 can be used to predict DM intake and LW gain, within the ranges of this experiment (25 – 50 kg LW, 1.10 – 1.54 kg DM/day and 116 – 308 g LW gain/day) if the nutrient composition of the diet being offered is known. Normally, the chemical composition of the diet being offered is defined in terms of the soluble carbohydrate, crude protein, ADF and NDF as a % of the DM, as well as the DM digestibility and ME content of the diet. The concentration of NDF in the DM is the only variable used to predict DM and ME intake and is then used in the following equations to predict DM intake, ME intake and LW gain.

(4.9) Predicted DM intake (kg DM/lamb/day) = 1.899 (± 0.098) – 0.017 (± 0.003) NDF (% DM);
$R^2 = 0.86$, $Pr < 0.01$.

(4.10) Predicted ME intake (kg DM/lamb/day) = 25.09 (± 1.26) – 0.300 (± 0.040) NDF (% DM);
$R^2 = 0.93$, $Pr < 0.01$.

(4.11) Predicted LW gain (g/day) = -150.54 (± 22.84) + 24.43 (± 1.42) predicted ME intake (MJME/day);
$R^2 = 0.98$, $Pr < 0.01$.

Therefore a diet containing 45% NDF is likely to result in a daily DM intake and ME intake of 1.13 kg DM and 11.6 MJME and achieve a LW gain of 132 g/day. In contrast a decrease in the NDF concentration of the diet being offered to 35% will result in a higher daily DM and ME intake, and consequently greater LW gain of 1.30 kg DM, 14.6 MJME and 205 g, respectively. Figures 4.19 to 4.21 illustrate the excellent relationship between predicted and actual DM intake, ME intake and LW gain using prediction equations and data from this experiment.

FIGURE 4.19. Relationship between actual dry matter (DM) intake in this experiment and predicted DM intake using Equation 4.9.

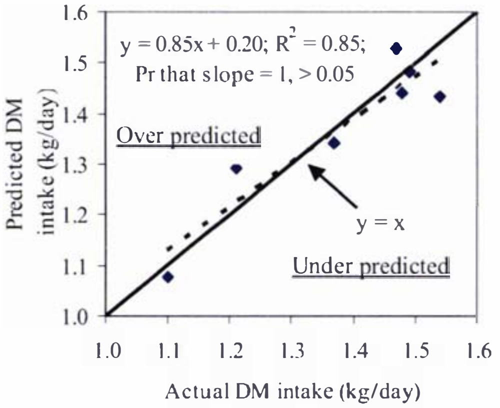


FIGURE 4.20. Relationship between actual metabolisable energy (ME) intake in this experiment and predicted ME intake using Equation 4.10.

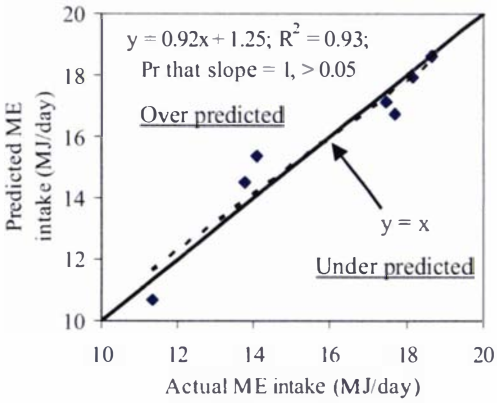
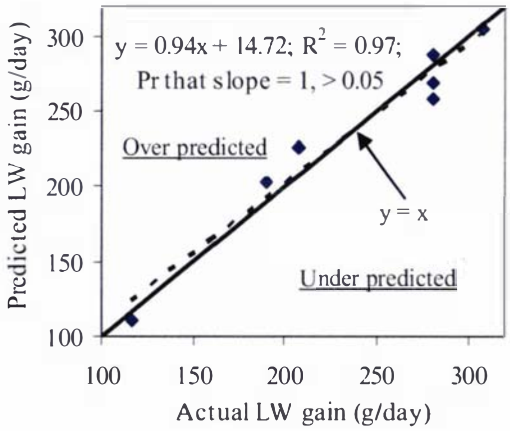


FIGURE 4.21. Relationship between actual liveweight (LW) gain in this experiment and predicted LW gain using Equation 4.11.



4.5 DISCUSSION

The seven diets fed to lambs resulted in large differences in daily gain. The pasture fed in this experiment resulted in significantly lower liveweight gains than of the diets containing legumes. When a high quality legume, such as sulla used in this experiment, was fed with pasture and lucerne LW gain was improved. There were improvements in carcass weight, dressing-out % and wool growth when lambs were fed legume-containing diets, which have lower fibre and higher soluble carbohydrate and CP contents. Intakes, dietary components and rumen parameters have been used to explain differences in lamb production.

Screening individual feeds for chemical composition, degradation and fermentation parameters using *in sacco* and *in vitro* techniques (Chapter 3) highlighted the positive nutritive attributes of legumes. Sulla, in particular, has concentrations of soluble carbohydrate, CP and NDF of 20 to 25% of the DM, whereas most forages contain higher proportions of fibre and lower concentrations of soluble carbohydrate. Sulla also contains CT which can reduce proteolysis and supply more AA to the animal. However, previous animal studies have shown mixed results when sulla has been fed to lambs. Stienezen *et al*. (1996) and Terrill *et al*. (1992a) reported high intakes and good production when sheep were fed sulla for short periods. A longer 17-week study (Douglas *et al*., 1999) reported lower growth rates when lambs were fed low and high allowances of sulla containing 8.8% CT in the DM (174 and 279 g/day) relative to sulla where CT had been deactivated by PEG (179 and 293 g/day). Forage legumes with high concentrations, or very astringent tannins can be more beneficial for ruminants when fed with other material to dilute the CT (Waghorn *et al*., 1998). Excellent production has been reported when *Lotus corniculatus* has been fed with pasture to sheep (Wang *et al*., 1996c) and cattle (Harris *et al*., 1998).

4.5.1 Animal production

High voluntary feed and ME intakes can be achieved on feeds with a high ratio of readily fermentable to structural carbohydrates, provided there is sufficient protein to meet the requirements for MP. Sulla and white clover were superior to lucerne which, in turn, was superior to pasture as a sole diet, and the addition of 50% sulla significantly improved the LW gain of lambs fed pasture and lucerne. No significant differences were observed between lambs fed sulla or white clover as sole diets or 50:50 mixtures of white clover:sulla or lucerne:sulla.

When daily gain was evaluated in terms of feed composition, 93% of variation was explained by NDF content of the DM, whereas intake of NDF accounted for 79% of variation between treatments. Inclusion of CP intake and DM digestibility with NDF did explain more of the variation between treatments (19%), but overall production was dominated by ME intake (LW gain vs. ME intake, $r = 0.99$). Protein supply with diets containing sulla or white clover appeared to exceed requirements for production. Combining these regression equations have shown that daily LW gain will increase by 24.4 g if lambs consume an extra MJME per day. The increase in daily ME intake is predominantly a result of a 3.3% unit decrease in the NDF concentration in the DM. This diet that had a lower NDF concentration in the dietary DM was 2% more digestible and contained an extra 1.3% units of CP, 1% units of soluble carbohydrates and 0.35 MJ ME units and 1% less ADF units.

It is well known that the production of animals fed white clover may be twice that of animals fed pasture (Ulyatt, 1981). Results from this study support and have added to the comparative feeding value data compiled by Ulyatt (1981) where lamb LW gain were ranked (Chapter 2; Burke *et al*., 2002b; Burke, 2003). Sulla has a similar feeding value to white clover and the feeding value of pasture and lucerne is improved when combined with sulla. Expected LW gains of lambs fed pasture:sulla (52:48) and white clover:sulla (47:53) were 208 and 295 g/day compared with observed LW gains of 190 and 281 g/day, respectively. The expected LW gain of lambs fed lucerne:sulla (50:50) was less (258 g/day) than the observed LW gain of lambs fed lucerne:sulla (281 g/day), mainly because feed intake for the lucerne:sulla diet was significantly greater (1.54 kg/day) than the expected feed intake (1.42 kg/day). These results highlight the benefits of feeding rapidly digestible and high quality forages to ruminants.

Previous *in sacco* and *in vitro* incubations of ryegrass, white clover, sulla and lucerne (Chapter 3) showed rapid degradation of white clover relative to the other three forages. Rapid degradation (often associated with low fibre concentration) provides a superior nutrient supply and also enables a rapid clearance of feed from the rumen which enables high intakes, observed with white clover and sulla in this trial. Indoor and field trials with lambs fed sulla (Terrill *et al.*, 1992a; Stienezen *et al.*, 1996; Douglas *et al.*, 1999; Bermingham *et al.*, 2001) have all shown sulla to be highly palatable for lambs, which selected both leaf and young stem fractions. The relatively low fibre and high soluble carbohydrate content of sulla are likely to have resulted in the increased feed intakes of pasture:sulla and lucerne:sulla relative to pasture and lucerne fed alone.

The anticipated benefits of CT in sulla to lower proteolysis were not evident in LW gain or wool growth. Despite the reduction in rumen proteolysis, these lambs were limited by ME rather than MP supply, and so extra amino acids absorbed from the small intestine would be surplus to requirements. Intakes of DM may have been greater if diets did not contain as much water as some of the diets fed in this study. Verite and Journet (1970) and John and Ulyatt (1987) showed a negative relationship between voluntary intakes and moisture content of forage with DM contents below 18%; sulla averaged 13.8% DM and white clover 14.6%. Lambs fed sulla or diets with white clover consumed 10.1 to 10.7 kg wet material per day.

Interpretation of lamb production data has been weakened by the inability of the alkane markers to estimate intake of individual lambs. The prediction (detailed in Appendix 4.2) suggests diet type affected the accuracy of the intake prediction, and data from lambs fed pasture were acceptable. Indoor trials and careful observations of lambs on the feed-pad did not suggest tablets were lost through regurgitation, but a rapid dissolution in the reticulum could result in a digesta bolus containing a high concentration of C32 passing out of the rumen to the abomasum and intestines. This would result in small portions of fæces having a high concentration of alkane, but the majority having a lower concentration because the marker was not distributed evenly through the digesta. This scenario could explain over-estimation of intakes from lambs sampled twice daily (on the feed-pads) but cannot account for the variable recovery from lambs held indoors. Indoor feeding enabled total faecal collection and accurate sub-sampling, so any effects of a 'bolus' of C32 passing to the intestine without proper mixing in the rumen should have been avoided. We are unable to account for the variability between individuals in alkane recovery or prediction of DM intake.

4.5.1.1 Carcass characteristics

Purchas and Keogh (1984) and Terrill *et al*. (1992a) showed that lambs fed forages containing CT were leaner than lambs fed non-CT forage or PEG supplemented diets, but this was not supported by Douglas *et al*. (1999) after adjustment for LW in this study. At slaughter carcass GR ranged from 7 to 10 mm for all treatments, except pasture (3.9 mm; Table 4.6).

GR fat-depth measured by ultrasound scanning on day 54 of the trial was 30% of GR fat-depth measured on day 61 at slaughter and suggests the ultrasound scanning on live lambs may not be a suitable method for detecting differences in carcass composition. Several researchers have questioned the benefit of ultrasound scanning for predicting composition (Leymaster *et al*., 1985; Fortin and Shrestha, 1986; Edwards *et al*, 1989) while others have found the technology useful (Bass *et al*., 1982; Faulkner *et al*., 1990; Porter *et al*., 1990). Fernandez *et al*. (1997) reported better correlations between ultrasound EMA and carcass EMA ($r = 0.88$) than between carcass and ultrasound back fat-depth ($r = 0.74$). The correlation between ultrasound and carcass GR fat depth ($r = 0.81$) was better than the correlation between ultrasound and carcass back fat-depth ($r = 0.71$; McEwan *et al*., 1989) so ultrasound predictions appear more suitable for EMA than fat-depth.

The use of ultrasound technology has been less satisfactory for lean and young animals than fat and older animals (McEwan *et al*., 1989; Hopkins *et al*., 1993). The presence of wool can make site identification difficult and compression of fat layers when the operator places the probe on the animal can cause ultrasound values to be less than actual values (Purchas and Beach, 1981; Hamby *et al*., 1986; McEwan *et al*., 1989). Despite these doubts about absolute values for fat-depth, relative changes in EMA, GR and back-fat depth over time can provide a useful comparison between treatments.

Lambs fed pasture and pasture:sulla had lower growth rates and the changes in EMA and fat-depth determined by ultrasound (Table 4.6) was not convincing. Hopkins *et al*. (1993) also reported very little change in carcass characteristics of lambs that had poor growth rates over 77 days and differences in carcass composition were difficult to detect using ultrasound technology when growth rates were small.

Dressing-out percentage in this study were mid-way between those of lambs fed high and low allowances of sulla and PEG-supplemented sulla reported by Douglas *et al.* (1999). Fatter animals have a higher proportion of carcass and less non-carcass components than lean animals and dressing-out percentage will be highly correlated with carcass GR (Kirton and Morris, 1989).

When poor quality diets (diets that have a high concentration of NDF, a low concentration of CP and a low digestibility) are fed MP is limiting with insufficient AA available for protein accretion so absorbed energy surplus to maintenance need is stored as fat. A similar situation is evident for high energy diets where energy surplus to protein synthesis and maintenance requirements is stored as fat and this fat deposition tends to be exacerbated in diets that have a high (A+B):P ratio (Gill *et al.*, 1984; Black *et al.*, 1987). However, in this study there were no obvious differences in the fat content of live lambs or carcasses at a constant liveweight because the high quality legume diets were not limited by energy, but had surplus protein.

4.5.2 Rumen fermentation

The relative high concentration of soluble carbohydrate to structural carbohydrate in legumes, particularly sulla, provides a ready source of ME for increasing microbial growth and flow from the rumen (Dellow *et al.*, 1988). The legume-containing diets had decreased A:P and (A+B):P ratios than pasture-fed lambs, suggesting a more efficient energy capture which increase the potential for gluconeogenesis and lactose synthesis in lactating animals. The impact of a low A:P ratio has been effectively demonstrated in lactating sheep where a lower A:P ratio increased milk protein and decreased fat concentration (Wang *et al.*, 1996b) and in lactating cattle (Woodward *et al.*, 1999). The effect of the VFA ratio on carcass in this trial was minor and masked by differences in intake and LW gain.

CT from one plant species is able to bind with and precipitate protein from another species both *in vitro* and *in vivo* (Waghorn and Jones, 1989; Min, *et al.*, 2000). Measurements of NH_3 concentration from initial *in vitro* studies (Chapter 3 and

Appendix 4.1) demonstrated this when sulla was mixed with pasture and white clover, but the effect was not apparent when lucerne was mixed with sulla.

Sub-samples of the feeds used in this experiment incubated *in vitro* suggested a reduction in proteolysis when sulla was combined with pasture and lucerne, but effects were minor with white clover (Appendix 4.5). Calculations of MP available for absorption by the lambs fed pasture:sulla and lucerne:sulla compared to lambs fed pasture and lucerne showed that a greater proportion of the metabolisable protein came from microbial growth (75%, 70%, 66% and 58%, respectively) than undegraded protein.

The absence of an effect of sulla on rumen NH_3 concentration when lambs were fed white clover:sulla was unexpected based on *in vitro* studies prior to conducting the experiment (Appendix 4.1), but the effects of sulla on rumen parameters *in vivo* were in line with *in vitro* studies using sub-samples of the diets fed in this experiment (Appendix 4.5).

Minor VFA are products of protein (AA) breakdown in the rumen (El-Shazly, 1952; Van Soest, 1994). Reductions in proteolysis and minor VFA concentrations have been attributed to CT when *Lotus pedunculatus* (8% CT in DM, assuming a butanol-HCl extraction; Waghorn *et al*., 1994b) and *Lotus corniculatus* (containing 3% CT; Waghorn *et al*., 1987a, b) were fed to sheep, but the only meaningful response on minor VFA in this trial occurred when sulla was fed with pasture (Table 4.10). The absence of synergistic effects when sulla was fed with white clover and lucerne in this experiment suggests dietary CT concentration of 2.6% DM in this study was insufficient to affect a significant reduction in proteolysis with diets containing 22 to 24% CP.

In any case, decreased proteolysis in the rumen will only be of benefit if there is a deficit of amino acids for protein synthesis by the animal. In this experiment, sufficient MP was already reaching the small intestine as microbial and undegraded dietary protein, so that any extra is likely to be deaminated and converted into urea in the liver.

4.6 CONCLUSIONS

Lamb production was improved by feeding forage legumes but ME intake was first limiting for production in all instances. Sulla has good potential to supplement medium quality pasture, but greatest benefits for LW gain were achieved when fed with lucerne. Compared to medium quality pasture, improved production of lambs fed diets containing legumes was due mainly to higher ME intake achieved with forages having low NDF, high CP and high soluble sugar concentrations. This was especially true when sulla was combined with pasture and lucerne. The presence of CT in sulla did affect rumen proteolysis in some instances, but growing ram lambs did not respond to the increase in MP supply. Rumen parameters, *in sacco* and *in vitro* incubations and simple modelling of nutrient supply can explain intake differences and interactions between nutrient supply and animal requirements when mixed forages are fed. Higher intakes would accentuate differences between diets. Lactating animals may be a better measure of feeding value for high quality forages because dietary effects on nutrient use can be observed through milk composition differences.

CHAPTER 5

PROTEIN SYNTHESIS AND FRACTIONAL SYNTHESIS RATES OF LAMBS FED FORAGE-BASED DIETS

CHAPTER 5: PROTEIN SYNTHESIS AND FRACTIONAL SYNTHESIS RATES OF LAMBS FED FORAGE-BASED DIETS

5.1 ABSTRACT

Four lambs from a larger experiment (Chapter 4) fed either pasture, lucerne, sulla or lucerne:sulla were used to determine the effects of diet on absolute whole body protein synthesis (WBPS; g/day) and protein fractional synthesis rates (FSR; %/day) in viscera, muscle and other tissues. The diets varied in quality and feeding value and the dietary composition of sulla was expected to increase protein flow to the intestine for absorption when fed as a sole diet and in a forage mixture. The four lambs fed each diet were housed indoors in metabolism crates for a period of 8 to 10 days and fed *ad libitum.*

Whole body protein synthesis was calculated from cysteine and sulphate kinetics following a continuous infusion of ^{35}S-cysteine and ^{35}S-sulphate to calculate cysteine oxidation and cysteine irreversible loss rate (ILR). Concentration and specific radioactivity of cysteine and sulphate were determined after 6, 7 and 8 hours of infusion to calculate fluxes through a two-pool model to estimate absolute WBPS (g/day). ^{3}H-valine was infused in conjunction with ^{35}S-cysteine to determine FSR rates in the duodenum and ileum (intact and scraped to remove the mucosal layer), rumen, abomasum, liver, pancreas, muscle and skin tissues at slaughter. Blood samples were collected after 6, 7 and 8 hours to measure valine concentration and ^{3}H-valine radioactivity in plasma. Tissues were sampled at slaughter and both plasma and tissue intracellular pools were used to calculate tissue FSR.

Principal diet effects on protein synthesis applied to lambs fed pasture. Pasture resulted in a lower cysteine ILR (21.6 μmol/min), oxidation to sulphate (2.5 μmol/min) and absolute WBPS (92.6 g/day) compared to other diets. The WBPS (g/day) for lambs fed the other diets were; lucerne (150.7), sulla (152.3), and lucerne:sulla (180.0), which corresponded to higher liveweight (LW) gains (g/day) for lambs fed the legume diets

(116 for pasture; 207, 308 and 281, respectively) in the larger experiment. Cysteine ILR for sulla and lucerne based diets ranged from 34.7 – 41.3 μmol/min and oxidation was 3.4 – 5.1 μmol/min.

Mean tissue FSR calculated from the plasma precursor pool (FSR_P; %/day) across diets, ranked tissues (from greatest to least): abomasum (104.3), duodenum (30.8), pancreas (25.3), ileum (22.7), rumen (13.7), liver (10.9), scraped (mucosal layer removed) duodenum (10.0), scraped ileum (7.1), skin (6.7) and muscle (2.3). Diet affected the FSR_P of duodenum, scraped duodenum, scraped ileum, rumen, pancreas and muscle, but there were no effects on ileum, abomasum, liver or skin FSR_P. Muscle FSR_P paralleled LW gain, with the slow growing pasture-fed lambs having a FSR_P of 1.6 %/day whilst the fastest growing lambs fed sulla and lucerne:sulla had muscle FSR_P of 2.7 and 2.8 %/day. Lambs fed diets containing CT had a lower rumen FSR (11.9 %/day) compared to diets without condensed tannins (15.5 %/day). This work showed dietary treatment affected both absolute WBPS and FSR of tissues and results are consistent with effects of the diets on LW gain.

5.2 INTRODUCTION

Proteins are dynamic components of body tissues (Waterlow *et al.*, 1978) undergoing synthesis and degradation at different rates for specific tissue types. Protein synthesis is energetically expensive and is affected by the supply (absorption) of amino acids and energy. Protein is the principal component of muscle and wool and net accretion, which is the difference between synthesis and degradation, is influenced by age, nutritional, environmental, hormonal and genetic factors. Net synthesis (protein deposition) has been measured in terms of the whole animal and in individual tissues (Lobley *et al.*, 1980), and measurements are calculated from irreversible loss rates (ILR) of radioactive amino acids. Protein synthesis rates are calculated from the specific activity and concentration of amino acids in blood and tissues. Fractional synthesis rates (FSR) of muscle protein declines from about 23 %/day in newborn lambs to 6 %/day at weaning (Attaix *et al.*, 1988), and continues to decline to 3 %/day or less as the lamb reaches 30 – 40 kg (Lobley, 1993). Fewer data are available for cattle, but between 1 and 8 years the muscle FSR of cattle declines from approximately 2 – 2.5 %/day to 1 %/day (Lobley *et al.*, 1980; Eisemann *et al.*, 1989). Whole body protein synthesis (WBPS) comprises the sum of values from individual tissues and their contribution to whole body protein. Splanchnic tissues (the gastro-intestinal tract and liver) exhibit the highest rates of protein synthesis (25 to 35 %/day; Lobley *et al.*, 1994) compared to skin (10 %/day; Lobley *et al.*, 1992) and muscle (3 %/day; Lobley, 1993; Lobley *et al.*, 1994). Splanchnic tissues account for about 10% of whole body protein compared to skin (15%) and muscle (45%; Lobley *et al.*, 1980; Davis *et al.*, 1981).

Liveweight (LW) gain is one measure of animal production that can be linked to protein deposition (Lobley *et al.*, 1987), but fat, water, bone and protein all contribute to whole body gain and composition. In growing animals, muscle dominates protein gain as it represents the largest protein store, but the proportion of fat in gain is affected by dietary feeding value, and increases with poor quality feeds (Hegarty *et al.*, 1999), animal maturity and condition (Lobley, 1993). In growing sheep and cattle the daily FSR of protein in muscle, gastro-intestinal tract (GIT), skin and liver contribute 18 – 20%, 26 – 35%, 14 – 20% and 4 – 8%, respectively, to whole body protein synthesis (Lobley, 1993).

Feed intake affects amino acid (AA) supply, absorption and entry into portal blood (Tagari and Bergman, 1978) as well as protein synthesis in tissues (Lobley *et al.*, 1992, 1994). Feed quality will affect nutrient supply (Oddy and Sainz, 2002), but few analyses have measured effects on WBPS or tissue FSR. The diets fed to lambs in this study differed in energy and protein content, and CT in the sulla may increase AA absorption from the intestine (Waghorn *et al.*, 1994b; 1997; Stienezen *et al.*, 1996; Bermingham *et al.*, 2001). McNabb *et al.* (1993) showed that the CT in *Lotus pedunculatus* increased sulphur-AA entry into the bloodstream of sheep.

Measurements made here complement data from these and other lambs fed seven contrasting diets in terms of feed intake, LW gain, wool growth, and carcass composition (Chapter 4). The pasture diet was typical of conventional grazing for lambs, but is not ideal to meet nutrient requirements for optimum production (Chapter 2), whereas lucerne and sulla are legumes that contained less fibre and more protein, both of which were more rapidly degraded (Chapter 3) compared to pasture.

Consequently sulla and lucerne have a higher feeding value than pasture (Chapter 4). Sulla has a higher feeding value than lucerne (Chapter 4) because it has a relatively high concentration of soluble carbohydrates (18 – 25% DM), low concentration of NDF (c. 18 – 22% DM) and contains condensed tannins (CT; typically 5 – 9% DM). Condensed tannins have the potential to reduce protein degradation in the rumen (McNabb *et al.*, 1996; Waghorn *et al.*, 1997) and could increase AA supply to the small intestine (Bermingham *et al.*, 2001) for absorption. Although growing ram lambs did not respond to additional amino acid availability attributable to CT, marked responses have been measured in lactating sheep (Wang *et al.*, 1996b) and cattle (Woodward *et al.*, 1999). However, results in Chapter 4 suggested that the CT was not effective in increasing the supply of undegraded protein from the rumen to the small intestine.

The choice of forage diets for measurement of WBPS and fractional protein synthesis were based on contrasting animal production in Chapter 4. Lambs gained 116 g/day when fed pasture, 207 g/day with lucerne and 308 g/day with sulla. Lambs fed a 50:50 mixture (on a DM basis) of sulla and lucerne had daily LW gains of 281 g/day.

The purpose of this experiment was to investigate the effects of feeding different forages as sole diets or in mixtures on absolute WBPS (g/day) and fractional protein synthesis (%/day) of individual tissues selected to represent a range of metabolic rates. Protein synthesis was expected to correspond with differences in LW gain of lambs given the four different diets and fractional protein synthesis in specific tissues may indicate the organs most affected by high quality diets and increased protein supply.

5.3 MATERIALS AND METHODS

5.3.1 Experimental design

The experiment described here was part of a larger trial (56 lambs; Chapter 4) which measured the effects of diet on feed intake, LW gain, wool production, carcass weight and composition, ruminal concentration of NH_3 and VFA as well as blood glucose and lactate values in lambs. Lambs described in Chapter 4 were fed either pasture, white clover, lucerne, sulla, or mixtures (50:50, DM basis) of pasture:sulla, white clover:sulla and lucerne:sulla. A subset of 16 lambs comprising four from dietary treatment groups fed pasture, lucerne, sulla and lucerne:sulla were transferred from the feeding pads to metabolism crates for this trial to measure protein synthesis. Lambs were selected based on the LW gain achieved in Chapter 4. They were slaughtered at a similar time to lambs in the larger trial and carcass characteristics were measured at slaughter.

The indoor experiment began on day 47 of the larger experiment and was completed in 18 days. Measurements were carried out on two lots of eight lambs (two from each dietary treatment) in a sequential design. Within each lot of eight lambs, measurements were staggered so only four lambs (one from each treatment) received a continuous 8 hour infusion at one time (Table 5.1). Each group of four lambs were held indoors for a period of 8 to 10 days. The intravenous infusion of ^{35}S-sulphate (SO_4) to determine sulphate ILR was given about day 5 of the indoor period and 5 days later on the day of slaughter the ^{35}S-cysteine infusion, to determine absolute WBPS, and ^{3}H-valine infusion, to determine FSR of several tissues was given. The sequence of events during the experiment is summarised in Table 5.1.

All procedures were reviewed and approved by the Crown Research Institute Animal Ethics Committee in Palmerston North, New Zealand according to the Animals Protection Act (1960) and Animal Protection Regulations (1987) and amendments.

TABLE 5.1. Schedule of events for lambs used for glucose tolerance tests and protein measurements.

Day	Event
1	Eight lambs (two per dietary treatment) were brought indoors and housed in metabolism crates and maintained on fresh forage fed *ad libitum*. One lamb from each dietary treatment was allocated to Group[1] 1 and the other to Group[1] 2.
2-3	Catheters were placed in both jugular veins of Group 1 and 2 lambs.
4	Intravenous infusion of ^{35}S-sulphate (SO_4) to Group 1.
6	Intravenous infusion of $^{35}S\text{-}SO_4$ to Group 2.
10	Intravenous infusion of both ^{35}S-cysteine and ^{3}H-valine to Group 1. Group 1 slaughtered.
11	Intravenous infusion of both ^{35}S-cysteine and ^{3}H-valine to Group 2. Group 2 slaughtered.
10-11	Another eight lambs (Groups 3 and 4) were brought indoors and housed in metabolism crates and fed fresh forage *ad libitum*. One lamb from each dietary treatment was allocated to Group[1] 3 and the other to Group[1] 4.
11 - 12	Catheters were placed in both jugular veins of Group 3 and 4 lambs.
13	Intravenous infusion of $^{35}S\text{-}SO_4$ to Group 3.
14	Intravenous infusion of $^{35}S\text{-}SO_4$ to Group 4.
17	Intravenous infusion of both ^{35}S-cysteine and ^{3}H-valine to Group 3. Group 3 slaughtered.
18	Intravenous infusion of both ^{35}S-cysteine and ^{3}H-valine to Group 4. Group 4 slaughtered.

[1] Group is the sequence in which lambs were subjected to measurements as not all 16 lambs could be manipulated at one any time. Lambs were divided into four groups of four lambs (one from each dietary treatment).

5.3.2 Animals and feeds

The 16 lambs which were selected on the basis of temperament to represent LW gain typical of their dietary treatment group, were brought indoors, weighed and housed in metabolism crates for the experiment. One lot of eight lambs was brought indoors on day 47 of the feeding trial and the others on days 55 and 56. The four lambs in Group 1 were an isotope infusions ($^{35}S\text{-}SO_4$; ^{35}S-cysteine and ^{3}H-valine) on days 4 and 10, with slaughter on day 10; those in Group 2 were given an isotope infusion on days 6 and 11 with slaughter on day 11 (Table 5.1). The second lot of eight lambs (Groups 3 and 4) was brought indoors on days 10 and 11. Isotope infusions were given to Group 3 lambs on days 13 and 17, and Group 4 lambs on days 14 and 18, after which both groups of four lambs were slaughtered on days 17 and 18, respectively (Table 5.1).

Lambs were fed fresh pasture, comprised of 86% ryegrass and 14% white clover (DM basis), lucerne, sulla or a 50:50 mixture (DM basis) of lucerne and sulla. Feeds were harvested daily by 1000 hours and chopped to about 50 mm in length as described in Chapter 4. All feed was held at 4°C and about 60% of the daily feed was placed on an overhead feeder at 1600 hours, and the remainder at 0900 hours the following day. Feed and water were provided *ad libitum* with feeding at hourly intervals. Intakes of individual lambs were measured daily. Lambs were weighed at the start (prior to feeding) of the indoor period and again at slaughter.

DM content of feed offered and refused was determined daily by drying representative forage samples at 95°C for 24 hours. Samples of feed offered and refused were taken daily, frozen at –20°C and refusals bulked over the experiment for each lamb. Samples were freeze-dried prior to analysis by Near Infra Red Spectroscopy (NIRS) to determine composition.

5.3.3 Surgical procedures

All lambs were fitted with catheters (single lumen polyvinyl chloride tube; 1.0 mm i.d., 1.5 mm o.d; Chritchley Electrical Products Pty Limited, Auburn, New South Wales, Australia) in each jugular vein, 2 – 4 days before infusions and sampling. One catheter was used to administer the glucose bolus and to infuse radioactive sulphate, cysteine and valine and the other was used for blood sampling. Sterile saline containing 150 i.u heparin/mL and 1% procaine penicillin (Bomacillin, Bomac Laboratories Ltd, Auckland, New Zealand) was used to maintain patency of catheters between infusions and sampling periods.

5.3.4 Whole Body Absolute Protein Synthesis Rates

Absolute WBPS for all lambs was calculated by measuring the ILR of cysteine and oxidation of cysteine to sulphate (SO_4) using radioactive isotopes (^{35}S-cysteine and ^{35}S-SO_4).

5.3.4.1 Isotopes and infusion rates

Cysteine ILR was calculated from specific radioactivity (SRA) and infusion of ^{35}S-cysteine (supplied by NEN^{TM} Life Science Products Inc, Boston), and oxidation of cysteine from measurement of sulphate ILR using ^{35}S-SO_4 (supplied by NEN^{TM} Life Science Products Inc, Boston). These calculations, with concentration of cysteine in whole body protein (MacRae *et al.*, 1993), enabled absolute WBPS (g/day) to be calculated.

The infusion of ^{35}S-SO_4 (55.5 MBq/lamb) was given 4 – 5 days prior to ^{35}S-cysteine infusion (46.3 MBq/lamb) to enable clearance of ^{35}S from the body prior to cysteine infusion. Isotopes were given to four lambs simultaneously, using a peristaltic pump (Watson-Marlow Bredel, Inc.) at about 34.0 g/h. The isotopes were prepared as follows: 46.3 MBq of ^{35}S-cysteine (39.8 TBq/mmol) was added to 380 mL sterile saline containing 0.5 mL of 0.2 mmol/L inert L-cysteine (BDH)/L as a carrier, and 55.5 MBq of ^{35}S-$NaSO_4$ (20.64 kBq/mmol) was added to 380 mL sterile saline containing 2 mL of 2 mmol/L inert $NaSO_4$ as a carrier. At the commencement of the cysteine infusion a 35 mL bolus containing 4.26 MBq was given, and a 35 mL bolus containing 5.11 MBq was given at the commencement of the inorganic sulphate infusion. Cysteine and inorganic sulphate were infused at 32.3 kBq/min and 49.8 kBq/min, respectively.

5.3.4.2 Sampling

A sample of infusate given to each lamb was retained to determine radioactivity and blood samples (background) were taken prior to each infusion and at 6, 7 and 8 hours of the infusion. The protocol for sampling blood was similar for all samples; about 7.5 mL was collected using a LH S-Monovette® (Sarstedt, Aktiengesellschaft and Co, Germany), gently mixed (by inversion) and held on ice prior to centrifuging (3,000 x *g* for 15 minutes at 4°C) to obtain plasma. Plasma samples were either stored or processed further prior to analysis being undertaken after completion of the experiment. Table 5.2 illustrates the sampling and processing procedures for plasma to determine radioactivity and metabolite concentrations.

5.3.4.3 Processing and analysis

Plasma collected on the day of the ^{35}S-cysteine infusion was deproteinised by treating with sodium dodecyl sulphate (SDS; 0.75% w/v SDS and 9 mmol/L EDTA; 0.5 mL) to denature proteins, trichloroacetic acid (TCA; 30% w/v; 0.5 mL) to precipitate proteins, an antioxidant (100 µl of 80 mmol/L dithiothreitol; DTT) and an internal standard (3 mmol/L norleucine in 0.1% phenol; 50 µL; Appendix 5.1). Deproteinized plasma was centrifuged (3,000 x *g* for 15 minutes at 4°C), filtered (0.45 µm) and the resulting supernatant stored at –85°C for analysis.

Plasma collected on the day of the ^{35}S-SO$_4$ infusion was frozen at –20°C prior to measurement of radioactivity, and processed (deproteinized) further to determine sulphate concentration.

Cysteine concentration

Plasma cysteine concentration was determined by an automated method using acid ninhydrin modified from Gaitonde (1967). Deproteinized plasma was reduced with DTT and reacted with acid ninhydrin and cysteine concentration determined by measuring absorbency at 570 nm using a continuous flow analyser (Technicon Autoanalyser II).

Sulphate concentration

Plasma sulphate concentration following ^{35}S-SO$_4$ infusion, was determined after centrifugation through a 10,000 molecular weight filter (0.5 mL in a vivaspin; Viviascience Ltd, Auckland, New Zealand) at 12,000 x *g* for 45 minutes. 50 µL of the resulting filtrate was stored in autosampler vials at –20°C for analysis by HPLC using an ion exchange column (Wescan #269.001, 4.1 mm x 250 mm with guard column) and the method developed by Sinclair and Tavendale (unpublished; Appendix 5.2).

Plasma sulphate concentration, following ^{35}S-cysteine infusion, was measured after treatment with an antioxidant (25 µL of 80 mmol/L DTT) and 15 µL of 3 mmol/L norleucine (in 0.1% phenol) as an internal standard. Samples were stored (-85°C) prior to centrifugation to remove proteins using the Centrisart® system (10,000 molecular weight filter) at 28,000 x *g* for 60 minutes and filtrate was held at -85°C until analysis by HPLC as above (Appendix 5.2).

Radioactivity

Total radioactivity of ^{35}S-cysteine and ^{35}S-SO$_4$ in infusates, plasma or processed plasma were determined by mixing 100 µL of sample with 2 mL of scintillation mixture (Starcint, INSUS Systems) and counting for 10 minutes in a Packard Tricarb Model 1500 scintillation counter (Lee *et al.*, 1993). All radioactivity measurements were corrected to the day of infusion using Equation 5.1.

EQUATION 5.1:

Radioactivity in sample corrected to day of infusion = $R\,(\exp^{-(\text{Days})(\text{Ln}(2)/\text{half-life})})$

Where:

R = radioactivity of sample analysed on day y,

Days = days between infusing radioactive label into animal on day x and radioactivity of sample analysed on day y.

Half-life = half-life of the radioactive label; ^{35}S = 87 days and ^{3}H = 4301 days.

The proportion of total radioactivity attributed to ^{35}S-cysteine and ^{35}S-SO$_4$ in infusates, plasma and processed plasma were determined by ion-exchange HPLC and ß-radioactivity using an inline scintillation counter (Model 2, ßram IN/US systems Inc., New Jersey, USA) coupled to a HPLC (LC4a, Shimadzu, Kyoto, Japan) as described by Lee *et al.* (1995) and Lee *et al.* (1999).

TABLE 5.2 . Processing of plasma and tissue samples taken from lambs infused with ^{35}S-sulphate (SO_4), ^{35}S-cysteine and ^{3}H-valine.

Infusion	Sample	**Processing Plasma**	Analysis (Method)
^{35}S-sulphate	0 h	Stored at –20°C; Total radioactivity.	Scintillation counter
	6, 7, 8 h	Stored at –20°C; Total radioactivity.	Scintillation counter
		Activity of ^{35}S-SO_4.	HPLC + ß-ram
		Concentration in plasma following protein removal (vivaspin).	HPLC
^{35}S-cysteine	0 h	Addition of DTT, SDS, internal standard, TCA; stored at –85°C.	
		Total radioactivity.	Scintillation counter
	6, 7, 8 h	^{35}S-Sulphate: Addition of DTT, internal standard; stored at –85°C.	
		Concentration following protein removal (Centrisart).	HPLC
		^{35}S-Cysteine: Addition of DTT, SDS, internal standard, TCA; stored at –85°C.	
		Total radioactivity.	Scintillation counter
		Activity of ^{35}S-cysteine and ^{35}S-SO_4.	HPLC + ß-ram
		Concentration following ninhydrin & DTT reduction.	Spectrophotometer
^{3}H-valine	0 h	Addition of DTT, SDS, TCA & internal standard, stored at –85°C.	
		Total radioactivity.	Scintillation counter
	6, 7, 8 h	^{3}H-valine: Addition of DTT, SDS, TCA & internal standard, stored at –85°C;	
		Total radioactivity.	Scintillation counter
		Activity of ^{3}H.	HPLC + ß-ram
		Concentration; derivatised, analysed by C18 reverse phase picotag.	Picotag
		Processing Tissues	
Tissue extracts (^{3}H-valine)	Tissue free valine	Addition of DTT, SDS, internal standard, TCA to supernatant; stored at –20°C.	
		Total radioactivity.	Scintillation counter
		Activity of ^{3}H; separation by HPLC.	HPLC + ß-ram
		Concentration; derivatised, analysed by C18 reverse phase picotag.	Picotag
	Tissue bound valine	FD pellet hydrolysed; Total radioactivity.	Scintillation counter
		Activity in ^{3}H.	HPLC + ß-ram
		Concentration; ion exchange chromatography with ninhydrin derivatising agent.	Shimadzu

SDS, sodium dodecyl sulphate; Internal standard, norleucine; DTT, dithiothreitol; TCA, trichloroacetic acid; FD, freeze-dried. Analytical methods are described in section 5.3.5 and 5.3.6.

5.3.4.4 Calculations

Specific radioactivity (SRA; dpm/µmol)

The SRA (Equation 5.2) of cysteine and sulphate in plasma were used to demonstrate steady state plateau values after 6 h of infusion and to calculate fluxes.

EQUATION 5.2: Specific radioactivity (SRA)

$$\text{SRA (dpm/}\mu\text{mol)} = \frac{\text{radioactivity of } ^{35}\text{S in sampled pool (dpm/mL)}}{\text{Concentration of cysteine or sulphate in the sampled pool } (\mu\text{mol/mL})}$$

Irreversible loss rate (ILR; µmol/min)

Irreversible loss rate of sulphate and cysteine measured at plateau SRA is the rate at which plasma cysteine or sulphate leaves the sampled pool and does not return within the time course of the experiment. This is calculated by expressing the rate of infusion relative to the SRA of the sampled pool (dpm/µmol; Equation 5.3).

EQUATION 5.3: Irreversible loss rate (ILR)

$$\text{ILR } (\mu\text{mol/min}) = \frac{\text{Infusion rate (dpm/min)}}{\text{SRA of pool (dpm/}\mu\text{mol)}}$$

Transfer quotient

Transfer quotient (TQ) measured at plateau SRA is the proportion of radioactive label (^{35}S) transferred from the primary pool (cysteine) to the secondary pool (sulphate from cysteine).

EQUATION 5.4: Transfer quotient (TQ)

$$\text{TQ} = \frac{\text{SRA of secondary pool}}{\text{SRA of primary pool}}$$

Total flux

Total flux measured at plateau SRA, is the combined total of all outflows (μmol/min) from a plasma pool to all processes. When deriving equations to calculate total flux through a pool, all outflows must equal all inflows and the total flux represents a steady state situation. Figure 5.1 illustrates the two-pool model (Nolan *et al.*, 1976) of fluxes through the cysteine and sulphate pools, with equations used to calculate fluxes.

Whole body protein synthesis (WBPS; g/day)

Cysteine (μmol/min) leaving the plasma pool for productive purposes is used to calculate WBPS (g protein/day) by expressing the flux as g/day and assuming a cysteine concentration in whole body protein for sheep of 36.2 g/kg total amino acid (MacRae *et al.*, 1993).

EQUATION 5.5:

$$\text{Protein synthesis (g/day)} = \frac{\text{Cysteine flux to production } (\mu\text{mol/min}) \times 10^{-6} \times 1440 \times 121.6}{0.0362}$$

5.3.4.5 Criteria to accept or reject values

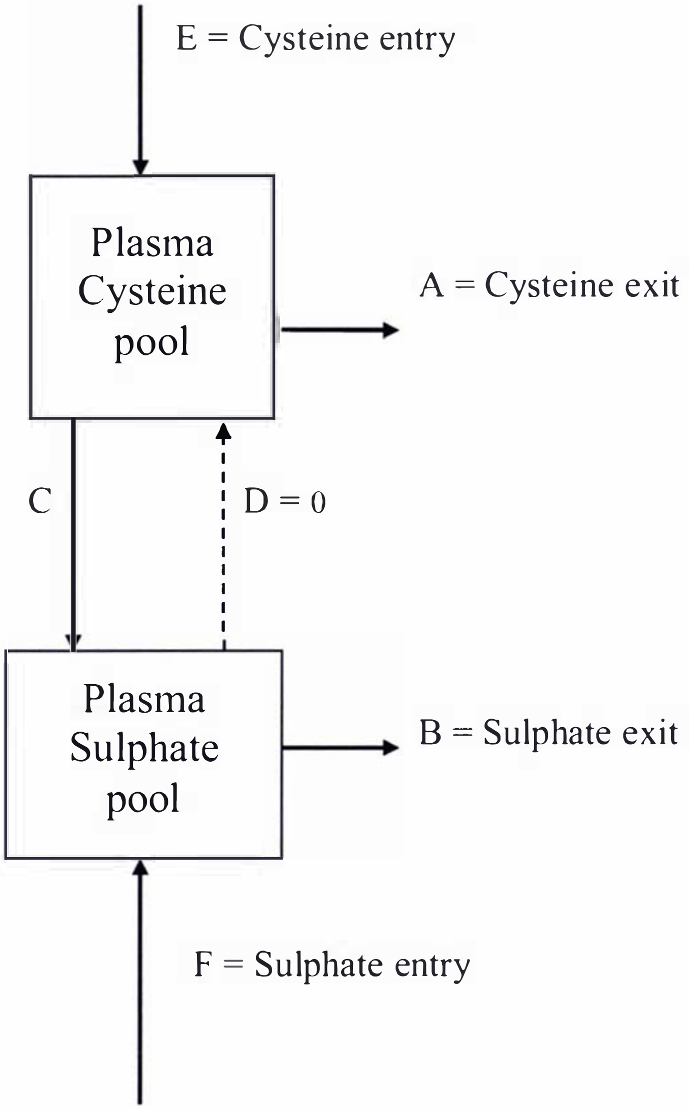
All data were examined to determine whether steady state SRA had been achieved. The criteria for determining whether to accept or reject values (6, 7 and 8 h) or to reject the likelihood that steady state had been reached were as follows:

If radioactivity (dpm/g) of ^{35}S between 6 and 8 hours increased more than 20%, the 8 hour value was assumed to be when steady state was achieved. Values that declined more than 10% were rejected.

If a concentration of SO_4 or cysteine at 6, 7 or 8 hours deviated more than 10% from the average of the remaining two concentrations it was removed.

Only data at corresponding times were used to calculate SRA of each lamb, for example if radioactivity at 7 and 8 hours and concentrations at 6 and 8 hours were accepted, the data at 8 hours was used to calculate SRA of that lamb. If radioactivity and concentration at more than one sampling time were used to determine SRA, the average radioactivity and average concentration were used to calculate the average SRA of cysteine, SO_4 and cysteine-SO_4 in each lamb.

FIGURE 5.1. Fluxes and equations through the cysteine and sulphate pools when ^{35}S-sulphate and ^{35}S-cysteine were infused.



Equations to calculate fluxes (µmol/min):

$A = ILR_{cysteine} - C$
$B = ILR_{sulphate}$
$C = TQ \times B$
$D = 0$
$E = A + C$
$F = B - C$

Where:

A = Cysteine leaving the plasma pool for maintenance and productive purposes.
B = Sulphate leaving plasma, primarily excreted in urine.
C = Cysteine irreversibly oxidised to sulphate, water, ammonia and carbon dioxide.
D = Plasma sulphate re-assimilated to cysteine; within time course of infusion it is assumed that there is zero flux in mammalian tissues.
E = Cysteine entry to plasma (includes cysteine from transulphuration of methionine, protein degradation, and absorption from the gastrointestinal tract).
F = Sulphate entering plasma (sulphate from all sources including the oxidation of methionine, and absorption from the gastrointestinal tract).

5.3.5 Tissue Protein Fractional Synthesis Rates

Calculations of FSR (%/day) for the duodenum and ileum (with and without the mucosal layer), rumen, abomasum, liver, pancreas, muscle and skin were based on a continuous infusion of tritiated [3,4-^{3}H] valine over an 8 hour period (92.5 MBq/lamb; Amersham Pharmacia Biotech UK Limited) prior to slaughter. FSR of tissue proteins were calculated from both the plasma and intracellular unbound (free) ^{3}H-valine precursor pools and from ^{3}H-valine incorporated into tissue proteins (bound fraction).

5.3.5.1 Isotopes and infusion rates

Each lamb was infused with ^{3}H-valine (in conjunction with ^{35}S-cysteine; Section 5.3.4; Table 5.1) to measure FSR of protein in tissues. The infusate was prepared by adding 92.5 MBq of [3,4-^{3}H] valine (1.04 TBq/mmol) to 380 mL sterile saline containing 1 mL of 0.2 mmol/L of inert L-valine. A 35 mL bolus containing 8.52 MBq ^{3}H-valine was given at the commencement of the 8 hour continuous infusion and the remainder infused at 89.7 kBq/min.

5.3.5.2 Sampling

A sample of infusate for each lamb was retained to determine radioactivity. Blood samples were collected prior to the infusion and after 6, 7 and 8 hours using LH S-Monovette®, gently mixed (by inversion) and held on ice prior to centrifuging (3,000 x *g* for 15 minutes at 4°C) to obtain plasma. Plasma samples were either stored (–20°C) for analysis or underwent further processing (deproteinized) before being analysed for radioactivity and valine concentration (Table 5.2).

At the completion of the blood sampling, but while ^{3}H-valine was still being infused, the lambs were euthanased by an intravenous overdose of sodium pentobarbitone (300 mg/mL; 0.5 mL/kg LW; Pentobarb 300). Lambs were weighed immediately and samples from the following tissues were excised within 15 minutes of death: duodenum, ileum, rumen, abomasum, liver, pancreas, muscle (biceps femoris) and skin. Samples of duodenum and ileum were stored intact or after scraping the mucosal layer off the luminal surface using a glass microscope slide. The tissues were rinsed in 0.9% chilled saline to remove traces of digesta or blood, frozen in liquid nitrogen and stored at – 20°C.

5.3.5.3 Tissue extraction

The intracellular (free) and protein-bound valine was obtained from pulverised tissue (in liquid nitrogen) homogenised with an extraction buffer (20 mmol/L TRIS pH 7.8; 2.5 mmol/L EDTA; 0.3% SDS; Appendix 5.3). The homogenate was centrifuged (28,000 g; 4°C for 30 minutes) to yield a supernatant containing intracellular unbound (free) AA (valine), peptide AA and soluble protein-bound AA, and a pellet containing AA (valine) incorporated into tissue protein (bound-protein). The pellet was rewashed in extraction buffer and centrifuged for a further 30 min. The pellet (protein-bound fraction) was freeze-dried and stored at –20°C for AA determination and ^{3}H-valine radioactivity.

The supernatant (2 mL) obtained from initial centrifugation was mixed with SDS/EDTA (1 mL; 0.75% SDS; 9 mmol/L EDTA) to denature soluble proteins, deproteinised with TCA (1 mL of 30% TCA) and an antioxidant (200 μL of 80 mmol/L DTT; pH 8.0) and internal standard (100 μL of 3 mmol/L norleucine) were added (Appendix 5.3). Samples were centrifuged (3,300 x *g* for 15 min at 4°C) and the resulting supernatant containing intracellular AA was filtered (45 μm) and stored at -20°C until analysis for AA concentrations and total radioactivity.

5.3.5.4 Processing and analysis

Processing of plasma

Plasma collected for ^{3}H-valine analyses and determination of SRA was processed (deproteinized) using methods described in section 5.3.5.3. This involved 1 mL plasma treated with 0.5 mL SDS (0.75% w/v SDS and 9 mmol/L EDTA) to denature the protein, 0.5 mL TCA (30% w/v) to precipitate the proteins and 100 μL DTT (80 mmol/L) as an antioxidant and 50 μL of an internal standard (3 mmol/L norleucine in 0.1% phenol; Appendix 5.1). Samples were centrifuged (3,300 x *g* for 15 minutes at

4°C), filtered (0.45μm) and the resulting supernatant stored at –85°C for analysis of ^{3}H radioactivity and valine concentration.

Valine concentration

Concentration of valine in deproteinized plasma and the tissue free pool (Appendix 5.4 and 5.5) were determined using the method described by Bidlingmeyer *et al.* (1984), including pre-column derivatisation and chromatography. Reverse-phase HPLC separated phenylisoothiocyanate derivatives, using a Waters Pico-Tag® column (3.9 x 300 mm, Waters Corporation, Milford, NMA 01757, USA) and a Shimadzu LC-10A HPLC system (Shimadzu Scientific Instruments Ltd., Columbia, MD 21046, USA) to measure valine concentration.

The concentration of AA and radioactivity in tissue bound-proteins were determined after hydrolysing approximately 50 mg of freeze-dried material by heating in 4 mL of 7.5 mol/L HCl at 110°C for 22 h. The hydrolysates were filtered, rotary evaporated to near dryness, washed in distilled deionised water and re-concentrated before dissolving in 1 mL of 0.2 mol/L sodium citrate buffer (pH 2.2) and 100 μL of 5 mol/L NaOH. The concentrations of AA (valine) in the hydrolysates were determined by ion exchange chromatography (Shimadzu Scientific Instruments Limited, Columbia, MD 21046, USA) with a post-column reaction using ninhydrin as the derivitising agent.

Radioactivity

Total radioactivity was measured in the infusate, plasma and intracellular pool by adding 100 μL of prepared sample to 2 mL of scintillation mixture (Starcint, INSUS Systems). The radioactivity in protein bound samples were determined by mixing 200 μL of hydrolysed pellet with 2 mL of scintillation mixture (Starcint, INSUS Systems).

The proportion of total radioactivity attributed to ^{3}H-valine was determined by ion-exchange HPLC and ß-radioactivity using an inline scintillation counter (Model 2, ßram IN/US systems Inc., New Jersey, US coupled to a HPLC (LC4a, Shimadzu, Kyoto, Japan).

5.3.5.5 Calculations

Specific radioactivity (dpm/μmol) of valine in plasma (SRA_P), tissue intracellular pool (SRA_I) and tissue bound-protein (SRA_B) were calculated by dividing the radioactivity by the valine concentration in the appropriate pool (Equation 5.3). Both the intracellular and plasma valine SRA were used as precursor pools for the estimation of FSR. The SRA of intracellular ^{3}H-valine of each tissue was assumed to reflect the steady-state SRA of the true precursor pool, valine-tRNA, for protein synthesis. The estimates of FSR calculated from the intracellular pool (FSR_I) were compared to the FSR obtained using the SRA of free ^{3}H-valine in plasma as the precursor pool (FSR_P).

Tissue FSR was calculated by determining the amount of ^{3}H-valine incorporated into protein from precursor pools (plasma or intracellular pool; Equation 5.6). Plasma samples were taken after 6, 7 and 8 hours of infusion to demonstrate plateau SRA and tissue FSR was calculated according to Wykes *et al.* (1996) on the basis of valine SRA and the infusion period. Conventional estimation of tissue FSR is based on the rate at which the isotope reaches plateau values, which was not possible for the tissue precursor pool, so estimates presented here assume a plateau SRA over the 8 hour infusion period and probably underestimates true FSR.

EQUATION 5.6: Fractional synthesis rate of tissues (FSR)

$$\text{FSR}\,(\%\,\text{d}^{-1}) = \frac{\text{SRA Valine}_{(\textit{protein bound})}}{\text{SRA Valine}_{(\textit{precursor pool})} * \text{period of infusion (day)}} * 100$$

Criteria for assessment of plateau radioactivity and valine concentration for each lamb have been defined in section 5.3.5.5.

5.3.6 Statistical Analyses

5.3.6.1 Whole body protein synthesis

SRA and ILR were calculated at 6, 7 and 8 hours and averaged for each animal in order to calculate fluxes and WBPS (g/day) of each lamb. Statistical analyses were performed using the GLM procedure within SAS (1996) to determine effects of diet on SRA, ILR, fluxes (A, B, C, E and F; Figure 5.1) and WBPS. Feed intake and LW were analysed using the mixed procedure within SAS (1996) designed to account for repeated measures. Results were presented as LS means and SEM when treatments were balanced, and when treatments were unbalanced the pooled SD was reported. The fluxes and WBPS of one lamb fed lucerne was much higher (> 2 SD) than the three other lambs fed lucerne and data from that lamb was removed. Contrasts were also used to compare the SRA, ILR, fluxes and WBPS rate of lambs fed pasture to lambs fed all other diets (lucerne, sulla and lucerne:sulla), and to compare lambs fed diets with CT (sulla and lucerne:sulla) with lambs fed diets without CT (pasture and lucerne). Probability values of these comparisons are reported. Probability (Pr) values less than 0.10 indicated a significant difference and values between 0.10 and 0.15 indicated a trend. All data were checked for normality and homogeneity.

5.3.6.2 Fractional protein synthesis

The effect of diet on the SRA and concentration of ^{3}H-valine bound in tissue (SRA_B, $Valine_B$) and intracellular free (SRA_I, $Valine_I$) and plasma (SRA_P; $Valine_P$) pools were determined using the GLM procedure within SAS. Diet effect on tissue FSR calculated from plasma (FSR_P) and intracellular pools (FSR_I) were determined using proc GLM and the LS means and SEM are reported. Contrasts were used to compare tissue FSR based on plasma (FSR_P) and intracellular pools (FSR_I) of lambs fed pasture with lambs fed all other diets (lucerne, sulla and lucerne:sulla), and to compare lambs fed diets with CT (sulla and lucerne:sulla) and diets without CT (pasture and lucerne). Probability values of these comparisons are reported. Probability (Pr) values less than 0.10 indicated a significant difference and values between 0.10 and 0.15 indicated a trend. All data were checked for normality and homogeneity.

5.4 RESULTS

All lambs settled into the metabolism crates easily with similar feed intakes and daily LW gains to lambs fed outdoors on sawdust feed pads (Chapter 3).

5.4.1 Feed intake and composition

Table 5.3 illustrates the contrasting composition of the four diets offered to and eaten by the lambs. Lambs offered pasture, made up of 86% ryegrass and 14% white clover DM, consumed relatively less soluble carbohydrate (119 g/kg DM) and more NDF (486 g/kg DM) than lambs fed the other diets. Lambs fed sulla and lucerne:sulla consumed a relatively high soluble carbohydrate (224 and 186 g/kg DM) and low fibre concentration (171 and 227 g/kg DM) compared to lambs fed pasture and lucerne. Crude protein concentration in the diet eaten was highest in lambs fed lucerne (273 g/kg DM) and lowest for lambs fed sulla and pasture (214 and 221 g/kg DM). Condensed tannin concentration of sulla was 56 g/kg DM and lucerne:sulla was 28 g/kg DM. The lambs were fed *ad libitum* and the diets consumed contained a higher concentration of protein and soluble carbohydrate, with less fibre, than the material offered for all diets. The increased metabolisable energy (ME) content of the material eaten relative to the material offered resulted in a range from 10.3 (pasture) to 12.7 (sulla) MJME/kg DM eaten (Table 5.3).

Differences in diet composition were reflected in feed intake and LW gain over the period of indoor feeding (Table 5.4). Lambs fed pasture consumed less feed (1.03 kg DM/lamb/day) and had the smallest LW gains (78 g/day) compared to lambs fed other diets with average DM intakes of 1.60 to 1.66 kg DM/lamb/day and LW gains of 235 to 335 g/day.

TABLE 5.3. Dry matter (DM) content and composition (g/kg DM) of diets offered and consumed by lambs fed pasture, lucerne, sulla and lucerne:sulla. Results presented are least-square means ± standard error of the mean (SEM).

	Pasture[1]	Lucerne	Sulla	Lucerne:Sulla[2]	SEM	Pr
Number	4	4	4	4		
DM content of diet offered (%)	22.5^{d}	18.4^{c}	13.8^{a}	16.0^{b}	0.30	< 0.01
Condensed tannin Offered (g/kg DM)	-	-	56	28	-	-
Soluble carbohydrate						
Offered	108^{a}	111^{b}	197^{d}	156^{c}	0.4	< 0.01
Consumed	119^{a}	136^{a}	224^{c}	186^{b}	5.8	< 0.01
Crude protein						
Offered	200^{b}	259^{d}	193^{a}	225^{c}	0.67	< 0.01
Consumed	227^{a}	273^{b}	214^{a}	241^{a}	5.1	< 0.01
Neutral detergent fibre						
Offered	464^{d}	333^{c}	241^{a}	282^{b}	1.4	< 0.01
Consumed	422^{d}	284^{c}	194^{a}	227^{b}	11.4	< 0.01
Ash						
Offered	91^{a}	100^{b}	109^{d}	105^{c}	0.14	< 0.01
Consumed	93^{a}	98^{ab}	110^{c}	102^{b}	2.1	< 0.01
Metabolisable energy (MJME/kg DM)						
Offered	9.7^{a}	10.0^{b}	11.9^{d}	11.0^{c}	0.02	< 0.01
Consumed	10.3^{a}	10.3^{a}	12.7^{c}	11.7^{b}	0.12	< 0.01

[1] 86:14% ryegrass:white clover in diet offered (DM basis).

[2] 48:52% lucerne:sulla in diet offered (DM basis).

[a, b] LS means within rows with a common superscript letter do not differ significantly ($Pr < 0.05$).

TABLE 5.4. Average dry matter (DM) intake and starting and finishing liveweights of lambs fed pasture, lucerne, sulla and lucerne:sulla. Least-square (LS) means ± standard error of the mean (SEM) are presented.

	Pasture	Lucerne	Sulla	Lucerne: Sulla	SEM	Pr
Number	4	4	4	4		
DM intake (kg/lamb/day)	1.03[a]	1.63[b]	1.60[b]	1.66[b]	0.052	< 0.01
Liveweight (kg)						
Start	30.7[a]	35.8[b]	38.4[bc]	40.0[c]	0.87	< 0.01
Finish	32.4[a]	39.8[b]	42.5[bc]	43.1[c]	0.96	< 0.01
Liveweight gain (g/day)[1]	78[a]	328[b]	335[b]	235[b]	64.2	0.05

[1] 1.0 kg was added to initial liveweight because lambs had not received their morning feed prior to weighing and placement in crates, but lambs were fully fed at their final weighing.

[a, b] LS means within rows with a common superscript letter do not differ significantly ($Pr < 0.05$).

5.4.2 Absolute whole body protein synthesis

Previous research indicated an intravenous infusion of ^{35}S-cysteine and ^{35}S-SO$_4$ would have reached plateau SRA after about 6 hours (Lee *et al.*, 1995), and this was used as a basis for sampling at 6, 7 and 8 hours. Figures 5.2 and 5.3 suggest plateau SRA had been achieved with ^{35}S-cysteine (with the possible exception of lambs fed lucerne:sulla) and ^{35}S-SO$_4$. Lambs fed pasture had significantly higher ^{35}S-cysteine and ^{35}S-SO$_4$ SRA than lambs fed other diets (Table 5.3; Figures 5.2 and 5.3; Appendix 5.6) because lambs fed pasture had lowest plasma cysteine and sulphate concentrations and were lighter than lambs fed other diets.

FIGURE 5.2. Plasma ^{35}S-cysteine specific radioactivity (SRA; dpm/μmol) following a continuous infusion of ^{35}S-cysteine into lambs fed pasture, lucerne, sulla and lucerne:sulla. Each point represents an average ± SEM of four lambs.

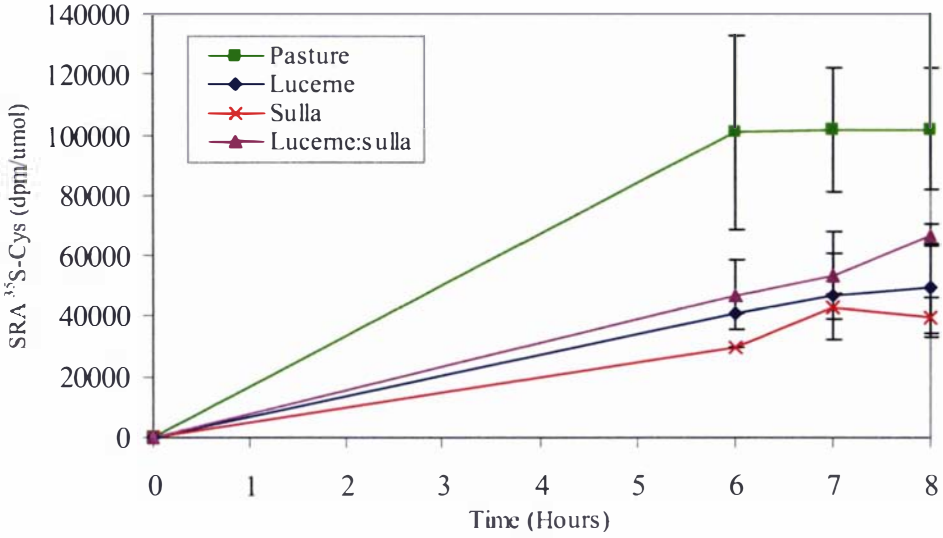
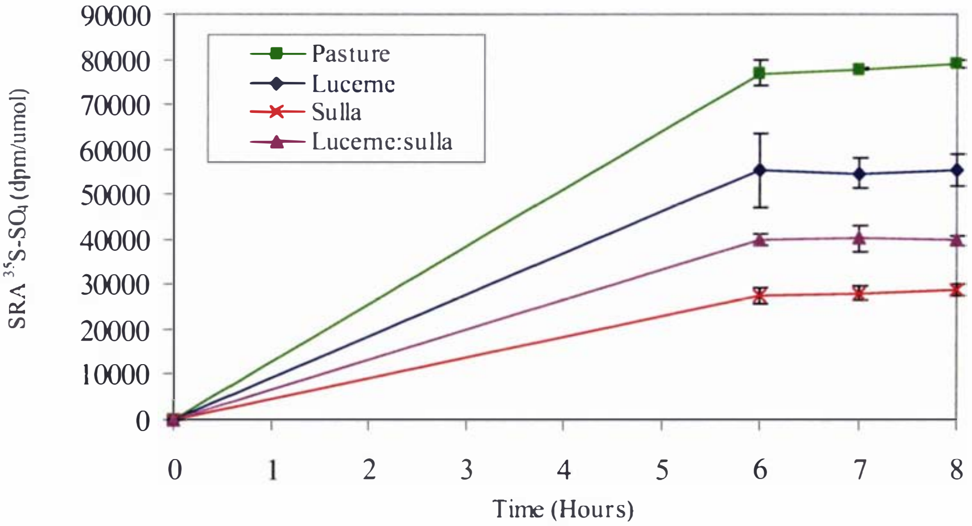


FIGURE 5.3. Plasma ^{35}S-sulphate (SO_4) specific radioactivity (SRA; dpm/μmol) following a continuous infusion of ^{35}S-SO_4 into lambs fed pasture, lucerne, sulla and lucerne:sulla. Each point represents an average ± SEM of four lambs.



5.4.2.1 Cysteine

Lambs fed pasture had significantly ($Pr < 0.10$) higher ^{35}S-cysteine SRA than lambs fed other diets (Table 5.5) and there was no difference between lambs fed lucerne, sulla or lucerne:sulla. Cysteine ILR (μmol/min; from the plasma pool) was lower ($Pr = 0.01$) in lambs fed pasture compared to lambs fed other diets and oxidation of cysteine to sulphate was significantly lower ($Pr = 0.03$) in lambs fed pasture compared to lambs fed sulla and lucerne:sulla, but not lambs fed lucerne. Lambs fed pasture had a lower flux of cysteine to productive purposes and lower absolute WBPS than lambs fed other diets (Table 5.5).

5.4.2.2 Inorganic sulphate

Plasma inorganic sulphate concentration was significantly higher in lambs fed sulla than those fed pasture and lucerne (Table 5.5). Lambs fed pasture had significantly higher $^{35}S\text{-}SO_4$ SRA (78.0 dpm/nmol) than lambs fed other diets where values for lambs fed lucerne, lucerne:sulla and sulla were 53.6, 39.7 and 28.1, respectively (Table 5.5; Figure 5.3). Sulphate ILR was affected by diet and ranged from 36.6 μmol/min in pasture-fed lambs to 105.4 μmol/min in sulla-fed lambs. Lucerne- and lucerne:sulla-fed lambs had sulphate ILR intermediate at 57.7 and 76.2 μmol/min ($Pr < 0.01$). These dietary treatment differences were predominantly due to lower amounts of sulphate entering the plasma sulphate pool from methionine and absorption from the gastrointestinal tract (F; Figure 5.4) with lowest values for lambs fed pasture (34.1 μmol/min) and highest with the sulla diet (100.4 μmol/min; Table 5.5; Figure 5.4).

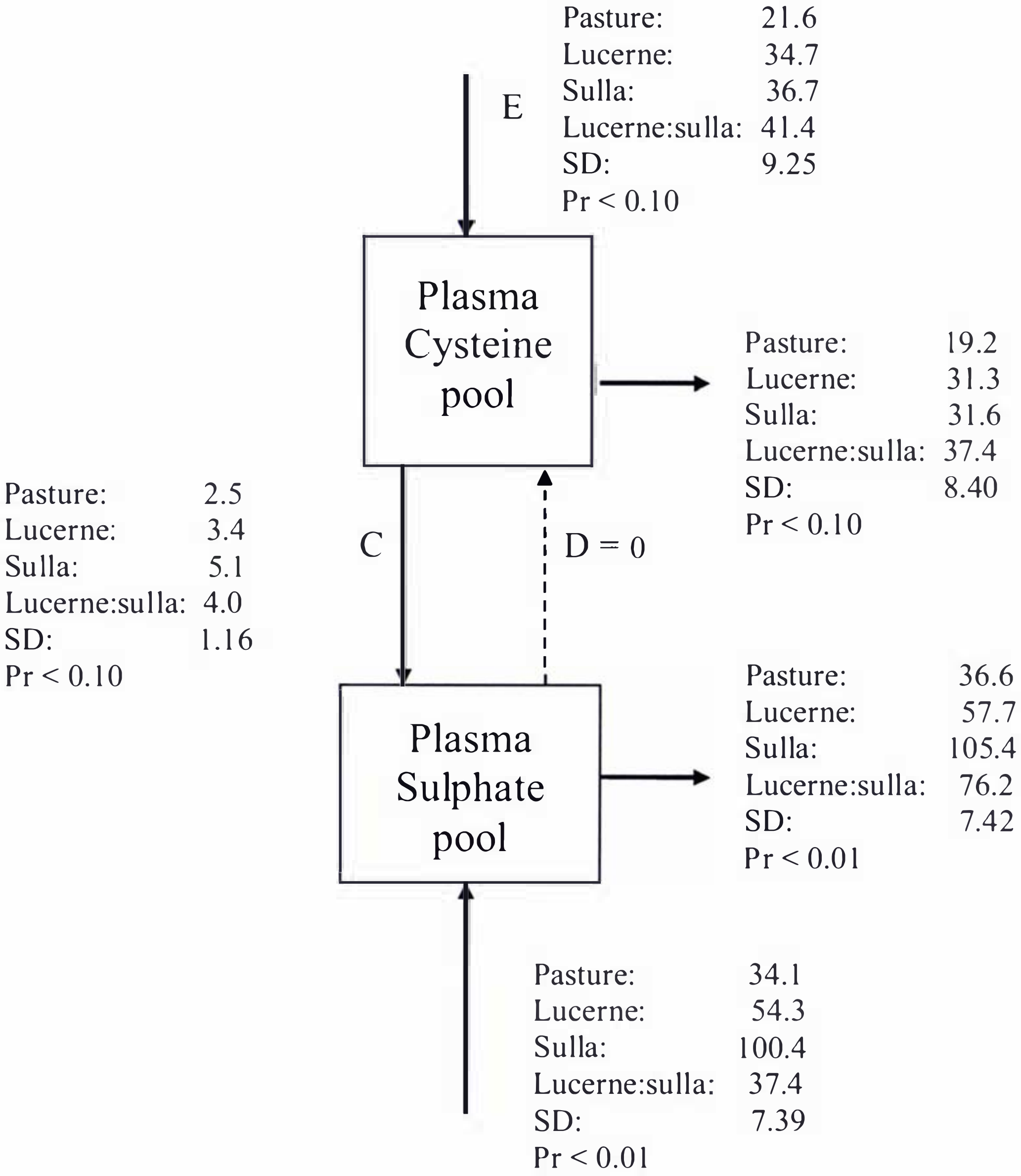
TABLE 5.5. Effect of diet on plasma cysteine and sulphate concentrations, specific radioactivity and fluxes enabling calculations of whole body (WB) protein synthesis in lambs fed pasture, lucerne, sulla and lucerne:sulla. Data are presented as least square (LS) means ± pooled standard deviation (SD).

	Pasture	Lucerne	Sulla	Lucerne:Sulla	Pooled SD	Diet effect	Pasture vs. others	CT vs. no-CT
						Pr	Pr	Pr
Number of animals	4	3	4	4				
Concentration (µmol/mL)								
Cysteine	0.068	0.107	0.106	0.103	0.0352	NS	0.097	NS
Sulphate	1.20^{a}	1.28^{a}	1.66^{b}	1.46^{ab}	0.272	0.153	0.127	0.048
Specific radioactivity (dpm/nmol)								
Cysteine	98.5^{a}	48.1^{b}	41.6^{b}	56.8^{b}	27.71	0.060	0.011	0.123
Sulphate	78.0^{a}	53.6^{b}	28.1^{c}	39.7^{d}	40.49	< 0.01	< 0.01	< 0.01
Transfer quotient								
Sulphate from cysteine	0.066	0.061	0.048	0.053	0.0192	NS	NS	NS
Flux: (µmol/min)[1]								
Cysteine entry (E); ILR	21.6^{a}	34.7^{b}	36.7^{b}	41.4^{b}	9.25	0.060	0.013	0.045
Sulphate entry (F)	34.1^{a}	54.3^{b}	100.4^{c}	72.2^{d}	7.39	< 0.01	< 0.01	< 0.01
Cysteine to sulphate (C)	2.45^{a}	3.40^{ab}	5.05^{b}	4.03^{b}	1.157	0.052	0.028	0.022
Cysteine to productive purposes (A)	19.2^{a}	31.3^{b}	31.6^{b}	37.4^{b}	8.40	0.0621	< 0.01	< 0.01
Sulphate leaving (B); ILR	36.6^{a}	57.7^{b}	105.4^{c}	76.2^{f}	7.42	< 0.01	< 0.01	< 0.01
WB Protein synthesis (g/day)	92.6^{a}	150.7^{b}	152.3^{b}	180.0^{b}	40.50	0.062	0.015	0.058

[1] See Figure 5.1

[a, b] LS means within rows with a common superscript letter do not differ significantly ($Pr < 0.05$). NS = non-significant, $Pr > 0.15$.

FIGURE 5.4. Effect of diet on whole body cysteine and sulphate fluxes (μmol/min) based on infusion of ^{35}S-cysteine and ^{35}S-sulphate in lambs fed pasture (n = 4), lucerne (n = 3), sulla (n = 4) and lucerne:sulla (n = 4). Results are represented as least-square means and associated pooled standard deviation (SD).



Where:

A = Cysteine leaving the plasma pool for maintenance and productive purposes.
B = Sulphate leaving plasma, primarily excreted in urine.
C = Cysteine irreversibly oxidised to sulphate, water, ammonia and carbon dioxide.
D = Plasma sulphate re-assimilated to cysteine; zero flux in mammalian tissues.
E = Cysteine entry to plasma (includes cysteine from transulphuration of methionine, protein degradation, and absorption from the gastrointestinal tract).
F = Sulphate entering plasma (sulphate from all sources including the oxidation of methionine, and absorption from the gastrointestinal tract).

5.4.3 Fractional Protein Synthesis

5.4.3.1 Valine concentrations

The concentration of valine in plasma differed across diets ($P < 0.01$) with the lowest value in lambs fed pasture (184 nmol/mL) and highest concentration in lambs fed lucerne:sulla (342 nmol/mL; Table 5.6). The differences between dietary treatments in intracellular valine concentration (45 – 80 nmol/mL) were much smaller than for plasma and diet did not affect values for duodenum, ileum, rumen, abomasum, pancreas and skin tissues (Table 5.6). Dietary treatment significantly affected the intracellular valine concentration in the muscle ($Pr < 0.05$) and liver ($Pr < 0.10$) with lowest values in lambs fed pasture.

The protein-bound concentration (mg/g DM) of valine was lowest for abomasal tissue (3.2) compared to all other samples (13 – 21) and only muscle and rumen were affected by dietary treatment (Table 5.6). The lowest concentrations in muscle and liver were in lambs fed sulla with 20.9 and 19.6 mg/g DM, respectively. Valine comprised 24.0 – 25.5 mg/g DM in muscle of lambs fed the other diets and dietary effects on rumen tissues (21.2 – 22.7 mg/g DM) were relatively minor (Table 5.6).

TABLE 5.6. Effect of diet on the concentration of valine in plasma and the intracellular pools (nmol/mL) and protein-bound pool (mg/g DM) in tissues of lambs fed pasture, lucerne, sulla and lucerne:sulla. Data are presented as least-square (LS) means ± SEM.

	Pasture	Lucerne	Sulla	Lucerne:sulla	SEM	Pr
Valine concentration (nmol/mL)						
Plasma	183.7^{a}	238.9^{b}	285.5^{c}	341.7^{d}	18.36	< 0.01
Valine concentration in the intracellular pool (nmol/mL)						
Duodenum	73.4	109.2	86.1	89.1	10.72	NS
Duodenum (scraped)[1]	62.0	61.1	59.5	37.5	9.18	NS
Ileum	82.4	67.5	91.0	80.6	8.58	NS
Ileum (scraped)[1]	37.0	46.8	60.1	49.0	7.85	NS
Rumen	37.7	44.6	48.9	40.0	6.03	NS
Abomasum	68.5	67.2	62.8	56.9	7.76	NS
Liver	46.6^{a}	66.8^{b}	72.0^{b}	67.7^{b}	6.55	0.07
Pancreas	67.6	47.6	55.4	49.4	13.28	NS
Muscle	43.3^{a}	58.3^{ab}	67.7^{b}	74.0^{b}	6.44	0.03
Skin	86.9	73.2	85.4	73.0	9.83	NS
Valine concentration in the protein-bound pool (mg/g DM)						
Duodenum	22.3	23.7	19.6	13.2	1.84	NS
Duodenum (scraped)[1]	16.8	16.1	16.8	16.0	0.96	NS
Ileum	19.4	21.0	18.9	20.5	1.43	NS
Ileum (scraped)[1]	19.6	17.0	19.2	18.7	1.48	NS
Rumen	22.7^{a}	22.1^{a}	19.6^{b}	21.2^{ab}	0.77	0.08
Abomasum	3.3	3.1	3.1	3.2	0.15	NS
Liver	13.3	14.0	12.3	13.2	1.15	NS
Pancreas	15.3	15.6	14.6	16.6	1.01	NS
Muscle	24.2^{a}	24.0^{a}	20.9^{b}	25.5^{a}	1.09	0.06
Skin	22.5	19.7	21.0	19.2	1.37	NS

[1] Scraped tissue duodenum and ileum without mucosal layer.

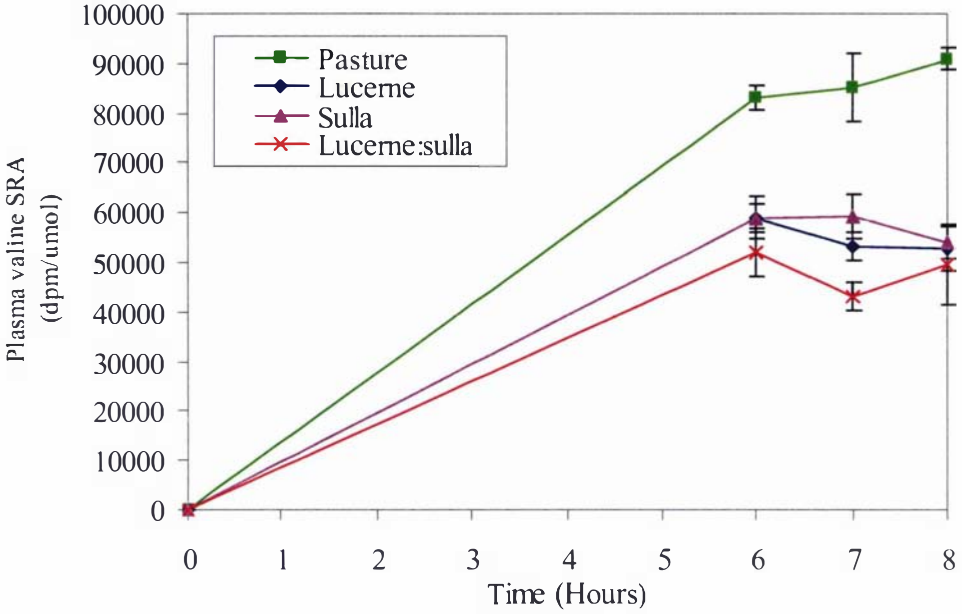
[a, b] LS means within rows with a common superscript do not differ significantly (Pr < 0.10). NS = non-significant, Pr > 0.15.

5.4.3.2 Specific radioactivity of valine

Plasma valine SRA (SRA_p) reached a plateau 6 hours after the start of the infusion (Figure 5.5). SRA_P averaged 85.0, 54.2, 57.9 and 51.4 (SEM = 4.33) dpm/nmol at plateau for lambs fed pasture, lucerne, sulla, and lucerne:sulla, respectively ($Pr < 0.01$). These values were usually three- to five-fold higher than SRA of valine in intracellular tissue pools (SRA_I), except for ileum (scraped) which was similar to plasma (Table 5.7). Dietary treatment affected the SRA_I of the duodenum, muscle ($Pr < 0.05$), ileum, ileum (scraped) ($Pr < 0.10$) and duodenum (scraped) ($Pr = 0.14$) tissues with values often higher in lambs fed pasture than other diets.

The protein-bound valine SRA (SRA_B) was substantially lower than SRA_I in all tissues except the abomasum (Table 5.7) which had a very low valine concentration (Table 5.6). The average SRA_B values for valine ranged from about 0.44 dpm/nmol (muscle) to 21.7 dpm/nmol (abomasum). SRA_B values in duodenum and ileum were much lower when they had been scraped to remove the mucosal layer. Diet affected SRA_B with significantly higher values in the rumen, abomasum and pancreas ($Pr < 0.05$) and skin ($Pr < 0.10$) of lambs fed pasture compared to other diets.

FIGURE 5.5. Plasma ^{3}H-valine specific radioactivity (SRA; dpm/μmol) at 6, 7 and 8 hours of infusion for lambs fed pasture, lucerne, sulla and lucerne:sulla. Each point represents an average ± SEM of four lambs.



5.4.3.3 Fractional synthesis rates of tissues

Estimates of tissue fractional protein synthetic rates (Table 5.8) were three-fold higher when calculated from the intracellular precursor pool (FSR_I) than the plasma pool (FSR_P) for all tissues across all diets, except for scraped ileum where FSR_P and FSR_I were similar (7.1 %/day and 7.7 %/day). Estimates of synthesis based on the plasma SRA_P pool resulted in less variation between lambs than calculations based on SRA_I (Appendix 5.7), and values based on the plasma pool resulted in protein synthetic rates in line with lamb growth and energy intake. Comparisons between tissues and between dietary treatments have been based on calculations using the plasma precursor pool to determine FSR (FSR_P).

Mean FSR_P (%/day) across diets ranked tissues (from greatest to least) as abomasum (104.3), duodenum (30.8), pancreas (25.3), ileum (22.7), rumen (13.7), liver (10.9), scraped duodenum (10.0), scraped ileum (7.1), skin (6.7) and muscle (2.3; Table 5.9). The difference between duodenum and ileum which were scraped to remove secretory protein (mucosal protein), and duodenum and ileum not scraped, indicates the FSR of the underlying tissues and the effect of synthesised proteins for secretion. There was no significant diet x tissue interactions but Table 5.9 illustrates the differences between tissue FSR_p within each diet and differences between average FSR_p of tissues. FSR_p of abomasal tissue (92.4 – 127.4 %/day) was significantly greater than FSR_p of all other tissues. On average, pancreas, duodenal and ileal tissue (22.6 – 30.8 %/day) had FSR_P significantly lower than abomasal tissue, but significantly greater than all other tissues, and FSR_p of all other tissues (muscle, skin, scraped ileum, scraped duodenum, liver, rumen) ranged from 2.3 to 13.7 %/day.

Diet had a significant effect on the FSR_P of scraped duodenum ($Pr < 0.05$) and a lesser effect on rumen and pancreas FSR_P ($Pr < 0.15$). There were no effects on the duodenum, scraped ileum, ileum, abomasum, liver, muscle or skin FSR_P (Table 5.9). The FSR_P of duodenum (scraped) were lower in lambs fed pasture (7.7 %/day) and lucerne:sulla (4.6 %/day) than lucerne (16.3 %/day; $Pr < 0.10$). Pasture also resulted in a lower FSR_P in duodenal tissue than other forages ($Pr = 0.12$), but CT did not affect duodenal protein synthetic rates. Pasture-fed lambs had a lower FSR_P in scraped ileum tissue than lambs given other diets ($Pr = 0.09$). In contrast to intestinal tissues, lambs fed pasture had a higher rumen FSR_P (15.9 %/day) than lambs fed other diets, and diets containing CT had a lower FSR_P than pasture and lucerne ($P = 0.03$). The pasture diet resulted in a higher FSR_P for pancreas (38 %/day) compared to other diets (24 – 31 %/day).

Muscle contains the largest amount of protein in lambs and the slow growing lambs fed pasture had the lowest FSR_P in muscle (1.6 %/day) compared to other diets ($Pr = 0.09$). Muscle FSR_P for lambs fed lucerne (2.1 %/day) was intermediate between lambs fed pasture and lambs fed sulla and lucerne:sulla (2.7 and 2.8 %/day; Table 5.9). Diets containing sulla increased muscle FSR_p in line with high average daily gains (Tables 4.4 and 5.2).

TABLE 5.7. Specific radioactivity (SRA; dpm/nmol) of valine in plasma and in intracellular (I) and bound protein (B) pools of tissues in lambs fed pasture, lucerne, sulla or lucerne:sulla (n = 4). Results are presented as least-square (LS) means and SEM. Probability (Pr) values are given for comparisons of diet.

							Diet effect
Tissue	Precursor Pool	Pasture	Lucerne	Sulla	Lucerne:Sulla	SEM	Pr
Plasma SRA	Plasma	85.0^{b}	54.2^{a}	57.9^{a}	50.4^{a}	4.34	< 0.01
Duodenum	I	25.6^{b}	9.8^{a}	17.5^{ab}	15.3^{a}	3.44	0.05
	B	5.4	5.0	4.5	4.8	0.58	NS
Duodenum scraped	I	24.0^{b}	15.4^{ab}	24.9^{b}	13.0^{a}	4.03	0.14
	B	2.3^{b}	2.9^{b}	2.2^{b}	0.7^{a}	0.59	0.11
Ileum	I	21.1^{b}	16.4^{a}	12.3^{a}	17.1^{a}	2.99	0.09
	B	6.1	4.9	4.1	3.0	0.90	NS
Ileum scraped	I	74.0^{b}	59.9^{ab}	44.4^{a}	46.7^{a}	7.34	0.06
	B	1.37	1.48	1.33	1.30	0.233	NS
Rumen	I	16.7	21.3	14.6	19.4	4.04	NS
	B	4.5^{b}	2.8^{a}	2.2^{a}	2.0^{a}	0.35	< 0.01
Abomasum	I	23.4	20.4	19.1	13.8	3.72	NS
	B	36.6^{b}	17.2^{a}	17.7^{a}	15.4^{a}	3.83	< 0.01
Liver	I	21.8	13.3	24.2	19.2	3.74	NS
	B	3.3	1.3	2.6	1.9	0.77	NS
Pancreas	I	31.4	26.4	19.3	21.3	6.18	NS
	B	10.6^{b}	5.4^{a}	6.0^{a}	4.0^{a}	0.94	< 0.01
Muscle	I	29.2^{b}	13.5^{a}	24.3^{b}	12.8^{a}	2.42	< 0.01
	B	0.44	0.37	0.52	0.43	0.063	NS
Skin	I	18.8	17.6	10.5	17.8	3.42	NS
	B	1.95^{b}	1.27^{a}	1.14^{a}	0.97^{a}	0.234	0.06

[a, b] LS means within rows with a common superscript letter do not differ significantly (Pr < 0.10).
NS = non-significant, Pr > 0.15.

TABLE 5.8. Fractional synthetic rates (FSR; %/day) of protein in tissues using the plasma (P) and intracellular pool (I) as precursor pools in lambs fed pasture, lucerne, sulla or lucerne:sulla (n = 4). Results are presented as least-square (LS) means and SEM. Probability (Pr) values are given for comparisons of diet, pasture with other diets and diets containing condensed tannins (CT) with diets containing no CT.

							Diet effect	Pasture vs. others	CT vs. no CT
Tissue	Precursor Pool	Pasture	Lucerne	Sulla	Lucerne:Sulla	SEM	Pr	Pr	Pr
Duodenum	P	19.3	27.8	23.3	30.7	3.96	NS	0.11	NS
	I	68.7	165.4	123.8	96.1	33.05	NS	0.14	NS
Duodenum scraped	P	7.7^{ab}	16.3^{c}	11.5^{bc}	4.6^{a}	2.34	0.02	NS	0.12
	I	27.0	58.9	31.1	34.4	11.9	NS	NS	NS
Ileum	P	21.8	27.3	21.9	19.6	4.91	NS	NS	NS
	I	76.5	91.4	138.4	57.4	28.2	NS	NS	NS
Ileum scraped	P	4.9	8.2	6.8	8.4	1.33	NS	0.09	NS
	I	5.7	7.7	9.2	8.2	1.30	NS	0.10	0.15
Rumen	P	15.9^{b}	15.1^{b}	11.3^{a}	12.4^{ab}	1.42	0.13	0.10	0.03
	I	95.4	58.6	45.4	39.3	17.89	NS	0.04	0.08
Abomasum	P	127.4	97.3	92.4	100.1	21.20	NS	NS	NS
	I	474.1	271.0	397.9	400.0	114.18	NS	NS	NS
Liver	P	11.5	7.1	13.4	11.7	3.83	NS	NS	NS
	I	44.6	47.0	40.9	34.1	17.11	NS	NS	NS
Pancreas	P	37.6^{b}	29.9^{ab}	31.1^{ab}	24.4^{a}	3.56	0.13	0.05	0.12
	I	134.9	70.8	128.4	62.1	31.0	NS	NS	NS
Muscle	P	1.6^{a}	2.1^{ab}	2.7^{b}	2.8^{b}	0.428	NS	0.09	0.05
	I	4.6^{ab}	8.6^{a}	6.8^{a}	11.3^{b}	1.71	0.09	0.05	NS
Skin	P	7.0	7.4	6.1	6.2	1.50	NS	NS	NS
	I	36.6	21.6	47.6	17.2^{b}	11.23	NS	NS	NS

$^{a, b}$ LS means within rows with a common superscript letter do not differ significantly ($Pr < 0.10$).

NS = non-significant, $Pr > 0.15$.

TABLE 5.9. Differences between fractional synthetic rates (FSR; %/day) of tissues in lambs fed pasture, lucerne, sulla and lucerne:sulla (n = 4) using the plasma (P) as the precursor pools and average FSR$_P$ of tissues. Results are presented as least-square (LS) means and SEM. Probability (Pr) values are given for comparisons of tissues within diets.

Tissue	Pasture	Lucerne	Sulla	Lucerne:Sulla	SEM (Diet)	Tissue average
Duodenum	19.3^{cd}	27.8^{b}	23.3^{b}	30.7^{b}	3.96	25.3^{c}
Duodenum scraped	7.7^{abc}	16.3^{ab}	11.5^{ab}	4.6^{ab}	2.34	10.0^{ab}
Ileum	21.8^{d}	27.3^{b}	21.9^{b}	19.6^{ab}	4.91	22.6^{c}
Ileum scraped	4.9^{ab}	8.2^{a}	6.8^{a}	8.4^{ab}	1.33	7.1^{ab}
Rumen	15.9^{bcd}	15.1^{ab}	11.3^{ab}	12.4^{ab}	1.42	13.7^{b}
Abomasum	127.4^{f}	97.3^{c}	92.4^{c}	100.1^{c}	21.20	104.3^{d}
Liver	11.5^{abcd}	7.1^{a}	13.4^{ab}	11.7^{ab}	3.83	10.9^{b}
Pancreas	37.6^{e}	29.9^{b}	31.1^{b}	24.4^{ab}	3.56	30.8^{c}
Muscle	1.6^{a}	2.1^{a}	2.7^{a}	2.8^{a}	0.43	2.3^{a}
Skin	7.0^{ab}	7.4^{a}	6.1^{a}	6.2^{ab}	1.50	6.7^{ab}
SEM (Tissues)	5.08	7.56	5.20	10.06		3.51
Tissue effect	< 0.01	< 0.01	< 0.01	< 0.01		< 0.01

[a, b] LS means within columns with a common superscript do not differ significantly ($Pr < 0.10$).

NS = non-significant, $Pr > 0.15$.

5.5 DISCUSSION

The four diets fed to lambs differed in composition and feeding value, resulting in a three-fold range in daily gain over the eight-week outdoor experiment (Chapter 4) and similar results were observed with lambs held in crates for protein synthesis measurements. Dietary composition (g/kg DM) of NDF (241 – 464), soluble carbohydrates (119 – 224) and CP (227 – 273) affected both feed intake and nutrient supply. The improved ME and nutrient supply for lambs fed sulla and lucerne:sulla relative to ryegrass, should enable genetic potential for protein synthesis and LW gain to be expressed with the diets containing sulla. Measurements of WBPS undertaken in this study demonstrated significant differences between diets for WBPS and tissue FSR.

5.5.1 Diet nutrient supply

Intakes of lambs fed pasture were probably limited by their size (30.7 vs. 35.8 – 40 kg) and dietary fibre content (422 g/kg DM eaten). Daily intakes of fibre (g) by lambs fed pasture, lucerne, sulla and lucerne:sulla lambs were 434, 463, 310 and 376 respectively and intake (g/day) of soluble carbohydrates for the respective treatments was 122, 221, 358 and 308 with CP intakes of 233, 445, 342 and 400. Lambs fed pasture and lucerne had higher NDF intakes and slower NDF degradation rates (11 %/h; Appendix 4.5) than sulla (20 %/h) and lucerne:sulla (14.5 %/h) and had lower DM and ME intakes. Principal differences in nutrient supply included the high soluble carbohydrate: crude protein ratio for sulla (1.05) compared to lucerne (0.50) and pasture (0.52) and the high intakes of CP by lambs fed legumes. The CP in pasture and lucerne would undergo extensive degradation in the rumen relative to sulla and lucerne:sulla (McNabb *et al*., 1996) resulting in extensive NH_3 absorption and urea synthesis with the lucerne diet, whereas the CT in sulla and lucerne:sulla allows more AA to pass to the intestine (Waghorn *et al*., 1987; Bermingham et al., 2001) for absorption and entry into the portal blood (Targari and Bergman, 1978). This is demonstrated by expressing the concentration of rumen NH_3-N (mg/L) in relation to the concentration of N in the diet eaten (g/kg DM) and shows higher values for pasture (91) and lucerne (94) than sulla (59) and lucerne:sulla (61). These data show a lower rumen NH_3 concentration arising from forages containing sulla and the CT may have increased total protein supply to the intestine to a greater extent than suggested by modelling calculations. The values used to estimate MP availability (g/day) for lambs in Chapter 4 (Appendix 4.6) suggests MP supply to the lambs fed pasture, lucerne, sulla and lucerne:sulla was 105, 162, 166 and 172 g/day, respectively, without benefits of CT. Analysis in Chapter 4 showed MP was not limiting lamb production, but absorbed AA will contribute to pools and fluxes associated with whole body and tissue protein synthesis.

5.5.2 Absolute whole body protein synthesis

Liveweight gain is a measure of net accretion of protein, fat, bone and water. The gain in protein is a balance between protein synthesis and degradation, and an increase in protein synthesis relative to protein degradation results in net gain. In the whole animal, protein synthesis and degradation both increase in response to dietary protein intake and ME intake, but the increase in protein synthesis exceeds that for protein degradation (Kelly *et al.*, 1993), resulting in increased protein accretion. However, when ME intake exceeds nutrient requirements for maintenance and protein synthesis, excess energy is deposited as fat (Oddy and Sainz, 2002).

Whole body protein synthesis of lambs fed pasture (93 g/day) was less than lambs fed lucerne, sulla and lucerne:sulla (150 – 180 g/day) and estimates of WBPS of lambs in this study were comparable to reports summarised in Table 5.10. However, when Davis *et al.* (1981) and Abdul-Razzaq and Bickerstaffe (1989) estimated WBPS using leucine and tyrosine (respectively) they obtained substantially higher values compared to sulphur-AA, which casts doubt upon labelling procedures. Methodology as well as effects of diet, age and intake contributes to differences in estimates of WBPS. The importance of specific labels for estimating WBPS are highlighted in the studies where the same wethers have been infused with different labelled amino acids and absolute estimates of WBPS differ substantially, (Table 5.10; Cronje *et al.*, 1992a, b; McNabb *et al.*, 1993; Wang *et al.*, 1994b; Lee *et al.*, 1995). When either ^{35}S-cysteine or ^{35}S-methionine were used to estimate WBPS in lambs fed fresh forages estimates ranged between 26 and 372 g/day (McNabb *et al.*, 1993; Wang *et al.*, 1994b Lee *et al.*, 1995).

The estimates of WBPS in this study correspond with previous measurements made with ^{35}S-cysteine in lambs fed *Lotus spp.* or ryegrass pasture (McNabb *et al.*, 1993; Lee *et al.*, 1995). Measurements by these authors and by Wang *et al.* (1994b) demonstrated a similar degree of cysteine oxidation (10 – 15%, to sulphate) as reported here. The cysteine ILR in lambs fed ryegrass (21.6 µmol/min) were similar to values reported by Lee *et al.* (1995) for 30 kg sheep fed ryegrass (745 g DM/day) and the ILR in faster growing lambs fed lucerne, sulla and lucerne:sulla (34.7 – 41.4 µmol/min) were similar to 39.8 µmol/min reported by McNabb *et al.* (1993) in 44 kg sheep fed *Lotus pedunculatus* (1200 g DM/day).

TABLE 5.10. Estimates of absolute whole body protein synthesis (WBPS) reported in other studies with sheep.

	Animal type	Liveweight (kg)	Intake (g DM/animal/day) and diet offered	Amino acid infused	WBPS (g/day)
1	Lambs	12	Maintained on a milk diet	^{14}C-leucine	122 – 207
2	Wethers	16	725 of ryegrass/clover pasture	^{3}H-leucine	610
3	Lambs	19 – 21	709 of concentrate (barley)	^{3}H-tyrosine	347 – 400
4	Wethers	20 – 25	660 to 980 of wheat straw with 60 or 110 g protein/day	^{35}S-methionine ^{14}C-lysine ^{3}H-leucine	54 – 104 43 – 61 42 – 110
5	Wethers	26 – 35	300, 600 or 900 of grass pellets 300 and 900 of grass pellets	^{3}H-phenanlynine ^{13}C-leucine	144; 191; 239 161; 281
6	Wethers	30	614 of *L. penduculatus;* 745 of ryegrass; 900 of *L. corniculatus*	^{35}S-cysteine ^{35}S-methionine	70 – 104 111 – 372
7	Wethers	35	671 or 1427 of lucerne pellets + concentrate	^{14}C-leucine	227 – 281
8	Wethers	35 – 47	0.4 x M and 1.1 x M of milled oaten hay with lupin seed	^{3}H-Phenanlynine	156 – 308
9	Ewe	32 – 69	Maintained with intra-ruminal VFA and casein infusion	^{3}H-leucine	250 – 283
10	Wethers	44	1200 – 1300 of *L. pedunculatus* (with and without PEG)	^{35}S-cysteine ^{35}S-methionine	84 – 177 100 – 166
11	Wethers	40 – 50	927 – 1400 lucerne hay	^{14}C-tyrosine	330
12	Wethers	48	985 *L. corniculatus* (with and without PEG)	^{35}S-cysteine ^{35}S-methionine	26 – 58 143 – 177
13	Ewes	53	Lucerne and barley	^{13}C-leucine	280
	This study	32 – 40	*Ad lib* pasture, lucerne, sulla and lucerne:sulla	^{35}S-cysteine	93 – 180

[1] Oddy *et al.* (1987); [2] Davis *et al.* (1981); [3] Abdul-Razzaq and Bickerstaffe (1989); [4] Cronje *et al.* (1992a), Cronje *et al.* (1992b); [5] Harris *et al.* (1992); [6] Lee *et al.* (1995); [7] Caine and Mathison (1992); [8] Adams *et al.* (2000); [9] Inkster *et al.* (1989); [10] McNabb *et al.* (1993); [11] Harris *et al.*, (1989); [12] Wang *et al.* (1994b); [13] Krishnamurti and Janssens (1988);
PEG, polyethylene glycol to remove the effects of condensed tannins in *Lotus spp*. For definitions of abbreviations see List of Abbreviations.

Validity of the WBPS data can be tested against net costs of protein synthesis, reported at 4.5 kJ/g protein synthesised (MacRae and Lobley, 1986; or 28 kJ/g N synthesised; Lobley *et al.*, 1987) which is similar to stoichiometry estimates for the cost of peptide bond synthesis (Kelly *et al.*, 1993). The lambs in this study consumed 10.0 – 20.3 MJME/day, and synthesis of 93 g and 180 g protein/day (pasture and sulla, respectively) required between 3.4% (sulla) and 4.2% (pasture and lucerne:sulla) of ME intake. This value is much lower than suggestions that protein synthesis accounts for about 20% of energy expenditure in young ruminants (MacRae and Lobley, 1986). The low energy expenditure in this study may be due to the partitioning of cysteine between the extracellular and intracellular free amino acids (Simon *et al.*, 1978; Lobley *et al.*, 1980). Our estimates of WBPS were based on the extracellular pool rather than intracellular pools, and estimates of protein synthesis calculated from extracellular pools are considered minimum values (Lobley, 1993). This was supported by Davis *et al.* (1981) where WBPS calculated from leucine SRA (200 g/day) was one third the rate calculated from intracellular free leucine SRA (600 g protein/day). Muscle is the largest component of whole body protein (45%; Lobley *et al.*, 1980) and the slow turnover rate in conjunction with a short infusion (8 hours) may underestimate absolute WBPS. The choice of amino acid may have also contributed to the discrepancy between estimated WBPS and protein energetic costs as different AA give different estimates of protein synthesis (Cronje *et al.*, 1992a, b; Table 5.10).

Daily gain (g) of lambs during the protein synthesis measurement (Table 5.4) was similar to those fed outdoors, at about 110 g/day for pasture and 220 – 300 g/day for other diets. Protein content of LW gain in lambs is about 150 – 180 g/kg empty LW (Abdul-Razzaq and Bickerstaffe, 1989; MacRae *et al.*, 1993; Adams *et al.*, 2000) and net protein accretion is about 17, 31, 46 and 42 g/day for pasture, lucerne, sulla and lucerne:sulla diets, respectively (Table 5.11). These data, with estimates of WBPS, enable calculation of protein degradation and the values for lambs fed pasture are in line with expectations based on feeding level (Lobley, 1993). The low protein degradation rate for lambs fed sulla, despite a high LW gain may be a consequence of low protein intake (and associated reductions in degradation; Harris *et al.*, 1992). The high concentration of soluble carbohydrate, low fibre and low protein degradation characteristic of sulla suggests more efficient use of nutrients for protein accretion compared to lambs fed other diets.

TABLE 5.11. Estimates of absolute whole body protein accretion (PA), protein synthesis (PS[1]), protein degradation (PD[2]) in g/day relative to metabolisable energy (ME; MJME/day) and crude protein (CP; g/day) intake of lambs fed pasture, lucerne, sulla and lucerne:sulla.

Diet	Intake		LW	LW gain	PA	PS[1]	PD[2]
	ME	CP	(kg)	(g/day)		g/day	
Pasture	10.0	233	32.4	116	17.4	92.6	75.2
Lucerne	16.8	445	39.8	207	31.1	150.7	119.7
Sulla	20.3	342	42.5	308	46.2	152.3	106.1
Lucerne:sulla	19.4	400	43.1	281	42.2	180.0	138.9

[1] Assume 150 g/kg empty LW gain

[2] PD values are the difference between PS and PA.

5.5.2 Fractional synthesis rates of tissues

The contrasting diets fed to lambs affected differences in voluntary feed intake and LW gain and consequently a range of WBPS rates were anticipated. Although protein gain associated with growth of lambs fed legumes will contribute to differences in WBPS with the four diets, values for individual organs may indicate differences in function associated with specific diets and help explain any effects of diet on the efficiency of feed utilisation for LW gain. In theory, the sum of protein synthesis for all organs should equal WBPS.

The choice of tissues sampled for estimating FSR included the largest protein masses (muscle and skin) as well as metabolically active visceral organs because they have a higher FSR which may be affected by digesta load (Lobley *et al.*, 1994) and diet composition (especially dietary protein content; Wykes *et al.*, 1996; Lescoat *et al.*, 1997). Dietary CT increase intestinal mucous secretion in pigs and poultry (Waghorn, 1996) but FSR of intestinal tissues with and without the mucosal layer were similar for lambs fed sulla and lucerne:sulla suggesting the CT did not appear to increase mucous secretion.

The secretion of mucous proteins from gut tissues (duodenum) increases FSR relative to gut with secreted mucous proteins removed (unscraped vs. scraped tissues; Lobley *et al.*, 1994). McDougall (1966) and Fauconneau and Michel (1970) have estimated synthesis of secretory proteins at 20 – 200 g/day.

Measurements of tissue FSR have focussed on plasma precursor pools rather than the intracellular precursor pool (Tables 5.8 and 5.9) for protein synthesis, partly because large differences between lambs in estimates based on the SRA_I lessened confidence in these data. The most appropriate pool would be that of amino acyl tRNA. Measurement of this pool is not technically feasible because tRNA has a very short half-life and very low tissue concentration (Davis *et al.*, 1989). Most investigators have assumed that amino acid SRA in plasma or intracellular pools represents the amino acid acyl tRNA, but argue that in some tissues the plasma precursor pool may underestimate FSR while the intracellular pool may overestimate tissue FSR, especially in liver (Davis *et al.*, 1989; Lobley 1993; Connell *et al.*, 1997).

The precursor pool used to calculate FSR for tissues is a function of the nature of the protein being synthesised in the tissue, for example constitutive or export proteins. The true precursor pool for tissues involved in the synthesis of export proteins, for example duodenum, ileum, abomasum, rumen, liver and skin is more likely to be the plasma pool and FSR_P will provide an adequate estimate for these tissues (Davis *et al.*, 1989; Lobley, 1993). Tissues involved in constitutive protein synthesis (eg. muscle) will provide better estimates of synthesis from the intracellular pool than the plasma pool, but muscle has a relatively slow turnover rate, so the differences between FSR estimated from both pools should be relatively small (Davis *et al.*, 1989). However, most studies report FSR from both pools (Table 5.12) due to the uncertainty as to which is correct, if either (Davis *et al.*, 1989; Lobley *et al.*, 1993). FSR estimated from both pools give the minimum (FSR_P) and maximum estimates (FSR_I) to illustrate the approximate bounds within which the true rate of protein synthesis is assumed to lie (Davis *et al.*, 1989; Lobley, 1993).

Tables 5.12a and b summarises tissue FSR from studies using both precursor pools and shows the data measured in this trial to be comparable with published estimates for sheep fed a range of diets, except FSR of abomasal tissue which was much higher in this trial compared to other studies. The FSR_I were typically two to five fold higher than FSR_P estimates in all tissues, except scraped ileum where estimates were similar (7.7 vs. 7.1 %/day).

The mass of body protein, with FSR_P, have been summed (Table 5.13) and compared with estimates of WBPS based on ^{35}S-cysteine infusion. The calculations include published FSR_P of tissues not measured in this study (eg. large intestine, bone, lungs, kidney etc.) and values ranged from 182 (pasture) to 256 (lucerne:sulla) g/day. Estimates based on FSR_P for individual tissues are 97% higher than WBPS for pasture, 57% for lucerne and 56% for sulla and lucerne:sulla. The differences in protein synthesis estimated by the two techniques is significant but the values derived from tissue FSR are in line with data presented in Table 5.10 for lambs of similar LW fed at or above maintenance.

TABLE 5.12a. Estimates of fractional protein synthesis rates (%/day) of tissues in sheep reported in the literature.

	Animal	Daily intake and diet	Pool	Duodenum		Ileum		Rumen	Abomasum	Liver	Muscle	Skin
				Unscraped	Scraped	Unscraped	Scraped					
1, 2	4 kg lambs	Ad lib milk	P	78		75		26	50	98	18	20
			I	86		84		30	56	115	23	24
3	20 kg lambs	NA	I					168		240	25	
4	20 kg lambs	*Ad libitum* fresh grass	P					14		15	3	12
			I					79		54	4.5	35
5	32 kg lambs	709 g barley concentrate	I	Entire PSI = 99.2 – 115.8				29 – 34	23 – 28	45 – 52	3.0 – 3.2	
6	33 kg sheep	300 – 900 g grass pellets	P								0.9 – 3.0	
7	34 kg wethers	1.2 x M mixed concentrate pellets	P	55 – 108		34 – 164				25 – 54		
			I	64 – 121		42 – 219				45 – 84		
8	35 – 38 kg wethers	720 and 1440 g of grass pellets	I	60 – 68	42 – 45	47 – 57	31 – 41	22 – 35	24 – 28	21 – 23		
9	35 – 47 kg wethers	0.4 x M – 1.8 x M oat hay with lupin seed	I	Gut = 31 – 51				9 – 14		21 – 22	1.2 – 2.9	11 – 21
10	40 – 45 kg wethers	300 – 900 g grass pellets	P							15 – 18	0.8 – 2.3	5 – 10
			I							37 – 57	1.5 – 2.8	32 – 45
11	40 – 50 kg wethers	NA	P							10	1.7 – 1.8	
12	52 kg wethers	1200 g grass pellets	P							9 – 10		
13	58 kg wethers	1500 g barley and dried grass pea pellets	P					11		19	4.1	
			I					13		21	4.5	
	Range			55 – 121	42 – 45	34 – 219	31 – 41	9 – 168	23 – 56	9 – 240	0.8 – 25	5 – 45
	This study			19 – 165	5 – 60	20 – 138	5 – 9	11 – 95	92 – 474	7 – 47	1.6 – 11	6 – 48

[1] Attaix and Arnal (1987) and [2] Attaix *et al.* (1988), ^{3}H-valine; [3] Buttery *et al.* (1977), ^{3}H-lysine; [4] Davis *et al.* (1981), ^{3}H-leucine; [5] Abdul-Razzaq and Bickerstaffe (1989), ^{3}H-tyrosine; [6] Harris *et al.* (1992), ^{14}C-phenylalanine and ^{13}C-leucine; [7] Southorn *et al.* (1992), ^{3}H-phenylalanine; [8] Lobley *et al.* (1994), ^{13}C-valine; [9] Adams *et al.* (2000), ^{2}H-phenylalanine; [10] Lobley *et al.* (1992), ^{14}C-phenylalanine and ^{13}C-leucine; [11] Buttery *et al.* (1975), ^{3}H-lysine; [12] Connell *et al.* (1997), ^{2}H-phenylalanine; [13] Schaefer *et al.* (1986), ^{3}H-leucine;

Abbreviations: M, maintenance; P, plasma precursor pool; I, intracellular precursor pool; PSI, proximal small intestine; GIT, gastrointestinal tract; Unscraped tissue is tissue with mucosal layer; Scraped tissue is tissue without mucosal layer.

No estimates for pancreas FSR were found.

TABLE 5.12b. Estimates of fractional protein synthesis rates (%/day) of tissues in goats and cattle reported in the literature and lambs in this study.

	Animal	Daily intake and diet	Pool	Duodenum		Ileum		Rumen	Liver	Muscle	Skin
				Unscraped	Scraped	Unscraped	Scraped				
[1]	50 kg goats	370 and 1350 g meadow hay and pellets	P	21 – 28				16 – 23	11 – 13	1.9 – 2.1	1.6 – 3.0
			I	28 – 40				40 – 50	26 – 31	3.3 – 4.2	6 – 11
[2]	286 kg steers	6.6 kg concentrate-based pellets	P	18 – 21		14 – 15			8 – 9	1 – 1.5	
			I	88 – 109		92 – 73			24 – 27	1.6 – 2.8	
[3]	236 – 263 kg heifers	Ad lib mixed concentrate diet	P	GIT = 23 – 28					10 – 11	1.6 – 1.8	
			I	GIT = 52 – 53					26 – 38	1.8 – 2.1	
	Range			21 – 109		14 – 92		16 – 50	9 – 31	0.8 – 25	1.6 – 11
	This study	Lambs		19 – 165	5 – 60	20 – 138	5 – 9	11 – 95	7 – 47	1.6 – 11	6 – 48

[1] Baracos *et al.* (1991), ^{3}H-phenylalanine; [2] Eisemann *et al.* (1989), ^{14}C-leucine; [3] Lobley *et al.* (1980), ^{3}H-leucine.

Abbreviations: P, plasma precursor pool; I, intracellular precursor pool; GIT, gastrointestinal tract; Unscraped tissue is tissue with mucosal layer; Scraped tissue is tissue without mucosal layer.

No estimates for pancreas or abomasum FSR were found.

TABLE 5.13. Estimate of whole body protein synthesis (g/day) using tissue synthesis rates estimated from the plasma precursor pool (FSR_P) in Tables 5.8 and 5.9.

	Pasture	Lucerne	Sulla	Lucerne:sulla
LW (kg)	32.4	39.8	42.5	43.1
Empty LW[1] (kg)	28.4	35.8	38.2	39.8
Muscle in carcass[2]	22.4	37.1	51.2	54.0
Skin (includes wool)[3]	27.8	37.1	32.9	33.9
Stomach[4]	35.2	36.6	34.3	37.9
Small intestine[5]	16.2	27.4	24.1	27.3
Liver[6]	9.0	7.0	14.2	6.7
Pancreas[7]	4.6	4.5	5.0	4.1
Total (g/day)	**115.2**	**149.6**	**161.9**	**163.9**
Plus other tissues				
Large intestine[8]	5.9	7.5	8.1	8.2
Kidneys[9]	1.9	2.4	2.6	2.6
Other tissues[10]	14.9	18.8	20.2	20.5
Bone[11]	36.7	46.3	49.8	50.6
Hooves and head[12]	7.7	9.7	10.4	10.6
Total (g/day)[13]	**182.4**	**234.4**	**252.9**	**256.4**

[1] Digesta is assumed to be 4 kg (Abdul-Razzaq and Bickerstaffe, 1989)

[2] 29% of carcass muscle (Carcass is 64% muscle and carcass was 45% of empty LW (Davies, 1989); 17% protein (Adams *et al.*, 2000); FSR in Table 5.8.

[3] 10% of empty LW (Fogarty *et al.*, 1992; Adams *et al.*, 2000); 14.5% protein (Adams *et al.*, 2000); FSR in Table 5.8.

[4] Rumen + abomasum are 3.6% of empty LW (Burrin *et al.*, 1990); 9% protein (Adams *et al.* , 2000; Lobley *et al.*, 1994); FSR from Table 5.8 (Rumen:abomasum = 5:1; Burrin *et al.*, 1990)

[5] 2.5% of empty LW (Burrin *et al.*, 1990); 11.1% protein (Adams *et al.*, 2000; Eisemann *et al.*, 1989; Lobley *et al.*, 1994); average FSR of duodenum and ileum with mucosa in Table 5.8.

[6] 2% of empty LW (Burrin *et al.*, 1990); 13.8% protein (Lobley *et al.*, 1994; Adams *et al.*, 2000; FSR from Table 5.8.

[7] Estimated at 0.3% of empty LW; estimated at 14% protein; FSR from Table 5.8.

[8] 1.7% of empty LW (Burrin *et al.*, 1990); 8.2% protein (Lobley *et al.*, 1994); 15% FSR (Baracos *et al.*, 1991).

[9] 0.3% of empty LW (Baldwin, 1995); 14% protein (estimated to be same as liver); 16% FSR (Baracos *et al.*, 1991).

[10] Heart, lungs, spleen, nervous tissue etc were 5% of empty LW (Jenkins and Leymaster, 1993; Baldwin, 1995); estimated at 15% protein; 7% FSR (Bermingham, unpublished, Baracos *et al.*, 1991)

[11] 13% empty LW (Grace, 1983); 20% protein (Young and Sykes, 1985); 3% FSR assumed based on carcass FSR reported by Lobley *et al.* (1980).

[12] Hooves and head (6% of empty LW; Jenkins and Leymaster, 1993); estimated at 15% protein; 3% FSR based on carcass FSR reported by Lobley *et al.* (1980).

[13] Blood (5% of empty LW) was not included in calculations. These estimates account for 78% of empty LW and the remaining 22% is predominantly fat (14% of empty LW; Broad and Davies, 1980; Baldwin, 1995).

5.5.2.1 Effect of diet

There were no significant effects of diet on FSR for most tissues, in part because of animal variation. Notable exceptions were muscle and rumen tissues, although FSR of scraped duodenum of lambs fed pasture were lower than other diets and pancreas FSR were higher in lambs fed pasture than lambs fed other diets. Diets containing sulla appeared to have lower rumen FSR compared to lucerne or pasture, but this result contradicts studies where high intakes (Lobley *et al.*, 1994) and low acetate:propionate ratios (Abdul-Razzaq and Bickerstaffe, 1989) have increased rumen FSR.

Muscle protein synthesis is very responsive to intake (Lobley *et al.*, 1992) and was lower in lambs fed pasture compared to the other feeds. Liveweight gain is largely attributable to muscle growth and differences in muscle protein FSR should be comparable to differences observed in whole body protein synthesis and LW gain.

Abdul-Razzaq and Bickerstaffe (1989) showed diets resulting in low acetate:propionate ratios increased muscle FSR. They attributed high muscle FSR to elevated blood glucose fluxes which stimulated insulin release and increased protein synthesis (Chrystie *et al.*, 1977; Bassett, 1978; Abdul-Razzaq and Bickerstaffe, 1989). The low acetate:propionate ratio in lambs fed sulla support this theory, but feed intake of these lambs was also high, especially compared to pasture. Muscle FSR was probably a response to all of these factors. FSR calculated from both SRA_P and SRA_I pools support significantly higher muscle FSR in lambs fed legumes than pasture.

5.6 CONCLUSIONS

Diets with contrasting composition and feeding value affected protein synthesis. WBPS (g/day) and muscle FSR (%/day) were lower in lambs fed pasture than lucerne, sulla and lucerne:sulla and estimates were comparable to differences in feed intake and LW gain. Muscle and rumen were the only tissues significantly affected by diet quality and feed intake, and CT did not affect FSR of tissues. Combining sulla with lucerne did not elicit any advantages in terms of protein synthesis above that of lucerne, possibly because energy intake limited the utilisation of extra protein supply.

CHAPTER 6

DIGESTION KINETICS OF FRESH FORAGES AND MIXTURES IN LACTATING DAIRY COWS

CHAPTER 6: DIGESTION KINETICS OF FRESH FORAGES AND MIXTURES IN LACTATING DAIRY COWS

6.1 ABSTRACT

In sacco and *in vitro* techniques have been used to define the digestion and fermentation kinetics of forages with diverse structural and chemical compositions to identify forages able to be fed with pasture and optimise dairy cow production. That work has involved one cow fed one diet, and overseas research has shown that both the cow and diet can affect the microbial populations in the rumen and influence rate of digestion. There is no information about cow and diet effects on *in sacco* and *in vitro* digestion kinetics of fresh forages. The aim of this study was to compare the digestion kinetics of pasture (P), sulla (S), maize silage (M) and mixtures of pasture:maize silage (P:M), pasture:sulla (P:S) and pasture:maize silage:sulla (P:M:S) in rumen fistulated cows fed each of these diets. All forages and mixtures were minced to a particle size similar to chewed material, and incubated *in sacco* and *in vitro* using fistulated cows fed pasture and pasture mixtures. Rates of digestion were determined *in sacco* and rumen inoculum from the same cows fed the four diets were used to determine net ammonia (NH_3) released by proteolysis and volatile fatty acid (VFA) yields and changes in pH. Degradation rates for DM, protein and fibre were fastest for S (16.3, 22.1 and 9.4 %/h), slowest for M (3.6, protein not determined and 3.3 %/h) and pasture in between (7.1, 11.7 and 5.8 %/h). Including M in the P diet reduced degradation rates of all components. Dry matter, protein and fibre *in sacco* degradation rates, averaged across all forages and forage mixtures were slowest when the P:M diet was fed (7.7, 12.1 and 5.4 $/h, respectively). In contrast DM, protein and fibre degradation rates (%/h) averaged for all forages were 9.0, 16.1 and 6.5 in cows fed pasture, 8.4, 13.9 and 6.6 for cows fed P:S and 7.9, 13.5 and 6.9 when cows were fed P:M:S. *In vitro* incubations showed the average net NH_3 production from each forage and forage mixture incubated over 24 hours was always less with rumen inoculum from cows fed P:M than other diets. Digestion kinetics of fresh forages were affected by cow-diet and lowered when M was included in the diet. Use of the kinetic and fermentation data for CNCPS

demonstrated poor predictive capacity of CNCPS for intake and the importance of small changes in forage composition for limiting nutrients.

6.2 INTRODUCTION

The productivity of ruminants can be optimised by balancing nutrient requirements with nutrient supply. In North America, total mixed rations (TMR) are formulated for high producing dairy cows using ration balancing models that require information on feed composition, digestion and fermentation in the rumen, and the more advanced models require information on the utilisation of nutrients. This information is readily available overseas, but in New Zealand where grazed pasture and fresh forages are the main diet of dairy cows, very little of this information exists. Recent research in New Zealand has used *in sacco* and *in vitro* techniques to define the digestion and fermentation kinetics of forages with diverse structural and chemical compositions (Chapter 3; Barrell *et al*., 2000), but this work has involved one cow fed one diet (Chapter 3). Weimer *et al*. (1999) demonstrated effects of both cow and diet on the the population of cellulolytic bacteria in the rumen and this influenced rate of digestion *in vitro* (Mertens *et al*., 1998), and Waghorn and Caradus (1994) showed small differences between cows in the disapperance of fresh white clover DM from nylon bags.

Dairy cow production can be improved if pasture is supplemented with forages with nutritional characteristics that give them a higher feeding value than pasture. Several studies have shown that feeding forages with medium quality pasture can improve production at specific times of the year (Table 2.11 in Chapter 2; Burke *et al*., 2002a, b; Waghorn, 2002), but the challenge is to determine which forage or forages will optimise dairy cow production. Degradation kinetics, products of fermentation and use of ration-balancing models enabled formulation of diets to meet nutrient demands, but information is needed to identify whether the effects are additive or synergistic when forages are mixed together. In Chapter 3 the Cornell Net Carbohydrate and Protein system (CNCPS) was used to predict the production of late lactation dairy cows fed medium quality pasture and mixtures containing two-thirds pasture with one-third maize silage (P:M), sulla (P:S) or maize silage and sulla (P:M:S) using nutrient composition and protein and neutral detergent fibre (NDF) degradation rates of ryegrass, white clover, maize silage and sulla measured in Chapter 3. Potential milk production from cows fed pasture (P; 80% ryegrass:20% white clover), P:M, P:S and P:M:S in late lactation was predicted to be 16.4, 16.2, 15.9 and 16.5 kg milk/day, respectively (Table 3.24). The limiting nutrient in the P:M diet was predicted to be metabolisable protein (MP) while metabolisable energy (ME) was the limiting nutrient for the P:S diet. Predicted production from cows fed the P and P:M:S diets were similar for ME and MP suggesting these diet supplied the ideal balance of ME and MP for optimal milk production.

The objectives of this study were to

1. determine milk production and composition differences between cows fed forage mixtures;
2. compare the digestion and fermentation kinetics of individual forages and mixtures of forages, and;
3. to identify cow-diet effects on degradation and fermentation kinetics of forages and mixtures.

6.3 METHOD

6.3.1 Experimental procedure

Thirty-two multiparous Friesian cows with an average liveweight of 514 kg (included 16 with rumen fistulae) were fed four dietary treatments and milk yield and composition were measured in a trial undertaken by Woodward *et al*. (unpublished) at Dexcel, Hamilton, New Zealand. The work described here was complementary to the production measurements and focussed on the cows with rumen fistulae. Rumen pH, ammonia (NH_3) and volatile fatty acid (VFA) concentrations were measured in the 16 fistulated cows, and degradation and fermentation kinetics of the dietary treatments were measured using one fistulated cow from each dietary treatment. The trial composed an initial 'uniformity period' followed by two 'feeding periods' when supplements were provided with pasture. All procedures were reviewed and approved by the Crown Research Institute Animal Ethics Committee in Hamilton, New Zealand according to the Animals Protection Act (1960) and Animal Protection Regulations (1987) and amendments.

6.3.2 Animals and treatments

Thirty-two mid-to late-lactation Friesian cows were allocated to four dietary treatments and balanced for current milk yield and liveweight (LW). The four diets (on a DM basis) were pasture only (P; 100% of diet), pasture:sulla (P:S; 66:34), pasture:maize silage (P:M; 66:34) and pasture:maize silage:sulla (P:M:S; 66:17:17) and measurements were made during two feeding periods of 13 days each (Period A = 25 February to 9 March and period B = 10 March to 22 March 2001). For the first five days of the feeding period cows grazed outside with S, M and M + S provided, after which they were brought indoors and fed their allocated treatment for eight days. Measurements were taken on the last five days of each indoor period.

Before cows were fed their allocated dietary treatment they were grazed together on pasture for seven days (uniformity period). On the last two days of this uniformity period, milk yield and composition were measured for all cows. Rumen pH and NH_3 concentration were measured in the 16 fistulated cows and *in sacco* dry matter (DM) loss of lucerne hay was measured in one fistulated cow to be allocated to each treatment (4 cows). These data were used to compare cows prior to feeding the four dietary treatments.

Four fistulated cows were included in each group of eight cows fed each dietary treatment. Cows were re-allocated to treatments between feeding periods A and B (balanced for milk yield and LW) with only two cows (including one fistulated cow) from each treatment remaining on the same diet during both periods. The one fistulated cow that remained on the same treatment in both periods was used for *in sacco* and *in vitro* incubations.

Digestion and fermentation kinetics were made for each dietary mixture fed to the cows and for components of the diets: pasture (P), sulla (S), maize silage (M) and mixtures (pasture:maize silage; P:M, pasture:sulla; P:S and pasture:maize silage:sulla; P:M:S). Measurements were made from both *in sacco* and *in vitro* using the four fistulated cows. *In vitro* and rumen parameters included pH, NH_3 and VFA concentrations in association with *in sacco* incubations from four fistulated cows, and during the second feeding period (B) from 16 fistulated cows on two occasions at 0800, 1300 and 1600 hours, rumen pH and rumen NH_3 and VFA concentrations were determined. The sequence of events for the trial are given in Table 6.1.

TABLE 6.1. Schedule of events for the uniformity and indoor feeding trial from 19 February to 22 March 2001.

Day	Event
Uniformity period - 19 to 24 February 2001 (Days 1 – 8)	
7-8	Milk yield and composition measured on all 32 cows
	In sacco DM loss of lucerne hay measured on 4 fistulated cows, and rumen pH and ammonia (NH_3) measured on 16 fistulated cows.
Dietary treatment period	
Period A - 25 February to 9 March 2001 (Days 9 – 21)	
9 to 13	All cows allocated to treatments and grazed with appropriate supplements.
14 to 21	Cows brought indoors and fed treatments; dry matter intake was recorded.
17 to 21	Milk yield and composition measured over a 5-day period.
16 to 19	*In sacco* (72 h) and *in vitro* (24 h) incubation of pasture (P), sulla (S), and maize silage (M) in four fistulated cows.
18 to 21	*In sacco* (72 h) and *in vitro* (24 h) incubation of pasture:maize silage (P:M), pasture:sulla (P:S) and pasture:maize silage:sulla (P:M:S) in four fistulated cows.
Period B - 10 to 22 March 2001 (Days 22 – 34)	
22 to 26	All cows reallocated to treatments and grazed with appropriate supplements.
27 to 34	Cows brought indoors and fed treatments; dry matter intake was recorded.
30 to 34	Milk yield and composition measured over a 5-day period.
29 to 32	*In sacco* (72 h) and *in vitro* (24 h) incubation of P:M, P:S and P:M:S in four fistulated cows.
31 to 34	*In sacco* (72 h) and *in vitro* (24 h) incubation of P, S, and M in four fistulated cows.
32 and 33	Rumen samples taken from all fistulated cows and rumen pH, ammonia, volatile fatty acid concentrations and particle size distribution measured.

6.3.3 Feeding regimens

6.3.3.1 Grazing

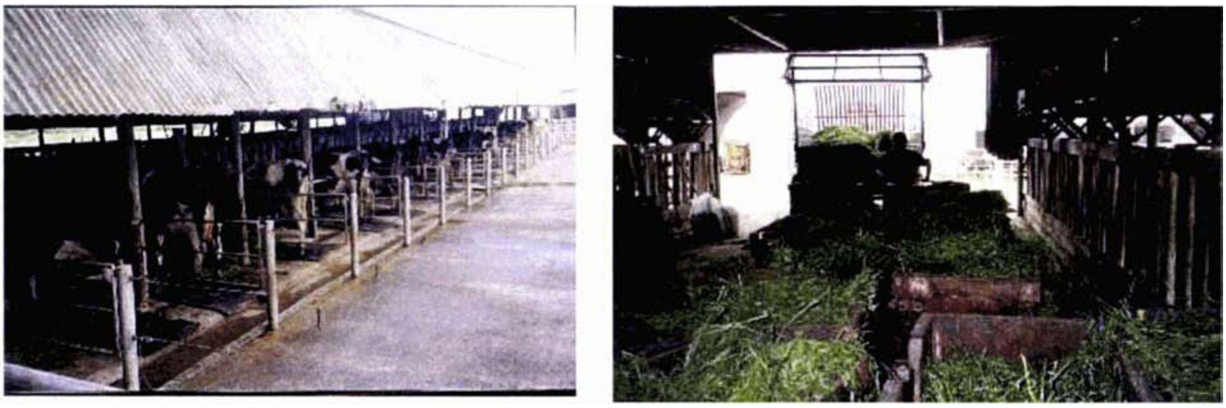
During the first five days of each feeding period, cows grazed ryegrass/white clover pasture and were given access to appropriate supplements. Cows allocated to the P treatment grazed swards all day, whereas those on P:S grazed an area of S which provided 5 kg DM/cow for three hours after the morning milking before returning to P for the remainder of the day. Maize silage (5 kg DM/cow) was fed to cows on the P:M treatment in portable feed troughs while cows were grazing P. Cows fed P:M:S were able to graze S for approximately 2 hours after the morning milking (about 2.5 kg S DM/cow) and were given access to M in portable feed troughs throughout the day while grazing P.

6.3.3.2 Indoor feeding

For the last eight days of each period cows were brought into a covered barn and individually fed from large bins (Photograph 6.1). Feed was available *ad libitum* with allocation of pasture and supplements based on a rapid (microwave) estimate of herbage DM (Appendix 6.1). Dry matter intake of cows was calculated by weighing herbage offered and refused at each feeding, with DM measurements, and effects of treatment on DM intakes were based on the last five days of each indoor period. Sulla and maize silage were not separated from the P:M, P:S and P:M:S refusals but care was taken to obtain a representative sample for DM determination.

Cows were fed P twice daily (0800 and 1700 h) while indoors. Sulla and/or M were fed once per day (0800 h) and were placed on top of the P in the feed bins. Fresh P was cut twice daily, fresh S was cut once per day and M was transported once daily from the silage stack.

PHOTOGRAPH 6.1: Indoor feeding of cows.



6.3.3.3 Collection and analysis of herbage, milk and rumen contents

DM content of all dietary components, offered and refused were determined by oven drying at 95°C for 48 hours. Dietary components offered were also sampled daily and bulked over the measurement period, dried at 60°C for 48 hours, and chemical composition (crude protein, CP; soluble carbohydrate; lipid; acid detergent fibre, ADF; neutral detergent fibre, NDF; organic matter digestibility, OMD; and metabolisable energy, ME) determined by Near InfraRed Spectroscopy (NIRS). Lignin concentration (acid detergent lignin, ADL) of forages and mixtures was determined by sequential extraction described by Chaves *et al*. (2002b).

Milk yield and concentrations of fat and protein were determined daily (pm + am sample) during the five day measurement period and on two days of the uniformity period. Samples were analysed for fat and protein concentration using an infrared milk analyser (Milkoscan 133B, Foss Electric, Hillerod, Denmark).

Digesta from the mid-rumen were collected from 16 fistulated cows at 0900, 1300 and 1600 hours on two days during the uniformity period and on day 32 and 33 of the trial. Rumen digesta was squeezed through cheesecloth to obtain liquor for pH, NH_3 and VFA measurements. Samples of rumen contents were also frozen and stored to determine particle size distribution of the DM as described in Chapter 3.

6.3.4 Digestion and fermentation kinetics

6.3.4.1 Uniformity period

The ruminal characteristics of all 16 fistulated cows were determined prior to treatment allocation by measuring pH and NH_3 concentrations at 0900, 1300 and 1600 hours on two consecutive days during the uniformity period (Table 6.1). Digestion kinetics were determined in the four cows to be fed the same diet throughout both feeding periods by incubating a minced lucerne hay standard. The *in sacco* digestion of minced lucerne hay in dacron bags (100 x 100 mm) required eight bags to be placed in each of four fistulated cows and four bags were removed after 6 and 24 hours to determine DM loss.

6.3.4.2 Dietary treatment period

The individual forages, P, M, S and mixtures of P:M, P:S and P:M:S were incubated *in sacco* in each of the four fistulated cows and *in vitro* using rumen inocula from each of the four fistulated cows during both feeding periods. *In sacco* and *in vitro* incubations were carried out simultaneously, with three forages being evaluated in two sequential time periods. Pasture, M and S were evaluated on days 16 to 19 and 31 to 34 of the trial and the mixtures were evaluated on days 18 to 21 and 29 to 32 of the trial. The same fistulated cow from each treatment was used for all *in sacco* incubations and provided rumen inoculum for *in vitro* incubations during feeding periods A and B. The experimental design for the digestion and fermentation kinetics was a split-plot design with cow-diet as the main-plot and forage and feeding period as the sub-plots.

Sample preparation

Forages were prepared using methods described in Chapter 3. Briefly, representative samples of fresh P, S and M were frozen immediately after collection and maintained frozen until used in *in sacco* and *in vitro* incubations. Frozen P and S were chopped to about 30 mm to facilitate mincing (whilst frozen) in a Kreft Compact meat mincer R70 (Kreft, Gmbh), but M was already 30 mm or less in length. Chopped P, M and/or S were mixed in the same ratios as that fed to cows (DM basis) before being minced. The frozen minced material was either sealed into dacron bags for *in sacco* incubation, or weighed into bottles for *in vitro* incubation.

Samples of minced P, M and S were retained to measure DM content (95°C for 24 h), chemical composition and particle size distribution by wet sieving (Chapter 3; Waghorn, 1986). Chemical composition was determined by drying P, S and M samples at 60°C for analysis by NIRS. DM content and chemical composition of mixtures were calculated from DM content and chemical composition of individual forages (P, M and S) and the ratio of each forage in mixtures.

In sacco

About 30 g of minced forage (approximately 6 g DM) was placed in 100 x 100 mm dacron bags (mean pore size of 35 μm). Ten bags of each forage were placed in the rumen of the fistulated cows on each diet and duplicate bags removed at 2, 6, 12, 24 and 72 hours. Duplicate samples of minced lucerne hay were included as a standard in all *in sacco* incubations of forages and were removed after 12 and 24 hours to monitor variation between periods. Removal at each time was facilitated by putting two bags of each forage or mixture in lingerie bags (6 dacron bags/lingerie bag; 8 bags at 12 and 24 h). Immediately after removal from the rumen (including the 0 h bags not placed in the rumen), bags were hand-rinsed with cold water until no further colour appeared. Bags were dried at 60°C for 48 hours, weighed and residues removed for analysis. Residues and forages were analysed by NIRS to estimate CP, soluble carbohydrate and fibre (ADF and NDF) contents (Table 3.1 in Chapter 3; Appendix 3.3).

In vitro

About 2.5 g of freshly minced forage (approximately 0.5 g DM) was weighed into 50 mL bottles (14 bottles for each forage) and warmed to 39°C with 12 mL of buffer, 0.5 mL of reducing agent and 3 mL of strained rumen liquor from each cow on each treatment, as described in Chapter 3. Bottles were placed in a shaking incubator (90 oscillations/minute) for the duration of the incubation. Duplicate bottles of each forage were sampled at 0, 2, 4, 6, 8, 12 and 24 hours of incubation with bottles at 2, 4 and 6 hours being opened for sampling and re-sealed for sampling again at 8, 12 and 24 hours, respectively. Freeze-dried and ground (FDG) lucerne was used as a standard and was included in each *in vitro* incubation to monitor variations between incubations by measuring pH and NH_3 at 2 and 8 hours and VFA concentration at 8 hours.

The pH was measured at 8, 12 and 24 hours of incubation. Ammonia concentration was determined at 0, 2, 4, 8, 12 and 24 hours of incubation and VFA concentration measured in samples taken at 6, 12 and 24 hours of incubation. Rumen liquor used in the incubation was also sub-sampled to determine pH and rumen NH_3 and VFA concentrations. The amount of NH_3 and VFA in rumen liquor was subtracted from quantities in *in vitro* bottles so that net production from fermentation could be determined.

6.3.4.3 Chemical analyses

The pH of strained rumen liquor and *in vitro* incubation media were measured using a MeterLab® (PHM210, Radiometer Pacific Limited, Copenhagen). The meter was recalibrated immediately prior to each set of measurements.

Ammonia concentration was determined in sub-samples (1 mL) of incubation media and rumen liquor that were acidified with 15 μL of concentrated hydrochloric acid (HCl), mixed and centrifuged (14,000 x *g*; 15 minutes; HermleZ160M). The supernatant was transferred and frozen prior to enzymatic reaction using a Sigma Chemical's kit (Cat. # 171-C) (Bergemeyer and Beutler, 1985) and colourimetric determination (Cobas Fara II; Hoffman LaRoche, Basel; Appendix 6.2). Ammonia concentration from *in vitro* incubations was expressed as net production per forage nitrogen (N) incubated.

Sub-samples (1.5 mL) of strained rumen liquor and incubation media combined from duplicate *in vitro* bottles, were centrifuged (14,000 x *g*; 15 minutes) and the supernatant frozen for analysis. VFA concentrations were determined by gas liquid chromatography described by Attwood *et al.* (1998) (Appendix 3.6) and expressed in relation to forage DM incubated and as molar ratios.

6.3.5 Statistical analysis

Analyses evaluated effects of diet (P, P:M, P:S, P:M:S) on milk production of the 32 cows and rumen parameters of the 16 rumen fistulated cows.

Constraints imposed by the experimental design did not allow dietary treatment effects to be separated from cow effects for any parameter involving the four fistulated cows during feeding periods A and B, so all data from these animals are termed cow-diet effects. Cow-diet effects on rumen parameters of the four cows used for *in sacco* and *in vitro* incubations and *in sacco* degradation rates and products of *in vitro* fermentation were evaluated. These analyses included *in sacco* losses of a minced lucerne hay standard during the uniformity period (cow-diet) and during both feeding periods (cow-diet, feeding period and interaction). The forages and mixtures (P, M, S, P:M, P:S, P:M:S) were also compared for *in sacco* and *in vitro* parameters.

Analyses of milk production and composition used the restricted maximum likelihood (REML) procedure of Genstat (Version 6.2). The model included feeding period, treatment, feeding period x treatment and the effect of cow as a fixed effect (Woodward *et al.*, unpublished). The uniformity data collected prior to diet evaluation were used as covariates in the analysis of milk production and composition.

All data were checked for normality and homogeneity and satisfied ANOVA assumptions. Cow-diet and forage effects on *in sacco* and *in vitro* incubations were compared using the GLM procedure within SAS (1996).

6.3.5.1 Cow Ruminal Characteristics

Uniformity period

To compare cows before they were fed the dietary treatments, rumen parameters (pH and NH_3 concentrations) of the 16 fistulated cows were analysed separately from the 4 fistulated cows that were to be used for *in sacco* and *in vitro* diet evaluations. For the 16 cows, the effect of cow-diet, time sample was taken and interaction were included in the model, and for the four cows the effect of cow-diet and time sample was taken were included in the model.

Dietary treatment period

The effect of dietary treatment on rumen pH, NH_3 and VFA parameters of the 16 fistulated cows during feeding period B was determined with cow-diet, time sample was collected and interaction of cow-diet x sampling time included in the model.

pH, NH_3 and VFA concentrations of rumen inoculum from the four fistulated cows used for all *in vitro* incubations during both feeding periods were compared to determine the effects of cow-diet, feeding period and cow-diet x feeding period.

6.3.5.2 *In sacco* digestion kinetics

Kinetic parameters for DM, CP and NDF disappearance from *in sacco* bags were predicted by fitting data from bag residues for each forage in each cow to a non-linear regression model (Ørskov and McDonald, 1979). The Marquardt procedure in SAS (1996) was used to fit both non-lag and lag equations described in Equation 1 and 2. Residual mean squares and degrees of freedom from fitting both models were recorded and an F-test conducted to determine which equation fitted each forage or mixture in each cow and each feeding period the best.

EQUATION 6.1. $PD = A + B\,(1-e^{(-kt)})$
EQUATION 6.2. $PD = A + B\,(1-e^{-k\,(t-Lag)})$

Where:
PD = potential degradation of DM, CP or NDF (%)
A = soluble fraction (%DM, % CP or %NDF)
B = degradable insoluble fraction (%DM, %CP or %NDF)
k = fractional disappearance rate of DM, CP or NDF per hour (%/h)
t = incubation time (h)
Lag = lag time (h). This is the period when there is either no digestion or digestion occurs at a greatly reduced rate (McDonald, 1981).

Effective degradability (ED) of forages and mixtures were calculated according to Sinclair *et al*. (1993) using Equation 6.3.

EQUATION 6.3: $ED_x (\%) = A + [(B\ k)/(k + c)]\ (1\text{-}e^{-(k+c)t})$

Terms are defined above with:
ED_x = effective degradability of x,
c = rumen outflow rate of each constituent at 6 %/h (Kolver *et al*., 1998b).

Effects of cow-diet and forage were compared for degradation of DM, CP and NDF using model components A, B, k and Lag. Cow-diet effects required data from all forages to be combined and forage effects were based on all cow-diet data. The effect of forage incubated (forage), diet the cow was fed (cow-diet), feeding period and interactions between cow-diet x feeding period and forage x cow-diet were included in the model. Effects due to feeding periods were accounted for by treating cow-diet x feeding period as a block and using it as an error term to test the main effects of feeding period and cow-diet. Least-square (LS) means ± standard error of the means (SEM) are presented.

It was necessary to choose either a non-lag (Equation 6.1) or lag (Equation 6.2) model for data analysis because parameters cannot be combined from both models in an analysis. Data were fitted against both the non-lag and lag models and although a better fit was obtained with the non-lag model in many instances, there were a number of data that could not be explained by the non-lag model. The need for consistency demanded the lag model to be used to define degradation data and this was used throughout.

One requirement of the Marquardt procedure was convergence of predicted degradation at 72 hours to signify completion of fermentation. When M was incubated the DM and NDF of M did not converge and predictions for A and B pools were unrealistic for 4 out of 8 DM curves and 5 out of 8 NDF curves using the programme in Appendix 6.3. In those cases where convergence was not reached the SAS programme in Appendix 6.3 was modified to obtain realistic degradation parameters (Appendix 6.4), by setting the A + B pools to the actual average loss of DM and NDF after 72 hours incubation (rather than A + B being predicted from the data).

The model of Orskov and McDonald (1979) assumes non-linearity, but the CP component of M demonstrated linear degradation and neither the lag or non-lag models were appropriate for analysis. CP degradation from M was not included in analysis of cow-diets and forage effects.

6.3.5.3 *In vitro* incubations

In vitro pH was analysed using the effect of cow-diet, forage type, and interaction of cow-diet x forage type.

In vitro NH_3 concentrations and VFA yields from *in vitro* fermentation were analysed to determine the effects of forage type, cow-diet, feeding period and time of incubation, as well as interactions: forage type x cow-diet; time x cow-diet; time x forage type and time x cow-diet x forage type. Analyses of VFA yields excluded feeding period because samples had been bulked across periods according to the forage or mixture incubated. Results were presented as LS means and SEM when treatments were balanced, and when treatments were unbalanced the pooled SD was reported.

6.4 RESULTS

6.4.1 Chemical composition and particle size distribution of forages

The chemical composition of the diets fed to cows during the 5-day measurement periods and forages and mixtures used for incubations were similar and are summarised in Table 6.2. The P fed in this study had been irrigated and appeared very leafy, but ADF and NDF concentrations were higher than expected at 31% DM and 52% DM, respectively. The composition of M and S used in this study were similar to other reported studies (Kolver *et al.*, 2001; Chapter 3). Combining one-third M, S or M:S with two-thirds P provided diets with contrasting chemical composition. Most noticeable was the lower CP and higher soluble carbohydrate concentration when M was included in mixtures (Table 6.2). Addition of S lowered NDF and increased soluble carbohydrate concentrations.

The fresh mincing technique was developed to mimic particle size reduction by chewing (Chapter 3) and the data presented in Table 6.3 illustrate a similar particle size distribution in rumen contents of the cows used here. Minced samples used for *in sacco* and *in vitro* incubations showed a relatively consistent particle size distribution within sieve sizes for the six dietary mixtures and match digesta samples taken from the mid-rumen of cows (3 – 5 h after eating). The overall similarity of minced material and rumen digesta of cows lends creditability to the mincing preparation for fresh forages.

TABLE 6.2. Dry matter (DM) content and composition (% of DM) of P, P:M, P:S and P:M:S[1] diets fed to cows, and P, M, S, P:M, P:S and P:M:S[1] forages used for *in sacco* and *in vitro* incubations[2].

Feed	% of P[4]	DM (%)	SC	CP	ADF	NDF	ADL	Ash	CT	OMD (%)	MJME/kg DM
Diets fed to cows[3]											
P – Period A	100	17.5	6.0	19.5	31.5	54.2	6.3	9.4	-	64.6	9.1
P – Period B	100	19.1	8.8	17.8	30.2	49.9	6.3	8.9	-	66.2	9.3
P:M – Period A[5]	66	23.4	17.0	15.2	29.5	50.2	5.8	7.7	-	-	9.6
P:M – Period B[5]	71	24.0	17.0	14.8	28.9	47.6	5.8	7.7	-	-	9.7
P:S – Period A[5]	66	15.3	9.7	20.0	29.3	44.7	7.0	9.9	0.7	70.0	9.9
P:S – Period B[5]	69	16.8	11.7	17.7	28.4	42.2	7.0	9.2	0.7	70.1	9.9
P:M:S – Period A[5]	66	19.4	13.4	17.6	29.3	47.3	6.4	8.8	0.4	-	9.7
P:M:S – Period B[5]	68	20.7	15.3	15.9	28.4	44.2	6.4	8.4	0.4	-	9.9
Forages and mixtures incubated[2]											
P	100	16.6	5.4	19.7	29.7	48.8	6.3	9.1	-	69.3	9.6
M	100	34.8	34.6	7.3	24.8	43.2	4.8	4.7	-	-	10.7
S	100	15.9	16.6	22.0	22.2	25.9	8.5	10.5	2.2	85.0	12.4
P:M[5]	66	20.8	15.1	15.6	28.1	46.9	5.8	7.7	-	-	10.0
P:S[5]	66	15.9	8.6	19.3	28.9	42.1	7.0	9.6	0.7	73.6	10.1
P:M:S[5]	66	17.9	10.8	17.6	28.8	46.7	6.4	8.6	0.4	-	9.9

[1] P, pasture; M, maize silage; S, sulla; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

[2] Forages were collected on 1 March 2001, prepared and used for all incubations. Data presented here are the means of two sub-samples of minced forage and mixtures taken on incubation days.

[3] Data presented here are the means of five feed samples collected during both feeding periods on the five measurement days.

[4] Calculated from weights of pasture, maize silage, sulla offered to cows in feed barn.

[5] Composition was calculated from the percentage of P, M, S and M:S in the diet.

Abbreviations: SC, soluble carbohydrate; CP, crude protein; ADF, acid detergent fibre; NDF, neutral detergent fibre; ADL, acid detergent lignin; CT, condensed tannin; OMD, predicted organic matter digestibility; ME, metabolisable energy.

TABLE 6.3. Particle size distribution (% of dry matter) of minced forages and mixtures used for incubations and digesta taken from the mid-rumen of cows fed P, P:M, P:S, and P:M:S[1] diets.

Feed	> 4 mm	1 – 2 mm	< 1 mm	Soluble
Minced				
P	19	19	29	33
P:M	19	24	27	31
P:S	24	23	25	28
P:M:S	17	23	27	34
M	12	26	24	38
S	18	23	20	39
Mean	18	23	25	34
Rumen contents (n = 4 cows/treatment)				
P	27	22	30	21
P:M	23	25	27	25
P:S	24	12	27	37
P:M:S[2]	32	20	26	23

[1] P, pasture; M, maize silage; S, sulla; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

[2] Rumen samples taken from three fistulated cows.

Data for individual cows are presented in Appendix 6.5.

6.4.2 Animal production

TABLE 6.4. Daily dry matter (DM) intake (kg/cow/day), milk, milkfat, milk protein and milksolids[1] yield (kg/cow/day) and milk composition (%) for cows fed P, P:M, P:S, and P:M:S[2]. Least-square (LS) means ± SEM are presented.

	P	P:M	P:S	P:M:S	SEM	Pr
Number of cows	8	8	8	8		
DM intake in Period A	14.3^{a}	13.0^{b}	13.8^{a}	14.2^{a}	0.32	< 0.01
DM intake in period B	19.6^{a}	15.9^{b}	16.3^{bc}	16.7^{c}	0.32	< 0.01
Average DM intake	16.9^{a}	14.5^{b}	15.1^{b}	15.5^{b}	0.24	< 0.01
Supplement (% dietary DM offered)	0	31.7	32.7	32.9	0.85	NS
Milk yield (kg/cow/day)	10.96^{a}	11.29^{a}	12.37^{b}	13.23^{c}	0.232	< 0.01
Milkfat (kg/cow/day)	0.50^{a}	0.48^{a}	0.56^{b}	0.58^{b}	0.012	< 0.01
Milk protein (kg/cow/day)	0.37^{a}	0.38^{a}	0.41^{b}	0.44^{c}	0.007	< 0.01
Milksolids (kg/cow/day)	0.87^{a}	0.86^{a}	0.97^{b}	1.02^{c}	0.018	< 0.01
Milk fat	4.55^{b}	4.31^{a}	4.53^{b}	4.42^{ab}	0.062	< 0.01
Milk protein	3.36	3.37	3.33	3.30	0.027	NS

[1] Milksolids yield = milkfat + milk protein yield (kg/day)

[2] P, pasture; M, maize silage; S, sulla; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

[a, b] LS means within rows with a common superscript do not differ significantly (Pr < 0.05).

NS = non-significant (Pr > 0.05).

Table 6.4 illustrates differences in intake and milk production when cows were fed P, P:M, P:S and P:M:S (Woodward *et al.*, unpublished). Cows fed P and P:M produced significantly less milksolids (0.87 and 0.86 kg/day) than cows fed P:S (0.97 kg/day) and P:M:S (1.02 kg/day). These differences in milk production did not match feed intakes and illustrate the effects of diet quality. Cows fed P consumed significantly more feed than cows fed other diets but milk production was the lowest. Intakes of P:M, P:S and

P:M:S were similar, averaging 15 kg DM/cow/day. The average daily DM intake of the four cows used for incubations was 13.5 kg during feeding period A and 17.4 kg during feeding period B. These were similar to herd averages. Higher feed intakes in feeding period B was due to a decrease in the fibre content of the diets offered. Feeding period did not affect milk production or composition. Milk fat concentration was reduced when M was included in the diet (Table 6.4).

Regression analysis of feed intake, milk production (kg/cow/day) and nutrient composition (% DM) in this experiment showed a 1% increase in digestibility from 69 to 70% in late lactation increased milk production by 0.2 kg milk/cow/day, but cows were required to consume an extra 11 kg DM of feed. This is an unreasonably high increase and is due to the high DM intake of cows fed pasture and poor milk production in the experiment. Nevertheless, the increase in milk production of 0.2 kg milk/cow/day required the dietary DM to contain 1.7% less NDF, 1.0% less ADF, 2.9% less protein and an increase in ME content by 0.14 MJME and soluble carbohydrate content by 5.3% of the DM.

6.4.3 Cow ruminal characteristics

6.4.3.1 Uniformity period

The average rumen pH and NH_3 concentrations measured in all 16 fistulated cows during the uniformity period had average values of 6.68 and 10.9 mmol NH_3/L. Mean values (Table 6.5) for the 16 cows prior to feeding of dietary treatments demonstrated similar pH and NH_3 concentrations. The four cows used for incubations also had similar rumen pH and NH_3 concentrations when grazing pasture (Table 6.6).

After 6 hours of *in sacco* incubation in the four fistulated cows, the mean DM loss of lucerne hay was 53.8% and this increased to 76.8% after 24 hours (Table 6.6). There were differences between cows in rates of DM loss ($Pr < 0.001$), especially after 6 hours of incubation. DM loss was slowest ($Pr < 0.05$) from the cow to be fed P:M, and rates were also slow for the cow to be fed P:S. After 24 hours the cow to be fed P had a higher loss (79.4%) than the others (Table 6.6). Despite the differences between cows in loss of lucerne hay DM at either 6 or 24 hours, DM loss during the uniformity period was not used as a covariate in the analysis because insufficient numbers of the lucerne hay samples had been incubated over time to estimate degradation rate and ED.

TABLE 6.5. Rumen pH, ammonia (NH_3) and volatile fatty acid (VFA) concentrations and molar percentage for 16 cows grazing pasture during the uniformity period[2] and when fed either P, P:M, P:S or P:M:S[1] in period B[3]. Molar ratios are given for acetate:propionate (A:P) and (acetate + butyrate):propionate ((A+B):P). Least-square (LS) means ± SEM are presented.

	Samples/ Treatment	Future treatment allocation^{2} or diet fed^{3}				SEM	Pr
		P	P:M	P:S	P:M:S		
Number of cows		4	4	4	4		
Uniformity period^{2}							
pH	12	6.75	6.63	6.71	6.63	0.041	NS
NH_3	12	10.0	10.7	10.7	12.0	0.59	NS
Feeding period B^{3}							
pH	24	6.27^{a}	6.53^{b}	6.54^{b}	6.44^{ab}	0.061	< 0.01
NH_3 (mmol/L)	24	10.0^{c}	5.0^{a}	8.0^{b}	6.3^{ab}	0.56	< 0.01
Total VFA^{4} (mmol/L)	4	117.0^{b}	82.9^{a}	92.1^{a}	92.7^{b}	5.37	< 0.01
Acetate %	4	58.9	56.7	62.5	58.5	1.65	NS
Propionate %	4	21.1	22.4	20.5	20.6	0.74	NS
Butyrate %	4	14.3^{b}	14.5^{b}	12.0^{a}	14.3^{b}	0.68	0.08
Minor %	4	4.0^{ab}	4.6^{bc}	3.6^{a}	5.0^{c}	0.31	0.04
A:P ratio	4	2.8	2.5	3.1	2.9^{a}	0.18	NS
A+B:P ratio	4	3.5	3.2	3.6	3.6^{a}	0.17	NS

[1] P, Pasture; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

[2] Uniformity period: cows were grazing pasture before being allocated to dietary treatments. All 16 fistulated cows (4 per treatment) were sampled at 0900, 1300 and 1600 hours on one day during the uniformity period.

[3] Feeding period B: cows were fed dietary treatments on day 32 and 33 of the trial. All 16 fistulated cows (4 per treatment) were sampled at 0900, 1300 and 1600 hours on two consecutive days of the feeding period.

[4] One sample per cow was analysed. The six rumen samples collected were bulked.

[a, b] LS means within rows with a common superscript do not differ significantly ($Pr < 0.05$).

NS = non-significant, $Pr > 0.10$.

TABLE 6.6. Rumen pH, ammonia (NH_3) concentration and *in sacco* DM losses from four cows grazing pasture during the uniformity period. Least-square (LS) means ± SEM are presented.

	Number per treatment	Future treatment allocation				SEM	Pr
		P	P:M	P:S	P:M:S		
Number of cows		1	1	1	1		
Rumen parameters[1]							
pH	3	6.74	6.60	6.75	6.68	0.050	NS
NH_3	3	10.5	11.4	10.5	10.9[a]	1.10	NS
In sacco DM loss of lucerne hay[2] (%)							
6 h	4	58.4[a]	47.2[b]	52.7[c]	57.0[a]	1.45	< 0.01
24 h	4	79.4[a]	74.6[b]	76.6[b]	76.6[b]	0.73	< 0.01

Abbreviations: P, pasture; M, maize silage; S, sulla; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

[1] Cows (1 per treatment) were sampled at 0900, 1300 and 1600 hours on one day during the uniformity period.

[2] Eight dacron bags of lucerne hay were placed into the one fistulated cow on each dietary treatment and four bags were removed out of each cow at 6 and 24 h.

[a, b] LS means within rows with a common superscript do not differ significantly ($Pr < 0.05$).

NS = non-significant, $Pr > 0.05$.

6.4.3.2 Dietary treatment period

Effects of diet were demonstrated by rumen parameters of the 16 fistulated cows sampled during period B (Table 6.5). The four cows used for incubations also indicated effects of diet, especially as rumen pH and NH_3 concentration were similar for all animals during the uniformity period (Table 6.6). These cows were given the same diet during feeding periods A and B and were able to demonstrate feeding period effects.

Dietary effects on 16 cows

During period B rumen pH was lower than for the uniformity period but NH_3 concentrations in cows fed P were the same at both times. Cows fed P had lower rumen pH and higher VFA concentrations ($Pr < 0.05$) than cows fed P with supplements (Table 6.5). There were no effects of specific supplements on VFA concentration but M resulted in lower rumen NH_3 concentration than sulla.

Average effects of diet on proportions of VFA were not significant except for minor VFA, where inclusion of M increased values relative to P or S (Table 6.5). The ratio of A:P was lower with the P:M diet than the P:S diet (2.5 vs. 3.1) however the A+B:P ratio was similar for all dietary treatments (Table 6.5).

Cow-diet effects on four cows

Analysis of ruminal data from periods A and B demonstrated cow-diet and period effects but no interaction between cow-diet and period.

Rumen pH was lowest for P:S and P:M diets about 2 hours after feeding, with values of 6.52 and 6.58 compared to 6.72 for both the P and the P:M:S diets. Cow-diet effects showed higher rumen NH_3 concentration in the cow fed P than other diets (Table 6.7) and the lowest concentration was in the cow fed P:S (17.3 vs. 10.2 mmol/L). Cow-diet did not have a significant effect on VFA concentration, but supplemented diets tended to have a lower proportion of acetate and lower A:P ratio, compared to P (Table 6.7). Including butyrate in the ratio reduced some of the treatment effects.

Period effects were evident for pH and NH_3, but not VFA concentrations. Average NH_3 concentration was higher during period A (15.5 ± 0.68 mmol/L) than B (12.2 ± 0.68 mmol/L; $Pr < 0.05$). Rumen pH 2 hours after feeding was higher in period A than B (6.75 vs. 6.52; $Pr < 0.05$).

TABLE 6.7. Effect of cow-diet and feeding period on rumen pH, ammonia (NH$_3$) and volatile fatty acid (VFA) concentrations and molar percentages for four cows fed either P, P:M, P:S and P:M:S[1] and used for *in sacco* and *in vitro* incubations. Molar ratios are given of acetate:propionate (A:P) and (acetate + butyrate):propionate ((A+B):P). Least-square (LS) means ± SEM are presented.

Cow-diet	Number per cow	P	P:M	P:S	P:M:S	SEM	Effect	
							Cow-diet	Period
Rumen NH$_3$ (mmol/L)								
Feeding period A	4	19.7	15.3	11.3	15.7	1.37		
Feeding period B	4	15.0	11.6	9.1	12.9	1.37		
Feeding period effect (Pr)		< 0.05	< 0.10	NS	NS			
Average	8	17.3^{c}	13.4^{b}	10.2^{a}	14.3^{b}	0.97	< 0.01	< 0.01
Rumen pH								
Feeding period A	2	6.93	6.52	6.83	6.72	0.069		
Feeding period B	2	6.50	6.52	6.61	6.44	0.069		
Feeding period effect (Pr)		< 0.01	NS	< 0.10	< 0.05			
Average	4	6.72^{a}	6.52^{b}	6.58^{b}	6.72^{a}	0.049	< 0.05	< 0.01
Rumen VFA (mmol/L)								
Feeding period A	2	73.7	58.7	59.1	78.1	8.38		
Feeding period B	2	71.4	61.9	56.4	70.7	8.38		
Feeding period effect (Pr)		NS	NS	NS	NS			
Average Total (mmol/L)	4	72.5	60.3	57.7	74.4	5.92	NS	NS
Molar percentage: Acetate	4	68.2^{b}	60.0^{a}	62.4^{a}	65.4^{ab}	2.01	< 0.10	< 0.05
Propionate	4	14.6	17.5	17.8	17.0	0.99	NS	< 0.10
Butyrate	4	9.8^{a}	14.2^{c}	12.6^{bc}	10.9^{b}	0.82	< 0.05	< 0.05
Minor	4	7.5	8.4	7.2	6.8	0.59	NS	NS
Molar ratios: A:P	4	4.71^{b}	3.52^{a}	3.58^{a}	3.91^{ab}	0.32	< 0.10	< 0.10
(A+B):P	4	5.38^{b}	4.34^{ab}	4.30^{a}	4.56^{ab}	0.33	NS	< 0.10

[1] P, pasture; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

[a, b,] LS means within rows with a common superscript do differ significantly ($Pr < 0.05$). NS = non-significant ($Pr > 0.10$).

6.4.4 *In sacco* digestion of forages and mixtures

6.4.4.1 Lucerne hay digestion across feeding periods

In sacco digestion of lucerne hay was not affected by cow-diet or feeding period after 24 h (Table 6.8), but after 12 hours losses across treatments were greater during period B (65.4%) than period A (59.6%; Pr < 0.01). Diet fed to cows had minimal effects on DM digestion and there was no cow-diet x feeding period interaction, although the cow fed P during feeding period A had a greater loss at 12 hours than other treatments (Table 6.8).

TABLE 6.8. *In sacco* DM loss of lucerne hay at 12 and 24 hours when incubated in cows fed P, P:M, P:S and P:M:S[1] during feeding periods A and B. Data in each feeding period are the least square (LS) means ± SEM of four values, n = 4.

	Feeding period A	Feeding period B	SEM	Pr
DM loss at 12 hours				
P	64.9^{a}	66.7	2.29	NS
P:M	59.6^{ab}	65.4	2.29	< 0.10
P:S	57.8^{b}	65.2	2.29	< 0.05
P:M:S	56.0^{b}	64.1	2.29	< 0.05
Cow-diet x feeding period effect (Pr)	NS			
Average	59.6	65.4	1.14	<0.01
DM loss at 24 hours				
P	75.5	74.1	1.07	NS
P:M	74.6	74.5	1.07	NS
P:S	75.0	75.1	1.07	NS
P:M:S	75.0	76.0	1.07	NS
Cow-diet x feeding period effect (Pr)	NS			
Average	75.3	74.8	0.53	NS

[1] P, pasture; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

[a, b] LS means, within feeding period, within columns with common superscripts do not differ significantly (Pr < 0.05). NS = non-significant, Pr > 0.10.

6.4.4.2 Forage effects

In sacco DM, CP and NDF degradation characteristics were significantly different between forages and mixtures (Table 6.9; Figure 6.1). The percentage of soluble DM (A) was greatest in M (49.6%) and least in P (33.9%), while the percentage of slowly degradable DM (B) was least in M (31.7%) and greatest in P (49.5%). Based on the percentage of each forage in the mixtures and the proportion of DM in the A and B pools for individual forages, the proportion of DM in the A and B pools for the P:M:S mixture was as expected, but was lower for P:M and higher for P:S.

The percentage of CP in the soluble (A) fraction (excluding M) was greatest for P (55.8%) and least for S (42.6%). Adding S and/or M to pasture reduced the percentage of CP in the soluble fraction. Although degradation kinetics could not be determined for M, the CP concentration was very low and most was soluble (about 70%) with only 10 to 15% CP left to degrade over 24 hours. The majority of NDF in forage mixtures was degradable and insoluble but the concentration in *in sacco* residues at 0 hours showed that up to 15% of fibre was solubilised by mincing.

Degradation rates are predicted for the B fraction of minced forages and although degradation rates for M DM was very slow (3.6 %/h), most DM was soluble so this degradation applied to about one-third of the original DM (32% DM; Table 6.9). Other forages contained about 42 – 50% of DM in this fraction, which formed a significant source of nutrient supply for the animal. The low-fibre S degraded most rapidly (16.3 %/h) and pasture degraded at 7.1 %/h. Pasture:sulla degraded more slowly than S at 10.7 %/h, but addition of M reduced DM degradation of forages and mixtures (Table 6.9). Maize silage and P:M had significantly greater lag periods for DM loss (4.9 and 3.6 h, respectively) than other forages and mixtures, but there was no difference in lag between P:M:S, S, P:S and P (0 – 0.9 h).

The differences between forages and mixtures in CP and NDF degradation rates reflected DM degradation. The CP degradation rate in S (22.1 %/h) and P:S (15.8 %/h) were significantly faster than P:M, P:M:S and P (9.6 %/h, 10.3 %/h and 11.7 %/h). Lag periods for protein degradation were shorter than DM from 0.1 to 1.9 hours (Table 6.9).

Sulla and P:S had the most rapid NDF degradation rates (9.4 and 8.9 %/h, respectively), M was the slowest (3.3 %/h) and P intermediate (5.8 %/h). NDF degradation of P:M and P:M:S were intermediate relative to the individual forages. Lags were largest for M and P:M (6.7 and 6.8 h, respectively) relative to others (0.4 – 1.8 h).

Effective degradability (ED) of DM, CP and NDF were positively correlated with degradation rates (ED vs. DM: r = 0.89; CP: r = 0.82; NDF: r = 0.72). Effective DM degradability ranged from a high value for sulla (78%) to a low value for P:M (55%). Pasture:maize silage DM had a smaller ED value than that of either P or M alone (Table 6.9). Effective degradation of protein exceeded 70% for all forage mixtures and ED of NDF ranged from 31% for M to 51% for P:S.

TABLE 6.9. Effect of forage and forage mixtures on dry matter (DM), crude protein (CP) and netural detergent fibre (NDF) degradation parameters[2] derived from the lag equation for P, M, S, P:M, P:S and P:M:S. Data presented are the least-square (LS) means ± SEM of four cows over two feeding periods (n = 8).

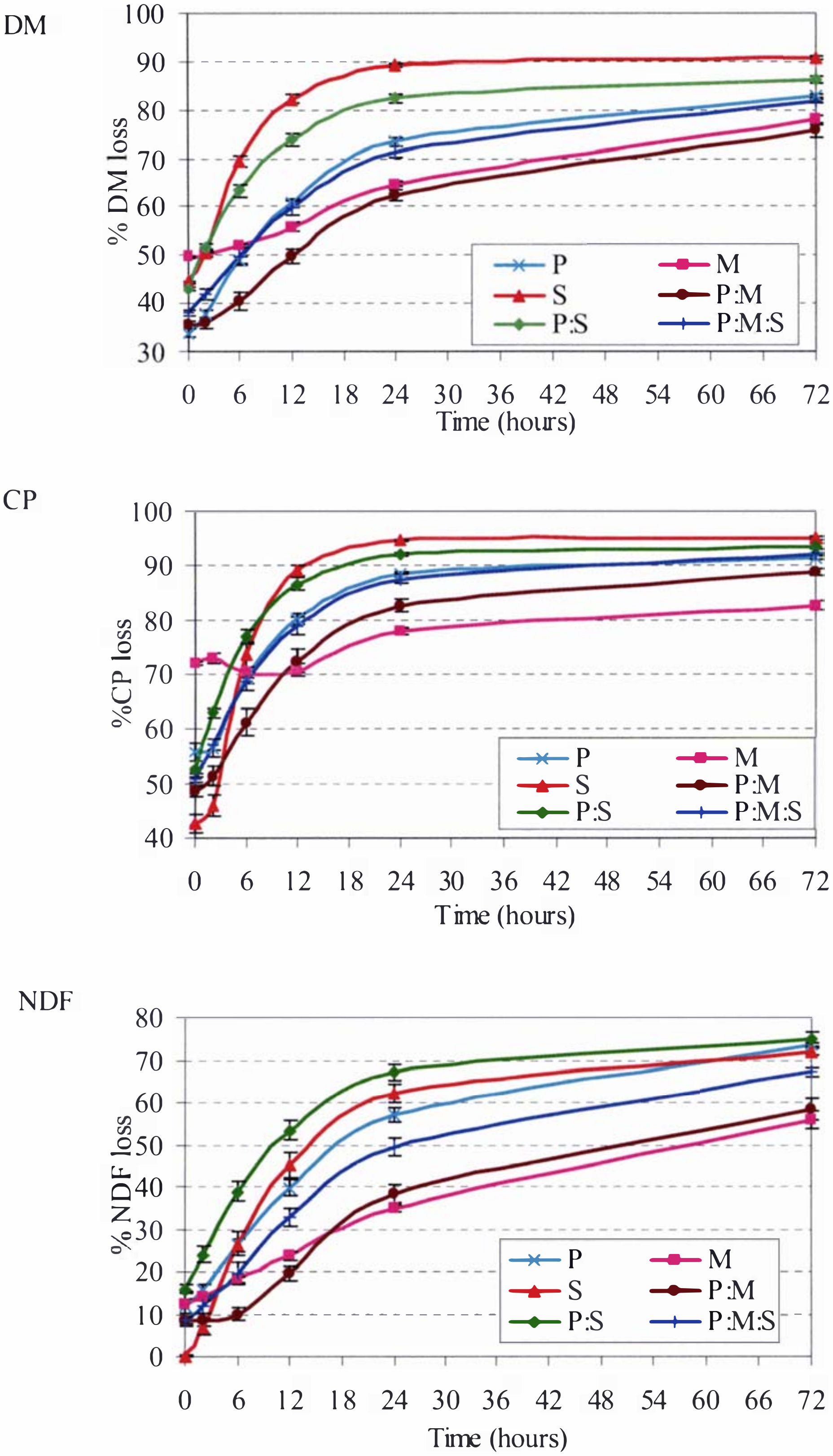
	A	B	k	Lag	ED
DM loss	% DM	% DM	%/h	h	% DM
M	49.6^{f}	31.7^{a}	3.6^{a}	4.9^{b}	61.3^{b}
P	33.9^{a}	49.5^{d}	7.1^{b}	0.9^{a}	60.6^{b}
S	44.5^{e}	46.2^{c}	16.3^{d}	1.1^{a}	77.9^{d}
P:M	35.7^{b}	41.6^{b}	5.3^{ab}	3.6^{b}	54.9^{a}
P:S	43.2^{d}	43.0^{bc}	10.7^{c}	0^{a}	70.4^{c}
P:M:S	38.1^{c}	44.4^{c}	6.3^{b}	0.9^{a}	60.5^{b}
SEM	0.43	0.80	0.65	0.63	0.72
Pr	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Protein loss	% CP	% CP	%/h	h	% CP
M	-	-	-		-
P	55.8^{e}	35.5^{a}	11.7^{a}	1.9^{b}	79.1^{c}
S	42.6^{a}	52.5^{c}	22.1^{c}	1.9^{b}	83.8^{e}
P:M	48.5^{b}	40.4^{b}	9.6^{a}	1.8^{b}	72.5^{a}
P:S	52.4^{c}	41.2^{b}	15.8^{b}	0.1^{a}	81.8^{d}
P:M:S	50.8^{d}	41.5^{b}	10.3^{a}	0.3^{a}	76.3^{b}
SEM	0.25	0.78	1.03	0.25	0.50
Pr	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
NDF loss	% NDF	% NDF	%/h	h	% NDF
M	14.4^{c}	49.5^{a}	3.3^{a}	6.7^{b}	31.3^{a}
P	11.8^{b}	63.6^{b}	5.8^{b}	1.6^{a}	42.5^{c}
S	0.2^{a}	72.3^{c}	9.4^{c}	1.0^{a}	43.0^{c}
P:M	8.7^{b}	52.4^{a}	5.3^{ab}	6.8^{b}	32.2^{ab}
P:S	15.9^{c}	59.3^{b}	8.9^{c}	0.4^{a}	50.8^{d}
P:M:S	8.5^{b}	61.3^{b}	5.3^{ab}	1.8^{a}	36.4^{b}
SEM	0.84	1.88	0.70	1.23	1.59
Pr	< 0.001	< 0.001	< 0.001	< 0.01	< 0.001

[1] P, pasture; M, maize silage; S, sulla; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

[2] A, soluble fraction; B, degradable insoluble fraction; k, fractional degradation rate (%/h); ED, effective degradability at an outflow rate of 6 %/h.

[a, b] LS means, within DM, protein or NDF losses, within columns with common superscripts do not differ significantly ($Pr < 0.05$).

FIGURE 6.1. Forage and forage mixture (P, M, S, P:M, P:S and P:M:S[1]) on *in sacco* dry matter (DM), crude protein (CP) and fibre (NDF) disappearance curves averaged across four cows over two feeding periods. Means ± SE bars are presented.



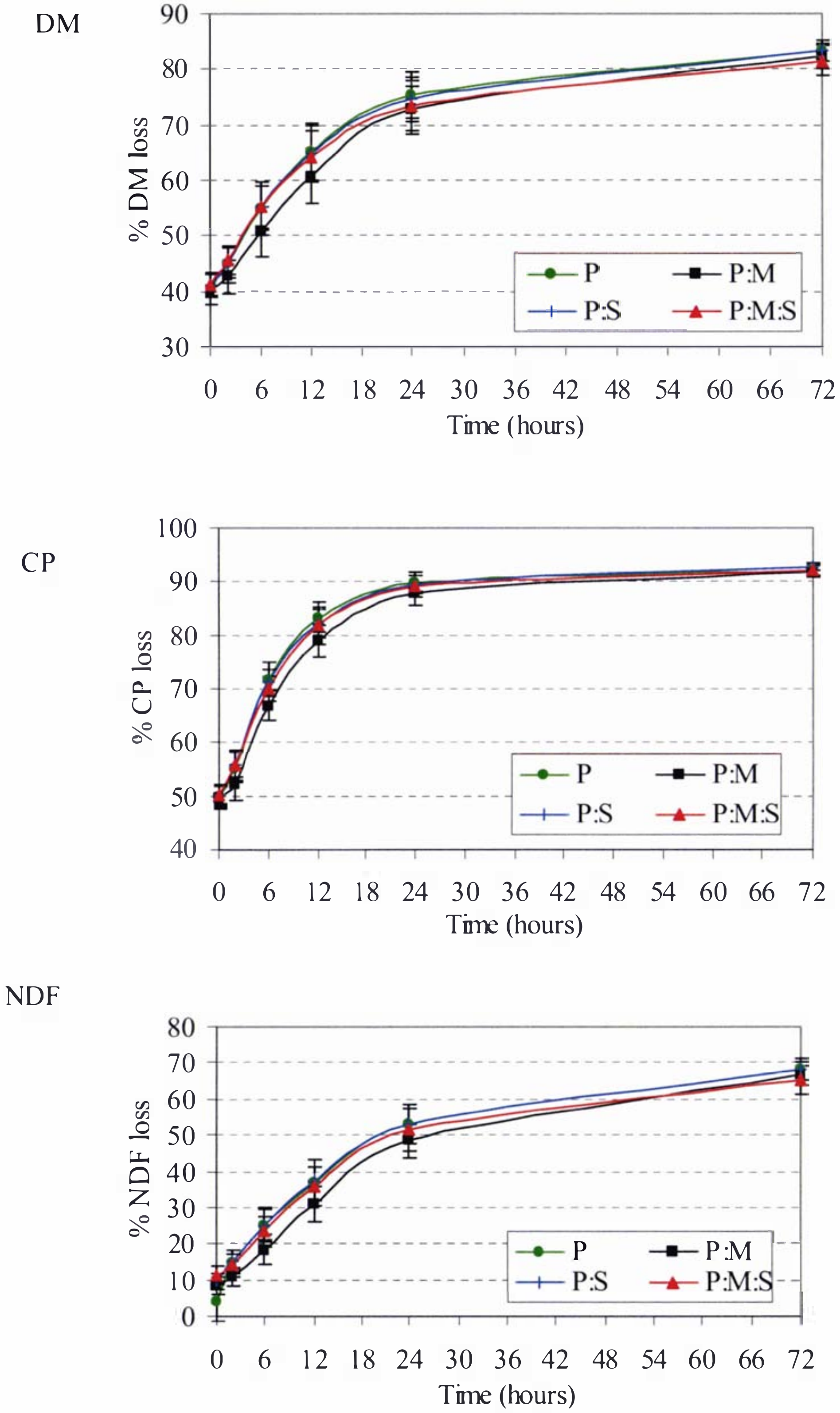
[1] P, pasture; M, maize silage; S, sulla; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

6.4.4.3 Cow-diet effects

This study has shown that the degradation kinetics of forages and mixtures are influenced by cow-diet, especially when P:M was fed. Cow-diet did not affect the soluble (A) fraction of DM, CP or NDF because this rapidly degradable fraction was created by mincing the forage (Table 6.3) and measured by washing sealed *in sacco* bags without placement in the rumen. The B fraction of the DM, CP and NDF is largely dependent on the loss of soluble material, so the distribution of these fractions in A, B and undegraded (U) pools were similar for all cow-diets. In contrast cow-diet did influence ED and to a lesser extent rates of degradation (Figure 6.2; Table 6.10).

Rates of digestion were similar for DM, CP and NDF across cow-diets, but the P:M diet was associated with a lower ED than the other diets. There was a significant cow-diet effect on the lag period for DM (0.8 – 3.6 h) and CP degradation (0.8 – 1.4 h; Table 6.10), but lags for NDF digestion were similar (2.3 – 3.7 h). When the non-lag equation was fitted to the data, the DM, CP and NDF degradation rates of forages and mixtures were also significantly slower when incubated in the cow fed P:M, with corresponding differences in ED (Table 6.10).

FIGURE 6.2. Cow-diet (P, P:M, P:S and P:M:S[1]) *in sacco* dry matter (DM), crude protein (CP) and fibre (NDF) disappearance curves averaged for all incubations. Means ± SE bars are presented.



[1] P, pasture; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

TABLE 6.10. Effect of cow-diet on degradation rates (k) determined with and without a lag period and effective degradability (ED) of dry matter (DM), crude protein (CP) and neutral detergent fibre (NDF) for forages and mixtures incubated in cows fed P, P:M, P:S and P:M:S[1]. Data presented for DM and NDF are the least-square (LS) means ± SEM of six forages incubated over two feeding periods (n = 12); data presented for CP are the LS means ± SEM of 5 forages incubated over two feeding periods (n = 10).

Lag model	DM[2]			CP[2]			NDF[2]		
Cow-diet	k (%/h)	Lag (h)	ED[3] (%)	k (%/h)	Lag (h)	ED[3] (%)	k (%/h)	Lag (h)	ED[3] (%)
P	9.0	1.9^{b}	65.3^{c}	16.1^{b}	1.4^{b}	80.1^{c}	6.5	2.2^{a}	39.8^{b}
P:M	7.7	3.6^{c}	63.6^{a}	12.1^{a}	1.6^{b}	77.2^{a}	5.4	3.6^{b}	36.9^{a}
P:S	8.4	1.4^{a}	64.7^{b}	13.9^{ab}	0.8^{a}	78.9^{b}	6.6	3.0^{b}	40.9^{b}
P:M:S	7.9	0.8^{a}	63.5^{a}	13.5^{ab}	1.0^{a}	78.6^{b}	6.9	3.5^{b}	40.0^{b}
SEM	0.34	0.22	0.12	0.60	0.08	0.26	0.50	0.22	0.59
Pr	NS	< 0.05	< 0.01	< 0.10	< 0.05	< 0.05	NS	< 0.10	< 0.06

No lag model	DM	CP	NDF
Cow-diet	k (%/h)	k (%/h)	k (%/h)
P	7.7^{c}	12.2^{b}	5.7^{b}
P:M	5.8^{a}	8.8^{a}	4.1^{a}
P:S	7.2^{b}	11.2^{b}	5.6^{b}
P:M:S	7.2^{b}	11.3^{b}	5.4^{b}
SEM	0.12	0.38	0.14
Pr	< 0.01	< 0.05	< 0.05

[1] P, pasture; M, maize silage; S, sulla; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

[2] For DM and NDF, disappearance curves for all forages and mixtures could be fitted; for CP, disappearance curves for only five forages and mixtures could be fitted, M could not be fitted.

[3] ED at a passage rate of 6 %/h.

[a, b] LS means within columns with common superscripts do not differ significantly (Pr < 0.05). NS = non-significant (Pr > 0.10).

6.4.4.4 Forage x cow-diet interaction

When forages and mixtures were incubated *in sacco* there were no significant ($Pr < 0.05$) forage x cow-diet interactions for the degradation rates and lag periods for DM, CP and NDF. The DM, CP and NDF disappearance curves for forages and mixtures incubated in cows fed different cow-diets are illustrated in Appendices 6.5, 6.6 and 6.7. The cow fed P:M had longer lag periods and slower degradation rates for all constituents of the forage mixtures (P:M, P:S and P:M:S; Appendix 6.6 – 6.8) indicating slower digestion in this cow. There were no obvious differences between cow-diets when P, S and M were incubated *in sacco*.

6.4.5 *In vitro* fermentation of forages and mixtures

6.4.5.1 Lucerne standard fermentation across feeding periods

In vitro fermentation data for the freeze-dried lucerne standard used in feeding periods A and B are summarised in Table 6.11. Net conversion of forage N to NH_3-N was greater in period B than period A ($Pr < 0.01$) with an average of 10 and 22% appearing as NH_3-N after 2 and 8 hours of incubation. The mean pH of buffered media after 8 h of incubation was similar for both periods. A similar percentage of DM was released as VFA after 8 hours of incubation in both periods equivalent to about 19% of lucerne DM. There was also a cow-diet effect on *in vitro* pH at 8 hours and conversion of lucerne N to NH_3 after 2 and 8 hours of incubation. The effect of the cow-diet are illustrated in Table 6.14 and discussed in section 6.4.5.3.

TABLE 6.11. Effect of feeding period on *in vitro* pH, and *in vitro* ammonia (NH_3) and volatile fatty acid (VFA) yields of freeze-dried lucerne standard using rumen inocula from each of the four cows fed contrasting cow-diets (P, P:M, P:S, P:M:S[1]). NH_3 and pH data are based on two incubations from each cow in each period (n = 16), but VFA are based on a single (bulked) sample from each cow in each period (n = 8). Least-square (LS) means ± SEM are presented.

	Feeding period A	Feeding period B	SEM	Pr
In vitro NH_3 concentration (% forage nitrogen recovered as NH_3)				
2 hours	9.1	11.9	0.45	< 0.01
8 hours	19.0	24.2	0.45	< 0.01
In vitro pH at 8 hours	6.35	6.38	0.027	NS
Total VFA produced after 8 hours (mmol/g DM)	2.78	2.73	0.26	NS

[1] P, pasture; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

NS = non-significant, Pr > 0.05

6.4.5.2 Forage effect

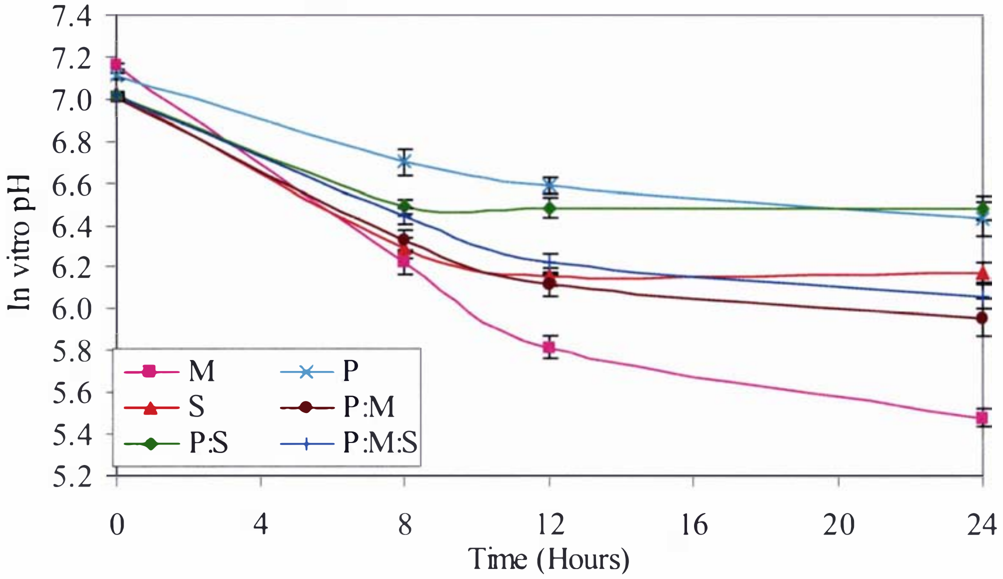
In vitro pH

Compared to other forages and mixtures, the *in vitro* pH for M had the largest decline, falling to 5.8 after 12 hours and 5.5 after 24 hours. *In vitro* pH of other forages and mixtures after 24 hours remained above 6.0 (Figure 6.3).

Ammonia

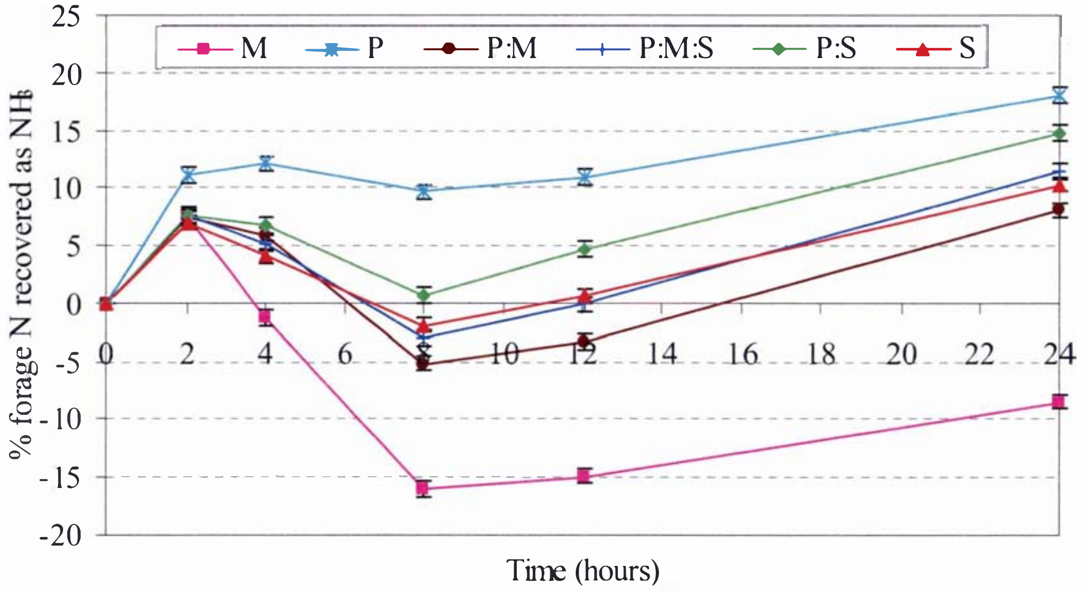
Incubation of forages and mixtures resulted in contrasting rates of NH_3 production (Figure 6.4) and the proportion of plant N appearing as NH_3-N peaked after about 2 hours of incubation for M, S, P:M, P:S and P:M:S. Up to 7% of N in these forages (except P) and mixtures was converted to NH_3 at 2 h, after which net NH_3 production relative to forage N declined and at 8 hours NH_3 concentrations were similar to 0 hour values. This was followed by a gradual increase with 8 to 15% of the forage N from S, P:M, P:S and P:M:S appearing as NH_3 at 24 hours. When M was incubated, net NH_3 yield decreased with a net utilisation of maize N after 4 hours of incubation. Pasture differed from other forages and mixtures, with 11 – 12 % of the forage N appearing as NH_3-N at 2 – 4 hours and increasing to 18% by 24 h. The net yield of NH_3 for each forage and mixture after 8, 12 and 24 hours are given in Table 6.13.

FIGURE 6.3. *In vitro* pH changes over 24 hours when P, M, S, P:M, P:S and P:M:S[1] were incubated. Data are from two incubations in each feeding period for each of the four cows (n = 16). LS means ± SEM bars are presented.



[1] P, pasture; M, maize silage; S, sulla; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

FIGURE 6.4. Net ammonia (NH_3) production (% forage nitrogen, N, recovered as NH_3) during 24 hour *in vitro* incubation of P, M, S, P:M, P:S, and P:M:S[1]. Data from two incubations for each cow in each feeding period (n = 16). LS means ± SEM bars are presented.



[1] P, pasture; M, maize silage; S, sulla; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

TABLE 6.12. Effect of forage and forage mixture on *in vitro* pH at 12 hours and net ammonia (NH_3) yield at 8, 12 and 24 hours when P, M, S, P:M, P:S and P:M:S[1] were incubated using rumen inocula from cows fed four dietary treatments (P, P:M, P:S and P:M:S[1]). Least-square (LS) means ± SEM are presented.

Forage incubated	*In vitro* pH 12 h	Crude Protein (% DM)	Ammonia (% forage nitrogen recovered as NH_3)		
			8 hours	12 hours	24 hours
Number	16	-	16	16	16
M	5.81^a	7.3	-16.0^a	-14.9^a	-8.5^a
P	6.58^c	19.7	9.6^e	11.0^e	18.0^c
S	6.15^b	22.0	-1.9^c	0.6^c	10.3^d
P:M	6.11^b	15.6	-5.1^b	-3.3^b	8.1^b
P:S	6.49^c	19.3	0.7^d	4.7^d	14.7^e
P:M:S	6.22^b	18.0	-3.0^c	-0.1^{bc}	11.4^c
SEM	0.042	-	0.66	0.66	0.66
Pr	< 0.001	-		< 0.001	

[1] P, pasture; M, maize silage; S, sulla; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

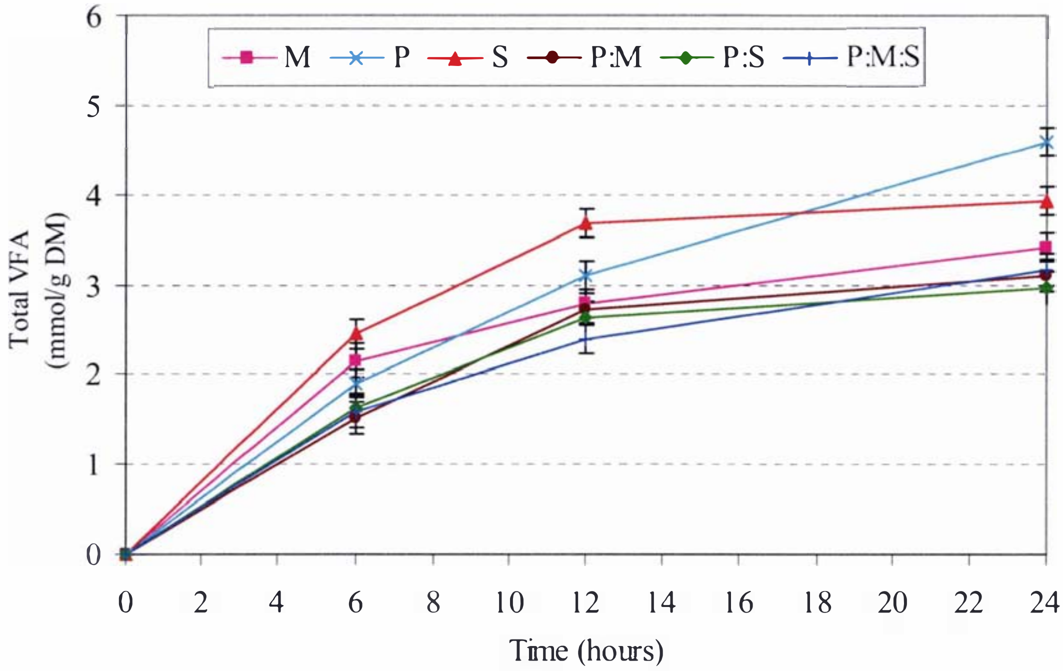
[a, b] LS means within columns with different subscripts are significant at Pr < 0.05.

VFA

The VFA yield from forage incubations are presented in Figure 6.5 and Table 6.13 and show P and S differed from the other forages and mixtures. The total yield of VFA from S was greatest at 6 and 12 hours (2.46 and 3.73 mmol/g DM), after which there was no further net production, whereas VFA yield from P continued at a relatively constant rate for the entire 24 hours. Pasture resulted in the greatest VFA yield (4.69 mmol/g DM) after 24 hours. Mixtures of P and S, and P, S and M resulted in a lower VFA yield than either P or S alone at all time points (Figure 6.5; Table 6.13).

Pasture and S had the highest percentage of acetate (61 and 59%, respectively) and lowest percentage of propionate (24 and 26%, respectively) and butyrate (11 and 13%, respectively) relative to M and mixtures. Diets containing M (M, P:M and P:M:S) had the lowest percentage of acetate (50 – 51%) and greatest percentage of propionate (28%) and butyrate (16 – 17%). The acetate:propionate (A:P) ratio averaged 1.86 for M and 2.64 for P (Table 6.13), but there was less difference in the (A+B):P ratio for M (2.47) and P (3.10). The percentage of minor VFA produced when S was incubated was significantly less than all other forages and mixtures at 2.8% suggesting reduced proteolysis. Maize silage and diets containing M had the highest percentage of minor VFA (4.5 – 4.9), while P and P:S (3.9 and 4.3%, respectively) were intermediate.

FIGURE 6.5. Cumulative volatile fatty acid (VFA) production from *in vitro* incubations of P, M, S, P:M, P:S and P:M:S[1]. Data are from bulked samples from each incubation and both feeding periods giving one sample from each of 4 cows (n = 4). LS means ± SEM bars are presented.



[1] P, pasture; M, maize silage; S, sulla; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

TABLE 6.13. Effect of forage and forage mixture on *in vitro* volatile fatty acid (VFA) yields and molar percentages for P, M, S, P:M, P:S and P:M:S[1] incubated with inocula from cows fed P, P:M, P:S and P:M:S[1]. Molar ratios are given for acetate:propionate (A:P) and (acetate + butyrate):propionate ((A+B):P). Least-square (LS) means ± SEM are presented.

Forage	Time (Hours)	Number	Total (mmol/g DM)	Percentage of				A:P ratio	(A+B):P ratio
				Acetate	Propionate	Butyrate	Minor[2]		
M	6	4	2.15^{bc}	57.0	25.7	13.6	4.1	2.27	2.81
	12	4	2.79^{de}	47.9	30.5	17.3	4.1	1.58	2.15
	24	4	3.41^{g}	51.0	27.9	17.2	4.6	1.82	2.45
P	6	4	1.90^{ab}	61.2	24.2	10.9	3.9	2.53	2.97
	12	4	3.11^{e}	61.9	22.9	11.6	3.5	2.72	3.22
	24	4	4.60^{i}	61.6	23.9	10.4	4.4	2.68	3.11
S	6	4	2.44^{c}	58.8	28.3	12.3	2.1	2.12	2.55
	12	4	3.67^{f}	61.5	24.1	12.1	2.1	2.54	3.05
	24	4	3.92^{h}	57.6	25.1	14.0	4.0	2.37	2.94
P:M	6	4	1.52^{a}	49.3	29.3	16.8	4.5	1.70	2.28
	12	4	2.72^{de}	54.8	26.0	15.5	3.8	2.13	2.73
	24	4	3.10^{g}	51.5	26.8	16.5	5.3	1.94	2.55
P:S	6	4	1.62^{a}	54.0	28.0	14.3	3.8	1.95	2.48
	12	4	2.62^{d}	57.8	25.0	13.0	4.0	2.33	2.86
	24	4	2.96^{g}	53.8	26.5	14.5	5.3	2.12	2.67
P:M:S	6	4	1.58^{a}	51.5	28.8	15.8	4.0	1.80	2.35
	12	4	2.40^{d}	50.0	29.0	16.5	4.3	1.76	2.34
	24	4	3.17^{g}	52.8	26.5	15.0	5.8	2.02	2.60
SEM			0.175	0.32	1.43	0.97	0.32	0.21	0.21
Time x forage effect			< 0.01	NS	NS	NS	NS	NS	NS

[1] P, pasture; M, maize silage; S, sulla; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

[2] Minor VFA comprise iso-butyrate, valerate and iso-valerate.

[a, b, c] LS means with common superscripts within columns do not differ significantly ($Pr < 0.05$) within 6 hours ([a, b, c]), 12 hours ([d, e, f]) or 24 hours ([g, h, i]).

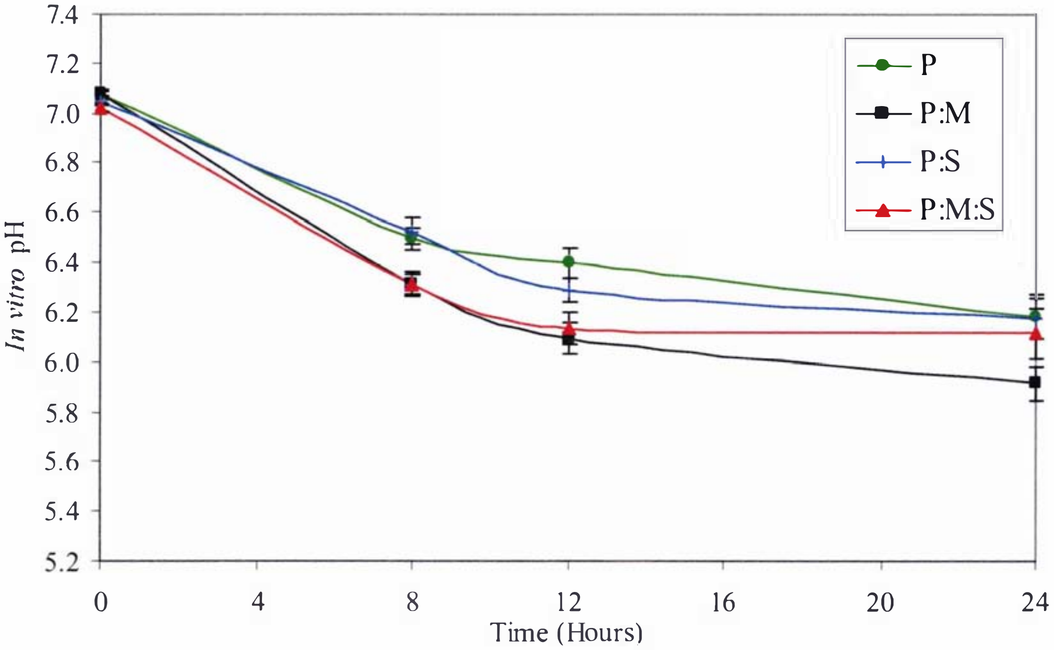
NS = non significant ($Pr > 0.05$).

6.4.5.3 Cow-diet effect

In vitro pH

Inoculum from cows fed diets containing M (P:M and P:M:S) resulted in lowest *in vitro* pH values (Figure 6.6). The pH was lower throughout the incubation with inoculum from the cows fed M in the diet and highest when inoculum from cows fed P and P:S were used. These effects were based on incubation of three forages and three mixtures and show that inoculum was able to affect the *in vitro* environment when a range of substrates were incubated. The P-fed cow also had the highest *in vitro* pH when lucerne hay standards were incubated (Table 6.14).

FIGURE 6.6. Effect of cow-diet on *in vitro* pH when inoculum from cows fed P, P:M. P:S and P:M:S[1] were used to incubate P, M, S, P:M, P:S and P:M:S[1] for 24 hours. Data are the average pH of duplicate bottles for each of six forages at each time from both feeding periods (n = 24). LS means ± SEM bars are presented.



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[1] P, pasture; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

Ammonia

Net NH_3 production when the freeze-dried lucerne standard was incubated *in vitro* was significantly lower after 2 and 8 hours when inoculum from cows fed P:M was used compared to using inoculum from other cows (Table 6.14). When forages and forage mixtures were digested using inoculum from cows fed P, P:M, P:S and P:M:S diets, slower NH_3 release from the P:M cows was evident after 2, 4, 12 and 24 hours of incubation (Table 6.15; Figure 6.7).

TABLE 6.14. Effect of cow-diet on *in vitro* pH and *in vitro* production of ammonia (NH_3) and volatile fatty acid (VFA) from freeze-dried lucerne when incubated using rumen inoculum from each of four cows fed either P, P:M, P:S and P:M:S[1]. Ammonia (NH_3) and pH data are duplicates of two incubation runs during both periods for each of four cows; VFA data are based on a single (bulked) sampled from each incubation run in both periods. Least-square (LS) means ± SEM are presented.

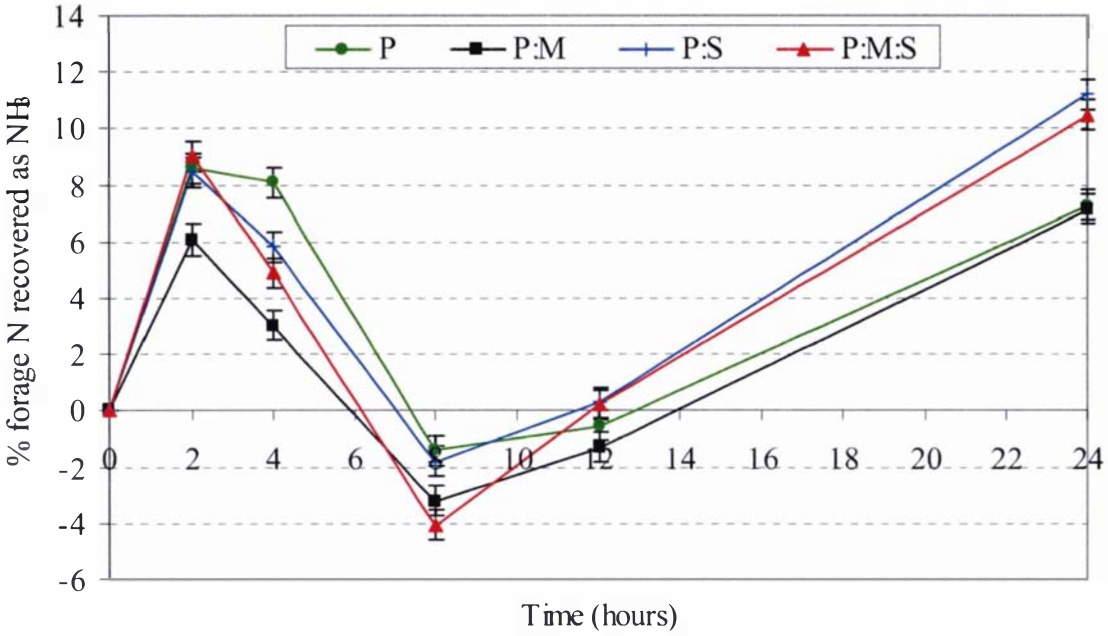
		Diet fed to cows					
Cow-diet	Number	P	P:M	P:S	P:M:S	SEM	Pr
NH_3 (% forage nitrogen recovered as NH_3)							
2 hours	8	11.0^{bc}	8.0^{a}	10.5^{b}	12.5^{c}	0.31	<0.01
8 hours	8	21.7^{b}	19.3^{a}	22.4^{b}	22.9^{b}	0.31	< 0.01
Average	16	16.3^{b}	13.6^{a}	16.4^{b}	17.7^{c}	0.22	< 0.01
In vitro pH							
8 hours	8	6.53^{b}	6.27^{a}	6.37^{a}	6.31^{a}	0.039	< 0.01
VFA (mmol/g DM)							
8 hours	4	2.61	2.63	2.59	3.00	0.196	NS

[1] P, pasture; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

[a, b] LS means within rows with common superscripts do not differ significantly ($Pr < 0.05$).

NS = non-significant ($Pr > 0.05$).

FIGURE 6.7. Effect of cow-diet on net ammonia (NH_3) production (% forage nitrogen, N, recovered as NH_3) when forages were incubated *in vitro* using rumen inoculum from cows fed P, P:M, P:S and P:M:S[1]. LS means ± SEM bars are presented.



[1] P, pasture; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla

TABLE 6.15. Effect of cow-diet on *in vitro* pH at 12 hours and net ammonia (NH_3) yield at 2, 4, 8, 12 and 24 hours when P, M, S, P:M, P:S and P:M:S were incubated using rumen inocula from cows fed four dietary treatments (P, P:M, P:S and P:M:S[2]). Least-square (LS) means ± SEM are presented.

Cow-diet	*In vitro* pH	Ammonia (% forage nitrogen recovered as NH_3)				
	12 hours	2 hours	4 hours	8 hours	12 hours	24 hours
Number	24	24	24	24	24	24
P	6.40^{a}	8.6^{b}	8.1^{c}	-1.4^{b}	-0.5^{b}	7.3^{a}
P:M	6.09^{b}	6.1^{a}	3.1^{a}	-3.2^{a}	-1.3^{b}	7.1^{a}
P:S	6.29^{c}	8.4^{b}	5.8^{b}	-1.8^{ab}	0.3^{a}	11.2^{b}
P:M:S	6.14^{b}	9.0^{b}	4.9^{b}	-4.1^{a}	0.2^{a}	10.5^{b}
SEM	0.034	0.54	0.54	0.54	0.54	0.54
Pr	< 0.001	< 0.001				

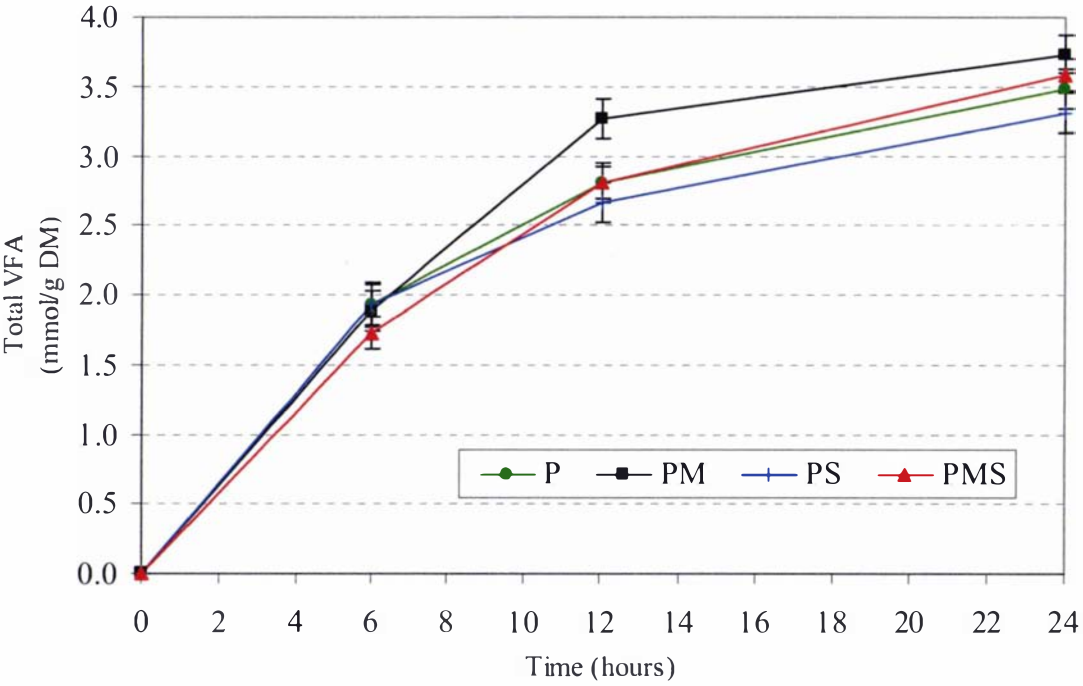
[1] P, pasture; M, maize; S, sulla; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

[a, b] LS means within columns with common superscripts do not differ significantly ($Pr < 0.05$).

VFA

The *in vitro* incubations using P:M inoculum (Figure 6.6) were associated with a higher VFA yield at 12 hours (Figure 6.8) suggesting a more active fermentation. The VFA concentration at 24 hours was also elevated with the P:M inoculum compared with P:S inoculum but not the other cow-diets. Cow-diet effects were not significant. There was no cow-diet effect on total VFA production after the lucerne hay standard was incubated for 8 hours (Table 6.14). Source of inoculum did not affect the molar percentage of acetate and propionate during *in vitro* incubations (Table 6.16) but the percentage of butyrate was higher with P and P:S inoculum (15 – 16% of VFA) compared with inoculum from cows fed diets containing M (12 – 14%). After 24 hours of incubation, the production of VFA (% of DM) was P 24%, P:M 25.7%, P:S 22.8% and P:M:S 24.7%.

FIGURE 6.8. Effect of cow-diet on total VFA yield (mmol/g DM incubated) for forages incubated *in vitro* using rumen inoculum from cows fed P, P:M, P:S and P:M:S[1]. LS means ± SEM bars are presented.



[1] P, pasture; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

TABLE 6.16. Effect of cow-diet on volatile fatty acid (VFA) yield, molar percentage and ratio of acetate:propionate (A:P) and (acetate + butyrate):propionate ((A+B):P) when forages and forage mixtures[1] were incubated using rumen inoculum from cows fed P, P:M, P:S and P:M:S[2]. Data are the least-square (LS) means ± SEM of 6 values, n = 6.

	Time (Hours)	Number	Total (mmol/g DM)	Percentage of				A:P ratio	(A+B):P ratio
				Acetate	Propionate	Butyrate	Minor[3]		
P	6	6	1.92	56.2	26.9	13.7^{a}	3.7	2.20	2.70
	12	6	2.81	54.0	25.8	16.5^{c}	3.3	2.15	2.78
	24	6	3.48	51.2	27.3	17.0^{f}	4.7	1.91	2.54
P:M	6	6	1.89	54.8	29.0	12.8^{a}	3.5	1.92	2.36
	12	6	3.27	58.0	26.3	12.7^{b}	3.0	2.20	2.72
	24	6	3.73	56.2	26.2	13.7^{de}	4.2	2.47	2.73
P:S	6	6	1.93	56.2	25.7	14.7^{a}	4.3	2.22	2.80
	12	6	2.66	54.2	26.3	15.3^{c}	4.3	2.12	2.71
	24	6	3.31	53.3	26.5	15.3^{ef}	5.2	2.04	2.62
P:M:S	6	6	1.73	53.2	28.0	14.4^{a}	4.2	1.98	2.43
	12	6	2.81	56.3	26.6	12.8^{b}	4.1	2.18	2.69
	24	6	3.58	57.6	24.5	12.5^{d}	5.1	2.04	2.99
SEM			0.175	0.32	1.43	0.97	0.32	0.21	0.21
Time x cow-diet effect			NS	NS	NS	< 0.05	NS	NS	NS

[1] Forages incubated are defined in Table 6.2.

[2] P, pasture; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

[3] Minor VFA compose iso-butyrate, valerate and iso-valerate.

^{a,b,c} LS means with common superscripts within columns do not differ significantly ($Pr < 0.05$) within 6 hours (^{a}), 12 hours ($^{b,\ c}$) or 24 hours ($^{d,\ e,\ f}$).

NS = non significant ($Pr > 0.05$).

6.4.5.4 Diet x forage interaction

There were no cow-diet x forage interactions for, pH, net NH_3 production or VFA yield. The profiles of net NH_3 production for forages and mixtures over the 24 hour incubation period were similar when P and S were incubated in rumen inoculum from all cows, but there were some noticeable differences due to inocula source when M, P:M, P:M:S and P:S were incubated (Appendix 6.9). The total VFA yield of forages and mixtures incubated *in vitro* using inoculum from cows fed P, P:M, P:S and P:M:S are illustrated in Appendix 6.10.

6.5 DISCUSSION

This study has evaluated the digestion kinetics of forages and forage mixtures in cows fed different forage-based diets, using a minced preparation specific for fresh forages (Barrell *et al.*, 2000). Digestion and fermentation kinetics of individual forage DM, protein and fibre (Chapter 3; Barrell *et al.*, 2000; Burke *et al.*, 2000) and ryegrass at different maturities (Chaves *et al.*, 2001, 2002a) have been reported but all studies were conducted using one cow fed one diet (lucerne hay), which enabled comparisons between forages, without the confounding effects of the cow or the cow-diet. However, digestion kinetics are a function of the intrinsic properties of feeds and microbial populations of the cow (Mertens *et al.*, 1997; Wohlt, 1997; Weimer *et al.*, 1999), and diet will affect digestion (Horton *et al.*, 1980; Mertens *et al.*, 1998).

The main findings in this study were that forages and forage mixtures differed in their degradation parameters and that cow-diet did affect rates and products of digestion. The low level of supplementation (33% dietary DM) limited the impact of M and/or S in the diet. However, the dietary treatments achieved a 4% unit range in dietary CP and a 10% unit range in both soluble carbohydrate and NDF concentrations.

There were significant dietary treatment effects on cow production. Cows fed P had the highest DM intake, but milksolids production was highest for cows fed the P:M:S diet. When milksolids yield was expressed in terms of DM intake (g MS/kg DM) values were

P (51), P:M (59), P:S (64), P:M:S (66) whereas yield per MJME (g MS/kg ME intake) were 5.6, 6.1, 6.5 and 6.7 for the respective diets.

The rumen parameters measured from the fistulated cows grazing P during the uniformity period had similar rumen pH and NH_3 concentrations. Comparison of *in sacco* incubation rates of minced lucerne hay standard during the uniformity period showed a more rapid degradation rate for the cow to be fed P and a slower initial degradation for the cow to be fed P:M, and these differences suggest a minor effect of individual cows on rumen degradation parameters. Researchers have shown that the cow can influence rumen degradation (Figroid *et al.*, 1972; Nelson *et al.*, 1972; Ayres, 1991; Waghorn and Caradus, 1994; Mertens *et al.*, 1998; Weimer *et al.*, 1999), hence the recommendation that two or three cows or rumen inoculum from two or three cows be used in incubations (Broderick, 1999). However, other studies (Weakley, 1983; Nocek, 1985; Cohen and Doyle, 2001) have shown that rumen digestion and fermentation does not differ between cows. Constraints of the experimental design and resources did not enable separation of cow from dietary effects in the work presented here, but this should be done in future studies.

6.5.1 Forage effects

Forage *in sacco* degradation was slowest for M and fastest for S, with values for P intermediate. The rapid degradation of S can be explained by the low NDF content and high concentration of soluble carbohydrates, but this argument does not apply to the slow degradation of M and P. Maize silage comprises two distinct fractions, and provided the integrity of the grain has been damaged, rapid digestion should take place, but the stover is predominantly fibre and of poor quality and will be slowly degraded.

Studies of fresh ryegrass leaf digestion have shown variations in both lag periods and fermentation kinetics despite similar chemical composition (Table 6.17). Results from this trial showed DM degradation of 7.1 %/h with a short lag period, in contrast to previous data in Table 3.6 (Chapter 3) and Barrell *et al*. (2000) and Chaves *et al*. (2001, 2002a). The ryegrass in all studies was minced in a similar manner and had an average DM degradation of about 9.6 %/h with a 2.1 h lag. Protein degradation rates in all studies ranged between 11 to 15 %/h, and a 4.6 hour lag period was reported in earlier evaluations in Chapter 3. The use of fresh mincing will prevent inhibition by cuticular waxes and the similar chemical composition of the ryegrass leaf in the five datasets (Table 6.17) suggest other factors must have accounted for the slow DM degradation.

TABLE 6.17. *In sacco* degradation parameters (A, B, k, Lag) and composition of ryegrass leaf in studies where it has been prepared by mincing fresh forage.

	A	B	K	Lag	DM	SC	CP	NDF
	(%)	(%)	(%/h)	(h)	(%)	(% DM)		
[1]DM	34	49	7.1	0.9	16.6	5.4	19.7	48.8
CP	43	52	11.7	1.9				
NDF	12	63	5.8	1.6				
[2]DM	44	50	10.6	4.2	18.8	9.1	15.5	48.7
CP	52	41	15.0	4.6				
NDF	20	71	9.3	3.9				
[3]DM	37	49	11.0	0	17.9	8.1	22.5	48.3
CP	31	67	11.0	0				
[4]DM	39	50	10.5	4.4	21.9	8.7	18.9	49.2
[5]DM	38	58	8.7	1.1	21.6	7.8	13.8	49.7
CP	48	48	14.0	0.2				
NDF	20	73	11.0	2				

[1] This chapter; [2] Chapter 3; [3] Barrell *et al.* (2000); [4] Chaves *et al.* (2001); [5] Chaves *et al.* (2002a) and Chaves (2003).

Abbreviations: A, soluble fraction; B, degradable insoluble fraction; k, degradation rate; h, hour; DM, dry matter; SC, soluble carbohydrates; CP, crude protein; NDF, neutral detergent fibre.

Differences in DM digestion rates for ryegrass leaf are important as it forms the basis for the majority of ruminant diets in New Zealand. The variation reported in Table 6.17 is greater than observations for other diets reported here and by Chaves *et al.* (2001, 2002a) and Waghorn (unpublished). The inconsistent digestion kinetics of ryegrass was supported by net NH_3 production. Barrell *et al.* (2000) reported linear NH_3 production over 24 hours, whereas results reported here and in other studies (Chapter 2; Chaves, *et al.*, 2001, 2002a) found NH_3 production rapidly increased for the first 6 to 8 hours, after which there was no further increase and production remained static or decreased (Burke *et al.*, 2000; Chaves *et al.*, 2001, 2002a). This difference in net NH_3 yield might suggest variations between incubations in microbial growth, which in turn affects DM digestion.

Degradation kinetics should be considered with products of digestion. Lower NH_3 concentration in the rumen contents of cows fed mixtures compared to P, and in *in vitro* media of S, P:S and P:M:S compared to P, suggest less wastage and greater utilisation of CP with diets containing S and M. *In vitro* yield of VFA was highest for the P and S diets. *In vitro* pH was relatively low and N availability for microbial growth was limited relative to mixtures, but the VFA production was similar between M and the mixtures.

Lower *in vitro* pH and NH_3 when P:M was incubated relative to P, and when P:M:S was incubated relative to P:S may explain the reduced *in sacco* digestion of DM and NDF of P:M and P:M:S relative to P and P:S, respectively. Decreased NDF digestion can occur when supplements that are rich in starch are fed due to reduced pH (Grant and Mertens, 1992c), and low NH_3 concentration may prevent the growth of fibre-degrading bacteria because NH_3-N is the only source of N for cellulose digesting bacteria (Russell *et al.*, 1992). The supply of NH_3 is the more likely reason for reduced NDF degradation because the *in vitro* pH of mixtures did not fall below 6.0.

6.5.2 Protein degradation and supply

Sulla contained only 2.2 % CT in the DM which would not impair feed intake (Waghorn *et al.*, 1997). Rapid *in sacco* disappearance of protein from S was probably a consequence of extensive cell rupture, but the *in vitro* NH_3 yield was less than that of high protein P (19.7% CP). These data suggest either an effect of the CT in S to lower protein degradation or the soluble carbohydrate in S and M enabling increased N capture in microbial protein. The low concentration of CT in the diets suggests that the soluble carbohydrate content was responsible for the more efficient use of the forage N in diets containing M and S. Therefore, the higher milk protein yield of cows fed P:M:S and P:S relative to cows fed P and P:M was due to the milk yield per se. and not due to the milk protein concentration.

Lower milk fat concentration when M was fed with P was unexpected because of the low proportion of M (and therefore maize grain) in the diet and the high rumen pH. Milkfat depression is common when cows are fed TMR rations, containing about 40% grain (Kolver *et al.*, 2002), but the grain in the P:M diet was about 15% of the DM. Nevertheless the molar ratio of VFA in rumen contents of cows fed P:M, and from *in vitro* incubations did show a lower A:P ratio than for the P diet. Low A:P and (A+B):P ratios suggest efficient energy capture and potential for good production requiring gluconeogenesis (lactose and milk production). Consequently, when M and S were combined with pasture, nutrients were more efficiently utilised with improved effects on milk yield and composition, compared to the other diets.

6.5.3 Effect of cow-diet

This study has shown that digestion and fermentation kinetics of forages were similar when incubated in cows fed different forage diets, but inclusion of M with P appeared to affect *in sacco* and *in vitro* incubations.

Cow-diet affects digestion by changing the rumen environment and microbial population (Weimer *et al.*, 1999), and differences are thought to be due to effects of ruminal pH on microflora (Mertens *et al.*, 1997, 1998). Low *in vitro* and rumen pH, bought about by readily fermentable substrates, does reduce fibre digestion (Robinson *et al.*, 1986; Grants and Mertens, 1992c), but in this study *in vitro* pH was not lowered sufficiently to depress fibre digestion.

Dry matter, CP and NDF degradation rates were slower, lag periods greater and net yield of NH_3 was less when forages and mixtures were incubated *in sacco* and *in vitro* using inoculum from the P:M cow compared to cows fed other diets. Other studies have reported reduced digestion rate of NDF and increased lag times when starch was included in the diet (Mertens and Loften, 1980; Lindberg, 1981; Aitchison *et al.*, 1986). Mertens *et al.* (1998) reported diets based on M reduced rates of both M and lucerne degradation relative to diets based on lucerne. If non-cell wall substrates (eg. starch) comprise a significant proportion of the diet, microbial populations develop an affinity for non-cell wall substrates rather than fibre (Russell and Baldwin, 1979). Results from this study suggest that the microbial population in the rumen inoculum of the cow fed the P:M diet was more dominant in amylolytic bacteria and had less cellulolytic and proteolytic bacteria, than the other diets (Mould and Orskov, 1983; Barrio *et al.*, 1986).

The availability of nitrogen may have limited fibre digestion when forages and mixtures were incubated in the cow fed P:M. Low NH_3 concentration and the presence of starch can lower fibre digestion (El Shazly *et al*., 1961) and this has been demonstrated when pasture was fed with beet pulp to cattle (Carey *et al.*, 1993; Bach *et al.*, 1999).

In this study *in vitro* pH was between 5.9 and 6.2 after 12 and 24 hours when inoculum was taken from cows fed diets containing M, whereas inoculum from P and P:S diets resulted in *in vitro* pH greater than 6.2 throughout the entire incubation. These effects, with significantly lower *in sacco* degradation rates and long lag times for the cow fed the P:M diet suggest a reduced rate of fermentation, yet VFA yield after 12 hours of incubation was greater from the P:M cow than from cows receiving any other diet.

It appears that providing one-third of DM intake as M with pasture affects the degradation of DM, CP and NDF with minor reductions in DM intake, and minor improvements in the efficiency of milk production (g MS/kg DM and g MS/MJ ME). However, when offered at one-sixth of the diet with P and S, animal production per unit of feed intake was improved. The fast degradation rate, CT and high and consistent VFA yield characteristic of S make it a desirable forage, and similar to some forages identified in Chapter 3 and 4. Future work could focus on two levels of M supplementation, with inclusion of S, or other legumes, to achieve a ration that increased and balanced the supply of ME and MP to the dairy cow and optimised the efficiency of milk production.

6.5.4 Evaluation of forage diets using CNCPS

CNCPS was used to evaluate the forages mixtures. A 514 kg Friesian cow in late lactation (210 days since calving and 120 days pregnant) with actual milk production and milk fat and protein concentrations for each dietary treatment presented in Table 6.4, together with the nutrient composition, soluble protein fraction and degradation rates of the pasture and mixtures in Tables 6.2 and 6.9. The predicted outcomes of feeding P, P:M, P:S and P:M:S are illustrated in Table 6.18 and compared with actual intakes and milk production. Initial evaluations of dietary components highlighted the potential of feeding maize silage and sulla with medium quality pasture to increase the supply of ME and MP (Chapter 3), but evaluation of the mixed diets in this study did not demonstrate the same potential value because sulla in this experiment had different degradation rates to the sulla in chapters 3 and 4. The NDF and protein of sulla in this experiment degraded at 9.4 and 22.1 %/h, respectively, compared to 16.4 and 11.7 %/h, respectively, in chapter 3. The percentage of protein in the soluble fractions was lower in this experiment (42.6% vs. 49.5%) and the faster degradation rate was associated with a lower CT concentration in this experiment (2.2 % DM) compared to that in Chapter 3 and 4 (5.6% of DM). CNCPS showed that MP limited milk production for all diets in this experiment (Table 6.18) including sulla fed as a sole diet. This is in contrast to results reported in Chapter 3 and 4 where ME was limiting and thus, the expected benefits of feeding sulla with P and M, which were also limiting in MP, were not observed.

Actual intakes of cows fed pasture was 3.4 kg DM/day greater than CNCPS predictions and 1 – 1.5 kg DM/day greater for the mixed diets (Table 6.18). Actual milk production did not match predicted values, with higher production by cows fed P:M and P:M:S diets (0.7 – 1.0 kg/day) and lower values (0.3 – 0.7) for cows fed P:S and P. Metabolisable protein was limiting for all diets, in contrast to predictions summarised in Table 3.24. These results demonstrate a poor predictive capacity of CNCPS for intake because they are based solely on liveweight and highlight the importance of small changes in forage composition and degradation rates for limiting nutrients.

TABLE 6.18. Evaluation of pasture and mixed forage diets fed to late lactating dairy cows using the CNCPS model.

	P[1]	P:M[1]	P:S[1]	P:M:S[1]
Actual milk production (kg/day)	11.0	11.3	12.4	13.2
Actual intake (kg DM/cow/day)	16.9	14.5	15.1	15.5
Predicted intake (kg DM/cow/day)	13.5	13.5	13.9	14.1
Diet nutrient composition				
ME (MJ/kg DM)	8.4	9.0	9.2	8.8
CP (g/100g DM)	18.7	15	18.9	16.8
Soluble CP (% CP)	56	49	52	51
NDF (g/100 g DM)	52.1	48.9	43.5	45.8
peNDF (g/100 g DM)	31	33	26	29
Total NFC (g/100 g DM)	15	25	23	25
Requirements				
ME requirements (MJ/day)	124	120	130	132
MP requirements (g/day)	1372	1261	1309	1389
Production predictions				
Milk production based on ME supply (kg/day)	14.4	13.5	14.1	14.2
Milk production based on MP supply (kg/day)	12.7	10.6	12.7	12.2
Daily weight change due to reserves (kg/day)	0.6	0.3	0.3	0.2
ME supplied (MJ/day)	142	130	138	137
Total MP supplied (g/day)	1461	1225	1323	1337
MP from bacteria (g/day)	705	768	780	773
MP from undeg. feed (g/day)	756	457	543	564
MP from undeg. feed (%MP total)	48	37	41	42
MP supplied/CP eaten (%)	46	56	46	51
Urea cost (MJ/day)	5.9	1.7	4.7	3.2
Cost of urea (% ME intake)	4.2	1.3	3.4	2.3
Predicted ruminal pH	6.46	6.46	6.46	6.46

[1] P, pasture; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla. Data used for CNCPS evaluation of mixtures was based on data derived from the actual mixtures analysed in Tables 6.2 and 6.9.

Abbreviations: ME, metabolisable energy; CP, crude protein; MJ, megajoules; MP, metabolisable protein; NDF, neutral detergent fibre; peNDF, effective fibre; NFC, non-fibre carbohydrates; undeg., undedgradable.

6.6 CONCLUSIONS

Production of cows fed a medium quality pasture diet can be improved by the addition of high quality forages. Maize silage and S are two forages that have the potential for feeding with medium quality P because of the rapidly fermentable starch and soluble carbohydrate in M and S, respectively. The CT in S was insufficient to increase the MP supply in this trial. Feeding M and S with P improved milk production per unit of DM and ME intake above that of P and mixtures of P:M and P:S. Conventional P:M gave mediocre responses in milksolids yield when M was fed with medium quality pasture. Results from *in sacco* and *in vitro* techniques can be used to explain animal production differences and to develop diets with optimal diet composition. By defining the digestion and fermentation kinetics of forages, in terms of degradation rate, products and consequences of digestion (eg. k, pH, NH_3 and VFA yields) forages with complementary characteristics can be combined for best results.

Digestion and fermentation of P, M and S were different from each other with S being digested faster than P, and P being digested faster than M. When forages were mixed together DM and CP digestion was in between that of individual forages, but mixing forages together affected the NDF digestion of P:S and to some extent P:M relative to the individual forages.

In sacco incubations suggested a cow-diet effect with a slower rate of digestion in the cow fed P:M relative to P or diets with S. However, this study did not separate the effects of the cow and the diet from the cow-diet effect, and it is important to obtain a better understanding of the effects of the cow and the diet on the degradation and fermentation kinetics of forages.

CHAPTER 7

OVERALL DISCUSSION AND CONCLUSIONS

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7.1 OVERALL DISCUSSION

The research presented in this thesis has described the chemical composition, degradation kinetics and the nutritive characteristics of contrasting forages and identified those with a high nutritive value for feeding with medium quality ryegrass and other forages. Selected forages were fed alone or in mixtures to lambs and cows to determine effects of diet on digestion, metabolism and production.

The nutritive characteristics of the forages have been described in terms of their chemical composition, as well as digestion and fermentation kinetics using *in sacco* and *in vitro* methods. *In sacco* and *in vitro* techniques allowed several forages to be evaluated relatively quickly and cheaply and these methods have enabled a simple comparison between forages (Chapter 3). *In sacco* incubations allowed the degradation parameters of all forage components (dry matter, DM; crude protein, CP; and fibre) to be defined in terms of soluble (A) and degradable fractions (B), degradation rates (k) and lag periods (L). *In vitro* incubations measured the products of fermentation as net ammonia (NH_3) and volatile fatty acid (VFA) yields over 24 hours.

In sacco and *in vitro* methods have been used to describe the characteristics of feeds used in total mixed rations (TMR; typically diets made up of concentrates and chopped conserved forage), but relatively little research had used these techniques to describe pasture-based fresh forages. Most studies have used freeze-dried and ground (FDG) dietary components prior to evaluation, but the FDG method did not simulate grazed pasture. Fresh forages have been chopped (Hoffman *et al.*, 1993; Kolver *et al.*, 1998b) and more recently minced to replicate material chewed by animals grazing fresh forage (Waghorn and Caradus, 1994; McNabb *et al.*, 1996). Barrell *et al.* (2000) and Cohen and Doyle (2001) showed the effect of the different preparation methods on *in sacco* degradation and *in vitro* fermentation parameters, and concluded that mincing was the most appropriate preparation for fresh forages. The particle size distribution of all forages examined in Chapters 3 and 6 were similar to reports for chewed boli and rumen contents of sheep and cattle fed fresh forages.

The analyses of forages (Chapter 3) utilised a single cow fed the same diet throughout to obtain relativity between forages. Differences between individual cows in digestion kinetics (Mehrez and Orskov, 1977; Grant and Mertens, 1992b; Waghorn and Caradus, 1994) are acknowledged but for the purpose of ranking, a single cow fed the same lucerne hay throughout provided a consistent rumen environment. The effect of diet on digestion kinetics of forages (Grant and Mertens, 1992b; Weimer *et al*., 1999) also requires that a consistent feed type be used in comparisons. This could not be achieved with pasture, so lucerne hay was chosen because it provided both stalky (stem) and good quality (leaf) components.

The experiment described in Chapter 6 attempted to address the effect of cow-diet on degradation kinetics and showed that diets containing maize silage reduced digestion rates compared with diets of pasture and pasture:sulla. This experiment (Chapter 6) used only one fistulated cow fed each diet, so cow and diet effects could not be separated, but the results have highlighted the need for more comprehensive analysis of cow and diet effects, especially as maize silage is an important component of many cow diets in New Zealand.

Evaluation of degradation and fermentation parameters between incubation runs required the animal(s), diet composition, intake, feeding schedule and characteristics of the rumen inocula (rumen pH, NH_3 and VFA concentrations) to be defined. The rumen inocula used for *in sacco* and *in vitro* incubations (Chapter 3 and 6) was defined with *in sacco* and *in vitro* ryegrass standards. However, evaluation of several ryegrass preparations (here and Waghorn, per com.) identified occasional variations which suggested ryegrass to be a poor choice of standard. Small variations in rumen inocula pH, NH_3 and VFA concentrations were noted.

The data described in Chapter 3 compare and rank contrasting forages in terms of their DM, CP and fibre degradation and fermentation kinetics. Compared with perennial ryegrass, *in sacco* incubations have shown that legume and herb DM, protein and fibre components are degraded faster, and that C4 plant material (kikuyu, paspalum and maize silage) are degraded more slowly. Rates of DM degradation ranged from 3 to 26 %/h. Condensed tannins (CT) in legumes were associated with lower proportions of soluble protein and *in sacco* protein degradation rates, but had no effect on potential (PD) and effective (ED) degradability, compared with legumes without CT. The effects of CT were clearly evident *in vitro* with only 5 – 25% of plant nitrogen (N) appearing as NH_3 in legumes containing CT and between 35 – 49% in equivalent legumes without CT (Chapter 3).

The ranking of *in sacco* degradation rates with contrasting forages complement animal studies (Ulyatt, 1981; Brown, 1990; Stevens *et al*., 1993; Johnson and Thomson, 1996; Harris *et al*., 1997) and the lamb experiment undertaken here (Chapter 4). Animals fed rapidly digested forages (eg. white clover, sulla, chicory) have higher feed intakes and better production compared with those fed grasses which were relatively slow to degrade. Forages that were degraded slowly generally contained more structural fibre and less rapidly degraded cell contents (soluble carbohydrates and protein) than legumes and herbs. The positive relationship between animal production data and rates of degradation *in sacco* supports the use of this technique to indicate the feeding value of forages.

The products of *in vitro* fermentation supported digestion kinetic data. The high crude protein content of legumes was degraded rapidly *in sacco,* and resulted in a high net NH_3 accumulation, with rapid rates of VFA production *in vitro.* Net NH_3 concentrations underestimated true proteolysis because some NH_3 is utilised for microbial growth. Condensed tannin in high protein legumes reduced rumen proteolysis *in vitro* and *in vivo* in lambs (Table 4.9 in Chapter 4; Waghorn *et al*., 1987a, b; 1994b; Stienezen *et al*., 1996). Rumen NH_3 concentrations were lower in the lambs fed sulla, pasture:sulla and lucerne:sulla compared to other diets and relative to CP eaten (Chapter 4).

Incubations of perennial ryegrass in this and other studies have resulted in inconsistent degradation and NH_3 production detailed in 6.5.1. Protein degradation of ryegrass-based pastures is extensive, limits amino acid availability for absorption, and requires significant urea synthesis (Beever, 1993), especially when production is limited by ME intake. The net yield of NH_3 measured by Chaves *et al*. (2001, 2002a) and from incubations reported in Chapter 3 of this thesis indicate a rapid proteolysis for the first six hours of incubation, after which the concentration declined or remained static. However, in earlier work with ryegrass Barrell *et al*. (2000) reported rapid and linear accumulation of NH_3 for the entire incubation, similar to white clover. In other instances digestion has been very slow for about six hours, after which NH_3 and VFA accumulated at an apparently normal rate (Waghorn, unpublished). These results suggest inconsistent digestion and possible inhibition of ryegrass digestion in some circumstances. Table 6.17 illustrates the differences in degradation parameters especially in lag and k values for five incubations with ryegrass leaf, but there were only small differences in fibre, CP and soluble carbohydrate concentrations. The basis for the variations between ryegrass incubations is not understood, but may have practical consequences if they occur during digestion. A better understanding of the way which nutrient composition affects digestion of the individual components (DM, CP and NDF) of pasture may explain poor animal production in some instances.

The data in Chapter 3 provided a nutritional basis for formulating mixed rations from forages that have characteristics that complement each other, using nutrient composition, digestion and fermentation parameters derived from *in sacco* and *in vitro* incubations as inputs to the Cornell Net Carbohydrate Protein Synthesis (CNCPS V5.0) model. The ideal diet for high performing ruminants requires a low but adequate concentration of rapidly digested structural fibre enabling high intakes (Chapter 2) and protein should be less degradable than that of most fresh forages, to provide more amino acids to the small intestine when MP supply is limiting. Animal production from high quality pastures (< 45% NDF; > 25% CP) are limited by ME (Chapter 3; Chapter 4; Kolver and Muller, 1998) and would be best fed with forages that supply more energy as rapidly digested fibre, soluble sugars and/or starch. High quality pastures are only available for about three months of the year in spring, and low to medium quality pastures (> 45% NDF; < 25% CP) are grazed for about six months (October to March) which coincides with the majority of the lactation. Modelling with CNCPS using digestion data from Chapter 3 identified high quality forage legumes for feeding with medium quality pasture. However, CNCPS does not model the effects of CT forages satisfactorily because the effects of CT on protein degradation were partly evident *in sacco*, but the *in sacco* method and CNCPS does not take into account the effect of tannin on the soluble protein fraction, which would also reduce the digestion of the soluble fraction.

Several forages were identified as having potential for meeting the energy and protein requirements of dairy cows. Sulla, was one that appeared to have potential and meet many of these criteria, but lucerne, red clover and white clover also had promise according to CNCPS. Sulla has potential because it contains at least 20% soluble carbohydrate, 20% CP, 25% NDF and 2 – 8% CT in the DM. The *in sacco* degradation and *in vitro* fermentation kinetics appeared ideal, and sulla is highly palatable with high DM yields. These factors prompted the use of sulla with lambs to determine the effects of feeding alone, with pasture, lucerne and white clover. It was envisaged that the CT in sulla would bind with protein from lucerne and white clover and reduce protein degradation of the mixtures, and the soluble carbohydrate content and rapidly digested NDF would provide an energy source to maximise microbial protein synthesis. The overall objective of this thesis was to identify forage mixtures suitable for optimising nutrient intake of high producing dairy cows, but lambs were used as a model to reduce costs.

Higher feed intakes and better production were reported in sheep fed sulla compared with pasture (Terrill *et al.*, 1992a), but the effects of CT on production depend on dietary concentration. In a four-month trial with growing lambs, sulla containing 8.8% CT in the DM was detrimental to lamb growth (Douglas *et al.*, 1999) but the CT did lower both NH_3 concentrations and acetate:propionate (A:P) ratios in the rumen (Terrill *et al.*, 1992a; Stienezen *et al.*, 1996; Douglas *et al.*, 1999). The CT increased flux of sulla N to the intestine for absorption (Bermingham *et al.*, 2001) and the low A:P ratios suggested a better supply of glucogenic precursors for lactating dairy cows. *In vitro* incubations and ruminal measurements in lambs fed sulla (Table 4.9; Chapter 4) suggested a reduction in proteolysis in response due to either the CT or rapidly digested carbohydrates. Trials with lactating cows fed *Lotus corniculatus* containing CT have shown good production relative to pasture (Woodward *et al.*, 1999, 2002) and *Lotus corniculatus* where the CT was denatured by polyethylene glycol (Woodward *et al.*, 1999).

Feeding sulla with pasture or lucerne on a 50:50 DM basis demonstrated synergistic effects on feed intakes, LW gains, carcass weights, wool growth and rumen parameters (Chapter 4). Lamb growth rate was lowest in lambs fed pasture as a result of lower DM intakes compared to diets containing legumes. The results of the experiment in Chapter 4 may also have been obtained with other forages that supplied similar energy. Spreadsheet models suggested animal production to be limited by energy supply and the supply of metabolisable protein was in excess of requirements. This was borne out by the strong relationship between ME intake and liveweight gain. No effect of the CT in sulla was observed, as sufficient metabolisable protein was already reaching the small intestine as microbial and undegraded dietary protein. In this study carcass fat was not affected by the sulla, although the CT in *Lotus corniculatus* has increased milk protein and lowered milkfat concentrations in sheep (Wang *et al.*, 1996b) and dairy cows (Woodward *et al.*, 1999).

Within the ranges of LW, intake and LW gain achieved in the experiment described in Chapter 4 a LW gain increase of 100 g/day is achievable if the daily DM and ME intake is increased by 0.23 kg and 40.2 MJ. Regression analysis found that DM and ME intake were most affected by concentration of NDF in the dietary DM, and LW gain was most affected by ME intake. Knowledge of these relationships allows the potential intake and LW gain to be predicted.

Metabolisable protein supply for lambs fed pasture, lucerne, sulla and lucerne:sulla was estimated as 105, 162, 166 and 172 g/day (Chapter 4), respectively, and comparable to the estimates of whole body protein synthesis (WBPS) for respective treatments (93, 151, 152 and 180 g/day). The WBPS and FSR of protein in muscle was higher in lambs fed lucerne, sulla or lucerne:sulla compared to pasture.

The lamb study (Chapter 4 and 5) was followed by a trial with lactating dairy cows to evaluate forage mixtures on production and rumen digestion in late lactation. Maize silage and sulla were fed with medium quality pasture. In Chapter 3 CNCPS identified that the supply of ME and MP was balanced when maize silage and sulla were combined with two-thirds pasture (Table 3.24), and therefore a dairy cow trial was conducted to measure the effect feeding a diet containing 67% ryegrass pasture and the remaining DM being either maize silage (P:M), sulla (P:S) or maize silage and sulla (P:M:S).

Milksolids responses were greatest from the P:M:S diet followed by P:S. Feeding maize silage with medium quality pasture did not increase milksolids production and rumen digestion rates were slowest with this diet. *In sacco* incubations showed that P:M reduced DM and fibre degradation rate and NH_3 availability may have limited microbial growth *in vitro*. Optimal ratios of sulla and maize silage with pasture, as well as other high quality forages, need to be determined for high, medium and poor quality pastures to identify optimal nutrient mixtures as well as maximum intakes. CNCPS evaluation suggests that the appropriate time for maize silage supplementation is in early lactation when pasture contains high concentrations of protein, but sulla could be best at other times when protein is limiting, but the principal determinant of production will be DM intake.

Animal trials have focused on feeding sulla with pasture and other legumes but CNCPS modelling showed red clover, chicory, *Lotus corniculatus* and plantain may also be ideal forages for feeding with pasture, especially when MP is limiting. Legumes that contain condensed tannins (CT), particularly *Lotus corniculatus,* may have greater potential in the pasture-based system than sulla and have proven valuable for dairy cows (Woodward *et al*., 1999). The CT in *Lotus pedunculatus* appears to be detrimental to animal production when fed alone, but could be effective when fed with non-CT forages by reducing proteolysis. Optimal forage mixtures will vary with the quality and type of pasture available at different times of the year and modelling will allow mixtures that balance the supply of ME and MP with animal requirements to be identified.

This thesis has demonstrated the benefits of *in sacco* and *in vitro* techniques for comparing contrasting forages and forage mixtures. Composition and degradation kinetics enable forage mixtures to be evaluated (eg. AFRC, 1992; SCA, 1990; CNCPS V5.0, 2003) to optimise nutrient supply. These systems do not predict intake and forage factors affecting intake of rations need to be developed. In addition to identifying forages with potential for feeding with pasture of varying quality, a further challenge will be incorporating them into grazing systems. The work presented in this thesis provides information for future refinement of ryegrass pasture-based feeding systems for dairy cows.

7.2 SUMMARY AND CONCLUSIONS

This thesis has shown that the nutrient composition and digestion of forages varies between forages and with stage of maturity. In Chapter 3, legume and herb species were digested faster than temperate grass species with tropical species having the slowest digestion rates. The *in sacco* technique was used to determine the soluble, slowly degradable and undegradable fractions and the degradation rate of DM, CP and fibre (NDF and ADF) components. These data were used in the CNCPS model to identify which forages had potential to optimise animal production as a sole diet or as a forage mixture. Modelling identified whether MP or ME was first limiting but did not predict intakes, which have a major effect on production.

Results from Chapter 3 indicated that white clover, lucerne and sulla were appropriate for feeding with medium quality pasture to maximise production. These were fed to lambs as sole diets or as 50:50 mixtures of pasture:sulla, white clover:sulla and lucerne:sulla. Lamb production was improved by feeding forage legumes that had low NDF, high protein and/or high soluble sugar concentrations with the fastest LW gains in lambs fed sulla, white clover, white clover:sulla and lucerne:sulla, and the slowest LW gains in pasture-fed lambs. Sulla has good potential to supplement pasture, but greatest benefits for LW gain were achieved when fed with lucerne. The presence of CT in sulla did affect rumen proteolysis in some diets, but animals did not respond to the increase in protein supply and the improved production of lambs fed diets containing legumes was due mainly to an increase in ME intake. Measurements of WBPS and FSR in Chapter 5 showed greater WBPS and muscle FSR in lambs fed lucerne, sulla and lucerne:sulla than lambs fed pasture. Rumen parameters, *in sacco* and *in vitro* incubations and spreadsheet modelling of nutrient supply explained differences between nutrient supply and animal requirements when mixed forages were fed.

The potential of feeding sulla (S) and maize silage (M) with medium quality pasture (P) was tested in a dairy cow trial during February and March. A diet containing one-third sulla or one-third maize silage:sulla (1:1 mix of maize silage and sulla) with two-thirds of pasture (P:S and P:M:S, respectively) improved milk production relative to cows fed pasture (P) and pasture with one-third maize silage (P:M). *In sacco* and *in vitro* incubations demonstrated effects of forage mixtures on digestion. Maize silage appeared to slow digestion and fermentation rates. The cow-diet effect, with a slower digestion in the cow fed P:M relative to diets without M has consequences for *in vitro* and *in sacco* evaluations.

The digestion and fermentation kinetics of forages, in terms of degradation rate, products and consequences of digestion (eg. k, pH, NH_3 and VFA yields) have been used in the CNCPS model to identify forage mixtures able to meet cow requirements for metabolisable energy and protein. Production, whether it be liveweight gain or milk production, can be increased when ryegrass-based pastures are combined with forages that increase and/or balance the supply of ME and MP. Results from this thesis identified high quality legumes as one option to increase animal production. Sulla and lucerne were two high quality legumes capable of improving liveweight gain and/or milk production. The benefits of these two high quality legumes is that they increase feed intake and supply more metabolisable energy and protein to the animal for productive purposes.

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APPENDICES

APPENDICES

APPENDIX 3.1. Method for measuring particle size distribution

1. Weigh about 30 g of wet minced feed into silver pie tins.
2. Record weight of filter paper on recording sheet and label filter paper according to size of sieve.
3. Put set amount of water into plastic measuring container (1400 – 1600 mL) and pour into plastic bottle on the sieving apparatus.
4. Turn on particle sieve and ensure no water leaks out of each sieve.
5. Tip weighed out feed into top sieve.
6. Use wash bottle to ensure no feed gets on side of sieves and stir top sieve plate continuously.
7. Run machine for 4 – 5 minutes or until you think the sample has been distributed evenly.
8. Scrape feed off each sieve plate onto filter paper under vacuum. Use water to rinse all feed off sieve plate.

Solubles

9. Drain water out of the machine into a tared container and weigh.
10. Stir liquid and pour a representative sample into a centrifuge bottle (1-litre) and make sure all bottles weigh the same so the centrifuge machine is balanced.
11. Spin centrifuge for 20 mins at 2.5 x *g*.
12. Pour liquid gently out of centrifuge bottles and filter through fluted filter paper. You will need to use additional water to completely rinse the bottles.
13. Put all filter papers in pink oven upstairs (60°C) weigh again after 24 hours.

APPENDIX 3.2. Methods for *in sacco* and *in vitro* incubations.

Days before incubation

Prepare cow by feeding cow two-three days prior to use.

Prepare forages by chopping frozen forage into short lengths. Re-freeze forage and mince using a Kreft Compact meat mincer R70 (Kreft, Gmbh). Use cold mincer parts by cooling them in the freezer.

In sacco

Day before incubation

1. Label 100 x 100 mm dacron bags.
2. Place about 30 g of minced forage (approximately 6 g DM) into 100 x 100 mm dacron *in sacco* bags (mean pore size 35 μm). Place 6 g DM of a standard forage (eg. lucerne hay, ryegrass or white clover) into dacron bags for removal at times (eg. 12 h) to enable variation between runs to be monitored.
3. Seal *in sacco* bags with a heat sealer.
4. Fill enough *in sacco* bags for duplicate bags at each time to be removed from cow (eg. 0, 2, 6, 12, 24 and 72 hours).
5. Place *in sacco* bags into freezer overnight.

Day of incubation

6. Remove bags from freezer so that the bags are thawed out enough before they are incubated in the cow.
7. Label lingerie bags with different colour ear tags. Put *in sacco* bags of forage from time 2 h and onwards into weighted lingerie bags. Record which *in sacco* bags are in which lingerie bag to avoid confusion when bags are removed from the cow. *In sacco* bags at 0 h are not incubated in the cow but are hand-rinsed under cold water.
8. When the cow is bought up to the crush to collect rumen liquor place lingerie bags into the rumen of the cow through the fistula after collecting rumen liquor sample.
9. Remove lingerie bags from the rumen at times throughout the incubation and hand-rinse *in sacco* bags with cold water until no further colour appears.
10. Dry *in sacco* bags at 60°C for 48 hours. Remove residues from *in sacco* bags, grind with coffee grinder and get analysed by NIRS for nutrient composition.

In vitro

Day before incubation

1. Record weights of empty bottles without lids. NB: When washing bottles never use detergent.
2. Weigh about 2.5 g of freshly minced forage (approximately 0.5 g DM) into 50 mL bottles. Place 0.5 – 0.6 g DM of a standard forage (eg. freeze-dried lucerne) into bottles to measure ammonia and volatile fatty acids at 2 and 8 hours.
3. Cap bottles and place bottles in freezer overnight.
4. Turn incubator on the day before the incubation so the temperature is stable for incubation.
5. Make up McDougal's buffer (artificial saliva) and mix overnight.
6. Label eppendorf tubes for ammonia and VFA samples.

Day of incubation

1. Feed cow first thing in the morning so the rumen inoculum is taken from a cow two-three hours after eating.
2. Gas buffer for 45 minutes and warm to 40°C in a bucket of hot water.
3. Put samples in incubator (up to 90 minutes before adding rumen inoculant).
4. Make reducing agent – enough for 0.5 mL/sample.
5. Add buffer and reducing agent to bottles. Remove two bottles from the incubator at a time. Place one bottle under the CO_2 gas flow whilst buffer and reducing agent is being added to the other bottle that has already been gassed. 12 mL of buffer is added to bottles using an automatic pipette (2 x 6 mL squirts) followed by 0.5 mL of reducing agent. Bottles are weighed, recapped and returned to the incubator. Two people will be needed for this to minimise exposure of the bottles to oxygen from this time onwards. One person will be needed to collect and return the bottles to the incubator while the other person is needed to purge bottles with CO_2, add buffer and reducing agent to the bottles and weigh and recap bottles.
6. Allow reducing agent to work for about 15 minutes whilst getting rumen contents.
7. Put hot water into a thermos flask and bring the rumen fistulated cow into the crush. Take bucket, cheese cloth, thermos flask and funnel to the cow. Open up the fistula and take some rumen contents (c. 4 kg) from within the rumen and squeeze through doubled-over cheesecloth into bucket. Pour rumen liquor into flask through a funnel. It is important to fill the flask to minimise exposure of the rumen liquor to oxygen.

8. Go directly back to the incubation room with the rumen liquor and measure the pH of the rumen liquor collected using a calibrated pH meter.

9. Pipette 3 mL of rumen liquor into each bottle at the incubator. It is important to do this fast to minimise the amount of air getting into the gassed bottles. NB: Cut off pipette tip to facilitate handling of the rumen liquor and keep stirring rumen contents whilst sampling. Depress pipette before immersing the tip into the rumen liquor. Place tip 5 cm below surface to obtain a 3 mL sample of rumen liquor.

10. Close incubator and turn on the shaker. Monitor incubator temperature throughout the incubation.

11. Take two samples of rumen contents for ammonia (NH_3) analysis and two samples of rumen contents for volatile fatty acids (VFA).

12. Remove bottles at designated times throughout the incubation and record pH of the *in vitro* bottle, take samples for rumen NH_3 and VFA according to protocol.

13. It is important to label eppendorfs appropriately and make sure they are consistent with the data sheet.

McDougal's Buffer (artificial saliva)

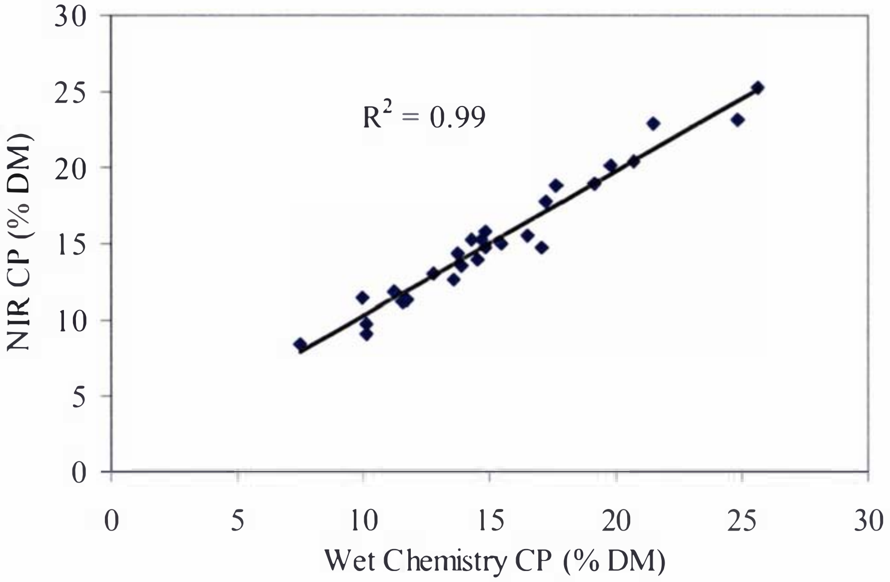
Per litre	
NaHCO3	9.8 g/L
Na_2HPO_4	3.6 g/L or $Na_2HPO_4.12H_20$ = 9.3 g/L
NaCl	0.47 g/L
KCl	0.57 g/L
$CaCl_2$ anhydrous	0.04 g/L
$MgCl_2$ anhydrous	0.06 g/L

Reducing agent

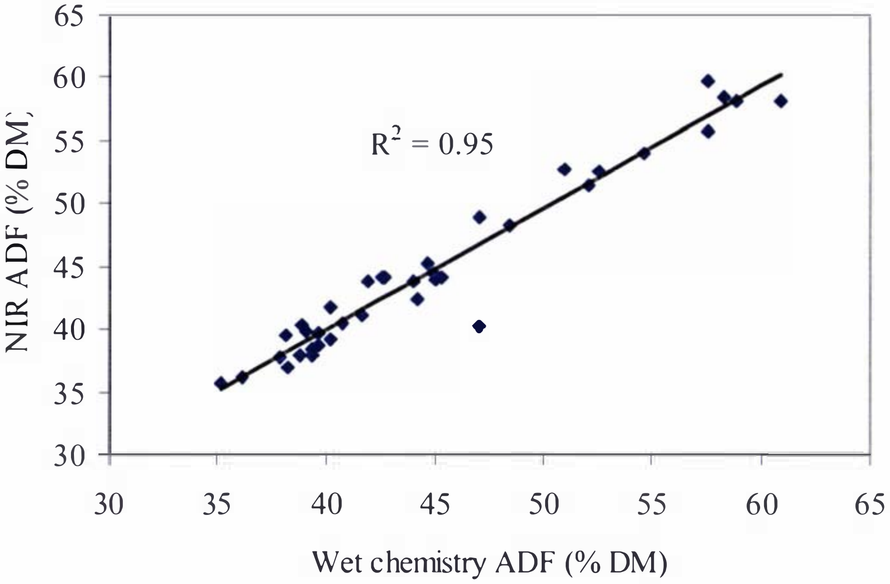
Per 50 mL	
Cysteine HCl	315 mg
Na sulphide	315 mg
Water	48 mL
1 mol/L NaOH	2 mL

APPENDIX 3.3. Comparison of crude protein (CP), acid detergent fibre (ADF) and neutral detergent fibre (NDF) estimated by wet chemistry and Near InfraRed Spectroscopy (NIRS).

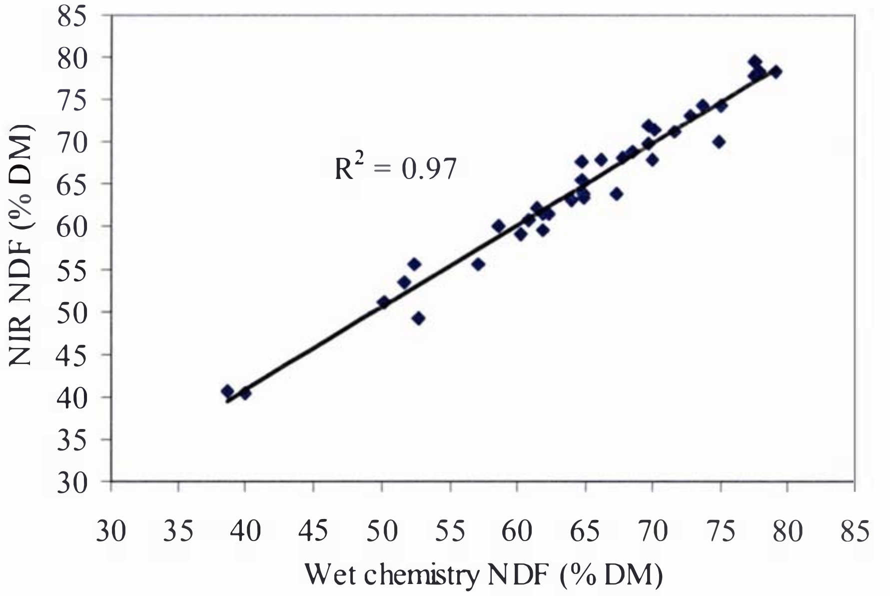
Crude protein



ADF



NDF



APPENDIX 3.4. Method for measuring pH and collecting samples for ammonia and volatile fatty acid (VFA) analysis.

pH

Use pH meter to determine pH of samples collected from rumen contents squeezed through cheesecloth for *in vitro* incubation; rumen contents obtained from stomach tubing lambs; or *in vitro* incubation bottles. Immerse probe directly into *in vitro* bottle or rumen content samples.

Ammonia

Rumen contents squeezed through cheesecloth

1. Take sample of squeezed rumen contents from thermos flask.
2. Add 1 mL of rumen liquor to a microcentrifuge tube containing 15 μL of concentrated HCl.
3. Shake to mix acid with sample and facilitate protein precipitation.
4. Centrifuge for 15 minutes at 14,000 x *g* (any microcentrifuge)
5. Pipette off supernatant and transfer to another microcentrifuge tube. Freeze at -20°C.

Rumen contents from stomach tubing

1. Suck sample from rumen using a lavage (stomach) tube.
2. Spin about 10 mL for 15 mins at 28,000 x *g* using the Sorval centrifuge.
3. Take 1 mL of supernatant and add it to a microcentrifuge tube containing 15 μL of concentrated HCl.
4. Shake.
5. Centrifuge for 15 minutes at 14,000 x *g*.
6. Pipette off supernatant and transfer to another microcentrifuge tube. Freeze at -20°C.

Liquid from in vitro incubation

1. Take 1 mL sample from *in vitro* bottle and add to a microcentrifuge tube containing 15 μL of concentrated HCl.
2. Shake.
3. Centrifuge for 15 minutes at 14,000 x *g*.
4. Pipette off supernatant and transfer to another microcentrifuge tube. Freeze at -20°C.

Volatile Fatty Acids

Rumen contents squeezed through cheesecloth

1. Take sample of squeezed rumen contents from thermos flask.
2. Add 1.5 mL of rumen liquor to a microcentrifuge tube.
3. Centrifuge for 15 minutes at 14,000 x *g* (any microcentrifuge)
4. Pipette off supernatant and transfer to another microcentrifuge tube. Freeze at -20°C.

Rumen contents from stomach tubing

1. Suck sample from rumen using a lavage (stomach) tube.
2. Spin about 10 mL for 15 mins at 28,000 x *g* using the Sorval centrifuge.
3. Take 1.5 mL of supernatant and add it to a microcentrifuge tube.
4. Centrifuge for 15 minutes at 14,000 x *g*.
5. Pipette off supernatant and transfer to another microcentrifuge tube. Freeze at -20°C.

Liquid from in vitro incubation

1. Take 1.5 mL sample from *in vitro* bottle and add to a microcentrifuge tube.
2. Centrifuge for 15 minutes at 14000 x *g*
3. Pipette off supernatant and transfer to another microcentrifuge tube and freeze.

APPENDIX 3.5. Method for measuring ammonia concentration (Chaney and Marbach, 1962).

This procedure is based on Chaney and Marbach (1962) and is a colourimetric procedure based on a combination of reagents for the catalysed indophenol reaction for the determination of ammonia, which produces a stable blue colour.

Preparation

1. Prepare solution A and B at least 24 hours before using. Make sure you use the same solution A and B for each run.

Solution A

Phenol	1 g
Na. Nitroprusside	5 mg

Dissolve in 100 mL of milliQ water. Store in dark bottle as reagent is light sensitive. Store in fridge at 4°C and do not keep for more than 1 week.

Solution B

Sodium hydroxide	0.5 g
Sodium hypochlorite (Janola)	1.7 mL

Dissolve in 100 mL of milliQ water. Store in brown bottle as reagent is light sensitive. Store in fridge at 4°C and do not keep for more than 1 week.

2. Use a standard (rumen contents) that will be used for all plates to monitor variation between plate and day variation. Prepare 100 μL eppendorfs of rumen liquor. Dilute 20μL of rumen contents with 80 μL of acidified artificial saliva (McDougal's buffer).

3. Prepare standard curve that ranges from 0 to 1122.5 ng of NH_3/20 μL. Prepare a standard stock solution and dilute it to achieve a range in concentrations.

Standard stock solution

4. Pre-weigh a 250 mL beaker.
5. Weigh out 802.35 mg of NH_4Cl and add it to the beaker.
6. Add about 200 mL of artificial saliva made with milliQ water and add 5 mL of concentrated HCl.
7. Dissolve and make up to 250 mL with artificial salvia.
8. Record final weight of beaker.

Concentration of standard stock solution was 60.0 mmol NH_4Cl/L or 3.21 mg/g
(Weight of NH_4Cl = 0.8374; final weight = 250.0 g; molecular weight = 53.49 g).

Standard curve

9. Prepare standard curve by adding *x* μL of stock solution to *x* μL of dilution buffer according to Table 3.5.1.

TABLE 3.5.1. Preparation of samples for development of standard curve.

Number	Stock (μL)	Dilution buffer (μL)	Total (μL)	mmol NH_3/L	ngNH_3/uL μgNH_3/mL	ng NH_3/20uL
1	0	4000	4000	0	0	0
2	20	3980	4000	0.3	5.102	102.042
3	40	3960	4000	0.6	10.204	204.084
4	60	3940	4000	0.9	15.306	306.126
5	80	3920	4000	1.2	20.408	408.168
6	100	3900	4000	1.5	25.511	510.210
7	120	3880	4000	1.8	30.613	612.252
8	140	3860	4000	2.1	35.715	714.294
9	160	3840	4000	2.4	40.817	816.336
10	180	3820	4000	2.7	45.919	918.378
11	200	3800	4000	3	51.021	1020.420
12	220	3780	4000	3.3	56.123	1122.462

Method

10. Remove samples for ammonia analysis from deep freeze). Defrost samples, shake, spin for 15 minutes at 14,000 x *g*.

11. Prepare a series of diluted (5, 10 or 20 fold) samples collected at different times to determine how much to dilute the sample before measuring ammonia concentration. Once the appropriate dilution factor is determined dilute the samples that ammonia concentration will be determined on.

12. Remove standard samples from the deep-freeze.

13. Put microtitre plate on ice.

14. Add 20 μL of standards, samples and diluted rumen contents into wells of microtitre plate.

15. Add 100 μL of solution A followed by 100 μL of solution B using automatic pipette.

16. Remove from ice and note time.

17. Shake on microtitre shaking plate for a few minutes.

18. Store in warm drying cupboard for 90 minutes after last well had solution B added. A series of measurements were undertaken prior to measuring ammonia

concentration of samples to determine the best method and time required for the reaction to stabilise.

19. Turn on spectrophotometer.

20. At 60 minutes shake again on microtitre shaking plate.

21. Read absorbency at 625 nm using a spectrophotometer.

NB. Chaney and Marbach (1962) found that the maximum blue colour was produced at room temperature after 30 minutes, but preliminary work found that it took longer for the blue colour to stabilise. It is thought that cysteine may interfere with the reaction and the blue is slower to develop and stabilise. Cysteine sulphide reducing agent was added to the *in vitro* bottles and therefore samples taken from the *in vitro* bottles for ammonia analysis contained a small amount of cysteine sulphide. By allowing 90 minutes for the reaction to take place, placing plate in a warming cupboard warmer than room temperature and diluting the samples rectified the effect of the cysteine. However, if cysteine is a major factor affecting the reaction it may be worth increasing the concentration of the reagent by 5 (see example) and decreasing the amount of sample used on the microtitre plate (5 μL cf. 20 μL)

Eg.

Solution A

Phenol	5
Na. Nitroprusside	25 mg

Dissolve in 100 mL of milliQ water. Store in dark bottle as reagent is light sensitive. Store in fridge at 4°C and do not keep for more than 1 week.

Solution B

Sodium hydroxide	2.5 g
Sodium hypochlorite (Janola)	8.5 mL

Dissolve in 100 mL of milliQ water. Store in dark bottle as reagent is light sensitive. Store in fridge at 4°C and do not keep for more than 1 week.

Calculating moles of ammonia per plant nitrogen using an excel spreadsheet

Column	Title of column	Units	Equation
A	Forage		
B	Sample ID		
C	Bottle ID		
D	Eppendorf ID		
E	Sample wet weight	grams	
F	Sample dry wet	grams	DM% x sample wet wt (E)
G	Protein	% DM	See NIR results
H	Nitrogen (N)	% DM	Protein (G)/6.25
I	N per flask	mg N/flask	N (H)/100 x sample dry wt (F) x 1000
J	Moles N per flask	mmol N/flask	mgN/flask (l)/molecular wt N (14 g)
K	Buffer weight	grams	
L	Water pool	mL	Buffer wt (K)+ 3 + 0.5 + (sample wet wt (E) x 0.5*)
M	Plate reading	ng NH_3/20 µL	
N	Dilution factor of unknown sample (DF)		
O	Moles NH_3 in water pool	mmol NH_3/L water pool	Plate (M) x $2.94e^{-6}$ x DF (N) x 1000
P	Moles NH_3/flask	mmol NH_3/ flask	mmol NH_3/L water pool (O) x (water pool (L)/1000)
Q	Moles NH_3/flask adjusted	mmol NH_3/flask	Time – Time 0 (average)
R	NH_3 per plant N	µmol NH_3/mmol plant N	$\frac{\text{mmol NH}_3\text{/flask (Q)}}{\text{mmol N/flask (J)}}$

* depends on type of forage and preparation of forage as to how much of the sample is part of the water pool.

Time	Minced	Chopped
0 – 4 hours	50% of water pool	10% of water pool
5-12 hours	70% of water pool	30% of water pool
13-24 hours	90% of water pool	50% of water pool

Notes:

Background NH_3 = 3.0 mL (rumen contents) x NH_3 concentration
= total NH_3 at time zero in flask (use this)

These two should be equal.

Average time 0 within a run or day.
Make adjustment to the total NH_3 in the flask (P)

a. Freeze-dried material – assume no water pool.
A better estimate of plant miscible water pool = (wet wt – dry wt)/2
As the plant degrades the proportion or water which is miscible (ie. In equilibrium with all buffer etc) increases to c. 90% at 24 hours so change the water pool equation to account for this as described above.

Perhaps look at non NH_3-N in rumen liquor in the future.
Be careful with sequential/multiple sampling because samples reduce pool size in flask.
Adjust for bacterial growth where possible.

APPENDIX 3.6. Method for measuring volatile fatty acid concentration.

End products of fermentation (VFA concentrations) were determined by gas chromatography as described Attwood *et al*. (1998).

The GLC used a nitroterephthalic acid modified polyethylene glycol column (DB – FFAP, 30 m x 0.53 mm x 1 µm film thickness; J and W Scientific, Ca, USA) attached to a Hewlett Packard 6890 series system. Helium was the carrier gas at a flow rate of 5mL/minute. The oven temperature started at –85°C, ramped to 200°C at 10°C/min, was held at 200°C for 10 minutes, then decreased to 50°C and held for 5 minutes before the next sample was injected. Peaks were detected using a flame ionisation detector, identified by comparison with standards, and integrated using HP chemstation software (Version 4.02). The n-caproic acid (10mmol/L final concentration) was used as an initial standard.

Procedure

1. Label wide mouth, yellow lid vials and put on ice.

2. Defrost VFA samples, shake and spin for 15 mins at 14,000 x *g*.

3. Add 100 µL of n-caproic acid to vials.

4. Add 100 µL of phosphoric acid to vials.

5. Add 1 mL of sample then screw on lid.
   Note it is important that minimal volatilisation occurs (ie. replace eppendorf lid and screw on yellow vial lid as quickly as possible).

6. Shake vial to ensure good mix.

Note: The n-caproic and phosphoric acids should be stored at 4°C, and in large batches it may cool to room temperature and separate out.

APPENDIX 3.7. Particle size distribution of minced forages used for *in sacco* and *in vitro* incubations.

Forage	> 4 mm	2 mm	1 mm	0.50 mm	0.25 mm	Residues	Solubles
Fresh							
Perennial ryegrass	14.0	11.9	12.9	6.8	8.7	10.8	34.9
Cocksfoot	6.2	9.7	17.3	11.9	19.2	14.2	21.6
Tall fescue	9.3	17.0	7.9	7.5	9.9	7.6	41.0
Yorkshire fog	12.7	16.1	8.1	6.8	6.6	7.0	42.8
Prairie grass	8.0	9.3	12.9	9.4	9.4	10.1	41.0
Grasslands Tama	16.8	8.6	10.9	4.7	5.0	6.5	47.4
Kikuyu	14.1	17.8	9.4	10.8	14.8	7.5	25.5
Paspalum	10.7	23.5	11.8	13.5	15.0	6.3	19.2
White clover	8.4	18.0	15.1	12.0	8.8	3.8	34.0
Lucerne	11.1	16.7	10.4	9.0	8.7	6.5	37.8
Red clover	8.3	17.3	19.0	12.8	11.6	7.5	23.6
Lotus corniculatus	3.1	12.6	16.3	9.6	8.7	14.0	35.6
Lotus pedunculatus	2.3	11.3	13.6	11.7	18.1	13.3	30.0
Sulla	3.3	9.8	17.3	10.7	12.7	10.1	36.1
Chicory	4.1	10.2	21.6	9.4	10.6	7.4	36.8
Plantain	1.9	4.8	21.0	15.7	18.3	9.5	28.9
Conserved							
Pasture silage	13.6	12.2	13.2	8.1	9.5	4.9	38.5
Oat silage	20.2	13.3	8.8	8.1	4.5	4.0	41.2
Lucerne silage	25.3	21.5	8.0	7.7	7.9	4.7	25.0
Sulla silage	4.1	12.7	14.3	10.5	11.9	10.3	36.2
Maize silage	9.6	8.8	14.7	9.4	16.6	284	12.5
Lucerne hay	16.4	22.7	17.1	8.7	8.7	2.0	24.3
Maize grain	1.4	40.4	25.9	8.3	6.4	6.6	11.0

APPENDIX 3.8. Particle size distribution in swallowed boli and rumen contents of sheep and cattle fed contrasting diets compared to the average particle size distribution of contrasting forages in this study (summarised by Waghorn, unpublished).

		Particle size (sieve aperture; mm)		
Source	Feed type	> 2	2 – 0.5	< 0.5
Sheep: chewing during eating[a]				
1	Early vegetative perennial ryegrass[a]	48	14	38
1	Early bloom perennial ryegrass[b]	33	32	35
2	Fresh perennial ryegrass[b]	43	16	41
2	Poor quality meadow hay[a]	49	29	23
3	Young ryegrass (50% NDF)	47	14	39
3	Mature ryegrass 60% NDF)	31	32	39
Sheep: rumen contents				
3	Young ryegrass (51% NDF)	15	18	67
3	Mature ryegrass (61% NDF)	13	46	41
4	Tropical grass leaf	12	70	18
4	Tropical grass stem	12	57	31
Cattle: rumen contents				
4	Tropical grass leaf	15	40	45
4	Tropical grass stem	20	46	34
5	Fresh lucerne after a 2 h meal	29	31	40
5	Lucerne hay after a 2 h meal	27	38	35
6	Fresh perennial ryegrass	51	12	37
6	Fresh lucerne	39	24	37
Mean	Sheep: chewed during eating	42	23	35
Mean	Sheep: rumen contents	13	48	39
Mean	Cow: rumen contents	30	32	38
Minced forage in this study				
Mean	Grasses	12	35	54
Mean	Legumes	5	41	54
Mean	Conserved grasses	11	32	53

[a] Assume 3 and 6% passing 0.5 and retained on 0.25 mm sieve for vegetation and pre bloom respectively.

[b] From original data set

[1] Ulyatt *et al.*, 1986; [2] Ulyatt, 1983; [3] Dellow *et al.*, unpublished; [4] Poppi *et al.*, 1985; [5] Waghorn *et al.*, 1986; [6] Waghorn, 1989.

APPENDIX 3.9. ***In sacco*** **forage dry matter (DM) degradation characteristics (% of DM) ± SEM as defined by soluble (A) and degradable insoluble (B) fractions, fractional degradation rate (k, %/h) and lag time (hours).**

Forage	A	B	k	Lag
Fresh				
Perennial ryegrass	44.1 ± 0.90	50.8 ± 1.51	10.6 ± 1.04	4.2 ± 0.40
Cocksfoot	49.3 ± 2.93	43.7 ± ± 5.94	11.6 ± 4.68	4.9 ± 1.25
Tall fescue	48.6 ± 1.32	45.3 ± 2.82	7.9 ± 1.82	9.0 ± 1.22
Yorkshire fog	48.5 ± 0.95	46.7 ± 1.67	8.7 ± 0.99	4.0 ± 0.55
Prairie grass	41.4 ± 0.94	50.4 ± 1.29	9.2 ± 0.59	0.7 ± 0.33
Grasslands Tama	55.5 ± 0.84	41.5 ± 1.41	10.1 ± 1.00	4.7 ± 0.43
Kikuyu	31.0 ± 1.17	55.3 ± 1.07	7.1 ± 1.20	7.8 ± 1.07
Paspalum	26.3 ± 1.70	57.8 ± 3.24	6.4 ± 1.04	4.2 ± 0.97
White clover	39.9 ± 1.10	54.1 ± 1.31	21.1 ± 1.47	1.2 ± 0.17
Lucerne	49.8 ± 1.47	38.6 ± 1.92	13.5 ± 1.85	0.7 ± 0.51
Red clover	21.8 ± 5.61	64.1 ± 7.32	13.4 ± 3.98	1.1 ± 1.07
Lotus corniculatus	50.5 ± 1.17	39.4 ± 1.51	15.0 ± 1.54	1.1 ± 0.33
Lotus pedunculatus	43.7 ± 0.73	40.9 ± 1.21	11.4 ± 1.09	4.8 ± 0.34
Sulla	51.7 ± 2.43	43.6 ± 3.21	12.3 ± 2.61	0.2 ± 0.88
Chicory	41.6 ± 1.13	52.4 ± 1.26	26.0 ± 1.87	0.4 ± 0.18
Plantain	39.1 ± 3.32	51.3 ± 3.95	33.2 ± 8.70	1.4 ± 0.37
Conserved				
Pasture silage	41.6 ± 1.76	49.5 ± 3.09	8.4 ± 1.61	4.2 ± 0.95
Oat silage	44.5 ± 1.19	47.1 ± 1.76	6.5 ± 0.56	1.0 ± 0.57
Lucerne silage	47.0 ± 0.84	37.5 ± 1.32	16.2 ± 2.39	4.0 ± 0.45
Sulla silage	49.1 ± 1.79	39.8 ± 2.87	5.9 ± 1.08	0
Maize silage	27.5 ± 3.14	54.4 ± 5.65	4.2 ± 1.05	0.4 ± 2.00
Lucerne hay	27.4 ± 1.58	46.5 ± 2.34	7.3 ± 0.93	0
Maize grain	17.5 ± 2.28	76.9 ± 3.05	6.6 ± 0.63	0.4 ± 0.69

[1] Calculated using an assumed fractional passage rate of 6 %/h.

APPENDIX 3.10. Crude protein (CP) concentration in the DM and *in sacco* degradation characteristics ± SEM (% of CP) of forages as defined by soluble (A) and degradable insoluble (B) fractions, fractional degradation rate (k, %/h) and lag time (hours).

Forage	CP	A	B	k	Lag
Fresh					
Perennial ryegrass	15.5	52.2 ± 0.29	43.5 ± 0.46	15.0 ± 0.60	4.6 ± 0.12
Cocksfoot	23.7	55.0 ± 2.42	41.1 ± 3.80	15.9 ± 5.2	5.0 ± 0.86
Tall fescue	16.4	53.0 ± 1.94	43.1 ± 3.18	7.5 ± 1.7	3.2 ± 1.35
Yorkshire fog	23.7	54.8 ± 1.63	43.6 ± 2.28	8.7 ± 1.1	1.0 ± 0.66
Prairie grass	19.9	52.4 ± 0.48	43.0 ± 0.61	17.1 ± 0.70	0.9 ± 0.12
Grasslands Tama	21.3	56.4 ± 1.22	41.7 ± 1.97	13.9 ± 2.4	4.3 ± 0.58
Kikuyu	16.4	47.9 ± 0.74	41.5 ± 1.12	12.5 ± 0.60	10.7 ± 0.42
Paspalum	13.5	29.1 ± 2.44	20.9 ± 6.54	7.8 ± 1.2	11.6 ± 1.93
White clover	26.9	38.4 ± 1.45	58.0 ± 1.81	19.1 ± 1.64	1.3 ± 0.22
Lucerne	29.9	52.0 ±1.48	41.5 ± 1.81	15.3 ± 1.7	0
Red Clover	27.4	30.1 ± 5.42	65.4 ± 7.33	10.4 ± 3.2	0
Lotus corniculatus	22.2	51.1 ± 1.46	44.3 ± 1.88	15.0 ± 1.7	1.0 ± 0.37
Lotus pedunculatus	21.5	33.0 ± 1.35	58.0 ± 2.24	11.4 ± 1.4	4.8 ± 0.45
Sulla	23.0	49.5 ± 2.34	46.4 ± 3.11	11.7 ± 2.3	0
Chicory	19.3	29.3 ± 1.14	65.4 ± 1.38	27.3 ± 2.1	0.8 ± 0.15
Plantain	24.7	33.3 ± 7.80	57.1 ± 8.57	34.9 ± 13.1	1.6 ± 0.60
Conserved					
Pasture silage	17.2	72.1 ± 0.85	22.8 ± 1.43	10.4 ± 1.9	5.5 ± 0.66
Oat silage	17.8	73.3 ± 0.84	21.7 ± 1.19	7.8 ± 1.0	0.9 ± 0.78
Lucerne silage	23.3	52.5 ± 0.75	40.0 ± 1.21	14.1 ± 1.5	5.0 ± 0.30
Sulla silage	21.2	55.4 ± 1.83	39.4 ± 3.31	7.4 ± 2.00	3.2 ± 1.60
Maize silage	7.6	17.4 ± 1.69	62.0 ± 4.98	3.4 ± 0.70	3.8 ± 1.48
Lucerne hay	24.2	37.6 ± 0.97	48.0 ± 1.64	10.2 ± 1.30	3.0 ± 0.63
Maize grain	10.2	0	90.2 ± 9.24	7.8 ± 3.20	9.5 ± 1.94

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[1] Calculated using an assumed fractional passage rate of 6 %/h.

APPENDIX 3.11. Neutral detergent fibre (NDF) concentration in the DM and *in sacco* degradation characteristics ± SEM (% of NDF) of forages as defined by soluble (A) and degradable insoluble (B) fractions, fractional degradation rate (k, %/h) and lag time (hours).

Forage	NDF	A	B	k	Lag
Fresh					
Perennial ryegrass	48.7	20.7 ± 1.80	71.6 ± 3.10	9.3 ± 1.31	3.9 ± 0.66
Cocksfoot	47.5	40.0 ± 1.57	49.5 ± 2.48	9.9 ± 1.71	4.0 ± 0.72
Tall fescue	41.6	15.6 ± 2.48	74.8 ± 5.33	6.4 ± 1.60	9.1 ± 1.44
Yorkshire fog	39.9	22.9 ± 1.39	68.6 ± 2.48	7.7 ± 0.87	3.9 ± 0.60
Prairie grass	44.8	18.3 ± 1.65	68.4 ± 2.47	7.1 ± 0.65	0
Grasslands Tama	36.5	36.2 ± 0.93	58.7 ± 1.66	7.8 ± 0.62	5.1 ± 0.38
Kikuyu	47.7	0	79.6 ± 4.00	5.8 ± 0.91	5.6 ± 0.71
Paspalum	57.8	8.3 ± 2.41	72.2 ± 4.31	7.8 ± 1.44	3.9 ± 0.99
White clover	25.6	21.2 ± 1.25	62.3 ± 1.51	28.3 ± 2.35	1.0 ± 0.15
Lucerne	29.5	39.9 ± 1.76	33.2 ± 2.73	17.0 ± 5.86	4.2 ± 0.99
Red Clover	33.6	6.0 ± 4.57	68.0 ± 6.84	13.0 ± 6.33	2.0 ± 2.83
Lotus corniculatus	28.2	0	73.1 ± 1.71	12.3 ± 1.14	1.0 ± 0.28
Lotus pedunculatus	33.1	1.7 ± 1.60	67.6 ± 2.83	8.1 ± 1.01	4.6 ± 0.61
Sulla	22.4	0	84.5 ± 5.94	16.4 ± 6.54	0.8 ± 0.88
Chicory	23.8	0	82.1 ± 2.02	33.9 ± 3.76	0
Plantain	28.3	12.1 ± 3.05	75.4 ± 3.60	24.1 ± 3.00	0
Conserved					
Pasture silage	50.3	11.9 ± 1.74	76.6 ± 2.93	4.7 ± 0.46	0.9 ± 0.70
Oat silage	53.2	24.4 ± 1.34	62.4 ± 2.44	7.3 ± 0.89	3.6 ± 0.69
Lucerne silage	30.5	5.3 ± 3.41	56.2 ± 4.03	11.2 ± 1.73	0.5 ± 0.78
Sulla silage	36.2	29.4 ± 2.58	43.2 ± 4.00	6.3 ± 1.60	0
Maize silage	40.5	0	63.7 ± 7.43	4.1 ± 1.38	0.4 ± 1.95
Lucerne hay	39.1	0	52.8 ± 4.62	4.1 ± 0.94	0
Maize grain	10.9	0	69.0 ± 2.46	7.8 ± 0.72	7.8 ± 0.47

[1] Calculated using an assumed fractional passage rate of 6 %/h.

APPENDIX 3.12. Acid detergent fibre (ADF) concentration in the DM and *in sacco* degradation characteristics ± SEM (% of ADF) of forages as defined by soluble (A) and degradable insoluble (B) fractions, fractional degradation rate (k, %/h) and lag time (hours).

Forage	ADF	A	B	k	Lag
Fresh					
Perennial ryegrass	25.5	10.3 ± 1.89	79.7 ± 3.20	10.2 ± 1.4	4.1 ± 0.57
Cocksfoot	23.6	5.0 ± 3.09	81.8 ± 4.80	10.0 ± 1.8	4.2 ± 0.80
Tall fescue	23.8	9.1 ± 5.60	80.0 ± 25.0	6.7 ± 0.02	9.5 ± 1.41
Yorkshire fog	19.3	5.7 ±2.41	83.9 ± 7.55	8.1 ± 0.006	4.4 ± 0.24
Prairie grass	23.1	0	81.0 ± 3.25	10.1 ±1.7	3.8 ± 0.70
Grasslands Tama	16.2	5.6 ± 1.40	85.6 ± 2.66	11.1 ± 1.7	7.8 ± 0.75
Kikuyu	29.5	0	79.7 ± 1.77	6.2 ± 0.50	4.9 ± 0.35
Paspalum	33.7	0	79.8 ± 7.35	10.6 ± 5.3	7.9 ± 2.48
White clover	19.0	0	81.6 ± 1.85	24.1 ± 2.75	1.5 ± 0.18
Lucerne	21.4	24.6 ± 1.74	45.6 ± 2.30	12.3 ± 1.5	1.8 ± 0.44
Red Clover	26.6	0	72.1 ± 11.61	9.0 ± 4.9	1.8 ± 2.09
Lotus corniculatus	19.6	3.0 ± 2.08	70.1 ± 3.15	11.2 ± 1.2	1.4 ± 0.40
Lotus pedunculatus	22.2	0	58.8 ± 2.95	10.4 ± 1.6	5.3 ± 0.57
Sulla	17.7	0	84.1 ± 5.71	14.6 ± 4.4	1.3 ± 0.71
Chicory	21.2	0	83.2 ± 0.76	26.3 ± 1.0	0
Plantain	24.3	24.6 ± 1.90	61.4 ± 2.24	39.0 ± 4.4	1.9 ± 0.12
Conserved					
Pasture silage	33.5	10.0 ± 1.66	78.0 ± 2.67	5.1 ± 0.40	0.9 ± 0.61
Oat silage	32.6	19.9 ± 1.34	66.4 ± 2.44	7.2 ± 0.90	3.1 ± 0.71
Lucerne silage	23.1	10.5 ± 1.59	51.2 ± 2.51	15.5 ± 2.90	4.4 ± 0.55
Sulla silage	29.5	23.3 ± 2.33	54.9 ± 4.23	4.6 ± 0.90	0
Maize silage	24.5	0	59.9 ± 3.43	5.1 ± 0.70	0
Lucerne hay	32.5	0	59.9 ± 6.15	3.4 ± 0.70	0
Maize grain	4.1	0	43.3 ± 2.50	7.2 ± 0.50	13.0 ± 0.50

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[1] Calculated using an assumed fractional passage rate of 6 %/h.

APPENDIX 3.13. ***In vitro*** **pH of individual forages evaluated over 24 hours (h).**

Forage	0 h	6 h	12 h	24 h
Fresh				
Perennial ryegrass	7.4	6.7	5.9	5.4
Cocksfoot	7.0	6.8	6.3	5.4
Tall fescue	7.0	6.7	6.3	5.5
Yorkshire fog	7.3	6.8	6.5	6.1
Prairie grass	7.4	6.8	6.3	5.9
Grasslands Tama	7.4	6.6	6.3	5.8
Kikuyu	7.3	6.9	6.9	6.8
Paspalum	7.5	6.9	6.7	5.7
White clover	7.4	6.6	6.8	6.6
Lucerne	7.3	6.6	6.4	6.0
Red Clover	7.4	7.0	7.0	7.1
Lotus corniculatus	7.4	6.9	6.9	6.8
Lotus pedunculatus	7.4	6.8	6.6	6.2
Sulla	7.4	6.8	7.2	7.2
Chicory	7.4	6.7	6.8	6.9
Plantain	7.3	7.0	7.0	7.1
Conserved				
Pasture silage	7.3	6.9	6.6	5.6
Oat silage	7.3	7.0	6.7	5.9
Lucerne silage	7.2	6.3	5.9	5.6
Sulla silage	7.2	6.4	6.5	6.0
Maize silage	7.3	6.9	6.2	5.4
Lucerne hay	7.4	6.9	6.7	6.6
Maize grain	7.4	7.0	5.8	4.9

APPENDIX 3.14. Net ammonia production ± SEM (% forage nitrogen released as NH_3) of forages evaluated *in vitro* for 24 hours (h).

Forage	Time of incubation						
	2 h	4 h	6 h	8 h	10 h	12 h	24 h
Fresh							
Perennial ryegrass	4.3 ± 0.48	6.8 ± 0.23	6.8 ± 0.46	4.7 ± 0.18	-0.9 ± 0.18	-3.3 ± 0.51	3.3 ± 0.48
Cocksfoot	3.7 ± 0.18	7.6 ± 0.08	10.1 ± 0.09	9.7 ± 0.37	10.7 ± 0.28	10.2 ± 0.31	16.5± 0.44
Tall fescue	4.5 ± 0.30	9.6 ± 0.31	10.2 ± 0.26	9.2 ± 0.69	5.9 ± 0.29	6.2 ± 0.62	16.2 ± 0.30
Yorkshire fog	5.3 ± 0.41	11.4 ± 0.25	13.7 ± 0.37	15.3 ± 0.39	15.3 ± 0.31	16.7 ± 0.17	30.9 ± 0.08
Prairie grass	3.3 ± 0.55	5.8 ± 0.25	9.2 ± 0.50	8.2 ± 0.30	9.8 ± 0.55	5.5 ± 0.60	18.7 ± 1.56
Grasslands Tama	4.4 ± 0.11	8.0 ± 0.25	12.0 ± 0.45	9.1 ± 0.39	9.4 ± 0.52	8.5 ± 0.17	23.1 ± 0.30
Kikuyu	4.3 ± 0.60	7.8 ± 0.27	11.9 ± 0.36	8.1 ± 1.21	11.0 ± 0.51	10.8 ± 0.47	4.1 ± 0.62
Paspalum	3.5 ± 0.45	5.1 ± 0.25	4.0 ± 0.61	2.8 ± 0.55	2.0 ± 1.35	-4.7 ± 0.30	-5.1 ± 0.20
White clover	4.3 ± 0.11	7.7 ± 0.29	13.7 ± 0.45	15.7 ± 1.03	21.7 ± 0.31	24.5 ± 0.23	49.2 ± 0.99
Lucerne	2.0 ± 0.04	4.4 ± 0.67	5.5 ± 0.83	8.4 ± 0.61	8.7 ± 1.30	15.0 ± 0.87	34.6 ± 0.39
Red Clover	5.5 ± 0.33	10.4 ± 0.25	12.2 ± 0.25	19.1 ± 0.59	23.0 ± 1.00	28.6 ± 0.92	45.5 ± 0.97
Lotus corniculatus	4.7 ± 0.24	8.1 ± 0.21	9.9 ± 0.45	10.9 ± 0.61	12.9 ± 0.40	14.7 ± 0.33	25.2 ± 1.77
Lotus pedunculatus	6.0 ± 0.24	8.8 ± 0.28	9.0 ± 0.41	8.1 ± 0.15	8.5 ± 0.15	8.9 ± 0.29	9.2 ± 0.82
Sulla	5.3 ± 0.25	9.0 ± 0.31	7.6 ± 0.22	5.6 ± 0.55	7.1 ± 0.41	8.1 ± 0.76	7.6 ± 1.26
Chicory	3.6 ± 0.31	3.1 ± 0.47	4.7 ± 0.44	4.2 ± 1.02	4.7± 0.85	6.6 ± 1.40	17.0 ± 1.16
Plantain	3.9 ± 0.23	5.7 ± 0.18	7.5 ± 0.14	9.2 ± 0.21	8.1 ± 0.21	9.0 ± 0.57	-1.4 ± 0.57
Conserved							
Pasture silage	3.7 ± 0.11	7.7 ± 0.43	20.7 ± 0.55	29.8 ± 0.74	32.6 ± 0.39	33.9 ± 0.57	37.1 ± 0.51
Oat silage	2.2 ± 0.12	5.3 ± 0.14	12.9 ± 0.24	23.3 ± 0.76	29.1 ± 0.32	30.1 ± 0.17	37.2 ± 0.71
Lucerne silage	0.9 ± 0.05	2.1 ± 0.13	5.1 ± 0.09	12.2 ± 0.87	15.5 ± 1.08	19.5 ± 0.34	23.9 ± 0.86
Sulla silage	4.6 ± 0.19	11.4 ± 0.33	17.9 ± 0.43	19.5 ± 071	22.0 ± 0.83	20.1 ± 0.32	29.9 ± 0.40
Maize silage	8.8 ± 0.62	13.5 ± 0.33	13.7 ± 0.40	-2.5 ± 0.98	-15.5 ± 2.80	-17.4 ± 0.26	-17.4 ± 0.26
Lucerne hay	4.2 ± 0.21	8.3 ± 0.56	11.9 ± 1.04	20.4 ± 1.03	24.7 ± 0.18	27.2 ± 0.46	38.0 ± 0.29
Maize grain	6.5 ± 0.24	14.0 ± 0.68	16.0 ± 1.06	-1.2 ± 0.83	2.3 ± 2.71	-9.8 ± 0.17	-10.2 ± 0.10

APPENDIX 3.15. Net ammonia production (mmol/L) of forages evaluated *in vitro* over 24 hours (h).

	Time of incubation						
Forage	2 h	4 h	6 h	8 h	10 h	12 h	24 h
Fresh							
Perennial ryegrass	2.61	4.13	3.88	2.62	-0.63	-2.07	1.62
Cocksfoot	4.02	8.49	10.71	10.37	11.38	11.14	17.42
Tall fescue	2.86	6.02	6.47	5.66	3.56	3.73	9.72
Yorkshire fog	3.47	7.53	8.72	9.75	9.94	10.21	19.22
Prairie grass	1.30	3.75	5.66	5.11	6.07	3.39	11.28
Grasslands Tama	2.81	5.08	7.15	5.43	5.77	5.05	13.62
Kikuyu	2.05	3.84	5.69	3.71	5.19	5.30	1.13
Paspalum	2.01	2.97	2.18	1.46	1.03	-2.72	-2.95
White clover	3.35	6.04	10.41	11.94	16.56	15.97	36.29
Lucerne	3.94	7.94	11.43	19.93	23.37	26.56	36.16
Red Clover	3.03	5.78	6.62	10.54	12.47	15.49	25.05
Lotus corniculatus	3.51	6.01	7.01	7.79	9.20	10.53	17.21
Lotus pedunculatus	3.36	4.86	4.76	4.29	4.56	4.64	4.65
Sulla	2.43	4.14	3.11	2.38	3.04	3.51	1.54
Chicory	2.00	1.77	2.45	2.12	2.45	3.45	8.64
Plantain	2.41	3.60	4.48	5.52	4.80	5.27	-1.03
Conserved							
Pasture silage	2.73	5.79	15.20	21.85	23.87	24.96	26.41
Oat silage	1.38	3.45	8.28	15.01	18.48	19.21	23.32
Lucerne silage	1.16	2.59	6.28	15.0	19.13	23.85	29.47
Sulla silage	3.42	8.40	12.55	13.88	15.56	14.65	20.46
Maize silage	3.62	4.42	2.05	-2.36	-5.05	-5.14	-4.26
Lucerne hay	3.94	7.94	11.43	19.93	23.37	26.56	36.16
Maize grain	2.69	5.92	6.35	-0.51	0.91	-4.16	-4.43

APPENDIX 3.16. Evaluation of forages with effective fibre concentrations of 40% and 60% for early and late lactating dairy cows using the CNCPS model.

TABLE 3.16.1. CNCPS evaluation of grasses with effective fibre concentrations of 40% NDF for cows in early lactation.

	Ryegrass	Cocksfoot	Fescue	Yorkshire fog	Prairie Grass	Tama ryegrass	Kikuyu	Paspalum
Effective fibre (%NDF)	40	40	40	40	40	40	60	60
Pasture intake (kg DM/cow/day)	17.1	17.1	17.1	17.1	17.1	17.1	17.1	17.1
Diet nutrient composition								
ME (MJ/kg DM)	9.8	10.3	10.5	11.1	10.4	10.6	10	8.9
CP (g/100g DM)	15.5	23.7	16.4	23.7	19.9	21.3	16.4	13.5
Soluble CP (% CP)	52	55	53	55	52	56	48	29
NDF (g/100 g DM)	48.7	47.5	41.6	39.9	44.8	36.5	47.7	57.8
peNDF (g/100 g DM)	19	19	17	16	18	15	29	35
Total NFC (g/100 g DM)	22	15	27	22	21	28	22	15
Performance predictions								
Milk production based on ME supply (kg/day)	20.6	21.2	22.6	23.6	21.9	22.2	21	17.9
Milk production based on MP supply (kg/day)	17.6	22.7	19.9	26.4	18.7	21	16.5	16
Daily weight change due to reserves (kg/day)	-0.4	-0.3	0	0.2	-0.1	-0.1	-0.3	-0.9
MP from bacteria (g/day)	988	774	898	802	829	850	881	824
MP from undeg. feed (g/day)	579	1058	783	1159	796	829	669	787
MP from undeg. feed (%MP total)	37	58	47	59	49	49	53	49
Total DIP (% CP)	81	81	73	75	80	81	74	63
Ruminal N balance (% of reqt.)	142	276	150	261	205	222	154	130
Total bacterial nitrogen (g/day)	263	206	240	214	221	227	235	220
Urea cost (MJ/day)	1.7	7.4	2.1	7.8	5.3	6.3	2.3	0
Excess N excreted (g/day)	112	364	121	376	234	279	127	66
Predicted ruminal pH	6.25	6.23	6.13	6.1	6.18	6.04	6.46	6.46
Cost of urea (% ME intake)	1.01	4.20	1.17	4.11	2.98	3.48	1.35	0.00

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre; undeg., undegraded; reqt, requirement.

TABLE 3.16.2. CNCPS evaluation of grasses with effective fibre concentrations of 6 60% NDF for cows in early lactation.

	Ryegrass	Cocksfoot	Fescue	Yorkshire fog	Prairie Grass	Tama ryegrass	Kikuyu	Paspalum
Effective fibre (%NDF)	60	60	60	60	60	60	60	60
Pasture intake (kg DM/cow/day)	17.1	17.1	17.1	17.1	17.1	17.1	17.1	17.1
<u>Diet nutrient composition</u>								
ME (MJ/kg DM)	10	10.5	10.5	11.2	10.5	10.6	10	8.9
CP (g/100g DM)	15.5	23.7	16.4	23.7	19.9	21.3	16.4	13.5
Soluble CP (% CP)	52	55	53	55	52	56	48	29
NDF (g/100 g DM)	48.7	47.5	41.6	39.9	44.8	36.5	47.7	57.8
peNDF (g/100 g DM)	29	29	25	24	27	15	29	35
Total NFC (g/100 g DM)	22	15	27	22	21	28	22	15
<u>Performance predictions</u>								
Milk production based on ME supply (kg/day)	21.2	21.6	23	23.9	22.3	22.4	21	17.9
Milk production based on MP supply (kg/day)	18.1	22.8	21.1	27.3	19.3	22.7	16.5	16
Daily weight change due to reserves (kg/day)	-0.3	-0.2	0	0.2	0	0	-0.3	-0.9
MP from bacteria (g/day)	1026	812	989	898	889	982	881	824
MP from undeg. Feed (g/day)	551	1016	747	1102	757	777	669	787
MP from undeg. Feed (%MP total)	35	56	43	55	46	44	53	49
Total DIP (% CP)	83	82	74	76	81	82	74	63
Ruminal N balance (% of reqt.)	139	266	139	237	194	196	154	130
Total bacterial nitrogen (g/day)	274	216	264	239	237	262	235	220
Urea cost (MJ/day)	1.5	7.3	1.5	7.5	5.1	5.5	2.3	0
Excess N excreted (g/day)	106	361	102	367	224	253	127	66
Predicted ruminal pH	6.46	6.46	6.46	6.44	6.46	6.35	6.46	6.46
Cost of urea (% ME intake)	0.88	4.07	0.84	3.92	2.84	3.03	1.35	0.00

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre; undeg., undegraded; reqt, requirement.

TABLE 3.16.3. CNCPS evaluation of legumes with effective fibre concentrations of 40% NDF for cows in early lactation.

	White clover	Lucerne	Red Clover	*Lotus corniculatus*	*Lotus pedunculatus*	Sulla	Chicory	Plantain
Effective fibre (%NDF)	40	40	40	40	40	40	40	40
Pasture intake (kg DM/cow/day)	17.1	17.1	17.1	17.1	17.1	17.1	17.1	17.1
Diet nutrient composition								
ME (MJ/kg DM)	10.5	11.1	10.2	9.9	8.7	9.9	10.2	9.6
CP (g/100g DM)	26.9	29.9	27.4	22.2	21.5	23	19.3	24.7
Soluble CP (% CP)	38	52	30	51	33	50	29	33
NDF (g/100 g DM)	25.6	29.5	33.6	28.2	33.1	22.4	23.8	28.3
peNDF (g/100 g DM)	10	12	13	11	13	9	10	11
Total NFC (g/100 g DM)	34	29	26	36	32	41	43	33
Performance predictions								
Milk production based on ME supply (kg/day)	21	22.6	19.9	19.6	16.2	19.7	20.9	18.3
Milk production based on MP supply (kg/day)	24.6	26.9	33.7	18.9	21.1	21	16.4	16.9
Daily weight change due to reserves (kg/day)	-0.3	0	-0.5	-0.6	-1.2	-0.5	-0.3	-0.8
MP from bacteria (g/day)	667	630	690	721	670	679	764	663
MP from undeg. Feed (g/day)	1196	1356	1666	905	1158	1052	712	884
MP from undeg. Feed (%MP total)	64	68	71	56	63	61	48	57
Total DIP (% CP)	79	74	68	79	71	76	81	84
Ruminal N balance (% of reqt.)	424	570	391	337	256	363	248	372
Total bacterial nitrogen (g/day)	178	168	184	192	179	181	204	177
Urea cost (MJ/day)	10.8	12.3	11	7.6	6.3	7.9	5.9	10.2
Excess N excreted (g/day)	595	826	627	337	281	363	248	484
Predicted ruminal pH	5.86	5.92	5.99	5.9	5.98	5.8	5.83	5.9
Cost of urea (% ME intake)	6.02	6.48	6.31	4.49	4.23	4.67	3.38	6.21

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre; undeg., undegraded; reqt, requirement.

TABLE 3.16.4. CNCPS evaluation of legumes with effective fibre concentrations of 60% NDF for cows in early lactation.

	White clover	Lucerne	Red Clover	*Lotus corniculatus*	*Lotus pedunculatus*	Sulla	Chicory	Plantain
Effective fibre (%NDF)	60	60	60	60	60	60	60	60
Pasture intake (kg DM/cow/day)	17.1	17.1	17.1	17.1	17.1	17.1	17.1	17.1
Diet nutrient composition								
ME (MJ/kg DM)	10.9	11.2	10.2	10	8.7	9.9	10.5	9.7
CP (g/100g DM)	26.9	29.9	27.4	22.2	21.5	23	19.3	24.7
Soluble CP (% CP)	38	52	30	51	33	50	29	33
NDF (g/100 g DM)	25.6	29.5	33.6	28.2	33.1	22.4	23.8	28.3
peNDF (g/100 g DM)	15	18	20	17	20	13	14	17
Total NFC (g/100 g DM)	34	29	26	36	32	41	43	33
Performance predictions								
Milk production based on ME supply (kg/day)	22.4	23.1	20.1	20.3	16.2	19.8	22.1	18.9
Milk production based on MP supply (kg/day)	27.5	28.5	34.7	21.1	22.3	22.2	19.5	18.8
Daily weight change due to reserves (kg/day)	0	0.1	-0.5	-0.4	-1.2	0.5	-0.1	-0.7
MP from bacteria (g/day)	851	773	828	875	798	784	938	810
MP from undeg. Feed (g/day)	1129	1283	1577	852	1094	1004	673	823
MP from undeg. Feed (%MP total)	57	62	66	49	58	56	42	50
Total DIP (% CP)	79	75	70	80	72	77	81	85
Ruminal N balance (% of reqt.)	338	470	333	230	220	263	183	310
Total bacterial nitrogen (g/day)	227	206	221	233	213	209	250	216
Urea cost (MJ/day)	1[illegible].4	12	10.6	6.6	5.5	7.3	4.7	9.3
Excess N excreted (g/day)	582	815	614	305	258	343	208	455
Predicted ruminal pH	6.07	6.17	6.28	6.14	6.26	5.99	6.03	6.14
Cost of urea (% ME intake)	5.58	6.27	6.08	3.86	3.70	4.31	2.62	5.61

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre; undeg., undegraded; reqt, requirement.

TABLE 3.16.5. CNCPS evaluation of conserved feeds and over-grazed (OG) and well-managed (WM) ryegrass with effective fibre concentrations of 40% NDF for cows in early lactation.

	Pasture silage	Sulla silage	Lucerne silage	Maize silage	Oat silage	Lucerne hay	Pasture-ryegrass OG[1]	Pasture-ryegrass WM[1]
Effective fibre (%NDF)	90	92	80	81	85	90	40	60
Pasture intake (kg DM/cow/day)	17.1	17.1	17.1	17.1	17.1	17.1	17.1	17.1
<u>Diet nutrient composition</u>								
ME (MJ/kg DM)	9.4	10.4	9.2	9.7	9.3	8.3	11.4	11.4
CP (g/100g DM)	17.7	21.2	23.3	7.6	18.4	24.2	26.3	26.3
Soluble CP (% CP)	70	55	53	17	71	38	43	43
NDF (g/100 g DM)	50.3	36.2	30.5	40.5	53.2	39.1	43.4	43.4
peNDF (g/100 g DM)	45	33	24	33	45	35	17	26
Total NFC (g/100 g DM)	28	30	40	46	21	30	14	14
<u>Performance predictions</u>								
Milk production based on ME supply (kg/day)	18.4	21.6	17.8	20.5	18.5	14.5	24.1	24.4
Milk production based on MP supply (kg/day)	8.6	17.1	14.5	12.3	11.1	16	31.5	31.7
Daily weight change due to reserves (kg/day)	-0.8	-0.2	-0.9	-0.4	-0.8	-1.5	0.2	0.3
MP from bacteria (g/day)	753	772	851	713	805	656	768	834
MP from undeg. Feed (g/day)	402	751	606	631	467	937	1432	1369
MP from undeg. Feed (%MP total)	35	49	42	47	37	59	65	62
Total DIP (% CP)	76	72	69	43	72	67	74	75
Ruminal N balance (% of reqt.)	207	226	238	65	191	321	321	300
Total bacterial nitrogen (g/day)	201	206	227	190	215	175	205	222
Urea cost (MJ/day)	5	5.7	6.2	0	4.4	7.8	9.6	9.4
Excess N excreted (g/day)	215	260	314	-104	196	389	525	520
Predicted ruminal pH	6.46	6.46	6.46	6.46	6.46	6.46	6.16	6.46
Cost of urea (% ME intake)	3.11	3.21	3.94	0	2.8	5.5	4.9	4.8

[1] High quality pasture ryegrass from the CNCPS library, either defined as over-grazed (OG) or well-managed (WM). The only difference between the two ryegrasses is the effective fibre concentration. The effective fibre values for OG ryegrass was 40% NDF and for WM ryegrass was 60%.

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre; undeg., undegraded; reqt, requirement.

TABLE 3.16.6. CNCPS evaluation of grasses with effective fibre concentrations of 40% NDF for cows in late lactation.

	Ryegrass	Cocksfoot	Fescue	Yorkshire fog	Prairie Grass	Tama ryegrass	Kikuyu	Paspalum
Effective fibre (%NDF)	40	40	40	40	40	40	40	40
Pasture intake (kg DM/cow/day)	14.63	14.63	14.63	14.63	14.63	14.63	14.63	14.63
Diet nutrient composition								
ME (MJ/kg DM)	10	10.5	10.6	11.2	10.5	10.7	10.1	9
CP (g/100g DM)	15.5	23.7	16.4	23.7	19.9	21.3	16.4	13.5
Soluble CP (% CP)	52	55	53	55	52	56	48	29
NDF (g/100 g DM)	48.7	47.5	41.6	39.9	44.8	36.5	47.7	57.8
peNDF (g/100 g DM)	19	19	17	16	18	15	29	35
Total NFC (g/100 g DM)	22	15	27	22	21	28	22	15
Performance predictions								
Milk production based on ME supply (kg/day)	16.1	16.3	17.7	18.4	17.1	17.2	16.4	13.7
Milk production based on MP supply (kg/day)	14.3	17.9	16.2	21.2	14.9	16.8	13.3	12.9
Daily weight change due to reserves (kg/day)	0.2	0.2	0.5	0.6	0.4	0.4	0.2	-0.2
MP from bacteria (g/day)	867	679	786	702	727	745	772	724
MP from undeg. Feed (g/day)	458	839	633	931	632	656	537	638
MP from undeg. Feed (%MP total)	35	55	45	57	47	47	41	47
Total DIP (% CP)	82	81	74	76	81	82	75	65
Ruminal N balance (% of reqt.)	142	277	150	261	205	223	154	130
Total bacterial nitrogen (g/day)	231	181	210	187	194	199	206	193
Urea cost (MJ/day)	1.5	7.4	2.1	7.6	4.7	6	2	0
Excess N excreted (g/day)	97	345	115	354	205	259	111	58
Predicted ruminal pH	6.25	6.23	6.13	6.1	6.18	6.04	6.46	6.46
Cost of urea (% ME intake)	1.03	4.82	1.35	4.64	3.06	3.83	1.35	0.00

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre; undeg., undegraded; reqt, requirement.

TABLE 3.16.7. CNCPS evaluation of grasses with effective fibre concentrations of 60% NDF for cows in late lactation.

	Ryegrass	Cocksfoot	Fescue	Yorkshire fog	Prairie Grass	Tama ryegrass	Kikuyu	Paspalum
Effective fibre (%NDF)	60	60	60	60	60	60	60	60
Pasture intake (kg DM/cow/day)	14.63	14.63	14.63	14.63	14.63	14.63	14.63	14.63
Diet nutrient composition								
ME (MJ/kg DM)	10.1	10.5	10.6	11.3	10.6	10.7	10.1	9
CP (g/100g DM)	15.5	23.7	16.4	23.7	19.9	21.3	16.4	13.5
Soluble CP (% CP)	52	55	53	55	52	56	48	29
NDF (g/100 g DM)	48.7	47.5	41.6	39.9	44.8	36.5	47.7	57.8
peNDF (g/100 g DM)	29	29	25	24	27	22	29	35
Total NFC (g/100 g DM)	22	15	27	22	21	28	22	15
Performance predictions								
Milk production based on ME supply (kg/day)	16.5	16.6	17.9	18.6	17.4	17.3	16.4	13.7
Milk production based on MP supply (kg/day)	14.7	18	17.2	22	15.4	18.2	13.3	12.9
Daily weight change due to reserves (kg/day)	0.3	0.3	0.5	0.6	0.4	0.4	0.2	-0.2
MP from bacteria (g/day)	899	711	865	785	778	859	772	724
MP from undeg. Feed (g/day)	434	804	603	883	599	613	537	638
MP from undeg. Feed (%MP total)	33	53	41	53	43	42	41	47
Total DIP (% CP)	84	82	75	77	82	83	75	65
Ruminal N balance (% of reqt.)	138	267	139	237	194	196	154	130
Total bacterial nitrogen (g/day)	240	190	231	209	208	229	206	193
Urea cost (MJ/day)	1.3	7.3	1.9	7.4	4.6	5.6	2	0
Excess N excreted (g/day)	92	343	107	346	200	247	111	58
Predicted ruminal pH	6.46	6.46	6.46	6.44	6.46	6.35	6.46	6.46
Cost of urea (% ME intake)	0.88	4.75	1.23	4.48	2.97	3.58	1.35	0.00

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre; undeg., undegraded; reqt, requirement.

TABLE 3.16.8. CNCPS evaluation of legumes with effective fibre concentrations of 40% NDF for cows in late lactation.

	White clover	Lucerne	Red Clover	*Lotus corniculatus*	*Lotus pedunculatus*	Sulla	Chicory	Plantain
Effective fibre (% NDF)	40	40	40	40	40	40	40	40
Pasture intake (kg DM/cow/day)	14.63	14.63	14.63	14.63	14.63	14.63	14.63	14.63
Diet nutrient composition								
ME (MJ/kg DM)	10.5	11.1	10.2	9.9	8.8	9.9	10.2	9.6
CP (g/100g DM)	26.9	29.9	27.4	22.2	21.5	23	19.3	24.7
Soluble CP (% CP)	38	52	30	51	33	50	29	33
NDF (g/100 g DM)	25.6	29.5	33.6	28.2	33.1	22.4	23.8	28.3
peNDF (g/100 g DM)	10	12	13	11	13	9	10	11
Total NFC (g/100 g DM)	34	29	26	36	32	41	43	33
Performance predictions								
Milk production based on ME supply (kg/day)	16	17.3	15	14.9	11.9	14.8	16	13.7
Milk production based on MP supply (kg/day)	19.2	21.1	26.9	14.7	16.4	16.4	12.7	12.8
Daily weight change due to reserves (kg/day)	0.2	0.4	0	0	-0.6	0	0.2	-0.2
MP from bacteria (g/day)	583	550	604	629	585	591	667	578
MP from undeg. Feed (g/day)	946	1084	1339	717	930	840	562	690
MP from undeg. Feed (%MP total)	62	66	69	53	61	59	46	54
Total DIP (% CP)	80	75	70	80	72	77	82	85
Ruminal N balance (% of reqt.)	425	569	393	276	259	301	222	374
Total bacterial nitrogen (g/day)	155	147	161	168	156	158	178	154
Urea cost (MJ/day)	10.3	11.6	10.5	6.8	6	7.4	5.2	9
Excess N excreted (g/day)	542	741	570	297	261	331	218	424
Predicted ruminal pH	5.86	5.92	5.99	5.9	5.98	5.8	5.83	5.9
Cost of urea (% ME intake)	6.71	7.14	7.04	4.69	4.66	5.11	3.48	6.41

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre; undeg., undegraded; reqt, requirement.

TABLE 3.16.9. CNCPS evaluation of legumes with effective fibre concentrations of 60% NDF for cows in late lactation.

	White clover	Lucerne	Red Clover	*Lotus corniculatus*	*Lotus pedunculatus*	Sulla	Chicory	Plantain
Effective fibre (%NDF)	60	60	60	60	60	60	60	60
Pasture intake (kg DM/cow/day)	14.63	14.63	14.63	14.63	14.63	14.63	14.63	14.63
Diet nutrient composition								
ME (MJ/kg DM)	10.9	11.2	10.2	10.1	8.7	9.9	10.5	9.8
CP (g/100g DM)	26.9	29.9	27.4	22.2	21.5	23	19.3	24.7
Soluble CP (% CP)	38	52	30	51	33	50	29	33
NDF (g/100 g DM)	25.6	29.5	33.6	28.2	33.1	22.4	23.8	28.3
peNDF (g/100 g DM)	15	18	20	17	20	13	14	17
Total NFC (g/100 g DM)	34	29	26	36	32	41	43	33
Performance predictions								
Milk production based on ME supply (kg/day)	17.1	17.7	15.2	15.4	11.9	14.9	16.9	14.2
Milk production based on MP supply (kg/day)	21.7	22.6	27.8	16.7	17.5	17.4	15.5	14.5
Daily weight change due to reserves (kg/day)	0.4	0.5	0	0.1	-0.6	0	0.3	-0.1
MP from bacteria (g/day)	740	674	723	763	695	682	816	704
MP from undeg. Feed (g/day)	891	1024	1264	674	877	801	530	641
MP from undeg. Feed (%MP total)	55	60	64	47	56	54	39	48
Total DIP (% CP)	80	76	71	81	73	78	82	85
Ruminal N balance (% of reqt.)	340	470	334	231	222	301	184	311
Total bacterial nitrogen (g/day)	197	180	193	203	185	182	218	188
Urea cost (MJ/day)	9.9	11.2	10.1	6.3	5.6	7.1	4.3	8.2
Excess N excreted (g/day)	530	730	557	281	248	321	186	399
Predicted ruminal pH	6.07	6.17	6.28	6.14	6.26	5.99	6.03	6.14
Cost of urea (% ME intake)	6.21	6.84	6.77	4.26	4.40	4.90	2.80	5.72

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre; undeg., undegraded; reqt, requirement.

TABLE 3.16.10. CNCPS evaluation of conserved feeds and over-grazed (OG) and well-managed (WM) ryegrass with effective fibre concentrations of 40% NDF for cows in late lactation.

	Pasture silage	Sulla silage	Lucerne silage	Maize silage	Oat silage	Lucerne hay	Pasture-ryegrass OG[1]	Pasture-ryegrass WM[1]
Effective fibre (%NDF)	90	92	80	81	85	90	40	60
Pasture intake (kg DM/cow/day)	14.63	14.63	14.63	14.63	14.63	14.63	14.63	14.63
Diet nutrient composition								
ME (MJ/kg DM)	9.5	10.4	9.3	9.7	9.5	8.4	11.5	11.5
CP (g/100g DM)	17.7	21.2	23.3	7.6	18.4	24.2	26.3	26.3
Soluble CP (% CP)	70	55	53	17	71	38	43	43
NDF (g/100 g DM)	50.3	36.2	30.5	40.5	53.2	39.1	43.4	43.4
peNDF (g/100 g DM)	45	33	24	33	45	35	17	26
Total NFC (g/100 g DM)	28	30	40	46	21	30	14	14
Performance predictions								
Milk production based on ME supply (kg/day)	14.2	16.7	13.4	15.7	14.2	10.6	18.8	19
Milk production based on MP supply (kg/day)	7.2	13.7	11.7	9.9	9.4	12.5	25.2	25.4
Daily weight change due to reserves (kg/day)	-0.1	0.3	-0.3	0.1	-0.1	-0.8	0.7	0.7
MP from bacteria (g/day)	665	679	743	621	708	575	673	730
MP from undeg. Feed (g/day)	336	603	495	525	399	758	1147	1093
MP from undeg. Feed (%MP total)	34	47	40	46	36	57	63	60
Total DIP (% CP)	76	73	69	44	72	68	75	76
Ruminal N balance (% of reqt.)	201	225	235	64	186	319	323	395
Total bacterial nitrogen (g/day)	177	181	198	166	189	153	179	195
Urea cost (MJ/day)	4.2	5	5.3	0	3.6	6.8	9.2	9.1
Excess N excreted (g/day)	181	225	270	-92	163	337	484	479
Predicted ruminal pH	6.46	6.46	6.46	6.46	6.46	6.46	6.16	6.46
Cost of urea (% ME intake)	3.02	3.29	3.90	0	2.6	5.5	5.5	5.4

[1] High quality pasture ryegrass from the CNCPS library, either defined as over-grazed (OG) or well-managed (WM). The only difference between the two ryegrasses is the effective fibre concentration. The effective fibre values for OG ryegrass was 40% NDF and for WM ryegrass was 60%.

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre; undeg., undegraded; reqt, requirement.

APPENDIX 3.17. CNCPS evaluation of forages. Data were used to derive Tables 3.21 and 3.22.

TABLE 3.17.1. Evaluation of grasses in early lactation using the CNCPS model.

	Ryegrass	Cocksfoot	Fescue	Yorkshire fog	Prairie Grass	Tama ryegrass	Kikuyu	Paspalum
Effective fibre (%NDF)	60	60	60	60	60	60	60	60
Pasture intake (kg DM/cow/day)	17.1	17.1	17.1	17.1	17.1	17.1	17.1	17.1
Diet nutrient composition								
ME (MJ/kg DM)	10	10.5	10.5	11.2	10.5	10.6	10	8.9
CP (g/100g DM)	15.5	23.7	16.4	23.7	19.9	21.3	16.4	13.5
Soluble CP (% CP)	52	55	53	55	52	56	48	29
NDF (g/100 g DM)	48.7	47.5	41.6	39.9	44.8	36.5	47.7	57.8
peNDF (g/100 g DM)	29	29	25	24	27	15	29	35
Total NFC (g/100 g DM)	22	15	27	22	21	28	22	15
Requirements								
ME requirements (MJ/day)	178	184	178	184	182	182	179	177
MP requirements (g/day)	1813	1824	1819	1766	1819	1761	1864	1954
Production predictions								
Milk production based on ME supply (kg/day)	21.2	21.6	23	23.9	22.3	22.4	21	17.9
Milk production based on MP supply (kg/day)	18.1	22.8	21.1	27.3	19.3	22.7	16.5	16
Daily weight change due to reserves (kg/day)	-0.3	-0.2	0	0.2	0	0	-0.3	-0.9
ME supplied (MJ/day)	171	180	180	192	180	181	171	152
Total MP supplied (g/day)	1577	1828	1736	2000	1646	1759	1550	1611
MP from bacteria (g/day)	1026	812	989	898	889	982	881	824
MP from undeg. Feed (g/day)	551	1016	747	1102	757	777	669	787
MP from undeg. Feed (%MP total)	35	56	43	55	46	44	53	49
MP supplied/CP eaten (%)	59	45	62	49	48	48	55	70
Urea cost (MJ/day)	1.5	7.3	1.5	7.5	5.1	5.5	2.3	0
Cost of urea (% ME intake)	0.88	4.07	0.84	3.92	2.84	3.03	1.35	0.00
Predicted ruminal pH	6.46	6.46	6.46	6.44	6.46	6.35	6.46	6.46

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre.

TABLE 3.17.2. Evaluation of legumes in early lactation using the CNCPS model.

	White clover	Lucerne	Red Clover	*Lotus corniculatus*	*Lotus pedunculatus*	Sulla	Chicory	Plantain
Effective fibre (%NDF)	40	60	60	60	60	40	40	40
Pasture intake (kg DM/cow/day)	17.1	17.1	17.1	17.1	17.1	17.1	17.1	17.1
Diet nutrient composition								
ME (MJ/kg DM)	10.5	11.2	10.2	10	8.7	9.9	10.2	9.6
CP (g/100g DM)	26.9	29.9	27.4	22.2	21.5	23	19.3	24.7
Soluble CP (% CP)	38	52	30	51	33	50	29	33
NDF (g/100 g DM)	25.6	29.5	33.6	28.2	33.1	22.4	23.8	28.3
peNDF (g/100 g DM)	10	18	20	17	20	9	10	11
Total NFC (g/100 g DM)	34	29	26	36	32	41	43	33
Requirements								
ME requirements (MJ/day)	188	189	187	183	182	184	183	187
MP requirements (g/day)	1768	1763	1794	1808	1915	1816	1796	1841
Production predictions								
Milk production based on ME supply (kg/day)	21	23.1	20.1	20.3	16.2	19.7	20.9	18.3
Milk production based on MP supply (kg/day)	24.6	28.5	34.7	21.1	22.3	21	16.4	16.9
Daily weight change due to reserves (kg/day)	-0.3	0.1	-0.5	-0.4	-1.2	-0.5	-0.3	-0.8
ME supplied (MJ/day)	180	192	174	171	149	169	174	164
MP supplied (g/day)	1863	2056	2405	1727	1892	1731	1476	1547
MP from bacteria (g/day)	667	773	828	875	798	679	764	663
MP from undeg. Feed (g/day)	1196	1283	1577	852	1094	1052	712	884
MP from undeg. Feed (%MP total)	64	62	66	49	58	61	48	57
MP supplied/CP eaten (%)	41	40	51	45	51	44	45	37
Urea cost (MJ/day)	10.8	12	10.6	6.6	5.5	7.9	5.9	10.2
Cost of urea (% ME intake)	6.02	6.27	6.08	3.86	3.70	4.67	3.38	6.21
Predicted ruminal pH	5.86	6.17	6.28	6.14	6.26	5.8	5.83	5.9

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre.

TABLE 3.17.3. Evaluation of conserved forages and ryegrass in early lactation using the CNCPS model.

	Pasture silage	Sulla silage	Lucerne silage	Maize silage	Oat silage	Lucerne hay	Pasture-ryegrass OG[1]	Pasture-ryegrass WM[1]
Effective fibre (%NDF)	90	92	80	81	85	90	40	60
Pasture intake (kg DM/cow/day)	17.1	17.1	17.1	17.1	17.1	17.1	17.1	17.1
Diet nutrient composition								
ME (MJ/kg DM)	9.4	10.4	9.2	9.7	9.3	8.3	11.4	11.4
CP (g/100g DM)	17.7	21.2	23.3	7.6	18.4	24.2	26.3	26.3
Soluble CP (% CP)	70	55	53	17	71	38	43	43
NDF (g/100 g DM)	50.3	36.2	30.5	40.5	53.2	39.1	43.4	43.4
peNDF (g/100 g DM)	45	33	24	33	45	35	17	26
Total NFC (g/100 g DM)	28	30	40	46	21	30	14	14
Requirements								
ME requirements (MJ/day)	182	183	183	177	181	185	186	186
MP requirements (g/day)	1871	1808	1872	1872	1862	1935	1753	1747
Production predictions								
Milk production based on ME supply (kg/day)	18.4	21.6	17.8	20.5	18.5	14.5	24.1	24.4
Milk production based on MP supply (kg/day)	8.6	17.1	14.5	12.3	11.1	16	31.5	31.7
Daily weight change due to reserves (kg/day)	-0.8	-0.2	-0.9	-0.4	-0.8	-1.5	0.2	0.3
ME supplied (MJ/day)	161	178	157	166	159	142	195	195
MP supplied (g/day)	1155	1523	1457	1344	1272	1593	2200	2203
MP from bacteria (g/day)	753	772	851	713	805	656	768	834
MP from undeg. Feed (g/day)	402	751	606	631	467	937	1432	1369
MP from undeg. Feed (%MP total)	35	49	42	47	37	59	65	62
MP supplied/CP eaten (%)	38	42	37	103	40	38	49	49
Urea cost (MJ/day)	5	5.7	6.2	0	4.4	7.8	9.6	9.4
Cost of urea (% ME intake)	3.1	3.2	3.9	0	2.8	5.5	4.9	4.8
Predicted ruminal pH	6.46	6.46	6.46	6.46	6.46	6.46	6.16	6.46

[1] High quality pasture ryegrass from the CNCPS library, either defined as over-grazed (OG) or well-managed (WM). The only difference between the two ryegrasses is the effective fibre concentration. The effective fibre values for OG ryegrass was 40% NDF and for WM ryegrass was 60%.

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre.

TABLE 3.17.4. Evaluation of grasses in late lactation using the CNCPS model.

	Ryegrass	Cocksfoot	Fescue	Yorkshire fog	Prairie Grass	Tama ryegrass	Kikuyu	Paspalum
Effective fibre (%NDF)	60	60	60	60	60	60	60	60
Pasture intake (kg DM/cow/day)	14.63	14.63	14.63	14.63	14.63	14.63	14.63	14.63
Diet nutrient composition								
ME (MJ/kg DM)	10.1	10.5	10.6	11.3	10.6	10.7	10.1	9
CP (g/100g DM)	15.5	23.7	16.4	23.7	19.9	21.3	16.4	13.5
Soluble CP (% CP)	52	55	53	55	52	56	48	29
NDF (g/100 g DM)	48.7	47.5	41.6	39.9	44.8	36.5	47.7	57.8
peNDF (g/100 g DM)	29	29	25	24	27	22	29	35
Total NFC (g/100 g DM)	22	15	27	22	21	28	22	15
Requirements								
ME required (MJ/day)	140	146	141	146	143	144	141	139
MP required (g/day)	1350	1361	1356	1312	1356	1308	1394	1471
Production predictions								
Milk production based on ME supply (kg/day)	16.5	16.6	17.9	18.6	17.4	17.3	16.4	13.7
Milk production based on MP supply (kg/day)	14.7	18	17.2	22	15.4	18.2	13.3	12.9
Daily weight change due to reserves (kg/day)	0.3	0.3	0.5	0.6	0.4	0.4	0.2	-0.2
ME supplied (MJ/day)	148	154	155	165	155	157	148	132
MP supplied (g/day)	1333	1515	1468	1668	1377	1472	1309	1362
MP from bacteria (g/day)	899	711	865	785	778	859	772	724
MP from undeg. Feed (g/day)	434	804	603	883	599	613	537	638
MP from undeg. Feed (%MP total)	33	53	41	53	43	42	41	47
MP supplied/CP eaten (%)	59	44	61	48	47	47	55	69
Urea cost (MJ/day)	1.3	7.3	1.9	7.4	4.6	5.6	2	0
Cost of urea (% ME intake)	0.88	4.75	1.23	4.48	2.97	3.58	1.35	0.00
Predicted ruminal pH	6.46	6.46	6.46	6.44	6.46	6.35	6.46	6.46

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre.

TABLE 3.17.5. Evaluation of legumes in late lactation using the CNCPS model.

	White clover	Lucerne	Red Clover	*Lotus corniculatus*	*Lotus pedunculatus*	Sulla	Chicory	Plantain
Effective fibre (% NDF)	40	60	60	60	60	40	40	40
Pasture intake (kg DM/cow/day)	14.63	14.63	14.63	14.63	14.63	14.63	14.63	14.63
Diet nutrient composition								
ME (MJ/kg DM)	10.5	11.2	10.2	10.1	8.7	9.9	10.2	9.6
CP (g/100g DM)	26.9	29.9	27.4	22.2	21.5	23	19.3	24.7
Soluble CP (% CP)	38	52	30	51	33	50	29	33
NDF (g/100 g DM)	25.6	29.5	33.6	28.2	33.1	22.4	23.8	28.3
peNDF (g/100 g DM)	10	18	20	17	20	9	10	11
Total NFC (g/100 g DM)	34	29	26	36	32	41	43	33
Requirements								
ME requirements (MJ/day)	149	150	149	145	144	146	144	148
MP requirements (g/day)	1318	1314	1339	1352	1444	1361	1342	1381
Production predictions								
Milk production based on ME supply (kg/day)	16	17.7	15.2	15.4	11.9	14.8	16	13.7
Milk production based on MP supply (kg/day)	19.2	22.6	27.8	16.7	17.5	16.4	12.7	12.8
Daily weight change due to reserves (kg/day)	0.2	0.5	0	0.1	-0.6	0	0.2	-0.2
ME supplied (MJ/day)	154	164	149	148	127	145	149	140
MP supplied (g/day)	1529	1698	1987	1437	1572	1431	1229	1268
MP from bacteria (g/day)	583	674	723	763	695	591	667	578
MP from undeg. Feed (g/day)	946	1024	1264	674	877	840	562	690
MP from undeg. Feed (%MP total)	62	60	64	47	56	59	46	54
MP supplied/CP eaten (%)	39	39	50	44	50	43	44	35
Urea cost (MJ/day)	10.3	11.2	10.1	6.3	5.6	7.4	5.2	9
Cost of urea (%ME intake)	6.71	6.84	6.77	4.26	4.40	5.11	3.48	6.41
Predicted ruminal pH	5.86	6.17	6.28	6.14	6.26	5.8	5.83	5.9

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre.

TABLE 3.17.6. Evaluation of conserved forages and model ryegrass in late lactation using the CNCPS model.

	Pasture silage	Sulla silage	Lucerne silage	Maize silage	Oat silage	Lucerne hay	Pasture-ryegrass OG	Pasture-ryegrass WM
Effective fibre (%NDF)	90	92	80	81	85	90	40	60
Pasture intake (kg DM/cow/day)	14.63	14.63	14.63	14.63	14.63	14.63	14.63	14.63
Diet nutrient composition								
ME (MJ/kg DM)	9.5	10.4	9.3	9.7	9.5	8.4	11.5	11.5
CP (g/100g DM)	17.7	21.2	23.3	7.6	18.4	24.2	26.3	26.3
Soluble CP (% CP)	70	55	53	17	71	38	43	43
NDF (g/100 g DM)	50.3	36.2	30.5	40.5	53.2	39.1	43.4	43.4
peNDF (g/100 g DM)	45	33	24	33	45	35	17	26
Total NFC (g/100 g DM)	28	30	40	46	21	30	14	14
Requirements								
ME requirements (MJ/day)	143	143	144	139	142	145	148	148
MP requirements (g/day)	1400	1349	1408	1405	1393	1460	1301	1296
Production predictions								
Milk production based on ME supply (kg/day)	14.2	16.7	13.4	15.7	14.2	10.6	18.8	19
Milk production based on MP supply (kg/day)	7.2	13.7	11.7	9.9	9.4	12.5	25.2	25.4
Daily weight change due to reserves (kg/day)	-0.1	0.3	-0.3	0.1	-0.1	-0.8	0.7	0.7
ME supplied (MJ/day)	139	152	136	142	139	123	168	168
MP supplied (g/day)	1001	1282	1238	1146	1107	1333	1820	1823
MP from bacteria (g/day)	665	679	743	621	708	575	673	730
MP from undeg. Feed (g/day)	336	603	495	525	399	758	1147	1093
MP from undeg. Feed (%MP total)	34	47	40	46	36	57	63	60
MP supplied/CP eaten (%)	39	41	36	103	41	38	47	47
Urea cost (MJ/day)	4.2	5	5.3	0	3.6	6.8	9.2	9.1
Cost of urea (% ME intake)	3.02	3.29	3.90	0	2.6	5.5	5.5	5.4
Predicted ruminal pH	6.46	6.46	6.46	6.46	6.46	6.46	6.16	6.46

[1] High quality pasture ryegrass from the CNCPS library, either defined as over-grazed (OG) or well-managed (WM). The only difference between the two ryegrasses is the effective fibre concentration. The effective fibre values for OG ryegrass was 40% NDF and for WM ryegrass was 60%.

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre.

APPENDIX 3.18. CNCPS evaluation of forage mixtures. Data were used to derive Tables 3.23 and 3.24.

TABLE 3.18.1. Evaluation of forage mixtures in early lactation using the CNCPS model.

	Ryegrass: white clover 20%	Ryegrass: white clover 50%	Ryegrass: white clover 80%	Pasture[1]: lucerne	Pasture[1]: sulla	Pasture[1]: *Lotus C*	Pasture[1]: *Lotus P*	Pasture[1]: plantain
Effective fibre (%NDF)	60:40	60:40	60:40	60:40	60:40	60:60	60:60	60:40
Intake of forage no. 1 (kg DM/cow/day)	13.65	8.53	3.41	8.53	8.53	8.53	8.53	8.53
Intake of forage no. 2 (kgDM/cow/day)	3.41	8.53	13.65	8.53	8.53	8.53	8.53	8.53
Total intake (kgDM/cow/day)	17.1	17.1	17.1	17.1	17.1	17.1	17.1	17.1
Diet nutrient composition								
ME (MJ/kg DM)	10.1	10.4	10.7	10.7	10	10.1	9.4	9.9
CP (g/100g DM)	17.8	21.2	24.6	23.8	20.4	20	19.6	21.2
Soluble CP (% CP)	48	43	40	51	49	50	40	39
NDF (g/100 g DM)	44.1	37.2	30.2	36.8	33.2	36.1	38.6	36.2
peNDF (g/100 g DM)	25	20	14	22	17	21	23	18
Total NFC (g/100 g DM)	24	28	32	27	33	30	28	29
Requirements								
ME requirements (MJ/day)	180	182	185	184	182	181	181	183
MP requirements (g/day)	1799	1777	1750	1782	1808	1805	1857	1815
Production predictions								
Milk production based on ME supply (kg/day)	21.4	21.8	22.2	22.3	20.6	20.8	18.8	20.2
Milk production based on MP supply (kg/day)	20.3	23.6	25.3	24.6	21.5	21.2	21.3	19.6
Daily weight change due to reserves (kg/day)	-0.2	-0.2	-0.1	-0.1	-0.4	-0.3	-0.7	-0.5
ME supplied (MJ/day)	173	178	183	183	171	173	161	169
MP supplied (g/day)	1676	1821	1880	1878	1745	1727	1784	1658
MP from bacteria (g/day)	1008	976	830	911	900	973	904	900
MP from undeg. Feed (g/day)	668	845	1050	967	845	754	880	758
MP from undeg. Feed (%MP total)	40	46	56	51	52	44	49	46
MP supplied/CP eaten (%)	55	50	45	46	50	50	53	46
Urea cost (MJ/day)	2.9	5.3	8.6	7.1	5	4.6	4.2	6.1
Cost of urea (% ME intake)	1.68	2.98	4.70	3.88	2.92	2.66	2.61	3.60
Predicted ruminal pH	6.46	6.26	6.02	6.34	6.15	6.32	6.38	6.2

[1] Pasture is made up of 80% ryegrass and 20% white clover

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre.

TABLE 3.18.2. Evaluation of forage mixtures in early lactation using the CNCPS model.

	Pasture[1]: chicory	Pasture[1]: red clover	Pasture: Lucerne silage	Pasture[1] sulla silage	Pasture[1]: oat silage	Pasture[1]: lucerne: maize silage	Pasture[1]: red clover: grain	Pasture[1]: maize silage
Effective fibre (%NDF)	60:40	60:60	60:80	60:92	60:85	60:60:81	60:60:80	60:81
Intake of forage no. 1 (kgDM/cow/day)	8.53	8.53	8.53	8.53	8.53	8.53	8.53	11.38
Intake of forage no. 2 (kgDM/cow/day)	8.53	8.53	8.53	8.53	8.53	4.27	4.27	5.69
Intake of forage no. 3 (kgDM/cow/day)	-	-	-	-	-	4.27	4.27	-
Total intake (kgDM/cow/day)	17.1	17.1	17.1	17.1	17.1	17.1	17.1	17.1
Diet nutrient composition								
ME (MJ/kg DM)	10.3	10.2	9.7	10.3	9.7	10.4	10.8	10.1
CP (g/100g DM)	18.5	22.6	20.5	19.5	18.1	18.3	18.2	14
Soluble CP (% CP)	38	37	51	52	60	46	36	43
NDF (g/100 g DM)	33.9	38.8	37.3	40.1	48.6	39.5	32.9	43.7
peNDF (g/100 g DM)	17	23	25	29	35	25	20	29
Total NFC (g/100 g DM)	34	25	32	27	23	31	37	31
Requirements								
ME requirements (MJ/day)	181	183	182	181	180	179	180	177
MP requirements (g/day)	1787	1798	1836	1804	1832	1801	1760	1814
Production predictions								
Milk production based on ME supply (kg/day)	22.7	20.8	19.6	21.5	19.9	22.3	23.8	21.9
Milk production based on MP supply (kg/day)	21.7	27.5	17.1	18.7	15.6	22	23.4	19.7
Daily weight change due to reserves (kg/day)	-0.2	-0.3	-0.6	-0.2	-0.5	-0.1	0.2	-0.1
ME supplied (MJ/day)	176	174	166	176	166	178	185	173
MP supplied (g/day)	1654	2038	1551	1599	1470	1767	1797	1664
MP from bacteria (g/day)	978	918	930	890	934	1001	974	1066
MP from undeg. Feed (g/day)	676	1120	621	709	536	766	823	598
MP from undeg. Feed (%MP total)	41	55	40	44	36	43	46	36
MP supplied/CP eaten (%)	52	53		48	47	56	58	70
Urea cost (MJ/day)	3.7	6.5	4.7	4.3	3.6	2.6	2.9	0
Cost of urea (% ME intake)	2.10	3.73	2.83	2.44	2.17	1.46	1.57	0.00
Predicted ruminal pH	6.16	6.39	6.46	6.46	6.46	6.46	6.26	6.46

[1] Pasture is made up of 80% ryegrass and 20% white clover

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre.

TABLE 3.18.3. Evaluation of forage mixtures in early lactation using the CNCPS model.

	Pasture[1] sulla	Pasture[1]: maize silage: sulla	White clover: sulla	Lucerne: sulla	Lucerne: chicory	Lucerne :plantain	Lucerne: sulla silage
Effective fibre (%NDF)	60:40	60:81:40	40:40	60:40	60:40	60:40	60:92
Intake of forage no. 1 (kg DM/cow/day)	11.38	11.38	8.53	8.53	8.53	8.53	8.53
Intake of forage no. 2 (kgDM/cow/day)	5.69	2.84	8.53	8.53	8.53	8.53	8.53
Intake of forage no. 3 (kgDM/cow/day)	-	2.84	-	-	-	-	-
Total intake (kgDM/cow/day	17.1	17.1	17.1	17.1	17.1	17.1	17.1
Diet nutrient composition							
ME (MJ/kg DM)	10	10.1	10.2	10.5	10.8	10.5	10.8
CP (g/100g DM)	19.1	16.6	25	26.5	24.6	27.3	25.6
Soluble CP (% CP)	49	47	44	51	43	44	53
NDF (g/100 g DM)	37.6	40.6	24	26	26.7	28.9	32.9
peNDF (g/100 g DM)	21	25	10	13	14	15	26
Total NFC (g/100 g DM)	29	30	38	35	36	31	30
Requirements							
ME requirements (MJ/day)	181	179	186	187	185	187	185
MP requirements (g/day)	1809	1811	1795	1790	1768	1795	1787
Production predictions							
Milk production based on ME supply (kg/day)	20.8	21.4	20.3	21.4	22.7	21.2	22.5
Milk production based on MP supply (kg/day)	21.3	20.4	22.5	24.7	23.1	23.2	23
Daily weight change due to reserves (kg/day)	-0.3	-0.2	-0.4	-0.2	0	-03	0
ME supplied (MJ/day)	171	173	174	180	185	180	185
MP supplied (g/day)	1738	1696	1787	1890	1789	1820	1801
MP from bacteria (g/day)	978	1021	668	731	803	736	794
MP from undeg. Feed (g/day)	760	675	1119	1159	986	1084	1007
MP from undeg. Feed (%MP total)	44	40	63	61	55	60	56
MP supplied/CP eaten (%)	53	60		42	43	39	48
Urea cost (MJ/day)	3.8	1.8	9.2	9.7	8.1	10.4	8.1
Cost of urea (% ME intake)	2.22	1.04	5.27	5.40	4.4	5.8	4.4
Predicted ruminal pH	6.29	6.46	5.83	5.99	6.00	6.04	6.46

[1] Pasture is made up of 80% ryegrass and 20% white clover

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre.

TABLE 3.18.4. Evaluation of forage mixtures in late lactation using the CNCPS model.

	Ryegrass: white clover 20%	Ryegrass: white clover 50%	Ryegrass: white clover 80%	Pasture[1]: lucerne	Pasture[1]: sulla	Pasture[1]: *Lotus C*	Pasture[1]: *Lotus P*	Pasture[1]: plantain
Effective fibre (%NDF)	60:40	60:40	60:40	60:40	60:40	60:60	60:60	60:40
Intake of forage no. 1 (kg DM/cow/day)	11.7	7.31	2.92	7.31	7.31	7.31	7.31	7.31
Intake of forage no. 2 (kgDM/cow/day)	2.92	7.31	11.7	7.31	7.31	7.31	7.31	7.31
Total intake (kgDM/cow/day)	14.63	14.63	14.63	14.63	14.63	14.63	14.63	14.63
Diet nutrient composition								
ME (MJ/kg DM)	10.3	10.5	10.8	10.7	10.1	10.1	9.5	10
CP (g/100g DM)	17.8	21.2	24.6	23.8	20.4	20	19.6	21.2
Soluble CP (% CP)	48	43	40	51	49	50	40	39
NDF (g/100 g DM)	44.1	37.2	30.2	36.8	33.2	36.1	38.6	36.2
peNDF (g/100 g DM)	25	20	14	22	17	21	23	18
Total NFC (g/100 g DM)	24	28	32	27	33	30	28	29
Requirements								
ME requirements (MJ/day)	142	144	147	146	144	143	143	144
MP requirements (g/day)	1339	1323	1302	1328	1351	1347	1392	1356
Production predictions								
Milk production based on ME supply (kg/day)	16.6	16.7	17	17.1	15.7	16	14.2	15.4
Milk production based on MP supply (kg/day)	16.4	18.9	20	19.7	17.1	16.9	17	15.5
Daily weight change due to reserves (kg/day)	0.3	0.3	0.3	0.4	0.1	0.2	-0.1	0.1
ME supplied (MJ/day)	151	154	158	157	148	148	139	146
MP supplied (g/day)	1409	1522	1552	1564	1456	1445	1491	1379
MP from bacteria (g/day)	882	854	723	796	785	849	790	785
MP from undeg. Feed (g/day)	527	668	829	768	671	596	701	594
MP from undeg. Feed (%MP total)	37	44	53	49	46	41	47	43
MP supplied/CP eaten (%)	54	49	43	45	49	49	52	44
Urea cost (MJ/day)	2.9	5.5	8.3	7	5	4.5	4.3	5.5
Cost of urea (% ME intake)	1.9	3.6	5.3	4.5	3.4	3.0	3.1	3.8
Predicted ruminal pH	6.46	6.26	6.02	6.34	6.15	6.32	6.38	6.2

[1] Pasture is made up of 80% ryegrass and 20% white clover

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre.

TABLE 3.18.5. Evaluation of forage mixtures in late lactation using the CNCPS model.

	Pasture[1]: chicory	Pasture[1]: red clover	Pasture[1]: lucerne silage	Pasture[1]: sulla silage	Pasture[1]: oat silage	Pasture[1]: lucerne: maize silage	Pasture[1]: red clover: grain	Pasture[1]: maize silage
Effective fibre (%NDF)	60:40	60:60	60:80	60:92	60:85	60:60:81	60:60:80	60:81
Intake of forage no. 1 (kg DM/cow/day)	7.31	7.31	7.31	7.31	7.31	7.31	7.31	9.76
Intake of forage no. 2 (kgDM/cow/day)	7.31	7.31	7.31	7.31	7.31	3.66	3.66	4.87
Intake of forage no. 3 (kgDM/cow/day)	-	-	-	-	-	3.66	3.66	-
Total intake (kgDM/cow/day	14.63	14.63	14.63	14.63	14.63	14.63	14.63	14.63
Diet nutrient composition								
ME (MJ/kg DM)	10.4	10.2	9.8	10.3	9.8	10.5	11.0	10.2
CP (g/100g DM)	18.5	22.6	20.5	19.5	18.1	18.3	18.2	14
Soluble CP (%CP)	38	37	51	52	60	46	36	43
NDF (g/100 g DM)	33.9	38.8	37.3	40.1	48.6	39.5	32.9	43.7
peNDF (g/100 g DM)	17	23	25	29	35	26	20	29
Total NFC (g/100 g DM)	34	25	32	27	23	31	37	31
Requirements								
ME requirements	142	145	143	143	142	142	142	139
MP requirements	1332	1339	1373	1344	1367	1342	1305	1352
Production predictions								
Milk production based on ME supply (kg/day)	16.7	15.9	15	16.7	15.4	17.2	18.7	17
Milk production based on MP supply (kg/day)	16.1	22	13.8	15	12.9	17.9	19.0	16.2
Daily weight change due to reserves (kg/day)	0.3	0.2	0	0.3	0.1	0.4	0.6	0.3
ME supplied	152	149	143	151	143	154	161	149
MP supplied	1387	1696	1312	1345	1258	1489	1502	1414
MP from bacteria (g/day)	854	803	813	781	819	876	865	934
MP from undeg. Feed (g/day)	533	893	499	564	439	613	637	480
MP from undeg. Feed (%MP total)	38	53	38	42	35	41	42	34
MP supplied/CP eaten (%)	51	51	44	47	48	56	56	69
Urea cost (MJ/day)	3.6	6.5	4	3.8	3.1	3	3.4	0.3
Cost of urea (% ME intake)	2.4	4.4	2.8	2.5	2.2	2.0	2.1	0.2
Predicted ruminal pH	6.16	6.39	6.46	6.46	6.46	6.46	6.26	6.46

[1] Pasture is made up of 80% ryegrass and 20% white clover

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre.

TABLE 3.18.6. Evaluation of forage mixtures in late lactation using the CNCPS model.

	Pasture[1]: sulla	Pasture[1]: maize silage: sulla	White clover: Sulla	Lucerne: sulla	Lucerne: chicory	Lucerne: plantain	Lucerne: sulla silage
Effective fibre (% NDF)	60:40	60:81:40	40:40	60:40	60:40	60:40	60:92
Intake of forage no. 1 (kg DM/cow/day)	9.76	9.76	7.31	7.31	7.31	7.31	7.31
Intake of forage no. 2 (kgDM/cow/day)	4.87	2.44	7.31	7.31	7.31	7.31	7.31
Intake of forage no. 3 (kgDM/cow/day)	-	2.44	-	-	-	-	-
Total intake (kgDM/cow/day)	14.63	14.63	14.63	14.63	14.63	14.63	14.63
Diet nutrient composition							
ME (MJ/kg DM)	10.1	10.2	10.2	10.6	10.8	10.5	10.8
CP (g/100g DM)	19.1	16.6	25	26.5	24.6	27.3	25.6
Soluble CP (% CP)	49	47	44	51	43	44	53
NDF (g/100 g DM)	37.6	40.6	24	26	26.7	28.9	32.9
peNDF (g/100 g DM)	21	25	10	13	14	15	26
Total NFC (g/100 g DM)	29	30	38	35	36	31	30
Requirements							
ME requirements (MJ/day)	143	141	148	148	147	149	147
MP requirements (g/day)	1350	1351	1342	1338	1319	1342	1333
Production predictions							
Milk production based on ME supply (kg/day)	15.9	16.5	15.3	16.3	17.3	16.1	17.3
Milk production based on MP supply (kg/day)	17.1	16.6	17.5	19.4	18.2	18.1	18.3
Daily weight change due to reserves (kg/day)	0.2	0.3	0	0.2	0.4	0.2	0.4
ME supplied (MJ/day)	148	149	149	155	159	154	158
MP supplied (g/day)	1456	1432	1470	1561	1483	1498	1500
MP from bacteria (g/day)	854	893	582	636	699	641	695
MP from undeg. Feed (g/day)	602	539	888	925	784	857	806
MP from undeg. Feed (%MP total)	41	38	60	59	53	57	54
MP supplied/CP eaten (%)	52	59	40	40	41	38	40
Urea cost (MJ/day)	3.9	2	8.8	9.3	7.9	9.9	7.9
Cost of urea (% ME intake)	2.6	1.3	5.9	6.0			
Predicted ruminal pH	6.29	6.46	5.83	5.99	6.00	6.04	6.46

[1] Pasture is made up of 80% ryegrass and 20% white clover

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre.

APPENDIX 4.1. Results from preliminary *in sacco* and *in vitro* incubations of ryegrass, white clover, lucerne, sulla and mixtures of ryegrass:sulla, lucerne:sulla and white clover:sulla

In sacco incubations

FIGURE 4.1.1. Dry matter (DM) loss of ryegrass, sulla and ryegrass:sulla.

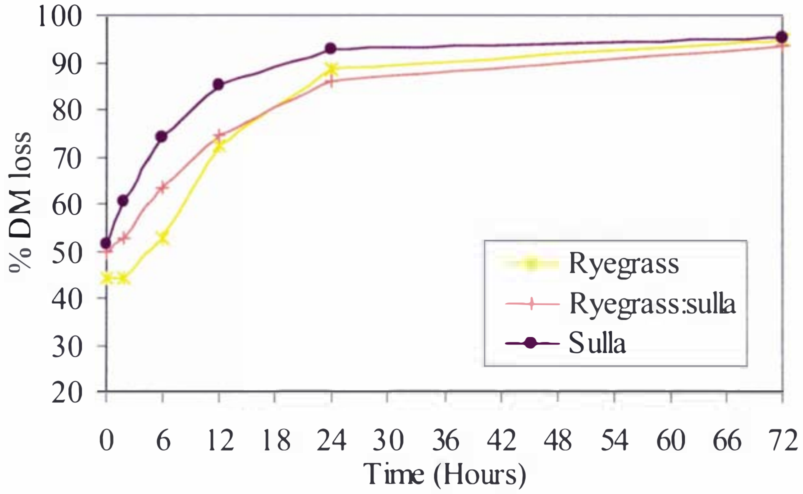

FIGURE 4.1.2. Dry matter (DM) loss of white clover, sulla and white clover:sulla.

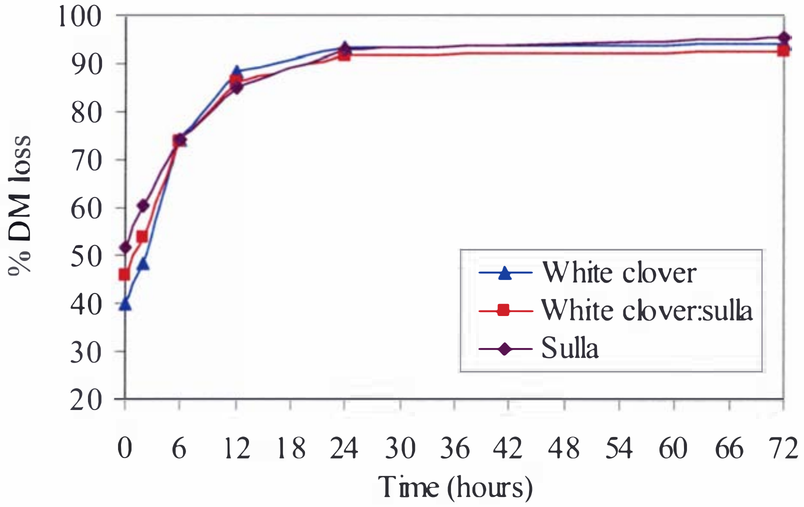

FIGURE 4.1.3. Dry matter (DM) loss of lucerne, sulla and lucerne:sulla.

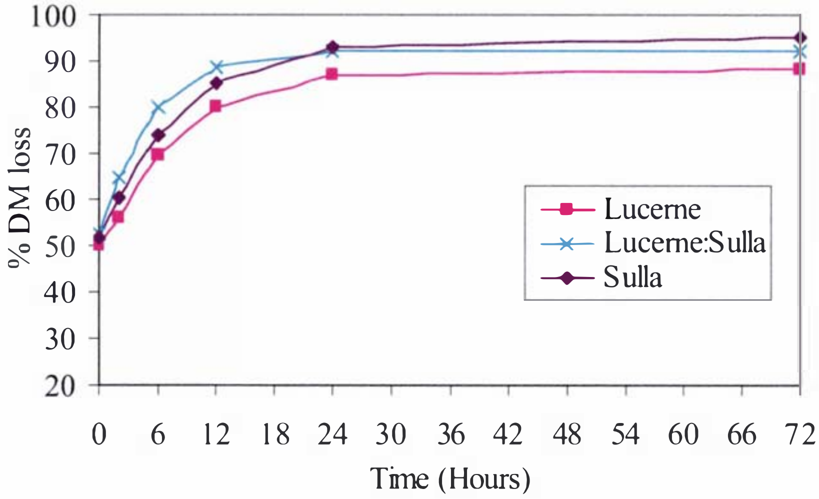

TABLE 4.1.1. Dry matter (DM) degradation parameters of pasture, white clover, lucerne, sulla and mixtures of pasture:sulla, lucerne:sulla and white clover:sulla.

Feed	A (% DM)	B (% DM)	k (%/h)	Lag (h)	ED (% DM)
Ryegrass	44.1	50.8	0.106	4.20	76.2
White clover	39.9	54.1	0.211	1.18	82.0
Lucerne	49.8	38.6	0.135	0.67	76.5
Sulla	51.7	43.6	0.123	0.18	81.0
Ryegrass:sulla	49.6	44.1	0.075	0.95	73.4
White clover:sulla	45.8	46.8	0.181	1.00	81.0
Lucerne:sulla	52.6	39.6	0.206	0.26	83.3

TABLE 4.1.2. Crude protein (CP) degradation parameters of pasture, white clover, lucerne, sulla and mixtures of pasture:sulla, lucerne:sulla and white clover:sulla.

Feed	A (% CP)	B (% CP)	k (%/h)	Lag (h)	ED (% CP)
Ryegrass	52.2	43.5	15.0	4.6	83.3
White clover	38.4	58.0	19.1	1.3	82.5
Lucerne	52.0	41.5	15.3	0	81.8
Sulla	49.5	46.4	11.7	0	82.5
Ryegrass:sulla	53.3	41.7	9.7	0.9	79.1
White clover:sulla	43.2	52.3	17.7	1.3	82.3
Lucerne:sulla	61.0	35.1	22.2	0.8	88.6

TABLE 4.1.3. Neutral detergent fibre (NDF) degradation parameters of pasture, white clover, lucerne, sulla and mixtures of pasture:sulla, lucerne:sulla and white clover:sulla.

Feed	A (% NDF)	B (% NDF)	k (%/h)	Lag (h)	ED (% NDF)
Ryegrass	20.7	71.6	9.3	3.9	64.2
White clover	21.2	62.3	28.3	1.0	72.6
Lucerne	39.9	33.2	17.0	4.2	64.4
Sulla	0	84.5	16.4	0.8	61.9
Ryegrass:sulla	13.2	72.0	8.0	3.7	54.3
White clover:sulla	0	76.3	18.4	0.8	57.5
Lucerne:sulla	7.5	67.4	22.1	0	60.5

In vitro incubations

FIGURE 4.1.4. Ammonia (NH_3) production (% NH_3 per forage nitrogen, N) from ryegrass, sulla and ryegrass:sulla.

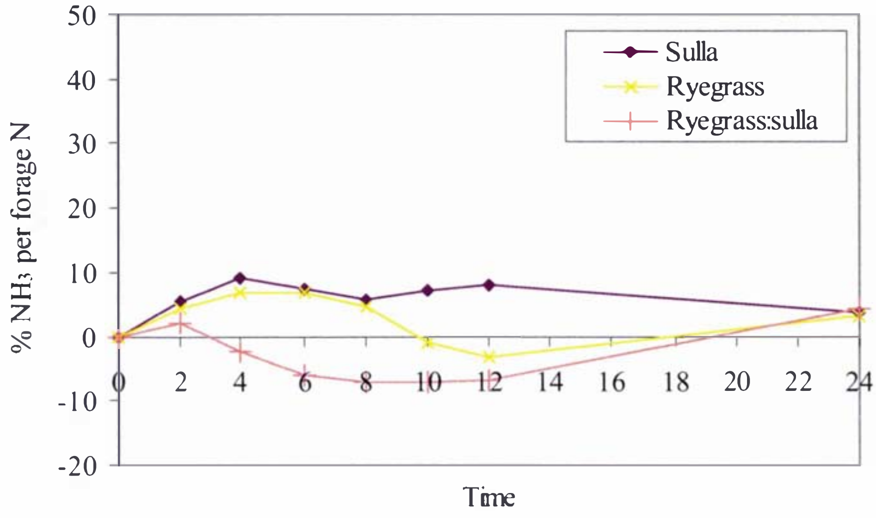

FIGURE 4.1.5. Ammonia (NH_3) production (% NH_3 per forage nitrogen, N) from white clover, sulla and white clover:sulla.

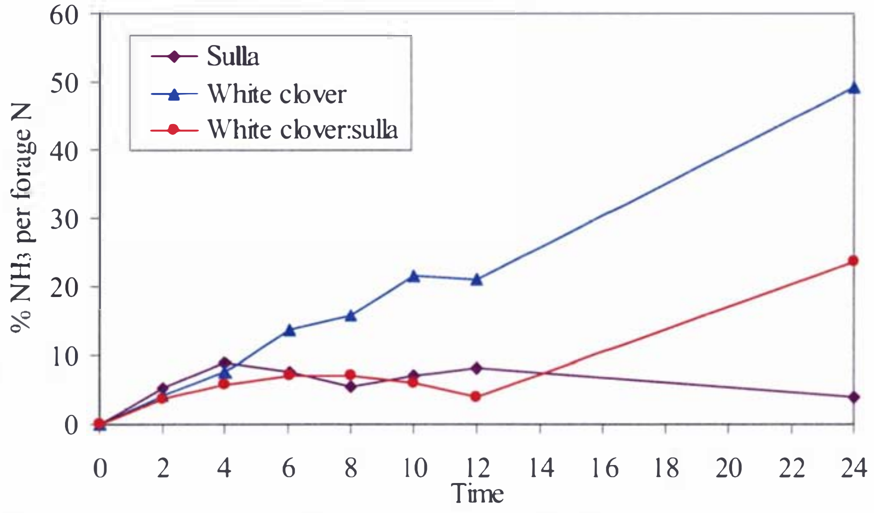

FIGURE 4.1.6. Ammonia (NH_3) production (% NH_3 per forage nitrogen, N) from lucerne, sulla and lucerne:sulla.

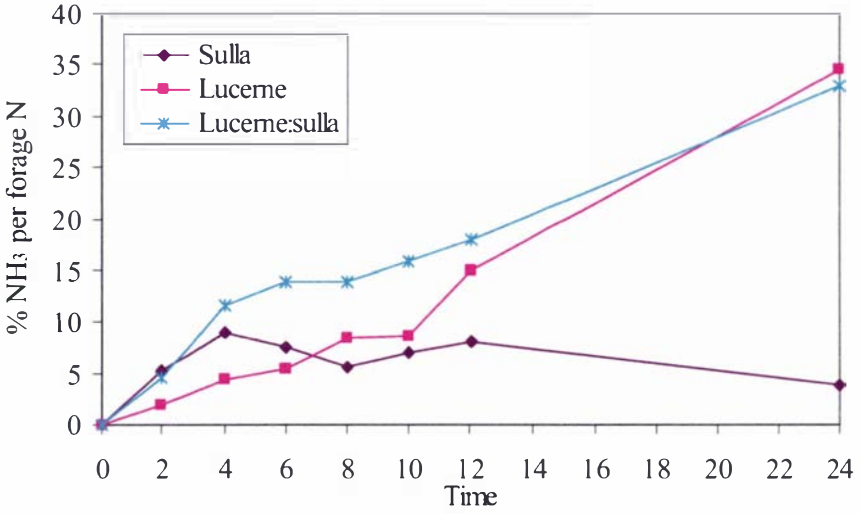

TABLE 4.1.4. Evaluation of diets using data from Appendix 4.1 and the CNCPS model.

	Individual feeds				Forages mixed together pre trial		
	Ryegrass	White Clover	Lucerne	Sulla	Ryegrass: Sulla	White clover: sulla	Lucerne: sulla
Effective fibre (% NDF)	60	40	60	40	60:40	40:40	60:40
Pasture intake (kg DM/cow/day)	17.1	17.1	17.1	17.1	17.1	17.1	17.1
Diet nutrient composition							
ME (MJ/kg DM)	10	10.5	11.2	9.9	9.0	10.0	10.6
CP (g/100g DM)	15.5	26.9	29.9	23	16.2	21.1	25.0
Soluble CP (% CP)	52	38	52	50	53	43	61
NDF (g/100 g DM)	48.7	25.6	29.5	22.4	35.7	24.2	25.1
peNDF (g/100 g DM)	29	10	18	9	18	10	13
Total NFC (g/100 g DM)	22	34	29	41	34	41	37
Requirements							
ME requirements (MJ/day)	178	188	189	184	179	184	186
MP requirements (g/day)	1813	1768	1763	1816	1889	1805	1789
Production predictions							
Milk production based on ME supply (kg/day)	21.2	21	23.1	19.7	17.9	20.3	22.7
Milk production based on MP supply (kg/day)	18.1	24.6	28.5	21	16.8	18.4	21.5
Daily weight change due to reserves (kg/day)	-0.3	-0.3	0.1	-0.5	-0.9	-0.4	-0.2
ME supplied (MJ/day)	171	180	192	169	154	171	180
MP supplied (g/day)	1577	1863	2056	1731	1590	1586	1579
MP from bacteria (g/day)	1026	667	773	679	915	727	742
MP from undeg. Feed (g/day)	551	1196	1283	1052	675	859	837
MP from undeg. Feed (%MP total)	35	64	62	61	42	54	53
MP supplied/CP eaten (%)	59	41	40	44	57	44	37
Urea cost (MJ/day)	1.5	10.8	12	7.9	2.4	6.9	9.6
Cost of urea (% ME intake)	0.88	6.02	6.27	4.67	1.56	4.04	5.33
Predicted ruminal pH	6.46	5.86	6.17	5.8	6.18	5.83	5.96

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre.

APPENDIX 4.2. Using alkanes to determine feed intake of lambs fed forages.

INTRODUCTION

The pre- and post-grazing difference method and the indigestible faecal marker technique using chromium oxide (Cr_2O_3) have been used to determine feed intake of grazing animals, but both techniques have some disadvantages. Pre- and post-grazing does not provide accurate estimates of group intake, especially when a high allowance is given and analysis of chromium has posed some analytical problems recently (Langlands, 1975). The chromium oxide marker cannot be analysed as a discrete compound and incompatibility in their analysis in herbage and faeces can lead to errors in the estimation of digestibility and intake (Langlands, 1975).

The use of indigestible plant components as markers for digestibility determinations in grazing ruminants offers potential advantages over other methods, in that digestibility can be directly estimated *in vivo*. Long chain n-alkanes are present in the cuticular wax of herbage species of predominantly odd-chain in the range of C25 to C35 with C29, C31 and C33 being the most abundant. Mayes *et al.* (1986) described a technique based on the measurement of faecal concentrations of the odd-chain n-alkanes derived from the cuticular wax of herbage, and orally administered even-chain alkanes. Recovery of all types of alkanes are not complete, but alkanes of adjacent chain length have similar recoveries, therefore errors arising from the incomplete recovery of odd- and even-chain alkanes cancel out in the calculation of intake (Mayes *et al.* 1986). A major advantage of the alkane technique is that it accommodates differences in diet digestibility between individual animals, rather than relying on a group mean estimate of digestibility. There is evidence to suggest that estimates based on C33/C32 are the most accurate and are also more accurate than those based on the chromium/*in vitro* procedure (Dove *et al.* 2000). Alkane markers were used in the lamb feeding study (Chapter 4) to estimate the intake of lambs fed pasture, white clover, lucerne, sulla and mixtures of pasture:sulla, white clover:sulla and lucerne:sulla.

METHOD

Intakes of individual lambs fed the seven forage diets on feed pads (Chapter 4) were determined during week six of the trial using alkane markers (Dove and Mayes, 1991). Tablets containing 32.5 mg of C32 alkane were administered twice daily to all lambs over a 10-day period (day 35 to day 44). For the last five days of this period (day 39 to 44) faecal samples were collected twice daily. Alkane concentrations in feed and faeces were determined and individual lamb intakes calculated according to the equation of Dove and Mayes (1991).

At the time of alkane administration (from day 35 to 44) three lambs from pasture, sulla, lucerne and lucerne:sulla treatments were removed from the feed pads and placed indoors in metabolism crates. A further sub group of 12 lambs (three lambs from the same treatments) were placed indoors from days 44 to 53. Both groups of lambs indoors were given alkane markers for 10 days and faecal samples were collected from total faecal collections over the last five days of each period to determine alkane recovery.

Recovery data indicated whether C31 or C33 (or both) herbage markers should be used for estimating intakes. Actual intakes of lambs in crates were also measured and compared to alkane marker intakes. The second group of lambs had intakes measured by alkane markers both outdoors and indoors, enabling indoor data from both groups to be adjusted to the outdoor situation.

EQUATION 4.2.1.

$$\text{kg DM intake/day} = \left(\frac{F_i}{F_j} \times D_j \right) \div \left(H_i \times \frac{F_i}{F_j} \times H_j \right)$$

Where F_i = concentration of C31 or C33 alkane in faeces

F_j = concentration of C32 alkane in faeces

H_i = concentration of C31 or C33 alkane in herbage

H_j = concentration of C32 alkane in herbage

D_j = daily dose of C32 alkane in tablet

Feed samples were taken daily, and refusals taken every 2 days and held at –20°C until being freeze-dried and analysed. Faecal samples from lambs on feed pads and 10% aliquots from those 24 lambs indoors were frozen and freeze-dried at 60°C and alkane concentrations (C31, C32 and C33) determined by gas chromatography. Alkane concentrations (C31, C32 and C33) of each forage were used to predict DM intake of lambs.

RESULTS AND DISCUSSION

Forage concentrations

The concentration of C31 and C33 alkanes in forages was used to determine which plant alkane (C31 or C33) should be used to estimate intake (Table 4.2.1). C33 could not be used to measure intake in lambs fed lucerne or white clover because this alkane was present in low concentrations relative to C31 (4.8% for lucerne and 17% for white clover), compared to 42% for ryegrass and 33% for sulla. Therefore, on this basis C31 alkane should be used to predict intake of lambs fed white clover and lucerne, and either C31 or C33 or an average of both markers could be used to predict intake of lambs fed pasture, sulla, pasture:sulla. Using the same rational, intakes of lambs fed lucerne:sulla and white clover:sulla could be predicted using the C31 marker.

Intake prediction

Comparisons between actual and predicted intakes of the lambs fed in metabolism crates showed all markers (C31, C33, mean of C31 and C33) predicted mean intakes of each dietary treatment group within 6% of true values for sulla, 12% of true values for pasture and over predicted intakes by about 22% for lucerne and 8% for lucerne:sulla diets. On this basis, the closest prediction was obtained from C33 for pasture and lucerne:sulla and C31 for lucerne. Sulla intakes could be predicted from either alkane or their mean, but there were large variations between predicted and actual intakes for individual lambs fed sulla (Table 4.2.2). Predictions for individual lambs, compared to true values were also at variance when lucerne was fed (especially with the C33 marker) and only the pasture diet resulted in an accurate prediction of intake with an acceptable variance between true and predicted intakes for individual lambs (Table 4.2.2).

TABLE 4.2.1. Concentrations in forage DM of C31, C32 and C33 plant alkanes fed to sheep as pure species or as mixtures

	Alkane concentration (mg/100g DM)		
Diet	C31	C32	C33
Grass (100%)	180.8	6.2	77.1
Lucerne (100%)	221.3	6.5	10.6
White clover (100%	39.5	0.0	6.9
Sulla (100%)	160.9	8.4	53.3
Grass:White clover (80:20)	152.6	5.0	63.1
Sulla:Pasture[a] (43:57)	156.1	6.5	58.9
Sulla:White clover (52:48)	102.6	4.4	31.0
Sulla:Lucerne (51:49)	190.5	7.5	32.4

[a] Pasture = On a DM basis, 80% ryegrass and 20% white clover

TABLE 4.2.2. Comparison between actual intakes (kg DM/lamb/day) measured indoors and values predicted using C31 or C33 plant alkanes with C32 alkanes for sheep fed pasture, sulla, lucerne or lucerne:sulla diets

	Pasture		Sulla		Lucerne		Lucerne:Sulla	
	mean ± SD		mean ± SD		mean ± SD		mean ± SD	
Based on C31	1.27	0.168	1.50	0.393	1.76	0.312	1.84	0.226
Based on C33	1.10	0.117	1.41	0.372	1.90	0.419	1.73	0.148
Based on C31 + C33	1.22	0.133	1.48	0.387	1.76	0.316	1.83	0.211
TRUE (indoors)	1.12	0.044	1.50	0.071	1.47	0.168	1.67	0.043
Deviance from true								
C31	0.16	0.158	0.00	0.347	0.24	0.250	0.17	0.175
C33	-0.02	0.126	-0.09	0.325	0.44	0.371	0.06	0.141
C31 + C33	0.11	0.127	-0.02	0.341	0.30	0.254	0.15	0.164

Alkane recoveries

Alkane recoveries were determined from the lambs fed indoors (Table 4.2.3). There were large variations in recoveries between individual lambs fed the same diet, especially for sulla and lucerne. The mean recoveries (84 – 93%) across diets obscures very large differences between individual animals (Table4.2.3), especially for sulla (71.1 – 110.3%), but also for lucerne (78.9 – 105.5%) and lucerne:sulla (85.6 – 110.4%). This variability can only arise from inadequate sub-sampling of faeces for alkane analyses, variations in chemical analyses or a true result of between sheep variability in digestion (or indigestion of alkanes). It is important to realise that equations for predicting intake (Dove and Mayes, 1991) assume losses of all alkanes during digestion are equal and therefore do not affect predictions, but the range between individuals (Table 4.2.3) especially for diets containing sulla and/or lucerne do give cause for concern.

TABLE 4.2.3. Recovery of C32 alkane from faeces of individual sheep. Data are based on a daily dose of 64.6 mg with adjustment for C32 in forage

Pasture		Sulla		Lucerne		Lucerne:Sulla	
Sheep No	Recovery (%)	Sheep No	Recovery (%)	Sheep No	Recovery (%)	Sheep No	Recovery (%)
2	80.8	4	110.3	23	85.0	1	88.2
3	89.2	11	71.1	26	105.5	25	92.0
14	71.5	32	81.2	36	95.2	30	90.6
29	81.0	38	85.5	43	87.6	33	110.4
42	78.1	44	100.0	46	78.9	35	92.8
55	94.2	53	102.0	60	87.9	41	85.6
Mean	84.5		91.7		90.0		93.3

Using alkanes to predict individual intakes on feed pads

Alkane concentrations in plants formed the basis for using C31 alkanes for predicting DM intakes of lambs fed white clover, lucerne, white clover:sulla and sulla with C33 used to predict DM intakes of lambs fed pasture, lucerne:sulla and pasture:sulla. However it was also necessary to consider effects of housing in metabolism crates when extrapolating indoor intakes of these lambs to their expected intakes outdoors on the sawdust feed pads. Effects of the indoor environment on intakes of the first group of 12 lambs were calculated on the basis of alkane predicted intakes indoors and outdoors of the second group (B) of 12 lambs fed the same four diets. Intakes of the group B lambs suggested that the indoor environment reduced DM intake by 19% for pasture, 8% for sulla, 11% for lucerne, and by 23% for the lucerne:sulla mixture. The indoor intakes of the group A lambs were increased by these amounts for calculation of intakes on the sawdust feed pads.

Comparing the actual intake of the 24 lambs fed indoors with predictions using the appropriate alkane marker (Table 4.2.2) showed good mean values for pasture, sulla and lucerne:sulla, but when lucerne was fed the C31 and C33 markers over-predicted actual intake by 0.24 to 0.44 kg/day, respectively.

Predicted intakes of lambs on feed pads were adjusted on the basis of under or over predictions with alkanes: pasture and sullla, no change; lucerne, reduced by 16%; lucerne:sulla reduced by 10%.

TABLE 4.2.4. Predicted dry matter (DM) intake (kg DM/lamb/day) using alkane markers[1], group intake of lambs during week 4 and 5 adjusted for over and under prediction of the alkane marker[2], DM intake of lambs over the eight week trial[2], live weight (LW) gain and efficiency of LW gain per group DM intake of lambs fed seven forage diets.

Diet	Predicted DM intake/lamb using alkane marker[1]	Group DM intake/lamb during alkane period[2]	Predicted DM intake – group DM intake
Pasture	1.35	1.05	+0.30
White Clover	1.41	1.60	-0.29
Lucerne	2.00	1.38	+0.62
Sulla	1.56	1.47	+0.09
Pasture:Sulla	1.48	1.24	+0.24
White clover:Sulla	1.46	1.56	-0.10
Lucerne:Sulla	1.44	1.66	-0.22
Diet effect (Pr)	P < 0.01	Pr < 0.01	
SEM	0.101	0.0917	

The intakes of all lambs in the trial, determined using predictions from alkane faecal markers (with adjustments for being held indoors) were compared with mean values for each treatment group at the same time as the alkane measurement (Table 4.2.4). Mean DM intake based on alkane measurements did not match group-adjusted intakes. With the lucerne diet alkane markers overestimated DMI by 0.62 kg/day (45%) and alkanes over estimated intake of pasture by 29%, pasture:sulla by 19% sulla by 6%. In contrast alkanes underestimated intakes by 6% for white clover:sulla, 12% for white clover and 13% for lucerne:sulla (Table 4.2.4).

We cannot explain the poor predictions of intake using alkane markers. The tablet preparation used in this study dissolved rapidly so there was little opportunity for lumps of C32 to pass into the faeces, with a high likelihood of low average concentrations (and overestimation of intakes). Regurgitation of tablets would also result in an overestimation of intake, but this is minimised by rapid disintegration (within 5 minutes) for this preparation. Of greatest concern is the variation between individuals in recovery, which may be real, and the effect of diet upon variance in both recovery and intake prediction. Data present here supports poor results from diets other than pasture. The alkane technique was developed using pasture, therefore when alkanes are used to predict intake of animals fed forages other than pasture, the technique becomes questionable.

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APPENDIX 4.3. Average dry matter content (DM), percentage of sulla fed, and composition (% of DM) of the seven diets offered to lambs over eight weeks.

Nutrient	DM%	Sulla fed (%)	Soluble Carbohydrate	Crude Protein	ADF[1]	NDF[2]	Lipid	Condensed Tannin	ME[3] (MJ/kg DM)	OMD[4]	Intake as % DM offered
Pasture											
Offered	24.0		12.1	15.5	29.5	48.0	2.8	0	10.1	70.0	77
Refusal			8.7	13.2	32.4	54.9	2.9		9.3	63.8	
Intake			13.2	16.3	28.6	45.8	2.8		10.3	71.9	
White Clover											
Offered	14.6		14.5	27.9	21.4	26.4	3.1	0	11.8	83.3	85
Refusal			6.3	27.7	26.9	34.0	3.7		10.6	75.8	
Intake			15.3	27.5	21.0	26.1	3.0		11.8	83.6	
Lucerne											
Offered	21.2		12.3	24.4	27.8	32.3	2.8	0	10.0	70.2	83
Refusal			4.7	20.9	35.9	45.0	2.7		8.8	61.1	
Intake			14.3	25.6	25.6	28.7	2.9		10.3	72.5	
Sulla											
Offered	13.8	100	21.8	19.2	22.2	21.5	2.2	5.6	12.2	84.2	84
Refusal			12.3	15.6	32.9	40.0	1.9	2.6	10.3	71.2	
Intake			23.9	20.1	19.6	17.0	2.3	6.4	12.7	87.5	
Pasture:Sulla											
Offered	19.2	48	16.7	17.3	26.0	35.3	2.5	2.7	11.1	76.7	86
Refusal			9.3	14.5	31.8	50.8	2.9	1.1	9.6	66.4	
Intake			17.9	17.9	25.0	32.7	2.4	2.9	11.4	78.5	
White Clover:Sulla											
Offered	14.2	53	18.5	23.0	21.8	23.9	2.6	3.0	12.0	83.7	92
Refusal			10.8	16.7	32.4	40.2	2.4	2.8	10.2	70.3	
Intake			19.2	23.6	20.8	22.4	2.6	3.0	12.2	84.9	
Lucerne:Sulla											
Offered	17.6	50	17.0	21.8	25.1	26.9	2.5	2.8	11.1	77.1	86
Refusal			9.5	14.1	34.2	43.3	2.3	1.8	9.5	64.7	
Intake			18.8	23.0	22.7	22.8	2.6	3.1	11.5	80.1	

[1] ADF, Acid detergent fibre; [2] NDF, Neutral detergent fibre; [3] ME, Metabolisable energy; [4] OMD, Predicted organic matter digestibility.

APPENDIX 4.4. Liveweight-adjusted fat depth at the 11th and 12th rib (GR; mm) and eye muscle area (mm^2)[1] estimated by ultrasound, and carcass GR fat depth and back fat depth of lambs fed seven forage diets over eight weeks. Least-square (LS) means ± SEM are presented.

Measurement	GR			Carcass GR	Back fat depth			Eye muscle area [1]		
Day	6	30	54		6	30	54	6	30	54
Pasture	2.9 ± 0.23	2.5 ± 0.20	2.2 ± 0.20	6.8 ± 0.82	2.0 ± 0.23	1.8 ± 0.20	2.1 ± 0.20	1060 ± 45.9	1014 ± 40.3	949 ± 39.3
White Clover	3.1 ± 0.22	2.3 ± 0.20	2.5 ± 0.27	8.3 ± 0.64	2.4 ± 0.23	2.4 ± 0.20	2.5 ± 0.28	1118 ± 45.8	1150 ± 40.1	1173 ± 56.2
Lucerne	2.7 ± 0.22	2.2 ± 0.20	2.4 ± 0.24	7.4 ± 0.84	2.3 ±0.23	2.1 ± 0.20	2.1 ± 0.21	1030 ± 45.3	1109 ± 39.7	1105 ± 40.8
Sulla	3.3 ± 0.24	2.6 ± 0.20	2.3 ± 0.24	7.6 ± 0.58	2.5 ± 0.24	2.5 ± 0.20	2.4 ± 0.24	1084 ± 49.0	1213 ± 39.7	1195 ± 48.4
Pasture:Sulla	3.2 ± 0.23	1.9 ± 0.20	2.4 ± 0.21	8.5 ± 0.59	2.0 ± 0.24	1.8 ± 0.20	2.1 ± 0.21	1079 ± 47.0	1115 ± 39.5	1001 ± 42.5
White Clover:Sulla	3.1 ± 0.22	1.9 ± 0.20	2.5 ± 0.26	7.4 ± 0.59	2.1 ± 0.23	2.0 ± 0.20	2.0 ± 0.27	1044 ± 44.9	1154 ± 39.7	1177 ± 54.5
Lucerne:Sulla	3.0 ± 0.22	1.9 ± 0.20	2.3 ± 0.25	7.4 ± 0.54	2.4 ± 0.23	2.6 ± 0.26	2.6 ± 0.26	1049 ± 45.0	1124 ± 39.4	1117 ± 51.5
Diet effect (Pr)		NS		NS		< 0.05			< 0.1	
Diet x day		NS		-		NS			< 0.01	
Liveweight		< 0.01		-		< 0.01			< 0.01	
Carcass weight		-		< 0.01		-			-	

[1] Eye muscle area calculated as 0.77 x width (A; mm) x depth (B; mm).

APPENDIX 4.5. Results from *in sacco* and *in vitro* incubations using pasture, white clover, lucerne, sulla and mixtures of pasture:sulla, lucerne:sulla and white clover:sulla fed to lambs in Chapter 4.

In sacco incubations

FIGURE 4.5.1. Dry matter (DM) loss of pasture, sulla and pasture:sulla.

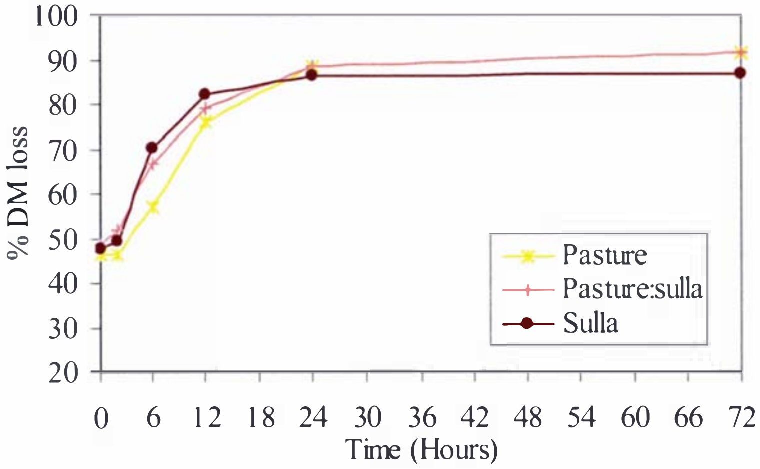


FIGURE 4.5.2. Dry matter (DM) loss of white clover, sulla and white clover:sulla.

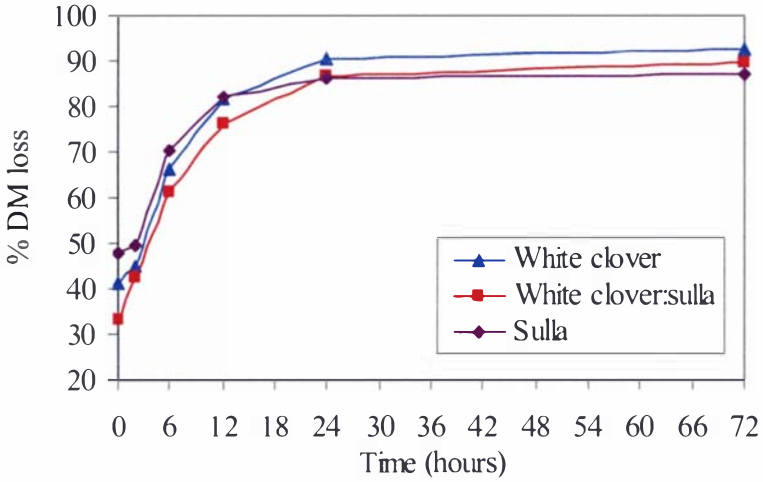


FIGURE 4.5.3. Dry matter (DM) loss of lucerne, sulla and lucerne:sulla.

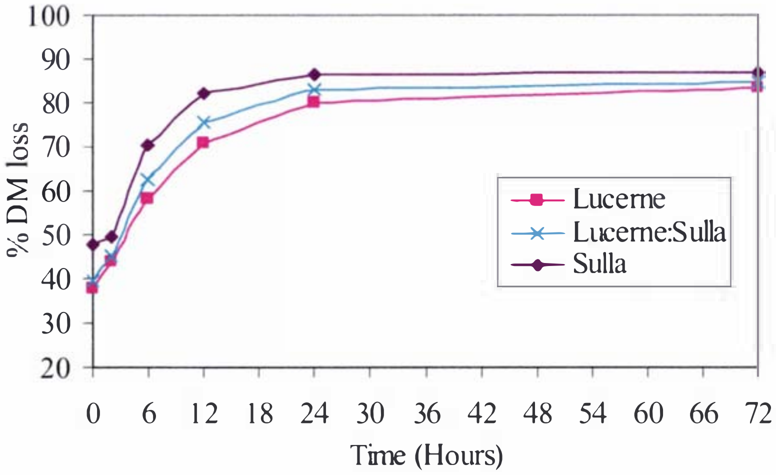


TABLE 4.5.1. Dry matter (DM) loss degradation parameters for feeds fed to lambs in Chapter 4.

Feed	A (% DM)	B (% DM)	k (%/h)	Lag (h)	ED (% DM)
Pasture	46.5	45.0	13.7	4.04	75.0
White clover	41.3	51.1	14.8	1.50	76.6
Lucerne	38.0	45.6	11.2	0.72	67.2
Sulla	47.7	39.2	20.4	1.78	77.2
Pasture:sulla	48.6	43.0	11.6	1.27	76.1
White clover:sulla	33.5	55.9	12.3	0.66	71.1
Lucerne:sulla	39.4	45.2	14.6	1.08	70.8

TABLE 4.5.2. Crude protein (CP) loss degradation parameters for feeds fed to lambs in Chapter 4.

Feed	A (% CP)	B (% CP)	k (%/h)	Lag (h)	ED (% CP)
Pasture	57.9	22.6	11.9	1.9	81.2
White clover	56.5	37.7	13.6	0.6	82.4
Lucerne	58.4	31.2	8.5	0	76.7
Sulla	68.9	23.3	10.7	0.3	83.8
Pasture:sulla	64.2	29.8	9.6	0	82.5
White clover:sulla	53.4	39.1	12.8	0	80.8
Lucerne:sulla	66.8	22.6	11.9	1.9	81.2

TABLE 4.5.3. Neutral detergent fibre (NDF) degradation parameters for feeds fed to lambs in Chapter 4.

Feed	A (% NDF)	B (% NDF)	k (%/h)	Lag (h)	ED (% NDF)
Pasture	6.5	73.3	11.0	4.3	48.8
White clover	0	87	13.6	1.7	58.3
Lucerne	0	68.9	10.6	1.0	43.0
Sulla	8.6	66.9	20.0	1.5	58.9
Pasture:sulla	10.5	75.3	10.6	1.3	57.2
White clover:sulla	0	80.2	12.0	1.6	51.7
Lucerne:sulla	0	70.7	14.5	1.3	48.8

TABLE 4.5.4. Evaluation of diets using data from Appendix 4.1 and the CNCPS model.

					Mixed *in sacco*			Mixed using model		
	Pasture (P)	White clover (WC)	Lucerne (L)	Sulla (S)	P:S	WC:S	L:S	P:S	WC:S	L:S
Effective fibre (% NDF)	60	40	60	40	60:40	40:40	60:40	60:40	40:40	60:40
Intake of first forage (kg DM/cow/day)	17.1	17.1	17.1	17.1	17.1	17.1	17.1	8.6	8.6	8.6
Intake of second forage (kg DM/cow/day)								8.6	8.6	8.6
Diet nutrient composition										
ME (MJ/kg DM)	10.2	10.3	10.6	11.5	9.3	10.1	10.2	11.2	11	11.5
CP (g/100g DM)	15.5	27.9	25.6	19.2	17.3	23	21.8	17.4	23.6	22.4
Soluble CP (% CP)	58	57	58	69	60	53	67	64	62	63
NDF (g/100 g DM)	48	26.4	32.3	21.5	35.3	23.9	26.9	34.8	24	26.9
peNDF (g/100 g DM)	29	11	19	9	18	10	13	19	10	14
Total NFC (g/100 g DM)	23	32	30	46	34	39	38	34	39	38
Requirements										
ME requirements (MJ/day)	178	188	185	183	180	185	184	179	185	183
MP requirements (g/day)	1793	1779	1809	1685	1870	1801	1816	1708	1729	1711
Production predictions										
Milk production based on ME supply (kg/day)	21.9	20.4	22	25.3	18.5	20.2	20.9	25.2	23.0	25.2
Milk production based on MP supply (kg/day)	18.7	24.0	25.1	18.3	17.2	20.1	17.3	22.4	21.1	24.1
Daily weight change due to reserves (kg/day)	-0.1	-0.4	-0.1	0.5	-0.8	-0.4	-0.3	0.4	0.1	0.4
ME supplied (MJ/day)	174	177	182	196	158	172	175	182	187	196
MP supplied (g/day)	1589	1844	1932	1463	1591	1669	1544	1695	1645	1781
MP from bacteria (g/day)	1016	639	819	820	923	694	790	1109	738	910
MP from undeg. Feed (g/day)	573	1204	1113	643	668	975	754	587	907	871
MP from undeg. Feed (%MP total)	36	65	58	44	42	58	49	35	55	49
Urea cost (MJ/day)	1.5	11.7	8.3	5.7	3.2	8.2	7.1	2.2	8.6	6.4
Predicted ruminal pH	6.46	5.87	6.24	5.79	6.17	5.83	5.99	6.22	5.83	6.02

Abbreviations: CP, crude protein; DIP, degradable intake protein; DM, dry matter; ME, metabolisable energy; MJ, megajoules; MP, metabolisable protein; N, nitrogen; NDF, neutral detergent fibre; NFC, non-fibrous carbohydrates; peNDF, effective fibre.

In vitro incubations

FIGURE 4.5.4. Ammonia (NH_3) production (% NH_3 per forage nitrogen, N) from pasture, sulla and pasture:sulla over 24 hours of incubation.

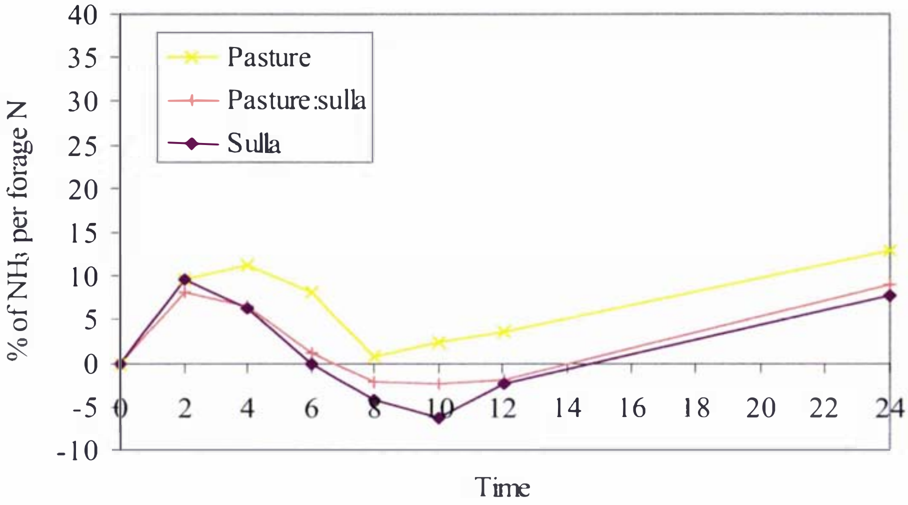


FIGURE 4.5.5. Ammonia (NH_3) production (% NH_3 per forage nitrogen, N) from white clover, sulla and white clover:sulla over 24 hours of incubation.

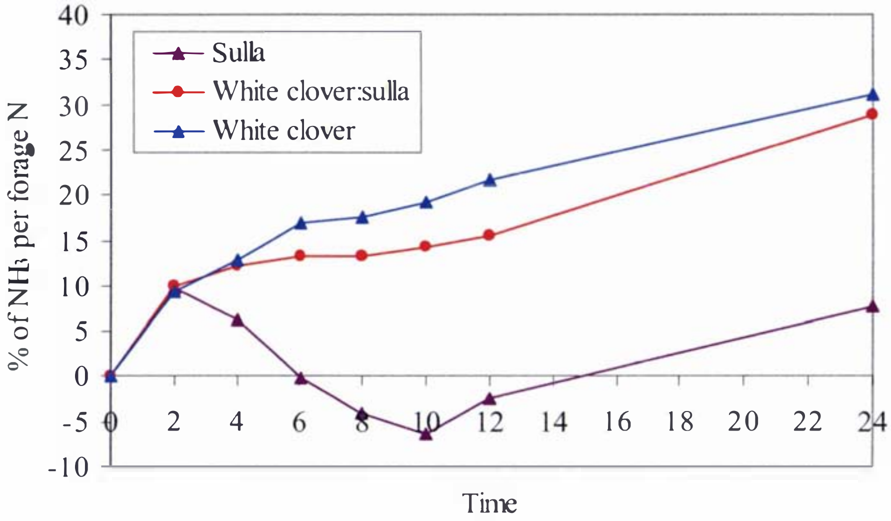
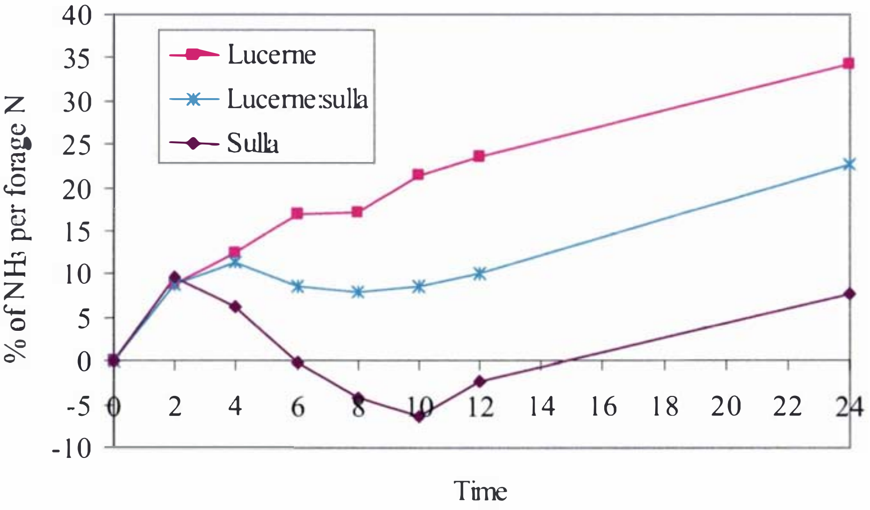


FIGURE 4.5.6. Ammonia (NH_3) production (% NH_3 per forage nitrogen, N) from lucerne, sulla and lucerne:sulla over 24 hours of incubation.



APPENDIX 4.6. Metabolisable energy and protein requirements and supply for lambs fed diets.

Metatbolisable energy requirements and supply

	DM intake	ME diet	Av LW	LW^0.75	LWG	NEbasal	Kmaint	MEmaint	NEgain/kg	NEgain	Kgain	MEgain/kg	MEgain	ME support	MEreqt	ME Intake	Reqt/Intake	MElprod
Pasture	1.10	10.3	31.45	13.28	0.116	3.61	0.71	5.11	19.38	2.25	0.42	45.8	5.31	0.53	10.95	11.33	0.97	6.22
White Clover	1.48	11.8	35.80	14.64	0.281	3.98	0.74	5.40	21.82	6.13	0.53	41.0	11.52	1.15	18.08	17.46	1.04	12.06
Lucerne	1.37	10.3	34.10	14.11	0.207	3.83	0.71	5.43	20.90	4.33	0.42	49.3	10.21	1.02	16.66	14.11	1.18	8.68
Sulla	1.47	12.7	35.25	14.47	0.308	3.93	0.75	5.21	21.75	6.70	0.60	36.5	11.23	1.12	17.57	18.67	0.94	13.46
Pasture:sulla	1.21	11.4	32.95	13.75	0.190	3.74	0.73	5.13	20.38	3.88	0.50	40.5	7.72	0.77	13.62	13.79	0.99	8.66
White Clover:sulla	1.49	12.2	36.15	14.74	0.281	4.01	0.74	5.38	21.94	6.16	0.56	39.1	11.00	1.10	17.48	18.18	0.96	12.79
Lucerne:sulla	1.54	11.5	36.10	14.73	0.281	4.00	0.73	5.48	21.92	6.15	0.51	43.0	12.07	1.21	18.76	17.71	1.06	12.23

Metabolisable protein requirements

	DM intake	CP diet	Av LW	LW^0.75	LWG	MPmain	MFP	LWG	NPgain	NPwool	NPret	MPgain	MPwool	MPretain	MPreqt
Pasture	1.10	16.3	31.45	13.28	0.116	29.05	16.50	0.116	15.36	4.54	19.90	26.03	17.45	43.48	89.0
White Clover	1.48	27.5	35.80	14.64	0.281	32.02	22.20	0.281	36.55	6.66	43.21	61.95	25.60	87.55	141.8
Lucerne	1.37	25.6	34.10	14.11	0.207	30.87	20.55	0.207	27.12	5.71	32.83	45.96	21.97	67.93	119.4
Sulla	1.47	20.1	35.25	14.47	0.308	31.65	22.05	0.308	40.18	7.02	47.19	68.10	26.99	95.09	148.8
Pasture:sulla	1.21	17.9	32.95	13.75	0.190	30.08	18.15	0.190	25.05	5.51	30.56	42.47	21.17	63.64	111.9
White Clover:sulla	1.49	23.6	36.15	14.74	0.281	32.25	22.35	0.281	36.54	6.65	43.19	61.92	25.59	87.51	142.1
Lucerne:sulla	1.54	23.0	36.10	14.73	0.281	32.22	23.10	0.281	36.51	6.65	43.16	61.88	25.58	87.47	142.8

Metabolisable protein supply

																UDPdig	0.75	
	a	b	c	FME/ME	LoF	r	CP Intake	RDP Reqt	MicrobMP	QDP	SDP	dg	effdg	EffRDP	Undg	UDP	UDP MP	MP Supply
Pasture	0.58	0.23	0.119	0.95	2.14	0.056	179	109	70	0.58	0.16	0.74	0.62	111	0.26	47	35	105
White Clover	0.57	0.38	0.136	0.95	3.35	0.084	407	185	118	0.57	0.23	0.80	0.69	281	0.20	80	60	178
Lucerne	0.58	0.31	0.084	0.95	3.07	0.079	351	147	94	0.58	0.16	0.74	0.62	219	0.26	91	68	162
Sulla	0.69	0.23	0.107	0.95	3.37	0.085	295	198	126	0.69	0.13	0.82	0.68	201	0.18	54	40	166
Pasture:sulla	0.64	0.3	0.096	0.95	2.65	0.069	217	139	89	0.64	0.17	0.81	0.69	149	0.19	40	30	119
White Clover:sulla	0.53	0.39	0.128	0.95	3.25	0.082	352	191	122	0.53	0.24	0.77	0.66	233	0.23	82	61	183
Lucerne:sulla	0.67	0.23	0.119	0.95	3.42	0.086	354	188	120	0.67	0.13	0.80	0.67	237	0.20	70	52	172

Equations and abbreviations for modelling

DM intake (kg DM/day) = DM intake for lambs in Chapter 4 (Table 4.4).
ME diet (MJME/kg DM) = metabolisable energy content of the diet (Table 4.3)
Av LW (kg) = (Start LW + End LW) ÷ 2
LWG (kg/day) = LW gain during the trial

Metabolisable energy requirements and supply

Metabolisable energy requirements were calculated from SCA (1990).

NE Basal (MJ/day) = (1 x 0.28 x $e^{(-0.03*1)}$) x $LW^{0.75}$
Kmaint = (ME diet x 0.02) + 0.5
MEmaint (MJ/day) = NE basal ÷ Km
NEgain/kg (MJ/kg) = ((6.7 + (((0.92 x (LWG x 1000)) ÷ ((4 x $66)^{0.75}$))-1))+(20.3-(((0.92 x (LWG x 1000)) ÷ ((4 x $66)^{0.75}$))-1)) ÷ (1 + $e^{(-6 \times ((Av\ LW/66)-0.4)))}$)
NEgain (MJ/day) = LWG x NEg/kg
Kg = 0.072 x ME diet - 0.318
MEgain/kg (MJ/kg) = NEg/kg ÷ Kg
MEgain (MJ/day) = MEgain/kg x LWG
MEsupport (MJ/day) = MEgain x 0.1
MEreqt (MJ/day) = MEmaint + MEgain + MEsupport
ME intake (MJ/day) = DM intake x ME diet
Reqt/Intake = MEreqt ÷ ME intake
MEIprod = ME intake - MEmaint

Metabolisable protein requirements

CPdiet = CP content (% CP) of diets fed to lambs in Chapter 4 (Table 4.3)
MPmaint (g/day) = 2.1875 x $LW^{0.75}$
MFP = DM intake x 15
LWG (kg/day) = LW gain
NPgain (g/day) = (LWG x (160.4 - (1.22 x Av LW) + (0.0105*(Av LW^2))))

NPwool (g/day) = (NPgain x 0.1) + 3

NPret (g/day) = NPgain + NPwool

MPgain (g/day) = NPgain ÷ 0.59

MPwool (g/day) = NPwool ÷ 0.26

MPretain (g/day) = MPgain + MPwool

MPreqt (g/day) = MPmain + MFP + MPgain + MPwool

Metabolisable protein supply

a = quickly degradable fraction (Table 4.5.2 in Appendix 4.5)

b = slowly degradable fraction (Table 4.5.2 in Appendix 4.5)

c = degradation rate per hour (Table 4.5.2 in Appendix 4.5)

FME/ME (Fermentable ME of a diet)

LoF = MPreqt ÷ MPmaint

$r = (-0.024 + (0.179 \times (1 - e^{(-0.278*\mathrm{LoF})})))$

CP intake (g/day) = DM intake x 1000 x CP content (% DM) ÷ 100

$\mathrm{RDPreqt}\ (\mathrm{g/day}) = (7 + (6 \times (1 - e^{(-0.35*\mathrm{LoF})}))) \times \mathrm{ME\ intake} \times \mathrm{FME/ME}$

MicrobMP (g/day) = RDPreqt x 0.75 x 0.85

QDP = a

SDP = (a x b) ÷ (c + r)

dg = QDP + SDP

Effdg (g/day) = (QDP x 0.8) + SDP

EffRDP (g/day) = CP intake ÷ Effdg

Undg = 1 - dg

UDP (g/day) = CP intake x Undg

UDPdig = 0.75

UDP MP (g/day) = UDP x UDPdig

MP Supply (g/day) = microbMP + UDP MP

Reqt/Supply = MPreqt ÷ MPsupply

MPprod (g/day) = MPsupply – MPFP - MPmaint

DM intake (kg DM/day) = DM intake for lambs in Chapter 4 (Table 4.4).
ME diet (MJME/kg DM) = metabolisable energy content of the diet eaten (Table 4.3)
Av LW (kg) = (Start LW + End LW) ÷ 2
LWG (kg/day) = LW gain during the trial

NE = net energy
Kmaint = efficiency utilisation of ME for maintenance
MEmaint = metabolisable energy for maintenance
NEgain/kg = net energy for gain per kg of gain
NEgain = total net energy gain
Kg = efficiency of utilisation of ME for gain
MEgain/kg (MJ/kg) = metabolisable energy for gain per kg of gain
MEgain = metabolisable energy for gain
MEreqt = metabolisable energy requirements
ME intake = metabolisable energy intake
MElprod = ME intake - MEmaint

CPdiet = CP content (% CP) of diets fed to lambs in Chapter 4 (Table 4.3)
MPmaint = metabolisable protein for maintenance
MFP = metabolic faecal protein, cost of processing the food eaten.
NPgain = net protein for gain
NPwool = net protein for wool
NPret = net protein retained in gain and wool
MPgain = metabolisable protein for gain
MPwool = metabolisable protein for wool
MPretain = metabolisable protein retained
MPreqt = metabolisable protein required
FME/ME = Fermentable ME of a diet
LoF = level of feeding
r = rumen digesta outflow rate
CP intake = crude protein intake
RDPreqt = rumen degradable protein requirement
MicrobMP = microbial metabolisable protein
QDP = quickly degradable protein
SDP = slowly degradable protein
dg = Extent of degradation of protein
Effdg = effective degradation of protein
EffRDP = effective rumen degradable protein
Undg = Undegradable protein proportion
UDP = undegradable protein
UDPdig = digestibility of the UDP
UDP MP = UDP metabolisable protein
MP Supply = microbMP + UDP MP
Reqt/Supply = MPreqt ÷ MPsupply
MPprod = MPsupply – MPFP - MPmaint

APPENDIX 5.1. Method for processing plasma to measure cysteine concentration and ^{35}S-cysteine radioactivity

Plasma collected on the day of the ^{35}S-cysteine infusion (1 mL) was added to a pre-weighed centrifuge tube and weighed. In order to denature plasma proteins 0.5 mL of a solution containing 0.75% w/v sodium dodecyl sulphate (SDS) and 9 mmol/L EDTA, 100 µL DTT (80 mmol/L) was combined with plasma and 50 µL of norleucine (3 mmol in 0.1% phenol) was added as an internal standard. The samples were re-weighed after each addition, and then left at room temperature for 15 mins before adding 0.5 mL of trichloroacetic acid (TCA; 30% w/v) to precipitate plasma protein. The tubes were reweighed and centrifuged (3,300 x *g*, 15 mins at 4°C) with the resulting supernatant filtered (0.45 µm) and stored at -85°C for analysis of cysteine concentration and radioactivity.

APPENDIX 5.2. Method to determine sulphate concentration (Sinclair and Tavendale, unpublished).

Standards of K_2SO_4 (1 mmol/L) in buffer were used about every 10 samples. Standard HPLC vials were used for the standard.

Samples were prepared using a "Vivaspin" unit (Vivacscience product number VS0102; 10,000 MW cut off PES membrane). 0.5 mL plasma was placed into the inner membrane part of the vivaspin and centrifuged at 12,000 *g* for 45 minutes. 50µL of the filtrate in the outer tube of the vivaspin was placed into an autosampler vial (1.1 mL v-shaped code 1.1STVG) and cap (with septa code 8-ST101, Teflon side against the vial) and 1.1 mL vial frozen at –20°C.

Deproteinized plasma (treated as in Appendix 5.1) underwent further processing with 25 µL of 80 mmol/L DDT and 15 µL of 3 mmol/L norleucine in 0.1% phenol, centrifuged at 28,000 x *g* for 60 minutes in a Centrisart® filter (10,000 molecular weight cut off) and stored at –85°C.

Sulphate concentration was determined by HPLC with a guard column 4.1 mm x 250 mm (Wescan #269.001) at 35°C and a conductivity detector. Sample injections of 5 µL were in a mobile phase p-Hydoxybenzoic acid (5 mmol/L; MW 138.1; pH 8.4) buffer set at a flow rate of 1.6 mL/min.

APPENDIX 5.3. Method for extraction of tissues.

Sub-samples (4 – 5 g) of frozen tissue were pulverised in liquid nitrogen using a modified French Cell press as described by Lee *et al*. (1993). The pulverised tissue was then stored at -20ºC until further processing. Approximately 1 g of smashed tissue was homogenised in extraction buffer (20 mmol/L Tris pH 7.8; 2.5 mmol/L EDTA; 0.3% SDS). The homogenate was centrifuged at approximately 28,000 *g* at 4°C for 30 mins and the supernatant containing intracellular peptides, AA and soluble protein was removed and the resulting pellet (protein-bound fraction) used to determine SRA of ^{3}H valine. The pellet (bound fraction) was freeze-dried (FD) and both the FD pellet and supernatant were stored at –20ºC until required for further treatment and analysis.

The supernatant (2 mL) was mixed with 1 mL of 0.75% (9 mmol/L) SDS/EDTA to denature proteins, 200 µL of 80 mmol/L DTT (pH 8.0) as an antioxidant and 100 µL of 3 mmol/L norleucine as an internal standard. The samples were mixed and left to stand at room temperature for 15 mins and then deproteinised with 1 mL of 30% TCA, centrifuged at 4°C at 3,300 *g* for 15 min. The resulting supernatant containing free pool AA was filtered (45 µm) and stored at -20°C until analysed for AA concentration and total radioactivity of ^{3}H by HPLC and liquid scintillation counting as described previously (Table 5.2).

APPENDIX 5.4. Method for processing plasma and tissue free pool to measure valine concentration.

The concentration of valine in plasma was determined by treating 0.5 mL of plasma with 25µL of 80 mmol/L dithiothreitol (DTT) as an antioxidant, 15µL of 3 mmol/L norleucine (in 0.1% phenol; as an internal standard) and centrifuging (28,000 *g* for 60 mins) in a Centrisart® filter (10,000 molecular weight cut off). The remaining filtrate containing free AA was stored at -20°C for measuring valine concentration (Table 5.2).

A 50 μL aliquot of the filtrate (plasma) and the supernatant containing unbound AA were dried under vacuum before the addition of 20 μL redry solution (2:2:1, methanol: 1M Na acetate: triethylamine (TEA; under $N_{2(g)}$) mixed by vortex and again dried down. The derivatising reagent (20 μL containing 7:1:1:1 ratio of methanol, MilliQ water, TEA (under $N_{2(g)}$), phenylisothiocyanate (PITC; under $N_{2(g)}$)) was added to each dried sample which was then vortexed and incubated at room temperature for 10 min after which dried down under vacuum. Dried derivatised samples were resuspended in 200 μL diluent containing 5 % CH_3CN and 95 % phosphate buffer (5 mmol/L Na_2HPO_4 adjusted to pH 7.40 using 10 % (v/v) H_3PO_4). Samples were vortexed and transferred to 1.5 mL microcentrifuge tubes and centrifuged at approximately 14,000 *g* for 5 min. The supernatant was transferred to autosampler vials. A stock standard solution containing 0.5 mmol/L AA was prepared using 200 μL Pierce A/N (Pierce C Chemicals, Lab supply Pierce (NZ) Ltd, Auckland, New Zealand) and 200 μL Pierce B (Pierce Chemicals, Lab supply Pierce (NZ) Ltd, Auckland, New Zealand) standard solutions together with 0.5 mmol/L norleucine in a final volume of 1 mL of 0.1 mol/L HCl. Stock standards were diluted 10-fold using 0.1 mol/L HCl before use. Derivitised samples (50 μL) were injected onto a Picotag C_{18} reverse phase column in an oven set to 46°C, with a 90 min run time between each injection (Appendix 5).

APPENDIX 5.5. Chromatography method to determine concentration of amino acids in plasma and tissue free pool.

Derivitised plasma and tissue free pool samples (50 μL) were injected onto a Picotag C18 reverse phase column in an oven set to 46°C, with a 90 minute run time between each injection. The elution system consisted of 2 mobile phases, buffer A (70 mmol/L sodium acetate.$3H_2O$ adjusted to pH 6.5 using glacial acetic acid, containing 1.8% acetonitrile and 2.5 μL of EDTA) and buffer B (15% aqueous methanol, containing 45% acetonitrile). Buffer B was run in a gradient starting with an increase from 0% at 13.5 minutes to 0.5% at 13.51 minutes, followed by an increase with a number 3 curve to 2% at 24 minutes, then by a linear increase to 6% at 30 minutes, then a -2curve to 23.5% at 50 minutes, and finally a linear increase to 36% at 62 minutes. Buffer B remained at 36% until 70 minutes when the gradient ended in a wash step of 100% B to remove residual sample from the column. The flow rate was set to 1.0 mL/minute. Separated AA was detected by a UV detector set at 254 nm.

APPENDIX 5.6. Results for individual lambs when infused with ^{35}S-sulphate and ^{35}S-cysteine.

	Pasture				Lucerne				Sulla				Lucerne:sulla			
	3	14	29	42	23	36	43	46	4	11	32	44	1	25	33	35
Sulphate infusion																
Infusion rate (dpm/h)	1.67E+08	1.91E+08	1.85E+08	1.43E+08	1.83E+08	1.79E+08	1.85E+08	1.85E+08	1.69E+08	1.74E+08	1.85E+08	1.78E+08	1.80E+08	1.81E+08	1.83E+08	1.80E+08
Radioactivity (dpm/g)	75927.1	94957.1	88239.3	115345.5	65464.3	61799.1	65087.7	75190.4	49016.6	50897.0	48191.9	34821.9	64075.5	60154.1	50870.6	55673.1
Sulphate conc (µmol/g)	0.99	1.20	1.12	1.50	1.09	1.28	1.04	1.52	1.67	2.11	1.69	1.15	1.65	1.41	1.24	1.53
SRA (dpm/µmol)	76694.1	79462.0	78785.1	77154.2	60243.2	48280.5	62886.7	49630.6	29351.3	24160.0	28459.8	30368.0	38833.7	42561.9	41135.3	36308.5
ILR (umol/min)	36.2	40.1	39.2	30.8	50.6	61.9	49.0	62.2	95.7	119.7	108.5	97.7	77.2	71.0	74.1	82.5
Cysteine infusion																
Infusion rate (dpm/h)	9.08E+07	1.43E+08	1.38E+08	9.89E+07	1.83E+08	1.35E+08	1.04E+08	5.26E+07	1.17E+08	8.06E+07	1.27E+08	5.03E+07	1.26E+08	1.90E+08	1.21E+08	8.54E+07
Radioactivity (dpm/g)	3643.1	9658.9	5640.8	7611.9	7332.0	5608.8	6981.6	2173.5	3320.0	4223.5	7124.8	2805.6	9948.3	5881.8	3954.3	3101.9
Cysteine conc (µmol/g)	0.0656	0.0614	0.0665	0.0789	0.1795	0.0740	0.1680	0.0801	0.0625	0.1207	0.1539	0.0873	0.1341	0.0891	0.0637	0.1239
SRA (dpm/µmol)	55562.6	157240.8	84857.2	96438.5	40846.6	75834.2	41562.3	27150.8	53117.0	35001.1	46297.2	32123.3	74159.7	65984.2	62115.8	25033.8
ILR (µmol/min)	27.24	15.13	27.12	17.10	74.68	29.78	41.89	32.28	36.56	38.38	45.61	26.12	28.21	48.07	32.41	56.87
Sulphate-cysteine																
Radioactivity (dpm/g)	3905.5	9632.0	9949.0	8127.7	6993.1	4357.9	4283.8	2197.7	3683.1	2630.8	5455.1	1861.8	4847.3	7622.0	3757.0	2334.6
Sulphate conc (µmol/g)	1.02	1.28	1.22	1.62	1.18	1.37	1.13	1.64	1.79	1.80	1.83	1.25	1.78	1.53	1.31	1.70
SRA (dpm/µmol)	3812.4	7549.2	8136.6	5016.1	5921.0	3186.0	3785.3	1339.7	2062.6	1460.9	2988.9	1495.3	2727.8	4991.6	2859.2	1376.3
TQ	0.0686	0.0480	0.0959	0.0520	0.1450	0.0420	0.0911	0.0493	0.0388	0.0417	0.0646	0.0465	0.0368	0.0756	0.0460	0.0550
a (µmol/min)	24.8	13.2	23.4	15.5	67.3	27.2	37.4	29.2	32.8	33.4	38.6	21.6	25.4	42.7	29.0	52.3
b (µmol/min)	36.2	40.1	39.2	30.8	50.6	61.9	49.0	62.2	95.7	119.7	108.5	97.7	77.2	71.0	74.1	82.5
c (µmol/min	2.5	1.9	3.8	1.6	7.3	2.6	4.5	3.1	3.7	5.0	7.0	4.5	2.8	5.4	3.4	4.5
d (µmol/min)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
e (µmol/min)	27.2	15.1	27.1	17.1	74.7	29.8	41.9	32.3	36.6	38.4	45.6	26.1	28.2	48.1	32.4	56.9
f (µmol/min)	33.7	38.2	35.4	29.2	43.3	59.3	44.5	59.2	92.0	114.7	101.5	93.2	74.4	65.6	70.6	78.0
Protein Synthesis (g/day)	119.3	63.7	112.6	74.7	324.6	131.0	180.4	140.8	158.3	160.9	186.1	104.0	122.3	205.8	139.8	252.2

APPENDIX 5.7. Results for individual lambs when infused with ^{3}H-valine.

	Pasture				Lucerne				Sulla				Lucerne:sulla			
	3	14	29	42	23	36	43	46	4	11	32	44	1	25	33	35
Valine$_P$ (µmol/L)	161.3	261.9	123.6	188.0	249.7	219.9	264.8	221.1	373.7	319.2	301.4	372.5	300.8	286.5	277.1	277.7
SRA$_P$	73.1	91.0	88.5	87.5	47.1	60.8	49.6	59.2	56.4	53.1	61.3	60.8	69.4	41.2	49.6	41.6
Duodenum																
Valine$_I$ (µmol/L)	80.0	113.9	59.2	40.4	129.7	95.4	116.8	95.1	90.4	97.5	57.3	99.4	102.4	71.3	88.7	93.9
Valine$_B$ (mg/g DM)	22.55	21.39	19.49	25.66	25.71	20.66	22.29	26.33	22.00	19.17	16.51	20.79	18.06	20.78	18.10	30.43
SRA$_I$ (dpm/nmol)	22.1	25.1	19.7	35.5	6.3	11.3	7.9	13.9	25.8	14.3	25.7	4.4	8.7	15.7	16.4	20.4
SRA$_B$ (dpm/nmol)	5.8	6.8	5.6	3.4	4.4	5.8	5.4	4.4	4.8	4.7	4.1	4.3	2.7	6.0	6.3	4.1
FSR$_I$ (dpm/nmol)	79.4	80.9	85.5	28.9	208.7	153.1	204.9	95.0	55.4	98.9	47.7	293.1	94.0	114.8	115.1	60.3
FSR$_P$ (dpm/nmol)	24.0	22.3	19.0	11.7	27.9	28.4	32.4	22.3	25.3	26.6	20.0	21.5	11.8	43.7	38.0	29.5
Duodenum (scraped)																
Valine$_I$ (µmol/L)	54.9	98.9	40.0	54.2	93.3	47.5	56.2	47.6	67.9	75.0	47.9	47.4	38.8	33.4	34.1	43.8
Valine$_B$ (mg/g DM)	14.45	17.12	18.86	16.59	17.75	15.99	14.58	15.93	17.09	13.09	17.56	19.47	16.79	15.18	14.34	17.82
SRA$_I$ (dpm/nmol)	20.9	20.9	21.8	32.3	16.3	13.4	10.8	20.9	14.5	19.9	28.0	37.2	2.3	5.7	22.9	21.3
SRA$_B$ (dpm/nmol)	0.4	4.1	0.3	4.2	2.8	2.1	3.0	3.7	2.8	2.2	1.4	2.4	0.4	1.2	0.7	0.5
FSR$_I$ (dpm/nmol)	6.3	58.6	4.1	38.9	51.8	47.6	83.6	52.4	57.1	32.9	14.8	19.6	56.1	65.5	8.8	7.2
FSR$_P$ (dpm/nmol)	1.8	13.5	1.0	14.4	18.0	10.5	18.3	18.5	14.7	12.3	6.8	12.0	1.8	9.0	4.1	3.7
Ileum																
Valine$_I$ (µmol/L)	85.2	107.2	56.6	80.4	70.9	55.7	87.8	55.7	108.0	103.1	83.7	69.2	85.4	75.7	96.9	64.6
Valine$_B$ (mg/g DM)	21.44	16.64	19.90	19.76	28.25	20.00	17.25	18.33	19.87	18.86	18.73	18.17	19.82	19.65	19.48	23.21
SRA$_I$ (dpm/nmol)	21.1	22.6	32.1	20.7	15.7	13.9	12.9	23.0	7.1	7.0	19.7	15.4	11.0	27.7	14.5	15.2
SRA$_B$ (dpm/nmol)	7.0	2.2	7.9	7.2	4.0	3.5	5.5	6.6	5.2	5.4	2.6	3.5	1.5	2.3	3.0	5.1
FSR$_I$ (dpm/nmol)	99.4	29.1	73.8	103.9	77.3	75.2	127.2	85.7	216.9	230.0	38.8	67.9	41.6	24.4	62.3	101.1
FSR$_P$ (dpm/nmol)	28.7	7.2	26.8	24.5	25.7	17.2	33.1	33.2	27.4	30.3	12.5	17.2	6.6	16.4	18.3	37.0
Ileum (scraped)																
Valine$_I$ (µmol/L)	37.0	35.1	39.5	36.3	79.0	32.7	35.7	39.7	46.6	86.2	58.0	49.5	41.1	52.4	66.6	35.8
Valine$_B$ (mg/g DM)	18.49	20.38	18.07	21.50	21.49	17.36	18.53	10.50	18.19	20.94	18.25	19.53	14.48	19.20	19.81	21.44
SRA$_I$ (dpm/nmol)	70.0	100.6	55.6	69.9	70.2	56.5	45.7	67.1	41.2	38.3	65.4	32.7	29.1	57.4	55.9	44.4
SRA$_B$ (dpm/nmol)	1.6	1.5	1.1	1.2	1.0	1.2	1.6	2.0	0.8	0.9	2.0	1.6	0.6	1.3	1.9	1.4
FSR$_I$ (dpm/nmol)	6.9	4.6	6.0	5.2	4.4	6.3	10.8	9.1	6.1	7.2	9.0	14.5	6.3	6.6	10.4	9.4
FSR$_P$ (dpm/nmol)	6.6	5.1	3.8	4.2	6.5	5.9	10.0	10.4	4.5	5.2	9.6	7.8	2.6	9.1	11.8	10.0
Rumen																
Valine$_I$ (µmol/L)	25.5	60.7	21.0	43.6	42.4	52.3	48.3	35.3	55.7	33.2	56.1	50.7	31.9	36.1	52.8	39.3
Valine$_B$ (mg/g DM)	20.40	24.56	22.41	23.49	25.10	19.72	21.52	22.00	20.22	19.47	19.56	19.34	20.61	20.96	20.34	22.82
SRA$_I$ (dpm/nmol)	17.0	7.9	23.1	14.8	29.8	15.1	32.0	8.2	15.8	15.4	16.3	10.7	9.9	30.7	14.8	22.3
SRA$_B$ (dpm/nmol)	4.6	4.1	5.8	3.3	2.0	3.3	2.3	3.5	2.6	2.1	2.1	1.9	2.6	2.2	1.6	1.7
FSR$_I$ (dpm/nmol)	81.3	158.4	75.4	66.4	20.3	65.6	21.3	127.0	50.1	40.2	38.5	52.9	79.3	21.3	33.4	23.2
FSR$_P$ (dpm/nmol)	18.9	13.7	19.7	11.2	12.9	16.3	13.8	17.6	14.1	11.7	10.2	9.3	11.3	15.9	10.0	12.5

APPENDIX 5.7 continued. Results for individual lambs when infused with ^{3}H-valine.

	Pasture				Lucerne				Sulla				Lucerne:sulla			
	3	14	29	42	23	36	43	46	4	11	32	44	1	25	33	35
Abomasum																
Valine$_I$ (µmol/L)	83.4	82.3	58.9	49.4	81.7	55.3	74.0	57.7	87.8	51.3	40.0	72.2	67.6	50.0	54.2	55.8
Valine$_B$ (mg/g DM)	3.29	3.05	3.37	3.39	3.52	3.04	2.92	2.95	3.43	2.94	3.06	2.86	3.07	3.10	2.85	3.84
SRA$_I$ (dpm/nmol)	13.2	28.4	23.9	28.2	14.6	15.9	30.7	20.2	26.6	7.6	15.9	26.3	6.9	19.0	18.1	11.1
SRA$_B$ (dpm/nmol)	21.5	41.7	43.0	40.2	20.9	6.6	15.7	25.4	12.7	23.0	18.5	16.5	13.2	18.7	6.9	23.0
FSR$_I$ (dpm/nmol)	488.8	440.8	539.7	427.0	427.7	124.0	153.6	378.6	143.2	911.7	348.3	188.5	569.2	293.9	114.2	622.9
FSR$_P$ (dpm/nmol)	88.4	137.6	145.7	137.9	133.0	32.5	95.0	128.9	67.6	130.2	90.5	81.5	57.0	135.9	41.7	165.7
Liver																
Valine$_I$ (µmol/L)	48.9	62.5	41.4	33.5	83.8	66.8	73.6	42.9	60.6	81.1	65.9	80.3	81.6	68.3	66.7	54.1
Valine$_B$ (mg/g DM)	8.60	12.21	16.98	15.25	14.07	14.61	13.23	14.19	15.00	13.44	9.83	10.86	11.93	14.85	13.30	12.72
SRA$_I$ (dpm/nmol)	15.7	18.7	22.8	30.0	3.8	18.0	16.3	15.2	26.4	26.9	9.7	33.6	10.6	19.5	21.1	25.8
SRA$_B$ (dpm/nmol)	1.3	3.5	3.9	4.6	1.6	1.7	0.6	1.2	6.4	0.5	2.5	0.9	2.1	1.6	2.4	1.7
FSR$_I$ (dpm/nmol)	25.5	55.9	51.2	46.0	123.6	29.1	11.7	23.7	72.4	6.0	77.2	8.2	59.0	23.9	34.4	19.2
FSR$_P$ (dpm/nmol)	5.5	11.5	13.2	15.8	10.0	8.6	3.8	6.1	33.9	3.1	12.2	4.5	9.0	11.3	14.6	11.9
Pancreas																
Valine$_I$ (µmol/L)	37.2	113.7	97.9	21.7	60.6	62.6	22.9	44.4	51.4	51.8	76.2	42.4	61.6	45.0	61.9	29.1
Valine$_B$ (mg/g DM)	14.76	17.60	13.82	15.17	19.26	13.99	12.33	17.01	14.67	15.67	13.63	14.46	15.43	15.97	19.37	15.61
SRA$_I$ (dpm/nmol)	21.2	30.4	16.3	57.8	20.0	16.1	28.9	40.5	32.3	15.4	7.9	21.6	12.3	24.6	20.7	27.4
SRA$_B$ (dpm/nmol)	8.8	7.7	15.4	10.7	4.5	6.5	5.4	5.2	4.9	6.9	6.8	5.2	4.0	3.1	4.7	4.0
FSR$_I$ (dpm/nmol)	124.5	75.5	283.7	55.8	67.2	121.5	56.3	38.2	45.7	133.7	261.3	72.7	97.6	38.0	68.6	44.1
FSR$_P$ (dpm/nmol)	36.1	25.2	52.2	36.9	28.6	32.2	32.8	26.1	26.2	38.8	33.5	25.8	17.3	22.7	28.7	29.1
Muscle																
Valine$_I$ (µmol/L)	41.7	58.5	30.7	42.4	55.5	59.3	41.4	77.0	67.5	62.4	68.9	72.2	48.4	85.8	78.1	83.6
Valine$_B$ (mg/g DM)	21.49	25.36	23.16	26.89	26.86	23.47	23.41	22.10	24.01	19.06	19.72	20.80	26.62	25.27	22.75	27.53
SRA$_I$ (dpm/nmol)	29.1	34.5	28.1	24.9	14.4	8.2	13.5	17.9	25.7	20.9	32.5	17.8	16.2	17.1	7.9	10.0
SRA$_B$ (dpm/nmol)	0.7	0.4	0.5	0.2	0.3	0.3	0.4	0.4	0.5	0.6	0.5	0.5	0.3	0.4	0.3	0.6
FSR$_I$ (dpm/nmol)	7.0	3.1	5.3	2.8	6.7	11.5	8.9	7.1	6.3	8.2	4.5	8.3	5.7	7.5	13.2	18.8
FSR$_P$ (dpm/nmol)	2.8	1.2	1.7	0.8	2.1	1.5	2.4	2.2	2.9	3.2	2.4	2.4	1.3	3.1	2.1	4.5
Skin																
Valine$_I$ (µmol/L)	87.8	113.9	74.6	59.6	98.0	62.4	76.1	56.3	93.7	105.7	75.8	66.4	52.7	96.8	78.4	64.2
Valine$_B$ (mg/g DM)	25.96	19.45	23.20	21.32	20.18	18.04	21.04	19.63	23.09	15.19	24.17	21.72	19.03	16.16	20.74	20.76
SRA$_I$ (dpm/nmol)	10.1	13.1	18.1	34.0	17.3	17.4	19.7	15.9	21.8	6.2	9.5	4.5	21.7	19.1	16.0	14.3
SRA$_B$ (dpm/nmol)	2.0	1.6	1.7	2.5	1.7	0.9	1.5	1.0	0.9	2.1	0.8	0.7	0.6	1.0	1.3	1.0
FSR$_I$ (dpm/nmol)	60.6	35.6	28.1	22.0	29.8	14.8	23.3	18.4	11.9	104.1	26.0	48.4	8.5	16.3	23.5	20.5
FSR$_P$ (dpm/nmol)	8.4	5.1	5.8	8.6	11.0	4.2	9.3	5.0	4.6	12.1	4.0	3.6	2.6	7.6	7.6	7.1

APPENDIX 6.1. Method to measure dry matter content using the microwave.

1. Take a representative sample of the forage being tested
2. Weigh between 100 – 200 g of the sample on an accurate scale. This is the wet weight.
3. Place the sample on a shallow plate that is suitable for use in the microwave oven.
4. Put a half full glass of water in the back corner of the microwave. Keep the water level constant during microwave use.
5. Place the sample in the microwave oven for 3 – 4 minutes. If the forage feels almost dry, weigh it and record the weight. Stir the sample, rotate the plate and then put the sample back into the oven for 1 minute.
6. Continue procedure No. 5 reducing the time to 30 seconds until drying does not reduce the weight more than 2 g or the sample begins to char. If charring occurs, use the previous weight for calculating moisture content. Dry weight is the last recorded weight after which the sample does not decrease more than 2 g and charring has not occurred.
7. To calculate the percentage of dry matter in the forage sample, divide dry weight by wet weight and multiply by 100.

NB. Microwave oven power can vary greatly and for this reason there may be variation in drying times.

APPENDIX 6.2. Method for analysing ammonia concentration of rumen samples.

The ammonia assays were performed using kits purchased from Sigma Chemicals (Cat # 171-C). The assay utilises the enzyme glutamate dehydrogenase to convert 2-oxoglutarate, NH_3 and NADH to glutamate and NAD. The reaction is quantified by measuring absorbance at 340 nm (Bergemeyer, and Beutler, 1985). Samples were analysed on a Cobas Fara II manufactured by Hoffmann LaRoche, Basel. The samples were diluted up to 25-fold with distilled water prior to analysis.

APPENDIX 6.3. Example of a SAS programme used to determine digestion parameters

Non lag model

```
options ls=96 ps=57 nocenter;
data insitu;
title 'dm disappearance';
        infile 'c:\phd\drc trial\insacco\sulldm.prn' firstobs=2;
        input feed hour run DM prot adf ndf;
run;
proc print;
run;
proc sort;
        by feed run;
data insitu;
        set insitu;
proc sort;
        by feed run;
PROC NLIN BEST=5 METHOD=MARQUARDT CONVERGENCE=.0000001 ITER=200 outest=est;
        BY FEED run;
        PARAMETERS A=15 TO 60 BY 2 K=.01 TO .2 BY .02 B=25 TO 98 BY 2;
        BOUNDS K>=0;
        E=EXP(-K*HOUR);
        MODEL DM=A+B*(1-E);
        DER.A=1;
        DER.B=(1-E);
        DER.K=(HOUR)*B*E;
        OUTPUT OUT=dmn PREDICTED=PR RESIDUAL=RS STUDENT=STD H=HI PARMS=A K B
STDP=sem STDI=sei;
PROC PRINT DATA=dmn;
        BY FEED run;
PROC PLOT DATA=dmn;
        BY FEED run;
PLOT dm*HOUR PR*HOUR='X'/OVERLAY;
PLOT RS*PR='R' STD*PR='S' HI*PR='H'/VREF=0 VPOS=25;
PROC CORR DATA=dmn;
        BY FEED run;
        VAR dm PR;

TITLE 'PREDICTED VS ACTUAL';
DATA dmn;
        SET dmn;
ED=A+B*(K/(K+.06));
PROC SORT;
        BY FEED run;
PROC MEANS MEAN ;
        BY FEED run;
        VAR A K B ED;
        OUTPUT OUT=NN MEAN=AN KN BN ED;
proc print;
run;
```

Lag model

```
DATA INSITU;
SET INSITU;
PROC SORT;
        BY feed run;
PROC NLIN BEST=5 METHOD=MARQUARDT CONVERGENCE=.0000001 ITER=200 outest=estL;
        BY feed run;
```

```
	PARAMETERS A=40 TO 50 BY 2 K=0.06 TO .26 BY .02 B=40 TO 50 BY 2 L=0 TO 3 BY .2;
	BOUNDS K>=0;
	BOUNDS 0<=L<3;
	E=EXP(-K*(HOUR-L));
	IF L>=HOUR THEN E=1;
	MODEL dm=A+B*(1-E);
	DER.A=1;
	DER.B=(1-E);
	DER.K=(HOUR-L)*B*E;
	DER.L=B*(-E)*K;
	OUTPUT OUT=dmL PREDICTED=PR RESIDUAL=RS STUDENT=STD H=HI PARMS=A K B
L;
run;
PROC PRINT DATA=dmL;
	BY feed run;
PROC PLOT DATA=dmL;
	BY feed run;
PLOT dm*HOUR PR*HOUR='X'/OVERLAY;
PLOT RS*PR='R' STD*PR='S' HI*PR='H'/VREF=0 VPOS=25;
PROC CORR DATA=dmL;
VAR dm PR;

TITLE 'PREDICTED VS ACTUAL';
DATA dmL;
	SET dmL;
PROC SORT;
	BY feed run;
PROC MEANS MEAN;
	BY feed run;
	VAR A K B L EDL;
	OUTPUT OUT=DL MEAN=AL KL BL LL;
proc print;
run;
```

APPENDIX 6.4: Example of adjusted SAS programme for maize silage DM and NDF where convergence did not occur.

<u>Non lag model</u>

```
options ls=96 ps=57 nocenter;
data msdm;
title 'dm disappearance';
     infile 'c:\phdc\drc trial\insacco\msdm.prn' firstobs=2;
     input feed hour run diet$ DM prot adf ndf;
run;
proc print;
run;
proc sort;
     by feed run;
data msdm;
     set msdm;
proc sort;
     by feed run;
PROC NLIN BEST=5 data=msdm (where=(feed=3021)) METHOD=MARQUARDT
CONVERGENCE=.0000001 ITER=2000 outest=est;
     BY FEED run;
     PARAMETERS A=15 TO 50 BY 2 K=.01 TO .3 BY .02 B=25 TO 98 BY 2;

          B=78.9-A;

     BOUNDS K>=0;
     E=EXP(-K*HOUR);
     MODEL DM=A+B*(1-E);
     DER.A=1;
     DER.B=(1-E);
     DER.K=(HOUR)*B*E;
     OUTPUT OUT=dmn PREDICTED=PR RESIDUAL=RS STUDENT=STD H=HI PARMS=A K B
STDP=sem STDI=sei;
PROC PRINT DATA=dmn;
     BY FEED run;
          var hour dm pr rs std hi a k b;
          run;
PROC PLOT DATA=dmn;
     BY FEED run;
PLOT dm*HOUR PR*HOUR='X'/OVERLAY;
PLOT RS*PR='R' STD*PR='S' HI*PR='H'/VREF=0 VPOS=25;
PROC CORR DATA=dmn;
     BY FEED run;
     VAR dm PR;

TITLE 'PREDICTED VS ACTUAL';
DATA dmn;
     SET dmn;
ED=A+B*(K/(K+.06));
PROC SORT;
     BY FEED run;
PROC MEANS MEAN ;
     BY FEED run;
     VAR A K B ED;
     OUTPUT OUT=NN MEAN=AN KN BN ED;
proc print;
run;
```

Lag model

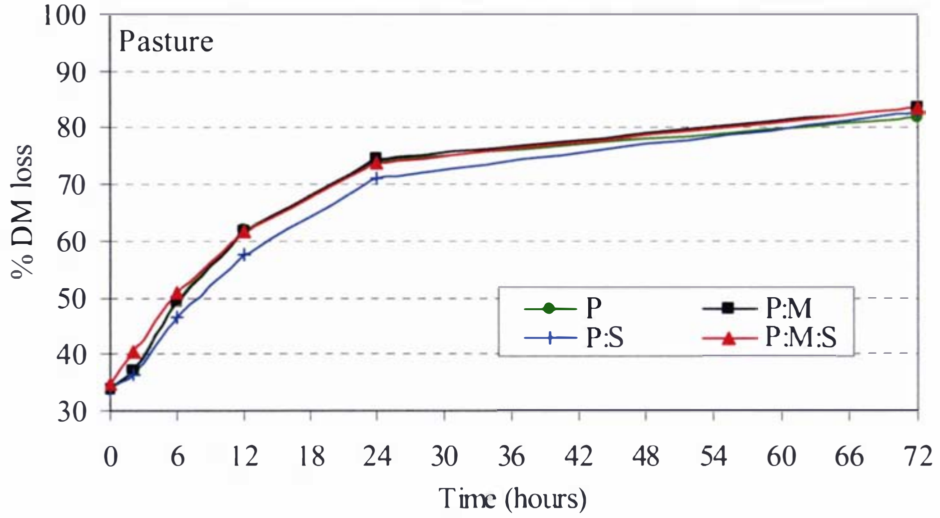
```
DATA msdm;
SET msdm;
run;
PROC SORT;
    BY FEED run;
PROC NLIN BEST=5 Data=msdm (where=(feed=3021))  METHOD=MARQUARDT
CONVERGENCE=.0000001 ITER=2000 outest=estL;
    BY FEED run;
    PARAMETERS A=15 TO 50 BY 2 K=.01 TO .3 BY .02 B=25 TO 98 BY 2 L=0 TO 12 BY .5;

    B=78.9-A;

    BOUNDS K>=0;
    BOUNDS 0<=L<=12;
    IF L>=HOUR THEN do;
    MODEL DM=A;
    DER.A=1;
    DER.B=0;
    DER.K=0;
    DER.L=0;
END;
ELSE DO;
IF L>0.02 THEN DO;
E=EXP(-K*(HOUR-L));
MODEL DM=a+b*(1-e);
DER.a=1;
DER.B=(1-E);
DER.K=(HOUR-L)*B*E;
DER.L=B*(-E)*K;
END;
ELSE DO;
L=0;
E=EXP(-K*HOUR);
MODEL DM=A+B*(1-E);
DER.A=1;
DER.B=(1-E);
DER.K=HOUR*B*E;
END;END;
OUTPUT OUT=DML PREDICTED=PR RESIDUAL=RS STUDENT=STD H=HI PARMS=A K B L;
PROC PRINT DATA=DML;
    BY FEED run;
var hour dm pr rs std hi a k b l;
PROC PLOT DATA=DML;
    BY FEED;
PLOT DM*HOUR PR*HOUR='X'/OVERLAY;
PLOT RS*PR='R' STD*PR='S' HI*PR='H'/VREF=0 VPOS=25;
PROC CORR DATA=DML;
VAR dm PR;
TITLE 'PREDICTED VS ACTUAL';
DATA DML;
    SET DML;
PROC SORT;
    BY FEED run;
PROC MEANS MEAN;
    BY FEED run; VAR A K B L;
    OUTPUT OUT=DL MEAN=AL KL BL LL;
run;
proc print;
run;
```

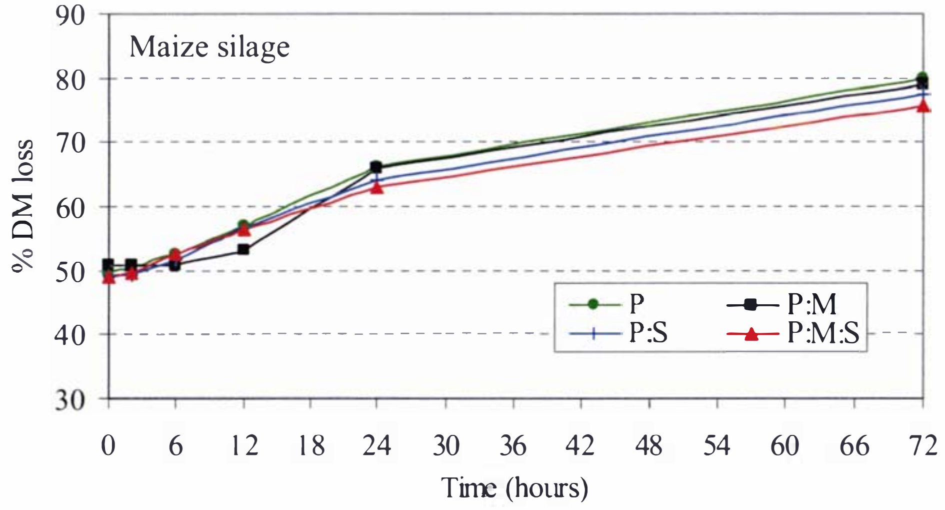
APPENDIX 6.5. Particle size distribution of rumen digesta for individual cows fed P, P:M, P:S and P:M:S[1]. Data are on a DM basis (g/100 g DM).

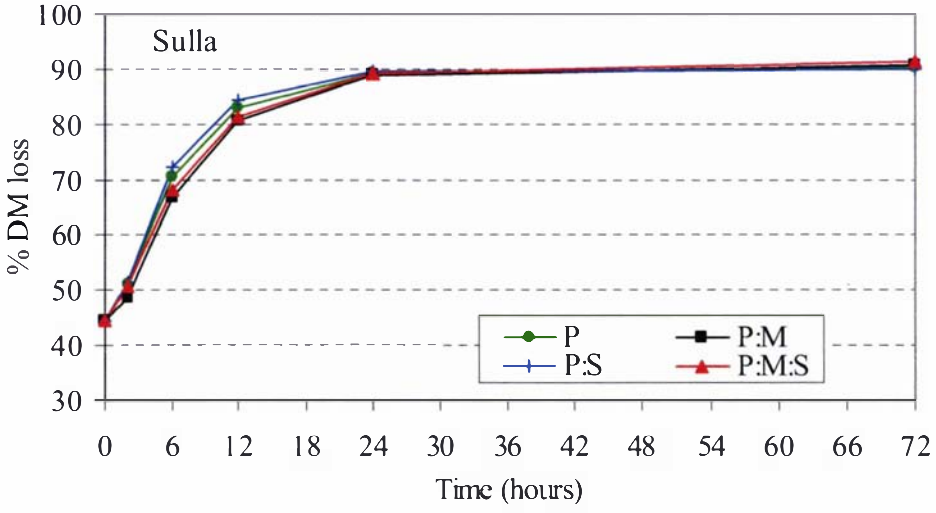
Cow	Treatment	> 4 mm	1 – 2 mm	<1 mm	Solubles
2801	P	27.3	19.6	28.7	24.4
710	P	27.3	28.5	26.0	18.2
5774	P	24.5	18.7	28.7	28.1
2760	P	27.4	20.2	37.4	15.0
4328	P:M	19.3	26.6	27.2	27.0
302	P:M	22.0	22.2	27.6	28.2
3823	P:M	32.0	25.2	27.1	15.8
3779	P:M	19.3	25.5	24.5	30.7
3772	P:S	23.7	9.6	28.4	38.3
6936	P:S	23.9	17.1	26.5	32.5
5775	P:S	30.0	9.8	27.8	32.4
3788	P:S	19.3	9.9	25.5	45.3
1765	P:M:S	29.6	18.8	29.1	22.5
5756	P:M:S	41.4	20.1	18.9	19.6
6919	P:M:S	23.4	19.6	31.2	25.8

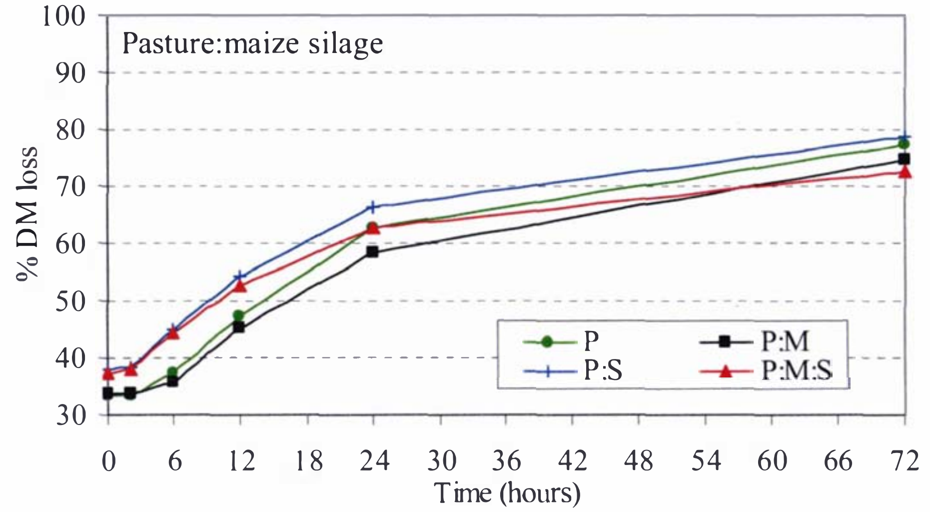
[1] P, pasture; M, maize silage; S, sulla; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

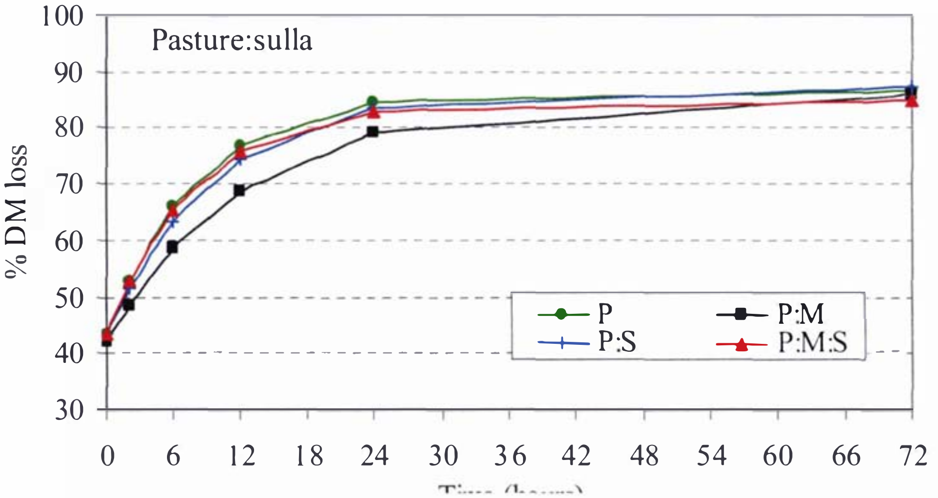
APPENDIX 6.6. Dry matter (DM) disappearance curves for P, M, S, P:M, P:S and P:M:S incubated *in sacco* in cows fed P, P:M, P:S and P:M:S[1]. Each figure summarises data for one forage or mixture when incubated in cows fed each of the four diets.

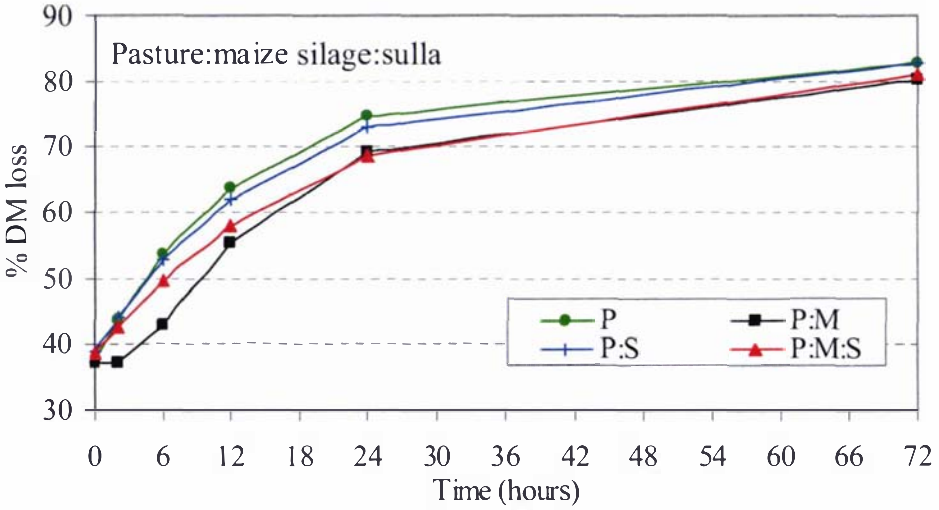






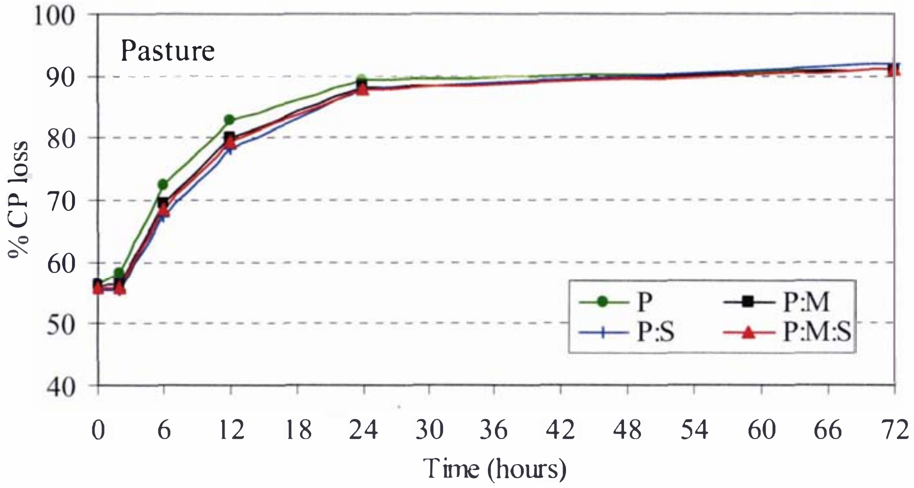


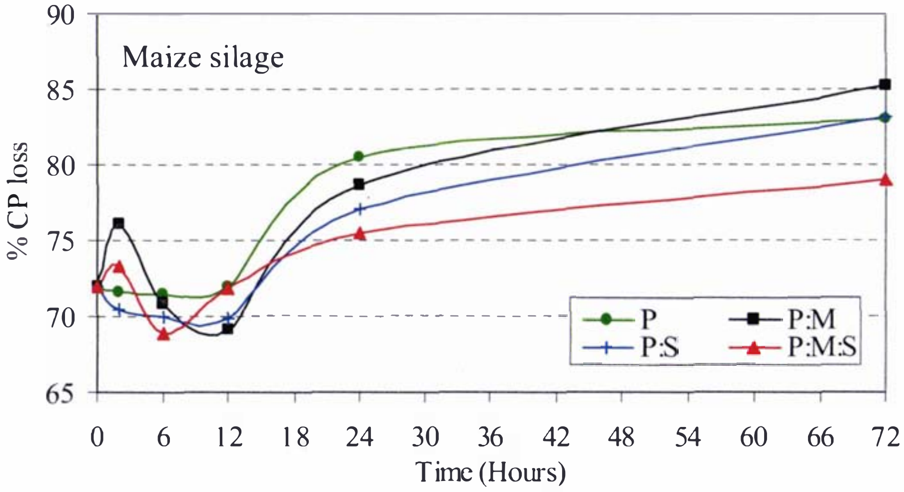


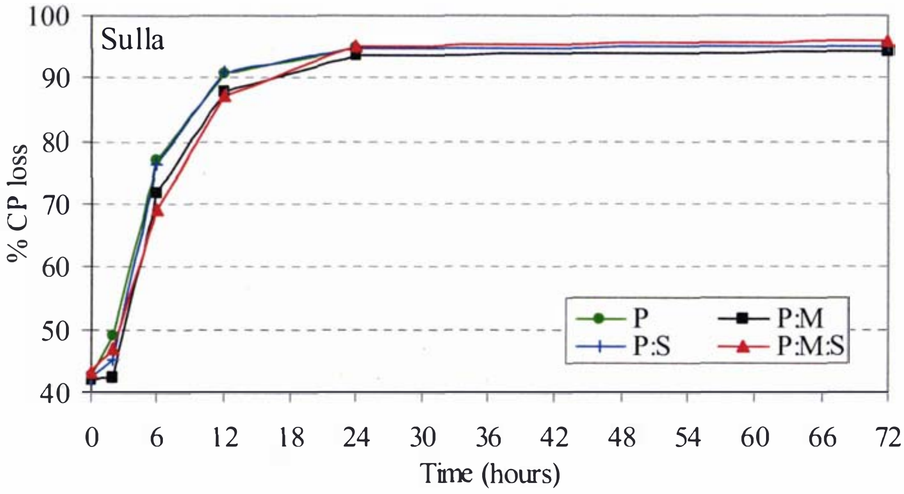


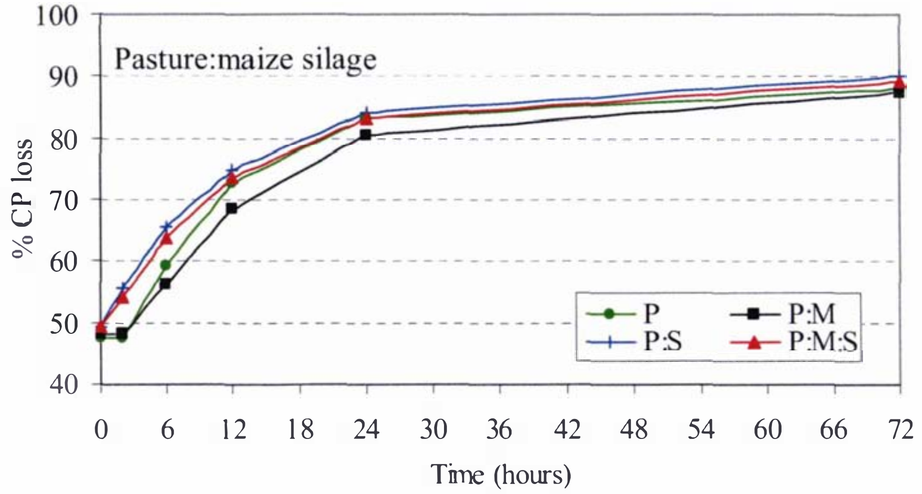
[1] P, pasture; M, maize silage; S, sulla; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

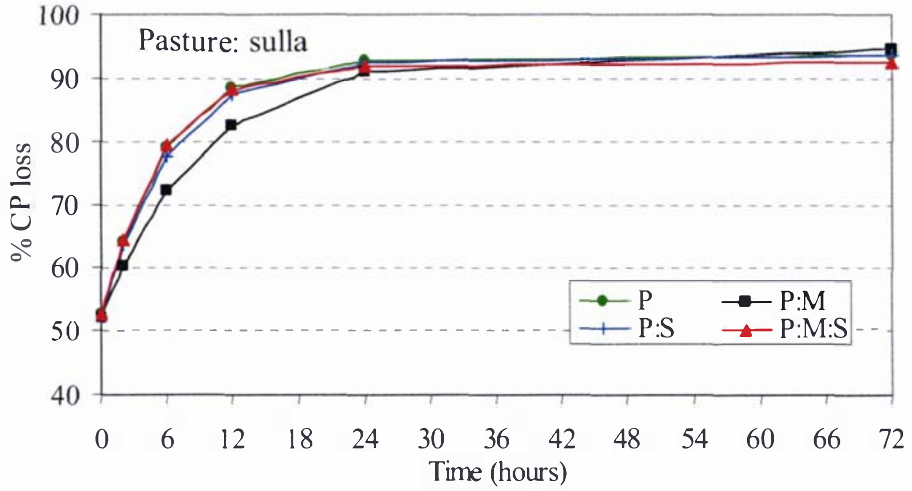
APPENDIX 6.7. Crude protein (CP) disappearance curves for P, M, S, P:M, P:S and P:M:S incubated *in sacco* in cows fed P, P:M, P:S and P:M:S[1]. Each figure summarises data for one forage or mixture when incubated in cows fed each of the four diets.

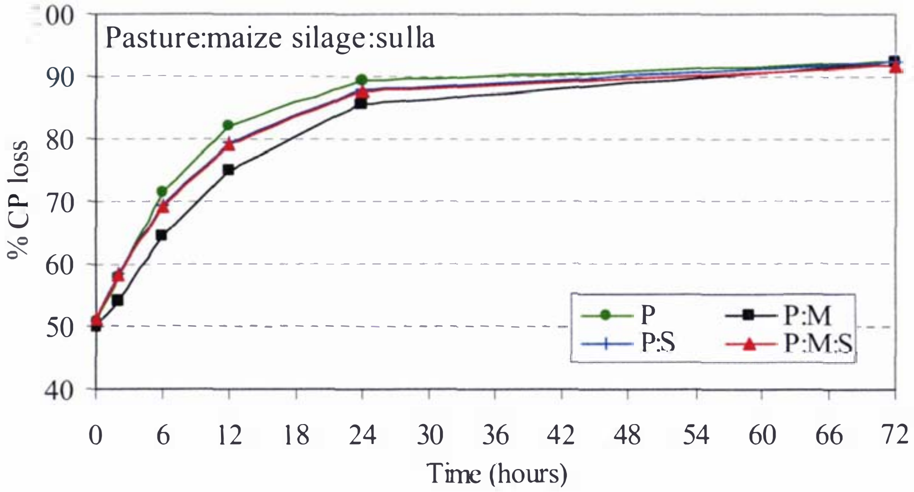






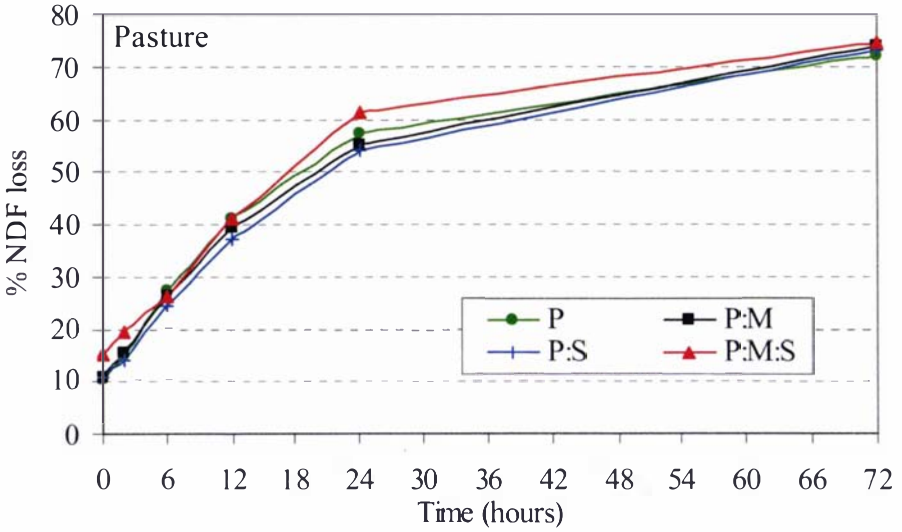


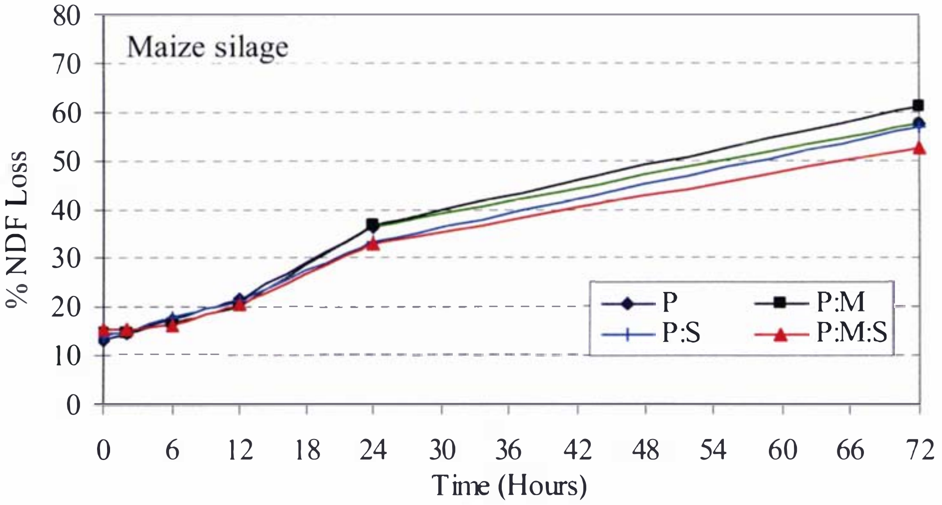


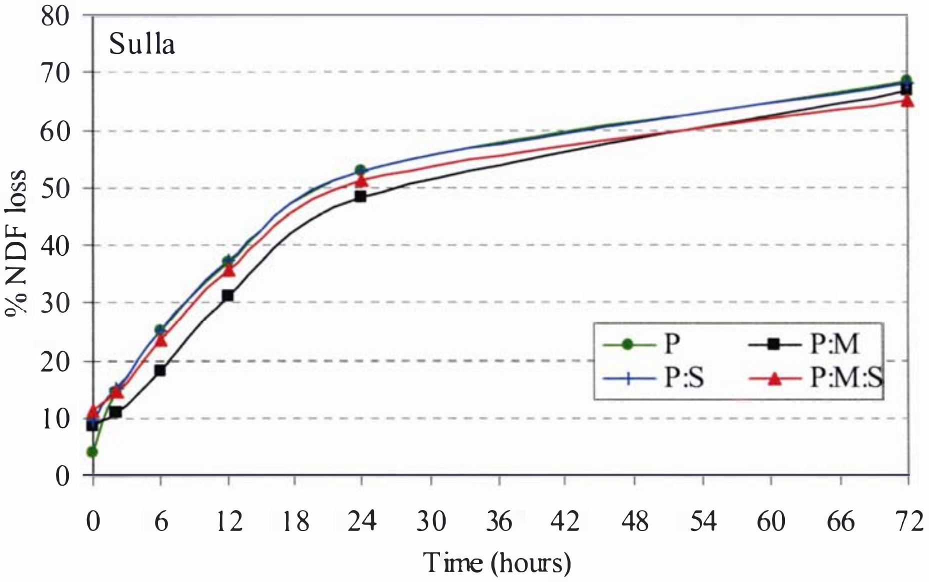


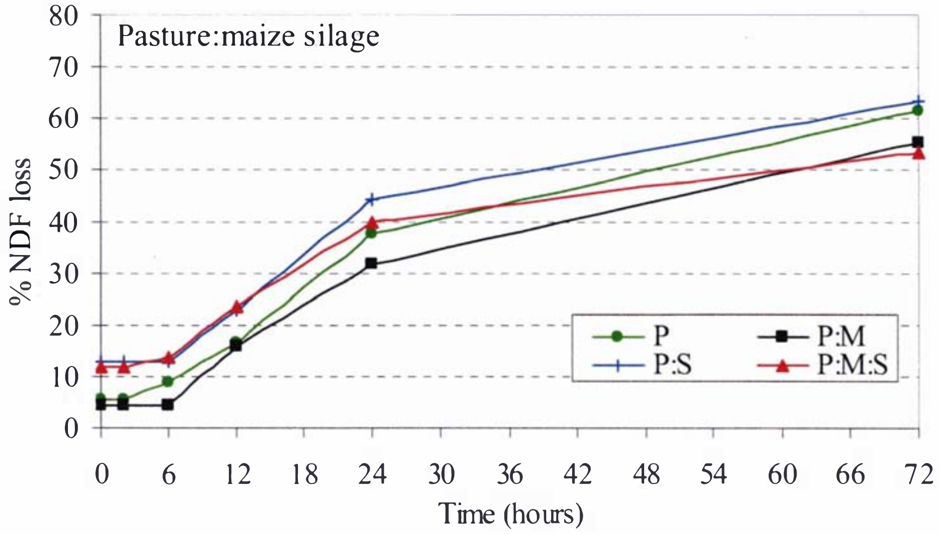
[1] P, pasture; M, maize silage; S, sulla; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

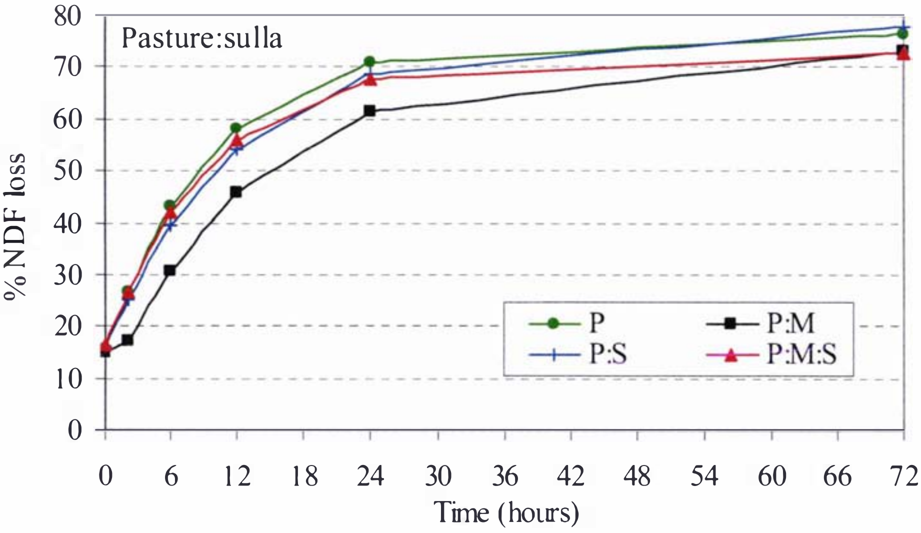
APPENDIX 6.8. Neutral detergent fibre (NDF) disappearance curves for P, M, S, P:M, P:S and P:M:S incubated *in sacco* in cows fed P, P:M, P:S and P:M:S[1]. Each figure summarises data for one forage or mixture when incubated in cows fed each of the four diets.

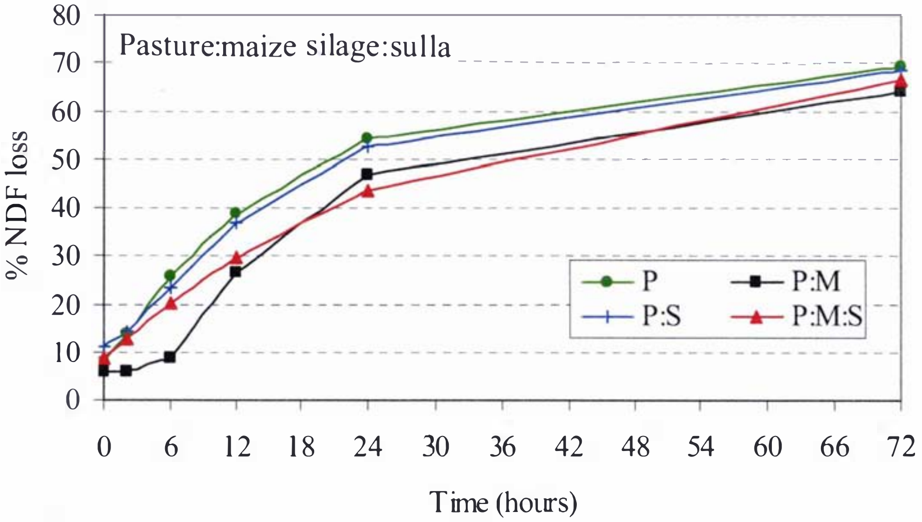






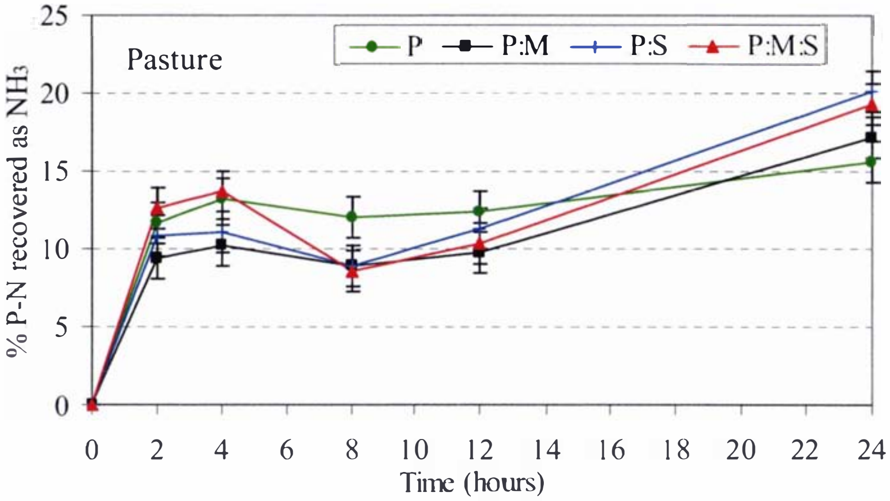


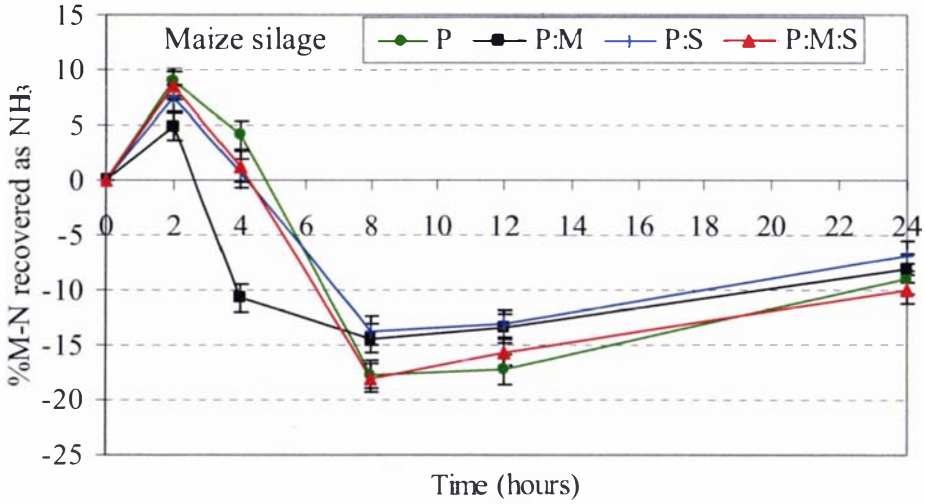


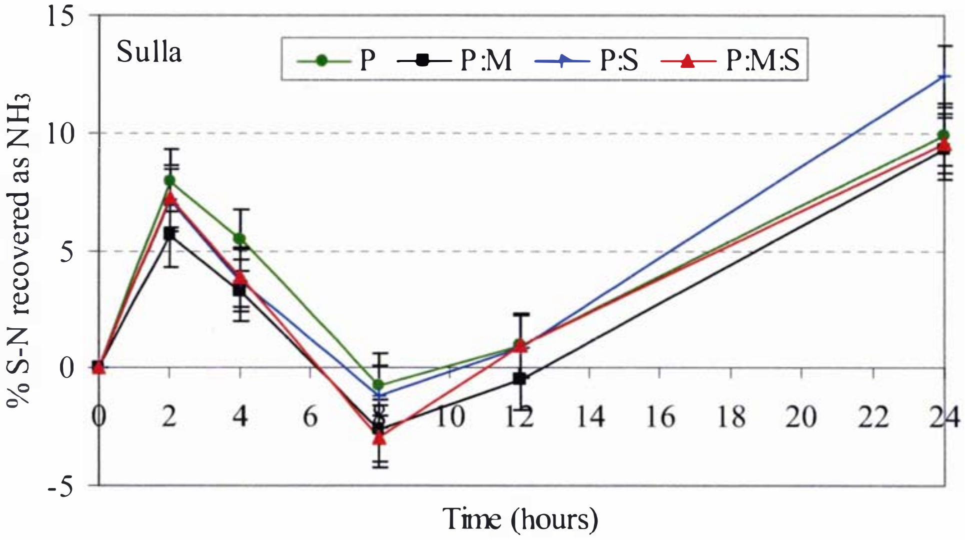


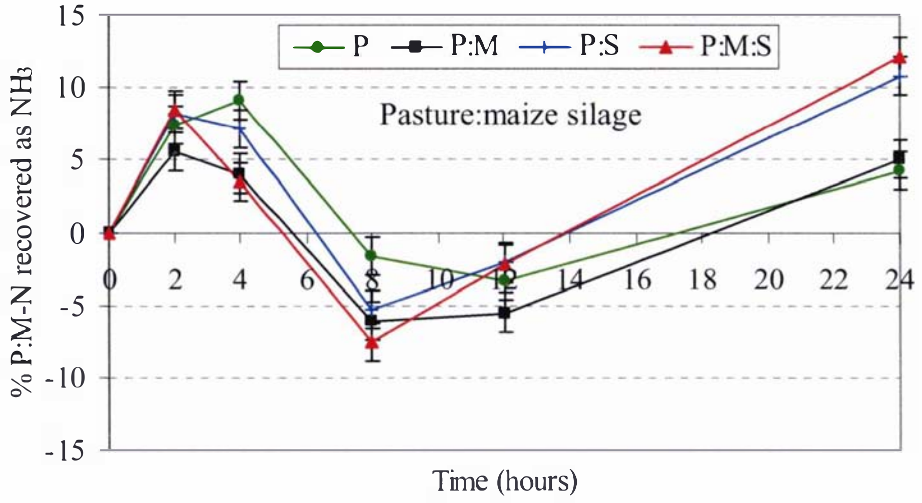
[1] P, pasture; M, maize silage; S, sulla; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

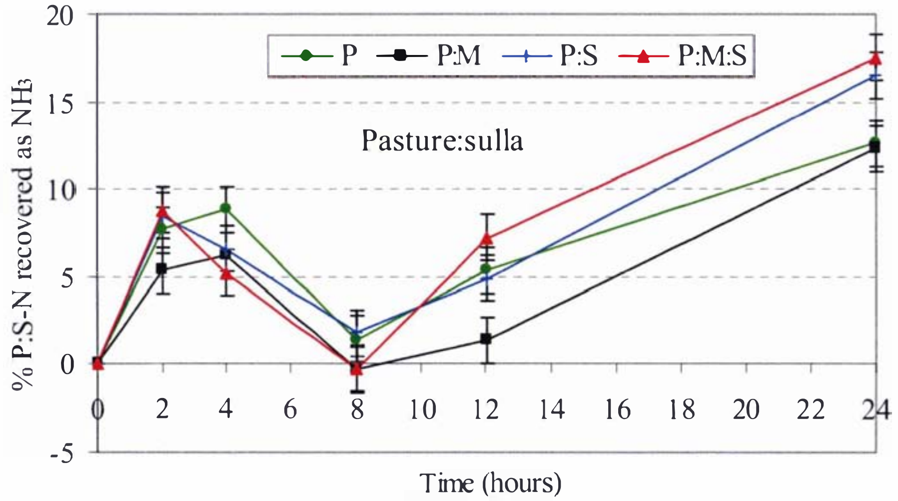
APPENDIX 6.9. Net ammonia (NH_3) production from P, M, S, P:M, PM, P:S, P:M:S incubated *in vitro* using rumen inoculum from cows fed P, P:M, P:S and P:M:S[1]. Each figure summarises data for one forage or mixture when incubated using inoculum from each cow-diet.

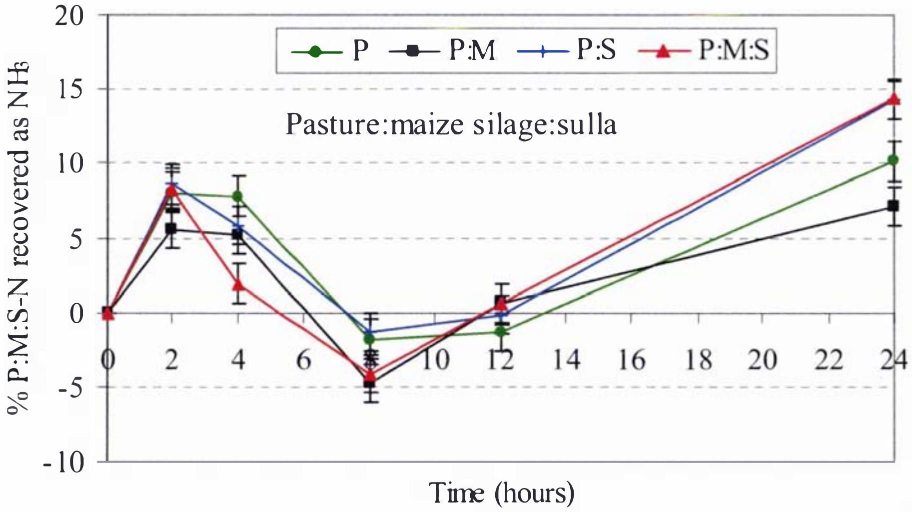






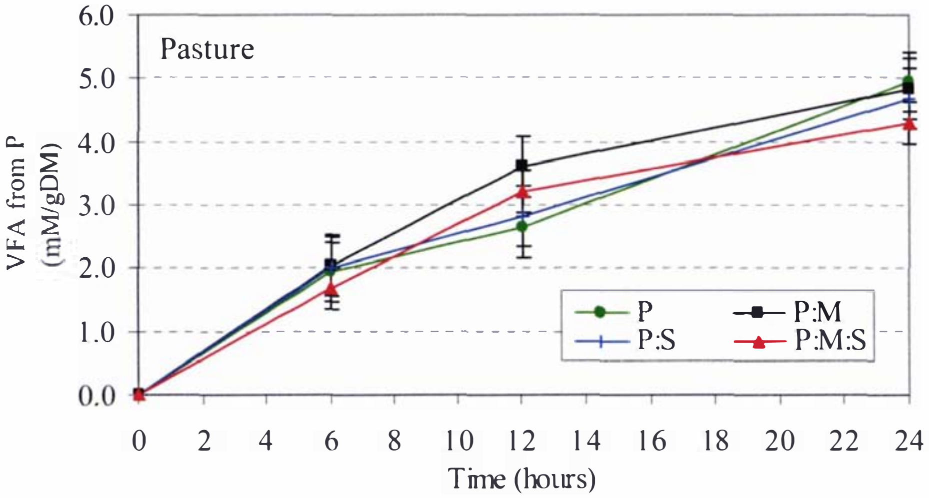


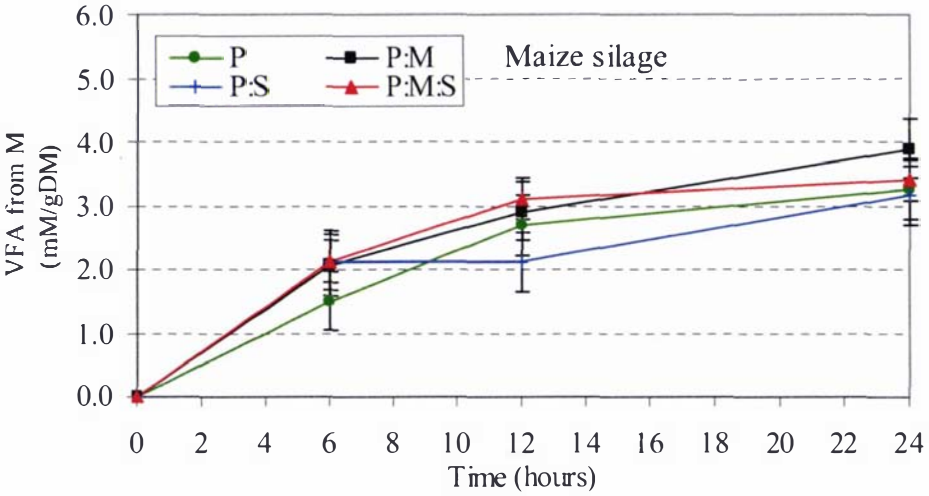


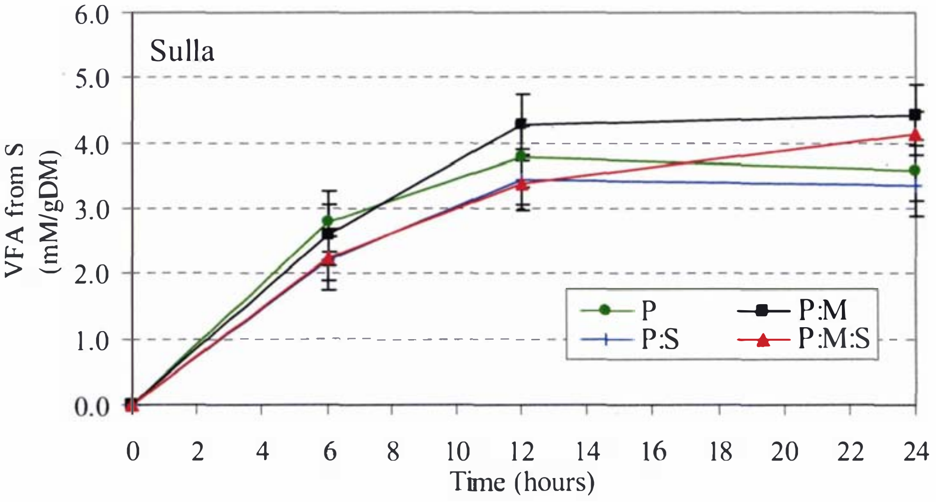


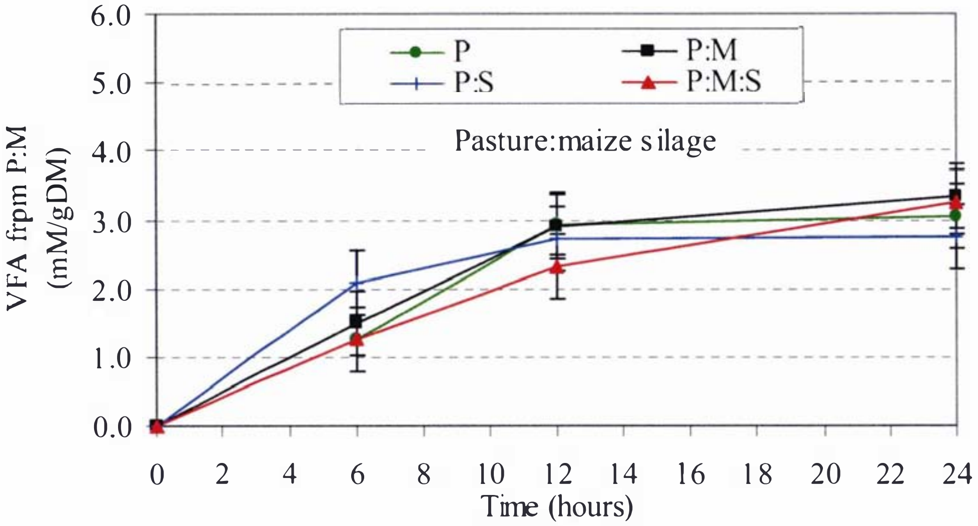
[1] P, pasture; M, maize silage; S, sulla; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.

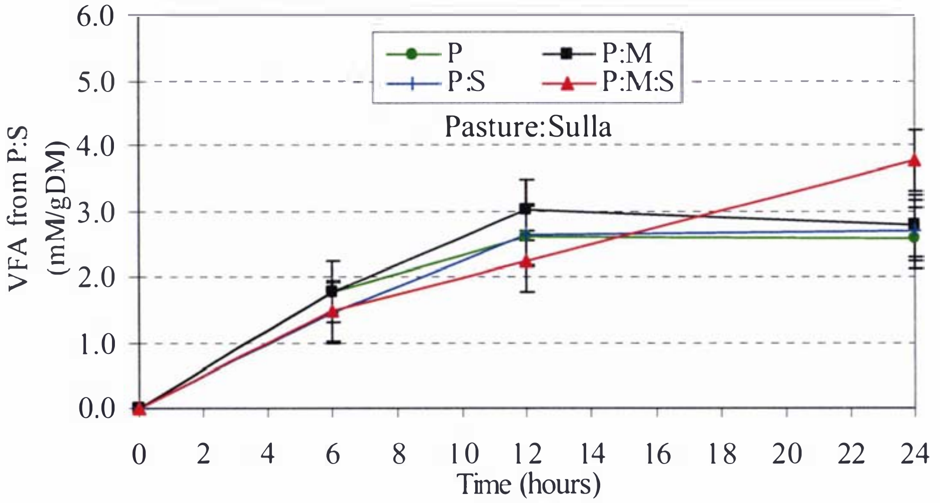
APPENDIX 6.10. Total volatile fatty acids (VFA) produced when P, M, S, P:M, P:S and P:M:S were incubated *in vitro* using rumen inoculum from cows fed P, P:M, P:S and P:M:S[1]. Each figure summarises data for one forage or mixture incubated with inocula from each cow-diet.

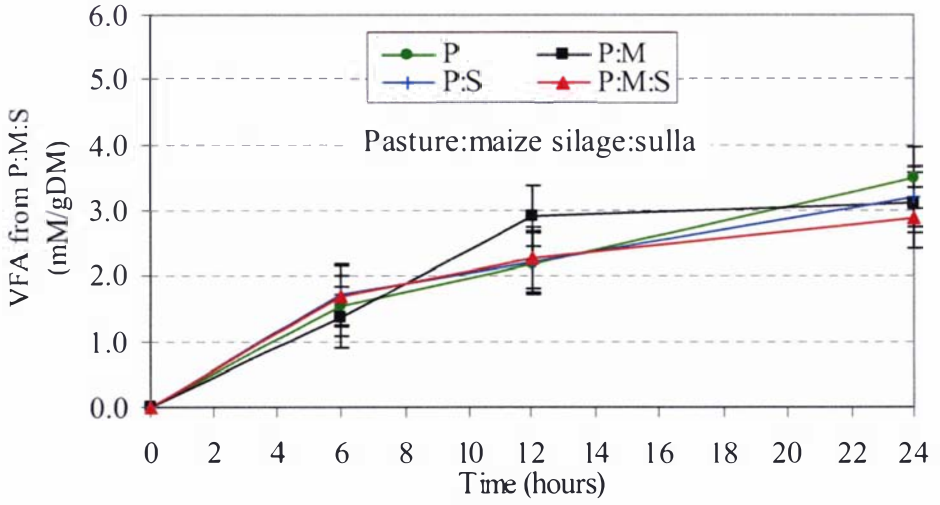












[1] P, pasture; M, maize silage; S, sulla; P:M, pasture:maize silage; P:S, pasture:sulla; P:M:S, pasture:maize silage:sulla.