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Milk Production from Kikuyu

(Pennisetum clandestinum)

Grass Pastures

by

Megan Reeves

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

Department of Animal Science
The University of Sydney
Camden NSW 2570

NSW Agriculture
Wollongbar Agricultural Institute
Bruxner Highway
Wollongbar NSW 2477

Fac. of Vet. Sc.

July 1997
Declaration

I certify that the contents of this thesis have not been submitted in any previous application for a degree or currently being submitted for another degree elsewhere.

Megan Reeves
July 1997
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Summary

The tropical grass kikuyu (*Pennisetum clandestinum*) is an important pasture species grazed by dairy cows in various coastal regions of Australia and throughout the world. Under favourable conditions, its high DM yields can support high stocking rates and milk production per hectare. However, under present management, the quality of kikuyu forage is generally low and consequently, milk production per cow is also low. The studies reported in this thesis aimed to develop a management system for kikuyu grass pasture that would optimise forage quality, in terms of cow requirements and hence, milk production per cow. The initial strategy was to monitor changes in the quality and agronomic characteristics of kikuyu with respect to time of day, regrowth, season and fertiliser application in order to identify criteria on which appropriate pasture management practices could be based. A comparison of the nutrients in well-managed kikuyu and ryegrass was made to establish inherent nutrient deficiencies in this species limiting milk production. Supplementation strategies then aimed to overcome the nutrient limitations of kikuyu pasture for milk production.

In the introduction (Chapter 1), the problems associated with dairy production from kikuyu grass pastures are outlined. A review of literature follows (Chapter 2), which examines agronomic characteristics of kikuyu and the milk production obtained in previous studies from cows grazing kikuyu pastures with or without supplementation. The general methods, common to more than one study in this thesis, are outlined in Chapter 3, while specific methods are described in the relevant experimental chapters.

Chapter 4 examined the stage of regrowth phenologically, in terms of leaves per tiller in relation to changes in plant components and key nutrients. The proportion of green leaf available above the 5cm stubble height declined while the proportion of stem and dead material increased markedly after 4.5 leaves per tiller appeared. Associated with the decline in green leaf, there was a substantial decline in herbage organic matter digestibility (OMD) and crude protein (CP) content of samples above 5cm stubble height. The nitrogen (N), phosphorus (P) and potassium (K) concentration of individual leaves declined; calcium (Ca) and magnesium (Mg) increased and sodium (Na)
concentrations remained constant with regrowth time, and this was reflected in total herbage above the 5cm stubble. The results of these studies indicated that the most desirable stage of regrowth to graze lactating dairy cows, in terms of quality, was 4.5 leaves per tiller. The effect of regrowth time on nutrient concentration was consistent, but there were seasonal differences in absolute concentrations, with higher concentrations of OMD, K and P in the early season, whereas, Ca and Mg and nitrate contents were highest late in the season.

A distinct within-day variation in water soluble carbohydrate (WSC) concentrations in kikuyu were observed. WSC increased at a rate of about 5g/kg DM per hour, reaching maximum concentrations during mid-afternoon. Concentrations remained constant until dark and then declined through the night reaching a minimum before sunrise.

In Chapter 5, the effect of application of N on the regrowth of kikuyu was examined. Although N application increased the leaf appearance rate, this was not significant. High rates of N application (≥ 50kg N/ha.month) appear undesirable as they tended to promote stem elongation and increase nitrate concentrations above moderate application rates (3.12 v 0.67 g/kg dry matter (DM), for application of 100 v 25 kg N/month, respectively). Concentrations of plant nitrate increased linearly when N content exceeded 36.8 g/kg DM (equivalent to 230g CP/kg DM). Although this concentration of nitrate would not be expected to be toxic to cattle, it is probably sufficient to reduce microbial activity, as excess nitrates are converted to the more toxic nitrite. A within-day variation in non-protein nitrogen (NPN) was apparent with minimal concentrations in mid-afternoon, corresponding with maximum WSC content, indicating grazing at this time of the day would optimise the WSC:NPN ratio, in terms of rumen microbial requirements.

In Chapter 6, samples of kikuyu and perennial ryegrass plucked to simulate forage grazed by milking cows, were obtained in the midst of the growing season for each species and the nutritive value compared. Kikuyu contained significantly lower (P<0.01) concentrations of OMD (0.733 v 0.842) than ryegrass but higher concentrations of neutral detergent fibre (NDF) (600 v 400 g/kg DM) and this would presumably be a
major factor limiting DM intake (DMI) and lower milk production from kikuyu. The relatively high concentrations of CP and low concentrations of WSC in kikuyu, compared with ryegrass, resulted in a much lower WSC to CP ratio (0.36 v 0.9, respectively) and would be expected to contribute to reduced microbial activity in the rumen. The concentration of methionine and cysteine, considered to be the most limiting amino acids in cows grazing ryegrass pastures, were 68 and 57% lower in kikuyu than ryegrass. Furthermore, only 71% of the N in kikuyu was in the amino acid form, compared to 92% in ryegrass. Kikuyu contained less Ca than ryegrass (3.1 v 5.9 g/kg DM), and much of this would be bound to oxalates (the oxalic acid concentrations in kikuyu and ryegrass were 6.8 v 1.2 g/kg DM, respectively) and therefore largely unavailable to grazing stock. The extremely low Na concentration of kikuyu compared with ryegrass (0.2 v 3.7 g/kg DM, respectively), is well below recommended concentrations for milking cows, particularly during the hot summer months, when requirements rise substantially.

An accurate estimate of intake of kikuyu was vital to explain the responses to supplementation and grazing management practices on milk production. In Chapter 7, the accuracy and precision of the relatively new alkane technique used to predict the DMI of individual dairy cows grazing kikuyu pasture, was examined. Analysis showed that kikuyu contained sufficient concentrations of appropriate chain length alkanes to use this technique, with mean (± standard error of mean (s.e.)) concentrations of 132 (2.3), 8 (0.2), 198 (3.9), 100 (4.4) and 4 (0.1) mg/kg DM for C₃₁, C₃₂, C₃₃, C₃₅ and C₃₆, respectively. In milking cows, the actual DMI and in vivo digestibility of harvested kikuyu were compared with estimates obtained from using dosed-even chain, and naturally occurring odd-chain alkanes present in kikuyu pasture. Concentrations of dosed alkanes (C₃₂ and C₃₆) and the ratios of dosed- and naturally-occurring adjacent alkane pairs (C₃₂/C₃₁, C₃₂/C₃₃ and C₃₆/C₃₅) reached a steady state 6 days after placing controlled release devices (CRD) containing alkanes in the rumen. The mean (± s.e.) faecal recovery rates (%) of C₃₁, C₃₂, C₃₃, C₃₅ and C₃₆ were 93.6 (2.7), 94.2 (4.9), 98.6 (2.8), 107.3 (3.1) and 90.5 (5.3), respectively. The discrepancies between actual and estimated DMI using alkane pairs ranged from -2.58 to 1.77 kg DM/cow.day. Overall, the C₃₂/C₃₁ and C₃₂/C₃₃ alkane pairs gave the most accurate estimate of DMI as these
alkane pairs had similar faecal recovery rates. The actual in vivo dry matter digestibility (DMD) of kikuyu was 0.633, and the closest estimate was 0.631 obtained using the naturally-occurring C_{35} alkane. These results indicate that the C_{32}/C_{31} or C_{32}/C_{33} alkane pairs should be used to estimate DMI, and the naturally-occurring C_{35} to estimate DMD in stock grazing kikuyu pastures.

Chapter 8 compared the precision of estimating the DMI of cows grazing kikuyu pasture fed 0, 3 or 6 kg of cereal-based concentrate/cow.day using either the alkane technique, a rising plate meter (RPM), or calculated from feeding standards of known requirements for maintenance, production and liveweight change (RS).

In Study 1, herbage DMI estimates obtained using the RPM and RS techniques over a 45 day period were compared. RS estimates were based on the metabolisable energy (ME) of the components of the ration derived from in vitro OMD values. Calibration equations for the RPM were determined at 2-weekly intervals and were either used as such, or pooled if they were not significantly different (P<0.01). Estimates of total DMI were lower using the RPM than the RS technique for unsupplemented cows grazing kikuyu (12.5 v 14.8 kg DM/cow.day) or fed 3 kg (10.4 v 12.9 kg DM/cow.day) of concentrate/cow.day, and higher for those receiving 6 kg/cow.day (10.5 v 7.8 kg DM/cow.day).

In Study 2 (12 days duration), herbage DMI derived using the alkane technique was compared with those estimated using the RPM and RS techniques. The C_{32}/C_{31} alkane pair gave the closest estimate of herbage intake compared to that obtained using RPM and RS techniques. Whole diet in vivo DMD (mean 0.700), determined by the alkane method, was similar for cows in all 3 groups, suggesting that digestibility of the kikuyu declined with increasing concentrate supplementation. In confirmation of this, the use of the in vivo DMD estimate of kikuyu (0.695) rather than the OMD determined in vitro (0.639), in RS calculations, resulted in intake estimates being reduced by 17%. The RS intake estimates for the unsupplemented cows grazing kikuyu aligned closely to predictions using the RPM and alkane techniques (12.3 v 13.5 and 12.6 kg DM/cow.day, respectively). The inclusion of concentrates in the diet lowered herbage DMI estimates
using the RS technique to a greater extent than RPM and alkane methods. This was most evident at the 6 kg rate of supplementation where kikuyu DMI estimated using RS (in vivo-derived DMD) was substantially lower than using either RPM or alkane techniques (6.5 v 12.4 and 9.2 kg DM/cow.day, respectively). These results are in line with the hypothesis that carbohydrate-based concentrate substantially lowers the digestibility of the basal forage, in this case kikuyu grass. The alkane technique provided a direct and precise method of measuring the intake of individual cows grazing well-managed kikuyu pastures. The RS technique can provide reasonable pasture DMI estimates if measurements are taken over an extended period (>12 d) to provide accurate liveweight data, and provided accurate animal production and feed quality parameters are available, although often many assumptions are required using this technique. The RPM technique is useful for obtaining herd estimates of pasture intake and for the determination of pasture parameters associated with intake.

The production response of cows grazing well-managed kikuyu pastures to supplementation with a cereal grain-based concentrate, with and without the inclusion of formaldehyde-treated protein meal, was examined in Chapter 9. Study 1 was a 3-farmlet study conducted over 45 days involving cows 5-6 months into lactation, fed 0 (R₀), 3 (R₃) and 6 (R₆) kg crushed barley/cow.day. Study 2 was an extension of Study 1, with animals 7 months into lactation. The concentrate fed was 72% barley and 24% formaldehyde-treated sunflower meal.

Mean milk yields (litres (L)/cow.day) for cows in Study 1 were 14.2, 18.3 and 18.0, and in Study 2 were 12.5, 18.5 and 17.4 for treatments R₀, R₃ and R₆, respectively. Milk fat (3.77 v 3.26%), but not protein, content of R₀ cows was significantly higher (P<0.05) than R₆ cows only in Study 1. No significant liveweight changes were detected, and therefore results indicate actual production responses from kikuyu grass.

Study 3 was a 3 x 4 factorial design plus a ‘control’ group (0.5 kg barley/cow.day used as a carrier for minerals) to examine the milk production response to 3 rates of concentrate feeding (3, 6 and 9 kg/cow.day) with 4 levels of formaldehyde-treated canola meal (FTCM; 0, 20, 40 and 60% of basal barley replaced).
isoenergetic within rates of concentrate fed. Cows were 3-4 months into lactation. Cows in the ‘control’ group had significantly lower (P<0.001) milk production (17.2 L/cow.day), milk protein (2.90%), plasma urea (PU) (5.90 mmol/L) and β-hydroxy butyrate (β-OHB) (0.525 mmol/L) than cows in the other treatment groups. The mean milk production response of 0.6 L milk/kg concentrate fed in Study 3 when feeding 3 kg/day, was lower than observed in Studies 1 and 2 (1.4 and 2.0 L/kg concentrate, respectively), and this may be due in part to the higher digestibility of pastures in Study 3. The concentration of ME, but not protein, in the concentrate in Study 3 had a significant influence (P<0.001) on milk production, milk fat and milk protein concentrations. Plasma glucose (P<0.05) and β-OHB (P<0.01) concentrations significantly increased with the incorporation of FTCM into the concentrate. Non-esterified fatty acid concentrations dropped significantly (P<0.05) below the concentrations of other treatments when the inclusion of FTCM was lowest. PU concentrations generally increased in response to increasing ME and FTCM concentrations in the concentrate, with an interaction between them.

Milk urea (MU) (mmol/L) concentrations showed a significant linear relationship to PU concentrations (mmol/L) as follows:

\[ MU = 0.167 + 0.272PU \]  \( (r^2 = 0.44; P<0.001) \).

Chapter 10 sought to explain the apparent decline in digestibility of the basal kikuyu forage when cows were supplemented with cereal-based concentrates. The effect of feeding buffers with concentrates was examined using 6 cannulated Hereford steers in a three period, three treatment cross-over design, in which steers grazed well-managed kikuyu and were fed 0, 2.5 (with buffers) or 2.5 (without buffers) kg barley/steer.day. In terms of intake and rumen capacity, these levels of intake were considered to be equivalent to intakes of 0, 3 and 6 kg/day in milking Friesian cows. Kikuyu DMI, determined using the alkane technique, was significantly higher (P<0.001) for steers fed kikuyu alone (4.3 kg DM), compared to those fed barley, with or without buffer inclusion. Between the concentrate groups, steers receiving buffer ate significantly more (P<0.001) kikuyu than those without buffer (3.74 v 3.42 kg DM), with a lower estimated rate of substitution of pasture for concentrate (0.26 v 0.40 kg DM kikuyu/kg DM barley).
As observed in Chapter 8, the whole diet DMD of steers fed kikuyu alone was significantly higher (P<0.05) than those fed concentrates (with or without buffer), indicating a fall in kikuyu digestibility with concentrate input. However, a significant decline in kikuyu degradation in sacco, was only observed in steers fed concentrate without buffer. Rumen pH concentrations of steers grazing kikuyu alone were significantly higher (P<0.001) than those fed concentrates with buffer, which in turn were higher than those without buffer. These results indicate that a reduction in rumen pH may have partially contributed to reduced kikuyu DMD. Treatment did not affect the rate of change of rumen pH post-feeding or digesta flow rates.

The studies reported in this thesis indicate that kikuyu pasture should be grazed when 4.5 leaves per tiller are visible. This stage of regrowth precedes a substantial reduction in forage quality resulting from stem elongation and leaf senescence. At this stage of regrowth, the concentrations of OMD, N, K, Ca, Mg and the WSC:CP ratio, are more appropriate for milk production. Even when kikuyu pastures are managed in a way which optimises nutrients in terms of cow requirements, milk production per cow (without liveweight change) is still restricted to 15-16 L/cow.day. However, this is well above the 12 L/cow.day considered to be the potential from other C4 tropical grasses. The most likely nutrients to be limiting are ME, NDF and the minerals Ca, Na, P and Mg. The importance of ME is indicated by the excellent response to the first 3kg of an energy-based supplement, raising milk yields to 18-19 L/cow.day. The inclusion of buffers in the diet is advisable when feeding rapidly degrading energy-based supplements to prevent a large decline in rumen pH, which is detrimental to kikuyu degradability.
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The effect of barley supplementation on the rumen degradability of dietary components and rumen pH, and the effect of buffer inclusion, in steers grazing kikuyu pastures

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<th>Definition</th>
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<tbody>
<tr>
<td>ADF</td>
<td>acid detergent fibre</td>
</tr>
<tr>
<td>AMZ</td>
<td>Australian milking zebu</td>
</tr>
<tr>
<td>AN</td>
<td>ammonium nitrate</td>
</tr>
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<td>AR</td>
<td>analytical reagent</td>
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<td>cm</td>
<td>centimetres</td>
</tr>
<tr>
<td>Co</td>
<td>cobalt</td>
</tr>
<tr>
<td>CP</td>
<td>crude protein (CP = N x 6.25)</td>
</tr>
<tr>
<td>CRD</td>
<td>controlled release device</td>
</tr>
<tr>
<td>Cu</td>
<td>copper</td>
</tr>
<tr>
<td>DGL</td>
<td>dry green leaf</td>
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<td>DOMD</td>
<td>digestible organic matter in dry matter</td>
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<td>DMD</td>
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<td>ethylenediaminetetra-acetic acid</td>
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<td>Fe</td>
<td>iron</td>
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<td>FCM</td>
<td>fat corrected milk</td>
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<td>formaldehyde-treated cottonseed meal</td>
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<td>GLC</td>
<td>gas liquid chromatograph</td>
</tr>
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<td>h</td>
<td>hours</td>
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<td>ha</td>
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<td>kilograms</td>
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<td>LAN</td>
<td>limestone ammonium nitrate</td>
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<td>M &amp; R</td>
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<td>ME</td>
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<td>neutral detergent fibre</td>
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<tr>
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<tr>
<td>RDP</td>
<td>rumen degradable protein</td>
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<tr>
<td>RGL</td>
<td>residual green leaf</td>
</tr>
<tr>
<td>RPM</td>
<td>rising plate meter</td>
</tr>
<tr>
<td>RS</td>
<td>energy standards in reverse</td>
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</table>
S - sulfur
sec - seconds
SBM - soyabean meal
SCM - solids corrected milk
Se - selenium
s.e. - standard error of mean
SN - sodium nitrate
SS - single superphosphate
TEA - triethylamine
TNC - total non-structural carbohydrates
U - urea
UDP - rumen undegradable protein
WSC - water soluble carbohydrates
Zn - zinc
β-OHB - β-hydroxy butyrate
μg - micrograms
μl - microlitres
Chapter 1
Introduction

Kikuyu (*Pennisetum clandestinum* Hochst. ex. Chiov.) was introduced into Australia from the Belgian Congo in 1919, became naturalised and is now a prominent pasture species in coastal New South Wales (NSW), Queensland and the irrigated regions in the south of Western Australia. The ingression of kikuyu into coastal NSW has been substantial and it now occupies 30% of the total milking area of the state and it is estimated that 70% of milk production in summer is derived from kikuyu pastures (Anon. 1992). Its presence is significant on 1200 dairy farms in NSW and the introduction of seeding varieties (eg. Whittet) has ensured that kikuyu is readily dispersed, and difficult to eradicate.

Very few studies (Colman and Kaiser 1974; Hughes *et al.* 1988; Henning *et al.* 1995) have examined the use of kikuyu specifically for dairy production, although it has sometimes been used as the base pasture in supplementation studies with dairy cattle (Ashwood and Kellaway 1982; Ashes and Hamilton 1983; Hamilton *et al.* 1992). However, various agronomic studies on kikuyu have been conducted in Hawaii (Whitney 1974a; Whitney 1974b), South Africa (Cross 1979a; Cross 1979b; Dugmore *et al.* 1986; Dugmore and du Toit 1988), and Australia (Mears 1970; Jeffery 1971; Drummond 1975; Awad *et al.* 1976; Awad and Edwards 1977; Colman and O’Neill 1978; Awad *et al.* 1979; Cook and Mulder 1984a).

Under favourable temperature and moisture conditions, kikuyu dry matter (DM) production can be extremely high (up to 30 000 kg DM/hectare (ha) per annum (Colman and O’Neill 1978)). However, under these conditions of virtually unrestricted kikuyu growth, the quality of the pasture on offer\(^1\) is generally low due to a high proportion of low quality stem, compared with higher quality leaf material. In addition, the mat of kikuyu formed facilitates a high wastage of leaf material, in terms of utilisation by cows, through senescence.

\(^1\) Throughout thesis, on offer refers to the herbage mass available to cows pre-grazing.
<table>
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<tr>
<th>Reference</th>
<th>Milk Fat (g/kg milk)</th>
<th>Milk Protein (g/kg milk)</th>
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<td>full lactation</td>
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<th>Reference</th>
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<th>Kikuyu pasture parameters</th>
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<td>mid-lactation</td>
<td>DOMD&lt;sup&gt;g&lt;/sup&gt; 0.60</td>
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<td>Olney and Albertson (1984)</td>
<td>9.1</td>
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<td>-</td>
<td>Friesian</td>
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<td>grab sample DMD&lt;sup&gt;c&lt;/sup&gt; 0.678</td>
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<td>6.0</td>
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<td>Friesian/Guernsey</td>
<td>various</td>
<td>CP&lt;sup&gt;h&lt;/sup&gt; 150 DMD&lt;sup&gt;c&lt;/sup&gt; 0.550 green leaf DM&lt;sup&gt;a&lt;/sup&gt; availability of 800 kg/ha</td>
<td>Rotational grazing - 1 day per paddock</td>
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<td>43.5 range 42.0 - 45.0</td>
<td>32.5 range 31.3 - 33.4</td>
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<td>CP&lt;sup&gt;h&lt;/sup&gt; 140 DMD&lt;sup&gt;c&lt;/sup&gt; 0.550 green leaf DM&lt;sup&gt;a&lt;/sup&gt; availability of 1200 kg/ha</td>
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<tr>
<td>Hamilton et al. (1992)</td>
<td>14.7</td>
<td>36.2</td>
<td>27.9</td>
<td>Friesian</td>
<td>1-3 months</td>
<td>pluck sample CP&lt;sup&gt;h&lt;/sup&gt; 156 OMD&lt;sup&gt;d&lt;/sup&gt; 0.66 mean green leaf on offer 2440 kg/ha</td>
<td>Strip grazing</td>
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<td>Henning et al. (1995)</td>
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<td>37.0</td>
<td>32.0</td>
<td>Friesian</td>
<td>various</td>
<td>OF&lt;sup&gt;e&lt;/sup&gt; sample DOMD&lt;sup&gt;d&lt;/sup&gt; 0.57 2 cows/ha</td>
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<td>34.0</td>
<td>32.0</td>
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<td>Rotational grazing - 4 day grazing</td>
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<sup>a</sup>DM = Dry Matter; <sup>b</sup>CP = Crude Protein; <sup>c</sup>DMD = Dry Matter Digestibility; <sup>d</sup>DGL = Dry Green Leaf; <sup>e</sup>RGL = Residual Green Leaf; <sup>f</sup>DOMD = Digestible Organic Matter in Dry Matter; <sup>g</sup>OMD = Organic Matter Digestibility; <sup>h</sup>OF = Sample from Oesophageally Fistulated animals.
The majority of experiments have found milk production from kikuyu grass pastures to be below 11 kg/cow.day, with the exception of Cross (1979b) and Hamilton et al. (1992) who reported yields of 15.3 and 14.7 kg/cow.day, respectively. However, in the studies of Cross (1979b) production was only predicted (involving a variety of assumptions) from results of a trial conducted in 1949, and therefore, these yields were not actually recorded. The high yields recorded by Hamilton et al. (1992) were from cows much earlier in lactation than those in other reports, and the genetic potential of the animals also was not indicated.

*Milk production from kikuyu pastures, on a per cow basis, is low and dependant on both the quantity and quality of forage DM on offer, and these aspects of kikuyu are discussed in the following review.*

### 2.3 Characteristics of Kikuyu Grass Pastures

Kikuyu is a C₄ tropical grass species which originated in the highlands of Kenya. In subtropical regions, kikuyu growth is very vigorous during the warm and moist summer/autumn period, but declines to low levels over the cool winter months. The annual change in growth rate of kikuyu, compared to perennial ryegrass/white clover pastures, under grazing conditions, at Wollongbar on the north coast of NSW are illustrated in Figure 2.1. Similar growth rate patterns were reported by Murtagh and Moore (1987).

There are 4 varieties of kikuyu available in Australia; *Common* is a mixture of 3 distinct ecotypes (Molo, Rongai and Kabete) originating from Kenya (Quinlan et al. 1975) and forms a dense sward, is non-seeding and propagates by runners; *Breakwell* is a free-seeding type with smaller leaves and flatter growth than other varieties, more suited to soil conservation than dairy pastures; *Whittet* is a free-seeding and highly productive variety which is taller, with broader leaves than the *Common* variety and is the variety most commonly sown; and *Noonan* which is shorter and has lower yields than *Whittet* (B. Clarke, personal communication) and is the only variety displaying some resistance to the kikuyu yellows (*Verrucalvus flavofaciens*) fungal infection. The *Common* and
Whittet varieties are likely to be most prevalent in dairy pastures of Australia.

Figure 2.1 Mean monthly growth rates (kg DM/ha.day) of dryland kikuyu and irrigated ryegrass at Wollongbar Agricultural Institute (W. J. Fulkerson.) (standard error of mean (s.e.) bars are indicated as vertical lines).

2.3.1 Morphology

The kikuyu plant is highly stoloniferous and rhizomatous and roots strongly at the nodes (Figure 2.2), allowing it to spread rapidly throughout an area, often invading other pasture/cropping areas if unchecked.

Figure 2.2 The morphology of the kikuyu plant (source: O'Reilly 1981).
This colonising characteristic of kikuyu grass makes it ideal for the control of soil erosion. However, the vigorous nature of kikuyu growth over the summer/autumn period in a subtropical environment, generally precludes its co-existence with legumes (Mears 1970).

The morphology of a pasture plant influences both its availability and nutritional value for a grazing cow. Tropical grasses tend to grow taller than their temperate counterparts, and hence, require greater amounts of stem material to maintain erectness, which in turn, lowers leaf density. The growing habit of kikuyu is less erect than many other tropical species such as *Digitaria decumbens* (Pangola grass) and *Cenchrus ciliaris* (Buffel grass), therefore, its comparatively higher leaf density should allow greater intake per bite by stock (Stobbs 1973). It would be expected that as kikuyu matures, associated stem elongation would reduce leaf density, and presumably reduce intake per bite, therefore increasing the time and energy required by the animal to harvest leaf material.

Similarly, the leaf:stem ratio has a marked effect on ingestive behaviour (Hodgson 1982), as grazing animals tend to select more leaf, and less stem than present in the total herbage *on offer* (Chacon and Stobbs 1976). Such behaviour is presumably associated with the ease of comminution (ie. mechanical breakdown) of leaves compared to stems, which in turn is related to structural strength and the shear force required to chew the herbage. Therefore, offering stock kikuyu pasture containing a high leaf:stem ratio should lower the comminution energy required, and hence enhance intake.

*The vigour and morphology of the kikuyu plant often precludes its co-existence with pastures of higher quality, and in such cases is best managed as a monosward. Management practices should aim to prevent the accumulation of large amounts of stem material in the sward, since the quality of stem is lower than leaf and also, to minimise the amount of energy required by the grazing animal to harvest kikuyu DM.*

### 2.3.2 Anatomy and Physiology

Differences in the leaf anatomy of tropical grasses, such as kikuyu, and temperate grass species can be related to biological and physiological differences between plants (Norton
Tropical and subtropical grasses utilise both the Hatch-Slack (C₄) photosynthetic pathway, and the Calvin (C₃) cycle for carbon fixation. This combination results in more efficient photosynthesis and water and nitrogen (N) use, under conditions of high radiation or temperature, compared to species which use only the C₃ cycle (ie. legumes and temperature grasses). Consequently, C₄ pasture grasses tolerate higher temperatures and require a higher temperature for optimal growth. Figure 2.3 illustrates the differences in the leaf anatomy of tropical and temperate grass species.

Figure 2.3 The leaf anatomy of tropical and temperate grass species (source: Norton 1982).

The C₄ pathway is distinguished in the leaf anatomy by a radial arrangement of chlorenchyma cells (bundle sheath) around vascular bundles, structural (granal or agranal) and size dimorphism of chloroplast in the bundle sheath and surrounding mesophyll cells and the presence of more and larger mitochondria in the bundle sheath cells than found in mesophyll cells (Laetsch 1974).

*The higher proportion of bundle sheath and vascular tissue in both leaf and stem, and less of the thin walled mesophyll cells in tropicales compared to temperates, render them more resistant to mechanical and microbial breakdown in the rumen. This results in increased retention time in the rumen, restricting voluntary intake, and consequently production, by the grazing animal (Norton 1982).*
2.3.3 Chemical Composition of Kikuyu and its Nutritional Value for Dairy Cows

To obtain optimal levels of production, the nutrient content of pasture must satisfy the requirements of the grazing animal. If these requirements are not met, production will be sub-optimal and supplementation may be required to enhance production to desired levels. The nutrient values for kikuyu and the factors affecting their concentration, are outlined below.

2.3.3.1 Dry Matter

Low forage DM content may negatively affect the intake of grazing cows when grazing young, actively growing pasture (Meissner et al. 1992), and would presumably restrict nutrient intake and consequently, milk production. The moisture content can be reduced by *topping* the pasture and allowing it to wilt prior to grazing. However, this technique is labour intensive and there has been no comparison of intake levels between this method and regular grazing. In addition, wilting would be expected to reduce the concentrations of soluble carbohydrates in the forage, offsetting any gain from increased DM.

In kikuyu, reported concentrations of DM range from 13% in young pasture (Marais et al. 1990) to 61.2% in mature plants experiencing drought conditions (Gomide et al. 1969a). Gomide et al. (1969a) and Said (1971) observed that DM content increased with plant maturity, indicating that intake levels have the potential to rise as kikuyu pasture matures. *The optimal stage of maturity to graze kikuyu has not yet been determined.* However, the fact that the DM content of temperate grasses is lower than kikuyu, indicates that DM content is unlikely to be a key factor in lower productivity.

2.3.3.2 Organic Matter

The reported organic matter (OM) concentrations of kikuyu range from 866 (Said 1971) to 907 (Dugmore and du Toit 1988) g/kg DM. Minson (1973) found the OM content of kikuyu was affected by N fertilisation. Kikuyu fertilised with low rates of N had significantly higher OM than that receiving high N (901 v 882 g/kg DM, respectively). Although only a small difference, these results suggest application of N increases growth, but not necessarily mineral uptake resulting in a dilution effect on OM.
The OM content of herbage can be reduced with soil contamination. However, in dairy systems, kikuyu is rarely grazed to ground level due an impenetrable stoloniferous mat at the base of the canopy.

2.3.3.3 Protein

a. Protein Requirements of Dairy Cattle

Dietary protein must satisfy both the requirements of the cow, and the microbe population in the rumen of the cow. The protein requirement of a dairy cow fluctuates according to her physiological state. When the animal is experiencing a balanced nutrient status, there is only a relatively small increase in protein requirements per MJ energy, as milk production increases. However, when in negative energy balance (eg. early lactation) dietary protein requirements increase substantially as the animal mobilises body reserves liberating proportionally higher levels of energy compared to protein (Orskov 1982).

In the cow, protein is obtained either directly from dietary sources which have escaped rumen fermentation (rumen undegradable protein (UDP)), or from microbes which pass from the rumen into the small intestine. Feeds also contain rumen degradable protein (RDP), the extent of which depends on intrinsic qualities of the feed and the rumen environment (Kellaway and Porta 1993). Dietary non-protein nitrogen (NPN) can also yield microbial protein in the rumen and is often considered to be part of the RDP component of the diet. Jones et al. (1996) stated that the quantity of amino acids absorbed from the small intestine is dependant on the following;

- rumen degradability of dietary protein
- the extent of microbial protein synthesis in the rumen
- the amino acid content and intestinal digestibility of undegraded and microbial protein.

Once the protein requirements of microbes for maintenance and synthesis are satisfied, excess RDP is converted into ammonia and excreted as urea in urine, and to a lesser
extent in milk (Figure 2.4). This conversion of ammonia to urea and its subsequent excretion, involves both a protein and energy cost to the animal. Although the UDP content of pasture may limit milk production, RDP is rarely limiting when pastures are in the vegetative state, as the protein present is generally high and very degradable.

Jones et al. (1996) provided an overview and comparison of various protein modelling systems (INRA 1978, 1989; ARC 1980, 1984; Hulme et al. 1986; NRC 1989; AFRC 1992, 1993) which can be used to predict the protein (and energy) requirements of dairy cattle. The models were observed to differ mainly in methods used for calculating the energy required for rumen fermentation, protein degradation in the rumen and protein digestibility in the small intestine. Most of these systems evidently require information on the rumen degradability of protein in feedstuffs. Few degradability studies have been undertaken on kikuyu. As a basic guide, the requirements for crude protein (CP) as
recommended by NRC (1989) for a 600 kg Friesian cow producing 20 kg of milk per
day (assuming the cow achieves NRC predicted DM intakes) of 150 g/kg DM have been
compared to reported CP concentrations of kikuyu pasture in Table 2.2. Whether or not
these CP concentrations actually satisfy cow requirements is dependant on the factors
mentioned above.

b. Factors Affecting the Protein Content of Kikuyu

The CP of a feedstuff can be separated into true protein (TP) and non-protein N (NPN)
fractions. The NPN fraction contains ammonium salts, nitrates, free amino acids,
peptides, heterocyclic compounds (including nucleic acids) and lignified nitrogenous
substances, some of which are incorporated into rumen microbial protein. Tropical
grasses contain lower concentrations of CP than their temperate counterparts due to their
use of the C₄ pathway for photosynthesis, the high proportion of stem in the plant, and
large vascular bundles (Wilson and Minson 1980). Reported concentrations of the CP
content of kikuyu range from 62 g/kg DM in plants not fertilised with N (Jeffery 1971),
to 378 g/kg DM (Rees and Little 1980) in N fertilised kikuyu (see Table 2.2). These
concentrations of N and the response to N fertiliser application in kikuyu are high
compared to other tropical grass species. Various studies have found that the N content
of kikuyu varies depending on plant maturity, plant component, fertiliser application and
season, and the effects of these parameters are outlined below.

(i) Plant Maturity

Minson (1990) reported the CP content in the kikuyu plant peaked 2 weeks after
fertilisation and then declined rapidly due to increased growth rates. Kikuyu can
maintain comparatively high concentrations of CP (above 12%) after the initial rapid
decline, compared to other tropical grass species (Milford and Haydock 1965; Gomide
et al. 1969a; Said 1971; Wilson and Haydock 1971; Aii and Stobbs 1980; Dugmore et
al. 1986). Ware (1978) found that the CP content of kikuyu declined to below 10% after
9-10 weeks of regrowth as stem and dead material accumulated.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Crude Protein (g/kg DM)</th>
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<th>ME (MJ/kg DM)</th>
<th>Fibre (g/kg DM)</th>
<th>Pasture Parameters</th>
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\(^{a}\) DMD: Dry Matter Digestibility

\(^{b}\) OMD: Organic Matter Digestibility

\(^{c}\) CF: Carbohydrate Fraction

\(^{d}\) ADF: Acid Detergent Fibre

\(^{e}\) NDF: Neutral Detergent Fibre

\(^{f}\) NPK: Nitrogen, Phosphorus, Potassium

\(^{g}\) NL: Nitrogen, Lime

\(^{h}\) AS: Azotobacter Subtilis
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<td>150-600N over season</td>
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<td>-</td>
<td>0.71(^f)</td>
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<td>mean</td>
<td>-</td>
<td>0.65(^f)</td>
<td>-</td>
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<tr>
<td></td>
<td>mean</td>
<td>-</td>
<td>0.72(^f)</td>
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<td>Crude Protein (g/kg DM)</td>
<td>Digestibility In vitro</td>
<td>Digestibility In vivo</td>
<td>ME(^{1}) (MJ/kg DM)</td>
<td>Fibre (g/kg DM)</td>
</tr>
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<td>DMD(^{a})</td>
<td>OMD(^{b})</td>
<td>DMD(^{a})</td>
<td>OMD(^{b})</td>
<td>CF(^{c})</td>
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<td>mean 101</td>
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<td>Crude Protein (g/kg DM)</td>
<td>Digestibility</td>
<td>ME* (MJ/kg DM)</td>
<td>Fibre (g/kg DM)</td>
<td>Pasture Parameters</td>
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<td>Plant part and cutting height (cm)</td>
<td>Age of regrowth (weeks)</td>
</tr>
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<td>OMD^b</td>
<td>DMD^a</td>
<td>OMD^b</td>
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<td>-</td>
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<td>0.53</td>
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<tr>
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<td>mean 119</td>
<td>-</td>
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<td>-</td>
</tr>
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<td>mean 115</td>
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<tr>
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<td>Crude Protein (g/kg DM)</td>
<td>Digestibility</td>
<td>ME (MJ/kg DM)</td>
<td>Fibre (g/kg DM)</td>
<td>Pasture Parameters</td>
<td></td>
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<td></td>
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<td>In vivo</td>
<td>CF</td>
<td>ADF</td>
<td>NDF</td>
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<td>-</td>
<td>10.6</td>
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<td>-</td>
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<td>0.66</td>
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<td>Marais et al. (1992)</td>
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<td>-</td>
<td>0.71</td>
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</tr>
<tr>
<td>mean</td>
<td>0.58</td>
<td>-</td>
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<td>-</td>
<td>7.8/10.6</td>
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<td>-</td>
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<td>-</td>
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<td>Jackson et al. (1996)</td>
<td>mean</td>
<td>166</td>
<td>0.67</td>
<td>-</td>
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</table>

* reported as IVD, presumed values to represent DMD; ^1 calculated from SCA (1990) equations; ME=0.17*DMD% - 2.0 or ME=0.16*OMD% - 1.8 when not reported. ^2 DMD - Dry Matter Digestibility; ^3 OMD - Organic Matter Digestibility; ^4 CF - Crude Fibre; ^5 ADF - Acid Detergent Fibre; ^6 NDF - Neutral Detergent Fibre; ^7 AS - Ammonium Sulphate; ^8 U - Urea; ^9 AN - Ammonium Nitrate; ^10 NPK - Nitrogen, Phosphorus and Potassium; ^11 NL - Nitrolime; ^12 SN - Sodium Nitrate; ^13 SS - Single Superphosphate; ^14 LAN - Limestone Ammonium Nitrate; ^15 OF - sample from oesophageally fistulated animal.
(ii) Plant Component

In a glasshouse study, Wilson and Sandland (1976) found that the N content of kikuyu DM above ground level declined as plant size increased, however, the N content of new leaves in kikuyu remained high. This suggests that the decline in quality was due to a change in plant components rather than a decline in leaf quality, and this is supported by studies showing that green leaf material contains higher concentrations of CP than the stem (mean concentrations of 176 v 120 g/kg DM, respectively) (Drummond 1975; ’t Mannetje 1975; Forde et al. 1976; Taylor et al. 1976; Marais et al. 1987 & 1992; Marais 1990a; Dugmore et al. 1991). In addition, the ratio of protein to NPN in plant parts differ. Marais (1990a) found that 77% of the CP in leaves was protein N compared to only 59% in the stem. Additionally, in kikuyu plants containing very high concentrations of N, the NPN portion in the stem was as high as 52% of the CP.

These findings indicate that pasture management strategies should aim to maximise the leaf:stem ratio of kikuyu on offer to grazing animals to obtain benefits from the higher concentrations of CP in leaf material, compared to stem.

(iii) Season

Seasonal variation in CP concentrations have been reported in regularly grazed and fertilised kikuyu swards with peaks in the autumn, possibly due to accumulation of N as growth rates slow (Marais et al. 1987; Dugmore and du Toit 1988). Similarly, in cutting trials, Whitney (1974a) found that the CP content of kikuyu tended to be higher during cooler temperatures, particularly in younger regrowth. In contrast, Rethman and de Witt (1988) found that the CP of kikuyu declined through the growing season with concentrations of 119, 110 and 77 g/kg DM in early summer, late summer and autumn, respectively and Awad et al. (1979) found that CP fell rapidly from mid-autumn to a minimum concentration in mid-winter. In a trial examining kikuyu over the winter months, Hughes et al. (1988) found that the CP content of kikuyu tended to decline over winter into spring. However, it is difficult to compare and interpret seasonal changes in kikuyu CP concentrations as they are influenced by N fertilisation regimes and climatic variations.
(iv) N Fertilisation

There is a high response in kikuyu CP to application of N fertiliser (Gomide et al. 1969a; Mears 1970; Jeffery 1971; Minson 1973; Whitney 1974a; Whitney 1974b; Tainton et al. 1982; Dugmore et al. 1986; Rumball 1991). Gomide et al. (1969a) observed that the CP response to N input was greatest early in the regrowth period compared to later (5.6 v 2.4 % difference between 0 and 200 kg N/ha at 4 and 36 weeks regrowth, respectively).

N is lost from pastures through leaching from the soil as $\text{NO}_3^-$ and volatilisation of fertiliser, faecal and urinary N into the atmosphere as $\text{NH}_3$. McKenzie and Tainton (1993) found levels of volatilisation from N fertiliser applied to kikuyu pastures was relatively low (3.7% of applied N) compared to temperate grass species (Ryden 1986) and levels were insignificant in practice.

The application of N fertiliser is an essential aspect of management if kikuyu is grown as a mono-culture without a legume.

c. Components of Protein

In South Africa, Dugmore and du Toit (1988) found the NPN component of kikuyu ranged from 4.1 to 10.5 g/kg DM with a mean of 7.8 g/kg DM. These workers also found a negative relationship between NPN and DM intake of steers. The most likely inhibitory factor in the NPN fraction of kikuyu is nitrate. Nitrates play an important role as an intermediate in plant metabolism where they become integrated into amino acids and other organic nitrogenous compounds. During this process nitrate is converted to nitrite, which is subsequently converted to ammonia ($\text{NH}_3$) by the action of nitrate and nitrite reductase, respectively. Nitrate accumulation in plant material occurs when uptake exceeds assimilation (conversion to plant protein) of nitrates. This imbalance may be due to any factors which inhibit growth other than protein deficiency. Thus, during periods of rapid growth, when temperature and moisture are favourable, the nitrate is rapidly assimilated and therefore its content in the plant is low (Marais et al. 1987). As growth rates decline, perhaps due to moisture or other stressors, the requirements for organic N are lowered, and there is a rapid increase in plant nitrate.
Deficiencies in calcium (Ca), potassium (K), magnesium (Mg), iron (Fe), manganese (Mn) and molybdenum (Mo) have all been shown to increase nitrate concentrations (Hewit 1971).

The nitrate concentration in kikuyu has been shown to be related to N content (Marais et al. 1990) and therefore, application of N fertiliser (Whitney 1974b; Dugmore et al. 1986). In fact, the proportion of nitrate in the plant rises rapidly following the application of N fertiliser and then declines at a rate which is dependent on the quantity of fertiliser applied (Minson 1990).

Nitrate absorption, translocation and accumulation in kikuyu is also enhanced by high concentrations of soil K (Marais et al. 1987) as it acts as a counter ion for N uptake in the plant, which results in a concomitant rise in plant K concentrations (Marais 1990a). The overall nutrient status of the soil needs consideration, since low concentrations of soil sulfur (S) and Mo have also been found to favour nitrate accumulation in plant tissue (McClure and Hunter 1983).

Nitrate concentrations are also dependant on the physiological state of the plant in terms of stage of development and the portion of plant under examination. Whitney (1974b) found that the concentration of nitrate was highest in 2 week-old kikuyu regrowth and tended to decline to 10 weeks of regrowth. In contrast, Marais et al. (1987) found lower nitrate concentrations in rapidly growing young leaves compared to more mature leaves which have much slower growth rates. These workers also found that the stem of kikuyu is the main nitrate storage organ. In another study, Marais et al. (1990) found that leaf nitrate concentrations did not exceed 4.5 g/kg DM (mean 1.7 g/kg DM) whilst stem nitrate concentrations reached up to 13 g/kg DM (mean 6.8 g/kg DM).

A possible diurnal variation of nitrate in kikuyu has not yet been reported in kikuyu pastures, however, it is common practice to feed high nitrate forage in the afternoon in the belief that nitrate concentrations will be lowest as the plant has used most nitrates to synthesise protein. A higher nitrate accumulation in the stem, compared to leaf, is another reason why the leaf:stem ratio should be maximised.
d. N Solubility

Stobbs et al. (1977) postulated that despite high concentrations of CP in N fertilised kikuyu, protein may still be a factor limiting animal production. These workers postulated that if a large proportion of plant N is in a soluble form it would be rapidly deaminated in the rumen to NH₃ and lost, reducing the effective protein content of the pasture. In support of this, Marais et al. (1990) found that rumen NH₃ concentrations in sheep grazing kikuyu grass containing 22.5% CP was twice that of those grazing kikuyu containing 16.9% CP. However, it could be argued that the deamination of NH₃ and subsequent loss may be due to a shortage in the diet of readily fermentable energy, causing inefficient N fixation into microbial protein. Thus, the diet could be considered energy deficient, but is exhibited by the animal as a shortage in protein supply.

High CP content may not automatically mean high N solubility levels. Despite containing the highest concentrations of CP of the tropical pasture species examined by Aii and Stobbs (1980), the N solubility of kikuyu (35.1%) was intermediate between Bracharia mutica (Brachiara) and Setaria anceps (Setaria) which had N solubilities of 43.3 and 26.3%, respectively. These workers found that the N solubility of kikuyu did not alter between 2-6 weeks of regrowth and also confirmed that stem N solubility was significantly higher than that of the leaf (66 v 24%, respectively).

e. CP Digestibility

The digestibility of CP has been found to decline with plant maturity. Said (1971) found that whilst CP concentrations declined 38% from 237 to 148 g/kg DM between 5 and 11 weeks old regrowth, the whole tract digestibility of CP (DCP) fell by a greater extent (50%) from 185 to 92 g/kg DM. The decreased availability of CP with plant maturity, may be associated with an increase in the number and thickening of cell walls, making it more difficult for microbes to degrade the material. However, the CP digestibility in sheep fed ad lib was higher (by 12%) for older (by 3 weeks) compared to younger, growth (Joyce 1974), although the actual age of regrowth was not detailed in this study.

The apparent degradability of N in the rumen (determined using in sacco techniques)
was much lower in older, stoloniferous material compared to young leaves in kikuyu (Hart 1984). These workers found levels of degradability in kikuyu forage were higher than for paspalum or oats (81 v 73%, respectively). Jeffery (1971) and Dugmore et al. (1986) found there was a significant positive relationship between the CP content of kikuyu and the digestibility of CP.

f. N and DMD in Kikuyu
Positive and negative relationships between kikuyu digestibility and N content have been reported in the literature. For example, Minson (1973) found no relationship between N and dry matter digestibility (DMD), whilst Dugmore et al. (1986) found CP concentrations were negatively correlated with the digestibility of both fibre and nitrogen free extract (NFE) fractions. Since these latter fractions constitute a large proportion of the kikuyu plant (Dugmore and du Toit 1988), the overall digestibility of the plant would be expected to decline. Marais (1990a) found that after the extraction of soluble substances from plant material, high concentrations of protein were associated with high digestibility and postulated that the negative effect of a soluble inhibitor (eg. nitrate) may actually conceal a positive effect of protein on digestibility.

Stage of regrowth may be responsible for these variable results. Drummond (1975) found the DMD of unfertilised (CP 167 g/kg DM) kikuyu grass was only slightly lower than that in fertilised (CP 226 g/kg DM) after 3 weeks of regrowth. However, after 6 and 9 weeks of regrowth, DMD of fertilised kikuyu dropped well below that of that in unfertilised. Additionally, after 6 weeks, the CP of the fertilised pasture also fell below that of the unfertilised grass, suggesting altered digestibility may be associated with the form of N present in the plant.

Pasture management can markedly affect the CP content of kikuyu and its nutritional value to grazing cows through control of plant maturity, leaf content and N fertiliser application. A high proportion of leaf and a high CP content in a kikuyu sward can be obtained from kikuyu in a young, vegetative state providing there is an adequate rate of N fertiliser application.
2.3.3.4 Digestible Energy

The energy content of a feed is usually expressed in terms of metabolisable energy (ME) which can be calculated from digestibility using established equations (SCA 1990). Digestibility is generally determined by either in vitro or in vivo techniques and reported concentrations in kikuyu are presented Table 2.2, with ME values calculated (if not already), using equations of SCA (1990). Unfortunately, only Sriskandarajah et al. (1980) reported digestible organic matter in dry matter (DOMD) (range 0.56-0.64), which is a more accurate measure of digestible energy as it accounts for the concentration of OM in DM.

a. Dry Matter Digestibility

The reported in vitro DMD concentrations in kikuyu range from 0.50 (Royal and Hughes 1976) to 0.74 ('t Mannetje 1975), with a similar range reported for in vivo DMD determination (0.48 (Jeffery 1971) to 0.74 (Marais et al. 1992)). Calculated ME values rarely exceed the NRC (1989) recommendations for lactating cows, indicating that the energy content of kikuyu presents a substantial limitation to milk production.

The DMD generally varied with stage of maturity and plant part. Said (1971) observed that the apparent in vivo DM digestion coefficient of kikuyu declined from 0.72 to 0.63 as plant age increased from 5 to 11 weeks. However, in a study by Joyce (1974), the DMD of kikuyu did not vary when the age of regrowth varied by 3 weeks. The initial age of the kikuyu was not indicated and may have been younger than that in the previous study, and therefore DMD may have been greater earlier in the regrowth period.

The reported DMD of kikuyu leaf was generally higher than stem (Taylor et al. 1976; Reid and Stevenson 1983; Pearson et al. 1985; Marais 1990a; Marais et al. 1992). However, 't Mannetje (1975) found the reverse in 4 week old regrowth. This may be related to stage of regrowth as some reports indicate that at an immature stage of regrowth, the DMD of leaf and stem are similar, but the stem DMD is generally much lower as the pasture matures (Minson 1990).
b. Organic Matter Digestibility

A wide range of OMD have been reported for kikuyu with \textit{in vitro} OMD ranging from 0.52 (Pastrana \textit{et al.} 1990) to 0.67 (Jackson \textit{et al.} 1996), and \textit{in vivo} OMD in the range of 0.54 to 0.73 reported by Jeffery (1971). As in the case of DM, the mean apparent OM digestion coefficients decline with increasing plant maturity (Said 1971). The addition of N fertiliser to kikuyu did not appear to have a substantial effect on OMD (Jeffery 1971). Studies are being undertaken to introduce the Brown mid-rib gene into kikuyu (D. Luckett, personal communication) which is linked to the gene for improved digestibility.

The relatively low energy concentrations of kikuyu appear to be a major constraint to milk production from kikuyu pastures. However, the literature indicates a very wide variation in ME concentrations, based on stage of maturity and plant component, indicating improvement may be attained through plant breeding or pasture management.

2.3.3.5 Fibre

Dietary fibre is negatively correlated to digestibility, and therefore, the energy content of a feed. However, a certain amount of dietary fibre is required otherwise chewing time is reduced, resulting in a decline in saliva secretion, rumen fluid pH and in the acetate:propionate ratio of the rumen fluid. These changes cause a disruption to normal rumen fermentation and a subsequent reduction in DM intake and milk fat concentrations (NRC 1989).

Dietary fibre is normally expressed as crude fibre (CF), acid detergent fibre (ADF) or neutral detergent fibre (NDF), and the reported concentrations of these constituents in kikuyu are shown in Table 2.2. However, ADF and NDF are considered to be more accurate measures of the fibre component of feed than CF (NRC 1989).

CF, ADF and NDF contain varying concentrations of cellulose and lignin, with or without hemicellulose. Cellulose is a structural carbohydrate contributing mostly to the rigidity and strength of plant structures, which is 100% digestible in its pure form,
although degradation is reduced when it is bound within plant tissue. Hemicellulose is bound into the cell wall and protected by coatings of lignin, and when isolated has a digestibility of 0.9. However, within plant tissue, the digestibility of hemicellulose declines to around 0.7 (Pearson and Ison 1989), whilst lignin (which is not a structural carbohydrate) is virtually indigestible (Minson 1990).

As expected, the cellulose (Gomide et al. 1969a; Whitney 1974a) and lignin (Whitney 1974a) content of kikuyu increases with plant age, to provide strength to support new growth. The increase in lignin with maturity may also be associated with increased proportions of stem material, since the stem fraction contains higher concentrations of lignin than leaf material (Marais 1990a).

Findings by Whitney (1974a) suggest that under high N fertilisation, kikuyu undergoes rapid vegetative growth (which is indicated by the low concentrations of cellulose and lignin at 2 weeks of regrowth in high, compared with low, N application treatments), followed by lignification and development of stem tissue (indicated by comparatively high concentrations of lignin at 10 weeks). These findings suggest that kikuyu fertilised with high rates of N should be grazed regularly to minimise stem elongation and fibre content of the forage on offer. However, Dugmore et al. (1986) did not find a significant difference in lignin or ADF concentrations due to N fertilisation.

a. Crude Fibre

CF represents the fibre fraction which is resistant to degradation in acid and alkali (NRC 1989). CF is generally reported to be negatively correlated to DMD in grasses and consequently high concentrations restrict voluntary intake (Minson 1990). However, Dugmore et al. (1986) found a positive correlation between CF and the in vivo OMD of kikuyu herbage. In a latter study, Dugmore et al. (1991) found that when kikuyu CF concentrations were below 300 g/kg DM, steers selected herbage containing higher CF than that for herbage and leaf material on offer, which may have been associated with improved digestibility of the pasture. The variation in results may indicate the importance of a minimum concentration of fibre required for efficient digestibility, whilst excess concentrations have detrimental effects on intake and digestion.
Tropical grasses contain higher CF concentrations than temperate species (Norton 1982), however, the mean CF content of kikuyu was lower, and less affected by advancing age than other tropical grasses examined by Gomide et al. (1969a). N fertilisation appears to affect kikuyu CF concentrations with non-significant increases between 3 and 6 weeks of regrowth at 200 and 400 kg N/ha (260 v 271 and 244 v 253 g/kg DM, respectively) observed by Dugmore et al. (1986). These results indicate that CF concentrations are lower in pastures receiving higher rates of N fertilisation, which may be related to increased growth rates.

In sheep, the apparent digestibility of CF in kikuyu fell by 10% between 5 and 11 weeks of regrowth, indicating the increased difficulty experienced by microbes at degrading fibre fractions with increased lignification (Said 1971).

The CF measurement has been superseded by ADF, NDF and lignin components, which categorize fibre into various components and perhaps provide a more comprehensive indicator of the nutritional value of the fibre fraction of herbage.

b. Acid Detergent Fibre
The ADF fraction of forage contains lignin, cellulose, indigestible protein and insoluble ash. The rate of ADF degradation is slow and concentrations have been found to be negatively correlated with intake (van Soest 1965). Although there is little variation in ADF concentrations in young regrowth, increasing concentrations of ADF would be expected to restrict intake as the plant matures and the level of lignification of cell walls increases. Joyce (1974) reported that the ADF content of kikuyu cut when 8-13 cm high was similar to pasture cut 3 weeks later when at a height of 20-30 cm high (330 v 329 g/kg DM). Similar results were also obtained by Dugmore et al. (1986) in kikuyu fertilised at either 200 or 400 kg N/ha, for 3 (344 and 339 g/kg DM) and 6 (355 and 347 g/kg DM) week old regrowth, although there was a trend for ADF to increase with age of regrowth.

Whitney (1974a) found a much greater affect of maturity on ADF concentrations between 2 and 10 week old kikuyu regrowth, with an additional affect of N fertiliser.
In 2 week old regrowth, ADF concentrations in very highly N fertilised (874 kg N/ha.annum) kikuyu were substantially lower than those receiving low to moderate rate (117 and 291 kg N/ha.annum, respectively) of fertilisation. However, by 10 weeks of regrowth, the high N treatment contained higher ADF than the lower N fertiliser treatments and this is probably due to initial rapid vegetative growth followed by the production of higher amounts of stem. In contrast, no effect of N fertiliser application on ADF concentrations was apparent in a study by Dugmore et al. (1986), however, the initial age of regrowth in this study (3 weeks) was greater than the previous study (2 weeks), which may have avoided the initial dilution effects of rapid regrowth in response to N fertiliser application.

In contrast to most other observations, Dugmore et al. (1986) found a positive correlation between ADF and the calculated digestible organic mater (DOM) content of kikuyu, which was similar to findings by Pattinson (1981). This discrepancy may be associated with use of predictive equations to estimate DOM for temperate species on tropical grass species.

c. Neutral Detergent Fibre

Extraction of NDF from plant material removes highly digestible cell-contents leaving the cell-wall fractions. NDF consists of ADF plus hemicellulose and is considered to be negatively correlated with feed energy concentrations and positively related to the gut fill effect of the diet. As with ADF, this fraction is also negatively correlated to intake (van Soest 1965). The reported concentrations of NDF in kikuyu (426 - 713 g/kg DM) (Table 2.2) significantly exceed recommended concentration of 280 g/kg DM for dairy cows (NRC 1989).

Kikuyu pasture contains adequate ADF for grazing dairy cows, however, high concentrations of NDF are likely to restrict DMI, and therefore, milk production potential. The adoption of pasture management strategies which allow kikuyu to be grazed before there was a significant rise in fibre accumulation, especially lignification, should improve animal production.
2.3.3.6 Non-Structural Carbohydrates

The main non-structural carbohydrates (NSC) found in grasses are sugars (glucose, fructose and sucrose), fructosan and storage polysaccharides (starch). The total amount of NSC in a plant is dependent on the rate of carbohydrate (CHO) production via photosynthesis, and its rate of utilisation for respiration and synthesis of plant components. Therefore, a within-day variation in NSC would be expected with a minimal concentration at daybreak and reaching maximal concentrations by early afternoon (Smith 1973).

Tropical grasses accumulate mainly starch as storage CHO in their leaves, whereas temperate grasses accumulate fructosan, mainly in the base of tillers (Jones and Wilson 1987). Low concentrations of NSC can retard the efficiency of N incorporation into microbial protein within the rumen and limit N and energy retention by the animal, which is reflected in elevated rumen NH$_3$ concentrations (Marais et al. 1990). Increasing the concentration of soluble CHO in forage consumed by ruminants should increase the efficiency of RDP usage, as well as increase the proportion of propionic acid in the rumen, reduce methane losses and increase the quantity of protein leaving the rumen (Minson 1990). Table 2.3 shows concentrations of NSC reported in kikuyu grass pastures.
Table 2.3 Concentration of total non-structural carbohydrates (TNC), acid soluble carbohydrates (ASC), water soluble carbohydrates (WSC) and starch concentrations of kikuyu grass pasture. Concentrations reported with either means or ranges; dashes represent information not given.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Plant part</th>
<th>Carbohydrates (g/kg DM)</th>
<th>Cutting height (cm)</th>
<th>Age of regrowth (weeks)</th>
<th>Time of cutting (24 hour time)</th>
<th>N fertilisation (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joyce (1974)</td>
<td>whole</td>
<td>-</td>
<td>27.4-40.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Forde et al. (1976)</td>
<td>lamina</td>
<td>-</td>
<td>38.5</td>
<td>5</td>
<td>1100-1700</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>sheath</td>
<td>-</td>
<td>37.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Taylor et al. (1976)</td>
<td>lamina</td>
<td>-</td>
<td>92.0</td>
<td>5</td>
<td>-</td>
<td>NPK ^4 500 NL ^5 100N</td>
</tr>
<tr>
<td></td>
<td>sheath</td>
<td>-</td>
<td>82.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Betteridge (1979)</td>
<td>whole</td>
<td>-</td>
<td>80' (Feb-Apr)</td>
<td>estimated sheep grazing height</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>156'' (Oct-Nov)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>64'' (Apr-May)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fernando and</td>
<td>whole</td>
<td>-</td>
<td>20.7-106.0</td>
<td>2.5</td>
<td>morning</td>
<td>90-270 U ^C, AN ^D or SN ^E</td>
</tr>
<tr>
<td>Jayaratne (1980)</td>
<td>leaf</td>
<td>62.9</td>
<td>-</td>
<td>5</td>
<td>4</td>
<td>LAN ^D 250 per annum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>39.0-89.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>stem</td>
<td>71.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>53.8-95.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>whole</td>
<td>66.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45.9-92.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Marais et al. (1990)</td>
<td>whole</td>
<td>48.5</td>
<td>-</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Jackson et al. (1996)</td>
<td>leaves</td>
<td>-</td>
<td>43.0-66.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

^1 Value is the sum of WSC and starch; ^2 Value is the sum of WSC, starch and pectin; ^4 NPK - nitrogen, phosphorus and potassium; ^5 NL - nitrolime; ^6 U - urea; ^7 AN - ammonium nitrate; ^8 SN - sodium nitrate; ^D LAN - limestone ammonium nitrate.
a. Total Non-Structural Carbohydrates

Marais (1990a) observed that the total NSC in kikuyu stem was higher than that of the leaf which appears to contradict the results of Jones and Wilson (1987) who found that tropical grasses store most NSC in their leaf portion. Marais (1990a) found a significant negative correlation between N and NSC concentration of kikuyu swards (with CP concentrations ranging from 99 - 306 g/kg DM), however, this was not evident in latter studies (Marais et al. 1990) of kikuyu containing a lower variation of CP concentrations (169 and 225 g/kg DM).

b. Water Soluble Carbohydrates

Glucose, fructose, sucrose and fructosan are the main water soluble carbohydrates (WSC) and are a readily available source of fermentable energy for rumen microbes. WSC change with age of regrowth and Joyce (1974) reported that the soluble sugar content of kikuyu cut when 8-13 cm high was higher than pasture cut 3 weeks later when at a height of 20-30 cm high (40 v 27 g/kg DM). Forde et al. (1976) observed that the lamina and sheath of kikuyu contained similar concentrations of WSC (mean 37.9 g/kg DM).

Of the grasses examined in this study, perennial ryegrass contained the highest concentrations of WSC, and the concentration was higher in the leaf sheath compared to lamina (100.6 v 87.9 g/kg DM), which is most likely a reflection of fructosan storage in the sheath material.

c. Starch

Starch accumulates in the leaves of tropical grass species (Smith 1973) and is also rapidly digested in the rumen, with similar feeding value to WSC (Norton 1982). There is very little data in the literature reporting concentrations of starch in kikuyu or the factors which influence starch accumulation. This information could enhance understanding on the optimal time to graze kikuyu in terms of the CHO:protein ratios.

The NSC concentration in kikuyu is low compared to temperate grass species and management strategies which aim to graze kikuyu pastures when NSC concentrations
are highest and provide the highest possible NSC:RDP ratio, would be expected to provide optimal nutrient utilisation by grazing ruminants.

2.3.3.7 Minerals

Various publications are available which contain recommended mineral requirements for lactating dairy cattle (ARC 1984; NRC 1989; AFRC 1993). Table 2.4 compares the mineral requirements as recommended by NRC (1989) for a 600 kg dairy cow producing 20 kg milk per day, to concentrations reported in kikuyu pasture since 1970. Also considered in Table 2.4 are recommendations by Beede (1988) for cows producing up to 20 kg milk/day, determined from work conducted in Florida, USA, where heat stress is a major factor influencing feed requirements. Cows were found to increase excretion of sodium (Na) and K in urine when experiencing heat stress, and under these conditions, increasing the concentration of these nutrients in the ration has been found to increase milk production (Huber et al. 1988).

a. Calcium

Ruminants only absorb sufficient Ca from feed to meet their requirements. These requirements are very high in lactating dairy cows, particularly during early lactation when milk yields are highest. Concentrations of Ca in kikuyu are marginal for milk production, and reported concentrations range from 1.5 (Awad et al. 1979) to 8.4 (Betteridge 1979) g/kg DM (see Table 2.4). Concentrations of Ca in kikuyu rarely exceed recommendations.

Within a kikuyu plant, the Ca concentration varies considerably between different plant components. Laredo (1974) observed that kikuyu leaf contained higher concentrations of Ca than stem (5.6 and 2.5 g/kg DM, respectively). Similarly, Marais et al. (1992) found that the Ca content of leaf lamina was more than twice that of the stem and leaf sheath. This indicates that as kikuyu matures and the proportion of stem in the whole plant increases, then overall plant Ca concentration should decline. To support this, Gomide et al. (1969b) found that the Ca content of the whole plant declined slightly
Table 2.4 Comparison of the mineral content of kikuyu and the minimum requirements of a 600kg cow producing 20 L milk/day as recommended by NRC (1989) or Beede (1993). Concentrations are reported with either means or ranges and dashes represent the information not given.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Minerals (g/kg DM)</th>
<th>Minerals (ppm)</th>
<th>Pasture parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ca</td>
<td>P</td>
<td>Mg</td>
</tr>
<tr>
<td>NRC</td>
<td>5.1</td>
<td>3.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Beede</td>
<td>5.0</td>
<td>4.2</td>
<td>3.5</td>
</tr>
<tr>
<td>Gomide et al.</td>
<td>4.2</td>
<td>1.8</td>
<td>3.2</td>
</tr>
<tr>
<td>(1969b)</td>
<td>3.2-5.8</td>
<td>1.1-2.6</td>
<td>1.9-4.7</td>
</tr>
<tr>
<td>Said (1971)</td>
<td>4.6</td>
<td>3.6</td>
<td>2.5</td>
</tr>
<tr>
<td>Whitney (1974a)</td>
<td>2.3-5.6</td>
<td>-</td>
<td>3.9-7.0</td>
</tr>
<tr>
<td>Joyce (1974)</td>
<td>4.7</td>
<td>3.7</td>
<td>2.9</td>
</tr>
<tr>
<td>Laredo (1974)</td>
<td>5.6</td>
<td>2.6</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>3.1</td>
<td>-</td>
</tr>
<tr>
<td>Kaiser (1975)</td>
<td>3.5</td>
<td>3.1</td>
<td>4.0</td>
</tr>
<tr>
<td>Russell (1976)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sherrell (1978)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Awad et al. (1979)</td>
<td>1.5-6.2</td>
<td>-2.0-5.8</td>
<td>1.6-2.6</td>
</tr>
<tr>
<td>Betteridge (1979)</td>
<td>4.5-8.4</td>
<td>3.8-4.2</td>
<td>1.7-2.1</td>
</tr>
<tr>
<td>Reference</td>
<td>Ca (g/kg DM)</td>
<td>P (g/kg DM)</td>
<td>Mg (g/kg DM)</td>
</tr>
<tr>
<td>---------------------------</td>
<td>--------------</td>
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</tr>
<tr>
<td>NRC</td>
<td>5.1</td>
<td>3.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Beede</td>
<td>5.0</td>
<td>4.2</td>
<td>3.5</td>
</tr>
<tr>
<td>Cross (1979b)</td>
<td>2.2-5.2</td>
<td>2.0-5.1</td>
<td>4.0-4.5</td>
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<tr>
<td>Fernando and Jayaratne (1980)</td>
<td>4.1-5.9</td>
<td>4.8-6.6</td>
<td>-</td>
</tr>
<tr>
<td>Rees and Little (1980)</td>
<td>-</td>
<td>3.9</td>
<td>-</td>
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<td>Tainton et al. (1982)</td>
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<td>1.3</td>
</tr>
<tr>
<td>Cook and Mulder (1984b)</td>
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<td>3.8</td>
<td>-</td>
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<tr>
<td>Pearson et al. (1985)</td>
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<td>3.2-4.6</td>
<td>0.9-1.6</td>
</tr>
<tr>
<td>Marais et al. (1987)</td>
<td>-</td>
<td>-</td>
<td>33.6</td>
</tr>
<tr>
<td>Hughes et al. (1988)</td>
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<td>2.8</td>
<td>3.6</td>
</tr>
<tr>
<td>Reference</td>
<td>Ca</td>
<td>P</td>
<td>Mg</td>
</tr>
<tr>
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<tr>
<td>NRC</td>
<td>5.1</td>
<td>3.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Beede</td>
<td>5.0</td>
<td>4.2</td>
<td>3.5</td>
</tr>
<tr>
<td>Marais (1990a)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marais (1990b)</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.7-3.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.4-2.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pastrana et al. (1990)</td>
<td>3.2</td>
<td>2.6</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>3.7</td>
<td>1.7</td>
<td>2.3</td>
</tr>
<tr>
<td>Rumble (1991)</td>
<td></td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>Ca (g/kg DM)</td>
<td>P</td>
<td>Mg</td>
</tr>
<tr>
<td>------------------</td>
<td>--------------</td>
<td>---</td>
<td>----</td>
</tr>
<tr>
<td>NRC</td>
<td>5.1</td>
<td>3.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Beede</td>
<td>5.0</td>
<td>4.2</td>
<td>3.5</td>
</tr>
<tr>
<td>Davison et al. (1991b)</td>
<td>2.6</td>
<td>4.0</td>
<td>3.9</td>
</tr>
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<td>Evans and Hacker (1992)</td>
<td>3.8</td>
<td>2.9</td>
<td>2.0</td>
</tr>
<tr>
<td>Marais et al. (1992)</td>
<td>4.0</td>
<td>10.3</td>
<td>7.4</td>
</tr>
<tr>
<td>Miles et al. (1995)</td>
<td>2.2-3.3</td>
<td>2.7-3.9</td>
<td>2.5-3.5</td>
</tr>
</tbody>
</table>
from 5 to 4 g/kg DM between 4 and 36 weeks of regrowth, whilst little variation was observed by Said (1971) and Joyce (1974) for shorter regrowth periods.

Miles et al. (1995) found that Ca concentrations are lowest when kikuyu growth rates peak during summer. Pearson et al. (1985) observed that Ca concentrations tended to increase with N fertilisation, whilst no effect was found by Tainton et al. (1982). Marais et al. (1992) found that when low concentrations of Ca are supplied to kikuyu grown in nutrient solution, DM yields were relatively low indicating the important role of Ca in plant growth.

In kikuyu, the availability of Ca to the ruminants is reduced, as much is bound to oxalate to form insoluble calcium oxalate (Ca(COO)$_2$) crystals (McKenzie and Shultz 1983). The leaf material of kikuyu contains 3 times more oxalate than the stem, however, insoluble oxalate comprises only 41% of the total oxalate in leaf tissue compared to 95% of that in stem tissue (Marais 1990b). This study also found a higher proportion of insoluble:soluble oxalate in kikuyu than Blaney et al. (1982) found in Cenchrus ciliaris or Setaria sphacelata, indicating that a higher proportion of Ca may be bound up in kikuyu compared to these other grass species.

The absorption of Ca bound to oxalate is higher in cows with a negative, compared to positive, Ca balance (Blaney et al. 1982). These workers determined the amount of Ca absorbed by steers fed setaria hay with a high oxalate content and compared this to wheaten hay with a much lower oxalate content (1.13 and 0.12% of total DM, respectively). The oxalate present in the setaria inhibited Ca absorption by 20% and workers suggest the maximum availability to cattle in tropical grass species should be considered to be 50%. Marais (1990b) calculated a much lower mean concentration of Ca availability in a kikuyu sward. Using the molar ratios of oxalate and Ca within plant tissues, he estimated the availability of Ca in kikuyu to cattle was only 5%, assuming that each mole of insoluble oxalate contains 1 mole of Ca and none of this is available to the ruminant through microbial breakdown.

A variety of factors influence oxalate concentration in plant tissue. Jones and Ford
(1972) found that setaria contained higher concentrations of oxalates when fertilised with N or K. Studies by Marais (1990b) substantiated this when he found a significant correlation between insoluble leaf oxalate and total N in tissue, as well as the protein N content of all kikuyu plant parts.

**Grazing management practices may influence the concentration of Ca ingested by the animal. For instance, pasture with a high proportion of leaf material has a higher Ca content than predominantly stemmy pasture. The presence of oxalates in kikuyu grass pasture inhibit the availability of Ca in kikuyu grass pastures and should be compensated for when balancing nutrient requirements through supplementation.**

b. **Phosphorus**

Phosphorus (P) is an essential mineral for dairy cattle for bone development, growth, reproduction and energy transfer and is rapidly mobilised from bone during periods of temporary dietary deficiency. Concentrations of P in kikuyu range from 1.1 (Gomide *et al.* 1969b) to 10.3 (Marais *et al.* 1992) g/kg DM (Table 2.4), with mean concentrations barely reaching the requirements of dairy cattle (NRC 1989; Beede 1988).

Concentrations of P in kikuyu have been reported to decline with advancing plant maturity by Gomide *et al.* (1969b) and Said (1971) after 4 and 5 weeks of age, respectively. However, Joyce (1974) found little difference in the P content of kikuyu cut at a height of 8-13 cm compared to that cut 3 weeks later when at a height of 20-30 cm (3.6 v 3.8 g/kg DM). The age of regrowth was not reported in this case and the pasture may have been cut at an age younger than the other studies. Miles *et al.* (1995) observed P concentrations were highest in mid-summer, whilst over the winter period, P concentrations in the green leaf material tended to decline until the following spring (Hughes *et al.* 1988). Slightly higher concentrations (4.0 g/kg DM) were obtained from the green herbage (leaf plus stem) compared to the remainder of kikuyu plant (Kaiser 1975).

Phosphorus concentrations in kikuyu can be influenced by fertiliser application. Awad
et al. (1979) and Pearson et al. (1985) found that application of N resulted in a depression in P concentration in kikuyu herbage, which may be related to a dilution effect from increased growth rates. However, Tainton et al. (1982) found that N fertiliser did not affect P concentrations with a mean of 3.0 g/kg DM. Awad et al. (1979) also found that the application of lime increased P concentrations.

Reported P concentrations in kikuyu are variable and tend to be marginal for high milk yields, and supplementation may be required to ensure adequate P intake by lactating cows. Appropriate management practices which maximise the amount of green leaf on offer, without compromising forage quality, may assist in the optimisation of P concentrations in kikuyu in terms of cow requirements.

c. Potassium

Forages generally contain higher K concentrations than required by dairy cattle (NRC 1989) and kikuyu pasture is no exception (see Table 2.4). Heat stress increases the requirement for K through losses from sweating and Beede et al. (1983) suggest that during the summer, dietary K should be at least 12.5 g/kg DM, which is still well below the concentrations found in kikuyu (29 g/kg DM).

Excess dietary K is undesirable as it interferes with Mg metabolism and absorption (Kemp et al. 1961) from the rumen. The concentration of K declines in forages with maturity and this has been observed in kikuyu by Gomide et al. (1969b) and Joyce (1974), suggesting that grazing more mature kikuyu may assist in avoiding problems of hypomagnesaemia.

Reported K concentrations of kikuyu vary with plant component, season and fertilisation. Kaiser (1975) found higher concentrations (36.6 g/kg DM) in the green herbage (leaf plus stem) of kikuyu than the remaining material. However, Marais (1990a) observed higher concentrations of K in the stem compared to leaf portions (58.2 and 40.5 g/kg DM, respectively). In agreement with Awad et al. (1979), these workers also observed a positive correlation between N and K concentrations in the sward and as mentioned earlier, suggesting that K acts as a counter ion for the uptake of nitrate.
In contrast, Tainton et al. (1982) found that the concentration of K was not affected by N fertiliser rates.

_Moderate, rather than heavy, applications of N fertiliser to kikuyu pastures may result in more favourable K concentrations in kikuyu. Allowing sufficient time for K concentrations to decline with regrowth time would also be of benefit._

d. Magnesium

Magnesium is a constituent of bone and is also involved in neuromuscular transmission and activities, and enzyme systems. The Mg reserves present in the bones are mobilised slowly and therefore a dietary deficiency may result in hypomagnesaemia relatively rapidly. The reported concentrations of Mg in kikuyu range from 0.9 (Pearson et al. 1985) to 7.4 (Marais et al. 1992) g/kg DM (Table 2.4), and in approximately half of the reports, mean concentrations are below the concentrations recommended under general conditions (NRC 1989) and for animals subject to heat stress (Beede 1988).

Reported changes in Mg content of kikuyu regrowth vary. Gomide et al. (1969b) observed that Mg concentrations of kikuyu declined with plant age, whilst Said (1971) found little variation. Joyce (1974) found lower concentrations of Mg in kikuyu cut when 8-13 cm high compared to pasture cut 3 weeks later when at a height of 20-30 cm (2.5 v 3.2 g/kg DM). Tainton et al. (1982) and Pearson et al. (1985) found that Mg concentrations in kikuyu tended to increase as N fertiliser rates increased.

_The concentrations of Mg in kikuyu are marginal for milk production and supplementation to some extent would be necessary to ensure it was not limiting milk production. The variable Mg concentrations reported in relation to regrowth make it difficult to establish the ideal time to graze kikuyu in terms of Mg content, although the addition of N fertiliser appears to raise Mg concentrations._

e. Sodium

Sodium is the principle cation in the maintenance of body-fluid balance, osmotic pressure regulation, acid-base balance, glucose uptake of cells, amino acid transport and
nerve transmission. Cows have the ability to reduce Na losses in urine, sweat and faeces to very low concentrations and also reduce Na (whilst increasing K) in saliva, when on diets low in Na. However, as mentioned earlier, when cows are suffering from heat stress, they increase the excretion of Na in urine. Kikuyu contains very low concentrations of Na with reported concentrations ranging from 0.01 (Said 1971) to 6.3 (Marais et al. 1992) g/kg DM. The mean reported concentrations are generally below NRC (1989) requirements and are considerably lower than concentrations suggested by Beede (1988) in warm climatic conditions (5.0 g/kg DM).

The Na content of kikuyu when cut at 8-13 cm height was slightly lower than pasture cut 3 weeks later at a height of 20-30 cm (3.5 v 4.7 g/kg DM) (Joyce 1974), indicating a possible small amount of accumulation with regrowth. However, supplementation with Na is essential to prevent dietary Na inadequacies. The high dietary concentrations of Na recommended by Beede (1988) may lead to concentrations in supplements so high as to be unpalatable to stock.

The Na content of kikuyu is well below recommended concentrations and supplementation appears to be essential, especially during summer when cows are experiencing heat stress.

f. Chlorine and Sulfur

Chlorine (Cl) is the major anion in extracellular fluid and assists in the maintenance of acid-base balance, osmotic regulation, O₂ and CO₂ transport and is found in gastric secretions. The only reported concentration of Cl in kikuyu (31 g/kg DM) (Russell 1976) is more than adequate for dairy cattle and especially if NaCl is added to the diet to correct Na deficiencies (NRC 1989).

Sulfur is an essential component of protein, of particular importance is its presence in the amino acid methionine and the B vitamins thiamin and biotin, which cannot be synthesized by animal tissue. Rumen microbes require a supply of S for production and synthesis, and if deficient, microbial activity is reduced which reduces DMD (Minson 1990). The S concentrations in kikuyu appear to be marginal for milk production (mean 43
2.3 g/kg DM) and S supplementation may enhance the efficiency of rumen microbes in cows grazing kikuyu pastures.

g. Iron, Cobalt, Copper, Manganese, Zinc, Molybdenum and Selenium
Micro-minerals are essential for various functions throughout the body and few reports on the concentrations of micro-mineral concentrations in kikuyu are available. The Fe content of kikuyu is considerably higher than requirements, even in mature pasture. Studies by Gomide et al. (1969b) found that the Fe content tended to decline with plant age from 306 to 232 ppm between 4 and 36 weeks of regrowth, although it still remained above the recommended requirement concentrations.

NRC (1989) recommends a dietary copper (Cu) intake of 10 ppm, but for cattle grazing pastures or consuming feeds high in substances which interfere with Cu absorption (e.g. Mo, S), requirements may be higher. Gomide et al. (1969b) and Kaiser (1975) found the Cu content of kikuyu exceeded 10 ppm with mean concentrations of 12 and 13 ppm, respectively.

Manganese concentrations in kikuyu reported by Gomide et al. (1969b) and Awad et al. (1979) were generally well above recommended concentrations of NRC (1989). Additionally, Gomide et al. (1969b) found that N fertilisation increased the Mn content of kikuyu.

The mean concentration of zinc (Zn) in kikuyu of 32 ppm found by Gomide et al. (1969b) is below the recommended concentration of 40 ppm for dairy cattle (NRC 1989) and may require supplementation. Molybdenum is an essential component of xanthine oxidase which is found in milk and animal tissues, yet a deficiency has never been observed in dairy cattle. NRC (1989) suggest that requirements are quite low and therefore deficiencies probably would not occur in practical situations.

Selenium (Se) is an essential element for ruminants required in only very low concentrations with the maximum tolerable concentration for dairy cows being 2 ppm (NRC 1989). The Se concentrations reported by Pastrana et al. (1990) were low (0.1-0.2
ppm) indicating that Se deficiencies may occur in dairy cows grazing kikuyu pastures without supplementation.

The mineral content of kikuyu is often insufficient to meet the demands of lactating cows. In many studies, the environmental and plant physiological conditions are generally not specified. In particular, sampling time during the day and the regrowth stage in relation to the plants regrowth cycle are not given, and these can often affect mineral concentrations. This may explain much of the variability observed and should be considered in determining the optimal stage at which to graze kikuyu pastures.

2.4 Factors Affecting Growth and Yield of Kikuyu

The rate of growth and DM production of a pasture is influenced by grazing management. Kikuyu is capable of producing high quantities of DM under favourable environmental and managerial conditions. The conditions include temperature, moisture, soil nutrient concentrations (especially N), soil pH and defoliation practice.

2.4.1 Temperature

As a C₄ grass species, DM production of kikuyu in a subtropical environment is markedly summer/autumn-dominant corresponding to periods of high temperatures (Murtagh and Moore 1987; Rumball 1991; Minson et al. 1993) and humidity.

Under field conditions, with no irrigation and available soil moisture of approximately 60%, Colman and O’Neill (1978) recorded a maximal growth rate for kikuyu of 237 kg DM/ha.day at a mean daily temperature of 22°C. They recorded significant growth (>20 kg DM/ha.day) of kikuyu at 13°C with growth ceasing at a mean screen temperature of 10°C.

Ivory and Whiteman (1978) reported the day and night temperatures to obtain maximal growth of kikuyu under glasshouse conditions were 29.4 and 25.6°C, respectively. Kikuyu growth ceased at 8°C which is equivalent to the mean critical daily temperature recorded by Ivory (1976) and the mean daily screen temperatures recorded by Coleman.
and O'Neill (1978) under field conditions.

Temperature has been found to affect the growth rate of different plant parts and plant development, affecting both the quality and quantity of kikuyu on offer throughout the season. Ivory (1976) found the optimal temperature for leaf growth and development was higher than for the growth of the whole plant, in various tropical grass species (which included kikuyu). However, in a latter study, Ivory and Whiteman (1978) found that the optimal temperatures for total plant, leaf, stem and root growth were similar. Leaf size and tiller growth were also found to be maximal at higher daily temperatures, compared to leaf and tiller number which were higher at low temperatures.

Murtagh et al. (1987) found that increasing temperatures shortened leaf appearance intervals. In this experiment, there were 2.7 ± 0.1 emerging leaves per tiller at any one time regardless of temperature and the total number of green lamina increased (8.9, 9.1, 10.0 and 11.1 green lamina per tiller) with increasing temperatures (15, 20, 25 and 30°C, respectively). This is in contrast to ryegrass, in which only one leaf expands at any one time (W. J. Fulkerson, personal communication).

Unlike Ivory and Whiteman (1978), Ivory (1976) noted that the optimal temperatures for stem growth were lower than for growth of the whole plant. However, Murtagh et al. (1987) found there was a rapid increase in stem yield at 30°C between 14 and 22 days regrowth compared to lower temperatures.

These findings suggest that pasture management should involve close observation of seasonal pasture growth in order to control excessive stem elongation and thus prevent the pasture becoming rank and declining in quality.

The optimal rate of tillering was found to occur at a lower temperature (mean 23.4°C) than for total plant growth (Ivory and Whiteman 1978), and Ivory (1976) observed that tillering in kikuyu declined as temperature lowered, and an inverse relationship existed between tillering and the growth rate of individual tillers. Similarly, Murtagh et al. (1987) found that tiller density was greater at low (15 and 20°C) compared to high (25
and 30°C) temperatures. Therefore, emphasis on vegetative propagation in kikuyu (as with ryegrass (W. J. Fulkerson, unpub. data)) occurs at lower temperatures than for optimal growth, which may be a contributing factor to the formation of a thick stemmy mat at the base of kikuyu pasture when temperatures decline with the onset of autumn.

The growth response of kikuyu to temperature appears to be intermediate between a temperate and tropical pasture species, a reflection of its origin in the highlands of Kenya. The variation in response to temperatures by plant parts indicates that management needs to consider these changes if we want to optimise pasture quality in terms of cow requirements, especially in periods of high temperatures when rapid leaf growth, stem elongation and leaf senescence are encountered.

2.4.2 Moisture
Various reports suggest that kikuyu can withstand dry periods to a certain extent (Mears 1970; Campbell et al. 1996) which is presumably due to an extensive and relatively deep root system, compared to temperate species in a similar environment. Despite this, under dryland conditions and adequate N fertilisation, Rethman and de Witt (1988) observed a 44% reduction in DM production when annual rainfall declined by 300 mm.

2.4.3 Soil Nutrient Concentrations and Fertilisation
Mears (1970) reported that kikuyu pastures require highly fertile soils for maximum production and are especially responsive to N fertilisation. Requirements for nutrients depend strongly upon the method of pasture utilisation. Under cutting and removal (silage, forage harvesting), fertilisation requirements will be substantially higher than under grazing situations where a large amount of nutrients are recycled in dung and urine (Cross 1979a) and therefore recommendations derived from cutting trials can be extrapolated to grazing situations only with caution (Miles 1991).

2.4.3.1 Nitrogen
Many studies have examined the response of kikuyu pastures to the application of N and the interaction with other factors. The degree of response of kikuyu to N is closely related to temperature, defoliation interval and, to a lesser extent, soil moisture (Colman
and O’Neill 1978) and other soil nutrient concentrations (Cross 1979a), and as a consequence, annual DM yields are difficult to compare between experiments due to variations in these factors, as well as discrepancies in residual soil N.

With adequate moisture, annual yields have ranged from 0.5 t/ha with nil fertiliser (Kemp 1975) to over 30 t DM/ha with application of greater than 800 kg N/ha (Whitney 1974a; Colman and O’Neill 1978). Current recommendations for N fertiliser application range from 200-400 kg N/annum at no more than 50 kg N/ha.month over the summer growing period (W.J. Fulkerson and K. Lowe, personal communication). In cutting trials, the response to N application varies between experiments. Initially, a linear response to N is apparent up to approximately 700 - 800 kg N/annum, after which the rate declines as indicated by data from Kemp (1976). Under grazing, Tainton et al. (1982) found a linear response to N application from 150 - 600 kg N over the growing season.

The N-use efficiency of kikuyu ranged from 13-27 kg DM/kg N applied, in studies reviewed by Mears (1970). In comparison to other tropical grass species, Kemp (1975) found that kikuyu had a much lower N-use efficiency (mean 13.3 kg DM/kg N applied) than either setaria or broadleaf paspalum (means of 21.8 and 23.2 kg DM/kg N, respectively). Rumball (1991) found the N-use efficiency in the cool season was extremely low compared to the warm season in northern New Zealand (0.8 v 17.5 kg DM/kg N applied), and similar observations were made by Pearson et al. (1985).

Generally, the N-use efficiency of a plant declines as N application increases. However, data obtained by Kemp (1975) showed the N-use efficiency of kikuyu actually increased by 21% when N application increased from 170-680 kg N/ha.annum, unlike setaria and broadleaf paspalum whose N-use efficiency declined by 30 and 32%, respectively. Similarly, at the one site in Hawaii, Whitney (1974a) found an increase in N-use efficiency by kikuyu when N fertiliser increased from 291-795 kg N/ha.annum, however, these workers observed a decline at another experimental site.

On the other hand, when comparing total yields over 16 weeks, Colman and O’Neill
(1978) found no difference in N-use efficiency of response between rates of N application and calculated efficiency values ranging from 12.5 - 48.6 kg DM/kg N applied. N-use efficiencies of similar range were also found by Whitney (1974a). These observations suggest that the response of kikuyu to N fertilisation is virtually linear within the current recommended rates of application.

In soils considered to have low fertility, the DM yield of Whittet kikuyu was the lowest of the tropical grasses examined at application rates of 250, 500 and 1000 kg N/ha.annum, with yields only 45, 52 and 57%, respectively of Chloris gayana cv. Katambora (Rhodes grass), the highest yielding grass examined (Cook and Mulder 1984a). Wilson and Sandland (1976) found that kikuyu had lower yields than Panicum maximum var. trichoglume (green panic) under various N and P regimes due to higher relative growth rates of the latter species. This indicates that kikuyu is less suited to relatively infertile soils compared to other tropical grass pastures.

Henzell and Oxenham (1973) found a quadratic relationship between the N content of the youngest fully expanded leaf (considered less variable than the whole plant top) and the relative growth rate of top herbage (it is unclear in the authors methods if this is above the 5 cm stubble height or ground level), however, the concentrations of leaf N in kikuyu required to achieve optimum growth rates was not stated.

N application appears also to affect the amount of DM accumulation in the lower canopy of the kikuyu sward. Cook and Mulder (1984a) found that stubble yield (DM between 0-10 cm above ground level) increased with an increase in N application from 250 to 500 and 500 to 1000 kg N/ha.annum, and this may be related to an increase in tiller number. In this regard, Wilson and Sandland (1976) found that as N application increased, tiller number also increased in regularly defoliated plants as they matured.

The incorporation of legumes into kikuyu-based pasture would result in an increase in the overall quality of the sward and reduce the requirement for N fertilisation. However, it has been extremely difficult to maintain legumes within kikuyu-based dairy swards (Mears 1970). As a consequence, there is almost complete reliance on fertilisation as
Apart from increasing the protein content of kikuyu, the application of N fertiliser increases the DM production potential of kikuyu, although its efficiency of use of N is generally lower than other tropical grass species. N application may affect the component growth of kikuyu and therefore would be an important aspect to consider in kikuyu management.

2.4.3.2 Phosphorus and Potassium

Kikuyu DM production responses to P and K are much lower than for N. In a trial conducted in the midlands of Natal, South Africa, Cross (1979a), observed that at low rates of N application (208 kg N/ha.season), increasing P and K application above 22 and 106 kg/ha.season, respectively had little effect on seasonal yields. Significant DM increases (2 tonnes DM/ha.season) in response to applied P and K (424 kg K and 88 kg P/ha.season, respectively) only occurred at high N application rates (360 kg N/ha.season) in conjunction with high rates of the alternate nutrient.

Moderate increases in kikuyu DM production in response to P application were also reported by Miles (1991), which may be related to an increase in tiller number as observed by Wilson and Sandland (1976). However, Miles (1991) found the response of kikuyu was considerably lower than ryegrass in particular, and clover. The author suggests that such variation may be related to lower soil temperature in the growth period of ryegrass compared to the kikuyu and clover since a decrease in soil P availability with a decline in soil temperature has been reported (Sutton 1969). Similarly, in glasshouse conditions, Wilson and Haydock (1971) observed that temperate grasses had a greater response to P application in above ground growth, root growth and tiller number, than tropica, whilst the latter increased more in top:root ratios.

Application of N to kikuyu gives the largest responses to fertilisation in terms of kikuyu DM production. However, soil P and K concentrations are important to support the higher potential DM production.
2.4.4 Defoliation Interval

Little work has been undertaken on the effect of defoliation practice on kikuyu grass production and quality. As expected, the DM production of kikuyu is increased by decreasing the cutting frequency (Whitney 1974a; Kemp 1976; Rethman and de Witt 1988) due to an increase in leaf area and subsequent light interception and photosynthetic activity on less frequently harvested plots. However, the increase in production is associated with herbage wastage through higher leaf senescence and a decline in herbage quality associated with senescence and an increased proportion of stoloniferous material.

*Few studies have examined the association between defoliation interval, growth and forage quality of the kikuyu plant to the grazing animal.*

2.4.5 Comparison with the Growth of C₃ Grasses

Wilson and Haydock (1971) compared the growth of various tropical grasses (including kikuyu) in summer and temperate grasses in winter (including *Lolium perenne* (perennial ryegrass)) in unshaded glasshouse conditions in a subtropical environment. They found that the tropicals grew almost twice as fast and grew better when soil nutrient concentrations were limiting (and vice versa), than the temperate grasses. Under these management conditions, kikuyu had a much higher top:root ratio than the ryegrass (2.63 and 1.44, respectively) and these workers suggest that the higher ratio would be associated with more rapid growth, maturation and senescence of above ground DM and consequent depletion of N. Wilson and Sandland (1976) found kikuyu to have a shoot:root ratio of 3.96 which was similar to green panic (3.83).

2.5 Pasture Intake

A knowledge of the amount of herbage consumed by grazing animals is an important aspect in the evaluation of production from pastures. It allows evaluation of the effects of treatment (eg. supplementation) on intake and pasture utilisation. Dry matter intake (DMI) levels can be used to determine optimum grazing management strategies and are necessary when balancing the rations of grazing cows and for pasture budgeting in farm
Herbage intake of grazing ruminants is difficult to measure and various approaches have been taken (in rotational grazing situations) which include;

- **pasture-based methods** - estimates of herd intake using pasture-based techniques would suffice for farm management decisions or when continuous estimates are required over an extended period (ie. months) on a whole herd basis (eg. Rising plate meter (RPM)).

- **animal-based methods** - when accurate determinations of DMI are required (eg. effects of concentrate feeding, substitution etc) for individual animals. Animal-based estimates are based on using indigestible markers to determine faecal output (FO) and together with pasture digestibility measurements (eg. alkanes or chromium markers).

- **prediction from animal performance** - animal performance data may provide a guide to comparative intakes between animals, but care must be taken interpreting estimates, as the equations used for calculations are based on average performance of many animals in various experimental situations. Comparison must be over a relatively long period (ie. months) to obtain an accurate measure of body weight change.

The technique chosen to predict herbage intake will depend on the resources available, and the accuracy, precision and type of intake estimate required. Various reviews and detailed information on these techniques is available and the reader is referred to Langlands (1975 & 1987), Leaver (1985) and Minson (1990) for further reading.

The determination of the intake of kikuyu using pasture-based techniques is difficult due to the high proportion of non-available stoloniferous material compared to temperate species. Fulkerson and Slack (1993) improved the accuracy and precision of estimating kikuyu mass using a rising plate meter by developing calibration equations for forage above 5 cm stubble height, rather than from ground level (which includes stoloniferous material). In reasonably well-managed dairy systems, it is thought that cows do not graze kikuyu below this height.
Chromic oxide was used as a faecal output marker to estimate kikuyu intake by Hamilton et al. (1992) and Henning et al. (1995) who determined means of 12.6 kg DM and 14.2 kg OM/cow.day, respectively, for cows grazing kikuyu pasture alone. However, determination of intake using the chromic oxide method requires an estimation of pasture digestibility, which is generally determined using in vitro techniques. These estimations disregard variability between and within animals under differing conditions (supplements, feed base etc.). This can be overcome by total faecal collection using bags and harnesses, however, the procedure is difficult with cows under grazing situations. These parameters are accounted for in a relatively new technique using alkanes (plant waxes) as relatively indigestible markers (Dove and Mayes 1991 & 1996). However, the method does not give completely independent estimates of intake for individual animals since herbage alkane levels in samples, representative of herbage ingested by groups of animals, are normally used in the estimations.

In experimental terms, availability of an accurate method of estimating intake of individual cows grazing kikuyu is essential to enable the establishment of a maximal intake level and determination of the degree of substitution resulting from supplementation.

2.6 Pasture and Grazing Management

The adoption and implementation of sound pasture and grazing management strategies can improve the quality of the pasture on offer. Consequently, production from the grazing animal will be enhanced, using an inexpensive feed-base, resulting in increased financial returns to the farmer.

In subtropical regions, tight pasture management practices are important since pasture growth rates can be very high and pasture quality can change rapidly. Despite this, typical grazing management practices for kikuyu do not successfully control growth.

Few studies have been undertaken which compare different grazing management systems for kikuyu. Henning et al. (1995) examined the effect of grazing cycle lengths
of 15, 30 or 60 days grazed for 1, 2 or 4 day periods, respectively, and found that the 30
day cycle provided the highest milk yields (regardless of grazing duration). Milk fat
and protein, body weight, OM intake and OMD did not differ significantly between
treatments. However, cycle length and duration of grazing were confounded with cows
grazing the 15 day cycle grazed for longer than in other treatment groups and the authors
suggested that this was due to low DM availability on short rotations. This study
disregarded changes in the length of the regrowth cycle of kikuyu with season. Quinlan
et al. (1975) strongly recommended that kikuyu should be grazed rotationally (preferably
using strip-grazing) allowing an interval of 21 days between grazings for periods of
rapid growth, with a lengthened interval (up to 6 weeks) when growth rates decline, thus
recognising the need to be seasonally flexible. Using a 3-week grazing interval, these
workers suggested stocking rates of 2.5 and 5 beasts (type unknown) when pastures are
fertilised with 150 and 300+ kg N/ha.annum, respectively.

2.7 Supplementary Feeding

2.7.1 Responses to supplementary feeding
Kellaway and Porta (1993) reviewed concentrate feeding on various pasture types in
Australia and identified the major factors which contribute to the wide range of
production responses to be;

- **body condition score** - cows with poor body condition give smaller milk
  production responses to supplements compared to cows in good
  condition. This is the result of poor cows partitioning energy into
  liveweight gain in preference to milk production.

- **substitution effect** - a decrease in pasture intake may occur when
  supplementary feeding cows, and the effects are greatest when pasture
  availability (see Figure 2.5) and digestibility are high, and large amounts
  of starch rich concentrates are fed. The rapid digestion of grain starch
  causes a decrease in rumen pH , reducing the number of cellulolytic
  bacteria and therefore fibre digestion and pasture intake. This effect is
  greater when starchy, rather than fibrous, supplements are fed. Even if
  a decline in rumen pH is prevented through the use of buffers, starch
appears to preferentially degraded over fibre (Mould et al. 1983).

Figure 2.5 Responses to supplementation at low, average and high levels of pasture on offer (source: Davison et al. 1982)

- **the rate at which the supplement is fed** - milk responses to supplementary feeding are curvilinear so marginal responses decrease as the rate of supplementary feeding increases.
- **stage of lactation** - effects the degree of milk response with the greatest responses to supplements occurring early in lactation. Later in lactation, milk responses decline as energy is partitioned into body condition in preparation for the next lactation.
- **genetic potential of the cow** - governs the ability of the cows to partition more energy into milk production and to increase their feed intake.
- **pasture and supplement quality** - response are likely to be greater when the quality of forage is poor rather than high, as the concentrates may provide nutrients which are limiting in the forage.
2.7.2 Responses to Supplementation of Cows Grazing Kikuyu Grass Pasture

A few studies have investigated the response of dairy cows grazing kikuyu pastures to supplementary feeding and these are summarised in Table 2.5. Responses ranged from 0.28 to 1.40 kg milk/kg supplement DM fed.

For example, responses to energy supplementation ranged from 0.38 kg milk/kg supplement fed from a group of mixed breed cows in mid lactation fed either 4 or 6 kg of rolled barley (Sriskandarajah et al. 1980), to 1.14 kg milk/kg supplement fed from Friesian cows in early lactation fed a lower rate (2.8 kg/cow.day) of barley (Hamilton et al. 1992).

2.7.2.1 Responses to Energy Concentrates

Energy is considered to be the major factor limiting milk production from tropical grass pastures (Royal and Jeffery 1972). To support this, supplementation with energy concentrates increased milk production of cows grazing kikuyu pasture (Colman and Kaiser 1974; Sriskandarajah et al. 1980; Kaiser and Ashwood 1981; Ashes and Hamilton 1983; Olney and Albertson 1984; Hamilton et al. 1992).

Cereal grains can be physically or chemically treated to increase the availability and utilisation of starch in the grain. In Australia, the most common methods of on-farm mechanical processing in Australia are rolling and hammer milling (Kellaway and Porta 1993). Physical treatment exposes grain starch to rumen microbes, resulting in rapid fermentation which may cause abnormal rumen function and often buffers are fed to reduce these effects (see later section).

Rapid fermentation of starch concentrates such as cereal grains, reduces rumen pH, altering rumen acetate:propionate concentrations, causing a reduction in milk fat concentrations. The milk fat concentration of cows grazing kikuyu grass pasture was reduced by energy supplementation (Kaiser and Ashwood 1981; Hamilton et al. 1992), as well as energy and protein supplementation (Hamilton et al. 1992). Generally, this was more than compensated for by the increase in overall fat yield associated with increased milk production. However, Ashwood and Kellaway (1982) and Olney and
Albertsen (1984), found no significant effect of untreated-barley supplementation on milk fat concentrations.

It is postulated that milk protein concentrations increase with energy supplementation due to increased yield of microbial protein in the rumen (Kellaway and Porta 1993), and such a response was observed by Kaiser and Ashwood (1981), Olney and Albertsen (1984) and Hamilton et al. (1992). However similar or reduced milk protein concentrations were observed by Sriskandarajah et al. (1980) between unsupplemented animals and those given rolled or NaOH-treated barley.

The responses to supplementation indicate that energy is a major factor limiting milk production from kikuyu grass pastures, and this is in line with the observation that supplementation with energy-based concentrates can enhance milk production of individual cows. In the majority of studies, the kikuyu pastures had poor digestibility and relatively low protein content, but much of this was due to inappropriate pasture management. In this regard, the response to supplementation of cows grazing kikuyu managed to optimise forage quality to meet cow requirements, is unknown.
<table>
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<td>Coleman and Kaiser (1974)</td>
<td>N fertilised rotationally grazed 3 week old regrowth 5 cows/ha</td>
<td>crushed oats</td>
<td>varied with mean of 1.6</td>
<td>Jersey, Guernsey, FCM</td>
<td>7.2, 9.7, 0.36</td>
<td>48.2, 45.6, 35.6</td>
<td>35.7</td>
<td>-</td>
</tr>
<tr>
<td>Royal and Hughes (1976)</td>
<td>N fertilised rotationally grazed</td>
<td>forage oats</td>
<td>DMD = 0.76, CP = 15.6</td>
<td>7</td>
<td>Guernsey</td>
<td>7.2, 9.7, 0.36</td>
<td>48.2, 45.6, 35.6</td>
<td>35.7</td>
</tr>
<tr>
<td>Royal and Hughes (1976)</td>
<td>N fertilised rotationally grazed</td>
<td>ryegrass</td>
<td>DMD = 0.50, CP = 10.6</td>
<td>7</td>
<td>yellow lupins</td>
<td>9.6, 0.34</td>
<td>46.7</td>
<td>38.3</td>
</tr>
<tr>
<td>Srikumaranjaya et al. (1980)</td>
<td>N fertilised fresh pasture offered daily</td>
<td>rolled barley</td>
<td>DMD = 0.59, CP = 106</td>
<td>4</td>
<td>Friesian, Guernsey, Australian Milking Zebu</td>
<td>7.8, 9.3, 0.38</td>
<td>39.4, 36.6, 35.4</td>
<td>35.5, 33.9, 35.4</td>
</tr>
<tr>
<td>Srikumaranjaya et al. (1980)</td>
<td>N fertilised fresh pasture offered daily</td>
<td>N. O. millet</td>
<td>DMD = 0.59, CP = 106</td>
<td>4</td>
<td>N. O. whole barley</td>
<td>10.2, 0.60</td>
<td>37.1, 37.1</td>
<td>37.1, 37.1</td>
</tr>
<tr>
<td>Reference</td>
<td>Pasture Management</td>
<td>Supplement Type and Quality</td>
<td>Amount of Supplement Fed (kg DM/day)</td>
<td>Breed and Stage of Lactation</td>
<td>Milk Production (kg/cow.day)</td>
<td>Milk Response (kg milk/kg supplement)</td>
<td>Milk Fat (g/kg milk)</td>
<td>Milk protein (g/kg milk)</td>
</tr>
<tr>
<td>--------------------</td>
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<td>-------------------------</td>
</tr>
<tr>
<td>Kaiser and Ashwood (1981)</td>
<td>N fertilised kikuyu ad lib DOMD = 0.55 CP = 124</td>
<td>rolled oats CP = 124</td>
<td>3.5</td>
<td>Friesian, Guernsey, Friesian/ Guernsey crosses and AMZ</td>
<td>-</td>
<td>14.7</td>
<td>-</td>
<td>36.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rolled oats SBM = 509</td>
<td>8.2</td>
<td></td>
<td>16.0</td>
<td>-</td>
<td>32.3</td>
<td>32.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rolled oats SBM = 509</td>
<td>3.1</td>
<td></td>
<td>14.1</td>
<td>-</td>
<td>39.7</td>
<td>31.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rolled oats SBM = 509</td>
<td>2.7</td>
<td></td>
<td>15.0</td>
<td>-</td>
<td>38.0</td>
<td>31.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rolled oats SBM = 509</td>
<td>2.3</td>
<td></td>
<td>15.1</td>
<td>-</td>
<td>35.9</td>
<td>31.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rolled oats SBM = 509</td>
<td>1.9</td>
<td></td>
<td>14.0</td>
<td>-</td>
<td>36.8</td>
<td>32.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rolled oats SBM = 509</td>
<td>7.8</td>
<td></td>
<td>18.3</td>
<td>-</td>
<td>32.6</td>
<td>32.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rolled oats SBM = 509</td>
<td>7.4</td>
<td></td>
<td>17.5</td>
<td>-</td>
<td>34.2</td>
<td>32.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rolled oats SBM = 509</td>
<td>7.0</td>
<td></td>
<td>18.4</td>
<td>-</td>
<td>31.5</td>
<td>31.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rolled oats SBM = 509</td>
<td>6.6</td>
<td></td>
<td>18.8</td>
<td>-</td>
<td>35.2</td>
<td>32.0</td>
</tr>
<tr>
<td>Reference</td>
<td>Pasture Management</td>
<td>Supplement Type and Quality</td>
<td>Amount of Supplement Fed (kg DM/day)</td>
<td>Breed and Stage of Lactation</td>
<td>Milk Production (kg/cow.day)</td>
<td>Milk Response (kg milk/kg supplement)</td>
<td>Milk Fat (g/kg milk)</td>
<td>Milk protein (g/kg milk)</td>
</tr>
<tr>
<td>--------------------------</td>
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<td>-----------------------------</td>
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<td>---------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Ashes and Hamilton (1983)</td>
<td>CP = 120</td>
<td>barley</td>
<td>2.5</td>
<td>10.3</td>
<td>12.1</td>
<td>0.72</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>barley casein</td>
<td>2.0, 0.5</td>
<td>10.9</td>
<td>12.6</td>
<td>0.68</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>barley casein</td>
<td>2.0, 0.5</td>
<td>10.5</td>
<td>12.2</td>
<td>0.68</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>barley casein</td>
<td>2.0, 0.5</td>
<td>10.5</td>
<td>13.1</td>
<td>1.04</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>barley casein</td>
<td>2.0, 0.5</td>
<td>10.9</td>
<td>13.2</td>
<td>0.92</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Olney and Albertson (1984)</td>
<td>N fertilised and irrigated continuous grazing IVD grab sample 678</td>
<td>barley 5 cows/ha</td>
<td>4</td>
<td>9.1 SCM</td>
<td>11.5 SCM</td>
<td>0.60</td>
<td>39.5</td>
<td>39.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>barley 7 cows/ha</td>
<td>4</td>
<td>6.0 SCM</td>
<td>10.5 SCM</td>
<td>1.13</td>
<td>41.6</td>
<td>40.1</td>
</tr>
<tr>
<td>Hughes et al. (1988)</td>
<td>N fertilised, rotationally grazed, short IVD = 550 CP = 150</td>
<td>sweet yellow forage lupins</td>
<td>1.7</td>
<td>6.8</td>
<td>8.1</td>
<td>0.77</td>
<td>43.0</td>
<td>41.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IVD = 625 CP = 125</td>
<td>4.0</td>
<td>9.1</td>
<td>0.58</td>
<td>43.3</td>
<td>31.4</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>winter-spring</td>
<td>2.1</td>
<td>9.2</td>
<td>9.9</td>
<td>0.33</td>
<td>43.5</td>
<td>42.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N fertilised, rotationally grazed, long IVD = 550 CP = 140</td>
<td>4.3</td>
<td>10.4</td>
<td>0.28</td>
<td>40.3</td>
<td>32.3</td>
<td>-</td>
</tr>
<tr>
<td>Reference</td>
<td>Pasture Management</td>
<td>Supplement Type and Quality</td>
<td>Amount of Supplement Fed (kg DM/day)</td>
<td>Breed and Stage of Lactation</td>
<td>Milk Production (kg/cow.day)</td>
<td>Milk Response (kg milk/kg supplement)</td>
<td>Milk Fat (g/kg milk)</td>
<td>Milk protein (g/kg milk)</td>
</tr>
<tr>
<td>-----------------</td>
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<td>-------------------------</td>
</tr>
<tr>
<td>Hamilton et al. (1992)</td>
<td>strip grazed over summer</td>
<td>barley</td>
<td>2.8</td>
<td>Friesian</td>
<td>14.7</td>
<td>17.9</td>
<td>1.14</td>
<td>24.6</td>
</tr>
<tr>
<td>OMD = 0.66 CP = 156</td>
<td></td>
<td>barley SFM&lt;sup&gt;6F&lt;/sup&gt;</td>
<td>1.9 + 1.1</td>
<td>2+ lactations</td>
<td>17.8</td>
<td>1.03</td>
<td>19.1</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>barley SFM&lt;sup&gt;5F&lt;/sup&gt;</td>
<td>1.9 + 1.1</td>
<td>1-3 m into lactation</td>
<td>18.9</td>
<td>1.40</td>
<td>18.0</td>
<td>15.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>barley SFM&lt;sup&gt;7F&lt;/sup&gt;</td>
<td>1.9 + 1.1</td>
<td></td>
<td>18.4</td>
<td>1.23</td>
<td>18.4</td>
<td>16.1</td>
</tr>
</tbody>
</table>

SFM - Sunflower meal; SBM - Soyabea meal; FCM - Fat corrected milk; SCM - solids corrected milk;<sup>6F</sup> - Untreated;<sup>5F</sup> - 0.5% treated with formaldehyde;<sup>7F</sup> - 0.7% treated with formaldehyde.
2.7.2.2 Responses to Protein Concentrates

When an imbalance in dietary nutrients occurs, it is often reflected in inefficient use of energy for milk production. At high rates of grain feeding, nutrient partitioning may result in energy being used for body tissue instead of milk production when dietary protein is limiting (Kellaway and Porta 1993).

Stage of lactation is an important factor influencing milk production responses to protein supplementation. If specific nutrients are limiting intake, dairy cows have the ability to mobilise body reserves to sustain a certain milk yields. Mobility of body reserves liberates comparatively greater amounts of energy compared to protein, resulting in relative protein deficiency. Consequently, most responses to protein supplementation occur within the first 100 days of lactation (Kaiser and Ashwood 1981; Davison et al. 1991a; Hamilton et al. 1992).

Untreated protein sources did not enhance milk production (Kaiser and Ashwood 1981) and composition (Hamilton et al. 1992) of cows grazing kikuyu unless fed in conjunction with high levels of energy. This suggests that N fertilised kikuyu pasture supplies sufficient RDP for milk production, and energy may be the limiting factor for microbial protein synthesis. To support this, rumen NH₃ concentrations 2 hours following feeding were by far the highest in animals fed untreated protein meal (Hamilton et al. 1992), indicating that the RDP may be in excess of microbial requirements and is subsequently converted to NH₃.

Protein supplementation enhanced milk production above levels obtained from energy sources when it was protected to some degree from rumen degradation (Ashes and Hamilton 1983; Hamilton et al. 1992). Such treatment of the protein increases the amount of UDP leaving the rumen and enhances the supply of amino acids to the small intestine. Optimal protection (0.5% formaldehyde) of the protein source is essential so as to reduce rumen degradation, but allow the subsequent release and absorption of essential amino acids in the small intestine. When protein intake is deficient (which is rare on N fertilised kikuyu pasture), additional protein may indirectly increase milk fat content through an increase in roughage intake (Kellaway and Porta 1993), and also

N fertilised kikuyu appears to contain adequate concentrations of RDP for reasonable milk yields and this is reflected in the lack of response to unprotected protein supplementation. However, milk production increases have been observed in response to supplementation when protein was protected from rumen degradation.

2.7.2.3 Responses to Forage Supplementation
Supplementation with higher quality forages is an alternative to feeding concentrates. Responses to supplementation with forage oats, ryegrass and lupins (Royal and Hughes 1976; Hughes et al. 1988) were low (0.27 - 0.36 kg milk/kg forage DM), when kikuyu was offered ad-lib. However, responses up to 0.77 kg milk/kg DM forage lupin, were observed by Hughes et al. (1988) from cows grazing ‘short’ kikuyu, due to restricted levels of kikuyu intake.

2.7.3 Effect of Supplements on Kikuyu Intake
Relatively low levels of substitution were observed by Hamilton et al. (1992) which is probably a reflection of the low (<3 kg DM/cow.day) rates of supplementation used and possibly the low levels of pasture on offer to the animals.
3.1 Location

All experiments were located on Wollongbar Agricultural Institute situated on the north coast of NSW (lat. 28° 48' S and long. 153° 20' E; 165 m above sea level).

3.1.1 Climatic Conditions

The long term average (132 years) annual rainfall at the site is 1613 mm, with the mean monthly long-term rainfall, evaporation and maximum and minimum temperatures, and the same data for the 3 years of the study are shown in Figures 3.1 and 3.2, respectively. Frosts occur infrequently, with an average probability of a frost in the coldest month (August) being 7%.

Figure 3.1 Monthly climatic data for the past 132 years at Alstonville Tropical Fruit Research station located 10 km east of Wollongbar Agricultural Institute for (a) mean and range of maximum and minimum temperatures (°C) and the period of likely frosts is indicated and (b) mean rainfall and evaporation (mm).
Rainfall/Evaporation (mm) and maximum and minimum temperatures (°C) at Alstonville Tropical Fruit Research station located 10 km east of Wollongbar Agricultural Institute during (a) 1993, (b) 1994 and (c) 1995.

<table>
<thead>
<tr>
<th>Month</th>
<th>Rainfall</th>
<th>Evaporation</th>
<th>Maximum Temperature</th>
<th>Minimum Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>65</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>65</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J</td>
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<td>A</td>
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<td>S</td>
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<td>O</td>
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<tr>
<td>N</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.1.2 Soil
The soil was a red kraznozem of basaltic origin. The mean (± s.e.) soil analysis taken at random from various sites within the experimental area was Ca = 0.890 ± 0.03 %; K = 0.380 ±0.02 milliequivalents/100 g soil; P (Colwell) = 64 ± 8 ppm and pH (CaCl₂) = 5.1.

3.1.3 Pastures
Both grazing and cut-plot trials were conducted on long-established 'common' kikuyu pastures.

3.2 Pasture Management
3.2.1 Management for Grazing Experiments
When required, the kikuyu was mulched using a Nobili BNU-195 mulcher fitted with wire blades (Cotello-01) to a 5 cm stubble height, within 2 days of grazing, to prevent the development of a stoloniferous mat. Urea was applied to the pasture at a rate of 100 kg/ha, 4 times during the kikuyu growing season (equivalent to 182 kg N/season). Pastures were grazed before a substantial proportion of stem protruded above the 5 cm stubble height resulting in an 18-24 day grazing interval.

Cows were offered a fresh block of pasture daily (unless otherwise stated) and a back-fence was used to prevent re-grazing on recently grazed blocks.

3.2.2 Management for Cut-plot Experiments
Cut-plot studies were irrigated as required to replace evapotranspiration losses. Each plot was hand-mown to a 5 cm stubble height using a rotary mower and the regrowth above this height examined (with the exception of Study 1b in Chapter 4) as it was the portion deemed available to grazing dairy cattle.

3.3 Pasture Sampling for Chemical Analysis
3.3.1 Sampling for Quality Analysis in Grazing Experiments
Pre-grazing pasture pluck samples were obtained each day by hand-plucking the pasture
to an approximate post-grazing height based on the previous day's grazing. Samples were oven dried at 80°C and then milled through a 1 mm sieve in preparation for analysis.

3.3.2 Sampling for Quality Analysis in Cut-plot Experiments
Samples were obtained using hand-shears to a 5 cm stubble height (with the exception of Study 1b in Chapter 4). Samples to be analysed for N, in vitro OMD, minerals, amino acids, ADF and NDF were dried at 80°C for 24 hours (h); those analysed for WSC, starch, oxalates and nitrates were dried at 60°C for 24 h and for NPN, the samples were freeze-dried. All samples were ground through a 1 mm sieve.

3.4 Chemical Analysis of Pasture Samples
3.4.1 Dry Matter
DM was determined by recording the weight of an empty Petri dish and then adding approximately 10 g of sample and recording the combined weight. The dish containing the sample was placed in an oven at 105°C overnight (16 h) and removed, allowed to cool in desiccator and the combined weight recorded. The DM % was then calculated as follows;

\[
DM \,(\text{g/kg}) = \frac{\text{Combined Dry Wt} - \text{Petri Dish Wt}}{\text{Combined Wet Wt} - \text{Petri Dish Wt}} \times 100
\]

Equation 1

3.4.2 Organic Matter
Organic matter was determined by ashing the sample. The weight of a dry crucible was recorded and approximately 10 g of sample (previously dried at 105°C for 16 h) added and the combined weight recorded. The crucible was placed in a muffle furnace at 500°C overnight (16 h), removed and allowed to cool in a desiccator and then the combined weight was recorded. The OM% was calculated as follows;

\[
OM(\text{g/kgDM}) = 100 - \left( \frac{\text{Combined Dry Wt} - \text{Crucible Wt}}{\text{Combined Wet Wt} - \text{Crucible Wt}} \times 100 \right)
\]

Equation 2
3.4.3 Nitrogen, Phosphorus and Potassium

Concentrations of N, P and K in plant material were determined using semi-micro digestion (Havilah et al. 1977) similar to that of the Macro Kjeldahl Method.

3.4.3.1 Digestion

Reagents

1. Standard Kjeldahl catalyst tablets (AJAX, product) with low Se (equivalent of 0.01g of Se) and 1g of sodium sulphate anhydrous.
2. Concentrated sulphuric acid (H$_2$SO$_4$)

Procedures

Samples (0.200 g) were weighed and placed into 50 ml digestion tubes containing 1 Kjeldahl catalyst tablet. Concentrated H$_2$SO$_4$ (5 ml) was added to digestion tube then placed on digestion block (150°C) in a fume cupboard for 1 h, followed by 1 h at 250°C and then at 350°C until the solution cleared. The flask was then heated for a further 30 min. The solution was then cooled prior to the addition of 20 ml of de-ionised H$_2$O and mixed on a vortex mixer, and allowed to cool. De-ionised H$_2$O was added up to 50 ml and the solution thoroughly shaken.

3.4.3.2 Nitrogen Determination

Reagents

1. Sodium Hydroxide (NaOH) 40% (w/v) Solution
   400 g of NaOH pellets were dissolved in approximately 500 ml of de-ionised H$_2$O, allowed to cool and made up to 1000 ml with de-ionised H$_2$O.
2. Salicylate Solution
   Sodium nitroprusside (0.34 g) and sodium salicylate (48.00 g) were dissolved in approximately 2000 ml of de-ionised H$_2$O and then made up to 2500 ml using de-ionised H$_2$O.
3. Cyanurate Solution
   Sodium dichloroisocyanurate (2.28 g) was dissolved in approximately 900 ml of de-ionised H$_2$O and sodium hydroxide 40% A.R. solution (138 ml) was added, and made up to 1800 ml.
Standards

1. Double Strength Diluent
Ten Kjeldahl catalyst tablets were placed in a 500 ml Kjeldahl flask, to which was added 50 ml of H₂SO₄ (A.R.). The contents were boiled for 2 h and allowed to cool prior to the addition of approximately 70 ml of de-ionised H₂O. The solution was again allowed to cool and then made up to 250 ml in a volumetric flask.

2. Stock Standard - N 1000 μg/ml
Ammonium sulphate ((NH₄)₂ SO₄) (A.R.) (1.1791 g) was dissolved in 100 ml of de-ionised H₂O and then the solution was made up to 250 ml in a volumetric flask.

3. Working Standards
The following aliquots of the stock solution were added to 50 ml of double strength diluent and made up to 100 ml with de-ionised H₂O.

<table>
<thead>
<tr>
<th>μg/ml N</th>
<th>0</th>
<th>25</th>
<th>50</th>
<th>100</th>
<th>150</th>
<th>200</th>
<th>250</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml 100 μg/ml N</td>
<td>0</td>
<td>2.5</td>
<td>5.0</td>
<td>10.0</td>
<td>15.0</td>
<td>20.0</td>
<td>25.0</td>
</tr>
</tbody>
</table>

Procedures
Aliquots of 0.1 ml of standards in duplicate (blank done in triplicate) and sample digests, followed by 8.9 ml of salicylate reagent and 1.0 ml of cyanurate reagent were added to spectrophotometer tubes, which were then capped and mixed well. Colour was then allowed to develop for at least 30 min before reading on a Shimadzu 160 A UV-VIS Spectrophotometer (697 nm). The N (%) was then calculated as follows:

\[
\% N = \frac{Absorbance - Blank}{Slope} \times \frac{A}{B} \times 10^4 \quad \text{Equation 3}
\]

\[
A - \text{Final Volume (50 ml)}
\]
\[
B - \text{Weight of sample (0.2 g)}
\]
\[
Slope - \text{Slope of calibration equation}
\]
3.4.3.3 Phosphorus Determination

Reagents
1. Murphy and Riley (M & R) Reagents
60 g of ammonium molybdate \((\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\) was dissolved in approximately 4000 ml of de-ionised H₂O and then 620 ml of concentrated H₂SO₄ (AR) was added. The solution was then allowed to cool and made up to 5000 ml with de-ionised H₂O.

2. Modified M & R Reagents
The M & R reagents were made up fresh daily and the amount made was dependant on the number of samples and standards to be used. Ascorbic acid (1.5 g) was dissolved in every 100 ml of M & R reagent used.

Standards
1. Double Strength Diluent
As for N determination (see section 3.4.3.2)

2. Stock Standard - P 1000 μg/ml
Dissolved 4.394 g of potassium di-hydrogen orthophosphate \((\text{KH}_2\text{PO}_4\) in de-ionised H₂O and made up to 1000 ml.

3. Working Standards
The following aliquots of the stock solution were added to 50 ml of double strength diluent and made up to 100 ml with de-ionised H₂O.

<table>
<thead>
<tr>
<th>μg/ml P</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml 100 μg/ml N</td>
<td>0</td>
<td>0.5</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
<td>4.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Procedures
Aliquots of 0.2 ml of standards in duplicate (blanks done in triplicate) and sample digests were added to spectrophotometer tubes, then 1.0 ml of modified M & R Reagent and 8.8 ml of de-ionised H₂O was added to each tube which were then capped and mixed well. Colour was then allowed to develop for at least 1 h in a water bath (60°C) before reading on a Shimadzu 160 A UV-VIS Spectrophotometer (827 nm). The P (%) was then calculated as follows:
3.4.3.4 Potassium Determination

Standards

1. Double Strength Diluent
As for N determination (see section 3.4.3.2)

2. Stock Standard - K 1000 μg/ml
Potassium chloride (KCl) (1.907 g) was dissolved in de-ionised H₂O and made up to 1000 ml.

3. Working Standards
The following aliquots of the stock standard solution were added to 50 ml of double strength diluent and made up to 100 ml with de-ionised H₂O.

<table>
<thead>
<tr>
<th>µg/ml K</th>
<th>0</th>
<th>50</th>
<th>100</th>
<th>150</th>
<th>200</th>
<th>250</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml 100 µg/ml K</td>
<td>0</td>
<td>2.5</td>
<td>5.0</td>
<td>15.0</td>
<td>20.0</td>
<td>25.0</td>
</tr>
</tbody>
</table>

Procedures

Samples were read directly on a Varian Spectra AA 300 Atomic Absorption Spectrophotometer and the K (%) was calculated as follows;

\[
\% K = \frac{\text{Absorbance} - \text{Blank}}{\text{Slope}} x \frac{A}{B} x 10^4 \quad \text{Equation 5}
\]

\[A - \text{Final Volume (50 ml)}\]
\[B - \text{Weight of sample (0.2 g)}\]
\[\text{Slope} - \text{slope of calibration equation}\]
3.4.4 Sodium, Calcium, Magnesium, Iron, Copper, Zinc and Manganese

The "wet-ashing" procedure (Johnson and Ulrich 1959) was used for the determination of the Na, Ca, Mg, Fe, Cu, Zn and Mn.

Reagents
1. Concentrated nitric acid (HNO₃) (A.R.)
2. 70% perchloric acid (HClO₄)

Standards - Macro Nutrients
1. 3 % KCl Solution (de-ionisation buffer)

57.24 g KCl (A.R.) was dissolved in de-ionised H₂O and made up to 1000 ml.

2. Stock Standards - Macro nutrients
   (i) Na - 100 µg/ml
   57.24 g of dried sodium chloride (NaCl) (A.R.) was dissolved in de-ionised H₂O and made up to 1000 ml.
   (ii) Ca - 100 µg/ml
   100 ml of 1000 µg/ml calcium nitrate (Ca(NO₃)₂) (B.D.H. Standard) solution was diluted in de-ionised H₂O and made up to 1000 ml.
   (iii) Mg - 100 µg/ml
   100 ml of 1000 µg/ml magnesium nitrate (MgNO₃) (B.D.H. Standard) solution was diluted in de-ionised H₂O made up to 1000 ml.

3. Working Standards - Macro Nutrients
To each 1000 ml volumetric flask, 100 ml of 3 % KC solution, 20 ml of 70 % HClO₄ and the following amounts of the stock standards were added, and then the solution was made up to 1000 ml final volume with de-ionised H₂O.

<table>
<thead>
<tr>
<th>µg/ml Na, Ca and Mg</th>
<th>0</th>
<th>1.0</th>
<th>5.0</th>
<th>10.0</th>
<th>15.0</th>
<th>20.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml of 100 µg/ml each stock</td>
<td>0</td>
<td>2.5</td>
<td>5.0</td>
<td>10.0</td>
<td>15.0</td>
<td>20.0</td>
</tr>
</tbody>
</table>

Standards - Micro Nutrients
1. Stock standards - Micro nutrients
   (I) Fe - 100 µg/ml
100 ml of 1000 µg/ml ferric nitrate (B.D.H. Standard) solution was diluted in de-ionised H₂O and made up to 1000 ml.

(ii) Cu - 100 µg/ml

100 ml of 1000 µg/ml cupric nitrate (B.D.H. Standard) solution was diluted in de-ionised H₂O and made up to 1000 ml.

(iii) Zn - 100 µg/ml

100 ml of 1000 µg/ml zinc nitrate (B.D.H. Standard) solution was diluted in de-ionised H₂O and made up to 1000 ml.

(iv) Mn - 100 µg/ml

100 ml of 1000 µg/ml manganese nitrate (B.D.H. Standard) solution was diluted in de-ionised H₂O and made up to 1000 ml.

2. Working standards - Micro nutrients

To each 1000 ml volumetric flask 20 ml of 70 % HClO₄ and the following amounts of the stock standards were added, and then the solution was made up to 1000 ml final volume with de-ionised H₂O.

<table>
<thead>
<tr>
<th>µg/ml Fe</th>
<th>0</th>
<th>2.5</th>
<th>5.0</th>
<th>7.5</th>
<th>10.0</th>
<th>12.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml of 100 µg/ml Fe</td>
<td>0</td>
<td>25.0</td>
<td>50.0</td>
<td>75.0</td>
<td>100.0</td>
<td>125.0</td>
</tr>
<tr>
<td>µg/ml Cu</td>
<td>0</td>
<td>0.5</td>
<td>1.0</td>
<td>1.5</td>
<td>2.0</td>
<td>2.5</td>
</tr>
<tr>
<td>ml of 100 µg/ml Cu</td>
<td>0</td>
<td>5.0</td>
<td>10.0</td>
<td>15.0</td>
<td>20.0</td>
<td>25.0</td>
</tr>
<tr>
<td>µg/ml Zn</td>
<td>0</td>
<td>0.5</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
<td>4.0</td>
</tr>
<tr>
<td>ml of 100 µg/ml Zn</td>
<td>0</td>
<td>5.0</td>
<td>10.0</td>
<td>20.0</td>
<td>30.0</td>
<td>40.0</td>
</tr>
<tr>
<td>µg/ml Mn</td>
<td>0</td>
<td>2.0</td>
<td>4.0</td>
<td>6.0</td>
<td>8.0</td>
<td>10.0</td>
</tr>
<tr>
<td>ml of 100 µg/ml Mn</td>
<td>0</td>
<td>20.0</td>
<td>40.0</td>
<td>60.0</td>
<td>80.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Procedures

(i) Digestion

When only Na, Ca and Mg analysis was required, 0.2000 g of sample including duplicates, references and blanks was weighed (0.500 g if trace elements were being determined as well) and placed into 50 ml graduated pyrex digestion tubes, each
containing 2 glass beads. In a fume cupboard, either 2.0 or 5.0 ml of concentrated nitric acid was added to tubes containing 0.2 and 0.5 g of sample respectively. Tubes were then allowed to stand for at least 2 h (preferably overnight) to dissolve and enable foaming to cease to ensure all of the sample was wetted and nearly completely dissolved prior to the commencement of digestion.

Tubes were then placed on a digestion block at 60°C for 1 h. Samples were then heated at 90°C for 20 min, 120°C for 20 min and 150°C for 20 min or until the volume of nitric acid was below half of the starting volume. Samples were then removed from the heating block and allowed to cool. Then 1 or 2 ml of 70 % HClO₄ was added for 0.2 and 0.5 g samples, respectively, and placed on digestion block at 240°C to allow the remaining nitric acid to boil off in a white cloud. The boiling point of HClO₄ is reached at approximately 200-210°C which is signified by a white reflux cloud in the neck of the tube. The digestion was left to proceed for 30 min after the initiation of the reflux. Tubes were then cooled in a fume cupboard prior to the addition of 25 ml of de-ionised H₂O, mixed on the vortex and left to cool. De-ionised H₂O was used to make up to 50 ml volume and samples were corked and mixed well.

(ii) Analysis

Na, Ca and Mg - The concentration of Ca, Mg and Na in the digests were too high for direct analysis and therefore the samples were diluted. The deionisation buffer 3 %KC was added at a rate of 1 ml per 10 ml of dilution volume.

Fe, Cu, Zn and Mn - These samples were read directly.

All samples were read using an atomic absorption spectrophotometer and concentrations were calculated as follows;

\[
\% \text{ Na, Ca, Mg} = \frac{\text{Absorbance} - \text{Blank}}{\text{Slope}} \times \frac{\text{Final Volume (50 ml)}}{\text{Sample Weight (g)}} \times \text{Dilution} \times 10^4
\]

Equation 6
Equation 7

\[
ppm \text{ Cu, Zn, Fe, Mn} = \frac{\text{Absorbance} - \text{Blank}}{\text{Slope}} \times \frac{\text{Final Volume (50 ml)}}{\text{Sample Weight (g)}}
\]

*Slope - Slope of calibration equation*

### 3.4.5 Acid Detergent Fibre

ADF content of plant material was determined using the method of van Soest (1963).

**Reagents**

1. Acid-Detergent Solution
   
   28 ml of concentrated H$_2$SO$_4$ was added to approximately 900 ml of de-ionised H$_2$O and made to 1000 ml, then 20.00 g of cetyltrimethylammonium bromide was added and dissolved by constant stirring.

2. Decahydonaphthalene (Dekalin) (A.R.)

3. Acetone (Laboratory reagent)

**Procedure**

Approximately 0.6000 g of sample (including duplicates, references and blanks) was weighed out and placed into a 250 ml Quickfit round bottom flask. 100 ml of ADF solution was added, followed by 2 ml Dekalin to each flask. The flasks were placed on heaters, connected to soxhlet condensers and cooling water allowed to flow through condensers. Flasks were slowly heated to boiling (3/4 heat for 5-10 min) and heat was reduced when boiling began to avoid foaming and adjusted to a slow, even boiling rate. Reflux continued for 1 h following the onset of boiling.

Glass fibre filters (Whatman GF/D) were placed into vitreosil sintered crucibles (tall form, porosity one, 40 mm plate, volume approximately 50 ml) and the combined unit weighed to 4 decimal places. The crucible was placed in a vacuum manifold and contents of the 250 ml flask was poured into it. The flask was rinsed a couple of times with hot de-ionised H$_2$O to ensure all fibre was recovered from the flask. Approximately 40 ml of acetone was used to rinse the flask and crucible.
The crucible was then removed from the manifold and acetone allowed to evaporate off the sample in the fume cupboard. Crucibles were then placed in the oven at 100°C overnight. On removal from oven, crucibles were placed in desiccator to cool and then weighed to 4 decimals. The ADF content of the sample was calculated as follows;

\[
\% \ ADF = \frac{C - B}{A} \times \frac{100}{1} \quad \text{Equation 8}
\]

\( A \) - Initial weight of sample (g)
\( B \) - Weight of crucible plus filter paper (g)
\( C \) - Final weight of sample, crucible and filter paper (g)

3.4.6 Neutral Detergent Fibre

Forage samples were analysed for NDF using the method of van Soest and Wine (1967) and was not corrected for protein.

**Reagents**

1. NDF solution

The following were added to a 1000 ml volumetric flask which was then made up to volume using de-ionised H₂O. The solution was stirred at low heat to dissolve.

- 30 g Sodium lauryl sulphate
- 18.61 g Ethylenediaminetetra-acetic acid (EDTA), disodium salt (disodium salt, \([\text{CH}_2\text{N(\text{CH}_2\text{COOH})\text{CH}_2\text{COONa})}_2\cdot 2\text{H}_2\text{O}\) )
- 6.81 g Sodium tetraborate (Na₂B₄O₇·10H₂O)
- 4.56 g Disodium hydrogen orthophosphate, anhydrous (Na₂HPO₄)
- 10 ml 2-Ethoxyethanol (C₂H₅OCH₂CH₂OH)

2. Sodium sulphite, anhydrous (Na₂SO₃)
3. Acetone ((CH₃)₂CO)
4. Decahydropnaphthalene (Dekalin)

**Procedure**

0.5 - 0.7 g of sample (to 4 decimal places) including duplicates, references and blanks were weighed out and placed in to 250 ml Quickfit round bottom flasks. The following
substances were added to the samples in a fume cupboard in order;
   i. 100 ml of NDF solution
   ii. 2 ml of Dekalin
   iii. 0.5 g of sodium sulphite
   iv. 0.05 ml heat stable amylase

Flasks were then connected to soxhlet condensers, water supplied to cooling condensers and heated to boiling in 5-10 min. Heat was then reduced to avoid foaming and adjusted boiling to a slow even level and refluxed for 1 h from the onset of boiling.

Glass fibre filters (Whatman GF/D) were placed in Vitreosil sintered crucibles (same as used for ADF), the combined weight recorded (4 decimal places) and then attached to a vacuum manifold. After the completion of heating, the contents of the flask were filtered quickly to prevent gelatinisation of the sample. The flask was rinsed with boiling de-ionised H₂O to ensure total fibre recovery from the flask. Acetone (approximately 40 ml) was used to rinse the flask and crucible.

Crucibles were placed in the fume cupboard for 30 min to evaporate acetone and then placed in a drying oven overnight at 100°C. On removal from the oven, crucibles were placed in a desiccator to cool and then weighed (4 decimals). The NDF content of the sample was calculated as follows;

\[
\% \text{ NDF} = \frac{C - B}{A} \times \frac{100}{1} \quad \text{Equation 9}
\]

\[
A - \text{Initial weight of sample (g)}
\]

\[
B - \text{Weight of crucible plus filter paper (g)}
\]

\[
C - \text{Final weight of sample, crucible and filter paper (g)}
\]

3.4.7 In vitro Organic Matter Digestibility

*In vitro* OMD was determined using the 2 stage procedure of Tilley and Terry (1963) as modified by Minson and McLeod (1972).
Procedure

Dry, ground forage samples were re-dried overnight and a 1 g sub-sample was obtained for OM analysis. A sample of 0.5 g was incubated for 48 h in rumen inoculum (obtained from donor sheep fed lucerne pellets) made up in artificial salivary buffer, followed by a 48 h acid-pan digestion. The undigested residue was ashed in a muffle furnace (550°C for 16 h) and in vitro digestibility determined on an OM basis.

3.4.8 Metabolisable Energy

The ME of a feed was calculated from either DMD (Equation 10) or OMD (Equation 11) using equations established by SCA (1990).

\[ ME \left( \text{MJ ME/kg DM} \right) = \left[ 0.17 \times \text{DMD(\%)} \right] - 2.0 \]  
Equation 10

\[ ME \left( \text{MJ ME/kg DM} \right) = \left[ 0.16 \times \text{OMD(\%)} \right] - 1.8 \]  
Equation 11

3.4.9 Water Soluble Carbohydrates

The WSC concentrations in plant material were determined using a modification of the method of Smith (1969).

Reagents

1. 0.2 % Benzoic acid/water - Extracting solution

10 g benzoic acid was dissolved in 20-25 ml of 95 % ethanol and slowly added to 2500 ml of distilled H₂O whilst stirring, and then made up to 5000 ml in a volumetric flask.

2. 1N Hydrochloric acid (HCl) - hydrolysing solution

Slowly added 100 ml 32 % HCl to approximately 600 ml water whilst stirring. When the solution was miscible and cool, it was made up to 1000 ml with water. Brij 35 (0.5 ml) was added and the solution mixed thoroughly and stored in bottle with a ground glass stopper.

3. Alkaline potassium ferricyanide solution

Potassium ferricyanide (K₂Fe(CN)₆) (0.25 g), sodium carbonate (Na₂CO₃) (50.00 g) and sodium chloride (NaCl) (9.00 g) were dissolved in approximately 600 ml of distilled H₂O and made up to 1000 ml with distilled H₂O. This solution was prepared weekly and stored in a light resistant container in a refrigerator.
Standards

1. 1.2% Sugar solution - Stock standard

Sucrose ($C_{12}H_{22}O_{11}$) (2.400 g) was dissolved in approximately 150 ml of 0.2% benzoic acid and made up to 200 ml.

2. Working standards

These were made up fresh for each run. The following aliquots were taken from the 1.2% sugar solution and made up to volume in 100 ml volumetric flasks with 0.2% benzoic acid.

<table>
<thead>
<tr>
<th>ml 1.2% sugar solution</th>
<th>2.50</th>
<th>5.00</th>
<th>10.00</th>
<th>20.0</th>
<th>25.0</th>
<th>50.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>% sucrose</td>
<td>0.03</td>
<td>0.06</td>
<td>0.12</td>
<td>0.24</td>
<td>0.30</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Procedure

Approximately 0.5000 g of plant sample was weighed into a 30 ml plastic container, then 20.0 ml of 0.2% benzoic acid extracting solution was added before placing on a reciprocal shaker for 1 h. Solution was allowed to settle for 30 min, followed by decanting solution and filtering for analysis into a small test tube. Each filtration was done by pushing a seraclear filter into the test-tube and pouring the filtered solution out of the centre and into an AA cup.

The prepared extractions were then put through the autoanalyser system using a modified method of Smith (1969).

3.4.10 Starch

Starch was extracted from plant material using high temperature amylase and amylloglucosidase as outlined below and then treated as for WSC.

Reagents

1. HT (high temperature) Amylase solution

0.200 g of concentrated HT Amylase was added to 50 ml distilled H$_2$O and stored in the refrigerator.

2. Citrate buffer
6.47 g of Na$_3$ Citrate and 3.80 g of citric acid was dissolved in approximately 500 ml of distilled H$_2$O and made up to 1000 ml with distilled H$_2$O and stored in a refrigerator.

3. Amyloglucosidase solution

**Standards**
The same standards were used as for WSC (see Section 3.4.9).

**Procedure**
2.0 g of dry, ground sample was weighed into a 2.5 mm test tube and 0.50 ml of HT Amylase solution was added. Then 1 ml absolute ethanol and 15 ml of distilled H$_2$O were added and the solution mixed well using a vortex. The solution was then heated in a boiling water bath for 2 min, vortexed, then heated again for 10 min and vortexed again. The screw caps were then loosely attached prior to incubation at 80-85°C for 1 h in water bath. Samples were then cooled and mixed on the vortex. 2.4 ml of citrate buffer was then added and the solution mixed again (vortex), and then 50 µL of amyloglucosidase solution was added. Samples were incubated in a water bath at 55°C for 1 h followed by filtration through Whatman N. 40 filter paper. Samples were stored in refrigerator prior to analysis on the autoanalyser system using a modified method of Smith (1969).

Samples are also analysed for WSC content (see Section 3.4.9), with starch content calculated as the difference between the value obtained from the above starch method and that obtained from WSC analysis.

3.4.11 Nitrate

The nitrate content of forage was determined using Griess-Ilosavy method as described in Rayment (1992).

**Procedure**
0.5 g of dried (85°C), plant material was boiled in deionised water for 1 h and nitrate was measured colorimetrically using a segmented flow analyser.
3.4.12 Non-Protein Nitrogen
NPN was determined as the difference between total N (see Section 3.4.3.2) and protein N. Protein N was determined using the method described by Faichney and White (1983) as described below.

Reagents
1. Trichloroacetic acid 5 % w/v (TCA)
2. Sulphuric acid 1.07 N.

Procedure
Weighed 0.5 g forage sample into 50 ml stoppered centrifuge tubes. Then added 20 ml of 5 % TCA to each tube which were shaken vigorously and placed into a shaking water bath at 60°C for 1 h (and given a vigorous shake by hand at 15 min intervals). Tubes were then cooled in an ice slurry for 15 min, then centrifuged for 20 min at 100 g. The supernatant was then decanted and the precipitate washed and centrifuged twice with 20 ml 5 % TCA. The precipitate was then dislodged using a small amount of de-ionised water and transferred to a Kjeldahl digestion flask. Sample was then treated as for total N and protein N was calculated as mg/g DM. NPN was then calculated as the difference between total N and protein N of the sample.

3.4.13 Oxalates
Oxalate concentrations in plant material were determined using the method of Roughan and Slack (1973).

3.4.13.1 Total Oxalates
Reagents
1. Anhydrous CH₂OH - Prepared by Grignard reaction and carefully stored to exclude moisture. 75 ml of anhydrous CH₂OH was placed in a 2 L flask fitted with a reflux condenser. 5 g of clean Mg turnings and 0.5 g of re-sublimed I₂ was added. Flask was gently warmed until the initiation of H₂ evolution and reflux continued until most of the Mg was converted to Mg(OCH₃)₂. Approximately 900 ml of distilled CH₂OH was added and refluxed for 30 min. Solution was then distilled with the exclusion of atmospheric H₂O.
2. Dry CHCl₃ - CHCl₃ was distilled through an aqueous solution of sodium thiosulphate. The dry CHCl₃ was recovered from the azeotropic distillate by freezing and filtering, and then stored over anhydrous CaCl₂.

3. Concentrated H₂SO₄ - Previously unopened bottle.

4. Esterification mixture - One volume of anhydrous CH₃OH was mixed with 2 volumes of dry CHCl₃, and then 5% by total volume of concentrated H₂SO₄ was carefully added. The solution was stored in a sealed brown bottle equipped with a 10 ml dispenser.

5. Sodium oxalate A.R. - Oven dried and cooled in a desiccator.

**Standards**

1. Oxalic acid - Stock Standard
   Sodium oxalate was used to prepare an aqueous solution containing the equivalent of 4 mg anhydrous oxalic acid/ml.

2. Working standards
   A series of aliquots outlined below were pipetted into culture tubes and dried in a forced air oven at ≤ 100°C.

<table>
<thead>
<tr>
<th>Stock standard (ml)</th>
<th>1.0</th>
<th>2.0</th>
<th>3.0</th>
<th>4.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>oxalic acid in sample (%)</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
<td>4.0</td>
</tr>
</tbody>
</table>

**Procedure**

0.4 g of dried herbage was weighed into a 20 ml capacity culture tube. 10 ml of esterification reagent was dispensed into sample and standard tubes and cap securely fastened. These stood overnight at 25°C or heated at approximately 60°C for 1 h in a water bath (being careful not to overheat). Tubes were allowed to cool if required and 2 ml of distilled water added. The cap was replaced and tubes vigorously shaken for 15 sec. Layers were then allowed to separate. The lower, chloroform layer was directly injected into a G.L.C. (Set up as outlined below), and the quantity injected was recorded (approximately 2 µl).

**G.L.C. Setup:**

1. Varian 2800
2. Column: 6' (glass). 17 % D.E.G.S. on Gas Chrom. Q (80-100 mesh)
iii. Detector: F. I. D.
iv. Oven temperature: 110°C
v. Injection port temperature: 200°C
vi. Detector temperature: 250°C
vii. Carrier gas flow rate: 15 ml N₂/min

Under these conditions, a plant sample containing 0.1 % oxalic acid produced a peak equal to 10 % of full scale deflection for an injection volume of 2 µl and electrometer setting of $4 \times 10^{-11}$ amps/mV. Retention time was approximately 5 min.

**Calculations**

After adjustment for differences in injection volumes, a standard graph was prepared and the amount of oxalic acid equivalent in sample tubes was determined, and the amount in original sample calculated.

3.4.13.2 Water Soluble Oxalates

**Procedure**

0.4 g of herbage sample was extracted overnight with distilled water, the sample filtered into culture tube and the water evaporated off. The remainder of the procedure followed that of total oxalates above.

3.4.14 Amino Acids

The amino acids present in herbage were determined using the Pico Tag Method (Cohen et al. (1989)).

3.4.14.1 Oxidative analysis for Cystine and Methionine

Cystine and methionine are destroyed by acid hydrolysis, therefore they have to be oxidised to cysteic acid and methionine sulphone before hydrolysis.

**Reagents**

1. Performic acid - 9 volumes of 90 % formic acid were mixed with 1 volume of 30 % hydrogen peroxide, and allowed to stand at room temperature for 1 h.
2. Re-dry solution - 200 µl of methanol (HPLC grade), 200 µl 0.2 M sodium acetate (NaOAc) and 100 µl triethylamine (TEA) (Fluka, Puriss) were mixed.

3. Derivatisation reagent - was freshly prepared for each analysis. 350 µl of methanol (HPLC grade), 50 µl water (HPLC), 50 µl triethylamine (Fluka, Puriss) and 50 µl PITC (phenol isothiocyanate) was added to a capped vial and mixed.

4. Sample diluent - 5mM sodium phosphate pH 7.6, 5 % methyl cyanide (MeCN).

Standards

1. Internal Standard (AABA)
1.0 ml (10 µmol) of AABA (10 µmol/ml 10mM HCl) was added to 4.0 ml of 10 mM HCl giving a solution of 10 000 nmol/5 000 µl).

2. Pierce H. Stock Standard
100 nmol/400 µl 10 mM HCl containing equal amounts of Cystine and Methionine (100 nmol/400 µl 10 mM HCl).

3. Working Standards
Standards were prepared as outlined below, followed by the addition of 20 µl of internal standard (AABA) into each standard resulting in a concentration of AABA of 20 nmol/L.

<table>
<thead>
<tr>
<th>Pierce H. Stock Standard (µl)</th>
<th>80</th>
<th>40</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final Solution Concentration (nmol/L)</td>
<td>20</td>
<td>10</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Procedure
Approximately 5.00 mg of ground herbage was weighed (and weight recorded) (1.00 mg for reference egg white) into a screw-cap Eppendorf tube, and 600 µl of performic acid, was added and the mixture sonicated (dissolved using an ultrasonic bath) and stored overnight at room temperature. The mixture was then evaporated to dryness in a speedvac concentrator. 500 µl of 6 M HCl was then added, the mixture purged with N₂ and then sealed and heated at 110°C for 24 h. Samples were allowed to cool and then 500 µl of internal standard (AABA) was added, mixed on vortex and then filtered through Microspin Centrifuge Filter (Alltech 2490). 20 µl aliquots of filtrate (40 µl of reference sample) were then dispensed into Eppendorf tubes, and all sample, reference
and standard tubes evaporated to dryness in speedvac. 30 μl of the Re-dry solution was added to each tube (60 μl for 20 nmol standard only) and evaporated to dryness. 30 μl of derivatisation reagent was added to each tube, mixed, sealed and allowed to stand for 20 min. Solution was evaporated under vacuum for 20 min and then 30 μl of methanol was added to each tube, the solution mixed and then redried to ≤ 70 millitorr. 100 μl of sample diluent was added to each sample tube (200 μl was added to standards and reference tubes), which were vortexed and transferred to HPLC suited vials. Solutions were analysed by HPLC using a 30 cm x 3.9 mm Pico Tag (Waters) column with florescent detector at 38°C, by injection of 20 μl using the following gradient at 1.0 ml/min.

<table>
<thead>
<tr>
<th>Buffer</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>230 mM NaOAc</td>
<td>60 % Me CN</td>
</tr>
<tr>
<td></td>
<td>7mM TEA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 μg/L EDTA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 % MeCN</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH 6.0</td>
<td></td>
</tr>
<tr>
<td>0 min</td>
<td>100 %</td>
<td>0 %</td>
</tr>
<tr>
<td>20 min - wash and recycle 40 min</td>
<td>56 %</td>
<td>44 %</td>
</tr>
</tbody>
</table>

3.4.14.2 Amino Acid Analysis
This method was used to determine the amino acid content of herbage. Although, this method allowed the determination of cystine and methionine, the results are likely to be inaccurate due to oxidation and the method outlined above provides a more accurate measure of these amino acids.

Reagents
Standards

1. Internal Standard - α-amino butyric acid
   1 ml 433 mg/100 ml 10 mM HCl: 20 ml 6 M HCl

2. Stock standard - were prepared as outlined in Method 3.4.14.1

3. Working standards
   These were prepared as outlined below:

   | Pierce H. Stock Standard (µl) | 40 | 20 | 5 |
   | Final Solution Concentration (nmol/L) | 20 | 10 | 2.5 |

Procedure

Approximately 100 mg of herbage sample was weighed into a pyrolysed glass tube, and 200 ml of distilled 6 M HCl and sonicated. The solution was flushed with N₂, the tubes sealed and then heated at 110°C for 24 h. Tubes were then allowed to cool and 1.0 ml of internal standard was added, the solution mixed and approximately 200 µl filtered through a Millipore Ultrafree-MC 0.45 µm filter unit.

10 µl of filtered samples were dispensed into Eppendorf tubes (or 10 µl of the reference hydrolysate). 20 µl of internal standard was then dispensed into each tube to give a concentration of 20 nmol/L, and all aliquots evaporated to dryness using a Speedvac. 30 µl of re-dry solution was added to each tube and then evaporated to dryness. 30 µl of derivatisation reagent was then added to each tube, the content mixed, tubes sealed and left to stand for 20 min. Solution was then evaporated under vacuum for 20 min, then 30 µl of methanol added to each tube, contents mixed and redried to ≤ 70 millitor.

100 µl of sample diluent was then added to each tube which was then vortexed and transferred to WISP vials. Solution was analysed using a 30 cm x 3.9 mm Pico Tag column (Waters) with florescent detector at 44°C. 20 µl was injected using the following gradient at 1.0 ml/min.
### Buffer Composition

<table>
<thead>
<tr>
<th>Buffer</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>230 mM NaOAc</td>
<td>60 % Me CN</td>
</tr>
<tr>
<td></td>
<td>7 mM TEA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 μg/L EDTA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 % MeCN</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH 6.4</td>
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</tr>
<tr>
<td>0 min</td>
<td>100 %</td>
<td>0 %</td>
</tr>
<tr>
<td>1 min</td>
<td>100 %</td>
<td>0 %</td>
</tr>
<tr>
<td>21.2 min - wash and recycle 40 min</td>
<td>65 %</td>
<td>35 %</td>
</tr>
</tbody>
</table>

### 3.5 Alkane Technique for Intake and Whole Diet Digestibility Estimation

Individual intake was determined using the method described by Mayes *et al.* (1986a) as modified by Dove (1992).

#### 3.5.1 Delivery of Alkanes

Gelatin capsules were prepared and administered in the trial reported in Chapter 8, and controlled release devices (CRD) (Captech Pty. Ltd., University of New England, Armidale) were used in other experiments involving alkanes. CRD's were placed directly into the rumen of the animals using a modified dosing gun.

#### 3.5.2 Sample Collection and Preparation for Analysis

##### 3.5.2.1 Herbage

Pasture pluck samples representative of that consumed by the cows were obtained and frozen. At the completion of each trial, samples were freeze-dried and ground (1 mm sieve) in preparation for analysis.

##### 3.5.2.2 Faeces

Faecal samples were collected immediately prior to the morning and afternoon milking following excretion in the paddock, or per rectum after milking if this was not successful. Samples were immediately frozen and at the completion of each trial, were
freeze dried and ground (1 mm sieve). Equal proportions of dried faeces from each collection were bulked across the experimental period for individual cows in preparation for analysis.

3.5.3 Alkane Extraction and Analysis

Reagents

1. 1.5M potassium hydroxide (KOH) in ethanol - the solution was made either daily or every 2 days by adding 84.165 g KOH to a 1000 ml volumetric flask and then ethanol was added to volume whilst stirring.

2. Heptane

3. Distilled water

4. Mini silica gel columns - Kieselgel silica gel (70-325 mesh) was mixed with heptane and then poured into a Gilson 5 ml pipette tip plugged at the tip with a cotton plug normally used between pipette tip and pipettor.

Standard

C₃₄ standard solution - Approximately 0.3500 g of C₃₄ (tetracontane) and 140 g of C₁₁ (undecane) or heptane, was added to a 250 ml volumetric flask and actual weights were recorded. The concentration of the internal standard was then calculated using Equation 12 as follows;

\[
\text{Concentration of } C_{34} \quad \frac{Wt \; C_{34} (g)}{Wt \; C_{34} (g) + Wt \; C_{11} (g) *}
\]

* or heptane

The internal standard was stored in the freezer between use to prevent volatilisation of the C₁₁ or heptane.

Procedures

Approximately 1 g of freeze-dried faeces (or 2 g of freeze-dried herbage) was weighed into a screw top culture tube and the weight of the sample recorded (4 decimal places).
The DM and OM of a separate sample was determined at the completion of extractions. Approximately 0.2000 g of C_{34} internal standard was weighed into the culture tube and the weight of the standard recorded, and then 10 ml (or 12-14 ml to herbage samples) of 1.5 M KOH in ethanol was added. Samples were mixed carefully to prevent excess sample sticking to the sides of the tubes and caps screwed on firmly to obtain a good seal. Samples were heated in water bath (or oven) at 90°C for 3.5 h. Samples were then allowed to cool and 8 ml of heptane added, mixed and 5 ml of distilled water was added and the solution was mixed again. Phases were allowed to separate and tubes warmed if necessary to lower the surface energy and allow easier separation. The top phase was removed using a pasteur pipette and stored in vial. A further 5 ml of heptane was added to the culture tube which was shaken once again, and the top phase added to the vial. The solvent was evaporated from the vial with the aid of compressed air and heat.

2 ml of heptane was added to the vial and warmed to ensure all hydrocarbons were dissolved. The solution was transferred to the top of a mini-silica gel column using a pasteur pipette which filtered into another vial. The column was then eluted with 5 x 2 ml heptane to maximise recovery of hydrocarbons. Once elution was complete, the eluent was evaporated until approximately 1.5 ml remained at base of vial. This was transferred to a 2 ml screw cap vial and evaporated to dryness in preparation for analysis using a gas liquid chromatograph (GLC). Approximately 0.5 ml of heptane was added to the sample before injection into the GLC set up as outlined below.

**GLC Setup:**

- Gas chromatograph type: Varian 3400
- Mode: Packed Column
- Autosampler type: Varian 8035
- Injection system: "F/V" on column
- Injection volume: 1 or 2 µl
- Detector: FID
- Column: Varian DBI 15m x 0.53mm OD Megabore bonded phase
- Column Temperatures: Programmed 2.5 min at 240°C; at 3°C/min to 288°C; at 2°C to 298°C
Injector temperature: 300°C
Detector temperature: 300°C
Carrier gas: helium, 15ml/min
Time between runs: 23.5 min
Replicate injections: 1

Data collection system: “Data Acquisition Plotting & Analysis” (qv. Dr Peter Sheppard, Chemistry Dept., Curtin University, Perth)

3.5.4 Intake Calculations

Daily herbage intake was estimated using Equation 13 (Dove and Mayes 1991).

\[
\text{Daily Herbage Intake} = \frac{\frac{F_I}{F_j} \times D_j}{H_i - \frac{F_I}{F_j} \times H_j}
\]  

Equation 13

where;

\(H_i\) and \(F_i\) are the respective herbage and faecal concentrations of the odd-chain alkane;

\(H_j\) and \(F_j\) are the respective herbage and faecal concentrations of the even-chain alkane;

and \(D_j\) is the daily dose of even-chain alkane.

When supplements were fed, herbage intake was calculated using Equation 14.

\[
\text{Herbage Intake} = \frac{\frac{F_I}{F_j} \times (D_j + I_c \times C_j) - (I_c \times C_p)}{H_i - \left(\frac{F_I}{F_j} \times H_j\right)}
\]  

Equation 14

where;

\(C_i\) and \(C_j\) are the respective concentrations of the natural and dosed alkanes in the supplement;

and \(I_c\) was the daily supplement intake (kg DM).

3.5.5 Whole Diet Digestibility Calculations

3.5.5.1 Using dosed C_{36} alkane to estimate faecal output

The whole diet DMD was estimated using dosed C_{36} using Equations 15 and 16.
\[ FO = \frac{DR \times RR}{FC} \]  \hspace{1cm} \textit{Equation 15}\n
where;

\( FO \) is the faecal output (kg DM or OM/cow.day);
\( DR \) is the dose rate of C\(_{36}\);
and \( FC \) is the faecal concentration of C\(_{36}\) (g/kg DM or OM).

\( RR \) is the recovery rate of C\(_{36}\) determined using either total faecal collection (Chapter 7) or assumed rate of 0.95 (Chapter 8) based on results from sheep (Dove and Mayes 1991);

\[ WDD = \frac{\text{Intake (kg DM/day)} - \text{Faecal Output (kg DM/day)}}{\text{Intake (kg DM/day)}} \]  \hspace{1cm} \textit{Equation 16}\n
where;

herbage intake is derived from dosed C\(_{32}\) alkane and the naturally-occurring C\(_{31}\) or C\(_{33}\) alkanes;
DMI includes herbage and any supplements fed.

\textbf{3.5.5.2 Using naturally-occurring C\(_{35}\)}

Whole diet digestibility was estimated from herbage and faecal concentrations of C\(_{35}\) using Equation 17.

\[ WDD = 1 - \frac{H_{35} \times RR_{35}}{100 F_{35}} \]  \hspace{1cm} \textit{Equation 17}\n
where;

WDD is whole diet digestibility;
\( H_{35} \) is herbage concentration of the C\(_{35}\) alkane;
\( RR_{35} \) is the recovery rate of the C\(_{35}\) alkane;
and \( F_{35} \) is the faecal concentration of the C\(_{35}\) alkane.
Chapter 4

The effect of season, age of regrowth and time of day of defoliation on the quality of kikuyu

4.1 Introduction

Various environmental and managerial factors influence the nutritional value of a pasture to the grazing animal, and therefore, the quality of a pasture is continually changing in response to these variables. As discussed in Chapter 2, pasture management practices should aim to optimise forage quality in terms of cow requirements, utilisation by stock and the regrowth potential of the plant (Murphy 1990).

Appropriate grazing intervals are a key strategy of an effective management system. Set-time grazing intervals provide a simple recipe for grazing management, however, they disregard changes in the length of the regrowth cycle caused by changing climatic conditions (eg. moisture availability and temperature) and management practices (eg. fertiliser application, mulching). Dry matter on offer or pasture height are more appropriate methods for determining the time for grazing, as they at least integrate the effects of environment on regrowth (Frame 1992). However, pasture height is related more to nutrient supply, in particular N (Ryle 1964), and therefore it can only be used to compare pasture masses of uniform fertility, rather than to determine grazing readiness of the plant.

The 3-leaf stage of regrowth (the time taken for 3 new leaves per tiller to fully expand) has been used as a simple plant-related indicator to flag the time of grazing in ryegrass. Grazing intervals longer than this usually result in wastage through leaf senescence, and a concomitant decrease in herbage quality (Fulkerson and Slack 1994). Shorter intervals result in a gradual depletion of plant reserves (primarily carbohydrates), which may retard the plant’s ability to regrow following defoliation (Davies 1965; Fulkerson and Slack 1995), and ultimately, its survival (Donaghy et al. 1997).

This study aimed to establish the stage of regrowth of kikuyu grass, measured by the number of fully expanded new leaves per tiller, which was associated with optimal
herbage quality, as affected by season, age of regrowth and time of day.

4.2 Materials and Methods
A series of cut-plot studies were conducted to examine the changes in quality of kikuyu with season, stage of regrowth and time of day, and these are outlined below. Unless otherwise stated, plots were established and samples collected as described in Sections 3.2 and 3.3. Urea was applied to the plots at a rate of 100 kg/ha immediately following mowing (ie. day 0). Chemical analysis for nutrient content of samples was conducted as described in Chapter 3.

4.2.1 Study 1. Variation in Kikuyu Components and Quality during Regrowth
a. Relationship between leaves per tiller and changes in plant components, N and OMD content
Following mowing, 30 tillers of kikuyu were individually marked and the number of leaves per tiller and leaf length were monitored at 3- to 4-day intervals for 40 days in November-December 1995 (early-season). This process was repeated with 36 tillers over January-February 1995 (mid-season).

The graphs used to determine the age of regrowth at which full leaf expansion occurred for each of the first 4 newly-emerging leaves following mowing are shown in Figure 4.1.

Figure 4.1 Graphs used to estimate the time taken for individual leaves of 90 % of the kikuyu plants examined to fully expand following mowing to a height of 5 cm. Plots for the first 4 newly emerged leaves in the early and mid seasons are shown.

The stage when a given leaf was considered to be fully expanded was taken to be when
the relevant leaves of 90% of the plants examined had ceased to expand. The number of new leaves which had fully expanded after 40 days in the early and mid season were 4 and 5, respectively. The estimated age of regrowth at which the leaf was fully expanded was later in the early, compared to mid, season (leaf 1: 15 v 11 days; leaf 2: 19 v 16 days; leaf 3: 24 v 21 days: and leaf 4: 31 v 25 days), indicating slower growth rates in the early season.

Each time leaf number was monitored, pasture was cut to a height of 5 cm from 4 quadrats (10 x 30 cm) placed at random in areas not previously cut. The herbage was separated into leaf-blade, leaf-sheath, stem and dead material and each component analysed for DM, N and OMD content. The concentration of CP and OMD for the whole plant above 5 cm stubble height was calculated from the concentration in, and fraction of, each plant component present.

b. Changes in the quality of individual leaves per tiller and stem of kikuyu in relation to regrowth over the growing season

This study was conducted in 1993-1994 during the early (November-December), mid (January-February) and late (March-April) periods of the kikuyu growing season. Following mowing, quadrat cuts (10 x 30 cm) from 4 replicate plots were taken to ground level at 7-day intervals and separated into old leaves, remnant leaf (identified by a cut tip, and was the last leaf elongating before mowing), new leaves (ie. 1st leaf, 2nd leaf, 3rd leaf etc.) and stem. Each component was then analysed for N, OMD and minerals (the latter in the early and mid season only).

c. Effect of Stage of Regrowth and Growing Season on the Quality of Kikuyu above 5cm Stubble Height

This study was an extension of Study 1a, however in this study, the plants were not separated into components, and additional nutrient analysis were obtained. Using the same plots as in Study 1b, cuts to 5 cm stubble height were obtained from each of 4 replicates at 7-day intervals for 5 (early-season) or 6 (mid- and late-season) weeks. Samples of all the plant material present above 5 cm stubble were analysed for OMD and oxalates (on combined replicate samples), N, minerals and nitrates.
4.2.2 Study 2. Within-day Variation in WSC and N

Three-week old kikuyu regrowth (in 4 replicates) was cut to a 5 cm stubble height at 3 h intervals from 0600 to 1800 h over a single day in November (9.5 h sunlight), January (10 h sunlight) and March (7.4 h sunlight). Samples were analysed for WSC and N content.

4.2.3 Statistical Analysis

Quality comparisons were made using linear regression and analysis of variance. In Study 2, quality parameters were fitted using the linear model $y = x\beta + e$, where $y$ is the quality parameter, $x$ is a model matrix containing the terms for age, age$^2$, and interactions with season, $\beta$ is a vector of coefficients estimated by least squares regression, and $e$ is the error term. All terms were tested using analysis of variance.

4.3 Results

4.3.1 Study 1. Variation in Kikuyu Components and Quality during Regrowth

a. Relationship between leaves per tiller and changes in plant components, N and OMD content

The variation in the DM portion of leaf-blade, leaf-sheath, stem and dead material and the corresponding CP and OMD of kikuyu above the 5 cm stubble height for the early- and mid-season are shown in Figures 4.2 (a) and (b), respectively.

The proportion of green leaf available above 5 cm stubble height declined substantially following the expansion of 4 new leaves (which visually appears to be the 4.5-leaf stage of regrowth - see Figure 4.3). This coincided with an increase in the proportion of stem and dead material, and was associated with a prominent decline in quality (both N and OMD). The decline in the leaf component in the mid-season was greater than that in the early-season (49 v 30%).
The maximum CP concentrations were similar in both early- and mid-season (233 v 234 g/kg DM), and these declined to similar concentrations by the completion of measurement. However, OMD was initially much higher in the early, compared to mid, season (0.759 v 0.548), and declined at a much slower rate. The decline in CP and OMD could not be analysed statistically as the replicates were combined at each sampling date.
Figure 4.3 Illustrations of kikuyu plants at various stages of regrowth from 1 to 6 leaves per tiller.
b. Changes in the quality of individual leaves per tiller and stem of kikuyu in relation to regrowth over the growing season

The CP concentration in kikuyu components was significantly influenced (P<0.001) by season, plant part and age of regrowth. Changes in CP of individual new leaves throughout the growing season showed similar trends and the means across this period are presented in Figure 4.4.

Figure 4.4 Changes in the mean crude protein (CP) content of new leaves 1 to 6 and the mean for all leaves emerging during regrowth (combined data from the early, mid and late periods of the growing season).

Although the CP content of each leaf declined with age, the first 2 leaves maintained high concentrations for longer than subsequent leaves, and leaves 5 and 6 emerged with the lowest initial CP concentrations.

The OMD, P, K and Mg concentrations of kikuyu components were significantly influenced (P<0.05) by season and plant part, and the mean concentrations for stem, and new leaves, combined across each week are presented in Table 4.1. The OMD, P and K content of leaf tended to be greater than that of stem, although this was only significant during mid-season. OMD and K concentrations in the early season were significantly higher (P<0.05) than later in the season for both leaf and stem.
Table 4.1 The mean organic matter digestibility (OMD), phosphorus (P), potassium (K) and magnesium (Mg) (g/kg DM) concentrations observed for the stem and new leaves (combined weekly data for leaves 1 to 6) in the early-, mid- and late-season. Different superscripts indicate significant differences (P<0.05) in means of leaf and stem material within nutrients.

<table>
<thead>
<tr>
<th>Season</th>
<th>OMDstem</th>
<th>OMDleaf</th>
<th>Pstem</th>
<th>Pleaf</th>
<th>Kstem</th>
<th>Kleaf</th>
<th>Mgstem</th>
<th>Mgleaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>early</td>
<td>0.582</td>
<td>0.640</td>
<td>3.3</td>
<td>3.6</td>
<td>18.4</td>
<td>21.4</td>
<td>4.3a</td>
<td>2.4b</td>
</tr>
<tr>
<td>mid</td>
<td>0.430a</td>
<td>0.607b</td>
<td>2.1a</td>
<td>3.7b</td>
<td>7.0a</td>
<td>17.2b</td>
<td>2.9</td>
<td>2.6</td>
</tr>
<tr>
<td>late</td>
<td>0.502</td>
<td>0.574</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>l.s.d. (P&lt;0.05)</td>
<td>0.09</td>
<td>0.06</td>
<td>0.4 n.s.</td>
<td>4.5</td>
<td>2.9</td>
<td>0.5 n.s.</td>
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<td></td>
</tr>
</tbody>
</table>

Ca accumulated in leaves as they matured, whilst the concentration in stem material remained constant (Figure 4.5). The Ca concentrations in the early season tended to be higher than the mid season.

Figure 4.5 Changes in the concentration of calcium (Ca) (g/kg DM) in (a) new leaves (combined replicate data for leaves 1 to 6) and (b) stem of kikuyu in the early and mid seasons.
The changes in the mean mineral concentration of the first new leaf to emerge during regrowth are shown in Figure 4.6.

Figure 4.6 Concentrations of potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), and sodium (Na) (g/kg DM) in the first new leaf to emerge since regrowth began (combined data from the early, mid and late periods of the growing season).

As the leaf aged, the concentrations of Ca and Mg increased, Na was constant, and both P and K declined. Similar trends were observed in subsequent leaves following emergence.

c. Effect of Stage of Regrowth and Growing Season on the Quality of Kikuyu above 5cm Stubble Height

In contrast to Study 1a, no significant effect of age of kikuyu regrowth on OMD content was observed. However, OMD in the early season (0.663 ± 0.01 (mean ± s.e.)) was significantly higher (P<0.001) than in the mid and late season, with no significant difference between the latter (0.549 ± 0.02).

CP in kikuyu above 5 cm stubble fitted the model CP = a + bx + cx², where x is the age of regrowth, a is the intercept, and b and c are the coefficients, with a multiple $R^2$ of 0.81 (Figure 4.7).
The CP of kikuyu declined significantly (P<0.001) with regrowth time and varied (P<0.001) between seasons. Ca fitted the model \( Ca = a + bx \), where \( x \) is the age of regrowth, \( a \) is the intercept and \( b \) is the slope of the line, with a multiple \( R^2 \) of 0.86. The concentration of Ca significantly increased (P<0.001) with age of regrowth and from early to late season. The concentration of other minerals did not differ with age of regrowth, however, variations between seasons were apparent (Table 4.2).

Table 4.2 Mean mineral concentrations (g/kg DM) of kikuyu regrowth above 5 cm stubble during the early (Nov-Dec), mid (Jan-Feb) and late (Mar-Apr) periods of the growing season. Different superscripts indicate significant differences (P<0.05) between means seasons within nutrients.

<table>
<thead>
<tr>
<th>Season</th>
<th>P</th>
<th>K</th>
<th>Mg</th>
<th>Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td>3.90a</td>
<td>20.52a</td>
<td>3.10a</td>
<td>0.27a</td>
</tr>
<tr>
<td>Mid</td>
<td>3.89b</td>
<td>15.82b</td>
<td>3.14b</td>
<td>0.47b</td>
</tr>
<tr>
<td>Late</td>
<td>3.40b</td>
<td>11.79b</td>
<td>5.28b</td>
<td>0.32a</td>
</tr>
</tbody>
</table>
Kikuyu in the late season contained significantly higher (P<0.001) concentrations of nitrate (890 ± 188 (mean ± s.e.) ppm), than early and mid season (118 ± 28 and 190 ± 39 (mean ± s.e.) ppm, respectively). Nitrate concentrations in the early and late season declined significantly (P<0.05) with age of regrowth. The mean oxalate content of kikuyu was 6.78g/kg DM, and did not vary significantly with age of regrowth or season.

4.3.2 Study 3. Within-day Variation in WSC and N

No significant seasonal variation in the within-day pattern of WSC was observed and the data were subsequently combined (Figure 4.8).

Figure 4.8 The within-day variation of water soluble carbohydrate (WSC) content (g/kg DM) in kikuyu regrowth above 5 cm stubble height. Vertical bars represent standard errors.

WSC concentrations significantly increased (P<0.001) following the onset of daylight until a maximum concentration was reached by mid afternoon. Between 0900 and 1500 h, when the relationship was near linear, the rate of increase in WSC concentration was approximately 5 g/kg DM per h, although the N content did not vary throughout the day.

4.4 Discussion

The 4.5 leaves per tiller stage of regrowth appears to be the best time to graze kikuyu to
obtain optimal overall forage quality to meet the requirements of lactating cows. After this, the plant components, and consequently the quality, markedly change with a substantial decrease in the proportion of high quality leaf and increase in both stem, and in particular, dead material.

Despite a difference in the number of days taken to reach the 4.5-leaf stage of regrowth as a consequence of lower growth rates in the early, compared to mid season, component proportions above 5 cm stubble were similar. This highlights the benefit of relating the grazing readiness of the pasture to a plant-related factor like leaf number per tiller, rather than set-time grazing intervals.

At the 4.5 leaf stage of regrowth, the mineral concentration of mature leaves have reached concentrations more suited to the requirements of the lactating cow. Thus, the concentration of K in emerging leaves are well above cow requirements, but decline as the leaf ages. These concentrations are similar to, or below, those found in ryegrass at full elongation (W. J. Fulkerson, personal communication). High concentrations of K are undesirable as they adversely affect Mg absorption through the ruminal wall (Minson 1990). Conversely, Mg is often limiting for dairy cattle, therefore, grazing at this stage would be beneficial as Mg tends to accumulate in the maturing leaf. Supplementation of both P and Na would be essential to meet cow requirements (NRC 1989) as concentrations of Na remain very low through regrowth, whilst P concentrations tended to decline with plant maturity (see also Wilson and Sandland 1976).

The CP of N-fertilised kikuyu can reach high concentrations (as discussed in Chapter 2) even at the moderate rates of N applied in these studies. By grazing kikuyu at the 4.5 leaf stage, the CP content had declined due to a combination of leaf maturation and the emergence of leaves containing lower concentrations of CP. However, CP (in terms of RDP) concentrations may still exceed the requirements of the cow (NRC 1989), and result in increased concentrations of rumen NH₃, and reduced rumen microbe activity.

The WSC content in kikuyu rises during the day as the products of photosynthesis accumulate and then fall at night as they are used for respiration. This within-day
variation in the pasture WSC suggests that the timing of grazing kikuyu during the day may improve the nutritional value of the pasture as the WSC to protein ratio would be more amenable to growth of rumen microflora. Thus, it may be logical to feed concentrates and other supplements during the day and commence grazing in late afternoon when ambient temperature and WSC concentrations are more amenable. Although animals can be supplemented with high carbohydrate concentrates, gearing grazing to high WSC and starch concentrations is perhaps a cheaper, and probably a more effective, means of doing this.

Seasonal influences affect kikuyu with a substantial reduction in quality late in the growing season. Some researchers refer to this period as the autumn slump (Dugmore and du Toit 1988) when milk yields decline. It would be expected that this change would be more dramatic in poorly-managed pasture, allowed to grow unrestricted over the growing season, due to the accumulation of substantial quantities of poor quality stem.

OMD was lower at the end, compared with the beginning, of the growing season. Similar findings were reported by Henning et al. (1995) in South Africa under both tight and lax pasture grazing management regimes where OMD of kikuyu declined from 0.676 in December to 0.447 in May. Perhaps lower temperatures later in the season, favour stem and tiller development, rather than leaf growth (see Chapter 2). Reduced leaf growth during this time may explain the high CP and nitrate concentrations observed in Study 1c. However, as discussed in Chapter 2, reported trends in N in kikuyu over the growing season have been variable and difficult to interpret, due to the interaction of many environmental and management factors.

Calcium concentrations in kikuyu also peaked in autumn, and although the concentration of Ca in new leaves of kikuyu increased up to the 4.5 leaves/tiller stage, concentrations remain marginal for milk production of 20 L/cow.day. The presence of oxalates present in kikuyu can bind Ca as insoluble crystals, rendering it largely unavailable to the animal (Ward et al. 1979; Blaney et al. 1982). Oxalate concentrations did not vary with season, therefore, not only are Ca concentrations lower during spring/early summer, but its
availability is further reduced due to oxalates. Supplementation would be required to
meet the nutritional requirements of lactating cows grazing kikuyu throughout the
season, with particular emphasis during the spring/summer.

The recommendation to graze kikuyu at the 4.5 leaf stage of regrowth, considers the
overall quality of kikuyu on offer in relation to cow requirements. Since the nutritional
indicators of quality change in different directions, and varying rates, with age of
regrowth, it is difficult to achieve optimal levels of all nutrients, and thus the 4.5 leaf
stage of regrowth provides an appropriate balance of protein, OMD and minerals.

As yet, the effect of grazing kikuyu at the 4.5 leaf stage on plant regrowth, hence dry
matter production, is unknown. However, it is difficult to compromise kikuyu quality
by quantity, when the quality of kikuyu is already below the requirements of lactating
cows.

4.5 Conclusion
The 4.5 leaves per tiller stage of regrowth appears to be the optimal time to graze kikuyu
in terms of forage quality for lactating cows. After 4.5 leaves have expanded, there is
a gradual increase in the proportion of stem, and a marked rise in dead material. In
relation to requirements of the milking cow, concentrations of Ca and Mg rise, and K
and CP fall to more appropriate concentrations, only the OMD and P concentrations
deteriorate. The OMD tends to decline from early to late season. WSC concentrations
are critically low in kikuyu but rise in the day under sunlight, and for this reason, kikuyu
grazed in the afternoon may be of better quality than the that grazed in the morning.
Chapter 5
The effect of N fertiliser on regrowth and quality of kikuyu

5.1 Introduction
The application of N fertiliser may influence the optimal stage to graze kikuyu if this changes the proportion of plant components and/or plant quality.

Mears and Humphreys (1974) found that the proportion of stem in kikuyu to ground level tended to rise with increasing input of N fertiliser. However, cows rarely graze kikuyu below 5 cm stubble height, and in fact, Minson (1973) found that the rate of N fertilisation had no effect on the ratio of leaf to stem in regrowth above this height.

Increased application of N fertiliser would be expected to increase the concentration of CP in herbage. Studies on ryegrass/clover swards indicate concentrations of NPN, nitrates and soluble N in particular in the pasture are increased with N fertilisation (Ross et al. 1978; Mackle et al. 1996). These workers observed no influence of N fertiliser rate on the concentration of non-structural carbohydrates. Conversely, other studies have shown a negative relationship between plant protein and non-structural carbohydrate content (Smith 1973), which would reduce the nutritional value of the herbage for dairy cows (Fulkerson and Trevaskis 1997).

This study aimed to determine the influence of N fertilisation on plant components, rate of leaf emergence, WSC, NPN and nitrate concentration in kikuyu.

5.2 Materials and Methods
5.2.1 Study 1. The Effect of N Fertiliser Rate on Regrowth and Quality
This study was conducted during March and April 1995 on a 9 x 9 m kikuyu plot of N-deficient kikuyu (as determined visually and having not received N fertiliser for the previous 2 months). The plot comprised 3 replicates of 5 treatments (0, 50, 100 (the rate currently recommended for kikuyu pasture), 150 and 200 kg urea/ha), randomly allocated within each replicate. Each replicate was separated by a 1 m buffer, and within
each replicate, treatments were separated by a 0.5 m buffer. The plot was mown to a height of 5 cm on day 0 and fertiliser treatments applied.

The time of appearance and length of individual leaves (as described in Chapter 4) of 5 marked tillers within each treatment plot were monitored at 4-day intervals for 40 days following mowing, to determine the stage of regrowth of plants in relation to leaf number. The day of appearance and full elongation of each leaf were determined using interpolation. On the same day, two random quadrat samples (10 x 30 cm) were taken from each treatment at 11 am. One sample was immediately placed in the oven for drying, in preparation for WSC analysis. The other was sorted into leaf-blade, leaf-sheath, stem and dead material for component analysis of DM and recombined for N analysis. For those samples obtained prior to 25 days regrowth, equal portions of the replicate samples had to be combined in order to obtain sufficient sample material for chemical analysis.

5.2.2 Study 2. The Effect of N Fertiliser on the Within-day Variation of N, Nitrate, NPN and WSC

When Study 1 was completed, treatment plots which had received 200 and 100 kg urea/ha, either received an additional 200 kg urea/ha or no further fertiliser, respectively, in April 1995. Over a 24 h period in May 1995 (10.1 h sunlight), 10 x 30 cm quadrats placed at random within each plot were cut to a height of 5 cm at 2 h intervals from 0600 to 0200 h. Samples were immediately dried and prepared for N, nitrate, NPN and WSC analysis.

5.3.2 Statistical Analysis

Nutrient concentrations of the pasture and the rate of emergence of leaves were compared statistically using linear regression and analysis of variance techniques.
5.3 Results

5.3.1 Study 1. The Effect of N Fertiliser Rate on Regrowth and Quality

The mean estimated age of regrowth at which individual new leaves appeared, and the stage at which they were fully elongated at each rate of fertiliser application, are illustrated in Figure 5.1.

Unfertilised pasture had significantly longer (P<0.01) leaf appearance intervals than N fertilised pasture for leaves 1 to 4. The number of leaves to emerge over the 40 day measurement period was 4, 5 and 6 for unfertilised, 50 kg urea/ha and the remaining treatments, respectively.
The mean changes in the portions of leaf-blade, leaf-sheath, stem and dead material are illustrated in Figure 5.2.

**Figure 5.2** Changes in the proportion of leaf-blade, leaf-sheath, stem and dead components in total DM above 5 cm stubble height for kikuyu fertilised with 0, 50, 100, 150 and 200 kg urea/ha.

At the higher rates of N fertilisation (ie. 100, 150 and 200 kg urea/ha), there appeared to be a more rapid decline in the leaf portion and a corresponding accumulation of stem material, compared with lower rates of N fertilisation.

The CP and WSC content of kikuyu was significantly influenced (P<0.01) by N fertiliser application rate and age of regrowth (Figure 5.3 (a and b)).
5.3.2 Study 2. The Effect of N Fertiliser on the Within-day Variation of N, Nitrate, NPN and WSC

Kikuyu fertilised with N contained significantly higher (P<0.001) concentrations of N (41.1 ± 0.05 v 35.4 ± 0.35 g/kg DM), nitrate (3124 ± 151 v 672 ± 87 mg/kg DM) and NPN (16.1 ± 0.38 v 12.0 ± 0.2 g/kg DM) compared to unfertilised plots. N and NPN, but not nitrate, showed significant within-day variation (P<0.05), with maximum concentrations in early morning, and lowest concentrations in mid-afternoon (Figure 5.4).
A significant relationship (P<0.001) between N and NPN was found;

\[
NPN(\text{g/kg DM}) = -0.98 + 0.62 \times N(\text{g/kg DM}) \quad (r^2 = 0.85)
\]

The relationship between nitrate and N concentrations as shown in Figure 5.5.

Figure 5.5 The relationship between nitrogen (N) (g/kg DM) and nitrate (mg/kg DM) content of kikuyu pasture above the 5 cm stubble height with fitted regression line.
Above 33 g N/kg DM there was a significant linear relationship (P<0.001) between N and nitrate concentrations as described below;

\[
\text{Nitrate (mg/kg DM)} = -10428 + 323.9 \times N\text{(g/kg DM)} \quad (r^2 = 0.56)
\]

There was a very significant (P<0.001) within-day variation in WSC, with no interaction between time of day and N fertilisation (Figure 5.6).

Figure 5.6 The within-day variation of water soluble carbohydrates (WSC) (g/kg DM) for N fertilised or unfertilised kikuyu.

![Graph showing WSC variation](image)

N fertilised kikuyu contained higher WSC than unfertilised at most times of the day. As observed in Section 4.3.3, minimum concentrations of WSC were found at sunrise with concentrations reaching a peak by mid-afternoon.

5.4 Discussion

The application of N fertiliser to kikuyu affected the growth, morphology and quality of the pasture, all of which have implications for pasture management.

The application of N fertiliser increased the rate of new leaf emergence. In contrast, N application does not appear to affect leaf appearance interval in perennial ryegrass (Davies 1977).
The proportion of stem above the 5cm stubble height was increased with N fertiliser application. These results are similar to trends observed by Mears and Humphreys (1974), when they examined leaf to stem ratio to ground level using fertiliser rates ranging from nil to 672 kg N/ha per annum. However, Minson (1973), did not find a significant alteration in the proportion of leaf to stem in DM above the 5 cm stubble height, when applying N fertiliser at rates ranging from 58 to 230 kg N per month during the summer growing period.

A gradual decline in the CP content of N fertilised kikuyu with age of regrowth to concentrations found in unfertilised kikuyu in the present study, has also been observed by Drummond (1975). This decline would be expected as the initial high uptake of N would be diluted through correspondingly higher plant growth, as well as a depletion of N available from the soil.

The concentration of NPN and nitrate in kikuyu fertilised at high rates of N were higher than those receiving lower rates. Nitrate concentrations rose dramatically when the N content of kikuyu elevated above 37 g/kg DM (equivalent to 230 g CP/kg DM). This relationship confirms earlier work by Marais (1990a), although he recorded much higher nitrate concentrations and this may have been due to environmental (ie. temperature, moisture) and managerial (ie. fertiliser rate and type) differences. Although the concentrations of nitrate found in this study would not be considered to be toxic to cattle (Blood and Radostits 1989) at higher rates of N fertiliser application, they may still be sufficient to reduce the rate of digestion of kikuyu in the rumen. Evidently, excess nitrate in the rumen is reduced to nitrite, which has a detrimental effect on the rumen microbial population (Marais 1980: Marais et al. 1988). Inhibited microbial function would presumably restrict DMI through reduced feed digestibility and rumen throughput. In support of this suggestion, Dugmore and du Toit (1988), found the DMI of steers was suppressed by high concentrations of NPN in kikuyu. Mackle et al. (1996) found moderate N fertilisation of ryegrass/clover pasture, slightly lowered DMI of lactating cows.

Although the CP content of unfertilised and fertilised pastures in this study were similar
(230 v 221 g CP/kg DM), the fertilised pasture contained higher NPN (0.69 v 0.53 %), soluble N (0.95 v 0.81%) and nitrate (0.09 v 0.04 %) concentrations. This would presumably result in elevated rumen NH₃ of cows grazing high N pasture, the breakdown of which can inhibit the level of intake (as mentioned above). Such results were observed by van Vuuren et al. (1992) with cows grazing ryegrass pastures, and presumably similar effects on rumen NH₃ would be observed in highly N fertilised kikuyu pasture.

These studies provide further evidence of the potential benefit of grazing kikuyu pastures in the afternoon, rather than morning. NPN concentrations are highest in the early morning and fall to lower concentrations by mid-afternoon. This virtually mirrors the changes in WSC. Grazing kikuyu in the afternoon could then improve rumen microbe activity in terms of a more amenable WSC to soluble protein balance.

High rates of N fertiliser application did not appear to be detrimental to the WSC concentration in kikuyu. In fact, the overnight decline in WSC was not as pronounced in the high, compared to the low N, treatment. Changes in WSC with age of regrowth and N fertiliser application rates are difficult to interpret, in view of the highly variable solar radiation on sampling days. Controlled lighting may be required to eliminate such variation.

5.5 Conclusion

High rates of N fertilisation reduce the quality of kikuyu pasture by increasing the proportion of stem above 5 cm stubble height. Additionally, nitrate and NPN increase to concentrations which may reduce rumen microbial activity and hence, disrupt rumen function. This can result in reduced microbial breakdown of feed, slow rumen outflow and consequently, lower DMI. Moderate applications of N (approximately 50 kg N per application) provide sufficient protein to promote DM production and enhance protein concentrations to meet the requirements of the lactating cow, without the detrimental side-effects of higher N fertiliser rates. The intake of NPN can be minimised, and WSC maximised by grazing kikuyu mid-afternoon.
Chapter 6
A comparison of the nutrient content of kikuyu and perennial ryegrass pastures grown in a subtropical environment

6.1 Introduction
Although the DM yields of kikuyu pastures in the subtropics can be high, milk production per cow is comparatively low (Colman and Kaiser 1974). In a recent study (W. Fulkerson, Unpub. data) of unsupplemented Friesian cows of average genetic merit grazing well-managed kikuyu pastures, daily milk production was limited to 15 L/cow per day. In comparison, production obtained from perennial ryegrass pastures in the same study, was 22-24 L milk/cow per day. Cows in this study were in the fourth to sixth month of lactation, with no significant change in liveweight, and therefore, the milk produced could be assumed to be directly attributable to the forage consumed.

This study provided a comparison of the nutrients in well-managed kikuyu and perennial ryegrass, at the peak of their respective growing seasons, grown at the same location in a subtropical environment, with the aim of identifying the nutrients responsible for differences in milk production between the two pasture types.

6.2 Materials and Methods
Kikuyu and perennial ryegrass samples (the latter obtained from irrigated ryegrass/white clover pastures), were plucked at 0900 h to simulate grazing height of milking cows. Samples were obtained in the midst of the growing season for each species (ie. January-April for kikuyu and June-October for ryegrass). Forage samples were representative of the optimal stage of regrowth for grazing by dairy cattle. Ryegrass was plucked at about the 3 leaves per tiller stage of regrowth (Fulkerson and Slack 1995), and kikuyu before significant stem appeared above the 5 cm stubble height (interval varying from 18-25 days’ regrowth). The number of replicates varied depending on the analysis performed on the samples.
Samples were analysed for OMD, ADF, NDF, N, amino acids, NPN, nitrate, WSC, starch, minerals and oxalate content as described in Chapter 3, as were ME and CP calculations. Nutrient concentrations of the pastures were compared statistically using linear regression and analysis of variance techniques.

6.3 Results

The mean nutrient concentrations found in kikuyu and perennial ryegrass are listed in Table 6.1.

Table 6.1 Mean nutrient (g/kg DM unless otherwise specified) concentrations of well-managed kikuyu and ryegrass pluck samples. Within rows, significant differences (P<0.05) between species are indicated by different superscripts. Level of significance indicated in last column.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Kikuyu</th>
<th>Ryegrass</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME (MJ ME/kg DM)</td>
<td>9.9a</td>
<td>11.7b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OMD</td>
<td>0.733a</td>
<td>0.842b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ADF</td>
<td>230a</td>
<td>177b</td>
<td>0.007</td>
</tr>
<tr>
<td>NDF</td>
<td>602a</td>
<td>395b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CP</td>
<td>207a</td>
<td>252b</td>
<td>0.007</td>
</tr>
<tr>
<td>Total amino acid (g/16g N)</td>
<td>74</td>
<td>93</td>
<td>n.a.</td>
</tr>
<tr>
<td>Total NPN</td>
<td>7.0a</td>
<td>9.1b</td>
<td>0.001</td>
</tr>
<tr>
<td>NPN (g/kg N)</td>
<td>221</td>
<td>245</td>
<td>n.s.</td>
</tr>
<tr>
<td>Nitrate</td>
<td>0.26</td>
<td>0.46</td>
<td>n.s.</td>
</tr>
<tr>
<td>WSC</td>
<td>19.3a</td>
<td>91.0b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Starch</td>
<td>34.4a</td>
<td>66.0b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P</td>
<td>3.08</td>
<td>3.33</td>
<td>n.s.</td>
</tr>
<tr>
<td>Na</td>
<td>0.15a</td>
<td>3.67b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>K</td>
<td>30.72</td>
<td>34.37</td>
<td>n.s.</td>
</tr>
<tr>
<td>Mg</td>
<td>2.24</td>
<td>2.38</td>
<td>n.s.</td>
</tr>
<tr>
<td>Ca</td>
<td>3.05a</td>
<td>5.92b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total oxalic acid</td>
<td>6.80a</td>
<td>1.20b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Soluble oxalic acid</td>
<td>1.10</td>
<td>&lt;1.00</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

n.s., not significant; n.a., not available.
The ME, OMD, CP, WSC, starch, Na and Ca concentrations were significantly higher (P<0.01) in ryegrass than in kikuyu, whilst kikuyu contained significantly higher (P<0.01) concentrations of ADF, NDF and total oxalate. Although the absolute NPN value of ryegrass was significantly higher than kikuyu, the percentage of NPN in N was not and simply reflects the higher N percentage in ryegrass. The WSC to CP ratio determined at 0900 h in ryegrass (0.36) was 4 times higher than for kikuyu (0.09).

Table 6.2 Mean (± s.e.) amino acid concentration (g/kg DM or g/16g N) in representative kikuyu (n=16, except for methionine, cysteine and tryptophan where n=2) and ryegrass (n=1) samples.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Kikuyu g/kg DM</th>
<th>Ryegrass g/kg DM</th>
<th>Kikuyu g/16g N</th>
<th>Ryegrass g/16g N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>17 ± 1.0</td>
<td>17</td>
<td>8.14 ± 0.2</td>
<td>9.69</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>18 ± 1.0</td>
<td>19</td>
<td>8.57 ± 0.3</td>
<td>11.28</td>
</tr>
<tr>
<td>Serine</td>
<td>8 ± 1.9</td>
<td>8</td>
<td>3.88 ± 0.1</td>
<td>4.71</td>
</tr>
<tr>
<td>Glycine</td>
<td>8 ± 0.5</td>
<td>9</td>
<td>3.82 ± 0.1</td>
<td>5.09</td>
</tr>
<tr>
<td>Histidine</td>
<td>3 ± 0.2</td>
<td>3</td>
<td>1.37 ± 0.1</td>
<td>1.59</td>
</tr>
<tr>
<td>Arginine</td>
<td>10 ± 0.7</td>
<td>10</td>
<td>4.95 ± 0.2</td>
<td>5.48</td>
</tr>
<tr>
<td>Threonine</td>
<td>7 ± 0.4</td>
<td>7</td>
<td>3.37 ± 0.1</td>
<td>4.21</td>
</tr>
<tr>
<td>Alanine</td>
<td>13 ± 0.8</td>
<td>14</td>
<td>6.08 ± 0.2</td>
<td>7.74</td>
</tr>
<tr>
<td>Proline</td>
<td>8 ± 0.5</td>
<td>10</td>
<td>3.84 ± 0.1</td>
<td>5.73</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>6 ± 0.4</td>
<td>6</td>
<td>2.99 ± 0.1</td>
<td>3.48</td>
</tr>
<tr>
<td>Valine</td>
<td>10 ± 0.6</td>
<td>10</td>
<td>4.73 ± 0.1</td>
<td>5.67</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>8 ± 0.5</td>
<td>8</td>
<td>3.79 ± 0.1</td>
<td>4.35</td>
</tr>
<tr>
<td>Leucine</td>
<td>14 ± 0.9</td>
<td>14</td>
<td>6.67 ± 0.2</td>
<td>8.23</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>8 ± 0.6</td>
<td>9</td>
<td>4.02 ± 0.1</td>
<td>4.86</td>
</tr>
<tr>
<td>Lysine</td>
<td>9 ± 0.6</td>
<td>9</td>
<td>4.35 ± 0.2</td>
<td>5.21</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.6</td>
<td>3.9</td>
<td>1.38 ± 0.1</td>
<td>2.20</td>
</tr>
<tr>
<td>Cysteine</td>
<td>2.3</td>
<td>4.0</td>
<td>1.21 ± 0.1</td>
<td>2.30</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1.3</td>
<td>1.4</td>
<td>0.7</td>
<td>8.06</td>
</tr>
</tbody>
</table>

*Note: The N content for the kikuyu (mean ± s.e.) and ryegrass samples used in the Table 6.2 were 3.31 ± 0.1 and 2.80, respectively.*

The concentration of individual amino acids in kikuyu did not vary significantly...
throughout the growing season. The mean amino acid concentrations in kikuyu and ryegrass DM are presented in Table 6.2. The amino acid component of N in kikuyu was much lower than that of ryegrass with only 71% of N in kikuyu comprising amino acids compared to 92% in ryegrass. The comparison of individual amino acids (Table 6.2) shows that ryegrass has higher concentrations of each amino acid in the N fraction of the samples compared to kikuyu.

Amino acid concentrations (g/kg DM) in kikuyu were similar to those of ryegrass, except that methionine and cysteine, were 68 and 57%, respectively, lower than in ryegrass.

6.4 Discussion

In comparison to ryegrass, the nutrient content of kikuyu pasture appears less favourable for milk production. The ME content of kikuyu in this experiment was considerably higher than found in the literature (see Chapter 2). However, ME concentrations were significantly lower than in ryegrass, suggesting that even in well-managed pastures, the energy content of kikuyu is still a major factor contributing to the difference in milk production between the species. The high fibre content of kikuyu contributes to lower digestibility. Low digestibility may limit voluntary intake, which is the major factor controlling animal production (Minson 1990). In contrast, the concentrations of ADF in kikuyu appear to conform more closely to recommended concentrations (NRC 1989) than in ryegrass, which may be reflected in higher milk fat concentrations in cows grazing kikuyu pastures.

As with various other tropical grass pastures (Kellaway and Porta 1993), kikuyu contained much lower concentrations of some essential minerals than the temperate species. Concentrations of Na were well below recommendations (NRC 1989; Beede 1988), indicating that supplementation would be essential, in particular during the summer months. The Ca concentration of kikuyu was well below recommendations, and its availability to the cow would be expected to be further reduced by relatively high concentrations of oxalates (see Chapter 2). In ryegrass, Ca concentrations were
generally marginal for moderate milk yields (NRC 1989) and in both species, P and Mg concentrations were below recommendations of Beede (1988), for subtropical conditions. Concentrations of K in both species greatly exceeded recommended concentrations. Such high concentrations of K would be expected to adversely affect Mg absorption in the rumen leading to an enhanced requirement for Mg.

Although the CP content of N-fertilised kikuyu is not as high as ryegrass, concentrations still exceed requirements for reasonable milk yields (NRC 1989). The high NPN concentration found in both kikuyu and ryegrass, and hence the relatively low concentration of ‘true protein’ suggests that ‘crude protein’ (N x 6.25) values may overestimate the value of protein to the animal. The nitrate component of NPN in kikuyu was low (3.71 %) and nitrate concentrations in both grasses were well below those considered to potentially harmful to cattle, as discussed in Chapters 2 and 5.

The extremely low WSC content of kikuyu resulted in a relatively low WSC to protein ratio compared to the ryegrass sample (0.09 v 0.36, respectively), and would be expected to adversely affect rumen function, in particular in protein use efficiency (Jones and Wilson 1987). However, the WSC to protein ratio in ryegrass in this study was also suboptimal, and lower than the ratio obtained by Humphreys (1989) of 0.56 for ryegrass in its vegetative state and grown in a cool, temperate environment. This is expected in view of the higher carbohydrate use for respiration in a warmer, subtropical environment. Although actual starch concentrations in kikuyu were found to be approximately half those in ryegrass, the proportion of starch in the total carbohydrates is higher in kikuyu than ryegrass, as starch is the storage carbohydrate in tropical grasses (see Chapter 2). The total non-structural carbohydrates in kikuyu are still much lower than ryegrass (54 v 157 g/kg DM, respectively).

The similarities observed between the two grasses in their amino acid profiles are to be expected, since in both, the predominate protein (‘rubisco’ enzyme) is likely to be the same although, kikuyu appeared to be comparably deficient of methionine and cysteine. Methionine is considered to be one of the two most limiting amino acids (the other being lysine) in ruminants (Cole and van Lunen 1994), grazing pasture, even ryegrass-based
pasture. The concentration of amino acids in DM were similar between the 2 species and this may be associated with the lower N level in the ryegrass sample assessed compared to that of the kikuyu.

6.5 Conclusion
When compared to well-managed ryegrass, kikuyu pastures appear to have various innate nutrient deficiencies which would limit milk production. Low digestibility and ME, and a low WSC to protein ratio in kikuyu may be expected to inhibit rumen function and limit voluntary intake. Furthermore, the mineral requirements of a cow producing greater than 20L per day may not be met from kikuyu alone due to low levels of Na, inadequate availability of Ca, and marginal concentrations of P and Mg. Some of the nutrient deficiencies may be improved through plant breeding (ie. increase digestibility through reduced NDF concentrations), but in the short term, they can be minimised by appropriate grazing management practices (see Chapters 4 and 5) and supplementation of stock (see Chapter 9) grazing these pastures.
Chapter 7

The relative accuracy and precision of estimating intake and digestibility of kikuyu grass pasture using plant wax alkanes

7.1 Introduction

The estimation of herbage intake of grazing cows is an important, but difficult, parameter to obtain (see Chapter 2). Recently, naturally occurring alkanes, found in the cuticular wax of plants, have been used to estimate herbage intake of grazing animals (Dove and Mayes 1991 & 1996). Mayes and Lamb (1984) first examined the use of alkanes as markers for intake estimation, but found faecal recovery was not complete, declining as carbon chain length shortened. However, Mayes et al. (1986a) developed a double alkane technique in which animals are dosed with even-chain alkanes. Intake is then estimated from the daily dose rate and the dietary and faecal concentrations of dosed even-, and naturally occuring odd-, chain alkanes with adjacent chain lengths, using Equation 13 (see Section 3.5.4). The equation indicates that errors associated with incomplete faecal recovery are cancelled out, provided that alkanes of similar recovery are used.

Various validation studies have been undertaken which compare actual DMI of individual cows grazing temperate pasture species, with estimates obtained using alkanes (Dove and Mayes 1991 & 1996). The results of these studies show that alkanes are an accurate means of estimating intake of pasture by cattle. The highest reported discrepancy between known and estimated intake in cattle being only 2.6 % (Dove and Mayes 1996). However, no validation studies have been reported for kikuyu pasture.

A distinct advantage of the alkane technique for intake estimation is that it incorporates the \textit{in vivo} herbage digestibility of the individual animal, rather than relying on an \textit{in vitro} digestibility estimate of the forage consumed (as required when estimating intake using chromic sesquioxide as a marker). Another advantage of the method is that it
accommodates the feeding of known concentrations of other dietary components (Mayes et al. 1986a; Dove and Mayes 1991 & 1996; Dove et al. 1995) (eg. supplements, rumen modifiers).

In most reported studies, alkane capsules were dosed either once or twice daily. The recent availability of intra-ruminal controlled release devices (CRD) which deliver a uniform output of alkane to the rumen, minimises the possible diurnal variation in alkane concentrations (Stakelum and Dillon 1990), and have also been shown to improve the accuracy of estimating the intake of sheep (Dove et al. 1991).

This study aimed to compare actual intake by, and digestibility in, cattle fed kikuyu with predictions of the same using dosed-even chain alkanes delivered via CRD, and naturally occurring odd-chain alkanes present in the pasture.

7.2 Materials and Methods
The study was conducted in March 1995 using 8 Friesian cows (4 lactating and 4 non-lactating) with a mean liveweight of 544 ± 22 (mean ± s.e.) kg. Prior to the onset of the study, the lactating animals were 173 ± 37 (mean ± s.e.) days into lactation, and producing 15.3 ± 1.0 (mean ± s.e.) L milk/cow.day whilst grazing kikuyu pasture and supplemented with barley grain. At the onset of the experimental period, milk production had declined to 9.0 ± 2.2 (mean ± s.e.) L milk/cow.day.

7.2.1 Experimental procedure
The study comprised a 6-day adjustment period to allow dosed alkanes to equilibrate in the faeces followed by a 6-day experimental period. During the adjustment period, cows were fed harvested kikuyu and fitted with faecal collection harnesses (without collection bags), to accustom cows to them. The first 4 days of the experimental period were used to estimate alkane recovery rates and in vivo herbage digestibility. Intake comparisons were made over the whole experimental period.
7.2.2 Herbage Harvest and Feeding

Kikuyu pasture (predominately leaf material) was harvested using a flail-type forage harvester immediately prior to feeding. Cows were fed *ad libitum* in individual stalls 3 times-a-day at 0800, 1500 and 1900 h at rate to ensure some residue remained. Between feeds, cows were housed in a sheltered area with access to an unlimited water supply, but no additional feed. The amount of pasture offered and the subsequent residues were weighed, after which, grab samples were taken and analysed for DM to determine actual DMI. Additional grab samples (n=14) of herbage offered were prepared and analysed for alkane content and *in vitro* OMD as described in Chapter 3.

7.2.2.1 Estimation of Herbage Intake

The CRD's containing C_{32} and C_{36} alkanes (nominal release rate of 344 mg/day for each alkane; K. Ellis, personal communication; University of New England, Armidale, NSW) were administered to each cow, at the onset of the *adjustment* period. Faecal grab samples were obtained: prior to CRD administration; daily during the *adjustment* period; and twice daily during the *experimental* period (see Section 7.2.2.2). Faecal samples were pooled for days 1-4 and 5-6 of the *experimental* period. Samples were prepared, alkanes extracted, analysed, and subsequent intakes were estimated as described in Chapter 3. Intakes were calculated separately for days 1-4 and 5-6 and averaged to establish mean daily DMI over the experimental period.

7.2.2.2 Estimation of Alkane Recovery Rates and Diet Digestibility

Each cow was fitted with a faecal collection bag and harness (see Figure 7.1) for the first 4 days of the *experimental* period.

*Figure 7.1* The faecal collection harness and bag used to collect faeces.
Collection bags were emptied 5 or 6 times daily, wet faeces weighed and a grab sample from each retained for determination of total faecal DM output. Over each 24 h period, faeces from each animal was combined in a large bin and mixed. A grab sample was obtained twice daily and frozen in preparation for alkane extraction and analysis (see Chapter 3).

Alkane recovery rates were determined by comparing alkane intake of kikuyu and subsequent output in faeces over days 1-4 in the experimental period. Actual whole diet DMD was determined using actual DMI and FO. Estimated whole diet DMD was determined using two methods. The first determined FO by dose rate, recovery rate (determined using either total faecal collection or an assumed recovery rate of 0.95 (Dove and Mayes 1991 & 1996)) and faecal concentration of C_{36} substituted into Equations 15 and 16 (see Section 3.5.5.1). The second method used C_{35} as an internal marker (Dove et al 1990) as described in Section 3.5.5.2.

7.2.3 Statistical Analysis
Differences in alkane concentrations of herbage samples were detected using ANOVA. The alkane pair with the most accurate estimation of actual intake was assessed using methods given in Bland and Altman (1986). The alkane pair which gave an average difference from actual intake closest to zero, with data lying within 95% confidence limits, was considered to be the best estimation of actual intake.

7.3 Results
7.3.1 Concentration of Alkanes in Kikuyu Herbage
The concentrations of alkanes in kikuyu herbage are outlined in Table 7.1.

<table>
<thead>
<tr>
<th>Alkane Chain Length</th>
<th>C_{25}</th>
<th>C_{26}</th>
<th>C_{27}</th>
<th>C_{28}</th>
<th>C_{29}</th>
<th>C_{30}</th>
<th>C_{31}</th>
<th>C_{32}</th>
<th>C_{33}</th>
<th>C_{35}</th>
<th>C_{36}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.1 (0.23)</td>
<td>12.9 (4.91)</td>
<td>12.3 (5.8)</td>
<td>9.1 (2.61)</td>
<td>19.4 (0.67)</td>
<td>5.9 (0.29)</td>
<td>131.5 (2.29)</td>
<td>7.8 (0.20)</td>
<td>197.6 (3.89)</td>
<td>99.7 (4.38)</td>
<td>4.0 (0.07)</td>
</tr>
</tbody>
</table>

Note: The concentration of C_{34} is not included as it was used as an internal standard in analysis.

Kikuyu herbage contained relatively high concentrations of the odd-chain alkanes C_{31},
C$_{33}$ and C$_{35}$, and comparatively low concentrations C$_{25}$-C$_{30}$, C$_{32}$ and C$_{36}$.

7.3.2 Changes in Faecal Concentration of Alkanes

Changes in the faecal concentrations of alkanes C$_{33}$ and C$_{36}$ for individual cows during the adjustment and experimental periods are illustrated in Figure 7.2.

Figure 7.2 Changes in the concentration of dosed alkanes C$_{32}$ and C$_{36}$ (mg/kg DM) (analysed in duplicate) in the faeces of (a) lactating cows and (b) non-lactating cows following administration of an intra-ruminal controlled release device (CRD) into the rumen.
The concentration of dosed alkanes in the faeces of most cows appeared to have reached a steady state by day 6. Cow 1005 was an exception where concentrations declined substantially between 3-6 days after CRD administration, although concentrations appeared to plateau during the experimental period. The concentration of the dosed alkanes in the faeces of cows 770 and 1163 declined and inclined, respectively, during the experimental period. Figure 7.3 illustrates the changes in the faecal concentration of the naturally-occuring alkanes $C_{31}$, $C_{33}$ and $C_{35}$ during the adjustment and experimental periods.

Figure 7.3 Changes in the concentration of naturally-occuring alkanes $C_{31}$, $C_{33}$ and $C_{35}$ (mg/kg DM) (analysed in duplicate) in the faeces of (a) lactating cows and (b) non-lactating cows during the adjustment and experimental periods.
7.3.3 Herbage DMI

The actual and estimated daily DMI and the discrepancies between these 2 values are shown in Table 7.2.

Table 7.2 Comparison of, and discrepancies between, actual dry matter intake (DMI) with estimates of DMI using naturally-occurring odd-chain alkanes (C31, C33, and C35) and dosed even-chain alkanes (C32 and C36) for individual lactating (L) or non-lactating (NL) cows and the mean values (and standard errors (s.e.) in brackets) for L and NL treatments.

<table>
<thead>
<tr>
<th>Cow</th>
<th>Lactating or Non-lactating</th>
<th>Actual DMI (kg DM/day)</th>
<th>Estimated of DMI using alkane pairs</th>
<th>Discrepancy between actual and estimated DMI (kg DM/cow.day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C32/C31</td>
<td>C32/C33</td>
</tr>
<tr>
<td>582</td>
<td>L</td>
<td>11.08</td>
<td>9.31</td>
<td>10.05</td>
</tr>
<tr>
<td>965</td>
<td>L</td>
<td>10.85</td>
<td>10.00</td>
<td>10.72</td>
</tr>
<tr>
<td>1005*</td>
<td>L</td>
<td>10.24</td>
<td>15.97</td>
<td>15.04</td>
</tr>
<tr>
<td>596</td>
<td>NL</td>
<td>9.22</td>
<td>9.23</td>
<td>9.89</td>
</tr>
<tr>
<td>1078</td>
<td>NL</td>
<td>8.05</td>
<td>8.71</td>
<td>9.33</td>
</tr>
<tr>
<td>1163</td>
<td>NL</td>
<td>7.67</td>
<td>8.40</td>
<td>9.01</td>
</tr>
<tr>
<td>1192</td>
<td>NL</td>
<td>6.35</td>
<td>5.81</td>
<td>6.24</td>
</tr>
</tbody>
</table>

Mean of lactating cows

Mean of non-lactating cows

P-value (P<0.05) <0.05 n.s. n.s. n.s. <0.05 n.s. n.s.

*Not included in statistical analysis due to large influences on results

The actual DMI of lactating cows was significantly higher (P<0.05) than non-lactating cows (10.15 ± 0.60 and 7.82 ± 0.59 (mean ± s.e.) kg/cow.day, respectively). However, the difference was not detected using estimates determined from the alkane technique.

The mean actual and estimated DMI, and discrepancies between these values are also shown for lactating and non-lactating cows.

There was little difference in intake estimation using the C31/C32 or C35/C36 alkane pairs, with the average difference between estimate and actual intake being 0.29 and -0.33 kg DM/cow, respectively. The C35/C36 pair gave consistently higher estimates of actual
intake than other pairs (average difference of +1.53 kg DM).

7.3.4 Alkane Recovery Rates and Diet Digestibility

The percentage recovery of alkanes in faeces increased as alkane carbon chain length increased (see Table 7.3).

Table 7.3 Calculated recovery rates of alkanes in faeces of individual cows (analysed in duplicate) and corresponding mean (± s.e.).

<table>
<thead>
<tr>
<th>Cow</th>
<th>C_{23}</th>
<th>C_{25}</th>
<th>C_{27}</th>
<th>C_{29}</th>
<th>C_{31}</th>
<th>C_{33}</th>
<th>C_{35}</th>
<th>C_{36}</th>
</tr>
</thead>
<tbody>
<tr>
<td>596</td>
<td>350.9*</td>
<td>273.1*</td>
<td>176.5*</td>
<td>153.8*</td>
<td>230.0*</td>
<td>112.0</td>
<td>111.6</td>
<td>114.9</td>
</tr>
<tr>
<td>70.4</td>
<td>77.5</td>
<td>88.6</td>
<td>104.9</td>
<td>100.5</td>
<td>107.7</td>
<td>103.7</td>
<td>114.2</td>
<td>124.1</td>
</tr>
<tr>
<td>770</td>
<td>64.4</td>
<td>75.4</td>
<td>86.9</td>
<td>102.2</td>
<td>95.3</td>
<td>101.9</td>
<td>70.6</td>
<td>107.1</td>
</tr>
<tr>
<td>170.9*</td>
<td>257.6*</td>
<td>161.7*</td>
<td>137.3*</td>
<td>181.7*</td>
<td>99.8</td>
<td>78.1</td>
<td>102.3</td>
<td>113.1</td>
</tr>
<tr>
<td>965</td>
<td>65.6</td>
<td>73.1</td>
<td>79.7</td>
<td>92.7</td>
<td>88.9</td>
<td>94.2</td>
<td>99.4</td>
<td>99.6</td>
</tr>
<tr>
<td>65.0</td>
<td>68.2</td>
<td>77.2</td>
<td>94.1</td>
<td>86.1</td>
<td>92.4</td>
<td>101.3</td>
<td>98.0</td>
<td>106.4</td>
</tr>
<tr>
<td>1005</td>
<td>55.4</td>
<td>60.5</td>
<td>67.4</td>
<td>78.3</td>
<td>72.4</td>
<td>77.5</td>
<td>71.3</td>
<td>80.6</td>
</tr>
<tr>
<td>421.7*</td>
<td>289.2*</td>
<td>160.5*</td>
<td>125.8*</td>
<td>195.4*</td>
<td>81.9</td>
<td>63.8</td>
<td>81.8</td>
<td>88.9</td>
</tr>
<tr>
<td>1078</td>
<td>55.6</td>
<td>63.0</td>
<td>70.4</td>
<td>82.3</td>
<td>78.7</td>
<td>83.8</td>
<td>78.5</td>
<td>96.7</td>
</tr>
<tr>
<td>116.2*</td>
<td>126.4*</td>
<td>108.4*</td>
<td>100.3*</td>
<td>111.5*</td>
<td>87.1</td>
<td>75.1</td>
<td>89.1</td>
<td>95.8</td>
</tr>
<tr>
<td>1163</td>
<td>53.8</td>
<td>66.2</td>
<td>77.9</td>
<td>90.4</td>
<td>90.7</td>
<td>98.3</td>
<td>76.9</td>
<td>104.6</td>
</tr>
<tr>
<td>60.0</td>
<td>71.4</td>
<td>80.6</td>
<td>91.2</td>
<td>93.5</td>
<td>97.9</td>
<td>76.8</td>
<td>103.9</td>
<td>111.9</td>
</tr>
<tr>
<td>1192</td>
<td>56.0</td>
<td>64.5</td>
<td>72.9</td>
<td>82.6</td>
<td>85.0</td>
<td>88.4</td>
<td>110.2</td>
<td>93.8</td>
</tr>
<tr>
<td>53.7</td>
<td>60.8</td>
<td>71.3</td>
<td>82.1</td>
<td>84.1</td>
<td>88.2</td>
<td>108.0</td>
<td>93.5</td>
<td>101.9</td>
</tr>
<tr>
<td>mean</td>
<td>60.0</td>
<td>68.0</td>
<td>77.3</td>
<td>90.1</td>
<td>87.5</td>
<td>93.6</td>
<td>86.1</td>
<td>98.6</td>
</tr>
</tbody>
</table>

* Obvious outliers removed for calculation of mean ± s.e., due to large influence on results.

The adjacent alkanes with the most similar rates of recovery in faeces were C_{31} and C_{32}.

The faecal alkane recovery rate and diet digestibility for cow 582 could not be determined due to difficulties with the faecal collection apparatus.

The actual diet DMD determined using actual DMI and faecal output for individual cows is compared with estimates of DMD using either dosed C_{35} or naturally-occurring C_{35} in Table 7.4, using either calculated rates of recovery or an assumed recovery rate of 0.95 (Dove and Mayes 1991 & 1996).
Table 7.4 A comparison of the actual diet dry matter digestibility (DMD) with estimated DMD determined using (i) dosed C_{36} in conjunction with intake estimates using the C_{32}/C_{31} alkane pair, or (ii) naturally-occurring C_{35} using either calculated rates of C_{35} or C_{36} recovery or assumed recovery rates of 0.95. The mean (s.e. in brackets) DMD values and the difference between actual and mean values are also presented.

<table>
<thead>
<tr>
<th>Cow</th>
<th>Actual</th>
<th>Estimated using Dosed C_{36}</th>
<th>Estimated using Naturally - Occurring C_{35}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Using calculated recovery</td>
<td>Using 95% recovery</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>965</td>
<td>0.656</td>
<td>0.659</td>
<td>0.671</td>
</tr>
<tr>
<td>596</td>
<td>0.626</td>
<td>0.653</td>
<td>0.656</td>
</tr>
<tr>
<td>1078</td>
<td>0.671</td>
<td>0.721</td>
<td>0.649</td>
</tr>
<tr>
<td>1163</td>
<td>0.629</td>
<td>0.546</td>
<td>0.618</td>
</tr>
<tr>
<td>1192</td>
<td>0.583</td>
<td>0.729</td>
<td>0.633</td>
</tr>
<tr>
<td>Means</td>
<td>0.633</td>
<td>0.662</td>
<td>0.646</td>
</tr>
<tr>
<td></td>
<td>(0.15)</td>
<td>(0.23)</td>
<td>(0.08)</td>
</tr>
</tbody>
</table>

| Difference between mean estimated and actual DMD | 0.29 | 0.17 | 0.01 | 0.38 |
|                                                | (0.26) | (0.10) | (0.04) | (0.09) |

The best estimation of DMD was determined from the naturally-occurring C_{35} alkane.

7.4 Discussion

In this study, the use of naturally-occurring odd-chain alkanes, in conjunction with adjacent, dosed even-chain alkanes provided an accurate method to estimate the DMI of cattle fed freshly-harvested kikuyu herbage. The best estimate of DMI was obtained using the C_{32}/C_{31} alkane pair, which had the most similar faecal recoveries, and the C_{32}/C_{33} pair. The discrepancy between the recovery rates of C_{36} and C_{35}, resulted in a much greater error in the prediction of intake using this alkane pair.

In previous experiments (see Table 7.5), where housed animals were fed fresh temperate herbage, Mayes et al. (1986c) using beef cattle, and Dillon and Stakelum (1989) and Stakelum and Dillon (1990) using dairy cows, found the most accurate estimate of intake was achieved using the C_{32}/C_{33} alkane pair with the absolute discrepancies...
between actual and estimated intake being 0.07, 0.09 and 0.10 kg/cow.day, respectively. In this study, the discrepancies in this study between actual and estimated intakes (ranging from -1.89 to 1.31 kg DM/cow.day) were higher.

Table 7.5 A comparison of the difference (%) between the faecal recovery rates of dosed even-chain alkanes (C₃₂ and C₃₆) and adjacent naturally-occurring odd-chain alkanes (C₃₁, C₃₃ or C₃₅) in published data for cattle.

<table>
<thead>
<tr>
<th>Reference Animal Description</th>
<th>Feed Details</th>
<th>C₃₁ - C₃₂</th>
<th>C₃₂ - C₃₃</th>
<th>C₃₃ - C₃₆</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayes et al. (1986c) non-lactating beef cow perennial ryegrass</td>
<td>-17.6</td>
<td>-3.7</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Dillon and Stakelum (1990) dairy cows mid-lactation 1. Grass silage and concentrates</td>
<td>-1.0</td>
<td>-4.4</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>dairy cows early lactation 2. Grass silage and concentrates</td>
<td>-6.6</td>
<td>-1.2</td>
<td>-1.7</td>
</tr>
<tr>
<td></td>
<td>dairy cows non-lactating 3. Grass silage</td>
<td>1.4</td>
<td>-1.9</td>
<td>0.3</td>
</tr>
<tr>
<td>Stakelum and Dillon (1990) dairy cows chopped grass forage</td>
<td>-3.5</td>
<td>0.9</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>Ohajuruka and Palmquist (1991) dairy cows non-lactating grass hay and concentrate (50:50)</td>
<td>-4.2</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Dillon (1993) dairy cows late-lactation fresh herbage</td>
<td>-0.1*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. C. Robaina, C. Grainger, G. H. McDowell and H. Dove (unpub. data) dairy cows early lactation silage</td>
<td>2.1*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>early lactation pasture</td>
<td>-2.6*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. W. Mayes and A. Hameleers (unpub. data) dairy cows</td>
<td>-1.9*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Unspecified alkane pair used for intake estimation as reported by Dove and Mayes (1996).

The alkane profile of kikuyu differs slightly from that reported from various temperate grass species. Kikuyu contained relatively high concentrations of C₃₁, C₃₃ and C₃₅ compared to C₂₉, C₃₁ and C₃₃ in ryegrass (Dove and Mayes 1996; Dove et al. 1996). This allowed calculation of DMI using 3 alkane ratios (C₃₁/C₃₂, C₃₃/C₃₂ and C₃₅/C₃₆). Additionally, the high C₃₅ concentration in kikuyu provided the opportunity to examine
its potential use as a digestibility marker in cattle. In sheep, the use of C35 to estimate
digestibility has been accurate compared to alternative techniques (Dove et al. 1990;
Dove and Coombe 1992). Despite the mean recovery rate of C35 being greater than 100
% in this study, it was the most accurate means of estimating digestibility compared to
the use of C36, which had relatively low faecal recovery.

Discrepancies between actual and estimated DMI of kikuyu, may be due to the variation
in the ratio of leaf to stem consumed by cows. Investigations have shown plant parts can
differ in alkane concentrations, which can affect DMI calculations (Laredo et al. 1991;
Baker and Klein 1994; Dove et al. 1991 & 1996). Evidence in kikuyu has been
provided by J. P. Marais (personal communication) who found leaf contained higher
alkane concentrations than stem. Such differences can be used to determine the intake
of various plant parts in similar fashion to the estimation of botanical composition of the
diet of herbivores (Dove et al. 1991; Dove and Mayes 1991 & 1996). This emphasises the importance of obtaining representative samples of ingested herbage
throughout the duration of grazing experiments to minimise variation between actual
and estimated intake.

Actual intakes of lactating cows were significantly higher than non-lactating cows.
However, a significant difference was not detected using the alkane technique, although
lactating cows tended to have a higher DMI than non-lactating cows. This may have
been related to the low number of animals used for statistical analysis.

The accuracy of determining intake using the alkane technique depends on the
assumption that the faecal recovery of an adjacent pair of alkanes is similar. The
variation between estimates of intake using different alkane pairs are most likely
associated with the differences in the faecal recovery between the adjacent alkanes. It
has generally been believed that the faecal recovery rate of alkanes improve as carbon
chain length increases (Dove and Mayes 1991 & 1996). However, in the present study,
the recovery of naturally occurring odd-chain alkanes from C23 to C26 increased linearly
with increasing carbon chain length, after which, the recovery of even-chain alkanes
(both natural and dosed) fell relative to the recovery of adjacent odd-chain alkanes.
Other studies (Mayes et al. 1986b; Mayes et al. 1988; Dove et al. 1989; Dillon and Stakelum 1990; Stakelum and Dillon 1990; Vulich et al. 1991) also have not observed a trend of increasing recovery as alkane length rose. If alkane recovery rate increased with carbon chain length, it would be expected that all of the values in the Table 7.5 would be negative. However, at least one quarter of the studies showed positive differences for $C_{31} - C_{32}$ and $C_{32} - C_{33}$, and almost all of the relevant studies found that $C_{35}$ had a higher rate of recovery than $C_{36}$.

It is likely that the abnormal results from cows 770 and 1005 (which were subsequently removed for statistical analysis) during this experiment were due to misfunctioning of the CRD’s, and associated problems should be considered when assessing their use. However, other factors may have been influencing the results. In the day 1-4 faecal sample from cow 770, the naturally occurring alkane levels were unusually high, whereas day-to-day variations in intake would be expected to cause corresponding variations in faecal concentrations of dosed alkanes. The faecal concentrations of natural odd-chain alkanes would be expected to be affected to a lesser degree due to the effects of intake on digestibility. The most likely cause of variations in natural alkanes is through variation in the dietary alkane content and it is evident that six of the eight cows exhibited particularly high natural alkane levels in the day 1-4 pooled faecal samples.

Difficulties associated with the use of faecal collection harnesses resulted in only a 4 day faecal collection period. Such a limited period could have resulted in substantial errors in estimates of faecal recovery since end point errors (ie. those resulting from variation in the timing of defacations on the first and final days of the collection period) could be relatively large. Large day-to-day variation in intake, and hence faecal output would further increase end point errors. Day-to-day variation in dietary alkane concentrations could also affect faecal recovery value and would depend on the herbage sample concentrations used in the estimations. It is often assumed that on a particular day, faeces produced are relevant to a previous days food. In the analysis of results of this experiment it may have been more appropriate to use actual DMI and herbage alkane concentrations from days 1-3 and the FO and faecal alkane concentrations from days 2-
4. However, the errors mentioned above with the limited collection time may have been greater so data was used for days 1-4 in both intake and output assessment.

The relatively high discrepancy between the recovery of dosed even-chain alkanes and their adjacent naturally-occurring odd-chain alkanes, may be due to an overestimation of CRD release rates of alkanes and it is essential to have an accurate estimate of rates to determine intakes and digestibility.

Faecal collection through the adjustment period allowed changes in absolute concentrations and ratios of dosed: naturally-occurring alkanes to be monitored. This allowed problems associated with CRD release of alkanes to be identified, and these animals were legitimately removed from subsequent analysis. Although this procedure was helpful in determining CRD alkane release problems, it may not be practical in many situations.

7.5 Conclusion

The alkane technique provides a direct and accurate method of determining the DMI of individual dairy cows grazing kikuyu grass pasture. Ideally, animals should receive a CRD to continuously administer both C_{32} and C_{36} throughout the day. The most accurate estimate of kikuyu DMI was obtained using dosed C_{32} alkane combined with either naturally-occurring C_{31} or C_{33} alkanes, and using C_{36} alkane to estimate FO. The most accurate estimates of \textit{in vivo} diet DMD was made by the use of C_{35}. The alkane technique overcomes errors associated with cow and treatment variability which often arise when using other methods to determine intake and DMD (eg. the chromic oxide method to determine intake which relies on an \textit{in vitro} estimate of digestibility).
Chapter 8
A comparison of three techniques to determine the herbage intake of dairy cows grazing kikuyu pasture

8.1 Introduction

The technique chosen to estimate the intake of a grazing animal depends on the available resources, the accuracy, precision and type of estimate required (whole herd or individual cows, and short- or long-term evaluation).

The daily intake estimates of the herd, but not individual animals, can be determined using the difference between pre- and post-grazing pasture mass estimated by devices such as the Ellinbank rising plate meter (RPM) (Earle and McGowan 1979). The RPM is non-destructive and minimises errors arising from within paddock variability (Michell 1982) as many readings can be conveniently obtained. The determination of the intake of kikuyu using a RPM is likely to be difficult due to the high proportion of non-available stoloniferous material compared to temperate species. Fulkerson and Slack (1993) improved the accuracy and precision of estimating kikuyu mass by developing calibration equations for forage above 5 cm stubble height, rather from ground level which includes the stoloniferous material.

Herbage intake can also be estimated using, in reverse (RS), the accepted energy requirements for maintenance, production, liveweight change and physiological status (MAFF 1975; Neilson et al. 1981; Fulkerson et al. 1985; NRC 1989). However, intakes generated using the RS technique are derived from generalised equations and this may not be a true representation of the intake of individual animals.

Chapter 7 showed that the alkane technique provides an accurate estimate of the DMI of individual dairy cows fed cut kikuyu pasture, however, no studies have estimated the DMI of individual cows grazing kikuyu grass pastures.

The aim of this study was to compare the precision of the alkane technique with that
based on the measurement of pasture mass before and after grazing (RPM), and that calculated from feeding standards applied in reverse (RS) to determine the herbage intake of cows grazing kikuyu pasture.

8.2 Materials and Methods

Herbage intakes were estimated for 3 groups of 14 Friesian dairy cows grazing on separate farmlets, supplemented with 0, 3 or 6 kg of cereal-based concentrate (as fed)/cow.day. The kikuyu pastures were managed and samples of forage and concentrate were obtained for OMD analysis, ME calculation and alkane extraction as described in Chapter 3.

8.2.1 Comparison of Intake Measurement Techniques

Two comparative studies were undertaken.

Study 1 was conducted over a 45-day experimental period through March and April and compared the use of the RPM and RS techniques. The concentrate offered was crushed barley.

Study 2 involved a 6-day adjustment period for alkane concentrations to equilibrate in the faeces, and a 12-day experimental period which compared the RPM and RS techniques with the alkane technique. The concentrate offered contained 75% crushed barley and 25% formaldehyde-treated sunflower meal.

8.2.1.1 Rising Plate Meter

The RPM was calibrated at fortnightly intervals with DM available above the 5 cm stubble height (Fulkerson and Slack 1993) using about 50 x 0.09 m² quadrats per calibration for both pre- and post-grazing pasture mass. Post-grazing pasture mass was determined within 3 days of pre-grazing estimates (Figure 8.1 (a)). Pasture mass was adjusted for growth between the time of pre- and post-grazing assessment using the mean growth rate of the previous intergrazing interval (mean 64 and 69 kg DM/ha.day for Studies 1 and 2, respectively). Intake was then calculated as the difference between the pre- and post-grazing pasture mass.
Rising Plate Meter Calibrations in *Study 1*

The prediction equations for a combined March sampling, and for each of the subsequent sampling periods for pre- and post-grazing in *Study 1*, are presented in Table 8.1.

**Table 8.1** Calibration equations ($Y = bX + a$) for predicting pasture mass (x kg DM/ha), mean rising plate meter (RPM) readings (Y cm) and the errors associated with the RPM calibration equations ($r^2$, r. s. d and CV) using the RPM in *Study 1*.

<table>
<thead>
<tr>
<th>Date</th>
<th>$n$</th>
<th>$b$</th>
<th>$a$</th>
<th>$r^2$</th>
<th>r. s. d.</th>
<th>RPM reading (cm)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RPM</td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-grazing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 March &amp; 22 March</td>
<td>98</td>
<td>0.00557</td>
<td>5.53</td>
<td>0.75</td>
<td>1.88</td>
<td>12.5</td>
<td>5.5 - 23.5</td>
</tr>
<tr>
<td>5 April</td>
<td>45</td>
<td>0.00501</td>
<td>6.38</td>
<td>0.83</td>
<td>1.16</td>
<td>11.5</td>
<td>5.0 - 16.5</td>
</tr>
<tr>
<td>14 April</td>
<td>49</td>
<td>0.00539</td>
<td>6.97</td>
<td>0.91</td>
<td>1.30</td>
<td>14.2</td>
<td>7.0 - 23.0</td>
</tr>
<tr>
<td>Post-grazing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 April</td>
<td>48</td>
<td>0.00505</td>
<td>7.07</td>
<td>0.86</td>
<td>0.81</td>
<td>9.8</td>
<td>5.5 - 17.0</td>
</tr>
<tr>
<td>14 April</td>
<td>47</td>
<td>0.00311</td>
<td>8.35</td>
<td>0.86</td>
<td>1.09</td>
<td>12.4</td>
<td>7.0 - 19.0</td>
</tr>
</tbody>
</table>

Figure 8.1 Measuring pasture mass using a rising plate meter.
The 2 pre-grazing calibrations made in March did not differ significantly in slope or intercept from the full-model so the data sets were pooled into 1 prediction equation. The remainder of the prediction equations differed significantly (\( P<0.05 \)) from the full model. The mean predicted amount of pasture on offer in a ‘representative’ paddock and the standard error associated with individual measurements using the RPM in March was 1350 ± 175 kg DM/ha.

**Rising Plate Meter Calibrations in Study 2**

In *Study 2*, the intercepts from all 3 pre- and post-grazing regression equations did not differ significantly from the full model regression. No significant difference was found between the post-grazing calibrations in this period, therefore the data sets were pooled to form one post-grazing calibration equation (Table 8.2).

<table>
<thead>
<tr>
<th>Date</th>
<th>n</th>
<th>b</th>
<th>a</th>
<th>( r^2 )</th>
<th>r. s. d.</th>
<th>RPM reading (cm)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td><strong>Pre-grazing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 May</td>
<td>50</td>
<td>0.00459</td>
<td>6.55</td>
<td>0.84</td>
<td>2.58</td>
<td>18.8</td>
<td>9.0 -33.0</td>
</tr>
<tr>
<td>20 May &amp; 26 May combined</td>
<td>79</td>
<td>0.00437</td>
<td>5.25</td>
<td>0.78</td>
<td>3.21</td>
<td>17.7</td>
<td>7.5 -35.0</td>
</tr>
<tr>
<td><strong>Post-grazing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined</td>
<td>151</td>
<td>0.00342</td>
<td>6.90</td>
<td>0.87</td>
<td>1.50</td>
<td>13.6</td>
<td>6.5 -29.0</td>
</tr>
</tbody>
</table>

Similarly, no significant difference was found between the latter 2 pre-grazing equations so their data were pooled to form single pre-grazing equations for this period. The predicted amounts of pasture on offer in the 'representative' pre- and post-grazed kikuyu paddocks were 2400 ± 393 and 1026 ± 203 (mean ± s.e.) kg DM/ha.

To obtain an indication of the standard error associated with measuring pasture height using the RPM, about 50 individual meter readings were obtained from a paddock representative of kikuyu pasture at that time, for both pre- and then both pre-and post-
grazing, in *Studies 1* and 2, respectively.

8.2.1.2 Prediction of Intake using Standard Energy Requirements in Reverse (RS)
Milk production and composition, and liveweight were recorded at 2-7 day intervals and the ME requirements calculated using the equations of MAFF (1975). These equations were used to demonstrate the RS technique as they are a simple representation of more complex equations. Although SCA (1990) determined requirement equations that are more suitable to the Australian environment, in this instance, these could not be used as the maintenance requirement is influenced by level of intake, the factor we are attempting to determine. The RS calculations were based on an *in vitro* OMD for kikuyu of 0.652 and 0.639 in *Studies 1* and 2, respectively.

8.2.1.3 Alkanes
The animals were dosed with the C₃₂ and C₃₆ alkanes with the initial intention of calculating intake from C₃₂ and faecal output from C₃₆. It was subsequently found that kikuyu contained high concentrations of C₃₅, therefore the dosed C₃₆ alkane was also used for intake estimation. Alkanes were supplied using hard-shell gelatin capsules, which were prepared using the method of Dove *et al.* (1988), as described below.

*Reagents*
1. Hard shell gelatin capsules (size; 22-55 mm) (Willi Krüger KG Hösel, Germany)
2. Powdered cellulose
3. Alkane solution

600 g of dotriacontane (C₃₂) and 600 g of hexatriacontane (C₃₆) were dissolved in 1500 ml of n-heptane in large round bottom flask (with bung), with the aid of heat. The solution was kept at 55°C whilst making the capsules, to ensure the hydrocarbons were totally dissolved.

*Procedure*
Gelatin capsules were separated into lid and base sections, and approximately 1 g of powdered cellulose and 1 ml of the alkane solution was added to each base, which were placed in fume cupboard for 24 h to allow heptane to evaporate. Lids were reconnected
to bases and secured using a piece of adhesive tape. Fifteen capsules (selected intermittently throughout preparation) were retained for later analysis of alkane content.

Gelatin capsules were administered twice daily following milking. A modified drenching gun was used to place the capsule on the distal area of the tongue (see Figure 8.2 and 8.3); capsules were generally swallowed immediately. Animals were then observed for 30 min to ensure that capsules were not rejected.

Faecal collection began 6 days after the initial dosing to allow dosed alkanes to reach
stable concentrations in the faeces (Figure 8.4). Faecal samples were then collected (as outlined in Section 3.5.2.2) for the following 12 days. Morning and afternoon samples were kept separate each day. At the end of the collection period, all the morning and evening samples were bulked.

Figure 8.4 Collecting faecal samples in paddock.

Herbage, concentrate and faecal samples were prepared, alkanes extracted and analysed, and DMI and DMD calculated as described in Sections 3.5.3, 3.5.4 and 3.5.5, respectively.

a. Soxhlet extraction procedure for the extraction of alkanes from gelatin capsules
The alkanes contained in gelatin capsules were recovered using the soxhlet extraction technique outlined below.

Procedure
A few anti-bump granules were added to the bottom of each round-bottomed Soxhlet flask which were then placed in the oven to dry. Clean extraction thimbles were then labelled with a pencil. After the removal of sealing tape, the contents of one gelatin capsule was added to each thimble by tipping in its friable contents and cutting the capsule with sharp scissors while holding inside the thimble. Each thimble was then loosely plugged with cotton wool.
Flasks were removed from the oven and weighed (3 decimal places), allowed to cool and then assembled, thimbles in holders and condensers on Soxhlet apparatus. The condensors were turned on and 300 ml of petroleum spirit (40-60°C boiling point) was added to the top of each unit so that it trickled down through the thimble. The heat was turned on beneath the flasks and increased until each Soxhlet unit was refluxing. The extraction continued for 6 h. The heat was then removed and when cool, the flasks and contents were removed and weighed.

From each flask, 1.0 g of sample was removed (in duplicate) and weighed (4 decimal places) into separate scintillation vials. Approximately 0.8 g C₄₄ internal standard was also weighed into each vial (4 decimal places). Solvent was then evaporated from vials in preparation for analysis for alkane content as described in Section 3.5.3. The alkane content of the capsule was then calculated from the known weights of the contents of the Soxhlet flask and the weight of the sample removed therefrom.

8.2.2 Statistical Analysis
Linear regression analysis of Y (RPM reading in cm) on X (pasture mass in kg DM/ha) were conducted on calibration data to determine the most appropriate equations from which to determine pasture mass. The slopes and intercepts of the regressions for each time period, within each study, were compared with the regression of pooled data (full model) using the extra sums of squares principle (Draper and Smith 1981). An indication of the standard error associated with predicting pasture mass from RPM readings was obtained at 1 time within each study using the method of Kendall and Stuart (1967).

Group effects were determined using the general linear model (GLM) of Minitab (Ryan et al. (1985)) within each method used for intake estimation. The RS and alkane derived estimates of intake were also compared using the GLM. The RPM estimates which represent the mean of daily group measurements could not be statistically compared with RS or alkane-derived intakes which represent a mean of individual intakes.

The morning and afternoon intake estimates determined using the alkane technique were
tested for significant changes using the paired \( t \)-test.

8.3 Results

8.3.1 Study 1. Comparison of the RPM and RS Techniques to Estimate Herbage Intake over an Extended Period (45 days)

At an average of 831 ± 43 (mean ± s.e.) kg DM/ha of pasture \textit{on offer} above the 5 cm stubble height, the RPM estimated the pasture intake of the group receiving no concentrate to be significantly higher \((P<0.05)\) than those being fed either 3 or 6 kg concentrates/cow.day (Table 8.3).

<table>
<thead>
<tr>
<th>Concentrate fed (kg as fed/cow.day)</th>
<th>Mean amount of pasture \textit{on offer} (kg DM/ha)</th>
<th>Estimated herbage intake (kg DM/cow.day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>943</td>
<td>RPM 12.5 RS 14.8</td>
</tr>
<tr>
<td>3</td>
<td>777</td>
<td>RPM 10.4 RS 12.9</td>
</tr>
<tr>
<td>6</td>
<td>773</td>
<td>RPM 10.5 RS 7.8</td>
</tr>
<tr>
<td>l.s.d. ((P=0.05)) n.s.</td>
<td></td>
<td>RPM 1.6 RS 1.5</td>
</tr>
</tbody>
</table>

The RPM did not detect a significant difference in pasture intake between the latter 2 groups. The RS calculations estimated a significant reduction \((P<0.05)\) in pasture intake for each 3 kg increase in concentrate fed. The RPM estimated levels of intake 16 and 19\% lower than RS for groups fed 0 and 3 kg concentrates/cow.day respectively, and 35\% higher for those fed 6 kg concentrates/cow.day. The RS data implied a much higher rate of substitution of grain for pasture than did the RPM between the 3 and 6 kg rates of concentrate feeding. The RPM detected no decrease in estimated pasture intake whereas a decrease of 5.1 kg/cow.day was estimated using RS (a substitution rate of 1.91).

8.3.2 Study 2. Comparison of the RPM, RS and Alkane Methods for Estimating Herbage Intake over a 12-day Period
8.3.2.1 Intakes using Alkane Method

The mean alkanes content of gelatin capsules was 339 and 337 mg of C_{32} and C_{36}, respectively. The mean alkane profiles of the plucked kikuyu samples, and concentrate fed, are outlined in Table 8.4.

Table 8.4 Mean alkane content (mg/kg DM) of kikuyu (s.e. in brackets).

<table>
<thead>
<tr>
<th>Alkane chain length</th>
<th>C_{25}</th>
<th>C_{26}</th>
<th>C_{27}</th>
<th>C_{28}</th>
<th>C_{29}</th>
<th>C_{30}</th>
<th>C_{31}</th>
<th>C_{32}</th>
<th>C_{33}</th>
<th>C_{34}</th>
<th>C_{35}</th>
<th>C_{36}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kikuyu</td>
<td>10.9 (0.29)</td>
<td>* 16.4 (0.40)</td>
<td>* 26.2 (0.40)</td>
<td>8.3 (0.12)</td>
<td>204.7 (2.70)</td>
<td>10.4 (0.12)</td>
<td>240.7 (3.00)</td>
<td>105.9 (3.09)</td>
<td>4.9 (0.20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentrate</td>
<td>4.3 (0.07)</td>
<td>* 6.0 (0.40)</td>
<td>* 8.9 (0.88)</td>
<td>* 8.1 (0.79)</td>
<td>* 3.3 (0.74)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: C_{34} concentrations are not included as it was used as an internal standard in analysis.

* Concentrations of alkane below detection (< 2 mg/kg DM)

As intakes estimated from morning and afternoon faecal samples did not differ significantly, data for these times were pooled. Estimates of pasture intake were higher when estimated from alkanes of longer chain length. This trend was significant (P<0.01) at the 0 and 3 kg rate supplementary feeding (Table 8.5).

Table 8.5 Mean estimated herbage intake and whole diet DMD using the alkane technique.

<table>
<thead>
<tr>
<th>Concentrate fed (kg as fed/cow.day)</th>
<th>Estimated herbage intake (kg DM/cow.day)</th>
<th>Mean diet DMD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C_{25}/C_{32}</td>
<td>C_{26}/C_{32}</td>
</tr>
<tr>
<td>0</td>
<td>11.7</td>
<td>12.6</td>
</tr>
<tr>
<td>3</td>
<td>10.1</td>
<td>10.7</td>
</tr>
<tr>
<td>6</td>
<td>8.7</td>
<td>9.2</td>
</tr>
<tr>
<td>l.s.d. (P&lt;0.05)</td>
<td>1.2</td>
<td>1.1</td>
</tr>
</tbody>
</table>

The whole diet DMD determined using alkanes did not differ significantly between the 3 groups, and had a mean of 0.699. The effect of each rate of concentrate supplementation on herbage DMD was of particular interest and was estimated in a similar manner to Doyle et al (1988) using the equation:

\[
\text{Diet DMD (\%)} = ax + by
\]
where:

- \( a \) is the fraction of diet comprising concentrate (DM basis)
- \( x \) is the digestibility of concentrate (%)
- \( b \) is the fraction of diet comprising herbage (DM basis)
- \( y \) is the digestibility of herbage (%)

The equation assumes that the diet digestibility is the sum of the proportional contributions made by each constituent in the diet.

Table 8.6 shows the estimated herbage DMD for cows fed 3 and 6 kg of concentrate at nominated levels of concentrate digestibility.

Table 8.6 Kikuyu DMD values \((y_1, y_2)\) estimated using separate equations at the 3 and 6 kg rate of concentrate feeding \((0.2x + 0.8y_1 = 70.5\) and \(0.4x + 0.6y_2 = 69.8\) respectively) using hypothetical values of concentrate DMD \((x)\).

<table>
<thead>
<tr>
<th>Concentrate fed (kg as fed/cow.day)</th>
<th>Concentrate DMD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.86</td>
</tr>
<tr>
<td>3</td>
<td>0.666</td>
</tr>
<tr>
<td>6</td>
<td>0.589</td>
</tr>
</tbody>
</table>

Estimated herbage DMD differed by 0.077 units at a nominated concentrate digestibility of 0.860 but only 0.026 units when concentrate digestibility was reduced to 0.740.

### 8.3.2.2 Intakes Calculated from RS

The alkane procedure allowed us to calculate herbage intake, faecal output and *in vivo* kikuyu DMD (0.695) in individual, unsupplemented cows. Intakes derived from RS for all groups of cows were calculated using this value, and secondly using the value derived *in vitro* (0.639) during Study 2. A 17% reduction in predicted intake was observed at each rate of feeding when *in vivo* rather than *in vitro*-derived digestibilities were used in RS calculations (Table 8.7), however, significant \((P < 0.05)\) reductions were only evident at the 0 and 3 kg rates of concentrate feeding.
Table 8.7 Mean estimates of herbage intake using either *in vitro* or *in vivo* digestibilities for energy standards in reverse (RS), the rising plate meter (RPM) method and the alkane technique using the C_{33}/C_{32} pair of alkanes.

<table>
<thead>
<tr>
<th>Concentrate fed (kg as fed/cow.day)</th>
<th>Pasture on offer (kg DM/ha)</th>
<th>Estimated intakes of grazed herbage (kg DM/cow.day)</th>
<th>RPM</th>
<th>RS</th>
<th>Alkanes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>In vitro</em> OMD</td>
<td><em>In vivo</em> DMD</td>
<td>l.s.d. (P=0.05)</td>
</tr>
<tr>
<td>0</td>
<td>1772</td>
<td>13.5</td>
<td>14.3</td>
<td>12.3</td>
<td>2.0</td>
</tr>
<tr>
<td>3</td>
<td>1931</td>
<td>15.4</td>
<td>12.2</td>
<td>10.4</td>
<td>1.3</td>
</tr>
<tr>
<td>6</td>
<td>2004</td>
<td>12.4</td>
<td>7.6</td>
<td>6.5</td>
<td>n.s.</td>
</tr>
<tr>
<td>l.s.d. (P=0.05)</td>
<td>n.s.</td>
<td>n.s.</td>
<td>1.8</td>
<td>1.5</td>
<td>1.1</td>
</tr>
</tbody>
</table>

8.3.2.3 Intakes Calculated from RPM

The average amount of pasture *on offer* above the 5 cm stubble height in Study 2 was 1902 ± 99 (mean ± s.e.) kg DM/ha. This was significantly higher (P<0.01) than in Study 1. The RPM detected no significant differences in pasture *on offer* or pasture intake between the 3 groups during this study.

8.4 Discussion

This study compared RS, the difference between pre- and post-grazing mass determined by RPM and the use of synthetic and natural plant alkanes as methods for estimating intake of kikuyu grass pasture by Friesian dairy cows. Although the actual herbage intake was unknown, the study examined the precision of each method of estimation and provides a useful guideline on their use.

In most cases there was a significant difference in pre- and post-grazing calibration equations for the RPM although, in contrast to the findings of Stockdale (1984), the pre- and post-grazing estimates were of similar precision. The mean pre- and post-grazing CV's in this study were also lower (13 and 9 % respectively) than those obtained by Stockdale (1984) (18.6 and 12.1% respectively). In Study 1, the slope of the regression equations decreased and the Y intercept generally decreased throughout the growing season, reflecting increases in the stoloniferous content of the pasture in the autumn period (see also Fulkerson and Slack 1993). Such changes in pasture growth pattern of
kikuyu preclude the pooling of all data within season and result in the requirement for more than one calibration throughout the season (Michell 1982).

Although there was no significant difference observed in the amount of pasture on offer to groups in either Study 1 or 2, some variation was apparent. Pasture intake levels may have been influenced by the amount of pasture on offer in Study 1 since virtually all of the pasture on offer was consumed. However in Study 2, pasture was offered far in excess of requirements and is unlikely to have affected intake.

In the assessment of the three pasture intake measurement methods, differences in the interpretation of the errors associated with each must be considered. The RPM method is effectively a single measurement for each group of animals. The l.s.d. values are related to the measurement errors from a large number of individual RPM measurements, together with errors resulting from the derivation of prediction equations converting RPM measurements to estimates of herbage mass. For the RS method, the l.s.d. values are calculated from between cow variation in intake calculated from milk production and liveweight change data, along with errors from prediction equations. The l.s.d. derived in the alkane method accounts for between animal variation in the intake estimate, but not measurement error. Therefore, the error structure of each intake estimation method differ and may not fully reflect the true errors associated with each method.

It is generally accepted that pasture intake will decline as the rate of concentrate supplementation in the diet increases and animals substitute concentrate for pasture (Kellaway and Porta 1993). However, the only significant difference in herbage intake detected in either study using the RPM was between the groups offered 0 and either 3 or 6 kg concentrate/cow.day in Study 1. The large errors of estimating intake of kikuyu by the difference between pre- and post-grazing pasture meter measurements, may be one reason for the lack of difference between concentrate feeding rate.

In contrast, the RS technique predicted a significant reduction in herbage intake as concentrate rate in the diet increased, during both Study 1 and 2. However, this assumed
a constant herbage digestibility (assessed in vitro), which did not appear to be the case. Previously, RS have been used to determine the intake of stock grazing pasture alone (Fulkerson et al. 1986). If RS are adopted, accurate data on animal production parameters and feed quality are essential to restrict errors in intake estimates. For example, when RS are used over a short period, it is difficult to assess liveweight fluctuations accurately and consequently large errors can arise when determining energy requirements. Variation between individual animals in efficiency of feed utilization or changes in the efficiency of feed use with increasing amounts of concentrate will also produce errors in RS estimates. Additionally, in Study 2, the digestibility of the base pasture appeared to be depressed by concentrate input, but this was not taken into account in RS calculations.

As mentioned in the Section 7.4, obtaining a herbage sample representative of that consumed by the grazing animal is vital to reduce prediction errors. The plucked samples obtained in this study were considered to most accurately reflect the quality of pasture consumed; however, any variations would result in errors in predicting intake in both the RS and alkane techniques. This becomes more difficult when the pasture contains more than one species.

As found in the previous chapter, estimates of the intake of kikuyu pasture using the alkane technique increased with increasing carbon-chain length. The C_{32}/C_{33} alkane pair provided the closest estimate of the actual kikuyu intake of lactating cows, estimates obtained by the RS method using in vivo derived digestibilities appear to be the most accurate alternative method to determining herbage intake.

The differences in intake estimates between alkane pairs (Table 8.5) may have been due to variations in the faecal recovery of the alkanes. In Chapter 7 it was found that the difference in faecal recovery rates of C_{31} and C_{32}, C_{32} and C_{33} and C_{33} and C_{36} were -0.5, -4.4 and +16.8 %, respectively. Therefore, in such a case, intake estimations using dosed C_{32} in conjunction with naturally-occurring C_{31} should provide the most accurate intake estimate. It is possible that the difference in the results in this experiment to that described in Chapter 7 may be related to the different administration techniques of the
alkanes. In Chapter 7 alkanes were administered by the use of CRD's whereas this experiment used gelatin capsules. It is likely that the effective daily dose rates of C_{32} and C_{36} would be more accurate when administered by gelatin capsule than by CRD, since the contents of the gelatin capsules were determined by chemical analysis. If this was the case, then it would appear that the C_{32}/C_{33} alkane pair would be more appropriate as suggested by Dove and Mayes (1991).

If it is assumed that there is a difference of 16 % in the recovery of the C_{35} and C_{36} alkanes (as occurred in Chapter 7; 1.07 vs 0.91) there would have been approximately an 18.6 % error in intake estimates as calculated from Equation 11 (see Appendix 1 for calculations). This value more than explains the 6 % difference in intake at the 6 kg rate of concentrate feeding, but only part of the 25 and 23 % differences in herbage intake estimates at the 0 and 3 kg rate of concentrate feeding, respectively observed in this study. Dillon and Stakelum (1990) and Mayes et al. (1986b) found that the faecal recoveries of alkanes, in stall fed dairy cows and sheep respectively, were not affected by concentrate type or feeding rate.

In contrast to expectations, the total diet DMD (as determined by alkane technique) did not change as the ratio of concentrate to kikuyu in the total ration increased. Arriaga-Jordan and Holmes (1986) found similar results for cows fed predominantly perennial ryegrass herbage and varying rates of cereal-based pellets. The authors attributed the apparent decrease of the base forage digestibility with increasing concentrate rates to depressed cellulose digestibility. This may be partially attributable to micro flora adaption to the more readily fermentable energy source rather than to a reduction in rumen pH (Mould and Orskov 1983). This suggestion is in line with results of the present study which show that such an effect increases with increase in highly digestible and more fermentable energy sources of concentrate (Table 8.6).

8.5 Conclusion
The herbage intakes estimated using the RPM allows estimation of herd intakes and is accurate enough for farm management situations, where kikuyu is well managed and
highly utilised. The RPM is also essential to enable pasture parameters such as pre- and post-grazing mass to be related to intake estimations. Although it provides only crude group intake values, the RPM also has the advantage of providing rapid short-term intake assessments which could be useful in making management decisions. RS is used as a guide to approximate herbage intake levels over an extended period and can provide intake estimates assuming that all assumptions (eg. pasture digestibility, liveweight change) are known. However, considering the large number of potentially invalid assumptions which can be made using this method, it could be argued there is little point to making such intake calculations. Both the RPM and RS techniques do not provide precise intake data on individual animals in any particular study. The alkane technique provide a direct and precise estimate of pasture intake, which can be obtained on a daily basis if required, and overcomes some of the problems associated with the use of the RPM and RS to determine the intake of kikuyu grass pastures.
Chapter 9

Production responses of dairy cows grazing well-managed kikuyu pastures to energy and protein supplementation

9.1 Introduction

Poor pasture quality is often reflected in low milk yields from cows grazing kikuyu pastures (see Chapter 2) with milk production per cow generally below 12 L/day.

Energy is considered to be the first factor limiting milk production from N-fertilised tropical grass pastures (Royal and Jeffrey 1972; Davison et al. 1991b). However, despite the relatively high CP concentrations in some tropical species, not all of this protein is thought to be available to the animal. Thus, various workers (Flores et al. 1979; Davison et al. 1991b; Moss et al. 1992) have obtained responses to protein supplementation, above what would be expected from estimated concentrations of protein in forage, but the degree of response varied with stage of lactation, pasture quality and quantity and the feeding rate of an energy supplement.

The aim of this study was to determine the production responses to energy and protein supplements fed to lactating cows grazing well-managed kikuyu pastures.

9.2 Materials and Methods

Three studies were conducted in summer/autumn between February 1993 and March 1994. Each study utilised Friesian cows of average Australian Breeding Value (ABV) for fat and protein yield, calving from October to December with age ranging from 3.5 to 11 years in Studies 1 and 2 whilst first calf heifers were also used in Study 3. Within each study, treatment groups were matched for ABV, milk production at that time and the previous lactation, age and stage of lactation.

Study 1 evaluated production response to cereal-based energy supplementation over a 6 week period. Studies 2 and 3 were short term feeding trials to determine whether UDP
was the next limiting nutrient to production of cows grazing kikuyu pastures after energy.

9.2.1 Experimental Procedure

9.2.1.1 Study 1

Study 1 was conducted between March and April 1993, when cows were 5-6 months into lactation. Three treatment groups, each comprising 14 animals, grazed in separate farmlets and were fed either 0 (R₀), 3 (R₃) or 6 (R₆) kg (as fed) of crushed barley grain mix/cow.day. Minerals were added to the grain of the R₃ and R₆ groups (2% lime (388 g Ca/kg), 1% NaCl and 1% Trifos (207 g P and 150 g Ca/kg)) and the R₀ group was given access to a mineral lick containing 54% NaCl, 13.5% Ca, 0.6% P and trace elements.

9.2.1.2 Study 2

Study 2 was of shorter duration (6 day adjustment period and 12 days experimental period) in May 1993 using the same cows as in Study 1. The supplement comprised 72% barley and 24% formaldehyde-treated canola meal (as fed basis) with mineral supplements as shown in Table 9.1.

Table 9.1 Composition of concentrates fed in Studies 2 and 3 (g/kg DM unless otherwise stated).

<table>
<thead>
<tr>
<th></th>
<th>Study 2</th>
<th>Study 3b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentrate mix</td>
<td>Crushed barley</td>
</tr>
<tr>
<td>CP</td>
<td>168</td>
<td>146</td>
</tr>
<tr>
<td>OMD</td>
<td>0.862</td>
<td>0.889</td>
</tr>
<tr>
<td>Ca</td>
<td>2.13</td>
<td>0.40</td>
</tr>
<tr>
<td>P</td>
<td>4.53</td>
<td>3.20</td>
</tr>
<tr>
<td>K</td>
<td>6.30</td>
<td>4.23</td>
</tr>
<tr>
<td>Na</td>
<td>5.40</td>
<td>0.08</td>
</tr>
<tr>
<td>Mg</td>
<td>1.77</td>
<td>1.23</td>
</tr>
<tr>
<td>ash</td>
<td>41.5</td>
<td>18.7</td>
</tr>
</tbody>
</table>

a Concentrate mix contained 72% barley and 24% formaldehyde-treated protected sunflower meal, 2% lime (38.8 g Ca/kg), 1% NaCl, 1% Trifos (207 g P and 150 g Ca/kg).

b Concentration of minerals in the total diet were balanced within each treatment group in accordance with NRC (1989) recommendations, depending on feeding rate, composition of concentrates and predicted kikuyu intake, with dicalcium phosphate (210 g P and 237 g Ca/kg), NaCl, and lime (388 g Ca/kg).
Animals remained in the same groups as for Study 1 but were in their 7th month of lactation. Pasture intake of individual cows was estimated by dosing cow with gelatin capsules containing C32 and C36 alkanes and naturally occurring plant cuticular alkanes as internal markers (Mayes et al., 1986a) (as reported in Chapter 8) which also enabled the calculation of substitution rates.

9.2.1.3 Study 3

Study 3 extended over 40 days in January and February 1994 and evaluated the effect of various concentrations of UDP fed in conjunction with energy supplements. The first 10 days were used as a standardisation period when all of the 91 animals used in this study were fed 4 kg concentrate comprising 66.7% barley and 33.3% formaldehyde-treated canola meal (FTCM)/cow.day. The cows were allocated to 13 treatment groups (Table 9.2) using stratified randomisation based on milk and milk component yield during the standardisation period, age, calving date and ABV for milk, fat and protein yield.

<table>
<thead>
<tr>
<th>Feeding rate</th>
<th>Treatment group</th>
<th>% 'protein' replacement\textsuperscript{a}</th>
<th>Barley (kg as fed/cow.day)</th>
<th>Canola meal (kg as fed/cow.day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 kg</td>
<td>Control</td>
<td>0</td>
<td>0.4</td>
<td>0</td>
</tr>
<tr>
<td>3 kg</td>
<td>A\textsubscript{0}</td>
<td>0</td>
<td>2.8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>A\textsubscript{20}</td>
<td>20</td>
<td>2.3</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>A\textsubscript{40}</td>
<td>40</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>A\textsubscript{60}</td>
<td>60</td>
<td>1.2</td>
<td>2.2</td>
</tr>
<tr>
<td>6 kg</td>
<td>B\textsubscript{0}</td>
<td>0</td>
<td>5.9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>B\textsubscript{20}</td>
<td>20</td>
<td>4.8</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>B\textsubscript{40}</td>
<td>40</td>
<td>3.5</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>B\textsubscript{60}</td>
<td>60</td>
<td>2.4</td>
<td>4.5</td>
</tr>
<tr>
<td>9 kg</td>
<td>C\textsubscript{0}</td>
<td>0</td>
<td>7.9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>C\textsubscript{20}</td>
<td>20</td>
<td>7.0</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>C\textsubscript{40}</td>
<td>40</td>
<td>5.2</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>C\textsubscript{60}</td>
<td>60</td>
<td>3.5</td>
<td>6.5</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Diets remained iso-energetic within each feeding rate
\textsuperscript{b} % protein replacement is the % FTCM in the supplement
After a 10 day period of adjustment to their experimental rations, the experimental period followed for 20 days and all animals grazed together as a group.

9.2.2 Feeding of Supplements
In all studies, concentrates were fed during milking, whilst in Study 3, a portion was fed in individual bails immediately after morning milking. Feed residues were collected and weighed following each feeding in Study 3.

The sunflower and canola meal fed in Studies 2 and 3, respectively, was partially protected (approximately 35%) from degradability in the rumen by treatment with formaldehyde.

9.2.3 Animal Measurements
9.2.3.1 Milk and Liveweight Change
Milk production and composition (using composite morning and afternoon samples) were recorded every 4 days in Study 3, and weekly in Studies 1 and 2, as was liveweight for all studies. Milk fat, protein and lactose were measured using a Milkoscan 133B (N Foss Electric, Denmark).

9.2.3.2 Blood Metabolites and Milk Urea
Tail blood samples, collected on the final day of Study 3 (commencing 3 h after the completion of feeding and continued over a 2 hour period), were centrifuged and the plasma collected and frozen. The plasma samples were analysed for urea (PU), β-hydroxy butyrate (β-OHB) and glucose using a random access autoanalyser (Roch Cobas Mira), and for non-esterified fatty acid (NEFA) concentrations using an enzymatic colorimetric method (Wako NEFA kit, Wako Chemicals USA, Richmond, VA). On the same day that blood samples were collected, composite morning and afternoon milk samples were obtained and centrifuged and the fat removed prior to freezing. The defatted milk samples (1 ml) were mixed with 0.5 ml 36% trichloroacetic acid to precipitate the protein, the mixture centrifuged and the supernatant analysed for urea (MU), also using a random access autoanalyser.
9.2.4 Pasture Measurement
Pasture was managed and pre-grazing pasture samples plucked to simulate grazing height obtained at weekly (Study 1), or daily (Studies 2 and 3) intervals, and prepared and analysed as described in Chapter 3 for NDF, ADF, N, minerals, OMD and WSC. Animals were offered a fresh block of pasture either daily (Study 1 and 2) or following each milking (Study 3). Pastures were grazed at a stocking rate of 3.5 cows per ha on farmlets in Studies 1 and 2, whilst in Study 3, the stocking intensity was 91 cows/ha.day.

A RPM was used on a daily basis to determine the amount of pasture on offer, and the pasture residue remaining post-grazing. A lack of soil moisture restricted pasture growth in Study 1, resulting in a mean amount of pasture on offer (above 5 cm stubble height) of 911 ± 36 (mean ± s.e.) kg DM/ha and consequently, all farmlets were heavily grazed which eliminated the need to mulch post-grazing. In Studies 2 and 3 there was 1986 ± 91 and 1445 ± 105 (mean ± s.e.) kg DM/ha of pasture on offer, respectively.

9.2.5 Statistical Analysis
The GLM was used to test for the presence of significant differences between treatments in all studies and interactions between protein and energy supply in Study 3 and differences between individual means tested by least significant difference (l.s.d). In Study 3, milk and milk component yields obtained in the standardisation period, were used as a covariate factor to adjust the respective means in the experimental period, and in conjunction with intake levels, were used as covariates in the analysis of variance used to test for differences between treatments.

9.3 Results
9.3.1 Kikuyu Quality
The quality of the kikuyu pasture during each study is shown in Table 9.3. The mean OMD of daily pasture samples obtained in Study 3 (0.733 ± 0.01 (mean ± s.e.)) was significantly higher (P<0.01) than from the daily farmlet samples in Studies 1 and 2 (0.645 ± 0.01 (mean ± s.e.)). The CP, ADF, NDF and ash content of pasture were not significantly different between studies with means of 205 ± 3, 239 ± 2, 615 ± 8 and 79
± 1 (mean ± s.e.) g/kg DM, respectively.

Table 9.3 Mean nutrient content (g/kg DM unless otherwise stated) of pluck samples of kikuyu pastures in Studies 1, 2 and 3. Significant differences (P<0.01) between studies are indicated by differing superscripts.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Study 1 Mar-Apr 1993</th>
<th>Study 2 May 1993</th>
<th>Study 3 Feb 1994</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>201.4</td>
<td>203.2</td>
<td>207.5</td>
</tr>
<tr>
<td>OMD</td>
<td>0.652*</td>
<td>0.644*</td>
<td>0.734b</td>
</tr>
<tr>
<td>NDF</td>
<td>610.7</td>
<td>633.8</td>
<td>602.5</td>
</tr>
<tr>
<td>ADF</td>
<td>242.9</td>
<td>243.5</td>
<td>230.9</td>
</tr>
<tr>
<td>Ca</td>
<td>3.13*</td>
<td>4.96b</td>
<td>3.05*</td>
</tr>
<tr>
<td>P</td>
<td>2.70e</td>
<td>2.38*</td>
<td>3.08b</td>
</tr>
<tr>
<td>K</td>
<td>38.20</td>
<td>31.12</td>
<td>30.72</td>
</tr>
<tr>
<td>Na</td>
<td>0.44*</td>
<td>0.41*</td>
<td>0.15b</td>
</tr>
<tr>
<td>Mg</td>
<td>2.67*</td>
<td>3.42b</td>
<td>2.24*</td>
</tr>
<tr>
<td>ash</td>
<td>84.3</td>
<td>78.0</td>
<td>80.0</td>
</tr>
</tbody>
</table>

The Ca and Mg content of samples taken during Study 2 were significantly higher (P<0.01) than in Studies 1 and 3 (4.96 ± 0.13 v 3.09 ± 0.13 and 3.42 ± 0.09 v 2.45 ± 0.12 (mean ± s.e.) g/kg DM, respectively). In Study 3, the P content was significantly higher (P<0.01) than in Study 2, and the Na content significantly lower (P<0.01) than in Study 1 or 2. Pastures in Study 1 had significantly higher (P<0.01) WSC concentrations than those in Study 2.

9.3.2 Study 1
Milk yield and composition from Study 1 are shown in Table 9.4. The highest response to energy supplementation was 1.4 L milk /cow.day per kg (as fed) at 3 kg barley fed. Unsupplemented cows had the highest milk fat, but the lowest milk lactose content. There was no significant difference in liveweight change between treatment groups with a mean increase in liveweight of 7.5 ± 1.3 (mean ± s.e.) kg, over the 45 day experimental period.
Table 9.4 Mean milk production and composition from cows in Study 1.

<table>
<thead>
<tr>
<th>Concentrate (kg as fed/cow.day)</th>
<th>Milk production (L/cow.day)</th>
<th>Milk composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fat</td>
</tr>
<tr>
<td>0</td>
<td>14.2</td>
<td>3.77</td>
</tr>
<tr>
<td>3</td>
<td>18.3</td>
<td>3.51</td>
</tr>
<tr>
<td>6</td>
<td>18.0</td>
<td>3.26</td>
</tr>
<tr>
<td>l.s.d. (P&lt;0.05)</td>
<td>1.0</td>
<td>0.34</td>
</tr>
</tbody>
</table>

9.3.3 Study 2

The production responses to supplementation in Study 2 (see Table 9.5) were similar to Study 1, but cows were in a later stage of lactation.

Table 9.5 Mean milk production and composition, liveweight change and pasture intake estimated by alkane technique in Study 2.

<table>
<thead>
<tr>
<th>Concentrate (kg as fed/cow.day)</th>
<th>Milk prod. (L/cow.day)</th>
<th>Milk composition (%)</th>
<th>Liveweight change (kg/cow)</th>
<th>Pasture intake (kg DM/cow.day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fat</td>
<td>Protein</td>
<td>Lactose</td>
</tr>
<tr>
<td>0</td>
<td>12.5</td>
<td>3.93</td>
<td>3.17</td>
<td>4.74</td>
</tr>
<tr>
<td>3</td>
<td>18.5</td>
<td>3.89</td>
<td>3.38</td>
<td>4.95</td>
</tr>
<tr>
<td>6</td>
<td>17.4</td>
<td>3.82</td>
<td>3.34</td>
<td>4.97</td>
</tr>
<tr>
<td>l.s.d. (P&lt;0.05)</td>
<td>2.1</td>
<td>n.s.</td>
<td>n.s.</td>
<td>0.19</td>
</tr>
</tbody>
</table>

There were some significant deviations in liveweight, with cows fed 3 kg barley having significantly greater (P<0.05) liveweight loss than those not supplemented. Pasture intake declined significantly (P<0.05) as the rate of supplement increased from 0 to 6 kg/cow.day. The substitution rate at the 3 kg rate of feeding was similar to 6 kg (0.59 v 0.63 kg DM pasture/kg DM concentrate fed, respectively).

9.3.4 Study 3

After covariate adjustment for milk production within the standardisation period, the control group produced significantly less (P<0.001) milk (17.2 L/cow.day), milk protein (2.90%), PU (5.90 mmol/L) and β-OHB (0.525 mmol/L) than the treatment groups.
The concentration of ME, but not FTCM, fed in the concentrate had significant influence (P<0.001) on milk yield and milk fat and protein concentrations (Figure 9.1).

Figure 9.1 Mean (a) milk production (L/cow.day), (b) milk fat (%) and (c) milk protein (%) in response to increasing concentrations of metabolisable energy (ME) (MJ/cow.day) fed in concentrates in Study 3.

Plasma glucose (P<0.05), β-OHB (P<0.01) and NEFA (P<0.05) concentrations were significantly affected by the proportion of FTCM in the concentrate (see Figure 9.2).

Figure 9.2 Mean plasma (a) glucose (b) β-hydroxybutyrate (B-OHB) and (c) non-esterified fatty acid (NEFA) concentrations (mmol/L) in response to increasing proportions of formaldehyde-treated canola meal (FTCM) fed in concentrates to cows in Study 3.
PU concentrations were significantly influenced (P<0.001) by the concentrations of ME and FTCM in the concentrate and the interaction (P<0.01) between them (see Figure 9.3).

Figure 9.3 The relationship between plasma urea concentrations (mmol/L) and the proportion of formaldehyde-treated canola meal (FTCM) fed in the concentrate at low, medium and high concentrations of energy supplementation in cows in Study 3.

A significant linear relationship (P<0.001, r²=0.44) was found between PU and MU as follows;

\[ MU (\text{mmol/L}) = 0.167 + 0.272 \times PU (\text{mmol/L}) \]

The liveweight change over the experimental period did not differ significantly between treatment groups with a mean of -5.1 ± 1.1 (mean ± s.e.) kg/cow.

### 9.4 Discussion

Unsupplemented cows of average genetic merit, grazing well-managed kikuyu pastures, produced 17.3 L/cow.day at 3 to 4 months, 14.2 L/cow.day at 5 to 6 months and 12.5 L/cow.day at 7 months of lactation, without detectable liveweight change in Studies 1 and 3, or at lower liveweight loss in Study 2, than supplemented animals. This production is substantially higher than the 9.1 L/cow.day reported by Olney and Albertsen (1984) for Friesian cows at 6 to 7 months of lactation under conditions of continuous grazing of kikuyu, or the 14.1 L/cow.day in early summer and 10.5 and 9.2
L/cow.day in the late summer and autumn, respectively, reported by Henning et al. (1995) under rotational grazing. Minson and Kondos (1969) and Hamilton et al. (1992) reported higher yields (16.5 and 14.2 L/cow.day, respectively), when cows were strip-grazed on N-fertilised kikuyu pastures. However, the genetic merit of the cows used in these 2 studies was not reported, nor was liveweight change by Minson and Kondos (1969). Hamilton et al. (1992) reported a liveweight loss of 0.10 kg/cow.day in unsupplemented cows compared to a significant liveweight gain of supplemented cows 2 months into lactation.

At the completion of Study 1, it was postulated that the lack of response to supplementation above the 3 kg/cow.day rate may have been due to an inadequate supply of UDP (as predicted by the computer program - Camdairy (Hulme et al. 1986)). However, inclusion of a low rate of formaldehyde-treated sunflower meal into the ration in Study 2 still did not enhance the milk production of cows fed 6 kg of concentrate above that of those fed 3 kg concentrate. A lack of milk production response to increased UDP supplementation at relatively low rates of supplementation, has also been reported by Kaiser and Ashwood (1981) and at various rates of supplementation by Davison et al. (1990).

The response to feeding 3kg concentrate in Study 3 (0.59 L milk/kg concentrate fed) was considerably lower than that obtained in Studies 1 and 2 (1.4 and 2.0 L milk/kg concentrate fed, respectively). This may be partially attributable to the higher digestibility of pastures in Study 3, as well as a balanced mineral intake resulting in increased rates of substitution (Mayne and Wright 1988).

Milk yields were influenced by ME, not FTCM, fed in the concentrate indicating that energy is the factor limiting milk production from kikuyu pastures, even at high rates of supplementation.

At a metabolic level, PU concentrations reflect the extent to which the diet is balanced with nitrogenous compounds to meet the requirements of the rumen microbes (Orskov 1982). In the present study, PU and MU concentrations were correlated, but not as
closely as observations by Ide et al. (1966), Oltner and Wiktorsson (1983) and Gustafsson and Palmquist (1993). Some of the variation observed in this trial may be partially attributable to the time taken to collect blood from all of the animals (approximately 2 - 3 h).

At each rate of energy supplementation, PU concentrations generally increased as the proportion of FTCM fed in the concentrate increased, indicating increased wastage of dietary protein. The slow rate of increase of PU at the low rate of energy supplementation in response to increasing FTCM compared to the medium and high rates, indicates most of the protein supplied at the low rate of feeding could be utilised by the animal.

In contrast to many studies, plasma glucose, β-OHB and NEFA were not influenced by the concentration of ME, but rather by the proportion of FTCM fed in the concentrate. Glucose is generally considered a poor indicator of energy status (Kaupinnen 1983) and rising concentrations in response to increasing proportions of FTCM in the concentrate, may reflect the fate of excess glucogenic amino acids absorbed by the small intestine and therefore be more of an indication of dietary composition, than nutritional status.

Similarly, Erfle et al. (1974) and Fisher et al. (1975) found β-OHB to have little or no use as an indicator of nutritional status and Miettinen and Huhtanen (1989) suggest acetoacetate may be a more appropriate ketone indicator of energy balance as it is less influenced by feeding and its production is more sensitive to energy deficiency (Kauppinen 1983). Nachtomi et al. (1991) found that cows on a high-CP diet had elevated concentrations of β-OHB and suggested it is the result of low concentrations of glucose in the blood, however no negative relationship was observed in Study 3.

At high rates of FTCM in the diet, excess ketogenic amino acids absorbed from the small intestine may have resulted in elevated plasma β-OHB concentrations as a result of oxidative degradation in the liver. Further more, Amaral-Phillips et al. (1993) suggest that increased β-OHB concentrations may also be due to an increase in butyrate production in the rumen which is then converted to β-OHB in the ruminal wall and liver.
Preston and Leng (1987) report that feeds containing high sugar concentration enhance butyrate production in the rumen. Canola meal typically contains 3 times the amount of WSC than barley (Jarrige 1989) and it is possible that higher concentrations of butyrate may be produced in the rumen of cows receiving a higher proportion of FTCM in the concentrate.

Plasma NEFA concentrations have been found to be negatively correlated to energy supply in lactating cows (Fisher et al., 1975; Miettinen and Huhtanen, 1989; Nachtomi et al., 1991). In this study, the ME content of the diet did not significantly affect NEFA concentrations, however concentrations were significantly lower in groups receiving 20% canola meal replacement than other treatment groups.

The absence of a milk production response above the 3 kg rate of feeding in studies 1 and 2, indicated a problem in concentrate feeding at this rate. In Study 2, when intake was measured by alkane techniques, it was found that the in vivo digestibility of the whole diet did not differ significantly between groups fed 0, 3 or 6 kg of concentrate (see Chapter 8). Similarly, Arriaga-Jordan and Holmes (1986) found no difference in the whole diet digestibility of dairy cows fed either perennial ryegrass alone or supplemented with 4-5 kg cereal-based concentrates and in fact observed that supplementation resulted in a decline in cell-wall digestibility. This is contrary to expectations if it is assumed that when the ratio of concentrate to herbage changes, the individual digestibilities of the concentrate and of the herbage remain constant.

Different sources of energy supplementation (eg. sugar v starch v fat v fibre) have been shown to disrupt normal rumen function by depressing rumen degradation of the fibre fraction of the forage (Doyle 1987). In this regard, a decline in ruminal pH, normally associated with cereal-grain supplementation, results in a reduction in cellulolytic activity (Mould and Orskov 1983). The decline in ruminal pH may be reduced with the addition of buffers to the concentrate mix. However, the use of buffers (Mould and Orskov 1983) or the absence of a decline in pH (Henning et al. 1980) does not totally prevent a decline in cell-wall degradability. Therefore, other factors, such as competition for essential nutrients amongst cellulolytic and amylolytic bacteria (Hoover
1986) may be responsible for a decline in the activity of cell-wall digesting bacteria (El-Shazly et al. 1961). If this is true then the results of studies showing decreased response to concentrate feeding attributable to substitution (of concentrate for pasture) may in fact be due to a reduction in forage digestibility due to intake of concentrate.

The interpretation of the results from Studies 1 and 2 may be limited to a degree due to what could be interpreted as a lack of replication of treatment groups. However, the design of Studies 1 and 2 blocked the cows in each group and paddocks were randomly allocated to each group. It is possible that production responses included possible plot effects, although these would probably be minimal as pasture nutrient composition and digestibility were very similar between groups. Similarly, intake responses may have been influenced by plot effects, but treatment (particularly the effects of substitution) effects would have been more likely.

With individual cows considered as replicates for each treatment in all studies it is also possible that some production responses were influenced by other animals in the group (eg. through aggressive grazing behaviour.). This is less likely to cause an influence in Study 3 as cows in all treatment groups grazed together.

9.5 Conclusion
Milk production from well-managed kikuyu can be maintained at 15 L/cow.day. Production above this level, appears to be related to energy input, as substantial responses are achieved when cereal-based concentrates are fed up to the 3kg rate of feeding. Responses to UDP are not apparent until high rates of supplemental energy are fed. Imbalances of energy and protein are indicated by elevated concentrations of PU, which suggest excess RDP in rumen.
Chapter 10

The effect of barley supplementation on the rumen degradability of dietary components and rumen pH, and the effect of buffer inclusion, in steers grazing kikuyu pastures

10.1 Introduction

In Chapter 9, low or even zero milk production responses were observed at medium-high rates of energy supplementation when cows of average genetic merit grazed well-managed kikuyu grass pastures. A zero response appears to reflect a substitution rate of 1:1 of grain for pasture. Evidence provided by the alkane technique in Chapter 8 indicated that the lower than expected response to barley supplementation of cows grazing kikuyu may also be the result of a substantial reduction in forage digestibility. Similar results have been found in cows grazing ryegrass pasture and fed various rates of cereal-based pellets (Arriaga-Jordan and Holmes 1986).

The adverse effect of CHO-based concentrates on the digestibility of forage component of the diet may be due to a decline in rumen pH. In this regard, buffers have been used to prevent the decline of pH concentrations in the rumen associated with feeding concentrates (Kellaway and Porta 1993). However, the use of buffers to reduce pH (Mould and Orskov 1983; Meissner et al. 1991) or even the absence of a decline in pH (Henning et al. 1980) may not totally prevent a decline in cell-wall degradability. Therefore, other factors may be contributing to a decline in forage digestibility, and some workers have suggested a possible shift in microbe nutrient source preference from fibre to carbohydrates, when these are available.

The aim of this study was to determine the effect of feeding barley-based supplements, with and without buffer, on the rumen digestibility of individual dietary components (kikuyu and supplement).
10.2 Materials and Methods

The trial was conducted during November/December 1995 using 6 rumen-cannulated cross-bred Hereford steers with a liveweight of 317 ± 12 (mean ± s.e.) kg.

10.2.1 Preliminary Period

Steers grazed kikuyu grass pasture and were individually fed barley at a rate of 1 kg/steer.day for 2 weeks to accustom animals to stall-feeding.

10.2.2 Experimental Design and Treatments

Six steers were fed 3 treatment diets (Table 10.1) in a three period, crossover design.

Table 10.1 Amount of barley (kg/steer.day) and buffer inclusion in the diets of steers in each treatment.

<table>
<thead>
<tr>
<th>Treatment diet</th>
<th>Barley (kg (as fed)/steer.day)</th>
<th>Buffer¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>2.5</td>
<td>+</td>
</tr>
<tr>
<td>C</td>
<td>2.5</td>
<td>-</td>
</tr>
</tbody>
</table>

¹ Buffer comprised of 15 g sodium bicarbonate and 8 g magnesium oxide per kg of concentrate fed in the diet (Kellaway and Porta 1993).

Steers were randomly allocated into 1 of the 3 treatment groups (2 steers per treatment) during Experimental Period I, and then allocated to a different treatment in Experimental Periods II and III, as outlined in Table 10.2.

Table 10.2 Allocation of treatment diets (A, B or C) to individual steers in Experimental Periods I, II and III.

<table>
<thead>
<tr>
<th>Steer Identification</th>
<th>Experimental Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
</tr>
<tr>
<td>4</td>
<td>B</td>
</tr>
<tr>
<td>6</td>
<td>C</td>
</tr>
<tr>
<td>13</td>
<td>A</td>
</tr>
<tr>
<td>14</td>
<td>B</td>
</tr>
<tr>
<td>16</td>
<td>C</td>
</tr>
</tbody>
</table>
Each period consisted of a 7 day “wash-out” time to allow adjustment to next treatment and a 7 day measurement time.

10.2.3 Feeding of Steers
Throughout the trial, the treatment groups grazed together on kikuyu pastures offered _ad lib_, and were managed as described in Chapter 3. During the adjustment period, the feeding rate was increased by 0.5 kg every 2 days until the desired rate was reached. All steers were removed from pasture twice daily (8am and 4pm) and placed in individual stalls where those receiving supplementation were fed to reflect the common method of supplementary feeding of dairy cows. The mean chemical composition of the kikuyu and barley offered to the steers is shown in Table 10.3.

<table>
<thead>
<tr>
<th>Nutrient (g/kg DM)</th>
<th>Kikuyu</th>
<th>Barley</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>201 (6)</td>
<td>94 (3)</td>
</tr>
<tr>
<td>ADF</td>
<td>234 (4)</td>
<td>25 (12)</td>
</tr>
<tr>
<td>NDF</td>
<td>588 (12)</td>
<td>229 (25)</td>
</tr>
<tr>
<td>P</td>
<td>2.6 (0.1)</td>
<td>3.2 (0.1)</td>
</tr>
<tr>
<td>K</td>
<td>34.5 (2.3)</td>
<td>3.0 (0.2)</td>
</tr>
<tr>
<td>Ca</td>
<td>2.8 (0.1)</td>
<td>0.3 (0.03)</td>
</tr>
<tr>
<td>Mg</td>
<td>2.8 (0.1)</td>
<td>1.4 (0.03)</td>
</tr>
<tr>
<td>Na</td>
<td>0.4 (0.1)</td>
<td>0.1 (0.05)</td>
</tr>
</tbody>
</table>

10.2.4 Steer Measurements and Sampling
10.2.4.1 Feed Intake and Digestibility Estimation using Alkanes
A CRD containing C₃₂ and C₃₆ alkanes (assumed release rate of 365 mg/day of both C₃₂ and C₃₆ (K. Ellis, personal communication)) was placed directly in the rumen of each steer at the beginning of each adjustment period. Herbage, concentrate and faecal samples were collected daily and the alkane content and intake estimates were obtained for each steer within each treatment were carried out as described in Chapter 3. The mean alkane concentrations found in kikuyu are outlined in Table 10.4.
Table 10.4 Mean (s.e. in brackets) alkane content (mg/kg DM) of kikuyu on offer and concentrate fed to steers.

<table>
<thead>
<tr>
<th>Alkane chain length</th>
<th>C_{21}</th>
<th>C_{22}</th>
<th>C_{27}</th>
<th>C_{28}</th>
<th>C_{29}</th>
<th>C_{30}</th>
<th>C_{31}</th>
<th>C_{32}</th>
<th>C_{33}</th>
<th>C_{35}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kikuyu</td>
<td>9.9</td>
<td>•</td>
<td>18.5</td>
<td>•</td>
<td>27.3</td>
<td>7.9</td>
<td>194.0</td>
<td>10.6</td>
<td>269.7</td>
<td>122.7</td>
</tr>
<tr>
<td></td>
<td>(0.82)</td>
<td>•</td>
<td>(0.97)</td>
<td>•</td>
<td>(1.25)</td>
<td>(0.47)</td>
<td>(9.9)</td>
<td>(0.78)</td>
<td>(22.0)</td>
<td>(14.6)</td>
</tr>
<tr>
<td>Concentrate</td>
<td>2.3</td>
<td>•</td>
<td>2.4</td>
<td>•</td>
<td>6.5</td>
<td>6.63</td>
<td>3.33</td>
<td>•</td>
<td>3.33</td>
<td>•</td>
</tr>
<tr>
<td></td>
<td>(0.13)</td>
<td>•</td>
<td>(0.24)</td>
<td>•</td>
<td>(0.50)</td>
<td>(0.81)</td>
<td>(0.65)</td>
<td>•</td>
<td>(0.65)</td>
<td>•</td>
</tr>
</tbody>
</table>

Note: C_{34} concentrations are not included as it was used as an internal standard in analysis
* Concentrations of alkane below detection (< 2 mg/kg DM)

Estimates of intake were determined using the C_{32}/C_{31} alkane pair, as these were found to be the most accurate in Chapter 7.

10.2.4.2 In sacco Degradation of Feeds

The rate of degradation of DM in both fresh kikuyu and barley, were determined for each steer using the in sacco technique outlined below.

Procedure

In sacco digestibility was determined using the technique described by Orskov et al. (1980) and Lindberg (1985) with modifications adopted by Opatpanakit (1994). Fresh kikuyu cut above the 5 cm stubble height was obtained prior to the beginning of the experiment, cut into 1-2 cm lengths and stored at -20°C. Nylon bags were prepared on the evening prior to placement in the rumen. Samples of approximately 5-6 g of frozen kikuyu were weighed into the bags (size: 80 x 125 mm; pore size: 37 μm; sewed with polyester thread and stitches sealed with silicone rubber). This was carried out in batches to minimise thawing of the kikuyu samples and intermittent samples were retained for DM analysis. Similarly, samples of approximately 4 g of barley were weighed into the bags. There were 2 bags of each sample per time per animal, with kikuyu samples being incubated for 0, 2, 4, 8, 12, 24, 48 and 72 h and for barley for 0, 2, 4, 8, 12, 24 and 48 h after initial feeding.

The bags were tied with small plastic cable ties at the open end and connected to a perspex plate (size: 50 x 40 x 7 mm). Then the bags were attached to 50 cm of nylon
string which allowed movement of bags in the rumen. The perspex plates were weighted with a steel nut and bolt (weight: approximately 200 g) to keep the bags down in the ventral sac of the rumen. Immediately after preparation, all bags (kikuyu and barley) and apparatus were stored at -20°C until the following morning.

The bags were suspended in the rumen of each steer just prior to morning feeding. After removal, bags were dipped in 90 % ethanol to cease microbial activity and stored at -20°C. When all bags had been removed, they were washed with cold water in a washing machine for approximately 8 and 16 minutes for grain and forage bags, respectively. The bags were then dried at 60°C for 48 h, weighed and stored for subsequent analysis.

Calculation
The rate of disappearance of DM from nylon bags was fitted to the non-linear model described by Orskov and McDonald (1979) (Equation 17), using the NEWAY computer programme (Rowett Research Institute, Aberdeen;UK)

\[ p = a + b(1 - e^{-ct}) \]  
Equation 17

where \( p \) is effective degradability,
\( a \) is the fast degradable and soluble fraction,
\( b \) is the progressively degradable fraction,
\( c \) is the degradation rate,
\( t \) is incubation time.

10.2.4.3 Rumen pH
On days 1-3 of each Experimental period, rumen liquor samples were obtained directly via the rumen cannula 3 h after the morning feed of barley and immediately analysed for pH. On day 5 of the Experimental period, rumen liquor samples were obtained prior to morning feeding, then 2, 4, 6 and 8 h post-feeding and immediately analysed for pH.

10.2.4.4 Digesta Rates of Passage
Rates of passage were determined using chromium (Cr) - mordanted fibre and cobalt (Co) - EDTA as particulate and liquid markers, respectively, using the methods of Uden
et al. 1980 (as modified by Opatpanakit 1994).

Prior to morning feeding on day 1 of each Experimental period, steers were dosed via the cannula with Cr-mordanted fibre (36 g/steer with the exception of Experimental Period II where dose rate was 35 g/steer and steer 16 received no fibre) and a pulse dose of Co-EDTA (10 g/steer).

Rumen fluid samples were collected from each steer 3, 6, 9, 24, 28 and 32 h after dosing, centrifuged, the liquid phase removed and stored at -20°C in preparation for Co analysis. Faecal samples were obtained (from defacations if timely or rectal grab samples) at 3, 6, 9, 12, 24, 28, 32, 48, 52, 56, 72, 80, 96, 120 and 144 h post-dosing and dried at 60°C and ground through a 1 mm sieve in preparation for Cr analysis.

The rate of liquid passage from the rumen was calculated by linear regression of the natural logarithm of Co concentrations in the rumen fluid against time after dosing. The rate of particle passage was estimated by linear regression of the natural logarithm of Cr concentrations in the faeces (for points following the peak concentration) against time after dosing.

Mean retention time (MRT) in total digestive tract was calculated using the trapezoidal rule as described by Faichney (1993) as follows;

$$MRT = \frac{\sum_{i=1}^{n} c_i \cdot t_i \cdot \Delta T_i}{\sum_{i=1}^{n} c_i \cdot \Delta T_i}$$

where \( C_i \) is the Cr concentration at time \( T_i \) after dosing so that \( c_i = (C_i + C_{i+1})/2 \), \( t_i = (T_i + T_{i+1})/2 \) and \( \Delta T_i = (T_i - T_{i+1}) \).

10.2.5 Statistical Analysis
Estimated intake, whole diet DMD, effective degradability data, liquid and particulate rates of passage and mean retention times were fitted by models given in Jones and
Kenwood (1990). The models included animal, period, treatment and carryover effects.

The two-hourly rumen pH data were fitted to a similar model which included terms for treatment, period and carry-over effects of treatments into the next period. The model also included linear time and treatment x time terms as well as random animal effects and a cubic spline smoothed response to time which was allowed to change for each animal and each treatment. Likelihood ratio tests were used to decide on the statistical significance of the random parts of the model while approximate F-ratios were used to test the significance of the fixed effects.

The daily rumen pH data were analysed using a “split-plot in time” analysis of variance. This approach was used because the structure of the experiment provides two levels of variation; variation between steers and variation within steers. Direct treatment, period and carryover effects were tested at the between steer level while day effects and two-way interactions were tested at the within steer level.

10.3 Results

10.3.1 Intake and Diet Digestibility

The mean (± s.e.) kikuyu intake and whole diet DMD estimated using the alkane technique are shown in Table 10.5. Steers grazing kikuyu alone consumed significantly more (P<0.001) herbage DM than those fed concentrates. Between the concentrate groups, steers receiving buffer had significantly higher (P<0.001) herbage intake than those fed barley without buffer. The whole diet DMD of steers fed kikuyu alone was significantly higher (P<0.05) than those fed concentrates (with or without buffer). The mean calculated rate of substitution of barley for kikuyu was over 50% higher in steers which had no buffer in their diet, however, there was no difference in whole diet DMD estimations between these 2 groups which indicates a fall in forage digestibility of the kikuyu component of the diet assuming the digestibility of the supplements did not differ.
Table 10.5  The mean (s.e. in brackets) intake of steers (kg DM/day) and whole diet dry matter digestibility (DMD) of kikuyu estimated using alkanes, and calculated rate of substitution (kg DM kikuyu/kg DM barley).

<table>
<thead>
<tr>
<th>Rate of Barley Fed (kg (as fed)/steer.day)</th>
<th>Intake (kg DM/steer.day)</th>
<th>Rate of Substitution (kg DM kikuyu/kg DM barley)</th>
<th>Mean Diet DMD (g/kg DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kikuyu</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4.32</td>
<td>4.32</td>
<td>0.420</td>
</tr>
<tr>
<td>(as fed)/steer.day</td>
<td>(0.1)</td>
<td></td>
<td>(0.03)</td>
</tr>
<tr>
<td>2.5</td>
<td>3.74</td>
<td>5.97</td>
<td>0.310</td>
</tr>
<tr>
<td>(with buffer)</td>
<td>(0.1)</td>
<td></td>
<td>(0.03)</td>
</tr>
<tr>
<td>2.5</td>
<td>3.42</td>
<td>5.65</td>
<td>0.320</td>
</tr>
<tr>
<td>(without buffer)</td>
<td>(0.1)</td>
<td></td>
<td>(0.03)</td>
</tr>
</tbody>
</table>

10.3.2 *In sacco* Degradation of Feeds

10.3.2.1 Kikuyu Degradation

The mean kikuyu *in sacco* DM degradation was determined assuming a rumen outflow rate of 3% per hour (as determined in section 10.3.2.2), and means for each treatment are outlined in Table 10.6.

Table 10.6. *In sacco* dry matter degradation (DMD) of kikuyu according to the equation; 
\[ p = a + b \left(1-e^{-ct}\right) \] (where; \(a = \) readily degradable DM; \(b = \) slowly degradable DM; \(c = \) rate of digestion of \(b\) and \(t = \) time of degradation ) in steers grazing kikuyu alone (Treatment A), or being supplementary fed barley with (Treatment B), or without (Treatment C) buffer. Differing superscripts within rows indicate a significant difference between treatments (P<0.05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Treatment</th>
<th>Standard Error of the Difference</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMD (p)</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>0.542</td>
<td>0.528</td>
<td>0.498</td>
<td>0.02</td>
</tr>
<tr>
<td>a</td>
<td>25.30</td>
<td>25.30</td>
<td>n.a.</td>
</tr>
<tr>
<td>b</td>
<td>65.24</td>
<td>67.90</td>
<td>3.46</td>
</tr>
<tr>
<td>c</td>
<td>0.0434</td>
<td>0.0313</td>
<td>0.005</td>
</tr>
<tr>
<td>lag</td>
<td>0.133</td>
<td>1.115</td>
<td>-1.021</td>
</tr>
</tbody>
</table>

n.a. Not applicable ; n. s. Not significant

The *in sacco* DM degradability of kikuyu in steers fed concentrate without buffer was significantly lower (P<0.01) than other treatments. A similar (but not significant) trend was observed in the potentially degradable insoluble fraction (b). The soluble fraction
(a) of kikuyu did not vary between treatments as the washing loss data was combined and the mean value used for each treatment period. The fractional rate of forage digestion (c) or lag time did not differ between the treatments.

10.3.2.2 Barley Degradation

The mean *in sacco* DM degradation for barley is outlined in Table 10.7.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Standard Error of the Difference</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>DMD (p)</td>
<td>0.638</td>
<td>0.639</td>
</tr>
<tr>
<td>b</td>
<td>58.34</td>
<td>60.99</td>
</tr>
<tr>
<td>c</td>
<td>0.09*</td>
<td>0.12b</td>
</tr>
<tr>
<td>lag</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

There was no significant difference between treatment groups for *in sacco* DMD, soluble fraction (a), potentially degradable insoluble fraction (b) or lag time of barley. The treatment group receiving buffer had significantly higher (p<0.001) barley DM degradation rate (c) than other treatments. There was no lag time for the initiation of barley degradation observed in any treatment group.

10.3.3 Rumen pH

The analysis of variance indicated significant (P<0.001) treatment and day effects on the rumen pH 3 h post-feeding. Therefore, the treatment and day means are presented in Table 10.8.
Table 10.8 The mean (s.e. in brackets) rumen pH concentrations 3 h post-feeding.

<table>
<thead>
<tr>
<th>Rate of Barley Fed (kg/steer.day)</th>
<th>Rumen pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day 1</td>
</tr>
<tr>
<td>0</td>
<td>7.57 (0.17)</td>
</tr>
<tr>
<td>2.5 (with buffer)</td>
<td>6.97 (0.18)</td>
</tr>
<tr>
<td>2.5 (without buffer)</td>
<td>6.33 (0.17)</td>
</tr>
</tbody>
</table>

The results indicate that steers grazing kikuyu alone had significantly higher (P<0.001) rumen pH concentrations than those fed concentrates plus buffer, who in turn had higher rumen pH than those fed concentrates without buffer.

Dropping non-significant terms sequentially from the original model led to a final model which consisted of period, treatment and linear time effects as well as random animal effects and a smoothed response to time. Effectively, this model fitted parallel treatment curves to the response of rumen pH to time from feeding, and therefore treatment did not affect the rate of change of rumen pH (Figure 10.1). Differences in rumen pH concentrations behaved similarly to the 3 h results above.

Figure 10.1 Fitted and observed changes in rumen pH from 0 to 8 h post-feeding.
10.3.4 Digesta Rates of Passage

There was no significant effect of treatment on the rate of liquid passage out of the rumen, particulate rate of passage into the faeces, MRT of liquid in the rumen or the MRT of particulates in the digestive tract. The treatment means of these variates are presented in Table 10.9.

Table 10.9 The mean (s.e. in brackets) liquid rate of passage out of the rumen, particulate rate of passage into faeces (% per hour), liquid retention time in the rumen and particulate retention time (h) in the digestive tract for each treatment.

<table>
<thead>
<tr>
<th>Rate of Barley Fed (kg/steer.day)</th>
<th>Rate of Passage (% per h)</th>
<th>Mean Retention Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>liquid</td>
<td>particulate</td>
</tr>
<tr>
<td>0</td>
<td>9.8</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>(0.4)</td>
<td>(0.2)</td>
</tr>
<tr>
<td>2.5 (with buffer)</td>
<td>8.3</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>(0.4)</td>
<td>(0.2)</td>
</tr>
<tr>
<td>2.5 (without buffer)</td>
<td>8.6</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>(0.4)</td>
<td>(0.2)</td>
</tr>
</tbody>
</table>

10.4 Discussion

The inclusion of barley in the diet of steers grazing kikuyu depressed herbage intake, although total DMI increased. The reduction in herbage DMI was associated with the effects of substitution of concentrate for pasture and an effect on degradability of kikuyu presumably due to reduced rumen pH and an additional factor independent of pH. The inclusion of buffers in the diet restricted the extent of substitution, and appeared to improve the effective degradability of kikuyu. However, whole diet DMD was not improved, indicating that factors other than low rumen pH may affect diet degradation.

The rates of substitution observed in this study were low (mean 0.3) compared to previous studies reported in this thesis on kikuyu (see Chapter 9) and on other pastures (eg. Kellaway and Porta (1993) reported a range of 0.3-0.9), and may be associated with various factors. Firstly, Kellaway and Porta (1993) observed that the rate of substitution tends to be lower on low, compared to high, quality pastures. Kikuyu is considered to
be of low quality and in such cases, concentrates are used more as a “supplement” rather than a “substitute”. When the supplement simply replaces herbage of similar quality, production responses become minimal (Arriaga-Jordan and Holmes 1986).

Secondly, the highest rate of substitution occurred when rumen pH was lowest. Kellaway and Porta (1993) suggested that lowered rumen pH, reduces the number of cellulolytic bacteria and therefore the extent of fibre digestion. This in turn increases the retention time (Scharp 1983) and restricts DMI. In this study, DMI was reduced in response to low rumen pH, however, no differences were observed in the MRT of particulate material in the digestive tract.

Most studies have found that a substantial reduction in fibre digestion occurs if rumen pH falls below 6.0, (Terry et al. 1969; Mould and Orskov 1983; Hoover 1986; Istasse et al. 1986). However, rumen pH concentrations in this study ranged from 6.0 to 7.9. The highest values were observed in unsupplemented steers and were much higher than expected. The high pH levels are unlikely to be due to slow sampling time, since pH measurements were conducted immediately following sample collection, and random calibration checks were undertaken on samples to validate pH concentrations. The high rumen pH levels are probably the result of the high fibre, and low WSC content of kikuyu. Since WSC is rapidly degraded in the rumen, the low dietary levels of WSC in kikuyu would mean that the reductions in rumen pH associated with WSC fermentation would be small. There appears to be no published data reporting rumen pH concentrations of cows specifically grazing kikuyu, however, pH in cows grazing grass/clover mixes ranged from 5.7 (Holden et al. 1994) to 6.8 (Mackle et al. 1996), which are well below the concentrations reported in this study for steers grazing kikuyu alone.

An inherent decline in rumen pH post-feeding was observed in all steers, regardless of the rate of concentrate inclusion. It is difficult to explain the 8-hour rumen pH changes of steers which received no concentrates, although the decline was expected in the remaining steers. Post-feeding reductions in rumen pH of cows fed pasture alone have also been observed by Mackle et al. (1996) and Holden et al. (1994) where pH declined
by up to 0.8 and 0.6 units, respectively. It is possible that these pH changes could be related to grazing activity throughout the day, since all steers were removed from the pasture for sampling and may have grazed intensely on their return to the pasture. Furthermore, the changes may have been related to the increasing WSC content of the pasture as the day progressed.

The inclusion of buffers in the diet only partially prevented the decline in rumen pH associated with concentrate feeding. Although recommended rates of buffer inclusion were used (Kellaway and Porta 1993), higher concentrations may be more effective in reducing the decline in rumen pH. An alternative option may be through the inclusion of virginiamycin in the diet to reduce lactic acid production (Clayton et al. 1997). Even so, Mould et al. (1983) observed that when buffers prevented a decline in rumen pH, concentrate feeding still negatively affected fibre digestion. These workers suggest that cellulolytic bacteria may degrade starch in preference to fibre. Similarly, Piwonka et al. (1994) suggests that although pH is a major factor affecting fibre digestion, there may be a shift in the population of cellulolytic microbes when sugars and starches are included in diets. Further evidence is provided by Howard et al. (1982), who found no correlation between any pH variables and rumen digestion of the cell wall. Alternatively, Henning et al. (1980) has postulated that an inhibitory substance (apart from low pH or limiting nutrients) reduces fibre digestion.

The addition of the buffers NaHCO₃ and MgO may have had other effects apart from that on rumen pH. Kikuyu is low in Na and Mg and their inclusion may have caused responses not associated with rumen pH.

The whole diet DMD reported in this study is well below that observed in Chapter 8 for lactating cows. This may be partially associated with the relatively small rumen volume and content of steers compared to lactating cows. Orskov (1994) suggested animals with larger rumen volume and content may experience higher levels of degradability due to increased rates of rumen retention. However, the difference observed in rumen degradation rates is more likely to be related to the fractional turnover rate, which tends to be substantially greater in lactating cows than steers being fed for maintenance.
Assuming that the digestibility of the dietary components was additive, the overall diet DMD would be expected to increase with the addition of cereal grains to a kikuyu-base diet. In contrast, barley supplementation substantially reduced whole diet DMD in this study. Similarly, the addition of starch concentrates did not increase overall diet DMD of lactating cows grazing kikuyu in studies reported in Chapter 8, or ryegrass pastures (Arriaga-Jordan and Holmes 1986). In the latter study, cereal concentrates reduced cellulose digestibility which presumably lowered herbage digestibility, and limited diet digestibility. This evidence indicates that supplementation with concentrates reduces the digestibility of kikuyu herbage.

The use of buffers, in this study, did not prevent a decline in whole diet DMD, although the effective degradability was improved. Meissner et al. (1991) made similar observations in sheep grazing high quality ryegrass, and suggested the reason for this was that rumen pH did not decline below 6.0. Therefore, the observed reduction in cell wall degradation were perhaps due to factors other than a fall in rumen pH. In the same study, the rate of NDF disappearance in kikuyu (and other forages with NDF concentrations <550-600 g/kg DM) was reduced through supplementation. However, in agreement with Vadiveloo and Holmes (1979), van Soest (1967) and Mertens and Ely (1979), poorer quality forages (NDF > 600g/kg DM)) were not adversely affected by supplementation. This may be associated with a high demand for essential nutrients by amylolytic bacteria at the expense of slower growing cellulolytic bacteria. In forages with higher concentrations of NDF, cellulolytic activity is comparatively lower, therefore there is less competition for nutrients between the two types of organisms.

In agreement, El-Shazly et al. (1961) found using in vitro techniques that a depression in cellulolysis with the inclusion of starch in a ration is predominantly due to competition for essential nutrients (in particular N) resulting in a preferential proliferation of amylolytic bacteria. These workers found that the inclusion of urea in the ration, could partly overcome the inhibition of cellulose digestion. However, the kikuyu in this study appeared to contain adequate concentrations of RDP to prevent such competition, and maybe other nutrients (eg. Na, S) were limiting.
Supplements with slower rates of ruminal degradation than that of barley may be more suited to the slowly degrading pastures such as kikuyu. The degradation of starchy supplements varies somewhat depending on type and physical processing. Through observations of rates of gas production (which was determined to be an accurate indicator of VFA production and pH changes), Opatpanakit et al. (1994) ranked the order of gas production to be wheat > triticale, oats > barley > maize > rice, sorghum.

In a latter study, Opatpanakit et al. (1995) observed that NDF degradation in ryegrass and lucerne was inhibited by the more rapidly degrading wheat and barley, and to a lesser extent maize, whereas, slowly degrading sorghum actually enhanced NDF degradation. Therefore, slower degrading rice or sorghum may be more appropriate to supplement cows grazing kikuyu pastures, than the more rapidly fermentable grains such as barley and wheat. In support of this, investigations by L Trevaskis (unpub. data), have found that higher milk production responses from cows grazing kikuyu pastures can be obtained using rice compared to barley and sugar supplements.

Although the degradability of kikuyu in this study was quite low, it is possible that freezing the kikuyu samples prior to placing in the rumen may have caused plant cells to rupture, rendering them more available to microbes to breakdown, thus over-estimating the degradation rate and overall plant digestibility. This has been found to in protein degradability studies when pasture is freeze-dried prior to analysis (Cohen et al. 1995).

The micro environment within bags may be another source of error in the experimental technique. The bags containing kikuyu could be expected to have low starch levels within and immediately surrounding the bag, compared to those containing barley. Therefore, the degradation results may have been influenced by the differing microbial numbers and types within and surrounding the bags.

10.5 Conclusion
The inclusion of buffers in the diet of steers grazing kikuyu, prevented a substantial
decline in rumen pH, but did not completely alleviate the decline. The effective degradability of kikuyu was partially improved by the use of buffers, whilst whole diet DMD was not. Although low rumen pH appears to be the major factor inhibiting kikuyu degradation, other factors still adversely affect degradability of kikuyu when cereal-based concentrates are fed. Slower degrading CHO-based supplements may be required to create a synergistic, rather than inhibitory effect on the degradation of kikuyu pasture.
**Chapter 11**

**Discussion**

11.1 The optimal stage of regrowth at which to graze kikuyu

The 4.5 leaves per tiller stage of regrowth appears to be the time to graze kikuyu in order to optimise forage quality in terms of cow requirements, and utilisation of DM. In addition, the regrowth potential of the plant at this stage is unlikely to be inhibited by a depletion of plant reserves (primarily carbohydrates), as shown indicated by W.J. Fulkerson (personal communication). Following this stage of regrowth, there is a rapid decline in the proportion of high quality leaf and an increase in both stem, and in particular, dead material, and consequently, the quality of kikuyu regrowth markedly decreases. Not only do these changes represent a decline in the quality of pasture on offer, but also indicate DM wastage through leaf senescence. These benefits are comparable to those observed when ryegrass is grazed at the 3 leaf stage of regrowth (Davies 1965; Fulkerson and Slack 1994; Fulkerson and Slack 1995). In both cases changes in herbage quality and growth were related to the morphological progress of regrowth as reflected in leaves per tiller.

The 4.5 leaf stage of regrowth represents the stage at which the nutrient content of kikuyu is most suited to meet the requirements of the lactating cow. The concentration of both CP and K in leaves are too high in young regrowth (approximately 250-300 and 25-30 g/kg DM, respectively) and remain well above requirements for a 600kg cow producing 20L milk/day (150 (NRC 1989) and 12.5 (Beede et al. 1983) g/kg DM, respectively). However, in agreement with the studies reported in Chapter 2, the concentration of both these nutrients declines with leaf age, and overall plant maturity, to more favourable concentrations (150-200 g/kg of CP and 15-20 g/kg DM of K) at the 4.5 leaf stage.

The reduction in CP concentrations in kikuyu with plant maturity would also produce a more favourable carbohydrate to RDP ratio for microbial activity in the rumen (Fulkerson and Trevaskis 1997). Excess dietary protein results in high rumen NH₃ concentrations and substantial amounts of energy are required for subsequent conversion
of NH₃ to urea and its excretion (Blaxter 1962). This may result in reduced milk production and reproductive performance may be reduced (Elrod and Butler 1993). Apart from grazing at the appropriate stage of regrowth, the carbohydrate to protein ratio can be further enhanced by grazing kikuyu in the afternoon, when WSC have peaked and NPN concentrations are minimal, as shown in the present study.

The concentration of Mg tends to rise to the 4.5 leaves per tiller stage of regrowth, but even then still remain only marginal for milk production (NRC 1989). The combination of rising Mg and falling K would be expected to reduce the incidence of hypomagnesaemia, since high K inhibits Mg uptake in the digestive tract of ruminants (Kemp et al. 1961). Similarly, Ca accumulates in kikuyu with maturity. Unfortunately, the presence of substantial amounts of oxalate in kikuyu can bind Ca into insoluble crystals, rendering it largely unavailable to the ruminant (Ward et al. 1979; Blaney et al. 1982). Although the degree to which oxalates reduce Ca availability was not determined in this study, Marais (1990b) speculates that availability is as low as 5% and thus, available Ca in kikuyu would be definitely inadequate to meet the demands of lactating cows, even at the appropriate regrowth stage.

The concentration of Na remains extremely low throughout regrowth, well below that of ryegrass and the requirements of lactating cows. These low Na concentrations would result in acute problems, particularly during times of heat stress when the cow excretes additional Na in sweat and urine (Beede 1988). Unfortunately, the already marginal concentrations of P tend to decline with regrowth as found in previous investigations on kikuyu (Gomide et al. 1969b; Said 1971). Thus, the results of these studies highlight the need to supplement lactating dairy cows grazing kikuyu pastures with Ca, P, Mg and Na, particularly if production is in excess of 20 L milk/cow.day.

The application of N is required to achieve acceptable DM yields of kikuyu. However, if N fertilisation is excessive (> 50 kg N/ha.month), the associated high concentrations of CP (>200 g/kg DM) result in dramatic increases in the concentration of nitrate in the plant, which can cause problems of excess N in the ruminant. Although the concentrations found in this study were below those considered toxic to the cow (Blood
and Radostits 1989), the build up of nitrite, formed during the breakdown of nitrate in the rumen, inhibits microbial function and consequently, the rate of rumen digestion (Marais 1990a; Marais et al. 1988). High rates of N application also tend to increase the proportion of stem to leaf above the 5 cm stubble height. Similar observations have been obtained by Mears and Humphreys (1974) and W. J. Fulkerson (personal communication).

11.2 Nutrient deficiencies of high-quality kikuyu
Apart from the mineral imbalances discussed, there are additional nutrients which would be expected to limit milk production even in well-managed kikuyu pasture in comparison to ryegrass.

Firstly, the substantially lower digestibility (and correspondingly lower ME) limits the utilisation of kikuyu by the ruminant. The high NDF concentrations characteristic of C₄ tropical grass species (Minson 1990), presumably limit the rate of passage through the rumen due to the reduced accessibility of microbes to degradable portions of the plant material. Consequently, this restricts DMI which is the major factor driving milk production. There has been considerable efforts to select C₄ grasses with reduced fibre content using conventional means, although this has not been successful. However, efforts to produce a more digestible kikuyu grass, through the incorporation of the brown mid-rib gene which is linked to genes for lower fibre, are currently being conducted (D. Luckett and A. Kaiser, personal communication).

Secondly, the absolute concentration of WSC, and even total non-structural carbohydrate (including both WSC and starch) to protein ratio, are more favourable for rumen microbial activity in ryegrass than kikuyu. However, some benefit can be gained in the knowledge that WSC concentrations peak in the afternoon as a result of photosynthesis, while NPN concentrations tend to fall. Reduced NPN is related to an increase in protein synthesis associated with greater energy availability in the plant. Therefore, production benefits may be gained in the timing of grazing. Similarly, WSC concentrations are critically low for the silage making process, and delaying harvest until the afternoon
should see concentrations rise by about 4% in DM. This practice has been adopted on farm (W. J. Fulkerson, personal communication), however more research is required in this area to validate this theoretical benefit of time of day on defoliation, on milk production or silage quality.

Thirdly, the CP component of ryegrass comprises a greater proportion of amino acids than kikuyu. Methionine and lysine are considered to be the two most limiting amino acids for milk production in a pasture-based diet (Cole and van Lunen 1994), and the concentrations of these in kikuyu are considerably lower than those found in ryegrass. However, if there is sufficient protein per unit of energy to support the microbial synthesis and activity, (which is the case in N fertilised kikuyu) amino acid supply from microbial protein alone should be sufficient to support milk production of 20 L/cow.day (Oldham 1981). Therefore, at the milk yields reported in these studies, energy is more likely to be limiting milk production than essential amino acids, and this has been confirmed in supplementation studied in this thesis. In this regard, the feeding of amino acids in the rumen protected form, has not been found to be beneficial to cows grazing pasture (L. Trevaskis; unpub. data).

11.3 Measurement of kikuyu intake
In order to determine the “true” responses to supplementation and various grazing management practices, DM intakes were monitored in these studies. The estimation of DMI is inherently difficult for any pasture species grazed by stock, and is even more difficult for kikuyu due to the large stoloniferous mat at the pasture base, which is unavailable to grazing cows (Fulkerson and Slack 1993). The alkane technique (Dove and Mayes 1991 & 1996) has provided a direct and accurate means of determining the DMI of individual cows grazing kikuyu. The most accurate estimates were obtained using the C31/C32 and C33/C32 alkane pairs, with the average difference between estimated and actual intake being 0.29 and -0.33 kg DM/cow.day, respectively.

A major advantage of the use of alkanes to determine DMI is that between animal variation is accounted for within the estimate. Such factors include intrinsic animal

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differences in digestion, health, the effects of supplements and rumen modifiers. When chromic oxide is used as an alternative FO marker, DMD is estimated by *in vitro* techniques which do not take these factors into consideration. A similar problem arose in these studies when the RS technique was used to predict DMI. When *in vitro* estimates of digestibility of the basal kikuyu forage were used, RS predicted a dramatic reduction in DMI of kikuyu as the rate of concentrate in the diet increased. However, this did not actually appear to be the case. When *in vivo* estimates of digestibility were used estimates of DMD (which were approximately 6% higher in this study) improved the accuracy of RS estimations of DMI, when compared to the alkane technique. A second factor compounding the problems of RS estimates, is that digestibility estimates are assumed constant, and the effect of concentrate on forage digestibility is not incorporated. This factor will be discussed in greater detail later in the discussion.

If RS are used to estimate DMI, it is essential to obtain accurate information on these forage quality factors, as well as animal production data. It is difficult to assess factors such as liveweight change in cows over a short period of time, and RS may be a more appropriate guide to DMI over extended periods for individual cows. However, it may be worthwhile considering alternative methods to determine DMI considering the host of assumptions which are likely to be used in the RS method.

The alkane technique provides an excellent tool to determine DMI of cows grazing kikuyu in experimental situations. A more practical approach to estimating the DMI of a herd grazing well-managed kikuyu is via the assessment of differences in pasture mass pre- and post-grazing using the RPM (Earle and McGowan 1979). The RPM technique was unable to detect differences between experimental treatments and would therefore be more appropriate for farm management situations. This technique has the advantage of obtaining pre- and post-grazing pasture parameters. The detected difference in pre- and post-grazing calibration curves for kikuyu reflect the influence of the stoloniferous mat formed at the base of the kikuyu sward (see also Fulkerson and Slack 1993).

Using the alkane technique, the mean estimated DMI of unsupplemented cows grazing kikuyu was 12.6 kg DM/cow.day. These levels are at, or below, those found by
Hamilton et al. (1992) and Henning et al. (1995) who determined DMI levels of cows grazing kikuyu to be 12.6 and 14.2 kg DM/cow.day, respectively, using the chromic oxide method. These levels of intake are below the 15 kg DM/cow.day cited by Holmes and Wilson (1984) as acceptable requirements for cows grazing temperate pastures, and well below the potential DMI of 24-28 kg/cow.day for cows with unlimited access to highly digestible feed and appropriate supplements (Lean and Westwood 1997).

11.4 Milk production from high-quality kikuyu pasture

These low DMI levels were reflected in relatively low milk production, ranging from 12.5 to 17 L/cow.day from cows grazing kikuyu grass alone in this study. However, these production yields exceed those observed in previous studies for cows grazing kikuyu pastures (Stobbs 1972; Colman and Kaiser 1974; Royal and Hughes 1976; Murtagh et al. 1980; Sriskandarajah et al. 1980; Ashwood and Kellaway 1982; Olney and Albertsen 1984; Hughes et al. 1988; Henning et al. 1995) in which milk production was rarely greater than 11 L/cow.day. Hamilton et al. (1992) reported production in cows in early lactation of 14.2 L/cow.day. However, this was still 2.8 L below the maximum yield observed in this study (with no liveweight change) and the cows were reported to be losing condition.

11.5 Responses of cows grazing high-quality kikuyu to energy and protein supplementation

Milk production was further enhanced through supplementation. In agreement with Royal and Jeffery (1972), energy appears to be the first factor limiting production from kikuyu. High responses (up to 2.0 L milk/kg concentrate) to an energy-based supplement were observed when 3kg of concentrate was fed. This compares favourably to previously reported responses to energy supplementation at similar feeding rates (refer to Table 2.5) which range from 0.4 (Sriskandarajah et al. 1980) to 1.1 (Hamilton et al. 1992) kg milk/kg concentrate fed. However, in the present study, at feeding rates greater than 3kg concentrate/cow.day, responses to additional energy supplementation were low or even zero. This is in agreement with studies of Arriaga-Jordan and Holmes (1986) in cows grazing predominantly ryegrass pasture, supplemented with cereal-based
concentrates. Similarly, the milk production responses of cows grazing pastures based on ryegrass in winter and kikuyu in summer, fell from 1.5 (at a feeding rate of 2.9 kg concentrate/cow.day) to 0.8 (at a feeding rate of 6.3 kg concentrate/cow.day) L milk/kg concentrate fed (Fulkerson et al. 1997).

The inclusion of concentrates in the diet depressed kikuyu intake, although total DMI generally increased. The reduced herbage intake was partially associated with the effects of substitution for pasture, particularly in relation to factors responsible for reducing kikuyu digestibility, as there was no difference in the whole diet DMD of cows receiving increasing amounts of concentrates. This finding is contrary to expectations if the digestibility of dietary constituents are presumed additive, and it appears that kikuyu digestibility was inhibited by the inclusion of concentrates in the diet.

The inhibitory effects of carbohydrate-based concentrates on the digestibility of the forage component of the diet has been shown in lambs by Doyle et al. (1988). Various factors appear to associated with the depression in forage digestibility resulting from carbohydrate supplementation, the most predominant being a reduction in rumen pH which inhibits fibre digestion (Terry et al. 1969; Mould and Orskov 1983; Hoover 1986; Istasse et al. 1986). The inclusion of buffers in the diet of supplemented steers grazing kikuyu in these studies only partially prevented a reduction in rumen pH, and maybe higher rates of buffers, or the inclusion of virginiamycin (Clayton et al. 1997), may have been more effective in neutralising the rumen pH.

Although low rumen pH is acknowledged to be a major factor limiting cellulolytic activity in the rumen, fibre degradation has been inhibited even when a decline in rumen pH has been prevented (Mould et al. 1983; Henning et al. 1980). Therefore, other factors including competition for essential nutrients amongst cellulolytic and amylolytic bacteria (Hoover 1986), a shift in the population of cellulolytic microbes when readily fermentable carbohydrates are included in diets (Piwonka et al. 1994) or the presence of an inhibitory substance (Henning et al. 1980), may also reduce fibre degradation. There appears to be validation in these claims as the inclusion of buffers in the diet of steers in this study did not improve whole diet DMD.
Supplements with slower rates of ruminal degradation which better match kikuyu breakdown in the rumen, may be an appropriate alternative supplement of cows grazing kikuyu. Rice degrades very slowly in the rumen compared to barley (Opatpatanakit et al. 1994), and in a recent study, higher milk production responses were observed in cows fed a rice-, rather than barley- or sugar-based concentrate, when grazing kikuyu pastures (L. Trevaskis, personal communication). Perhaps more investigation into the suitability of concentrates to different types of pasture would be beneficial, based on rumen degradation characteristics.

In contrast to milk production, responses to supplementation at the metabolic level were more influenced by the rates of protected protein, rather than the energy content, of the supplement. As expected, PU concentrations increased in response to higher proportions of FTCM in the concentrate, and presumably resulting in greater N wastage. This was reflected in MU concentrations which were significantly related to circulating PU.

Elevated glucose and \( \beta \)-OHB concentrations in the blood were related to high concentrations of protected protein supplementation in these studies. This is contrary to expectations that these metabolic indicators would reflect the energy composition of the diet. There are various explanations for this phenomenon. Firstly, it may reflect the fate of excess glucogenic amino acids absorbed from the small intestine, therefore being more of a reflection of dietary composition than nutrient status. Secondly, it may be related to increased butyrate production in the rumen which is converted to \( \beta \)-OHB in the rumen wall and liver (Amaral-Phillips et al. 1993). The higher sugar content of canola meal, compared to barley (Jarrige 1989) may promote higher concentrations of butyrate production (Preston and Leng 1987). The third scenario may be related to body tissue mobilisation related to an increased demand for energy to process, and excrete excess dietary N.
Appendix 1.

Determination of the error in intake estimation when faecal recovery rates of adjacent alkanes differ

All being equal, with the exception of the faecal recovery rates of 2 adjacent alkanes, the alkane intake equation (Equation 11 in Chapter 3) virtually amounts to the following:

\[
I = \frac{\frac{F_i}{F_j} \cdot \text{constant}}{H_i - (\frac{F_i}{F_j} \cdot H_j)}
\]

since dose, intake of supplement and supplement alkanes are the same in the 2 cases of equal v non-equal recoveries.

Embedded in the \( \frac{F_i}{F_j} \) is the assumption of equal recoveries i.e. \( \frac{q_i}{q_j} = 1 \) therefore, the equation really is;

\[
I = \frac{\frac{F_i}{F_j} \cdot 1 \cdot \text{constant}}{H_i - (\frac{F_i}{F_j} \cdot 1 \cdot H_j)}
\]

but suppose that the ratio of recoveries is not 1 but 1.186 (i.e. \( \frac{1.07}{0.91} \)), with the faecal recovery rate between alkanes being 16% (i.e. 1.07-0.91).

Then,

\[
I' = \frac{\frac{F_i}{F_j} \cdot 1.186 \cdot \text{constant}}{H_i - (\frac{F_i}{F_j} \cdot 1.186 \cdot H_j)}
\]
for $H_j \gg H_p$, as it usually is, the effect of the 1.186 in the denominator is much less than its effect in the numerator, and for the sake of demonstration can be ignored.

Thus, $I' \approx 1.186*I$, i.e. the estimate obtained when recoveries differ is different from that obtained when recoveries are equal, by an amount approximately equal to the ratio of recoveries.

Therefore, in the above example, the 16 % difference in recovery results in about an 18.6% increase in the ratio of recoveries which is translated into an 18.6% error in the intake estimate.
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