Digestion characteristics of forages, including perennial ryegrass at different stages of maturity, and supplementary feeding for dairy cows grazing pasture

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Abstract

This thesis defines digestion kinetics for perennial ryegrass (*Lolium perenne* L.), which is the main component of diets fed to dairy cows in New Zealand. Chemical composition and digestion kinetics were measured in fresh minced ryegrass as it matured and leaf, stem and inflorescence of several grass species. *In sacco* and *in vitro* incubations were used to define rates of degradation and nutrient release. Two short-term grazing trials were used to evaluate contrasting silages as supplements for cows fed restricted amounts of summer pasture. The minced preparation of ryegrass resulted in a similar distribution of dry matter (DM) between particle size fraction and rumen digesta from cows fed pasture. Mincing released 0.46 – 0.80 of crude protein into the soluble fraction, with highest proportions for mature grasses which had low CP concentrations (about 8 g CP/100 g DM). In contrast, the majority of fibre remained in the insoluble fraction but rates of degradation (k) approximately halved as grass matured. *In vitro* yield of VFA was similar for immature and mature minced ryegrass (after 12 hours VFA was equivalent to about 30% of DM), even though ammonia concentration declined to very low values for stem and mature grass. This suggests the rapid initial microbial growth was able to sustain a high level of DM degradation to VFA with mature grass. The summer pasture used for silage supplementation was of uncharacteristically good quality so the expected contrasts between maize, pasture, sulla (*Hedysarum coronarium*), lotus (*Lotus corniculatus*) and sulla/maize silage mixtures were less than expected. Milk responses to lotus silage supplements were greater than other silages (e.g.: 290 g milksolids from 54 MJ ME by lotus versus 110 g milksolids from about 50 MJ ME supplied by other silages). Pasture substitution was low (0.06 – 0.33). The Cornell Net Carbohydrate and Protein System (CNCPS) was chosen for evaluation of cow trial data because it uses feed degradation parameters as input variables to estimate nutrient supply. Model prediction of milk yield matched observed values when cows maintained liveweight. Milk yield was underestimated at low intakes and overestimated at high intakes because no allowance is made for nutrient partitioning between milk production and liveweight change.

Acknowledgments

What does PhD mean?

A PhD means inspiration, commitment, responsibility, education, teamwork and fun as well (not writing this thesis though!).

Firstly I must thank my parents, particularly my mother who encouraged me to receive all this education, and also my father who challenged me all the time and supported me during my whole life.

NZODA for providing the opportunity and financial support for me to make this dream become reality. A dream that I have had since my first year of University, back in 1989. Eleven years later that dream began to become true.

Dr. Waghorn, Garry, "The old man": my mentor and godfather here in New Zealand, a good one. Energetic, hard working, committed with students, family and animals, funny, supportive, and of course, with his good and "bad" days. Garry has always been ready and keen to listen and help. I am not sure if I could have made it this far without you. *Muito obrigado,* Garry*. De verdade*.

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Contents

List of illustrations

Abbreviations

AA: Amino acids.

AAC: Australian Agriculture Council.

ADF: Acid detergent fibre.

ADL: Acid detergent lignin.

ADFIN: Acid detergent fibre insoluble nitrogen (the N present in ADF).

BW: Body weight (kg).

^{o}C: Degree centigrade.

CP: Crude protein, being total N x 6.25 (or x 6.38 for milk).

CPI: Crude protein intake.

CS: Condition score.

CV: Coefficient of variation.

DE: Digestible energy. GE of the feed minus the GE of the corresponding faeces.

DIP: Degradable intake protein (%CP).

DM: Dry matter.

DMD: Dry matter digestibility.

DMI: Dry matter intake.

DOMI: Digestible organic matter intake.

g: Grams.

GE: Gross energy. Synonymous with heat of combustion.

h: Hour(s).

ha: Hectare.

INRA: Institut National de la Recherche Agronomique.

in sacco: In bag.

in vitro: In glass.

in vivo: In animal.

k_i (subscript): The net efficiency of use by the animal of ME (i.e., NE/ME) for energy maintenance (k_m), for NE gain in growth and fattening (k_g), as milk produced (k_L) and pregnancy (k_{pr}).

kg: Kilograms.

LIC: Livestock Improvement Corporation.

LW: Live weight.

LWC: Live weight change.

LWG: Live weight gain.

MCP: Microbial crude protein.

ME: Metabolisable energy.

MEI: Metabolisable energy intake.

ME_m: The ME required by the animal for maintenance, or the maintenance (support) metabolism.

mg: Milligram (10^{-3}g).

mL: Millilitres.

mm: Millimetres.

MP: Metabolisable protein.

MS: Milk solids (milk fat + milk protein).

MSE: Mean square error.

NAN: Non-ammonia nitrogen (total N in digesta minus ammonia-N).

NDF: Neutral detergent fibre.

NDFIN: Neutral detergent fibre insoluble nitrogen (the N present in NDF).

NE: Net energy. The NE value of a feed (MJ/kg DM) varies with the purpose for which its ME is used because of differences between the k_m, k_g and k_L values for that feed.

NFC: non-fibrous carbohydrates (calculated by difference: 100 – CP – lipid – NDF – ash).

ng: Nanogram (10^{-9}g).

NIRS: Near infrared reflectance spectroscopy.

NPN: Non-protein nitrogen.

NSC: Non-structural carbohydrates.

NV: Nutritive value.

NZ: New Zealand.

OM: Organic matter.

OMD: Organic matter digestibility.

OMI: Organic matter intake.

pH: Whole number referring to the number of hydrogen ions present in a solution. Negative logarithm of the hydrogen ion concentration.

RDP: Rumen degraded protein; that part of the CPI which is fermented and may be used by the microbial population in the rumen, yielding MCP (synonymous of DIP).

RUD: Rumen undegraded protein.

SE: Standard error.

SR: Substitution rate (kg pasture/kg supplement) = (Pasture DMI in control (unsupplemented) group – pasture DMI in supplemented group)/supplement DMI.

STDEV: Standard deviation.

TMR: Total mixed ration.

UDP: Undegradable dietary protein (synonymous of RUD).

µg: Microgram (10^{-6}g).

µL: Microliter (10^{-6}L).

VFA: Volatile fatty acids.

Forage common and scientific names

Birdsfoot trefoil, lotus	*Lotus corniculatus*
Browntop:	*Agrostia capillaris*
Chicory:	*Cichorium intybus*
Foxtail:	*Alopecurus arundinaceus*
Italian ryegrass:	*Lolium multiflorum*
Kentucky bluegrass:	*Poa pratensis L.*
Kikuyu:	*Pennisetum clandestinum*
Lotus major:	*Lotus penduculatus*
Lucerne, alfalfa:	*Medicago sativa*
Maize, corn:	*Zea maize*
Meadow bromegrass:	*Bromus biebersteinii* Roem & Schreb.
Orchardgrass or cocksfoot:	*Dactylis glomerata* L.
Paspalum:	*Paspalum dilatatum*
Red clover:	*Trifolium pratense*
Phalaris, reed canarygrass:	*Phalaris arundinacea* L.
Ryegrass (perennial):	*Lolium perenne* L.
Smooth brome grass:	*Bromus inermis* L.
Sulla:	*Hedysarum coronarium*
Tall fescue:	*Festuca arundinacea* Schreb.
Timothy:	*Phleum pratense*
Yorkshire fog:	*Holcus lanatus*
White clover:	*Trifolium repens*

Chapter 1 - General introduction

The productivity and nutritive value of perennial ryegrass dominant pasture is a constraint to future increases in productivity in New Zealand dairy systems (Clark *et al*., 2001). Over the last 50 years, annual pasture production has not increased significantly on research or commercial farms. Cows have a rapid decline after peak milk production relative to cows fed roughage with concentrates and short lactation lengths (266 - 268 days between 1997 and 2002) which is dependent upon growing conditions and availability of silage and supplements.

In 2001/02 New Zealand had over 3.5 million dairy cows; herd size averaged 271 milking cows on 103 hectares giving a stocking rate of 2.7 cows per hectare (Livestock Improvement Corporation (LIC), 2002). National cow production averaged 307 kilograms of milksolids per lactation (North and South Island, 301, 338 kg milk solids/year respectively).

In 2001/02, three co-operatively owned dairy companies processed over 13 billion litres of milk in New Zealand. Over 1.1 billion kilograms of milk solids was processed from seasonal supply units into products predominantly for export (LIC, 2002).

The trend of increased production per cow over the last 9 years (Figure 1.1) is due to genetic gain and improvements in farm management (LIC, 2002). However animal improvements are influenced by the effect of the weather on the quality and quantity of pasture available for the cows. Unfavourable weather conditions in 1998/99 resulted in the lowest production per cow since 1992.

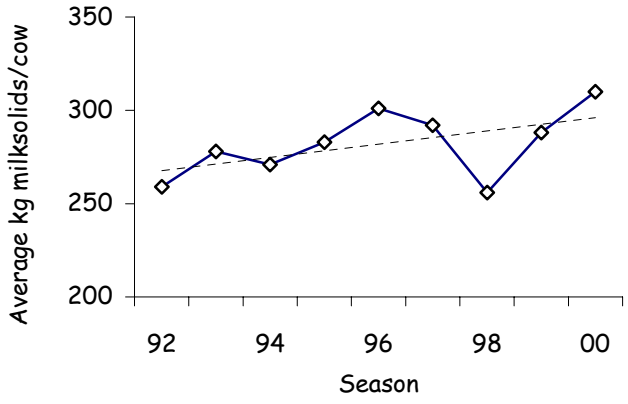


FIGURE 1.1 – Trend in milksolids production per cow since 1992. Data from LIC (2002).

The quality and quantity of nutrients available to grazing animals is extremely variable because weather conditions affect growth, growth rate, onset of grass maturation, sward composition and feeding management practices. Ryegrass pastures can have low dry matter and high fibre concentrations which may restrict feed intake so cow nutrient requirements are often not met (Waghorn, 2002).

Grasses commence spring growth with vigorous leaf production, which has a high feeding value (nutritive value x voluntary intake) for grazing cattle, but as daily temperatures rise, an increasing proportion of stem and inflorescence appears. Although grazing management can control the extent to which grass is allowed to produce seed heads, nutritive value declines because of changes in chemical composition. The principal changes are increased proportions of fibrous stem and decreased concentrations of leaf protein (Wilman and Agiegba, 1982), so that the maturing plant has higher proportions of fibre and lower proportions of protein in the dry matter. These changes reduce the amount of amino acids available to ruminants and may increase the proportions of acetate: propionate available for absorption (Russell and Strobel, 1993).

However, the most significant effect of grass maturation is that the rate of digestion and clearance of residual forage fibre from the rumen is reduced, because mature forages are slower to digest and require more chewing to reduce the particle size of plant fragments to a size able to pass out of the rumen (Ulyatt *et al*., 1986). The slower rate of passage from the rumen reduces feed intake. Hence, maturation in a grass-dominant sward results in lowered intake as well as declining nutritive value (Kennedy and Murphy, 1988).

Grass maturation has a major impact upon dairy cow productivity because grass-dominant pasture is unable to provide sufficient nutrients to match the genetic merit of New Zealand dairy cows, as evidenced by low milk production compared to cows fed concentrate diets and anoestrus coinciding with pasture maturation in some situations (Verkerk *et al*., 2000).

Improved nutrition for dairy cows requires an understanding of digestion kinetics, including the rate of degradation of plant constituents and the nutrients released from digestion. This is especially important with grass dominant pastures where composition changes with the time. Burke *et al*. (2000) defined the degradation kinetics of immature leafy material from a range of grasses, legumes and herbs to be used as a basis for formulating forage mixed rations. That work determined degradation rates for dry matter, protein, soluble carbohydrate, neutral detergent fibre (NDF; cellulose,

hemicellulose and lignin) and acid detergent fibre (ADF; cellulose and lignin). It also indicated the amount and proportions of volatile fatty acids (VFA) produced, and have provided a mathematical basis for comparing contrasting feed types, but only for immature forages.

Accurate prediction of performance for animals fed ryegrass diets and options for supplementation with high-quality forages requires a greater understanding of how digestion processes are influenced by the stage of maturity. The objective of Chapter 3 was to analyse and compare changes in composition and digestion of DM, CP NDF and ADF of ryegrass growing to maturity in spring.

The work described in Chapter 4 examines grasses, which have not been grazed and are in an advanced stage of maturity, not senescent but with stems and nearly mature flowers. This study aimed to determine the digestive characteristics at the opposite end of the range to that of Burke *et al.* (2000), using five very mature grass species. This involved the separation of the five grass species into leaf, inflorescence and stem (including sheath) fractions for incubation *in vitro* and *in sacco*. *In vitro* incubations were conducted to determine the products of degradation (ammonia from proteolysis and VFA), whilst the *in sacco* technique was conducted to determine the rate at which dry matter (DM) and its constituent chemical fractions are degraded through microbial digestion.

Since the early 1970's, a large number of studies have quantified the response in milk production to supplementation with grazing dairy cows. The principal factors controlling the response to supplementation have been identified as the quantity and quality of the herbage allowed, milk yield potential and stage of lactation of cow plus the quantity and nature of the supplement (Bryant and Trigg, 1982; Holmes, 1987; Thomson *et al*., 1998; Woodward *et al*., 2002; Holmes *et al*., 2002; Penno, 2002).

Chapter 5 and 6 summarises experiments designed to improve the nutrition of cows in summer when pasture allowances are often insufficient to meet nutrient needs. Supplementation will increase and sustain milk production through to the end of lactation but responses will depend on pasture quality and type of supplement offered. This work emphasises the importance of meeting cow protein requirements especially as maize silage, commonly used as a forage supplement, has a very low protein concentration and is not suitable for feeding with low quality summer pasture. Sulla is of interest because it is a high yielding legume containing condensed tannins (CT) and high concentration of readily fermented carbohydrates, which offer good potential for high quality silage production (Niezen *et al*., 1998).

Simulation through mathematical modelling has shown to be a powerful tool for effectively integrating and utilising current knowledge of digestion kinetics (e.g.: Baldwin *et al.*, 1987; Dijkstra *et al.*, 1992). Such models can be used theoretically to predict rumen fermentation and nutrient absorption for improving animal performance. Chapter 7 describes the use of a dairy nutrition model (the Cornell Net Carbohydrate and Protein System) to develop strategies for high milk production within a grazing system and predictions are evaluated against data from animal feeding trials at Dexcel, Hamilton, New Zealand described in chapters 5 and 6.

Chapter 2- Literature review

Introduction

This review will define the nutritional problems experienced by dairy cows grazing pasture and indicate options for use of supplements for cows fed pasture. The focus will include effects of grass maturation and the need to complement changing pasture quality in order to maintain production.

In New Zealand, cows that calve in spring can experience a very rapid decline in milk production post peak lactation. Do New Zealand dairy cows have the potential to achieve high levels of milk production from pasture based seasonal systems? Work by Kolver *et al.* (2002) compared performance of overseas (OS) and New Zealand (NZ) Holstein Friesian (HF) dairy cows fed either a pasture diet (predominantly perennial ryegrass) or a total mixed ration (TMR) in the New Zealand environment (Table 2.1). The aim of this study was to test the potential of both genetics (NZ and OS) and diet type when fed a balanced diet (TMR) or when fed only pasture, and the likely consequences of using overseas genetics in New Zealand systems. Pasture was fed generously to NZ and OS throughout lactation with a daily allowance of > 60 kg dry matter (DM)/cow. Feed availability was not restricted for any treatment.

The NZ Friesian cows fed TMR produced 38% more milk, 29% more milk solids (MS) and weighed 12% more than NZ cows grazing pasture at the end of lactation. The findings of Kolver *et al.* (2002) support previous observations summarised by Ulyatt and Waghorn (1993) which clearly show a high genetic merit for milk production by New Zealand cows. The rapid decline of milk production after peak lactation of cows grazing pasture is a consequence of feeding and diet.

TABLE 2.1 – Average annual milk production, efficiency of milksolids production, liveweight and reproduction performance of New Zealand (NZ) and overseas (OS) Holstein Friesians grazing pasture (Grass) or fed total mixed ration (TMR) during the 2000/2001 season (Kolver *et al.*, 2002).

Genotype	NZ		OS		
Diet	Grass	TMR	Grass	TMR	P diet
Annual milk production (kg/cow)	5300	7304	5882	10097	<0.001
Milksolids (MS: kg/cow)	465	602	459	720	<0.001
Efficiency (kg MS/kg $LW^{0.75}$)	4.42	5.26	3.97	5.72	<0.001
Mean LW (kg/cow)	495	556	565	634	<0.001
Gain LW during lactation (kg/cow)	44	92	-20	77	<0.001
Empty rate (%)	7	14	62	29	0.1

The principal causes for the rapid post peak decline in milk production appear to be the cow's inability to consume sufficient nutrients in early lactation, compounded by lower feeding value of pasture in late spring. The decline in feeding value is a consequence of lower nutritive value of grass as it matures, compounded by a lower intake of mature forage. Low intakes may be due to increased concentrations of fibre in ryegrass as it initiates stem development. The fibre requires physical breakdown to pass out of the rumen, and thus limits intake. The increase in proportions of structural fibre is also associated with lower concentrations of protein, rapid digested soluble carbohydrates, and a low ME content of pasture DM. Hence the low feeding value of pasture is due to the dominance of grasses as well as effects of flowering, but under some conditions there may be insufficient feed in late spring/summer and supplementation will be essential in order to maintain milk production (Wilson and Moller, 1993; Moller, 1997). Intakes and pasture quality are inter-related and supplements must complement the pasture quality on offer.

This review provides a brief summary of pasture and forage supply for dairy production, examines pasture and forage changes with maturation and consequences for dairy cow nutrition. Results of trials where supplements have been fed to dairy cows have been summarised. Opportunities for using *in vitro* and *in sacco* techniques are presented, to identify principal factors required for model inputs and also to design appropriate supplements for pasture based diets fed to cows. The review concludes

with a synopsis of the CNCPS model for predicting cow performance when pasture is supplemented with forage species.

2.1 - Impacts of pasture quality and forage supply on dairy production

Pastures are managed swards, usually based on improved cultivars of grasses and legumes. Management will optimise feed quality and supply in a sustainable manner. New Zealand pastures are usually dominated by grasses but can include a range of forages including herbs, weeds as well as legumes. Forage is the edible part of plants, other than separated grain, that can provide feed for grazing animals, or that can be harvested for feeding.

Pastures fed in New Zealand dairy systems are predominantly but not exclusively based on ryegrass. For example, a typical dairy botanical pasture composition in Northland and Waikato regions is illustrated in Figure 2.1.

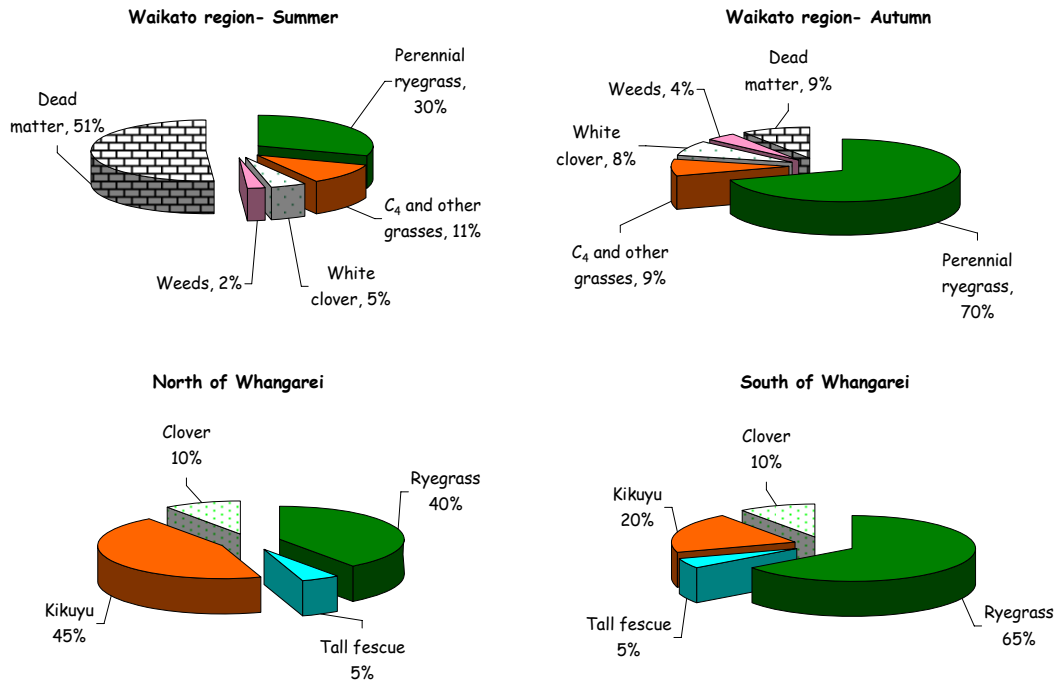


FIGURE 2.1 – Typical botanical pasture composition in Waikato regions (summer and autumn) and in north and south of Whangarei – Northland – Sources Simons *et al.* (1998) and Freeman (unpublished).

New Zealand pasture is characterised by the change in composition over the grazing season and by variability in supply. The principal dairying regions have been located in areas of reliable rainfall and good pasture growth (Waikato, Taranaki) but improved farming practices, availability of supplements (maize and pasture silage; green crop and hay) has enabled dairying to expand from Northland to Southland. These regions have very different practices, climates and needs for supplementation. For example, farming in Northland includes C_4 grasses (kikuyu; paspalum[1]) and can be influenced by toxins such as grass endophytes (Easton and Couchman, 1999) or facial eczema (Towers, 1986). In this region there is a requirement for substituting grass with supplements which are free of toxins. In contrast, dairying in Southland is sustained largely by ryegrass, with reliable rainfall but short growing seasons. There is a significant requirement for supplements in that region to prolong lactation.

These examples demonstrate the diversity of pasture diets and suggest provision of supplements to achieve an optimal balance of nutrients for the lactating cow is not a simple matter. The situation is made more complex by the changes in grass composition over a growing season and the need to balance cow requirements with feed availability and pasture growth. Forage quality decreases as plants mature. Increased proportions of fibre result in lower quality forage with fewer and more slowly released nutrients. Figure 2.2 demonstrates seasonal changes in pasture nutritive value averaged over three years in the Manawatu region.

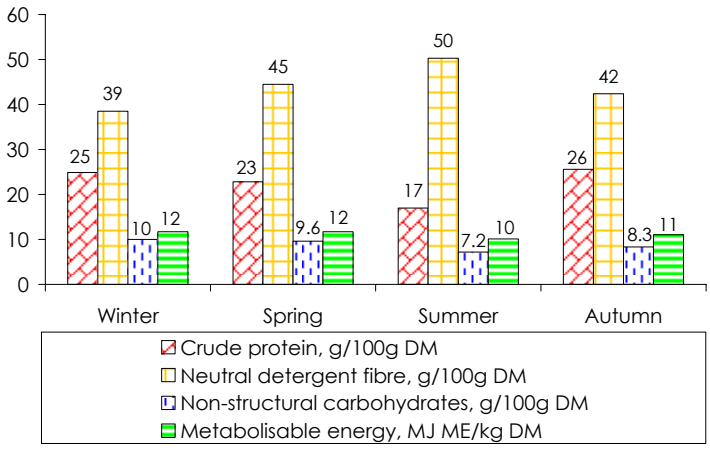


FIGURE 2.2 – Changes in pasture composition thoughtout the season. Adapted from García (2000).

[1] Please see page xiii for all scientific names used in this thesis.

Overall, the nutritive value of pasture is lowest during summer and reductions in quality are usually accompanied by reduced pasture growth under dry conditions. The relationship between pasture growth and feed demand will depend on location, but the general relationship is illustrated in Figure 2.3. It is apparent that supplementation is essential to maintain both diet quality and quantity, and the type and extent of supplementation will differ for all environments. The objective of the research present here is to formulate a system to accommodate changes in grass quality and to devise supplementation strategies most appropriate to the dairy cow.

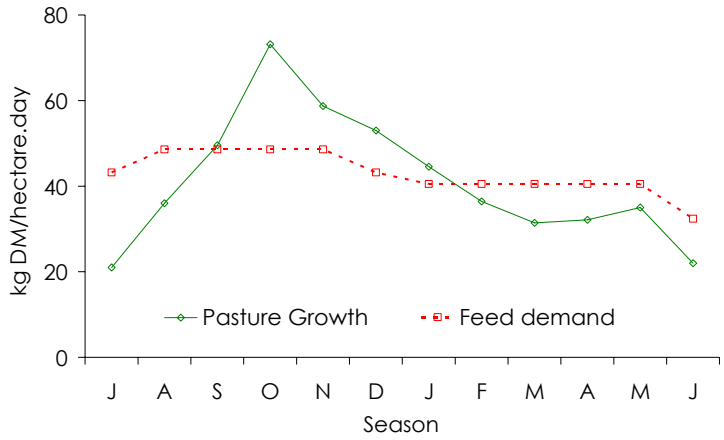


FIGURE 2.3 – Annual pattern of pasture growth and demand for a spring calving herd stocked at 2.7 cows/hectare (Brookes, 2003).

2.1.1 - Feed supply

Factors associated with feed supply on farms include herd size, effective area, grazing management over the lactation and dry period, as well as bought in replacements and sources of supplementary feed. Sourcing supplementary feed outside the farm, use of off-farm winter grazing and purchase of supplements can give a falsely high value for stocking rate (cows per hectare). The management and effective utilisation of forages is more relevant to good dairy practice than comparisons of stocking rates.

Producers try to match as closely as possible the seasonal pattern of feed production with feed required by the dairy herd (Holmes *et al.*, 2002). Most seasonal dairy farms calve in winter (late July-August; called "spring calving") to ensure peak lactation coincides with peak pasture growth. This system results in an inadequate feed supply in the first few weeks of lactation with a surplus in late spring which needs to be

controlled to maintain pasture quality. Summer pasture growth is often insufficient to meet feed requirements of the herd (Figure 2.3).

Herbage mass will influence intake per animal together with pasture quality (pre-grazing) to affect herbage utilisation (pre-grazing minus post-grazing). Table 2.2 suggests targets for average pasture cover and pre-grazing mass throughout the season (Matthews *et al.*, 1999). The post-grazing herbage mass indicates differences in pasture management needed to achieve optimum utilisation at different times of the year for a ryegrass based pasture. Low post-grazing herbage mass indicates an insufficient feed supply and hard grazing may slow pasture re-growth.

TABLE 2.2 – Seasonal pasture targets for a seasonal supply dairy farm. Adapted from Matthews *et al.* (1999).

Season	Average pasture cover	Pre-grazing herbage mass	Post-grazing herbage mass
		kg DM/ha	
Winter	1900-2100	2500-2700	800-1000
Early spring	1900-2000	2500-2700	1300-1400
Late spring/summer	2000-2200	2500-2700	1500-1600
Late autumn	1750-2000	2400-2700	1200-1400

The relation between intake and forage allowance is generally curvilinear (Holmes, 1987; Poppi *et al.*, 1987). Pasture intake increases with an increasing allowance until it reaches a plateau. Hodgson (1990) suggested that the herbage allowance should be two to three times the maximum daily herbage intake of the animals, but higher allowances result in pasture wastage associated with low utilisation.

Figure 2.4 shows a relationship between pasture allowance and intake summarised from recent publications (Bargo *et al.*, 2002; Dalley *et al.*, 1999; Delaby *et al.*, 2001; Kolver and Muller, 1998; Stockdale, 2000a; Wales *et al.*, 2001; Wales *et al.*, 1999b).

Low allowances, when growth is slow (early spring), during drought (summer) or with overstocking reduces feed intakes and lowers the quality of feed consumed. The diet will contain a high proportion of sheath and stem (Hodgson, 1990; Minson, 1990) and ryegrass endophyte toxins from hard grazing in summer. Cows which are last to be milked, especially young animals, will be most disadvantaged because they may reach the paddock 1-2 hours after the first cows have commenced grazing.

A high allowance is synonymous with a low utilisation, but high residual DM does not always equate to wastage. In fact allowances below about 40 kg DM/cow.day in spring are likely to limit intakes even though only 40% of available feed may be utilised (Table 2.2). High allowances will benefit cow health and production but stocking rate may be increased to manage pasture quality and prevent wastage (Brookes, 2003).

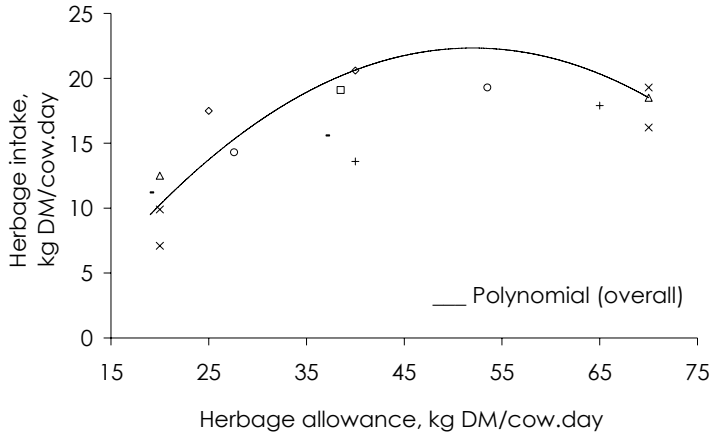


FIGURE 2.4 – Pasture dry matter intake of lactating cows over a range of pasture allowance. Each symbol (Δ, ○, ◊, +, x, -) represents one study. Pre-grazing pasture mass ranged between 2000 – 4900 kg DM/ha. Pasture types: mainly perennial ryegrass but included smooth bromegrass, cocksfoot, Kentucky bluegrass and weeds.

When excess forage accumulates, this is normally conserved as silage or hay. Conservation can be used to manage pastures but mature (flowering) grass provides a low quality feedstuff which will not achieve high intakes or productivity by lactating cows. Forage conservation must be designed to achieve high quality silage/hay as well as good pasture management, and this is often difficult to achieve.

With lower stocking rates (SR) MacDonald *et al.* (2001) showed that there was a need for increased topping and pasture conservation (Table 2.3). The lowest SR were topped 2.1 times and 1.54 t DM/ha was conserved as silage compared with no topping and little conservation at the highest SR ($P < 0.02$). Pasture utilisation increased as SR increased from 2.2 to 4.3 cows/ha ($P < 0.001$). Also, as SR increased, milksolids per cow declined but the efficiency of pasture utilisation increased.

TABLE 2.3 – The effect of stocking rate on lactation length, annual milksolids (MS) per cow and per hectare, the amount of pasture conserved and topped, and estimated economic farm surplus. Adapted from trials carried out in the Waikato region, New Zealand by MacDonald *et al.* (2001).

	Stocking rate (cows/hectare)				
	2.2	2.7	3.2	3.7	4.3
Comparative stocking rate (kg liveweight/t DM on offer)	62	76	90	103	120
Days in milk	296	278	260	238	222
Cow performance (kg MS/cow)	435	380	353	309	274
Productivity (kg MS/hectare)	967	1043	1105	1145	1168
Silage conserved (t DM/ha)	1.54	1.37	0.94	0.37	0.10
Percentage of farm area topped (%)	210	100	40	10	0
Pasture eaten:					
t DM per cow	5.06	4.65	4.24	4.01	3.71
t DM per hectare	11.1	12.5	13.6	14.9	16.0
Feed required for cow maintenance (t DM/ha)	4.7	5.7	6.7	7.7	8.7
Pasture utilisation (%)	63	70	72	81	81
Feed conversion (kg MS produced / t DM eaten)	86	82	83	77	74
EFS - Economic farm surplus ($/ha)[1]	2884	2960	3054	2940	2751

[1]EFS calculated at $4.50/kg milksolids.

Stocking rate is an important variable determining pasture intake and responses of cows to supplementary feeds. Stocking rate should be high enough to ensure that the herd eats a high proportion of the pasture growth in spring, without the need for excessive pasture conservation or topping (Figure 2.2; Table 2.3) and without limiting intakes. Conserved feeds could be used to improve lactation in summer and extend lactation in autumn (MacDonald, 1999; Pinares and Holmes, 1996).

The poor match between typical pasture growth and cow requirements are further complicated by climate. Droughts are an important problem and vary in severity, duration and timing. Other problems include high or persistent winds which can reduce pasture growth, whilst excessive rain will cause pasture damage by pugging. Menneer *et al.* (2001) reported a 40% decline in pasture re-growth due to excessive rain. Low temperatures limit growth rate, and farmers may use nitrogen fertilizers to stimulate growth, but forage nitrogen concentration may be excessive and clover growth is likely to be suppressed by rapid grass growth.

The net effect of climate and seasonality is a requirement for supplementary feed. The choice of supplement will depend on pasture quality and quantity, cow requirements and cost. In a recent review, Bargo *et al.* (2003) has indicated that high producing dairy cows fed pasture-based diets need to be supplemented to achieve their genetic potential for pasture intake, and substitution rate (the reduction in pasture

intake per kilogram of supplement) was a major factor explaining the variation in milk response to supplementation.

2.1.2 - Pasture quality

Pasture quality can be maintained by good management, but ryegrass will enter a reproductive phase in late spring (October-November) and have lower nutritive value (NV) than vegetative grass. The impact of flowering on NV of the sward will be influenced by grass cultivar, proportion of grass versus legumes and other species, climate and farmer management. Pastures which are dominated by grasses will have greater changes in NV over the cow's lactation than pastures with a higher (and sustainable) proportion of legumes and herbs. Ulyatt (1981) used growing lambs to rank grasses in terms of feeding value (Table 2.4) and Burke *et al*. (2002b) ranked forage legumes using the same methodology (Table 2.5). Legumes were almost twice as effective for lamb growth as perennial ryegrass. Grasses showed a considerable range in feeding value. Increasing the legume content of the diet is the most effective way to improve the feeding value of New Zealand pastures (Harris *et al.*, 1997a; Woodward *et al.*, 1999; Marotti *et al.*, 2002; Burke, 2003).

TABLE 2.4 – Comparative feeding value in terms of sheep live weight gain of some pasture species grown in New Zealand. Adapted from (Ulyatt, 1981).

Forage	Feeding value[1]	Number of studies
Italian ryegrass cultivar Paroa	83	1
Timothy	67	5
Perennial ryegrass cultivar Ariki	58	2
Perennial ryegrass cultivar Ruanui	52	16
Browntop in spring	52	1
Browntop in summer	43	1

[1]All values relative to white clover.

TABLE 2.5 – Comparative feeding value in terms of sheep live weight gain, forage dry matter (DM) content, composition (g/100g of DM), and metabolisable energy concentration for fresh species. Adapted from Burke *et al.* (2002b).

Forage	Feeding value[1]	DM(%)	CP[2]	NSC[3]	NDF[4]	ADF[5]	Lignin[6]	CT[7]	ME[8]
White clover	100	15	27	12	26	19	5.9		11.5
Sulla	100	12	23	18	22	18	8.6	5	12.7
Chicory	95	14	19	11	24	21	7.0		12.5
Lotus	87	16	22	13	28	20	7.1	4	11.0
Lotus major	84	16	22	12	33	22	17.1	5-9	12.0
Lucerne	82	24	30	9	30	21	6.1		10.9
Ryegrass	52	19	16	9	49	26	2.9		11.0
Maize silage		35	8	42	41	25	4.4		10.7

[1]All values relative to white clover.

[2] Crude protein.

[3]Non-structural carbohydrates.

[4]Neutral detergent fibre (cellulose + hemicellulose + lignin).

[5]Acid detergent fibre (cellulose + lignin).

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

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

[8]Metabolisable energy (MJ ME/kg DM).

Moller (1997) monitored a range of NV parameters in pastures throughout the year. Pasture samples were collected at 2-4 week intervals from four sites (two each in the Manawatu and Waikato) and analysed to predict digestibility (OMD), CP, ADF, NDF and non-structural carbohydrate (NSC) concentrations. Pasture digestibilities, which are strongly associated with energy intakes, averaged 74% - 80% in spring and autumn but were lower in summer (70%). Pasture CP and NSC followed a similar pattern to digestibility and were inversely related to fibre concentration (ADF and NDF). The author suggested that the low NSC concentrations in pasture may be insufficient to capture ammonia arising from pasture breakdown in the rumen. Together with increasing structural fibre, these factors combine to lower intakes of energy and protein, and may contribute to an accelerated decline in lactation performance following peak production in October.

The proportion of plant components also change during growth. Studies with ryegrass under controlled conditions show an increasing proportion of stem at the expense of leaf as the plants enter the reproductive stage (Table 2.6). Hoogendoorn (1986) reported similar results for ryegrass pasture (Figure 2.5). When grazed in late winter, grass leaf accounted for about 52% of DM after 8 or 16 weeks of re-growth. Lax grazing decreased the legume content of the sward and increased the amount of dead matter. When grazed in late spring, the lax grazing again resulted in more dead matter in re-growth than intensive grazing, but it also reduced the amount of leaf to 34% of DM versus 46% of DM in more intensively grazed swards. Pasture management does have an influence on proportions of leaf and stem in ryegrass.

TABLE 2.6 – Changing composition of Italian ryegrass components (% of plant DM) and *in vitro* digestibility of green and dead leaf, stem and inflorescence harvested at two week intervals after an initial cutting. Source Wilman and Agiegba (1982).

	Weeks after cutting						
	2	4	6	8	10	12	14
DM distribution (%) in:							
green leaf	82	63	37	25	15	8	4
dead leaf	0	0	3	6	8	11	14
Stem	18	37	57	63	67	65	64
Inflorescence	0	0	3	6	10	16	18
DM digestibility (%):							
green leaf	64	66	65	61	65	61	58
dead leaf				52	44	49	47
Stem	64	68	63	62	59	57	49
Inflorescence				65	62	65	63
Estimated metabolisable energy[1] (MJ ME/kg DM)	12.0	11.5	11	10.5	10.1	9.6	9.2

[1]For the whole plant, based on expected *in vivo* digestibility.

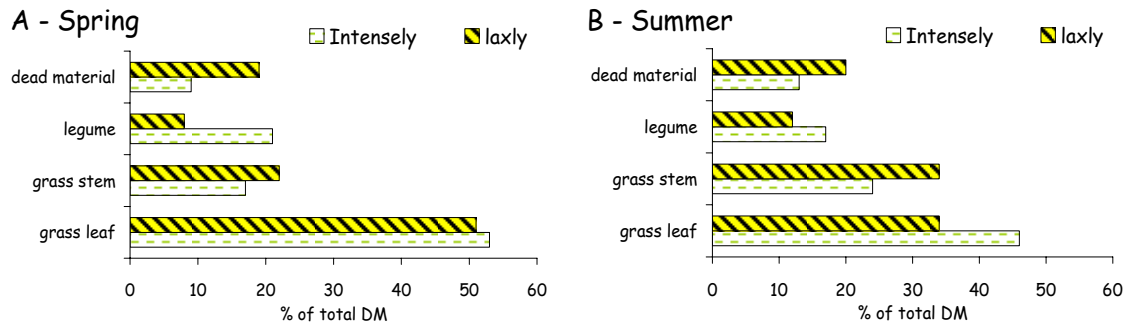


FIGURE 2.5 – Composition of New Zealand pasture in spring and summer. Adapted from Hoogendoorn (1986).

In contrast with grasses, the digestibility of white clover is less affected by maturity despite an increase in proportion of petiole. Legumes with stems, such as lucerne and red clover become less digestible as they mature, in association with increasing proportion of stem (Table 2.7).

The botanical composition and proportions of grass versus legume and of leaf, stem and dead material in pasture (Figures 2.2 and 2.5) will determine the nutritive value over the lactation. Providing feed availability is not limiting, the impact of changes in plant components on nutritive value of pasture will depend on the extent and duration of elevated proportions of stem, inflorescence and dead matter. If climate, management and grass species enable a short flowering period then dairy nutrition could be maintained by substituting poor quality pasture for high quality legume or other supplement over that period. If flowering is prolonged, the effect on pasture feeding value will be more severe. The principal objective of pasture management could be to minimise the amount of stem and dead matter (Figure 2.5).

Grass and legume leaf both contain high concentrations of CP, but the concentration of cell wall structural fibre (NDF) is much higher in grasses than legumes. These differences are even greater for C_4 grasses. Legume leaves contain higher concentration of readily fermentable DM (pectin, organic acids, and soluble sugar) than grasses and are more rapidly degraded in the rumen (Moore and Hatfield, 1994).

TABLE 2.7 – Changing composition and *in vitro* digestibility of legume components harvested at two week intervals after cutting. Source Waghorn and Barry (1987).

	Weeks after cutting					
	3	5	7	9	11	13
Structural components (% of whole plant)						
White clover						
leaf (%)	75	68	61	55		
petiole (%)	15	23	29	37		
inflorescence (%)	8	11	11	12		
Red clover						
leaf (%)	71	62	54	45	40	
petiole (%)	25	26	28	30	34	
stem (%)	4	11	16	19	17	
flower (%)			2	6	9	
Lucerne						
leaf (%)	70	50	44	39	33	
stem (%)	30	50	56	61	67	
Digestibilities						
white clover	82	81	80	76	74	70
red clover	77	77	71	67	64	54
lucerne plant	75	72	63	62	61	60
lucerne leaf	76	76	75	74	72	72
lucerne stem	73	65	58	54	54	52

2.1.3 - Interaction between availability and quality

When pasture is offered as a sole diet, even under true *ad libitum* conditions, feeding value will be limited by both the balance of nutrients in pasture (compared to cow requirements) and by voluntary feed intake. The implications of high concentrations of rapidly degradable protein and low concentration of readily fermentable carbohydrate (Moller, 1997) are confounded by a high water content in spring growth (Ulyatt and Waghorn, 1993), excess concentration of slowly degradable structural fibre in early summer and in some situations inadequate forage supply in summer. High bulk, slow rates of fibre breakdown and insufficient forage are all associated with insufficient nutrient intake and a low feeding value of pasture for dairy cows. These limitations may be compounded by fungal toxins such as ryegrass endophytes (Thom *et al.*, 1999) or facial eczema which become more important as cows are forced to eat a high proportion of feed on offer, typically during summer dry periods.

The effect of low DM percentage spring pasture on voluntary feed intake was identified by Vérité and Journet (1970) who suggested water contents above 84% would lower feed intake. A relationship between intake and pasture DM content was identified by John and Ulyatt (1987) $r^2 = 0.79$, confirming observations of Vérité and

Journet (1970). Moisture content is affected by rapid growth and forage species, but impacts upon feed intakes and lactation performance in New Zealand dairy systems have not been defined.

In contrast, effects of maturation and summer drought on feeding supply are well documented. The drop in pasture quality in October/November coincides with the reproductive phase of ryegrass growth, rising soil temperature and increasing day length, and as long as ryegrass remains the base species of dairy pasture there would appear to be limited opportunity to manipulate this with grazing management. Rotational grazing (short rotation lengths), topping of seed heads, maintaining good fertility, taking paddocks out of the system for renewal or feed conservation (silage or hay) when the herbage mass is about 3000 kg DM/ha are the standard methods used for quality control but they can only reduce rather than prevent effects of flowering. Choosing later flowering ryegrass varieties may help slow the decline in quality.

Easton *et al.* (2001) evaluated new perennial ryegrass cultivars from 17 trials undertaken throughout New Zealand since 1991 and concluded that the new cultivars released to the market yielded on average 6% more herbage annually, and 9% in summer, than previous cultivars.

Use of legumes that are active in cooler conditions, can be grazed without damage or fed fresh, as well as silages (e.g. cultivars of red clover, lucerne and lotus silages) would alter opportunities for maintaining diet quality, providing they are persistent and productive.

Kolver *et al.* (1999) showed that mowing pasture before or after grazing in summer improved pasture quality and milksolids production. The mowing gives a more uniform pasture for the next round of grazing, reducing the amount of stems and inflorescences.

Although fungal endophyte is not normally considered a nutritive characteristic of pasture, presence of deleterious endophytes have major effects on dairy production, especially in areas affected by summer drought. In many cases, pastures have flowered, have reduced quality associated with high proportions of stem and low growth rates and are in short supply. Cows are forced to graze for longer periods and to low residuals but may not achieve adequate intakes. Feeding value is low and intakes of stem and sheath will often include endophyte fungus (Easton *et al.*, 2001). The ergovaline present in both ryegrass and fescue infected with wild type endophyte causes heat stress in cattle and further depresses intake, production and profit (Table 2.8). Blackwell and Keogh (1999) found that in December, cows grazing endophyte-

free pasture produced 24% more milk than the cows grazing pasture with endophyte. This difference between performances increased progressively until mid-April.

TABLE 2.8 – Effect of ryegrass endophyte on annual milk solids production in Northland. Adapted from Blackwell and Keogh (1999).

	Total season	Milk solids[1] (kg/cow)	Milk value[2] ($/cow)
Cows grazing endophyte free pasture	335	251	828
Cows grazing pasture containing endophyte	295	211	696
Difference	40	40	132
% Difference		+ 19%	+ 19%

[1]Milk solids produced between 1 October and 29 April.

[2]Calculated using $3.30/kg milk solids.

Inclusion of dead matter and litter in the diet of cows given insufficient feed is believed to further reduce feeding value. Quantitative data concerning NV of senescent forage is scarce, but dead matter is likely to contain other pathogenic fungi (Waghorn *et al.*, 2002).

2.2 - Implications of forage maturation on feeding value for dairy cows

Feeding value is a combination of intake (availability) and nutritive value. These factors are linked in the New Zealand situation, because climate and growth physiology of grasses alter nutritive value, which in turn limits the potential intake even when feed allowance is high. In reality, allowance is also reduced in some situations and this will further limit milk production.

2.2.1 - Nutritive value

In a survey of pasture composition from the principal dairy regions in the North Island Prewer (unpublished) demonstrated a small increase in DM content, decline in concentration of NSC and a significant increase in NDF concentration in the DM in late August (corresponding with stem elongation) which continued through February-March (Figure 2.6). Predicted ME concentration and OMD declined from October through March and indicate a need for supplementation to maintain NV.

The data in Figure 2.6 also demonstrate wide ranges in values for pastures throughout the growing season, suggesting good opportunities for matching performance with feed quality.

The changes in pasture composition (Figure 2.6) correspond to changes in proportion of leaf, stem, petiole and inflorescence of grasses and legumes (Tables 2.6 and 2.8), but effects are dominated by changes in grasses (Table 2.9). The increase in structural fibre (cellulose, hemicellulose and lignin) at the expense of other components reduces the rate and extent of physical breakdown and clearance from the rumen which in turn reduces supply of both energy (VFA) and protein to the animal. Lower protein content has important implications for nutrient supply for both young growing and lactating ruminants.

The physical structure of grasses may have a greater influence on feeding value than suggested by chemical analyses. The stem of ryegrass comprises about 20% thick-walled vascular bundle and sclerenchyma cells, in contrast to about 14% in leaf (Table 2.10). These long narrow fibrous cells develop thick secondary walls which lignify with maturity and account for a high proportion of the force required to shear leaves and stems despite the relatively small cross section of these tissues (Table 2.10). Vascular tissues have important functions for plants, in transport of nutrients and maintaining plant vertical structure so leaves are able to intercept incoming energy for photosynthesis. However they also represent a significant inhibition to nutritive value for ruminants.

TABLE 2.9 – Composition (% of dry matter) and digestibility of ryegrass at 4 stages of maturity. Adapted from Waghorn and Barry (1987).

	Young leaf	Mature leaf	Head emergence	Seed setting
Non-structural carbohydrates	14	12	11	10
Organic acids	4	5	5	3
Protein	15	12	11	6
Non-protein nitrogen	4	4	3	3
Pectin	2	2	2	2
NDF	40	45	47	60
ADF	24	26	28	34
Lignin	3	4	4	7
Ash	8	8	7	6
Lipid	9	8	7	5
Digestibility of dry matter (%)	86	83	79	62

Abbreviations see Table 2.5.

TABLE 2.10 – Comparison of anatomy and nutritive characteristics of leaf and stem of C_3 and C_4 grasses and C_3 legumes. Adapted from Wilson (1991).

	Tissue types (% cross-sectional area basis)		Nutritive characteristics (% of dry matter)		
	Thin-walled [a]	Thick-walled [b]	NDF	ADL	OMD
Leaf					
C_4 tropical grass	68	32	57	2.4	61
C_3 temperate grass	86	14	43	1.8	72
C_3 temperate legume	96	4	11	1.4	81
Stem					
C_4 tropical grass	80	20	65	6.8	50
C_3 temperate grass	80	20	69	5.6	50
C_3 temperate legume	75	25	42	9.9	58

[a] Thin walled cells comprise epidermis, mesophyll and parenchyma.

[b] Parenchyma bundle sheath, sclerenchyma and vascular tissue.

NDF=neutral detergent fibre; ADL=acid detergent lignin; OMD=organic matter digestibility.

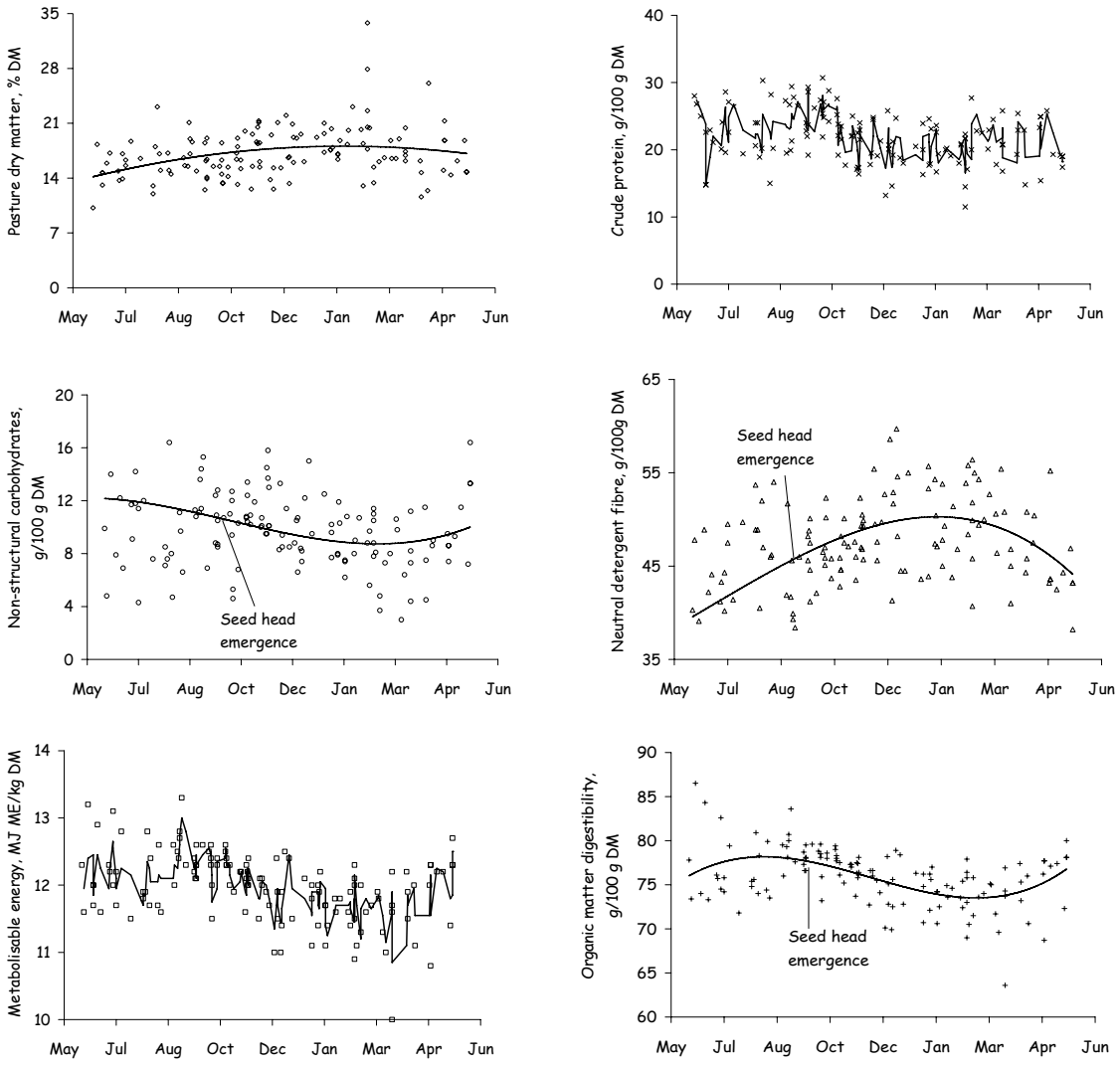


FIGURE 2.6 – Changes in pasture chemical composition thought the season in the North Island. Adapted from Prewer (unpublished).

Tropical C_4 grasses are increasing in pastures and have moved steadily south over the past decade (Bell and Keene, 1996; Davies and Hunt, 1983). They now comprise nearly half of the pastures north of Whangarei (Figure 2.1) and it is important to appreciate the very considerable differences in anatomy and feeding value of temperate and tropical grasses, as the latter are likely to become increasingly dominant in North Island pastures. They differ in basic biochemical pathways for photosynthesis, and carbon fixation as well as anatomical structures (Table 2.11).

TABLE 2.11 – Differences between C3 and C4 forages in habit and biochemistry.

	C_3 Plant	C_4 Plant
Examples of forages	Ryegrass, forage legumes; temperate grasses	Maize, tropical grasses
Temperature	Optimum 15-25 °C Minimal 0 °C	Optimum 25-40 °C Photosynthesis decreases below 10 °C
Annual production DM	22 ± 3.3 t/ha year	39 ± 17 t/ha year
Transpiration (g H_2O/g DM synthesis)	450 to 900 g H_2O/g DM	250 to 350 g H_2O/g DM
Light Intensity	Saturated at 1/3 of maximum radiation summer	Rarely saturated
Photo-respiration losses	1/3 total gross process	Very low or absent
Energetic requirements to fix CO_2 (high energy phosphate bonds ~P)	9~P	11~P

Source: Starr and Taggart (1989).

Leaves of C_3 grasses contain a high proportion of thin walled cells, which are loosely configured, so 10-35% of the tissue volume comprises intracellular air space (Hanna *et al.*, 1973; Wilson *et al.*, 1991). The thin walls and limited lignification enable rapid bacterial colonisation and rapid digestion (Chesson, 1988). In contrast, C_4 leaves and stems of both C_3 and C_4 grasses contain higher proportions of thick walled cells (scherenchyma, vascular tissue and parenchyma bundle cells in C_4 grasses) that are closely packed with only 3-12% intracellular air (Wilson, 1993) and have lignified cell walls (Table 2.10). This structure inhibits bacterial access to cell contents and to the secondary (interior) cell wall, resulting in slow rates of degradation. Bacteria are unable to digest lignin and cannot penetrate the primary (outer) cell wall unless it has been physically damaged allowing bacterial access to degrade internal secondary cell wall tissues.

Both the leaf and stem of C_4 grasses contain a high proportion of thick walled cells, similar to that of ryegrass stems. The implications of structural fibre, lignification and thick walled cells for feeding value is substantial, because of a lower nutrient release, slower digestion and especially because extensive chewing is required to enable clearance from the rumen. Maize silage is often used to supplement pasture,

but the stover will not provide a good source of nutrient for the cows fed poor quality grass. Maize stover is equivalent to any other C_4 grass and has a low NV.

The proportion of soluble dry matter (DM) is highest in actively growing forage tissue and declines as plants become mature and dormant. Soluble nutrients move from leaves to roots, and may be leached by rain. The loss of soluble DM from leaves increases the percentage of cell wall. Cell wall thickness also increases as plants mature; organic matter digestibility is lowered and rate of fermentation decreases with both the proportion and thickness of the fibre. The net effect of maturation is a decreased nutrient availability for the ruminant.

The effect of maturation is relatively minor for legumes (Table 2.7) and of little practical significance in dairy pasture because legumes usually account for less than 15% of the DM. The low proportions of legumes are usually a consequence of rapid grass growth and shading. Legumes have a high feeding value and because cows are able to achieve high intakes, they have a high NV. Hoffman *et al.* (1998) showed dairy cows fed lucerne silage produced more milk and had a high DM intake compared with cows fed ryegrass silage. In recent work Broderick *et al.* (2002) indicated that, relative to ryegrass silage, feeding alfalfa silage stimulated much greater feed intake, which supported greater milk production.

2.2.2 - Digestion of plant cells

Study of histological changes in forage leaves after *in vitro* incubation showed that leaf mesophyll cells were the first component to be digested due to thinner walls, absence of lignin and less cutinised nature of these cells compared to other leaf cells (Hanna *et al.*, 1973). The large intercellular spaces in mesophyll tissue of ryegrass enable easy access for rumen flora through damaged surfaces. Digestion begins adjacent to damaged areas and proceeded toward the more compact cells.

C_4 grass leaves contain lower proportions of mesophyll cells and the parenchyma bundle sheath cells in these grasses are lignified and slow to digest. Parenchyma bundle sheath cells may account for 20% of C_4 leaf cross-sectional area and surround vascular tissues further limiting microbial access to these fibres. Unlike mesophyll cells, the parenchyma bundle sheath cells become more lignified with age so the nutritive value of tropical grass leaf declines rapidly with maturity, whereas ryegrass leaf digestibility does not change significantly until very mature (Wilman and Agiegba, 1982).

Akin (1979) ranked the digestibility of cell walls in the order mesophyll = phloem = undifferentiated parenchyma > epidermis > parenchyma bundle sheath > sclerenchyma > lignified vascular tissue. The initial three cell types are usually rapidly and completely digested, as are the epidermis cells, except for the wall adjacent to the cuticle. The structure of parenchyma bundle sheath cell walls in C_4 grasses and their rate of digestion vary greatly between different taxonomic groups. It is mainly the cell walls of the sclerenchyma, vascular tissue, and sometimes the stem parenchyma and stem epidermis, which are digested very slowly and contain a substantial indigestible component. The degree of indigestibility depends largely on the chemical nature of their lignin polymers and linkage with fibre components (Waghorn and McNabb, 2003), but also on the extent of cell rupture.

2.2.3 - Maturation on rate and products of digestion

Ruminants have evolved digesting forage plants. They consume fibrous feeds that are not suitable for consumption by humans and single stomached animals and convert them into nutritious feeds such as meat and milk. The rumen provides a site where the rumen microorganisms can digest structural and soluble carbohydrates, proteins, and fibre. Through this digestion process, energy (mainly volatile fatty acids) and microbial protein are produced that are utilised by the animal (Figure 2.7). However, very successful selection for improved milk production has exceeded the capacity of dairy cows to consume sufficient forage feeds (Kolver *et al.*, 2002). This is especially true when forages mature.

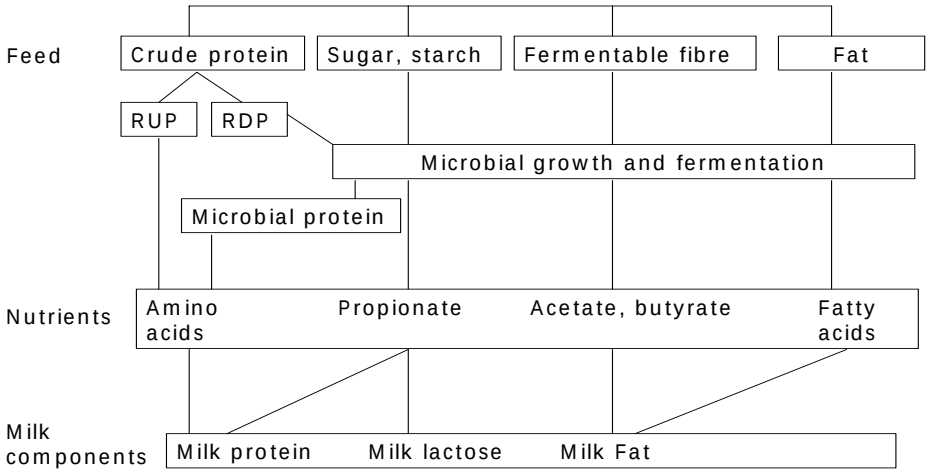


FIGURE 2.7 – Feed, nutrient flow from rumen, and milk components. Adapted from Ishler *et al.* (1996).

Principal factors that limit forage intake include availability, palatability, protein to energy ratio, distension of the reticulum and cranial sac of the rumen, and perhaps hormonal and chemical factors associated with nutrients (Forbes, 1986). There is general agreement that intake of poor quality forages containing large amounts of lignocellulose is limited by the capacity of the reticulum-rumen and the requirement to reduce particle size to pass out of the rumen (Mertens, 1994; Wilson and Mertens, 1995). Murphy (1990) concluded that when distension of the reticulum-rumen is limiting feed intake, either rate of digestion or passage from the organ must increase before further increases in consumption can occur. This means that chemical and physical properties of lignocellulose which affect digestibility and passage will determine intake and nutrient availability.

The resistance of lignocellulose to particle size reduction is an important property, because digesta must be made small enough to pass from the rumen and alleviate the inhibition caused by distension on voluntary feed intake. Also reduction of digesta particle size by chewing and rumination increases the relative surface area exposed to microbial attack and digestion increases the functional specific gravity of the particles, which enhances their probability of passing from the rumen (Ulyatt *et al.*, 1986; Waghorn and Barry, 1987).

Additional chemical factors affecting clearance and particle breakdown include stiffness or brittleness, silica, and physical factors such as the orientation of cellulose fibrils in cell walls. Waghorn *et al.* (1986) indicated that the effectiveness of chewing was greater during eating than rumination for sheep fed chopped lucerne hay, perhaps because of the brittle nature of the feed and differences in feed and digesta composition. In addition, they reported that rumination up to 14 hours post feeding was more effective than rumination between 14 hours post-feeding and subsequent feeding. This result might suggest digesta particles become more resistant to reduction with time of fermentation but it remains to be seen whether this effect is due to lignocellulose composition and whether it can be correlated with other physical measures such as maximum bending stress. The combined effects of increasing fibre content and declining digestibility causes a dramatic decline in feed value of the whole plant and lower intake by grazing animals (Waghorn, 2002).

Increased rumination chewing and gut motility to enhance particle passage may be equally important for affecting voluntary feed consumption (Beauchemin *et al.*, 2003). However, these functions will only increase supply of digestible energy if the rate of digestion is sufficiently high relative to passage (or disappearance) rates, so that particles flowing to the intestines have a low content of potentially digestible fibre.

The only option for overcoming the effects of fibre which limits the feeding value of mature grasses is to substitute a portion of the diet with a rapidly digestible feedstuff. Hence supplements may be used as an additional source of nutrients, when pasture is in short supply and also as an essential alternative to mature pasture in order to maintain nutrient intakes.

2.2.4 - Carbohydrate digestion

When carbohydrates, both structural (neutral detergent fibre) and non-structural (sugars and starches), undergo microbial fermentation, VFA are produced as the principal metabolites. The primary VFA in descending order of abundance are acetic, propionic, butyric, isobutyric, valeric and isovaleric acids. The VFA can provide up to 80 percent of the energy needs of the animal.

Acetic acid (CH_3COOH) can constitute 50 to 70 percent of the total VFA and predominates in a high forage diet. Acetate is an energy source and a major precursor for lipogenesis in adipose tissue and the mammary gland. Acetate production is essential to maintain adequate quantities of milk fat (France and Siddons, 1993).

Propionic acid (CH_3CH_2COOH) can make up 18 to 22 percent of the total VFA when forages are fed, but accounts for a higher proportion of VFA in cattle fed high grain diets. Propionic acid is converted to glucose in the liver and is essential for lactose and protein synthesis.

Butyric acid ($CH_3CH_2CH_2COOH$) accounts for 10 to 15 percent of the total VFA in forage fed animals. It is largely converted to B-hydroxybutyric acid (BHBA) during absorption through the rumen epithelium. BHBA is used for fatty acid synthesis in adipose and mammary gland tissues and as an energy source (Ishler *et al.*, 1996).

Fibre-degrading bacteria are the principal sources of acetate, propionate and butyrate, where as valerate and iso-acids are derived largely from amino acid digestion. The proportion of VFA is greatly influenced by diet. Despite differences in the microbial population and in feed intake, ruminal VFA proportions are fairly stable among forage diets, but differ for forage versus concentrate based diets. As the forage to concentrate ratio decreases, the acetate to propionate ratio also decreases (Agnew and Newbold, 2002), indicating a change in microbial populations.

Although proportions of VFA produced from contrasting diets have been characterised, ruminal conditions (e.g.: ratio of fibre: readily fermentable substrates) can affect microbial populations and their production of VFA from dietary components. This is illustrated in Table 2.12 which shows that the acetate to propionate ratio resulting

from fermentation of hemicellulose in a high forage diet was 3.2, but only 2.2 when fermented in a high grain diet. The acetate to propionate ratio from cellulose fermentation also varied with diet (7.3 for a forage diet and 13.2 for a grain diet) and rates of production were much higher for hemicellulose digestion (Murphy *et al.*, 1982).

TABLE 2.12 – Proportions of VFA arising from digestion of contrasting carbohydrate fractions as affected by forage and concentrate diets. Adapted from Murphy *et al.* (1982).

Substrate	Diet [b]	Proportion of carbohydrate converted to [a]			
		Acetate	Propionate	Butyrate	A : P ratio
Soluble carbohydrate [c]	F	0.69	0.21	0.11	3.3
	C	0.45	0.21	0.30	2.1
Starch	F	0.59	0.14	0.21	4.2
	C	0.40	0.30	0.20	1.3
Cellulose	F	0.66	0.09	0.23	7.3
	C	0.79	0.06	0.07	13.2
Hemicellulose	F	0.57	0.18	0.21	3.2
	C	0.56	0.26	0.11	2.2

[a] Ratios do not add up to 100 because valerate and the iso-acids are not taken into account.
[b] F=forage diets; C=diets containing more than 50 percent of a cereal-based concentrate diet.
[c] Soluble carbohydrate fraction includes organic acids and pectin in this analysis.

2.2.5 - Protein digestion

The utilisation of structural fibre by ruminants is dependent on microbial degradation, and therefore on microbial growth. The rumen fauna includes a diverse range of bacterial species, many which have proteolytic activity so there is extensive and rapid degradation of forage protein to peptides and amino acids. The high solubility of protein in fresh forages makes it vulnerable to degradation by a range of bacteria, including hyper-ammonia producing species (Attwood *et al.*, 1998). About 70% of plant protein is degraded in the rumen when fresh forages are fed (Ørskov, 1999).

Amino acids may be used for microbial growth but most bacteria utilise ammonia released from plant amino acids (AA) for microbial protein synthesis. Degradation of de-animated AA yield branched chain and other VFA. The rate of ammonia absorption through the rumen wall increases at higher ruminal pH values, but concentrations above 1000 mg/L or 20 mg/L blood are toxic (Annison *et al.*, 2002).

Ammonia production in excess of bacterial utilisation is converted to urea at a net metabolic cost of about 12 kJ/g NH_3-N (Baldwin, 1995).

Although protein degradation provides substrates for bacterial growth, it can account for an excessive loss of forage protein and reduces AA available for absorption. Microbial protein has amino acid composition that is very close to casein and has higher biological value (BV) than plant protein. The BV of microbial protein has been reported to be from 66 to 87 BV. Protein supplements which are fed with concentrates may be heat treated to increase the proportion of undegradable protein (UDP). Inclusion of condensed tannins (CT) in forage diets have reduced the degradation of plant protein (McNabb *et al.,* 1996) and increased the flow of plant protein to the abomasum from 0.30 to 0.44 of intake in sheep (Waghorn *et al.*, 1994). About 80% of microbial nitrogen is protein.

When a good quality pasture is fed, with an OMD of about 75% and containing 25% CP in the DM the loss to degradation is about 175 g CP/kg DM or 233 g CP/kg digestible organic matter intake (OMI). Microbial growth in ruminants fed pastures is lower than for concentrate diets, where CP content is about 18% of the DM and comprises approximately 35% UDP. About 100 g microbial CP is synthesised per kg pasture digestible OMI (ARC, 1980). Equivalent values for concentrate diets are about 130 g microbial CP/kg digestible OMI. Table 2.13 summarises losses and availability of CP for cows fed pasture in spring and summer, compared to those given TMR, but it should be appreciated that predictions of microbial CP from cows fed pasture using National Research Council (NRC, 2001; pages 55-56) information suggest microbial CP will be lower than values from Agriculture Research Council (ARC, 1980). Data from NRC (2001) suggest only about 67 and 86 g microbial CP will be synthesised from each kilogram digestible organic matter from spring and summer pasture respectively. The excess ammonia will be excreted at an added cost to the cow.

Data summarised in ARC (1980) and NRC (2001) suggest microbial growth is about 80 g microbial amino acid/kg pasture digestible OM, but the range could extend from 60 to 90 g. Values for concentrate based diets are about 100 g microbial AA/kg digestible OMI.

The impact of high CP concentration in spring pasture and the high degradability of CP in both spring and summer forage is emphasised by comparison with the TMR diet (Table 2.13). Net absorption of AA required for metabolism and milk production is substantially less when grass is fed with similar quantities from plant and microbial origin, compared with TMR ration. Care must be exercised when supplements

are chosen at any time during lactation to complement the quality and CP content of pasture available to cows. Energy is often considered to be the first limiting nutrient for milk production in pasture based systems, but metabolisable protein supply can be limiting at some times.

2.2.6 - Lipids digestion

Rumen microbes rapidly and extensively modify dietary lipids. Hydrolysis of galactolipids (from plant leaves) and triglycerides (from seeds) releases glycerol and galactose which are fermented to VFA. Liberated fatty acids may adhere to the surfaces of bacteria and feed particles but are not degraded by rumen microbes in significant amounts. Some fatty acids are incorporated into cells but most are hydrogenated prior to absorption from the intestine.

TABLE 2.13 – An illustration of nitrogenous fluxes and microbial growth in lactating cows fed spring and summer pasture typical of New Zealand farming and a total mixed ration (TMR).

	Spring pasture	Summer pasture	TMR [a]
Milk (kg/day)	25	15	30
Milk solids (kg/day)	2.10	1.20	2.40
Dry matter intake (kg/day)	16	12	18
Organic matter digestibility (g/100g DM)	75	67	75
Digestible organic matter intake (DOMI; kg/day) [b]	11.16	7.48	12.56
Crude protein (g/100g DM)	25	15	18
Crude protein intake (kg/day)	4.00	1.80	3.24
Rumen degradable protein intake (kg/day)	2.80 (70%)	1.26 (70%)	1.13 (35%)
Microbial crude protein (g/kg DOMI) [c]	98	98	123
Microbial crude protein (g/day)	1094	733	1545
Microbial AA absorbed [d]	700	469	989
Plant AA absorbed [e]	840	378	1474
Total AA absorbed	1558	847	2463
Faecal crude protein [f]	800	630	972
Milk crude protein [g]	900	540	1080
Urinary N [h]	368	101	190
Cost of urea synthesis (MJ/day)	4.41	1.21	2.28

[a] Intakes and performance from Kolver *et al.* (2002). Begin of lactation for TMR cows.

[b] Assume organic matter = 0.93 of dry matter and 0.66 of digestion occurs in the rumen.

[c] Microbial crude protein yield assumes 186 g CP (29.7 g N)/kg OM fermented in the rumen, or about 123 g CP/kg DOMI for diets where N flow to the intestines equal N intake. Calculations here assume 0.20 of N is lost as ammonia absorption for cows fed pasture and there is no net loss for the TMR diet (NRC, 2001) but predictions from NRC (2001) page 56 suggest values for spring, summer and TMR would be about 67, 86 and 147 g/kg DOMI respectively.

[d] 0.80 of microbial CP is amino acids, of which 0.80 are absorbed.

[e] 0.70 of undegradable dietary protein is absorbed.

[f] Assume 0.80 digestibility for spring pasture, 0.65 for summer and 0.70 for TMR.

[g] Milk protein is 3.6 g/100 g.

[h] Assume no gain or loss of N to body weight and a cost of 12 kJ/g NH_3-N for urea synthesis (Baldwin, 1995).

2.3 - Consequences of forage maturation for the lactating cow

The potential for productivity by New Zealand cows has been illustrated by Kolver *et al.* (2002) (Table 2.1) who showed that a TMR supplied *ad libitum* was able to sustain lactation more efficiently than pasture given at a high allowance of 60 kg DM/cow.day (Figure 2.8).

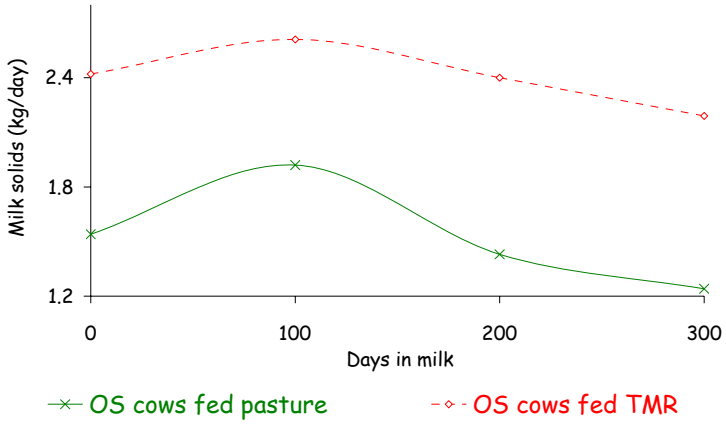


FIGURE 2.8 – Milk solids (kg/day) production in early, mid and late lactation for overseas Holsteins (OS) cows fed pasture or total mixed ration (TMR) diet. Source Kolver *et at.* (2002).

The low peak lactation of cows fed pasture may be a consequence of pre-calving nutrition, an inability to consume sufficient nutrients from lush spring grass, or the nutrient balance of the diet, but these effects are confounded by grass maturation in late spring. The impact of maturation is often made worse by insufficient supply (Figure 2.1), typical of many dairying regions which rely on rainfall, rather than irrigation.

The impact of lowered NV (and possible feed shortage) typical in late spring/summer in many regions is made worse by substantial body weight losses after calving. Low feeding value can result in post-partum anoestrus (period of non-cycling) which has major implications for calving in the following season (Macmillan, 1997; Verkerk *et al.*, 2000) as well as low milk production.

The timing and choice of supplements will be crucial if cow performance is to be maintained and the opportunity to complement mature summer pasture is a focus of this thesis.

The rapid decline in milk production post peak lactation (Figure 2.8) is likely to result from limited ability to mobilise body tissue as well as lower NV of feed available to the cow. The mobilisation of body reserves during early lactation can result in daily live weight losses up to 1 kg, which are clearly unsustainable. Maximum energy deficit occurs within 2 - 3 weeks after calving and cows may achieve a positive energy balance approximately 60 days after calving (Holmes *et al.*, 2002). The loss of body weight is indicated by condition score (CS) and in the post-calving period one body CS is about 25 kg of live weight for a mature Jersey cow and more likely 40 kg for a large Holstein-Friesian dairy cow (Grainger and McGowan, 1982).

Although it is normal for cows grazing pasture to lose weight after calving, a loss of more than one CS during early lactation indicates the feeding programme or management are not adequate to allow cows to achieve high milk production and remain healthy (Figure 2.9).

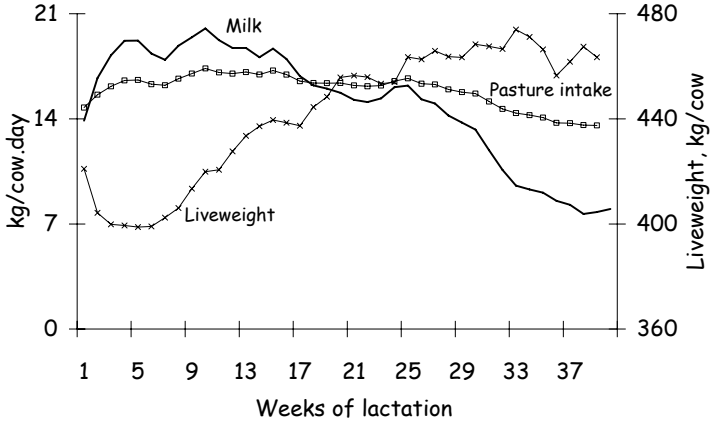


FIGURE 2.9 – Phases of lactation cycle for New Zealand dairy cows fed ryegrass/white clover pasture *ad libitum* (> 60 kg pasture DM/cow.day). Adapted from Holmes *et al.* (2002). Pasture intake (kg DM/cow.day) was estimated by: (0.372 x FCM + 0.0968 x $LW^{0.75}$) x (1 - $e^{(-0.192x(WOL+3.67))}$) where FCM = four percent fat corrected milk (kg/day), LW = liveweight (kg), and WOL = week of lactation (NRC, 2001).

If a lactating cow is neither gaining nor losing body weight (BW), its feed requirements will depend on its live weight and on its milk yield (Table 2.14). For example, to gain 1 body CS in 70 days, the feed requirement for a Friesian cow producing 1.4 kg MS/day is 15.8 kg leafy pasture DMI/day.

TABLE 2.14 – Feed requirements of lactating cows with various levels of milk production, losing, gaining or maintaining a constant level of body condition (kg leafy pasture DM eaten/day). Adapted from Holmes *et al.* (2002).

		Live weight of cow (kg)		
Condition score (CS)	Milk solids (kg/day)	370 (Jersey)	450 (Friesian)	550 (Holstein)
No change in body condition	0.7	8.6	9.6	10.5
	1.4	12.6	13.9	14.9
	2.1	16.6[a]	18.2[a]	19.2[a]
Losing 1 CS in 70 days	1.4	11.6	12.6	13.6
	2.1	15.6[a]	16.7[a]	17.9[a]
Gaining 1 CS in 70 days	0.7	9.9	11.4	12.3
	1.4	13.9	15.8	16.7

[a] Cows may be unable to eat these amounts of feed, especially in the first month of lactation.

A primary goal is to manage the feeding program to properly manipulate body condition loss and minimize the duration and extent of negative energy balance. High milk yield does not cause excessive weight loss if the feeding program is well-tuned, but this can be difficult when grass is the only dietary component. Successful feeding management during spring will enable cows to get in calf and be gaining bodyweight at the beginning of summer.

The genetic potential of modern cows exceeds the ability of all dairying systems in the world to meet nutrient requirements – the cow is designed to loose body condition in early lactation. This is due in part to the bulk of ryegrass (Ulyatt and Waghorn, 1993; Waghorn, 2002) as well as its rate of clearance from the rumen, microbial growth and composition of absorbed nutrients (Mertens, 1992b).

Hodgson and Brookes (1999) described three main factors affecting pasture intake:

1. nutrient requirements of the cow;

2. factors associated with distension of the alimentary tract, digestibility and rate of digestion and passage of the feed;

3. limitation to the potential pasture intake resulting from a combination of pasture and animal factors affecting grazing behaviour.

Several researchers have reviewed the factors controlling pasture intake by ruminants (Demment *et al.* 1995; Dulphy *et al.* 1989; Forbes, 2000; Mertens, 1994; Poppi,

1996) but none explain the constraints linked with intake of fresh ryegrass, although Waghorn (2002) identified the high moisture content and physical bulk of pasture as a likely constraint to intake by New Zealand dairy cows. He showed that rumen fill was up to 22% of bodyweight (BW) compared to 15-20% of BW in North American cows fed forages and suggested limited increases in milk production could be expected from current feeding practices. Low pasture DMI has been identified as a major factor limiting milk production of high genetic merit cows under grazing conditions (Clark *et al.* 2001; Clark *et al.* 1997b; Kolver and Muller, 1998; Waghorn, 2002).

2.2.7 - Options for maintaining cow performance

It is essential to improve the intake and nutritive value of diets for dairy cows to reduce the post peak decline in milk production. Careful management of pastures is essential to minimise the intensity and extent of flowering that impacts on rumen fill and to provide sufficient forage during periods of poor growth. Use of supplements will be necessary to overcome detrimental aspects of grass growth on animal performance. The overriding considerations associated with dietary supplementation must be economic viability, and in New Zealand fresh or conserved forage crops will be the main source of supplements. Principal variables will be type of supplements, time of supplementation and extent to which the supplement complements or substitutes for pasture. Supplements vary in nutritive characteristics, including protein content and degradability, DM content, ME content and acceptability, so appropriate choices should be made to optimise provision of nutrients. Table 2.15 summarises cow responses to a range of forage-based supplements evaluated in New Zealand.

Supplements may be used to provide more ME, more protein or both. Provision of additional ME will be possible if pasture supply is inadequate, but when a high pasture allowance is available, substitution is likely. When the supplement has a high water or fibre content, it may not increase intake, but use of silage (e.g.: maize silage) may increase total intake of lactation cows (Table 2.15).

TABLE 2.15 - Lactation responses of New Zealand cows in post peak lactation when fed supplements with pasture or fed high quality legumes. Adapted from Burke (2003).

Diet	DMI (kg)	ME (MJME/ kg diet DM)	CP (g/100g DM)	Milk yield (kg/ day)	Milk solids yield (kg/day)	Response (g milk solids/ kg DM fed)
[1] Ryegrass + 20%WC	10.9	10.3	21.5	8.5	0.80	74
Ryegrass + 50% WC	12.1	10.5	22.6	10.0	0.93	77
Ryegrass + 80% WC	12.0	10.6	23.8	9.8	0.93	77
[2] Ryegrass	12.1	9.5	14.3	10.2	0.96	79
Ryegrass + 25% WC	13.1	10.1	16.4	12.5	1.17	90
Ryegrass + 50% WC	14.8	10.5	18.4	13.6	1.24	84
Ryegrass + 75% WC	15.8	10.7	21.9	13.7	1.26	82
[3] Pasture	-	9.7	24.3	10.4	0.87	-
Pasture + turnips	-	9.8	23.2	11.3	0.99	34
Pasture + chicory	-	9.9	24.1	10.8	0.93	32
[4] Pasture	9.6	10	19.7	8.6	0.73	76
Pasture + turnips	12.1	10.7	17.1	10.6	0.93	82
Pasture + sorghum	11.0	9.7	17.4	9.2	0.82	80
[5] Pasture	12.4	10.4	11.6	12.4	0.93	75
Grass + 75% WC	15.0	11.3	19.1	16.6	1.26	84
Grass + 75% Lotus C	13.8	11.8	20.8	18.3	1.38	100
[6] Ryegrass	14.2	10.6	18.2	10.0	0.83	59
Lotus C. – CT	16.7	11.4	25.6	13.8	1.13	68
Lotus C.+ CT	16.8	11.4	25.6	16.5	1.40	83
[7] Pasture (restricted)	12.5	10.0	17.4	13.2	1.00	80
Pasture (full)	18.5	10.1	18	17.0	1.11	70
Pasture + PS	17.0	10.3	16.9	14.3	1.11	65
Pasture + MS	16.6	10.1	14.4	13.7	1.12	68
Pasture + Lotus CS	17.2	10.3	19.1	13.7	1.29	75
Pasture + SS	15.7	10.0	16.7	13.7	1.10	70
[8] Pasture	16.9	9.2	19.7	11.0	0.87	52
Pasture + MS (66:34)	14.5	9.8	15.6	11.3	0.86	59
Pasture + SS (66:34)	15.1	9.6	19.3	12.4	0.97	64
Pasture + MS:SS (66:16:16)	15.5	9.7	18	13.2	1.02	66
Mean						73
Range						32-100

Abbreviations: DMI: DM intake; WC: white clover; Lotus C: *Lotus corniculatus*; MS: maize silage; SS: sulla silage; Lotus CS: *Lotus corniculatus* silage; CT: condensed tannin (– CT indicates inactivation by daily administration of polyethylene glycol).

[1]Harris *et al.* 1997b: *ad libitum* feeding indoors; [2]Harris *et al.* 1997a: *ad libitum* grazing; [3]Waugh *et al.* 1998: 4-8 kg DM supplement + 25 kg DM pasture offered/cow; [4]Clark *et al.* 1997a: 4 kg supplement + 25 kg DM pasture offered/cow; [5]Harris *et al.* 1998a: *ad libitum grazing*; [6]Woodward *et al.* 1999: *ad libitum* feeding indoors; [7]Woodward *et al.* 2002: 5kg DM supplement + 25 kg DM pasture allowance/cow; [8]Woodward *et al.* unpublished: *ad libitum* feeding indoors.

Substitution rate has been reviewed by Penno (2002) and Bargo *et al.* (2003) for cows grazing pasture. In general terms, when cows are consuming high quality forages, SR increases as the energy intake of the cow increases from either forage or the supplement. Penno *et al.* (1998) found a greater MS response to supplements during winter and summer than spring and autumn with cows grazing pasture but stage of lactation did not affect MS response. They suggested supplementary feeding decisions were based on the level of under-feeding and in Waikato farming the response to supplement was directly proportional to the increase in ME supply by the supplement (Penno *et al.*, 1998). They also concluded that the full lactation responses to supplements were two fold greater than those measured in short term feeding trials.

The results of trials conducted by Penno *et al.* (1998) are due in part to an inadequate supply of pasture, so the supplements provide more ME, but efficient dairy production must provide sufficient feed and the choice of supplement should focus on dietary nutritive value as well as sufficiency. The high CP content of spring pasture and losses to ammonia become a substantial metabolic cost which can exceed 4.4 MJ/day (Table 2.13).

Forage legumes that contain condensed tannins (CT) (*Lotus corniculatus*, *Lotus pedunculatus*, *Hedysarum coronarium*) may have potential in the pasture-based system because the CT can reduce protein degradation in the rumen and allow a greater passage of undegraded protein to the small intestine for absorption (McNabb *et al.*, 1996). These forages could improve the UDP supply when used to supplement spring pastures and at others times of the year. Woodward *et al.* (1999) showed that cows fed *Lotus corinculatus* produced 51% more milk than pasture-fed cows, with the response due to the effect of CT (Table 2.15).

The reasons why New Zealand farmers currently do not supplement with legumes (fresh or ensiled) are due to failure of legumes to produce competitive yields of dry matter, difficult agronomic or management requirements to maintain pure or mixed swards, and the high cost of ensiled these forages.

It is clear from past experiments (Table 2.15) and the changing quality of pastures that the choice and supply of supplements is complex and must be based on the chemical composition, rates of digestion, and nutrient supply from existing pasture as well as characteristics of the supplement. These values can be measured in cow feeding trials, but these are very expensive and time consuming. An alternative procedure is to characterise both pasture (at different maturity and composition) and supplements using *in vitro*, *in sacco* and chemical analyses to predict cow responses.

This form of evaluation is indirect but cost effective. Results from indirect evaluations must be validated against cow production trials where supplements are given with pasture and a good relationship could form the basis for a predictive model to provide appropriate supplements for cows given a wide variety of pastures.

2.4 - Techniques for evaluating feeding value of pasture and supplements

2.4.1 - Chemical composition

Measurements of chemical composition by wet chemistry or prediction with NIRS have a role to play in determining nutritive value, but they are not discussed here and on their own are unable to predict animal performance.

2.4.2 - *In vitro* incubations

In vitro systems to study rumen fermentation have been used extensively for more than 40 years. They involve fermentation of different feeds, often with rumen fluid (microbial inoculum) but some times with enzymes in a buffered anaerobic environment. These systems differ in their degree of complexity and a brief indication of alternative methods with their principal attributes and weaknesses is given below, with several factors affecting the outcome of these systems.

Many *in vitro* incubations are batch cultures where incubations proceed for set time periods without addition or removal of material from vessels. A common evaluation is that of protein degradation which can be calculated from the ammonia nitrogen released from protein degradation with losses to both ammonia nitrogen (NH_3-N) and utilisation by ruminal microbes. Interpretation of proteolysis is affected by the nature of the substrate, incubation time and feed preparation.

The problem of N incorporation into bacteria has been avoided through the use of chloramphenicol and hydrazine sulphate to inhibit microbial protein synthesis and prevent AA and ammonia nitrogen utilisation by ruminal microbes (Broderick, 1987). This method can give degradation rates similar to *in vivo* observations (Broderick and Albrecht, 1997) but values were about 30% lower than uninhibited systems (Hristov and Broderick, 1994).

It is more logical to allow a normal microbial growth, without artificial inhibition and to use $^{15}NH_3$ to quantify microbial nitrogen utilisation (Hristov and Broderick, 1994).

Protein degradation rates are computed as appearance of NH_3 plus net microbial protein synthesis.

A major limitation of the Tilley and Terry (1963) method is the need for rumen fistulated animals to provide rumen fluid. This can be avoided by using enzymatic method and good correlations can be obtained between enzymatic digestion and *in vivo* or *in vitro* digestibility for a variety of forages. Jones and Theodorou (2000) summarised enzymatic methods and indicated potential problems, especially with feeds having high levels of ammonia and free AA (as in forage silages). The breakdown of slowly degraded residual proteins (i.e. less than 0.01 h^{-1}) must be computed from the appearance of additional ammonia and AA in the presence of high background nitrogen and is not accurate. Microbial activity tends to decrease over time, especially if pH is not maintained. The accumulation of ammonia and AA may also result in end-product inhibition of enzymes (Calsamiglia *et al.*, 2000).

It is important to realise that these problems apply equally to enzymatic or microbial systems. The value of either system will depend on research objectives so it is easier to obtain a ranking of plant material than to obtain absolute values which match *in vivo* degradation rates.

2.4.2.1 - Continuous culture

The most physiologically appropriate approach to determining constituent degradation *in vitro* would be to design a system that carefully simulates ruminal fermentation. Various continuous-culture fermentation systems have been designed to simulate the ruminal environment, enabling the study of ruminal microbial ecology and digestion of nutrients, for example dual-flow continuous culture (Hoover *et al.*, 1976) or single-flow Rusitec (Czerkawski and Breckenridge, 1977). Advantages of these systems compared with *in vivo* measurements include reduction in cost, time and variation among experimental units. Continuous cultures avoid problems of products inhibition or altered pH but the microbial population will not mimic *in vivo* over long periods. Compared to *in vivo*, there are no complications from endogenous N sources, and digesta flow markers are not required because passage rates are regulated and measured directly. However, similar problems exist as with *in vivo* measurements; reliable techniques are required for isolation of microbial cells and for differentiation of effluent digesta into microbial and dietary fractions. These systems are tools for research and modelling of ruminal fermentation, but are elaborate and expensive, require inoculation with ruminal digesta and may not be suitable for routine analysis of microbial digestion for individual feed ingredients (Calsamiglia *et al.*, 2000).

2.4.2.2 - Products of fermentation

In vitro incubation enables the products of digestion to be quantified. Measurement of VFA and ammonia production during microbial fermentation can indicate the nutritive value (NV) of forages in terms of protein losses to degradation and yields of VFA. Measuring ratios of VFA especially the proportion of glucogenic: lipogenic precursors (propionate: acetate and butyrate) also help understanding the NV of feeds.

The measurements of ammonia yield indicate net proteolysis but omits the quantity of ammonia-N incorporated into bacteria, which Barrell *et al.* (2000) reported to be 10% of white clover N and almost 20% of degraded N after 24 hours. Hence a measure of microbial growth would add value to data sets because it will indicate the extent of N capture and also the nutritive value of the substrate for bacterial growth.

The proportion of plant N converted to ammonia indicates susceptibility to degradation and can indicate effects of CT on proteolysis. Ammonia production will indicate relative ability to meet bacterial N requirements, the amount of NH_3 needing to be detoxified post-absorption and the amount of protein which has not been degraded and is potentially available for AA absorption *in vivo*. Hence proportional loss of plant N to ammonia will indicate the likelihood of insufficient N for bacterial growth, for example when plant CP concentrations are less than 9% of the DM *in vivo*. Conversely a large ammonia production *in vivo* results in a large toxic load to be converted to urea for excretion. The measurements of net ammonia production during *in vitro* incubation provides a relative measure of protein breakdown in the rumen, nevertheless this does not account for the nitrogen taken up by the bacteria.

2.4.3 - *In situ* or *in sacco* incubations

The *in sacco* technique is synonymous with *in situ*, dacron or nylon-bag technique. *In sacco* incubations measure the disappearance of feed components from a bag containing the test diet after incubation, for a variable period, in the rumen of an animal fitted with a rumen fistula. Degradability of DM, CP, fibre and energy can be measured against time. The technique was first used to provide a dynamic assessment of protein degradation by Mehrez and Ørskov (1977).

Adesogan *et al.* (2000) suggest the *in sacco* rumen degradability technique to be theoretically superior to *in vitro* digestibility techniques because it provides information on the dynamics of forage digestion. Digestion kinetics are important, but

such information will be affected by the way material is prepared for incubation and no information is obtained on the products of digestion when the *in sacco* method is used.

Several authors (Weiss, 1994; Nozière and Michalet-Doreau, 2000; Ørskov, 2000) have reviewed the methodology of the *in sacco* incubations and identified several important sources of variation in results from this technique. These are briefly described below, as they influence the interpretation of data obtained in this study.

2.4.3.1 - Animal and diet

Mehrez and Ørskov (1977) concluded that three cannulated animals should be used and that replication within animals and between days was not worthwhile and made little difference to the total variance. However, if the objective is to rank the feed potential of forage selection for forage or quality, then only one animal needs to be used (Ørskov, 2000).

2.4.3.2 - Host dietary effects

It is important to standardise diets of animals use for *in sacco* incubations (and provision of inoculum for *in vitro* incubations). Mould and Ørskov (1984) fed cattle a high-quality roughage diet with about 25 g nitrogen (N) kg^{-1} digestible DM. Reasons for different degradation rates observed with contrasting rations of roughage and grains are not well defined but probably relate to a combination of ruminal microbial (Weimer *et al.*, 1999) and physical factors that are subject to dietary changes.

Animals should be fed twice daily at a maintenance level or slightly above, with a minimum interval between meals of 8 hours.

Weakley *et al.* (1983) showed clearly that diet affects the DM and N disappearance from dacron bags in the rumen for cows fed different hay: grain ratios. When a soybean meal was incubated, cows fed a high grain diet resulted in the slowest DM and N losses. The type of forage fed to the recipient animal can also affect *in sacco* disappearance of forages, but results have been inconsistent (Weiss, 1994).

2.4.3.3 - Bag type and sample size

The type of material used for the *in sacco* bags is polyester, nylon or dacron, with the latter being readily available from the Ankon® Corporation. The principal requirement for bags, aside from indigestibility, is a consistent pore size, so a welded monofilament mesh is preferable to a woven multifilamentous mesh (Australian Agricultural Council (AAC), 1990). The pore size must permit free exchange of fluid and

microorganisms between the bag and the ruminal liquor and be small enough to prevent loss of indigestible particles or the entry of feed particles. The pore size recommended for *in sacco* incubations is between 30 and 50 µm (Hvelplund and Weisbjerg, 2000; Nocek, 1988).

Sample behaviour and digestion kinetics will also be affected by the quantity in the bag. There should not be an excess of sample in the bag because this could affect the rate at which bacteria enter the bag and colonise the test material but, equally, sufficient sample needs to be incubated so sufficient residues are available for analyses. The latter point can be accommodated by using bags of different sizes (e.g.: 5 x 10 cm versus 10 x 20 cm) and this will be affected by sample preparation. Fresh minced forages may contain as little as 10% DM, so a substantial bulk must be placed in a bag, compared to the conventional use of freeze dried and ground material. Hvelplund and Weisbjerg (2000) recommend *in sacco* incubations contain 10 - 16 mg DM/cm^2 of bag surface.

2.4.3.4 - Bag placement and incubation sequence

The rumen environment is often compacted and layered with a raft or mat in the dorsal aspect with a liquid phase in the ventral rumen. This heterogeneous environment makes it difficult to obtain a consistent placement of bags, so typically they are contained within a large aperture lingerie bag and weighted to reduce mobility and prevent floatation. Without the weight the bags may float on the surface of the rumen and not become properly incorporated into the digesta. A cord length at least equal to the distance from the cannula to the bottom of the rumen is recommended for attachment to the cannula.

For comparison of degradation rates, it is probably better to introduce all the bags at the same time in the rumen because there will be a similar microbial environment for all bags during initial stages of digestion, compared to the sequential placement method (Michalet-Doreau and Ould-Bah, 1992).

2.4.3.5 - Washing and drying procedure

For washing, cold water should be used. Hvelplund and Weisbjerg (2000) recommend an automatic machine washing as preferable for standardisation, with a washing time of 10 - 15 minutes. Different methods of washing bags post incubation gave extremely variable results (Huntington and Givens, 1995). There appears to be a need for further research in order to standardise this procedure and more work is required to elucidate the optimum process. However, Cherney *et al.* (1990) compared

and evaluated effects of length of time of machine and hand rising on DM disappearance, DM remaining and their standard error (SE). Machine rinsing twice for two minutes or hand rinsing bags resulted in a similar SE. Machine rinsing twice for five minutes was too long and resulted in an under-estimate of DM remaining after incubation.

2.4.3.6 - Microbial contamination

Microbial contamination arises from bacteria adhering to plant residues, which are not removed by washing. The extent of contamination is dependent upon incubation time and the extent to which forages have been degraded, so contamination is not constant. Correction for attachment is more important with high microbial contamination values and adds an additional variable to the assay. Attachment is measured using microbial ^{15}N-labelled markers (Hvelplund and Weisbjerg, 2000) but such measurements are not practical during routine *in sacco* incubations.

The importance of microbial contamination appears to be minor with concentrates (Nocek, 1985) but Mathers and Aitchison (1981) demonstrated about 20% of the residual N in lucerne samples arose from microbial contamination after 24 hours in the rumen. For lucerne hay, Blair and Cummins (1983) cited by Nocek (1985) reported 4.1, 11.7 and 8.7% of total N was microbial after 12, 18 and 30 hours of ruminal exposure with a subsequent water wash, respectively. These effects can influence calculation of degradation kinetics, with reductions of 0.5 – 5 hours in lag time of hay corrected for microbial N contamination compared with non-corrected ruminal N digestion (Nocek and Grant, 1987). The effects of correction on rates of forage digestion were variable and although microbial contamination of residues does underestimate N degradation, removal can be expensive, laborious or inaccurate (Olubobokun *et al.*, 1990).

2.4.3.7 - Modelling *in sacco* degradation kinetics

A number of methodological factors affecting the experimental measurements of *in sacco* disappearance have been considered (Huntington and Givens, 1995; Nocek, 1988; Nozière and Michalet-Doreau, 2000) and the choices of mathematical models used to fit curves and estimate rumen degradation parameters have been described by López *et al.* (1999).

The characteristics of feed degradability have been defined as the soluble fraction, A; the insoluble but degradable fraction, B and the speed at which the B fraction is degraded (k). These rate constants have been used in an attempt to

develop a system that can predict not only feed nutritive value but consumption as well (Ørskov, 2000).

McDonald (1981) was the first to define a lag phase indicating that the microbes take time to adhere to the substrate and for a time at the beginning of the *in sacco* incubation (up to 2 - 4 hours) there is no loss in DM. In fact for some feeds there may be a small increase in DM. The lag phase will be used in all digestion kinetics studies described in this thesis.

The characteristics of the degradation curve are described for DM by equation (i):

$P = A + B\ (1-e^{-k\ (t-L)})$ Equation (i) where:

P = potential degradability (%)

t = incubation time (hour)

A = soluble DM (% of DM washed out of bags at t = 0h)

B = degradable insoluble DM (%)

L = lag phase (hours)

k = the fractional disappearance rate (k, %. $hour^{-1}$).

1 – (A+B) = the undegradable portion of a sample (C; %).

In the example given above and in Figure 2.10, the degradation referred to DM, but fibre (NDF and ADF) and protein are also evaluated in terms of digestion kinetics. Figure 2.10 illustrates the degradation of a typical forage.

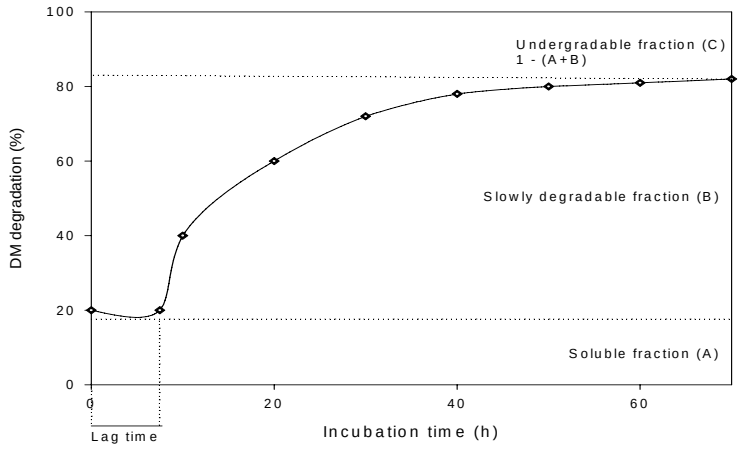


FIGURE 2.10 – Typical *in sacco* degradation curve of forage DM.

Both the lag time (L) and soluble (A) fraction are affected by the incubation and preparation procedure, so it is important that preparation is well defined and repeatable. The largest component of plant degradation is represented by the parameters B and k which result from microbial-plant interactions.

2.4.3.8 - Determination of effective degradability

Effective degradability takes into account both extent and rate of digestion, to enable comparison between forages, and indicate relative nutritive value.

To estimate the extent of ruminal digestion, mathematical methods for interpretation of the *in sacco* degradation profile may include a fixed turnover rate to indicate rate of outflow. Ørskov and McDonald (1970) proposed an integration of the *in sacco* degradation profile in relation to the ruminal particulate outflow rate.

The effective degradability (E) can also be calculated from the kinetic parameters obtained from equation (ii):

$E = A + ((B * k) / (k + k_d))$ Equation (ii)

Where A, B and k are defined for equation (i) and k_d is fractional passage rate (or rumen small particle outflow rate; %.hour^{-1}). The value for k_d has been set up 6%.hour^{-1} for analysis of forage digestion.

The main methods used for fitting data to the kinetic model are:

a) Logarithmic transformation followed by linear regression (lnLIN);

b) Non-linear least square regression (NLIN).

Both are procedures of SAS program (SAS, 2001). In most cases, NLIN regression methods are preferred because they result in the smallest residual sum of squared deviations from the model (Nozière and Michalet-Doreau, 2000).

For the last 20 years, the *in sacco* technique has proved to be useful and robust technique for estimation of the feeding value of forages, and effects of the rumen environment and degradation. Description of degradation characteristics is inexpensive, repeatable but must relate to *in vivo* performance to be useful.

2.4.4. Sample preparation

The type of feed preparation has very significant effects on degradation kinetics, in terms of constituent disappearance, proteolysis, VFA production and microbial growth. Although conventional feed preparation involves freeze drying and grinding, this is not appropriate for fresh forages which have been both minced and freeze dried for comparison *in sacco* (McNabb *et al.*, 1996). Incubation of masticated feeds would be the optimal preparation for *in sacco* studies, but it is difficult to obtain masticated material. Barrell *et al.* (2000) and Cohen and Doyle (2001) demonstrated degradation kinetics of fresh chopped, minced and also freeze dried and ground material and suggested the minced preparation to be most appropriate for fresh grasses and legumes. *In vitro* and *in sacco* incubations should be carried out with feed prepared in a way that best mimics chewing by ruminants.

Particle size has an influence on the accessibility of dietary components to the microflora enzymes. A few studies have shown that the lag time significantly increased with an increase in particle size (Barrell *et al.*, 2000; Emanuele and Staples, 1988). This maybe overcome by incubating material of a similar size, rather than by passing through a single mesh to allow a wide range in particle sizes to be included in the incubator. However the technique must also relate to chewed material *in vivo* to reproduce the range of mean particle sizes in a swallowed bolus. Barrell *et al.* (2000) demonstrated that mincing fresh forage with a 12 mm screen aperture simulated particle distribution achieved by mastication in ruminants (Ulyatt *et al.*, 1986; Waghorn *et al.*, 1989). For wet materials, e.g. forage and silages, Ørskov (2000) also suggested that a mincing machine is the most appropriate form of preparation.

2.5 - Simulation modelling

The purpose of a model is to describe mathematically the response of each compartment or several connected compartments to a variable or combination of variables. A model is considered mechanistic when it simulates behaviour of a function through elements at a lower level (Gill *et al.*, 1989). Most biological responses are integrated and nonlinear and change over time (Sauvant, 1991 cited by Fox and Barry, 1994). Mechanistic models provide a useful means of integrating knowledge and formulating hypotheses. Thus mechanistic modelling is an integral part of a research programme, with experimental and modelling objectives highly inter-related (Dijkstra and France, 1994).

Prediction of requirements and feed utilisation by ruminants is unique to each production setting, so models 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). Several models used for ration balancing (Udder, GrazFed, Camdairy, feedTECH) are empirical rather than causal, and often they are not interactive or user friendly. Models used for ruminal studies, including Molly (Baldwin *et al.*, 1987) and Dijkstra's rumen model (Dijkstra *et al.*, 1992), are complex, difficult to use and are not designed for interpreting cow performance in relation to feed characteristics.

In most application systems (AFRC, 1993; AAC, 1990; Institut National de la Recherche Agronomique (INRA), 1989) the prediction of metabolism of nutrients is not as advanced as the prediction of ruminal fermentation, because the fate of nutrients in the animal is less well understood than their production in the rumen. Metabolic routes connect tissues and metabolic compartments, involve interactions among nutrients and include metabolic regulations which drive the partioning of absorbed nutrients between tissue maintenance and production (Sauvant, 1991 cited by Fox and Barry, 1994). Therefore a combination of mechanistic and empirical approaches must be used. Systems are generally steady state and static, and use statistical representations of data that represent the aggregated response of whole compartments. This approach was used in developing the Cornell Net Carbohydrate and Protein System (CNCPS) for evaluating cattle diets as described and validated by Russell *et al.* (1992); Sniffen *et al.* (1992); Fox *et al.* (1992); O'Connor *et al.* (1993); Pitt *et al.* (1996) and Tylutki and Fox (1997). The CNCPS model has been used in the interpretation of data obtained from experiments described in this thesis.

A problem with nutrition models is that they cannot predict intake well consequently giving a weak prediction of performance. They are designed to estimate nutrient supply not predict cow performance (St-Pierre and Thraen, 1999).

The CNCPS (version 5.00.20) systems will be tested in Chapter 7 to determine the accuracy and utility of this model to predict and formulate diets that were based on pasture and forage supplements using data obtained *in sacco* and *in vitro* incubations and two dairy cow trials conducted in mid-lactation, where pasture was complemented with contrasting silages.

2.6 – Conclusion

Dairy cow performance is affected by feed quality and availability. In New Zealand the ryegrass based pastures show a significant decline in quality in late spring in association with flowering. This change is bought about by increasing proportions of stem and reductions in leaf with a net lowering of both ME content and cow intake. The changes in ryegrass have a significant input upon cow performance, and this thesis quantifies these changes as ryegrass matures. The hypothesis is that both degradation rate and yield of nutrients diminish from ryegrass as it matures and these can be quantified to develop forage mixtures, which provide balanced diets for dairy cows.

Chapter 3

Chemical composition, *in vitro* and *in sacco* digestion kinetics of perennial ryegrass as influenced by stage of maturity[1]

[1] A small portion of these data has been previously published in the *Proceedings of the New Zealand Society of Animal Production*, 2002, 157-162.

3.1- Abstract

Animal performance from grass-dominant pastures is affected to a large extent by pasture quality and especially maturation in relation to flowering. Maturation in late spring/summer lowers feed quality due to the changing proportions of leaf, stem, inflorescence and dead matter and the associated changes in chemical composition. Perennial ryegrass of increasing maturity has been used for *in vitro* and *in sacco* incubations to determine the net production of ammonia from protein degradation and yields of volatile fatty acids (VFA) and to determine rates of digestion of the dry matter (DM), crude protein (CP) and fibre fractions of the dry matter (DM). The principal finding was a rapid decline in crude protein content from about 24 g CP/100 g of DM harvested at 22 days to 10 g CP/100 g of DM for that harvested after 60 days. These changes were associated with increases in the neutral detergent fibre (NDF) fraction of the DM (43 to 54 g NDF/100 g of DM) and in lignin concentration (2.7 to 4.9 g lignin/100 g of DM). Changes were more rapid in late-cut than early cut forages. The principal consequences of increased maturity were slower degradation rates of DM (k = 0.11 to 0.03 h^{-1}) and NDF (k = 0.14 to 0.03 h^{-1}) and no effect on CP degradation rates. There was a significant change in the pattern of ammonia and VFA production when ryegrass matured.

Keywords: ryegrass; forage maturity; digestion kinetics; *in sacco*; *in vitro*; dairy cows.
Short title: Digestion kinetics of ryegrass

3.2 - Introduction

The effect of maturity on digestion and animal performance arises mainly from changes in plant morphology and in cell wall components, which affect dry matter (DM) intake and digestibility (Van Soest, 1994).

Maturity is the most important factor affecting pasture quality. From a nutrition perspective, forage quality relates to the feeding value or the ability to utilise feed for production (e.g. milk, meat, wool). Forage quality is never static; plants continually change in quality as they mature. As plant cell-wall content increases, indigestible lignin accumulates and in late spring, grass maturity changes so rapidly that it is possible to measure a significant decline in forage quality every two or three days (Cherney *et al.*, 1993). Changes occur in both the proportions of leaf, stem and inflorescence as well as the chemical composition of these structures. Both the extent and the rate of change in components are important and farmers face a considerable challenge

when they manage pastures to ensure a high nutrient intake for high producing animals.

Accurate prediction of performance for animals fed ryegrass diets and options for supplementation with high-quality forages requires a greater understanding of how digestion processes are influenced by the stage of maturity. The objectives of this experiment were to analyse and compare changes in composition, digestion and its products when ryegrass (*Lolium perenne* L.) grows to maturity in spring. The rate of change in quality is also important and the effect of cutting date on these components was included in the evaluation.

The hypothesis to be tested here was that ryegrass maturation will alter rates of degradation and products of digestion and these changes will be affected by initial cutting dates of the sward. Experiments were designed to create a dataset for use in dairy nutrition for grazing cows in New Zealand.

3.3 – Material and methods

A one-year-old pure perennial ryegrass sward (*Lolium perenne* L. cv. Grasslands Samson) was grown at AgResearch Grasslands Aorangi Research Station. A 15 m by 25 m plot was divided into three equal areas separated by trimmed pathways and mown on either 21/08/2000 (area 1), 11/09/2000 (area 2) or 21/09/2000 (area 3) as illustrated in Figures 3.1 and 3.2. After the initial cut, the plots were allowed to grow for the duration of the trial until 11 December with about 2 kg of forage harvested from each area at 7 - 14 day intervals for analysis. Table 3.1 indicates the schedule of cutting dates and days of re-growth (age) of the forage harvested from each area.

TABLE 3.1 – Cutting schedule days of re-growth and harvesting dates of ryegrass from three areas. The samples were used for *in vitro* and *in sacco* incubations.

Area	1	2	3
		Initial harvest date	
Sample dates	21 August	11 September	21 September
11 September	21 [a]		
21 September	31 [a]	10 [a]	
5 October	45 [f]	24 [f]	14 [f]
13 October	53 [b]	32 [b]	22 [b]
24 October	64	43 [g]	33 [g]
3 November	74 [c]	53 [c]	43 [c]
10 November	81	60	50 [g]
17 November	88 [d]	67 [d]	57 [d]
27 November	98	77	67
4 December	105 [e]	84 [e]	74 [e]
11 December	112	91	81

Superscripts indicate samples analysed *in vitro* (A to E) and *in sacco* (A to G).

Samples were cut with electric clippers approximately 5 cm above the soil level. Ryegrass re-growth was harvested on 11 occasions from area 1, ten occasions from area 2 and nine occasions from area 3 (Table 3.1). The areas harvested decreased as the ryegrass matured to maintain approximately the same amount of sample harvested for each period of ryegrass re-growth, and forage from each area was stored in plastic bags and frozen at -16°C for chemical analysis, *in vitro* and *in sacco* incubations. The area and weight of forage harvested were measured throughout the trial and the height of the sward determined using a ruler to estimate mean length of leaf or leaf and stem.

This design enabled the nutritive value of ryegrass to be monitored in relation to age (length of re-growth period), harvest dates and in relation to herbage mass (t DM/ha). Although the effects of plant age (maturity) on nutritive characteristics are well known, this study was designed to determine whether the effects of initial cutting date and rate of growth affects changes in nutritive value.

The chemical, *in vitro* and *in sacco* analyses provided information on the composition of the ryegrass, the ammonia and VFA production during *in vitro* digestion and the rate of DM, CP, NDF and ADF disappearance during *in sacco* digestion. I*n vitro* and *in sacco* analyses were undertaken with 15 and 21 samples respectively (Table 3.1). All the samples were submitted for chemical analyses (wet and NIRS) and DM determination (AOAC, 1990).

Once the grass was harvested and frozen it was maintained below 0°C throughout all preparations, including chopping, mincing, weighing into *in vitro* bottles and dacron *in sacco* bags. The material was thawed when *in vitro* incubations commenced or immediately prior to placement in the rumen for *in sacco* incubation.

FIGURE 3.1 – View of the ryegrass plots midway through the maturation trial.

FIGURE 3.2 – The organization of plots showing areas 1, 3 and 2 (left to right) with newly mown paths separating each area.

3.3.1 - Preparation of fresh forage for incubations

Ideally the sample preparation for incubations should mimic the particle distribution resulting from chewing during eating and rumination. Frozen forages were chopped into approximately 2 cm lengths (scissors) and minced in a Kreft Compact meat mincer R70 (Kreft, GmbH) fitted with a screen plate with 12 mm holes (Barrell *et al.*, 2000; Burke *et al.*, 2000; Chaves *et al.*, 2001). The mincer components (screen plate, housing, screw, cutter and loading tray; Figure 3.3) were placed in a freezer prior to mincing to ensure the grass remained frozen and this enabled the forage to be macerated rather than squeezed and prevented excessive cell wall rupture during mincing. The process was designed to mimic effects of chewing by ruminants as far as possible (Ulyatt *et al.*, 1986; Waghorn *et al.*, 1989). This method is described by Waghorn and Caradus (1994) and Barrell *et al.* (2000), and is similar to the method used by Cohen and Doyle (2001).

FIGURE 3.3 – Mincer used for fresh forage preparation.

Approximately 500 g of wet material were used for each ryegrass sample for *in sacco* and *in vitro* incubations, chemical and particle size analysis. Mincing took place

within 1-3 days of incubation. The mincing procedure involved assembly of cold mincer parts and mincing the chopped frozen material. Care was taken to ensure the sample did not thaw, so aliquots of about 200 g were removed from the freezer, minced and the minced material returned to the freezer. With very mature forage, only 250 g could be minced before the mincer parts become warm. When this occurred the mincer parts were dismantled, washed with cold water, dried with paper tower and returned to the freezer. Once cold, the process was continued to provide sufficient material for analysis.

Minced material was stored at -16°C in sealed plastic bags until the day prior to incubations. *In vitro* incubations required about 0.5 g DM (approximately 1.5 – 3.0 g wet weight (ww), depending on DM content) to be placed in incubation bottles and 5.0 g DM (15 - 30 g ww) into 100 x 100 mm dacron bags (35 μm pore size; Figure 3.4) for *in sacco* incubation.

FIGURE 3.4 – Visual aspect of the ryegrass after mincing.

3.3.2 - Source of rumen fluid for *in vitro* and *in sacco* incubations

One non-lactating Friesian cow fitted with a permanent rumen fistula was used for all the *in sacco* incubations and provided rumen liquor for *in vitro* incubations (Figure 3.5). Three forage samples were incubated as one batch and both *in vitro* and *in sacco* incubations commenced at the same time with rumen liquor removed immediately prior to placement of bags in the rumen. The liquor was used for inoculum of *in vitro* bottles within 20 minutes of collection.

A single cow was used for all incubations to avoid effects of variation between animals in studies on digestion kinetics (Waghorn and Caradus, 1994; Nozière and Michalet-Doreau, 2000; Ørskov, 2000). Differences in microbial populations between individual cows can exceed differences attributable to contrasting diets (Weimer *et al.*, 1999).

The effects of diet on microbial population (Nocek, 1988; Weiss, 1994) were minimised by feeding a single diet of good quality lucerne hay to the cow 10 days prior

and during each incubation. The hay was fed at maintenance (Madsen and Hvelplund, 1994), at 07:00 and 19:00 h, with water available *ad libitum*.

FIGURE 3.5 – The Friesian cow used in all incubation runs. Collecting rumen samples.

3.3.3 - Chemical analyses and particle distribution

Samples of minced forage were retained for chemical analysis by Near Infrared Reflectance Spectroscopy NIRS (Corson *et al.*, 1999), measurement of dry matter content by drying at 60°C for 24 hours and particle size distribution by wet sieving (Waghorn *et al.*, 1986).

3.3.4 - *In vitro* incubations

The *in vitro* incubations provided measures of net ammonia production, yield and proportions of VFA (acetate, propionate, butyrate, iso-butyrate, valerate and iso-valerate) and changes in pH over each incubation period. The net ammonia production and yield of VFA were expressed in terms of plant material incubated (e.g.: µMol NH_3-N/mMol plant N) and plotted over time.

In vitro incubations were undertaken in 50 mL Schott bottles with bicycle valves fitted into the lid (Figure 3.6) enabling fermentation gases to escape. Bottles were placed in an incubator (Gallenkamp orbital incubator. Cat. No. IOC400.XX1.C, Made in UK) with good temperature control which had been fitted with a rack to handle simultaneous incubations of up to 94 bottles (Figure 3.7). Incubator temperature was

maintained at 39 ± 0.5°C and the rack was set at 90 oscillations per minute. Three forage samples were incubated simultaneously, with standards. Each minced forage was weighed into 24 Schott bottles and at each sample time three bottles of each forage were removed for sampling and analysis. Sampling times were 0, 2, 4, 6, 8, 10, 12 and 24 hours.

On the day before the incubation, 1.5 - 3.0 g of sample, corresponding to 0.5 g DM frozen minced material was weighed into incubation bottles. On the day of incubation, the bottles containing forage substrate were warmed to 39°C in the incubator for 60 minutes, gassed with CO_2 before adding 12 mL of artificial saliva (buffer, Appendix 1) saturated with carbon dioxide at 39°C and 0.5 mL cysteine sulphide reducing agent (Appendix 1). This process took about 40 seconds for each bottle, which was then capped and returned to the incubator at 39°C. Rumen liquor was obtained from the cow, strained through cheese cloth (Figure 1.1A, Appendix 1) into a two litre thermos® flask and 3 mL dispensed into each bottle. Addition of rumen liquor to 76 bottles took approximately 12 minutes. Appendix 1 shows details for incubation procedure.

FIGURE 3.6 – Bottle used for *in vitro* incubation.

FIGURE 3.7 – Gallenkamp incubator used for *in vitro* incubations.

The cow was fed 120 - 150 minutes before the rumen liquor was collected. The pH was measured at the time of collection and sub-samples of rumen liquor were taken for determination of ammonia and VFA concentrations. Three bottles of each forage preparation were removed from the incubator for determination of ammonia and pH concentrations at each sampling time. VFA concentrations were measured in pooled samples from three bottles at 0, 6, 12 and 24 hours. Incubation residues were retained for future measurements of microbial DNA to indicate microbial growth. Changes in pH were used to indicate the ability of the buffer to maintain the *in vitro* environment despite production of VFA. Low pH values (below 5.6) demonstrated an atypical situation for forage fermentation and data from these bottles were removed from the analysis.

3.3.4.1 - Standards for *in vitro* incubations

Freeze dried and ground lucerne standards were included with all *in vitro* incubations to monitor variation between runs. Three bottles of the standards were removed at 2 and 8 hours of incubation.

Freeze dried and ground lucerne was used as an internal standard because ryegrass occasionally demonstrated substantial microbial inhibition. It is most important that the internal standard demonstrates consistency of incubation characteristics.

3.3.4.2 - Determination of ammonia

The procedure for sampling bottles was as follows: nine bottles were removed from the incubator and pH was measured. Sub-samples of 1 mL were added to 1.5 mL microcentrifuge tubes containing 15 µL concentrated HCl and centrifuged at 14000 rpm (14000 g) for 15 minutes (eppendorf – 5415 D Centrifuge ; Figure 3.8) to precipitate particulate matter. The supernatant was transferred to a 1.5 mL microcentrifuge tube and frozen for ammonia analysis (Chaney and Marbach, 1962; Appendix 2).

FIGURE 3.8 – Equipment for sampling *in vitro* bottles.

3.3.4.3 - Determination of VFA

A further 1 mL of liquor was removed and centrifuged for determination of VFA concentrations. Two samples of approximately 0.4 mL were taken from each bottle and combined (from the triplicate bottles of each forage sample) to provide one sample for VFA analysis and a spare. Samples were centrifuged as above and the supernatant frozen for analysis by gas liquid chromatography described by Attwood *et al.* (1998) – Appendix 3.

3.3.4.4 – Fermentation kinetics

Fermentation kinetics for each data set were used to evaluate *in vitro* incubations. Changes in pH were plotted against incubation time to remove data when pH concentrations dropped below 5.6. Excessively low values (below 5.6) probably mean that the data are no longer representative of *in vivo* digestion and were discarded.

During the incubations, ammonia is produced by the microbes as a product of protein degradation during fermentation. Some of this ammonia is used by bacteria for growth. Thus ammonia measurements from *in vitro* incubations represent the net amount of ammonia produced rather than the gross amount. The measurements of net ammonia production during *in vitro* incubation provided a relative measure of protein breakdown in the rumen; nevertheless this does not account for the nitrogen taken up by the bacteria. Both ammonia concentration and net NH_3-N production as a proportion of plant N were plotted against time to indicate rate of proteolysis for each incubation run (A, B, C, D and E; Table 3.1).

Measurement of VFA and ammonia production during microbial fermentation can indicate the nutritive value of a feed in terms of protein losses to degradation and yields of VFA from fermentation. Measuring ratios of VFA contribute to the understanding of the nutritive value of feeds, especially proportions of propionate: acetate. Concentrations of VFA expressed per gram of DM incubated were plotted against incubation time (0, 6, 12 and 24). VFA data have been combined across areas to give values for young (under 33 days), medium (43 - 57 days) and mature (over 67 days) ryegrass.

3.3.5 - *In sacco* incubations

The *in sacco* incubations allowed the rates of disappearance of DM, CP, NDF and ADF to be calculated. Approximately 5 g DM was weighed into dacron bags, sealed and held at -16°C prior to incubation. Bags were incubated for 0, 2, 6, 12, 24 and 72 hours, with duplicate bags at each time for each forage sample. The procedure for incubation required duplicate bags for each forage sample to be placed in a weighted (350 g) lingerie bag, so that five lingerie bags were placed in the rumen at the commencement of incubation. The 0h bags were not placed in the rumen. Minced ryegrass standards were incubated and removed at 2 and 12 hours to monitor between-run variation. After removal from the rumen, dacron bags containing forage residues were washed under cold water until no further colour appeared (i.e.: all soluble material was removed) prior to drying at 60°C for 24 hours. Dried bags were weighed and analysed to determine disappearance of DM, residues were removed, ground and analysed by NIRS to determine CP, NDF and ADF concentration in the DM.

Calibration curves had been developed for NIRS from samples of bags residues collected after 2 to 72 hours of incubation and analysed by wet chemistry during initial incubation runs (See Table 6.1A Appendix 6 for details). The quantity of DM digested, with composition of minced plant material less residues enabled loss of ryegrass constituents to be calculated and plotted to enable a visual appraisal and calculation of digestion kinetics.

The use of reference samples as internal standards enabled inspection for variability between runs. Minced ryegrass collected from a different site was used as an internal standard for *in sacco* digestions.

In most instances the three samples used for *in sacco* and *in vitro* incubations runs were from the three different areas (mowing dates 1, 2 and 3), and the 7

sequential incubation runs gave 21 data sets to define ryegrass maturation (7 ages for each area).

3.3.5.1 - Digestion kinetics

Digestion curves for each data set were used to evaluate *in sacco* degradation kinetics of ryegrass in the rumen. The disappearance of DM, CP, NDF and ADF were analysed using a non-linear model (model no. 1) described by López *et al.* (1999) to determine fractional disappearance rate (k, %/hour) and potential degradation (P) according to:

$$P = A + B\ (1\text{-}e^{-k\ (t\text{-}L)})$$

Where A = soluble fraction (% of each constituent, washed out of bags at t = 0h), B = insoluble degradable fraction (%), t = time in hours, L = lag phase (hours).

The effective degradability (E) was calculated from the kinetic parameters obtained from exponential adjustment assuming a fractional passage rate (k_p) of 0.06 h^{-1}:

$$E = A + B * (k / (k + k_p))$$

The turnover rate (k_p) of 0.06 used here is based on measurements by Van Vuuren *et al.* (1993) who reported values ranging from 0.041 to 0.067/h for dairy cows fed ryegrass, and is commonly used to evaluate forages (Elizalde *et al.*, 1999; Kolver *et al.*, 1998).

The metabolisable protein system (AFRC, 1992) for defining ruminal degradation was used to calculate protein degradability parameters:

Quickly degradable protein (QDP, g/100g DM) = A * [CP] where [CP] is CP concentration (g CP/100g DM).

Slowly degradable protein (SDP, g/100g DM) = $[(B * k) / (k + k_p)] * [CP]$,

Effective rumen degradability of crude protein (ERDP, g/100g DM) = [(0.8 * QDP) + SDP],

Rumen degradable protein (RDP, g/100 g DM) = QDP + SDP,

Rumen undegradable protein (RUP, g/100g DM) = [CP] – RDP.

Model parameters were estimated with the non-linear (NLIN) procedure of (SAS, 2001), which is appropriate for situations that do not have long digestion lag times. The model is able to reduce the residual deviations from the model equation for both

degradation rate and A and B estimates (Nocek and English, 1986). Separate curves were calculated for each stage of maturation for each area.

3.3.6 - Statistical analyses

3.3.6.1 - Fixed effects model analysis for grass growth and chemical composition

The effect of initial mowing dates (areas 1, 2 and 3) were analysed in the context of a fixed effect model using PROC GLM (SAS, 2001). Linear, quadratic and interactions effects were tested using the following model:

$Y = b_o + b_{1i}$*mowing date + b_{2i}*X + b_{3i}*mowing date*X + b_{4i}*X^2 + b_{5i}*mowing date*X^2 + e [equation 1], where:

Mowing data is a categorical variable so b_o is in fact the intercept for mowing date 3 (20 September). b_{1i} is then the difference for mowing date 1 and 2. Similarly for interaction terms. X = days of re-growth (age) or herbage mass (HM). The complete outputs from the models using PROC PRINT option (SAS, 2001) are presented in the CD appendix.

Statistical analyses for herbage mass and days of re-growth (age)

Ryegrass production per hectare (herbage mass; HM) was regressed against age (days of re-growth) using the fixed effects model to check if the growth rates change with maturity between and within areas (mowing dates). It is important to express data in relation to both parameters (age and HM) because relationships between them may differ under contrasting climatic and environmental conditions, i.e., ryegrass cut at different dates may have different growth rates (or herbage mass) in different parts of New Zealand.

Statistical analyses for chemical composition

Using the same basis as HM and age, the NIRS chemical composition (CP, NSC, Lipid, NDF, ADF, Ash, OMD and ME) was regressed against both age and HM independently. In order to compare slopes and intercepts from the three areas (for each component) against age and against HM concentrations were analysed using the PROC GLM (SAS, 2001) described above.

When there were no differences between slopes and intercepts for individual chemical components for the three areas, the fixed model analysis was used to

calculate overall slope and intercept for each component against age and against HM across all areas.

Results from fitting a fixed model with plant age (10 days ≤ age ≤ 112 days) and herbage mass variables using the GLM procedure (SAS, 2001) are presented as equations (slopes, intercepts, r^2, error, significance level) and plotted to show relationships. See appendix CD (Chapter 3) for details of SAS procedures.

3.3.6.2 - Statistical analyses for *in vitro* data

In vitro incubations were usually carried out for samples taken from each area on the same harvest date (Table 1.1A - Appendix 1). Incubation runs are a continuous variable and we arbitrarily chose five time points.

Ammonia concentrations in each incubation bottle were used to calculate net ammonia production (Appendix 2) and analyses were based on net conversion of plant N to ammonia N. The conversion of plant N to NH_3-N was evaluated by comparing the effects of plant age between areas within the same incubation run and interactions of age x incubation time according to the model:

$Y = b_o + b_{1i}*age + b_{2i}*time + b_{3i}*age*time + e$ [equation 2], where:

Y = response variable (e.g.: NH_3-N, acetate, propionate), b_o = intercept, age = age effect, time = incubation time effect, age*time = interact terms and e = error term.

VFA analyses were based on single samples for each of the four periods for each forage age. VFA production was expressed as net production mMol/g grass DM incubated. Data are expressed as net production from 0 - 6, 6 - 12 and 12 - 24 hour, and as molar proportion including rates of acetate: propionate (A: P). Statistical analyses enabled a comparison of net VFA production between age at harvest and time of incubation using a similar model to that for testing ammonia concentration (equation 2, above). Data were analysed using general linear models procedures of SAS (SAS, 2001). Differences among means were detected using F tests. Significance was declared at $P < 0.05$ unless otherwise noted. See appendix CD for procedures details.

Acidity (pH) was used primarily to monitor incubations and the rumen inoculum. When pH ≤ 5.6 data were not included in any analysis as this situation no longer represents *in vivo* rumen fermentation of animals fed forages. Mean pH values for each forage at each time point have been determined and plotted over incubation time for all 15 forage incubations.

3.3.6.3 - Statistical analyses for *in sacco* data

Data from *in sacco* incubations were expressed as degradation curves using a non-linear least-square procedure (PROC NLIN; SAS, 2001) to provide estimates for A, B and k. Observations were weighted using the term 1/standard error^2 to give a higher weighting to data having least variance about the curves, and therefore more precise estimates (Koong *et al.*, 1975; Murphy *et al.*, 1982).

Evaluations were made for DM, CP, NDF and ADF for soluble (A) and degradable (B) pool, and rate of degradation (k) according to López *et al.* (1999). These analyses were made against area, age at harvest date and lignin concentration using a general linear model (Appendix CD). When treatment effects were significant for any parameter, those data were fitted using a fixed effects model analysis [equation 1] to determine slopes and intercepts and to determine if mowing date effect was statistically significant. For example k for DM was regressed against age at harvest for each area. Appendix CD (Chapter 3) shows details for statistical analysis SAS procedures and complete data set outputs.

3.4 - Results

3.4.1 – Growth of ryegrass sward

Ryegrass grew to 70 - 90 cm in height over the experiment (Figure 3.9; Table 3.2). Herbage mass (above the 5 cm cutting height) increased from about 120 to over 4500 kg DM/ha (Table 3.2) and both height and mass were greatest for the first area mowed (Figure 3.9 and 3.10).

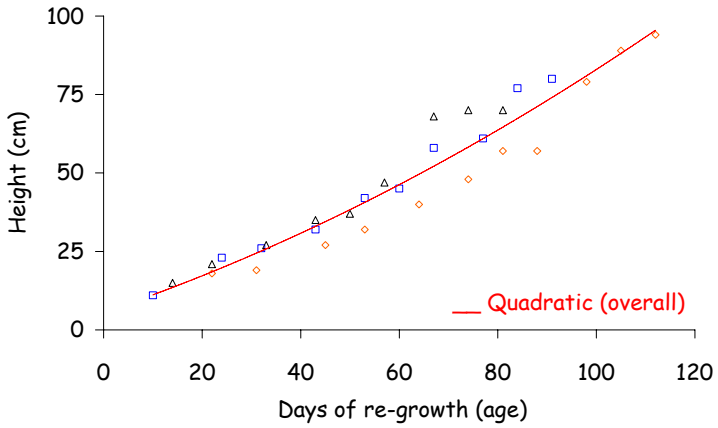


FIGURE 3.9 – Relationship between ryegrass height (cm) and days of re-growth (age) for area 1 (◊), area 2 (□) and area 3 (Δ). Height (cm) = 5.6 + 0.5age + 0.002age^2 (r^2 = 0.92).

The fixed effects model used to evaluate herbage mass (HM; t/DM) and chemical composition (g/100 g DM) of pasture samples from the three areas (Table 3.3) enabled both linear and quadratic relationships to be tested. Analysis of herbage mass with age (days of re-growth) showed that a significant amount of variation was accommodated by the model ($P < 0.0001$). The relationship was not linear ($P = 0.14$; Table 3.4) but a quadratic relationship accounted for 81% of variation in herbage mass with days of re-growth: HM = - 0.5 + 0.034age + 0.0002age^2 (r^2 = 0.81; Figure 3.10).

The interaction (age^2*mowing date) showed there was no effect of initial mowing date on herbage mass, in relation to days of re-growth ($P = 0.25$). The ryegrass in each area grew at similar rates irrespective of initial cutting date (Figure 2).

Model outputs are calculated for the three areas (Appendix CD) and values for area 1 and 2 are tested in relation to area 3. The parameter estimates provided by the model have been presented for area 3 in Table 3.5 and show: HM = - 0.44 - 0.028age + 0.0011age^2 (Table 3.5).

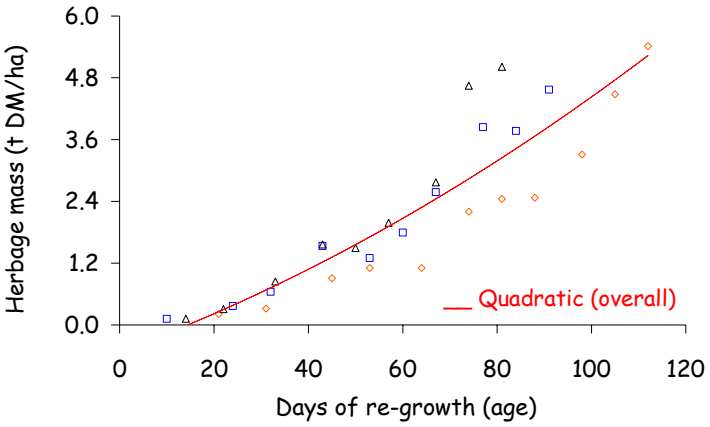


FIGURE 3.10 – Relationship between ryegrass herbage mass (t DM/ha) and days of re-growth (age) for area 1 (◊), area 2 (□) and area 3 (Δ).

The height of the sward canopy can be used as an indicator for monitoring grazing management. The relationship between herbage mass and the height of the ryegrass sward showed linear relationship with maturity (Figure 3.11). Estimation of herbage mass based on the height of the pasture, with 92% of the variation between height and HM explained by the model.

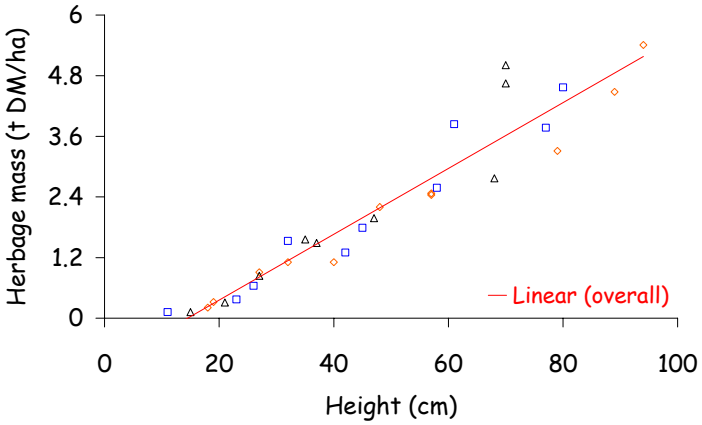


FIGURE 3.11 – Relationship between herbage mass (HM: t DM/ha) and height (cm) for area 1 (◊), area 2 (□) and area 3 (Δ). HM = - 0.95 + 0.065height (r^2 = 0.92)

3.4.2 - Chemical composition

Changes in chemical composition with maturity are tabulated (Table 3.3) and presented in Figure 3.12 with regression relationships to define changes over time. Single curves are presented when effects of initial mowing date were not significant; otherwise the curves relate to each data set.

Crude protein (CP) concentration (g/100 g DM) declined rapidly with maturity (Table 3.3) from about 23.7 at 22 days to 6.8 after 112 days of re-growth in area one, 20.9 at 10 days to 6.5 after 91 days of re-growth in area two, and 18.5 at 14 days to 8.2 after 82 days of re-growth in area three. These changes were associated with increases in the neutral detergent fibre (NDF) fraction of the DM (42.7 to 64.8; 48.1 to 62.3 and 49.5 to 59.7 g/100 g DM for area one, two and three respectively) as well as the acid detergent fibre (ADF) fraction of the DM (Table 3.3).

The concentration of acid detergent lignin (ADL) in the DM increased by a small amount as the ryegrass matured from about 2.74 up to 24 days to about 5.25 g/100 g DM at the final harvest (Table 3.3). Non-structural carbohydrate concentration increased with maturity until about 60 days of re-growth after which it declined. Both ash and lipid concentrations decreased with maturity. Predictions of organic matter digestibility (OMD; g/100 g DM) suggested decreases from about 84 at early stages of re-growth to about 62 in the last harvest date across the areas.

The nutritive value of mature ryegrass was also interpreted in relation to the predicted metabolisable energy (ME content) of the DM. As ryegrass matured, the ME (MJ ME/kg grass DM) decreased from 12.3 MJ/kg at 22 days of age to 8.8 MJ/kg at 112 days in area one. Immature grass in area three had consistently lower nutritive value than immature grasses in areas one and two at similar days of re-growth (age).

3.4.2.1 – Chemical composition and days of re-growth (age)

Model analysis of all nutritive value parameters (CP, NSC, Lipid, NDF, ADF, Ash, OMD and ME) have been fitted against days of re-growth (age) to demonstrate either linear or quadratic relationships and to identify significant differences between data sets (initial mowing dates) in Table 3.4. In all cases except NSC the model accounted for a significant amount of variation in the data ($P < 0.0001$; Table 3.4) but mowing date (age^2*mowing date) only affected fibre concentration and predicted OMD (Figure 3.12). Significant fits to the data were made with a linear effects model for lipids and ash, and quadratic effects for CP, NSC, NDF, ADF, OMD and ME (Table 3.4; Figure 3.12; Appendix CD). Residual (predicted – actual values) plots from fitted data have been

presented in Figure 3.13 to indicate patterns associated with chemical composition and indicators of NV. These data relate to the choice of linear or quadratic fit to the data indicated in Table 3.4. Interpretation of data has been made on the basis of best (linear or quadratic) fit for chemical components (all g/100 g in the DM) in relation to days of re-growth (age) as follows:

The decline in CP concentration with maturation was curvilinear (Figure 3.12) and the rate was not affected by initial mowing dates (P = 0.25). The overall model best able to predict CP concentration in ryegrass swards in relation to days of re-growth will be: $CP = 25 - 0.29age + 0.001age^2$ ($r^2 = 0.82$)

Model results for non-structural carbohydrate (NSC) concentrations demonstrated curvilinear relationship with age but there were no significant difference between initial mowing dates. When non-significant terms were removed from the model only 28% of the total variance in NSC concentrations and days of re-growth (age) can be explained by the model (P = 0.012):

$NSC = 5.5 + 0.014age - 0.001age^2$ ($r^2 = 0.28$)

Changes in fibre concentrations due to maturity showed similar patterns for NDF and ADF. Mowing date 2 and 3 suggests a slightly more rapid increase in fibre concentration during maturation compared with the first mowing date (Table 3.4; Figure 3.12). The effect of initial mowing date on fibre concentration has little practical significance because the lower values for the first mowing date are due to the very early cutting in relation to flowering. The overall model to predict fibre from ryegrass pastures using days of re-growth will be:

$NDF = 46.6 - 0.016age + 0.0016age^2$ ($r^2 = 0.80$)

$ADF = 25.5 + 0.013age + 0.0011age^2$ ($r^2 = 0.79$)

Both lipid and ash concentration in the DM did not demonstrate significant quadratic relationships with age (Table 3.4; Figure 3.12 and 3.13), and when the non-significant terms where removed from the models, the overall linear predictions for lipid and ash were:

$Lipid = 4.3 - 0.02age$ ($r^2 = 0.73$)

$Ash = 13.5 - 0.07age$ ($r^2 = 0.76$)

Concentration of metabolisable energy (MJ ME/kg DM) decreased when ryegrass matured but the difference between initial mowing dates were non-significant (P = 0.16). Data were explained using the quadratic component of the model (P < 0.0001) and ME concentration in ryegrass swards was predicted by:

$ME = 11.6 + 0.009age - 0.0003age^2$ ($r^2 = 0.73$)

Interpretation of chemical composition in relation to NV requires that analyses account for the majority of the DM. Mertens (1992b) reported that the concentrations of non-fibrous carbohydrates [NFC; calculated by difference: 100 – CP – lipid – NDF – ash] and non-structural carbohydrate [NSC; as measured by enzymatic methods] are not equal for many feeds and the terms should not be used interchangeably (NRC, 2001). Pectin should be included in NFC but not in NSC. When the principal components (CP + NSC + lipid + NDF + ash) predictions by the model are added, the values total about 84 g/100 g DM. Data are given in Table 3.6 to illustrate the extent to which NIRS analyses account for DM in ryegrass at 31 and 60 days of re-growth compared with model predictions. As expected, model predictions were similar to measured values but constituents only accounted for about 78 - 85% of DM. Some of the discrepancy will be due to pectin and organic acids which were not used in calibration of the NIRS (i.e. are not part of the NSC calibration) and represent a weakness in the data set for NSC. Typically pectin accounts for about 1.5 ± 0.2 g/100 g of DM in ryegrass and organic acids 8 ± 1.5 g/100 g of DM (Ulyatt, 1984). When these components are added to model predicted values, total constituents are 94.2 g/100 g of DM for ryegrass at 31 days of re-growth but only 87.3 g/100 g of DM at 60 days of re-growth (Table 3.6).

3.4.2.2 – Chemical composition versus herbage mass (HM)

Concentrations of CP, NSC, lipid, NDF, ADF, etc. have been evaluated in relation to HM (Table 3.7 and 3.8) and have been plotted in Figure 3.14. Overall, results were similar to relationships with age but all the components had a curvilinear response with HM as the quadratic term (HM^2) and/or interaction with initial mowing date (HM^2*mowing date) showing significant effects (Table 3.7). The equations enabling predictions of chemical composition from herbage mass are given in Table 3.8.

This chapter has focused on relationships between herbage quality and age, but data in Table 3.8 demonstrate a good relationship with herbage mass as well. This has important implications for using the model, as either days of re-growth (age) or herbage mass could predict ryegrass quality.

Table 3.9 compares the prediction models for NV using HM and NIRS analyses. This comparison leads to a conclusion that HM would be a better predictor of NV than age (Table 3.6). Also the goodness of fit for all constituents and OMD were slighter higher when regressed against HM (Table 3.8) than age (Table 3.5), but the differences were small.

TABLE 3.2 - Sward height (cm) and estimates values of total herbage dry matter production per hectare (herbage mass; t DM/ha).

Area	1	2	3	1	2	3
Initial mowing dates	21/08	11/09	21/09	21/08	11/09	21/09
Sample dates	Height (cm)			Herbage mass (t DM/ha)		
11 September	18			0.21		
21 September	19	11		0.32	0.12	
5 October	27	23	15	0.91	0.37	0.12
13 October	32	26	21	1.11	0.64	0.31
24 October	40	32	27	1.11	1.53	0.84
3 November	48	42	35	2.20	1.30	1.56
10 November	57	45	37	2.44	1.79	1.49
17 November	57	58	47	2.47	2.58	1.98
27 November	79	61	68	3.31	3.84	2.77
4 December	89	77	70	4.48	3.77	4.65
11 December	94	80	70	5.41	4.57	5.01

TABLE 3.3 - Chemical composition (g/100 g dry matter (DM)), and predicted nutritive value of ryegrass cut at different days of re-growth (age) and initial mowing dates (21/08 for area 1, 11/09 area 2 and 21/09 area 3).

Area	Date	Age	CP	NSC	Lipid	NDF	ADF	ADL	Ash	OMD	ME
1	11.9	22	23.7	10.9	4.2	42.7	21.8	2.66	11.6	87.7	12.3
1	21.9	31	19.1	9.0	4.0	45.9	24.8	2.38	10.9	83.8	11.9
1	5.10	45	16.8	9.4	3.8	48.7	26.4	2.50	10.8	81.6	11.6
1	13.10	53	18.0	6.1	4.0	51.5	29.2	2.50	12.3	80.4	11.3
1	24.10	64	12.5	11.4	3.1	48.0	27.4	3.91	9.3	80.5	11.7
1	3.11	74	12.6	9.9	3.2	51.8	30.2	2.62	9.2	76.0	11.0
1	10.11	81	9.5	10.3	2.8	53.7	32.1	5.04	8.5	72.6	10.6
1	17.11	88	8.9	9.8	2.5	55.2	33.8	3.02	8.3	71.1	10.4
1	27.11	98	9.4	9.7	2.7	55.7	34.2	5.04	7.4	68.5	10.1
1	4.12	105	8.0	8.6	1.9	60.7	38.8	4.35	6.7	61.2	9.1
1	11.12	112	6.8	7.3	2.4	64.8	39.4	5.31	7.1	59.9	8.8
2	21.9	10	20.9	7.5	4.1	48.1	25.8	2.68	11.7	82.7	11.7
2	5.10	24	18.6	3.3	3.9	44.8	30.1	2.94	15.3	76.8	10.4
2	13.10	32	17.4	8.1	3.6	49.3	27.9	2.66	11.7	82.6	11.7
2	24.10	43	16.7	10.4	3.5	47.1	26.3	2.70	10.5	83.2	11.8
2	3.11	53	13.0	9.7	3.3	49.8	28.9	2.62	10.4	79.8	11.4
2	10.11	60	10.1	11.3	2.8	50.8	29.7	4.62	9.0	77.0	11.2
2	17.11	67	9.7	10.7	2.8	51.1	30.9	3.07	9.3	76.0	11.0
2	27.11	77	7.9	9.5	2.5	56.5	34.4	5.12	7.3	68.0	10.0
2	4.12	84	7.3	8.9	2.1	60.2	37.4	3.74	6.6	63.2	9.4
2	11.12	91	6.5	8.3	2.3	62.3	37.9	5.40	6.5	61.5	9.1
3	5.10	14	18.5	7.6	3.7	49.5	28.5	2.95	12.7	82.2	11.5
3	13.10	22	19.5	8.4	3.9	48.9	26.1	2.49	11.4	82.4	11.7
3	24.10	33	13.5	8.9	3.0	47.4	28.4	2.44	11.8	79.3	11.1
3	3.11	43	13.1	10.9	3.0	48.8	28.2	2.49	9.6	79.4	11.5
3	10.11	50	10.2	11.4	2.7	51.8	29.6	2.65	9.1	77.8	11.3
3	17.11	57	10.5	11.1	2.9	50.4	30.0	2.81	9.2	76.7	11.1
3	27.11	67	8.9	10.8	2.8	53.7	31.7	4.94	8.0	71.4	10.5
3	4.12	74	8.9	9.1	2.4	56.7	35.6	4.56	6.9	65.2	9.6
3	11.12	81	8.2	8.5	2.3	59.7	37.2	5.04	7.6	63.6	9.4
Mean [a]		59	12.8	9.2	3.1	52.2	30.8	3.5	9.6	75.1	10.8

Abbreviations: CP, crude protein; NSC, non-structural carbohydrates; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; OMD, organic matter digestibility; ME, metabolisable energy (MJ ME/kg DM).

[a] Mean across the three areas.

TABLE 3.4 – Significance levels of fixed model analysis for height, herbage mass and chemical components of nutritive value against days of re-growth (age).

	Model Pr > F	Difference between intercepts	Linear term age	Interaction age*mowing date	Quadratic term age^2	Interaction age^2*mowing date
HM	<.0001	0.66	0.14	0.46	<.0001	0.25
Height	<.0001	0.49	0.10	0.16	0.0003	0.46
CP	<.0001	0.08	<.0001	0.32	0.02	0.24
NSC	0.09	0.24	0.01	0.21	0.01	0.19
Lipid	<.0001	0.75	0.01	0.63	0.87	0.54
NDF	<.0001	0.09	0.01	0.12	<.0001	0.04
ADF	<.0001	0.02	0.12	0.10	<.0001	0.06
Ash	<.0001	0.64	0.20	0.52	0.44	0.56
OMD	<.0001	0.12	0.01	0.17	<.0001	0.04
ME	<.0001	0.28	0.01	0.36	<.0001	0.16

Abbreviations see Table 2 (and text).

TABLE 3.5 – Parameter estimates of regression for height, herbage mass and nutritive value against days of re-growth (age) defined by the model for area (mowing date) 3.

	intercept	coefficient age	coefficient age^2	Error	r^2
HM	- 0.44±0.54 ^{ns}	- 0.028±0.025 ^{ns}	0.00105±0.0003 **	0.33	0.97
Height	7.60±6.05 ^{ns}	0.44±0.28 ^{ns}	0.005±0.003 ^{ns}	3.68	0.98
CP	24.32±1.93 **	- 0.36±0.09 **	0.002±0.001 *	1.18	0.96
NSC	3.76±2.55 ^{ns}	0.28±0.12 *	- 0.003±0.001 *	1.55	0.43
Lipid	4.27±0.36 **	- 0.04±0.02 *		0.22	0.92
NDF	52.45±2.63 **	- 0.27±0.12 *	0.004±0.001 **	1.60	0.94
ADF	29.85±2.21 **	- 0.19±0.10 ^{ns}	0.003±0.001 **	1.34	0.94
Ash	14.15±1.53 **	- 0.11±0.07 ^{ns}		0.93	0.86
OMD	79.78±3.22 **	0.22±0.15 ^{ns}	- 0.005±0.002 **	1.96	0.96
ME	11.61±0.41 **	0.009±0.015 ^{ns}	-0.0003±0.0001 *	0.52	0.73

Estimates ± standard error (SE).

ns = not significant; ** P < 0.01; * P < 0.05; t-test for intercept and regression coefficient differ from zero.

Error = Error of the model (error of prediction). Root mean square error.

Abbreviations see Table 2 (and text).

The use of single or double asterisk in this and other tables denotes significance of estimates parameters from zero at 5 and 1% levels respectively. This terminology applies to all equations in this chapter. ns = non significant.

TABLE 3.6 – Example of model predictions of nutritive value against age compared with NIRS analyses at 31 and 60 days.

	Model predictions		NIRS analyses	
Days of re-growth	31	60	31	60
CP	17.2	11.7	19.1	10.1
NSC	4.9	2.4	9.0	11.3
Lipid	3.7	3.1	4.0	2.8
NDF	47.6	51.3	45.9	50.8
Ash	11.4	9.3	10.9	9.0
Sub-total	84.7	77.8	88.9	84.0
Pectin and organic acids [a]	9.5	9.5	9.5	9.5
Total	94.2	87.3	98.4	93.5

Abbreviations see text.

[a] Ulyatt (1984).

TABLE 3.7 – Significance levels of fixed model analysis for chemical components of nutritive value against herbage mass (HM).

	Model Pr > F	Difference between intercepts	Linear term HM	Interaction HM*mowing date	Quadratic term HM^2	Interaction HM^2*mowing date
CP	<.0001	0.27	<.0001	0.88	<.0001	0.89
NSC	<.01	0.03	0.0002	0.04	0.0002	0.07
Lipid	<.0001	0.10	<.0001	0.77	0.003	0.99
NDF	<.0001	0.05	0.04	0.05	0.05	0.09
ADF	<.0001	0.003	0.005	0.01	0.28	0.02
Ash	<.0001	0.35	<.0001	0.72	0.02	0.76
OMD	<.0001	0.01	0.003	0.01	0.06	0.01
ME	<.0001	0.02	0.31	0.01	0.01	0.02

Abbreviations see Table 2 (and text).

TABLE 3.8 – Parameter estimates of overall regression of chemical composition (g/100g in the DM) against herbage mass (HM; t/hectare) across all initial mowing dates (areas).

	intercept	coefficient HM	coefficient HM^2	Error	r^2
CP	21.21±0.76 **	-6.48±0.75 **	0.76±0.14 **	1.75	0.88
NSC	7.37±0.64 **	2.36±0.63 **	-0.45±0.12 **	1.47	0.35
Lipid	4.10±0.12 **	-0.72±0.12 **	0.07±0.02 **	0.27	0.84
NDF	46.26±0.87 **	2.45±0.86 *	0.17±0.16 ns	2.01	0.87
ADF	25.46±0.73 **	2.37 ±0.73 **	0.04±0.14 ns	1.70	0.87
Ash	12.90±0.40 **	-2.33±0.39 **	0.23±0.07 **	0.92	0.83
OMD	84.30±1.04 **	-3.89±1.03 **	-0.15±0.19 ns	2.42	0.91
ME	11.76±0.17 **	-0.27±0.17 ns	-0.055±0.03 ns	0.39	0.85

Abbreviations see Table 2 and 4 (and text).

TABLE 3.9 – Example of model predictions of nutritive value against herbage mass compared with NIRS analyses at 31 and 60 days (0.32 and 1.79 t DM/ha, respectively).

	Model predictions		NIRS analyses	
Herbage mass (t DM/ha)	0.32	1.79	31 days	60 days
CP	19.2	12.1	19.1	10.1
NSC	8.1	10.2	9.0	11.3
Lipid	3.9	3.0	4.0	2.8
NDF	47.1	50.7	45.9	50.8
Ash	12.2	9.5	10.9	9.0
Sub-total	90.4	85.4	88.9	84.0
Pectin and organic acids[a]	9.5	9.5	9.5	9.5
Total	99.9	94.9	98.4	93.5

Abbreviations see text.

[a] Ulyatt (1984).

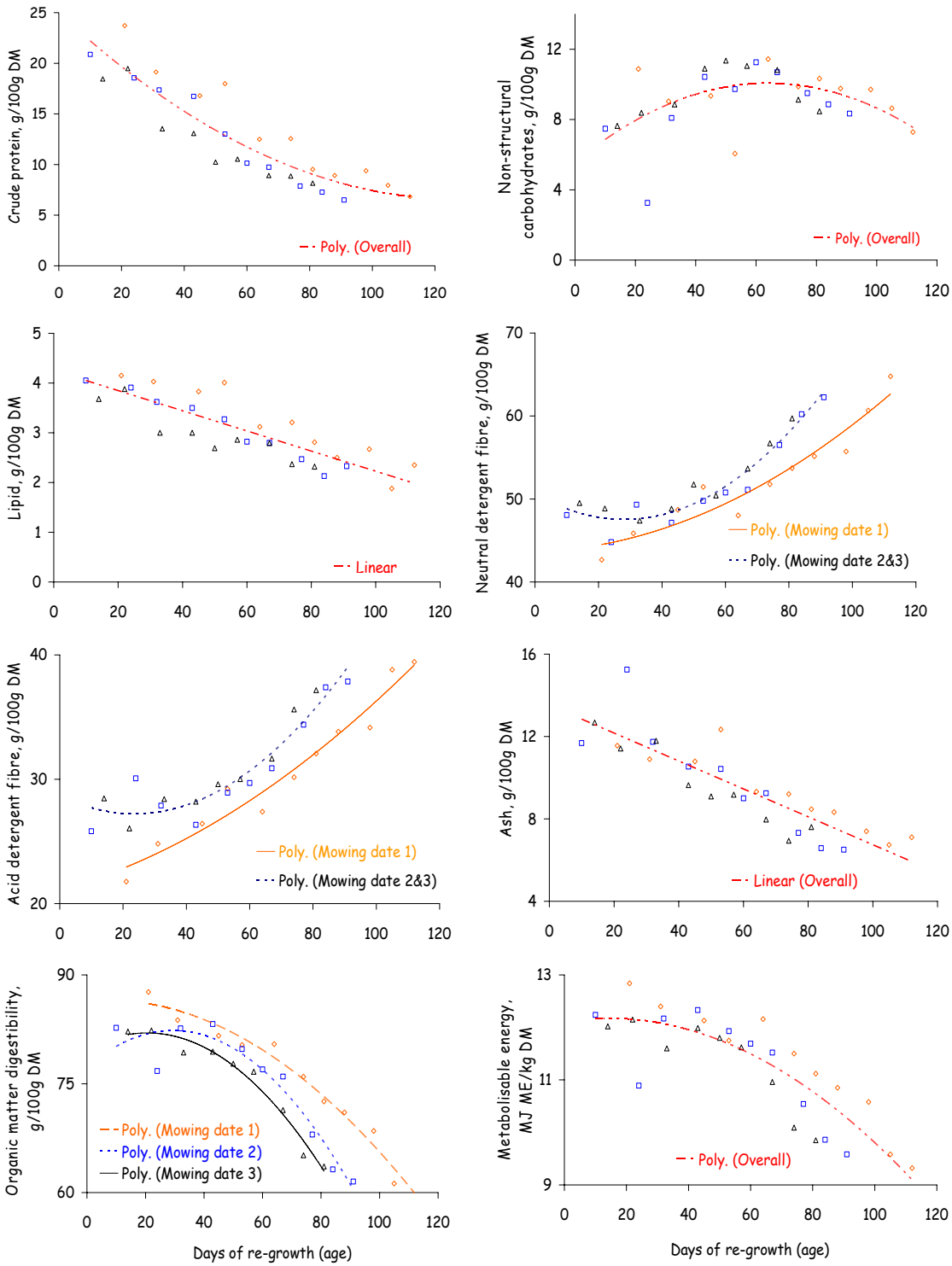


FIGURE 3.12 – Plots of chemical composition against days of re-growth (age) for area 1 (◊), area 2 (□) and area 3 (Δ). Regression equation for area 3 is showed in Table 3. Poly. means quadratic.

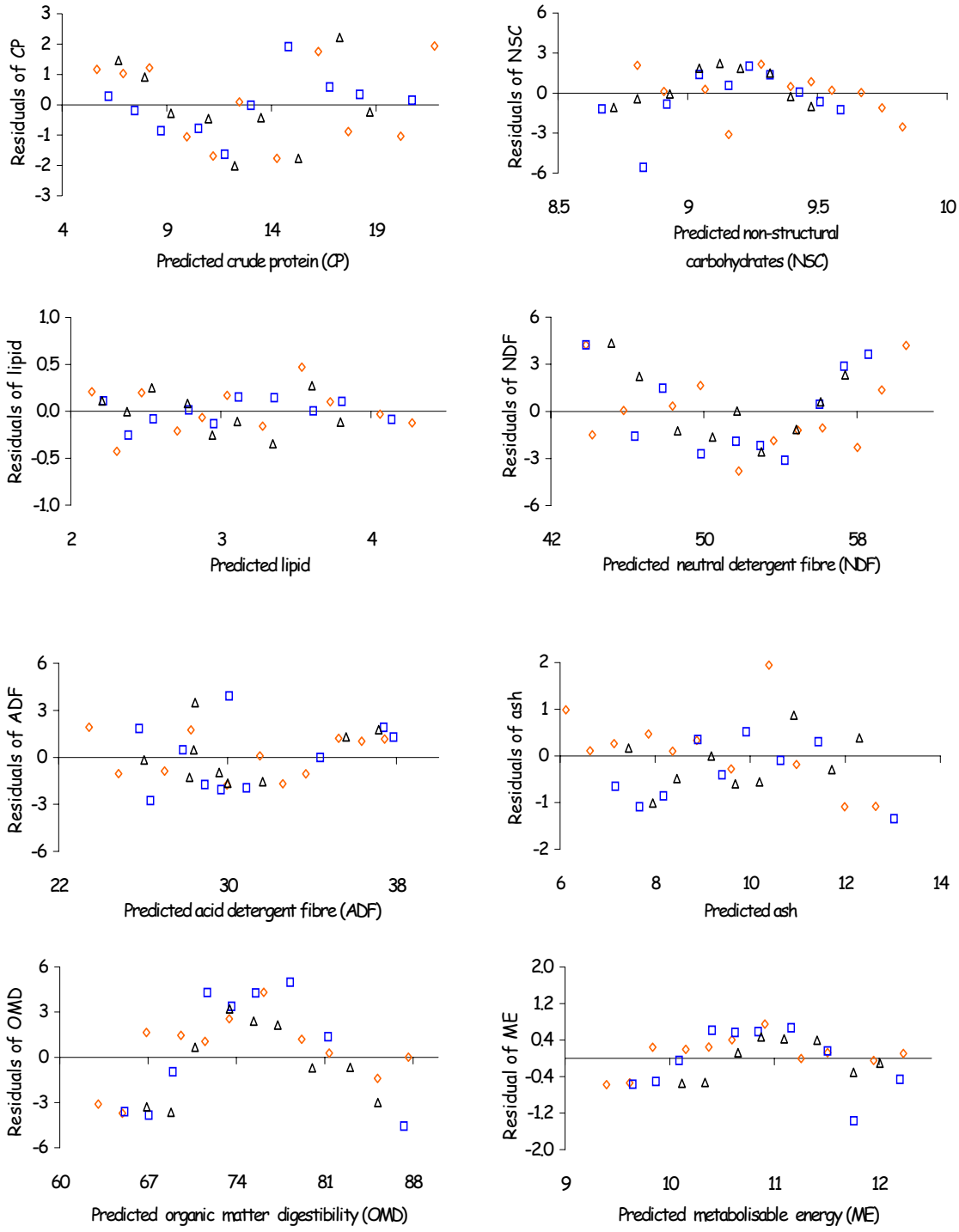


FIGURE 3.13 – Residual plots from the fixed model analysis to demonstrate tendency (linear or quadratic) in change in nutritive value with increase of days of re-growth (age) for area 1(◊), area 2(□) and area 3(Δ).

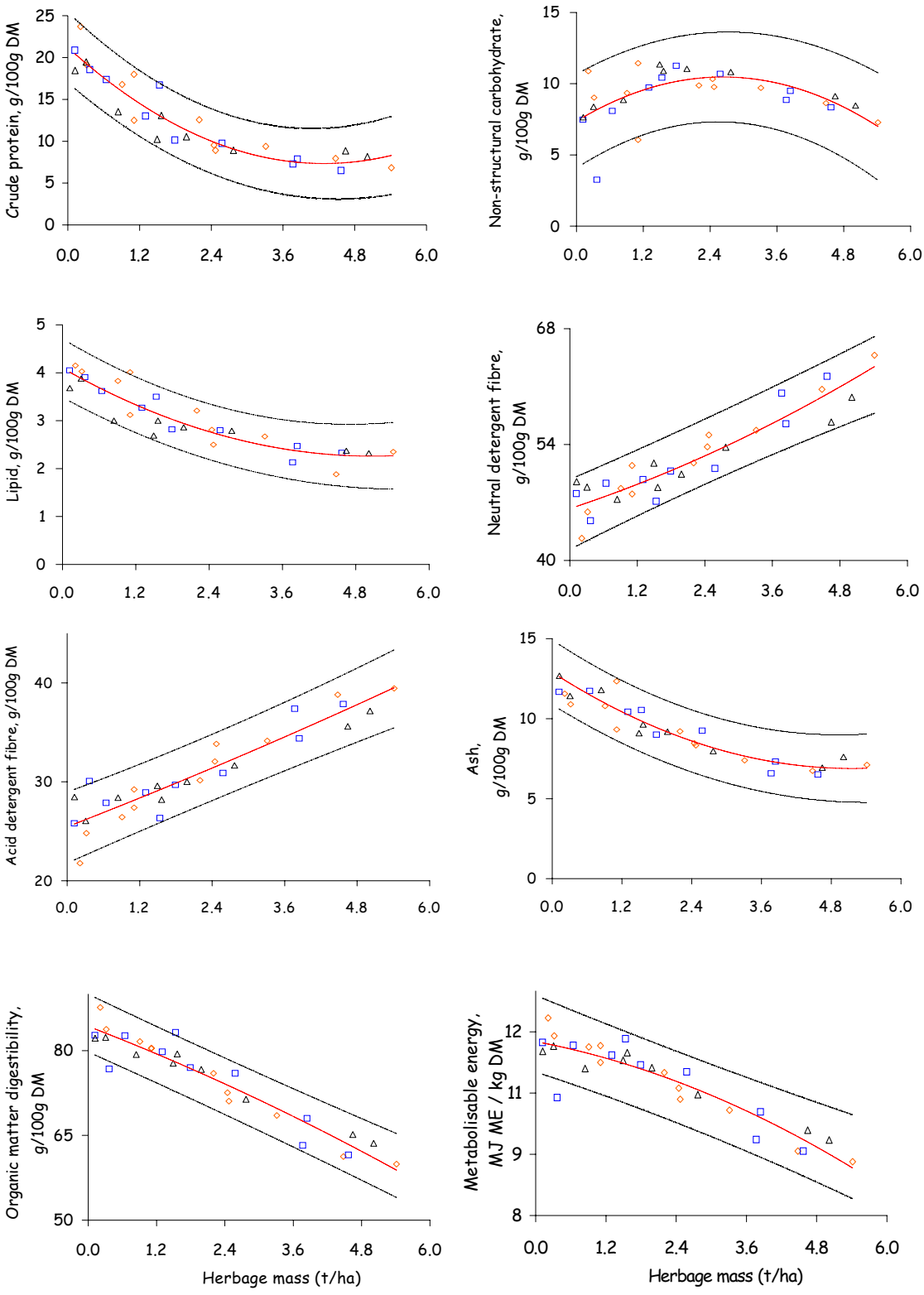


FIGURE 3.14 – Plots of chemical composition against herbage mass (HM) for area 1 (◊), area 2 (□) and area 3 (Δ). Estimates overall regression parameters across areas are presented in Table 6. Dot lines represent 95% confidence limits for individual predicted value.

3.4.3 - *In sacco* results

The *in sacco* incubation measured rates of disappearance of DM, CP and fibre during digestion. The ryegrass had been minced to replicate effects of chewing, and the extent to which cells were ruptured and broken into small particles will influence the accessibility of plant components for bacteria.

The particle size distribution of minced DM (Table 3.10) shows that about 31% was retained on sieves with 2 mm or greater aperture size and 35 - 38% was able to pass through sieves with 0.25 mm apertures. Particle size distribution was similar for the three areas (initial mowing date) and no effects were evident for changing maturity (age). The absence of changes with maturity for soluble DM (A fraction) indicated that the mincer performed in a consistent manner, although more mature grasses required a much greater mechanical effort to mince.

The digestion of minced material reduced the quantity of residues in the *in sacco* bags, indicated by the rates of DM loss and differential rates of disappearance for soluble constituents, fibre and CP. Digestion will alter the composition of remaining (residual) DM and may affect microbial growth and products of digestion after 12 or 24 hours of incubation, relative to initial degradation (0 to 6 hours). The changing composition of residual DM is summarised in Table 3.11 and Appendix 6 (Table 6.2A). Most apparent was the loss of CP with washing minced forage (initial – 0h) and the decline in CP concentration in residues to 24 hours, after which it increased (at 72 hours). The fibre content increased as a consequence of CP and soluble DM loss during the incubation, reaching 70 - 80% of the DM with ryegrass of all ages.

The decrease in CP concentration with digestion was greatest with young ryegrass (20.3 to 6.5% of DM at 24 hours) but the pattern was similar for all levels of maturity (Table 3.11).

Fibre, indicated by NDF, showed a substantial (17 - 21 percentage units) increase in concentration with washing (initial – 0 hours) followed by small increases to 24 hours. Overall, the NDF concentration increased from about 49% of DM in the ryegrass, to about 78% at 24 hours, whilst ADF concentration increased by a lesser amount from about 27% to 45% of DM at the end of the incubation (Table 3.11).

3.4.3.1 – DM digestion kinetics

The DM digestion kinetics have been summarised in Table 3.12 and Figure 3.15 for ryegrass harvested from the three areas (initial mowing date) and maturity (age).

The percentage DM in the soluble "A" fraction of grasses harvested from the three blocks averaged 41% of DM and was not affected by age (P = 0.39) or by initial mowing date (P = 0.63). Thirty four percent of the variation in the value for "A" (DM) was explained by age, mowing date and lignin concentration.

The percentage of slowly degradable DM in the "B" fraction of ryegrass harvested from area one, two and three averaged 56, 51 and 52 respectively (Table 3.12) and was not affected by initial harvest date, days of re-growth or lignin concentration (P > 0.30). The model explained only 20% of the variation in the B fraction of ryegrass. Neither initial harvest date nor age had a major effect upon the proportion of DM in either A or B fraction, so the absence of a significant effect explained by the model may be expected.

In contrast to the distribution of DM in the A and B pools, maturity had a major (P < 0.001) impact on the fractional disappearance rates (k) which declined from 0.11 h^{-1} to 0.03 h^{-1} with mature ryegrass. Rates of DM loss were not affected by either initial harvest date or lignin concentration and the model accounted for 66% of the variation in k.

The DM effective degradability (E), which takes into account the effect of passage from the rumen, was calculated assuming an out flow rate of 0.06 h^{-1},and averaged 65, 67 and 67 for grasses harvested from areas one, two and three respectively. Values declined from about 77% from immature grass to 49% with mature grass harvested from area 3.

In sacco dry matter disappearance data is illustrated in Figure 6.1A (Appendix 6). The impact of forage maturation on rates of disappearance is clearly evident with separate lines defining disappearance of each "age" over 72 hours. Also it shows similar DM degradation rates for mature ryegrass from each area. Differences in DM disappearance for grass harvested at different ages were most apparent at the 12 and 24 hour incubation times, which would affect rumen clearance. Total digestion (after 72h of incubation) was similar for all materials tested (averaging 92.6 %).

3.4.3.2 – CP digestion kinetics

Table 3.13 presents kinetic data for *in sacco* digestion of ryegrass CP. Soluble CP ranged from 46 to 80% of total CP. In contrast to DM, the percentages of CP in the "A" fraction (average 61% of CP) was higher in older plants (P < 0.001) with 73 - 80% released by mincing on the most mature ryegrass (Table 3.13 and Figure 3.16). Initial mowing date did not affect the proportion of solubilised protein (P > 0.09) and the

model explained 78% of the variation in soluble CP. Parameters used to predict quantities of degradable protein (QDP, SDP, ERDP, and RDP) were higher in immature ryegrass because protein concentrations were highest in young material. Rumen degradable protein averaged 12.1 g/100 g DM (83% of total CP) across all ages with a range from 19.4 to 5.6 g/100 g DM and the percentage of protein in the RDP fraction was not affected by days of re-growth.

The slowly degradable "B" fraction of CP had much smaller values than the soluble and rapidly degradable A fraction, averaging about 46% of total CP in immature forage and declining to 25% in mature ryegrass. Initial mowing date did affect the proportion of CP in this fraction ($P < 0.01$) and the model explained 88% of the variation in the proportion of CP in the "B" pool.

Rates of CP degradation ($k = 0.12\ h^{-1}$) were about twice that for DM but rates were not affected by maturity (Table 3.13). Maturation, initial harvest date or lignin concentration did not affect degradation rates for CP ($P > 0.16$) and only 24% of variance was explained by the model.

The effective degradability for CP averaged 83% across the three areas and the rapid and extensive degradation is illustrated in Figure 6.2 (Appendix 6).

3.4.3.3 – Fibre digestion kinetics

The concentration of fibre increased with plant maturation from less than 50% to about 70% of the DM, and after mincing the distribution between soluble and insoluble but potential degradable pools was similar for all levels of maturity (age). The distribution of NDF and ADF between soluble and potential degradable pools, and degradations rates (k) are presented in Tables 3.14 and 3.15, and Figures 3.17 and 3.18. Both NDF and ADF were present mainly in the insoluble and potential degradable ("B") pool. The distribution of NDF was similar for initial cutting date. About 21% of fibre was soluble and 10% undegradable. In contrast to the similar distribution of fibre fractions between pools for all samples, the rate of ADF degradation (k) declined as ryegrass matured ($P < 0.001$; Table 3.15).

The degradation curves for both NDF (Figure 6.3A) and ADF (Figure 6.4A – Appendix 6) show clear effects of age on degradation rates and lag time. Principal effects were reduction in k values from about 0.11 h^{-1} with immature forage to about 0.04 h^{-1} for mature ryegrass. The model accounted only for 21% of the variation in degradation rates for NDF and 65% for ADF, but lignin concentration did not affect rates of fibre degradation or distribution of ADF between pools. Interpretation of rates

were complicated by lag times, which varied in accordance with the model fit to the data but averaged 4.2 and 4.9 hours for NDF and ADF, respectively. When equations incorporated a substantial lag period (e.g.: Figures 3.17 and 3.18) rates of degradation post lag were quite rapid, but when the fit did not demonstrate a substantial lag, rates of digestion were slower. Nevertheless, the fibre digestion was slow ($k = 0.08\ h^{-1}$) relative to CP ($0.12\ h^{-1}$) and shows that the microbial population were either slow to colonise or slow to degrade the fibre fractions of ryegrass, especially when mature (Figure 6.3A and 6.4A). The lags tended to be longer for ADF fraction (which has a higher proportion of lignin than NDF) but lignin did not affect the distribution of fibre between A and B pools ($P > 0.77$; Table 3.15).

The effective degradability incorporates effects of both A and B pools and degradation rate, and calculations for NDF and ADF ranged from about 59% for ryegrass up to 35 days of re-growth to less than 40% for mature forage. These calculations assume a passage rate of $6\%.h^{-1}$, and would probably be lower with very mature forage.

TABLE 3.10 – Particle size distribution of ryegrass dry matter for *in sacco* and *in vitro* incubations indicated by sieve aperture size either retaining or enabling material to pass.

Area	Date	Age	> 2mm	0.25 – 1mm	< 0.25 mm
1	11/09/00	22	36.7	26.7	36.6
1	21/09/00	31	35.9	28.8	35.4
1	5/10/00	45	27.9	30.8	41.3
1	13/10/00	53	36.8	25.6	37.6
1	3/11/00	74	34.5	27.9	37.6
1	3/11/00	88	22.4	50.4	27.3
1	4/12/00	105	19.0	49.9	31.1
1	Average		30.4	34.3	35.3
1	STDEV		7.4	10.9	4.7
2	21/09/00	10	27.8	34.4	37.8
2	5/10/00	24	28.6	30.1	41.2
2	13/10/00	32	39.1	25.1	35.8
2	24/10/00	43	28.4	31.7	39.9
2	3/11/00	53	36.7	23.2	40.2
2	17/11/00	67	34.9	37.2	27.9
2	4/12/00	84	22.6	37.1	40.2
2	Average		31.1	31.3	37.6
2	STDEV		5.9	5.6	4.7
3	5/10/00	14	38.6	25.0	36.3
3	13/10/00	22	42.9	24.7	32.4
3	24/10/00	33	33.3	24.1	42.6
3	3/11/00	43	31.2	31.7	37.1
3	10/11/00	50	25.5	32.4	42.1
3	17/11/00	57	35.1	30.9	33.9
3	4/12/00	74	20.1	45.7	34.2
3	Average		32.4	30.7	36.9
3	STDEV		7.7	7.5	4.0
Average all areas			31.3	32.1	36.6
STDEV all areas			6.7	8.1	4.3

STDEV: standard deviation.

TABLE 3.11 – Composition of ryegrass (0 hour) and *in sacco* residues over the 72 hours digestion period, averaged for young, medium and mature forage. Data derived from appendix 6 (Table 6.2A) and averaged for area 1, 2 and 3. Units all in g/100 g in the DM.

			Duration of incubation (hours)					
	Age	Initial	0	2	6	12	24	72
CP	22-24 days	20.3	17.0	14.3	11.2	10.2	6.5	9.6
	43-45 days	15.5	11.1	9.8	8.4	5.7	5.8	10.8
	67-74days	10.4	5.0	5.2	5.5	4.5	4.2	7.3
NDF	22-24 days	45.7	62.3	65.4	66.9	73.2	78.0	73.0
	43-45 days	48.2	69.3	70.7	66.3	74.1	76.2	69.2
	67-74days	53.2	70.4	72.0	74.6	77.4	79.8	77.9
ADF	22-24 days	26.0	35.8	38.3	42.8	43.7	46.1	46.4
	43-45 days	27.0	39.1	39.9	39.0	44.0	44.9	42.4
	67-74days	32.2	42.3	43.3	46.2	48.4	49.5	46.6

Abbreviations see text.

TABLE 3.12 - Ryegrass dry matter (DM) degradation characteristics (% of total DM) as defined by soluble (A), degradable insoluble (B), undegradable residue (C = 100 – A – B) as well as fractional disappearance rate (k, h^{-1}), lag time (Lag, hour), and effective degradability (E) which takes in account the effect of passage from the rumen[1].

Area	Age	A	B	C	k	Lag	E
1	21	42	57	2	0.07	0.9	71
1	31	38	60	2	0.06	0.0	67
1	45	51	45	4	0.10	3.1	77
1	53	36	57	6	0.08	0.9	69
1	74	43	50	7	0.05	0.9	65
1	88	28	67	5	0.04	0.0	57
1	105	34	56	11	0.03	1.4	52
2	10	46	49	5	0.11	9.8	67
2	24	44	52	4	0.11	1.1	77
2	32	42	53	6	0.08	4.6	67
2	43	51	45	4	0.10	3.1	77
2	53	42	48	10	0.07	1.7	66
2	67	40	55	6	0.04	3.0	60
2	84	36	54	10	0.03	0.0	55
3	14	46	51	3	0.10	1.4	77
3	22	33	62	5	0.08	1.4	66
3	33	50	46	4	0.11	2.7	78
3	43	42	51	7	0.06	1.7	65
3	50	48	48	4	0.07	1.4	72
3	57	45	50	5	0.05	4.2	65
3	74	31	56	12	0.03	3.1	49
Average	49	41	53	6	0.07	2.2	67
Model P		0.1284	0.4298		0.0012		
Age P		0.3927	0.8947		0.0017		
Area P		0.6259	0.3005		0.6457		
Lignin P		0.2698	0.5745		0.9033		
r^2		0.34	0.20		0.66		

[1] Passage rate set at 0.06 h^{-1}.

P: P values assessing goodness of fit for the overall model and tests (age, area and lignin concentration). Area relates to initial mowing date.

TABLE 3.13 - Ryegrass crude protein (CP) degradation characteristics (% of total CP) as defined by soluble (A), degradable insoluble (B), undegradable residual (C = 100 – A – B) pools as well as fractional disappearance rate (k, h^{-1}), lag time (L, hour), effective degradability (E) which takes in account the effect of passage from the rumen. Crude protein is also expressed in terms of quickly degradable protein (QDP), slowly degradable protein (SDP[a]), effective rumen degradability of CP (ERDP), rumen degradable protein (RDP) and rumen undegraded protein (RUP), all in g/100 g DM.

Area	Age	A	B	C	k	L	E[a]	QDP	SDP	ERDP	RDP	RUP
1	22	53	47	0	0.10	0.5	81	12.5	6.9	16.9	19.4	4.4
1	31	51	48	1	0.08	0.0	78	9.8	5.2	13.0	14.9	4.2
1	45	55	43	2	0.18	0.5	87	9.2	5.5	12.8	14.6	2.2
1	53	55	41	4	0.13	0.0	83	9.9	5.0	12.9	14.9	3.1
1	74	61	33	7	0.16	3.3	83	7.6	3.0	9.0	10.6	2.0
1	88	69	30	1	0.03	0.0	78	6.1	0.8	5.7	7.0	1.9
1	105	78	12	10	0.06	12.0	80	6.2	0.5	5.4	6.6	1.3
2	10	56	44	0	0.06	0.0	79	11.8	4.7	14.1	16.5	4.4
2	24	53	45	2	0.19	0.1	87	9.8	6.4	14.2	16.2	2.4
2	32	50	46	4	0.12	2.3	79	8.8	5.3	12.3	14.1	3.3
2	43	70	28	2	0.13	0.1	89	11.7	3.2	12.6	14.9	1.8
2	53	57	35	8	0.34	4.5	85	7.4	3.8	9.8	11.2	1.8
2	67	73	22	5	0.06	4.9	82	7.2	1.1	6.8	8.2	1.5
2[b]	84	77	22	1	0.01	6.0	79	5.6	0.0	4.5	5.6	1.7
3	14	50	49	2	0.20	1.0	86	9.2	6.9	14.2	16.1	2.4
3	22	46	49	4	0.13	0.0	81	9.0	6.7	13.9	15.7	3.8
3	33	54	44	2	0.16	0.0	86	7.3	4.3	10.1	11.6	2.0
3	43	57	35	8	0.20	5.0	82	7.5	3.5	9.5	11.0	2.0
3	50	64	31	5	0.13	0.0	85	6.6	2.2	7.4	8.7	1.5
3	57	75	22	3	0.05	0.5	84	7.9	1.0	7.3	8.9	1.6
3	74	80	15	5	0.02	12.0	81	7.0	0.3	5.9	7.3	1.5
Mean	49	61	35	4	0.12	2.5	83	8.5	3.6	10.4	12.1	2.4
Model P		<.0001	<.0001	0.3456								
Age P		0.0006	<.0001	0.9780								
Area P		0.0957	0.0079	0.6697								
Lignin P		0.1036	0.0699	0.1614								
r^2		0.78	0.88	0.24								

[a] Passage rate set at 0.06 h^{-1}.

[b] PROC NLIN failed to converge.

P: P values assessing goodness of fit for the overall model and tests (age, area and lignin concentration). Area relates to initial mowing date.

TABLE 3.14 - Ryegrass neutral detergent fibre degradation characteristics (% of total NDF) as defined by soluble (A), degradable insoluble (B), undegradable residue (C = 100 – A – B) as well as fractional disappearance rate (k, h^{-1}), lag time (Lag, hour), and effective degradability (E) which takes in account the effect of passage from the rumen[a].

Area	Age	A	B	C	k	Lag	E
1	22	29	64	7	0.14	9.4	61
1	31	20	77	2	0.05	0.8	55
1	45	19	75	6	0.10	1.5	65
1	53	23	66	11	0.09	3.6	58
1	74	30	61	10	0.05	2.2	53
1	88	19	70	12	0.07	10.1	41
1	105 [b]	16	71	13	0.03	2.0	37
2	10	30	63	7	0.09	10.7	53
2	24	17	76	7	0.11	1.9	65
2	32	27	64	9	0.08	7.1	54
2	43	21	73	6	0.09	1.3	63
2	53	24	65	11	0.04	1.3	50
2	67	19	68	13	0.07	10.1	40
2	84 [b]	20	38	42	0.08	1.5	40
3	14	26	69	5	0.10	1.5	67
3	22	13	78	9	0.08	3.3	53
3	33	22	73	5	0.10	1.2	65
3	43	25	67	9	0.04	2.6	50
3	50	24	69	6	0.07	1.6	60
3	57	24	67	9	0.07	9.0	47
3	74	7	77	16	0.03	3.9	29
Average	49	22	68	10	0.08	4.1	53
Model P		0.0962	0.2514		0.4448		
Age P		0.9811	0.1633		0.1431		
Area P		0.6126	0.2672		0.3597		
Lignin P		0.0377	0.9703		0.3734		
r^2		0.39	0.29		0.21		

[a] Passage rate set at 0.06 h^{-1}.

[b] PROC NLIN failed to converge.

P: P values assessing goodness of fit for the overall model and tests (age, area and lignin concentration). Area relates to initial mowing date.

TABLE 3.15 - Ryegrass acid detergent fibre degradation characteristics (% of total ADF) as defined by soluble (A), degradable insoluble (B), undegradable residue (C = 100 – A – B) as well as fractional disappearance rate (k, h-1), lag time (Lag, hour), and effective degradability (E) which takes in account the effect of passage from the rumen[a].

Area	Age	A	B	C	k	Lag	E
1	22	17	75	8	0.15	10.2	54
1	31	20	73	7	0.08	8.8	48
1	45	20	74	6	0.09	1.6	63
1	53	19	69	12	0.10	4.1	58
1	74	29	62	10	0.04	2.7	52
1	88	19	70	11	0.08	10.4	41
1	105	19	74	6	0.02	3.0	37
2	10	21	71	8	0.10	11.6	45
2	24	30	64	6	0.10	1.7	69
2	32	21	69	10	0.08	7.4	51
2	43	19	75	6	0.08	1.1	61
2	53	24	66	11	0.04	1.7	49
2	67	18	69	13	0.07	10.5	39
2	84	22	41	38	0.05	1.3	40
3	14	31	63	6	0.10	2.5	67
3	22	4	86	10	0.08	4.0	48
3	33	28	67	5	0.09	1.1	66
3	43	23	68	9	0.04	3.3	48
3	50	25	69	6	0.07	1.6	59
3	57	22	69	9	0.07	9.2	46
3	74 [b]	10	77	13	0.03	6.0	28
Average	49	21	69	10	0.08	4.9	51
Model P		0.9863	0.2825		0.0024		
Age P		0.9855	0.2814		0.0008		
Area P		0.8651	0.2618		0.0567		
Lignin P		0.9271	0.7704		0.6133		
r^2		0.02	0.27		0.65		

[a] Passage rate set at 0.06 h^{-1}.

[b] PROC NLIN failed to converge.

P: P values assessing goodness of fit for the overall model and tests (age, area and lignin concentration). Area relates to initial mowing date.

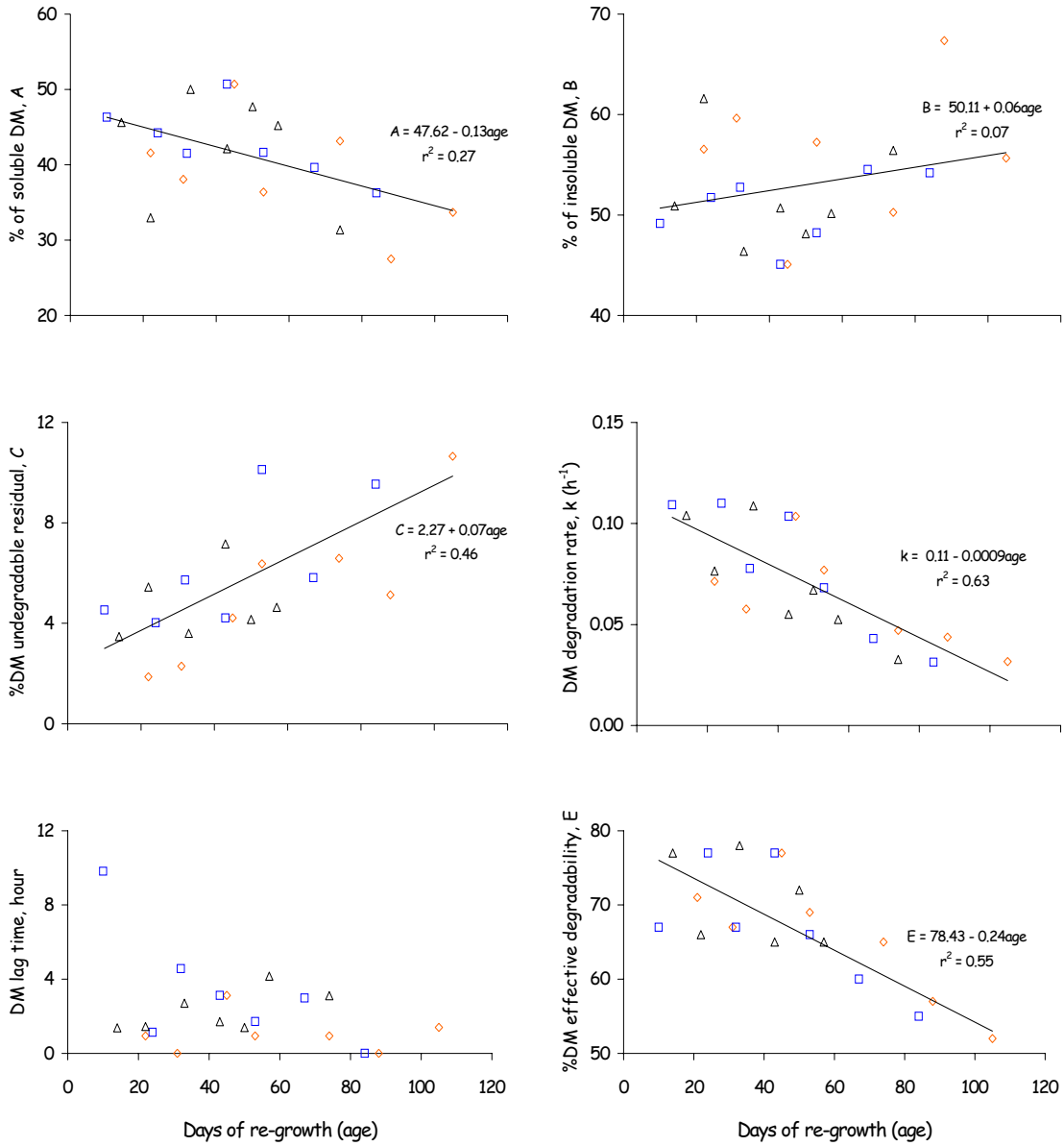


FIGURE 3.15 - Dry matter (DM) degradation parameters during *in sacco* incubations of ryegrass in different ages (days of re-growth) for areas 1 (◊), 2 (□) and 3 (Δ).

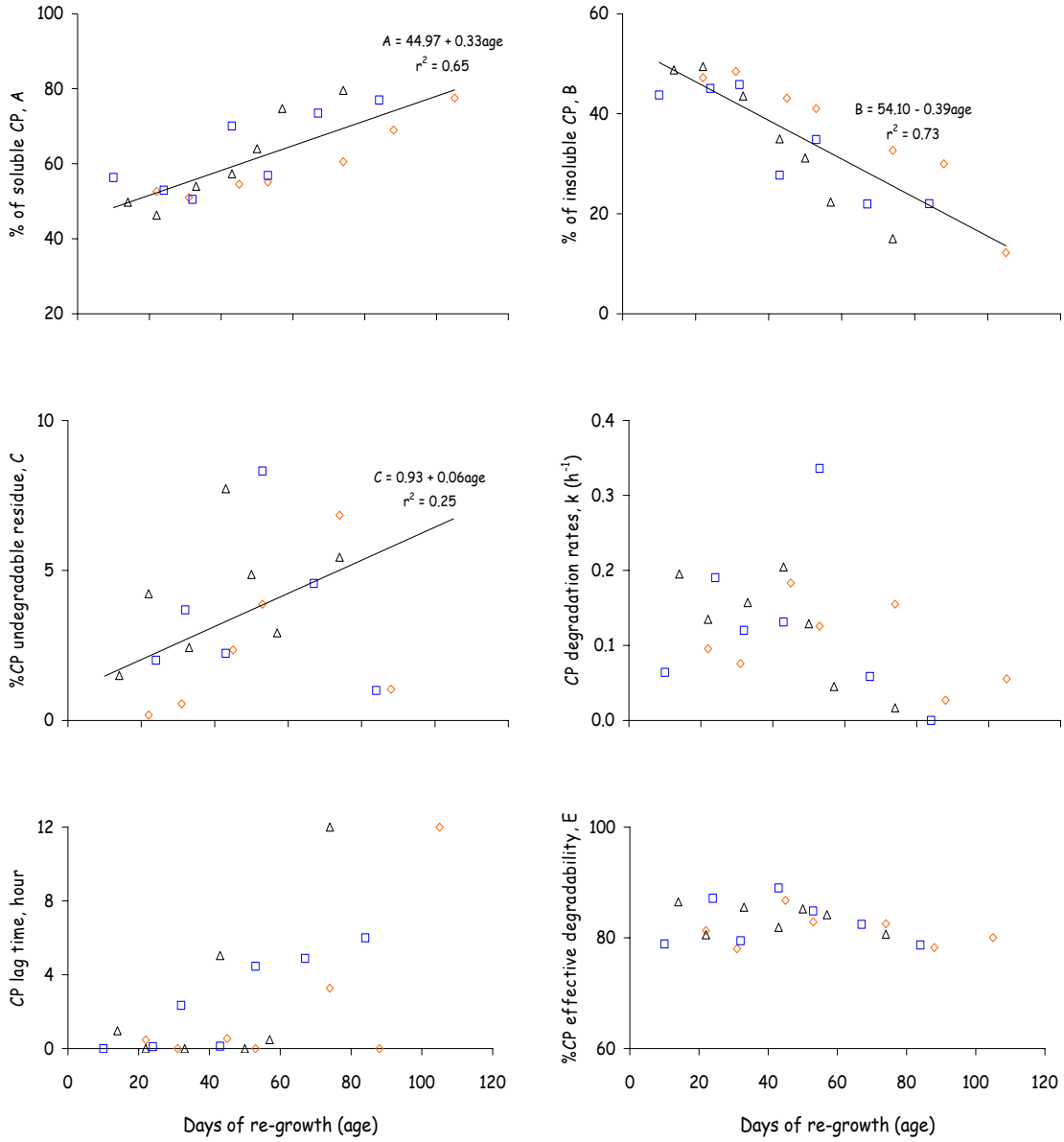


FIGURE 3.16 – Crude protein (CP) degradation parameters during *in sacco* incubations of ryegrass in different ages (days of re-growth) for areas 1 (◊), 2 (□) and 3 (Δ).

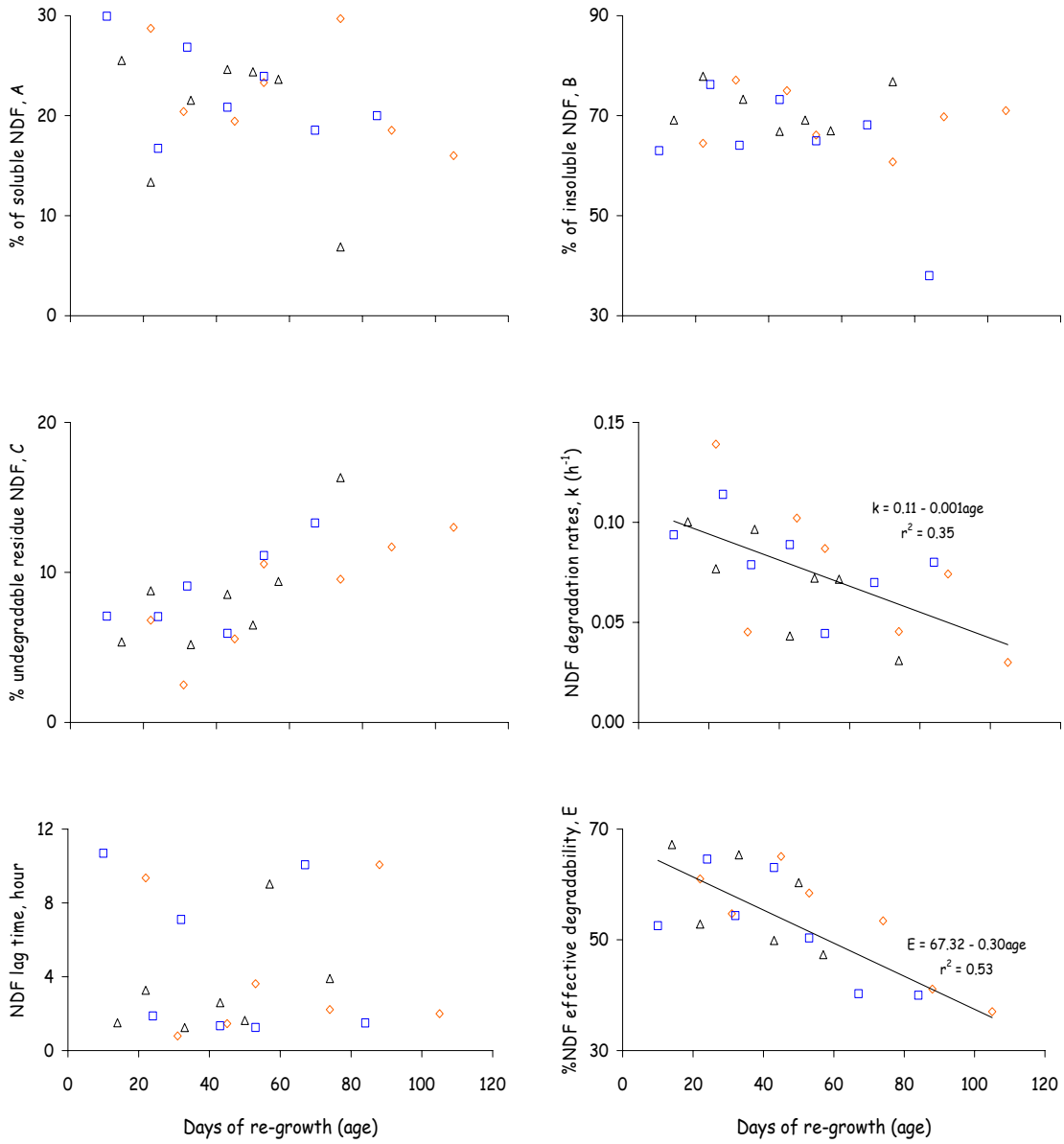


FIGURE 3.17 – Neutral detergent fibre (NDF) degradation parameters during *in sacco* incubations of ryegrass in different ages (days of re-growth) for areas 1 (◊), 2 (□) and 3 (Δ).

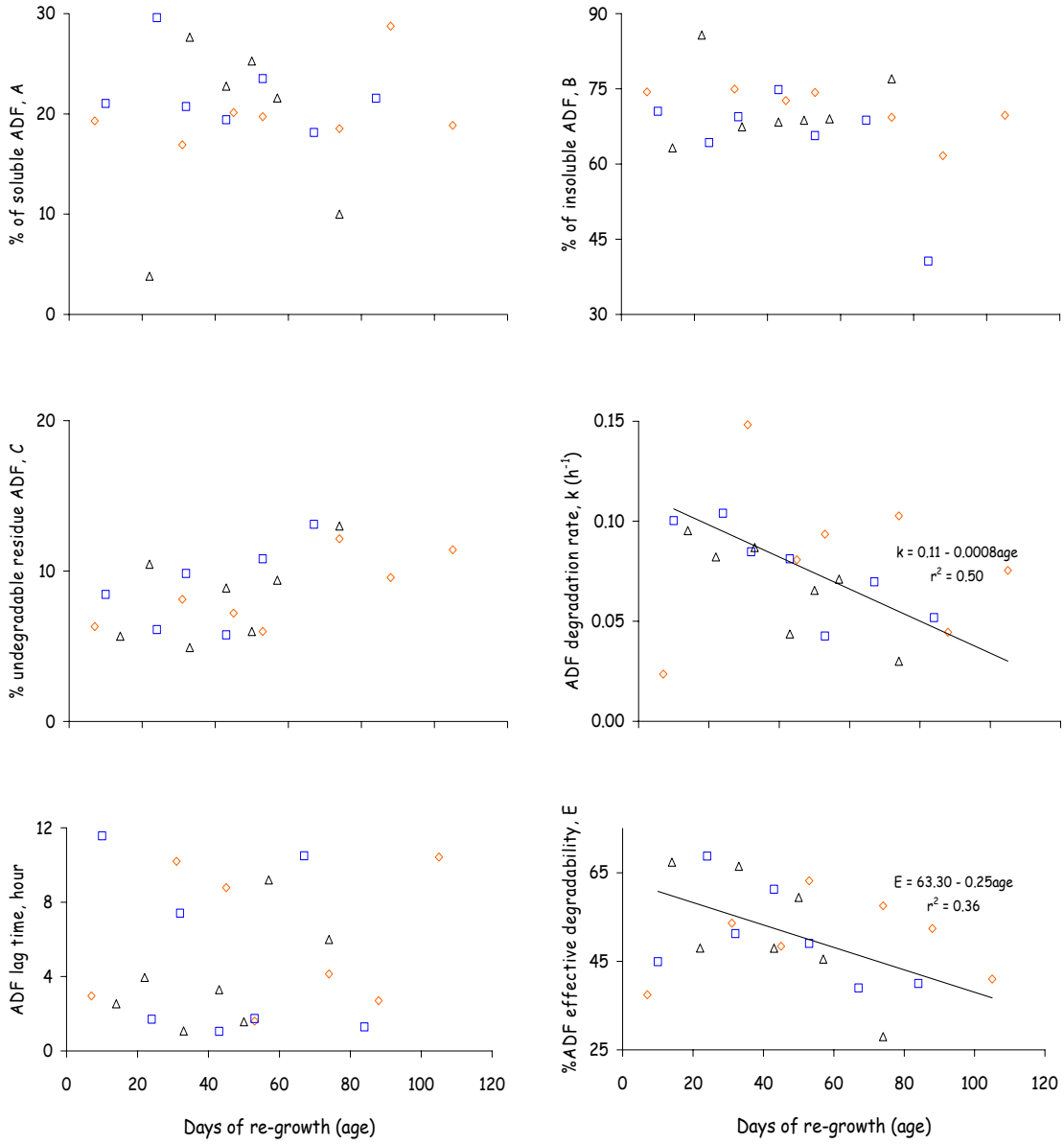


FIGURE 3.18 – Acid detergent fibre (ADF) degradation parameters during *in sacco* incubations of ryegrass in different ages (days of re-growth) for areas 1 (◊), 2 (□) and 3 (Δ).

3.4.4 - *In vitro* results

The buffered incubation media used for *in vitro* runs enabled the net yield of ammonia to be measured as well as both the production and proportions of VFA. All incubations included about 0.5 g minced ryegrass DM and yield of metabolites have been expressed in terms of plant N and plant DM. Calculations require the contribution of rumen inoculum to ammonia and VFA (Table 3.16) pools be subtracted from yields determined over the incubation period (Appendix 4). Net production of ammonia indicates both the extent to which protein degradation exceeds the capacity for microbial utilisation and the likelihood that plant N is insufficient for microbial growth. The *in vitro* incubations complement the rates of disappearance of feed components from *in sacco* bags.

The *in vitro* incubations were buffered to minimise changes in pH and values over the first 12 hours of incubation always remained above 5.6, so the environment was similar to that of cows fed forage diets (normal range about 5.8 – 6.6). The decline in pH over the incubation was due to production of VFA in excess of the capacity for buffering. Most the incubations remained above 5.6 for all time points (Figure 3.19) but data from bottles where pH declined below 5.6 were discarded because the data are probably not representative of forage digestion. Few data were omitted in response to the pH criteria, all from 24 hour samples especially from run D where pH averaged 5.4 after 24 hour.

3.4.4.2 – Net ammonia production *in vitro*

The concentration of ammonia exceeded 0 hour values for about 10 hours of incubation with young forages, harvested up to 32 days of re-growth (runs A and B), but older ryegrass resulted in an ammonia deficit after 3 - 4 hours of incubation (Figure 3.20). The mature ryegrass in runs C, D and E (averaging 57, 71 and 88 days of re-growth) had peak ammonia concentrations after 2 hours of incubation (Table 3.17) and the lowest concentrations after 6 hours of incubation (Figure 3.21). Immature ryegrass, with CP concentrations ranging from 14 to 24 g/100 g of the DM, appeared to have sufficient N to meet microbial requirements for up to 10 hours of incubation and CP concentrations below 10 g/100 g DM in mature ryegrass were inadequate for sustained microbial growth. Increasing ammonia concentration after 12 hours suggests bacterial lysis is exceeding utilisation for growth. Microbial yield of VFA may have been restricted by ammonia insufficiency when pasture over 32 days of age was incubated because microbial growth would be limited.

The extent of CP degradation was determined *in sacco* and these data can be used to provide an estimate of microbial growth *in vitro* when ammonia concentration in the buffered media declined to the 0 hour value. This is calculated (Table 3.17) by multiplying the proportion of plant protein degraded *in sacco* (at the time *in vitro* ammonia concentrations declined to the 0 hour value; Figure 6.2A – Appendix 6; 0.60 – 0.99) by the amount of plant CP incubated *in vitro*. The net release of plant N, assumed to be utilised for microbial growth, ranged from 0.28 to 0.85 mMol N. The calculation could not be made for highly degradable young (22 days) ryegrass, because the ammonia concentration remained elevated over the entire 24 hour of incubation. Microbial growth was not sufficient to utilise all of the N released from immature ryegrass.

In summary, there was a changing pattern of NH_3 production as ryegrass matured; young grass with CP concentration over 17 g/100g of DM (Table 3.3) had sufficient N to sustain microbial growth for at least 10 hours. However older material, even with similar CP concentrations (e.g.: 53 days of re-growth from Area 1), released insufficient CP to maintain ammonia concentrations after 2 hours of incubation (Figure 3.20). The relationship between N incorporated into microbial CP and days of re-growth is presented in Figure 3.24.

TABLE 3.16 – pH, ammonia, volatile fatty acid concentrations and proportion of acetate to propionate in the rumen liquor of the cow used for *in sacco* and *in vitro* incubation runs.

	Runs:				
Rumen inoculum	A	B	C	D	E
pH	6.20	6.59	6.60	6.41	6.39
NH_3 concentration (mMol/L)	21.60	21.40	15.70	20.20	29.30
Total VFA concentration (mMol)	109.00	90.90	72.17	109.10	99.93
Acetate: propionate ratio	4.01	3.90	4.29	3.34	2.94

TABLE 3.17 – Time for peak ammonia concentration and decline to initial (0 hour) values in vitro with an estimate of plant N incorporated to microbial N (in mMol).

			Time after start (h)				
Run	Area	Age	peak NH_3	Return to 0h NH_3 conc.	Plant N (mMol)	Proportion of N degraded [1]	Plant N to microbial N (mMol) [2]
A	1	22	6	NA	1.38	0.99	NA
	1	31	6	12	1.08	0.76	0.82
	2	10	10	12	1.17	0.73	0.85
B	1	53	2	9.0	0.92	0.82	0.75
	2	32	6	10	0.98	0.79	0.77
	3	22	6	10	0.97	0.8	0.78
C	1	74	2	3.5	0.65	0.64	0.42
	2	53	2	3.5	0.69	0.62	0.43
	3	43	2	3.0	0.74	0.6	0.44
D	1	88	2	4.5	0.63	0.72	0.45
	2	67	2	3.5	0.47	0.74	0.35
	3	57	2	4.5	0.53	0.78	0.41
E	1	105	2	3.0	0.32	0.79	0.25
	2	84	2	3.0	0.36	0.77	0.28
	3	74	2	3.5	0.36	0.77	0.28

NA – not applicable as *in vitro* ammonia remained elevated for the 24 hour incubation period.

[1] Based on *in sacco* degradation after incubation times required for *in vitro* ammonia concentrations to return to initial (0 h) values.

[2] Product of plant N and proportion degraded.

3.4.4.3 – VFA production *in vitro*

Concentrations of VFA (Appendix 4) have been summarised for young, medium and mature ryegrass (Figure 3.22) and demonstrate higher total concentrations per gram DM incubated from mature versus young ryegrass. This was apparent for acetate and n-butyrate, but concentrations of propionate were similar for all three maturities at the three sampling times and the concentration of minor VFA were highest from young ryegrass. The rates of acetate: propionate average 3.1 for ryegrass having less than 51 days of re-growth and 3.4 for more mature forage.

When expressed as rates (Table 3.18; Figure 3.23), VFA production declined from an average of 2.17 mMol total VFA/g DM from 0 - 6 hours to 1.14 between 6 - 12 and 0.84 between 12 - 24 hours. During the first 6 hours of incubation VFA production was higher for mature (over 57 days) forage, at 2.69 mMol/g DM than medium or young ryegrass (2.03 and 1.79 mMol//g DM respectively), although effects of maturity on VFA production had disappeared by 12 hours of incubation. Effects of maturity on rates of production were greatest for acetate and minor VFA, and least for propionate. Ratios of A: P produced were similar throughout the 24 hours incubation for young ryegrass (3.19), highest for medium maturity (3.74) and mature ryegrass showed a decline in A: P produced from 3.5 at 0 - 6 hours to 1.9 at 12 - 24 hours.

The yield of VFA over 24 hours was most consistent for young ryegrass (1.79 declining to 1.19 mMol/g DM) and least consistent for mature ryegrass (Table 3.18). When the yield of each VFA was expressed as mg, the proportion of DM degraded and released as VFA was calculated (Table 3.19). These data show on average 14.6, 7.7 and 5.6% of DM converted to VFA after 0 - 6, 6 - 12 and 12 - 24 hours time period respectively and after 24 hours the percentage of young, medium and mature ryegrass DM released as VFA were 26.7, 26.3 and 30.4 respectively.

TABLE 3.18 – Rates of volatile fatty acid (mMol/g DM) production per time period. Data have been combined across areas to give values for young (under 33 days), medium (43 - 57 days) and mature (over 67 days) ryegrass.

Period	Acetate	Propionate	n-butyrate	Minor	Total	Ratio A:P
			Young			
0-6 h	1.12	0.39	0.22	0.06	1.79	3.01
6-12 h	0.66	0.21	0.11	0.03	1.01	3.10
12-24 h	0.82	0.25	0.07	0.04	1.19	3.47
			Medium			
0-6 h	1.31	0.46	0.24	0.02	2.03	3.01
6-12 h	0.73	0.23	0.17	0.03	1.16	4.33
12-24 h	0.50	0.14	0.05	0.02	0.72	3.89
			Mature			
0-6 h	1.81	0.53	0.32	0.04	2.69	3.48
6-12 h	0.79	0.25	0.16	0.04	1.24	3.15
12-24 h	0.43	0.09	0.08	0.02	0.62	4.57

TABLE 3.19 – Amounts (mg) of volatile fatty acids produced per *in vitro* incubation time period. Approximately 0.5 g dry matter was used for all incubations.

Period	Acetate	Propionate	n-butyrate	Minor
		Young		
0-6 h	67.14	29.13	19.59	5.87
6-12 h	39.87	15.50	9.49	2.51
12-24 h	49.53	18.56	6.20	4.07
		Medium		
0-6 h	78.59	33.74	21.48	2.24
6-12 h	43.96	16.68	15.26	3.22
12-24 h	30.31	10.06	4.73	2.25
		Mature		
0-6 h	108.58	38.95	27.86	4.02
6-12 h	47.71	18.40	13.91	3.44
12-24 h	25.86	6.92	6.66	2.19

Abbreviations see Table 3.18.

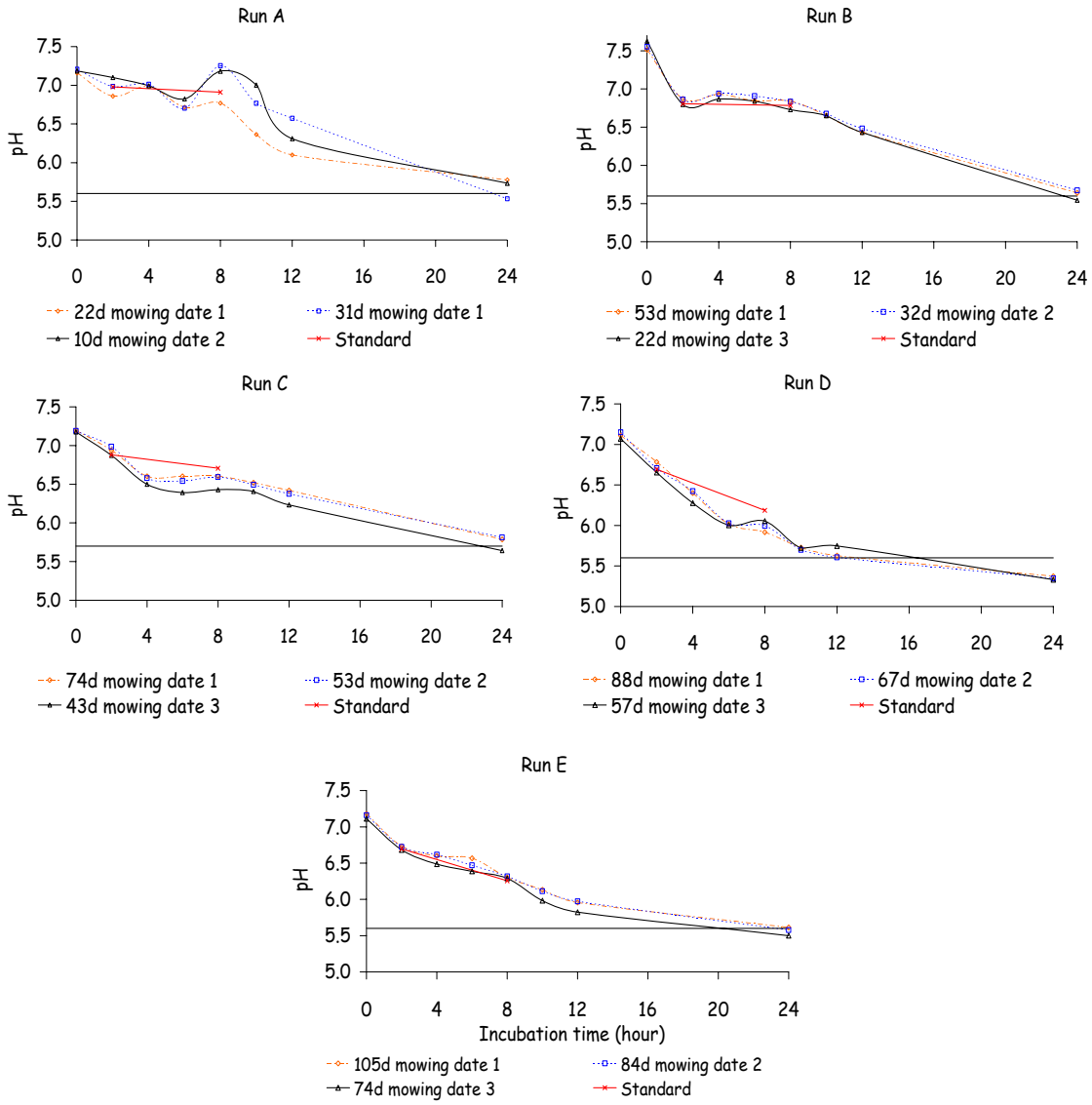


FIGURE 3.19 – pH during *in vitro* incubations for ryegrass at different ages (days – d) in five incubations runs (A to E).

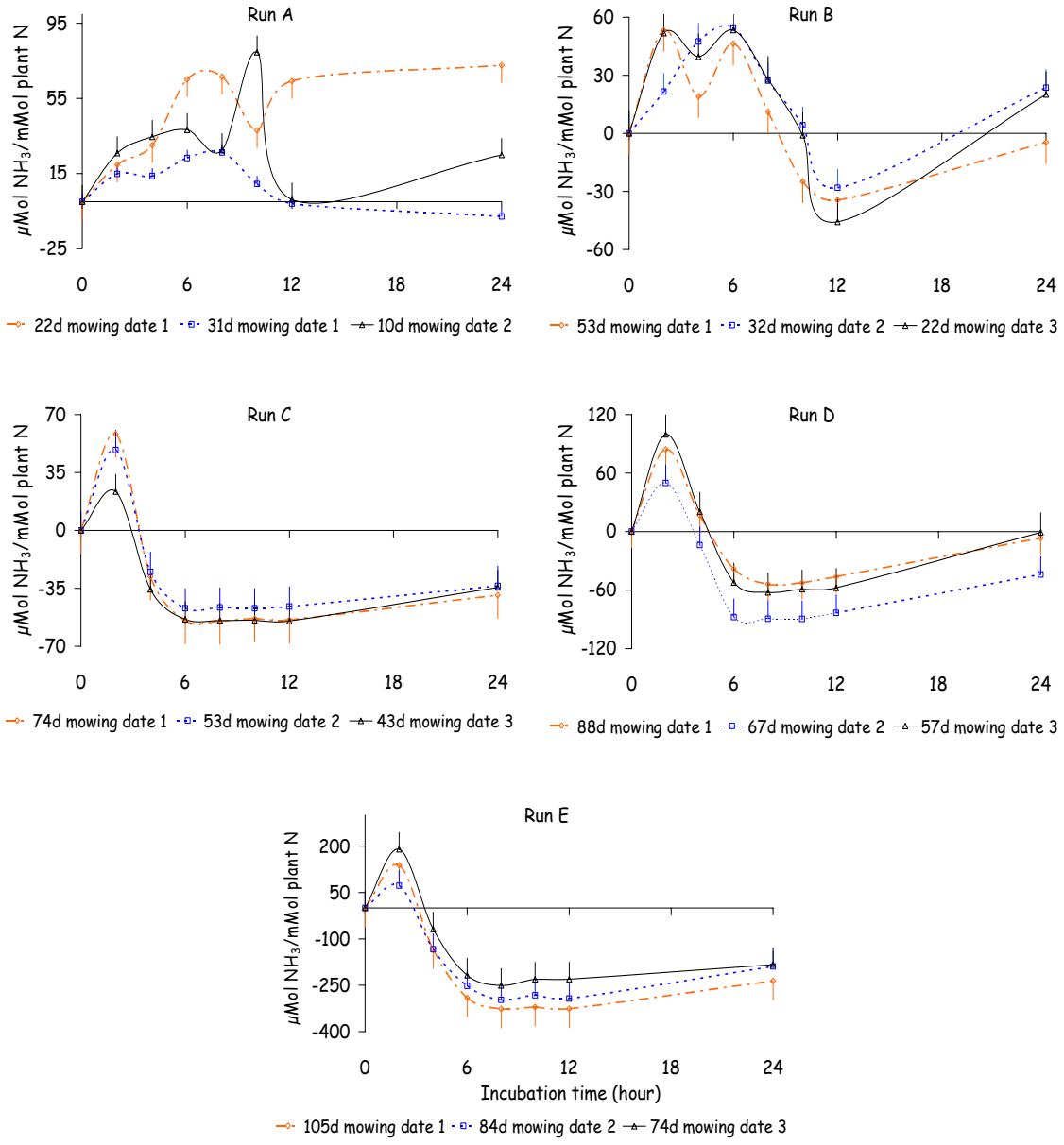


FIGURE 3.20 – Net ammonia production expressed in terms of plant N during five incubations runs (A to E).

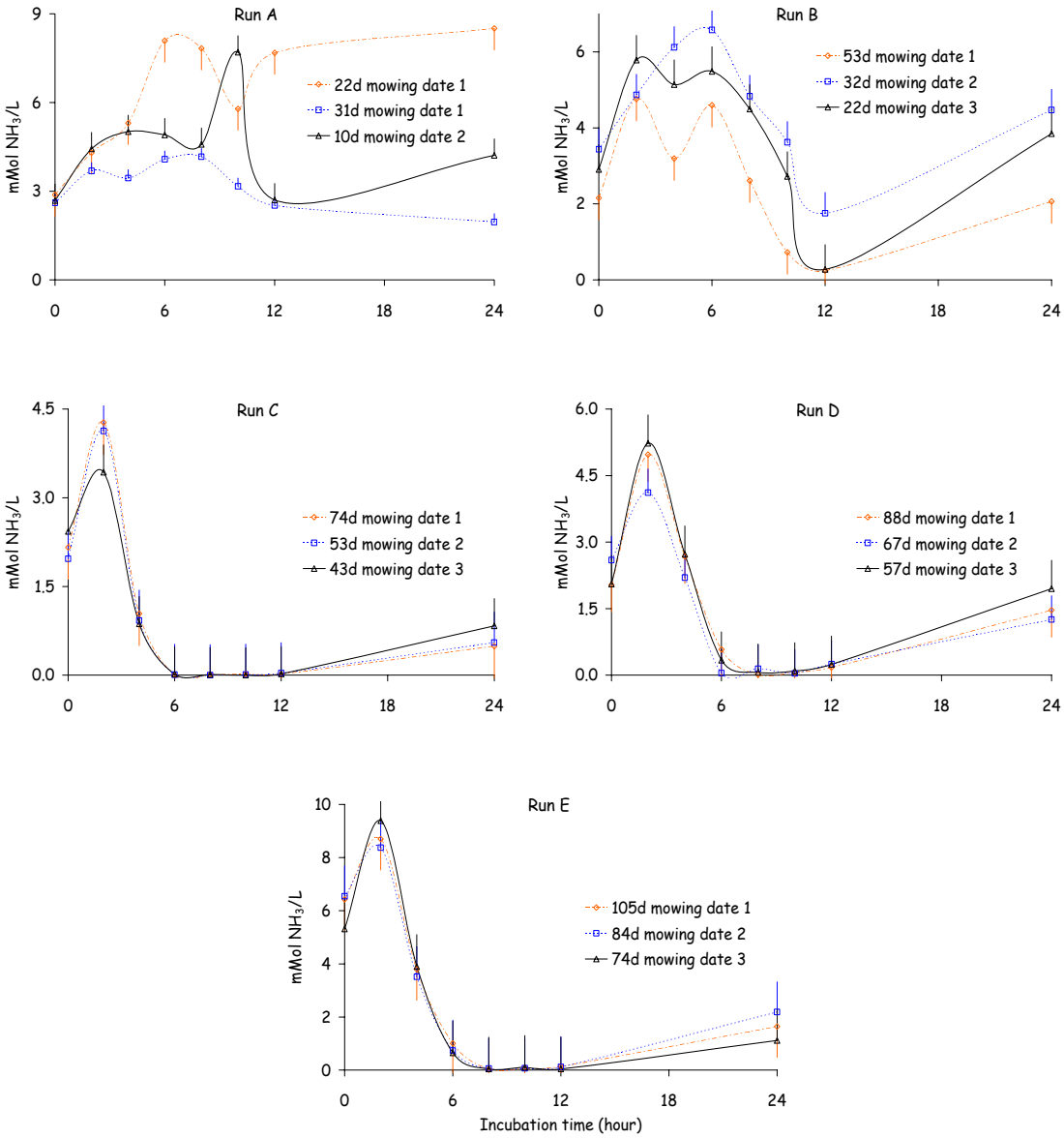


FIGURE 3.21 – Pattern of ammonia concentration mMol/L for *in vitro* incubations.

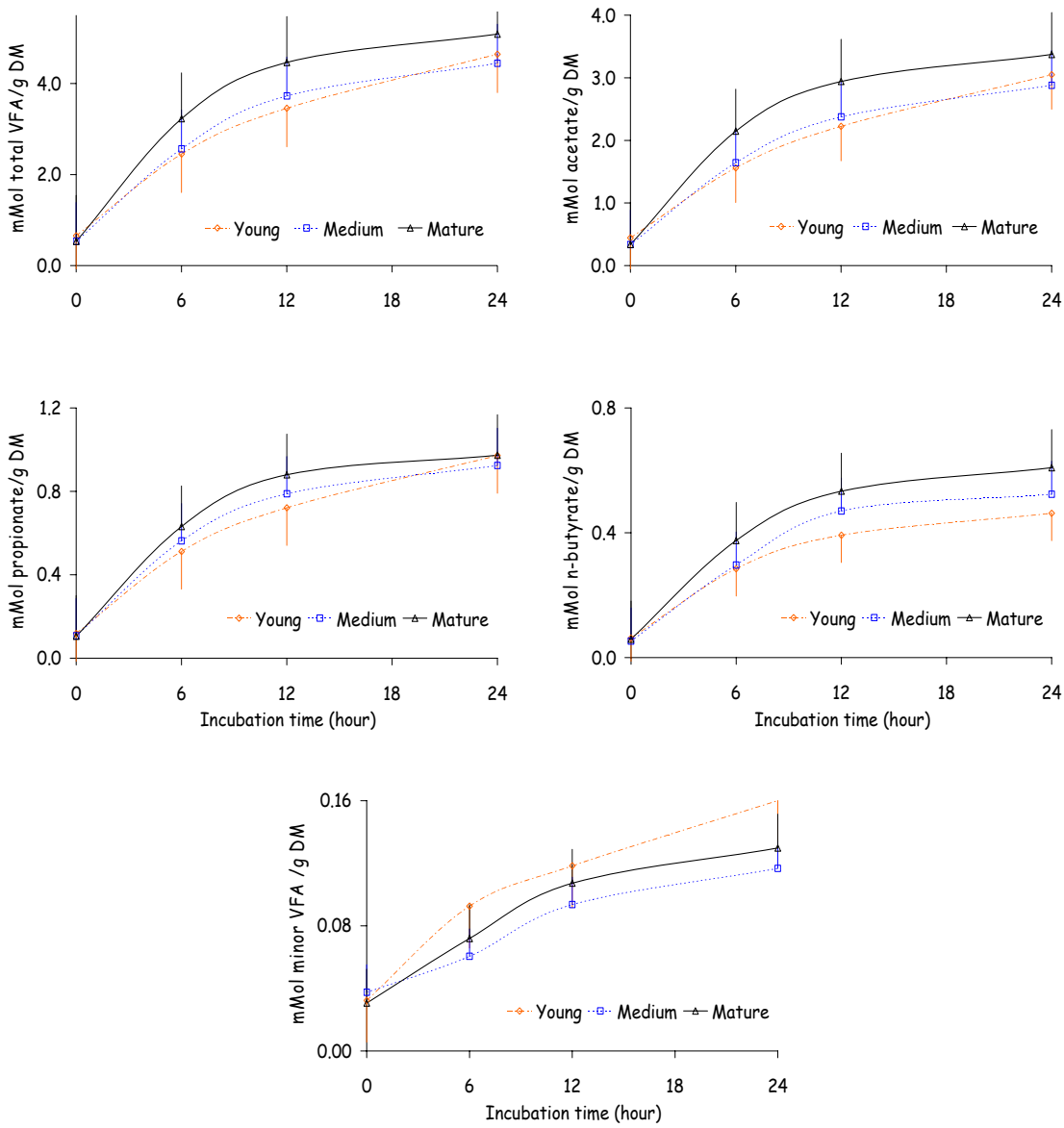


FIGURE 3.22 – Concentration of total VFA, acetate, propionate, n-butyrate and minor VFA (iso-butyrate, n-valerate and iso-valerate) expressed in terms of substrate DM after 6, 12 and 24 hours of incubation *in vitro*. Data have been combined across areas to give values for young (under 33 days), medium (43 - 57 days) and mature (over 67 days) ryegrass.

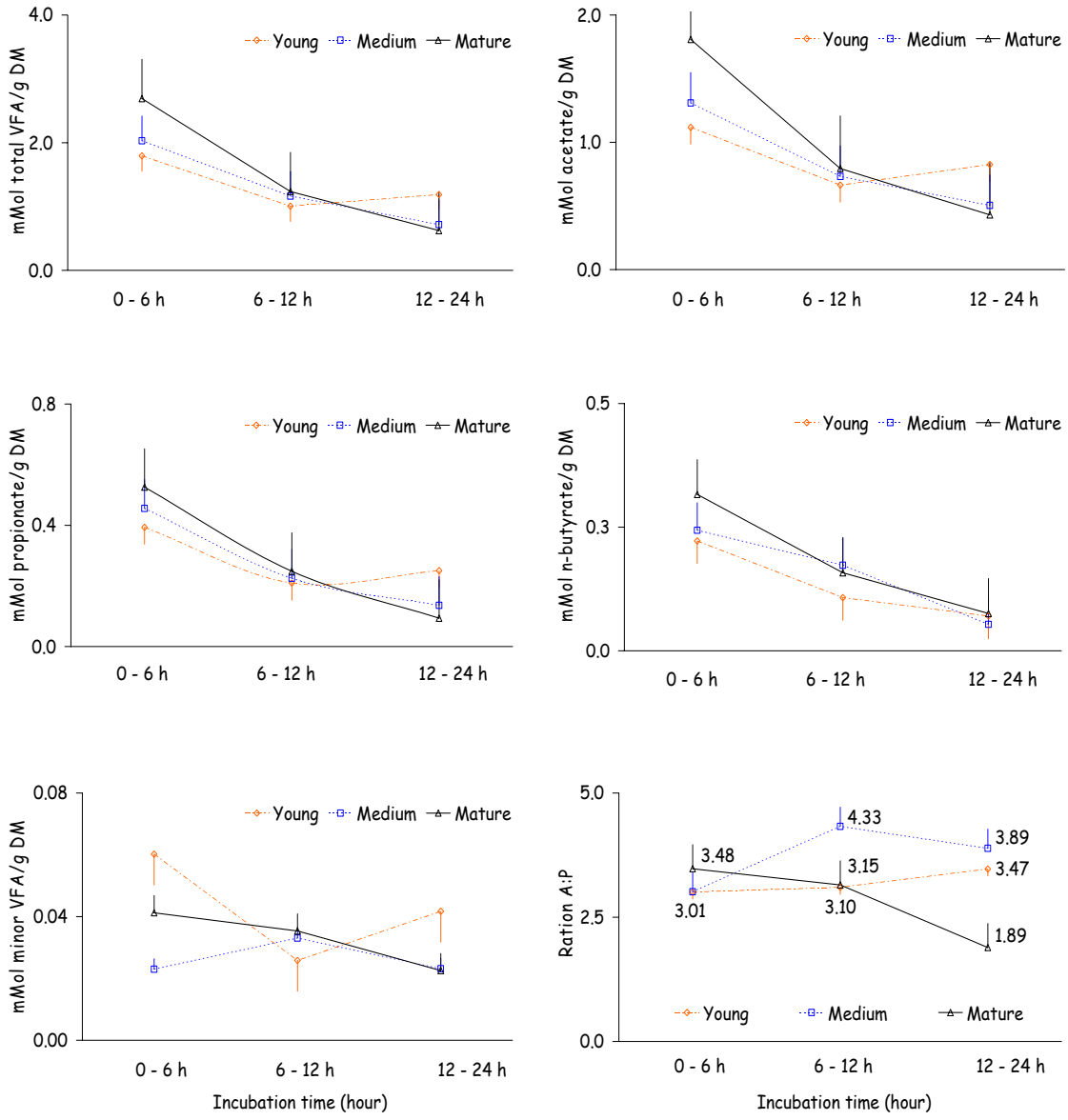


FIGURE 3.23 – Production of total VFA, acetate, propionate, n-butyrate, minor VFA (iso-butyrate, n-valerate and iso-valerate) and the ratio of acetate: propionate expressed in terms of substrate DM available for fermentation from 0 - 6, 6 - 12 and 12 - 24 hours of incubation. Data have been combined across areas to give values for young (under 33 days), medium (43 - 57 days) and mature (over 67 days) ryegrass.

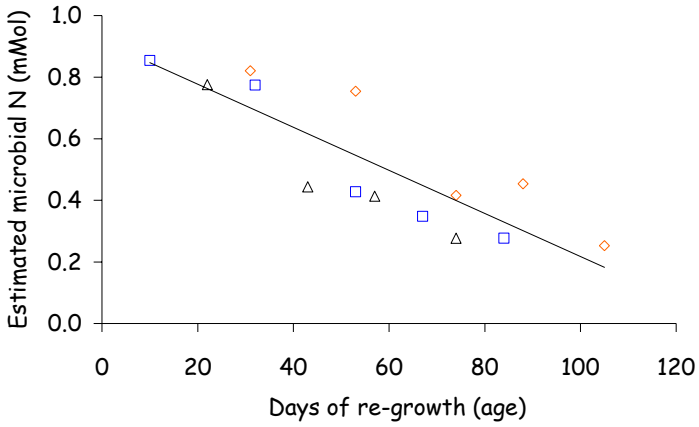


FIGURE 3.24 – The relationship between N incorporated into microbial CP and days of re-growth for areas 1 (◊), 2 (□) and 3 (Δ). Microbial N (mMol) = 0.92 – 0.007*age (r^2 = 0.73; P < 0.001). Data from *in vitro* incubations and assume that all N released from forages, where ammonia concentrations declined to 0 hour values, was incorporated into microbial N.

3.5 - Discussion

The primary purpose of this study was to define rates and products of digestion from ryegrass as it matures. The ryegrass used in this study was allowed to become very mature and normal grazing would be within 50 days of re-growth but a broad range of composition will provide a sound base for diet formulation. Furthermore, mature forage resembles summer pasture in many situations, so the entire data set will enable diet formulation using appropriate feedstuffs to complement ryegrass pastures. Our immediate plans are to apply this information to dairy cow nutrition.

Grass maturation can be defined as the proportion of leaf, stem, inflorescence and dead matter on the basis of chemical composition and in terms of digestibility of components. Data presented here complement work of Armstrong (1964; 1982), Elizalde *et al.* (1999), INRA (1989), Jacobs *et al.* (1998), Mambrini and Peyraud (1994), Waite *et al.* (1964) and Wilman and Agiegba (1982). Wilman and Agiegba (1982) described the changes in digestibility and proportion of leaf, stem, inflorescence and dead material from 2 – 14 weeks of re-growth. Elizalde *et al.* (1999) compared ruminal DM and CP degradation kinetics of fresh bromegrass, tall fescue and lucerne at different stages of maturity and showed an analogous relationship between forage quality and degradation parameters. Mambrini and Peyraud (1994) studying effects of two stages of ryegrass maturity (28 and 49 days of re-growth) on cow performance showed that Increasing maturity reduced grass N content, organic matter digestibility and non-ammonia N flow to the duodenum. Mambrini and Peyraud (1994) also found that total retention time in the gastrointestinal tract increased (43 - 48.8 hours) with a more mature grass and a longer chewing time was associated with coarse particles.

The increase in proportion of stem, with associated increase in fibre content of the DM, and reduction in digestibility are well known (Cowan and Lowe, 1998; Hodgson, 1990; Minson, 1990; Sheaffer *et al.*, 1998; Wilson *et al.*, 1995), but less information is available concerning the rates and products of digestion, composition of *in sacco* residues and the effects of initial cutting date (relative to flowering) on these changes. The data presented here are based on perennial ryegrass cultivar Grasslands Samson and are unique to New Zealand.

Although herbage mass and days of re-growth (age) were evaluated against chemical composition and rates of digestion, these may not be an appropriate base for ration balancing. Herbage mass will depend on soil fertility and growing conditions, so digestion characteristics of ryegrass grown at sites other than that used for this experiment must be based on composition rather than growth. This trial also showed

that the relationship between days of re-growth (age) and digestion was affected by initial mowing date (in relation to flowering), so effects of age should be interpreted in relation to flowering date.

Even though chemical composition has not been a good predictor of feeding value on its own (Cherney and Mertens, 1998; Poppi, 1996; Ulyatt, 1973) it provides the underlying basis for ration balancing. This is shown in formulation of total mixed rations, where chemical composition is supported by requirements for effective fibre, buffers and processing to create a ration able to match cow nutrient requirements and sustain stable rumen fermentation (Mertens, 1997). In a similar way, the chemical composition of ryegrass can form the basis of ration formulation but it needs to be supported by digestion kinetics and this will be affected by time from flowering.

Mathematical modelling, together with laboratory analysis of forage, forms the basis of ruminant feeding systems (Dynes *et al.*, 2003) but many models have been developed to formulate diets from chopped forages fed with grains. These models are essential to formulate balanced diets (Johnston and Shivas, 1999), but data are needed to improve model predictions when forages are grazed and form a major component of the diet (Alderman *et al.*, 2001). Kinetics of degradation and microbial growth will form a significant part of any system designed to predict cow performance from forage diets. Future systems will need to consider constraints to voluntary intake of ryegrass and especially implications of substitution when other forages are offered (Dixon and Stockdale, 1999; Stockdale, 1996; Wales *et al.*, 1999b). Prediction of chemical composition is also vital for model inputs and the use of NIRS (Adesogan *et al.*, 2000; Corson *et al.*, 1999; Givens and Deaville, 1999; Reeves III, 2000) has enabled economical determination of forage composition and products of digestion.

Chemical composition and nutritive value

Fibre was the principal component of ryegrass, with high concentrations of NDF and ADF in the DM even with young ryegrass (NDF and ADF ≥ 42.7 and 21.8 g/100 g DM, respectively). This is typical of pure perennial ryegrass species (Ulyatt, 1984), which differ from pastures that contain clovers and weed species. Cherney *et al.* (1993) measured changes in forage quality with increased maturation for five perennial grasses (tall fescue, foxtail, timothy, reed canarygrass and meadow bromegrass) and showed average NDF concentrations of fibre of young grasses was 40% and increased to 63% of DM after 42 days of re-growth. Chilibroste *et al.* (2000) reported a linear increase in NDF concentration of ryegrass over 30 days of growth from 44.6 to 53.2% at 6 to 30 days of re-growth respectively. The NDF forms a significant portion of ryegrass,

even when vegetative and the increase with maturation occurs before the appearance of the stem or inflorescence (Ulyatt *et al.*, 1988). Once flowering commences the proportion of leaf declines and fibre dominates ryegrass structure (Wilman and Agiegba, 1982).

Conversely, CP concentrations decreased from 23.7 g/100 g DM in young material to only 5 g/100 g DM in mature grass. High values are associated with a high proportion of leaf, and low values with a high proportion of stem. Typical concentrations in ryegrass leaf are 19 g/100 g DM, and in stem 8 g/100 g DM. The increase in stem contributed to reduced plant CP in addition to the changing concentration in plant structure.

Concentrations of NSC (excluding pectins and organic acids) measured in this study did not demonstrate consistent trends with age. The NIRS was calibrated to measure soluble sugars and starches based on chemical analysis (Englyst *et al.*, 1987) but calibration did not include pectin which accounts for about 1 – 2 % of DM or organic acids, fructans and sucrose. These are major components of NSC, particularly in pasture grasses with typical values of 9 – 14 % in DM for water soluble sugars and 5 – 9 % in DM for organic acids (Ulyatt, 1984). Hall (1998) showed that NSC should include all carbohydrates not found in neutral detergent fibre (NDF) so an alternative estimate of NSC could be derived from 100 – CP – NDF – lipid – ash, however this prediction sums errors associated with each analysis. Digestible NDF and NSC comprise the main energy sources available in the rumen.

Digestible nutrient pools

The distribution of DM and its constituents between soluble (A), insoluble but degradable (B) and insoluble undegradable (C) pools will depend on both the composition of the forage and the method of preparation. The absence of age or initial mowing date effects on DM distribution between pools suggests a similar cell rupture and breakage using fresh minced technique, but there were differences in the DM and fibre (NDF and ADF) degradation rate with maturity (k; $P < 0.001$). Rates of DM degradation for young ryegrass were similar to those reported for immature ryegrass (k = 0.114) by Burke *et al.* (2000). The fibre content of grass increased as it matured, but both fibre and DM degradation rates declined as grass matured (Tables 3.12 – 3.14), probably in association with lignification (Jung *et al.*, 1997). There were no meaningful effects of initial mowing dates on rates of fibre degradation.

Although lignin concentration increased from 2.4 to 5.4 g/100 g of DM with ryegrass maturation, concentration was not associated with changes in degradation

rate (k) of any constituent tested (DM, CP, NDF and ADF). Nevertheless, lignification increases the requirement for chewing to achieve the desired degree of cell rupture and increased bonding between lignin and structural carbohydrate appears to account for slower digestion of mature forage (Waghorn and McNabb, 2003). The increased bonding and presence of schlerenchyma and vascular tissues that are tough and resistant to microbial enzymes appear to reduce the effectiveness of chewing, rumen clearance and yield of nutrients during digestion.

The degradation rates for CP were not reduced with age ($P > 0.97$; Table 3.13), and the distribution in minced DM did not match initial expectations. The proportion of CP in the readily fermentable pool increased from about 50% with young ryegrass to about 75% with very mature material. This distribution shows that a higher proportion of protein was released from mature plant cells which were resistant to mincing, but once released (solubilised), plant age had no affects on rate of disappearance *in sacco*. However the amount of CP was affected by plant maturity and this has implications for microbial growth and feed quality.

Predicted OMD declined with maturity, from 83 to about 60 g OMD/100 g of DM. Similar values were summarized by Waghorn and Barry (1987) showing young leafy ryegrass digestibility decreased from 86% to 62% digestibility when mature. Low digestibility is associated with slow degradability and this is indicated by the *in sacco* data. Digestibility is also positively correlated with voluntary feed intake (VFI) (Minson and Wilson, 1980; Minson and Wilson, 1994). However this relationship is empirical and OMD accounts for only about 60% of the variation in VFI of forages for ruminants (Dynes *et al.*, 2003). These relationships are altered by changing particle size (Minson, 1990) and demonstrate the difficulty of predicting the intakes of ruminants fed roughages.

Forage preparation for *in sacco* and *in vitro* incubations

The implications of sample preparation for *in sacco* procedures have been recently reviewed by Cohen and Doyle (2001) who concluded that concentrates should be dried and ground, but fresh mincing was more suitable for fresh forages. This supports the conclusion of McNabb *et al.* (1996) that mincing was more suitable for evaluating protein solubilisation and degradation than drying and grinding fresh forages. Barrell (2000) compared *in vitro* and *in sacco* digestibility of fresh minced, chopped and freeze dried and ground forages and showed mincing minimized lag times relative to chopping or drying and grinding. Preparation affected the ranking of forages and the author concluded mincing was the preferred preparation. Our results from *in vitro* and *in sacco* incubations also indicate minimal lag times, but the extensive release of CP from mature grasses required a comparison between particle size distribution (Table 3.10) and chewed *boli* or rumen contents from ruminants fed immature versus mature forage to demonstrate the suitability of mincing.

While the general objective for sample preparation is to imitate chewing, this is obviously not possible because once forage is placed in the bags it can not be chewed further. The initial mincing is a compromise between initial masticating during eating and further chewing during rumination. The initial particle size of material can not be a perfect representation of *in vivo* particle size because it could be "over chewed" (i.e. smaller particle size than chewed during eating) which will result in degradation that may be too fast (compared to *in vivo*). However, the alternative of "under chewed" would result in an unrealistically slow degradation in the latter part of the *in sacco* period (i.e. after 12 hours of incubation). Ruminants chew plant material several times before it exits the rumen and *in sacco* preparations can only involve a single chewing.

Data in Table 3.20 summarise particle size data for either young or mature grass, leaf and stem or contrasting diets (e.g.: ryegrass versus legumes). This information, from sheep and cattle clearly demonstrates a similar degree of particle size reduction for immature grass versus mature and for legumes, stems and hay. Although the distribution of particle size between large (> 2.0 mm), medium and small (< 0.25 mm) pools differed for individual studies, it is clear that particle size of mature ryegrass was not larger than for immature ryegrass or legumes. These observations were supported by trials with 6 or 12 week re-growth of leaf or stem from tropical grasses which showed no difference in particle size distribution in rumen contents of sheep or cattle (Poppi *et al.*, 1985). Modulus of fineness measurements for rumen contents of sheep were 2.78

mm for 6 week re-growth, and 2.67 mm for 12 week re-growth. Comparative values for cattle were 2.98 and 2.90 mm, respectively.

The percentage of DM able to pass a 0.25 mm sieve after chewing during eating (38%) was similar to that for rumen content (35%; Table 3.20). These values are similar to mean particle size of minced ryegrass (Table 3.10) where 37% of DM passed a 0.25 mm sieve and show the mincer provided a good representation of rumen of diets and maturities.

Particle size distribution in rumen contents was similar to swallowed boli, but proportions on each sieve will depend on outflow rates, intake and time since eating (Ulyatt *et al.*, 1986; Waghorn, 1986). The similar particle size in swallowed boli and rumen contents of sheep and cattle fed diverse diets is a function of time spent chewing, with more chewing required for fibrous than succulent material. Dado and Allen (1994) showed that chewing time was a function of NDF content in diet fed to cows. The speed and ease with which the mincer ground young versus mature ryegrass in this study, showed maturity demanded a much greater energy input for similar quantities of DM but the particle size distribution of young and mature forages were similar (Table 3.10).

The mincing preparation used for incubations presented here showed a high degree of uniformity and the proportional distribution of DM across particle sizes resemble these for chewed forage and rumen contents (Table 3.20).

TABLE 3.20 – Particle size distribution in swallowed boli and rumen content of sheep and cattle fed contrasting diets to indicate effects of grass maturation or differences between forages containing high and medium concentrations of fibre. All perennial ryegrass (*Lolium perenne* L.) unless indicated.

Source	Feed type	Particle size (sieve aperture; mm) > 2	2 - 0.25	< 0.25
	Sheep chewing during eating			
1	Early vegetative ryegrass [a]	48	14	38
1	Early bloom ryegrass [a]	33	32	35
2	Vegetative ryegrass [b]	43	16	41
2	Poor quality (mature) meadow hay [b]	49	29	23
3	Young ryegrass (51 g NDF/100 g DM)	47	14	39
3	Mature ryegrass (61 g NDF/100 g DM)	31	32	36
	Sheep rumen contents			
3	Young ryegrass (51 g NDF/100 g DM)	15	18	67
3	Mature ryegrass (61 g NDF/100 g DM)	13	46	41
4	Tropical grass leaf [c]	12	70	18
4	Tropical grass stem [c]	12	57	31
	Cattle rumen contents			
4	Tropical grass leaf [c]	15	40	45
4	Tropical grass stem [c]	20	46	34
5	Fresh lucerne after a 2 hours meal	29	31	40
5	Lucerne hay after a 2 hours meal	27	38	35
6	Fresh ryegrass after a 2 hours meal	51	12	37
6	Fresh lucerne after a 2 hours meal	39	24	37

Abbreviations: NDF, neutral detergent fibre

[a] Assume 3 and 6% of DM passed a 0.5 mm and was retained in a 0.25 mm sieve for vegetative and pre bloom ryegrass, respectively

[b] Data from publication and also the original data set.

[c] Pangola (*Digitaria decumbens*) and Rhodes (*Chloris gayana*) grasses fed in separated trials, with no significant species effects and averaged for presentation.

1 - Ulyatt *et al.* (1986).

2 - Ulyatt (1982).

3 - Dellow *et al.* (unpublished).

4 - Poppi *et al.* (1985).

5 - Waghorn (1986).

6 - Waghorn *et al.* (1989).

Similar particle size distribution across grass maturities *in vivo* (Table 3.20) and in samples incubated here (Table 3.10) suggest the slower DM degradation rates with increasing maturity is primarily a function of chemical composition rather than particle size. This is also evident with fibre fractions but CP degradation rate was independent of maturity. The higher proportion of small losses (including soluble) DM arising from chewing or mincing does limit the use of *in sacco* procedures because up to 50% of DM (and up to 80% of CP) is able to leave the bags immediately and does not contribute to measurement of degradation kinetics. Soluble DM will degrade very rapidly with only a small fraction leaving the rumen unchanged (Ørskov, 2000). Use of *in vitro* fermentation in conjunction with *in sacco* digestion provides an indicator of products arising from digestion of soluble (A) and insoluble but degradable (B) DM and constituents.

The preparations used here support the idea of Michalet-Doreau and Ould-Bah (1992) who suggested inclusion of large particles were important in forage incubations and acknowledge the requirement to compromise between the mincing preparation and the need to mimic mastication by ruminants.

Mincing resulted in about 37% of DM able to pass a 0.25 mm sieve and 31% unable to pass a 2 mm sieve. Table 3.20 shows this distribution is similar to that of rumen contents in sheep and cattle fed forages but the soluble (A) fraction from ryegrass (Table 3.12) accounted for a slightly higher proportion of DM than identified by sieving (Table 3.10). The higher value for A fraction does support studies showing chewing ruptured about 60% of fresh forage cell (Reid, 1984; Waghorn *et al.*, 1986). The technique for measuring particle size and release of cell contents may contribute to these anomalies. DM loss from *in sacco* bags require washing through pores of about 35 µm, compared to the 0.25 mm sieve used to determine particles size; nevertheless the overall distribution of DM of minced ryegrass does resembles the *in vivo* situation.

The soluble fractions of forages are important for ruminant digestion as they represent readily fermentable energy, and proteins are very rapidly degraded (McNabb *et al.*, 1996). Data from this experiment suggest extensive protein degradation from mature ryegrass and this may be essential for maintaining the microflora when poor quality diets are fed. Differences in proportions of protein release from immature and mature ryegrass is supported by more extensive chewing when mature or fibrous forages are fed (Dado and Allen, 1994). Cows consuming early cut, high quality lucerne spend less time chewing than those fed full bloom lucerne and the higher intakes and milk production support the impact of energy cost associated with

fibre degradation (Brouk and Belyea, 1993; Martz and Belyea, 1986; Robinson and McQueen, 1997).

Particles retained on larger sieves are not soluble and further physical degradation size is important for increasing tissue surface area and exposure to adherent fibrolytic bacteria (Weimer, 1996). Continued particle size reduction, to less than 2 mm (Waghorn, 1986) is essential to enable passage from the reticulum-rumen and avoid constraints of rumen fill and to maximise forage intake. However simple reduction in particle size does not mean the residues will have rapid rates of digestion because many small particles deeply stain with acid phloroglucinol, indicating a high proportion of lignin in the DM and have limited digestibility despite a high surface area exposure (Pond *et al.*, 1984). The Figure 3.25 illustrates fractions affecting passage of forage particles through the ruminant digestive tract. Grazing animals consume forage plants which are broken down by mastication, whereas intensive feeding involves mechanical chopping before ingestion (e.g. maize silage) and these diets are characterised by high voluntary intakes.

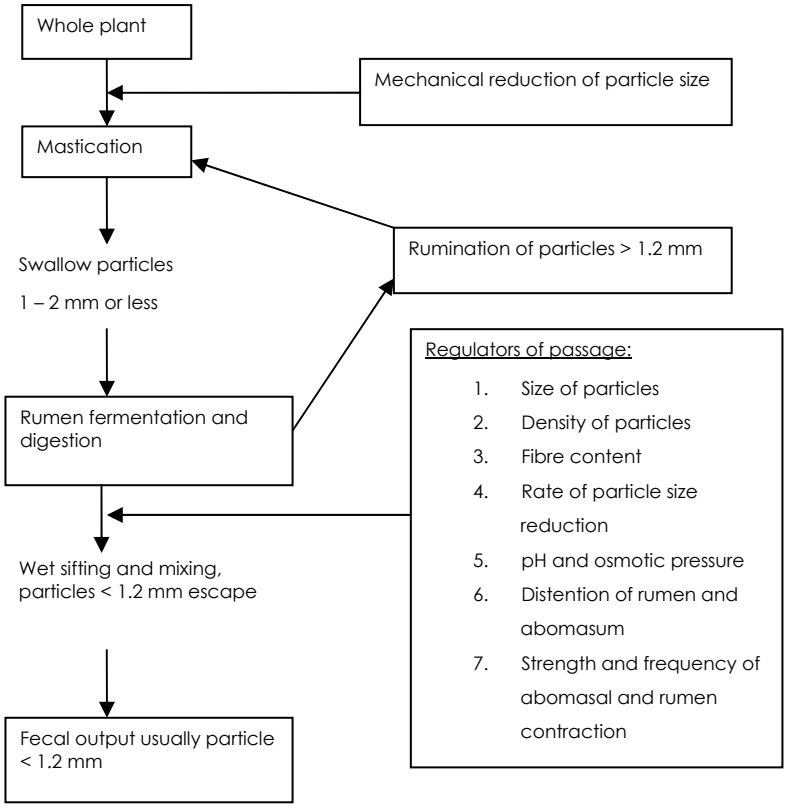


FIGURE 3.25 – Diagram of passage of forage particles in the ruminant. Adapted from Martz and Belyea (1986).

Crude protein degradation

Chewing during eating or mincing released about 40% of DM into soluble or very small particulate matter pools which included about 50% (or more) of CP and non-structural carbohydrates and less than 30% of fibre. These components would enable rapid microbial growth but the release of insoluble (B pool) protein was more rapid (12%.h^{-1}) than fibre (8%.h^{-1}), which is likely to result in a low efficiency of CP utilisation and excessive formation of ammonia when immature ryegrass is offered. The protein consumed by the animal should be partly degradable in the rumen; peptides and amino acids derived from proteolysis stimulate microbial growth and rumen fermentation (Beever and Cottrill, 1994). It is, therefore, important to define the CP degradability (Table 3.13) and ammonia production (Figure 3.20) for ryegrass at different maturities and provide appropriate readily fermentable carbohydrate to limit wastage.

It is difficult to quantify the metabolic cost of ammonia conversion to urea (Lapierre and Lobley, 2001) but there is no doubt that a rapid release of soluble protein from immature ryegrass is wasteful and energetically expensive. Excessive CP degradation may be the most limiting nutritional factor in high-quality temperate forages (Broderick, 1995). Analyses of protein kinetics across all levels of maturity (Table 3.13) showed the proportion of CP as RDP averaged 0.83 and maturation did not affect the RDP: RUP ratio. Regression analyses from 38 studies with dairy cows (206 treatments) were used to evaluate lactation responses to proportions of RDP and RUP (Figure 3.26; NRC, 2001). Those analyses, when applied to cows producing up to 32 kg milk/day suggested a dietary requirement of 12.2% RDP with 6.2% RUP in the DM. The relationship between milk production and dietary protein was:

Milk = - 55.61 + 1.15DMI + 8.79RDP – 0.36RDP^2 + 1.85RUP (r^2 = 0.52) where DMI and milk are kg.day^{-1}, and RDP and RUP are percent of diet DM.

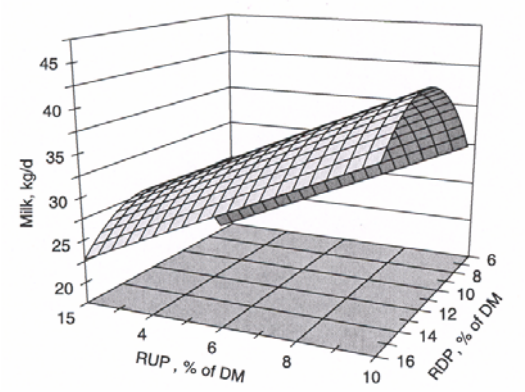


FIGURE 3.26 – Milk production response to rumen undegradable and degradable protein (RUP and RDP, respectively). Dry matter intake held constant at 20.6 kg/day. Source NRC (2001).

Although some success has been demonstrated in complementing rapidly degradable nitrogenous forages with readily fermentable energy (Carruthers *et al.*, 1997; Nocek and Russell, 1988) in most of cases there has been little benefit to cow performance (Huntington and Archibeque, 1999; Kolver *et al.*, 1998; Robinson and McQueen, 1994; Shabi *et al.*, 1998). An alternative strategy may be the reduction in protein solubility through complementing pasture with forage containing condensed tannins (CT). The CT from one forage have been shown to reduce protein degradation in other forages (Waghorn and Jones, 1989) and when fed as mixed diet (Waghorn and Shelton, 1995). Also CT have increased milk production from dairy cows with reductions in fat and increases in protein content in some instances (Harris *et al.*, 1998a; Woodward *et al.*, 1999).

As ryegrass matured the rate of CP degradation was not affected, but the proportion of protein released by mincing increased, so ration balancing would require different criteria to that for immature ryegrass. The lower concentration of CP in mature ryegrass suggests a need for supplementation with RUD to ensure adequate supply for lactation. The *in vitro* incubations of ryegrass older than about 50 days of re-growth demonstrated a very rapid disappearance of CP released by mincing so the microbial growth will not be sustained.

The *in vitro* incubations complemented *in sacco* data and indicated the extent of protein degradation, as well as the rates and proportions of VFA produced. *In vitro* ammonia concentrations represented a net production, so the short peak of NH_3 from mature forages (Figure 3.21) indicated the extent of release by mincing and the rate of

ammonia utilisation by bacteria. However the *in vitro* ammonia concentration was above 3.5 mMol/L, considered minimal concentration for growth (Satter and Slyter, 1974) for the initial 3 – 4 hours of incubation, even with mature forages. Calculations of microbial growth from Table 3.17 and OMD suggests digestion of medium – mature grasses could yield 80 – 120 g microbial CP/kg DOM, which is similar to estimates for cows grazing pasture (98 g/kg DOM; Table 2.13).

These calculations are supported by the similar amount and rate of *in vitro* VFA production from grasses at all stages of maturity (Figure 3.22), so the predominant limitation to performance of cows grazing mature ryegrass may be their capacity for particle size reduction, rumen fill and intake. Recycling of endogenous N would help maintain good levels of microbial growth *in vivo* (Lapierre and Lobley, 2001) but extensive and rapid protein degradation of both immature and mature ryegrass will limit amino acid availability, despite a liquid turnover of 9 – 18%.h^{-1} (Berzaghi *et al.*, 1996; Reis and Combs, 2000). There is a need for either supplementation with readily fermentable carbohydrates or protected protein when cows are given immature pastures, or provision of RDP with mature pastures for milk production greater than 30 kg milk/cow.day. These options should be evaluated with feeding trials to determine to extent of response as pastures mature.

Fibre degradation and VFA production

NDF comprises cellulose, hemicellulose and lignin and represents the cell wall of plants. About 20% of the NDF appeared in the soluble "A" fraction after mincing, but the majority is digested slowly to provide a main portion of the energy derived from rumen fermentation. Rates of digestion of NDF are slow (3 to 14%/h; Table 3.14) compared to CP. The model demonstrated significant reductions in the rate of NDF digestion as ryegrass matured which will limit both the rate and supply of VFA for absorption and possibly feed intake.

The filling effect of mature grass (or higher fibre content diets) is due to resistance to particle size reduction during rumination or chewing (Dado and Allen, 1995; Dulphy *et al.*, 1989). However lactating cows often have high intakes that result in passage of particles at a similar rate as digestion rates of fibre (Mertens, 1992b). Some fibre is not digested and will pass out of the rumen before microbial fermentation is completed.

The impact of ryegrass fibre on rumen fill, function and passage requires careful evaluation. Although NDF usually accounts for 45 - 50% of ryegrass DM, which is well above the 35% upper limit recommended for lactating dairy cows, the effective fibre

(eNDF) content in pastures has been estimated as 43% to maintain rumen pH between 6.0 – 6.2 even though pasture samples ranged from 17 to 78% across studies (Kolver and de Veth, 2002). The fibre in ryegrass is flexible and resilient (Pond *et al.*, 1984) but does not have the "scratch factor" typical of chopped and stalky lucerne or maize, so the assessment of effective fibre given for North Hemisphere TMR do not seem relevant for ryegrass. The analyses reported here have shown the slow degradation of NDF in mature grass is likely to limit both feed intake and the rate at which VFA are supplied to the cow. Dietary NDF from ryegrass may pose a substantial limitation to production from cows grazing pasture (Kolver *et al.*, 2002; Waghorn, 2002).

The proportions and amounts of VFA produced from fermentation are a result of substrate (additional to NDF) and the rate at which degradation occurs. Cellulytic bacteria adhere to particles (Weimer *et al.*, 1999) so the extent to which forage is damaged during chewing (or mincing) will affect rates of VFA production. The *in vitro* incubations showed similar proportions of VFA over the 24 hours duration and this was not affected by grass maturation (Table 3.18). Typically, acetate accounted for about 63 – 67 % and propionate 19 – 22 % of total VFA, which is in accordance with *in vivo* concentrations and production (Berzaghi *et al.*, 1996; Kolver *et al.*, 1998; Reis and Combs, 2000). These data show that maturity does not affect proportions of VFA and the proportions are similar at the commencement of fermentation and after 12 hours.

Most surprising was the rapid rate of VFA production from mature ryegrass during the first six hours of incubation. Production was about 30% greater than immature ryegrass (Figure 3.22). It is possible that the mature grass had a higher proportion of ruptured cells relative to younger material, as indicated by the high proportion of CP released into the soluble (A) fraction or that high N content of immature grass limited microbial activity. These data suggest the changes in chemical composition associated with maturity (Table 3.3) affect the ease and extent to which cell wall are damaged rather than the potential degradability of the cell walls. When ground sufficiently, virtually all plant cell wall can be digested, including lignified structures (Wattiaux *et al.*, 1991).

Yield of VFA after 6 or 12 hours declined with all grass maturities. This was unlikely to be affected by pH of the media for the initial 12 hours of incubation, but low CP concentrations in mature forages must have limited bacterial growth. The soluble DM (especially CP and NSC) would enable a rapid initial fermentation; however incubation of mature forage after 6 hours would not represent *in vivo* conditions where endogenous urea would sustain bacterial growth. The cow is faced with a substantial task in chewing mature forages during eating and rumination This is time consuming,

energy demanding and likely to provide a major constraint to intake as well as release of nutrients.

3.6 – Conclusion

This research illustrated that DM and fibre digestion rates, along with other established forage quality parameters, clearly declined with increased maturity. Plant maturity had no effect on CP degradation rates or VFA yields but affected ammonia production *in vitro* and probably limited microbial growth when very mature. Particle size distribution of ryegrass provided by the mincer indicated a high degree of uniformity and the proportional distribution of DM across particle sizes resembled chewed forage and rumen contents *in vivo*. Changing maturity of ryegrass pastures demands a flexible system of supplementation to accommodate the excessive ammonia production in new growth and inadequate ammonia release from mature grass. The data provided here are able to set the foundation for a model able to predict optimal types and levels of supplementation for dairy cows grazing pasture in New Zealand.

The hypothesis was proven in part; maturation did alter rates of degradation but products of digestion were less affected by maturation and the proportion of N degraded was similar for all maturities. Maturation lowered microbial yield and lessened ammonia production but yield and proportion of VFA *in vitro* was not affected by maturation. Initial cutting dates did not affect rates or products of digestion after a similar number of days re-growth.

Chapter 4

Digestion kinetics of leaf, stem and inflorescence from five species of mature grasses.[1]

[1] A small portion of these data were previously published in the *Proceedings of the New Zealand Society of Animal Production*, 2001, 8-12.

4.1 - Abstract

Digestion kinetics were measured for mature (green and non-senescent) components of five grass species using *in sacco* and *in vitro* incubations to define rates of degradation and nutrient release. Mature perennial ryegrass, tall fescue, Yorkshire fog, phalaris and paspalum were hand separated into leaf, stem and inflorescence for incubations. Concentration of fibre (NDF) in dry matter (DM) fractions ranged from 49 - 68 (leaf), 63 - 72 (stem) and 50 - 68 g/100 g of DM (inflorescence). Crude protein concentrations in the DM of the respective fractions were 7.0 - 23.6, 3.3 - 7.7 and 7.5 - 12.0 g/100 g of DM. Soluble DM (% of the total) determined after mincing accounted for 31 - 54% of leaf, 26 - 56% of stem and 20 - 49% of inflorescence, and fractional (h^{-1}) degradation of the insoluble DM was very slow, ranging from 0.04 - 0.11 (leaf), 0.03 - 0.05 (stem) and 0.03 - 0.08 (inflorescence). After 24 hours of *in vitro* incubation plant nitrogen content become limiting for fermentation in most instances, especially with tall fescue and paspalum. Volatile fatty acid (VFA) production appeared to be similar for leaf, stem and flower fractions, but the proportion of plant DM released as VFA after 48 hours was only 7 – 12%, with a higher value (19%) for tall fescue. Nitrogen concentration in forage DM was not directly related to VFA yield *in vitro*.

Keywords: forages; digestion kinetics; *in sacco*; *in vitro*; plant maturity; dairy cows.

Short title: Digestion kinetics of mature grasses

4.2 - Introduction

A major issue facing the New Zealand dairy industry is the rapid decline in milk production after peak lactation and anoestrous corresponding with grass maturation in October-December. Grasses commence spring growth with vigorous leaf production, which has a high feeding value (nutritive value x voluntary intake) for grazing sheep and cattle, but as daily temperatures rise an increasing proportion of stem and inflorescence appears (Fulkerson *et al.*, 1998). Although the extent to which grass is allowed to produce seed heads can be controlled by grazing management, nutritive value declines because of changes in chemical composition. The principal changes are increased proportions of fibrous stem (Wilman and Agiegba, 1982) and decreased concentrations of leaf protein, so that the maturing plant has higher proportions of fibre and lower proportions of non-structural (readily fermentable) carbohydrate and protein in the dry matter. These changes reduce the amount of amino acids available to

ruminants and may increase the proportions of acetate: propionate available for absorption (Russell and Strobel, 1993). Nevertheless, a significant effect of grass maturation is that the rate of digestion and clearance of residual forage fibre from the rumen is reduced, because mature forages are slower to digest and require more chewing to reduce the particle size of plant fragments to a size able to pass out of the rumen (Chapter 3). The slower dry matter passage rate from the rumen may reduce feed intake. Consequently, maturation in pasture will result in lowered intake as well as declining nutritive value.

Supplementation of dairy cows on pasture is often undertaken without knowledge of the nutrients available from the pasture base. Burke *et al.* (2000) defined the degradation kinetics of immature leafy material from a range of forages to be used as a basis for formulating forage mixed rations (FMR). That work determined degradation rates for dry matter, protein and fibre. They also indicated the amount and proportions of volatile fatty acids (VFA) produced, and have provided a mathematical basis for comparing contrasting feed types, but only for immature leaves. The work described here examines grasses which have not been grazed and are in an advanced stage of maturity, not senescent but with stems and nearly mature flowers. This study aims to determine the digestive characteristics at the opposite end of the range to that of Burke *et al.* (2000), using five very mature grass species.

This experiment involved the separation of the five grass species into leaf, inflorescence and stem (including sheath) fractions for incubation *in vitro* and *in sacco*. *In vitro* incubations were conducted to determine the products of degradation (ammonia from proteolysis and VFA), whilst the *in sacco* technique was conducted to determine the rate at which dry matter (DM) and its constituent chemical fractions are degraded through microbial digestion.

The primary purpose of this work was to define the degradation kinetics and products of digestion from components of mature grasses. The grasses form a cross section of species found in New Zealand, and are also used in grazing systems overseas. Perennial ryegrass forms the dietary basis of New Zealand dairy systems. Tall fescue is used in New Zealand but covers over 14 million hectares in the USA. Tall fescue has many desirable agronomic and forage attributes, forms a dense persistent sward and is tolerant of a wide range of management regimes. Paspalum is a subtropical dairy pasture that occurs in Northland and dominates most irrigated swards during summer and autumn in northern Victoria (Australia). It is a poor quality forage (Stockdale, 1997) but with a wide spread distribution. Phalaris and Yorkshire fog are considered weed grasses in New Zealand, but phalaris (Reed canary grass) is used in

North America and Australia where it is able to produce high biomass and dominate native grasses (Carlson *et al.*, 1996). Yorkshire fog is common in wet, acid and infertile areas and frequently occurs in New Zealand pastures.

Measurements were made of rates and products of degradation from leaf, stem and flowers from five mature grass species to define nutritional responses to flowering.

4.3 – Material and methods

The mature grass species used in this study were perennial ryegrass (*Lolium perenne* L. cv. Grasslands Samson), tall fescue (*Festuca arundinacea*), Yorkshire fog (*Holcus lanatus*), phalaris (*Phalaris arundinacea*) and paspalum (*Paspalum dilatatum*). About 2 kg (fresh) of each species was harvested in the summer of 1999/2000, refrigerated and immediately separated (by hand) into leaf, inflorescence and stem (with sheath) fractions, and stored at -16°C until incubation. Dead matter was discarded. Approximately 500 g wet material was obtained for each plant fraction for *in sacco* and *in vitro* incubations of minced material as well as measurements of dry matter content, particle size distribution of minced fractions and chemical composition by near infra red reflectance spectroscopy (NIRS). These procedures have been described in detail in section 3.3.

Frozen forages were chopped into approximately 2 cm lengths (using scissors) and minced (whilst frozen) in a Kreft Compact meat mincer with 12 mm holes in the sieve plate. Minced material was stored at -16°C until the day prior to incubations when about 2.5 g wet weight (ww; 0.5 g DM) was placed in incubation bottles and 25 g ww (5.0 g DM) into 100 x 100 mm dacron bags for placement in the rumen of a fistulated cow. *In sacco* and *in vitro* incubations were carried out simultaneously for leaf, stem and inflorescence fractions of one species during each incubation. One ruminally cannulated non-lactating Friesian cow was fed lucerne hay for all incubations in order to maintain a similar rumen environment over the period of evaluation and inclusion of ryegrass standards enabled variations between runs to be monitored.

Fourteen bags of each forage constituent were placed in the rumen and duplicates removed after 0, 2, 6, 12, 24, 48 and 72 hours for washing, drying (60 °C), weighing and analysis of residues by NIRS. Disappearance of DM, CP, NDF and ADF fractions were analysed using the non-linear model (No. 1) described by López *et al.* (1999) to determine fractional disappearance rate (k, %.hour^{-1}), lag time (L, hour) and potential degradation (P) according to:

$$P = A + B\ (1\text{-}e^{-k(t-L)})$$

where A = soluble fraction (% of DM, CP, NDF or ADF at t = 0 hour), B = degradable insoluble fraction and t is time in hours. Incubations showed a significant initial lag period for the components so the effective degradability was calculated by incorporating a lag phase with a DM fractional passage rate (k_p) of 0.06 h^{-1} (Hoffman *et al.*, 1993) into the model. Analyses were also made for fibre fractions using a slower outflow rate which may be more typical of poor quality diet (k_p = 0.02 h^{-1}).

$$E = A + B*k/(k + 1/((1/k_p) - L))$$

Effective rumen degradability of CP (ERDP, g/kg DM) was calculated as: ERDP (g/kg DM) = CP [(0.8 x A) + (B x k)/(k + kp)] (AFRC, 1992). Relationships between degradability, nutritive characteristics of the forage components in *in sacco* samples and ERDP were analysed by regression (PROC GLM; SAS, 2001) using the model:

$$\text{ERDP (g/kg DM)} = a + bX$$

where X is the effective degradability of DM or NDF content of forage components for *in sacco* residues.

Twenty-one bottles were prepared with each forage constituent for *in vitro* incubations by adding 12 mL buffer, 0.5 mL reducing agent and 3 mL rumen fluid to the plant material to 50 mL bottles (Burke *et al.*, 2000; Chapter 3). Bottles were made anaerobic by flushing with carbon dioxide and held at 39°C in an oscillating incubator for the duration of each incubation. Triplicate samples were removed after 0, 2, 6, 8, 12, 24, 36 and 48 hours of incubation for determination of ammonia concentrations and VFA at 0, 6, 12, 24 and 48 hours. See appendices 1, 2 and 3 for incubation details, ammonia and VFA analyses.

Statistical analysis

Chemical composition and *in sacco* data from leaf, stem and flowers were analysed by the general linear model (PROC GLM; SAS, 2001). Fixed model analysis was designed to test and detect differences among means for each data set (i.e. concentrations and digestion parameters). The terms tested were: "Forage" (sum of leaf, stem and flower for each forage) and "Components" (means of leaves, stem and flower among forages). As all components of each forage were incubated together there may be run effects in addition to forage effects, so the term "Forage" is in reality a forage-run effect. For *in vitro* data, the following terms were added to the *in sacco* model: "Time" (effect of incubation time on concentration of products of

fermentation); and interactions "Forage*components" (differences among components for each forage specie), "Component*time" and "Forage*time".

Data from *in sacco* incubations were expressed as degradation curves using a non-linear least-square procedure (PROC NLIN; SAS, 2001) to provide estimates for A, B, C, k and lag time. Plots of DM, CP and fibre disappearance against incubation time (in hours) were averaged by component (leaf, stem and flower). No attempt was made to weight degradation estimate parameters, as was done for *in sacco* data in Chapter 3.

Ammonia concentrations in each incubation bottle were used to calculate net ammonia production (Appendix 2) and plotted against incubation time. Analyses were based on net conversion of plant N to ammonia N and also concentration of ammonia in media at each incubation time (mMol NH_3/L). The conversion of plant N to NH3-N was evaluated by comparing plant components, forages and interactions described above. VFA analyses were based on single samples for each of the five periods for each forage component. VFA production was expressed as net production mMol/g DM incubated and as molar proportion of acetate: propionate (A: P). Mean pH values for each forage component at each time point were determined and plotted over incubation time for the 15 forage components incubated. Appendix CD (Chapter 4) has details of SAS procedures used for statistical analyses and complete data outputs.

4.4 – Results

4.4.1 - Chemical composition

Although dead matter was discarded during the hand separation of leaf, stem and flower, the high DM percentage of most components, together with NDF concentrations in excess of 50 g/100g of the DM, demonstrate the maturity of the forages collected (Table 4.1). Paspalum had the highest NDF concentrations (over 65 g/100 g of the DM for all constituents), with a low crude protein (CP) concentration (below 8 g/100 g of the DM). Leaf of temperate forages (ryegrass, tall fescue, Yorkshire fog and phalaris) had at least 15 g CP/100 g of DM, with lower concentrations in flowers and low (< 10 g/100 g of DM) and variable CP concentrations in stem. Non-structural carbohydrate concentration was low (< 10 g/100 g of DM) in all plant components, except tall fescue flower (Table 4.1). Stem accounted for 40 - 60% of plant DM across all grasses but leaf ranged from 8 - 40% and flower 12 - 30% of the DM. Mature ryegrass

and Yorkshire fog had over 35% leaf when mature, whereas fescue and phalaris had less than 15% leaf and over 30% flower dry matter.

4.4.2 - Particle size

The particle size distribution of minced material (% of DM) showed that 20 - 26% of leaf, stem and flower fractions of perennial ryegrass, tall fescue and Yorkshire fog were retained on sieves with 2 mm or large aperture sizes, although a higher proportion of tall fescue flower (38% of DM) was retained on these sieves (Table 4.2). In contrast only 7 - 13% of paspalum fractions were 2 mm or larger in size. Dry matter passing sieves with a 0.25 mm aperture size (fine particulate and soluble DM) accounted for 29 - 57% of plant DM, with a narrow range across the five grass species for flowers (35 - 47%) relative to leaves (tall fescue 30% - paspalum 57%) and stems (tall fescue 30% - Yorkshire fog 46%). Intermediate sized DM (0.25 – 1.0 mm) accounted for 37 - 43% of DM across leaf, stem and flower fractions of the five grasses (Table 4.2).

TABLE 4.1 – Dry matter concentration (g DM/100 g material) at harvest, chemical composition (g/100 g of the DM), predicted organic matter digestibility (g/100 g) and metabolisable energy (ME; MJ/kg DM) content of leaf, stem and flower fractions of five mature grasses used for measurement of digestion kinetics.

Forage	DM	CP	NSC	Lipid	NDF	ADF	ADL	Ash	OMD	ME
Perennial ryegrass										
leaf	21.9	18.9	8.7	4.0	49.2	23.7	4.7	11.5	79	8.5
stem	30.2	7.7	6.5	1.6	62.6	38.0	5.7	8.9	56	8.2
flower	45.5	12.0	8.3	3.4	52.8	31.5	5.2	8.1	61	8.8
Tall fescue										
leaf	30.5	15.0	4.5	3.1	56.0	33.9	3.6	12.1	64	9.0
stem	36.1	6.0	7.1	1.4	65.7	39.0	6.3	6.5	53	7.9
flower	46.7	8.7	18.5	2.2	50.6	32.5	3.4	6.6	62	9.1
Yorkshire fog										
leaf	20.3	23.6	6.2	4.2	50.8	24.7	4.3	8.9	78	9.9
stem	34.1	5.0	4.8	1.4	68.3	42.1	6.5	6.8	52	7.7
flower	54.3	8.6	5.6	5.3	59.0	34.0	4.6	6.2	57	8.2
Phalaris										
leaf	32.2	21.6	5.5	4.3	49.7	30.8	1.6	12.0	71	9.7
stem	34.6	6.1	0.0	1.3	71.8	48.3	6.5	8.9	46	6.7
flower	37.6	10.1	8.3	4.3	50.1	32.3	4.6	8.8	59	8.4
Paspalum										
leaf	39.3	7.0	0.1	3.1	67.7	42.4	6.2	10.0	54	7.7
stem	28.6	3.5	6.6	1.3	65.4	41.5	5.6	8.5	56	8.2
flower	50.7	7.5	0.0	3.3	67.7	43.1	7.7	5.5	39	5.8
Mean across species										
leaf	28.8	17.2	5.0	3.7	54.6	31.1	4.1	10.9	69.1	8.9
stem	32.7	5.7	5.0	1.4	66.7	41.8	6.1	7.8	52.7	7.7
flower	47.0	9.4	8.2	3.7	56.0	34.7	5.1	7.1	55.7	8.0

Abbreviations: CP, crude protein; NSC, non-structural carbohydrates; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; OMD, organic matter digestibility; ME, metabolisable energy.

TABLE 4.2 – Dry matter particle size distribution of leaf, stem and flower fractions of five mature grasses for *in sacco* and *in vitro* incubations indicated by sieve aperture size either retaining or enabling material to pass.

Sieve size	≥ 2 mm	0.25 - 1 mm	< 0.25 mm
Perennial ryegrass			
leaf	22.2	37.6	40.2
stem	25.8	34.0	40.2
flower	24.1	28.8	47.1
Tall fescue			
leaf	19.9	50.6	29.6
stem	23.8	47.2	29.0
flower	38.0	22.1	39.9
Yorkshire fog			
leaf	26.0	21.4	52.6
stem	20.2	34.3	45.5
flower	20.6	32.3	47.1
Phalaris			
leaf	22.7	39.8	37.5
stem	13.1	47.7	39.2
flower	12.1	50.5	37.4
Paspalum			
leaf	9.6	33.8	56.6
stem	6.6	53.0	40.4
flower	12.5	52.4	35.1
Average	≥ 2 mm	0.25 - 1 mm	< 0.25 mm
leaf	20.1	36.6	43.3
stem	17.9	43.3	38.9
flower	21.5	37.2	41.3
STDEV	≥ 2 mm	0.25 - 1 mm	< 0.25 mm
leaf	6.3	10.6	11.1
stem	8.0	8.6	6.0
flower	10.6	13.5	5.5

STDEV = standard deviation.

4.4.3 - DM digestion kinetics

In sacco dry matter distribution across fractions and disappearance rates are summarised in Table 4.3 and illustrated in Figure 4.1. DM in the soluble (A) fraction averaged across forage species and components was 38% with flowers having lower values than leaves and stems ($P < 0.0001$). The model explained 44% of variation in soluble DM content for forage species and components.

The insoluble and slowly degradable DM fraction (B) differed between components with higher values for leaves followed by flowers and stems ($P < 0.0001$; Table 4.3). The model explained 57% of the variation in the B fraction for all forages. The undegradable (C) fraction was higher for stems and flowers (23%) compared to leaves (11%).

Kinetic data derived from curves fitted to *in sacco* data (Table 4.3) show rapid degradation rates of ryegrass, paspalum and fog leaf and very slow degradation of phalaris and fescue leaf. However the rapid degradation of paspalum leaf was preceded by a 10 hour lag period, whereas the model did not fit a lag period to phalaris leaf. Leaf, stem and flowers had similar, slow degradation rates for phalaris but stem degradation was preceded by an 8 hour lag. Differences in effective degradability (a prediction of degradation *in vivo*) incorporate effects of lag phase with degradation rate and outflow (0.06 h^{-1}) and show similar low values for both stem and flower relative to leaf. Effective degradability will be determined by particle size and release of soluble DM during mincing and/or chewing as well as the physical and chemical structure of leaf, stem and flower.

Table 4.3 and Figure 4.1 show similar rates of degradation for both flower and stem fraction DM averaged for the five forages which were about half that for leaf ($P < 0.0001$). The error bars indicate substantial differences between grass species in degradation rate of individual constituents, and this is further demonstrated by the DM disappearance from the leaf fraction of each species in Figure 4.2. The model accounted for sixty seven percent of the variation in rate of DM digestion (k).

Paspalum flower was predicted to be especially indigestible (E = 35%) compared to flower from temperate grasses (E = 51 - 59%). Most constituents of mature grasses had substantial lag periods prior to DM loss, suggesting a relatively slow colonisation of particles by rumen bacteria and fungi.

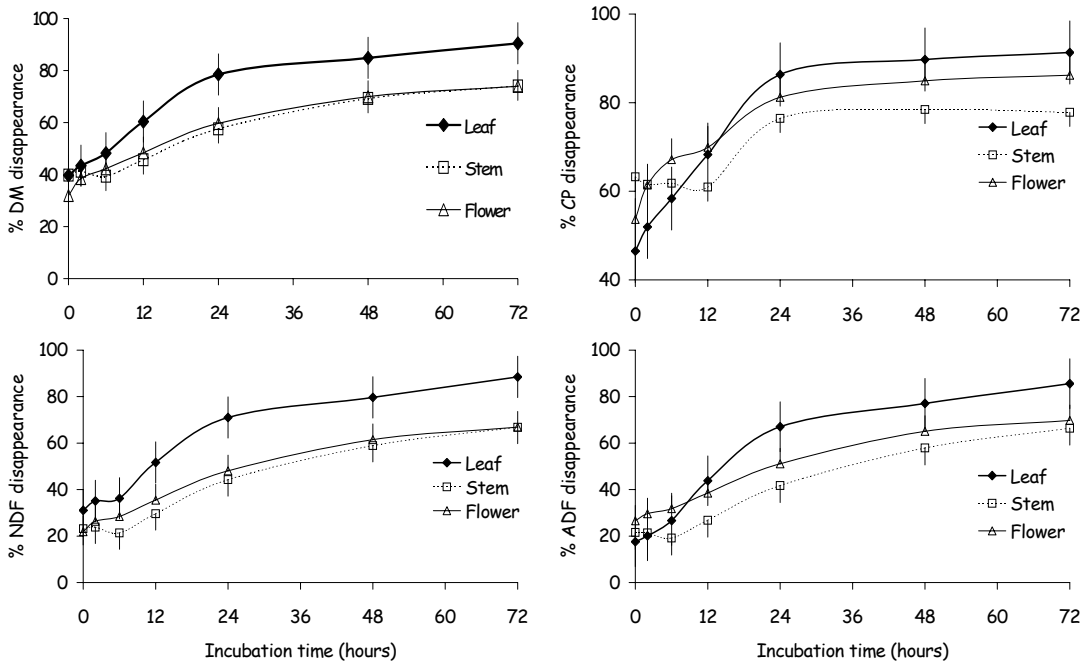


FIGURE 4.1 - *In sacco* dry matter (DM), crude protein (CP), neutral and acid detergent fibre (NDF and ADF) disappearance from leaf, stem and flowers from five mature grasses (mean ± standard error bar).

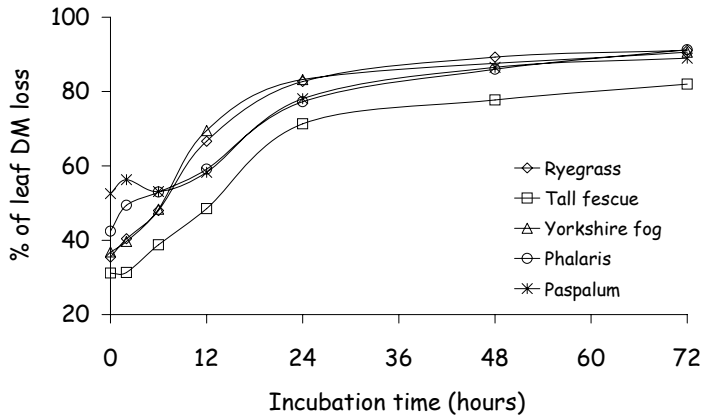


FIGURE 4.2 - Disappearance of leaf dry matter (DM) during *in sacco* digestion of five mature grasses.

TABLE 4.3 - Mature grass dry matter (DM) degradation characteristics (% of DM) defined as soluble (A), degradable insoluble (B) and undegradable residue (C = 100 – A – B) as well as fractional degradation rate (k, h^{-1}), lag time (Lag, hours) and effective degradability (E) which takes into account the effect of passage rate from the rumen.

Forage	A	B	C	k	Lag	E[1]
Perennial ryegrass						
leaf	38	53	9	0.09	3.7	67
stem	47	30	23	0.04	9.5	54
flower	49	34	17	0.04	4.6	59
Tall fescue						
leaf	31	51	17	0.06	4.1	54
stem	26	33	41	0.05	8.7	36
flower	26	47	27	0.08	0.0	53
Yorkshire fog						
leaf	38	51	11	0.11	4.0	68
stem	35	45	20	0.03	6.0	46
flower	44	29	26	0.05	3.2	56
Phalaris						
leaf	43	51	5	0.04	0.0	63
stem	36	46	18	0.03	8.3	46
flower	31	55	14	0.03	0.0	51
Paspalum						
leaf	54	35	11	0.09	10.4	66
stem	56	31	13	0.05	8.1	66
flower	20	43	37	0.04	4.6	35
Leaf	41±1.00	48±0.76	11	0.079±0.002	4.5±0.22	64
Stem	40±0.96	37±0.72	23	0.042±0.002	8.1±0.21	50
Flower	34±0.96	42±0.72	24	0.048±0.002	2.5±0.21	51

[1] Calculated using a fractional passage rate of $0.06h^{-1}$.

4.4.4 - CP digestion kinetics

The soluble CP (A) fraction varied from 35 – 61% for mature leaves, 41 – 74% in stems and 41 – 67% in flowers and averaged 55% across grass species and components. There were significant differences between components and forages ($P < 0.0001$; Table 4.4) in the % of CP released into the soluble pool. Appendix CD (Chapter 4) presents the complete data set outputs (e.g.: Least Squares Means for effect of Forages and Components).

The insoluble degradable (B) pool contained on average 46, 18 and 31% of CP for leaf, stem and flower across forages and also varied between forages ($P < 0.001$).

The distribution of CP between A and B fractions was similar for leaf of all grasses but when stem was minced most of the protein was released into the A fraction, except for paspalum. As with DM distribution, stems had higher values for undegradable CP (fraction "C" averaged 21% compared to flowers (13%) and leaves (7%). The model accounted for 41 and 55% of the variation in distribution of CP within the A and B fractions, respectively.

CP degradation rates differed across forages species and components (Table 4.4; $P < 0.001$). Tall fescue had lower degradation rates (k) for leaf CP (0.05 h^{-1}) and higher values for stem and flower compared to other forages. Lag phases resembled patterns for DM, with longer lags for stems than leaves or flowers.

Effective degradability (E; Table 4.4) calculated using a fractional passage rate of 0.06 h^{-1} shows similar values of about 71% for leaves, stems and flowers in all forages, despite differences in distribution between pools, degradation rates and lag times.

Protein degradability has been expressed in terms of DM (ERDP) and plotted against DM degradability and NDF content. Figure 4.3 demonstrates similar ERDP for stem of all species but diverse values for leaf, resulting in a poor positive relationship ($r^2 = 0.27$) between effective rumen degradability of CP and DM across forages and components ($P = 0.049$). A stronger relationship is evident when ERDP is plotted against NDF concentration for the five forages and components incubated *in sacco* ($P < 0.001$; Figure 4.4). Leaf, stem and flower varied in NDF content from 492 to 718 g/ kg DM and the concentration of NDF accounted for 56% of the variation in effective rumen degradability for protein across forage components (Figure 4.4).

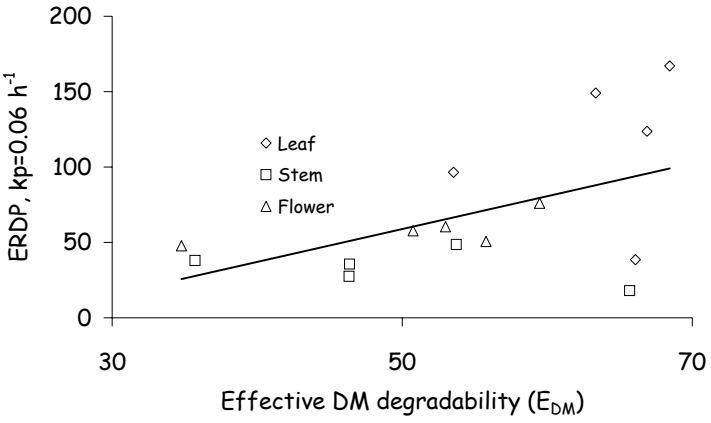


FIGURE 4.3 – Relationship between effective degradability of dry matter (E_{DM}) and crude protein (ERDP, g/kg DM) of forage components. When rumen DM outflow is 0.06 h^{-1}, the relationship is described as: ERDP (g/kg DM) = - 50.1 + 2.18 (±1.00) x EDM (r^2 = 0.27; Root MSE = 40.1; CV = 58.2%; P=0.0488).

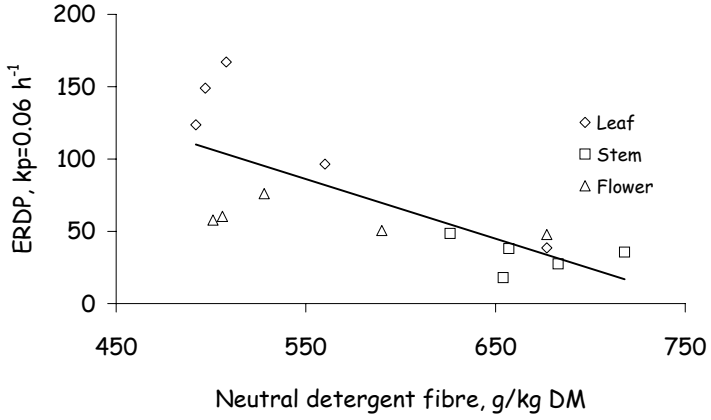


FIGURE 4.4 – Relationship between neutral detergent fibre content (NDF, g/kg DM) and effective rumen degradability of crude protein (ERDP, g/kg DM) of forage components. When rumen DM outflow is 0.06 h^{-1}, the relationship is described as: ERDP (g/kg DM) = 313 - 0.41(±0.10) x NDF (r^2 = 0.56; Root MSE = 30.9; CV = 44.8%; P = 0.0012).

TABLE 4.4 - Mature grass crude protein (CP) degradation characteristics (% of CP) defined as soluble (A), degradable insoluble (B) and undegradable residue (C = 100 – A – B) as well as fractional degradation rate (k, h^{-1}) and lag time (Lag, hours). These data are used predict effective degradability (E), which takes into account the effect of passage rate from the rumen and the effective rumen degradability protein (expressed on a dry matter basis (ERDP; g/kg DM).

Forage	A	B	C	k	Lag	E[1]	ERDP[1]
Perennial ryegrass							
Leaf	42	52	6	0.10	1.0	73	124
Stem	70	12	18	0.09	10.2	74	49
Flower	67	20	13	0.06	4.7	75	76
Tall fescue							
Leaf	53	46	1	0.05	0.0	75	96
Stem	74	6	20	0.13	0.0	78	38
flower	41	46	13	0.23	0.0	78	60
Yorkshire fog							
leaf	46	48	7	0.15	3.8	77	167
stem	59	19	22	0.04	11.9	76	27
flower	50	33	18	0.08	3.9	67	51
Phalaris							
leaf	61	37	2	0.07	0.0	81	149
stem	60	28	12	0.04	2.0	70	36
flower	56	41	3	0.03	0.0	68	58
Paspalum							
leaf	35	45	20	0.09	12.0	48	39
stem	41	29	31	0.12	12.0	51	18
flower	65	18	17	0.11	5.2	75	48
Leaf	47±1.18	46±1.12	7	0.09±0.03	3.4±0.27	71	115
Stem	61±1.13	18±1.07	21	0.08±0.03	7.2±0.25	70	34
Flower	56±1.13	31±1.07	13	0.10±0.03	2.8±0.25	73	59

[1] Calculated using a fractional passage rate of 0.06h^{-1}.

4.4.5 - Fibre digestion kinetics

Fibre accounted for 50 – 70% of DM for the five mature grasses and their components (Table 4.5). ADF accounted for a slightly lower percentage of leaf NDF (57%) compared to stem and flower (62%), and values were lowest for ryegrass and Yorkshire fog leaf (48%). Fibre digestion will contribute a major portion of energy for the animal but rate and extent of digestion will be affected by particle size and microbial colonisation.

In contrast to protein, an average of 26% of NDF and 22% of ADF were released into the "A" fraction by mincing. There were differences between forages and components in the distribution of both NDF and ADF (Table 4.5 and 4.6) with 65% and 53% of variation in "A" pool of the respective components explained by the model. The insoluble degradable (B) fraction accounted for about half of the NDF and a similar proportion of ADF, but with higher values for leaf than stem and flower (Table 4.6).

Both NDF and ADF degradation rates were twice as fast for leaves compared to stem and flower ($P < 0.0001$; Figure 4.2). The lag time was highest (8 – 9 hours) for stems compared to leaves and flowers and virtually all components of all plants exhibited a substantial lag period prior to degradation. When fractional outflow rate was 0.06 h^{-1} the net effect of lag and degradation rate resulted in an effective degradability for leaf NDF and ADF averaging 56 and 48% respectively, and lower values for stem and flower fibre (E; Table 4.5 and 4.6). Fibre was poorly degraded, but calculation using a slow outflow from the rumen (0.02 h^{-1}) increased degradation of NDF and ADF by 15 – 20 percentage units. One consequence of a slow outflow rate would be very low feed intake. The model explained 53% and 59% of variation in the "B" pool for NDF and ADF respectively.

TABLE 4.5 - Mature grass neutral detergent fibre (NDF) degradation characteristics (% of NDF) defined as soluble (A), degradable insoluble (B) and undegradable residue (C = 100 – A – B) as well as fractional degradation rate (k, h^{-1}), lag time (Lag, hours) and effective degradability (E) which takes into account the effect of passage rate from the rumen.

Forage	A	B	C	k	Lag	$E_{2\%}$	$E_{6\%}$
Perennial ryegrass							
leaf	28	59	13	0.08	4.0	75	58
stem	36	38	27	0.04	10.9	58	42
flower	31	46	23	0.03	5.0	59	44
Tall fescue							
leaf	18	51	30	0.11	8.2	61	43
stem	5	45	50	0.04	10.0	34	15
flower	16	55	29	0.03	0.0	49	34
Yorkshire fog							
leaf	31	54	14	0.09	3.5	76	62
stem	22	55	23	0.03	6.0	52	35
flower	35	32	33	0.04	3.9	56	46
Phalaris							
leaf	30	62	8	0.03	1.8	68	50
stem	24	58	17	0.03	5.5	55	37
flower	9	66	25	0.04	3.9	51	31
Paspalum							
leaf	53	40	7	0.06	9.9	81	65
stem	27	51	22	0.05	8.3	62	42
flower	27	39	34	0.03	4.8	51	39
Leaf	32±0.87	53±0.86	14	0.08±0.02	5.5±0.25	72	56
Stem	23±0.83	49±0.81	28	0.04±0.02	8.1±0.23	52	34
Flower	24±0.83	48±0.81	29	0.04±0.02	3.5±0.23	53	39

$E_{2\%}$ and $E_{6\%}$ were calculated using a fractional passage rate of 0.02 and 0.06h^{-1} respectively.

TABLE 4.6 - Mature grass acid detergent fibre (ADF) degradation characteristics (% of ADF) defined as soluble (A), degradable insoluble (B) and undegradable residue (C = 100 – A – B) as well as fractional degradation rate (k, h^{-1}), lag time (Lag, hours) and effective degradability (E) which takes into account the effect of passage rate from the rumen.

Forage	A	B	C	k	Lag	$E_{2\%}$	$E_{6\%}$
Perennial ryegrass							
leaf	6	77	17	0.09	4.2	69	48
stem	31	42	27	0.04	10.7	56	39
flower	30	49	21	0.03	4.6	60	44
Tall fescue							
leaf	5	59	35	0.12	9.1	55	34
stem	0	51	49	0.04	11.0	32	5
flower	26	57	17	0.02	0.0	53	39
Yorkshire fog							
leaf	9	72	19	0.11	3.7	69	52
stem	18	60	22	0.03	6.0	51	32
flower	31	39	30	0.04	1.8	56	45
Phalaris							
leaf	21	70	9	0.03	1.4	65	45
stem	26	57	17	0.03	6.0	57	39
flower	19	61	21	0.04	4.0	58	39
Paspalum							
leaf	53	37	11	0.08	11.2	80	64
stem	29	50	21	0.05	8.8	62	43
flower	33	37	30	0.04	7.0	56	43
Leaf	19±1.21	63±1.04	18	0.09±0.002	5.9±0.26	68	48
Stem	21±1.15	52±0.99	27	0.04±0.002	8.5±0.25	52	31
Flower	28±1.15	48±0.99	24	0.03±0.002	3.5±0.25	57	42

$E_{2\%}$ and $E_{6\%}$ were calculated using a fractional passage rate of 0.02 and 0.06h^{-1} respectively.

4.4.5 - *In vitro* incubations

The buffered media used for *in vitro* incubations showed moderate decreases in pH, suggesting a limited production of VFA. The only value below 5.6 was for tall fescue flowers after 12 hours of incubation (Figure 4.5). Tall fescue flower contained a high concentration of NSC (18.5 g NSC/100 g DM) relative to other grass components.

Protein degradation is indicated by ammonia concentration in the incubation media (mMol NH_3/L; Figure 4.6) and net ammonia production from plant components for each forage species (Figure 4.7 and 4.8). Concentrations generally peaked at or before 12 hours of incubation (except for phalaris leaf) and values above 0 hour suggest protein degradation is exceeding microbial utilisation. Inadequate ammonia yield from all components of tall fescue and paspalum are apparent (Figure 4.6 and 4.7; Table 4.7) after 12 hours whereas concentrations remained elevated for incubations of leaf from ryegrass, phalaris and Yorkshire fog.

When NH_3 concentrations are expressed as a proportion plant N, the values are affected by N in plant DM as well as proteolysis and incorporation into bacterial protein (Barrell *et al*, 2000). Some leaves contained sufficient N for microbial growth, but stem and flower had insufficient N, especially after 12 hours of incubation (Figure 4.8). Negative values indicate ammonia uptake from the rumen inoculum and this was apparent for all stem material except ryegrass. These data show insufficient N was released from all components of tall fescue and paspalum after 6 hours to sustain microbial growth. Nitrogen insufficiency may limit production of VFA *in vitro* and limit degradation rates *in situ*.

The rates of VFA production over 48 hours have been summarised for leaf, stem and flower in Figure 4.9. A rapid initial VFA yield was apparent for all constituents (to 12 hours) although propionate production appeared to be more rapid from flower than stem or leaf. Net production of all VFA were sustained over 48 hours when stem was incubated, whereas the yield did plateau or became negative after 24 hours for the leaf and flower fractions.

The average rates of VFA production were similar for leaf, stem and flower fractions from 0 – 12 hours (Table 4.8) with yields of 112, 105 and 119 µMol/g DM per hour for the respective fractions. Calculations of yield over the entire 48 hours incubation across the five species (Appendix 7 – Table 7.2A) averaged 1.61, 2.12 and 1.46 mMol/g DM for leaf, stem and flower respectively.

There was a much greater rate of VFA production from tall fescue and phalaris compared to ryegrass, Yorkshire fog and paspalum (Table 4.9; Appendix 7 Table 7.2A). The differences are illustrated in Table 4.9 at 12 hour, but the absence of replicates prevented a robust statistical validation of forage effects (not significant). By 48 hour, the net yield of VFA (% of DM assuming a mean molecular weight of 67) for the five grass species (assuming leaf, stem and flower each contribution are third of the DM) was perennial ryegrass, 6.8%; tall fescue, 18.9%; Yorkshire fog, 12.1%; phalaris, 10.5% and paspalum, 9.7%.

TABLE 4.7 – Mean values for net ammonia production and concentration above 0 hour values in the incubation buffer and from leaf, stem and flower and for the five grass species at intervals during the 48 hour incubation.

	mMol NH_3/mM plant N			mMol NH_3/L		
	2 - 6	8 - 12	24 - 48	2 - 6	8 - 12	24 - 48
Leaf	0.053	0.065	0.003	6.7	7.1	4.7
Stem	0.054	-0.030	-0.122	4.5	2.9	0.7
Flower	0.082	0.090	-0.045	6.8	6.8	2.3
Perennial ryegrass	0.071	0.107	0.022	4.6	6.0	2.4
Tall Fescue	-0.009	-0.107	-0.113	5.7	1.5	0.8
Yorkshire fog	0.074	0.081	-0.078	6.6	6.8	2.2
Phalaris	0.084	0.079	0.043	6.9	7.4	6.8
Paspalum	0.087	0.027	-0.154	5.9	5.8	0.6

TABLE 4.8 – Mean values for pH and rates of volatile fatty acid production from leaf, stem and flower and for the five grass species incubated for 48 hours. Data for VFA are mean hourly rates averaged for 0 – 6 and 6 - 12 hour incubation period. Main effects and interactions have been tested for significance.

		µMol/g DM.hour				
	pH	Acet	Prop	Buty	Minor	Total
Leaf	6.90	79.4	18.7	11.3	2.8 [ac]	112.1
Stem	7.08	74.5	25.1	13.1	1.2 [bc]	105.4
Flower	6.90	68.6	16.7	19.9	5.9 [a]	119.4
Perennial ryegrass	7.22	63.6	16.7 [b]	13.3	3.4	97.0
Tall Fescue	6.35	108.0	40.3 [a]	23.5	3.2	175.0
Yorkshire fog	7.22	57.5	12.8 [b]	9.0	2.0	81.2
Phalaris	6.95	86.5	14.2 [b]	14.7	5.3	120.7
Paspalum	7.05	55.2	16.7 [b]	13.1	2.7	87.6
Model P	0.000	0.976	0.223	0.651	0.518	0.947
Forage P	<.0001	0.678	0.058	0.311	0.593	0.476
Components P	0.167	0.946	0.449	0.242	0.046	0.947
Time P	0.000	0.777	0.080	0.080	0.973	0.466
Forage*components P	0.002	0.900	0.629	0.932	0.707	0.907
Components*time P	0.949	0.988	0.405	0.777	0.962	0.928
Forage*time P	0.150	0.815	0.225	0.850	0.473	0.884
r^2	0.82	0.47	0.82	0.69	0.73	0.52

Acet, acetate; Prop, propionate; Buty, n-butyrate; Total, total VFA.

Other abbreviations see text.

TABLE 4.9 – Total yield of volatile fatty acids from five grasses, assuming equal proportion of leaf, stem and flower in the DM after 6, 12, 24 and 48 hours of incubation, and rates of VFA production.

	Cumulative VFA (mMol/g DM)				Rates (µMol/g DM per hour)			
	6 h	12 h	24 h	48 h	0-6 h	6-12 h	12-24 h	24-48 h
Perennial ryegrass	0.83	1.16	1.57	1.01	138	55	34	-23
Tall Fescue	1.01	2.1	2.62	2.82	168	181	43	8
Yorkshire fog	0.46	0.82	1.34	1.8	77	60	43	19
Phalaris	0.77	1.45	1.92	1.57	128	68	39	15
Paspalum	0.67	1.05	1.03	1.45	112	63	-2	18

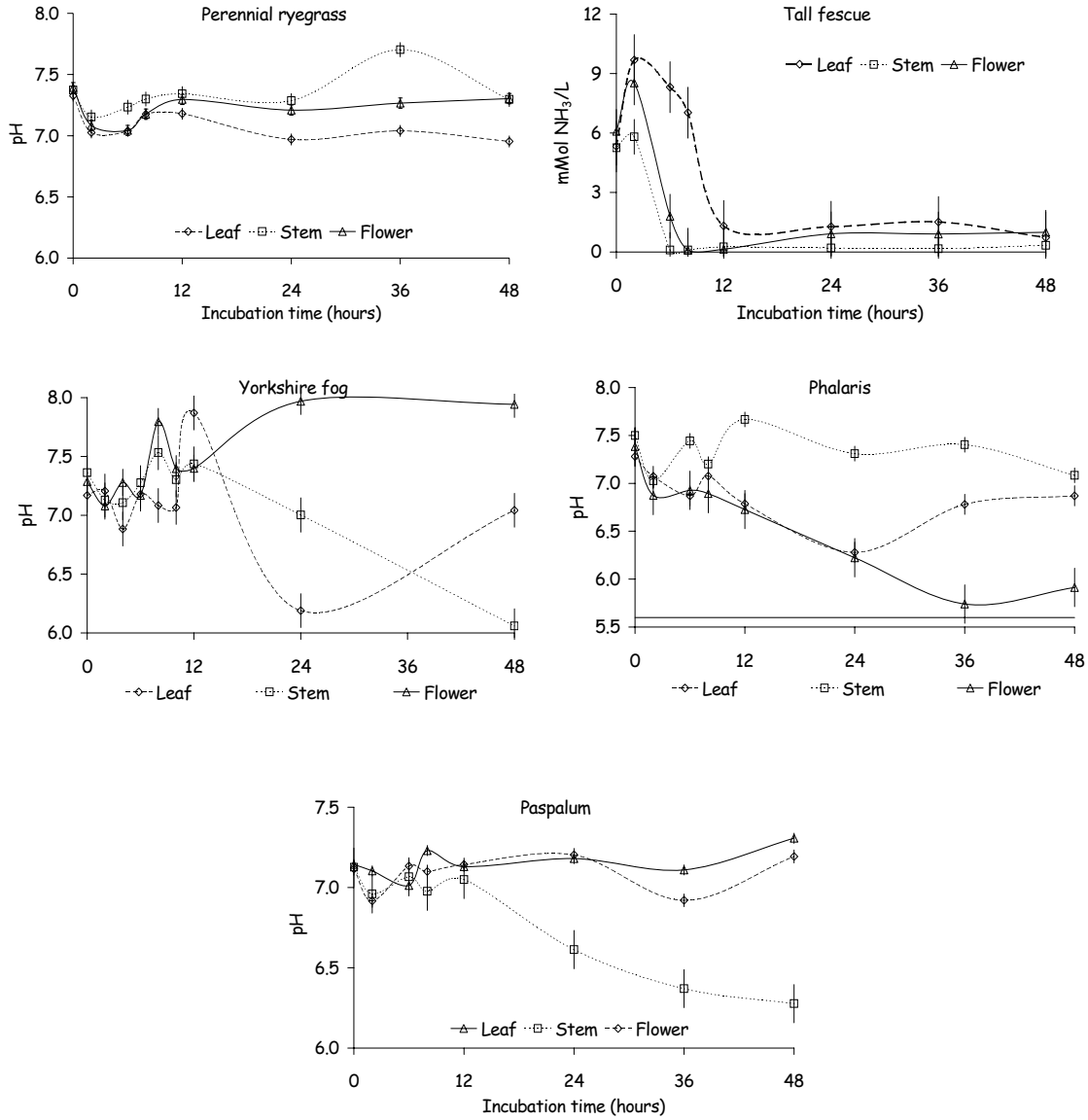


FIGURE 4.5 – pH during *in vitro* incubations of mature grass leaf, stem and flower.

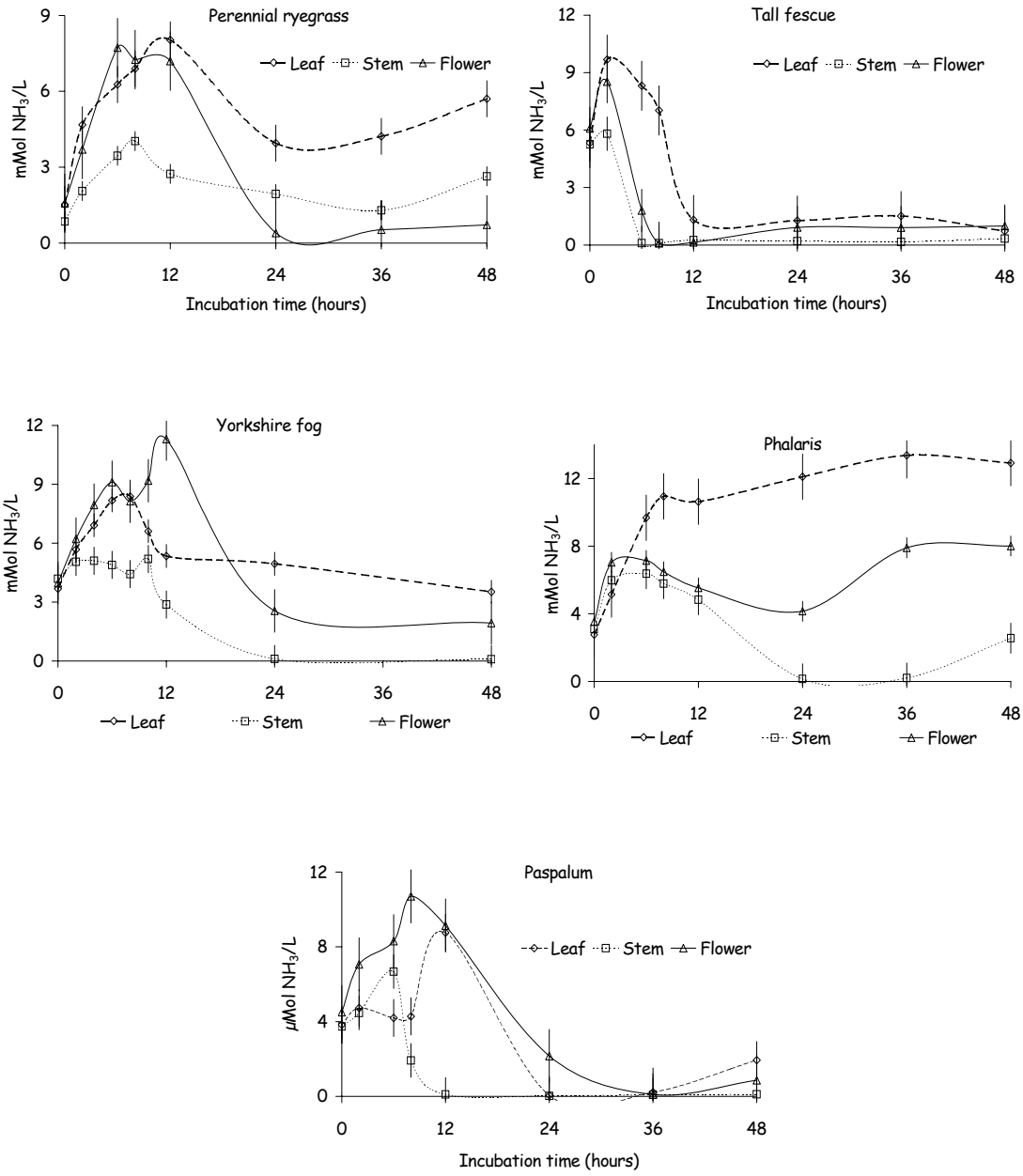


FIGURE 4.6 – Pattern of ammonia concentration over 48 hours expressed in mMol/L for *in vitro* incubations.

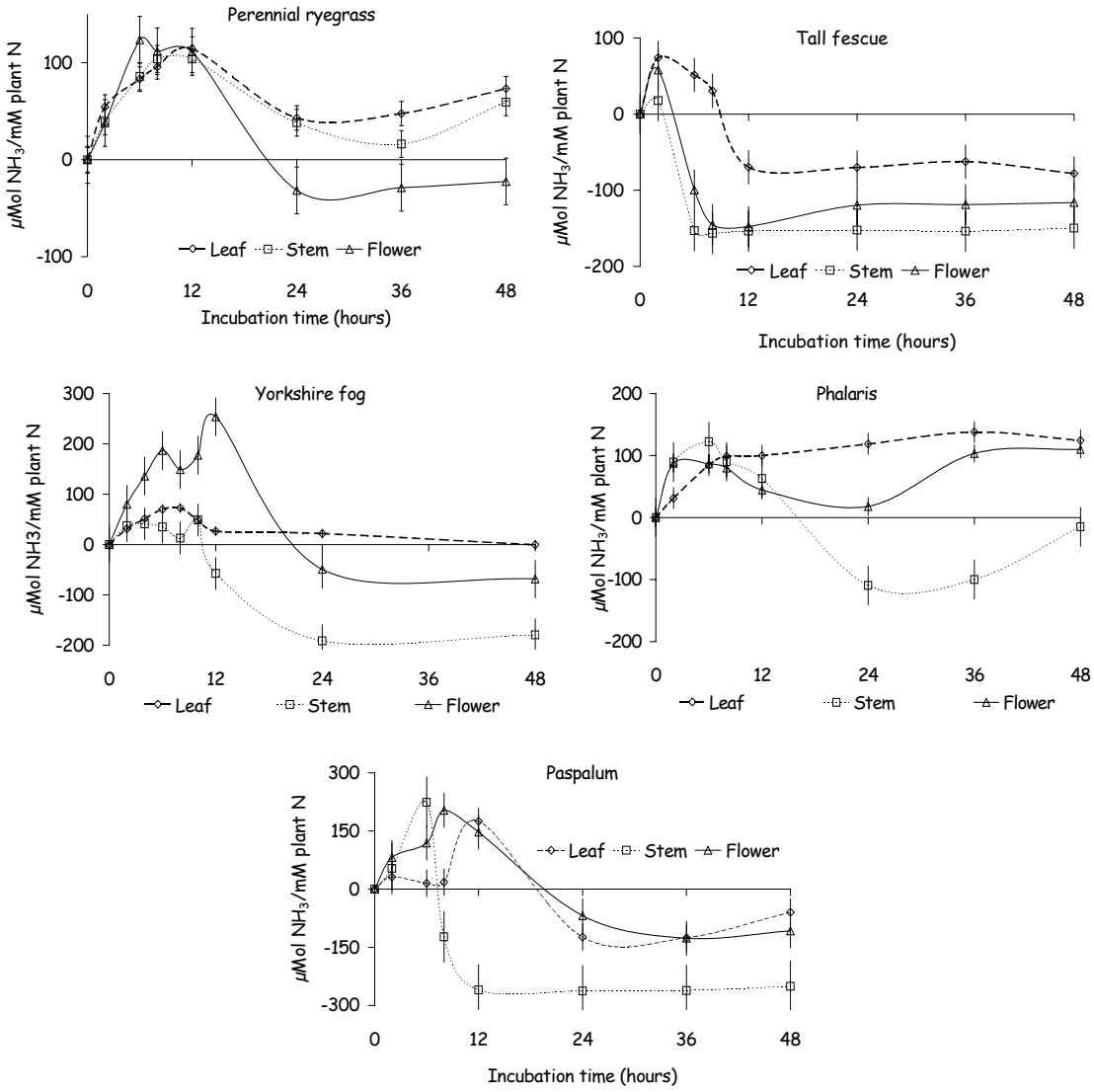


FIGURE 4.7 – Net ammonia production expressed in terms of plant nitrogen during in vitro incubations for mature grasses in five incubations runs.

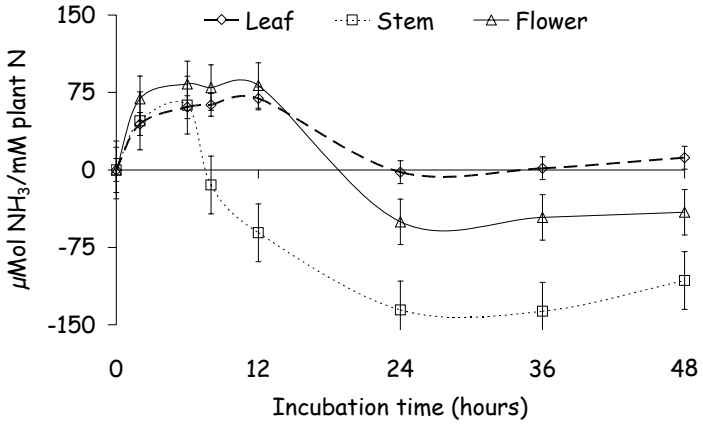


FIGURE 4.8 – Net ammonia production expressed in terms of plant nitrogen averaged by components (leaf, stem and flower) for five mature grasses during *in vitro* incubations.

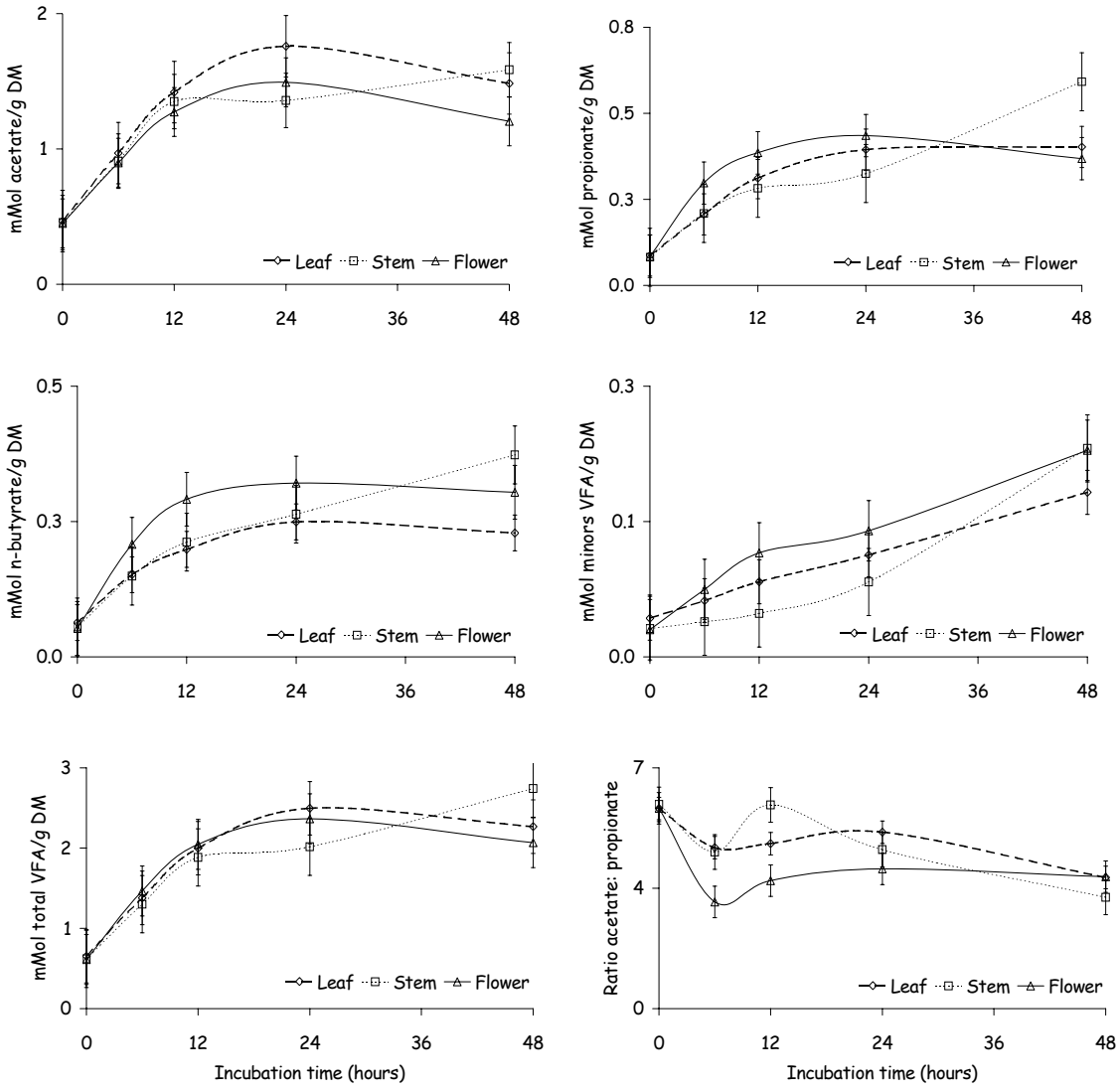


FIGURE 4.9 - Volatile fatty acids production expresses in terms of dry matter (mMol/g DM) and ratio acetate: propionate averaged by components (leaf, stem and flower) for five mature grasses during *in vitro* incubations.

4.5 - Discussion

The chemical composition and digestion data from the contrasting species, and their components are intended to complement the digestion kinetic data for ryegrass presented in Chapter 3. Separation into leaf, stem and flower constituents provides information on individual fractions of mature grass, excluding dead material.

The decline in nutritive value of mature grass is well known (Wilson, 1993; Wilson *et al.*, 1995), and will be a consequence of both changing proportions of leaf, stem and inflorescence and can alter composition of these components. Data presented here confirm the slow degradation of stem, but also suggest a relatively low nutritive value of the flower, despite moderate concentrations of non-structural carbohydrates in perennial ryegrass and tall fescue. In contrast, leaf from ryegrass and Yorkshire fog had high DM degradation rates (k = 0.09 and 0.11) which were similar to values reported by Burke *et al.* (2000) for respective species (k = 0.114 and 0.092). The chemical composition of young and old ryegrass and fog leaves was also similar, whereas tall fescue and paspalum leaves contained substantially more NDF and less soluble carbohydrate when mature (Table 4.1 and Burke *et al.*, 2000).

Reductions in nutritive value appear to be a consequence of decreasing proportion of leaf and increasing proportion of stem and flower with less effect due to composition of each component. Stockdale (1999b) reported chemical composition of leaf, stem and flower from ryegrass and paspalum grown under irrigation in Northern Victoria (Australia; Table 4.10), which were similar to values in this study. Stem and flower had a very different chemical composition to leaf. Under irrigation, the proportion of ryegrass and paspalum components differed throughout the year, but the nutritive characteristics of leaf, stem and flower of both species remained relatively constant. The proportion of stem in paspalum pasture reached 35% of the DM in summer and flower accounted for a further 10% (Stockdale, 1999b).

TABLE 4.10 - Chemical composition (g/100 g of the DM; mean ± standard error) and estimated metabolisable energy (ME; MJ/kg DM) content of leaf, stem and flower fractions of ryegrass and paspalum irrigated pastures in summer-autumn in northern Victoria (Australia).

	CP	NDF	ADF	ME
Ryegrass				
leaf	14.3±1.09	57.2±0.71	30.9±0.64	10.6±0.24
stem	7.4±0.39	64.7±0.55	30.8±1.11	9.9±0.30
flower	11.1±0.65	59.9	34.2	8.8±0.20
dead matter	12.0±0.65	66.4±0.88	43.5±0.72	7.1±0.17
Paspalum				
leaf	13.8±0.72	64.0±0.56	36.8±0.38	8.6±0.12
stem	5.8±0.57	68.2±0.95	37.1±0.49	9.7±0.18
flower	8.4±0.38	73.0±1.29	38.2±1.36	7.6±0.23
dead matter	10.6±0.43	69.0±0.73	45.8±0.44	5.9±0.13

From Stockdale (1999b).

The changing proportions of ryegrass leaf, stem and flower, and their nutritive value was reported by Wilman and Agiegba (1982; Table 2.6). Stem DM increased to over 60% of the DM, whilst green leaf declined to very low levels. *In vitro* digestibility of leaf declined by a small amount as grass matured (64 to 58%) whereas stem was 49% digestible when mature. As grasses flower and mature, there is a decline in forage quality caused by the translocation of soluble carbohydrates from stem and leaves to the flowers, and an increased lignification of cell walls (Hacker and Minson, 1981). Nitrogen concentration in leaves decline with maturity, but leaf always contains a higher protein concentration and less NDF (Tables 4.1 and 4.10) than stems and flowers. In general, leaf was more rapidly digested than stem and flower fractions, but with phalaris the difference among all three constituents was relatively minor.

In vitro incubations revealed insufficient nitrogen in paspalum and fescue leaves (and stem and inflorescence of all species) for sustained microbial growth, as evidenced by the very low ammonia concentration following 12 hours of incubations. Volatile fatty acid production was very low for all grasses except for tall fescue, despite the apparently inadequate supply of NH_3-N for microbial growth (Satter and Slyter, 1974). The slow rate of VFA production with ryegrass was particularly surprising given that adequate N was present in leaf and flower. Incubations of young ryegrass leaf have yielded 36% (Barrell, 2000) and 27% (Chapter 3) of DM as VFA after 24 hours. There was a poor relationship between feed quality characteristics and *in vitro* production of VFA in this study and reasons for this are not understood.

The principal factor affecting nutritive value of mature forages is the slow rate of physical degradation and clearance from the rumen of animals unable to select leafy material from the sward (Cherney *et al.*, 1993; Waghorn, 2002). A requirement to eat stem and flower is likely to restrict feed intake, and intake will be further affected by the extent to which animals chew and reduce particle size of the forage during eating and rumination. These factors were overcome by the mincing procedure to achieve a particle size distribution (Table 4.2) similar to that of rumen content (Table 3.20) but substantially more effort is required to chew mature versus immature forages, especially stem fractions. This was the main reason for the use of a slow (0.02 h^{-1}) outflow rate typical of a poor quality diet to calculate effective degradability (E) of fibre (Table 4.5 and 4.6), for comparison with higher outflow rates (0.06 h^{-1}) used in previous analyses (Chapter 3). When the outflow rate is 0.06 h^{-1} the effective degradability for leaf fibre reached about 52% but a longer residence in the rumen (i.e. slower passage rate: 0.02 h^{-1}) improves the degradability to about 70%. A rapid particle size reduction will facilitate both digestion and clearance of residues from the rumen.

4.6 - Conclusion

This assessment of grasses and their components showed stem and flower to have slower rates of digestion than leaf, with higher proportions of indigestible fibre. *In sacco* kinetics suggested slow colonisation of all components, but especially stem and this will limit the rate of nutrient production as well as voluntary intakes. Effective degradability was highest for leaf but rates *in vivo* will depend on the speed and extent of particle size reduction by chewing during eating and ruminating. High quality pastures are achieved by good pasture management that prevents flowering by removing the apical meristem from reproductive tillers using a rapid stock rotation or topping (Korte, 1982).

Chapter 5

Supplementing fresh pasture with maize, lotus, sulla and pasture silages for dairy cows in summer.[1]

[1] A small portion of these data were previously published in the *Proceedings of New Zealand Grassland Association*, 2002, 85-89.

5.1 - Abstract

Forages suitable for supplementing pasture-fed dairy cows over summer should provide adequate nutrients and increase milk yield and liveweight above that produced by cows grazing ryegrass/white clover pasture. A trial was conducted in January – February 2001 to compare benefits obtained from feeding four types of silage. There were two silages that contained condensed tannins (CT): lotus (*Lotus corniculatus*) and sulla (*Hedysarum coronarium*), maize silage or traditional ryegrass dominant pasture silage, all fed at 5 kg dry matter (DM)/cow.day with restricted pasture (RP). Cows on the RP (control) treatment and those fed the silage treatments were offered an allowance of 25 kg pasture DM/cow.day, while the full pasture (FP) cows were offered 50 kg pasture DM/cow.day. Silage supplementation, regardless of silage type, increased both DM intake and milk yield compared with cows given RP only. Cows on the lotus silage supplement and the FP treatment had significantly higher milk production than the other silage supplemented cows, all of which had similar milk yields. For cows given lotus silage, the high milk yield was probably due to a combination of the higher nutritive value of the silage and to the action of CT, because the total DM intake of cows fed the lotus silage was the same as that of cows given the pasture and maize silages. The high milk yield of the FP treatment was mainly a result of the cows having a higher intake of pasture than cows on all the other treatments. This study demonstrated the potential benefit of silage supplementation, particularly with lotus silage, for increased milksolids yield in summer when low pasture growth rates and quality may otherwise limit production.

Keywords: condensed tannins; dairy cows; grazing; pasture; silages.

Short title: Silages for grazing cows in summer.

5.2 - Introduction

In New Zealand most dairy farming is in regions with sufficient rainfall to maintain pasture growth but summers often have periods without adequate precipitation and pasture growth is reduced (McGrath *et al.*, 1998). Pasture quality can also be reduced by dry conditions after flowering, with reduced digestibility, lower protein content (Figures 2.5 and 2.6) and an increased proportion of dead matter (Gray and Lockhart, 1996). Dead matter can result in fungi growth with toxicity problems in some situations. Summer feeding often results in harder grazing, with lower residual pasture dry matter and cows may be forced to consume stems and leaf sheaths. Ryegrass endophyte

(*Neotyphodium lollii*) grows in the reproductive stem and leaf sheath, with peak toxicity during seed head emergence and during dry summers in response to water stress and high temperatures (Easton, 1999).

Supplements are usually provided to make up a shortfall in pasture supply, but they may also improve the nutritive value of pasture on offer. Supplements should be chosen to complement the pasture and to meet nutritional demands of lactating cows, but frequently maize and grass silage are chosen because it is available. In some instances supplements are given to reduce intakes of ryegrass infected with *Neotyphodium lollii* to avoid toxicity which can lead to an early end of lactation (Hamilton-Manns and Crothers, 1999). The type and extent of supplementation should be determined by the specific or dominant constraint to feeding and dairy production.

Trials undertaken here have required pasture supply to be constrained to mimic normal situations in many dairy regions. The experimental plan anticipated a dry summer (typical of the Waikato region) so legume silages were chosen for supplementation, as well as maize and pasture silages which are frequently used by farmers. These treatments were intended to alter diet composition and to provide sufficient feed to meet cow requirements. Issues associated with endophytes and/or other toxins were not addressed.

The objective of this work was to evaluate contrasting silages as supplements for cows fed restricted amounts of summer pasture. The use of lotus (*Lotus corniculatus*) and sulla (*Hedysarum coronarium*) silage was based on a need to provide adequate protein to lactating dairy cows, especially as summer pastures usually contain less than 16% CP in the DM (Moller *et al.*, 1996; Wilson *et al.*, 1995). The CT in these forages protects protein from degradation during ensiling and may reduce proteolysis in the rumen (Niezen *et al.*, 1998).

The hypothesis from this chapter was that provision of about 35% of ME from silages with contrasting chemical and botanical composition to cows grazing a restricted pasture allowance would result in a range of milk solids responses in a short-term trial.

Data obtained from this trial, with kinetic information, were used as inputs to the Cornell Net Carbohydrate and Protein System (CNCPS) model to determine the first limiting nutrient and provide information concerning rumen digestion parameters.

5.3 – Material and methods

This experiment was carried out at Dexcel's No. 5 dairy farm at Hamilton (Waikato region) and involved 60 cows in mid lactation. The trial comprised a seven day uniformity period when all cows were grazing a ryegrass/white clover pasture, followed by a treatment period when either pasture or pasture with supplements were offered for four weeks commencing 15th January 2001. The 60 Friesian cows were allocated to six treatments and balanced for milksolids yield and liveweight measured during the uniformity period.

5.3.1 - Treatments

During the uniformity period cows were grazed together with a pasture allowance of about 40 kg DM/day. Milk yield was measured on two consecutive days and liveweight on three mornings of the uniformity week. These data enabled allocation to treatments on the basis of performance and bodyweight and also a covariance analysis of treatment effects.

There were six dietary treatments, enabling the effects of either lotus or sulla silage to be compared with the more typical pasture or maize silages. One group of 10 cows was given a high (*ad libitum*) pasture allowance (full pasture) and another was given a pasture allowance similar to cows receiving silage supplements (restricted pasture):

1. Pasture only – full allowance (50 kg DM/cow.day)
2. Pasture only – restricted allowance (25 kg DM/cow.day)
3. Pasture (restricted) + pasture silage (5 kg DM/cow.day)
4. Pasture (restricted) + maize silage (5 kg DM/cow.day)
5. Pasture (restricted) + lotus silage (5 kg DM/cow.day)
6. Pasture (restricted) + sulla silage (5 kg DM/cow.day)

Pasture restricted and full allowance treatments enable comparisons between this trial and previous work (Harris *et al.*, 1998a), and calculation of substitution rates when silages were offered. Cows were grazed throughout the treatment periods in treatment groups of 10 animals but each group was divided into two groups for measurement on Tuesday, Wednesday and Thursday of each week. On these three days each herd of 10 cows were split into two herds of five (same cows in each group

each week) when milk and pasture measurements and samples were collected in order to replicate the treatments. On the remaining four days each week (Friday, Saturday, Sunday, Monday) the replicate groups were combined into treatment groups (six groups of 10 cows) for ease of management. Cows in each treatment group were grazing similar pastures in adjacent plots (Figure 5.1).

FIGURE 5.1 – Overall view of the random six treatments in separated paddocks.

Cow management

Each treatment group was given a new break of pasture on a daily basis with a back-fence using electric tape. Daily pasture allowances for each treatment group were estimated using visual assessment of pre-grazing herbage mass and allocation of break size accordingly. Silages were fed to cows on a group basis from portable feed troughs (one trough per five cows; Figure 5.2) once cows returned to the paddock after morning milking. Silage DM was determined by quick drying (microwave) confirmed by drying for 24 hours at 100^{o}C, and sufficient placed in troughs to provide five kg supplement DM/cow.day. Troughs were collected from paddocks when cows were at afternoon milking and any refusal weighed and sub-sampled for DM determination. Water was available *ad libitum*.

FIGURE 5.2 –Trough used to feed silage supplements (in detail: lotus silage).

5.3.2 - Measurements

Pasture intakes: pasture intakes of each treatment group were estimated by using a rising plate meter to measure pre- and post-grazing herbage mass (50 measures per 24 hour break for each group). This was done three times per week during the measurement period for each group of 10 cows to coincide with milk sampling days. Quadrants of pasture were cut one day of each week pre- and post-grazing (on representative pasture) to calibrate the rising plate meter (Hodgson *et al.*, 1999).

Silage intakes: silage intakes of each group were measured by weighing silage pre- and post-feeding. Pre-feeding weighing was done every day, but post-feeding weighing of refusals was done only during the measurement period to coincide with days on which pasture intakes were estimated. For all silages except sulla, refusals were very small, but cows did reject sulla stem.

Pasture quality: samples were collected for analyses of pasture quality using an electric clipper. Pre-grazing pasture samples were cut at 5 cm above ground level to replicate grazing height from each treatment break on each day during the measurement period (15 samples/break). Samples were bulked over the three days to provide one sample for each group of five cows per week.

Pasture was sub-sampled and dried to determine dry matter content (100^{o}C; 24 hours) and for NIRS analyses (60^{o}C; Corson *et al.*, 1999).

Silage quality: sub-samples of the silages offered were taken on measurement days and bulked to provide one sample per week. Silage refusals were sub-sampled to

determined DM refused. An additional sub-sample of lotus and sulla silages was freeze dried and the CT concentration measured using the butanol-HCl colorimetric procedure (Terrill *et al.*, 1992).

Milk yield and composition: Milk yield and composition (fat and protein concentration) were determined on the three measurement days (Tuesday PM – Friday AM) using an automated milk sampler and infrared milk analyser (Milkoscan 133B, Foss electric, HillerØd, Denmark). Milk yield and composition were also measured on two days during the uniformity period and milk solids yields were used in allocation of cows to treatments and for covariate analyses to determine treatment effects.

Liveweight: Liveweight (LW) was measured immediately before each AM milking for three consecutive days during each measurement period and during the uniformity period. Liveweight was also used to allocate cows to treatments and for covariate analyses.

5.3.4 - Statistical analysis

Two replicate groups of five cows on each of the six treatments provided 12 groups in total and enabled a statistical evaluation of treatments effects. Data were analysed using PROC GLM (SAS, 2001) with group to group variation used as the error term. The uniformity data collected before the feeding trial started was also used as a covariate for analysis of the milk parameters. See Appendix CD (Chapter 5) for a complete data set of results and SAS procedures used in the analyses.

5.4 - Results

The conditions which are typical of the Waikato region in summer did occur in January of the year when this trial was carried out so the quality of pasture was very representative of a large proportion of New Zealand dairy farms that manage pastures well during summer (Table 5.1). However, pasture quality declined over the experimental period, especially during the last week of the measurements (Figure 5.3). The quality of the pasture is indicated by the concentration of CP in the pasture offered to cows in each treatment group (17.8 – 19.0 g CP/100 g DM; Table 5.1). Concentrations of fibre were moderate for ryegrass and similar to ensiled pasture and the average predicted OMD for pasture exceeded 70%, which was adequate for dairy cows.

5.4.1 – Pasture and silage composition

The pasture composition was constant for the first three weeks of the treatment period, but dry conditions resulted in a decline in quality during week 4 (Figure 5.3). The pasture DM increased from 21.5 to 27.0 g DM/100 g material over the trial period, with a decline in CP (19.0 to 16.0 g CP/100 g DM) and increasing NDF concentration to 49.2 g NDF/100 g DM (Figure 5.3). The pasture quality in week 4 was reasonable but more typical of summer dairy pastures in New Zealand.

The chemical composition of silages (Table 5.2) demonstrates a wide variation in quality. Maize silage had a very low CP and high NSC concentration, in contrast with sulla silage that contained low-medium CP and CT concentrations (14.5 and 1.6 g/100 g of DM) compared to the lotus silage (23.4 and 3.4 g/100 g of DM). In this trial, the lotus silage was of excellent quality (odour, colour, nutritive value (NV)) where as the sulla silage was stemmy (50.8g NDF/100 g of DM) and had lower ME content and estimated DM digestibility. The sulla was of poor nutritional quality compared to the other silages and about 25% was rejected (stem) by the cows (Table 5.3). This happened because sulla was past optimum maturity when harvested for ensiling and the contractor did not have machinery for chopping the sulla prior to wrapping.

The low proportion of sulla stems consumed by the cattle resulted in a lower daily intake of sulla than other silages but the leaf fraction that was eaten had a higher quality than the silage on offer. Data from a separate trial showed the composition of ensiled sulla stem (g/100 g DM) was about 75 NDF, 52 ADF, 8 CP with a ME content of 8.6 MJ/kg DM. When components of the stem (rejected by cows) were subtracted from the material on offer, the sulla DM eaten comprised (g/100 g DM) 17 CP, 41 NDF and 36 ADF. Estimated ME content was 10.8 MJ/kg DM. These data were used to calculate the composition of the diet eaten by cows given restricted pasture with sulla silage (Table 5.4).

Pre-grazing pasture mass for all treatments was about 3600 kg DM/ha and the larger area offered to cows given the full pasture treatment resulted in higher post-grazing herbage mass (2150 kg DM/ha) than these given the other treatments. Pasture residual DM was similar for cows fed silages (1920 ± 560 kg DM/ha) and averaged 1790 kg DM/ha for cows given restricted pasture as a sole diet. The lower residual DM for cows given restricted pasture compared to full pasture resulted in a higher proportion of stem being eaten and a lower diet quality.

5.4.2 – Cow performance

Silage supplementation, regardless of type, increased both DMI and milk production when compared with the restricted pasture treatment (Table 5.3). Cows fed the lotus silage and full pasture treatments had significantly higher milk and milksolids (MS) yields than the other silage supplemented cows ($P < 0.001$). Cows fed pasture, maize and sulla silage supplements all had similar milk yields (Table 5.3) although intake of sulla silage was significantly less than other three.

The higher milk yield of the full pasture treatment compared with the restricted pasture treatment was associated with a higher DMI (Table 5.3) due to the high pasture allowance and the quality of the diet eaten by cows given a full pasture allowance would be substantially better than for cows fed restricted pasture which were forced to eat a higher proportion of feed on offer. However the difference in MS yield (0.29 kg/cow.day) was small compared to the 6 kg difference in DMI of the two groups fed pasture.

Cows given the lotus silage produced more milk than those receiving the other silages (Table 5.3) probably in response to an improved diet quality. DM intakes were similar for lotus, pasture and maize silages (16.6 – 17.2 kg/cow; Table 5.3).

Feeding value (intake x NV) of silages did not affect milk fat concentrations in any of the treatments over the four weeks of the trial. However, cows fed the lotus silage had a slightly higher milk protein concentration ($P < 0.05$) than those in other treatments over the entire trial period (Table 5.3). The lotus silage contained 23 g CP/100 g DM and the 3.4 g CT/100 g DM would limit protein degradation and improve amino acid supply for absorption.

Table 5.4 summarises the feeding value of the diets eaten by cows given the six treatments. The lotus silage showed the lowest dietary NDF among all treatments. The maize silage supplementation in this experiment was sufficient to increase dietary NSC levels compared to pasture, but concentrations were well below optimum level of 30 to 40 percent of the ration DM (Nocek, 1997).

Substitution rate (SR) defines the extent to which a supplement replaces pasture in the diet and is expressed as the decrease in pasture intake/kg of supplement fed. Estimation of SR requires a control or un-supplemented treatment (restricted pasture). SR (kg pasture/kg silage) is calculated as: (pasture DMI in restricted group – pasture DMI in supplemented group)/silage DMI. A zero value means that pasture intake remains the same, while a value of 1.0 means that the supplement completely replaces the

pasture. Low pasture SR must be achieved if feeding silage is to have an additive effect with pasture to increase milk production.

The pasture DMI of cows receiving silage supplements was similar to the pasture DMI of cows fed the restricted pasture (control) treatment, and all SR values were below 0.15. As a result, the silage supplements reduced pasture DMI only slightly, particularly for cows in the lotus silage treatment which had the lowest SR (Table 5.3). This result suggests pasture availability was limiting cow intake and although a pasture allowance of 40 kg DM/cow.day would avoid this limitation (Table 2.2; Figure 2.4) pasture shortages do occur in many summer situations.

The cows weighed 531 kg with a range of group means from 512 to 545 kg. Although statistical analysis did not show significant live weight changes between treatment (P = 0.06; Table 5.3), higher LW gains were obtained for cows receiving a high pasture allowance and those fed with pasture, lotus and sulla silages compared with cows fed low pasture allowance or maize silage.

TABLE 5.1 – Chemical composition of pasture dry matter (DM; g/100 g) cut to 5 cm for each dietary treatment averaged over the four weeks of grazing.

	Cows feeding treatments									
	FP	RP	PS	M	LS	SS	P	LSD[a]	r^2	SE
DM	23.6	23.4	24.9	25.0	23.2	23.0	0.2604	2.3	0.91	0.45
CP	18.8	18.5	17.8	18.0	18.6	19.0	0.0011	0.4	0.88	0.30
NSC	10.1	9.7	10.5	10.4	9.9	10.4	0.1935	0.8	0.95	0.12
Lipid	3.9	3.8	3.8	3.7	3.8	3.9	0.1100	0.1	0.75	0.04
NDF	46.4	47.3	46.8	47.4	45.8	45.5	0.1223	1.6	0.89	0.46
ADF	25.3	25.9	25.7	25.8	25.5	24.8	0.1154	0.8	0.84	0.27
Ash	8.9	9.1	8.7	8.6	8.9	8.9	0.1186	0.3	0.77	0.10
OMD	71.0	70.3	70.5	70.2	70.5	72.2	0.2593	1.9	0.92	0.40
ME [b]	10.1	10.0	10.1	10.1	10.1	10.3	0.4786	0.3	0.94	0.05

Abbreviations: FP, full pasture; RP, restricted pasture; PS, pasture silage; M, maize silage; LS, lotus silage; SS, sulla silage. Others see text and tables in previous chapter.

P: P values assessing difference between treatments.

[a] Least significant difference alpha 0.05. SE = standard error. [b] ME, MJ ME/kg DM.

TABLE 5.2 –Average dry matter (DM) content and nutritional composition of the feeds offered to cows in the six treatment groups. Units are all g/100g DM unless stated otherwise.

	Full pasture	Restricted pasture	Pasture silage	Maize silage	Lotus silage	Sulla silage
DM	23.6	23.4	32.6	33.7	33.1	35.4
CP	18.8	18.5	15.6	6.9	23.4	14.5
NSC	10.1	9.7	5.9	31.6	2.6	3.6
NDF	46.4	47.3	46.8	44.5	35.5	50.8
ADF	25.3	25.9	31.1	27.0	27.5	40.6
OMD	71	70.3	71	NA	69.2	63.4
ME (MJ/kg DM)	10.1	10.0	11.4	10.4	10.9	10.2
CT					3.4	1.6

Abbreviations see text and tables in previous chapter.

NA = not applicable.

TABLE 5.3 – Daily pasture and silage allowances, dry matter intakes (DMI), substitution rate (SR), milk production and live weight (LW, in kg) change over 4 weeks trial for cows given six dietary treatments. Units are all kg/cow/day unless stated otherwise.

	Cow feeding treatments							
	FP	RP	Pasture Silage	Maize Silage	Lotus silage	Sulla silage	LSD	P<
Pasture allowance	50	25	25	25	25	25		
Silage allowance	0	0	5	5	5	5		
Total DMI	18.5	12.5	17.0	16.6	17.2	15.7	0.89	0.001
Total DMI (%LW)	3.4	2.4	3.2	3.2	3.2	3.0		
Pasture DMI	18.5	12.5	12.0	11.8	12.2	12.1	0.93	0.001
Silage DMI			5.0	4.8	5.0	3.6	0.34	0.001
Milk fat, %	4.29	4.48	4.20	4.29	4.12	4.19		ns
Milk protein, %	3.29	3.23	3.24	3.21	3.37	3.23		0.05
Milk	17.0	13.1	15.0	15.0	17.2	15.1	0.54	0.001
Milk solids	1.29	1.00	1.11	1.12	1.29	1.10	0.07	0.001
LW change	9.5	1.6	4.8	0.7	5.8	8.2	7.50	0.06
LW	542	512	534	524	545	529	7.83	0.001
SR			0.10	0.15	0.06	0.11	0.09	0.001

Abbreviations see text and previous tables.

TABLE 5.4 – Composition of the diets eaten by cows. Units are all g/100g DM unless stated otherwise.

	Cow feeding treatments					
	Full pasture	Restricted pasture	Pasture silage	Maize silage	Lotus silage	Sulla silage
Dietary CP	18.0	17.4	16.9	14.4	19.2	18.9
Dietary NSC	10.2	9.7	8.6	16.0	7.6	8.3
Dietary NDF	45.3	46.5	46.6	45.9	43.3	45.9
Dietary ME (MJ/kg DM)	10.1	10.0	10.4	10.1	10.3	10.2

Abbreviations see text and previous tables.

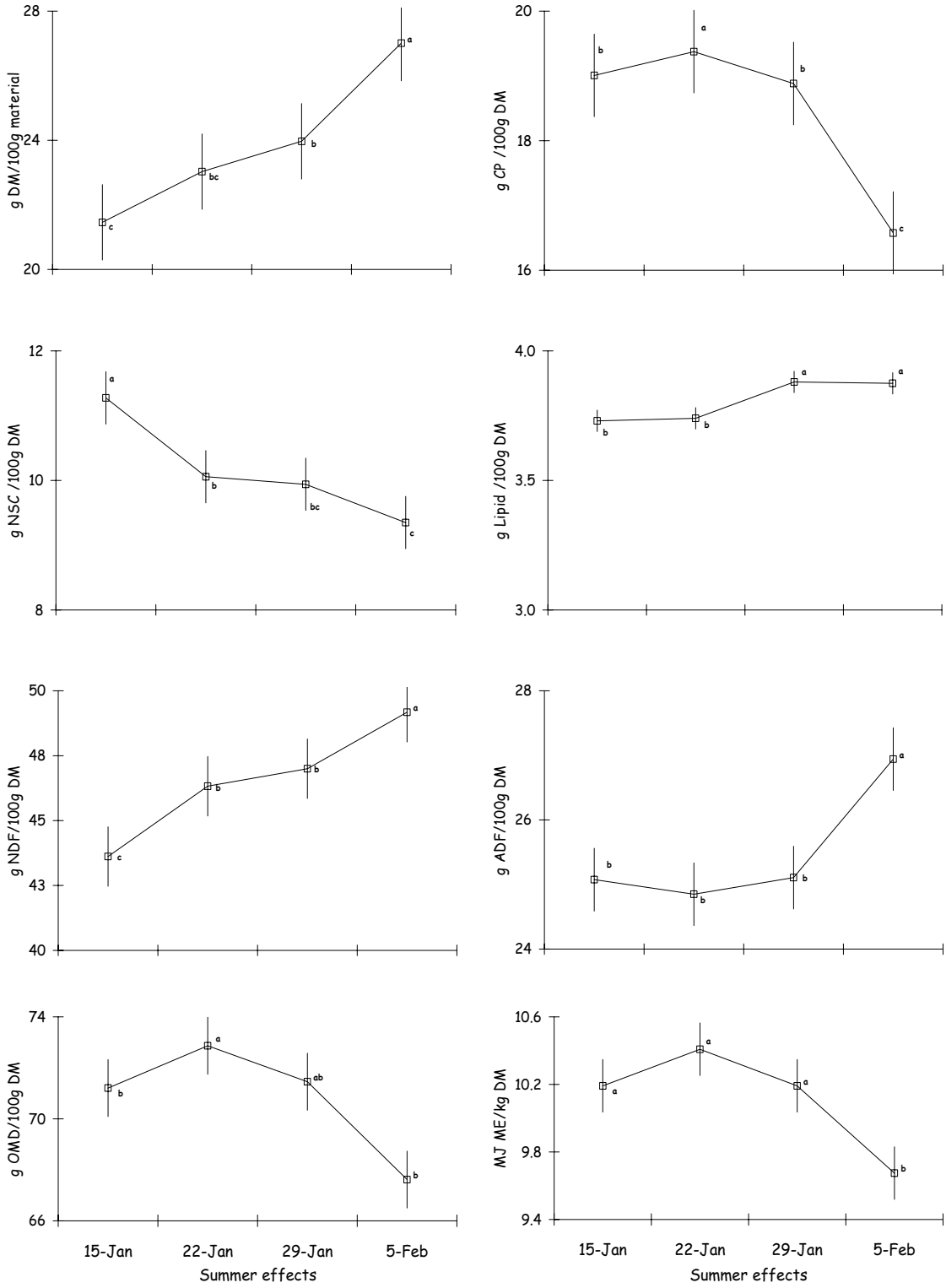


FIGURE 5.3 - Changes in chemical composition of pasture cut to 5 cm over four weeks of the grazing trial. Data are averaged across all treatment groups. $^{a, b, c}$ Least square means with different superscripts differ ($P < 0.05$) within plots.

5.5 - Discussion

The principal result from silage supplementation of restricted pasture was increased DM intakes with minimal substitution. The lotus silage provided a much greater increase in milk production compared to maize, pasture or sulla silages.

Silages accounted for only 23 – 30% of dietary DM, so effects on composition of the whole diet were minor. The principal response to silage supplementation supports the suggestion of Penno *et al.* (1998) that energy was limiting milksolids production. These authors used concentrate based supplements offered to cows consuming a similar intake of pasture as cows in this experiment and reported an increase of about 220 g milksolids from 50 MJ supplemental ME given to cows in mid lactation and grazing summer pasture. The response to pasture, maize and sulla supplements fed in this experiment was about 110 g MS from about 57, 50 and 40 MJ ME supplied by the respective silages.

Supplementation with lotus silage resulted in an additional 290 g milksolids from 54 MJ ME. Lotus appeared to complement pasture to improve both the nutritive value of the diet and cow intake. An outstanding feature of the lotus silage was its acceptability. The cows actually ran down the race to access lotus silage, ate all that was on offer and ate as much pasture as other treatments. The high acceptability of lotus silage has been reported previously by Woodward *et al.* (2001) when cows ate 38% more than moderate quality pasture silage, both given as a sole diet. Fresh lotus is also highly acceptable to dairy cows with intakes 8 - 15 % higher than pasture (Harris *et al.*, 1998a; Woodward *et al.*, 1999).

Pastures intakes and residual DM

Although summer feed shortage is common in several dairy regions (Clark, 1995; Clark *et al.*, 1997a; Penno *et al.*, 1998; Shaw *et al.*, 1997; Thom *et al.*, 1998) we anticipated a higher intake of pasture by cows on the restricted pasture treatment compared with those given silage supplements. Comparison with published reports support an allowance of 25 kg DM/cow.day to achieve a moderate, but not excessive restriction on pasture availability (Auldist *et al.*, 1998; Harris *et al.*, 1998b; Shaw *et al.*, 1997; Suksombat *et al.*, 1994) and the DM residuals after grazing of 1787 ± 368 kg/ha for restricted pasture and 1916 ± 350 kg/ha for silage treatments support a moderate restriction. These values exceed target post-grazing herbage mass for summer ryegrass based pasture of 1500 – 1600 kg/DM suggested by Matthews *et al.* (1999). Comparable residual herbage masses after grazing a moderate herbage allowance (18 - 30 kg

DM/cow) have ranged between 1120 and 2200 kg DM/ha (Suksombat *et al.*, 1994; Thomson, 1996).

Pasture quality and composition of residual DM will influence acceptability, but the high proportion of dead matter typical of dry summer pastures (Figure 2.1) was not evident in this study. Hence cows given restricted pasture could have consumed more DM but the balance between demand for nutrients and the effort required to harvest, or quality of pasture available has resulted in a low intake, low milk production and no change in live weight. The residues will have a higher proportion of stem than the pre-grazed sward and future studies need to measure the composition of residual DM, especially when cows respond to supplements and leave a substantial pasture residue.

Butler and Hoogendoorn (1987) showed herbage intake of dairy cows was better related to leaf allowance than to green or total herbage and suggested that differences in performance may be affected to a greater extent by the level of leaf mass and dead matter in pasture than by green grass stem. More recently Clark *et al.* (1999) and Thom *et al.* (1999) have demonstrated detrimental effects of toxins produced by fungal endophyte (*Neothphodium lolli*) on intake and milk production. Endophytic and other fungi are concentrated in the base of the sward and in dead forage (Easton, 1999) and will reduce intakes and lower productivity. These factors may have contributed to a reluctance by cows in all treatment groups given restricted pasture to consume more than 12.5 kg DM/day.

Irrespective of the reason for pasture intakes of only 11.8 – 12.5 kg DM/day, provision of silages did enable increased intakes, to levels approaching that of cows given unrestricted pasture. Pasture intake was not limited by rumen fill or metabolic factors and either medium quality leafy pasture or chopped silages was able to increase DM intakes by about 35% even though NDF accounted for 43 – 47% of the dietary DM. The chopping of pasture, maize and lotus silages, together with high DM percentage may have enabled high intakes despite the concentration of fibre because a substantial reduction in particle size and cell damage had occurred during processing and ensiling. The low substitution of silages contrasts with values of 0.3 – 0.4 when similar quantities of turnips or sorghum were fed to cows under a similar feeding regime (Harris *et al.*, 1998b) and with 0.14 – 0.40 kg DM reduction in white clover pasture intake per kg of maize silage eaten at two levels of pasture allowance (Stockdale, 1996).

The total intakes of cows given unrestricted pasture or silage supplements ranged from 30 – 34 g DM/ kg liveweight, which exceeds predicted maxima of about 14.0 kg/day on the basis of NDF concentrations (Figure 5.4; Cherney and Mertens, 1998).

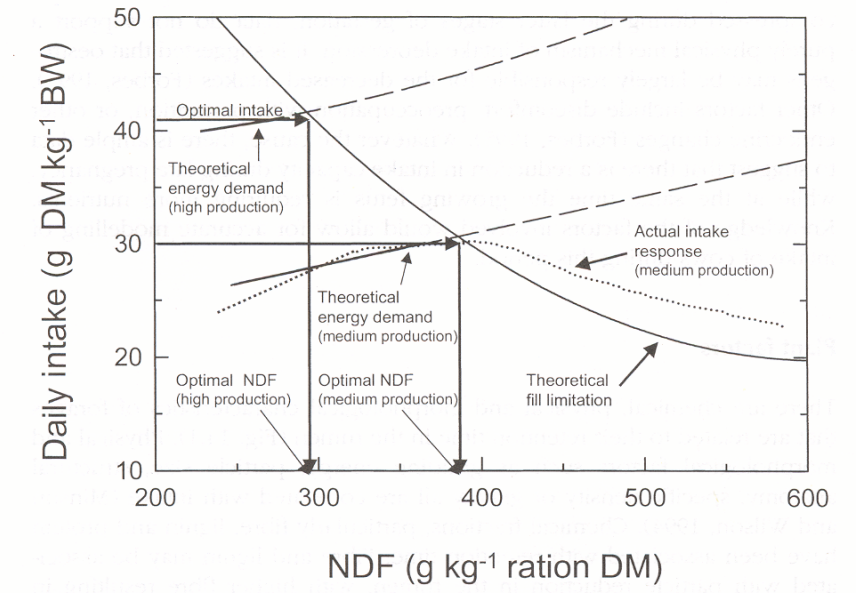


FIGURE 5.4 – Illustration of neutral detergent fibre NDF-Energy Intake System for predicting intake for optimal and low-fibre rations. BW = body weight of animal. Source Cherney and Mertens (1998).

Fibre based predictions of DM intake derived from Northern hemisphere models for cows fed TMR (Kolver *et al.*, 2002) do not apply to New Zealand pasture based diets but the NRC (2001) dairy model did predict intakes on the basis of milk production and body weight with reasonable accuracy (Figure 5.5).

$$DMI\ (kg/d) = (0.372 \times FCM + 0.0968 \times BW^{0.75}) \times (1 - e^{(-0.192 \times (WOL+3.67))})$$

Where FCM = four percent fat corrected milk (kg/day), BW = body weight (kg), and WOL = week of lactation.

Mean predictions of cow DMI for all six treatments based on the equation above were compared with actual values (Table 5.3) and are shown in Figure 5.5.

The mean bias, that represents the average inaccuracy of predictions across treatments, was only 0.4 kg/day DMI. So using NRC (2001) equation could lead the reader to conclude that predictions are precise for all treatments with silage

supplements. However restricted pasture showed the highest residual (predicted – actual) for DMI of 3.3 kg/day, because this group had insufficient feed to meet requirements.

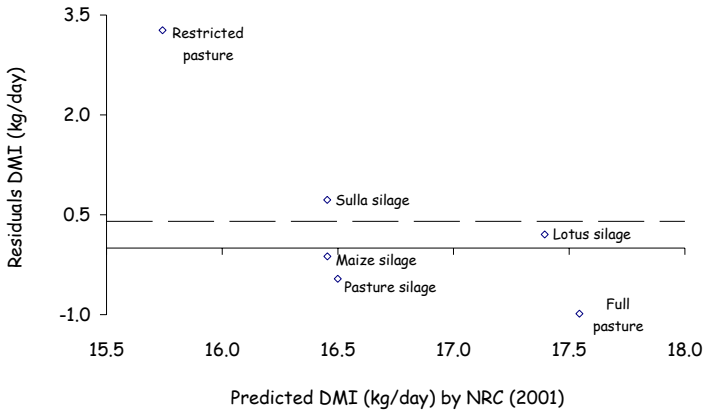


FIGURE 5.5 - Residuals (predicted – actual) versus predicted values of dry matter intake (DMI) from NRC (2001). Line (_ _ _) indicates mean bias.

Diet quality and cow performance

Pasture offered to cows in this study was better quality (19 g CP/100 g DM, 71 g OMD/100 g DM and 10.1 MJ ME/kg DM) than typical summer pasture (Figure 2.1) which would contain 14 – 16 g CP/100 g DM with a DM digestibility of about 65 g OMD/100 g DM (Clark, 1995; Clark *et al.*, 1997b; Simons *et al.*, 1998). The use of forage supplements limited opportunities for major changes in chemical composition of diets, but the maize silage resulted in a higher NSC and lower CP and a higher substitution rate than other diets. The lotus silage did increase CP and lowered NDF concentrations in the whole diet (Tables 5.3 and 5.4). These diets did affect cow performance, with maize silage resulting in the lowest milk protein concentration and no change in body weight whilst the lotus silage achieved the highest milk protein concentration ($P < 0.05$), the highest milk production and smallest increase in liveweight over the trial.

The lotus silage also contained condensed tannins which are able to protect dietary proteins from degradation in the rumen and increase amino acid flow to the intestine for absorption (Waghorn *et al.*, 1997; Wang *et al.*, 1996b). Even though lotus silage accounted for 29% of DM intake, the CT is able to affect both lotus and pasture protein (Min *et al.*, 2003; Waghorn and Jones, 1989) and this would complement the effect of this high protein silage supplement.

Wang *et al.* (1996a) demonstrated a 21% increase in milk production and a 14% increase in milk protein production in the second half of lactation due to the effects of CT in *Lotus corniculatus* grazed by sheep. Harris *et al.* (1998a) fed a high allowance (60 kg DM/cow.day) of either medium-poor pasture (11 g CP; 53 – 56 g NDF/100 g DM) or *Lotus corniculatus* (22 g CP; 25 g NDF/100 g of DM) to cows and reported a 8 – 15% increase in DM intakes with lotus and a 42 – 54% increase in milksolids production. Later studies identified the contribution of CT in fresh lotus to milk production. Woodward *et al.* (2001) showed CT in lotus did not affect voluntary intakes but accounted for 42% of the increase in milk yield and 57% of the increase in milk protein percentage, relative to ryegrass pasture. Cows fed lotus ate 15% more DM than cow fed medium quality (53 g NDF/100 g DM) ryegrass.

The impact of forage quality on intake and performance has been demonstrated for the lotus silage, with an additional 290 g milksolids/day compared to about 110 g MS/day for other silages. However, sulla silage quality was poor so the cows only ate 3.6 kg DM/day, and the response to silage (g MS/kg silage DM consumed) was 28 g compared to 58 g for lotus, 22 for pasture and 28 g for maize silages. We believe the sulla silage has been under-valued in this experiment because it was of poor quality. Sulla is able to yield in excess of 18 t DM/ha.year (Rys *et al.*, 1988) compared to a maximum of about 13 t DM/ha.year for *Lotus corniculatus* and this biannual crop requires further evaluation. Sulla frequently contains higher concentrations of CT than the 1.6 g CT/100 g DM reported here (Waghorn *et al.*, 1998), with good concentrations of CP and NSC. It ensiles very well (Niezen *et al.*, 1998) but techniques need to be developed to harvest and chop sulla on a farm scale.

CNCPS diet evaluation

The CNCPS model has equations for predicting nutrient requirements (first limiting nutrient: ME or MP), feed intake, and feed utilisation over wide variations in cattle (frame size, body condition and stage of growth), feed carbohydrate and protein fractions and their digestion and passage rates (Sniffen *et al.*, 1992). However, little research has been conducted to evaluate the CNCPS predictions in dairy cattle consuming fresh forages (Kolver *et al.*, 1996).

Data from this experiment were used in the CNCPS model to provide an explanation for cow responses to the six dietary treatments. Inputs to the model included feed composition collected in this experiment; degradation rates for carbohydrate and protein from CNCPS feed library and Burke (unpublished) (Table 5.5) and cow parameters (Table 5.3). CNCPS library parameters enabled diet composition

to be generated (Table 5.6) and to predict milk production and live weight change. The model also generated estimates of microbial growth, nitrogen kinetics and passage rate (Table 5.6).

TABLE 5.5 – Individual feed composition and degradation rates used in the CNCPS evaluation of pasture and silage diets fed to cows in this experiment.

General characteristics

Individual feeds	DM (%)	NDF (%DM)	peNDF (%NDF)	Lignin (%NDF)	Fat (%DM)	Ash (%DM)	Starch (%NFC)
Full pasture	17.4	55.9	40.0	7.23	3.9	11.7	48.0
Restricted pasture	23.4	47.5	40.0	9.31	4.1	11.0	48.0
Pasture silage	32.6	46.8	95.0	5.50	2.6	7.2	63.0
Maize silage	33.7	44.5	85.0	10.59	3.0	4.0	80.0
Lotus silage	33.1	35.5	80.0	20.30	3.2	10.0	64.0
Sulla silage	35.4	50.8	92.0	25.60	5.2	10.1	64.0

Energy and protein values

Individual feeds	CP (%DM)	UIP (%DM)	Sol-P (%CP)	NPN (%Sol-P)	NDFIP (%CP)	ADFIP (%CP)
Full pasture	19.0	22.4	55.0	4.76	9.1	3.04
Restricted pasture	17.4	22.4	55.0	4.76	9.1	3.04
Pasture silage	15.6	22.4	50.0	100.0	31.0	10.0
Maize silage	6.9	20.9	58.0	100.0	16.0	7.0
Lotus silage	23.4	17.6	50.2	28.0	13.0	9.0
Sulla silage	14.5	38.4	54.0	28.0	15.0	10.0

Degradation rates

Individual feeds	Degradation rates (%.h^{-1})					
	Carbohydrate			Protein		
	A	B1	B2	B1	B2	B3
Full pasture	85.3	19.2	14.0	200	12.0	2.00
Restricted pasture	85.3	19.2	14.0	200	12.0	2.00
Pasture silage	10.0	25.0	4.0	200	9.0	1.75
Maize silage	10.0	30.0	5.0	300	15.0	0.25
Lotus silage	10.0	25.0	9.0	150	9. 0	1.25
Sulla silage	10.0	25.0	9.0	150	9.0	1.25

peNDF, physical effective NDF; UIP, undegradable intake protein; Sol-P, soluble protein; NPN, non-protein nitrogen; NDFIP, neutral detergent fibre insoluble protein; ADFIP, acid detergent fibre insoluble protein. Carbohydrate A fraction relates to degradation of sugars and organic acids, B1: starch and soluble fibre and B2: available NDF; Protein B1 fraction: rapid degradable protein; B2: intermediately degraded protein and B3: slowly degraded protein. Other abbreviations see text and previous tables.

TABLE 5.6 – CNCPS predictions of nutrient composition, cow performance and rumen parameters of treatment diets.

	Feeding treatment					
	FP	RP	Pasture silage	Maize silage	Lotus silage	Sulla silage
Diet nutrient composition						
ME, MJ/kg DM	9.67	10.24	9.95	10.03	9.84	9.42
CP, g/100g DM	19.0	17.4	16.9	14.4	19.1	16.7
Soluble CP, %CP	55.0	55.0	53.6	55.4	53.3	54.8
NDF, g/100 g DM	55.9	47.5	47.3	46.6	44.0	48.3
peNDF, g/100 g DM	22	19	26	24	22	25
Total NFC, g/100 g DM	11.2	21.6	24.8	27.7	24.3	21.6
Total fat, g/100 g DM	3.9	4.1	3.7	3.8	3.8	4.4
Performance predictions						
ME allowable milk, kg/day	20.5	11.0	19.3	19.2	19.2	15.1
MP allowable milk, kg/day	24.7	14.0	18.5	17.5	19.5	15.3
Daily weight change due to reserves, kg/day	0.5	-0.4	0.7	0.8	0.3	0.1
Rumen digestion, metabolism and passage						
MP from bacteria, g/day	1013	764	978	1038	953	873
MP from undeg. feed, g/day	980	447	615	485	752	567
MP from undeg. feed, %MP total	49	37	39	32	44	39
Total DIP, %CP	78.0	79.7	75.3	78.1	75.9	77.7
Ruminal N balance, % of req.	160	146	146	125	167	151
Total bacterial nitrogen, g/day	270	204	261	277	254	233
Urea cost, MJ/day	5.0	2.0	3.0	1.0	4.2	2.3
Urea cost, %ME intake	2.8%	1.6%	1.8%	0.6%	2.5%	1.6%
Excess N excreted, g/day	221	101	150	94	190	124
Liquid passage rate, $\%.h^{-1}$	11.2	9.3	10.8	10.7	10.7	10.3
Pasture passage rate, $\%.h^{-1}$	6.51	5.54	6.46	6.42	6.45	6.20
Silage passage rate, $\%.h^{-1}$	NA	NA	5.02	5.30	5.83	4.73
Predicted ruminal pH	6.37	6.23	6.46	6.46	6.34	6.46

NFC, non-fibrous carbohydrates. DIP, degradable intake protein. Undeg, undegradble. Other abbreviations see text and previous tables.

Cows fed pasture and pasture with lotus or sulla silages had ME as the first limiting nutrient but MP was the first limiting nutrient with the pasture silage and maize silage treatments. The low CP in pasture and maize silages lowered dietary CP compared to the pasture alone, lotus silage and sulla silage treatments. Although the maize silage diet had the lowest CP%, it resulted in excellent microbial growth (277 g/day) due to high NFC content of the diet.

The CNCPS provides a system in which microbial protein production and undegraded feed protein values are predicted mechanistically, based on the integration of feed carbohydrate and protein fraction pool sizes, microbial growth on fibre and non fibre fractions, digestion and passage rates. A goal in formulation with the CNCPS is to have at least 50% of the total MP be of microbial origin (Fox *et al*., 2003). All diets fed in this trial achieved that goal. Maize silage supplementation resulted in the highest proportion of MP from bacteria (68%). All diets met requirements for ruminal microbial nitrogen with ruminal N balance ranging between 125 – 167% of requirements.

Diets with higher CP concentrations (full pasture and lotus silage treatments) resulted in higher cost for disposal urea and higher excess N excreted than other treatments. These diets also resulted in the highest milk production (actual and predicted; Tables 5.3 and 5.6).

Complementary effects (FP versus silages supplements)

The value of forage supplements to complement pasture can be determined by comparing FP with silage supplement diets in the CNCPS diets evaluations. FP provided more nutrients for greater milk production because cow grazing FP had a greater DMI compared to restricted pasture with silages. As a result ME and MP supplies were highest when cows were fed FP.

The effect of tannins in lotus and sulla was able to be modelled by using degradation rates determined *in sacco*, however the total DIP content of the diet was not changed markedly by the tannin-containing supplements. The lotus silage diet had a similar CP and DIP content to FR treatment and not surprising resulted in a similar loss of N as urea. Maize silage, which had lower CP content, had a lower level of urea excretion.

The model predicted that pasture would have a lower passage rate when fed with pasture, maize, lotus and sulla silages. This would have contributed to an improved MP yield from undegraded feed with the supplemented diets, but this effect was small compared to the generally lower CP and ME contents, and DMI of diets containing silage supplements.

If cows can be well fed on good quality pasture, similar to that used in this experiment, the complementary benefits of forage silages are minimal as nutrient supply and milk production would be higher on the pasture only diet.

Supplementary effects

The value of forage supplements to supplement pasture can be determined by comparing RP with the forage supplement diets. Increasing DMI by supplementing with forages increased ME and MP supply, and milk production. This was a function of higher DMI, as the pasture generally contained more ME and CP than the silage diets.

Lotus silage gave the greatest milk solids response (5.3 g MS/MJ ME) which would be expected for a cow restricted to 12.5 kg pasture DM intake and fed a high quality supplement (Penno, 2002). The responses of the pasture, maize and sulla silages were much lower than fro lotus (Table 5.7).

TABLE 5.7 – Cow responses to supplements expressed in grams of milk solids per silage dry matter intake (g MS/kg silage DMI) and in grams of milk solids per metabolisable energy consumed (g MS/MJ ME).

Response to supplement:	Pasture silage	Maize silage	Lotus silage	Sulla silage
Grams of milk solids per kg silage DM intake	22	25	58	28
Grams of milk solids per MJ ME	1.9	2.4	5.3	2.7

Cow responses showed the silages gave poor responses except for lotus silage. The greater response of lotus may have been due to an effect of tannin on pasture protein. However CNCPS predictions suggested MP was not first limiting nutrient for milk production on the lotus diet, and the model was unable to explain the response to lotus versus other silages.

5.6 - Conclusion

The benefits of lotus silage for milk production demonstrated in this experiment support previous observations with fresh lotus fed to sheep or cattle as a sole diet. The principal constraint to use of lotus in dairying systems is its inability to compete with other forages under high fertility situations and moderate DM yield. Future work should focus on ruminal digestion of lotus and supply of nutrients for absorption. The sulla silage used in this trial was of poor quality, but the crop has good potential to complement pasture because it is able to produce very high DM yields, with a low fibre and high readily fermentable carbohydrate concentration.

The hypothesis was proven, with lotus silage providing a significant greater response in milk and milk solids yield compared to pasture, maize and sulla silage supplements. The responses appear to been due to the high CP content of the silage, and possibly the effects of CT on protein degradability.

Chapter 6

Supplementation of grazing dairy cows with sulla and maize silages in summer.[1]

[1] A small portion of these data were previously published in the *Proceedings of New Zealand Grassland Association*, 2002, 125-128.

6.1 - Abstract

A trial was carried out at Dexcel in Hamilton to investigate the effects of silage supplementation of grazing dairy cows in summer. Forage mixtures used in the four week trial were based on previous trial results (Chapter 5) but inclusion of rumen fistulated cows in five treatments enabled rumen sampling and use of *in sacco* incubations to determine effects of diet on digestion kinetics. Sulla and maize silages were used to supplement pasture and to meet minimum requirements for dietary protein concentration. Five groups of ten cows were grazed on a restricted daily allowance of 18 kg dry matter (DM) pasture/cow to simulate a summer pasture deficit, and four groups received sulla silage (S) or maize silage (M) alone or in mixtures of 25M:15S or 15M:25S to make up 40% of total DM intake. A sixth group was given a relatively unrestricted (38 kg DM/cow.day) pasture allowance. The silage mixtures and pasture were incubated *in sacco* during the final week of the trial. The pasture was of high nutritive value and not typical of usual summer conditions, which favoured a response to quantity rather than quality of silage supplements. There were no differences in cow performance with the four silage supplements and the low milksolids (MS) production (about 1.0 kg/day) relative to full pasture (1.3 kg MS/day) showed the principal limitation to performance was feed quantity. Milk composition was not affected by silage type and the low level of pasture substitution (0.29) suggested ME was the principal limitation to performance. Samples of rumen liquor and *in sacco* data demonstrated significant effects of supplement. *In sacco* data showed highest DM degradation rates (k, h^{-1}) when cows were fed 6 kg sulla silage (0.08) whereas diets with a high proportion of maize silage were degraded slowly ($P < 0.01$). Supplementation with sulla may increase digestion rate and rumen clearance and reduce the effect of fibre in ryegrass diets.

Keywords: dairy cows; maize; pasture; silage; sulla.

Shot title: Sulla and maize silages for grazing dairy cows.

6.2 - Introduction

The previous cow trial (Chapter 5) which examined responses to maize, pasture, lotus and sulla silage supplementation showed clear benefits of lotus silage for milk production when pasture supply was restricted. Although maize silage did maintain milk production over the four week experimental period the cows did not gain weight (in contrast to other silages) and the dietary CP was less than cow requirements (NRC, 2001). The potential of sulla silage was not expressed because the long stalks and sub-

optimal quality resulted in substantial refusals. These silages provided a foundation for further evaluation, with more focus on achieving adequate dietary CP using fewer silages types.

The poor cow response to maize silage (M), in contrast with lotus silage (Chapter 5; Woodward *et al.*, 2002) emphasised the importance of meeting cow protein requirements especially as M has a very low protein concentration and is not suitable for feeding with low quality summer pasture. However when maize silage is fed in a balanced diet, it provides a low cost source of non-structural carbohydrates (NSC; mainly starch) which complements pasture for much of the year (Kolver *et al.*, 2001). Sulla is of interest to us because it is a high yielding legume (Waghorn *et al.*, 1998) containing condensed tannins (CT) and high concentration of NSC which offers good potential for high quality silage production (Niezen *et al.*, 1998). Balancing dietary protein deficiency by feeding sulla and improving readily fermentable carbohydrate intake with maize silage may optimise milksolids production from cows grazing poor-medium pasture in summer.

This trial also anticipated a dry summer with restricted pasture availability, typical of the Waikato dairy environment. Maize and sulla silages were fed alone or as mixtures to account for about 40% of DM intake. The use of maize silage as a sole supplement enabled data from this trial to be compared with results in Chapter 5 and literature reports (Kolver *et al.*, 2001; Phillips, 1988; Stockdale, 1995).

The hypothesis to be tested here was that mixtures of maize and sulla silages fed to cows grazing restricted pasture would provide a more balanced diet and improve milk solids production relative to either silage fed as a sole diet.

Data obtained from this trial, with kinetic information, were used as inputs to the CNCPS model to determine the first limiting nutrient and provide information concerning rumen digestion parameters.

6.3 - Material and methods

Sixty Friesian cows [15 primiparous and 45 multiparous including 10 with rumen fistulae; 483 kg liveweight; 14.3 kg milk /day; 156 days in milk] were allocated to six treatments and balanced for milksolids yield and liveweight. The overall design comprised a uniformity (covariance) period of one week, when all cows were grazed on pasture enabling their subsequent allocation to six groups to be fed the experimental diets for three weeks. Table 6.1 presents schedule of events during this experiment.

TABLE 6.1 - Schedule of events for uniformity and feeding trial from 24 December 2001 to 26 January 2002.

Day	Event
Uniformity period	24th December 2001 to 6th January 2002 (Days 1 - 14)
8 - 10	Milk yield and liveweight measured on all 60 cows
Diet adaptation	7th to 14th January 2002 (Days 15 - 22)
15 - 22	All cows allocated to treatments and grazed with appropriate silage supplements.
Diet evaluation	Week 3 – 14th to 21st January 2002 (Days 22 - 29)
22 - 29	All cows allocated to treatments and grazed with appropriate silages.
23 - 26	Liveweight, milk yield and composition measured over a 4-day period.
22 - 26	Pasture mass measured by rising plate meter, with silage intakes and sampling for dry matter and NIRS analyses.
	Rumen samples taken from fistulated cows to measure digesta rumen pH, ammonia, volatile fatty acid concentrations.
25 - 28	Sampling and preparing forages for *in sacco* incubation
	Week 4 – 21st to 26th January 2002 (Days 29 - 33)
30 - 33	Liveweight, milk yield and composition measured over a 4-day period.
29 - 33	Pasture mass measured by rising plate meter, with silage intakes and sampling for dry matter and NIRS analyses.
	Rumen samples taken from fistulated cows to measure digesta rumen pH, ammonia, volatile fatty acid concentrations.
	In sacco (72 hours) incubation of pasture and pasture – silage mixtures corresponding to diets fed to each of the 10 fistulated cows.

6.3.1 - Treatments

The six treatments enabled the effects of supplementing a pasture diet with either maize and/or sulla silages to be compared with un-supplemented pasture. Use of full and restricted pasture allowance allowed substitution rates to be calculated as well as impacts of pasture availability. The pasture allowance was intended to be 25 kg DM/cow.day, but calculations after sub-division of plots using a visual assessment of pasture mass in the last week of the trial resulted in less herbage DM mass on offer than expected.

1. FP: full pasture allowance (38 kg DM/cow.day)
2. RP: restricted pasture allowance (18 kg DM/cow.day)
3. PMS: pasture (restricted) + maize silage (4 kg) + sulla silage (2 kg)
4. PSM: pasture (restricted) + sulla silage (2 kg) + maize silage (4 kg)
5. PS: pasture (restricted) + sulla silage (6 kg)
6. PM: pasture (restricted) + maize silage (6 kg)

6.3.1.1 - Feeding

The dietary regime was similar to that used in the previous trial (Chapter 5) where pasture and milk yield measurements were made on Tuesday, Wednesday and Thursday of each week. On these three days each group of 10 cows were split into two groups of 5 cows (with the same cows in each group each week) in order to replicate the treatments. On the remaining four days of each week the replicate groups were combined into treatment groups (six groups of 10 cows).

The full allowance (38 kg DM/cow.day) of pasture was intended to provide unrestricted feed, while the restricted pasture allowance of 18 kg DM/cow.day was intended to mimic summer conditions with feed shortages. Cows in each treatment group were given a new break of pasture once daily using electric fences, and water was always available. Daily pasture allowances for each treatment group were estimated by visual assessment of pre-grazing herbage mass and allocation of the appropriate area to be grazed. Silage was fed to four groups of cows in portable feed troughs (one trough per 5 cows), after the cows returned to the paddock following the morning milking. Silage offered to cows was based on a rapid DM determination of DM using a microwave oven, to provide 6 kg silage DM/cow.day. Troughs were removed

from paddocks when cows were at afternoon milking and refusals weighed. The dry matter contents of the silages offered and refused were determined by drying at 100°C for 24 hours (See procedure details in Chapter 5).

6.3.2 - Measurements

Pasture intakes were determined by measurement with a rising plate meter before and after grazing as described in section 5.3.2. Silage DM intakes were also determined so total daily DM intakes for each sub-group of cows were calculated over the three day measurement period in weeks three and four of the trial.

Pasture quality: two types of samples were collected using an electric clipper, for analyses of pasture quality:

1. Pre-grazing pasture samples were cut to ground level from each of 12 breaks on each day during the measurement period. Samples from each break were bulked within weeks to provide one sample for each group of five cows per week to indicate the quality of feed on offer.

2. A second pre-grazing pasture sample was cut to estimated grazing height at about five cm above ground level from each break on each day during the measurement period. Samples were bulked to provide one sample for each group of five cows per week to indicate quality of pasture consumed. Both pasture sample types were sub-sampled to determine DM content (100°C; 24 hours) and for NIRS analyses (60°C; Corson *et al.*, 1999).

Silage quality: sub-samples of the silages offered were taken on measurement days and bulked to provide one sample per week. Sub-samples were taken of silage refusals over the same period and bulked for analyses. Silages and refusals were sub-sampled for dry matter measurement (100°C; 24 hours) and NIRS analysis.

Composition of silage offered and refused enabled calculations of the dietary composition (nutrient x intake) for each constituent (DM, CP, lipid, fibre and ME). Dietary composition = ((kg offered * concentration constituent offered) – (kg refused * concentration constituent refused)) / kg intake.

Liveweight (LW) was measured before milking on three mornings per week during weeks one, three and four of the trial. Milk yield was measured for each cow on three days per week, and sub-samples taken to measure fat, protein and lactose concentration as described in Chapter 5.

6.3.3 – *In sacco* incubation and digestion kinetics

Two rumen fistulated cows were included in each treatment except those given restricted pasture and enabled *in sacco* incubations of both pasture and of the dietary mixtures fed to each cow to be conducted.

Pasture used in incubations was obtained by cutting pasture five centimetres above ground level before grazing, and frozen prior to chopping and mincing for *in sacco* incubations. The pasture was incubated in all cows as a single constituent and also combined with sulla and maize silages in similar proportions to the diet eaten by cows. Samples of maize and sulla silages were collected and frozen in proportion for mixing with chopped pasture prior to mincing.

Mixtures used for *in sacco* incubations comprised about 60% pasture DM and 40% silage DM. The four silage mixtures (PM, PS, PMS and PSM) were only incubated in cows which were fed the same dietary mixture; i.e. the two cows fed pasture with maize silage were used to incubate pasture and maize silage; those fed PMS incubated PMS *in sacco*. Duplicate bags of dietary mixtures were removed at each time from all cows as well as duplicated bags of ryegrass pasture. The only exception were cows fed full pasture, where duplicate bags of pasture where removed at each sampling time from the two cows

In sacco incubations for each diet were prepared by chopping pasture and sulla silage to 2 cm length, mixing frozen chopped pasture, sulla and maize silages as required, and mincing to resemble chewed forage as described in section 3.3.1. Samples were weighed into *in sacco* bags and also used to determine DM, chemical composition and distribution of particle size. A total of 28 bags (four per lingerie bag) were placed in the mid-ventral rumen of cows, and were removed at 2, 6, 9, 12, 24, 48 and 72 hours. Bags, including 0 hour samples which were not put in the cow, were washed, dried (60°C) and analysed to determine DM, CP, NDF and ADF content to calculate rates of disappearance during digestion. Procedures for *in sacco* calculations and data analyses are given in section 3.3.5.

The disappearance of DM, NDF and ADF were analysed using a non-linear model described in Chapter 3. The effective degradability (E) was calculated from soluble and degradable pools and kinetic parameters, by fitting equations to *in sacco* data assuming a fractional passage rate (k_p) of both 0.06 and 0.08 h^{-1}. A k_p value of 0.08 h^{-1} was used (AFRC, 1992) for comparison with high producing dairy cows (Hoffman *et al.*, 1998; Kolver *et al.*, 1998) even though intakes in this study were relatively low.

Disappearance of CP was calculated using a similar procedure but additional definition was applied in relation to the degradability of protein and CP content of the DM. The RDP and RUP values for the diets (percent of CP) were estimated from the model describing degradation and ruminal escape of feed proteins (NRC, 2001; Ørskov and McDonald, 1970) using the equations:

$$RDP = A + B\ [k / (k + k_{DM})]$$

$$RUP = B\ [k_{DM} / (k + k_{DM})] + C$$

Where k is fractional rate of protein degradation and DM passage (k_{DM}), and C is the undegradable fraction. Equation for estimating K_{DM} (rate of DM passage from the rumen, %/h) of wet forages = $3.054 + 0.614X_1$ where X_1 = DMI, percentage of body weight (NRC, 2001).

The metabolisable protein system (AFRC, 1992) for defining ruminal degradation was used to calculate protein degradability parameters as described in section 3.3.5.1. Effective rumen degradability of CP (ERDP, g/kg DM) was calculated as: ERDP (g/kg DM) = CP $[(0.8 \times A) + (B \times k)/(k + k_p)]$. In this chapter, values for effective degradability of CP and ERDP were calculated using outflow rates of digesta DM from the rumen of 0.02, 0.05 or 0.08/h, which approximate to the outflow rates in dry cows at maintenance, cows producing less than 15 L milk/day, or cows fed a good quality diet that enabled a rapid passage through the rumen (Wales *et al.*, 1999a).

Relationships between degradability, nutritive characteristics of the diet *in sacco* samples and ERDP were analysed by regression (SAS, 2001) using the model:

$$ERDP\ (g/kg\ DM) = a + bX$$

where X is the effective degradability of DM or NDF content of diet *in sacco* residues.

6.3.4 – Rumen samples

Rumen fluid was also collected 5 times during the first day of the *in sacco* incubation to check the pattern of rumen metabolite concentration over 12 hours from 07:00 h (pre-feeding) to 19:00 h.

Rumen fluid samples were collected twice daily on the measurements days before morning and afternoon milking (Table 6.1). On each occasion, about 1 kg of rumen contents was taken from the mid-ventral rumen and strained though a cheese cloth to collect 100 mL of rumen fluid. Rumen pH was determined at collection

(PHM210, Radiometer Pacific Limited, Copenhagen) before samples were centrifuged and prepared to determine ammonia and VFA concentrations (sections 3.3.4.2 and 3.3.4.3).

6.3.5 - Statistical analysis

Procedures for statistical analyses of *in sacco* data are given in section 3.3.6.3. For each degradation parameter (A, B, k and E) fixed model effect tested differences between diets incubated, cow/diet effects upon pasture digestion kinetics and cow effects. A general linear model procedure of SAS (PROC GLM; SAS, 2001) was used for analysis of the milk parameters and liveweight change with uniformity period data as a covariate (section 5.3.4). Rumen ammonia and VFA concentrations were analysed using mixed model procedure of SAS (PROC MIXED; SAS, 2001) to calculate treatment means. Rumen pH analysis included day, treatment and treatment x day interaction. Pearson correlation coefficients were estimated using the CORR procedure of SAS. Effects were declared significant at $P < 0.05$ unless otherwise noted. See Appendix CD (Chapter 6) for a complete data set of results and SAS procedures used in these analyses.

6.4 - Results

This trial had similar responses to those in the previous year (Chapter 5) because pasture quality in both studies were very representative of well-managed summer pasture in the Waikato, Taranaki and South Island in most years. The quality of pasture on offer was better (Table 6.2) than the previous year (Table 5.1) and prevented any benefits associated with silage quality to be expressed even though feed availability was very low. The CP concentration in pasture exceeded that in sulla silage, which had a higher NDF concentration than pasture. The main effect of silage supplementation was through provision of additional ME although the NSC content of maize silage did alter diet composition.

6.4.1 – Pasture and silage composition

The chemical composition of pasture and silages are summarised in Table 6.2. Dry matter of pasture cut to 5 cm above ground level ranged from an average of 15.9 to 20.5 g DM/100 g across treatments. There were no significant changes in pasture quality over the four week duration of the trial, with average DM of 18.6 in week three and 17.9 g/100 g in week four. Crude protein was 21.1 and 21.4 g/100 g DM in weeks three and four with NDF contents of 47.3 and 43.3 g/100 g DM in the respective periods.

The mean values for the pastures offered to each treatment group were consistent, averaging 21.2 g CP/100 g DM and 45.3 g NDF/100 g of the DM (Table 6.2). Predicted organic matter digestibility exceeded 73 g/100 g for all pastures cut at 5 cm above ground level. Maize and sulla silages contrasted in chemical composition, with concentrations (g/100 g DM) of 6.6 and 15.7 (CP) and 39.1 and 50.0 (NDF) for maize and sulla silage respectively. The low NSC concentration in sulla silage (3.6 g/100 g DM) was due in part to the high lactic acid content. Sulla silage contained 3.5 g CT/ 100 g DM.

Pasture cut to 5 cm left about 1600 kg residual DM per hectare. Residual DM was poor quality with about 71 g NDF/ 100 g DM. Fibre (NDF) and CP concentrations of the sward cut to ground level averaged 51.0 and 19.6 g/100 g DM respectively.

TABLE 6.2 – Chemical composition of pasture dry matter (DM; g/100 g) cut to 5 cm above ground for each dietary treatment, and of maize and sulla silages averaged over the three week experimental period.

	Cows feeding treatments						Maize	Sulla
	FP	RP	PMS	PSM	PS	PM	silage	silage
DM	19.4	20.2	20.5	18.2	19.9	18.4	35.9	34.1
CP	21.7	21.5	21.0	21.7	21.0	20.6	6.6	15.7
NSC	9.2	9.1	8.5	8.9	9.4	9.0	41.4	3.6
Lipid	4.0	3.9	4.0	4.0	3.9	3.8	2.9	5.2
NDF	43.9	44.1	46.3	45.8	45.4	46.5	39.1	50.0
ADF	24.4	24.9	25.4	24.8	25.2	25.9	23.8	40.8
Ash	10.1	10.2	9.9	9.9	9.6	9.9	4.0	10.1
Lignin	4.0	4.2	3.7	4.0	4.0	4.3	3.4	8.0
OMD	75.6	74.9	74.1	74.8	73.6	74.6	NA	65.9
ME (MJ/kg DM)	10.7	10.6	10.5	10.6	10.5	10.6	10.8	10.5
pH							3.9	3.9
Lactic acid							2.3	11.4
Ammonia-N (% total N)							0.9	4.7
Total CT								3.5

Treatments: FP: full pasture; RP: restricted pasture; PMS (RP + 4 kg maize + 2 kg sulla silages/cow.day); PSM (RP + 4 kg sulla + 2 kg maize silages/cow.day); PS (RP + 6 kg sulla silage/cow.day); PM (RP + 6 kg maize silage/cow.day). Other abbreviations see text.

6.4.2 – Cow performance

Maize, sulla and silage mixtures increased DMI but milk and milksolids (MS) production were similar to the cows given restricted pasture as a sole diet (Table 6.3). The main impact of silage was to maintain liveweight. There was insufficient feed available to maintain both milk production and liveweight (LW) of cows given the RP treatment. Cows fed the full pasture treatments had significantly higher milk and MS yields than the silage supplemented cows ($P < 0.001$) and LW of all cows except those given RP were similar at the beginning and end of the trial.

Cows given the high pasture allowance ate 5.3 kg more pasture DM and produced additional 4.2 kg milk compared to RP cows, but the difference in milk production would have been greater if LW changes were similar for both groups. The RP treatment group lost 12 kg liveweight during the three week feeding period. Milk composition was not affected by treatment.

Pasture intake by cows given the four supplements were about 1.6 kg DM/day lower than these given RP. On average 5.5 kg silage DM was eaten so the substitution rate was about 1.6/5.5 = 0.29 (Table 6.3). All supplements had a similar substitution rate (0.26 - 0.33) which showed provision of silages resulted in a substantial increase in feed intake when pasture allowance was 18 kg DM/cow.day. The pasture 5 cm above ground level was of unusually high quality (Table 6.2) but stubble below 5 cm had a higher fibre content. This level of substitution is similar compared to grazing cows were offered grains or hay (Stockdale, 1999a) with pasture allowance of 32 kg DM/cow.day. Also the SR in this trial was comparable with a level of substitution of maize silage for white clover pasture of 0.14 and 0.40 kg DM reduction in pasture intake per kg DM of silage eaten at pasture allowance of 19 and 39 kg DM/cow.day (Stockdale, 1996).

TABLE 6.3 – Daily pasture and silage allowances, dry matter intakes (DMI), substitution rate (SR), milk production and live weight (LW, in kg) change over 4 weeks trial for cows given six dietary treatments. Units are all kg/cow/day unless stated otherwise. LSD and significance are for treatments within rows.

	FP	RP	PMS	PSM	PS	PM	LSD[a]	P
Pasture allowance	38	18	18	18	18	18		
Silage allowance	0	0	6	6	6	6		
Total DMI	15.7	10.4	14.6	14.4	13.9	14.3	2.3	0.001
Pasture DMI	15.7	10.4	8.8	9.0	8.7	8.8	1.6	0.001
Silage DMI			5.8	5.4	5.2	5.5	0.3	0.001
Pasture utilisation[b] (%)	41	58	49	50	48	49	3.8	0.003
Milk lactose	0.83	0.64	0.71	0.66	0.66	0.67	0.05	0.002
Milk fat	0.73	0.57	0.56	0.56	0.54	0.55	0.06	0.001
Milk protein	0.57	0.42	0.46	0.44	0.44	0.46	0.02	0.001
Milk	17.2	13.2	14.3	13.7	13.7	13.7	1.00	0.001
Milk solids	1.30	0.99	1.02	1.00	0.98	1.01	0.07	0.001
LW change	-3.8	-12.4	1.0	-4.5	-0.8	-1.4	3.4	0.06
LW, kg	497	471	480	485	475	489	3.5	0.001
Substitution rate			0.28	0.26	0.33	0.29		

[a] Least significant difference ($P < 0.05$)

[b] (kg DM pasture eaten/kg DM pasture offered) x 100.

Abbreviations see text and Table 6.2.

Pasture DM on offer averaged 3141 kg/ha for all treatments, with post grazing residuals of 1525 kg/ha for cows given a restricted allowance with silages and 1814 kg DM/ha for cows given a full pasture allowance. Cows given restricted pasture as a sole diet left 1298 kg residual DM. Pasture DM utilisation was 58% for RP and 49% across the four silage supplemented treatments (Table 6.3). Cows given full pasture allowance ate 41% of that on offer.

Although all silages were acceptable, with refusals of 0.2 – 0.8 kg DM/cow.day, the preferred supplement comprised 4 kg maize silage and 2 kg sulla silage. The refusals from this treatment group comprised mainly sulla stem and proportions refused increased with the amount of sulla silage offered (about 0.6 kg with 4 kg sulla DM and 0.8 with 6 kg sulla DM; Table 6.3). PS treatment had the lowest proportion of sulla silage eaten compared to other supplemented treatments ($P < 0.001$; Table 6.3). The sulla was harvested about 6 weeks later than optimal because of difficulties obtaining services of a contractor. It was quite stalky as indicated by the high fibre content, but

was well ensiled and had a high concentration of lactic acid. Crude protein concentration (Table 6.2) may have been higher with an earlier harvest.

There were significant differences between diets in the concentration of most constituents (Table 6.4). Provision of silages increased the dietary DM concentration (P = 0.005). The supplementary silages were intended to meet cow requirements for crude protein (CP), and the values in Table 6.4 show this was achieved with the exception of the PM diet which was only slightly less than a desirable 16 - 17% CP in DM for cows in mid lactation (NRC, 2001). The non-structural carbohydrates were highest when maize silages were used reaching 22% of dietary DM but were lowest with sulla. Dietary lipid averaged 4.0 g/100 g DM for all treatments and ash was lowest for cows fed elevated proportions of maize silage. There were no differences (P > 0.64) between diets in concentration of NDF or estimated ME (Table 6.4), although ADF concentrations were higher (P < 0.001) in treatments where sulla silage was used.

TABLE 6.4 – Average dry matter content and nutritional composition of diets eaten by cows. Data are based on composition of pasture cut to 5 cm and proportion of silages in the diet. Data are all in g/100 g DM unless stated otherwise.

Dietary composition	FP	RP	PMS	PSM	PS	PM	LSD[a]	P
DM, %	17.4	15.9	26.5	24.6	25.2	25.4	4.85	0.005
CP	21.7	21.5	16.4	18.4	19.0	15.4	3.91	0.035
NSC	9.2	9.1	16.8	11.8	6.9	22.0	0.78	<.001
Lipid	4.0	3.9	3.8	4.2	4.3	3.6	0.29	0.006
NDF	43.9	44.1	44.8	45.8	47.5	42.7	6.94	0.647
ADF	24.4	24.9	26.9	28.6	31.3	24.5	3.00	0.007
Ash	10.1	10.2	8.4	9.3	10.0	7.4	0.69	<.001
ME (MJ/kg DM)	10.7	10.6	10.6	10.6	10.6	10.6	0.49	0.994

Abbreviations see Table 6.2 and text.

Given that all silage treatments provided a similar level of nutrition, indicated by LW change and milk yield, and that 86 – 96 % of silage offered was eaten, the responses to performance of cows given full pasture were probably due to higher feed intakes. In contrast the cows offered 18 kg pasture DM/day (RP treatment) only ate 10.4 kg DM which was 58% of DM on offer and was probably too restricted. However pasture DMI is closely related to pasture allowance (Holmes *et al.*, 2002) and the

restrictions did mimic pasture deficits faced by farmers when pasture is in short supply and silages are given to increase feed availability.

Although there were no differences in responses between the individual silage supplement treatments, the PMS resulted in the highest milk yield without liveweight loss (Table 6.3). This diet also met cow requirement for CP and provided 16.9 g non-structural carbohydrates per 100 g of DM, suggesting a relatively good nutritive value despite its relatively high NDF concentration (44.8 g NDF/100 g DM). The PM diet also achieved a similar level of production, probably because the high pasture CP content enabled good complementation with low protein in maize silage.

6.4.3 – *In sacco* incubations

The complete data set for degradation characteristics for DM, CP and fibre (NDF and ADF) are presented in Appendix 8 (Tables 8.1A to 8.4A). Data presented here are averaged for incubation of pasture in each of the 10 fistulated cows and for pasture/silage mixtures incubated in two cows fed each treatment: PMS, PSM, PS and PM. The key tests of significance are indicated on each table as follows: fit of the model ("Model"), comparison between pasture/silage mixtures regardless of the cow diet ("Forage") and differences between cows based on *in sacco* digestion of pasture only ("Cow/diet"). Effects of individual cows on degradation rates were also tested in the model ("Cow").

6.4.3.1 – DM digestion

The amount of DM in the soluble "A" fraction was similar for all diets (Table 6.5; $P > 0.67$) and the slowly degradable B fraction was slightly higher for the PMS than other diets (43; $P = 0.06$). The PS was most rapidly degraded and the PM and PMS were slowly degraded (Table 6.5; Figure 6.1). The rate of pasture DM degradation rate ($k = 0.067$) was intermediate and there were no differences due to cow/diet ($P = 0.17$). There were significant differences in k values between cows ($P < 0.019$).

6.4.3.1 – CP digestion

The effective degradability of CP (Table 6.6) was higher than that of DM (Table 6.5) for all diets, probably because about 61% of CP was solubilised and the undegradable CP fraction was smaller that that for DM. Diets with a high proportion of sulla silage had slightly higher effective degradability and a high proportion of maize silage lowered effective degradability. Cow/diet had no effects on protein degradability, measured with pasture ($P = 0.5183$).

TABLE 6.5 – *In sacco* degradation characteristics of dry matter (DM), in cows fed full pasture and four silage supplements. Kinetics are defined by soluble (A), degradable insoluble (B) and undegradable residue (C = 100 – A – B) as well as fractional disappearance rate (k, h^{-1}), and effective degradability (E) which takes in account the effect of passage from the rumen[1].

DM	A	B	k	C	$E_{6\%}$	$E_{8\%}$
Pasture	44	39	0.067	17	64	61
PMS	42	43	0.049	15	61	58
PSM	43	38	0.068	19	63	60
PS	46	36	0.084	17	68	65
PM	45	36	0.054	19	62	59
Model P	0.67	0.18	0.010			
Forages P	0.67	0.06	0.004			
Cow/diet P		0.31	0.170			
Cow P		0.51	0.019			
r^2	0.17	0.89	0.98			

Abbreviations are given in Table 6.2.

[1] Passage rate set at 0.06 h^{-1} and 0.08 h^{-1}.

P: assessing goodness of fit for the overall model and tests of forage, cow/diet and cow effects.

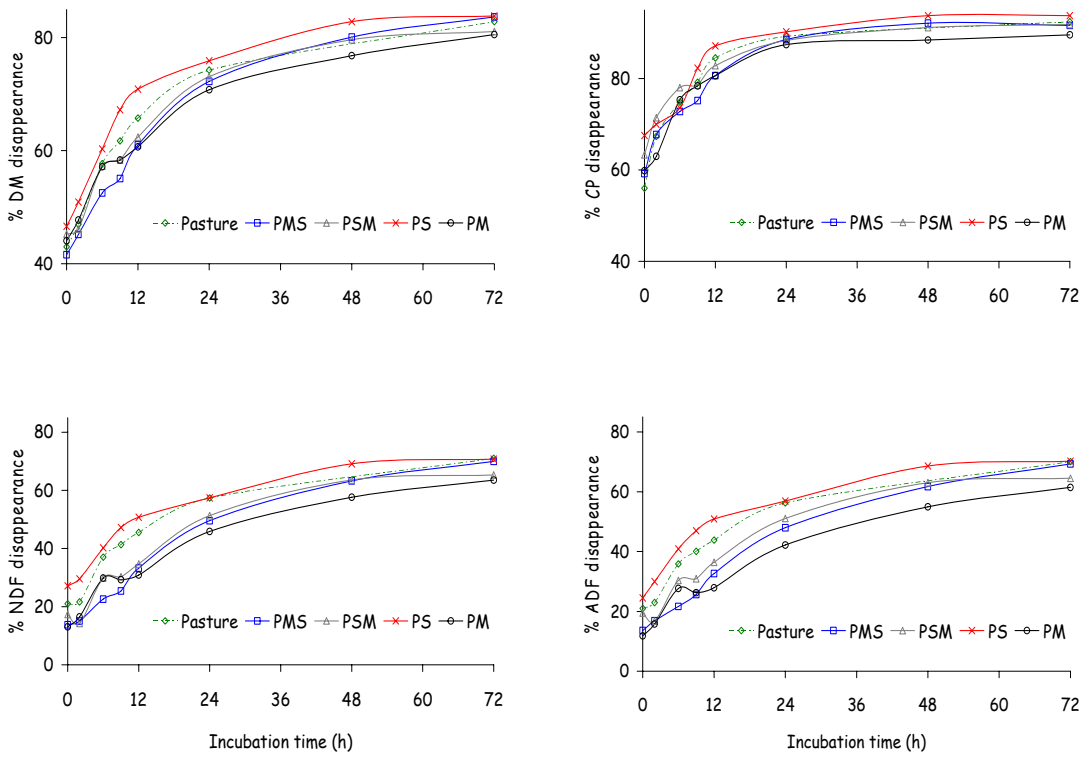


FIGURE 6.1 – Dietary dry matter (DM), crude protein (CP) and fibre (NDF and ADF) disappearance during *in sacco* incubations from fistulated cows fed full pasture and four silages supplements. Cow diets and forages *in sacco* bags are indicated on each figure.

TABLE 6.6 – Crude protein degradability coefficients (A, B and k) and effective degradability using fractional passage rates of 0.02, 0.05 or 0.08 h^{-1} ($E_{2\%}$, $E_{5\%}$ or $E_{8\%}$) for pasture and mixtures of pasture and silages incubated *in sacco*.

CP	A	B	k	C	$E_{2\%}$	$E_{5\%}$	$E_{8\%}$
Pasture	56	36	0.121	8	86	81	78
PMS	61	32	0.070	7	86	80	77
PSM	65	26	0.084	8	87	82	79
PS	66	28	0.090	6	89	84	81
PM	56	33	0.128	11	85	80	77
Model P	0.0009	0.1467	0.0675				
Forages P	0.0009	0.0464	0.0204				
Cows/diet P		0.1695	0.5183				
Cow P		0.8067	0.1272				
r^2	0.74	0.91	0.94				

Abbreviations are given in Tables 6.2 and text.

TABLE 6.7 – Rumen degradable protein (RDP) and rumen undegradable protein (RUP) as a percentage of crude protein concentration, and rate of dry matter passage from the rumen (k_{DM}) (NRC, 2001) for pasture and mixtures of pasture and silages incubated *in sacco*.

	% of crude protein		
	RDP	RUP	K_{DM}
Pasture	81.4	18.6	0.049
PMS	79.9	20.1	0.050
PSM	82.1	17.9	0.049
PS	83.8	16.2	0.049
PM	79.7	20.3	0.049
Model P	0.0336	0.0336	
Forages P	0.0099	0.0099	
Cow/diet P	0.1492	0.1492	
Cow P	0.1057	0.1057	
r^2	0.96	0.96	

Abbreviations are given in Tables 6.2 and text.

In contrast to DM disappearance, the rate of protein degradation *in sacco* was reduced when sulla was mixed with pasture (Table 6.6; P = 0.02), possibly in response to the protection conferred by condensed tannins in sulla. Reduced protein degradation rate is likely to increase protein availability for absorption and increase nutritive value for cows. Calculations from NRC (2001) showed an average of 81.4% RDP and 18.6% RUP across all diets including pasture. Diets containing sulla had the highest proportion of RDP (Table 6.7).

Crude protein degradability has also been evaluated by estimating dietary QDP, SDP, ERDP, RDP, RUP expressed as g/kg DM from pasture and mixtures of pasture, maize and sulla silage at three contrasting rumen outflow rates (Table 6.8). The slow, medium and fast (0.02, 0.05 and 0.08 h^{-1}) rates correspond to cows fed at maintenance and at medium and high levels of intake. Outflow rate of about 0.05 h^{-1} correspond to cows at mid lactation with limited fed availability, as with silage supplementation in this study. Expression in terms of dietary DM provides a realistic assessment of mixtures with varying proportions of supplement containing a range of CP concentrations.

Forage mixtures varied in NDF content from 430 to 479 g/ kg DM and the concentration of NDF accounted for 68% of the variation in effective rumen degradability for protein across diets. These data demonstrate a linear positive ($P < 0.0001$) relationship between dietary NDF concentration and ERDP across the five diets incubated *in sacco* (Figure 6.2).

There was also a significant positive relationship between the effective DM degradability of pasture/silage mixtures and ERDP (Figure 6.3) but the variation about the regression ($r^2 = 0.29$) confirmed forage differences between DM and CP degradation rates. Cow/diet may also affect the extent of pasture DM digestion *in sacco* (Appendix 8) and predicted degradability of pasture DM (Figure 6.3).

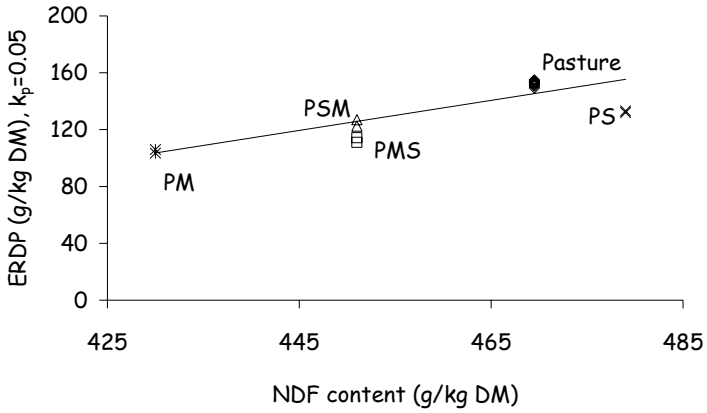


FIGURE 6.2 – Relationship between neutral detergent fibre content (NDF, g/kg DM) and effective rumen degradability of crude protein (ERDP, g/kg DM) of ryegrass pasture, and pasture plus sulla and/or maize silages. When rumen DM outflow is 0.05/h, the relationship is described as: ERDP (g/kg DM) = - 352 + 1.1(± 0.18) x NDF (r^2 = 0.68; Root MSE = 11.1; CV = 8.05%; P < 0.0001). Abbreviations are given in Table 6.2 and text.

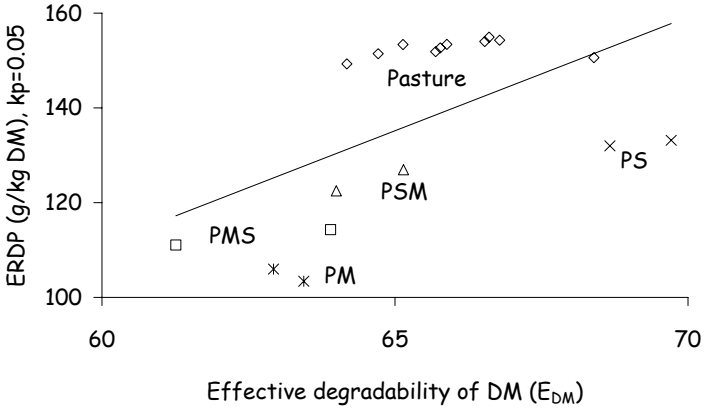


FIGURE 6.3 – Relationship between effective degradability of DM (E_{DM}) and crude protein (ERDP, g/kg DM) of ryegrass pasture and pasture plus sulla and/or maize silages. When rumen DM outflow is 0.05/h, the relationship is described as: ERDP (g/kg DM) = - 176.7 + 4.80 (± 1.88) x E_{DM} (r^2 = 0.29; Root MSE = 16.4; CV = 11.9%; P = 0.0212). Abbreviations are given in Table 6.2 and text.

TABLE 6.8 –Crude protein degradability parameters QDP, SDP, ERDP, RDP, RUP in g/kg DM (AFRC, 1992) for pasture and mixtures of pasture and silages incubated *in sacco*.

k_p=0.02	QDP	SDP	ERDP	RDP	RUP
Pasture	122	66	164	188	29
PMS	100	42	122	142	23
PSM	117	39	133	156	24
PS	124	43	142	167	21
PM	91	40	112	130	24
Model P	0.1018	0.0616	<.0001	<.0001	0.0063
Forages P	0.0186	0.0109	<.0001	<.0001	0.0012
Cow/diet P	0.5726	0.3766	0.0309	0.0263	0.0263
Cow P	0.9222	0.9306	0.7267	0.4764	0.4764
r^2	0.93	0.94	0.99	0.99	0.98

k_p=0.05	QDP	SDP	ERDP	RDP	RUP
Pasture	122	55	153	177	40
PMS	100	32	112	132	33
PSM	117	31	125	148	32
PS	124	33	133	158	30
PM	91	32	105	123	31
Model P	0.1018	0.0746	<.0001	<.0001	0.0038
Forages P	0.0186	0.0132	<.0001	<.0001	0.0006
Cow/diet P	0.5726	0.5420	0.0711	0.0942	0.0942
Cow P	0.9222	0.8344	0.0570	0.0949	0.0949
r^2	0.93	0.94	0.99	0.99	0.99

k_p=0.08	QDP	SDP	ERDP	RDP	RUP
Pasture	122	47	145	169	48
PMS	100	26	106	126	39
PSM	117	26	119	143	37
PS	124	27	127	152	36
PM	91	28	100	118	36
Model P	0.1018	0.0813	<.0001	<.0001	0.0055
Forages P	0.0186	0.0146	<.0001	<.0001	0.0009
Cow/diet P	0.5726	0.6376	0.0431	0.1327	0.1327
Cow P	0.9222	0.7518	0.0142	0.1361	0.1361
r^2	0.93	0.93	0.99	0.99	0.98

Abbreviations are given in Tables 6.2 and text.

K_p = fractional passage rate.

The parameters QDP (quick degradable protein), SDP (slow degradable protein), ERDP (effective rumen degradable protein), RDP (rumen degradable protein), RUP (rumen undegradable protein) all in g/kg DM were calculated as describe in the Material and methods of Chapter 3 (Item 3.3.5.1).

6.4.3.1 – Fibre digestion

Although there were no differences in NDF concentration between the pasture/silage mixtures (Table 6.4) when they were incubated *in sacco* there were differences in rates of NDF and ADF digestion ($P < 0.05$; Table 6.9). Both NDF and ADF where present mainly in the slowly degradable fraction (B) and undegradable residues. Rates of fibre degradation were slowest for mixtures containing a high proportion of maize silage and tended to be most rapid for PS (Table 6.9). The degradation curves for DM, NDF and ADF (Figure 6.1) show clear effects of maize on rates and extent of degradation of each constituent.

The fibre effective degradability for pasture and pasture supplemented with 6 kg DM sulla silage/cow.day were about 30% higher than other supplemented cows treatments (Table 6.9). Incubation of pasture showed that cow/diet did not affect rates of fibre degradation when pasture was incubated *in sacco* ($P = 0.3389$).

TABLE 6.9 – Neutral and acid detergent fibre (NDF and ADF) degradation characteristics (% of total DM) in cows fed full pasture and four silage supplements. Kinetics are defined by soluble (A), degradable insoluble (B) and undegradable residue (C = 100 – A – B) as well as fractional disappearance rate (k, h^{-1}), and effective degradability (E) which takes in account the effect of passage from the rumen[1].

NDF	A	B	k	C	$E_{6\%}$	$E_{8\%}$
Pasture	22	50	0.053	28	45	42
PMS	13	64	0.033	23	35	31
PSM	11	56	0.054	33	37	33
PS	27	43	0.058	30	49	46
PM	14	53	0.037	33	35	31
Model P	0.0001	0.0696	0.0803			
Forages P	<.0001	0.0152	0.0248			
Cow/diet P	0.0038	0.3919	0.3389			
Cow P		0.3947	0.1884			
r^2	0.94	0.94	0.94			

ADF	A	B	k	C	$E_{6\%}$	$E_{8\%}$
Pasture	23	49	0.050	28	44	40
PMS	14	62	0.031	25	34	30
PSM	14	52	0.053	33	38	34
PS	26	44	0.065	31	49	45
PM	13	53	0.030	34	32	29
Model P	0.0001	0.1367	0.0290			
Forages P	0.0002	0.0556	0.0085			
Cow/diet P	0.0009	0.2384	0.1666			
Cow P		0.3019	0.0825			
r^2	0.94	0.91	0.96			

Abbreviations are given in Tables 6.2 and text.

6.4.4 – Rumen pH, ammonia and VFA concentrations

Rumen liquor pH averaged across all cows (Figure 6.4) shows higher values ($P < 0.001$) prior to AM feeding (mean 6.7 ± 0.1) compared to values after the PM milking (16:00 h) which average 5.6 ± 0.1. There was no effect of diets on either morning or afternoon pH, or diurnal pattern (Figure 6.5).

Mean concentrations of rumen VFA were similar across treatments, averaged 101 mMol/L, with about 0.69 acetate, 0.17 propionate and 0.11 butyrate (Table 6.10). There were no treatment effects on concentration or molar proportion of VFA. The ratio of acetate: propionate averaged 4.1 and was similar for all diets. In contrast, the pasture diet resulted in highest concentrations of rumen ammonia (Table 6.10) and lowest values were measured when maize silage was included in the diet. Cows fed either pasture or pasture plus sulla silage had higher rumen NH_3 concentrations than other supplemented treatments ($P = 0.03$).

The diurnal variation in VFA concentrations (Appendix 8 – Figure 8.1A) showed peak concentrations about 6 hours after morning feeding. The diurnal range in total VFA concentrations was greatest with the PM diet and least with PMS. Dietary effects on the extent of diurnal variation was similar for acetate and n-butyrate but diets containing sulla appeared to have least diurnal variation in concentrations of minor VFA.

Ammonia concentrations followed a similar diurnal pattern as VFA but the variation was much smaller with PMS and PSM, than other diets. This can be explained by grazing behaviour because cows fed PMS and PSM chose to eat supplements first, followed by pasture while cows given PS or PM grazed pasture first and ate supplements after grazing.

TABLE 6.10 – Rumen pH and concentrations (mMol/L) of metabolites for individual cows grazing pasture with and without maize and/or sulla silage supplements.

			Volatile fatty acids					
Diet	Cow	pH	Acetate	Propionate	n-buty	Minor	Total	NH_3
Pasture	5272	6.1	69.4	17.5	11.7	4.0	102.6	12.0
Pasture	7915	6.2	71.9	17.8	11.7	4.3	105.6	10.8
PMS	3343	6.2	66.6	16.2	9.8	3.2	95.7	4.8
PMS	3792	6.1	64.7	15.6	9.4	3.4	93.2	6.2
PSM	5774	6.2	72.4	17.8	10.6	4.1	105.0	8.1
PSM	7920	6.2	70.8	16.1	10.3	3.2	100.5	5.9
PS	3788	6.3	66.5	15.8	9.8	3.7	95.7	9.2
PS	7926	6.2	66.5	18.7	11.1	4.2	100.5	8.6
PM	5756	5.9	76.4	17.3	11.7	4.2	109.7	7.0
PM	7912	6.1	71.6	16.0	10.3	3.2	101.1	5.1
	Mean	6.1	69.7	16.9	10.6	3.8	100.9	7.8
Diet P		0.4079	0.5551	0.9373	0.6278	0.9303	0.6063	0.0156
Week P		0.0008	0.0062	0.1297	0.7804	0.8746	0.0171	0.0351
Diet*week P		0.8832	0.9707	0.8701	0.7218	0.0482	0.9935	0.4806
NH_3 P		0.7739	0.0766	0.0163	0.0158	0.0003	0.0368	

Abbreviations are given in Tables 6.2 and text.

n-buty, n-butyrate; Total, total VFA.

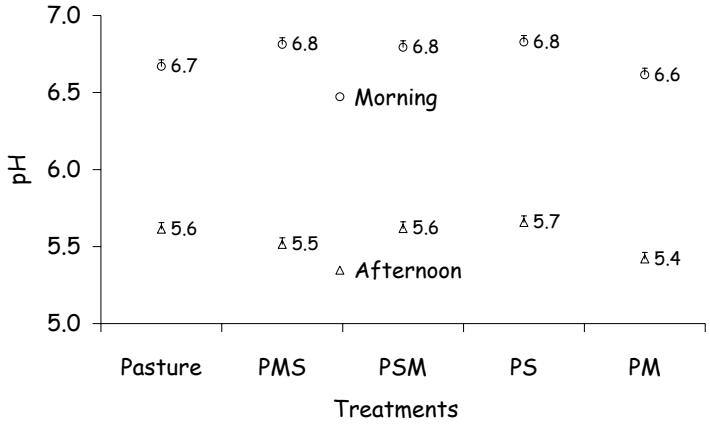


FIGURE 6.4 – Average morning (AM) and afternoon (PM) rumen fluid pH from fistulated cows fed full pasture and four silages supplements. Abbreviations are given in Table 6.2.

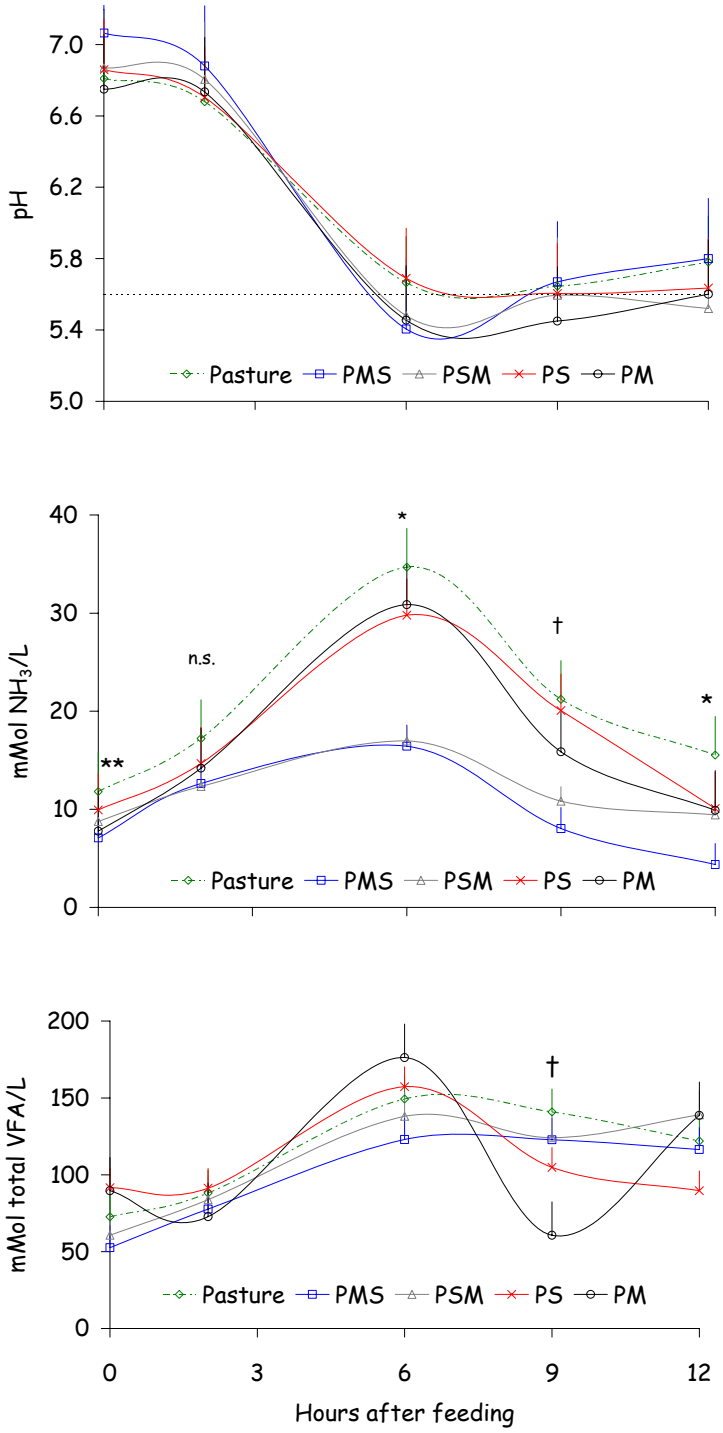


FIGURE 6.5 – Pattern of pH and rumen fluid ammonia and total VFA concentration between 07:00 h and 19:00 h, averaged from fistulated cows fed full pasture and four silages supplements on the first day of the *in sacco* incubation. †P < 0.10, *P < 0.05, and **P < 0.01. Abbreviations are given in Table 6.2.

6.5 - Discussion

The impact of silage supplementation has been examined in Chapters 5 and 6 in terms of production response and extent of pasture substitution by silage. Interpretation has been based on DM and nutrient intake and *in sacco* digestion kinetics. Both trials were constrained by the high quality pasture on offer, which exceeded the quality of silage supplements in some instances.

The extent of production response will depend upon the amount of pasture available, which was probably insufficient in the second study, and the nutritional value of the diet. Substitution of pasture by supplements can be an important consideration when optimising production against feeding costs and it is important to ask whether both the extent of substitution and the response to supplementation for several silages and mixtures can be addressed in a single trial. An initial requirement will be an accurate measurement of cow intakes.

Dry matter intake prediction

Estimation of cow intakes is difficult, whether on a group (Berchielli *et al.*, 2000) or on an individual basis using indigestible markers and faecal sampling. A brief evaluation of intakes measured in both experiments (Chapter 5 and 6) has been made by comparison with predictions of DMI using the AAC equations for dairy cows (AAC, 1990). Inputs for the model are cow breed, days of pregnancy, liveweight (LW) and LW change, age, condition score, milk production and composition, energy content and digestibility of the feeds, and initial estimate of DMI. DMI predictions were highly correlated with actual values for DMI ($P < 0.0001$; $r^2 = 0.61$) and showed that DMI was underestimated by only 0.12 kg across all diet treatments (Figure 6.6). Cows fed restricted pasture as a sole diet in these short term trials tended to maintain milk production but lost liveweight, so removal of these data from the analysis resulted in a more precise model prediction of DMI ($r^2 = 0.90$) for cows either grazing a high pasture allowance or given restricted pasture plus supplemental silages.

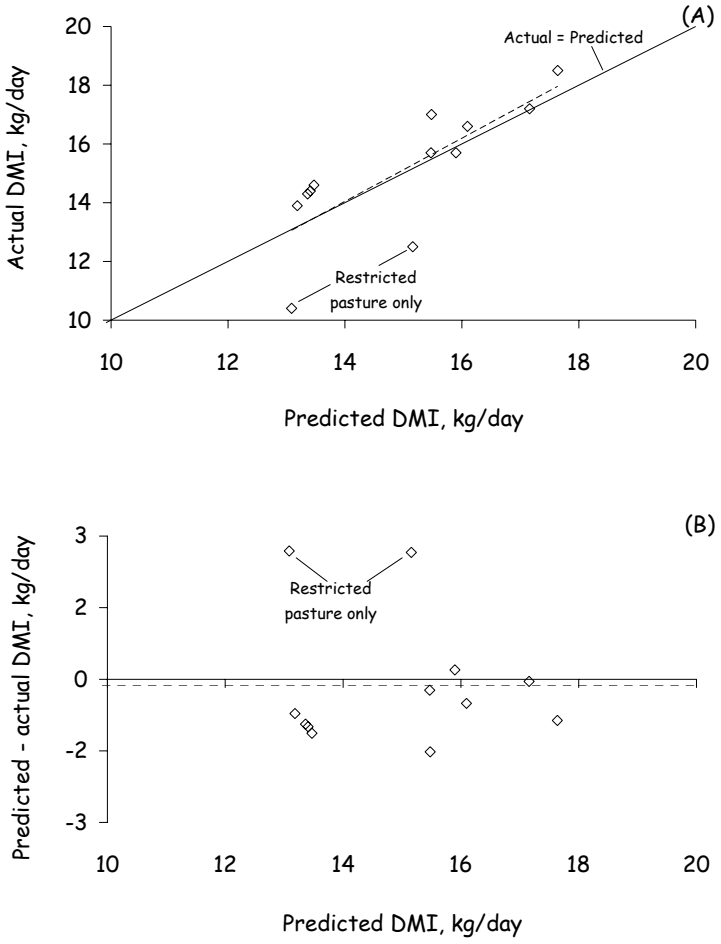


FIGURE 6.6 – Plot of actual and predicted values for dry matter intake (DMI) using the AAC (1990) model (A) and the mean bias calculated from the model prediction minus actual DMI (B). The relationship between actual and predicted values (without the restricted pasture treatment) is given by: Actual DMI = 2.72 + 0.86 (± 0.10) x Predicted DMI (r^2 = 0.90). Data are kg/day.

The over prediction of intakes for cows given restricted pasture as a sole diet (Figure 6.6) suggest an inability of the model to account for minimal residual DM when pasture allowance is low. When pasture on offer was only 18 kg DM/cow.day it would be impossible for cows to eat 15 kg pasture DM/day. Model prediction when pasture was offered as a moderate - high allowance or when grazing cows were offered silage supplements, are close to DMI measured in both experiments. This is reassuring and suggests the pre and post-grazing pasture cuts enabled an accurate estimate of group intakes. The allocation of very low quantities of pasture does not enable good model prediction and excessive feed restriction is inappropriate for maintenance of milk production.

Pasture allowance and substitution

Similar cow responses to all silage supplements in this experiment suggests ME was more limiting than other nutrients and differences in silage composition were small to effect milk production. The high quality pasture on offer probably contributed to the lack of response to specific silages.

The impact of pasture allowance has been summarised by Stockdale (2000b) using data from 17 Australian and 3 Irish studies. These data, with those from experiments conducted here have been plotted (Figures 6.7 and 6.8) to show the relationship between pasture allowance and both intake and substitution of pasture by supplement. Figure 6.7 shows a typical curvilinear relationship between pasture allowance and pasture DM intake without supplements but intakes of supplements with pasture were higher than of pasture alone, especially at very low allowances (Peyraud *et al.*, 1998). When 20 – 30 kg pasture DM was allowed per cow, with supplements, the data did not demonstrate any relationship between pasture intake and allowance, so intakes would have been determined by quality and quantity of feedstuffs and the cow's metabolic requirements.

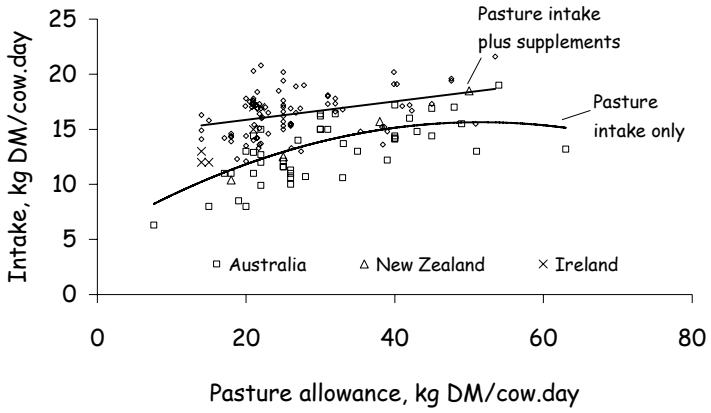


FIGURE 6.7 – Relationship between dry matter intake and pasture allowance (PA) of cows offered only pasture (PDMI; PDMI = 4.0 + 0.47 x PA - 0.0046 x PA^2; r^2 = 0.45) and total intake (TDMI) of cows offered pasture with supplements (symbol: ◊; TDMI = 14.2 + 0.08 x PA; r^2 = 0.13; P < 0.001). Data from Stockdale (2000b) and Chapters 5 and 6.

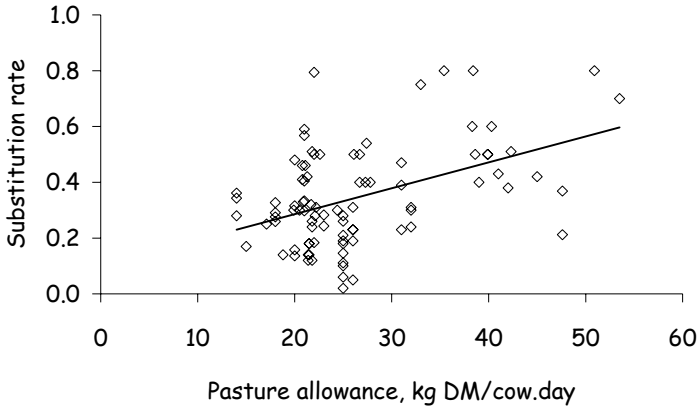


FIGURE 6.8 – Relationship between substitution rate (SR) and pasture allowance (PA) by cows grazing pastures in New Zealand, Ireland and Australia (SR = 0.1 + 0.009 (± 0.002) x PA; r^2 = 0.20, Root MSE = 0.16, CV = 46.4%, SR mean = 0.35, P < 0.0001).

Intake of supplements will also vary with quality and quantity of both the pasture and the supplements and the metabolic drive of cows to eat. Although substitution rate (SR) increased with pasture allowance (Figure 6.8) the coefficient of variation was large (46.4%). When pasture allowance was above 35 kg DM/cow.day, SR increased significantly. These relationships cannot define likely degrees of substitution for specific supplements and use of both low (20 – 30 kg DM/cow.day) and high (over 35 kg DM/cow.day) allowances may be required to define SR for contrasting supplements.

Absolute levels of substitution vary with stage of lactation, quality and quantity of both pasture and supplements. There was a lower substitution in the first experiment (0.06 – 0.15) when pasture was restricted to 25 kg DM/cow, compared to 0.26 – 0.33 when only 18 kg pasture DM/cow was available, but 5 and 6 kg silage DM were available in the respective trials. Effects of diet and supplement have been shown by Stockdale and Dellow (1995) where lactating cows fed white clover pasture with 3.5 – 5.0 kg maize silage had an average SR of 0.49. More recently, Walker *et al.* (2001) reported substitution of paspalum pasture for grain increased from 0.02 – 0.28 as concentrate increased from 3 – 10.4 kg DM/day (experiment 1) and when un-supplemented pasture intake was 15 kg DM/cow and 3 – 5.9 kg of concentrates were fed, the SR ranged from 0.23 – 047 kg/kg (experiment 3). They concluded that high rates of substitution were responsible for diminishing financial returns to farmers.

The acceptability of both supplements and pasture may provide an insight into the nutritional balance required by the cows, and suitability of silages for supplementing

pasture. In this study the cows ate the silage mixtures (PMS and PSM) in preference to pasture and this resulted in lower 12 hour fluctuations in rumen ammonia and VFA concentrations (Figure 6.5). In the previous experiment (Chapter 5) the cows actually ran to access the lotus silage. Lotus silage, maize and sulla silages mixtures were eaten before pasture whereas pasture was eaten before either maize or pasture or sulla silages in both experiments. Acceptability is likely to affect both DMI and the extent of substitution. Other factors include quality and quantity of pasture and supplements, cow demand for nutrients and consequences of grazing close to the ground or near faeces and urine patches when pasture availability is restricted.

Rumen degradation *in sacco*

When maize silage was added to pasture the extent and rate of DM degradation declined, whereas the reverse was true for sulla. With highly productive cows the rate and extent of DM degradation will affect performance and sulla silage may be more appropriate than maize silage, especially as CP degradation rate was reduced when sulla silage was incubated with pasture. Slower CP degradation is consistent with the presence of CT (Broderick and Albrecht, 1997; Burke *et al.*, 2002a; Messman *et al.*, 1996)

Pasture incubation in cows given five diets allowed cow/diet effects to be measured, and different diets affected degradability of pasture DM and fibre (NDF and ADF; Figure 6.9). There appeared to be less DM and fibre degradation when pasture was incubated in cows fed diets including maize silage but addition of sulla to cow diets increased effective degradability. Effects of diet on CP degradation were less apparent (Table 6.8). Impact of diet on degradation rates has been observed by Mertens and Waghorn (unpublished) where diets based on maize silage reduced rates of both maize and lucerne degradation relative to diets based on lucerne. Results here support this observation and show the effects of diet on effective rumen degradation were greater with higher intakes and/or high outflow rates (Tables 6.6 and 6.8). The effect of diet on *in sacco* degradation requires more investigation.

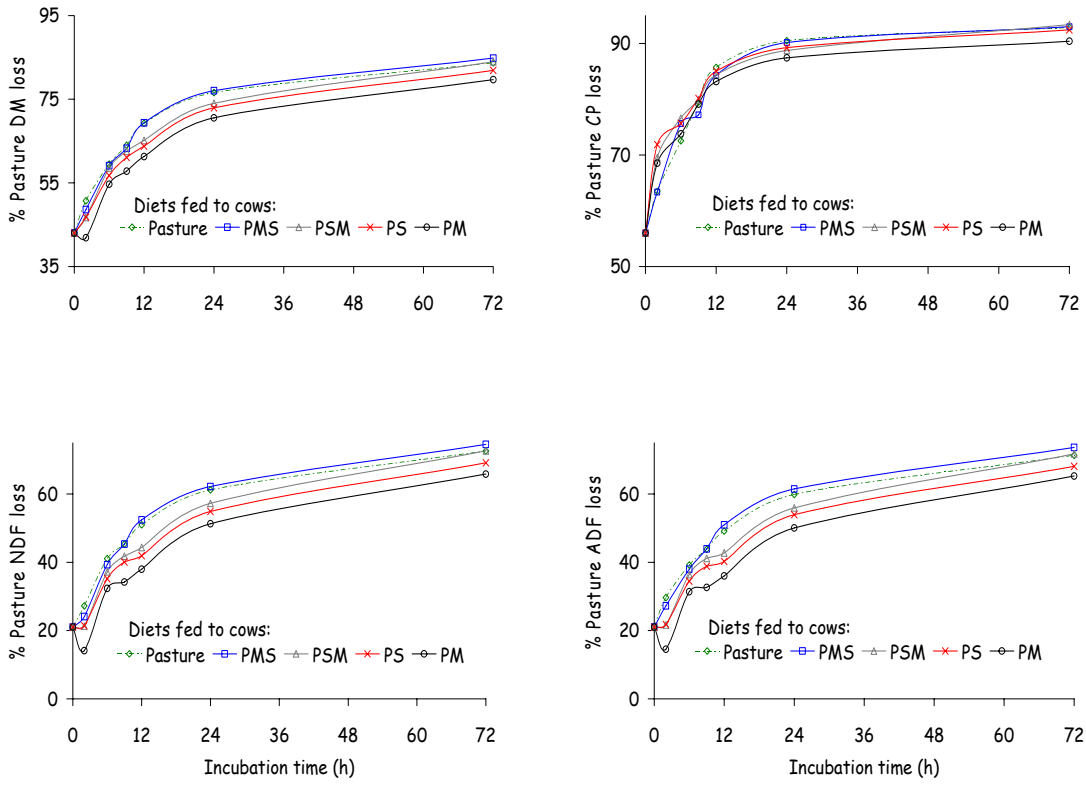


FIGURE 6.9 – Pasture dry matter (DM), crude protein (CP) and fibre (NDF and ADF) disappearance during *in sacco* incubations from fistulated cows fed full pasture and four silages supplements.

Why did this study use different ways to assess dietary protein? Metabolisable protein is an important component of diet quality and is the sum of digestible microbial true protein and digestible undegraded feed protein (AFRC, 1992). Digestible microbial true protein is calculated from the microbial CP supply which is, in turn, calculated from the total fermentable energy in the diet, providing there is sufficient effective rumen degradable protein (ERDP) for unrestricted microbial growth. The degradation coefficients (A, B and k) of feed proteins incubated in dacron bags in the rumen are required for the determination of ERDP (section 3.3.5.1).

Clark *et al.* (1997b) suggest there is limited understanding of the nutritional constraints to dairy production from summer pastures, but effects of grass maturation and pasture supply can limit MP for milk production. There is little information on rumen degradability of forage CP (Chapters 3 and 4; Barrell *et al.*, 2000 and Burke *et al.*, 2000) so several approaches (AFRC, 1992; NRC, 2001) were used to evaluate this problem. Proportion of RDP and RUP differed across diet treatments (Table 6.7 and 6.8) and

estimates of effective rumen degradability of crude protein (ERDP, g/kg DM) showed strong positive relationship with dietary CP content.

CP degradation characteristics reported here are in agreement with an Australian study of perennial pastures through the year (Wales *et al.*, 1999a). They showed that summer pasture in Victoria, Australia, provided a surplus of MP of 0.14 to 0.23 kg/cow.day compared to 0.48 to 1.21 kg/cow.day in spring and suggested MP was unlikely to limit milk production of cows eating 17 kg pasture DM/day and producing up to 30 kg milk/day. These data support the argument that the good quality pasture available for both trials presented here resulted in insufficient energy rather than protein for cows producing 1.0 – 1.2 kg milksolids/day.

Rumen digesta and cow feeding behaviour

Diets did not affect concentration or molar proportions of VFA (Table 6.10). The 84% increase in dietary NSC concentration when maize silage was added to pasture did not affect either propionate concentration or acetate: propionate ratio. In contrast cows fed pasture as a sole diet had a higher concentration ($P = 0.0156$; Table 6.10) of ammonia compared to other diets.

Although there were no effects of diet on rumen VFA, diets with pasture, maize and sulla silages (PMS and PSM treatments) provided a stable rumen environment possibly due to feeding behaviour. The lower variation over the 12 hours period from 07:00 – 19:00 h for VFA was also evident for NH_3 (Figure 6.5), probably because the cows given these treatments ate supplements first (PMS and PSM) and then grazed pasture. Cows grazed pasture first with PM and PS diets. The PMS treatment was the most acceptable diet for the cows, with least refusals and provided the highest NSC dietary concentration. Visual observations suggest the cows preferred mixtures of sulla and maize silages compared either silage fed alone, but the short duration of the trial and restricted pasture availability prevented further evaluation of silage effects on total DMI or performance.

Future progress to maximise milk production from cows grazing pasture and supplemented with silages should investigate the ability of diets to alter fermentation rate and be nutritionally balanced. Studies should also test both low and high pasture allowances to obtain information on choice of nutrient from pasture versus the supplement. This information will provide a better understanding of substitution than can be obtained with a single pasture allowance and a focus on choice and cow behaviour may provide a better understanding of rumen stability and cow performance.

CNCPS diet evaluation

The CNCPS model was used to evaluate dietary mixtures of maize and sulla silages with pasture to identify limitations to performance and predict rumen degradation parameters.

The model provided useful information concerning microbial growth, rumen passage rates, nutrient limitations and predictions of performance of cattle supplemented with single silages in Chapter 5. Silages mixtures were fed to provide a more balanced nutrient supply to dairy cows in this experiment and ruminal measurements indicated some effects of diet on fermentation. The CNCPS model has been used to further evaluate these diets and to identify sources of variation in cow performance. This information will enable improved dietary formulations for grazing cows given supplements. Table 6.11 presents feed composition collected in this experiment and degradation rates (CNCP feed library, section 6.4.3 and Burke, unpublished) used in diets evaluated with CNCPS.

TABLE 6.11 – Feed composition and degradation rates used into CNCPS evaluation.

General characteristics

Individual feeds	DM (%)	NDF (%DM)	peNDF (%NDF)	Lignin (%NDF)	Fat (%DM)	Ash (%DM)	Starch (%NFC)
Full pasture	23.60	45.30	40.0	7.23	3.90	11.70	48.0
Restricted pasture	15.90	45.30	40.0	9.31	4.10	11.00	48.0
Pasture - PMS	20.47	46.33	40.0	7.50	3.98	9.90	48.0
Pasture - PSM	18.19	45.80	40.0	6.22	4.03	9.91	48.0
Pasture - PS	19.92	46.53	40.0	7.40	3.83	9.87	48.0
Pasture - PM	18.42	45.43	40.0	8.02	3.90	9.55	48.0
Maize silage	33.00	49.00	90.0	10.59	3.00	4.00	80.0
Sulla silage	34.00	50.00	85.0	22.72	5.20	10.10	70.0

Energy and protein values

Individual feeds	CP (%DM)	UIP (%DM)	Sol-P (%CP)	NPN (%Sol-P)	NDFIP (%CP)	ADFIP (%CP)
Full pasture	18.00	22.4	55.0	4.76	9.1	3.04
Restricted pasture	21.90	22.4	55.0	4.76	9.1	3.04
Pasture - PMS	20.98	22.4	61.0	4.76	9.1	3.04
Pasture - PSM	21.70	22.4	65.0	4.76	9.1	3.04
Pasture - PS	20.63	22.4	66.0	4.76	9.1	3.04
Pasture - PM	21.00	22.4	63.0	4.76	9.1	3.04
Maize silage	9.50	20.9	58.0	100.0	16.0	7.0
Sulla silage	15.70	29.1	66.0	28.0	24.0	16.0

Degradation rates

Individual feeds	Degradation rates (%.h^{-1})					
	Carbohydrate			Protein		
	A	B1	B2	B1	B2	B3
Full pasture	85.3	19.2	14.0	200	12.0	2.0
Restricted pasture	85.3	19.2	14.0	200	12.0	2.0
Pasture (PMS, PSM, PS, PM)	85.3	19.2	14.0	200	12.0	2.0
Maize silage	10.0	30.0	5.0	300	15.0	0.25
Sulla silage	10.0	25.0	9.0	150	11.0	1.75

Abbreviations see text and Table 5.5.

TABLE 6.12 – CNCPS predictions of nutrient composition, cow performance and rumen characteristics of treatment diets.

	FP	RP	PMS	PSM	PS	PM
Nutrient composition						
ME, MJ/kg DM	10.41	10.53	10.03	9.99	9.52	10.34
CP, g/100g DM	18.0	21.9	17.2	18.6	18.8	16.6
Soluble CP, %CP	55.0	55.0	61.1	64.7	66.0	61.9
NDF, g/100 g DM	45.3	45.3	47.5	47.2	47.8	46.8
peNDF, g/100 g DM	18	18	28	28	28	28
Total NFC, g/100 g DM	22.7	19.7	25.2	23.2	21.7	27.4
Total fat, g/100 g DM	3.9	4.1	3.9	4.2	4.3	3.6
Performance predictions						
ME allowable milk, kg/day	18.2	8.1	16.7	15.4	13.4	16.7
MP allowable milk, kg/day	20.4	13	14.6	14.1	12.1	14.1
Daily weight change due to reserves, kg/day	1.3	-0.9	-1.9	0.3	0.0	0.5
Rumen digestion, metabolism and passage						
MP from bacteria, g/day	942	585	855	814	745	886
MP from undeg. feed, g/day	652	473	454	470	450	413
MP from undeg. feed, %MP total	41	45	35	37	38	32
Total DIP, %CP	78.2	80.4	79.3	79.9	79.5	80.5
Ruminal N balance, % of req.	123	207	151	168	177	142
Total bacterial nitrogen, g/day	251	156	228	217	199	236
Urea cost, MJ/day	4.8	3.6	2.3	3.3	3.4	1.8
Urea cost, %ME intake	2.9%	3.3%	1.6%	2.3%	2.6%	1.2%
Excess N excreted, g/day	204	167	118	151	154	103
Liquid passage rate, $\%.h^{-1}$	10.6	8.7	10.5	10.3	10.2	10.2
Pasture passage rate, $\%.h^{-1}$	6.40	5.22	6.23	6.14	6.07	6.12
Maize silage passage rate, $\%.h^{-1}$	NA	NA	4.83	4.76	NA	4.73
Sulla silage passage rate, $\%.h^{-1}$	NA	NA	4.90	4.82	4.78	NA
Predicted ruminal pH	6.19	6.19	6.46	6.46	6.46	6.46

NFC, non-fibrous carbohydrates. Other abbreviations see text and previous tables.

ME was the first limiting nutrient for cows fed pasture alone and MP was the first limiting nutrient when cows were fed pasture plus silages.

At least 50% of the total MP was of microbial origin for all diets, with highest percentage for the maize silage treatment (PM: 68% of MP of microbial origin; 886 g/day). The recommendation for ruminal N balance is 100 to 110% of requirements (Fox *et al.*, 2003) and this was easily achieved from all diets (123 to 207% of requirement).

Development of rations that will improve efficiency of nutrient use and reduce nutrient wastage on farm are requirements for economic and environment sustainability. Provision of silage mixtures attempted to balance nutrient supply with demand and the CNCPS simulations predicted nutrient requirements, balances,

excretion of N and urea cost for each treatment group. Higher concentration in CP in pasture resulted in high urea costs (2.9% and 3.3% of ME intake for FP and RP respectively). This represents an increased cow maintenance cost, associated with removal of excess nitrogen, and the high predicted MP from undegraded feed (652 g/day) with the FP diet was a association with the highest N excretion (204 g/day). This will have a negative impact on the environment in terms of nitrogen leaching and generation of nitrous oxides (Carran, 2002) relative to diets containing maize and sulla silages. However, the low CP% of the maize and sulla has caused MP to be the first limiting nutrient and milk production drooped by feeding these silage supplements compared to FP treatment.

Although CNCPS provided useful information concerning nitrogen fluxes with similar predictions of total DIP to effective CP degradability measured *in sacco* (Table 6.6), the predictions of mean rumen pH differed from observed values (Figures 6.4 and 6.5).

6.6 - Conclusion

Dairy farmers face the task of maintaining a desired level of production often when the quality of pasture is less than optimal. As pasture is likely to provide the cheapest source of nutrients, it is important to maximise feed intake at minimal cost. Silage supplements can be used to fill summer feed deficits when pasture quality declines due to maturation of ryegrass. To achieve positive responses from supplements, supplements should be of higher quality (nutritive value) than pasture and the supplements must be chosen to complement the pasture on offer. The differences between digestion kinetics of maize and sulla silage supplements demonstrate the importance of selecting an appropriate supplement to complement the pasture on offer.

The hypothesis was not proven, but the low pasture allowance may have prevented a response due to insufficient ME intake. Dietary mixtures did reduce diurnal variation in rumen fermentation parameters, without affecting changes in milk or milk solids production.

Chapter 7

Simulation models for ration balancing and an evaluation of the CNCPS model.[1]

[1] Part of these data was previously published in the *Proceedings of the New Zealand Society of Animal Production*, 2003, 91-95.

7.1 - Abstract

The importance of mechanistic models for ration balancing with forages is indicated and physical limitations to intake emphasised, because these limit nutrient supply to cows grazing forages, especially grass. Ration balancing models using fresh or ensiled forages to complement pasture will need to accommodate intake limitations, due to rumen fill, clearance, chewing or other criteria. The potential of the Cornell Net Carbohydrate and Protein System (CNCPS) model to predict milk production from diets based on pasture and forage supplements was tested using information presented in this thesis. Data were obtained from studies in which pasture was complemented with contrasting silages including maize, pasture, sulla, lotus and forage mixtures (Chapters 5 and 6), comprising 30 - 40% of dry matter intake (DMI). Twelve diets were used in this evaluation. DMI, live weight (LW), days in milk, and diet composition were determined during the trials and used as inputs in the model. Across all diets, a significant ($P < 0.01$) relationship existed between predicted and actual values for DMI ($r^2 = 0.63$), milk yield ($r^2 = 0.64$) and LW change ($r^2 = 0.57$) but there were still large unexplained sources of variation. No significant mean bias was observed for any of the variables, but the slope of residual differences against predicted values was significantly different from zero for milk yield, LW change ($P < 0.01$) and for DMI ($P < 0.06$). The results indicate a satisfactory prediction of milk production when cows are neither gaining nor losing weight, but that a systematic bias exists probably because of the CNCPS model's failure to account for nutrient partitioning.

Keywords: CNCPS; dairy cows; diet formulation; modelling; nutrients requirement.

Short title: Evaluation of the CNCPS for dairy cows.

7.2 - Introduction

Improvements to the nutrition of dairy cows fed pasture will require a balance between the amount of pasture and supplements eaten. The biggest problems for achieving a nutritionally balanced ration are the continuous change in pasture composition and the effects of fibre in pasture on voluntary feed intake. Changing composition will require monitoring (available through NIRS analytical services) and ration balancing will need models with a strong mechanistic component which are suitable for New Zealand pastoral feeding. Ration balancing, to optimise performance, cow welfare and profitability should be able to: 1) indicate optimal mixtures of diet components to achieve intended levels of production and 2) incorporate an intake

regulatory component, because feed type will affect potential intake as well as nutrient supply from feed degradation. Intakes of forages are likely to be regulated by physical constraints (chewing, rumen fill and rumen clearance) rather than metabolic feedback.

Ration models used in the dairy industry serve a number of functions. In the Northern Hemisphere they are used for ration balancing to formulate least cost diets. These models are designed to meet cow nutrient requirements for specific stages of lactation, and take into account pregnancy, age, liveweight, milk production and composition. These systems assume feed is not limiting and intake is regulated by metabolic rather than rumen fill parameters. These models focus on cow requirements and the National Research Council (NRC, 2001) dairy model and the Cornell Net Carbohydrate and Protein System (CNCPS) indicate whether metabolisable protein or energy is the first limiting nutrient for milk production.

The models are based on cow needs for specific nutrients as well as overall demands for protein and energy. The rumen component of the model uses feed composition, degradability and empirical formulae to calculate outputs such as ME, rumen microbial growth, nitrogen and peptide balance and predicted ruminal pH but does not predict VFA yield or methane production. The model is evolving to meet changing needs of the North America dairy industry, for example limiting excessive waste of nitrogen and phosphorus because of environmental concerns.

These mechanistic models have potential benefits for dairy nutrition, compared to empirical systems which tend to be specific to the data set used for model development, because they offer potential for accommodating diverse dairy feeding and production regimes. Modelling digestion parameters, especially supply of specific nutrients and consideration of rumen function enables diverse feed types to be accommodated, at least in theory. However no model can balance feed inputs with production indices when feed intake is limiting, as is often the case under grazing systems. The North America models do have constraints concerning intakes, but these do not apply easily to New Zealand pastures. Intake of cows fed pasture will be constrained by rumen outflow (clearance; Waghorn, 2002) and this has not been included as a regulatory component of the CNCPS or NRC models. The long, high fibre, leafy (some times stemmy) grass-based pastures grazed by New Zealand cows are very different from low fibre (below 35% NDF in the DM) chopped, grain and silage based diets for which North American models were developed.

Pasture based models have usually been empirical in nature, but they do accommodate feed supply, which has a major effect on performance of grazing animals. Examples of these systems include those developed in Australia (Camdairy; Udder), New Zealand (feedTECH), United States (Dafosym) and complex models which attempt to include metabolism and performance (Molly). These all have specific applications for the dairy industry, but many also have weaknesses. Some factors which are crucial for successful modelling to understand fresh forage utilisation include:

- Ready availability for evaluation, for example downloading from the Internet for a trial period (normally 30 days) or even available with purchase of feeding information (NRC, 2001). Models will only be useful if they easily accessed for evaluation.

- Model should be interactive and user-friendly. There is no point in developing a computer program (model) that is hard to use and does not interact with the user. The success of Microsoft Windows® is due to its interactive nature; people are discouraged by systems requiring specialist skills (e.g.: DOS system); difficult models are less likely to be tested and utilised than Windows based systems.

- The model should be based on scientific evidence. Although most dairy models are empirical, based on a summary of equations from different studies, mechanistic models need to be tested and information provided to explain the basis of their function prior to release.

- A mechanistic nutrition model needs to incorporate: feed composition and degradability parameters; animal characteristics (type, age, breed, liveweight, stage of lactation and pregnancy, milk production and composition) and environment factors. These should be interactive because each factor impacts on the others.

- The inputs required by the model should be readily available and be unambiguous. Inputs should not have to be derived from a unique data basis or involve expensive measurements. If inputs are too complicated, models frequently use default values which may not be appropriate for the feeding systems being tested, and the predictive capability will be compromised.

The NRC and CNCPS models offer good potential for predicting responses (e.g. milk yield) to feeding regimes (e.g.: use of silages supplements), and there is an increasing demand for this type of information in the New Zealand dairy industry. The industry needs systems for calculating production responses to supplementation, for

example enabling lactation to be extended into autumn or substitution of pasture in early lactation to achieve high energy intakes and persistent milk production. It is essential that appropriate supplements be given to complement ryegrass pastures and that responses to supplementation be predictable.

The research summarised in this thesis has provided information concerning the composition and degradation kinetics of pasture, especially in relation to maturation and this will ultimately provide a basis for model predictions. The rumen component in the CNCPS model makes this a logical choice for using the *in sacco* and *in vitro* data from these and other trials (Burke *et al.*, 2000). *In sacco* digestion kinetics comprise two principal fractions: 1) a soluble rapid degradable fraction released by eating and chewing (A) typically comprising about 40% of the DM, and 2) a slowly degradable insoluble fraction (B) which disappears in response to microbial activity (k). The proportion of insoluble degradable (B) fraction has a significant impact on predictions for performance. This is logical, but this fraction and its degradation rate will be influenced by particle size reduction by chewing to affect microbial colonisation and also rumen clearance.

These parameters apply to all components of the diet and suggest application to the CNCPS model should be straight forward, but the model requires inputs which are not readily obtainable for fresh forages. Values for these fractions are more available for grains and silages than fresh forages.

The model is valuable in that it can contribute to our understanding of nutrition, ration evaluation, research planning and understanding ruminal kinetics, rather than simply predicting cattle requirements (Fox *et al.*, 1992; Kolver *et al.*, 1996; Russell *et al.*, 1992; Sniffen *et al.*, 1992), but users must be aware of model limitations.

The CNCPS model had been validated against results from grazing trials (Chapters 5 and 6) to indicate strength and weakness in the current model format. There are numerous statistical tools available for evaluating the accuracy and precision of models that predict animal performance. These include plots of predicted and observed values (Kolver *et al.*, 1996), mean squares prediction error analysis (Bateman *et al.*, 2001; Kohn *et al.* 1998; Smoler *et al.*, 1998), and analysis of residuals (predictions from the models minus actual data) against predictions (Kohn *et al.*, 1998; St-Pierre, 2003).

The aim of this study was to determine the utility and accuracy of the CNCPS model to predict milk production based on pasture and silage supplements, using data obtained from the two dairy cow trials conducted in mid-lactation (Chapter 5 and 6)

when pasture was complemented with contrasting silages. Further evaluations were made by predicting performance of cows fed low, medium and high quality pasture with or without silage supplements to identify first limiting nutrients and indicate rumen digestion parameters. These evaluations utilized composition and kinetic data obtained in this thesis and from Burke (unpublished).

The hypothesis was that the Cornell model (CNCPS) would predict cow performance and indicate nutritional limitations from intake and digestion parameters when cows grazing pasture were supplemented with contrasting silages.

7.3 - Material and methods

7.3.1 - Cow trials used for model evaluation

The data against which the model predictions were tested were derived from twelve rations (treatments means) in two trials carried out in Hamilton (Chapters 5 and 6). Each trial comprised 60 Friesian cows (10/treatment) averaging 528 ± 17 kg live weight (LW); 17 ± 2.4 kg milk/day; 156 ± 15 days in milk. Cows grazed ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) pasture complemented by contrasting silage supplements contributing 30 - 40% of DMI. Each trial was four weeks in duration and silage supplements included maize (M; *Zea mays*), sulla (S; *Hedysarum coronarium*), pasture, lotus (LC; *Lotus corniculatus*) and mixtures of M and S.

Pasture intakes by each treatment group were estimated by using a rising-plate meter to estimate pre- and post-grazing herbage mass. This was done three times per week for each treatment group. Weekly pasture cuts (pre- and post-grazing of representative pasture) were made to ground level for calibrating the rising-plate meter and determining chemical composition (e.g.: protein, fibre, ME, minerals) of material on offer by NIRS analyses (Corson *et al.*, 1999).

Digestion kinetic data (rates and extent of digestion) were obtained from *in sacco* incubations (Burke *et al.*, 2000; Chapter 6). Both kinetic data and chemical composition of feeds were entered into the feed library of the model. Neutral and acid detergent fibre insoluble nitrogen required by the model to estimate the amount of slowly degraded and unavailable protein in each feed, ruminal rates of soluble carbohydrate and protein fermentation, and amino acid composition (Tedeschi *et al.*, 2000) for pasture and silages for all treatments were obtained from the CNCPS library files for similar feeds.

7.3.2 - CNCPS evaluation

In this study, principal outputs assessed from the model were: DMI, milk production predicted from the first limiting of either metabolisable energy (ME) or metabolisable protein (MP), LW change and dietary ME concentration.

Animal characteristics (animal type, age, breed type, days pregnant and since calving, number of lactation), milk production, LW, management practices, environmental aspects and feed composition (e.g.: protein, fibre, lignin, starch, mineral concentrations) from the twelve rations were used as inputs in the CNCPS model. The data were used to examine the model predictions for trial means (four weeks each trial) over all treatments.

7.3.3 - Model evaluation and statistical analysis

The model used in this evaluation was CNCPS Version 5.00.20 (updated August 2002). Model evaluation should include a rigorous statistical component and in this study three different methods have been used to evaluate the CNCPS predictions.

Method 1: Linear regression. Most often, predictions are evaluated by regressing actual values versus predicted responses.

Method 2: Measures of deviation. Alternatively, Kohn *et al*. (1998) showed that a measure of how well model predictions fit observed data can be calculated as the root mean square prediction error (RMSPE):

$$RMSPE = \sqrt{[\sum(\text{predicted} - \text{actual})^2/\text{number of observations}]}$$

This term is the square root of the estimate of variance of actual values about the predicted values. The RMSPE is comprised of two terms that identify systematic problems with models: the mean bias and the residual error. The mean bias represents the average inaccuracy of model predictions across all data and the residual error is the remaining error in model prediction after accounting for the mean bias. The residual error is also referred to as prediction error excluding mean bias.

$$\text{Mean bias} = \sum (\text{predicted} - \text{actual})/\text{number of observations}$$

$$\text{Residual error} = \sum[RMSPE^2 - (\text{mean bias})^2]$$

As a summary measure of the relative degree of deviation, either mean bias or RMSPE can be used (Mayer and Butler, 1993).

Regressions of the residuals (predicted values minus actual values) against the predicted values were used to identify whether or not the magnitude of the bias increases or decreases with the magnitude of the predicted values (Draper and Smith, 1981).

Method 3: Systematic bias. This method of evaluating model prediction is based on milk yield predicted from the first limiting of either ME or MP available. The difference between milk predicted from allowable ME or MP and actual milk (residual) was regressed against dietary variables affecting milk production. These included dry matter intake (DMI), LW change and dietary composition (crude protein (CP); fibre (NDF); ME; and fermentable carbohydrates).

7.4 - Results

Mean predictions (Pr) of cow performance, based on model simulations from dietary composition, DMI, and LW, compared with actual (A) values are shown in Tables 7.1 and 7.2. The model under-predicted mean DMI (13.69 vs. actual 15.07 kg DMI/cow.day) and mean dietary ME (9.98 vs. actual 10.5 MJ ME/kg DM) and over-predicted milk production based on ME or MP content of the diet (15.08 vs. actual 14.85 kg milk). ME was first limiting for cows fed restricted and unrestricted pasture allowance and pasture with lotus silage, whereas MP limited milk production by cows given pasture with maize silage. Predicted milk yields by the CNCPS model from diets with sulla or pasture silages was limited to a similar extent by ME and MP.

Method 1 – Linear regression of actual against predicted values

Predicted values were significantly ($P < 0.01$) correlated with actual values for DMI ($r^2 = 0.63$;), milk production ($r^2 = 0.64$;), and LW change ($r^2 = 0.57$; Figure 7.1), but predicted ME concentrations were not correlated with values measured by NIRS ($r^2 = 0.03$). However, there were still large unexplained sources of variation (residual variance or mean-square error (MSE)), and the slopes of the regression lines were significantly different than the theoretical value of 1.0 (Table 7.1). Information provided by simple regression analysis can be ambiguous and lack sensitivity (Mitchell, 1997; St-Pierre, 2001), and, thus, was not able to provide a proper interpretation of these relationships.

Method 2 – Deviation of predicted from actual values

When model predictions were tested using measures of deviation, mean bias was not statistically significant from zero for DMI, milk production, LW change or dietary

ME. The residual error terms represent the error in prediction after accounting for the mean bias (Table 7.1; Figure 7.2).

The slope of the regression line was significantly ($P < 0.01$) greater than zero for milk production, LW change and ME concentration, and this difference was close to significance ($P < 0.06$) for DMI. This indicates a systematic bias, in which the residual differences increase at higher predicted values. For instance, the model under-predicted milk production of cows fed restricted pasture (PR) and overestimated performance when fed unrestricted pasture (FP) and pasture silage. Predictions of milk production for high pasture allowance and maize silage supplements were inconsistent. The relationships are shown graphically in Figure 7.2.

Method 3 - Systematic bias

Examination of systematic bias provides an insight into factors responsible for the deviation between predicted and actual values. The regressions of the residuals of milk production (limited by energy or absorbed protein) against actual DMI and LW change are plotted in Figure 7.3. A significant slope (different from 0) for the regression indicates a systematic bias in the model prediction, and the r^2 represents the fraction of the error (excluding mean bias) that can be explained by the slope bias (Draper and Smith, 1981).

Significant biases for CNCPS predicted milk production were observed for DMI and LW change (Figure 7.3; $r^2 = 0.85$ and 0.67, respectively; $P < 0.01$). The differences between predicted and actual milk production increased by 1.19 kg milk/kg DMI and 11.91 kg milk/kg LW change (slope bias; Figure 7.3).

7.5 - Discussion

The CNCPS model is designed to predict nutrient supply, in terms of ME and MP, from rumen parameters, and recent data on digestion kinetics of fresh and conserved forages have been used as model inputs in this study. Because nutrient supply is difficult to measure in grazing animals, validation relies on a comparison of animal performance predicted from these estimates against that observed in practice.

The lack of a significant mean bias for any of the parameters examined would suggest very good model prediction. However, the analyses carried out showed that accurate prediction of mean values does not necessarily demonstrate good predictability for individual diets (residual error is large; Table 7.1), and may limit the

utility of the CNCPS model for fresh forages. These concerns are illustrated by predictions of milk production.

The mean actual and predicted milk yields were similar (14.85 and 15.08 kg/day) and the regression explained 64% of the variance across the diets. However, a regression equation with a slope of + 0.31 (theoretical value = 1.0) and an intercept of 10.14, (theoretical value = 0.0) has little biological meaning. When residuals were regressed against predicted milk production (Method 2), there was no significant mean bias, but residual differences increased for values above and below mean predicted milk production.

Further analysis (Method 3) demonstrated a systematic bias in predicted milk production with changes in DMI and LW change. Good predictions were obtained for a small number of diets, whilst a substantial under-prediction was evident for cows fed restricted quantities of pasture, and a substantial over-prediction occurred with high DMI. This approach assumes that actual DMI and LW change are measured without errors and attention should be taken to avoid flawed conclusions because it is difficult to obtain accurate measurements of both parameters, especially with outdoor grazing.

The inability of the model to predict milk production either side of the mean is a cause for concern (Table 7.1). The CNCPS model uses inputted milk production as a driving variable to calculate the ME, MP and other nutrients required to achieve that level of production. In this evaluation, the inputted milk production values were those observed for cows fed the experimental diets (Chapters 5 and 6). The nutrients available to meet the requirements for these milk yields are estimated from DMI and predicted dietary ME or MP concentrations, less the amounts required for maintenance and pregnancy. The predicted milk production is determined by the first limiting nutrient (ME or MP). If ME supply is insufficient to meet the inputted milk yield, then the extent of liveweight loss required to fill the ME deficit is calculated. However, the extent of liveweight loss that will actually occur is not predicted.

When the supply of available nutrients is insufficient to meet the specified milk yield inputted (for example by feeding a restricted pasture allowance), the model does not allow extra nutrients to be partitioned between body reserves and milk production. This inability of CNCPS to account for partitioning, accounts for the systematic bias in performance identified by the analysis. Model predictions of milk production are much closer when cows do not gain or lose LW (Figure 7.3B).

St-Pierre and Thraen (1999) highlighted that the CNCPS is a requirement system, not a response system. CNCPS will calculate the nutrients required to support a given

level of milk production and composition (Figure 7.4A). Milk production is an input and is used to estimate DMI but constraints of digesta clearance from the rumen and the ability of cows to convert body reserves into milk may account for poor DMI prediction. In addition, the production responses (e.g.: milk production, live weight change; Figure 7.4B) is a function of feeding value (nutritive value of feeds x intake), animal response (genetic merit), environment and management factors and interactions between those. Nutrient requirement systems (e.g.: CNCPS) are unable to predict responses because they cannot account for partitioning of nutrients between the various productive processes (e.g.: milk production; LW change).

The CNCPS model showed poor cow performance prediction when cows are gaining or losing weight, however when the energy balance is close to zero the model presented satisfactory prediction. The model is useful to evaluate diet composition (DM, ME, amino acids, calcium, phosphorus, potassium) in relation to requirements and it identifies the first limiting nutrient for milk production (ME, MP, methionine or lysine). An improved understanding of how effective (eNDF) and physical effective fibre (peNDF) varies with pasture quality and how it is correlated with pH in the rumen is necessary (Kolver and de Veth, 2002). Prediction of rumen parameters (e.g.: peptide balance) are not easily validated because of the difficulty in measuring actual production *in vivo*. Dry matter intake based on liveweight may not apply to a forage-based system. For example, intakes of low quality hay would be different to intakes of high quality ryegrass/white clover pasture for cows with the same liveweight.

CNCPS scenarios evaluation

Additional tests of model outputs are based on low, medium and high quality ryegrass pasture fed with and without silages. These pastures will differ in fibre degradation rates as well as fibre and crude protein content. Simulations will predict dairy cows response to pasture composition, typical of changes from spring to summer. Table 7.3 presents feed composition and degradation rates used as inputs to CNCPS.

Low, medium and high quality ryegrass pasture was fed alone at restricted (12 kg DM intake cow/day) and un-restricted allowances (18 kg DM intake cow/day). Restricted allowances of the three pasture qualities were also fed with 6 kg DM of pasture, maize, lotus and sulla silages as well as mixtures of maize and sulla silages (PMS, PSM).

Scenarios with pasture as a sole diet

Table 7.4 presents CNCPS simulations for cows fed low, medium and high quality pasture and at restricted and un-restricted allowances. ME was the first limiting nutrient for cows grazing ryegrass based-pasture, at both allowances irrespective of quality (Table 7.4).

With incremental increases in CP content (low to high quality pasture) the model showed a significant decrease of the percentage of metabolisable protein (MP) coming from bacterial flow and an increased proportion of MP from undegraded feed. Higher concentrations of CP in the pasture decreased total bacterial N production and resulted in large increases in cost of urea excretion, reaching 12 MJ/day (Tables 2.13; 7.4). Because ME was limiting, cow milk production only increased by a small amount in response to pasture protein concentration, so the cows fed high quality pasture had very high N losses to urea. The milk production response with increasing pasture quality was associated with a lower dietary NDF, but this caused a large increase in environmental pollution with urinary-N.

The total degradable intake protein (DIP, % of CP) increased with increased pasture quality in association with decreased forage passage rates. DIP estimation is similar to the CP effective degradability calculated in previous chapter (Table 6.6). Predicted pH values agree well with observed values in other pasture studies (Kolver and Muller, 1998; Kolver and de Veth, 2002) but were higher than values reported in Chapter 6 (Table 6.10).

Scenario of low pasture quality at restricted allowance and single silage supplementation

ME was the first limiting nutrient for cows fed pasture with lotus or sulla silages but MP was the first limiting nutrient with the pasture silage and maize silage treatments (Table 7.5). Pasture and maize silages had a low CP content relative to other silages and the maize silage diet provided only 91% of rumen N requirements (Table 7.5).

These simulations show no benefits in supplementing low quality pasture with any of the silages used in simulations. Provided the cow could eat 18 kg pasture (48% NDF) per day, predicted performance from pasture alone (Table 7.4) exceeded predictions with restricted pasture and silages (Table 7.5).

Scenario of medium pasture quality at restricted allowance and single silage supplementation

In this scenario, MP was the first limiting nutrient for cows fed medium quality pasture (MQP) with pasture silage, but ME was the first limiting nutrient for milk production with other silages (Table 7.6).

Again, maize silage resulted in the lowest CP and soluble-CP in the diet but highest NFC, which contributed to a higher daily total bacterial N (312 g) compared to other diets (275 – 293 g). The cows fed MQP supplemented with maize silage would excrete less urea than others treatments (150 versus 226 – 322 g/day) and could potentially produce most milk (Table 7.6).

Silages did not complement restricted pasture for milk production, compared to un-restricted MQP, but if pasture supply were insufficient, CNCPS suggested maize silage to be the optimal supplement (Table 7.6).

Scenario of high pasture quality at restricted allowance and single silage supplementation

When high quality pasture (HQP) was fed with single silages, MP was again the first limiting nutrient for all treatments except lotus silages (Table 7.7). HQP plus maize silage again had highest NFC and lowest soluble-CP contents compared to other silage treatments (Table 7.7). It is likely that this maintained good bacterial growth (278 g/day), compared to-un-restricted HQP with 263 g total bacterial N per day (Table 7.4). When HQP was fed with lotus silage, predicted cost for urea synthesis were 5.6% ME intake. This is similar to values for HQP fed alone (5.7% of ME intake) and probably overestimates true costs of urea disposal because the CT in lotus will divert feed N to faeces rather than urine (Waghorn et al., 1994).

None of the silages fed with HQP would result in better cow performance than un-restricted HQP fed alone (Table 7.4) according to CNCPS predictions.

Scenario of low, medium and high quality pasture (LQP, MQP and HQP) with mixture of maize and sulla silages.

In this scenario, MP was the first limiting nutrient for cows fed high quality pasture (HQP) with silage mixtures, but ME was the first limiting nutrient for milk production with LQP and MQP with silage mixtures (Table 7.8).

Again, diets with higher CP concentrations (HQP with PMS or PSM) resulted in higher cost for urea disposal and higher urinary N excretion than other treatments. All diets resulted in similar predicted milk production, passage rates from rumen and pH.

Future studies should include long-term trials with continuous assessment of actual and predicted DM intakes, milk production and live weight change in association with dietary composition to enable a rigorous evaluation of model predictions. This information may provide credibility for the model predictions and add confidence to predictions of rumen function in cows fed fresh forage diets.

7.6 - Conclusion

The need for mechanistic models to predict responses of lactating cows fed pasture and forage supplements have been indicated, and the CNCPS model was used to evaluate dairy cow performance. This evaluation included feed composition, soluble fraction (A) and degradation rates from *in sacco* data. The results of model predictions were analysed with a more rigorous statistical analysis than simple linear regression of actual versus predicted values and demonstrated systematic biases in the predictions. Milk production was either over- or under-estimated, depending on the level of feeding. This probably results from model inability to account for partitioning of nutrients between milk production and liveweight change.

Evaluation of low, medium and high quality ryegrass pasture fed alone and with supplements demonstrated contrasting outputs, especially for nitrogen. The poor suitability of feeding maize silage with low quality pasture was not demonstrated by model predictions of cow performance despite insufficient N for microbial growth.

The hypothesis was proven in part. The model was able to predict cow performance when intakes were restricted and it did indicate first limiting nutrients for all diets but and milk production predicted from pasture/silage diets appeared unrealistically high, compared to measurements presented in Chapter 5 and 6. The model predicted rumen parameters, especially nitrogen fluxes but the validity may be reduced if model behaviour differed substantially from cow performance.

TABLE 7.1 – Actual (A) and CNCPS predictions (Pr), regressions, correlations, bias and errors for dry matter intake (DMI), milk production, live weight (LW) change (all kg/cow.day) and dietary metabolisable energy (ME) concentration (MJ ME/kg feed DM). Predictions for milk production are based on the first limiting factor: allowable metabolisable energy (ME) or allowable metabolisable protein (MP) for all diets.

				Method 1 (Linear regression)				Method 2 (Measures of deviation)				
		Mean value	SD [a]	A versus Pr	r^2	MSE [b]	P^1	Mean bias [c]	Residual error [d]	RMSPE [e]	r^2	P^2
DMI	A	15.07	2.2									
	Pr	13.69		y = –12.79 + 2.04x	0.63	1.98	< 0.01	-1.40^{ns}	1.6	2.1	0.31	0.06
Milk production	A	14.85	1.6									
	Pr	15.08		y = 10.14 + 0.31x	0.64	0.96	< 0.01	0.23^{ns}	2.75	2.76	0.89	< 0.01
LW change	A	0.01	0.2									
	Pr	0.15		y = – 0.03 + 0.28x	0.57	0.02	< 0.01	0.14^{ns}	0.39	0.42	0.9	< 0.01
Diet ME concentration	A	10.5	0.3									
	Pr	9.98		y = 12.15 – 0.16x	0.03	0.62	ns	-0.54^{ns}	10.52	10.53	0.58	< 0.01

[a] Standard deviation.

[b] Mean square error (estimate of variance).

[c] Mean predicted minus mean actual. t-test $_{(5\%,\ n-2)}$ for mean bias different from zero.

[d] Model prediction error excluding that due to the mean bias.

[e] Root mean square prediction error.

P^1: P value of F-statistic for slope = 1.

P^2: P value of F-statistic for slope ≠ 0.

ns = not significant.

TABLE 7.2 – Actual and CNCPS predictions for dry matter intake (DMI), milk yield (MY) (both kg/cow.day), dietary metabolisable energy (ME) concentration (MJ ME/kg feed DM) and first limiting nutrient (ME or MP) for individual diets (Chapters 5 and 6).

	DMI		MY		ME		Limiting
	Actual	CNCPS	Actual	CNCPS	Actual	CNCPS	ME or MP
Chapter 5 treatments:							
FP	18.5	15.1	17.0	20.5	10.1	9.7	ME
RP	12.5	13.4	13.1	11.0	10.0	10.2	ME
PP	17.0	14.1	15.0	18.5	11.1	10.0	MP
PM	16.6	13.9	15.0	17.5	10.4	10.0	MP
PL	17.2	15.0	17.2	19.2	10.9	9.8	ME
PS	15.7	14.0	15.1	15.1	10.2	9.4	ME
Chapter 6 treatments:							
FP	15.7	14.4	17.2	16.0	10.7	10.4	ME
RP	10.4	12.8	13.2	7.5	10.6	10.5	ME
PMS	14.6	12.9	14.3	14.7	10.5	10.0	MP
PSM	14.4	13.0	13.7	14.2	10.6	10.0	MP
PS	13.9	12.6	13.7	12.0	10.6	9.5	MP
PM	14.3	12.9	13.7	14.2	10.6	10.3	MP
Average	15.1	13.7	14.9	15.0	10.5	10.0	

Abbreviations: Chapter 5 treatments, FP, full pasture (50 kg pasture DM/cow.day); RP, restricted pasture (25 kg pasture DM/cow.day); PP, RP + 5 kg DM of pasture silage; PM, RP + 5 kg DM of maize silage; PLS, RP + 5 kg DM of lotus silage; PS, RP + 5 kg DM of sulla silage. Chapter 6 treatments, FP: full pasture (38 kg pasture DM/cow.day); RP, restricted pasture (18 kg pasture DM/cow.day); PMS, RP + 4 kg maize + 2 kg sulla silages/cow.day; PSM, RP + 4 kg sulla + 2 kg maize silages/cow.day; PS, RP + 6 kg sulla silage/cow.day; PM, RP + 6 kg maize silage/cow.day. MP, metabolisable protein.

TABLE 7.3 – Feed composition and degradation rates used into CNCPS scenarios evaluation.

General characteristics

Feed name	DM (%)	NDF (%DM)	peNDF (%NDF)	Lignin (%NDF)	Fat (%DM)	Ash (%DM)	Starch (%NFC)
Pasture quality:							
Low	20.0	48.0	40.0	5.14	3.0	9.0	45.0
Medium	17.0	46.5	40.0	5.80	4.0	9.4	48.0
High	15.0	40.0	60.0	6.00	6.9	10.7	48.0
Silage:							
Pasture	32.6	46.8	95.0	5.50	2.6	7.2	63.0
Maize	33.7	44.5	85.0	10.59	3.0	4.0	80.0
Lotus	33.1	35.5	80.0	20.30	3.2	10.0	64.0
Sulla	35.4	36.2	92.0	20.00	5.2	10.1	64.0

Energy and protein values

Feed name	CP (%DM)	UIP (%DM)	Sol-P (%CP)	NPN (%Sol-P)	NDFIP (%CP)	ADFIP (%CP)
Pasture quality:						
Low	15.0	29.3	54.0	4.76	24.0	2.2
Medium	20.0	21.9	54.0	3.41	12.5	2.6
High	25.0	0.0	54.0	2.44	4.54	1.65
Silage:						
Pasture	15.6	22.4	55.0	100.0	31.0	10.0
Maize	6.9	20.9	10.0	100.0	16.0	7.0
Lotus	23.4	17.6	51.0	28.0	13.0	9.0
Sulla	21.2	38.4	55.4	28.0	15.0	10.0

Degradation rates

Feed name	Degradation rates (%.h^{-1})					
	Carbohydrate			Protein		
Pasture quality:	A	B1	B2	B1	B2	B3
Low	350	40.0	9.5	200	14.0	2.00
Medium	350	45.0	11.00	200	16.0	2.00
High	350	21.5	12.0	200	18.0	2.00
Silage:						
Pasture	10	25.0	4.7	200	10.4	1.75
Maize	10	30.0	4.1	300	3.4	0.25
Lotus	10	25.0	12.3	150	15.0	1.25
Sulla	10	25.0	6.3	150	7.4	1.25

Abbreviations see text and Table 5.5.

TABLE 7.4 – Pasture diets; CNCPS predictions of nutrient composition, cow performance and rumen parameters.

	Restricted pasture allowance			Un-restricted pasture allowance		
Simulated pasture DMI, kg/cow.day	12	12	12	18	18	18
Pasture quality	Low	Medium	High	Low	Medium	High
Nutrient composition						
ME, MJ/kg DM	10.7	11.0	12.0	10.3	10.7	11.8
CP, g/100g DM	15	20	25	15	20	25
Soluble CP, %CP	54	54	54	54	54	54
NDF, g/100 g DM	48.0	46.5	40.0	48.0	46.5	40.0
peNDF, g/100 g DM	19	19	24	19	19	24
Total NFC, g/100 g DM	28.6	22.6	18.5	28.6	22.6	18.5
Total fat, g/100 g DM	3.0	4.0	6.9	3.0	4.0	6.9
Performance predictions						
ME allowable milk, kg/day	12.3	12.8	14.6	23.6	24.4	27.3
MP allowable milk, kg/day	16.8	16.3	16.6	26.4	26.7	28.9
Daily weight change due to reserves, kg/day	0.0	0.0	0.3	1.8	1.9	2.3
Rumen digestion, metabolism and passage						
MP from bacteria, g/day	902	796	688	1296	1143	985
MP from undegraded feed, g/day	399	468	564	681	842	1060
MP from undeg. feed, %MP supplied	31	37	45	34	42	52
Total degradable intake protein, %CP	74.4	80.3	85.8	72.2	78.1	83.4
Ruminal N balance, % of requirement	103	157	266	104	156	264
Total bacterial nitrogen, g/day	240	212	183	346	305	263
Urea cost, MJ/day	1	3.3	6.9	3.3	6.8	12
Urea cost, %ME intake	0.8%	2.5%	4.8%	1.8%	3.5%	5.7%
Excess N excreted, g/day	42	151	339	123	285	563
Liquid passage rate, $\%.h^{-1}$	9	9	9	11.3	11.3	11.3
Forage passage rate, $\%.h^{-1}$	5.38	5.42	5.11	6.74	6.79	6.40
Predicted ruminal pH	6.24	6.21	6.44	6.24	6.21	6.44

Abbreviations see text and previous tables.

TABLE 7.5 - Low quality pasture (LQP) with single silages; CNCPS predictions of nutrient composition, cow performance and rumen parameters.

	LQP + pasture silage	LQP + maize silage	LQP + lotus silage	LQP + sulla silage
Simulated pasture DMI, kg/cow.day	12	12	12	12
Simulated silage DMI, kg/cow.day	6	6	6	6
Simulated total DMI, kg/cow.day	18	18	18	18
Nutrient composition				
ME, MJ/kg DM	10.13	9.80	10.00	9.99
CP, g/100g DM	15.2	12.3	17.8	17.1
Soluble CP, %CP	54.3	45.8	52.7	54.6
NDF, g/100 g DM	47.6	46.8	43.8	44.1
peNDF, g/100 g DM	28	25	22	24
Total NFC, g/100 g DM	29.9	33.3	29.4	29.2
Total fat, g/100 g DM	2.9	3.0	3.1	3.7
Performance predictions				
ME allowable milk, kg/day	23.3	22.4	22.4	22.6
MP allowable milk, kg/day	22.4	21.9	23.4	22.9
Daily weight change due to reserves, kg/day	1.7	1.5	1.6	1.6
Rumen digestion, metabolism and passage				
MP from bacteria, g/day	1192	1153	1147	1124
MP from undegraded feed, g/day	610	661	713	722
MP from undeg. feed, %MP supplied	34	36	38	39
Total degradable intake protein, %CP	71.3	65.9	73.8	71.8
Ruminal N balance, % of requirements	114	91	134	130
Total bacterial nitrogen, g/day	318	308	306	300
Urea cost, MJ/day	2.4	2.3	4.3	3.6
Urea cost, %ME intake	1.3%	1.3%	2.4%	2.0%
Excess N excreted, g/day	125	44	193	173
Liquid passage rate, $\%.h^{-1}$	11.3	11.3	11.3	11.3
Pasture passage rate, $\%.h^{-1}$	6.74	6.74	6.74	6.74
Silage passage rate, $\%.h^{-1}$	5.25	5.58	6.11	5.82
Predicted ruminal pH	6.46	6.46	6.37	6.44

Abbreviations see text and previous tables.

TABLE 7.6 - Medium quality pasture (MQP) with single silages; CNCPS predictions of nutrient composition, cow performance and rumen parameters.

	MQP + pasture silage	MQP + maize silage	MQP + lotus silage	MQP + sulla silage
Simulated pasture DMI, kg/cow.day	12	12	12	12
Simulated silage DMI, kg/cow.day	6	6	6	6
Simulated total DMI, kg/cow.day	18	18	18	18
Nutrient composition				
ME, MJ/kg DM	10.39	10.32	10.25	10.26
CP, g/100g DM	18.5	15.6	21.1	20.4
Soluble CP, %CP	54.3	47.5	52.9	54.5
NDF, g/100 g DM	46.6	45.8	42.8	43.1
peNDF, g/100 g DM	27	25	22	24
Total NFC, g/100 g DM	25.9	29.3	25.4	25.2
Total fat, g/100 g DM	3.5	3.7	3.7	4.4
Performance predictions				
ME allowable milk, kg/day	23.7	23.9	22.7	22.8
MP allowable milk, kg/day	22.5	24.5	24.1	23.4
Daily weight change due to reserves, kg/day	1.8	1.8	1.6	1.6
Rumen digestion, metabolism and passage				
MP from bacteria, g/day	1099	1171	1054	1030
MP from undegraded feed, g/day	696	739	827	830
MP from undeg. feed, %MP supplied	39	39	44	45
Total degradable intake protein, %CP	75.7	72.2	77.2	75.7
Ruminal N balance, % of requirements	150	117	181	174
Total bacterial nitrogen, g/day	293	312	281	275
Urea cost, MJ/day	5.2	2.9	7.5	6.9
Urea cost, %ME intake	2.8%	1.6%	4.1%	3.7%
Excess N excreted, g/day	226	150	322	292
Liquid passage rate, $\%.h^{-1}$	11.3	11.3	11.3	11.3
Pasture passage rate, $\%.h^{-1}$	6.79	6.79	6.79	6.79
Silage passage rate, $\%.h^{-1}$	5.25	5.58	6.11	5.82
Predicted ruminal pH	6.46	6.46	6.35	6.42

Abbreviations see text and previous tables.

TABLE 7.7 - High quality pasture (HQP) with single silages; CNCPS predictions of nutrient composition, cow performance and rumen parameters.

	HQP + pasture silage	HQP + maize silage	HQP + lotus silage	HQP + sulla silage
Simulated pasture DMI, kg/cow.day	12	12	12	12
Simulated silage DMI, kg/cow.day	6	6	6	6
Simulated total DMI, kg/cow.day	18	18	18	18
Nutrient composition				
ME, MJ/kg DM	11.12	11.06	10.98	10.99
CP, g/100g DM	21.9	19.0	24.5	23.7
Soluble CP, %CP	54.2	48.7	53.0	54.4
NDF, g/100 g DM	42.3	41.5	38.5	38.7
peNDF, g/100 g DM	31	29	25	27
Total NFC, g/100 g DM	23.2	26.6	22.7	22.5
Total fat, g/100 g DM	5.5	5.6	5.7	6.3
Performance predictions				
ME allowable milk, kg/day	25.6	25.9	24.7	24.8
MP allowable milk, kg/day	22.7	24.3	24.9	24.2
Daily weight change due to reserves, kg/day	2.1	2.1	1.9	2.0
Rumen digestion, metabolism and passage				
MP from bacteria, g/day	969	1041	924	900
MP from undegraded feed, g/day	800	829	967	963
MP from undeg. feed, %MP supplied	45	44	51	52
Total degradable intake protein, %CP	80.3	78.2	81.2	80
Ruminal N balance, % of requirements	214	162	268	257
Total bacterial nitrogen, g/day	258	278	246	240
Urea cost, MJ/day	8.9	6.5	11.1	10.5
Urea cost, %ME intake	4.4%	3.3%	5.6%	5.3%
Excess N excreted, g/day	377	268	515	473
Liquid passage rate, $\%.h^{-1}$	11.3	11.3	11.3	11.3
Pasture passage rate, $\%.h^{-1}$	6.4	6.4	6.4	6.4
Silage passage rate, $\%.h^{-1}$	5.25	6.58	6.11	5.82
Predicted ruminal pH	6.46	6.46	6.46	6.46

Abbreviations see text and previous tables.

TABLE 7.8 – Low, medium and high quality pasture (LQP, MQP and HQP) with mixture of maize and sulla silages. CNCPS predictions of nutrient composition, cow performance, and rumen parameters.

	LQP + PMS (4M2S)	MQP + PMS (4M2S)	HQP + PMS (4M2S)	LQP + PSM (4S2M)	MQP + PSM (4S2M)	HQP + PSM (4S2M)
Simulated pasture DMI, kg/cow.day	12	12	12	12	12	12
Simulated maize silage DMI, kg/cow.day	4	4	4	2	2	2
Simulated sulla silage DMI, kg/cow.day	2	2	2	4	4	4
Simulated total DMI, kg/cow.day	18	18	18	18	18	18
Nutrient composition						
ME, MJ/kg DM	10.04	10.30	11.04	10.02	10.28	11.01
CP, g/100g DM	13.9	17.2	20.6	15.5	18.8	22.1
Soluble CP, %CP	49.4	50.3	50.9	52.2	52.6	52.8
NDF, g/100 g DM	45.9	44.9	40.6	45.0	44.0	39.7
peNDF, g/100 g DM	25	25	28	24	24	28
Total NFC, g/100 g DM	31.9	27.9	25.2	30.6	26.6	23.9
Total fat, g/100 g DM	3.2	3.9	5.8	3.5	4.2	6.1
Performance predictions						
ME allowable milk, kg/day	22.9	23.6	25.5	22.8	23.2	25.2
MP allowable milk, kg/day	23.9	24.0	24.2	23.4	23.7	24.1
Daily weight change due to reserves, kg/day	1.6	1.8	2.1	1.6	1.7	2.0
Rumen digestion, metabolism and passage						
MP from bacteria, g/day	1218	1124	994	1171	1077	947
MP from undegraded feed, g/day	679	766	868	699	796	913
MP from undeg. feed, %MP supplied	36	41	47	37	42	49
Total degradable intake protein, %CP	68.3	73.6	78.9	70.2	74.8	79.5
Ruminal N balance, % of requirements	102	132	187	115	151	218
Total bacterial nitrogen, g/day	325	300	265	312	287	253
Urea cost, MJ/day	2.8	4.2	7.8	2.7	5.5	9.2
Urea cost, %ME intake	1.5%	2.3%	3.9%	1.5%	3.0%	4.6%
Excess N excreted, g/day	98	190	324	133	237	392
Liquid passage rate, $\%.h^{-1}$	11.3	11.3	11.3	11.3	11.3	11.3
Pasture passage rate, $\%.h^{-1}$	6.74	6.79	6.40	6.74	6.79	6.40
Maize silage passage rate, $\%.h^{-1}$	5.58	5.58	5.58	5.58	5.58	5.58
Sulla silage passage rate, $\%.h^{-1}$	5.82	5.82	5.82	5.82	5.82	5.82
Predicted ruminal pH	6.46	6.46	6.46	6.46	6.44	6.46

Abbreviations see text and previous tables.

FIGURE 7.1 - Actual versus predicted values for A: dry matter intake (DMI), B: milk production and C: live weight (LW) change using CNCPS. (◊) = individual treatments. PR = restricted pasture, FP = unrestricted pasture.

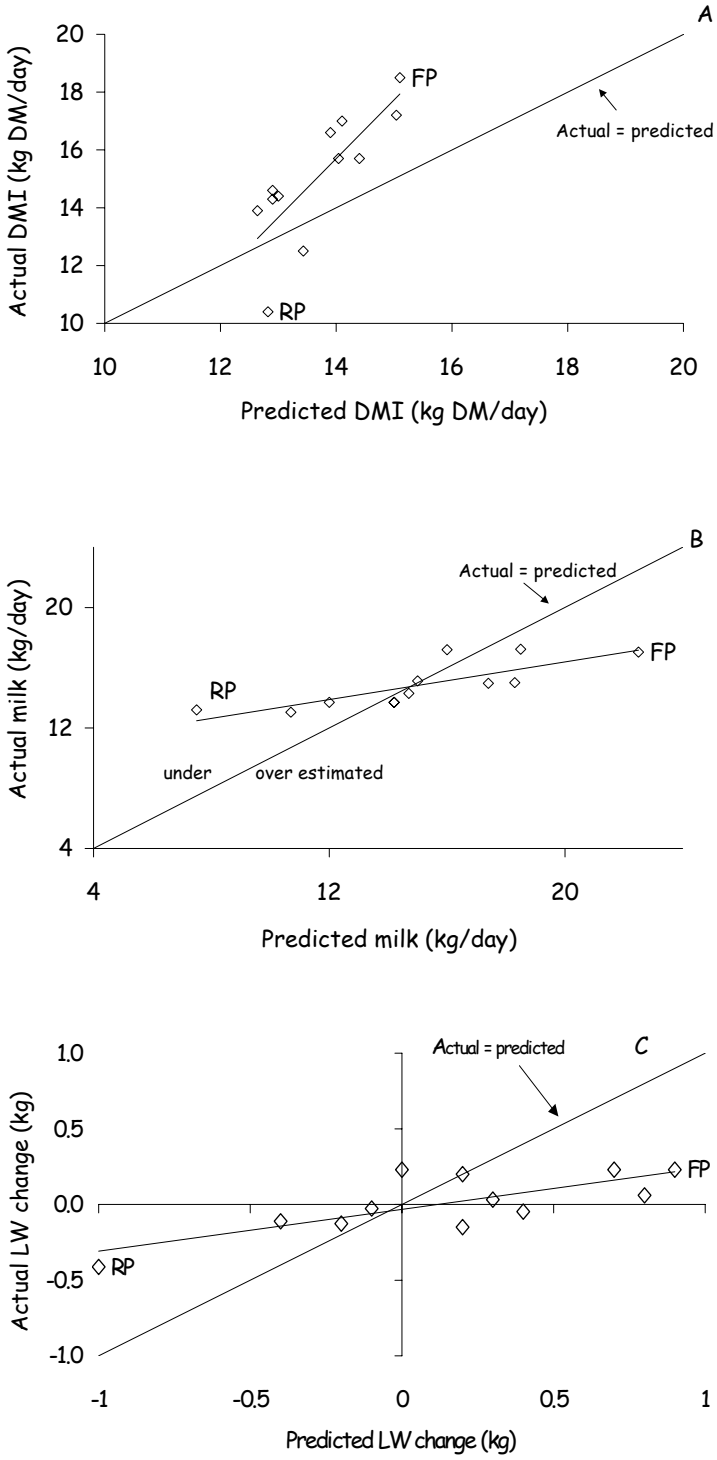


FIGURE 7.2 - Residual (predicted – actual) versus predicted values for A: dry matter intake (DMI), B: milk production and C: live weight (LW) change using CNCPS. (◊) = individual treatments. Line (_ _ _) indicates mean bias. PR = restricted pasture, FP = unrestricted pasture. P value of F-statistic for slope ≠ 0.

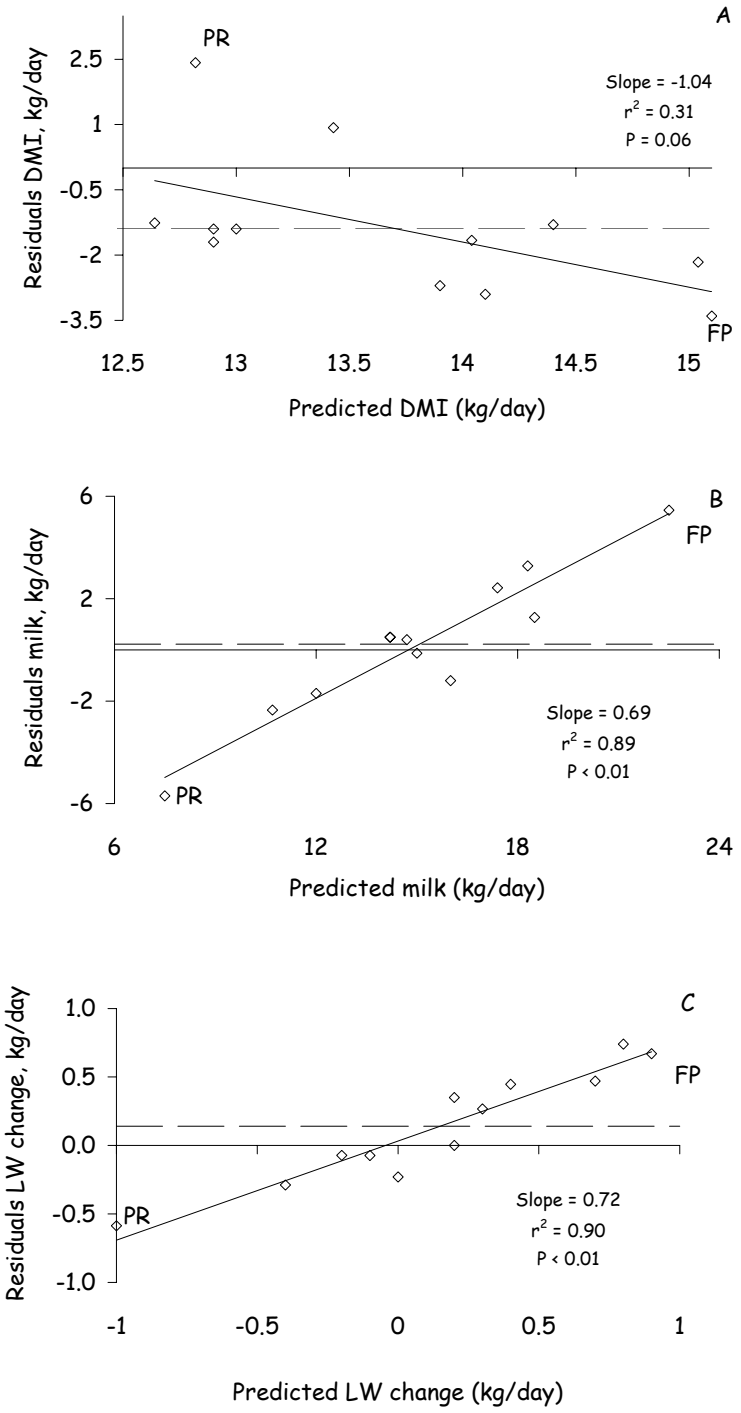
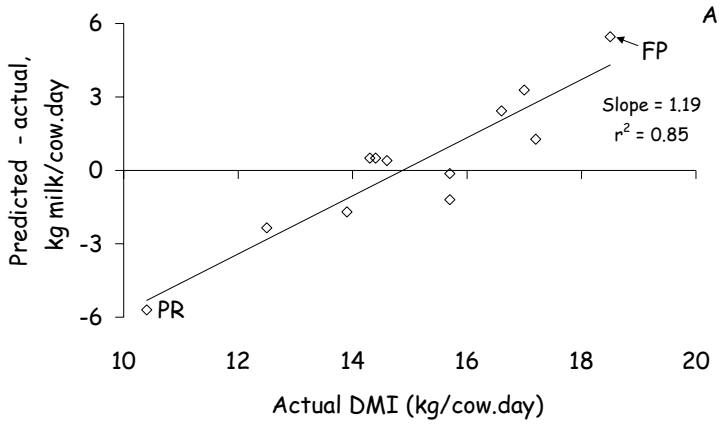


FIGURE 7.3 - Milk production (kg/cow.day) predicted by the CNCPS model minus actual milk production (Y axis) versus A: actual dry matter intake (DMI) and B: liveweight (LW) change. PR = restricted pasture, FP = unrestricted pasture.



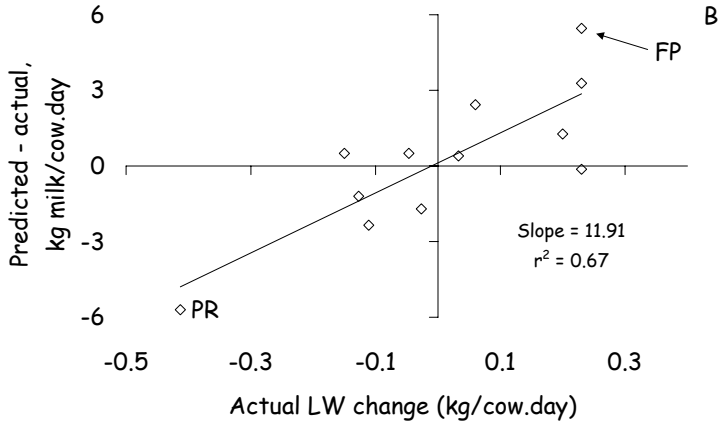
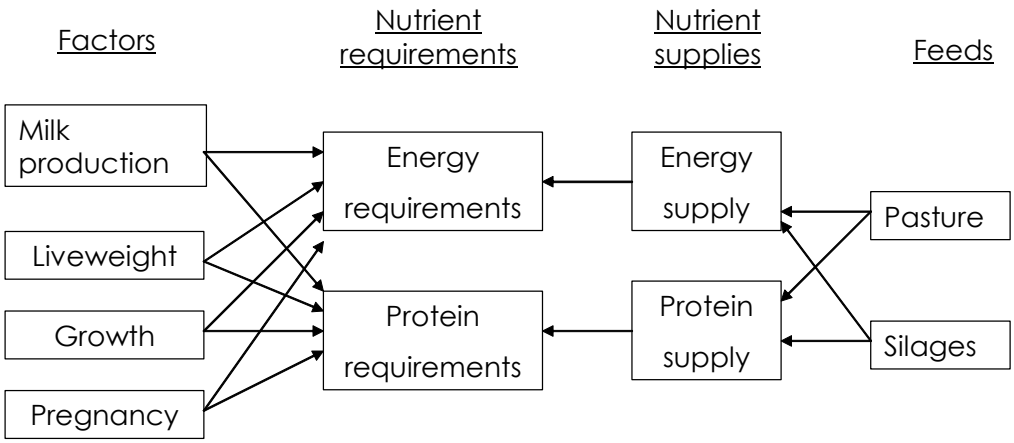
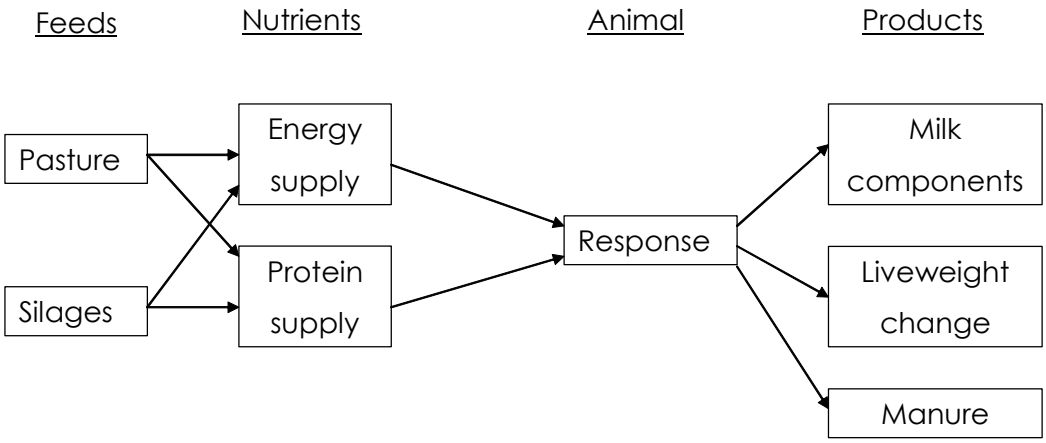


FIGURE 7.4 - Schematic representation of the CNCPS requirement-based system (A) and B: production response system. Adapted from St-Pierre and Thraen (1999).

(A)



(B)



Chapter 8 - General discussion

The research undertaken for this thesis represents a comprehensive analysis of ryegrass maturation on nutritive value, covering chemical composition, rates of degradation and products of digestion. This has formed a data base for New Zealand pastoral systems and is intended to provide information to improve supplementation of dairy cows. The well known and rapid changes in ryegrass composition and feeding value with maturity require constant re-evaluation of supplementation to meet feeding objectives. It is not possible to provide a balanced diet without knowing the digestion kinetics of ryegrass. Part of this research involved separation of leaf, stem and flower to provide basic data concerning the contribution of each component of ryegrass.

One objective of this work was to provide data enabling appropriate supplementation of summer ryegrass dominant pastures for dairy cows. Poor nutritive value (NV) of pasture in summer contributes to the decline in milk production and can also lead to liveweight losses and shortened lactation especially with restricted pasture availability. Two dairy trials were carried out to examine effects of contrasting silage supplements for lactating cows fed pasture in mid summer. The silages included conventional (maize, pasture) and legume (lotus and sulla) silages as single supplements and mixtures to provide data showing cow responses and substitution. These trials provided background information against which future supplements can be designed to complement pasture on offer.

There are several models to aid interpretation of cow grazing data, and the CNCPS system was chosen to compare measured and predicted data because it predicts the nutrient supply from digestion kinetics data. The evaluation showed energy to be a prime limitation when pasture was fed, but the model did not take into account effects of liveweight change. The model provides a good basis for diet evaluation using forages with contrasting nutritive characteristics but ruminal parameters in cows fed fresh forages have required detailed inputs of digestion kinetics which are not always available. Examples include a poor relationship between rumen effective fibre and pH, inadequate estimation of DM intake (based on liveweight) and complex feed degradability component inputs.

An important aspect of the *in vitro* and *in sacco* experiments was the use of the fresh mincing procedure to provide material for *in vitro* and *in sacco* incubations which mimicked cow digesta. The data from *in vitro* and *in sacco* incubations were complementary and provided a simple and logical data set to show the way maturation influenced microbial digestion. Chemical composition, *in sacco* digestion kinetics and *in vitro* products of digestion helped explain effects of maturation, and this was made more obvious when grass leaf, stem and flower were incubated separately. The slow degradation of mature grass components highlighted the importance of clearance of undigested residues from the rumen, and provided good evidence for diminished feeding value (FV) from flowering ryegrass. The energy required for mincing ryegrass of increasing maturity was not measured, but increasing effort would be required for particle breakdown of maturing ryegrass. Slow particle size reduction of mature forage will reduce microbial growth and outflow from the rumen, with lower intakes and nutrient yields per unit of intake. Cow productivity could only be maintained by substituting mature grass with more rapidly digested feeds. The information presented here will affect both the level and quality of supplements needed to maintain production from cows fed summer pasture.

Comparison of initial cutting dates showed minor effects on the rate of change in chemical composition, with late cut grass maturing slightly more rapidly than early cut grass. This affected fibre but not CP or NSC content. Protein degradation was not affected by maturation, but a higher proportion was released into the soluble (A) fraction with mature grass. This appeared to indicate more extensive cell rupture of mature grass and is likely to reflect the *in vivo* situation where more extensive chewing is needed to swallow mature, compared to succulent forages. In contrast to protein, fibre degradation rate was halved as ryegrass matured, probably as a result of cross linkages between fibre components and lignification. Estimated ME values for ryegrass were reduced from about 12.8 to 8.8 MJ/kg DM as ryegrass became mature, but effects of maturation on FV would be substantially greater because of effects on intake.

The ammonia production *in vitro* was a function of grass CP content, extent of release into the soluble fraction and microbial utilisation. One effect of maturation was a brief period of ammonia surplus followed by insufficiency for microbial growth, but the rate and amounts of VFA produced were not affected by forage CP content or ammonia concentration. This suggests forage CP may have a greater effect on ruminant performance through provision of plant and microbial amino acids for absorption, than through microbial VFA production.

Maturation, including effects of leaf, stem and flower components had little effect on proportions of VFA. Rates of VFA production were similar for young, medium and mature ryegrass, possibly because all were minced to a similar particle size distribution, but this will be similar to forage eaten by grazing cows. Chewing will have a significant impact on rate and extent of fermentation as well as clearance from the rumen.

The grazing trials showed exceptional responses with lotus silage fed to cows. Future research would benefit cow nutrition if the reasons for the large responses to lotus could be explained. The maize silage supplement resulted in a moderate increase in milk production, but this would have been lower with pasture containing less nitrogen, typical of a normal summer. In contrast, mixed silages such as maize and sulla silages gave good responses and a more stable rumen environment than either maize or sulla silage fed with pasture. Cows preferred the mixed silages to a single silage, except for lotus. *In sacco* incubations suggested a less active fermentation with increasing proportion of maize silage in the diet. This may have important implications for provision of nutrients for cows but more studies are needed to understand effect of *in vivo* environment on digestion rate and nutrient release.

The CNCPS model does provide a good method for diet evaluation, to reduce the numbers and enable a better design of cow trials. The model gives good predictions of milk production if cow energy balance is close to zero, as with the trials reported here. More investigations of model capability for use under grazing systems are necessary, especially using data from whole lactation trials. Long term, systems trials have the advantage of quantifying carry over effects associated with short trials.

Ryegrass maturation data will form the foundation for an improved understanding of the NV of ryegrass pastures. The data do not include impacts of grass quality on intake, and this is a serious limitation to use of the CNCPS model and to all studies of FV from pasture. However, the effects of maturation on degradation of protein and fibre have been clearly defined and show a surprising resilience for VFA production by bacteria when forage N is limiting. Cow trials have given some useful information about silage supplementation but future work should provide mixed supplements to form an appropriate proportion of the diet, dependent on the quality of available pasture.

There have been some limitations to the work presented in this thesis, due to insufficient knowledge in early stages of this work and insufficient time for more measurements. It would have been worthwhile to measure proportions of leaf, stem and flower from the pastures with increasing maturity, and this may be done in future. An additional useful measurement would be the energy required to mince grasses at different stages of maturity. These measurements may form the basis of future trials if intake is to be targeted as a topic for balancing pasture with supplements.

Analyses focused on a 6% fractional outflow rate from the rumen and although some analyses used contrasting values (e.g.: 2%), this aspect of rumen function probably requires more consideration, especially when comparing fibre and protein. Outflow rates affect both intake and nutrient supply through effective degradability. The CNCPS model is sensitive to feed supply and although intakes are not well defined by the model, the issues of feed availability under New Zealand grazing systems need to be accommodated when the model is used.

In vitro data presented here would be more valuable if it could be combined with microbial growth. Future *in vitro* studies with forages should incorporate microbial growth to give a more comprehensive and meaningful discussion of products of digestion. It is important that these factors be incorporated in the model to improve the rumen aspects, especially as DNA-based systems are showing promise for rapid and inexpensive measurements of bacterial numbers.

Future research should investigate sulla characteristics, especially agronomic requirements, harvesting and chemical composition. This Mediterranean legume has good potential to provide high yields of high quality forage, and is being used by several commercial farmers. However, little information has been published and more trials should test the benefits of both the NSC and condensed tannins for dairy cow nutrition and performance.

The information presented in this thesis showed that mixed forages (sulla, maize and lotus) are preferred by cows, to individual forage supplements and farmlet trials over a whole lactation should investigate benefit of mixtures on cow nutrition, performance and sustainability. Use of an autumn calving herd would enable effects of ryegrass maturation to be avoided and autumn pasture would provide a high quality diet for cows at peak lactation. Supplements would have a major role in winter when grass availability is low and could give ideal opportunities to compare contrasting silages. The CNCPS model might be used to balance diets in long term trials and

economic analyses would determine profitability and sustainability of farm systems. These analyses apply to all farming systems where choices of diets are available.

List of publications derived from this PhD project

Chaves, A.V.; Waghorn, G.C.; Brookes, I.M Woodward, S.L. 2003. Empirical evaluation of the NRC dairy cattle model to predict performance of dairy cows fed pasture with corn and sulla silages. *Proceedings of the VI International Symposium on the Nutrition of Herbivores*: *in press*.

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APPENDIX 1: *In vitro* incubation.

McDougall's buffer

Recipe for McDougall's artificial saliva used as a buffer in the *in vitro* incubations. The buffer was prepared the day prior to incubations to ensure the components were dissolved prior to gassing with CO_2 on the day of incubation. One litre was sufficient for 3 x 24 containers with six standards.

$NaHCO_3$	9.8 g/L
$Na_2HPO_4.12H_2O$	9.3 g/L
NaCl	0.47 g/L
KCl	0.57 g/L
$CaCl_2$ anhydrous	0.04 g/L
$MgCl_2$ anhydrous	0.06g/L

Reducing agent

The reducing agent was cysteine sulphide immediately prepared before the incubations as follows.
Mix in order:
0.315 g cysteine hydrochloride
48 mL water
2 mL 1N NaOH
0.315 g sodium sulphide (crystals to be rinsed in water and blotted dry to adjust weight)

In vitro incubation procedure

1. Prepare forages freeze and weigh about 2.5g wet weight (0.5g DM) into bottles.
2. Place bottle in freezer after weighing on the previous day. Put caps on bottles in freezer.
3. Bottles are weighed empty without caps. When washing bottles never use detergent. Feed cows first thing in morning so you sample liquor 2-3 hours after onset of eating.
4. Prepare Excel sheet with bottle weights for data entry.

5. Start numbering microcentrifuge tubes.

Day of incubation:

1. Turn incubator on previous day so the temperature is stable for incubation.
2. Place buffer in a bucket of hot water, warm to 40°C whilst gassing for 45 minutes before dispensing into bottles.
3. Put samples in incubator (up to 90 minutes before adding rumen inoculant).
4. The rumen samples, collected by hand from the rumen into a cheese cloth bag, were strained through cheesecloth into a warm thermos® flask using a funnel. The flask was filled to minimise exposure of the liquor to air and taken directly to the incubation room.
5. Make sufficient reducing agent for 0.5 mL/sample.
6. Add buffer to bottles by removing two at a time, then dispense 12 mL buffer and 0.5 mL reducing agent. Weigh and return bottles to incubator. This is the rate limiting step for incubations, and one bottle is always being gassed whilst other is having buffer/reducing agent and weighed. This require two people, one to collect bottles, weigh after addition of buffer and reducing agent and the other to purge the bottles with CO_2 and add buffer and reducing agent.
7. Allow reducing agent to work for about 15 minutes whilst getting rumen contents.
8. Bring cow in to yards; put hot water in flask; take bucket, cheese cloth (Figure 1.1A below), thermos and funnel to cow; obtain about 4 kg contents from the cow and squeeze into thermos.
9. *In sacco* bags are normally placed in the rumen immediately after rumen contents have been taken.
10. Dispensing rumen liquor into incubation bottle requires 2 people; one to open and close bottles and the other to pipette liquor.
11. Calibrate pH meter; measure pH of rumen contents.
12. Pipette 3 mL rumen liquor into each bottle. Cap and return to incubator.

NB: Cut tip off pipette to facilitate handling rumen contents; keep stirring rumen liquor whilst sampling. Depress pipette before immersing tip in liquor; sample 5 cm below surface.

13. Take 2 samples of rumen contents. One for ammonia analysis (1 mL + 15 µL concentrated HC1) and one for VFA determination (1 mL of rumen contents).
14. Microcentrifuge tubes should be labelled prior to incubations. For each run there are 3 x 24 samples plus 6 standards, so about 156 tubes are needed for samples used to determine ammonia concentrations (76 for acidified media and 76 for storing the supernatant). A further 32 are needed for VFA samples taken at 0, 6, 12 and 24 hours. Only two samples are taken from each sample type at each time (i.e. 3 bottles are sub-sampled into two tubes) but tubes are needed for the initial

centrifugation and for storage. All samples were stored at -20°C prior to ammonia and VFA analysis.

FIGURE 1.1A – The cheese cloth used to strain the rumen liquor has large holes enabling small particulate material pass into the liquor. From left to right, 2.5 kg roll of cheese cloth (stockinette), placement of cheese cloth in bucket to strain the liquor and the thermos flask to transport rumen liquor from cow to the incubator (bottles).

APPENDIX 2: Ammonia determination method (Chaney and Marbach 1962).

The procedure is based on Chaney and Marbach (1962) and is a colorimetric procedure based on a combination of reagents for the catalysed indophenol reaction for the determination of ammonia, which produces a stable blue colour.

Solution one:

0.11M Phenol 10 g/L

0.17 mM Sodium nitroprusside (sodium nitrosopentacyanoferrate (III)) 0.05 g/L

Dissolve in distilled water. Make about 100mL at any one time and store in a brown bottle.

Store reagent at 4^0C and do not keep for more than a week.

Solution two:

0.13 M Sodium hydroxide 5 g/L

Sodium hypochlorite 0.42 g/L (Commercial bleach 17mL/L)

Dissolve in distilled water. Make about 100mL at any one time and store in a brown bottle.

Store reagent at 4^0C and do not keep for more than one week.

STANDARDS

Solution three:

60 mMol/L NH_4Cl; pre-weigh a 250 mL beaker. Weigh 802.35 mg of NH_4Cl. Add it to the beaker. Add about 200 mL of artificial (made with distilled water) and 5 mL of concentrated HCl. Dissolve and make up to 250 mL with artificial saliva. Record final weight.

Stock; weight of NH_4 = 0.8374. Final weight = 250.31. MW of NH_4Cl = 53.49

Actual concentration was 62.5 mMol L^{-1} NH_4Cl (or 3.35 mg g^{-1}).

Solution four:

Dilution Buffer; Combine 245 mL of artificial saliva and 5 mL HCl.

Upper detection method limit for the assay is 80 nMol. The standard curve needs to be in the range of 0-75 nMol NH_3.

Prepare standards gravimetrically. Store at -20^0C.

0 nM NH3 (in 20 µL); 0 µL of stock NH4Cl (solution 3) and 4000µL of buffer
5 nM NH3 (in 20 µL); 17 µL of stock NH4Cl (solution 3) and 3983µL of buffer
10 nM NH3 (in 20 µL); 34 µL of stock NH4Cl (solution 3) and 3966µL of buffer
20 nM NH3 (in 20 µL); 67 µL of stock NH4Cl (solution 3) and 3933µL of buffer
30 nM NH3 (in 20 µL); 100 µL of stock NH4Cl (solution 3) and 3900µL of buffer
35 nM NH3 (in 20 µL); 117 µL of stock NH4Cl (solution 3) and 3883µL of buffer
40 nM NH3 (in 20 µL); 134 µL of stock NH4Cl (solution 3) and 3866µL of buffer
50 nM NH3 (in 20 µL); 167 µL of stock NH4Cl (solution 3) and 3833µL of buffer
55 nM NH3 (in 20 µL); 184 µL of stock NH4Cl (solution 3) and 3816µL of buffer
60 nM NH3 (in 20 µL); 200 µL of stock NH4Cl (solution 3) and 3800µL of buffer
70 nM NH3 (in 20 µL); 234 µL of stock NH4Cl (solution 3) and 3766µL of buffer
75 nM NH3 (in 20 µL); 250 µL of stock NH4Cl (solution 3) and 3750µL of buffer

Predicted concentration and the amount of NH_3 in each reaction when 20 µL of each standard is used.

0 nM/L and in 20 µL there is 0 nM (0 ng).
0.25 nM/L and in 20 µL there is 5 nM (273 ng).
0.5 nM/L and in 20 µL there is 10 nM (546 ng).
1.0nM/L and in 20 µL there is 20 nM (1075 ng).
1.5 nM/L and in 20 µL there is 30 nM (1605 ng).
1.75 nM/L and in 20 µL there is 35 nM (1878 ng).
2.0nM/L and in 20 µL there is 40 nM (2150 ng).
2.5 nM/L and in 20 µL there is 50 nM (2680 ng).
2.75 nM/L and in 20 µL there is 55 nM (2953 ng).
3.0nM/L and in 20 µL there is 60 nM (3209 ng).
3.5 nM/L and in 20 µL there is 70 nM (3755 ng).
3.75 nM/L and in 20 µL there is 75 nM (4012 ng).

Add 20 µL of standard or unknown (in duplicate) to each well in the plate.

Add 100 µL of Solution 1 to each well.

Add 100 µL of Solution 2 to each well.

Leave on plate to incubate at room temperature for 30 minutes.

Read absorbency at 625 nm using a spectrophotometer.

APPENDIX 3: VFA determination method.

VFA concentration was determined by gas chromatography (GC) as described by Attwood *et al.* (1998).

The GC used a nitroterephthalic acid modified polyethylene glycol column (DB – FFAP, 30m x 0.53mm x 1.0μm 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 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 final concentration) was used as an initial standard.

Basic procedure:

1. Label wide mouth, yellow lid vials and put on ice.
2. Defrost VFA samples, shake and spin for 15 minutes at 15000 rpm.
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 followed to vials and then screw on lid.

Note it is important that minimal volatilisation occurs (i.e. 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 4: *In vitro* data from Chapter 3.

TABLE 4.1A - Net ammonia production from *in vitro* incubation of maturing ryegrass[a]. Data are mean values for in µMol NH_3/mM plant N. Negative values indicate a net utilisation of ammonia present in rumen inoculum, with large values a consequence of low plant N concentrations.

Run A Incubation time (h)	0	2	4	6	8	10	12	24
22d mowing date 1	0	19.67	30.05	65.18	66.50	38.00	64.24	72.62
31d mowing date 1	0	14.79	13.63	23.23	26.25	9.43	-1.03	-7.86
10d mowing date 2	0	25.84	34.52	38.24	27.51	79.53	1.16	24.90
Standard		112.2			294.0			

Run B Incubation time (h)	0	2	4	6	8	10	12	24
53d mowing date 1	0	53.26	19.09	46.29	11.16	-24.86	-34.47	-4.62
32d mowing date 2	0	21.59	47.46	54.65	27.24	4.19	-28.09	23.62
22d mowing date 3	0	51.81	39.66	53.33	27.91	-1.05	-45.74	20.12
Standard		83.2			265.2			

Run C Incubation time (h)	0	2	4	6	8	10	12	24
74d mowing date 1	0	58.18	-27.92	-54.51	-54.75	-53.25	-54.01	-39.06
53d mowing date 2	0	48.59	-25.00	-46.99	-46.54	-46.99	-45.96	-33.50
43d mowing date 3	0	23.49	-35.65	-53.65	-54.37	-54.25	-54.74	-34.43
Standard		169.7			508.9			

Run D Incubation time (h)	0	2	4	6	8	10	12	24
88d mowing date 1	0	84.38	16.38	-38.32	-53.93	-52.49	-46.22	-6.56
67d mowing date 2	0	49.89	-13.81	-87.62	-89.42	-89.56	-83.41	-43.97
57d mowing date 3	0	99.68	20.38	-52.22	-62.35	-59.17	-57.65	-0.78
Standard		195.8			331.6			

Run E Incubation time (h)	0	2	4	6	8	10	12	24
105d mowing date 1	0	138.0	-134.6	-290.7	-326.6	-320.7	-325.9	-235.8
84d mowing date 2	0	71.9	-133.6	-252.3	-297.5	-282.2	-293.1	-189.3
74d mowing date 3	0	190.0	-68.5	-217.7	-250.6	-230.6	-230.4	-183.5
Standard		273.8			736.0			

[a] Runs A – E refer to *in vitro* incubation runs described in Chapter 3 (Table 3.1).

TABLE 4.2A - Concentrations (mMol/L) of volatile fatty acids production from *in vitro* incubation of runs A to E as described in section 3.3 – Chapter 3.

Run A

Time (h)	acetate	propionate	Iso-but	butyrate	Iso-val	valerate	Total
22 days mowing date 1							
0	13.4	4.4	1.8	0.2	0.3	0.5	20.8
6	53.2	18.2	9.5	0.8	1.0	1.5	84.2
12	80.5	27.7	13.0	1.3	1.8	2.4	126.7
24	92.6	30.1	15.0	1.2	1.7	2.4	143.0
31 days mowing date 1							
0	14.5	3.1	1.8	0.0	0.3	0.5	20.3
6	54.7	20.6	8.6	0.6	0.7	1.4	86.6
12	62.8	24.3	14.3	0.8	1.0	1.9	105.2
24	98.2	35.0	14.3	1.1	1.3	2.2	152.1
10 days mowing date 2							
0	14.5	3.7	2.1	0.0	0.4	0.6	21.2
6	48.0	16.8	7.8	0.7	0.9	1.4	75.5
12	66.5	22.7	10.3	0.8	0.9	1.6	102.7
24	91.6	32.1	14.0	1.4	1.5	2.4	143.0

Run B

Time (h)	acetate	propionate	Iso-but	butyrate	Iso-val	valerate	Total
53 days mowing date 1							
0	12.0	4.2	1.5	0.5	0.7	0.9	19.8
6	40.8	11.4	8.3	0.5	0.6	1.2	62.8
12	73.3	20.9	12.7	0.7	0.6	1.7	109.9
24	86.0	24.0	12.2	0.9	0.9	1.8	125.8
32 days mowing date 2							
0	14.3	4.7	2.3	0.4	0.5	0.7	22.8
6	41.0	11.5	8.4	0.5	0.7	1.3	63.4
12	70.2	18.8	12.0	0.6	0.6	1.7	104.0
24	94.6	24.6	13.1	1.3	1.2	2.2	137.0
22 days mowing date 3							
0	14.7	3.1	1.9	0.0	0.4	0.5	20.5
6	45.3	12.5	9.8	0.6	0.8	1.5	70.5
12	69.8	20.0	12.2	0.7	0.7	1.8	105.2
24	87.9	26.7	14.2	1.1	1.1	2.3	133.3

Run C

Time (h)	acetate	propionate	Iso-but	butyrate	Iso-val	valerate	Total
74 days mowing date 1							
0	10.21	3.18	1.28	0.15	0.20	0.27	15.29
6	48.10	18.30	8.90	0.00	0.38	0.92	76.60
12	65.20	27.10	14.90	0.75	0.60	1.30	109.85
24	73.10	27.70	16.90	0.83	0.77	1.50	120.80
53 days mowing date 2							
0	9.77	2.52	1.07	0.13	0.17	0.22	13.88
6	48.10	17.20	7.90	0.00	0.37	0.84	74.41
12	70.60	27.30	15.30	0.60	0.64	1.40	115.84
24	86.70	29.10	15.80	0.99	0.79	1.50	134.88
43 days mowing date 3							
0	9.77	3.40	1.70	0.00	0.41	0.45	9.77
6	50.20	18.90	8.20	0.00	0.36	0.90	50.20
12	63.40	26.80	16.30	0.71	0.58	1.40	63.40
24	81.60	32.40	18.90	1.00	0.83	1.80	81.60

Run D

Time (h)	acetate	propionate	Iso-but	butyrate	Iso-val	valerate	Total
88 days mowing date 1							
0	12.5	2.7	1.9	0.0	0.0	0.5	17.6
6	89.4	26.3	14.2	0.7	0.6	2.0	133.1
12	103.6	33.1	18.1	1.1	0.7	2.3	158.9
24	114.1	30.2	18.4	0.8	0.9	2.4	166.8
67 days mowing date 2							
0	13.4	2.6	1.6	0.2	0.3	0.5	18.5
6	85.1	24.9	13.5	0.7	0.5	1.8	126.5
12	101.1	29.4	16.3	0.7	0.7	2.1	150.3
24	118.9	34.1	19.7	1.1	0.9	2.4	177.1
57 days mowing date 3							
0	14.2	4.6	2.9	0.3	0.5	0.9	23.3
6	75.8	26.2	14.3	0.8	0.5	1.8	119.4
12	107.6	30.1	18.3	1.0	0.8	2.3	160.1
24	113.6	33.1	20.4	0.8	0.9	2.7	171.5

Run E

Time (h)	acetate	propionate	Iso-but	butyrate	Iso-val	valerate	Total
105 days mowing date 1							
0	11.6	5.8	2.9	0.3	0.5	0.8	21.9
6	71.9	18.8	12.9	0.0	0.7	1.8	106.1
12	112.0	28.8	19.1	0.0	0.9	2.4	163.2
24	122.0	32.0	21.1	1.0	1.0	2.6	179.7
84 days mowing date 2							
0	11.6	3.3	2.1	0.2	0.4	0.6	18.2
6	75.6	21.1	13.8	0.0	0.6	1.8	112.9
12	112.0	29.8	19.1	0.9	0.9	2.4	165.1
24	120.0	33.7	21.6	1.0	1.0	2.6	179.9
74 days mowing date 3							
0	11.6	4.3	2.6	0.3	0.5	0.7	20.1
6	69.7	20.0	13.3	0.0	0.6	1.7	105.3
12	106.3	31.3	21.2	0.9	0.9	2.5	163.0
24	130.0	37.7	24.5	1.3	1.1	2.9	197.5

Abbreviations: Iso-but, iso-butyrate; iso-val, iso-valerate.

APPENDIX 5: Equations used to describe relationships between height, herbage mass and nutritive characteristics of ryegrass due to maturation (age; days of re-growth).

The use of single or double asterisk in this and other equations denotes estimates parameters significantly different from zero at 5 and 1% levels respectively. This terminology applies to all equations in this chapter. ns = non significant.

Height (cm) = $5.59 \pm 5.29^{ns} + 0.53 \pm 0.19^{*} \times age + 0.002 \pm 0.002^{ns} \times age^2$ (r^2=0.92; CV=14.5%; Root MSE=6.79; mean height=47 cm).

HM = $- 0.53 \pm 0.56^{ns} + 0.034 \pm 0.02^{ns} \times age + 0.0002 \pm 0.00016^{ns} \times age^2$ (r^2=0.81; CV=34.2%; Root MSE=0.72 t/ha; mean HM=2.11 t/ha).

HM = $- 0.95 \pm 0.2^{**} + 0.065 \pm 0.004^{**} \times$ height (r^2=0.91; CV=23.1%; Root MSE=0.49 t/ha; mean HM=2.11 t/ha).

CP (g/100g in the DM) = $24.98 \pm 1.68^{**} - 0.29 \pm 0.06^{**} \times age + 0.001 \pm 0.0005^{*} \times age^2$ (r^2=0.82; CV=16.82%; Root MSE=2.16; mean CP=12.82 g/100g in the DM).

NSC (g/100g in the DM) = $5.51 \pm 1.21^{**} + 0.014 \pm 0.04^{**} \times age - 0.001 \pm 0.0004^{**} \times age^2$ (r^2=0.28; CV=16.84%; Root MSE=1.55; mean NSC=9.22 g/100g in the DM).

Lipid (g/100g in the DM) = $4.25 \pm 0.15^{**} - 0.02 \pm 0.002^{**} \times age$ (r^2=0.73; CV=11.28%; Root MSE=0.35; mean Lipid =3.07g/100g in the DM).

NDF (g/100g in the DM) = $46.58 \pm 1.94^{**} - 0.016 \pm 0.07^{ns} \times age + 0.0016 \pm 0.0006^{*} \times age^2$ (r^2=0.80; CV=4.76%; Root MSE=2.48; mean NDF=52.18g/100g in the DM).

ADF (g/100g in the DM) = $25.50 \pm 1.68^{**} + 0.013 \pm 0.06^{ns} \times age + 0.001 \pm 0.0005^{*} \times age^2$ (r^2=0.79; CV=7.01%; Root MSE=2.16; mean ADF=30.75g/100g in the DM).

Ash (g/100g in the DM) = $13.53 \pm 0.47^{**} - 0.068 \pm 0.007^{**} \times age$ (r^2=0.76; CV=11.28%; Root MSE=1.08; mean Ash =9.56g/100g in the DM).

ME (MJ ME/kg DM) = $11.61 \pm 0.41^{**} + 0.009 \pm 0.015^{ns} \times age - 0.0003 \pm 0.0001^{*} \times age^2$ (r^2=0.73; CV=4.85%; Root MSE=0.52; mean ME=10.80 MJ ME/kg DM)

APPENDIX 6: *In sacco* data from Chapter 3.

TABLE 6.1A – NIRS calibration estimates parameters for *in sacco* residues and lignin concentration in forages determined by wet chemical analyses. Means in g/100 g DM.

Constituent	N	Mean	SEC	RSQ	SECV	1-VR
CP	120	10.30	0.54	0.99	0.68	0.99
NDF	138	70.13	2.54	0.93	3.00	0.91
ADF	136	46.80	1.55	0.95	1.89	0.92
Lignin	86	4.65	0.30	0.99	0.45	0.98

Where

N = Number of samples

SEC = Standard Error of Calibration (e.g. NDF 70.13±2.54)

RSQ = R Squared

SECV = Standard Error of Cross Validation (e.g. NDF 70.13±3.00)

1-VR = Cross Validation RSQ

TABLE 6.2A - Composition of ryegrass (0 hour) and *in sacco* residues over the 72 hours digestion period. Data are for crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF).

				g CP/100 g DM					
Area	Date	Age	CP initial	0	2	6	12	24	72
1	11/09/2000	22	23.7	19.2	18.1	16.2	13.0	7.4	9.3
2	5/10/2000	24	18.6	15.7	12.3	8.5	6.2	5.7	9.0
3	13/10/2000	22	18.5	16.0	12.5	8.9	11.5	6.6	10.6
	Average	23	20.3	17.0	14.3	11.2	10.2	6.5	9.6
	STDEV		3.0	2.0	3.3	4.3	3.6	0.8	0.8
1	5/10/2000	45	16.8	13.8	11.4	8.2	6.1	6.3	9.7
2	24/10/2000	43	16.7	9.7	8.3	7.4	5.1	6.5	9.6
3	3/11/2000	43	13.1	9.8	9.8	9.8	5.9	4.7	13.0
	Average	44	15.5	11.1	9.8	8.4	5.7	5.8	10.8
	STDEV		2.1	2.3	1.6	1.2	0.6	0.9	1.9
1	3/11/2000	74	12.6	9.0	8.8	7.6	6.5	4.1	11.3
2	17/11/2000	67	9.7	4.0	4.5	4.8	3.8	4.7	5.3
3	4/12/2000	74	8.9	2.1	2.2	4.0	3.3	3.8	5.4
	Average	72	10.4	5.0	5.2	5.5	4.5	4.2	7.3
	STDEV		1.9	3.6	3.4	1.9	1.7	0.5	3.4
Total average		46	15.4	11.0	9.8	8.4	6.8	5.5	9.2
Total STDEV			4.7	5.7	4.7	3.5	3.3	1.2	2.5

g NDF/ 100g DM

Area	Date	Age	NDF initial	0	2	6	12	24	72
1	11/09/2000	22	42.7	55.5	58.6	66.6	69.9	79.6	76.6
2	5/10/2000	24	44.8	66.9	73.2	67.8	76.1	76.1	73.0
3	13/10/2000	22	49.5	64.6	64.3	66.2	73.8	78.3	69.4
	Average	23	45.7	62.3	65.4	66.9	73.2	78.0	73.0
	STDEV		3.5	6.0	7.4	0.8	3.1	1.8	3.6
1	5/10/2000	45	48.7	70.9	72.4	65.7	73.2	74.5	68.5
2	24/10/2000	43	47.1	72.3	75.4	67.1	75.2	75.6	69.8
3	3/11/2000	43	48.8	64.6	64.3	66.2	73.8	78.3	69.4
	Average	44	48.2	69.3	70.7	66.3	74.1	76.2	69.2
	STDEV		1.0	4.1	5.7	0.7	1.0	1.9	0.7
1	3/11/2000	74	51.8	64.9	65.9	69.9	74.0	79.7	73.5
2	17/11/2000	67	51.1	70.5	72.2	75.5	78.1	80.0	79.6
3	4/12/2000	74	56.7	75.9	77.9	78.6	80.0	79.8	80.6
	Average	72	53.2	70.4	72.0	74.6	77.4	79.8	77.9
	STDEV		3.1	5.5	6.0	4.4	3.1	0.2	3.9
Total average		46	49.0	67.3	69.4	69.3	74.9	78.0	73.4
Total STDEV			4.1	5.9	6.3	4.6	2.9	2.1	4.6

g ADF/ 100g DM

Area	Date	Age	ADF initial	0	2	6	12	24	72
1	11/09/2000	22	21.8	32.1	33.2	43.1	45.5	47.6	46.6
2	5/10/2000	24	30.1	38.0	40.6	40.3	44.4	44.9	46.4
3	13/10/2000	22	26.1	37.1	41.2	45.0	41.4	45.8	46.3
	Average	23	26.0	35.8	38.3	42.8	43.7	46.1	46.4
	STDEV		4.2	3.2	4.5	2.4	2.1	1.4	0.2
1	5/10/2000	45	26.4	38.3	39.5	37.9	42.6	43.7	43.9
2	24/10/2000	43	26.3	41.1	41.8	38.9	44.7	43.9	42.0
3	3/11/2000	43	28.2	38.0	38.3	40.3	44.6	47.2	41.5
	Average	44	27.0	39.1	39.9	39.0	44.0	44.9	42.4
	STDEV		1.1	1.7	1.8	1.2	1.2	2.0	1.3
1	3/11/2000	74	30.2	38.1	39.2	42.1	45.1	48.1	44.0
2	17/11/2000	67	30.9	42.9	43.5	46.3	48.7	49.5	47.7
3	4/12/2000	74	35.6	45.9	47.2	50.3	51.5	50.9	48.1
	Average	72	32.2	42.3	43.3	46.2	48.4	49.5	46.6
	STDEV		2.9	4.0	4.0	4.1	3.2	1.4	2.3
Total average		46	28.4	39.1	40.5	42.7	45.4	46.8	45.1
Total STDEV			3.9	3.9	3.8	4.0	3.0	2.5	2.4

STEDEV: standard deviation.

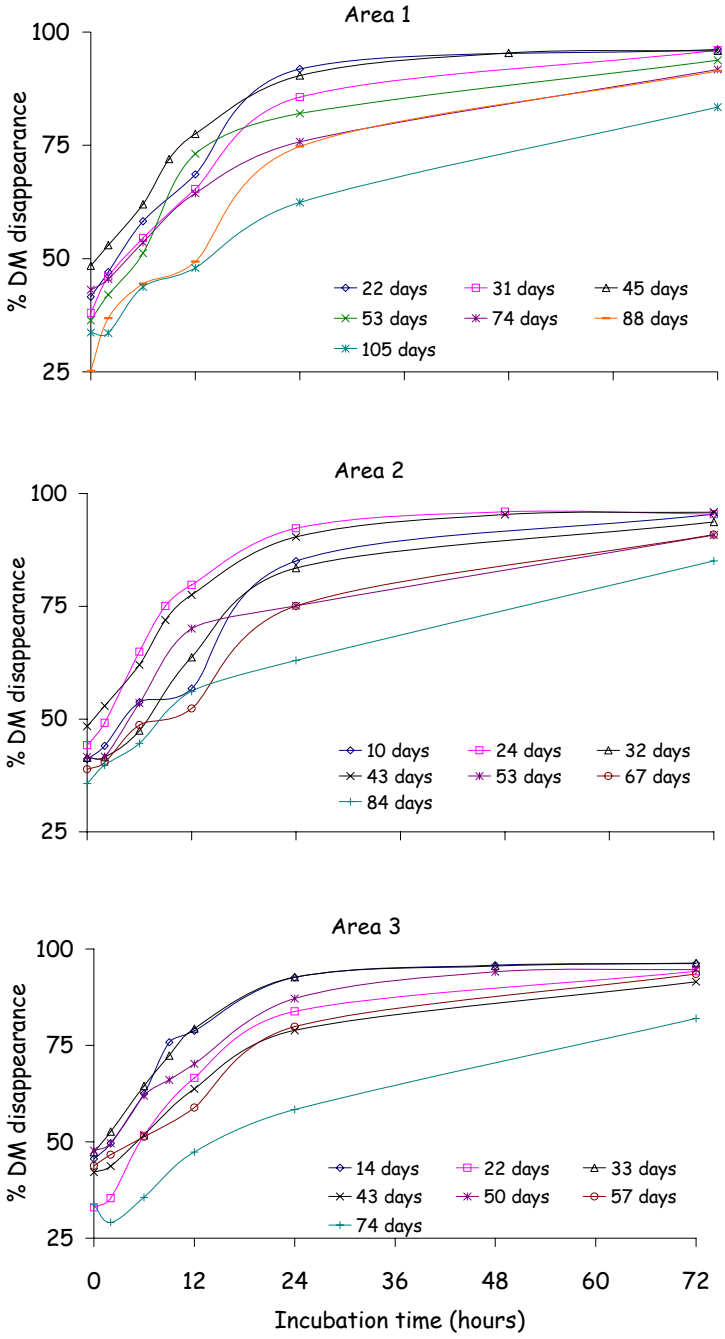


FIGURE 6.1A - Dry matter (DM) disappearance during *in sacco* incubations of ryegrass in different ages (days of re-growth) for areas 1, 2 and 3.

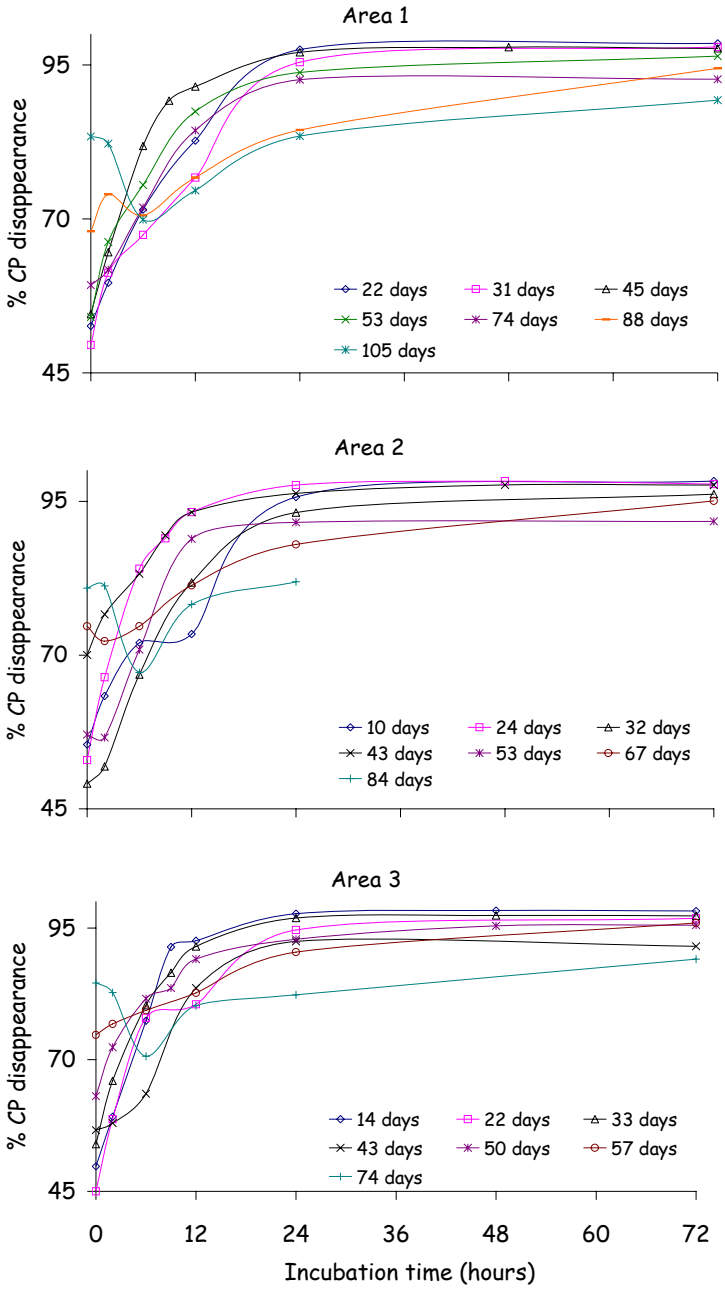


FIGURE 6.2A – Crude protein (CP) disappearance during *in sacco* incubations of ryegrass in different ages (days of re-growth) for the three areas.

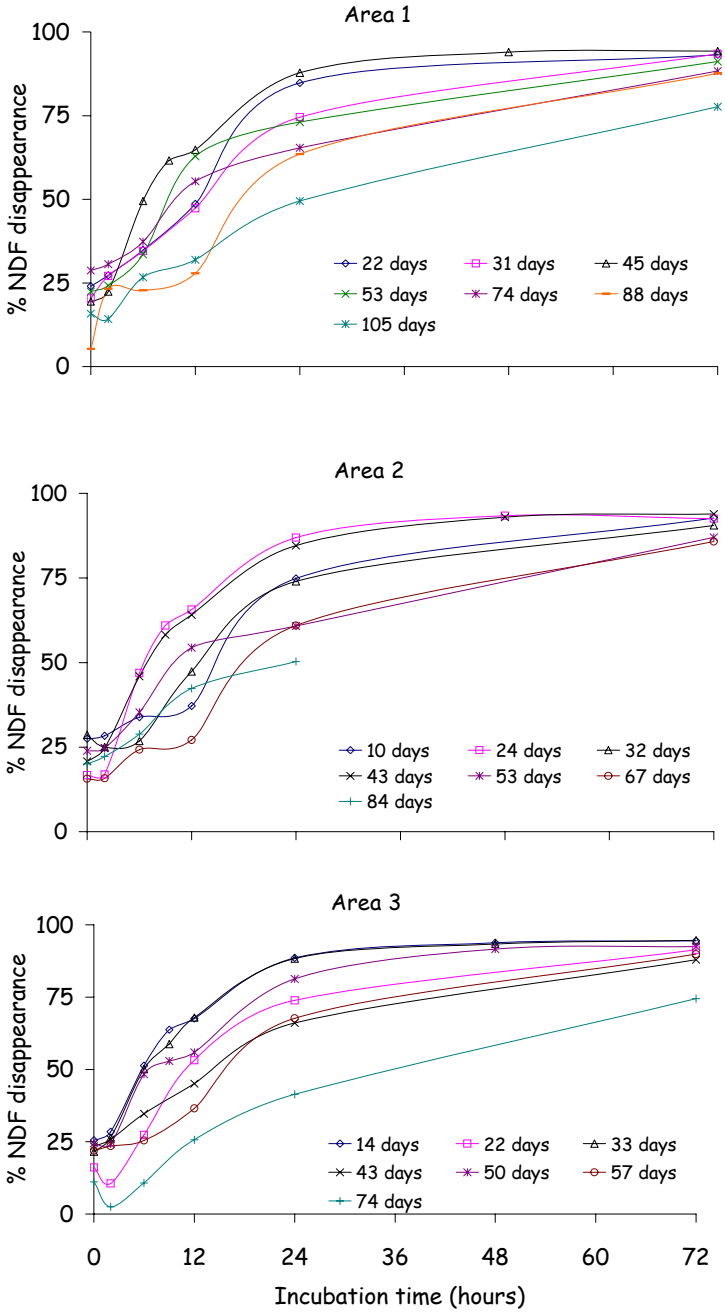


FIGURE 6.3A - Neutral detergent fibre (NDF) disappearance over time during *in sacco* incubations of areas 1, 2 and 3.

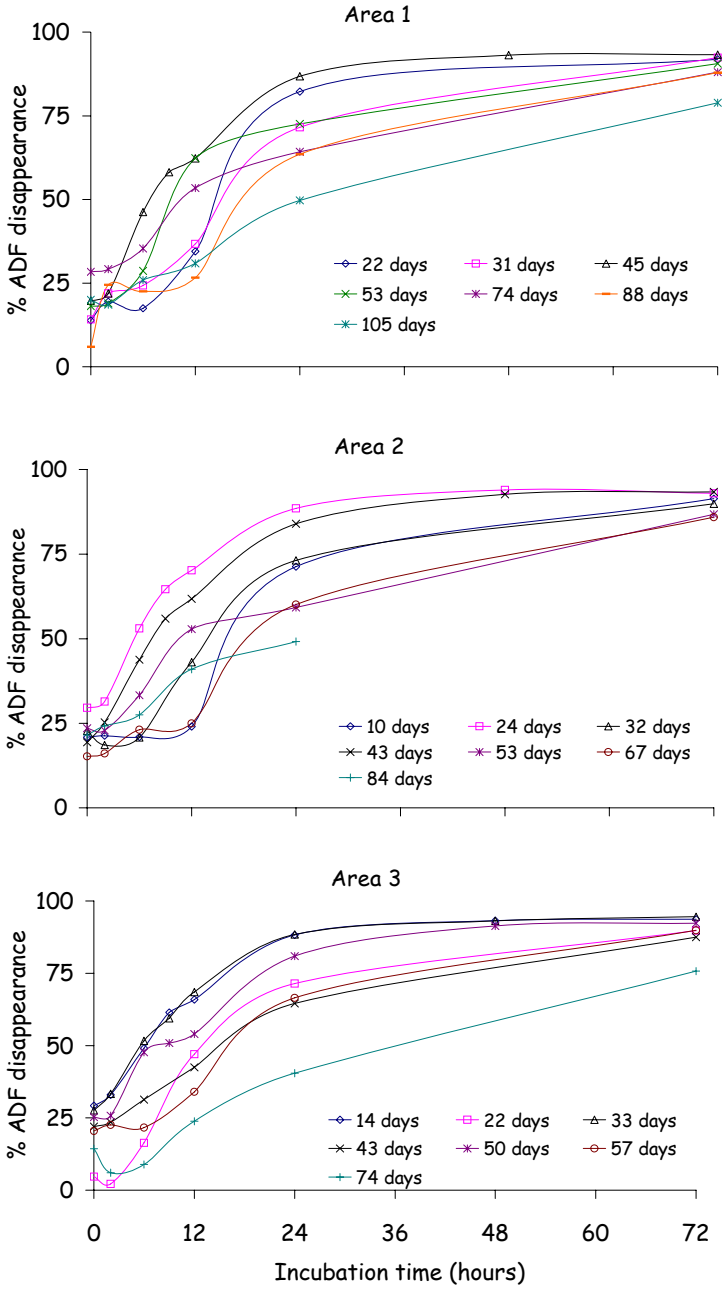


FIGURE 6.4A - Acid detergent fibre (ADF) disappearance over time during *in sacco* incubations of areas 1, 2 and 3.

APPENDIX 7: *In vitro* data from Chapter 4.

TABLE 7.1A - Net ammonia production from *in vitro* incubation from five mature grasses. Data are mean values for in µMol NH_3/mM plant N. Negative values indicate a net utilisation of ammonia present in rumen inoculum, with large values a consequence of low plant N concentrations.

Time h	0	2		6	8		12	24	36	48
Perennial ryegrass										
Leaf	0	54.4		83.1	95.8		114.2	43.1	47.6	73.3
Stem	0	39.6		85.9	103.9		103.9	38.1	16.2	59.4
Flower	0	38.0		123.8	111.7		111.4	-31.5	-28.8	-22.4
Tall fescue										
Time h	0	2		6	8		12	24	36	48
Leaf	0	74.0		51.3	30.4		-69.7	-70.1	-62.4	-78.0
Stem	0	17.8		153.0	156.5		153.6	152.4	153.8	149.7
Flower	0	57.9		-99.5	145.4		147.8	119.7	118.8	116.1
Yorkshire fog										
Time h	0	2	4	6	8	10	12	24		48
Leaf	0	31.4	51.5	70.2	72.6	48.2	26.6	21.9		-0.6
Stem	0	37.7	41.1	34.8	12.8	48.6	-57.3	191.2		179.2
Flower	0	80.0	136.0	186.7	148.9	177.6	253.7	-49.3		-68.4
Phalaris										
Time h	0	2		6	8		12	24	36	48
Leaf	0	31.3		84.6	99.0		99.9	118.6	137.9	124.3
Stem	0	89.4		122.3	90.0		63.0	109.4	-99.9	-14.9
Flower	0	87.7		86.5	79.9		44.6	18.0	103.4	110.0
Paspalum										
Time h	0	2		6	8		12	24	36	48
Leaf	0	31.3		14.9	17.8		175.0	124.0	125.6	-59.6
Stem	0	53.0		223.7	123.4		260.3	262.9	261.7	250.9
Flower	0	81.4		119.0	203.2		147.1	-68.9	126.8	107.6

TABLE 7.2A - Concentrations (mMol/g DM) of volatile fatty acids production from *in vitro* incubation from five mature grasses as described in section 4.4.5 – Chapter 4.

Forage	Component	Time	Acetate	Propionate	Butyrate	Minor	Total
P. ryegrass	Leaf	0	0.39	0.07	0.04	0.03	0.53
P. ryegrass	Leaf	6	0.64	0.18	0.08	0.03	0.92
P. ryegrass	Leaf	12	1.38	0.34	0.18	0.08	1.98
P. ryegrass	Leaf	24	1.62	0.22	0.21	0.07	2.12
P. ryegrass	Leaf	48	1.03	0.27	0.13	0.19	1.63
P. ryegrass	Stem	0	0.37	0.07	0.04	0.02	0.50
P. ryegrass	Stem	6	1.31	0.18	0.16	0.03	1.68
P. ryegrass	Stem	12	0.83	0.12	0.14	0.00	1.09
P. ryegrass	Stem	24	0.95	0.22	0.20	0.07	1.44
P. ryegrass	Stem	48	0.79	0.21	0.18	0.22	1.39
P. ryegrass	Flower	0	0.36	0.06	0.03	0.01	0.47
P. ryegrass	Flower	6	0.91	0.24	0.17	0.07	1.40
P. ryegrass	Flower	12	1.19	0.34	0.28	0.10	1.91
P. ryegrass	Flower	24	1.76	0.41	0.32	0.14	2.64
P. ryegrass	Flower	48	0.75	0.24	0.28	0.27	1.53
Tall fescue	Leaf	0	0.65	0.14	0.09	0.05	0.93
Tall fescue	Leaf	6	1.49	0.31	0.23	0.06	2.08
Tall fescue	Leaf	12	2.25	0.54	0.32	0.08	3.18
Tall fescue	Leaf	24	2.71	0.76	0.39	0.10	3.97
Tall fescue	Leaf	48	2.35	0.65	0.33	0.08	3.40
Tall fescue	Stem	0	0.61	0.12	0.08	0.03	0.83
Tall fescue	Stem	6	1.10	0.33	0.20	0.02	1.65
Tall fescue	Stem	12	1.54	0.47	0.29	0.05	2.35
Tall fescue	Stem	24	1.65	0.42	0.42	0.15	2.64
Tall fescue	Stem	48	1.81	0.96	0.56	0.18	3.52
Tall fescue	Flower	0	0.52	0.10	0.07	0.02	0.71
Tall fescue	Flower	6	1.06	0.42	0.26	0.04	1.78
Tall fescue	Flower	12	1.88	0.79	0.48	0.09	3.24
Tall fescue	Flower	24	2.19	0.89	0.53	0.11	3.73
Tall fescue	Flower	48	2.31	0.96	0.62	0.13	4.01
Yorkshire fog	Leaf	0	0.37	0.09	0.06	0.03	0.54
Yorkshire fog	Leaf	6	0.77	0.15	0.14	0.03	1.08
Yorkshire fog	Leaf	12	0.93	0.16	0.08	0.02	1.19
Yorkshire fog	Leaf	24	1.91	0.53	0.25	0.08	2.78
Yorkshire fog	Leaf	48	1.84	0.56	0.25	0.12	2.78
Yorkshire fog	Stem	0	0.32	0.08	0.04	0.02	0.46
Yorkshire fog	Stem	6	0.33	0.08	0.04	0.03	0.48
Yorkshire fog	Stem	12	0.92	0.29	0.17	0.04	1.42
Yorkshire fog	Stem	24	1.03	0.23	0.21	0.06	1.54
Yorkshire fog	Stem	48	1.99	0.36	0.37	0.17	2.90
Yorkshire fog	Flower	0	0.43	0.10	0.06	0.02	0.61
Yorkshire fog	Flower	6	0.98	0.22	0.19	0.05	1.45
Yorkshire fog	Flower	12	1.34	0.28	0.23	0.08	1.92
Yorkshire fog	Flower	24	0.84	0.17	0.22	0.09	1.32
Yorkshire fog	Flower	48	0.77	0.17	0.17	0.21	1.32

Phalaris	Leaf	0	0.52	0.05	0.05	0.04	0.65
Phalaris	Leaf	6	0.94	0.19	0.16	0.09	1.38
Phalaris	Leaf	12	1.30	0.27	0.23	0.10	1.90
Phalaris	Leaf	24	1.76	0.32	0.28	0.19	2.54
Phalaris	Leaf	48	1.54	0.26	0.26	0.16	2.21
Phalaris	Stem	0	0.52	0.05	0.05	0.04	0.66
Phalaris	Stem	6	0.83	0.18	0.12	0.04	1.17
Phalaris	Stem	12	1.90	0.16	0.19	0.06	2.31
Phalaris	Stem	24	1.40	0.19	0.15	0.00	1.74
Phalaris	Stem	48	1.26	0.44	0.29	0.25	2.24
Phalaris	Flower	0	0.55	0.05	0.05	0.04	0.69
Phalaris	Flower	6	1.13	0.35	0.22	0.08	1.78
Phalaris	Flower	12	1.50	0.24	0.26	0.14	2.14
Phalaris	Flower	24	2.16	0.29	0.34	0.18	2.98
Phalaris	Flower	48	1.58	0.25	0.27	0.15	2.25
Paspalum	Leaf	0	0.42	0.09	0.07	0.04	0.62
Paspalum	Leaf	6	1.01	0.21	0.16	0.05	1.43
Paspalum	Leaf	12	1.23	0.25	0.19	0.07	1.74
Paspalum	Leaf	24	0.79	0.14	0.12	0.02	1.08
Paspalum	Leaf	48	0.66	0.27	0.17	0.20	1.31
Paspalum	Stem	0	0.46	0.10	0.07	0.02	0.65
Paspalum	Stem	6	0.98	0.27	0.22	0.05	1.52
Paspalum	Stem	12	1.56	0.37	0.27	0.05	2.25
Paspalum	Stem	24	1.76	0.56	0.34	0.07	2.72
Paspalum	Stem	48	2.07	0.99	0.46	0.15	3.66
Paspalum	Flower	0	0.39	0.10	0.05	0.03	0.58
Paspalum	Flower	6	0.41	0.25	0.20	0.07	0.92
Paspalum	Flower	12	0.46	0.27	0.22	0.07	1.02
Paspalum	Flower	24	0.50	0.40	0.19	0.07	1.16
Paspalum	Flower	48	0.62	0.22	0.19	0.20	1.23

APPENDIX 8: *In sacco* data from Chapter 6.

TABLE 8.1A – Dry matter (DM) degradation characteristics (% of total DM) from fresh minced forages incubated in dacron bags in the rumen from individual fistulated cows fed full pasture and four silages supplements as defined by soluble (A), degradable insoluble (B), undegradable residue (C = 100 – A – B) as well as fractional disappearance rate (k, h^{-1}), and effective degradability (E) which takes in account the effect of passage from the rumen[1].

Cow	Diet	*In sacco*	A	B	C	k	E 6%	E 8%
5272	Pasture	Pasture	46	38	16	0.067	63	60
7915	Pasture	Pasture	47	36	17	0.077	64	61
5756	PM	Pasture	40	40	20	0.057	62	60
7912	PM	Pasture	38	41	21	0.079	67	64
3343	PMS	Pasture	47	40	13	0.066	64	61
3792	PMS	Pasture	45	39	16	0.071	64	61
3788	PS	Pasture	42	42	16	0.065	65	62
7926	PS	Pasture	41	39	21	0.078	65	62
5774	PSM	Pasture	43	38	19	0.086	65	62
7920	PSM	Pasture	43	42	15	0.058	64	61
5756	PM	PM	46	36	19	0.047	62	59
7912	PM	PM	43	36	20	0.062	62	59
3343	PMS	PMS	43	41	16	0.052	62	59
3792	PMS	PMS	39	47	14	0.045	59	56
3788	PS	PS	46	38	16	0.083	68	65
7926	PS	PS	46	36	18	0.085	67	65
5774	PSM	PSM	42	39	19	0.073	63	61
7920	PSM	PSM	46	38	16	0.045	62	59
		Average	44	39	17	0.067	64	61
	Model P		0.67	0.18		0.01		
	Forages P		0.67	0.063		0.004		
	Cow/diet P			0.31		0.17		
	Cow P			0.51		0.019		
	r^2		0.17	0.89		0.98		

[1] Passage rate set at 0.06 h^{-1} and 0.08 h^{-1}.

P: assessing goodness of fit for the overall model and tests (Forages: effect of diet incubated; Cow/diet: diet effects on forages *in sacco* and Cow P: cow effects.

TABLE 8.2A – Crude protein (CP) degradation characteristics (% of total DM) from fresh minced forages incubated in dacron bags in the rumen from individual fistulated cows fed full pasture and four silages supplements as defined by soluble (A), degradable insoluble (B), undegradable residue (C = 100 – A – B) as well as fractional disappearance rate (k, h^{-1}), and effective degradability (E) which takes in account the effect of passage from the rumen[1].

Cow	Diet	*In sacco*	A	B	C	k	E 2%	E 5%	E 8%
5272	Pasture	Pasture	56	37	7	0.104	87	81	77
7915	Pasture	Pasture	53	39	7	0.132	90	85	81
5756	PM	Pasture	54	35	11	0.136	87	82	78
7912	PM	Pasture	60	29	11	0.141	81	77	74
3343	PMS	Pasture	57	36	7	0.115	87	81	77
3792	PMS	Pasture	51	41	8	0.129	91	85	81
3788	PS	Pasture	58	33	9	0.156	85	81	78
7926	PS	Pasture	56	35	9	0.148	87	82	79
5774	PSM	Pasture	58	32	10	0.169	84	80	78
7920	PSM	Pasture	61	32	7	0.098	83	77	74
5756	PM	PM	62	27	10	0.093	85	80	77
7912	PM	PM	55	34	11	0.135	84	80	76
3343	PMS	PMS	60	32	8	0.099	86	81	77
3792	PMS	PMS	62	32	6	0.062	86	79	76
3788	PS	PS	67	28	5	0.080	89	84	81
7926	PS	PS	65	28	6	0.093	89	84	81
5774	PSM	PSM	65	26	9	0.124	88	84	81
7920	PSM	PSM	66	26	8	0.071	86	81	78
		Average	59	32	8	0.116	86	81	78
	Model P		0.0009	0.15		0.0675			
	Forages P		0.0009	0.0464		0.0204			
	Cow/diet P			0.17		0.52			
	Cow P			0.9		0.13			
	r^2		0.74	0.91		0.94			

Abbreviations see Table 7.1A.

TABLE 8.3A Neutral detergent fibre (NDF) degradation characteristics (% of total DM) from fresh minced forages incubated in dacron bags in the rumen from individual fistulated cows fed full pasture and four silages supplements as defined by soluble (A), degradable insoluble (B), undegradable residue (C = 100 – A – B) as well as fractional disappearance rate (k, h^{-1}), and effective degradability (E) which takes in account the effect of passage from the rumen[1].

Cow	Diet	*In sacco*	A	B	C	k	$E_{6\%}$	$E_{8\%}$
5272	Pasture	Pasture	26	48	26	0.056	44	41
7915	Pasture	Pasture	26	46	28	0.062	45	41
5756	PM	Pasture	17	53	30	0.039	42	38
7912	PM	Pasture	13	52	34	0.062	48	44
3343	PMS	Pasture	26	53	21	0.055	46	43
3792	PMS	Pasture	23	50	28	0.063	46	43
3788	PS	Pasture	20	55	25	0.049	46	42
7926	PS	Pasture	18	48	34	0.060	45	42
5774	PSM	Pasture	22	49	30	0.063	46	42
7920	PSM	Pasture	20	56	24	0.044	45	41
5756	PM	PM	14	54	32	0.035	34	31
7912	PM	PM	14	51	35	0.043	35	32
3343	PMS	PMS	15	60	25	0.036	37	33
3792	PMS	PMS	8	71	21	0.030	32	28
3788	PS	PS	26	47	28	0.064	50	46
7926	PS	PS	28	42	30	0.056	48	45
5774	PSM	PSM	10	56	34	0.058	38	34
7920	PSM	PSM	17	57	27	0.033	37	33
		Average	26	48	26	0.056	49	46
	Model P		0.0001	0.0696		0.0803		
	Forages P		<.0001	0.0152		0.0248		
	Cow/diet P		0.0038	0.3919		0.3389		
	Cow P			0.3947		0.1884		
	r^2		0.94	0.94		0.94		

Abbreviations see Table 7.1A.

TABLE 8.4A Acid detergent fibre (ADF) degradation characteristics (% of total DM) from fresh minced forages incubated in dacron bags in the rumen from individual fistulated cows fed full pasture and four silages supplements as defined by soluble (A), degradable insoluble (B), undegradable residue (C = 100 – A – B) as well as fractional disappearance rate (k, h^{-1}), and effective degradability (E) which takes in account the effect of passage from the rumen[1].

Cow	Diet	*In sacco*	A	B	C	k	$E_{6\%}$	$E_{8\%}$
5272	Pasture	Pasture	26	48	26	0.051	43	40
7915	Pasture	Pasture	28	44	29	0.056	42	39
5756	PM	Pasture	17	54	29	0.034	41	37
7912	PM	Pasture	13	52	35	0.059	47	43
3343	PMS	Pasture	26	52	22	0.053	45	42
3792	PMS	Pasture	25	47	28	0.056	44	41
3788	PS	Pasture	20	54	26	0.048	45	41
7926	PS	Pasture	18	47	36	0.056	44	40
5774	PSM	Pasture	22	47	31	0.062	45	42
7920	PSM	Pasture	20	56	24	0.041	44	40
5756	PM	PM	14	57	29	0.026	31	28
7912	PM	PM	12	51	37	0.039	32	29
3343	PMS	PMS	15	59	26	0.035	37	33
3792	PMS	PMS	9	72	19	0.026	31	27
3788	PS	PS	24	47	29	0.070	49	46
7926	PS	PS	26	42	31	0.062	48	45
5774	PSM	PSM	13	52	35	0.059	39	35
7920	PSM	PSM	18	55	27	0.031	37	33
		Average	19	52	29	0.048	42	38
	Model P		0.0001	0.1367		0.0290		
	Forages P		0.0002	0.0556		0.0085		
	Cow/diet P		0.0009	0.2384		0.1666		
	Cow P			0.3019		0.0825		
	r^2		0.94	0.91		0.960		

Abbreviations see Table 7.1A.

TABLE 8.5A – Particle size distribution of pasture, sulla and maize silages dry matter for *in sacco* incubations indicated by sieve aperture size either retaining or enabling material to pass.

	> 2mm	0.25-1mm	Fine
Pasture	35	40	25
Sulla silage	18	35	47
Maize silage	23	23	55
Average	25	33	42
STDEV	9	9	15

STDEV: standard deviation.

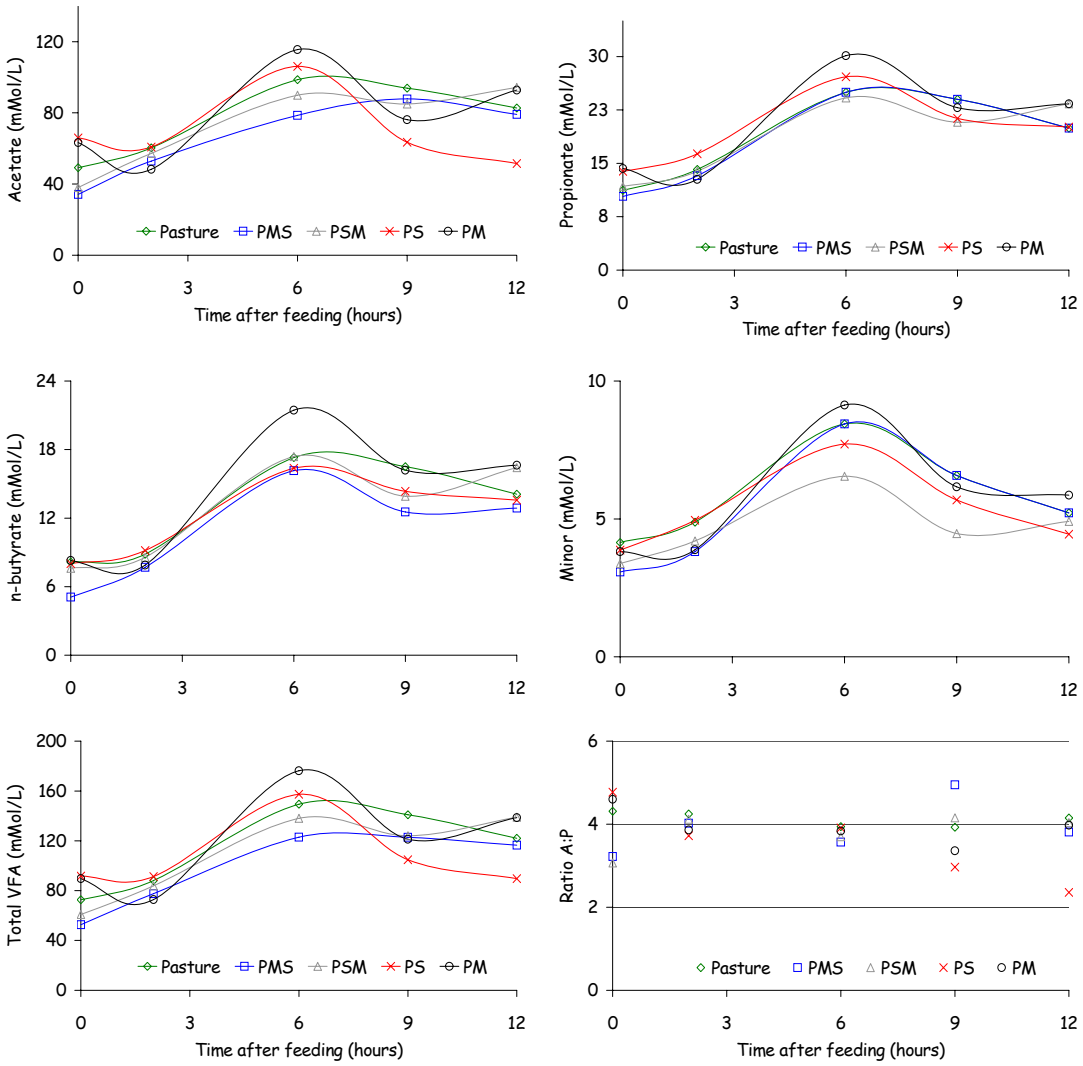


FIGURE 8.1A - Mean rumen fluid volatile fatty acids concentrations and acetate: propionate (A: P) ratios for cows fed pasture and pasture plus maize and sulla silages. Data on the X axis are times after AM feeding.

APPENDIX 9: A simplified method for lignin measurement in a range of forage species[1].

9.1A - Abstract

Lignin is the prime factor influencing the digestibility of plant cell wall material. As concentrations of lignin increase, digestibility, intake, and animal performance usually decrease. Presented is a simplified acid detergent lignin procedure which has been used to determine lignin concentration from a wide range of forages and also ryegrass at different stages of maturation. Forages used in this study included grasses, legumes, herbs and conserved material, with lignin concentration ranging from 2.02 to 21.1% of the DM. Legumes tended to have higher values than grasses, and ryegrass maturation was not accompanied by increased lignin concentration at 53 days of age. These results will be incorporated into a NIRS method for determining forage quality, and used in a dairy nutrition model to assist in ration formulation for dairy cows.

Keywords: fibre; forages; lignin; analytical method.

Short title: Lignin measurements in forages.

9.2A - Introduction

9.2.1A - Importance of lignin for ruminants

Lignin is a major anti-nutritional component of grasses. Lignin limits cell wall (fibre) digestion by providing a physical barrier to microbial attack and the concentration of both fibre and lignin increases as plants mature (Van Soest, 1978; Chaves *et al*., 2002). Ruminants can digest the cellulose and hemicellulose components of fibre but the lignin inhibits the rate and extent of digestion especially when the proportion of lignin in fibre begins to increase. Lignin precursors also have anti-microbial properties (Jung and Fahey, 1983). However it benefits plants by providing mechanical support, water impermeability and protection from insects. The consequences of lignification are summarised in Table 9.1A.

[1] Previous published in the *Proceedings of New Zealand Grassland Association*, 2002, 129-133. Best paper poster award at New Zealand Grasslands Conference 2002.

Abundant data show negative correlations between the lignin concentration in plants and both dry matter (DM) and fibre digestibility (Jung *et al*., 1997). Lignin is more prevalent in grass stem than leaf and absent from legume leaves. In contrast to grasses, there are minimal ferulate cross linkages between lignin and hemicellulose in legume stems (Hatfield *et al.*, 1999), so the lignin concentration is less detrimental to nutritive value of legumes, compared to grasses. This was demonstrated by more rapid degradation of legume fibre than grass fibre (Jung *et al*., 1997).

A good understanding of fibre, its composition, degradation and effects on energy availability is essential for maintaining a nutrient supply to high producing animals. The rate of digestion, breakdown by chewing and clearance from the rumen determine feed intake, and rumen capacity is likely to limit future increases in productivity from ryegrass based pasture (Waghorn, 2002). Flowering is associated with significant reductions in intake.

There are several chemical classifications of fibre, usually based on sequential removal of constituents by boiling in detergents or acids. Plant residues remaining after boiling in neutral detergent are not available for ruminant absorption unless they are degraded by microbial fermentation. The neutral detergent fibre (NDF; the residue after boiling in a neutral detergent) comprises hemicellulose, cellulose, lignin and ash, commonly referred to as the cell wall or fibre fraction of plants. Digestion of NDF in an acid detergent removes hemicellulose leaving the acid detergent fibre (ADF) fraction, and a further digestion in 12 M sulphuric acid removes cellulose leaving lignin and ash. Lignin is determined after the mineral (ash) fraction is measured by ashing.

Measuring lignin is complicated by the extensive cross linkages with cellulose and hemicellulose and the insolubility of this polymer, (Hatfield *et al*., 1994). A variety of methods have been developed to estimate lignin with the acid detergent lignin (ADL) procedure (Van Soest, 1967) usually employed for forage analysis.

This paper presents a method, which was adapted from Goering and Van Soest (1970) to simplify lignin measurements, together with data from a wide range of forages and also ryegrass of increasing maturity. These data will be incorporated into a NIRS (Near Infra Red Spectrometer) method for determining forage quality, enabling improved predictions of animal performance and formulation of mixed forage rations for dairy cows to avoid current limitations of rumen fill.

9.3A – Material and methods

9.3.1A - Measurement procedure

Lignin is usually determined as part of a sequential extraction comprising three refluxing and filtering steps; firstly with neutral detergent followed by acid detergent and lastly 12 M H_2SO_4 digestion to leave lignin and ash residues. The standard ADL determination uses about 1 g dried material, requires condensing and refluxing apparatus including 600 mL Berzelius beakers for boiling and 50 ml fritted glass crucibles (coarse porosity) to retain residues after refluxing. The main modifications of the ADL procedure of Goering and Van Soest (1970), presented here, are the removal of the neutral detergent fibre step and a reduction in the quantity of material required for analysis. Screw capped culture tubes (16 x 100 mm) have been used for digestion in both acid detergent and 12 M H_2SO_4 to obtain an acid-insoluble residue lignin and ash residue. Glass microfibre filters (Watman 55 mm GF/C) were used for collecting fibre residues from the digestion tubes after the final digestion and for ashing. The modified technique is presented in Table 9.2A.

This technique was tested to determine variability and precision and used to determine lignin concentration in a wide range of forage samples.

9.3.1.1A - Repeatability

Replicate analysis is the primary means of evaluating data variability or precision. The coefficient of variation (CV) was used as a measure of repeatability:

$$CV\ (\%) = (SD/\mu) \times 100$$

Where SD = sample standard deviation and μ = mean of replicate analyses.

Precision was monitored by a reference standard (ryegrass/white clover pasture) analyzed with every batch of 20 forage samples. Standard deviation (SD) was calculated from repeated analysis of control samples and duplicates of the forage samples.

The modified ADL method has been used to determine the lignin concentration of a range of forage types and also ryegrass of increasing (21 to 105 days) maturity. These data were required for model prediction of forage mixed rations for high producing dairy cows.

9.4A - Results

The technique developed for lignin measurements (Table 9.2A) resulted in good repeatability for a wide range of forage types (Table 9.3A). In most cases the CV was less than 5%. It is however important to be aware that the condensed tannins present in some forages have been shown in this laboratory to comprise part of the lignin fraction after acid digestion. True lignin will be calculated from 12 M sulphuric acid residue less the values for ash and tannins determined by a separate analysis.

The forages used for the lignin assay (Table 9.3A) are used in farming systems and were part of a systematic study of feeding values by Burke *et al*. (2000) and Chaves *et al*. (2001). All grasses except those in the "maturation" trial were leafy and vegetative, and had similar concentrations of lignin (about 3% of DM) except for cocksfoot and paspalum. Lignin concentration in clover and lucerne were higher than most of grasses but the values for Lotus species were elevated by the presence of tannins. Most surprising were the high values for lignin in chicory and plantain, both of which have very low fibre concentrations (Burke *et al*., 2000).

The concentration of lignin in ryegrass increased as the grass aged, and was strongly correlated with the decline in organic matter digestibility (Figure 9.1A; $r^2 = 0.90$). Lignin and NDF concentration increased simultaneously, but the relative rate of change showed lignin increased more rapidly than NDF. Mature ryegrass was digested much more slowly by rumen microflora after mincing (*in sacco*) than young ryegrass (Figure 9.1A; Chaves *et al*., 2002).

9.5A - Discussion

The data presented here show that a rapid lignin assay, based on fewer steps and smaller quantities of samples and reagents (compared to Goering and Van Soest, 1970) can give good repeatability. Analysis of diverse forage samples confirms the generally lower values in grasses than legumes, show higher values when ensiled and slow increases in concentration with ryegrass maturation.

Although the increases in lignin and NDF concentration in ryegrass correspond to slower *in sacco* degradation (Burke *et al*., 2000; Chaves *et al*., 2001), there was no increase in lignin concentration for the first 53 days of re-growth (Table 9.3A) when most grazing would take place. This suggests that either structural changes in the non lignin fibre components were responsible for slower degradation or that cross linkages between lignin and hemicellulose were responsible (Figure 9.2A). These changes cannot be detected by analyses of lignin concentration. Lignin concentration is not

the sole determinant of fibre degradation rate of ryegrass (Inoue *et al*., 1989) and the linkages with fibre constituents (Ralph *et al*., 1995; Wilson and Hatfield, 1997) have a major impact on fibre degradation. Other factors affecting fibre degradation include the extent of chewing (Waghorn, 2002), diet composition, especially inclusion of readily fermentable substrates, level of feeding and rumen pH. Future improvements in the nutritive value of ryegrass must also reduce the extent or effectiveness of cross linkages between lignin, hemicellulose and cellulose in manner that enables plants to retain their structural integrity. These changes may involve biotechnology and the identification of the brown midrib mutant of corn, with substantial higher digestibility (Barrière *et al*., 1992), provides an incentive for investigation. Casler (1997) demonstrated that a 20 year plant breeding effort could theoretically result in a new cultivar (for most perennial forage species) with an *in vitro* digestibility up to 10% higher than the original population.

The simplified procedure for lignin analysis and future prediction by NIRS (Corson *et al*., 1999) will improve estimation of feeding value for fresh and ensiled forages. The principal driver for this procedure was a need for including lignin values in the CNCPS (Cornell dairy nutrition) model to assist in ration formulation for dairy cows.

TABLE 9.1A - Consequences of lignification.

- Required by the plant to maintain structural integrity
- Lignin can be a significant component of grass leaves, stems, sheaths and legume stems
- Lignin concentration is higher in C4 grasses than C3 grasses
- Lignin concentration is higher in legumes than grasses
- Lignin concentrations can be dependent upon analytical technique, with Klason lignin values being 2 - 4 times as high as acid detergent lignin for grasses and 30 - 50% higher for legumes
- Lignification is especially intense in schlerenchyma and vascular tissues which form long indigestible fibres
- Lignin is not degradable by bacteria
- Slows rate of intake, digestion and increases the need for chewing and salivation
- Lignin is impermeable to bacteria; cell wall rupture is a prerequisite to digestion, from the cell lumen
- Limited rupture during eating limits initial rate of degradation
- Continual damage to cell walls by chewing increases surface area of polysaccharide for colonisation by fibrolytic bacteria
- Passage from the rumen (clearance) is a prerequisite for further intake and non degradable cell walls must be chewed to a size able to pass a 1 or 2 mm sieve aperture for sheep and cattle respectively
- Within feed types (e.g. temperate grasses) lignin concentration is poorly correlated with digestibility (*in vivo* and/or *in vitro*)
- Linkages via ferulic and p-coumeric acids between core lignin and cell wall polysaccharides appear to have a major impact on feeding value and are more important than concentration *per se*.
- Dietary lignin is determined by proportions of legume and grass, maturity and selection of leaf, stem and sheath constituents

- Limitations attributable to lignin apply to all forage fed animals
- The nutritive value of tropical grasses are further reduced by urinary nitrogen losses associated with excretion of metabolised phenolics
- The impact of lignin can be especially severe when animals have been selected for high genetic merit under good feeding regimens (e.g. total mixed ratio) and then fed pasture
- Dairy cattle are affected to a large extent by lignification, because the demands for milk production exceed capacity to provide nutrients from pasture. Consequences include significant bodyweight loss, failure to ovulate and rapid declines in productivity
- Solutions include selecting animals with more effective chewing, larger rumen capacity, ability to allow larger particles to escape from the rumen
- Breeders may modify lignin to enable easier cell rupture, less binding to polysaccharides, fewer lignified tissues or identify genotypes with brittle fibres more easily broken to pass from the rumen
- Lignin is the principal limitation to production from forages

TABLE 9.2A - Modified procedures for the determination of acid detergent lignin (ADL). (Procedure to obtain acid detergent fibre (ADF) for ADL analysis)

1. Dry 16 mL glass tubes at 100°C for at least an hour.
2. Weigh tubes to 4 or 5 decimal places in groups of 10 at a time[2].
3. Dry finely ground samples at 60°C and weigh about 250 mg into weighed tubes. (Grinding was carried out in a Wiley mill with a 1 mm screen (Mertens, 1992a)).
4. Add 10 mL acid detergent fibre solution (2% cetyl trimethylammonium bromide in 1 litre 0.5M H_2SO_4) to the sample. Put cap on tube. Vortex.
5. Reflux over a steady heat (water bath) for one hour at 95 - 100°C, vortex every 10 minutes.
6. Centrifuge for 10 minutes at 3000 rpm and remove supernatant by suction.
7. Add 15 mL hot distilled water to residue; vortex. Centrifuge for 10 minutes at 3000 rpm and discard supernatant. Repeat this step 3 times.
8. Add 15 mL acetone to the residue. Vortex. Centrifuge for 10 minutes at 3000 rpm and discard supernatant. Repeat this step 1 more time.
9. Evaporate residual acetone in water bath at 60°C.
10. Dry tubes at 90°C overnight.
11. Weigh tubes and residue using hot weighing technique to 4 or 5 decimal places, calculate ADF (Mertens, 1992a).

[2] When 24 tubes (or GMF) were removed from 105°C in a single batch, weight of first tube did not change over the time the 24th tube were weighed (~5 minutes) so can remove more than 10 at once.
[2] Include 8 additional GMF blanks with each batch of 22 forage samples to determine ashing losses from GMF.

(ADL procedure)

A protective mask MUST be worn when handling 12M H_2SO_4 in this assay.

12. Label glass microfibre filters (GMF) with marker pen (both sides), dry at 100°C over night and weigh 10 tubes at a time[1] to 5 decimal places.

13. Add 1.5 mL 12 M H_2SO_4 to tubes containing residues (in fume cupboard) and digest at 30°C for 60 minutes mixing carefully every 10 minutes.

14. Following digestion the acid-insoluble residue was collected by filtration using 45 mm Buchner funnels with pre-weighed 55 mm Whatman GF/C glass microfibre filters. An extensive washing with water and a final acetone rinse (twice) was used prior to drying the samples overnight at 100°C.

15. Weigh the filters and residue to 5 decimal places.

16. Ash at 450°C for 6 hours. Weigh GMF with ash to 5 decimal places.

17. ADL was determined as the difference in weight of the residue before

TABLE 9.3A - Comparative ADL concentration (% of DM) of different feeds.

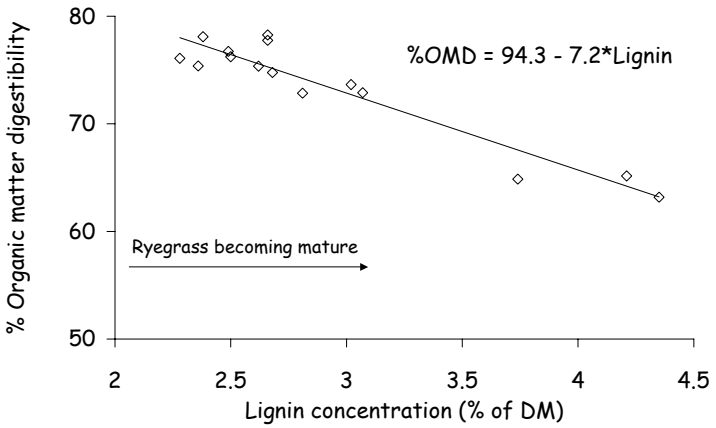
Feed	ADL[1] %	CV %
Fresh grasses		
Lolium perenne (Perennial ryegrass AR1[2])	2.92	1.14
Holcus lanatus (Yorkshire fog)	3.13	2.20
Lolium perenne (Perennial ryegrass Nil Endophyte)	3.38	2.23
Bromus willdenowii (Prairie grass)	3.78	0.92
Festuca arundinacea (Tall fescue)	3.78	2.97
Pennisetum clandestinum (Kikuyu)	3.82	2.05
Dactylis glomerata (Cocksfoot)	5.13	2.72
Paspalum dilatatum (Paspalum)	6.85	2.52
Legumes and herbs		
Trifolium repens (White clover)	5.87	2.04
Medicago sativa (Lucerne)	6.12	3.34
Trifolium pratense (Red Clover)	6.23	12.53
Cichorium intybus (Chicory)	6.97	6.96
Lotus corniculatus (Birdsfoot trefoil)	7.22[3]	4.40
Lotus pedunculatus (Lotus major)	16.97[3]	3.48
Plantago lanceolata (Plantain)	21.10	0.72
Conserved		
Zea maize (Maize grain)	2.02	2.72
Avena sativa (Oat silage)	4.29	4.03
Lolium perenne (Perennial ryegrass silage)	4.31	4.56
Zea maize (Maize silage)	4.36	3.87
Zea maize (Maize silage)	4.95	1.31
Medicago sativa (Lucerne silage)	7.33	0.06
Medicago sativa (Lucerne hay)	8.04	0.40
Perennial ryegrass maturation		
Perennial ryegrass 21 days	2.65	4.54
Perennial ryegrass 31 days	2.38	5.94
Perennial ryegrass 53 days	2.51	3.10
Perennial ryegrass 74 days	2.63	0.27
Perennial ryegrass 88 days	3.02	
Perennial ryegrass 105 days	4.35	
Pasture reference standard	5.34	3.78
Pasture reference standard	5.78	2.33

[1]Mean of values.

[2]AR1, AgResearch cultivar with endophyte (*Neotyphodium lolii)* selection for high in peramine but low in other key alkaloids.

[3]Lignin includes condensed tannins.

FIGURE 9.1A - Relationships between lignin, estimated organic matter digestibility (% OMD) of maturing perennial ryegrass. OMD and lignin concentration in the dry matter (DM) (r^2 = 0.90) and *in sacco* DM disappearance of ryegrass harvested at five maturation ages (P > 0.05).



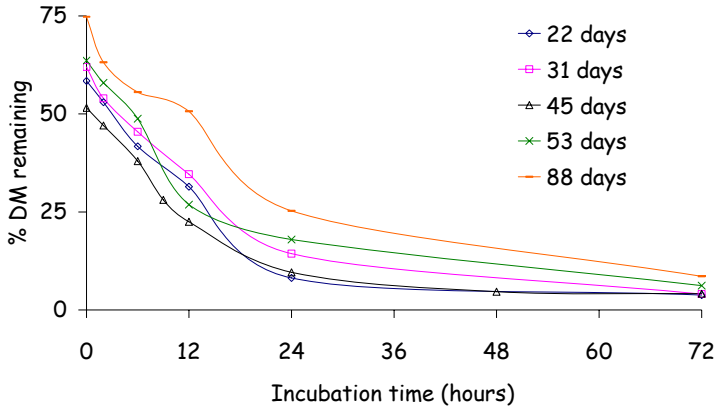
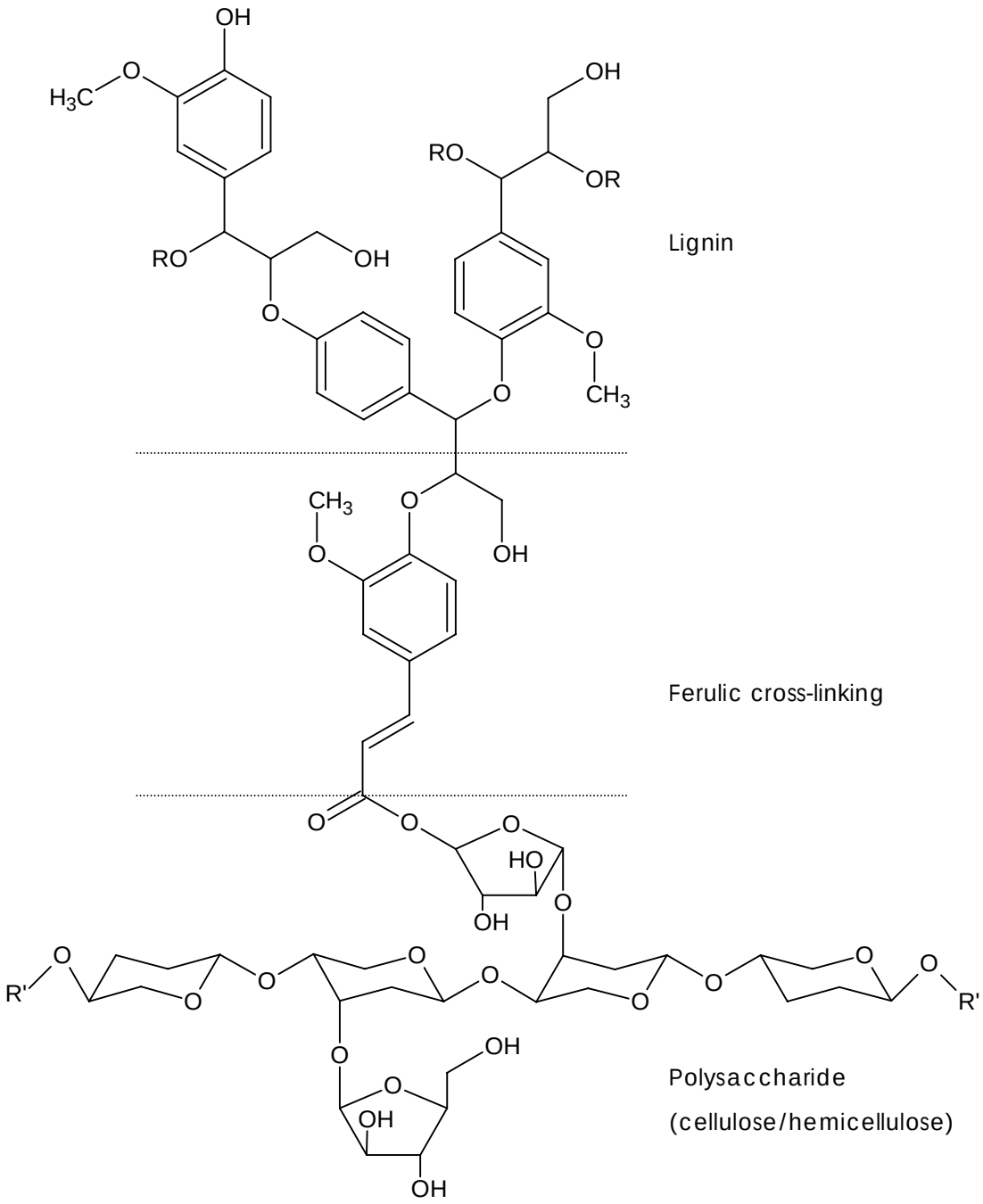


FIGURE 9.2A - Lignin-carbohydrate complex showing ferulate crosslinkages.



Adapted from Chesson (1988).

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