The Effect of Nitrogen Fertilization and Stage of Re-growth on the Nutritive Value of Kikuyu in the Midlands of KwaZulu-Natal

by

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requirements for the degree of
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ABSTRACT

Kikuyu pasture was fertilized at low and high levels of nitrogen (N), namely 50 and 200 kg N/ha, after mowing and clearing the plots, to induce low and high levels of N in the herbage. The subsequent growth was harvested at 20-, 30- and 40-d re-growth. These treatments were conducted in spring, summer and autumn. Treatments included level of N, stage of re-growth and season as variables in digestion trials using sheep and voluntary feed intake (VFI) trials using long yearling heifers in pens equipped with Calan gates. Nitrogen fertilization level had no impact on herbage dry matter digestibility (DMD). Stage of re-growth influenced digestibility in the spring and summer, the highest values recorded in the 30-d treatment. However, in the autumn, the 20-d re-growth recorded the greatest digestibility. Digestibility declined as the season progressed. Digestibility was not correlated to any of the chemical fractions measured in the herbage, including *in vitro* DM digestibility (IVDMD). Voluntary feed intake (VFI) followed a similar trend to digestibility, with peak values recorded for the 30-d treatment in the spring and summer, while the 20-d material induced the greatest intake in the autumn. Nitrogen fertilization had a negative impact on VFI over all seasons. Similarly to digestibility, VFI was not correlated to any of the chemical fractions measured, but was correlated to digestibility and moisture concentration of the herbage.

Nitrogen degradability was determined using the *in situ* bag technique. Differences ($p<0.05$) were recorded for the quickly degradable N (a) and potentially degradable N (b) fractions within season, but not for the degradation rate of the slowly degraded fraction (c) per hour. The effective degradability (dg) was not influenced by N fertilization level in the spring, while N fertilization increased the dg values in the summer and autumn. Stage of re-growth exerted a positive effect ($P<0.05$) on the dg values.

Rumen pH, rumen ammonia and blood urea nitrogen (BUN) levels were measured in rumen fistulated sheep. Rumen pH increased also with increasing level of N fertilization and declined with advancing stage of herbage re-growth in the autumn. Rumen ammonia increased with time of sampling post feeding to 4 hrs and then tended to decline by 6 hrs. Nitrogen fertilization level influenced rumen ammonia levels ($p<0.05$), with the low N level producing the lowest rumen ammonia levels. Rumen ammonia levels were highest at 20-d re-growth stage in summer and at the 40-d re-growth stage in autumn. DM concentration of the herbage had an inverse relationship with rumen ammonia. BUN levels were increased by high N fertilization and were positively correlated to rumen ammonia levels.

Five years of digestibility data (82 digestion trials) and three years of intake trials (38 trials) data was pooled. These data, chemical composition of the herbage and the daily maximum temperatures, rainfall and evaporation recorded at and prior to the digestion and intake trials at Cedara were analysed using multiple regression techniques. Rainfall and temperature in the period of cutting and fertilization had a negative effect on digestibility, irrespective of the stage of re-growth at harvesting, 20, 30 or 40 days later, and a combination of the two proved significant, accounting for the most variance in DDM. Temperature depressed DMD by 11.4 g/kg DM per degree rise in temperature (^{o}C). Temperatures recorded during the cutting and fertilization phase were highly negatively correlated to VFI, irrespective of stage of re-growth. The DM concentration of the herbage as fed accounting for 32% of the variance in DMD, the NPN content of the herbage accounting for only 12.2% of the variance and the ash concentration of the herbage accounting for 15.9% of the variance in digestibility. Non-protein nitrogen was negatively correlated to VFI. Both DMD and VFI were highly negatively influenced by the moisture concentration of the herbage.

Overall, the results of these trials demonstrated that environmental factors such as rainfall and temperature had a far greater impact on the digestibility of kikuyu herbage than the chemical composition, which had a minimal effect. Nitrogen fertilization did not influence herbage digestibility overall, but exerted a highly negative impact on voluntary intake.

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DECLARATION 2 - PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis:

Publication 1 Dugmore, T J & Nsahlai, I V. Kikuyu in South Africa – a review. Submitted to the South African Journal of Animal Science

Publication 2 Dugmore T J & Nsahlai, I V, 2010. Effect of environmental factors on the digestibility and the voluntary feed intake of kikuyu. Paper presented at the 43rd congress of the South African Society for Animal Science, July 2009. Sth. Afr. J. Anim. Sci. 40 (5) 414-417

Publication 3 Dugmore T J & Nsahlai, I V, 2010. Effect of herbage composition the digestibility and voluntary intake of kikuyu. Paper presented at the 43rd congress of the South African Society for Animal Science, July 2009. Sth. Afr. J. Anim. Sci. 40(5) 418-4212.

Publication 4 Dugmore, T J & Nsahlai, I V. The effect of nitrogen fertilizer and stage of re-growth on the digestibility and voluntary intake of kikuyu. In preparation for submission to the South African Journal of Animal Science

Publication 5 Dugmore, T J & Nsahlai, I V. The effect of nitrogen fertilizer and stage of re-growth on the rumen degradability of nitrogen in kikuyu. Planned for submission to the South African Journal of Animal Science

Publication 6 Dugmore, T J & Nsahlai, I V. The effect of nitrogen fertilizer and stage of re-growth on rumen fermentation parameters of sheep fed fresh kikuyu herbage. Planned for submission to the South African Journal of Animal Science

Signed: ___________________________

Contents

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In acknowledgment of the research that went ahead of me in the KZN midlands by my colleagues and mentors, part of the purpose of this thesis is to document the highlights of this invaluable work before it is lost to science, as much of it is not published in journals.

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Soli Deo Gloria

Abbreviations

A : effect of age
ADF : acid detergent fibre
ADG : average daily gain
BW : body weight
BUN : blood urea nitrogen
C : Crude fibre
CP : Crude protein
D : stage of regrowth
DM : dry matter
DDM : digestible dry matter
DOM : digestible organic matter
DMD : dry matter digestibility
DMI : dry matter intake
dg : degradability
EE : ether extract
edg : effective degradability
IVDOM : in vitro digestible organic matter
IVDMD : in vitro dry matter digestiobility
KZN : KwaZulu-Natal
LAN : limestone ammonium nitrate
LSD : least significant difference
N : nitrogen
NDF : neutral detergent fibre
NDFN : neutral detergent fibre digestibility
NFC : non-fibre carbohydrate
NFE : nitrogen-free extract
NO_3-N : nitrate
NPN : non-protein nitrogen
NIR : near-infrared spectroscopy
NSC : non-structural carbohydrate
OM : organic matter
PD : potential degradability
REML : residual maximum likelihood model
S : season
SDN : slowly degraded nitrogen
SR : stocking rate
QDN : quickly degraded nitrogen
VFI : voluntary feed intake

Chapter 1
Introduction

The grass *Pennisetum clandestinum* is called kikuyu after the Kikuyu tribe of Kenya. It occurs naturally only within the tropics of east, central and north-east Africa and even here it is confined to small areas (Edwards, 1937; Cameron, 1960; Pratt *et al.*, 1966). It occurs there in what Edwards (1935) described as areas of high moisture and low temperature at high altitudes. Altitudes range from 1950 to 2700 m with frequent mists occurring and an annual rainfall of 1000 to 1600 mm which is well distributed throughout the year, although with a distinct single or double wet season (Morrison, 1969). Kikuyu occurs as a natural stage in the plant succession of these areas. The climax vegetation is forest, with the destruction or clearing of the forest by fire or man, kikuyu comes in with a mixture of *Trifolium semipilosum* and with increased fertility the productive kikuyu-clover stage may be maintained (Edwards, 1935; van Rensburg, 1960; Pratt *et al.*, 1966; Morrison, 1969).

Kikuyu grass has spread throughout the semi-tropical areas of the world (Cameron, 1960). Hunters and travellers passing through these areas in the early part of the century were impressed with this grass and introduced it to various parts of the world. Forbes in 1909 brought back a single root, collected at Lake Naivasha, to South Africa which he planted on his farm near Athol in the Transvaal. In 1911 a cutting was sent to the Botanical Gardens in Pretoria, where it was vegetatively multiplied and flowered every year, but being a male sterile strain it formed no seed. From here it has spread to most areas of South Africa (Stapf, 1921). Although seeding of kikuyu has been observed in South Africa (Edwards, 1961) there is no evidence in the literature of any further introduction of any strains into South Africa, until the recent introduction of Whittet seed kikuyu from Australia. Kikuyu was introduced into then Rhodesia (Zimbabwe) in 1917, first from Potchefstroom, and then from Natal in South Africa, were it flourished (Walters, 1918).

Kikuyu was introduced to Australia from Kenya in 1908 but failed to survive (Parker, 1941). In 1919 seed was introduced from the then Belgian Congo, one seed germinated and was multiplied vegetatively and distributed throughout Australia, New Zealand and Fiji (Whittet, 1921; Parker, 1941; Wilson, 1975b). Further introductions were made, the most important of which was in 1960 from Kenya to the Grafton Research Station in New South Wales, from which the cultivar, Whittet, was finally released (Quinlan *et al.*, 1975). On the east coast of New South Wales, Australia, kikuyu provides some 75 % of the pasture grazed by dairy cows during the summer and autumn. Kikuyu is also well suited to 860 000 ha of Queensland (Minson *et al.*, 1993).

Kikuyu was first introduced into Hawaii in 1925, and in 1958 the Kabete strain was brought in from Kenya by the Hawaii Agricultural Experiment Station (Hosaka, 1958). Kikuyu is one of Hawaii's most important forage grasses, where it is the dominant species on approximately 80 000 ha of grassland from sea level to over 1500 m elevation (Whitney, 1974a).

Kikuyu has been listed as a promising introduction in the tropical highland areas near the equator and in humid subtropical climates (Mears, 1970), where it is considered a high quality grass for dairying and cattle finishing in the high-altitude areas (Skerman & Riveros, 1990; Hacker & Jank 1998). Although it is considered a serious weed in California (Youngner, 1961) and in New Zealand (Ballinger, 1962), it is utilized in other countries such as Brazil (de Araujo, 1952), Panama (McCorkle, 1968) and the Montane areas of Peru (2700 m altitude; Johnson & Pezo, 1975), Colombia (2250 m altitude; Benavides, 1977), Costa Rica (1600-2100 m altitude; van der Grinten *et al.*, 1992) and Guatemala (Hacker & Jank, 1998) in South/Central America. It is a major dairying grass in the cool mountain pastures of Columbia (up to 3500 m) and Costa Rica (Skerman & Riveros, 1990), as well as Sri Lanka (Ketth & Ranawana, 1971).

In Costa Rica (Herrero, 1993; pers. comm.) the dairy industry is well established on the highlands at altitudes of 2000 to 3000 m, with temperatures varying from 10° C at night to 20° C during the day, receiving an annual precipitation of 3000 to 3500 mm well distributed throughout the year. Kikuyu is the main pasture, grown on very steep slopes with very acid andisol soils. Rotational grazing is practiced, with one day of stay and a rest period of 35 days. Stocking rates are highly variable from 2 to 6 animals per ha with nitrogen fertilization levels varying from none to 500 kg N/ha/yr, with a mean of 150 kg N/ha/yr. Production levels from kikuyu plus concentrates vary from 4500 to 6500 kg milk per 305 day lactation for Holstein and 2800 to 4000 kg milk per 305 day lactation for Jersey cows.

The seasonal growth rhythm of kikuyu is determined by a combination of temperature and rainfall (Colman, 1971; Kemp, 1974). Temperature, however, had an overriding effect on the commencement and cessation of growth (Kemp, 1974). In its native habitat, mean minimum and maximum temperatures range from 2 to 8° C and 16 to 22° C, respectively (Morrison, 1969). Russel and Webb (1976) considered the optimum temperature for growth to be 16 to 21° C, with poor adaptation to high temperatures.

Kikuyu is markedly more frost tolerant than other commonly grown tropical grasses in the sub-tropics (Quinlan *et al.*, 1975). An advantage of kikuyu over most other tropical grasses is its ability to grow well during autumn with little deterioration in forage quality until frosts occur, or until June-July (mid-winter) in frost free areas (Murtagh, 1975). High nitrogen fertilization and an adequate water supply increased kikuyu's frost tolerance (Gartner & Everett, 1970; Wetherall, 1970). Kikuyu also maintains its nutritive levels in the dry season (Wetherall, 1970; Minson, 1972; Mears & Humphreys, 1974b; Cross, 1979b). The application of nitrogen has considerable impact on the length of the growing season (with growth greater than 5 kg DM/ha/day) of kikuyu. At zero N the growing season was only 2 to 3 months, at 170 kg N/ha/year the growing season was 6 months and at 680 kg N/ha/year the season was extended to 9 months. Colman (1971) also showed a substantial response by kikuyu to early spring nitrogen dressings, thereby increasing the growing season. Thus, the application of nitrogen may also be justified for the provision of dry season feed. These aforementioned factors explain the success of kikuyu as a foggaged pasture in the midlands of KwaZulu-Natal.

It is clear from the preceding discussion that kikuyu thrives at high altitude in the tropics, at altitudes exceeding 2000 m, where the elevation gives these areas a sub-tropical climate (Quinlan *et al.*, 1975), while in the sub-tropics it has become a successful introduction at lower altitudes, c. 1000 to 1500 m in the KwaZulu-Natal Midlands, at latitudes of approximately 28 to 30°S, similar to northern New South Wales in Australia. At higher latitudes, with lower temperatures it has found a niche along the Eastern Cape and Southern Cape coast at latitudes of c. 34°S, where moderate summer temperatures and mild winters suit its requirements. There are 3 distinct ecotypes of kikuyu in East Africa (Edwards, 1961), and with introductions to other countries being collected in different areas and grown in differing environments, kikuyu could respond differently in different areas, as noted between the kikuyu in the Eastern Cape at Alexandria and Cedara. These two populations required different near-infrared spectroscopy (NIR) calibrations to read the same elements (Thurtell, pers comm.).

Kikuyu is one of the most important summer pasture species in South Africa (Miles *et al.*, 1995). Kikuyu comprises 24% of the cultivated pasture area (123 000 ha) in KwaZulu-Natal, second only to *Eragrostis curvula* (32%) which is generally used for hay (Fotheringham, 1981). In the KwaZulu-Natal Midlands, 87% of the kikuyu produced was used for summer grazing on dairy farms (Heard *et al.*, 1984).

Dairying in KwaZulu-Natal, which produces *c*. 23.6% of South Africa's 2 .5 billion ℓ milk by *c*. 332 dairy farmers (Lacto Data, 2010), is centered in the midlands of KwaZulu-Natal. This area lies between 900 m (3 000 ft) and1500 m (5 000 ft) altitude, thereby ameliorating the temperatures in this sub-tropical zone, situated at a latitude of 28° to 30°S. The average maximum January/July temperatures are 26/19.3°C with THI's of 72/61 at Cedara. The mean maximum temperature in the midlands varies from 24° to 28°C in January and 16° to 22°C in July, while the mean minimum temperature vary from 10° to 14°C in January to 0° to 4°C in July. This area has relatively warm summers with a predominantly summer rainfall of between 750 and 1000 mm. Winters are cool, with clear sunny days and heavy frost occurring at night, but with average daily maximum temperatures of 20° to 22°C, a diurnal variation of approximately 20°C. Frost is a regular occurrence throughout the winter, with 20 to 40 frosts recorded, on average, over the winter period (Schulze, 1997).

The bioclimatic areas in which much of the dairying occurs are the Moist Midlands Mistbelt (BRG 5, Camp, 1997), the Moist Highland Sourveld (BRG 8, Camp, 1997) and the Moist Transitional Tall Grassveld (BRG 11; Camp, 1997). These bioresource groups (BRG) equate to bioclimatic groups 3, 4 and 6 (Phillips, 1973) for BRG's 5, 8 and 11 respectively. A survey of the province (Agriquest, 1981) revealed that 16 956 dairy cows (14.5 %) were found in the Mist-belt (bioclimate 3; Phillips, 1973), 24 065 (20.6 %) in the Highland Sourveld (bioclimate 4e) and 24 582 (21.1%) in the Moist Tall Grassveld (bio-climate 6; BRG 11 & 12). A further 24 330 dairy cows (20.9%) were found in the Dry Tall Grassveld (bioclimate 8; BRG 13) in which much less pasture was utilised.

The Moist Midlands Mistbelt comprises 520 212 ha (Camp, 1997). It lies in an altitude range of 900 m to 1400 m above sea level, is generally hilly rolling country with a high percentage of arable land, approximately 47 % being suitable for cropping. This bio-resource group has a humid climate, with an annual rainfall ranging from 800 to 1280 mm. Heavy mists are common and an important feature, providing additional moisture, particularly to forests. The mean annual temperature is 17° C. Climate hazards include occasional droughts, usually of short duration, occasional hail, frost which varies from slight to severe, and excessive cloudiness during the summer growing season. Hot north-westerly ("berg") winds, followed by sudden cold temperatures or cold fronts, make for unpredictable conditions, particularly in the spring and early summer. Few relic areas of the former *Themeda triandra* grassland veld remain. The palatable grass species have been replaced by hardy pioneer species such as *Aristida junciformis, Eragrostis plana, Sporobolus africanus* and *Hyparrhenia hirta,* due to excessive burning and poor management practices. *Aristida junciformis,* or Ngongoni grass, is only palatable in the very early stages of growth following fire. Mixed *Podocarpus* forests are a feature of this BRG, usually situated in the transitional area with the higher-lying BRG (highland sourveld).

The potential of the soils of this BRG (5) are high, in spite of the fact that the inherent nutrient status is very low. Particular problems are P-fixation and Al-toxicity. Soils are highly leached. This BRG is usually well watered, with streams rising in the south-facing hills where the BRG meets the Highland Sourveld. Due to the poor veld condition, high soil potential (37% of area) and potential arable land (47% of area) this BRG is most suited to intensive systems of farming. Dairy is an important enterprise, due to the poor veld being replaced by cultivated pastures, for which the area is ideally suited, forming the basis of livestock enterprises (Camp, 1997).

The Moist Highland Sourveld, which is 833 271 ha in extent, occurs above an altitude of 1400 m and generally below 1800 m. It lies between the Moist Midlands Mistbelt or Moist Transitional Tall Grassveld and the Montane Veld. The mean annual rainfall is from 800 mm to 1265 mm. Approximately 80% of the rain falls in the summer months of October to March. Mist is a frequent occurrence, particularly at the higher altitudes, and snow occurs occasionally, mostly in the highest ranges of the BRG. The mean annual temperature is 14.1°C. Summers are moderately warm, with a December mean of 18°C. Winters are cold and the mean minimum July temperature ranges from 0.1°C to 3.6°C in the west and south, whil`e those in the north and east are warmer, ranging from 1.8°C to 6.0°C. Severe frost can occur over a 6 month period, particularly in bottomland areas. Light frosts occur during the early and late summer months. The vegetation is a fire maintained grassland dominated by *Themeda triandra* and *Tristachya leucthrix.* In the absence, or reduction, of fire a development towards *Podocarpus* forests occurs. Forest patches occur mainly on the cooler and moister south-facing slopes, particularly where they have been protected from fire. This BRG is generally rich in water resources. Numerous streams arise in the area and streams and rivers rising in the adjacent mountains flow through this BRG. Suitable sites for farm dams are common and building material is generally suitable (Camp, 1997).

The soils of the Moist Highland Sourveld are relatively deep, highly leached and strongly acid. Soil fertility is low, but physical properties are favourable. Twenty four percent of the area is arable, while 20% of the BRG has high potential soils. The prevailing climatic conditions make this BRG difficult to farm. Its dry, cold and frosty winters cause a short growing season. In summer, hailstorms are a frequent occurrence. The soils are highly leached, requiring expensive fertilizer input. Despite these problems this BRG is suited to intensive farming systems including beef, dairy, sheep, timber and maize (Camp, 1997).

The Moist Transitional Grassveld (BRG 11, Camp, 1997) comprises an area of 743 770 ha. It is a transitional zone which lies between the drier Tall Grassveld and the Moist Highlands Sourveld. The altitude ranges from approximately 900 m to 1 400 m. The mean annual rainfall is 800 mm to 1116 mm with a mean annual temperature of 16.9° C. Mists occur frequently in the spring and early summer. Frosts are moderate, with the occasional severe frost. The vegetation is dominated by a *Themeda triandra - Hyparrhenia hirta* plant association. This is generally a well watered BRG with streams rising or flowing through it. It is an important maize and timber production area, while dairy and beef are important farming enterprises (Camp, 1997).

The Dry Tall Grassveld (BRG 13) covers 449 298 ha and occurs mainly as a transitional zone between the dry valley vegetation and the moist BRG's. The mean annual rainfall range is 660 mm to 745 mm and the mean annual temperature is 17.3° C. There are 4 to 5 dry months of the year and droughts are frequent occurrences. Summers are warm to hot and winters cool with cold spells. Moderate, with occasional severe, frosts occur in the winter. The grassland is dominated by *Hyparrhenia hirta* with occasional *Acacia sieberana* woodlands.

A survey of agricultural land usage (Agriquest, 1981) revealed that 123 009 ha were cultivated pasture, of which 29 294 ha of kikuyu was cultivated in the province; 7 400 ha in the Mist-belt (bioclimate 3; Phillips, 1973), 4 773 ha in the moist phase of the highland sourveld (bioclimate 4e) and 5 317 ha in the Moist Tall Grassveld (bioclimate 6). Irrigated temperate pastures (predominantly annual ryegrass) comprised 19 567 ha; 2 641 ha in the Mist-belt (bioclimate 3), 8 705 ha in the moist phase of the highland sourveld (bioclimate 4e) and 2 233 ha in the Moist Tall Grassveld (bioclimate 6). Maize for silage is grown on 26 975 ha; 2 491 ha in the Mist-belt (bioclimate 3), 5 712 ha in the moist phase of the highland sourveld (bioclimate 4e) and 6 464 ha in the Moist Tall Grassveld (bioclimate 6). *Eragrostis curvula* for hay, predominantly for beef herds, is cultivated on 39 802 ha; 2 482 ha in the Mist-belt (bioclimate 3), 13 442 ha in the moist phase of the highland sourveld (bioclimate 4e) and 6 096 ha in the Moist Tall Grassveld (bioclimate 6). Kikuyu pasture is the predominant summer pasture species. Paspalum (760 ha), Nile Grass (1 084 ha), Star Grass (2 744 ha) and other Cynodon pastures (1 218 ha) comprise the other recorded summer pastures.

As highlighted by the survey of agricultural land useage (Agriquest, 1981) milk production in KwaZulu-Natal is based on tropical pastures, almost exclusively kikuyu during the summer and irrigated annual ryegrass (*Lolium multiflorum*) in the winter, supplemented by maize silage and, to a lesser extent, *Eragrostis curvula* hay. The predominant form of feeding is grazing with concentrate supplementation after milking. Low soil pH and very high acid saturation levels make the cultivation and persistence of legumes problematic.

Kikuyu grass, being a tropical C_4 plant, tends to be relatively low in metabolizable energy (*c.* 9 MJ/kg DM) and therefore limits production to *c.* 10ℓ/day for a 500 kg cow (Bredon & Stewart, 1979). However, good responses to concentrate feeding (*c.*1.4ℓ/d) have been recorded for cows grazing kikuyu at Cedara (Dugmore, 1998). This response is moderated at high levels of concentrate supplementation due to the substitution effect of concentrate for roughage. The forages used in the winter months have substantially high energy levels, namely *c.*10 MJ/kg DM for both annual ryegrass (*Lolium multiflorum*) grazing and maize silage, milk yields of *c.*17ℓ/cow/day have been recorded for both ryegrass and maize silage. However, maize silage is deficient in crude protein (*c.* 70 g CP/kg DM) and annual ryegrass has a surplus of crude protein (> 200 g CP/kg DM). Consequently these two forages make a highly complimentary mixture in the diet (Bredon & Stewart, 1979). Tall fescue (*Festuca arundinacea*) has also been evaluated at the Cedara Agricultural Research Station, with 550kg cows in mid-lactation producing 16ℓ/day off fescue (ME > 9.4MJ/kg DM) and a production response to concentrate feeding of 0.9 kg/kg concentrate (Dugmore *et al.*, 1992a). The protein content of both kikuyu and ryegrass generally exceed 200 g CP/kg DM and have commonly been recorded at levels in excess of 250 g/kgDM due to the high annual nitrogen fertilization levels (350 kg N/ha/season for ryegrass and 310 kg N/ha/season for kikuyu). The ruminal protein degradability of both are high, *c.*80% for ryegrass and *c.* 75% for kikuyu, resulting in a surplus of protein in the system and consequently high blood urea nitrogen levels and high milk urea nitrogen (MUN) levels (> 400 mg milk urea/ℓ milk) recorded on kikuyu pastures at Cedara which can negatively impact on milk production and cow fertility (van der Merwe *et al.*, 2001).

Cows are calved throughout the year due to the majority of milk in South Africa being used as fresh milk. A few producers have shifted to seasonal calving, but these are the exception. Herd size has increased from an average herd size of 120 cows in herd in 1980 to *c.* 200 in 1998, and presently stands at *c.* 400 cows for herds where dairying constitutes the primary enterprise. The cost of concentrates has accounted for a consistent 56 to 58% of the variable cost over the past 20 years. Milk yields have improved by 27% over the past 20 years. Labour costs amount to 10 to 14% of total costs, a reduction from the 15 to 20% of total costs it constituted 30 years ago. Transport amounts to 3.3% of the variable costs of milk production. Nitrogen fertilizer costs account for *c.*17% of the variable costs of production (Tammac Consultants: www.tammac.co.za).

It is apparent from the preceding discussion that kikuyu is well adapted to the midlands off KwaZulu-Natal and comprises an important pasture species for dairying in KwaZulu-Natal. It is highly productive, especially with nitrogen fertilization, with high DM yields per ha (Cross, 1978). Unfortunately, animal production, relative to its nutritive value has been disappointing (Bredon, 1980). Atypical relationships have been observed between the digestibility of kikuyu and its herbage nitrogen and the fibre fractions (Pattinson, 1981; Dugmore *et al*., 1985). Therefore, the objective of this research is to examine the effect of nitrogen fertilizer and stage of re-growth of the herbage on its nutritional value. Nutritional value is considered a combination of intake and digestibility which determines energy intake. As the N fraction, or N fertilization, seems to be implicated in the numerous problems besetting kikuyu, from poor animal production to toxicity (kikuyu poisoning) as discussed in the literature review (Ch 2) for South Africa (with numerous examples elsewhere in the literature), very high levels of N fertilization were utilised to ensure that any potential problems would be induced in the animals, to try and understand the limitations of highly N fertilized kikuyu. There are indications in the literature that the negative factors in kikuyu are minimised in older herbage, therefore stage of re-growth was incorporated into the experimental design. Season is known to influence kikuyu, as indicated by the autumn slump with reduced milk yields, therefore trials were conducted in the 3 growing seasons, namely spring, summer and autumn. As minerals in kikuyu, namely a sodium deficiency and a low calcium concentration, particularly an inverse Ca:P ratio and poor K/(Ca+Mg) ionic balance which are known characteristics of kikuyu, a liming treatment was included to see if the mineral problems could be alleviated. Rumen degradation parameters and fermentation indicators were also determined to evaluate the efficiency of rumen fermentation.

The characteristics associated with kikuyu are presented in Appendix Table 1. Numerous trials reporting milk production off kikuyu are listed in Appendix Table 2. Data found in the literature on the chemical composition and digestibility of kikuyu is presented in Appendix Table 3. A summary of the mineral concentrations recorded in the literature is given in Appendix Table 4.

Chapter 2
Kikuyu in South Africa

INTRODUCTION

"Two years before Union there entered the Transvaal an unknown plant immigrant, brought down from Kenya in a biscuit tin by Mr David Forbes. Half of this was given to Mr Burtt-Davy. This was our now famous kikuyu, the ideal pasture grass, and destined, I think, one day to produce more wealth than maize has done and be much less of a gamble" (Hall, 1934). This introduction of kikuyu into South Africa was described by Burtt-Davy (1915).

This kikuyu originated from the East African highlands of Ethiopia and central Kenya, and from the Ngorongoro Crater which carry a natural grassland of *Pennisetum clandestinum* (Skerman & Riveros, 1990). The grass *Pennisetum clandestinum* is called kikuyu after the Kikuyu tribe of Kenya. It occurs naturally only within the tropics of east central and north east Africa and even here it is confined to small areas (Edwards, 1937; Cameron, 1960; Pratt *et al.*, 1966). It occurs there in what Edwards (1935) described as areas of high moisture and low temperature at high altitudes. Altitudes range from 1 950 to 2 700 m, with a rainfall of 1 000 to 1 600 mm well distributed throughout the year, although with a distinct single or double wet season. Frequent mists occur (Morrison, 1969).

Hunters and travellers passing through these areas in the early part of the century were impressed with this grass and introduced it to various parts of the world. Forbes in 1909 brought back a single root, collected at Lake Naivasha, to South Africa which he planted on his farm near Athol in the Transvaal. In 1911 a cutting was sent to the Botanical Gardens in Pretoria, where it was vegetatively multiplied. It flowered every year, but being a male sterile strain it formed no seed. From here it has spread to most areas of South Africa (Stapf, 1921). Although seeding of kikuyu has been observed in South Africa (Edwards, 1961) there is no evidence in the literature of any further introduction of any strains into South Africa, until the recent introduction of Whittet seed kikuyu from Australia. Kikuyu was introduced into Rhodesia (Zimbabwe) in 1917, first from Potchefstroom and then from Natal in South Africa, were it flourished (Walters, 1918). Kikuyu grass has also spread throughout the semi-tropical areas of the world (Cameron, 1960) where it is considered a high quality grass for dairying and cattle finishing in high-altitude areas of the tropical and subtropical world (Skerman & Riveros, 1990; Hacker & Jank 1998).

Kikuyu was first planted on Cedara in *c.*1915, but was gradually invaded by pioneer grasses due to inadequate inputs. Pioneering research on kikuyu commenced in 1929 at Cedara and by 1940 approximately 40 ha of kikuyu was established (Taylor, 1941). This area of kikuyu is still utilized by the Cedara dairy herd.

Kikuyu has become one of the most important summer pasture species in South Africa (Miles *et al.*, 1995). Kikuyu comprises 24% of the cultivated pasture area (123 000 ha) in KwaZulu-Natal, second only to *Eragrostis curvula* (32%) which is generally used for hay (Fotheringham, 1981). Kikuyu is well adapted to the high rainfall areas of KwaZulu-Natal, especially to the KwaZulu-Natal Midlands, and forms the bulk of summer grazing for milk production (Dugmore & du Toit, 1988; Marais *et al.*, 1990). In the KwaZulu-Natal Midlands, 87% of the kikuyu produced was used for summer grazing on dairy farms (Heard *et al.*, 1984).

The objective of this chapter is to focus on kikuyu research in South Africa, much of which is not published in journals. Other detailed reviews of kikuyu, by Mears (1970) and Marais (2001), have been published.

ANIMAL PRODUCTION

Dairying

In pioneering trials, commencing on Cedara in 1929, two grade Friesland cows were grazed on the pasture and rotated over three camps covering an area of 1⅓ acres (5396 m^2). The two cows yielded a total of 13740 lbs (6238 kg) over a nine month growing season, commencing on 1 September to 31 May, an average production of 11.34 kg/cow/d with no concentrates. This return represented a yield of over 1000 gallons (4548 kg) per acre (Taylor, 1931). Taylor (1941) coined the terms "Friesland acre" and "Jersey acre" which were stocked at two Friesland cows or 3 Jersey cows per acre on Cedara. The milk production for the Friesland acre, at a SR of 4.9 cows/ha, was 9 296 kg per ha with no supplements and 12 145 kg per ha with 1 918 kg of maize meal supplement per ha over a 7 month growing season. The Jerseys, stocked at 7.4 cows/ha, produced 12 820 kg milk/ha with 2 058 kg maize meal supplement per ha. Although milk production per cow was low, production per hectare was good.

Warren (1972) determined that kikuyu on Cedara could sustain a milk production of 11 kg FCM in spring, 9 kg FCM in summer and 6.8 kg FCM in autumn for 450 to 500 kg cows grazing kikuyu. Bredon and Hathorn (1974) tested various concentrate feeding systems and found the typical diminishing returns responses to energy, which they used to develop their feeding tables and philosophy outlined in the publication "Guide to Feeding of Dairy Cattle". No responses to additional protein on kikuyu grazing were found, except in the autumn. Similarly, no responses were found to fish meal as a source of bypass protein supplementation for cows grazing kikuyu on Cedara (Dugmore *et al.*, 1992b). Milk production responses to concentrates fed to lactating cows grazing kikuyu for the data of Bredon and Hathorn (1974) are 1.4 kg/kg concentrate fed (Dugmore, 1998). Dugmore and Walsh (1990) recorded a milk yield response of 4.1 kg milk to 0.5 kg bypass (prilled) fat fed to multi-parous cows grazing kikuyu and fed 10 kg concentrates per day, with no response by primi-parous cows. Henning *et al.* (1995) recorded milk yields of 9.5, 10.9 and 8.9 kg/d for Friesland (S A Holstein) cows grazing kikuyu at 15, 30 and 60 day cycles over the growing season, respectively, on the Bathurst Research Station in the Eastern Cape. Botha *et al.* (2008) recorded 15 kg/cow/d (5.05% BF) in spring, 14.4 kg/cow/d (4.41% BF) in summer and 12.1 kg/cow/d (4.95% BF) in autumn from Jersey cows grazing kikuyu pastures and fed 4 kg concentrate per day at Outeniqua Research Station in the Southern Cape. Malleson *et al.* (2009) recorded 17.3 kg FCM/cow/d for Jersey cows grazing kikuyu plus 6 kg concentrate and 19.4 kg FCM for cows when fish meal was included in the concentrate at Outeniqua Research Station. However, the ruminal ammonia concentration of the control treatment (4.74 mg/dl) was lower than the minimum level (5 mg/dl) of rumen ammonia recommended for maximal microbial protein synthesis by Satter and Slyter (1974) indicating that the response to fish meal may not be entirely attributable to the undegradable protein fraction in the fish meal supplemented.

Dugmore *et al.* (1995) found that Friesland cows calving in spring (Sep – Nov) onto summer kikuyu produced less milk (6.5%) per lactation than cows calving down later in summer, autumn or winter when annual ryegrass and maize silage formed a significant part of their diet at stages (autumn & winter) of their lactation. The spring calving cows, ranging in weight from 504 kg post-partum in their first lactation to 554 kg post-partum in their third lactation averaged 13.1 kg milk (3.46% BF) per day over a 300 day lactation period with no supplementation, apart from minerals.

Similar responses have also been recorded in Australia. Colman (1975) considered it unlikely that crude protein intake would limit production on a nitrogen fertilized pasture. Hence, the low production could be attributed to inadequate energy intake. Reeves *et al.* (1996b), in Australia, reported that with good management, *viz.* utilising pasture mulching to reduce stoloniferous mat formation, grazing before substantial stem protruded above the 5 cm stubble layer (18-24 day grazing interval), and offering a fresh block of pasture daily, they achieved milk productions of 17.3, 14.2 and 12.5 ℓ per day in early lactation, mid-lactation and late lactation, respectively, from larger Friesland cows. Significant responses were recorded for energy (barley) supplementation, but no significant response to undegradable protein supplementation, indicating that energy is still the factor limiting milk production from kikuyu pasture (Reeves *et al.*, 1996b). Granzin (2005) considered the high intakes of NDF and low ME to be the major limitations to milk production off kikuyu.

Dairy heifers on kikuyu

Du Plessis (1992) measured the average daily gain of Friesland heifers on kikuyu pasture at the Cedara Research Station over a period of three years and found that yearling Friesland heifers had an average daily gain (ADG) of 0.45 kg (range: 0.35 to 0.61 kg) during the summer period. These responses corroborate Australian data for dairy replacement heifers continuously grazed on kikuyu fertilized with 165 kg N, growing at ADG's ranging from 0.44 to 0.54 kg per day at stocking rates varying from 2.5 to 4.2 heifers/ha (Cowan *et al.*, 1976).

Henning (1994), at Bathurst in the Eastern Cape, found that Friesland heifers aged from 6 to 12 months grazed on kikuyu averaged 0.63, 0.57 & 0.51 kg/d at grazing cycles of 15, 30 & 60 days, except in March when the 60-d rotation was most productive. This is in contrast to the data of cows on kikuyu where the 30-d cycle was the most productive. Henning (pers comm) found that the blood glucose status of animals younger than a year old was highest when grazing on a 15-d cycle, whereas the glucose status of animals older than a year was found to be highest on a 30-d cycle. The higher growth rates for the kikuyu at Bathurst could also be a reflection of higher digestibility expected at higher latitudes, as indicated by Cowan and Lowe (1998).

Allwood (1994) recorded the growth rate responses of Friesland heifers grazing kikuyu to energy and protein supplementation at Baynesfield Estates and found positive responses to supplementary energy. Unsupplemented heifers on kikuyu gained 0.52 kg/d while those consuming 0.51 kg of an energy supplement daily gained at 0.71 kg/d. Responses to energy plus protein (1.18 kg supplement/d) and protein (0.46 kg supplement/d) supplements did not differ from that of energy supplementation alone, with gains of 0.69 kg/d and 0.64 kg/d respectively. The growth rates of the Friesland heifers on kikuyu grazing, ranging from 8 to 16 months of age and 200 to 350 kg body weight, were modelled and described by the following equation (Allwood, 1994):

$$\text{ADG (kg/d)} = 0.3904 + 0.01661\ (\text{MJ supplementary ME}) + 0.0000373\ (\text{months of age} * \text{kg body weight})$$

$$R^2 = 26.7\%; \qquad P<0.05$$

In an attempt to explain the relatively poor growth rates of Friesland heifers on kikuyu grazing, Horne (1996) and Fushai (1997) compared yearling Friesland (dairy breed) and Hereford (beef breed) heifers on kikuyu pasture (with and without energy supplementation) during the summer period at Cedara. Horne (1996) found that the Hereford heifers gained more weight than the Friesland heifers (0.81 *vs* 0.01 kg/d; $P < 0.05$) under rotational grazing of kikuyu pasture without supplementation (apart from a high calcium mineral lick). The ADG of the Friesland heifers increased from 0.01 kg/day to 0.60 kg/d with energy supplementation, while the Hereford heifers' ADG only increased from 0.81 kg/d to 0.97 kg/d. Fushai (1997) had similar, although less pronounced, findings to Horne (1996). The Hereford heifers in the study by Fushai had a greater average daily gain (1.2 versus 0.5 kg/d; $P < 0.05$) than the Friesland heifers when no energy supplementation was given. However, the Friesian heifers did not respond to energy supplementation with the average daily gain decreasing from 0.54 kg/day to 0.50 kg/day with energy supplementation. The difference in weight gain of the Hereford and Friesland heifers in both Horne's (1996) and Fushai's (1997) research was ascribed to the poor DMI of kikuyu herbage. Fushai (1997) found that the Hereford heifers, during both trial periods (with and without energy supplementation), had a greater intake than the Friesland heifers (111.1 *vs* 95.3 g DM/kg live weight $^{0.75}$, $P < 0.05$) on kikuyu, while on a continuous grazing system.

Beef cattle on kikuyu

At the Stockowners Co-op in the KZN midlands, Theron (1980) evaluated the economics of N fertilization of pasture on beef production. At N levels of 112, 224, 336 & 448 kg/ha and stocking rates of 6, 8, 11 & 12 steers per ha the average daily gain averaged 0.7 kg/d. Marginal returns per ha were maximised at 11 steers per ha and 336 kg N/ha. However, the lowest cost and greatest returns per animal were recorded on the low N treatment with 6 steers per ha, which resulted in largest return to total cost of all the treatments. In a comparative trial with K11 at Stockowners, the animal performance of steers on K11 exceeded that of steers on kikuyu.

Tainton *et al.* (1982) determined the effect of N fertilization on the growth rates of beef steers grazing kikuyu in the midlands of KwaZulu-Natal. The treatments were rotationally grazed to the same residual pasture height using filler animals. The mean ADG values were 0.80, 0.68, 0.61 & 0.64 kg/d on N fertilization levels of 150, 300, 450 & 600 kg N per ha. Tainton *et al.* (1982) also measured the stem to leaf ratios of the pasture on offer. The highest leaf to stem ratios measured, namely 0.6 and 0.62, were recorded on the 300 and 450 kg N treatments. The lowest leaf to stem ratio (0.3) was recorded on the highest N treatment. The low N (150 kg/ha) treatment, which resulted in the best animal performance, had an intermediate leaf to stem ratio of 0.52, indicating that the animal requires a balance between leaf and stem for optimum production. Pasture costs per ha were 170% higher for the highest N treatment relative to the low N treatment, while animal costs and total costs per ha for the highest N treatment were double those of the low N treatment. Pasture costs more than doubled per kg live-weight gain while animal associated costs were 30% higher for the high N treatment relative to the low N treatment. Marginal returns per ha favoured the low N treatment, with the high N being the least profitable treatment.

Louw and Bartholomew (1998) reported on the productivity of the Cedara beef cow herd on kikuyu pasture. The pasture was fertilised with 275 kg N/ha/season and other elements according to soil test. Simmentaler and Hereford cows that calved from August to October were rotationally grazed over 8 camps, with a 3.5 day period of stay. All herds had access to a high Ca mineral lick. The length of the grazing season on kikuyu was influenced by stocking rate (SR). The average length of the grazing season declined from 267 days at 3 cow+calf pairs per ha to 207 days at 7 cow+calf pairs per ha. The average weight gain for the calf to weaning was 0.89, 0.83, 0.76 and 0.70 kg/d for stocking rates of 3, 4.5, 6 and 7 cows/ha for Simmentaler cows, while Hereford calves gained 0.71, 0.68, 0.65 and 0.63 kg/d to weaning at stocking rates of 3, 4.5, 6 and 7 cows per ha. Weaning weights were 289, 261, 226 and 203 kg for the Simmentalers and 240, 222, 200 and 185 kg for the Herefords at stocking rates of 3, 4.5, 6 and 7 cow+calf pairs per ha. Economic analysis at the prevailing prices in 1998 indicated little or no profit was achievable for beef suckler cows on fertilised kikuyu pastures.

Using Simmentaler cross British beef steers over an 18 year period at the Nthabamhlope Research Station in the Highland Sourveld, Mappledorum (1998) reported that ADG's ranged from 0.81 kg/d, at a stocking rate of 4.13 yearling steers/ha, to 0.45 kg/d, at a stocking rate of 9.92 steers/ha, for a seasons growth (190 days). Seasonal differences were recorded with a mean ADG of 0.83 in spring, 0.75 in summer and 0.61 in autumn. The optimum stocking rate was 5 steers/ha.

Supplementary feeding of beef animals on kikuyu has had limited success in increasing animal production. Meaker (1998) summarised work on supplementary feeding conducted at Cedara and showed that 1 kg concentrate increased ADG by 178 g/d, from 402 g/d to 580 g/d, and a second kg of concentrate increased ADG by a further125 g/d, a typical diminishing returns response. Feeding an ionophore did not increase the ADG of steers on kikuyu.

Sheep on kikuyu

De Villiers (1998) showed that South African Mutton Merino and Döhne Merino wether lambs achieved growth rates of 84, 52, and 13 g/d at stocking rates of 20, 35 and 55 lambs/ha at Cedara. In excess of 85% of the lambs graded A2 or better on the low stocking rate, while the majority of lambs had poorer grades on the high stocking rates. Van Ryssen *et al.* (1976), also at Cedara, found that both molasses and maize supplementation of weaned lambs, at 10 g/kg live-weight for the molasses and 6.75 g/kg live-weight for the maize treatments, increased gain by approximately 18.4% and this translated into improved carcass grades at slaughter. At Elsenberg in the winter rainfall region of the Western Cape, Brand *et al.* (1999) supplemented South African Mutton Merino ewes on irrigated kikuyu in the summer during pregnancy and lactation with sweet lupin grain at a rate of 0.5% of live-weight. Supplemented ewes lost less weight than unsupplemented ewes, but increased birth weight of the lambs by 21%. Wool growth was not improved by supplementation. The growth of pre-weaned lambs (c. 180 g/d) reared by the ewes was not improved by supplementation.

At Bathurst, Zeeman and Venter (1995) conducted a trial over 8 years, running Döhne Merino sheep on kikuyu with 3 breeding cycles of 8 months each per 2 years. On average pre-weaning growth, with creep feed, was from 200 to 230 g/d. In comparison to an annual lambing system on kikuyu pasture, the annual mating system had 5 percentage units higher reproduction, higher lambing percentages, body weight and wool production. However, the 3 lambings in 2 years was 27% more profitable than the annual lambing systems.

Lyle *et al.* (2000), at the Kokstad Research station, found that ewes lambed onto late season kikuyu pastures in April and May lost weight (2.18 kg) while lactating, with lambs gaining 147 g/d to weaning. In contrast, ewes lambing onto oats pasture gained weight (9.8 kg), while their lambs gained 250 g/d to weaning.

Kikuyu foggage

Kikuyu for foggage is generally closed to grazing in January or February and allowed to grow out for an accumulation of herbage, which is usually grazed as a winter feed after frosting. Rautenbach *et al.* (2008) found that the CP, P, K, Cu and Se concentrations and DM degradability of kikuyu foggage initially decreased rapidly, as green leaf decreased in the stand, while ADF and NDF increased with time from mid-May for kikuyu foggage but stabilized with little change after *c.* 37 days in Northern KwaZulu-Natal.

Sheep wethers on foggaged kikuyu at Nooitgedacht on the Highveld gained 0.7 kg/d for the first week, due to their poor condition, 0.2 kg/d for the next 2 weeks, 0.3 kg per wether per week thereafter for 5 weeks after which the pasture was not able to maintain live-weight (Rethman & Gouws, 1973). At the Athole Research Station, young cows (395 kg) grazing kikuyu foggage from end-May to early August gained 0.58 kg/d over 70 days. At Nooitgedacht, weaner bull calves gained 0.43 kg/d from early June to mid-July.

Sheep on foggage at Nooitgedacht utilised 55% of the available material. Mature dry ewes (56 kg live-weight) on foggage lost on average 8.5% of live-weight and lambs (25 kg live-weight) on foggage lost 7.2% of live-weight over an 80 day grazing period. In another trial, Merino wether lambs (31 kg initial weight) on kikuyu foggage from late May to mid-August lost 0.7 kg body-weight (Barnes & Demsey, 1993). Oesophagealy fistulated sheep selected material with a digestibility of 54 to 59 (% IVDOM), while cut material ranged from 36 to 45%, an average improvement of 44% in the selected material. Barnes and Demsey (1993) concluded that foggaged kikuyu is at best a maintenance feed.

Dry ewes on foggaged kikuyu at Cedara lost between 4.7 and 10.3% of initial body-weight over an eight week grazing period (de Villiers *et al.*, 2002). Dry ewes initially gained weight for the first 2 weeks after the commencement of grazing in mid-July, maintained weight for another 2 weeks and then started to decline in weight from mid-August (de Villiers, 1998). This decline in weight of animals from August on foggage is common. Roos (1975) stated that the problems on foggage are encountered during August and September when the kikuyu starts to green, when the animals become extremely selective while grazing with a subsequent decrease in weight.

At Cedara, steers (217 kg live-weight) grazing kikuyu foggage, either by strip grazing or continuously, gained 0.29 and 0.22 kg/d for the strip and continuous grazing treatment over 96 days ending on 1 October. For the first 39 days the continuous grazing treatment gained 0.44 kg/d while the strip grazing treatment gained 0.26 kg/d (Gertenbach, 1998).

Kikuyu Silage

At Cedara, de Figueiredo (1998) investigated making silage of kikuyu, as a method of conserving summer surpluses. The ideal stage to cut was at 25 cm grass height, at this stage digestibility was not compromised, while the yield and DM content of the herbage were optimised. Kikuyu being very low in carbohydrates (4-6% NSC) required added sugars/molasses at ensiling to ensure good fermentation. It was also found that it was important to wilt the herbage to 30% DM prior to ensiling, due to its low NSC and high protein content (20-30% CP). During the silage fermentation process, half the true protein in the kikuyu was degraded to non-proteinic nitrogen, adding to the high NPN levels often found in high N fertilised kikuyu.

Feeding trials on beef animals showed that unsupplemented kikuyu silage was adequate for maintenance, at best. The addition of a rumen undegradable protein source, viz: 250 g fish meal (the same batch as used in the dairy trials at Cedara), 720 g cottonseed oilcake or 300 g maize gluten meal per day increased growth rates in beef weaners to 600 g/d. Full fat soybeans as a protein source resulted in gains of 365 g/d.

INTAKE & DIGESTIBILITY

Bredon (1980) highlighted the fact that although kikuyu was similar to ryegrass in terms of chemical composition, animal production trials showed that its production was disappointing relative to its digestibility. Bredon (1980) indicated that this problem had been overcome practically in the Cedara Feeding System (Bredon & Hathorn, 1974; Bredon, 1982) by modifying the energy value of kikuyu for estimating milk yield in line with actual milk production. Digestibility trials were initiated at Cedara in an effort to determine the cause of the poor production off kikuyu relative to its chemical composition.

Digestion trials at Cedara Research Station (Dugmore *et al.*, 1986; Dugmore & du Toit, 1988) showed that the relationship between the N and fibre fractions of kikuyu on its digestibility can be contrary to accepted relationships. The generally accepted relationship between the chemical fractions and digestibility is positive for N and negative for fibre. The fraction causing the atypical relationships considered to be the N fraction, with the inverse relationship between N and fibre influencing the relationship between fibre and digestibility. Pattinson (1981), at the Ukulinga Research farm of the University of KwaZulu-Natal, found that fistulated animals on kikuyu selected against herbage with a high CP concentrations and for herbage with a relatively high level of acid detergent fibre (ADF). The dry matter disappearance of herbage samples, collected by oesophageal fistula, from the nylon bags in the rumen was found to increase with increasing fibre content. Pattinson (1981) concluded that the low intake of kikuyu was unrelated to the conventional quality attributes such as CP, ADF and *in vitro* dry matter digestibility (IVDMD). This is in agreement with Austin (1980) who, also at Ukulinga, found that the only plant component in kikuyu associated with acceptability was the N fraction, which was negatively associated with acceptability. Acid detergent fibre was variably associated with acceptability, significantly positive in one trial, but with a slight positive, although non-significant correlation over all trials. Karnezos (1986) found that crude fibre (CF) levels in kikuyu herbage were positively correlated with ADG in steers grazing kikuyu and considered that this positive relationship was possibly associated with herbage availability on Ukulinga. Köster (1992), at the University of Pretoria, found that the cell wall contents of oesophageal extrusa were significantly higher than that of hand cut samples, while the IVDOM and N contents of the oesophageal and hand cut samples were not different for bana, kikuyu and sorghum herbage. Similarly, Dugmore *et al.* (1991) found that steers selected against N (in kikuyu with high N contents >144 g CP/kg DM) and for CF, relative to the herbage on offer at Cedara. The material selected also had a higher digestibility that than on offer.

Lower individual animal performance has been recorded with increased levels of nitrogen fertilization by Tainton *et al.* (1982) and Karnezos *et al.* (1986). Pienaar *et al.* (1993a,b) working both at Ukulinga and Irene, found that the nitrogen content of the leaves of kikuyu was negatively related to voluntary feed intakes. Similarly, Pienaar *et al.* (1993b) found that rumen ammonia levels were negatively associated with feed intake, all other factors, including moisture concentration, showing no significant association with voluntary feed intake. The lack of association between moisture content and voluntary intake found by Pienaar *et al.* (1993b) contrasts with the results obtained by Lesch *et al*, (undated), who cut kikuyu and dried it in a forced draft oven prior to reincorporating it into a 20:1 or 10:1 mixture with fresh (145 g DM/kg) kikuyu of the same stage of re-growth and fed to Merino wethers. Organic matter and CP digestibilities were not changed by the inclusion of the dried kikuyu. Trials were conducted in the early and late season. The inclusion of the dried material increased the voluntary feed intake of the sheep, as indicated in the regression obtained, viz: Voluntary feed intake (% of BW) = 2.06651 + 0.04881 (% DM in herbage).

Henning *et al.* (1995) found that the IVDMD of kikuyu did not differ for re-growths of 15, 30 and 60 days, although milk production was highest ($P<0.05$) for cows grazing on a 30-d rotation, as opposed to 15- and 60-d cycles. Similarly, Brand (1998) at the University of Stellenbosch, found that for kikuyu fertilized with 250 kg N/ha/year, split into three applications, intake was 20% lower for three week re-growth than for six week re-growth when cut and fed to lactating dairy cows and that the comparative intake of dwarf Elephant Grass was 23% greater than the kikuyu. The intake of dwarf Elephant Grass did not differ between the 3-

and 6-week stage of re-growth. It is of interest to note that Brand (1998) found that the dry matter digestibility of the kikuyu (0.7687) was superior to that of the dwarf Elephant Grass (0.6517). The voluntary DMI of dwarf Elephant Grass cut and fed to lactating dairy cows, above a basal 4.5 kg lucerne hay in the diet, was 7.11 kg DM for 3 weeks re-growth and 7.07 kg DM for 6 weeks re-growth, appreciably higher than that of kikuyu at 4.56 kg DM for 3 week re-growth and 5.73 kg DM for 6 week re-growth. Similarly, the milk yields of the cows on dwarf Elephant Grass were higher (24 kg/d) than that of the cows on kikuyu (21.3 kg/d) over the trial period.

Köster (1991) recorded mean organic matter intakes of 7.0 kg OM per mature ox per d over the season, significantly lower than the 8.37 kg OM per day measured for Bana grass, a Pennisetum hybrid, with the intake of kikuyu dropping dramatically in the autumn while that of Bana was at its highest in the autumn. The IVDOM for both the kikuyu and Bana were 64.8 vs 64.6 respectively, indicating that the differences were not attributable to digestibility. Köster (1991) recorded mean DOM intakes of 37.4 g/kg $W^{0.75}$/d for kikuyu relative to 43.8 g/kg $W^{0.75}$/d for Bana over the season.

Henning *et al*. (1995) recorded organic matter (OM) intakes of 14.2 kg OM per day on kikuyu for 550 kg Friesland cows at Bathurst in the Eastern Cape. The intakes measured by Henning *et al.* (1995) were appreciably higher, at *c*. 15.7 kg DM/d (2.8% of BW) than those determined by Hamilton *et al*. (1992) who recorded 12.6 kg DM/d from multi-parous Australian Friesian cows in early lactation grazing kikuyu utilizing the chromic oxide technique, Reeves *et al*. (1996) who recorded 13.7 kg DM per day for unsupplemented cows on kikuyu using the alkane technique, but are similar to those determined by Ketth and Ranawana (1971) of 2.7% of BW for stall fed lactating cows. Granzin (2003) recorded NDF intakes of 1.36 to 1.43% of liveweight on kikuyu and considered that rumen fill may have been the primary factor limiting intake. This is in contrast to Pienaar *et al*. (1993b) who found that rumen volume was not limiting intake in sheep grazing kikuyu.

Numerous other studies, in Australia and Hawaii, have shown similar atypical relationships to kikuyu. Holder (1967) found that the voluntary intake of sheep consuming kikuyu was reduced if the kikuyu was fertilized with N. Similarly, Milford and Minson (1965) found that high levels of N fertilizer negatively affected intake in kikuyu, with the intake of young heavily fertilized pasture containing 200 g/kg CP being 28% less than the intake for the same pasture after growing for a further 28 days and the CP concentrations had fallen to 115 g/kg. Jeffrey (1971b) found that N fertilization also negatively affected the digestibility of kikuyu. Campbell and Ho-a (1971) reported that the digestibilities of the organic matter (OM), crude fibre (CF) and nitrogen free extractives (NFE) were depressed in herbage cut at six-week re-growth stages over the growing season when fertilized with 336 kg N/ha, applied in four equal dressings, as opposed to lower rates of N (0, 112 & 224 kg N/ha). Minson (1973) found that fertiliser nitrogen affected both digestibility and intake over a season. High N fertilization (230 kg N/ha) significantly reduced the digestibility, relative to low N fertilization (57.5 kg N/ha) in mid-season (Feb) but not in the early season (Dec) or autumn (Apr), where the high N level resulted in higher digestibilities. Similarly, the higher level of N fertilizer reduced intake in mid-season (Feb), but increased intake in April.

Kamstra *et al.* (1966) concluded, from digestibility trials, that kikuyu should be allowed to grow out for 6 to 8 weeks and then grazed heavily. Jeffrey (1971b) found that stage of re-growth exerted a significant effect on the DMI and digestibility, with the earliest cut giving the poorest values. Soto *et al.* (1980) found that the VFI of sheep increased with herbage maturity when fed kikuyu of 39, 50 and 78 days re-growth. Castrillon and Montoya (2004), in Columbia, recorded DMI of 3.28 and 2.5 kg/100 kg live-weight of

Holstein cows for 62-d and 24-d re-growths of kikuyu, fertilised with 125 kg N/ha/yr, respectively.

Nitrates

The nitrate concentration of kikuyu increases rapidly with an increase in N concentration in the plant (Whitney, 1974b; Marais 1990b; Reeves *et al.* 1996). Concentrations of nitrate N in high-N kikuyu, recorded at Cedara, may reach 8.0 g/kg DM (Marais, 2001), although le Roux *et al.* (1984) only recorded nitrate levels of 0.7 g/kg at an N fertilization rate of 650 kg N/ha/yr in the Eastern Cape. Nitrate itself is relatively non-toxic, but is readily reduced to ammonia in the rumen with toxic nitrite as an intermediate (Marais 2001). Nitrate derived from high-nitrate pastures markedly reduces digestibility *in vitro* (Marais, 1980; Marais *et al.* 1988). Marais (1980) found that low levels of nitrate in kikuyu stimulated digestibility, with the optimal level being *c.* 1 mg/g. Higher levels of nitrate caused a rapid decrease in digestibility, with a 26% reduction in digestibility at 8.0 mg nitrate-N/g. Marais *et al.* (1988) found that the growth in pure culture of 3 of the 4 major cellulolytic bacteria found in the rumen, *viz. Ruminococcus flavefaciens, Butyrivibrio fibrisolvens* and *Bacteroides succinogenes* was reduced in the presence of nitrate. Nitrate levels, associated with high nitrogen levels in kikuyu may explain much of the negative effect of nitrogen on digestibility, intake and animal performance off kikuyu pasture. Austin (1980) found that nitrate levels in kikuyu were negatively correlated (-0.356; $P \leq 0.01$) to acceptability, as were herbage nitrogen levels, albeit weaker (-0.284; $P \leq 0.05$) indicating that nitrate was the causative agent, nitrate and N being highly correlated. Dugmore *et al.* (1986) found a negative correlation between NO_3-N and digestible organic matter concentration of kikuyu. The relationship between CP and NO_3-N was described as $CP = 13.4 + 5.7\ NO_3\text{-}N$; r = 0.779 (Dugmore *et al.*, 1986). Pienaar *et al.* (1993a) found that the nitrate concentration of kikuyu leaves was described by a quadratic equation, namely; $NO_3\text{-}N = 0.0091 + 0.0667x - 0.003438x^2$, $r^2 = 0.789$, where x represents the number of days of active growth following N fertilization.

FERTILIZATION

Fertilization trials at Cedara found that the kikuyu component of the sward increased in proportion to the level of N fertilization, displacing the pioneer species *Eragrostis plana* and *Sporobolus capensis* (Theron *et al.* 1960), thereby overcoming one of the problems encountered when kikuyu was first introduced at Cedara in 1915. This competitive ability of kikuyu in relation to other grasses by applying nitrogen has also been shown in its native habitat (Morrison, 1966) and in Australia (Gartner, 1966; Mears, 1974; Ivory & Jacobsen, 1977).

In trials conducted at Cedara College of Agriculture, the mean response to nitrogen was 37.5 kg DM/kg/N applied (Theron *et al.*, 1960) and 35 kg DM /kg/ N applied under favourable conditions (Miles, 1998). Kikuyu showed a near linear response to N fertilizer up to 356 kg N/ha. Subsequently, Miles and Manson (2000) showed a near linear response to N fertilizer in the highland sourveld of KwaZulu-Natal up to 400 kg N per ha per annum, a sharply diminishing response to 600 kg N per ha, with little response recorded at levels over 600 kg N per ha. The response to nitrogen fertilizer has also been determined by many research workers world wide (Morrison, 1966; Tamimi *et al.*, 1968; Wright, 1968; Gomide *et al.*, 1969; Campbell *et al.*, 1970; Colman, 1970; Gartner & Everett, 1970; Wetherall, 1970; Whitney, 1970; Jayawardana *et al.*, 1971; Mears, 1974; Mears & Humphreys, 1974a; Whitney, 1974a, 1974b; Kemp, 1975; Murtagh, 1975; Kemp, 1976; Ivory & Jacobsen, 1977) with yield varying from 13 to 43 kg DM/kg N applied, depending on location, soil, season and level of fertilization. Kikuyu fertilized with nitrogen is considered one of the most productive pastures in regions with a humid mesothermal climate (Colman, 1970).

Yields of kikuyu at Cedara peak at 15 000 to 16 000 kg DM/ha/year with an application of 300 kg N/ha/yr. However, under higher rainfall conditions calculated yields of 19 000 kg DM/ha/yr can be expected (Cross, 1978). Cross (1978) derived the following regression equations relating to the growth of kikuyu in the Midlands and South Coast of KwaZulu-Natal. These relationships clearly indicate that water and N fertilization are the main drivers of kikuyu pasture yield.

$$\text{Yield (kg DM/ha)} = 14\,477.93 - 248.512\ N + 1.731\ N^2 - 0.002964\ N^3\ ; \qquad R = 0.94689.$$

$$\text{Yield (kg DM/ha)} = 0.00097\ R^2 + 0.0412\ R.N + 0.065\ N^2\ ; \qquad R = 0.93865.$$

$$\text{Yield (kg DM/ha)} = 621.909 - 6.0113\ R + 49.555\ N + 0.057285\ R.N - 0.12819N^2\ ; \qquad R = 0.93719.$$

where: N = level of nitrogen applied in kg/ha per season,
R = Rainfall in mm from 1 August to 31 April.

Köster (1991), at the University of Pretoria, recorded yields of 20.2 ton DM/ha/yr for irrigated kikuyu, fertilized with 230 kg N/ha over the growing season.

At Cedara, Theron *et al.* (1960) also found that liming kikuyu increased the response to N, particularly at higher levels of N fertilization. Awad *et al.* (1976), in Australia, considered kikuyu sensitive to acidity. Liming of acid soils considerably reduced the nitrogen requirement for maximum yield. Awad and Edwards (1977) found that with liming, nitrogen requirements were reduced from 672 kg N/ha/year to 135 kg N/ha/year to give the same DM yield. In contrast, Miles and Manson (2000) stated that in the midlands of KwaZulu-Natal no growth response to liming was recorded at acid saturations up to 60%. The lower N requirements attributed to liming may be a consequence of the improved N, P and S supply due to accelerated mineralization of the organic matter by soil micro-organisms when lime is applied (Miles & Manson, 2000). However, Miles (1998) advocated liming when acid saturations exceed 40%, in order to improve the Ca levels in the leaves for animal production.

Austin (1980) found that nitrogen fertilization also reduced P levels, but increased K and Ca levels, as well as improving the Ca:P ratio in kikuyu herbage at the Ukulinga Research Farm of the University of KwaZulu-Natal. Austin (1980) found that liming increased the Ca:P ratio, while Ca levels in the herbage were increased by the application of phosphate. This increase in Ca due to P fertilization (Austin, 1980) confirm a similar response determined by Awad *et al.* (1976), who found that increasing levels of soluble P in the soil decreased soluble Al levels in the soil, with soluble Al in the soil negatively associated to Ca uptake by the plant.

MINERAL COMPOSITION

Calcium and phosphorus

Moses (1932) pointed out the low Ca concentrations of kikuyu, relative to other minerals and pasture species. Bredon and Hathorn (1974) highlighted the fact that kikuyu has an inverse Ca:P ratio. Miles *et al.* (1995) recorded low Ca:P ratios in midsummer relative to those recorded in spring and late summer/autumn for Ca and P in the KwaZulu-Natal Midlands at Cedara and Nthabamhlope. Similar trends were established for K/(Ca+Mg) cation ratios (Miles *et al.*, 1995) The low Ca concentration in the herbage in summer arises because plant growth responds more strongly to temperature than Ca uptake (Awad *et al.*, 1979). Kikuyu is unusual among tropical species in that it has a greater P requirement in spring/early summer than when growth is at its peak in mid-summer (Miles, 1986).

Austin (1980) investigating the effects of N, P, Ca and Mg (dolomitic lime) fertilization on the mineral concentrations of kikuyu at Ukulinga, found that Ca in the plant was increased by phosphate fertilization, while N fertilization improved the Ca:P ratio, possibly by reducing the phosphate levels in the herbage. Liming also increased the Ca:P ratio. Nitrogen fertilizer improved Mg levels, but also increased the potassium levels in the herbage. However, the acceptability of the pasture was not affected by any of the minerals recorded (Ca, P, K, Ca & Mg). This is in contrast to Karnezos (1986) who, also at Ukulinga, found that Ca was the only plant fraction that significantly influenced live weight gain in steers. Interestingly, the Ca:P ratio was not significantly related to animal performance (Karnezos, 1986).

The low Ca content of kikuyu is compounded by the accumulation of oxalic acid in kikuyu (Marais, 1990a). Oxalic acid forms complexes with many elements to form oxalates. The Ca in calcium oxalate is poorly available to the grazing herbivore (Barry & Blaney, 1987; Marais, 2001). Oxalates are present in high concentrations in some tropical grasses and have been suggested as a cause of Ca deficiency in Latin America (Kiatoko *et al.*, 1978) and transit tetany in Australia (Minson, 1990). The oxalate concentration in kikuyu is relatively low, ranging from 5 to 20g/kg DM (Barry & Blaney, 1987) and from 3.9 to 24.4 g/kg DM for kikuyu at Cedara (Marais, 2001). Oxalate accumulates during rapid growth, particularly following fertilization with potassium and nitrogen (Jones & Ford, 1972).

Sodium and potassium

Kikuyu is natrophobic, necessitating supplementation with sodium. Bredon (1995) reported symptoms of Na deficiency in dairy cows on Cedara. Miles *et al.* (1995) reported that Na and K concentrations in kikuyu were highly correlated, with Na concentrations extremely low (0.02-0.05 % of DM) with high K levels. Miles *et al.* (2005) found that unpalatable pastures contain very high levels of K and usually (but not always) high N. Similarly, Morton *et al.* (2001) in New Zealand found that bite rate was greatly reduced when cows grazed on spring pasture containing potassium levels above 37 g/kg DM.

The relationship between Na and K appears to be important, particularly for reproduction. Beringer (1988) recommended a K:Na ratio of 20:1 in the ration, with calving interval increasing by 5 days for each 10% increase in K:Na ratio above a ratio of 30. Fulkerson *et al.* (1998) recorded a mean K:Na ratio of 30:1 for kikuyu in Australia. These ratios are lower than those recorded for kikuyu at Cedara (Dugmore *et al.,* 1987; Miles *et al.*, 1995).

Well-established kikuyu pastures in the KwaZulu-Natal Midlands are typified by high K levels, even exceeding 50 g K/kg being recorded by Miles *et al.* (1995) and Miles (1998). High K intake can lead to interference with the uptake of other cations like Ca and Mg leading to metabolic problems (Leaver, 1972), especially when the intake of Na is low (Martens & Rayssiguier, 1980; Beringer, 1988). Kemp and t'Hart (1957) suggested that metabolic problems (grass tetany and milk fever) can be expected if the K/(Ca+Mg) equivalent ratio was greater than 2.2. Miles *et al.* (1995) recorded K/(Ca+Mg) ratios (ion concentrations expressed on an equivalents basis) varying from 2.21 to 3.15 at Cedara and Nthabamhlope. Liming has been advocated as a measure to improve these cation ratios in kikuyu herbage (Miles, 1998).

ANIMAL DISORDERS

Outbreaks of "kikuyu poisoning" have been recorded in South Africa, in the Eastern Cape (van Zyl, 1977), the Western Transvaal (van Heerden *et al.*, 1978) and KwaZulu-Natal (Bryson & Newsholme, 1978). Both the cases in the Western Transvaal and KwaZulu-Natal followed closely after infestation of the pastures by army worms (*Spodoptera exempta*). The outstanding clinical symptoms were; excessive salivation, partial paralysis of the tongue, localized fine muscular tremors, ruminal tympany and stasis and congested or cyanotic mucous membranes. The rumen was well filled with bright green, sloppy, well chewed ingesta. Last (1993) informed KwaZulu-Natal veterinarians and cattle farmers that outbreaks of kikuyu poisoning, unrelated to army worm, had been dealt with by the Allerton Veterinary Laboratories. It was believed that the kikuyu poisoning, unrelated to army worms, was identical to that of the army worm associated syndrome. Last (1993) suggested that the problem arose when drought stricken or over grazed pastures were heavily fertilized with N followed by irrigation, or rain, and heat. Rapid growth of the kikuyu occurred under these circumstances and it was believed that toxic nitrogenous compounds were produced during this period. Dehydration, over distension of rumen with watery contents and sham drinking were commonly presented clinical symptoms. MacFarlane (undated) confirmed these symptoms, suggesting that there was a nitrogen breakdown product in the rumen which was toxic. Army worm may have played a role in damaging the plant, but most outbreaks were not associated with army worm. Predisposing factors were considered to be: climate, usually a dry period followed by wet overcast conditions and rapid growth; fertilization, with a history of N applications a few weeks before outbreaks have occurred and time of year, with most cases were seen from October to December and again in April. In the Eastern Cape, problems with kikuyu poisoning peaked in April, usually occurring after periods of hot wet weather, particularly if following a drought period (van Zyl, 1977). Murtagh (1975b) found that an abrupt transition from dry to wet conditions soon after topdressing enhanced the response to nitrogen fertilization relative to the response during continuing wet conditions, which may be a predisposing factor in the animal health problems discussed.

There have also been reports of animal disorders after kikuyu pastures have been grazed in New Zealand and Australia. However, not all animals in the herd showed symptoms and some affected animals recovered (Quinlan *et al.*, 1975). Clinical symptoms were abdominal distension, incoordination of the hind legs followed by tetany and recumbency, sunken eyes, excessive drinking of water, salivation and death in extreme cases (Cordes *et al.*, 1969). The disorders followed when animals were put onto lush, rapidly growing kikuyu pasture when good rains occurred after a dry spell. In some cases, the paddocks had been invaded a few weeks earlier by army worms or black beetles (Cordes *et al.*, 1969; Healey, 1975). The toxic principle that develops in or on kikuyu has not been identified with certainty, but a fungus has been suspected (Healey, 1975). However, Black (1985) suggested that the toxin production may be by the plant itself, induced by the prior plant damage and the rapid growth phase induced by warm wet conditions. Botha (2010) stated that an endophyte, Fusarium torulosum, has been isolated from kikuyu grass samples collected during an outbreak and this may be causal agent of kikuyu poisoning.

Kikuyu can accumulate toxic levels of nitrate when soil nitrogen levels are high, adequate soil moisture is present and cloudy or cold conditions prevail (Marais, 2001). Pienaar *et al.* (1993a) found indications of saponins in kikuyu and that 30.8% (± 11.1) of the N in kikuyu was soluble, both of which could contribute to bloat.

CONCLUSION

Pattinson (1981) concluded that it appears that the low intake on kikuyu was unrelated to the conventional quality attributes such as CP, ADF & IVDMD and stated that kikuyu seems to be a special case in that the low intakes seem to be contrary to all the quality attributes determined. Similarly, none of the chemical components of kikuyu have proved to be accurate in predicting its nutritive value (Dugmore & du Toit, 1988) or DMI (Meissner *et al.*, 1988). The need to grow out kikuyu for better animal performance may be indicated by the data of Henning *et al.* (1995), where intakes of lactating dairy cows on kikuyu improved from 15 to 30-d re-growths and that of Brand (1998) where the intakes of lactating dairy cows on 42-d re-growths were higher than those on 21-d re-growth kikuyu indicate. Marais *et al*. (1990) speculated on anti-quality factors being formed in kikuyu herbage containing high levels on N.

However, it must be noted that even within South Africa, differences in kikuyu grown in different geographical regions must be considered. For example, kikuyu grown in the Alexandria district of the Eastern Cape responds differently to that in KwaZulu-Natal when analysed by Near Infrared Reflectance Spectroscopy (NIR) and has to be treated as a separate population for calibration and readings (Thurtell, Cedara Feed-laboratory, pers comm.). Geno-environmental interactions may make the results found for kikuyu in the KZN midlands unique to that particular environment.

Chapter 3
The Effect of Nitrogen Fertilization and Stage of Re-growth on the Digestibility and Dry Matter Intake of Kikuyu

Abstract

High levels of nitrogen fertilization have been implicated in nutritional disorders in kikuyu. Kikuyu pasture was fertilized at two levels of N, namely 50 and 200 kg N/ha, to induce low and high levels of nitrogen in the herbage, after mowing and clearing the plots. The subsequent growth was harvested at 20, 30 and 40 days of re-growth. Digestion trials using sheep and voluntary intake trials using long yearling heifers in pens equipped with Calan gates were conducted in the spring, summer and autumn. Nitrogen fertilization level had no significant impact on herbage digestibility. Stage of re-growth did influence digestibility in the spring and summer, with a quadratic response and the highest values recorded in the 30-d treatment. However in the autumn the 20-d re-growth recorded the highest digestibility. Digestibility declined as the season progressed. Digestibility was not correlated to any of the chemical fractions measured in the herbage, including *in vitro* digestibility. Voluntary intake followed a similar trend to digestibility, a quadratic response with peak values recorded for the 30-d treatment in the spring and summer, while the 20-day material recorded the highest intake in the autumn. Nitrogen fertilization, in contrast to its neutral effect on digestibility, had a negative impact on voluntary intake over all seasons. Similarly to digestibility, voluntary intake was not correlated to any of the chemical fractions measured, but was correlated to digestibility and moisture concentration of the herbage. In general, digestibility could not be predicted and the relationship between peak digestibility and stage of re-growth often varied between trials, indicating that other factors such as weather and moisture content affected the response. Similar trends were recorded for voluntary intake.

Key words: *Pennisetum clandestinum*, N fertilization, re-growth stage, digestibility, voluntary intake

INTRODUCTION

There is general concern over the long-term effects of using high rates of nitrogen fertilizer (300 kg N/yr) on soil acidity and possible nutrient imbalances in stock. Ruminant blood Ca and P levels have been reduced after 4 years of high nitrogen application (Ashwood *et al.* 1993).

A consistent factor to emerge in reviewing the literature (ch. 2) on kikuyu is the negative effect of nitrogen on the nutritive value of kikuyu (Milford & Minson, 1965; Holder,1967; Cambell & Ho-a, 1971; Jeffery, 1971b; Minson, 1973; Austin; 1980; Pattinson, 1981; Tainton *et al*, 1982; Karnezos, 1986; Dugmore *et al*, 1986; Dugmore & du Toit, 1988) and the variable effect of stage of re-growth on kikuyu digestibility and intake (Jeffrey, 1971a; Soto *et al*, 1980; Dugmore *et al*, 1986; Henning *et al*., 1995; Brand, 1998).

The lack of data on factors affecting the nutritive value of kikuyu was the motivation for this study to investigate the interaction between the stage of re-growth of kikuyu and nitrogen concentration in the herbage on its digestibility and VFI. As the hypothesis is that high N fertilization levels have a negative impact on animal production, the high N treatment was designed to test the limits of the animals ability to cope with the N and even to induce nutritionally related disorders in the animal. The relationship between the chemical fractions and nutritive value (digestibility & DMI) as well as factors affecting the nutritive value of kikuyu are examined.

MATERIALS AND METHODS

The experimental area is situated at Cedara (29° 32' S; 30° 17' E) in the KwaZulu-Natal Mistbelt. Cedara lies at an altitude of 1067 m and receives an average annual rainfall of 875 ±142 mm, falling predominantly in the summer months. The mean January max/min temperature is 25.3/14.8°C, with frost in winter.

Pasture

Kikuyu pasture at Cedara, established in *c*. 1930, was made available for cutting and feeding to sheep in digestion trials and long yearling cattle in concomitant VFI trials. Each treatment was allocated 10 plots, cut with a reciprocating mower and fertilized and subsequently harvested on consecutive days to allow for a supply of the same stage of re-growth over a 10-d period. Plot sizes were determined following pilot cutting trials to determine DM production for each N level and stage of re-growth. The area of the low N plots was 2.1 ha (1000, 600 & 500 m^2 for the 20-, 30- & 40-d) and 1.25 ha (600, 350 & 300 m^2 for the 20-, 30- & 40-d) for the high N plots. The plots were soil sampled prior to each season to allow for the correction of nutrient deficiencies according to the Cedara fertilizer advisory service recommendations (Manson *et al*., 2010). Nitrogen fertilizer was applied as LAN at two fertilization rates, namely 50 kg N and 200 kg N per ha for each cut to induce both low and high protein levels in the herbage.

Soils

The soils are of the Inanda form and are acid and highly weathered (clayey, kaolinitic, thermic Plinthustalf). Soil pH (KCl) ranged from 3.8 to 4.3. Soil acid saturation (extractable acidity as a percentage of total extractable cations) levels ranged from 10 to 14%. Soil clay ranged from 59 to 67%, with organic C levels exceeding 5%. Soil P (15 mg/l), K (140 mg/l), Ca (1104 mg/l) and Mg (200 mg/l) levels exceeded the target soil test for production (Manson *et al*., 2010).

Preparation of plots

Each treatment was allocated 10 plots, each of which was cut to 3 cm and fertilized on successive days. Plots were subsequently harvested on consecutive days to allow for a supply of material at the same stage of re-growth over a 10-d period. For instance, plot one was cut with a self propelled reciprocating mower and fertilized with N on day 1, plot 2 was cut and fertilized with N on day 2, etc, through to day 10 on which plot 10 was cut and fertilized. For the 20-d treatment, plot 1 was harvested on day 20, plot 2 on day 21, etc, through to plot 10 on day 29 ensuring that 20-d old re-growth was harvested each day. Commencing on day 30 and day 40, the 30- and 40-d the re-growths were harvested following a similar pattern described for the 20-d ones.

Experimental protocol

The nutritive value of kikuyu was obtained according to the experimental protocol in Table 3.1, in which its digestibility, VFI of kikuyu, as influenced by season, N level in the herbage and stage of re-growth was determined using wethers.

Table 3.1 Experimental protocol to determine the nutritive value of kikuyu

Stage of growing season	Nitrogen fertilization level (kg N/ha/cut)	Stage of re-growth (days after cutting and fertilization)		
Spring	50	20	30	40
	200	20	30	40
Mid-summer	50	20	30	40
	200	20	30	40
Autumn	50	20	30	40
	200	20	30	40

Digestion trials

The technique described by Juko *et al.* (1961), using faecal collection bags, was used for the digestion trials. Five Merino wethers, ranging in weight from 25 (at start) to 35 kg at the end of the growing season, were used per digestion trial, after a suitable adaptation period on kikuyu for the growing season. Each trial had a 6-d collection period following a 4-d preliminary, or change over, period between re-growth stages. Fresh green kikuyu was cut each day using a self propelled reciprocating mower, chaffed through a 25-mm screen and fed to appetite, allowing for approximately 20% orts. Samples of the fresh herbage (c. 300 g), orts and faeces were collected daily and dried in a forced draft oven at approximately 70 to 80°C for 24 hours, or until dry. Faeces and orts samples were pooled per sheep per trial, with a 10% sample taken per sheep daily. Dry matter digestibility was determined (Schneider & Flatt, 1975).

Voluntary feed intake

The accurate measurement of VFI of pasture by ruminants can only be achieved in pen-feeding experiments (Minson *et al.*, 1976). Therefore, individual animal intakes were measured using Calan feed gates (Broadbent *et al.,* 1970). Six Hereford or Sussex long-yearling heifers were used per treatment, following a suitable adaptation period, with a 6-d collection period following a 4-d preliminary, or change over, period between re-growth stages. The same group of heifers (n=12) was used for the growing season (Spring to Autumn), but redistributed between trials. The herbage was fed long, following cutting by a reciprocating sickle bar mower at 5-cm grass height, raking and filling wool bales, one per animal, with approximately 50 to 60 kg fresh herbage. Sufficient forage was offered to allow for a 15 to 20% excess, thereby ensuring ad libitum intakes (Minson *et al.*, 1976; Wickes, 1983). Intakes are expressed as percentage of live weight (kg/100kg BW) to standardize and avoid variation in intakes due to differing animal size and animal growth as the season progressed. The heifers were weighed at the commencement of each intake trial and ranged from 250 to 270 kg live weight at the start of the season and ended the season at 327 to 350 kg.

Chemical analysis

Feed samples were analyzed for DM, CP, EE (AOAC, 1980), ADF, ADF-N, NDF-N, (Goering & van Soest, 1970) as modified by van Soest and Robertson (1980), non-structural carbohydrates (NSC) (Marais, 1979), non-protein nitrogen by trichloroacetic acid procedure (Marais & Evenwell, 1983) in the Cedara Feed Laboratory. Gross energy was determined by bomb calorimeter. *In vitro* dry matter digestibility was determined using the Minson and McLeod (1972) modification of the Tilley and Terry (1963) technique. Minerals were determined by dry-ashing samples and atomic absorption spectroscopy using the Hunter method (Hunter, 1975). All batches sampled included a known control sample.

Statistical analysis

Analysis of variance and multiple regression techniques, using Genstat 8.1 (Genstat, 2005), were used to test for significant differences and to quantify the relative contribution of the factors tested to the variance accounted for in the regressions. Data were analysed within season, with N fertilizer level and stage of re-growth as the factors and then pooled over the seasons, if homogeneous, with season added as a factor. Where data sets were unbalanced, the residual maximum likelihood model (REML) of Genstats variance component analysis was used. A statistical model was developed to predict voluntary feed intake and digestibility using multiple regression analysis.

RESULTS

Chemical composition

The chemical components and the effects of season, nitrogen fertilization and stage of re-growth and the presence of interactions are presented in Table 3.2 & 3.3 for year 1 and in Tables 3.4 & 3.5 for year 2. These values fall within the ranges reported in Appendix Tables 2.2 and 2.3.

The N fraction of the herbage was affected by the N fertilization level, the higher level increasing the N concentration of the herbage by *c*. 21%, relative to that of the low N fertilization level. N levels in the herbage decreased linearly with increased stage of re-growth. Season affected N levels quadratically in year 1 (Table 3.3) with an interaction between N level and season recorded, with the least recorded in summer and the greatest in the autumn, spring being intermediate.

The fibre fractions responded differently to the N fertilization level. Acid detergent fibre was not affected by the level of fertilization, while NDF was reduced at the high N fertilization level. Acid detergent fibre levels in the herbage were lowest at the 30-d stage of re-growth, highest at the 20-d stage of re-growth, with the 40-d intermediate. Neutral detergent fibre followed a more traditionally accepted pattern, with the lowest NDF at the 20-d stage of re-growth and the 30- and 40-d re-growth not different. Season influenced the ADF levels, with the greatest values recorded in the summer and the lowest levels in spring, with autumn intermediate (Table 3.3). Neutral detergent fibre levels in the herbage declined over the seasons in year 1, commencing with a spring high, declining through summer to an autumn low (Table 3.3), while increasing from spring to summer in year 2 (Table3.4).

Non-structural carbohydrates were reduced at the higher N fertilization level. Stage of re-growth influenced NSC levels, the least recorded for the 30-d re-growth stage, with the greater levels recorded at the 20- and 40-d re-growth stages. NSC levels were not significantly affected by season. Gross energy content of the herbage was increased at the higher N fertilization level, declined as season progressed from spring through to autumn, but was not influenced by stage of re-growth.

Table 3.2 The chemical composition (g/kg DM) of fresh kikuyu herbage used in the digestion trials, fertilized at two levels of N (50 kg and 200 kg N per cut) and harvested at 20-, 30- and 40-d re-growth in spring, summer and autumn of year 1

Treatment	DDM	DM	ASH	ADF	NDF	CP	N	ADF-N.	NDF-N	NPN.	NSC.	FAT	In vitro	Nitrates
Spring														
high 20	643	200	115.3	387.5	556.2	237.6	38.0	2.5	11.5	14.7	39.0	23.5	772	2.38
low 20	627	205	118.7	390.5	550.4	226.8	36.3	1.7	9.0	14.1	37.3	18.0	623	1.29
high 30	639	171	120.3	317.0	677.5	239.0	38.2	3.2	8.4	16.5	28.3	21.4	758	2.46
low 30	702	193	116.2	308.8	827.4	205.7	32.9	3.3	8.5	15.4	38.0	15.6	697	0.73
high 40	786	240	121.1	311.1	672.3	213.4	34.1	2.6	8.5	16.4	32.9	-*	770	1.46
low 40	773	247	125.6	307.9	676.1	187.4	30.0	2.3	8.0	13.2	35.7	-	649	1.01
Summer														
high 20	608	209	110.3	347.9	663.9	222.4	35.6	2.4	8.8	16.1	32.6	16.8	-*	
low 20	635	230	95.0	358.5	695.4	168.5	27.0	1.8	7.7	12.4	43.9	22.8	-	
high 30	547	134	131.3	354.4	640.2	217.8	34.8	2.0	7.6	15.9	31.1	23.2	-	
low 30	541	144	125.0	338.8	653.1	189.7	30.4	1.9	7.1	12.9	35.7	18.0	675	
high 40	687	191	93.9	383.0	708.2	229.6	36.7	2.4	7.4	13.3	39.8	17.3	559	
low 40	696	216	99.8	381.3	746.6	146.9	23.5	1.8	8.1	10.4	46.2	16.2	603	
Autumn														
high 20	700	241	90.5	330.5	635.1	255.3	40.9	3.6	10.0	17.5	45.2	17.8	603	
low 20	701	235	99.4	398.8	659.8	226.9	36.3	3.0	9.2	15.4	46.5	21.3	626	
high 30	689	283	81.8	349.6	684.1	248.0	39.7	2.9	8.9	16.4	50.4	20.5	570	
low 30	745	261	97.0	323.5	679.0	194.1	31.0	2.2	7.0	14.3	-	19.3	575	
high 40	634	250	85.3	350.9	686.6	225.6	36.1	2.8	8.9	15.2	50.4	16.3	-	
low 40	529	222	119.5	324.8	681.9	177.0	28.3	2.2	7.8	14.9	-	14.6	-	

DM = dry matter, DDM = dry matter digestibility, ADF = acid detergent fibre, NDF = neutral detergent fibre, CP = crude protein, N = nitrogen, ADF-N = acid detergent nitrogen, NDF-N = neutral detergent nitrogen, NPN = non-protein nitrogen, NSC = non-structural carbohydrate, in vitro = in vitro DDM. * samples lost in a move to a new laboratory.

Table 3.3 The redicted means for the main effects, where significantly different (P>0.05), for the chemical components determined for the fresh kikuyu herbage harvested for the digestion trials, fertilized at two levels of N (50 kg and 200 kg N per cut) and harvested at 20-, 30- and 40-d re-growth in spring, summer and autumn of year 1

Component	Main effects								Interactions			
	Season			Nitrogen		Re-growth stage						
	Spr	Sum	Aut	Low	high	20	30	40	S.N*	S.D	N.D	S.N.D
Ash (g/kg DM)	119.5	108.7	87.6	NS		104.3	112.0	99.5	NS	<0.001	NS	<0.001
ADF (g/kg DM)	312.4	360.8	346.3	NS		347.8	328.6	343.2	NS	0.001	<0.001	0.002
NDF (g/kg DM)	696.9	683.4	671.1	697.8	669.8	662.6	693.5	695.3	0.018	<0.001	0.037	<0.001
CP (g/kg DM)	218.8	195.3	221.1	191.4	231.7	222.4	215.7	196.6	0.015	NS	NS	NS
log ADF-N	0.989	0.710	1.006	0.806	0.997	0.928	0.935	0.843	NS	<0.001	NS	NS
NDF-N (g/kg DM)	9.4	7.7	8.6	8.3	8.9	9.7	7.9	8.1	NS	<0.001	NS	NS
NSC (g/kg DM)		NS		40.7	34.5	40.9	33.3	38.6	NS	0.030	NS	NS
GE (MJ/kg DM)	19.53	18.83	18.65	18.86	19.14	NS			NS	0.030	NS	NS
log Ca (% DM)	-1.224	-	1.221	NS		NS			0.29	0.29	NS	NS
log Mg (% DM)	-1.071	-	1.402	0.112	0.219	-	0.129	0.201	NS	0.031	NS	NS
log Na (% DM)	-2.571	-	0.239	NS		NS			0.003	NS	0.055	NS
log P (% DM)	-1.06	-	1.08	0.026	-0.0035	-	0.051	-0.279	<0.001	NS	NS	NS
Cu (mg/kg DM)	11.06	-	9.58	9.97	10.67	NS			NS	NS	NS	NS
Zn (mg/kg DM)		NS		NS		NS			NS	NS	NS	NS
Mn (mg/kg DM)	106.4	-	120.5	NS		NS			NS	0.017	NS	NS
NPN Spr	Combined data not homogenous			15.18	16.89	17.31	15.98	14.82	-	-	0.002	-
Sum				11.93	15.12	14.26	14.45	11.87	-	-	NS	-
Aut				NS		NS			-	-	NS	-

*S = season; N = nitrogen; D = days of re-growth. NS = no significant differences within main effect. ADF = acid detergent fibre, NDF = neutral detergent fibre, CP = crude protein, N = nitrogen, ADF-N = acid detergent nitrogen, NDF-N = neutral detergent nitrogen, NPN = non-protein nitrogen, NSC = non-structural carbohydrate

Table 3.4 The predicted means for the main effects, where significantly different (P>0.05), for the chemical components determined for the fresh kikuyu herbage harvested for the digestion trials, fertilized at two levels of N (50 kg and 200 kg N per cut) and harvested at 20- and 30-d re-growth in spring and summer* in year 2

Component	Main effects						Interactions			
	Season		Nitrogen		Re-growth stage					
	Spr	Sum	Low	high	20	30	S.N*	S.D	N.D	S.N.D
Ash (g/kg DM)	99.1	80.5	NS		95.0	84.7	NS	NS	NS	NS
ADF (g/kg DM)	336.7	352.8	NS		332.4	357.1	<0.001	NS	NS	NS
NDF (g/kg DM)	765.3	842.6	813.5	794.4	788.7	819.2	NS	NS	NS	NS
CP (g/kg DM)	NS		183.5	223.5	220.1	186.6	NS	NS	NS	NS
NPN (g N/kg DM)	9.809	7.807	6.915	10.702	NS		NS	NS	NS	NS
Ca (g/kg DM)	NS		3.237	3.680	NS		0.005	NS	NS	NS
Mg (g/kg DM)	NS		3.983	4.4635	4.105	4.513	0.025	NS	NS	NS
K (g/kg DM)	NS		NS		29.22	23.92	NS	NS	NS	NS
Na (g/kg DM)	NS		0.3619	0.444	NS		NS	NS	NS	NS
P (g/kg DM)	3.182	3.479	3.461	3.20	NS		0.001	NS	0.005	0.038
K/(Ca+Mg)	NS		1.469	1.222	1.533	1.138	NS	NS	NS	NS
Cu (mg/kg DM)	NS		8.286	9.958	NS		NS	NS	NS	NS
Zn (mg/kg DM)	NS		34.17	37.48	NS		NS	NS	NS	NS
Mn (mg/kg DM)	113.5	97.8	101.2	119.1	NS		NS	0.002	NS	NS
Log Fe	6.806	6.160	NS		NS		NS	NS	NS	0.044

*S = season; N = nitrogen; D = stage of re-growth. Predicted means for autumn could not be generated due to missing values. ADF = acid detergent fibre, NDF = neutral detergent fibre, CP = crude protein, N = nitrogen, NSC = non-structural carbohydrate

Table 3.5 The dry matter intake (%BW), DMD and chemical composition (g/kg DM) of the kikuyu herbage fed during the VFI trials in year 2

Season	Treat	Re-growth	DMI	DMD	DM	ash	CP	ADF	NDF	NPN
Spring	High N	20 d	3.10	730	193	120	238	327	747	13.7
	Low N	20 d	3.15	746	210	107	205	318	746	8.5
	High N	30 d	3.09	765	234	102	198	360	772	9.5
	Low N	30 d	3.62	725	237	109	176	344	796	7.5
	High N	40 d	2.80	770	229	87	174	371	789	8.5
	Low N	40 d	3.07	740	226	90	148	345	797	7.4
Summer	High N	20 d	2.19	701	215	97	246	334	817	9.9
	Low N	20 d	2.68	683	203	101	192	353	855	5.9
	High N	30 d	3.24	764	246	84	212	356	843	9.7
	Low N	30 d	3.64	743	239	94	161	368	867	5.8
	High N	40 d	2.20	688	211	87	161	347	*	*
	Low N	40 d	2.72	735	217	88	160	351		
Autumn	High N	20 d	2.80	768	240	117	271	303		
	Low N	20 d	2.97	721	208	118	255	307		
	High N	30 d	1.85	692	155	104	266	331		
	Low N	30 d	2.19	666	141	103	244	343		
	High N	40 d	1.70	669	137	96	203	335		
	Low N	40 d	2.11	642	141	99	212	338		

* samples lost in move to new laboratory; ADF = acid detergent fibre, DMI = dry matter intake, DMD = dry matter digestibility, NDF = neutral detergent fibre, CP = crude protein, N = nitrogen, NPN = non-protein nitrogen, NSC = non-structural carbohydrate

Digestibility

The dry matter digestibility determined at 20, 30 and 40 days of re-growth for two levels of N fertilization are presented in Table 3.6.

Table 3.6 The dry matter digestibility (g/kg) of fresh kikuyu herbage, determined by sheep, for kikuyu fertilized at two levels of N (50 kg and 200 kg N per cut) and harvested at 20, 30 and 40 day re-growth in spring, summer and autumn in year 1

Season	Stage of re-growth (days post cutting and fertilization)					
	Low N			high N		
	20	30	40	20	30	40
Spring	627^{a} ± 41.0	702^{b} ± 38.5	773^{c} ± 8.5	643^{a} ± 28.1	639^{a} ± 38.7	786^{c} ± 16.2
Summer	635^{ad} ± 31.0	541^{b} ± 41.5	696^{c} ± 38.8	608^{a} ± 31.6	547^{b} ± 60.2	687^{cd} ± 34.9
Autumn	701^{a} ± 30.7	745^{a} ± 24.5	529^{b} ± 89.0	700^{a} ± 41.8	689^{a} ± 29.6	634^{a} ± 40.4

^{abc} indicate significant differences at $P \leq 0.05$ within row

Table 3.7 Correlation matrix for the chemical components and digestibility of kikuyu for year 1

	DDM	DM	ASH	ADF	NDF	CP	N	ADF-N	NDF-N	NPN	GE	NSC	FAT
DMD	1.000												
DM	0.613	1.000											
ASH	-0.287	-0.655	1.000										
ADF	-0.202	-0.102	-0.293	1.000									
NDF	0.254	0.086	-0.195	-0.400	1.000								
CP	0.066	0.044	-0.199	0.104	-0.410	1.000							
N	0.066	0.044	-0.199	0.104	-0.410	1.000	1.000						
ADF-N.	0.342	0.247	-0.309	-0.310	0.248	0.634	0.634	1.000					
NDF-N	0.121	0.190	-0.131	0.305	-0.454	0.570	0.570	0.463	1.000				
NPN	0.014	0.105	-0.004	-0.337	-0.186	0.789	0.789	0.708	0.405	1.000			
GE	0.331	-0.105	0.077	-0.189	0.275	0.290	0.290	0.354	0.161	0.220	1.000		
NSC	0.206	0.671	-0.873	0.397	0.152	-0.007	-0.007	0.177	0.228	-0.165	-0.133	1.000	
FAT	0.007	-0.146	0.096	0.288	-0.444	0.269	0.269	-0.058	0.232	0.138	-0.074	-0.182	1

DM = dry matter, DDM = dry matter digestibility, ADF = acid detergent fibre, NDF = neutral detergent fibre, CP = crude protein, GE = gross energy N = nitrogen, ADF-N = acid detergent nitrogen, NDF-N = neutral detergent nitrogen, NPN = non-protein nitrogen, NSC = non-structural carbohydrate.

In the spring N fertilization had no effect on digestibility, however, stage of re-growth exerted a linear effect on digestibility, with digestibility increasing with stage of re-growth (635, 671, & 779 for 20-, 30- & 40-d re-growths respectively), A N by stage of re-growth quadratic interaction was determined for the data, with the high N 30-d re-growth DMD depressed relative to the low N 30-d re-growth (639 vs 702 for the high and low N respectively) and not differing from the high N 20-d re-growth (643 vs 639 for the 20- and 30-d high N re-growths respectively). Similarly, during the summer N fertilization also had no significant effect on digestibility. Stage of re-growth exerted a significant quadratic effect on digestibility, with the 30-d re-growth having the lowest digestibility, 20-d intermediate and 40 day the highest (621, 544 & 692 for 20-, 30- & 40-d respectively). No N by stage of re-growth interactions were found for the summer period. During the autumn N fertilization also had no effect on digestibility, while stage of re-growth influenced digestibility, with the 40-d re-growth digestibility now depressed, contrary to the earlier season values (701, 717 & 581 for 20-, 30- & 40-d respectively). A N by stage of re-growth interaction on digestibility, both linear and quadratic proving significant, was found for the autumn growth. The DMD for the high N 40-d re-growth was not different from the 20- or 30-d high N re-growths, but was higher than the low N 40-d re-growth (634 vs 529 respectively for the high N and low N 40-d re-growth). Season also influenced the digestibility of the kikuyu, with the highest values in the spring, the lowest in the summer and autumn intermediate.

Examination of the correlation matrix for the chemical components and DMD (Table 3.7) indicates that none of the chemical components examined had correlations with digestibility, apart from the DM concentration of the pasture on offer. Further regression analysis, including multiple regression and quadratic combinations, confirmed these non-significant relationships with DMD. The DMD in sheep was only influenced by the moisture concentration of the herbage when fed:

$$\text{DMD} = 409 + 1.16\ (\text{DM}); \qquad R^2 = 0.336; \qquad P < 0.001 \qquad n = 18.$$

Of interest, was that *in vitro* dry matter digestibility was not associated with *in vivo* digestibility, the relationship quantified by the following regression equation:

$$\text{DMD} = 742 - 0.085\ (\text{in vitro DMD}); \qquad R^2 = -0.08; \qquad P = 0.744; \qquad n = 13.$$

Considering the effect of moisture concentration on digestibility, the lack of association between *in vitro* and *in vivo* digestibility could be due to the inhibiting effect of moisture, or an anti-quality factor, in fresh herbage which is eliminated or degraded in the drying process, prior to *in vitro* digestibility determination. The regression of DMD and DM on *in vitro* DMD confirms the assumption that drying influenced the *in vitro* DMD, namely:

$$\textit{In Vitro}\ \text{DMD} = -252.61 + 2.83\ \text{DMD} - 4.95\ \text{DM}; \qquad R^2 = 0.55; \quad P < 0.01; \quad n = 13.$$

The ash concentration of the dried sample used for the *in vitro* analysis had an influence on the *in vitro* DMD, as shown below, although positively correlated and considering ash was not related to DMD *in vivo*.

$$\textit{In Vitro}\ \text{DMD} = 242.36 + 3.795\ (\text{ash}) \qquad R^2 = 0.496; \qquad P < 0.01; \qquad n = 13.$$

The digestibilities, determined over the season, are given in Table 3.8 and the associated VFI's for Year 2 are given in Table 3.9.

Table 3.8 The dry matter digestibility (g/kg) of fresh kikuyu herbage, determined with sheep, for kikuyu fertilized at two levels of N (50 kg and 200 kg N per cut) and harvested at 20, 30 and 40-d re-growth in spring, summer and autumn in year 2

Season	Stage of Re-growth					
	20 day		30 day		40 day	
	low N	high N	Low N	high N	Low N	high N
Spring (Nov-Dec)	746^{ac} ± 18.4	730^{a} ± 12.0	725^{a} ± 26.4	765^{bc} ± 18.8	740^{ac} ± 17.3	770^{bc} ± 16.6
Summer (Jan-Feb)	684^{a} ± 22.0	701^{ac} ± 10.0	743^{b} ± 33.5	764^{b} ± 22.1	735^{bc} ± 35.8	688^{a} ± 13.9
Autumn (Mar-Apr)	721^{ac} ± 37.8	758^{a} ± 31.6	666^{b} ± 36.8	692^{bc} ± 45.7	642^{b} ±38.4	668^{b} ± 32.0

^{abc} indicate significant differences at $P = 0.05$ within row

In Year 2, nitrogen influenced the digestibility of the herbage during the spring, with the high N treatment expressing a higher digestibility (0.7552 vs 0.7373), in contrast to Year 1. Stage of re-growth did not affect the digestibility of the herbage. However, a stage of re-growth by N interaction occurred, with the high N 30- and 40-d herbage having a higher digestibility than the high N 20-d re-growth.

In the summer, in contrast to the spring but similar to year 1, N had no effect on the digestibility of the herbage, while differences were recorded between the stages of re-growth. A quadratic response was determined for stage of re-growth, with the 30-d material higher than the 20- or 40-d re-growths (692, 753 & 711 for the 20-, 30- & 40-d re-growths respectively). A significant interaction between N and stage of re-growth was found, with the high N 30-d digestibility higher than the high N 20- or 40-d material. For the low N treatment, both the 30- and 40-d re-growth were greater than the low N 20-d re-growth.

In the autumn, N fertilization tended to increase herbage digestibility ($P = 0.057$), while re-growth had a linear effect on herbage digestibility. Digestibility declined linearly with increasing stage of re-growth. There was no interaction between N and stage of re-growth for the autumn material as opposed to the spring and summer. Season had an influence on the digestibility of the kikuyu, with spring having the highest digestibility, declining to summer and autumn (746, 719 & 691 for spring, summer & autumn respectively).

The DMD was positively influenced by herbage moisture concentration, as in the preceding trial, and is described by the following equation:

$$\text{DMD} = 535 + 0.90\ (\text{DM}); \qquad R^2 = 0.701; \qquad P = 0.001$$

Voluntary intakes

The voluntary intake of the heifers expressed as percentile of BW is given in Table 3.9. Nitrogen fertilizer level, as a main effect, exerted a impact on VFI over all the seasons, the lower N level associated with higher intake, with predicted means of 3.27 vs 2.99 % BW in spring, 3.02 vs 2.56 % BW in summer and 2.43 vs 2.12% BW in autumn for low and high N levels respectively. The negative trends for N on VFI are in contrast to the effect of N fertilization on digestibility, N having a positive effect on digestibility in the spring and autumn, but a negative effect in the summer. The effect of stage of re-growth as a main effect on VFI is presented in Table 3.10. The effect of re-growth stage on VFI was quadratic, peaking at the 30-d stage of re-growth. No interaction was determined between N and stage of re-growth.

Table 3.9 Voluntary feed intakes (percentage of BW) of fresh kikuyu herbage, determined with long yearling heifers, for kikuyu fertilized at two levels of N (50 kg and 200 kg N/ha per cut) and harvested at 20-, 30- and 40-d re-growth in spring, summer and autumn of Year 2

Season	Stage of Re-growth						*P*
	low N			high N			
	20	30	40	20	30	40	
Spring (Nov-Dec)	3.15 ±0.27	3.62 ±0.43	3.07 ±0.47	3.10 ±0.18	3.09 ±0.36	2.81 ±0.36	N = 0.031 Stage = 0.030
Summer (Jan-Feb)	2.68 ±0.38	3.64 ±0.20	2.72 ±0.26	2.20 ±0.22	3.24 ±0.28	2.21 ±0.37	N ≤ 0.001 Stage ≤ 0.001
Autumn (Mar-Apr)	2.97 ±0.25	2.20 ±0.16	2.11 ±0.16	2.80 ±0.13	1.85 ±0.20	1.70 ±0.17	N ≤ 0.001 Stage ≤ 0.001
Mean	2.93	3.15	2.64	2.70	2.73	2.24	

A re-growth stage of 30 days appears optimum in spring and summer, with a 20 day re-growth the most appropriate in the autumn. In mid-summer no difference was recorded between the 20- and 40-d re-growth stages. It is of interest to note that at peak growth stages, namely mid-summer, a longer grazing interval would appear to be more appropriate, and at the slower growth rates of autumn, shorter grazing intervals are most suitable to maximize intake.

Table 3.10 Predicted means for the main effect of stage of re-growth on voluntary intake (% BW) of long-yearling heifers fed fresh kikuyu forage

Season	Stage of re-growth		
	20 day	30 day	40 day
Spring	3.12^a	3.35^a	2.93^b
Summer	2.47^a	3.44^b	2.46^a
Autumn	2.88^a	2.03^b	1.92^b

^{a},^{b} indicate significant differences at $P \leq 0.05$ within row

Voluntary feed intake as influenced by both DMD and moisture concentration of the herbage are described by the following equations:

$$\text{VFI} = -4.94 + 0.01\ (\text{DMD}); \qquad R^2 = 0.497; \qquad P < 0.001$$

$$\text{VFI} = 0.215 + 0.012\ (\text{DM}); \qquad R^2 = 0.592; \qquad P < 0.001$$

No interaction between DMD and DM as fed were determined.

Apart from the as-fed DM concentration of the herbage, no other correlations with VFI or DMD are found in the correlation matrix (Table 3.11). There are correlations between the ash concentration and CP as well as a negative correlation between ash and ADF in the herbage. Similarly, a negative correlation was found between ADF and CP in the herbage.

Table 3.11 Correlation matrix of the herbage components

	DMI	DMD	DM	ash	CP	ADF	NDF	NPN
DMI	1							
DMD	0.726	1						
DM	0.785	0.847	1					
Ash	0.133	-0.051	-0.141	1				
CP	-0.345	-0.157	-0.346	0.666	1			
ADF	0.190	0.115	0.223	-0.740	-0.702	1		
NDF	0.024	-0.299	0.406	-0.579	-0.276	0.590	1	
NPN	-0.216	0.184	-0.385	0.443	0.747	-0.464	-0.625	1

ADF = acid detergent fibre, DM = dry matter, DMD = dry matter digestibility, DMI = dry matter intake, NDF = neutral detergent fibre, CP = crude protein, NPN = non-protein nitrogen

DISCUSSION

Overall, a four-fold increase in nitrogen fertilization caused a 21% increase in herbage CP, with a similar increase of 22% in the herbage NPN levels. Nitrogen fertilization also impacted negatively on the NSC concentration of the herbage, reducing NSC by approximately 15 %. NDF, but not ADF, was also influenced by nitrogen fertilization, with high N fertilization decreasing the NDF concentration of the herbage by *c.* 2%. The effects of increased N fertilization on the N fractions are similar for CP, but not for NPN, to those determined by van der Merwe (1993) for annual ryegrass fertilized with the same levels of N fertilizer, also at Cedara. Van der Merwe (1993) recorded a 23.4% increase in CP and a 54.7% increase in NPN due to the greater level of fertilizer. However, no differences were recorded for NDF concentration, while NSC was decreased by 22.7 % at the higher level of N, possibly due to the greater levels of NSC in ryegrass, for van der Merwe's (1993) data. It is of interest to note that the same levels of N fertilization resulted in 40 to 50% higher protein levels in annual ryegrass, relative to kikuyu, possibly explaining the higher proportional increase in NPN in ryegrass.

In terms of VFI, there was a linear decline over season from spring to autumn. However, the high intakes of *c.* 3.5% of BW in spring and summer are very good and the *c.* 2% of body weight in autumn compare relatively well to the 2 to 2.5 % of live weight suggested as expected intakes by Ketth and Ranawana (1971), the 94 g/kd $W^{0.75}$ (2.97 % BW) by Mann and Stewart (2003) for Friesland bulls fed kikuyu or the 94 g/kd $W^{0.75}$ (2.97 % BW) for Friesland heifers grazing kikuyu (Fushai, 1997). However, Fushai (1997) recorded increased VFI for Hereford heifers of 113 g/kd $W^{0.75}$ (3.57 % BW) relative to the Friesland heifers on the same pasture using *n*–alkanes to determine intake.

Stage of re-growth had a quadratic influence on intake, with the 30 day re-growth generally producing the highest intakes in spring and summer, with the 20 day the highest in the autumn. These higher intakes at more mature stages of re-growth are consistent with the findings of Jeffrey (1971b) who, using sheep, found maximum intake at 37-d re-growth. Henning *et al.* (1995) recorded greater intakes for dairy cows grazing kikuyu on a 30-d rotation relative to those on 15- and 60-d rotations, while Brand (1988) found dairy cows fed 6-w re-growth had higher intakes than cows fed 3-w kikuyu re-growth. Pattinson (1981) also noted a low VFI of lush autumn kikuyu. Similarly, Johnson *et al.* (1968) found that DMI for *Panicum maximum* was not related to maturity.

Nitrogen fertilization had a negative effect on VFI. This is consistent with the conclusions of Pienaar *et al.* (1993b) who found that the N concentration of kikuyu leaves was negatively related to VFI and also that a high soluble N concentration in kikuyu limited VFI. Van Niekerk *et al.* (2002) similarly found that N fertilization resulted in lower intakes in *Panicum maximum* c. Gatton, and concluded that it may have been a result of an imbalance between rumen ammonia production and the availability of carbohydrates in the rumen. It must be born in mind that a high N in the herbage, with high rumen ammonia levels, may lead to an imbalance between N and soluble carbohydrate causing a negative effect on animal production as insufficient microbial protein may reach the small intestine because of extensive N losses occurring in the rumen via NH_3-N (Aii & Stobbs, 1980). A severe protein to energy imbalance has been identified in kikuyu, caused by a lack of readily digestible energy in the form of non-structural carbohydrates (Joyce, 1974; Mears & Humphreys, 1974a). The optimal non-structural carbohydrate to degradable protein ratio in the diet for rumen microbial synthesis is about 2:1 (Hoover & Stokes, 1991) and even up to 4:1 (NRC, 2001) in dairy diets. A ratio of only 0.6:1 was obtained for kikuyu (Fulkerson, *et al.*, 1998).

The voluntary intakes of kikuyu were correlated to the DDM concentration of the herbage for the present data. However, Milford and Minson (1965) concluded that intake and digestibility are not closely related in tropical forages, as in temperate species, and suggested that VFI may the most useful criterion for assessing the nutritive value of tropical species.

The non-significant relationship between *in vivo* and *in vitro* digestibility measured for these data may be atypical, but is not unique for tropical grasses. Olubajo *et al.* (1974) studying four tropical grass species in Nigeria, found no relationship between *in vivo* and *in vitro* digestibility and considered that the discrepancies between *in vivo* and *in vitro* digestibilities reflect a problem in understanding and evaluating tropical forages which had little parallel in temperate regions. Butterworth and Diaz (1970) concluded that correlations between chemical parameters and digestibility point out interesting features that contradict the generally conceived pattern of relationships between fibre components and digestibility in tropical grasses. Poor correlations between the chemical fractions in tropical herbage and digestibility have been reported (Jarrige & Minson, 1964; Sullivan, 1964; Minson, 1971). Milford (1960) and Butterworth (1967) commented on the fact that the fibre content of sub-tropical and tropical grasses were not related to their nutritional values. Locally, work by Pattinson (1981), Karnezos (1986), Dugmore (1985) and Dugmore and du Toit (1988) have also shown a lack of association between the traditional measures for quality in kikuyu and its nutritive value.

The lack of association between ADF and NDF and digestibility, as well as all the other chemical components, for these data are not necessarily unique for a tropical grass, considering the preceding discussion and are consistent with the findings of Boval *et al.* (2007) who found no significant correlations between ADF and NDF on intake or digestibility in a tropical pasture. Similarly, while kikuyu maintains high CP levels compared to other species (Milford & Haydock, 1969), it does not have a similarly higher feeding value (Jeffrey, 1971).

Herbage DM concentration, as fed, was positively associated with VFI, while high N fertilization was negatively associated with VFI. Other classically important factors, such as ADF and NDF, had no effect on VFI. Similarly, the DM concentration of the feed had an impact on digestibility. Deinum (1966) using perennial ryegrass found that high levels of water decreased herbage DM concentration and depressed the digestibility of herbage relative to dry treatments in pot experiments. Similarly, Snaydon (1972) found that the digestibility of lucerne was greater when water supply was restricted. Butterworth *et al.* (1961) found that the digestibility of Pangola grass in the wet season had a lower digestibility than in the dry season. Grant *et al.* (1974) found that wilting Napier grass markedly increased intake and digestibility, while Wilson (1981) concluded that in tropical species moisture stress increased digestibility relative to well watered pasture. The impact of herbage moisture content is discussed in more detail in chapter 7.

In conclusion, the high N treatment was sufficiently high enough to induce alkalosis in steers on concomitant digestibility trials in year one. In spite of the high levels of N fertilizer, the N fertilization level did not impact on the digestibility of kikuyu. However, herbage N and NPN, which are also affected by temperature, rainfall, sunlight or overcast conditions, had negative effects on digestibility and intake. Other chemical fractions in the herbage exerted minimal effect on digestibility and nutritive value. The effect of herbage moisture concentration was, surprisingly, the dominant factor influencing both digestibility and intake of kikuyu herbage negatively.

Chapter 4

The Effect of Liming and Stage of Re-growth on the Digestibility, Dry Matter Intake and Mineral Composition of Kikuyu

Abstract

Kikuyu pasture is known to be Ca deficient, particularly on acid soils, therefore kikuyu was fertilized with 4 ton lime/ha, spread on the soil surface to evaluate the effect of liming on the nutritive value of kikuyu. Herbage was harvested at 20-, 30- and 40-d of re-growth in the spring, summer and autumn. Digestion trials using sheep and voluntary intake trials using long yearling heifers in pens equipped with Calan gates were conducted on the cut herbage. Stage of re-growth influenced digestibility in the spring and summer. However, in the autumn, the 20-d re-growth recorded the highest digestibility. Digestibility declined as the season progressed. Digestibility was not correlated to any of the chemical fractions measured in the herbage, but was negatively correlated to the moisture concentration of the herbage. Voluntary intake followed a similar trend to digestibility, a quadratic response with peak values recorded for the 30-d treatment in the spring and summer, while the 20-d material recorded the highest intake in the autumn. Voluntary intake was not correlated to any of the chemical fractions measured, but was positively correlated to digestibility and negatively correlated to the moisture concentration of the herbage. The application of lime to the pasture neutralized the negative effects of soil acidity, improved herbage Ca levels, improved digestibility in the autumn but not voluntary intake. In general, digestibility could not be predicted and the relationship between peak digestibility and stage of regrowth often varied between trials, indicating that other factors such as weather and herbage moisture concentration affected the response. Similar trends were recorded for voluntary intake. Supplementation of Ca through a mineral lick or balanced concentrate is advocated in preference to liming to improve mineral balances in the herbage.

Key words: Pennisetum clandestinum, lime, chemical composition, minerals, re-growth stage

INTRODUCTION

Concern has been expressed over the long-term effects of using high rates of N fertilizer (300 kg N/ha/yr) on soil acidity. The adverse effects of soil acidity on kikuyu have been demonstrated by Awad (1976), Awad and Edwards (1977). The relatively low Ca levels in tropical grasses and the high levels of oxalic acid found in some tropical species suggest that Ca availability may limit forage quality (Norton, 1982). The uptake of Ca and Mg is also restricted in acid soils with very high levels of soluble aluminium (Duncan, 1999). In fact, reduced blood Ca and P levels have been demonstrated in cattle after 4 years of high N application (Ashwood *et al.,* 1993).

Deficiencies or imbalances in minerals such as Ca, P, S, Na, Mg and certain of the trace-elements may alter ruminal function, digestibility and intake of animals (Reid & Jung, 1982). Minson (1990) reported increased VFI of tropical grasses due to Ca supplementation, while Karnezos (1986) reported a positive relationship between the Ca concentration of kikuyu grass and live weight gain in steers. On the other hand, liming may increase the concentration and availability of minerals, increase digestibility, reduce excessive levels of minerals, and reduce other anti-nutritional factors that could be restricting intake and animal production (Richardson *et al.*, 2002). Miles (1986) noted an improvement in mineral balances due to liming. This study will examine the effect of liming on the digestibility, VFI and mineral balances of kikuyu grass at a high N fertilization level for the three stages of re-growth, namely, 20-, 30- and 40-d after cutting and fertilizing.

MATERIALS AND METHODS

The experimental area is situated at Cedara (29° 32' S; 30° 17' E) in the KwaZulu-Natal Mistbelt. Cedara lies at an altitude of 1067 m and receives an average annual rainfall of 875 ±142 mm, falling predominantly in the summer months. The mean long term January maximum/minimum temperature is 25.3/14.8 °C, while frosts occur during the winter.

Soils

The soils are of the Inanda form and are acid and highly weathered (clayey, kaolinitic, thermic Plinthustalf). Soil pH (KCl) ranged from 3.8 to 4.3. Soil acid saturation (extractable acidity as a percentage of total extractable cations) levels ranged from 10 to 14%. Soil clay ranged from 59 to 67%, with organic C levels exceeding 5%. Soil P (15 mg/l), K (215 mg/l)), Ca (900 mg/l) and Mg (250 mg/l) levels exceeded the target soil test for production (Manson *et al*., 2010).

Pasture

Before commencing the study, plots were soil sampled prior to each season to allow for the correction of nutrient deficiencies according to the Cedara fertilizer advisory service recommendations (Manson *et al*., 2010). A pilot cutting trial was conducted to determine DM production for each stage of re-growth within a lime level in order to determine the plot sizes needed for these studies. An area of 1.25 ha was used for the liming treatment and a further 1.25 ha for the control treatment. Plot sizes were 600, 350 and 300 m^2 for the 20-, 30- and 40-d respectively. Nitrogen fertilizer was applied as LAN at 200 kg N per ha for each trial (Spr, Sum & Aut) to ensure good pasture yields. The same re-growth of kikuyu pasture was cut and fed to sheep in digestion trials and to long yearling cattle in a VFI trial.

Preparation of plots

Each treatment was allocated a block with10 plots with low and high N separated to avoid cross over effects, each of which was cut to 3 cm and fertilized on successive days. Plots were subsequently harvested on consecutive days to allow for a supply of material with the same stage of re-growth over a 10-day period. For instance, Plot one was cut with a self propelled reciprocating mower and fertilized with N on Day 1, Plot 2 was cut and fertilized with N on Day 2, etc, through to Day 10 on which Plot 10, was cut and fertilized. For the 20-d treatment, Plot 1 was harvested on Day 20, Plot 2 on Day 21, etc, through to Plot 10 on Day 29 ensuring that 20-d old re-growth was harvested each day. Re-growths belonging to the 30- and 40-d treatments were subsequently fertilized and harvested following the same procedure.

Liming

The trial plots were cut and fertilized as usual, following the application earlier in the season of 4 ton dolomitic lime per ha as a top-dressing to half the plots. Fertilization and liming was by hand with distribution of previously weighed amounts to each plot. Only two cuts were done during this season, commencing later and ending earlier than previously to ensure sufficient availability of material from the restricted area. The trial was repeated for a second season in the following year, without a further lime application.

Digestion trials

The technique described by Juko *et al.* (1961) was used for the digestion trials. Five wethers, ranging in weight from 25 to 35 kg over the growing season, fitted with faecal bags were used per digestion trial after a suitable adaptation period on kikuyu. Each trial had a 6-d collection period following a 4-d preliminary or change over period between re-growth stages. Fresh green kikuyu was cut each day, chaffed through a 25-mm screen and fed to appetite, allowing for approximately 20% orts. Samples of the fresh herbage, orts and total faeces were collected and dried in a forced draft oven at approximately 70 to 80 °C for 24 hours, or until dry.

Voluntary feed intake

The accurate measurement of VFI of pasture by ruminants can only be achieved in pen-feeding experiments (Minson *et al.*, 1976). Therefore, individual animal intakes were measured using Calan feed gates (Broadbent *et al.,* 1970). The heifers were weighed at the commencement of each intake trial; they ranged from 200 to 300 kg live weight at the start of the season and from 300 to 360 kg at the end of the season. Six Hereford or Sussex long-yearling heifers used per treatment were adapted to kikuyu pasture for a minimum of 21 days. Following a 4-day preliminary or change over period between re-growth stages, VFI was determined for six days by collecting and weighing orts. The feed was fed long, following cutting by a reciprocating (sickle bar) mower at 3-cm grass height, raking and filling wool bales, one per animal, with approximately 50 to 60 kg fresh herbage. Sufficient was offered to allow for a 15 to 20% excess, thereby ensuring *ad-libitum* intakes (Minson *et al.*, 1976; Wickes, 1983). Intake was expressed as percentage of live weight (kg/100 kg BW) to standardize and avoid variation in intakes due to differing animal size, and growth as the season progressed.

Chemical analysis

Feed samples were analyzed for DM, CP (AOAC, 1980), ADF, NDF (Goering & van Soest, 1970) as modified by van Soest and Robertson (1980), NSC (Marais, 1979), NPN by trichloroacetic acid procedure (Marais & Evenwell, 1983) in the Cedara Feed Laboratory. Minerals were determined by dry ashing samples and atomic absorption spectroscopy using the Hunter method (Hunter, 1975).

Statistical analysis

Analysis of variance and multiple regression techniques, using Genstat (Genstat, 2005), were used to test for significant ($P \leq 0.05$) differences and to quantify the relative contribution of the factors tested to the variance accounted for in the regressions. Where data sets were unbalanced, the residual maximum likelihood model (REML) of Genstats variance component analysis was used. A statistical model was developed to predict VFI and digestibility using multiple regression analysis.

RESULTS

Chemical composition

The effect of liming on the chemical composition is presented in Table 4.1. The predicted means ($P<0.05$) for the main effects of liming and stage of re-growth over the season, for the year subsequent to the application of lime are presented in Table 4.2. Liming had no effect on any of the major parameters measured during the year of liming. In the year subsequent to liming, liming increased the Ca concentration of the kikuyu in summer, Na in autumn and P in spring. The K concentration of the herbage was increased by liming in both the spring and summer. Non-structural carbohydrates were not affected by liming, but declined dramatically in the autumn re-growths. Stage of re-growth did not influence the NSC concentration of the herbage. Crude protein also increased in the autumn re-growths, possibly affecting the NSC values negatively. Season did not influence ADF levels, but the summer material had the highest NDF concentration. The ionic balance of K/(Ca + Mg) declined in autumn after peaking in mid-summer. The non-limed treatment recorded the lowest ionic ratio. However, liming did improve the Ca concentration of the herbage.

Table 4.1 The chemical composition (g/kg DM for the chemical fractions and macro minerals and mg/kg for trace minerals) of fresh kikuyu herbage used in the digestion trials, limed (4 t/ha, applied in year 1) and unlimed and harvested at 20-, 30- and 40-d re-growth's

Season	Days	Treatment	Ash	CP	NPN	ADF	NDF	NSC	Ca	Mg	K	Na	P	K/(Ca+Mg)*	Zn	Cu	Mn	Fe
Year 1																		
Spring	20	limed	120.7	234.3	8.3	311.9	707.2	#	2.9	3.5	37.1	0.3	3.4	2.2				
	20	unlimed	127.0	232.7	7.6	303.9	688.3		2.8	3.4	38.3	0.3	3.6	2.3				
	30	limed	101.4	208.6	6.6	330.7	710.2		3.2	4.1	29.6	0.7	3.3	1.5				
	30	unlimed	98.5	212.3	7.0	325.7	710.8		2.9	3.9	28.7	0.6	3.3	1.6				
	40	limed	104.3	170.8	5.1	343.9	713.6		2.4	3.5	31.0	0.9	2.5	1.9				
	40	unlimed	84.4	175.1	4.9	352.2	733.9		2.5	4.1	22.6	0.9	2.5	1.3				
Autumn	20	limed	87.9	200.2	7.9	306.2	695.7	49.9	#									
	20	unlimed	83.3	211.6	9.1	325.2	692.2	52.1										
	30	limed	68.5	184.4	10.9	347.8	764.9	53.1										
	30	unlimed	58.2	184.0	5.7	358.6	779.1	45.9										
	40	limed	72.4	164.1	5.7	355.4	723.3	50.0										
	40	unlimed	55.0	164.9	5.2	380.5	758.7	49.9										
Year 2																		
Spring	20	Limed	110.3	240.0	11.2	296.9	641.0	38.5	2.98	3.59	34.21	0.30	3.17	1.97	32.27	8.92	79.60	2536.64
	20	Unlimed	87.5	252.3	11.4	305.8	646.8	40.3	2.81	3.41	28.84	0.29	3.27	1.76	35.00	10.22	105.70	693.90
	30	Limed	76.9	206.8	7.7	317.3	672.5	48.2	3.33	4.09	21.84	0.81	2.93	1.12	30.83	9.90	87.83	821.06
	30	Unlimed	70.4	228.4	11.0	298.5	672.5	50.9	3.14	3.75	20.81	0.44	2.91	1.16	32.88	11.29	98.69	569.33
	40	Limed	86.5	196.7	11.4	341.1	695.8	49.3	2.41	3.30	27.55	0.22	2.39	1.82	29.41	13.66	94.32	729.62
	40	Unlimed	71.3	200.5	9.0	337.4	714.4	52.0	2.79	3.94	18.79	0.18	2.40	1.05	32.28	16.65	112.30	552.46
Summer	20	Limed	101.8	242.7	11.0	303.2	641.7	38.0	3.19	3.17	33.87	0.29	3.16	2.06	28.26	11.85	99.81	596.94
	20	Unlimed	89.6	233.6	12.7	324.0	691.1	45.3	3.08	3.76	29.67	0.24	3.11	1.69	31.46	9.96	126.21	547.25
	30	Limed	88.4	223.5	10.2	330.3	702.5	43.3	3.14	3.49	26.97	0.20	2.66	1.55	29.85	8.81	102.55	871.66
	30	Unlimed	89.1	237.9	11.2	331.9	718.1	44.3	2.67	3.09	27.54	0.22	2.66	1.83	34.08	9.06	146.14	871.11
	40	Limed	82.9	184.4	9.8	321.4	697.7	54.0	2.33	3.19	23.19	0.32	2.10	1.58	31.41	7.55	119.51	889.09
	40	Unlimed	81.1	193.9	9.7	337.4	713.9	50.3	2.27	3.12	18.67	0.34	2.04	1.30	34.08	21.01	118.13	894.01
Autumn	20	Limed	95.0	267.2	10.3	290.9	652.4	42.7	3.96	4.03	25.30	0.40	2.77	1.26	32.67	10.71	117.94	1360.03
	20	unlimed	91.7	271.8	10.2	306.2	673.2	29.6	3.65	3.50	17.62	0.23	2.73	0.96	30.70	12.18	155.33	973.64
	30	limed	79.2	257.7	10.8	315.8	678.4	22.5	3.03	4.08	16.20	0.25	2.95	0.85	33.91	9.95	103.47	532.10
	30	unlimed	87.9	244.1	10.0	331.3	686.4	30.1	3.13	3.78	19.88	0.20	2.52	1.10	37.75	12.41	208.15	1299.02
	40	limed	92.8	266.5	11.1	337.5	662.3	19.0	2.93	4.03	25.27	0.26	2.65	1.37	33.81	10.76	135.47	565.13
	40	unlimed	73.8	266.2	12.0	331.6	681.4	24.0	2.78	3.85	20.73	0.20	2.60	1.17	33.39	10.45	149.63	549.29

* ionic balance; # samples lost in a laboratory move; ADF = acid detergent fibre, NDF = neutral detergent fibre, CP = crude protein, NPN = non-protein nitrogen, NSC = non-structural carbohydrate

Table 4.2 The predicted means for the main effects, where different ($P<0.05$), for the chemical components determined for the fresh kikuyu herbage harvested for the digestion trials, limed and unlimed, and harvested at 20-, 30- and 40-d re-growth's in spring, summer and autumn in the season following liming

Component	Main effects								Interactions			
	Lime		Season			Re-growth stage						
	Limed	unlimed	Spr	Sum	Aut	20	30	40	S.D	S.L	D.L	S.D.L
Ash (g/kg DM)	90.41	82.49	83.82	88.8	86.73	95.98	81.99	81.38	0.03	NS	0.01	NS
ADF (g/kg DM)	NS		NS			304.5	320.8	334.4	0.023	NS	NS	NS
NDF (g/kg DM)	671.7	688.8	674.2	694.2	672.4	658	688.4	694.3	0	NS	NS	NS
CP (g/kg DM)	NS		219.5	219.3	262.3	250	233.1	218.1	0	0.032	NS	0.016
NPN (g/kg DM)	NS		NS			NS			0.01	NS	NS	0.001
NSC (g/kg DM)	NS		46.5	45.86	27.97	NS			0	NS	NS	0.001
P (g/kg DM)	2.752	2.67	2.844	2.619	2.679	3.033	2.77	2.33	1	NS	NS	NS
K/(Ca+Mg)	1.51	1.336	1.481	1.671	1.118	1.618	1.271	1.382	0	NS	0	NS
Zn (mg/kg DM)	31.4	33.54	32.18	31.52	33.7	31.8	33.2	32.39	0.01	NS	NS	NS
log Fe	NS		NS			6.794	6.562	6.44	0	0.039	0.026	NS
Ca: Spr	NS		Combined data not homogenous over season			2.892	3.233	2.602	-	-	NS	-
Sum	2.886	2.674				3.133	2.907	2.299	-	-	NS	-
Aut	NS					3.81	3.08	2.85	-	-	NS	-
Mg: Spr	NS		Combined data not homogenous over season			3.504	3.92	3.619	-	-	0	-
Sum	NS					NS			-	-	NS	-
Aut	NS					NS			-	-	NS	-
K: Spr	27.87	22.81	Combined data not homogenous over season			31.52	21.32	23.17	-	-	0.033	-
Sum	28.01	26.43				31.77	27.25	22.64	-	-	0.015	-
Aut	NS					NS			-	-	NS	-
Na: Spr	NS		Combined data not homogenous over season			0.2934	0.4579	0.199	-	-	NS	-
Sum	NS					0.2664	0.213	0.331	-	-	NS	-
Aut	0.304	0.212				0.319	0.223	0.232	-	-	NS	-

ADF = acid detergent fibre, NDF = neutral detergent fibre, CP = crude protein, NPN = non-protein nitrogen, NSC = non-structural carbohydrate

Digestibility

The results of the digestion trials for the limed and unlimed kikuyu are given in Table 4.3.

Table 4.3 The digestibility of fresh kikuyu herbage, determined with sheep, for limed and unlimed kikuyu, fertilized 200 kg N/ha/season and harvested at 20-, 30- and 40-d re-growth

Season	Stage of re-growth					
	limed			Unlimed		
	20	30	40	20	30	40
Spr '90	694^{a}	621^{b}	774^{c}	674^{a}	580^{d}	672^{a}
(Dec)	± 22.67	± 39.93	± 21.59	± 18.24	± 36.75	± 23.68
Aut '91	553^{a}	543^{a}	522^{a}	545^{a}	521^{a}	411^{b}
(Feb-Mar)	± 34.23	± 19.42	± 25.14	± 37.79	± 9.62	± 35.17
Spr '91	682^{a}	664^{a}	584^{b}	659^{a}	650^{a}	598^{b}
(Nov-Dec)	± 25.54	± 14.42	± 37.46	± 12.95	± 9.89	± 18.54
Sum '92	573^{a}	549^{a}	488^{b}	593^{a}	$525^{a\ b}$	$535^{a\ b}$
(Feb-Mar)	± 45.40	± 19.25	± 49.51	± 19.71	± 40.05	± 23.45
Aut '92	593^{a}	519^{b}	557^{ab}	569^{ac}	540^{bc}	578^{ac}
(Mar-Apr)	± 33.46	± 45.76	± 55.56	± 34.53	± 38.68	± 21.46

^{abc} = significant differences within season

Liming improved the digestibility (696 vs 642 g/kg DM) of the kikuyu in the spring of the year lime was applied. Stage of re-growth also exerted an impact on digestibility, with the 40-d re-growth having the greatest digestibility overall. However, it was the limed 40-d treatment in the spring that had the highest digestibility. The 30-d re-growth had the lowest digestibility, although the limed treatment was better than the unlimed treatment. During the autumn of the year lime was applied, liming increased digestibility (539 vs 493 g/kg DM), whilst the digestibility decreased with the stage of re-growth.

Liming had no effect on the digestibility of the kikuyu in the spring herbage in the year subsequent to liming. Stage of re-growth had an effect on digestibility; the 20- and 30-d re-growths, whether limed or unlimed, did not differ from each other. The 40-d re-growths, both limed and unlimed, had lower digestibility than the other re-growths. Liming similarly exerted no effect on the summer re-growths, but the 20- day herbage had greater digestibility than the others. In the autumn, liming had no effect on digestibility, while the limed 30-d re-growth had a lower digestibility than the 20- (limed and unlimed) and 40-d (unlimed) re-growths. The stage of re-growth did not affect the digestibility for the unlimed treatments.

Overall, the DMD of the limed and unlimed treatments were influenced by moisture concentration. A regression analysis of the data showed that the digestibility of the herbage was dependant upon the moisture concentration of the herbage, DMD increasing with increasing DM concentration of the herbage within the range of the recorded values. The regression is as follows:

$$\mathrm{DMD} = 28.03 + 4.767.\mathrm{DM} - 0.0086.\mathrm{DM}^{2;} \qquad R^2 = 0.66; \qquad P < 0.0001 \qquad n = 31$$

The DM concentration of the herbage is given in Table 4.4.

Table 4.4 The dry matter concentration (g/kg) of the herbage harvested for the intake trials

Treatment	Early season			Late summer		
	Stage of re-growth			Stage of re-growth		
	20	30	40	20	30	40
Year 1						
Limed	174	160	238	143	173	160
Unlimed	191	163	192	150	184	175
Year 2						
Limed	138	168	175	150	153	169
Unlimed	134	177	192	164	153	173

Voluntary intake

The VFI of the limed and unlimed kikuyu is presented in Table 4.4. Surprisingly, considering that liming influenced the digestibility of the herbage, and that VFI was correlated to DMD, liming had no effect on VFI in the year of liming. However, for the year of liming, stage of re-growth influenced intake in that the 40-d material, both limed and unlimed, recorded greater intakes than the other treatments in the early season herbage, indicating other factors influenced VFI. In the year subsequent to liming, the 30-d limed treatment had a greater intake than the unlimed. Stage of re-growth had no effect on the early season material. However, for the late season material, the 20-d VFI had a greater intake than the 30- or 40-d material. Voluntary intakes were correlated to the DMD of the herbage and the DM concentration of the herbage, with CP showing a weak negative trend, as indicated in the following regressions:

VFI (% BW) = -0.178 + 0.0037.DMD; $R^2 = 0.417$; $P < 0.001$

VFI (% BW) = 0.64 + 0.015 DM; $R^2 = 0.521$; $P < 0.001$

VFI (% BW) = -1.1 + 0.002.DMD + 0.011.DM; $R^2 = 0.60$; $P < 0.001$

VFI (% BW) = 3.28 - 0.0066.CP; $R^2 = 0.08$; $P < 0.10$

Table 4.5 The voluntary feed intakes (DM, as a % of BW) of fresh kikuyu herbage by long yearling heifers, for limed and unlimed kikuyu pasture, fertilized with 200 kg N/ha/cut and harvested at 20-, 30- and 40-d re-growth in spring and late summer

Treatment	Early season			Late summer		
	Stage of re-growth			Stage of re-growth		
	20	30	40	20	30	40
Year 1						
Limed	Insufficient Re-growth	1.42^{a} ±0.32	3.19^{b} ±0.50	1.90^{c} ±0.25	1.90^{c} ±0.27	1.84^{c} ±0.19
Unlimed		1.47^{a} ±0.43	2.80^{b} ±0.58	2.01^{c} ±0.25	2.11^{c} ±0.44	1.81^{c} ±0.26
Year 2						
Limed	Insufficient Re-growth	2.36^{a} ±0.24	1.88^{b} ±0.33	1.99^{b} ±0.25	1.63^{c} ±0.21	1.62^{c} ±0.27
Unlimed		1.94^{b} ±0.33	2.01^{ab} ±0.42	1.90^{b} ±0.33	1.63^{c} ±0.18	1.77^{c} ±0.11

^{abc} = significant differences within year

DISCUSSION

Calcium levels in actively growing kikuyu, growing on acid soils, are low (Awad & Edwards, 1977; Miles, 1986). Kaiser (1975) and Bredon and Hathorn (1974) found that supplementation of growing animals on kikuyu with a high Ca lick increased animal performance, which agree with other observations that high Ca levels in the herbage is associated with improved animal performance (Karnezos, 1986). It is thus expected that liming, with the potential to improve Ca levels in the plant, would potentially be able to improve intake and animal performance.

Liming is known to have increased DMD in other tropical species. For instance, Rees and Minson (1976) found that fertilizing pangola grass (*Digitaria decumbens*), with Ca (liming) on a Ca deficient soil increased the digestibility and VFI of the herbage. They attributed this increase in intake to an 18% reduction in the time the DM was retained in the reticulo-rumen, and the increased digestibility to an increase in the digestibility of the hemi-cellulose fraction. Since feeding a Ca supplement had no effect on the digestibility or VFI, Rees and Minson (1976) concluded that the increases in digestibility and intake were the result of changes in the structural components of the grass and not a simple increase in the Ca concentration of the diet.

Liming had no consistent effect on VFI. Intake followed the same trends for stage of re-growth as in Chapter 3, with higher VFI at longer re-growth stages in the early season and intake declining with advancing stages of re-growth in the later part of the growing season. A trend (p=0.01) for VFI to decline with increasing CP concentration of the herbage is consistent with the negative effect of N fertilization on intake determined in Chapter 3. Similarly, the DM concentration of the herbage has an overriding effect on intake, accounting for 60% of the variation in VFI, VFI increasing with increasing DM concentration.

Awad *et al.* (1977) found that liming kikuyu on highly acid soils substantially increased Ca, N and Mo concentration in the kikuyu tops. While lime increased Ca concentration, the application of N fertilizer, within a lime treatment, decreased the Ca concentration of the herbage. Liming was also shown to reduce the N fertilizer requirements for plant growth, an important factor considering the negative aspects of high N levels on animal intake and performance. For these data, liming increased the digestibility in the year of application, but not in the subsequent year. However, these increases did not translate into increased VFI, except for the limed 30-d re-growth in year 2. The lack of VFI response to liming and Ca levels in the herbage is consistent with the findings of Austin (1980), that the acceptability of kikuyu pasture was not affected by the mineral composition. Minson (1990) pointed out that the VFI of a low Ca diet can be improved by Ca supplementation, but only if Ca is the first limiting element. Examining the composition of the present data, Ca levels exceeded the requirements of growing yearlings in all seasons, except for the spring and summer 40-day re-growths.

Liming increased the P levels of the herbage, increased Ca in summer, increased K levels in the kikuyu in spring and summer, increased the K/(Ca+Mg) ratio and improved Na levels in the autumn for the year following liming. In terms of the mineral requirements for growing yearling dairy animals (NRC, 1989), Ca was adequate to meet the requirements for the limed treatments, but marginally deficient for the un-limed treatments. The P levels were adequate to meet the requirements for both the limed and un-limed treatments. Magnesium levels were adequate for the animal requirements in terms of composition and were not affected by liming. However, the availability of Mg in fresh green herbage is low and, furthermore, the absorption of Mg is depressed by high levels of K and N in fresh herbage (Pickard, 1986).

The mean K levels in the herbage were 26 g/kg DM, with a maximum value of 38.3 g/kg DM and a minimum value of 16.19 g/kg DM, lower values than those recorded by Dugmore *et al.* (1987) and Miles *et al.* (1995), with levels exceeding 50 g/kg not unusual (Miles, 1998). Potassium levels were lower in older herbage. The minimum K value recorded exceeds the nutrient requirements of growing yearlings by a factor of 3. These values are well below the levels of 46 g K/kg DM at which the negative effects of potassium are encountered in practice (NRC, 2001; Miles *et al.*, 2005), although a maximum tolerable K concentration of 30 g/kg is reported for ruminants (NRC, 1980). Increasing dietary K levels from 7 to 30 g/kg linearly decreased energy and weight gain in lambs (NRC, 1985). Similarly, Morton *et al.* (2001) found that high K levels (> 37 g/kg DM) reduced the intake on ryegrass pasture. The increased K/(Ca + Mg) ionic balance recorded is in contrast to the findings of Miles (1986) who found that liming decreased the K/(Ca + Mg) ratio, albeit at inherently greater levels of K in the herbage. This ionic ratio, when in excess of 2.2 has been implicated with grass tetany (Kemp & t'Hart, 1957).

Liming did not affect the levels of Na in the herbage, except an improvement in the autumn due to liming. The Na levels of the kikuyu are far below the requirements of growing animals, varying from 10 to 50 % of the requirements. However, this is not surprising considering that kikuyu is a natrophobic plant (Marais, 2001). In terms of trace minerals, Fe levels exceed the requirements by at least tenfold. Manganese levels were adequate and Zn levels marginally low for animal requirements. The low Zn levels recorded are a consequence of the low soil test levels, as indicated by recommendations to include Zn in the fertilizer (Fertilizer Advisory Service, KwaZulu-Natal Department of Agriculture and Environmental Affairs). Copper levels in the herbage were reduced by liming and appear adequate for animal requirements. However, blood Cu levels in dairy cows at Cedara during the trial period indicated that the cows were deficient in Cu, possibly due to the antagonistic effects of the excessive Fe level (Pickard, 1986; NRC, 2001).

Using mineral composition data alone in assessing adequacy or deficiency, Ca, Zn, Na and Cu appear deficient, while Fe and K are far in excess of animal requirements. Liming would appear to improve the Ca concentration of the herbage to levels of adequacy.

In conclusion, liming increased the Ca, P and K concentration, of the herbage, adversely affecting the K/(Ca+Mg) ratio. The N fractions and NSC concentration in the herbage were not influenced by liming. However, even considering the improved Ca concentration in a pasture known for low Ca concentrations and the minimal effect of liming on intake and digestibility from these data, liming is not recommended on kikuyu pastures with relatively low acid saturation levels in the soil as measure of improving mineral balances in the herbage. Supplementation of adequate amounts of Ca and other deficient minerals can be applied more cost effectively in a mineral lick or balanced dairy concentrate.

Chapter 5

Seasonal Variation in the Degradation Properties of Kikuyu Fertilized with Two Levels of N and Harvested at Three Growth Stages

Abstract

The effect of N fertilizer and stage of re-growth on the degradability characteristics of the N fraction in kikuyu was determined. Kikuyu pasture was fertilized at two levels of N, namely 50 and 200 kg N/ha/trial, to induce low and high levels of N in the herbage, after clearing the plots. The subsequent growth was harvested at 20-, 30- and 40-d of re-growth. Digestion trials using sheep were conducted in the spring, summer and autumn with samples taken from the material on offer at each trial to determine N degradability in three lactating rumen fistulated Friesland cows using the *in situ* bag technique. Samples were taken at 07:30, cut into 5 to 10 mm lengths and the fresh material incubated in the rumen. Rumen pH was measured, rumen and blood samples taken to determine rumen ammonia and blood urea N levels. Differences were recorded for the quickly degradable N (a) and slowly degradable N (b) fractions within season, but not for the degradation rate of the slowly degraded fraction (c) per hour. However, the rate of degradation did vary over seasons. N fertilization level affected the a-fraction positively and the b fractions negatively in the summer and autumn, but not in the spring. Stage of re-growth influenced the a- and b-fractions in all seasons, higher degradability recorded with advancing maturity. The effective degradability (edg) was not influenced by N fertilization level in the spring, while N fertilization increased the edg values in the summer and autumn. Stage of re-growth exerted a positive effect on the edg values. Effective degradability could be predicted from the herbage N fraction.

Key words: Pennisetum clandestinum, N fertilization, stage of re-growth, degradability.

INTRODUCTION

Nitrogen fertilization of cultivated pastures has become an increasingly important aspect of dairy farming in KwaZulu-Natal over the past thirty years. These pastures typically have a crude protein concentration varying from 120 to 300 g/kg DM (Bredon, & Stewart, 1979). Theoretically, these high CP levels would be sufficient for the production of thirty litres, or more, over most of the growing season. However, most of the CP in pastures has, as a general rule, a high proportion of non-protein N (NPN) (Bredon & Stewart, 1979). There is a limit to the amount of NPN that can be utilized by rumen microflora. Thus, in practice, some of the CP obtained from pastures is not utilized. Grasses vary in the proportion of NPN to protein N concentration. In fact, kikuyu can sometimes contain more NPN than protein N (Bredon & Stewart, 1979). Le Roux *et al.* (1984) recorded levels of 3.18, 5.60, 5.62 and 7.51 g NPN/kg DM or 25%, 37.3%, 31% and 38.8% NPN as a fraction of total N for N fertilizer levels of 50, 250, 450 and 650 kg N/ha/yr, respectively, in the Tsitsikamma. Fulkerson *et al.* (1998) found that 26% of the CP in kikuyu was in the form of NPN.

In highly fertilized pastures and maize silage, where a large proportion of the CP is already in the form of NPN, the proportion of protein degradation in the rumen should be relatively high. Consequently, as most dairy herds in KwaZulu-Natal rely to a great extent on well fertilized pastures and feeds such as maize silage for their roughage needs, it is unlikely, under KwaZulu-Natal conditions, that medium (>12 kg milk/d) to high producing cows would obtain any benefit from urea. Urea (NPN) supplementation would, if anything, cause a depression in production (Bredon & Stewart, 1979). Bredon and Stewart (1979) concluded that in the case of the dairy cow, particularly the lactating cow on pasture, in most cases it would be best from a production and health point of view to avoid urea feeding.

The value of the protein, or N, fraction in kikuyu appears to be unclear in terms of animal production. Bredon and Stewart (1979) concluded that ideally protein requirements should be based on knowledge of the degradability of feed proteins. Unfortunately, little information was available on protein degradability locally (Bredon & Stewart, 1979), particularly the *in situ* derived edg values. The *in situ* technique is the commonly used and widely recommended technique (ARC, 1984; AAC, 1990; NRC, 2001). There is a need for more edg values for kikuyu under different conditions in order to fine tune diet formulation and expand our local feed database. Consequently, the rumen degradability parameters of the N fraction in fresh kikuyu herbage was determined over the growing season, with high and low N fertilization and at different stages of re-growth to evaluate protein quality.

MATERIALS AND METHODS

The experimental area is situated at Cedara (29° 32' S; 30° 17' E) in the KwaZulu-Natal Mistbelt. Cedara lies at an altitude of 1067 m and receives an average annual rainfall of 875 ± 142 mm, falling predominantly in the summer months. The mean January maximum/minimum temperature is 25.3/14.8°, while frosts occur during the winter.

Pasture

Soils in plots of Kikuyu pasture at Cedara, established in *c*. 1930, were sampled and analyzed prior to the onset of each season and nutrient deficiencies were corrected according to the Cedara fertilizer advisory service recommendations (Manson *et al*., 2010). These pastures were subsequently cut, sampled and the remainder fed to sheep in digestion trials; part of the sampled herbage was incubated in the rumen of lactating dairy cows. Each N-fertilization treatment was allocated 10 plots, which were cut, fertilized and subsequently harvested on consecutive days to allow for a supply of the same stage of re-growth over a 10 day period.

Dry matter digestibility, DM and N disappearance data, obtained from the incubation of kikuyu re-growth, were determined for kikuyu fertilized with limestone ammonium nitrate (LAN) at the equivalent of 50 and 200 kg N/ha/dressing and harvested at re-growth stages of 20, 30 and 40 days during the spring, summer and autumn of the growing season.

Preparation of plots

Each treatment was allocated 10 plots, and each plot was cut to 3 cm and fertilized with N on a day corresponding with the expected day of use in the digestibility study. For instance, plot one was cut and fertilized with N on Day 1, and Plot 2 on Day 2, and so on until Day 10 on which Plot 10 was cut and N fertilized. For the 20-day re-growth treatment, Plot 1 was harvested on day 20, Plot 2 on day 21, followed by intermediate plots until Plot 10 was cut on day 29 ensuring that a 20-d old re-growth was harvested each day. Harvesting of a 30-d or 40-d re-growth commenced on day 30 or day 40.

Digestion trials

The technique described by Juko *et al.* (1961) was used for the digestion trials. Five wethers were used per digestion trial, after a suitable adaptation period on kikuyu. Each trial had a 6-d collection period following a 4-d preliminary or change over period between re-growth stages. Fresh green kikuyu was cut each day, chaffed through a 25-mm screen and fed to appetite, allowing for approximately 20 % orts. Samples of the fresh herbage, orts and faeces were dried in a forced draft oven at approximately 70°C for 24 hours, or until weight loss ceased. Samples were then subsequently analyzed for DM in order to determine DMD.

Rumen pH, rumen ammonia and Blood Urea Nitrogen

Six rumen fistulated sheep, ranging in weight from 25 to 35 kg over the season, were randomly assigned to each of the N-fertilization treatments, making 3 animals per treatment. Sampling of rumen content and blood were conducted early, mid and late season, coinciding with the N degradability trials, thus utilizing the herbage from the same plots. Sampling of blood and rumen, and recording of ruminal pH (*in situ*) commenced at 08H00 each morning, immediately following feeding, with further sampling after 3 and 6 hours, and lasted for 3 consecutive days. Rumen pH was taken *in situ* using a pH probe (Hanna HI 9025 waterproof pH meter; HANNA Instruments) and comprised a mean of three readings taken in the fore, mid and posterior rumen.

Rumen fluid was taken from the different areas of the rumen, filtered through cheesecloth into a 250 ml beaker, sub-sampled with a 5 ml pipette and acidified with 1 ml 1N H_2SO_4 per 10 ml of filtrate. The filtrate was centrifuged and the supernatant frozen until required for the analysis of NH_4^+ ions using an auto-analyzer (AOAC method 990.02, semi-automated). Rumen ammonia was determined using the technique described by Weatherburn (1967).

Blood samples were collected from the jugular vein into 8.5 ml lithium heparin vacutainer® tubes, centrifuged immediately at 2000 rpm, to prevent rupture of red blood cells, for 10 minutes and plasma frozen until analysed for BUN using the technique of Chaney and Marbach (1962).

Determination of degradability

The DM and N degradability (dg) of fresh kikuyu herbage was determined using three lactating Friesland cows in mid-lactation, each fitted with a rumen cannula. The experimental cows were managed within the dairy herd and fed according to the Cedara feeding system (Bredon & Stewart, 1979), grazing kikuyu and fed 6 to 8 kg concentrates per day.

Nylon bags, with an average pore size of 53 µm, with internal dimensions of 200 x 100 mm and rounded corners were used to determine *in situ* degradability. Each bag was hand made as described by van der Merwe (1993). Kikuyu herbage was harvested by reciprocating (sickle bar) mower between 07:30 and 08:00 and immediately taken to the laboratory, mixed and representative samples taken randomly from the long material, chopped into 5- to 10-mm lengths with a paper-cutting guillotine. These fresh-chopped samples were then put into nylon bags, equivalent to approximately 5 g herbage DM, representing a sample size of approximately 12.5 mg herbage DM/cm² of bag surface area. The bags were then secured onto a stainless steel disc (approximately 90g, 60 mm diameter, 3 mm thick with 10 evenly spaced holes drilled through the periphery of the disc as described by Erasmus *et al*., 1988) tied onto two 700-mm nylon lines (Polyarn, 0.80-mm diameter with 27 kg breaking strength) which were tied to the cannula spacer ring acting as draw lines to retrieve the disc from the rumen. Bags were tied close to the disc with approximately 20mm of free line between bag and disc to prevent entanglement between bags. Bags were tied in a set order to simplify the removal of the bags after withdrawal of the disc from the rumen, i.e. bags nearest to the draw line were removed first. Both the disc and bags were pushed through the digesta mat towards the ventral sac of the rumen and the other end of the nylon line secured to the cannula spacer ring. The incubation periods of the herbage samples were 0, 5, 24, 48 and 72 hours. These times were decided upon after taking into account previous studies on herbage with similar incubation periods (Filmer, 1982; Hof, 1990), cow management as cow were grazing pasture some distance away, number of bags per cow for two treatments (bags of fresh herbage containing c 25 g were bulky) and the mean retention time of kikuyu in the rumen indicating the critical time was at, or after, 24 hours incubation. Indications from prior pilot studies on kikuyu and ryegrass at Cedara established that the initial rates of N disappearance, over the first 12 hours, were slow in kikuyu (7.88, 25.12, 39.7 & 48.84 for 0, 3, 6 & 12 hr incubations respectively) relative to ryegrass where N disappearance at 6 hours (82.53%) exceeded that of kikuyu at 24 hrs (72.08%). The edg determined for the kikuyu herbage incubated at 0, 6, 24, 48 & 72 hrs only overestimated the edg (k=0.03) by 2% (p=0.06) compared to incubations of 0, 3, 6, 12, 24, 48 & 72 hrs (van der Merwe *et al.*, 1991). Two additional bags filled with standard lucerne hay samples were tied at the bottom of the disc and removed after 24 and 72 hours, respectively to standard controls (Filmer, 1982, AAC, 1990). The bags for incubations at 48 and 72 hours were doubled (two per treatment) to ensure sufficient residue material for analysis. The disc plus bags usually remained located in the ventral rumen sac except in a few instances in which they were found in the anterior rumen sac near the reticulum or positioned near the ventral blind sac. Once removed, bags were placed in a bucket of cold water to arrest microbial activity (AAC, 1990), taken to the laboratory, hand-washed under running cold water for five minutes (Lindberg, 1985), oven dried at 65°C for 48 hours, removed and allowed to cool in a desiccator and weighed. Residues were removed by turning the bags inside out, and stored in sample bottles for N analyses.

Sample analytical procedures

Sample material was divided into two, part for DM determination and part for freeze drying. Dry matter determinations were done in triplicate by placing samples in aluminium foil pans, followed by oven drying at 65°C for 48 hours. The dried samples were pooled (within treatment), milled through a 2-mm screen and placed in glass bottles, pending chemical analyses. Samples were also placed in polythene bags and frozen at -20°C for 12 hours. The frozen samples were freeze dried under vacuum for 48 hours, milled through a 2-mm screen and stored in glass bottles.

Oven- and freeze-dried samples were subjected to total Kjeldahl N analysis (AOAC, 1980). Oven-dried N values were used on the recording sheet as an indication of N in the original sample. Bag residue samples were analysed for total Kjeldahl N.

Calculations and statistical analyses

The following model proposed by Orskov and McDonald (1979) was fitted to the DM and N-disappearance (%) data:

$$p = a + b\,[1 - e^{(-ct)}] \qquad (1)$$

where:

p = the percentage herbage DM or N degraded after time t (hours)

a = the soluble or rapidly degradable DM or N fraction. This value is the intercept of the degradation curve at time 0 hours.

b = the insoluble but slowly, or potentially, degradable DM or N fraction.

c = the fractional rate of degradation, indicating the degradation rate (/h) of the b fraction.

These non-linear rumen parameters (a, b and c) were estimated using the NONLIN procedure of Genstat 8.1 (Genstat, 2005).

The maximum extent of degradation, normally referred to as the potential degradability (PD) was estimated as (a+b). Using the above parameters, the edg of the DM and N fraction was then calculated using fractional rumen outflow rates, k = 0.03/h (in line with the mean retention time derived for kikuyu in other studies (Hart & Liebholtz, 1990; Pienaar *et al.*, 1993b; Mann, 2001), according to the equation proposed by Orskov and McDonald (1979) and modified by Orskov and Ryle (1990):

$$dg = \text{wash} + [(PD - \text{wash})*c \div (c+k)] \qquad (2)$$

The non-linear degradation parameters for DM and N dg were subjected to an analysis of variance procedure of Genstat 8.1 (Genstat, 2005) using the following statistical model:

$$Y_{ijk} = \mu + F_i + A_j + S_k + A_j{*}F_i + F_i{*}S_k + A_j{*}S_k + F_i{*}A_j{*}S_k + eijk;$$

Where Y is the dependent variable, μ is the overall mean, F is the effect of N-fertilizer level, A is the effect of age of re-growth, S is the effect of season and e is the random variation which is assumed to be homogenously distributed. Where data sets were unbalanced, the residual maximum likelihood model (REML) of Genstats variance component analysis was used. Differences between means were determined by the least significant difference (LSD) range test method. The LSD method was protected by F test (Snedecor & Cochran, 1980) and differences were only accepted as being significant when the overall F test was significant ($P<0.05$). Data was analysed within season, with N fertilizer level and stage of re-growth as the factors and then pooled over the seasons, if homogeneous, with season added as a factor.

RESULTS

The chemical composition of kikuyu herbage used is given in Table 5.1. Generally NDF increased from spring through summer reaching a maximum in winter. Neutral detergent fibre values did not necessarily increase with increased stage of re-growth. The N concentration is more variable and does not show large, or defined seasonal trends.

The DM degradation parameters and the edg values are given in Table 5.2. The edg was influenced by season and N fertilization level, but not by stage of re-growth.

In the spring, the level of N fertilizer did not affect the effective degradability of the DM. However, stage of re-growth did influence the effective degradability of the DM, declining with age. The a-value for DM loss was lowest for the 40-d, intermediate for the 20-d and highest for the 30-d re-growth. Nitrogen fertilization level had no affect on the a-value. The b fraction was not affected by N fertilization or stage of re-growth. The potential extent of degradation (PD = a+b) decreased with stage of re-growth, but was not affected by N fertilizer level. Level of N fertilizer did not affect the rate of degradation (c-value) of the DM. The 20- and 30-d re-growth were not different, while the 40-d re-growth reduced the c-values.

Table 5.1 The dry matter (g DM/kg fresh herbage) and N composition (g/kg DM) of kikuyu herbage (used for incubation), fertilized at two nitrogen levels and harvested after 20, 30 and 40 days of re-growth

Component		Stage of Re-growth					
		Low N (50 kg/ha/season)			High N (200 kg N/season)		
		20	30	40	20	30	40
Spring	DM	182	204	224	156	180	185
	N	28.3	32.4	25.6	38.5	33.9	36.9
Summer	DM	207	187	172	195	152	145
	N	25.3	27.9	19.9	36.9	31.8	37.1
Autumn	DM	188	188	181	174	182	152
	N	26.7	19.9	29.7	40.5	37.2	36.1

In summer, the a-value was greatest for the 30-d re-growth and differed from the 40-d re-growth, which in turn was greater than the 20-d re-growth. The age of re-growth affected both the rate of degradation and the b-value for the 30-d re-growth, which were less than the 20- and 40-d values.

The PD increased with the age of re-growth. N fertilizer level had no effect on any of the degradation parameters, apart from the DM edg which was greater for the high N treatment. Stage of re-growth influenced the DM edg, the 30-d re-growth the highest value, with the 40-d intermediate and the 20-d the lowest.

In the autumn the a-value increased with the age of re-growth, as did the effective degradability of the DM. Nitrogen fertilization increased the edg of the DM, while reducing the a-value. The b-fraction was not affected by N fertilization level, but declined with age of re-growth. However, the age of re-growth and N fertilizer level had no effect on the rate of degradation or PD in this season. The DM edg was highest for the 40-d re-growth, the 20- and 30-d re-growth not differing from each other.

The main effects of treatment over the growing season are given in Table 5.4. Over the growing season as a whole, the b fraction, rate of degradation and potential degradability were not affected by N level, while the greater N fertilizer level increased both the a-value and edg for the N fraction and increasing N fertilizer level increased the a-fraction while decreasing the edg of the DM. Stage of re-growth did not influence the b-fraction, increased the a-fraction from the 20- to 30-d re-growths, after which they did not differ. The rate of degradation was reduced at the 40-d re-growth stage relative to the 20- and 30-d re-growths, while the PD and DM edg was highest at the 30-d stage with the 20- and 40-d not differing.

In terms of season, the rate of degradation and DM edg declined as the season progressed from spring highs to autumn lows. Conversely, the potential degradability peaked in autumn, with spring and summer not differing. The a-fraction was greatest in the summer, the spring and autumn not differing. The b-fraction was lowest in the summer, with spring and autumn not different.

Interactions between all the main factors (N fertilizer, age of re-growth and season) were determined for the a-fraction, with the N x season interaction reducing the spring a-values for the high N relative to the low N, while the low N a-values in the autumn were very low relative to the high N treatment. For the N x d interaction, the 20-d low N values for the a-fraction were severely depressed, while for the season x days interaction depressed the spring 40-d re-growths and the autumn 20-d re-growths. Similar responses were found for the N x season x d interaction with low spring 40-d and low autumn 20-d values recorded. A season x d interaction was found for the PD values, with greatest 30-d values recorded in the autumn. Similarly, for the a-fraction, interactions between all the main factors were found for effective degradability. A season x N interaction depressed the autumn low N treatment value relative to the high N value. A season x d interaction reflected decreased 20-d values for DM edg in the summer and autumn, while a N x d interaction increased the low N 40-d values relative to the high N treatment. A N x season x d interaction increased the autumn 40-d edg values for the low treatment relative to the high N treatment. No interactions were recorded for the b-fraction or c-values.

Table 5.2 Non-linear parameters and effective Dry Matter (DM) degradability (edg) of the DM fraction (%) for kikuyu fertilized at two N levels (50 & 200 kg/ha/season) and harvested at 20-, 30- and 40-d of re-growth

Season	N Fert	Age (days)	Wash	a	b	c	PD (a+b)	DM edg
Spring	Low	20	13.0^{a} ± 1.45	11.76^{c} ± 0.461	71.4^{a} ± 2.984	0.0732 ± 0.011	83.50^{a} ± 2.633	62.55^{a} ± 3.785
	High	20	11.7^{a} ± 2.77	11.62^{c} ± 2.414	73.3^{a} ± 2.818	0.0898 ± 0.008	84.87^{a} ± 0.703	66.65^{a} ± 1.449
	Low	30	20.5^{c} ± 12.98	21.74^{b} ± 13.27	59.92^{bc} ± 12.97	0.0913 ± 0.044	81.66^{a} ± 2.116	65.93^{a} ± 3.170
	High	30	10.1^{a} ± 7.83	11.00^{c} ± 1.317	70.5^{a} ± 0.952	0.0733 ± 0.022	81.49^{a} ± 0.388	59.98^{a} ± 4.575
	Low	40	3.19^{b} ± 0.15	4.44^{c} ± 1.593	74.6^{a} ± 0.547	0.0484 ± 0.015	79.02^{ac} ± 1.502	49.19^{b} ± 4.499
	High	40	4.19^{b} ± 4.73	6.26^{c} ± 4.954	68.8^{ab} ± 4.497	0.0597 ± 0.012	74.99^{ac} ± 3.159	50.57^{b} ± 4.729
Summer	Low	20	0.15^{e} ± 0.03	-0.83^{a} ± 2.562	72.8^{a} ± 1.216	0.0466 ± 0.006	70.94^{c} ± 3.009	42.67^{c} ± 2.473
	High	20	20.80^{c} ± 1.88	19.60^{b} ± 2.110	56.0^{bc} ± 1.453	0.0542 ± 0.007	75.53^{ac} ± 0.538	55.93^{ab} ± 1.381
	Low	30	35.10^{d} ± 1.99	35.52^{b} ± 1.969	47.4^{b} ± 1.907	0.0541 ± 0.012	82.91^{a} ± 1.090	65.59^{a} ± 1.312
	High	30	30.9^{d} ± 0.86	30.93^{b} ± 1.812	53.8^{bc} ± 2.895	0.0586 ± 0.008	84.71^{a} ± 1.008	66.41^{a} ± 1.773
	Low	40	24.5^{c} ± 2.74	23.85^{a} ± 3.494	58.3^{b} ± 10.48	0.0481 ± 0.013	82.11^{a} ± 7.393	59.18^{a} ± 2.785
	High	40	11.71^{ac} ± 6.65	12.75^{b} ± 4.931	75.3^{a} ± 5.072	0.0362 ± 0.020	88.01^{a} ± 7.393	50.80^{b} ± 3.237
Autumn	Low	20	0.19^{e} ± 1.73	-4.12^{a} ± 0.731	87.3^{a} ± 8.848	0.0466 ± 0.013	83.35^{a} ± 7.742	43.65^{c} ± 3.021
	High	20	5.2^{ab} ± 0.57	6.40^{a} ± 1.243	70.2^{a} ± 1.602	0.0472 ± 0.001	76.61^{ac} ± 1.163	49.17^{b} ± 0.772
	Low	30	0.76^{e} ± 2.87	1.44^{b} ± 1.875	98.5^{a} ± 3.30	0.0253 ± 0.011	99.97^{b} ± 0.84	39.42^{c} ± 7.231
	High	30	9.5^{a} ± 3.21	11.23^{a} ± 2.280	72.6^{a} ± 21.84	0.0338 ± 0.009	83.85^{a} ± 16.05	47.47^{b} ± 4.374
	Low	40	17.7^{ac} ± 1.67	17.21^{a} ± 1.123	69.5^{ab} ± 1.798	0.0450 ± 0.012	86.73^{a} ± 0.768	58.46^{a} ± 3.783
	High	40	33.2^{d} ± 2.13	34.15^{c} ± 2.825	56.1^{bc} ± 1.742	0.0279 ± 0.012	90.28^{ab} ± 3.727	59.86^{a} ± 2.088
P levels								
	S		<0.001	<0.001	0.004	<0.001	0.049	0.021
	F		0.029	<0.001	0.319	0.718	0.012	0.002
	A		<0.001	<0.001	0.107	0.022	0.044	<0.001
	SxF		<0.001	<0.001	0.06	0.948	0.021	0.014
	SxA		<0.001	<0.001	0.02	0.07	0.007	<0.001
	FxA		<0.001	<0.001	0.153	0.194	0.288	<0.001
	SxFxA		<0.001	0.001	0.19	0.231	0.261	<0.001

Superscripts a, b, c, d indicate significant difference ($P<0.05$) within columns.

a = Quickly degradable material

b = Slowly degradable material

PD (a+b) = Total potentially degradable material

c = Degradation rate of the b fraction per hour

k = Fractional rumen outflow rate per hour

S = season (spr, sum, aut)

F = N fertilizer level

A = Age of re-growth (days)

Table 5.3 Non-linear parameters and effective degradability of the nitrogen (N) fraction (%) for kikuyu fertilized at two N levels and harvested at 20-, 30- and 40-d of re-growth

Season	N Fert	Age (days)	wash	a	b	c	edg k = 0.03	Edg K = 0.05
Spring	Low	20	1.82^{a} ± 2.07	1.5^{a} ± 2.72	91.1^{a} ± 1.894	0.094^{ab} ± 0.008	70.9^{a} ± 2.348	61.7^{a} ± 2.802
	High	20	3.65^{a} ± 2.69	1.6^{a} ± 2.819	92.0^{a} ± 1.445	0.094^{ab} ± 0.009	71.8^{a} ± 1.458	62.4^{a} ± 1.680
	Low	30	23.77^{b} ± 11.59	24.2^{b} ± 11.883	66.6^{b}± 11.817	0.117^{a} ± 0.027	77.1^{b} ± 0.978	70.8^{b} ± 0.976
	High	30	0.15^{a} ± 4.25	1.4^{a} ± 5.738	88.9^{a} ± 5.713	0.114^{a} ± 0.028	71.2^{a} ± 2.779	62.4^{a} ± 3.227
	Low	40	9.31^{a} ± 2.21	8.8^{c} ± 3.231	87.7^{a} ± 2.888	0.071^{b} ± 0.008	66.1^{a} ± 0.664	56.9^{ac}± 0.665
	High	40	8.84^{a} ± 7.29	9.5^{a} ± 7.431	79.8^{ab} ± 8.818	0.079^{b} ± 0.012	67.0^{ac} ± 3.601	58.0^{a} ± 4.314
Summer	Low	20	0.28^{a} ± 1.81	-2.7^{a} ± 1.521	87.6^{a} ± 3.104	0.071^{b} ± 0.010	59.8^{c} ± 4.835	50.1^{c} ± 4.835
	High	20	27.01^{bc} ± 7.81	25.3^{b} ± 7.553	65.2^{b} ± 6.501	0.074^{b} ± 0.015	73.1^{a} ± 2.140	64.8^{ab}± 2.710
	Low	30	30.05^{c} ± 4.26	28.7^{b} ± 3.271	63.4^{b} ± 2.960	0.074^{b} ± 0.001	74.2^{ab}± 0.951	67.1^{b} ± 1.241
	High	30	18.85^{b} ± 14.15	18.0^{ab} ± 15.587	75.1^{ab}± 15.898	0.084^{ab} ± 9.015	72.9^{a} ± 6.330	64.7^{ab}± 8.539
	Low	40	1.12^{a} ± 2.07	-0.2^{a} ± 4.623	88.9^{a} ± 7.235	0.073^{b} ± 0.013	62.8^{c} ± 1.730	52.6^{c} ± 2.217
	High	40	15.05^{ab} ± 12.92	15.1^{ab} ± 11.736	77.8^{ab}± 9.208	0.060^{cb} ± 0.015	69.3^{a} ± 3.315	60.9^{a} ± 3.660
Autumn	Low	20	8.30^{a} ± 1.80	8.3^{a} ± 1.609	82.5^{a} ± 1.611	0.064^{cb} ± 0.004	64.7^{c} ± 1.373	54.8^{c} ± 1.371
	High	20	15.16^{ab} ± 0.69	14.6^{ab} ± 0.811	74.8^{ab} ± 1.466	0.081^{ab} ± 3.263	69.1^{a} ± 2.617	60.8^{a} ± 3.263
	Low	30	3.05^{a} ± 2.84	3.4^{a} ± 1.395	81.9^{a} ± 11.718	0.051^{c} ± 0.005	54.7^{d} ± 5.559	44.5^{d} ± 4.172
	High	30	12.26^{ab} ± 3.09	14.5^{ab} ± 3.111	73.9^{ab} ± 13.325	0.064^{cb} ± 0.005	64.0^{c} ± 5.075	54.9^{c} ± 3.435
	Low	40	14.17^{ab} ± 11.40	14.4^{ab} ± 9.536	80.8^{a} ± 11.070	0.057^{c} ± 0.011	66.8^{ac} ± 5.314	56.9^{ac} ± 6.977
	High	40	36.45^{c} ± 4.62	36.4^{c} ± 4.650	57.3^{c} ± 5.180	0.061^{cb} ± 0.005	74.9^{ab} ± 1.060	68.0^{b} ± 1.488
P levels								
	S		0.018	0.15	0.123	0.003	0.007	0.004
	F		0.001	0.008	0.096	0.229	<0.001	<0.001
	A		0.017	0.009	0.044	0.002	0.531	0.316
	SxF		<0.001	<0.001	0.001	0.672	<0.001	<0.001
	SxA		<0.001	<0.001	0.005	0.448	<0.001	<0.001
	FxA		<0.001	<0.001	0.002	0.672	0.319	0.015
	SxFxA		0.009	0.01	0.166	0.559	0.042	0.037

Superscripts a, b, c, d indicate significant difference ($P<0.05$) within columns.

a = Quickly degradable material

b = Slowly degradable material

a+b = Total potentially degradable material

c = Degradation rate of the b fraction per hour

k = Fractional rumen outflow rate per hour

S = season (spr, sum, aut)

F – N fertilizer level

A = Age of re-growth (days)

Table 5.4 The predicted means for the main effects, where different (*P*>0.05), for the non-linear degradation parameters and the effective degradability for the dry matter (DM) and nitrogen (N) fraction of fresh kikuyu herbage harvested for the degradability trials, fertilized at two levels of N (50 kg and 200 kg N per cut) and harvested at 20-, 30- and 40- d re-growth in spring, summer and autumn

Parameter		Main effects								Interactions (*P* levels)$^{\#}$			
		Season			N		Re-growth stage						
		Spr	Sum	Aut	Low	High	20	30	40	S.N*	S.D	N.D	S.N.D
DM	a	11.13	20.14	11.05	12.22	15.99	7.24	18.64	16.44	<0.001	<0.001	<0.001	0.001
	b	69.7	60.6	72.9	NS			NS		0.060	0.019	0.153	0.190
	c	0.072	0.049	0.04	NS		0.057	0.056	0.044	0.948	0.070	0.194	0.231
	a+b	80.87	80.70	83.95	NS		79.08	85.14	83.53	0.060	0.007	0.288	0.261
	edg 0.03	59.15	56.77	86.80	56.3	54.05	79.08	85.77	54.68	0.014	<0.001	<0.001	<0.001
N	a	8.1	14.2	15.3	9.9	15.2	8.3	15.1	14.2	<0.001	<0.001	<0.001	0.003
	b	83.1	76.3	75.3	NS		82.2	75.0	77.7	<0.001	<0.001	<0.001	0.130
	c	0.096	0.073	0.063	NS		0.080	0.084	0.068	0.211	0.060	0.220	0.992
	a+b		NS		NS			NS		0.403	0.009	0.973	0.680
	edg 0.03	70.9	68.6	65.9	66.9	70.0		NS		<0.001	<0.001	0.319	0.042

$^{\#}$ = indicates if interactions between main effects were significant and at what level of significance ; * S = season; N = nitrogen; D = days of re-growth; NS = p>0.05.

a = Quickly degradable material, b = Slowly degradable material, a+b = Total potentially degradable material, c = Degradation rate of the b fraction per hour, edg 0.03 = effective degradability at a fractional rumen outflow rate of 0.03 per hour.

The non-linear parameters and degradability data for the N fraction in the herbage is presented in Table 5.3. The predicted means for the main effects are given in Table 5.4.

In the spring, the a-, or quickly degradable nitrogen (QDN), fraction of the herbage was depressed by the higher N fertilization level. For stage of re-growth the 30- and 40-d re-growths had higher QDN values than the 20-d re-growth. There was a quadratic interaction between days of re-growth and N fertilizer causing the low N QDN values to peak at 30 days, but no differences for the 20-, 30- and 40-d re-growths in high N treatment. The b, or slowly degradable nitrogen (SDN), fraction was affected by N fertilization level in the spring, with the higher N level increasing the SDN value. Stage of re-growth exerted an effect, with the 20-d re-growth SDN values greater than the 30- or 40-d re-growth values. There was a N by stage of re-growth interaction for the low N treatment with a quadratic response, the 30-d SDN values lower than both the 20- and 40-d re-growth values. The 40-d SDN value was intermediate, with the 20-d SDN values greater than the 30-d values. No differences were measured for 20-, 30- and 40-d SDN values in the high N treatment. The c-, or degradation rate of the SDN fraction per hour, was not affected by N fertilization level within the spring season. However, stage of re-growth influenced the c-value, with the 30-d the greatest (0.01156), the 20-d intermediate (0.0945) and the 40-d (0.0766) the lowest value.

In the summer, N exerted an effect on the QDN (a) fraction, with the greater fertilization levels inducing greater a-values (9.1 *vs* 19.5 for low and high). A quadratic response to stage of re-growth (days) was observed, with the 30-d herbage recording the highest QDN values, higher than the 20- and 40-d values which were not different (12.1, 23.4 and 7.4 for 20-, 30- and 40-ds respectively) for the low N treatment. An interaction was recorded between the N treatment and stage of re-growth, with the low N treatment QDN values peaking at 30d re-growth, relative to lower 20- and 40-d values. The high N treatment values for QDN were not different for stage of re-growth and did not differ from the 30-d low N value. Nitrogen fertilization level had no effect on the SDN (b) fraction during the summer (79.6 vs 72.7 for low and high N). Stage of re-growth exerted a quadratic response on the SDN values, with the 40-d values the greatest, 20-d intermediate and 30-d the lowest (75.8, 69.3 and 83.4 for the 20-, 30- and 40-d re-growth). A quadratic interaction was recorded between N fertilization and stage of re-growth for the low rate of N fertilization, with the 30-d values the lowest and the 20- and 40-d not differing from each other (86.5, 63.4 and 88.9 for 20-, 30- and 40-d). No differences were recorded for the SDN values at the high N level. The c, or degradation rate of the SDN fraction per hour, was not affected by treatment within the summer season.

In the autumn, N exerted an effect on the QDN (a) fraction, with the high fertilization levels inducing greater values (8.7 vs 21.9 for low and high). A quadratic response to stage of re-growth (days) was observed, with the 30-d herbage now recording the lowest a-values (11.5, 9.0 and 25.4 for 20-, 30- and 40-d re-growths respectively), contrary to the 30-d peak values recorded for the spring and summer. An interaction was recorded between the N treatment and stage of re-growth, with the 40-d greater than the 20- and 30-d values, which were not different (14.6, 14.6 and 36.5 for the 20-, 30- and 40-d high N re-growths respectively) for the QDN values. Nitrogen fertilization induced a lower SDN (b) fraction during the autumn (81.5 vs 68.7 for low and high N). No differences due to stage of re-growth or interactions recorded between N fertilization and days for either fertilization level were recorded in the autumn. In the autumn, N fertilizer level increased the c-, or degradation rate of the SDN fraction per hour, values (0.0577 vs 0.0689 for low and high N). Stage of re-growth had a negative effect on the c values, with the 20-d values (0.0729) greater than the 30- (0.0577) or 40-d (0.0594) values. No interactions were observed for the c-values in the autumn.

Season influenced the QDN fraction, with the spring values lower than the summer or autumn values, which did not differ (8.1, 14.2 and 15.3 for spring summer and autumn respectively). Over all seasons, N fertilization induced higher QDN values. For stage of re-growth the 20-d herbage had the lowest QDN values, with the 30- and 40-d not differing. Similarly, for the SDN fraction season exerted an effect, with the greatest values recorded in spring, with summer and autumn not differing. Over all seasons, N fertilization did not have an effect on the SDN values. However, for stage of re-growth the 30-d treatment had the lowest SDN values, with 40-d intermediate and 20- the highest values (82.2, 75.0 and 77.4 for 20-, 30- and 40-d re-growths respectively).

The c-, or degradation rate of the SDN fraction per hour, was influenced by season, with the highest values in the spring, declining through summer to autumn lows (0.0995, 0.0721 and 0.0631 for spring, summer and autumn respectively). Nitrogen fertilizer level did not have an impact on the c-values over all seasons. Overall, for the seasons, the 40-d stage of re-growth had the lowest c-value (0.06778) relative to the 30- (0.08407) and 20- (0.08001) re-growths. However, an interaction between season and stage of re-growth (days) was recorded for the c-fractions, with the spring 30-d values greater than the 20- or 40-d values, while the 30-d values in the autumn did not differ from the 40-d values. No other interactions were found between the c-value and any of the other main effects.

The edg of the N fraction was not influenced by N fertilization in the spring. However, for stage of re-growth the 40-d edg values were lower than those of either the 20- or 30-d re-growth. A quadratic interaction between N and stage of re-growth (days) was found for the low N treatment, with the 30-d re-growth greater than the 20- or 40-d re-growth. No differences occurred between the re-growth stages for the high N treatment.

In the summer, N fertilization level had no effect on the edg values, with the low N level tending (P = 0.076) to be lower (66.8) than the high N (70.4) treatment. There was a quadratic effect of stage of re-growth (days) on edg, with the 30-d (73.3) values greater than the 20 (66.6) or 40 (65.7) day values. A quadratic interaction between stage of re-growth and N fertilization level was found on edg, with the 30-d re-growth (73.8) higher than either the 20- (61.68) or 30-d (64.9) re-growth for the low N treatment, while no differences were determined for the high N treatment.

For the autumn, N fertilization level had an impact on edg, the greater N level associated with greater edg. Similarly, stage of re-growth had a negative quadratic association with edg, the 30-d re-growth now lower than the 20- or 40-d re-growths, as opposed to the spring and summer responses. No interaction between N and stage of re-growth was determined.

Overall, seasonal differences were found, with edg declining from a high in spring to a low in autumn. Nitrogen fertilization levels also exerted an effect on edg, with greater values recorded for the greater fertilization level. Stage of re-growth had no effect on edg over the season as a whole, whereas within season differences were determined for the spring, summer and autumn values. A season by N interaction was found for the low N treatment, the edg values declining from a spring high to an autumn low, however, no differences were noted for the high N treatment. No interaction between N and stage of re-growth was found. A season by stage of re-growth interaction was determined, with the edg of the 40-d re-growths increasing over season to peak in the autumn while the edg's for the 20- and 30-d re-growths declined to an autumn low. A quadratic interaction was found between season, N fertilizer level and stage of re-growth on edg, edg varying from a high of 77 for low N 30-d re-growth in the spring to a low of 54 for low N 30-d re-growth in autumn. The predicted means for the season, N fertilizer level and stage of re-growth interaction are given in Table 5.4.

The relationship between the DM and N degradability parameters were investigated. An association was determined between DM degradability and N degradability ($P < 0.0001$, $R^2 = 0.67$). In terms of the a-, b- and c-values, relationships were determined, albeit quite weak relationships for the a- and b-fractions, whereas the c-values were highly correlated ($R^2 = 0.80$). These relationships are quantified in the following regression equations:

a-fraction (N) = 3.2 + 0.65 (DM a fraction); $R^2 = 0.44$; $P = 0.001$; n = 18
b-fraction (N) = 33.36 + 0.67 (DM b fraction); $R^2 = 0.32$; $P = 0.008$; n = 18
c-value (N) = 0.032 - 0.85 (DM c value); $R^2 = 0.80$; $P < 0.0001$; n = 18
N edg = 40.16 + 0.512 (DM edg); $R^2 = 0.67$; $P < 0.0001$; n = 18

The relationship between DM and N loss was examined, with relationships determined for each incubation time. These relationships are quantified in the following regression equations:

0 hr N loss = 2.28 + 0.71 (0 hr % DM loss); $R^2 = 0.484$; $P = 0.001$; n = 18
5 hr N loss = 19.10 + 0.556 (5 hr % DM loss); $R^2 = 0.342$; $P = 0.006$; n = 18
24 hr N loss = 50.94 + 0.418 (24 hr % DM loss); $R^2 = 0.401$; $P = 0.002$; n = 18
48 hr N loss = 60.05 + 0.38 (48 hr % DM loss); $R^2 = 0.603$; $P < 0.0001$; n = 18
72 hr N loss = 59.68 + 0.385 (72 hr % DM loss); $R^2 = 0.587$; $P = 0.0001$; n = 18

The relationship between DM loss from the nylon bags and the *in vivo* DM digestibility (Table 5.8) was examined, with only the 24, 48 and 72 hour DM loss being significantly, but weakly, related to DM digestibility. DM digestibility was associated with the edg of the DM at an outflow rate of 0.03. The regression equations quantifying these relationships are as follows:

DMD = 0.772 - 0.0021 (24 hr DM loss); $R^2 = 0.336$; $P = 0.011$; n = 16
DMD = 0.896 - 0.0033 (48 hr DM loss); $R^2 = 0.363$; $P = 0.008$; n = 16
DMD = 1.007 - 0.0045 (72 hr DM loss); $R^2 = 0.307$; $P = 0.015$; n = 16
DMD = 0.781 – 0.00239 (DM edg); $R^2 = 0.781$; $P = 0.031$; n = 16

The a-fraction of the N was not correlated to either the N of the herbage. The a-fraction was also correlated to the 0 hr rumen incubation value for N and is quantified by the regression:

a = 0.59 + 0.95 (0 hr % N loss); $R^2 = 0.957$; $P < 0.0001$ n = 18.

The b-fraction for N was negatively associated to the N fraction ($P = 0.125$) in the herbage. The b-fraction was also highly correlated to the 0 and 5 hr rumen incubation values for N and is quantified by the regression:

b = 90.7 - 0.967 (0 hr % N loss); $R^2 = 0.881$; $P < 0.0001$; n = 18.
b = 108.8 - 0.838 (5 hr % N loss); $R^2 = 0.561$; $P = 0.0002$; n = 18.

The c-value for N was not correlated to any of the other parameters measured.

The edg values are highly correlated to the N concentration of the herbage. Regression analysis established that the N concentration of the herbage was the major determinant of protein degradability in the rumen and therefore dg is a function of N fertilization. The regression equations giving the response of edg to N concentration (g/kg DM) in the herbage are:

edg = 51.92 + 0.52 (N); $R^2 = 0.291$; *P* = 0.012; n = 18.
edg = -11.22 + 4.9 (N) - 0.07 (N^2); $R^2 = 0.488$; *P* = 0.002; n = 18.

The edg values for N were correlated to the rumen incubation values for N loss and are quantified by the following regressions:

edg = 64.9 + 0.286 (0 hr % N loss); $R^2 = 0.323$; *P* = 0.008; n = 18.
edg = 55.26 + 0.368 (5 hr % N loss); $R^2 = 0.497$; *P* = 0.0006; n = 18.
edg = 30.84 + 0.502 (24 hr % N loss); $R^2 = 0.477$; *P* = 0.0009; n = 18.
edg = -37.43 + 1.202 (48 hr % N loss); $R^2 = 0.683$; *P* = 0.00001; n = 18.
edg = -51.68 + 1.34 (72 hr % N loss); $R^2 = 0.492$; *P* = 0.0007; n = 18.

In terms of the rumen fermentation parameters determined (Table 5.7), pH ranged from 6.08 to 6.41 for the low N treatments and from 5.86 to 7.13 for the high N treatments. There was a decline in rumen pH with increasing stage of re-growth. In general, rumen pH ranged between the 5.5 and 7.0 required for microbial fermentation (Stevens *et al.*, 1980). The high N 20-d re-growth in spring tended to alkalosis, with a few animals suffering from alkalosis in the trials. The rumen ammonia levels ranged from 5.34 to 11.27 mmol/ℓ, higher for the high N treatments (P = 0.027) and also exhibiting a decline with stage of re-growth (P = 0.035). Blood urea N (BUN) values ranged from 7.8 to 10.69 mmol/ℓ, not differing over treatments.

In terms of the correlations between the parameters determined (Table 5.6), many weak associations between the variables are noted. The stronger correlations between variables were then subjected to further regression analysis to quantify the relationships. The NDF concentration of the herbage tended to be inversely related to rumen pH [pH = 8.31 - 0.0024 (NDF); *P* = 0.065; n = 16]. Blood urea nitrogen tended to be positively associated with rumen ammonia levels [BUN = 7.49 + 0.254 (ammonia); *P* = 0.07; n = 18]. Rumen fermentation parameters and degradability parameters were not associated.

The N concentration of the herbage DM tends to be negatively associated with the DM concentration of the herbage [N = 55.98 - 0.138 (DM); *P* = 0.087; n = 16], as is the rumen pH [pH = 7.7 – 7.57 ($DM_{\text{as fed}}$); *P* = 0.054; n=16].

Table 5.5 Dry matter digestibility (g/kg) values of the kikuyu used for determining the non-linear parameters and effective N degradability of the N fraction for kikuyu fertilized at two N levels and harvested at 20-, 30- and 40-d of re-growth

Season	Stage of re-growth					
	low N			high N		
	20	30	40	20	30	40
Spring	615^{ab} ± 34.5	712^{c} ± 24.5	654^{b} ± 46.0	593^{a} ± 27.9	640^{b} ± 9.7	601^{a} ± 17.6
Summer	-*	645 ± 26.2	650 ± 24.0	-*	578 ± 36.2	646 ± 61.4
Autumn	671^{b} ± 21.0	726^{c} ± 14.5	668^{b} ± 17.9	653^{ab} ± 37.5	695^{b} ± 13.5	616^{a} ± 48.2

* = insufficient material for digestion trial

^{abc}, figures in rows with differing superscripts differ at *P*=0.05

Table 5.6 Correlation matrix for rumen degradability parameters determined in lactating cows, rumen ammonia, rumen pH, blood urea N and herbage digestibility, determined with sheep, and herbage N concentrations

Parameters	N dg $_{0.03}$	A	B	C	DMD	Rumen PH	BUN	Rumen Ammonia	Herbage DM	Herbage N
N dg 0.03	1									
A	0.452	1								
b	-0.692	-0.897	1							
C	0.5	-0.28	0.056	1						
DMD	-0.436	0.191	-0.061	-0.212	1					
Rumen pH	0.148	-0.209	0.318	0.178	-0.341	1				
BUN	-0.023	-0.02	-0.02	0.224	-0.079	-0.507	1			
Rumen ammonia	0.209	0.163	-0.231	0.139	-0.361	-0.093	0.436	1		
Herbage DM	-0.198	-0.215	0.137	0.318	0.523	-0.626	0.235	-0.076	1	
Herbage N	0.665	0.388	-0.516	0.193	-0.367	0.321	0.205	0.322	-0.44	1

a = Quickly degradable material, b = Slowly degradable material, a+b = Total potentially degradable material, c = Degradation rate of the b fraction per hour, dg = degradability BUN = Blood urea nitrogen, DMD = dry matter digestibility,

Table 5.7 Nitrogen concentration (g/kg) of the herbage, edg (%), rumen pH, rumen ammonia levels (mmol/ℓ) and blood urea nitrogen concentration (mmol/ℓ) determined in sheep fed kikuyu fertilized at two N levels and harvested at 20-, 30- and 40-d of re-growth

Season		Stage of re-growth					
		Low N (50 kg N/ha)			High N (200kg N/ha)		
		20	30	40	20	30	40
Spring	N	28.1	32.2	25.5	38.4	33.9	36.9
	edg	70.9^{a}	77.1^{b}	66.1^{a}	71.8^{a}	71.2^{a}	67.0^{ac}
	rumen pH	6.41^{ab}	6.15^{ab}	6.08^{ab}	7.13^{a}	6.51^{ab}	5.86^{b}
	rumen ammonia	7.02^{ab}	7.27^{ab}	6.49^{ab}	7.08^{ab}	7.11^{ab}	7.89^{ab}
	BUN	10.01	9.23	9.85	8.89	9.50	10.07
Summer	N	25.3	27.8	19.9	36.9	31.8	37.1
	edg	59.8^{c}	74.2^{ab}	62.8^{c}	73.1^{a}	72.9^{a}	69.3^{a}
	rumen pH	6.29^{ab}	6.28^{ab}	-*	6.28^{ab}	6.37^{ab}	-*
	rumen ammonia	8.37^{abc}	5.65^{b}	5.34^{b}	11.27^{c}	8.63^{abc}	5.70^{b}
	BUN	9.66	9.11	8.50	10.69	9.71	8.39
Autumn	N	26.6	19.9	29.7	40.5	37.2	36.1
	edg	64.7^{c}	54.7^{d}	66.8^{ac}	69.1^{a}	64.0^{c}	74.9^{ab}
	rumen pH	5.85^{b}	6.48^{ab}	6.25^{ab}	6.53^{ab}	6.55^{ab}	6.30^{ab}
	rumen ammonia	8.08^{ab}	6.54^{b}	5.61^{b}	8.17^{ab}	6.04^{b}	7.13^{ab}
	BUN	7.80	8.50	9.60	9.11	10.69	9.14

* - pH probe broke, ^{abc}, figures for same component with differing superscripts differ significantly at *P*=0.05, edg = effective degradability, BUN = blood urea nitrogen.

DISCUSSION

Effective N dg was evaluated at fractional outflow rates of 0.03 and 0.05 as it is considered that these are the most appropriate for kikuyu. Pienaar *et al.* (1993b) established mean retention times of 33.11 hrs for kikuyu in sheep. Mann (2001) established mean retention times of 35.3 to 38.6 hrs for kikuyu when determined by chromium markers and 42.3 to 47 hours when determined by alkane markers in yearling Friesland bulls. Hart and Liebholz (1990) established mean retention times of 39.8 hrs for young kikuyu growth and 36.7 hrs for older stoloniferous growth in kikuyu using ytterbium nitrate as a marker. Mann (2001) determined that peak digesta flow occurred at 21 to 25 hours after feeding. Consequently, fractional outflow values of 0.03 and 0.05 were used as these approximate well with the digesta flow rates and mean retention times of kikuyu.

The edg values of kikuyu N determined for this study equate reasonably well with values in the literature. Erasmus (1990) determined an edg value of 0.701 at k= 0.05 for freeze dried kikuyu herbage, which is consistent with the upper values recorded for the present data. Hart & Liebholtz (1990), using markers, determined a true rumen degradability of 0.79 to 0.8, irrespective of stage of re-growth for oven dried kikuyu herbage. Brand (1998) determined a edg value of 0.5957 for 6-wk re-growth kikuyu N at k = 0.05, very similar to the 40-d values at k=0.05 for the present study (Table 5.4). Van der Merwe (1998) cited N dg values of 0.74, 0.55 and 0.57 for 20-, 30- and 40-d kikuyu re-growths in the autumn at Cedara for k = 0.05. It is of interest to note that while large differences were recorded for the low N treatment edg values over the seasons, minimal differences were observed for the edg's in the high N treatment.

The N loss values for 0 hrs (wash) for these data are highly variable over the season and stage of re-growth, however they were correlated to the N concentration and moisture concentration of the herbage which varied according to the prevailing climatic conditions, namely rainfall, temperature, sunshine or overcast. Dugmore (1985) showed that nitrate levels in kikuyu increased exponentially, with extremely rapid increases in nitrate at concentrations higher than *c.* 3% N in the herbage. This may explain the high a-values recorded at high N concentrations, as nitrate would form part of the soluble fraction. Pienaar *et al.* (1993a) recorded the solubility of kikuyu N as being 30.8% of the N fraction, which approximates well with the a- (soluble fraction) values recorded in the *in situ* N degradability determinations for the 40-d autumn high N re-growth, but greater than the other QDN (a) values recorded. Erasmus (1990) and Brand (1998) recorded QDN (a) values of 36.1 and 32.3 respectively for kikuyu, although this may be influenced by freeze drying which tends to increase the N disappearance at initial incubation times (van der Merwe *et al.*, 2005) and greater DM losses occurred after freeze drying than after oven drying below 60 °C (Lopez *et al.*, 1995; Philippeau & Michalet-Doreau, 1997). It is of interest to note that the N disappearance in annual ryegrass determined in the same environment during spring at Cedara for a 10-h incubation (van der Merwe *et al.*, 2005) were similar to the 24 hour incubation values for kikuyu. The QDN (a) values for these data are closer to the values cited by Fulkerson *et al.* (1998) of 21. However they found no clear seasonal trends for degradation rates over two years and subsequently pooled all their data over the sampling period, as opposed to the clear seasonal and re-growth effects determined for this study. Examining the results of Fulkerson *et al.* (1998), if the pasture was sampled at a relatively young stage of re-growth it would be consistent with our data, especially the high N treatment where no differences were observed between young (20-d) and older (40-d) re-growths. Similarly, the data is consistent to that of Hart and Liebholz (1990), where there were no differences between young leafy growth and older stoloniferous growth. In these data, generally, only the intermediate kikuyu (30-d) re-growth in the low N treatment was appreciably higher than the other treatments. The data for the low N treatment, where dg was not affected by stage of re-growth, is inconsistent with the generally accepted responses, namely that dg increases with age as noted by van der Merwe (1993) for ryegrass at Cedara.

The edg values (k=0.03) for the low N fertilization treatment, which had N fertilization levels slightly lower than those generally recommended for kikuyu in the KZN midlands varied from a high of 77 for 30-d re-growth in spring to a low of 54 for the 30-d treatment in the autumn. The edg values (k=0.03) for the high N treatment, with N fertilization levels far exceeding the recommended levels for kikuyu in the KZN midlands, did not vary appreciably, with a high of 75 down to a low of 64, both recorded in the autumn.

Rumen ammonia concentration for all treatments exceeded the minimum requirement of 3.6 mmol/ℓ (Satter & Slyter, 1974), or the 3 to 6 mmol/ℓ required to support maximum microbial output (Harrison & McAllan, 1980), indicating protein levels in kikuyu were not limiting for rumen fermentation. A few of the treatments marginally exceeded rumen ammonia levels of 8 mmol/ℓ, considered excessive by Roseler (1998), the summer 20-d high N treatment recorded levels of 11.27 mmol/ℓ. Excess ammonia is absorbed into the blood stream and converted to urea in the liver and excreted via the urine, leading to a net loss of CP (Minson, 1990) and energy (Marais, 2001). However, this process is influenced by rumen pH, with a high rumen pH resulting in the ammonia being in the NH_3 form which is rapidly transported across the rumen wall to be converted to blood urea by the liver. All the BUN values exceeded the 6.5 mmol/ℓ recommended by Roseler (1998).

BUN levels were not correlated to edg values of the herbage or other rumen fermentation parameters measured, although they tended to be positively associated with rumen ammonia levels. Minson (1990) stated that the concentrations of blood urea and rumen ammonia are related, but the correlation is low, possibly because the concentration of blood urea is also affected by the energy in the diet. Unfortunately, BUN and the rumen fermentation parameters measures are not associated to rumen degradability of the herbage, thereby eliminating a simple test for protein degradability of the herbage.

Ultimately, the concerns regarding the protein quality of kikuyu, relative to other grass species, appears to be unfounded. In fact, Pienaar *et al.* (1993a) found that microbial N, expressed as a fraction of total non-ammonia N measured in the abomasum varied between 0.5 and 0.7, with 0.54 suggested as an adequate representation of the microbial protein fraction as a fraction of NAN. The efficiency of microbial protein production was 43.2 (±13.3) g N per kg OM apparently digested in the rumen. This compares favorably with the value of 37.8 proposed for fresh grass and legumes (ARC, 1984).

The higher level of N fertilization increased the a-fraction by 54% and the edg by only 4% for kikuyu. The a-fraction increased with stage of re-growth from 20 to 30 days by 82%, after which it remained constant. The increase in the a-fraction with stage of re-growth was unexpected as van der Merwe (1993) showed a decline in the a-fraction with increasing stage of re-growth. Similarly the a-fraction was lower in spring than in the summer and autumn, which did not differ. The edg of the kikuyu herbage declined over the seasons, commencing with a spring high. Nitrogen fertilizer level impacted on the edg, with higher levels recorded for the high N treatment. Stage of re-growth had no impact on the edg values. The b- and c-fractions declined over the seasons and with increasing stage of re-growth, while N fertilization level did not influence these fractions.

In conclusion N fertilization, season and stage of re-growth had a considerable impact on the a-fraction of the herbage. However, N fertilization, season and stage of re-growth did not impact on the edg of kikuyu overall.

Appendix Table 5.1 DM disappearance (%) of the herbage DM after incubation the rumen of dairy cows for kikuyu fertilized at two N levels and harvested at 20-, 30- and 40-d of re-growth

Incubation time		Stage of Re-growth					
		Low N (50 kg N/ha)			High N (200 kg N/ha)		
	(hrs)	20 d	30 d	40 d	20 d	30 d	40 d
Spring	0	13.0 ± 1.45	20.5 ± 12.98	3.19 ± 0.15	11.7 ± 2.77	10.1 ± 7.83	4.19 ± 4.73
	5	33.8 ± 3.86	46.6 ± 1.21	22.7 ± 3.09	36.6 ± 5.28	33.9 ± 4.57	27.9 ± 6.95
	24	75.8 ± 0.71	69.9 ± 5.53	58.3 ± 1.87	77.3 ± 1.95	66.9 ± 8.24	60.3 ± 1.58
	48	83.0 ± 1.55	80.6 ± 3.97	74.1 ± 2.91	84.1 ± 0.22	77.8 ± 3.43	73.3 ± 2.40
	72	84.1 ± 0.86	83.0 ± 3.45	76.6 ± 1.75	84.2 ± 0.85	81.6 ± 1.72	79.6 ± 1.37
Summer	0	0.15 ± 0.03	35.1 ± 1.99	24.5 ± 2.74	20.8 ± 1.88	30.9 ± 0.86	11.7 ± 6.65
	5	13.3 ± 1.01	47.2 ± 0.44	35.0 ± 5.07	30.8 ± 1.93	44.6 ± 0.96	26.9 ± 4.22
	24	46.9 ± 3.04	69.4 ± 2.58	62.9 ± 7.27	61.1 ± 2.63	71.3 ± 3.33	50.3 ± 8.88
	48	64.4 ± 5.50	78.8 ± 2.61	76.0 ± 4.36	72.0 ± 1.99	82.1 ± 4.11	74.0 ± 4.95
	72	66.8 ± 1.89	81.7 ± 1.62	78.7 ± 3.88	73.3 ± 0.76	83.2 ± 1.34	77.0 ± 4.32
Autumn	0	0.19 ± 1.73	0.7 ± 2.87	17.7 ± 1.67	5.2 ± 0.57	9.5 ± 3.21	33.2 ± 2.13
	5	7.6 ± 0.94	11.0 ± 1.33	30.3 ± 4.92	21.9 ± 1.76	24.7 ± 0.24	42.9 ± 1.50
	24	40.2 ± 16.0	33.9 ± 5.00	62.5 ± 6.96	52.9 ± 1.24	48.3 ± 3.42	59.4 ± 4.01
	48	64.8 ± 3.48	53.3 ± 18.09	77.8 ± 5.94	69.2 ± 0.42	67.7 ± 12.4	75.4 ± 4.58
	72	70.2 ± 1.85	65.1 ± 19.00	82.7 ± 2.42	73.7 ± 1.04	76.1 ± 13.4	80.6 ± 2.40

Appendix 5.2 Nitrogen disappearance (%) of the N fraction after incubation the rumen of dairy cows for kikuyu fertilized at two N levels and harvested at 20-, 30- and 40-d of re-growth

Incubation time		Stage of Re-growth					
		Low N (50 kg N/ha)			High N (200 kg N/ha)		
	(hrs)	20	30	40	20	30	40
Spring	0	1.82 ± 2.07	23.77 ± 11.59	9.31 ± 2.21	3.65 ± 2.69	0.15 ± 4.25	8.84 ± 7.29
	5	33.10 ± 5.66	55.18 ± 2.19	33.60 ± 0.11	32.11 ± 4.02	42.81 ± 1.26	36.86 ± 5.47
	24	86.62 ± 2.59	84.69 ± 3.04	78.28 ± 0.11	89.19 ± 1.16	60.05 ± 6.32	76.35 ± 5.82
	48	90.64 ± 2.31	90.40 ± 1.44	87.99 ± 0.82	92.63 ± 1.46	90.53 ± 2.69	87.86 ± 2.82
	72	91.48 ±2.06	91.24 ± 1.81	88.18 ± 0.51	90.54 ± 4.57	91.50 ± 0.33	89.32 ± 1.37
Summer	0	0.28 ± 1.81	30.05 ± 4.26	1.12 ± 2.07	27.01 ± 7.81	18.85 ± 14.19	15.05 ± 12.92
	5	18.21 ± 2.96	45.96 ± 0.48	24.40 ± 10.37	42.43 ± 4.61	41.74 ± 17.66	35.99 ± 6.26
	24	69.29 ± 5.26	83.31 ± 1.78	73.73 ± 5.44	81.58 ± 2.24	82.34 ± 4.39	73.05 ± 2.65
	48	83.20 ± 2.28	90.07 ± 1.48	86.81 ± 1.44	88.64 ± 1.91	92.29 ± 1.78	88.99 ± 2.51
	72	82.57 ± 1.25	91.04 ± 0.97	86.88 ± 2.16	88.88 ± 0.85	92.51 ± 1.38	91.79 ± 2.28
Autumn	0	8.30 ± 1.80	3.04 ± 2.84	14.17 ± 11.40	15.16 ± 0.69	12.26 ± 3.09	36.45 ± 4.62
	5	31.48 ± 6.73	22.33 ± 3.88	34.70 ± 6.22	38.58 ± 6.53	38.33 ± 2.73	51.71 ± 2.81
	24	71.57 ± 8.44	61.36 ± 3.91	72.87 ± 9.53	78.91 ± 2.47	72.07 ± 1.42	80.07 ± 1.70
	48	87.33 ± 1.40	76.57 ± 11.06	90.49 ± 1.15	87.44 ± 0.24	89.71 ± 0.37	91.79 ± 0.97
	72	88.26 ± 2.89	84.20 ± 8.66	92.71 ± 0.25	89.64 ± 1.03	91.40 ± 5.14	92.63 ± 0.76

Chapter 6

The Effect of Nitrogen Fertilization and Stage of Re-growth of Kikuyu on Rumen Fermentation Parameters

Abstract

The effect of high N levels at different stages of re-growth on rumen fermentation parameters was determined for kikuyu. Kikuyu herbage was cut and fertilized with either 50 or 200 kg N/ha prior to harvesting at 20-, 30- or 40-d re-growth. These treatments were conducted in the summer and autumn. The kikuyu harvested was fed to rumen fistulated sheep, three per treatment, in metabolic crates at each of the re-growth stages for each season, with a further treatment with sheep on the high N material being supplemented 5 g of a yeast culture daily (Yea-Sacc). Sampling occurred at 0, 2, 4 and 6 hours after feeding. Rumen pH was determined in situ, while samples were taken for analysis of rumen ammonia. Blood samples were taken to determine blood urea nitrogen. Herbage samples were analysed for DM, N and NDF. Rumen pH increased also with increasing level of N fertilization and declined with advancing stage of herbage re-growth in the autumn. Rumen pH declined linearly with time post morning feeding in summer and autumn. The rumen pH decreased rapidly with time for the low N fertilization level, but relatively slowly for the high N fertilization level. The rumen pH decreased with increasing herbage DM and NDF concentrations. Rumen ammonia increased with time of sampling post feeding to 4 hrs and then tended to decline by 6 hours. Nitrogen fertilization level influenced rumen ammonia levels significantly, with the low N level producing the lowest rumen ammonia levels (8.86), Yea-Sacc the highest values (12.11) and the high N intermediate (10.54) in summer, while in the autumn, the low N treatment was the lowest (9.53), with the Yea-Sacc (11.7) and high N (12.57) not differing. Rumen ammonia levels were greatest at 20-d re-growth stage in summer and at the 40-d re-growth stage in autumn. DM concentration of the herbage had an inverse relationship with rumen ammonia. Blood urea nitrogen (BUN) levels were increased by high N fertilization and were positively correlated to rumen ammonia levels. Yea-Sacc markedly increased the intake of the sheep on the high N material.

Keywords: *Pennisetum clandestinum*, N fertilization, stage of re-growth, season, rumen pH, rumen ammonia, blood urea nitrogen.

INTRODUCTION

High levels of N in kikuyu herbage have been negatively associated with intake, digestibility and animal production (Milford & Minson, 1965; Holder, 1967; Cambell, 1971; Jeffery, 1971; Minson, 1973; Austin; 1980; Pattinson, 1981; Tainton *et al.*, 1982; Karnezos, 1986; Dugmore *et al.*, 1986; Dugmore & du Toit, 1988; Pienaar *et al.*, 1993b). Nitrites derived from high N pastures have been shown to markedly reduce digestibility *in vitro* (Marais *et al.*, 1988). The presence of readily digestible carbohydrates in the digesta tends to ameliorate the harmful effects of nitrite (Wright & Davison, 1964). Chemical analysis of kikuyu indicates a severe protein-to-energy imbalance, caused by a lack of readily digestible energy in the form of NSC (Marais, 2001). High rumen ammonia concentrations have been shown to be negatively related to VFI in kikuyu, due to a high load of ammonia on the liver (Pienaar *et al.*, 1993b). A relatively high rumen pH facilitates the rapid movement of ammonia across the rumen wall (Bloomfield *et al.*, 1963).

Monensin has been shown to have a protein-sparing effect due to a reduced proteolytic activity in the rumen (Poos *et al.*, 1979; Bergen & Bates, 1984); consequently it has been shown to reduce milk urea nitrogen levels in dairy cows grazing kikuyu pastures (van der Merwe *et al.*, 2001). Yeast cultures have been shown to improve VFI (Newbold 1995) and indirectly manipulate rumen pH (Lundeen, 2006). Increases in cellulolytic and total bacteria numbers, with associated increases in microbial protein flow from the rumen have been observed (Newbold, 1995). The effect of supplements expected to manipulate (monensin) or enhance rumen fermentation (Yea-Sacc, a yeast culture; Alltech Company, Nicholasville, Kentucky, USA) were tested in an attempt to reduce the negative effects of high N on rumen fermentation. This study was conducted to determine the impact of level of N-fertilizer application and stage of kikuyu herbage re-growth, and monensin and Yea-Sacc on rumen fermentation, rumen pH, rumen ammonia and BUN.

MATERIALS AND METHODS

The experimental area is situated at Cedara (29° 32' S; 30° 17' E) in the KwaZulu-Natal Mistbelt. Cedara lies at an altitude of 1067 m asl and receives an average annual rainfall of 875 ±142 mm, which falls predominantly in summer. The mean January maximum/minimum temperature is 25.3/14.8°C, while frosts occur during winter.

Pasture

Kikuyu pasture at Cedara, established in *c*. 1930, was cut and fed to sheep in this study. Each treatment was allocated 10 plots of pasture and plot sizes were determined in a pilot cutting trial in which herbage DM yields for each N level and stage of re-growth were determined. Soils in these plots were sampled and analyzed prior to each season so as to correct for nutrient deficiencies according to the recommendations of the Cedara fertilizer advisory service (Manson *et al*., 2005). Nitrogen fertilizer (in the form of LAN) was applied at two rates, namely 50 kg N and 200 kg N per ha for each cut to induce both low and high protein levels in the herbage. On Day 1, a plot belonging to each of the treatment combinations was cut with a reciprocating mower and fertilized; on Day 2, the second plot of each treatment combination was also cut and fertilized with N. This process of cutting and applying fertilizer continued until the 10th plot of each treatment combination was cut and fertilized on Day 10. Commencing on days 20, 30, and 40 for the respective re-growth stages, kikuyu herbage was cut and fed to sheep as the herbage reached the appropriate re-growth stages, which were 20-, 30- and 40-d re-growth. This procedure was followed to ensure a supply of herbage at the same stage of re-growth over a 10-day period. Consequently, data collection terminated on days 29, 39, and 49 for the 20-, 30- and 40-d re-growth stages, respectively.

Fistulated Sheep and experimental design

Twelve rumen fistulated sheep, ranging in weight from 25 to 35 kg over the growing season, were assigned to the four treatment groups of 3 balanced for initial weight. Within a re-growth stage, each of these animal groups was randomly assigned to the 4 treatments (low N, high N without or with either Yea-Sacc at 5 g/d or monensin at 0.15 g/d (carried in 5g molasses meal). The experiment was conducted early (summer) and late (autumn) in the season. Animals previously on kikuyu were adapted to the re-growths for 4 days before a 6-day data collection phase. Fresh herbage was distributed at 08h00 and topped up in the afternoon (15:00). Samples were collected on days 3 and 5 of the feeding period. Sampling of blood and rumen fluid, and measurement of rumen pH (*in situ*) commenced at 08h00, immediately after feeding, on the collection day and continued thereafter every 2 hours until 6 hours had elapsed from the time of feeding. Rumen pH was taken *in situ* using a pH probe, with 3 readings, taken in the fore, mid and posterior rumen.

Rumen fluid was taken from the different areas of the rumen, filtered through cheesecloth into a 250 ml beaker, sub-sampled with a 5 ml pipette and acidified with 1 ml 1N H_2SO_4 added per 10 ml of filtrate. The filtrate was centrifuged and the supernatant frozen until required for the analysis of NH_4^+ ions using an auto-analyzer (AOAC method 990.02, semi-automated). Blood samples were collected from the jugular vein into 8.5 ml lithium heparin vacutainer® tubes, centrifuged immediately at 2000 rpm, to prevent rupture of red blood cells, for 10 minutes and plasma frozen until analysed for urea using an auto-analyzer (Chaney & Marbach, 1962).

Chemical analysis

Feed samples were analyzed for DM, CP (AOAC, 1980), and NDF following the method of Goering and van Soest (970) as modified by van Soest and Robertson (1980).

Statistical analysis

Sheep on the monensin treatment experienced a drastic decline in intake to the extent that the monensin levels became toxic, consequently this treatment was terminated early in the first trial. Data were analyzed using the analysis of variance and multiple regression techniques of Genstat 8.1 (Genstat, 2005), with N fertilizer level, stage of re-growth and time after feeding as main effects. Time values was analysed as a repeated measure. Where data sets were unbalanced, the residual maximum likelihood model (REML) of Genstats variance component analysis was used. Differences between means were determined by the LSD range test method. The LSD method was protected by F test (Snedecor & Cochran, 1980) and differences were only accepted as being significant when the overall F test was significant ($P<0.05$). Voluntary feed intake data was analysed using Gensta 8.1 (Genstat, 2005).

RESULTS

The results are presented in Tables 6.1 to 6.3, with the predicted means for the main effects given in Table 6.4.

Rumen pH

Generally, rumen pH declined linearly with time post morning feeding in summer and autumn (Table 6.1). Rumen pH also increased with increasing level of N fertilization and declined with advancing stage of herbage re-growth in the autumn. Rumen pH was affected by the interaction effects of time of sampling x N fertilization level in both seasons, time x stage of re-growth in summer, and time x N fertilization x re-growth stage in autumn. In both seasons, the rumen pH decreased rapidly with time for the low N fertilization level, but relatively slowly for the high N fertilization level. In summer, the pH decreased linearly for the 30-d re-growth stage, but followed a curvilinear trend for the 20- and 40-d re-growth stages, apart from the 20-d high N treatment which fluctuated over time. In autumn, the pH decreased consistently faster over time for all re-growth stages receiving the low N than for the high N-fertilizer application. For re-growth stages receiving a high N application, the pH declined relatively slowly for the 30 and 40 days re-growth but remained fairly stable at close to neutral pH for the 20 day re-growth stage and Yea-sacc (6.63) treatments.

Stage of re-growth also influenced rumen pH levels, the highest rumen pH recorded on the 20-d treatment (6.68), the lowest on the 40-d treatment (6.38), with the 30-d treatment intermediate (6.55).

The rumen pH tended to decrease with increasing herbage DM ($P= 0.065$) and NDF ($P = 0.065$) concentrations. However, both these variables included in a multiple regression could explain 41% of the variation in rumen pH as indicated in the following equation:

$$\text{Rumen pH} = 10.03 - 0.0086\ (\text{DM}) - 0.0026\ (\text{NDF}); \qquad R^2 = 0.406; \quad P = 0.023; \quad n = 14$$

Rumen Ammonia

Overall, rumen ammonia increased with time of sampling post feeding to 4 hrs and then tended to decline by 6 hrs. Individual treatments did not differ for time post feeding, apart from the 20-d summer treatment which peaked at 4 hours post feeding. In the low N treatment there was a trend to peaking earlier at advancing stages of re-growth in both summer and autumn, the 30-d re-growth peaking at 2 hrs and the 40-d re-growth was maximal at 0 hrs. Apart from the summer Yea-Sacc 20-d re-growth treatment and the low N 20-d treatment, which increased linearly with time, the other treatments were not different for time. A trend was recorded ($P = 0.063$) for rumen ammonia levels increasing after feeding to peak at 4 hours after the morning feed in the autumn.

In the summer, N fertilization level influenced rumen ammonia levels, with the low-N level producing the lowest rumen ammonia levels (8.86 mmol/l) Yea-Sacc the highest values (12.11 mmol/l) and the high N intermediate (10.54 mmol/l). In the autumn, N exerted a effect on the rumen ammonia levels with the low N treatment the least (9.53 mmol/l), with the Yea-Sacc (11.7 mmol/l) and high N (12.57 mmol/l) not differing.

Stage of re-growth had a highly significant impact on rumen ammonia levels, with the 20-d treatments the greatest (15.4 mmol/l), the 30-d the lowest (7.65 mmol/l) and the 40-d intermediate (8.45 mmol/l) in summer. In the autumn, stage of re-growth exerted a quadratic effect on rumen ammonia levels with the 40 day recording the greatest values (13.01 mmol/l), the 30-d the least (9.81 mmol/l) and the 20-d intermediate (10.97 mmol/l) but not different from the 30-d treatment.

Time x re-growth and time x N x re-growth interactions were found for the summer treatments, with no interactions recorded for the autumn treatments. In the time X re-growth interaction, the 20-d treatment ammonia values increased to 6 hrs, while the 30- and 40-d peaked at 2 and 4 hrs respectively. For the time x N x re-growth interaction the Yea-Sacc treatment was consistently higher than the other treatments over all time intervals for the 40-d treatment, but not for the 20- and 30-d treatments.

Rumen ammonia was influenced by a combination of the N concentration and DM of the herbage, the effect of DM possibly due to its inverse relationship with rumen pH ($P = 0.063$). Increasing digestibility of the herbage ($P = 0.16$; $R^2 = 0.06$) tended to reduce rumen ammonia levels. Rumen ammonia tended to be negatively associated with rumen pH levels ($P = 0.138$). These relationships are given below:

$$\text{Rumen } NH_4 = 7.728 + 0.056\,(N) - 0.0146\,(DM); \quad R^2 = 0.41; \quad P = 0.01; \quad n = 18$$

The very strong exponential relationship between herbage N concentration and rumen ammonia is illustrated by the following equation:

$$\text{Rumen } NH_4 = 3.88 + 0.06\,(N^2); \qquad R^2 = 0.98; \qquad P < 0.0001; \qquad n = 18$$

Table 6.1 Rumen pH values of sheep fed freshly cut kikuyu herbage, fertilized with low (50 kg N/ha) or high (200 kg N/ha) levels of N and harvested at 20-, 30-, and 40-d re-growth and the effect of Yea-Sacc on the high N treatment (LSD summer = 0.51; LSD autumn = 0.47)

Rumen pH		Low N treatment				High N treatment				Yea Sacc Treatment			
		hrs post feed				hrs post feed				hrs post feed			
	Hrs	0	2	4	6	0	2	4	6	0	2	4	6
Round 1	20 day	6.52	6.23	5.99	5.93	6.40	6.58	6.41	6.61	6.57	6.37	6.15	6.03
Summer	SE	0.25	0.15	0.17	0.16	0.58	0.45	0.50	0.43	0.15	0.08	0.13	0.11
	30 day	6.72	6.31	6.01	5.73	6.45	6.37	6.19	6.17	6.87	6.55	6.60	6.43
	SE	0.35	0.36	0.29	0.22	0.19	0.31	0.39	0.37	0.14	0.13	0.10	0.07
	40 day	6.64	6.36	6.24	6.22	6.43	6.40	6.32	6.27	6.49	6.50	6.44	6.33
	SE	0.39	0.29	0.18	0.23	0.14	0.17	0.19	0.15	0.45	0.46	0.28	0.21
Round 2													
Autumn	20 d	6.92	6.44	6.18	5.96	7.09	7.03	7.01	6.90	6.89	6.67	6.60	6.50
	SE	0.33	0.24	0.14	0.18	0.38	0.35	0.43	0.48	0.66	0.59	0.52	0.48
	30 day	6.78	6.42	6.23	6.00	6.67	6.62	6.59	6.59	6.84	6.72	6.66	6.55
	SE	0.31	0.30	0.29	0.29	0.18	0.30	0.29	0.18	0.19	0.18	0.24	0.22
	40 day	6.23	6.07	5.92	5.72	6.77	6.60	6.60	6.59	6.75	6.50	6.45	6.40
	SE	0.72	0.49	0.44	0.35	0.39	0.27	0.24	0.23	0.34	0.25	0.28	0.25

SE = standard error

Table 6.2 Rumen ammonia concentration (mmol/l) of sheep fed freshly cut kikuyu herbage, fertilized with low (50 kg N/ha) or high (200 kg N/ha) levels of N and harvested at 20-, 30-, and 40-d re-growth and the effect of Yea-Sacc on the high N treatment (LSD summer = 4.63 : LSD autumn = 4.96)

		Low N treatment				High N treatment				Yea Sacc Treatment			
		hrs post feed				hrs post feed				hrs post feed			
Round 1		0	2	4	6	0	2	4	6	0	2	4	6
Summer	20 day	10.44	13.59	16.67	16.25	13.06	15.18	15.06	15.55	15.12	15.86	18.40	19.58
	SE	1.52	2.98	4.83	3.98	4.07	3.33	2.22	4.10	5.11	1.48	2.70	2.73
	30 day	4.93	7.03	6.23	5.93	9.43	8.20	7.87	8.06	8.62	9.36	8.21	8.40
	SE	1.17	1.37	1.20	2.00	2.50	1.66	2.26	2.23	1.59	1.77	1.88	2.64
	40 day	7.30	6.23	6.67	5.41	6.66	8.95	10.86	7.60	10.09	9.89	12.78	8.97
	SE	0.94	1.96	1.18	1.88	4.31	3.99	5.66	4.24	4.05	2.83	3.80	1.66
Round 2													
Autumn	20 d	11.75	9.39	12.32	9.90	10.68	10.18	12.36	13.10	8.91	10.13	13.12	9.82
	SE	1.86	2.25	1.78	2.25	3.13	4.58	5.21	6.84	3.08	4.95	4.90	2.89
	30 day	7.28	7.96	7.37	7.37	10.27	12.28	13.08	11.46	9.22	9.33	9.92	12.23
	SE	2.26	3.68	1.45	3.50	5.67	3.09	1.62	1.77	2.04	1.93	3.82	5.37
	40 day	10.90	9.45	10.50	10.16	13.19	14.30	14.88	15.01	13.62	13.56	15.59	14.99
	SE	5.44	2.93	3.12	3.31	3.94	2.93	2.26	2.41	2.76	3.24	3.99	3.57

SE = standard error

Table 6.3 Blood urea nitrogen concentrations (mmol/l) of sheep fed freshly cut kikuyu herbage, fertilized with low (50 kg N/ha) or high (200 kg N/ha) levels of N and harvested at 20-, 30-, and 40-d of re-growth and the effect of Yea-Sacc on the high N treatment (LSD summer = 2.66; LSD autumn = 2.26)

		Low N treatment				High N treatment				Yea Sacc Treatment			
		hrs post morning feed				hrs post morning feed				hrs post morning feed			
Round 1		0	2	4	6	0	2	4	6	0	2	4	6
Summer	20 day	5.15	5.44	5.51	5.56	8.18	7.97	7.60	7.64	7.61	7.51	7.69	7.34
	SE	0.91	1.39	1.36	1.29	2.30	2.19	1.70	1.80	0.99	1.06	1.27	1.31
	30 day	6.64	6.57	6.56	6.69	7.58	7.89	7.77	7.86	6.81	6.75	7.03	7.18
	SE	1.44	1.46	1.30	1.83	1.79	0.69	0.56	0.62	0.87	1.28	1.40	1.58
	40 day	5.01	5.18	5.45	5.24	6.19	6.55	6.60	6.47	7.92	7.54	8.03	9.71
	SE	1.54	1.43	1.30	1.06	1.19	1.25	1.28	1.17	2.95	3.11	2.74	3.41
Round 2													
Autumn	20 d	6.45	6.47	6.19	7.34	6.16	7.81	7.19	7.99	7.88	7.88	8.14	8.69
	SE	1.10	0.94	1.11	1.27	1.85	0.66	1.24	0.65	1.92	2.08	2.19	2.33
	30 day	7.60	8.31	8.69	7.61	9.00	8.42	9.41	7.93	8.73	8.84	9.51	9.67
	SE	1.40	0.95	1.31	0.93	2.22	2.37	2.47	1.16	2.84	2.84	2.29	1.97
	40 day	5.86	6.44	7.52	6.88	5.64	6.60	6.71	6.51	8.51	9.55	9.37	8.01
	SE	1.49	0.88	1.27	1.01	0.39	0.92	1.90	0.53	1.11	1.57	2.05	1.24

SE = standard error

Table 6.4 The predicted means for the main effects (time after feeding, N fertilization level and stage of re-growth) on the rumen pH, rumen ammonia levels (mmol/l) and blood urea nitrogen (mmol/l) levels of sheep fed kikuyu herbage, fertilized with either 50 kg N/ha or 200 kg N/ha and harvested at a 20-, 30- or 40-d re-growth in summer and autumn

Parameter	Time after feed (hrs)				Nitrogen			Re-growth			Significant Interactions
	0	2	4	6	L	H	HY$^{\#}$	20	30	40	
Summer											
pH	6.57^{a}	6.41^{b}	6.26^{c}	6.19^{d}	6.24	6.38	6.45	6.32	6.37	6.38	T.R* T.N.
Ammonia (mmol/ℓ)	9.50^{a}	10.45^{b}	11.42^{c}	10.64^{bc}	8.86^{b}	10.54^{ab}	12.11^{a}	15.4^{b}	7.65^{a}	8.45^{a}	T.R; T.N.R
BUN (mmol/ℓ)	6.77	6.82	6.91	7.07	5.75^{a}	7.55^{b}	7.58^{b}	6.93	7.09	6.66	-
Autumn											
pH	6.77^{a}	6.56^{b}	6.47^{c}	6.35^{d}	6.24^{b}	6.75^{a}	6.62^{a}	6.68^{a}	6.55^{ab}	6.38^{b}	T.N; T.N.R
Ammonia (mmol/ℓ)	10.65	10.73	12.13	11.56	9.53^{b}	12.57^{a}	11.70^{a}	10.97^{a}	9.81^{a}	13.01^{b}	-
BUN (mmol/ℓ)	7.29^{a}	7.79^{b}	8.04^{b}	7.81^{b}	7.02^{b}	7.45^{ab}	8.73^{a}	7.35^{a}	8.64^{b}	7.21^{a}	T.R; T.N.R

*; T = time after feeding in hrs; N = nitrogen fertilization treatment; R = stage of re-growth at harvesting.

$^{\#}$, HY = high N + Yea-Sacc treatment

^{a,b,c,d}, means with differing superscripts within main effect differ

Blood Urea Nitrogen

In the summer, sampling times post-morning feed did not affect BUN over all treatments and re-growth stages. Similarly, the 20-, 30- and 40-d stages of re-growth post fertilization did not influence the BUN values. However, N fertilization level did have an influence on the BUN values, with the low N treatment recording lower BUN values than the high N and Yea-Sacc treatments, with means of 5.75, 7.357 and 7.579 mmol/l for the low N, high N and Yea-Sacc treatments respectively. No interactions were found for the different factors recorded. For the Yea-Sacc 30- and 40-day re-growths there is a slight trend to increasing BUN with time post feeding.

In the autumn, differences were recorded between sampling times with the 0-hr treatment lower than the 2-, 4- and 6-hr post feeding recordings which did not differ from each other. The Yea-Sacc treatment was also greater than the low-N treatment, with the high N intermediate (8.731, 7.447 and 7.020 mmol/l for the Yea-Sacc, high N and low N respectively). Stage of re-growth also exerted a quadratic effect on the BUN values, with the 30-d treatment recording greater values than either the 20- or 40-d stage of re-growth (7.349, 8.644 and 7.206 mmol/l for the Yea-Sacc, high N and low N respectively). A quadratic interaction between time of sampling, N level and stage of re-growth was established, with the Yea-Sacc treatment recording greater BUN levels than both the high N and low N treatments at the 40-d stage of re-growth. Yea-Sacc maintained high BUN values at all the stages of re-growth relative to the decreases in BUN for the 40-d treatment for both low and high N.

Overall, BUN levels tended to be influenced by rumen ammonia levels, this relationship is as follows:

$$\text{BUN} = 7.495 + 0.25\ (\text{rumen NH}_4); \qquad R^2 = 0.137; \qquad P = 0.07; \qquad n = 18$$

Rumen pH levels tend to be positively associated ($P = 0.06$) with BUN levels.

Effect of Yea-Sacc on Dry Matter Intake

The DMI of the sheep consuming high N herbage, with and without Yea-Sacc, is presented in Table 6.5. YeaSacc improved the VFI of sheep, successfully negating the negative effects of high N fertilization on kikuyu herbage intake (chapter 3).

Table 6.5 Herbage intake of sheep fed fresh kikuyu herbage (*as fed*) fertilized with high N and cut at 20-, 30- and 40-d re-growth, with or without Yea-Sacc supplementation

Days re-growth	Kikuyu intake (kg fresh/day)	
	high N	high N + Yea-Sacc
Early season		
20	1.28 ± 0.25	4.16 ± 0.42
30	2.40 ± 1.06	4.10 ± 0.37
40	1.82 ± 0.58	3.49 ± 0.66
Mean	1.83	3.91
Late season		
20	2.53 ± 0.50	3.64 ± 0.71
30	3.57 ± 0.17	4.44 ± 0.48
40	2.59 ± 0.92	3.02 ± 1.50
Mean	2.89	3.70

DISCUSSION

The rumen pH values recorded were generally within the recommended values for good rumen fermentation. An exception to the general trends were high rumen pH levels for the high N treatment in the autumn 20-d re-growth, remaining stable but high with time after feeding. Alkalosis problems were experienced at times on this treatment. The Yea-Sacc treatment for the 20-d autumn re-growth followed the typical downward trend, which, considering that it was added to high N herbage, tended to ameliorate the negative effects of the high N treatment. Giger-Reverdin *et al*. (2004) showed that live yeast culture improved the ruminal buffering capacity and that rumen buffering capacity increased as the pH decreased.

The inverse relationship recorded between NDF + DM and rumen pH appears to be consistent with the NRC (2001) statement that "the concentration of NDF is inversely related to rumen pH because NDF generally ferments slower and is less digestible than NFC (i.e. less acid production in the rumen) and because the majority of dietary NDF is in the form of forage with a physical structure that promotes chewing and saliva production (i.e buffering capacity)". However, it would appear that the NRC was referring to acidity rather than pH, in which case the inverse relationship between rumen pH and NDF recorded is atypical. For kikuyu, in these data, it would appear that highly N-fertilized kikuyu with high moisture (low DM) and low NDF concentrations inherently results in alkalytic diets. Alkalosis was a factor at times with animals on high N treatments. Increasing DM and NDF concentration suppress this alkalytic tendency in kikuyu and reduce rumen pH to normal levels for these data. Pienaar *et al*. (1993b) found that rumen fill was affected by the moisture concentration of the kikuyu, high levels of rumen fill being recorded on drier kikuyu. Rumen fill was also greater on high-NDF material, compared to low-NDF material. Pienaar *et al*. (1993b) also found that low intake was associated with high rumen ammonia levels, rumen ammonia being negatively correlated (-0.138) with rumen pH for these data.

Pienaar *et al.* (1993b) recorded similar rumen ammonia values to the present data for sheep of 6 to 14 mmol/l. However, Marais *et al.* (1990b) recorded higher rumen ammonia values of 18 mmol/l for sheep fed kikuyu containing 36 g N/ kg DM (225 g/kg CP) and 8.5 mmol/l for kikuyu with an N concentration of 27 g N /kg DM (169 g/kg CP).

The rumen ammonia levels recorded are considered excessive, exceeding the 8 mmol/l recommended by Roseler (1998) at the 20-d re-growth stage even for the low N (50 kg N/ ha) dressing, possibly an explanation for the poor responses on the 20-d treatments in early to mid season recorded for the digestion and VFI trials. However, at the 30- and 40-d stage of re-growth the levels are acceptable, even for the high N fertilized level (200 kg N/ ha). The high rumen ammonia levels recorded in the autumn the high 40-d re-growth material (14-15 mmol/l) relative to the 20-d treatments with lower rumen ammonia levels (10-13 mmol/l) in autumn may explain why greater feed intakes were recorded for the 20-d herbage, in contrast to the spring and summer intakes in Chapter 3.

Rumen ammonia levels recorded in this trial were consistently high, exceeding the requirement for "maximal microbial protein production" (Satter & Slyter, 1974) but possibly low at times for "maximal rumen fermentation rates" (Mehrez & Orskov, 1977). Satter and Roffler (1975) suggested a minimum rumen microbial requirement of 2.9 mmol/l rumen fluid (5 mg/dl) for maximal microbial protein production, or the 1.6 mmol for roughage diets determined by Pisulewski *et al.* (1981). Wallace (1979) suggested that the optimal range for ruminal ammonia concentration was 1.1 and 11.74 mmol/l. However, Boniface *et al.* (1986) suggested that with the animal grazing forage, emphasis should rather be placed on the maximal rate of fermentation, as feed intake and nutrient supply both depend on the rate of fermentation, while microbial protein synthesis is an important but secondary consideration. Orskov (1982) concluded that for maximal rumen fermentation, the rumen ammonia concentration should be between 11.74 and 14 mmol/l rumen fluid. Nagadi *et al.* (2000), in an *in vitro* gas production study, found that an ammonia concentration of 1.7 mmol/l was sufficient for maximal NDF digestibility, whilst one of between 3.5 and 7.1 mmol/l ammonia was necessary to achieve the maximal potential fermentation rate for NDF in kikuyu. These are lower than the values of Orskov (1982) due to the lower fermentable energy value of tropical forages (Nagadi *et al.,* 2000). For these data, mean rumen ammonia levels were 10.5 in the summer and 11.27 in the autumn. The 20-d re-growth in summer elicited ammonia levels (15.4 mmol/l and at times peaking at over 16 mmol/l, even for the low N treatment) exceeding the upper value of 14 mmol/l ammonia recommendations by Orskov (1982), as did the 40 day high N and Yea-Sacc re-growth in autumn (approx 15 mmol NH_3/l rumen fluid). The higher rumen ammonia levels in the autumn 40 day re-growths may be associated with low digestibility and a lack of readily available carbohydrate in the rumen. Pienaar *et al.* (1993a) recorded rumen ammonia levels of between 6 and 14 mmol/l rumen fluid and found that rumen ammonia levels were negatively correlated to VFI (P=0.0107) in kikuyu, concluding that these high levels supported the theory that a high load of ammonia reaching the liver could inhibit VFI. In terms of optimal levels of rumen ammonia for fermentation rates, the values recorded by Pienaar *et al.* (1993b) fall within the range cited by Orskov (1982), but even at these “optimal” levels the rumen ammonia concentration was negatively associated with DMI, indicating a lack of fermentable energy or some other negative factor, possibly associated with high N as suggested by Last (1993) and Macfarlane (undated), present in kikuyu.

High rumen ammonia levels are aggravated by low rumen pH, as high rumen pH facilitates the rapid movement of ammonia across the rumen wall (Bloomfield *et al.*, 1963). Pienaar *et al.* (1993b) recorded a mean rumen pH of 6.53. The mean rumen pH recorded in this trial was 6.36 in summer and 6.54 in the autumn. Grant and Mertens (1992) stated that cellulolytic organisms grow optimally at a pH of 6.7 and that at a pH below 6.2 digestion rate is inhibited. The rumen pH values recorded therefore appear to be very close to the optimal levels for rumen fermentation. Nitrogen fertilization and stage of re-growth had no effect on the rumen pH in the summer. However, in the autumn, N fertilization and stage of re-growth impacted on rumen pH. The high N treatment with a predicted mean pH of 6.75 compared to 6.23 for the low N treatment. Rumen pH declined with an increasing stage of re-growth, the 20-d treatment peaking at a pH of 6.68, declining to 6.38 at 40 days. Besides these, an interaction between N, time after feeding and stage of re-growth produced rumen pH values exceeding 7 for the high N 20-d treatment. On several occasions during the trials, animals on the high-N treatment were diagnosed as suffering from alkalosis, indicating a potential problem. Consequently, high rumen pH combined with relatively high rumen ammonia levels could have caused high blood ammonia levels, negatively impacting on VFI, especially at the 20-d stage of re-growth. This impact of high rumen pH and ammonia should be reflected in high BUN nitrogen values as shown by the positive correlation for rumen ammonia and pH on BUN for these data, with all the BUN values exceeded the 6.5 mmol/ℓ recommended by Roseler (1998).

Feeding excess degradable protein can lead to an increase in ruminal ammonia and higher urea concentration in body fluids, including blood and milk. A high level of ammonia in the rumen will cause an increase in rumen pH, which increases the absorption rate of ammonia from the rumen. Ammonia is very toxic, and as a result excess ammonia is carried primarily to the liver and converted to urea which is excreted in the urine or recycled back to the rumen (Ahmadzadeh, 2001). Elevated BUN is correlated with infertility and overall poor reproductive performance, with 7.1 mmol/l considered the critical value above which fertility problems are associated (Ferguson, *et al.*, 1993; Butler, 1998, Ahmadzadeh, 2001).
The mean BUN levels recorded for these data are 6.89 for the summer and 7.73 for the autumn, similar values to the 7.38 mmol/l recorded by van der Merwe *et al.* (2001) for dairy cows fed concentrates on

kikuyu. These values fall within the range, 3.57 to 8.92 mmol BUN/l considered typical for mammalian plasma (Frandson *et al.,* 2003), although considering the preceding discussion reproductive inefficiency can be expected at the high end of the range. The BUN levels for the present data are greater than the recommended value of 6.5 mmol/l (Roseler,1998), especially for the high N and Yea-Sacc treatments (namely, 7.4 and 8.7 respectively) and for all stages of re-growth (6.3, 8.6 & 7.2 for 20-, 30- & 40-d re-growths respectively) in the autumn. The low N treatment recorded slightly lower BUN values than those recommended by Roseler (1998). Haaland *et al.* (1977) considered BUN to be an objective test, helpful in determining a protein or energy inadequacy. Haaland *et al.* (1977) found that a balanced high protein : high energy diet produced BUN values in the normal range of c. 4 mmol/l, a high protein : low energy diet c.7.3 mmol BUN/l, a low protein : high energy diet produced 0.96 mmol BUN/l and a low protein : low energy diet produced 2.3 mmol BUN/l. The BUN levels recorded for the present data indicate an unfavorable energy to protein ratio with excessive protein relative to the energy concentration which is potentially detrimental to reproductive performance. This lack of energy relative to protein in highly fertilized, high protein, kikuyu has been emphasized by Marais *et al.* (1990) who had to supplement the kikuyu with 330 g maize meal per kg DMI to reduce the excessive rumen ammonia levels (18 mmol/l) on the high N diet to the 8.5 mmol NH_3/l recorded on a lower N kikuyu diet.

Yea-Sacc supplementation tended to ameliorate the negative effects of the high N treatment as well as stimulating intake, even though it was associated with some of the highest rumen ammonia levels recorded in the trials. Giger-Reverdin *et al.* (2004) showed that live yeast culture improved the ruminal buffering capacity and that rumen buffering capacity increased as the pH decreased. Reviews of research trials on yeast supplements showed that an improvement in production when yeast is added to the diet can largely be explained by an increase in DMI (Newbold, 1995; Macgregor, 2000). It has been suggested that yeast may scavenge oxygen within the rumen, thereby stimulating the growth of anaerobic bacteria (Newbold, 1995). Martin and Nisbet (1992) also suggested that the metabolites in the yeast culture may stimulate bacterial growth. Yeasts have been shown to increase rumen solids turnover rate, rumen liquid dilution rate, total bacteria numbers, as well as cellulolytic bacteria numbers (Macgregor, 2000).

In conclusion, high levels of N fertilization of kikuyu results in herbage with high CP concentrations, however, not as high as those recorded on ryegrass at the same N fertilization levels by van der Merwe (1993), causing high rumen ammonia, low rumen pH and consequently high BUN levels in ruminants grazing kikuyu. These N fractions can peak at levels considered to be potentially dangerous for livestock health and detrimental to production and reproduction. The supplementation of a yeast culture in a potentially stressed rumen environment ameliorated the negative effects of high ammonia and rumen pH.

Chapter 7

Effect of Environmental Factors and Herbage Composition on the Digestibility and the Voluntary Feed Intake of Kikuyu

Abstract

The effect of temperature, rainfall and evaporation on the digestibility and intake of kikuyu was determined by pooling years of variable rainfall and temperatures data for analyses by multiple regression. Five years of digestibility data, amounting to 82 digestion trials, were pooled for digestibility. Voluntary intake data were pooled for the three years of intake trials, amounting to 38 intake trials. These data, chemical composition of the herbage and the daily maximum temperatures, rainfall and evaporation recorded at and prior to the digestion and intake trials at Cedara were pooled, analysed using multiple regression techniques, and regressed on DMD and VFI, to examine the influence of environment and herbage composition on the nutritive value of the herbage and to develop simple linear regression models for predicting kikuyu quality and intake. Rainfall and temperature in the period of cutting and fertilization had a negative effect on digestibility, irrespective of the stage of re-growth at harvesting, 20, 30 or 40 days later, and a combination of the two proved significant, accounting for the most variance in DMD. Temperature depressed DMD by 11.4 g/kg DM per degree rise in temperature (^{o}C). Temperatures recorded during the cutting and fertilization phase were highly negatively correlated to VFI, irrespective of stage of re-growth. Excluding the mineral fractions, only three of the chemical components of the herbage emerged as important, namely, the DM concentration of the herbage as fed accounting for 32% of the variance in DMD, the NPN concentration of the herbage accounting for only 12.2% of the variance and the ash concentration of the herbage accounting for 15.9% of the variance in digestibility. Of the macro-mineral components Ca ($P = 0.11$), Mg ($P = 0.064$) and P ($P = 0.147$) tended to be positively associated with DMD, while Na and K were related to DMD. Crude protein, albeit with a negative trend (P=0.18), and ADF, with a positive trend (P=0.129), were not correlated to VFI. Non-protein nitrogen was negatively correlated to VFI, although it did not account for much of the variability (11%) in VFI. Ca was also positively correlated to VFI, also accounting for very little of the variation in VFI (11.7%). Herbage Mg had a positive influence on VFI, accounting for 24% of the variation in VFI. Both DMD and VFI were highly negatively influenced by the moisture concentration of the herbage.

Keywords: *Pennisetum clandestinum*, DMD, VFI, composition, environmental temperature, rainfall.

INTRODUCTION

Climatic factors such as temperature, water availability and high evaporative demand could cause differences in DM digestibility (Minson & McLeod, 1970; Minson, 1990). The chemical composition of herbage has been used to predict digestibility (Minson, 1982). However, van Soest and Robertson (1980) stated that there was no solid theoretical basis for the relationship between the fibre fractions and digestibility, other than statistical association.

The ability to accurately predict the nutritive value of kikuyu herbage with a relatively easy and cheap chemical analysis would be of immense value to dairy farmers utilising kikuyu as a major, or sole, roughage source in the summer months in the KZN midlands, consequently the data over the trial period were regressed against DMD and VFI to establish if any good correlations existed between the chemical fractions and nutritive value. The effect of temperature, rainfall and evaporation on DMD and VFI were also evaluated.

MATERIALS AND METHODS

The data recorded for the *in vivo* digestibility and DM intake trials reported on in chapters 3 to 6 were pooled and analysed using multiple regression techniques. Five years of digestibility data, amounting to 82 digestion trials, were pooled for digestibility. Voluntary intake data were pooled for the three years of intake trials, amounting to 38 intake trials. The methodology for these trials is given in chapters 3 and 4.

The daily maximum temperatures, rainfall and evaporation recorded at and prior to the digestion and intake trials at Cedara was extracted from the database of the Institute for Soil, Climate and Water. The temperatures recorded are abbreviated to T_0 for the mean temperature of actual trial period, T_{-1} for the mean of the week prior to the trial period, T_{-2} for the mean of the second week prior to the trial period and T_{fert} for the mean temperatures at cutting and N fertilization. The rainfall recorded is abbreviated to R_0 for the mean rainfall of actual trial period, R_{-1} for the mean of the week prior to the trial period, R_{-2} for the mean of the second week prior to the trial period and R_{fert} for the mean rainfall at cutting and N fertilization. Similarly, evaporation figures are abbreviated as E_0, E_{-1}, E_{-2} and E_{fert}. The long term mean data for Cedara, comprising 91 years data for temperature and rainfall and 40 years for class A pan evaporation, is also presented.

The chemical composition data (from data presented in Chapters 3, 4, 5 & 6), daily maximum temperature, rainfall and evaporation recorded during and prior to the trial periods were regressed on DMD and VFI, using the multiple linear regression techniques, including the stepwise regression analysis procedure, of Genstat 8.1 (Genstat, 2005), to examine the influence of environment and herbage composition on the nutritive value of the herbage and develop simple linear regression models for predicting kikuyu digestibility and intake.

Table 7.1 The mean maximum temperatures (^oC), rainfall (mm/month) and evaporation (mm/month) recorded per month over the growing season for Cedara, relative to the long term mean (LTM) for Cedara

Growing season		Oct	Nov	Dec	Jan	Feb	Mar	Apr	Total
1	Rain	65.3	67.6	185	137.8	142	40.2	34.8	672.7
	Temp	21.7	22.8	22.9	24.5	23.3	25.2	22.8	-
	Evap	149.9	140.4	132.1	157.7	89.1	146.5	114.3	930
2	Rain	61.0	262.3	132.3	70.1	133	128.5	44.1	831.3
	Temp	21.8	21.8	25.1	25.0	23.6	22.9	23.0	-
	Evap	150.1	148.7	180.0	146.3	116.2	98.3	94.9	934.5
3	Rain	99.1	40.0	122.9	250.3	163.1	83.5	13.9	772.8
	Temp	20.6	24.6	22.9	25.9	25.4	23.3	24.7	-
	Evap	111.2	167.6	119.8	192.2	145.6	123.3	143.5	1003.2
4	Rain	209	198.1	149	104.6	119.9	95.3	50.8	926.1
	Temp	21.1	23.5	25.0	25.1	26.9	26.2	25.6	-
	Evap	100.5	150.4	180.6	171.7	168.6	189.0	138.0	1098.8
5	Rain	34.2	82.6	68.7	69.2	108.1	114.9	25.0	502.7
	Temp	24.2	24.5	26.1	26.7	25.1	25.0	23.8	-
	Evap	185.7	166.1	176.2	200.2	133.6	151.1	127.0	1139.9
LTM	Rain	83.2	110.8	131.0	135.3	122.1	110.6	50.9	743.9
	Temp	22.7	23.6	24.9	25.3	25.3	24.8	22.9	-
	Evap	138.2	137.2	158.8	149.9	134.2	130.0	105.8	954.1

RESULTS

Environmental effects

The mean temperature and rainfall recorded for Cedara over the trial years is given in Table 7.1. Rainfall and temperatures over the years were highly variable, ranging from very high rainfall seasons to a severe drought in growing season 5, when several trials could not be completed due to a lack of herbage. The autumn in growing season 4 was particularly hot, although rainfall was very close to normal.

The digestibility of the herbage was not affected by the temperature or rainfall during the duration of the digestion trials. However, the temperatures recorded in the week prior (T_{-1}) to the trial had a negative impact on digestibility, although only accounting for little of the variability in DMD, as quantified in the following equation:

$$DMD = 979 - 13.8\ (T_{-1}); \quad R^2 = 0.167; \quad P < 0.0001; \quad n = 82$$

Addition of the previous weeks temperature data to the regression proved significant and improved the variance in DMD accounted for as indicated in the following equation:

$$DMD = 1336.8 - 9.286\ (T_{-1}) - 7.477\ (T_{-2}) - 12.002\ (T_{fert}); \quad R^2 = 0.318; \quad P < 0.0001; \quad n = 82$$

Rainfall in the week prior to the trial had no impact on digestibility, while the rainfall in the second week prior to the digestion trial tended to improve digestibility, but accounted for very little of the variability in digestibility, as quantified in the following equation:

$$DMD = 629.5 + 0.479\ (R_{-2}); \quad R^2 = 0.03; \quad P = 0.051; \quad n = 82$$

A combination of the rainfall data proved significant and improved the variance in DMD accounted for as indicated in the following equation:

$$DMD = 643.8 + 0.396\ (R_{-1}) + 0.891\ (R_{-2}) - 1.059\ (R_{fert}); \quad R^2 = 0.307; \quad P = 0.001; \quad n = 82$$

Evaporation also exerted a significant effect on digestibility, although it accounted for little of the variation in DMD as described in the following equations:

$$DMD = 579.7 + 12.9\ (E_0); \quad R^2 = 0.039; \quad P = 0.04; \quad n = 82$$

$$DMD = 755.1 - 22.1\ (E_{-2}); \quad R^2 = 0.13; \quad P = 0.0005; \quad n = 82$$

High evaporation rates at cutting and feeding enhanced DMD. Evaporation rates 2 weeks prior to cutting and feeding have negative effects on DMD, the opposite effect to rainfall at 2 weeks prior to harvesting.

Rainfall and temperature in the period of cutting and fertilization had the largest effect on digestibility, irrespective of the stage of re-growth at harvesting (20, 30 or 40 days) and a combination of the two proved significant, accounting for the most variance in DMD. These equations are given below:

$$DMD = 672.7 - 0.83\ (R_{fert}); \quad R^2 = 0.168; \quad P < 0.001; \quad n = 82$$

$$DMD = 1063.6 - 17.7\ (T_{fert}); \quad R^2 = 0.23; \quad P < 0.001; \quad n = 82$$

$$DMD = 1090 - 0.826\ (R_{fert}) - 17.6\ (T_{fert}); \quad R^2 = 0.40; \quad P < 0.001; \quad n = 82$$

When combined, only the rainfall and temperature at fertilization proved significant factors in a multiple regression predicting DMD. This combination of rainfall and temperature at cutting and fertilization accounted for 40% of the variability found in the digestibility of the kikuyu. Including evaporation rates with the rainfall and temperature data increased the variability accounted for slightly, as indicated in the following equation:

$$DMD = 1141.8 - 0.74\ (R_{fert}) - 16.6\ (T_{fert}) - 15.6\ (E_{-2}); \quad R^2 = 0.465; \quad P < 0.001; \quad n = 82$$

In terms of temperature, the longer term mean temperature, namely for the month (mth 0) during which the trial was conducted and even the month prior to that (mth-1), was highly correlated to digestibility, as indicated in the following equation:

$DMD = 1325.4 - 28.17 (T_{mth\ 0})$; $R^2 = 0.23$; $P = 0.001$; $n = 82$

$DMD = 1242.6 - 24.6 (T_{mth-1})$; $R^2 = 0.22$; $P = 0.001$; $n = 82$

$DMD = 1533 - 19.6 (T_{mth\ 0}) - 17.01 (T_{mth-1})$; $R^2 = 0.23$; $P = 0.001$; $n = 82$

The negative effect of temperature on digestibility is consistent with, but higher than the response recorded by Minson and McLeod (1970) who found that DMD, determined with sheep, was depressed by 11.4 g/kg DM per degree rise in temperature (^{o}C) for 2 temperate and 2 tropical species.

The voluntary intake of kikuyu was not correlated to the temperature or rainfall recorded during the actual intake trial, similar to digestibility. Rainfall in the week prior to the trial tended to be positively related to VFI, but accounting for very little of the variability in intake, as quantified in the following equation:

$VFI = 2.10 + 0.008 (R_{-1})$; $R^2 = 0.07$; $P = 0.056$; $n = 38$

Rainfall occurring during the cutting and fertilization period was not correlated to VFI, unlike the effect on digestibility. The daily maximum temperatures recorded in the week prior to the intake trials was negatively correlated to intake, similar to the effect on digestibility, and is quantified by the following equation:

$VFI = 5.68 - 0.139 (T_{-1})$; $R^2 = 0.14$; $P = 0.01$; $n = 38$

Temperatures recorded during the cutting and fertilization phase were highly correlated to VFI, irrespective of stage of re-growth, accounting for some 40 % of the variability in intake as indicated in the following equation:

$VFI = 6.54 - 0.187 (T_{fert})$; $R^2 = 0.42$; $P = 0.001$; $n = 38$

A combination of the temperature data proved significant and improved the variance in DMD accounted for as indicated in the following equation:

$VFI = 8.57 - 0.097 (T_{-1}) - 0.173 (T_{fert})$; $R^2 = 0.497$; $P = 0.001$; $n = 38$

In contrast to digestibility, when temperature and rainfall data was combined to predict VFI, rainfall did not contribute to the regression equation.

Evaporation at harvesting and at cutting and fertilization was also related to VFI, as indicated below:

$VFI = 1.49 + 0.163 (E_0)$; $R^2 = 0.138$; $P < 0.012$; $n = 37$

$VFI = 1.31 + 0.196 (E_{fert})$; $R^2 = 0.206$; $P < 0.01$; $n = 37$

However, due to strong correlations between temperature and evaporation at cutting and fertilization and at harvesting these variables could not be included together in a regression.

Herbage composition

Over all the years and treatments, only five factors emerged that were correlated with digestibility. Excluding the mineral fractions only three factors emerged as important, namely, the DM concentration of the herbage as fed accounting for 32% of the variance in DMD, the NPN concentration of the herbage accounting for only 12.2% of the variance and the ash concentration of the herbage accounting for 15.9% of the variance in digestibility. However, a combination of the two factors, DM and NPN improved the correlation with DMD, accounting for 52% of the variation in DMD. Including ash in the regression improved the variance accounted for in DMD to 60.7%. These are expressed in the following equation:

$\text{DMD} = 397.5 + 1.31\ (\text{DM g/kg});$ $R^2 = 0.323;$ $P < 0.0001$ $n = 94$

$\text{DMD} = 720.8 - 6.978\ (\text{NPN g/kg});$ $R^2 = 0.122;$ $P < 0.004$ $n = 58$

$\text{DMD} = 404 + 1.55\ (\text{DM}) - 5.598\ (\text{NPN});$ $R^2 = 0.526;$ $P < 0.00001$ $n = 58$

$\text{DMD} = 266.4 + 1.485\ (\text{DM}) + 1.482\ (\text{ASH}) - 4.83\ (\text{NPN})$ $R^2 = 0.607; P < 0.00001$ $n = 58$

Crude protein levels in the herbage were correlated to the NPN concentration of the herbage quadratically as indicated in the following equation:

$\text{CP} = 139.4 + 11.27\ (\text{NPN}) - 0.38\ (\text{NPN}^2)$ $R^2 = 0.105;$ $P < 0.017$ $n = 58$

Of the macro-mineral components Ca (*P* = 0.11), Mg (P = 0.064) and P (*P* = 0.147) tended to be positively associated with DMD, while Na and K were related to DMD as indicated in the following regression equations:

$\text{DMD} = 591.9 + 116.3\ (\text{Na});$ $R^2 = 0.16;$ $P = 0.002;$ $n = 48$

$\text{DMD} = 532.2 + 4.54\ (\text{K});$ $R^2 = 0.069;$ $P = 0.038;$ $n = 48$

A combination of the minerals accounted for 29.6% of the variation in DMD as indicated in the following regression equation:

$\text{DMD} = 255.8 + 51.19\ (\text{Mg}) + 5.79\ (\text{K}) + 87.86\ (\text{Na});$ $R^2 = 0.296;$ $P = 0.0003;$ $n = 48$

The voluntary feed intake of the heifers for the combined data over all the seasons is expressed in the following regression equations:

$\text{VFI} = -1.037 + 0.0052\ (\text{DMD})$ $R^2 = 0.61;$ $P < 0.00001$ $n = 38$

$\text{VFI} = -0.60 + 0.0156\ (\text{DM}_{\text{as fed}})$ $R^2 = 0.68;$ $P < 0.00001$ $n = 38$

$\text{VFI} = 3.88 - 0.032\ (\text{DM}) + 0.000126\ (\text{DM}^2);$ $R^2 = 0.709;$ $P < 0.00001$ $n = 38$

$\text{VFI} = -1.94 + 0.022\ (\text{ash}) - 0.008\ (\text{CP}) + 0.005\ (\text{NDF});$ $R^2 = 0.564; P < 0.00001$ $n = 29$

$\text{VFI} = -3.21 + 0.0075\ (\text{NDF});$ $R^2 = 0.37;$ $P < 0.0002$ $n = 38$

$\text{VFI} = 2.82 - 0.043\ (\text{NPN});$ $R^2 = 0.11;$ $P < 0.038$ $n = 30$

$\text{VFI} = -0.407 + 0.728\ (\text{Mg});$ $R^2 = 0.24;$ $P < 0.008$ $n = 24$

$\text{VFI} = 0.467 + 0.0637\ (\text{Ca});$ $R^2 = 0.117;$ $P < 0.056$ $n = 24$

Crude protein, albeit with a negative trend (P=0.18), and ADF, with a positive trend (P=0.129), were not correlated to VFI. Non-protein nitrogen was negatively correlated to VFI, although it did not account for much of the variability (11%) in VFI. Ca was also positively correlated to VFI, also accounting for very little of the variation in VFI (11.7%). Herbage Mg had a positive influence on VFI, accounting for 24 % of the variation in VFI.

The DM concentration of the herbage in the trials ranged from 134 g/kg to 283 g/kg with a mean of 188.7 g/kg. The data was re-analyzed with all trials containing a DM lower than the mean value excluded from the analysis to determine if the effect of DM (moisture) was limited to the wetter herbage. Both DMD (P = 0.039) and VFI (P = 0.02) were still depressed by the moisture concentration (>188 g/kg) of the herbage, albeit not as strongly as with the greater moisture (lower DM) concentrations.

Year differences accounted for an appreciable amount of the variability in VFI (34.5%). Including seasonal effects with the year effects increased the amount of variability accounted for to 48.7%. Season had a negative effect, VFI declining as the seasons progressed from spring through summer to autumn. Days of re-growth did not contribute to the regression.

A combination of the environmental factors and the chemical composition of the herbage produced regression equations which accounted for an appreciable amount of the variation in DMD and VFI, as indicated in the following equations:

$$\text{DMD} = 855.5 + 0.29\,(\text{NDF}) - 0.84\,(R_{\text{fert}}) - 16.86\,(T_{\text{fert}}); \quad P < 0.001; \; R^2 = 0.465; \; n = 82$$

$$\text{DMD} = 487.7 + 2.09\,(\text{ash}) - 30.75\,(E_{-2}) + 21.94\,(E_0); \quad P < 0.001; \; R^2 = 0.457; \; n = 82$$

$$\text{DMD} = 310.2 + 1.225\,(\text{DM}) + 1.578\,(\text{ash}) - 3.83\,(\text{NPN}) - 19.44\,(E_{-2}) + 16.98\,(E_0); \quad P < 0.001; \; R^2 = 0.738; \; n = 57$$

$$\text{VFI} = 1.26 + 0.159\,(E_0) - 0.196\,(T_{-1}) + 0.0067\,(\text{NDF}); \quad P < 0.001; \; R^2 = 0.812; \; n = 29$$

$$\text{VFI} = 1.85 + 0.013\,(\text{DM}) - 0.103\,(T_{-1}) + 0.092\,(E_0); \quad P < 0.001; \; R^2 = 0.793; \; n = 37$$

$$\text{VFI} = -1.32 + 0.0146\,(\text{DM}) + 0.01\,(\text{ash}); \quad P < 0.001; \; R^2 = 0.732; \; n = 37$$

The variables, ADF, CP, E_{fert} and R_{fert} were correlated to the DM concentration of the herbage as fed and were therefore excluded from data sets for analysis containing DM. Similarly, T_{fert} is correlated to DM and ash and could not be included together in regressions.

DISCUSSION

Minson (1990) stated that seasonal differences in DMD could be a result of changes in temperature, water availability and light. The negative effect of temperature on digestibility recorded for this kikuyu data is greater than that recorded for other species, namely; 11.4 g/kg by Minson and McLeod (1970), -18 g/kg by Deinum and Dervin (1976) and -5.6 g/kg by Wilson and Minson (1980). The effects of temperature and rainfall appear to be implicated when the pasture is fertilized rather than at harvesting. A lag effect appears to exist between the effect of rainfall on the digestibility or intake of kikuyu, with rainfall the week prior to harvesting shown to be positively associated with both DMD and VFI. Rainfall in the week of harvesting had no effect on DMD or VFI, possibly due to rainfall during the harvesting period only contributing to extra-cellular water, rather than intracellular water. Minson and McLeod (1970) also noted that DMD was negatively correlated to mean daily temperature the month prior to cutting (r = -0.97), as was total evaporation (r = -0.91).

An examination of the correlations between the environmental factors and herbage DM concentration show that the rainfall at fertilization was negatively correlated to the DM of the herbage at harvesting, while the maximum daily temperatures at fertilization and in the weeks prior to harvesting are negatively associated with the DM concentration of the herbage at harvesting. The highest correlation was recorded at fertilization. Evaporation at fertilization and at harvesting were positively associated with the DM concentration of the herbage. Crude protein concentration of the herbage has been negatively associated with DMD and VFI. Both the temperature (0.21) and rainfall (0.19) at fertilization are positively associated with CP levels at harvesting, while the evaporation at fertilization was negatively associated (-0.41) with CP at harvesting. These factors are possibly indicated in the absorption of nitrates at fertilization, high rainfall and temperature positively associated with N uptake by the plant, while high evaporation reduced the uptake of N by the plant.

It is of interest to note that the factors generally positively associated with grass growth and productivity, namely, N fertilization, rainfall and heat (temperature) were negatively related to DMD and VFI. This poses a dilemma for the pasture farmer, as both the quantity of pasture grown and its quality are important for economic animal production. This data adds a new dimension to the conclusion by Holmes and McMillan (1982) that grazing management "is the art of successful dairying compromise", the producer needing to maximize grass growth without unduly sacrificing pasture quality, as the two appear diametrically opposed in kikuyu, as in other species. This data appears to confirm the commonly held belief that a dry year is good for individual animal production, relative to wet years, which initially will increase animal production per ha but decrease it in the longer term due to lower plant growth. Similarly, Wilson (1981b) concluded that for tropical species moisture stress increases digestibility over full moisture, as did Sequin et al. (2002) for Kura clover. Terrill *et al.* (1991) strongly suggested that DMI, DMD, and apparent digestibility of NDF, ADF, hemi-cellulose and cellulose in ryegrass was reduced in forage from flooded soils.

The effect of herbage moisture concentration on VFI is well documented, however, its negative effect on DMD recorded for this data appears to be the exception. Moisture concentrations exceeding 780 g/kg fresh material are generally considered as having a detrimental effect on VFI (Arnold, 1962; Davies, 1962; John & Ulyatt, 1987, Minson, 1990). However, there are exceptions to the generalized rule. Holmes and Lang (1963) concluded that neither internal water concentration nor external rain water restricted ruminant DMI of forages. Halley and Dougal (1962) reported a moderate degree of correlation between DM concentration and DMI of herbage by ruminants, while Johnson *et al.* (1968) found that DMI were not related to stage of maturity, season of harvest, DM concentration, crude fibre concentration or digestibility of guinea grass (*Panicum maximum*). Pasha *et al.* (1994) showed that high moisture forage exhibited both a faster rate of passage and a decreased intake compared with dry forage.

The effect of soil water on herbage digestibility is not conclusive, with most references finding that herbage moisture has no effect on digestibility (Garwood *et al.*, 1979; Danelon *et al.*, 2002). However, Reid *et al.* (1959) in a study of 28 forages representing a variety of temperate species found that increasing DM concentration of the herbage negatively impacted on herbage digestibility. In contrast, Yoelao *et al.* (1970) observed an increase in digestibility in lucerne or berseem due to wilting, and concluded that this may have been a result of the high dry season temperatures rather than of the wilting process. This observation by Yoelao *et al.* (1970) is consistent with the results of Danelon *et al.* (2002) who found no response to wilting in fermentation characteristics and digestibility of lucerne. However, Grant *et al.* (1974) found that wilting Napier grass markedly increased the intake and digestibility of the dryer forage as opposed to higher moisture forage. Similarly, Pasha *et al.* (1994) found that the digestibilities of DM, NDF and ADF were greater for dried herbage relative to high moisture frozen forage. Pasha found higher rates of passage for high moisture forage and suggested that the decrease in DMD for wet forage is due to its increased rate of passage. No significant interactions between forage maturity and moisture concentration were observed by Pasha *et al.* (1994) for either intake or digestibility. Pasha *et al.* (1994) indicated that greater ruminal NH_3N may be a possible inhibitor of intake of ruminants fed high moisture forages.

Wilson (1981a, b) found that, for tropical forage species, drought or slight moisture stress increased digestibility relative to well-watered forage. Snaydon (1972) concluded that increased water supply decreased the digestibility of lucerne, which was contrary to the general assumption that irrigated lucerne was more digestible than dry-land lucerne. Garwood *et al.* (1979) found no consistent effect of water on the digestibility of temperate grasses.

Cross (1978) examined the effect of N fertilization and rainfall on kikuyu growth rates and derived the following regression equations relating to the growth of kikuyu to N fertilization and rainfall in the Midlands and South Coast of Natal, indicating the overall importance of N fertilization on pasture growth and yield, albeit at the expense of herbage quality and individual animal performance:

$$\text{Yield (kg DM)} = 14\,477.93 - 248.512\,N + 1.731\,N^2 - 0.002964\,N^3\,; \qquad R = 0.94689.$$
$$\text{Yield (kg DM)} = 0.00097\,R^2 + 0.0412\,R.N + 0.065\,N^2\,; \qquad R = 0.93865.$$
$$\text{Yield (kg DM)} = 621.909 - 6.0113\,R + 49.555\,N + 0.057285\,R.N - 0.12819N^2; \; R = 0.93719.$$

where: N = level of nitrogen applied in kg/ha per season,
R = Rainfall in mm from 1 August to 31 April.

Holder (1967) found that the VFI of sheep consuming kikuyu was reduced if the kikuyu was fertilized with N. Similarly, Milford and Minson (1965) found that high levels of N fertilizer negatively affected intake of kikuyu, with the intake of young, heavily fertilized pasture containing 200 g/kg CP being 28% less than the intake for the same pasture after growing for a further 28 days and the CP concentration falling to 115 g/kg. Jeffrey (1971) found that N fertilization also negatively affected the nutritive value of kikuyu. Campbell and Ho-a (1971) reported that the digestibilities of the organic matter (OM), crude fibre (CF) and nitrogen free extractives (NFE) decreased in herbage cut at six-week re-growth stages over the growing season when fertilized with 336 kg N/ha, applied in four equal dressings, as opposed to lower rates of N (0, 112 & 224 kg N/ha). Minson (1973) found that fertilizer N affected both digestibility and intake over a season. High N fertilization (230 kg N/ha) reduced the digestibility, relative to low N fertilization (57.5 kg N/ha) in mid-season (Feb) but not in spring or autumn, where the high N level resulted in higher digestibilities (Dec & Apr). Similarly, the higher level of N fertilizer reduced intake in mid-season (Feb), but increased intake in April.

Chemical composition is the generally accepted measure of nutritive value for herbage. Pattinson (1981) concluded that the low intake of kikuyu was unrelated to the conventional quality attributes such as CP, ADF and *in vitro* dry matter digestibility (IVDMD). This is in agreement with Austin (1980) who found that the only plant component associated with acceptability in kikuyu was the N fraction, which was negatively associated with acceptability. Austin (1980) found that nitrate levels in kikuyu were negatively correlated (-0.356; $P \leq 0.01$) to acceptability, as were herbage N levels, albeit weaker (-0.284; $P \leq 0.05$) indicating that nitrate was the causative agent, nitrate and N being highly correlated. Pienaar *et al.* (1993b) found that the N concentration of the leaves of kikuyu was negatively related to VFI. Similarly, Pienaar *et al.* (1993b) found that rumen ammonia levels were negatively associated with VFI, all other factors, including moisture concentration, showing no relationship with VFI.

Acid detergent fibre was variably associated with acceptability, positive in one trial, with a slight positive correlation but non-significant correlation overall (Austin, 1980). Karnezos (1986) found that crude fibre levels in kikuyu herbage were positively correlated with ADG in steers grazing kikuyu and considered that this positive relationship was possibly associated with herbage availability. Köster *et al.* (1992) found that the cell wall contents (NDF) of oesophageal extrusa were greater than that of hand cut samples, however the IVDOM and N concentrations of the oesophageal and hand cut samples were not different for bana, kikuyu and sorghum herbage. The positive association between ADF and NDF on VFI for these data are consistent with the selection of material higher in fibre in oesophageal extrusa recorded by Pattinson (1981) and Dugmore *et al.* (1991). Similarly, these positive relationships between the fibre fractions and VFI are consistent with the findings of Milford and Minson, (1965) who recorded, that of seven tropical grass species tested, only kikuyu had a negative correlation coefficient between percentage leaf blade and VFI, and that of Tainton *et al.* (1982) who found improved ADG for animals grazing kikuyu with a higher proportion of stem, i.e. lower leaf:stem ratios.

Dugmore *et al.* (1986) recorded a negative correlation between CP (N x 6.25) and digestible organic matter, while a lower individual animal performance was recorded with increased levels of nitrogen fertilization by Tainton *et al.* (1982) and Karnezos *et al.* (1988). None of the chemical components of kikuyu have proved to be accurate in predicting its nutritive value (Dugmore & du Toit, 1988) or DMI (Meissner *et al.*, 1988). Pattinson (1981) concluded that kikuyu seems to be a special case in that the low intakes seem to be contrary to all the quality attributes determined, a conclusion which still appears to be valid.

Karnezos (1986) modeled the ADG rates of steers grazing kikuyu over a season at the Ukulinga Research farm of the University of KZN using herbage availability and chemical composition. The only chemical components which contributed to the model were Ca and N, with N negatively correlated to ADG in steers. Pasture availability (kg DM offered per animal) was positively correlated to ADG.

Austin (1980) investigating the effect of N, phosphate, Ca and Mg (dolomitic lime) fertilization on minerals in kikuyu found that Ca in the plant was increased by phosphate fertilization, while N fertilization improved the Ca:P ratio, possibly by its reduction of phosphate levels in the herbage. Liming also increased the Ca:P ratio. Nitrogen fertilizer improved Mg levels, but also increased the K levels in the herbage. However, acceptability of the pasture was not affected by any of the minerals recorded (Ca, P, K, Ca & Mg). This is in contrast to Karnezos (1986) who found that Ca was the only plant fraction that influenced live weight gain in steers. Interestingly, the Ca:P ratio was not related to animal performance (Karnezos, 1986).

Well established kikuyu pastures in the KwaZulu-Natal Midlands are typified by high K levels, up to 50 g K/kg DM being recorded by Miles *et al.* (1995). High K intake can lead to interference with the uptake of other cations like Ca and Mg leading to metabolic problems (Leaver, 1972), especially when the intake of Na is low (Martens & Rayssiguier, 1980; Beringer, 1988).

The relationship between Na and K appears to be important, particularly for reproduction. Beringer (1988) recommended a K:Na ratio of 20:1 in the ration, with calving interval increasing by 5 days for each 10% increase in K:Na ratio above a ratio of 30. The levels recorded by Fulkerson *et al.*, (1998) were a mean K:Na ratio of 30:1. These ratios are lower than those recorded for kikuyu on Cedara (Dugmore *et al.,* 1987; Miles *et al.*, 1995).

The positive effect of Na on digestibility is not unexpected, considering kikuyu is a natrophobe, with particularly low Na levels in the leaves. Sodium concentrations in the blood of dairy cows grazing kikuyu on Cedara were found to be marginally deficient, in spite of greater levels of Na being supplemented, relative to levels supplemented when grazing ryegrass, which is indicative of the low Na concentrations found in kikuyu at Cedara. Reason *et al.* (1989) found that cows grazing kikuyu were Na deficient, while Davison *et al.* (1980) found that supplementation with NaCl increased milk production on a tropical pasture.

The biometrical analysis of this data confirmed the observation by Butterworth and Diaz (1970) that interactions among the various plant constituents were so high as to obscure meaningful relationships. Butterworth and Diaz (1970) also concluded that there were so many exceptions to the widely accepted generalization that CP and ether extract positively influence digestibility, while fibre has a negative influence, as to render them of little value for tropical species. Also the accuracy of prediction of digestibility from proximate analysis was in most cases extremely low. These conclusions have been confirmed by these and other data cited for kikuyu and other tropical species.

Chapter 8
General Discussion

An overview of the digestion trials is given in Appendix Table 5. In general, the greatest DMD values were in either the 30- or 40-d treatments in the spring and summer, with the 20-d herbage consistently the greatest in autumn. The effect of N fertilization on DMD was variable, with high N having a negative impact in some years and no effect in others. Stage of re-growth consistently affected the DMD of the low N herbage. In the high N treatments, stage of re-growth had minimal impact on digestibility, possibly because the effects of N on DMD had already reached a plateau at this level of N fertilization. Seasonal effects also varied between years, some years recording a linear decrease in digestibility from spring, through summer to autumn. In other years a quadratic response was recorded, with a spring high, summer trough and an increase to an intermediate autumn digestibility. The linear decline in digestibility is supported by milk yields recorded off kikuyu grazing at Cedara, with a spring high of 11 kg in spring, 9 kg in summer and 7 kg in autumn (Bredon, 1976). Similar quadratic responses for digestibility over season, with the summer trough, have been recorded at Cedara (Dugmore & du Toit, 1988) and concur with the findings of Minson and McLeod (1970) that temperature negatively affects digestibility, resulting in a summer low for digestibility (Minson, 1990).

There was a linear decline of VFI over season from spring to autumn. The best intakes of c. 3.5% of body weight in spring and summer and c. 2% of body weight in autumn in Trial 2 are very good for a tropical grass species. However, the intakes of c.2.5% of body weight in the early season and c. 1.8% of body weight in the latter part of the season for the liming trials are possibly more representative of kikuyu grazing and close to the 2 to 2.5% of live weight suggested as expected intakes by Ketth and Ranawana (1971), or the 94 g/kg $W^{0.75}$ by Mann & Stewart (2003) for Friesian bulls fed kikuyu.

Nitrogen fertilization had a negative effect on VFI, also reflected in the negative impact of herbage NPN on intake for this data. The mean rumen ammonia levels were 10.5 in the summer and 11.27 in the autumn. Only the 20 day re-growth in summer contained ammonia levels (15.4 mmol/ℓ and at times peaking at over 16 mmol/ℓ, even for the low N treatment) exceeding the upper value of 14 mmol/ℓ ammonia recommended by Orskov (1982), while the 40 day re-growth in autumn (13 mmol NH_3/ℓ rumen fluid) almost reached this upper value. The higher rumen ammonia levels in the autumn 40 day re-growths may be associated with low digestibility and a lack of readily available carbohydrate in the rumen. Pienaar *et al.* (1993b) recorded rumen ammonia levels of between 6 and 14 mmol/ℓ rumen fluid and found that rumen ammonia levels were negatively correlated to VFI (P=0.0107) in kikuyu, concluding that these high levels supported the theory that a high load of ammonia reaching the liver could inhibit VFI. On several occasions during these trials, animals on the high N treatment were diagnosed as suffering from alkalosis, indicating a potential problem. High rumen pH combined with relatively high rumen ammonia levels could have caused high blood ammonia levels, negatively impacting on VFI, especially at the 20 day stage of re-growth. This impact of high rumen pH and ammonia are reflected in high BUN values.

The Ca concentration of the herbage was, overall for the pooled data, positively associated with VFI, indicating that, indirectly, liming should have a beneficial effect on intake. Another mineral associated with intake was Mg. Potassium had a positive influence on DMD, which is inconsistent with the findings of Morton *et al.* (2001) that high K levels reduced intake on ryegrass pasture. Herbage DM concentration was positively associated with VFI in the intake data, while high N fertilization was negatively associated with

VFI. Another classically important factor, ADF, had no effect on VFI, while NDF was positively associated with VFI in contrast to the accepted negative effect of fibre on intake.

A high moisture concentration in herbages fed to cattle has not always been associated with low VFI. Campling and Balch (1961) found that adding free water to the rumen did not reduce DMI, while Davies (1962) showed that once the water concentration of the herbage exceeded 80%, any further increase in moisture concentration led to a decrease in VFI, an effect confirmed in many subsequent studies (Minson, 1990). The decrease in intake was not caused by water *per se,* because adding water into the rumen via a ruminal fistula had no effect on VFI (Davies, 1962). Holmes and Lang (1963) concluded that neither internal water content or external rain water restricted DMI in forages. Halley and Dougal (1962) reported a moderate degree of correlation between DM content and DMI in herbages, while, Johnson *et al.* (1968) found that DMI was not related to stage of maturity, season of harvest, DM concentration, crude fibre concentration or digestibility for guinea grass (*Panicum maximum*).

It has been suggested (Minson, 1990) that the lower intake is caused by the wet forage being swallowed before it has been properly chewed and, hence, before maximal particle breakdown has occurred. Forage that is prematurely swallowed would require more break-down during rumination, be retained longer in the rumen and, hence, be eaten in smaller quantities (Minson, 1990). However, while this may explain a lower VFI, a longer rumen retention time should enhance digestibility, which it did not do for these data. Similarly, the aforementioned effect of wet forage on intake proposed by Minson (1990) may explain the inverse relationship between herbage DM concentration and rumen pH, possibly due to less chewing of wet material and consequently less buffering in the rumen from saliva.

Todd (1956) found the VFI by sheep of kikuyu exceeded 3.0% of body weight, however, the forage had been wilted overnight due to its high in situ moisture concentration of over 85%. Similarly, Pienaar *et al.* (1993b) found that the greatest intake by sheep in his trials was on wilted material and intake was reduced by a flush in growth following rain. Pienaar *et al.* (1993a) also postulated that a high soluble N concentration limited VFI in kikuyu grazing. This is supported by Marais (1980) who found that extracting kikuyu with water increased its digestibility *in vitro*, and that reintroducing the soluble nitrate fraction which had been extracted inhibited the digestibility *in vitro*.

The effect of herbage DM concentration on intake and digestibility could be associated with the fact that high herbage moisture would be linked to rainfall, particularly during the misty conditions encountered in the KZN mistbelt, which would reduce the amount of sunshine, thereby reducing photosynthesis and consequently the conversion of nitrates to protein in the plant. Nitrates occur normally in kikuyu, which may accumulate high concentrations under such conditions as high levels of N fertilizer, low light intensity and drought (Hegarty, 1982). The higher levels of nitrate (and NPN) in kukuyu are associated with lower digestibility (Marais, 2001) and intake. High moisture and soil N levels would promote the uptake of NPN, including nitrate.

As VFI is positively correlated to DM concentration of the herbage, increased rate of passage due to high moisture content cannot be responsible for the lower DMD. The possibility that the anti-quality factors in kikuyu exist in a water soluble state may explain the lower DMD at higher moisture concentrations. These factors may be linked to the N fraction in the herbage as suggested by MacFarlane (undated) and Last (1993), or even high levels of nitrates (Marais, 2001). Marais (1980) found that extracting the soluble elements in the kikuyu herbage with water prior to *in vitro* digestion improved the digestibility of the herbage.

The effect of moisture concentration of fresh material on digestibility is surprising, considering that for research reviewed by Schneider and Flatt (1975) there was no suggestion that a high moisture concentration would impact digestibility, and that fresh material, if anything, would have greater digestibility than dry material. Deinum and Dirven, (1974) stated that moisture supply (rain or irrigation), which increases soil moisture and consequently herbage moisture concentration would generally bring about small increases in digestibility. However, Yoelao *et al.* (1970) were similarly surprised when they unexpectedly found that wilting lucerne and berseen clover for 3 to 4 hours increased the organic matter, crude protein and energy digestibility coefficients *in vivo* by between 15 to 22 percent. In their trial fresh herbage was cut every morning and half fed fresh to buffalo heifers, while the other half was wilted for several hours to about 30% DM and then fed to buffalo heifers. The retention time of the undigested residues in the alimentary tract was not affected by wilting. Yoelao *et al.* (1970) concluded that the increases in digestibility could not be directly attributed to wilting. Examining the results of Yoelao *et al.* (1970), the increase in digestibility due to wilting appears highly proportional to the CP concentration of the herbage.

The negative effect of moisture concentration on digestibility indicates a possibility of an anti-quality factor in the soluble fraction of the kikuyu, which would harbor the NPN and nitrate fraction, as problems usually occur on well fertilized kikuyu growing actively following rain. The best digestibility and intake results obtained on kikuyu are those from wilted or dried pasture (Todd, 1956; Pienaar *et al.*, 1993b). The improved digestibility following extraction of kikuyu herbage with water by Marais (1980), and the subsequent reduction in digestibility when the soluble N fraction was reintroduced as nitrate would implicate a nitrogenous compound. Austin (1989) came to a similar conclusion, finding that N, particularly nitrate-N, depresses acceptability of kikuyu. Soil water, or heavy rainfall, and therefore by assumption moisture concentration of the herbage, followed by luxuriant growth are often associated with animal health problems. Last (1993) and MacFarlane (undated) suggested that, on previously stressed kikuyu, during periods of rapid growth following rain on N fertilized pasture, a form of kikuyu poisoning was encountered, and it was believed that toxic N compounds were produced during this period. Cordes *et al*. (1969) found that alkalosis and deaths of animals on a kikuyu pasture was associated with luxuriant growth following rain, following a dry period, in New Zealand and also cited an example of cattle dying following irrigation in the then Rhodesia (Zimbabwe). Black (1985) also suggested that the plant produced a toxin in response to stress. Macadam (1999) concluded that kikuyu poisoning occurred when cattle grazed rapidly growing kikuyu following rain or irrigation and is caused by some substance believed to cause cessation of rumen function. High moisture concentrations and lush kikuyu appear to be the common thread in kikuyu poisoning, negatively impacting on rumen function.

Examining the data on the effect of temperature on digestibility and intake from the present data show that digestibility is reduced by c. 4.4% for a 1°C increase in temperature at 25°C, with an estimated ME of 9.1 MJ/kg DM. However, at the same temperature intake is reduced by 14.8%. This reduction in intake is considerably higher than expected from changes in energy concentration alone. Figures from Bredon and Stewart (1979) indicate a 3.3% difference for the estimated intakes of dairy cows consuming diets with the same energy concentration, as predicted from the temperature data for 25°C and 26°C. Therefore, the effect of temperature is greater than that of changes in energy concentration alone on DMI. The DMI of lactating dairy cows is affected by environmental conditions outside the thermo neutral zone (5 to 20°C), intakes decreasing with temperatures above 20°C (NRC, 2001). Zemmelink (1986) stated that VFI is reduced in animals to reduce heat production in order to maintain thermal balance. Additionally, higher temperatures have a negative effect on forage quality and this will contribute to a greater heat load for the animal in many practical situations (Zemmelink, 1986).

The implications of the higher expected temperatures from climate change are severe, with estimated reductions in milk yield of 36% due to a combination of lower energy concentration and lower intakes, using the present data for cows grazing pasture alone without supplements. Strategies to alleviate the effects of high environmental temperature on dairy cows need to be formulated. These may include feeding indoors during the day and only grazing in the cooler times of the day and the use of diets, or ingredients, which do not generate high heats of fermentation or digestion.

The high NDF concentrations measured in certain trials are of concern. For instance the NDF measured for the 30-d low treatment in spring (Table 3.2), 827 g/kg DM vs 677 g/kg DM for the 30-d high treatment. The NDF-N vales were 8.4 g/kg DM and 8.5 g/kg DM respectively for the low and high 30-d treatment, indicating that residual bound N was not the cause. The ADF values were 308 and 317 g/kg DM for the low and high treatments respectively, indicating ADF was not affected. These values are the mean of six determinations, one for each day of the trial period, which were consistently high. The samples were dried together, analyzed together in batches of 12 with a control sample indicating that the analytical procedures were consistent for both treatments. The highest NDF recorded for these data was from freeze dried herbage samples taken for the protein degradability determination trials, indicating overheating during drying was not the issue with the high NDF values. These data points have been excluded from the data pool. Other unknown factors may have influenced the NDF determination of the kikuyu.

The NDF for these data was determined on the complete grass plant, including leaves, sheath and stem (which may have been the largest component by weight) with relatively older material at 40 days re-growth. The lower NDF concentrations reported by Miles *et al*. (2000) were for hand plucked samples, therefore leaf and at that leaf that broke off easily which could explain the lower NDF values. The other data mentioned would have been selectively taken from relatively young immature pasture. Similarly, the mean NDF values cited by Malleson *et al*. (2009) are lower than most of the present data, however the standard errors for that data were extremely high (60.3 ± 45.1% NDF) indicating a large range, or variation, in NDF concentrations.

Fibre forms the major component of kikuyu, as with most other tropical C_4 grasses, followed by the protein fraction and then a minimal contribution by EE and NSC. Consequently the NDF fraction is the greatest potential contributor to the digestibility or ME concentration of kikuyu. Therefore, the digestibility of the NDF fraction is a major determinant of total plant digestibility and the herbage ME concentration. Apparent digestibility coefficients of CF of 70% (in steers) and 62% (in sheep) and 57% for ADF have been determined for kikuyu by Dugmore *et al*. (1986) and Dugmore and du Toit (1988) indicating the major contribution of the fibre fraction to the energy concentration. Therefore, systems evaluating fibre digestibility, such as NDF digestibility (NDFN) advocated by the NRC (2001) could prove to be of great value in determining, or estimating, the ME concentration of kikuyu herbage. The NRC (2001) have suggested that the ADF system for predicting energy concentration be abandoned and be replaced with the summative method which calculates total digestible nutrients using actual analysis for digestible NDF, along with the other digestible nutrients (CP, fatty acids & non fibre carbohydrate). Neutral detergent fibre digestibility can be determined by in situ or in vitro methods with the NRC (2001) using the in vitro method as its basis for DNDF of forages (Hoffman *et al*., 2001).

The use of either an in situ or in vitro system (with rumen fluid) has its complications for routine laboratory analysis. It requires fistulated animals for in situ or donor animals for rumen fluid, it’s a relatively labour intensive and time consuming process with relatively low throughput. Whether NDFN determined in situ is any different from IVDMD is debatable, except possibly for predicting DMI, especially in kikuyu where

IVDMD did not prove accurate for these data, as for some other tropical species (Olubajo *et al.*, 1974). Further research is required to verify the applicability of NDFN for kikuyu, especially against known in vivo data sets which require large inputs of labour and time to generate.

Near infrared spectroscopy offers a potential solution to the routine analysis of kikuyu, and other forages, for NDFN, IVDMD and digestibility estimation. However, caution must be exercised in the use of NIRS technology as calibrations need constant development and checking against wet chemistry. Experience in the Cedara feed laboratory (Thurtell, pers comm.) has found that kikuyu samples from the eastern Cape required a different calibration curve to those from KwaZulu-Natal. Therefore laboratories should consider using calibrations determined elsewhere on different forage populations with great care, considering the accuracy and relevance for their environmental conditions.

The relationship between the chemical composition data and digestibility indicate that the traditional “proximate” type of analysis may not be entirely appropriate for determination of “quality” in kikuyu and even in other species as indicated by the NRC (2001). The use of lignin and silica assays on kikuyu, in addition to the more conventional fibre analysis, also proved unsuccessful in predicting quality (Dugmore, 1985) due to very low concentration of lignin in kikuyu, kikuyu being a prostrate creeping grass. The determination of the fibre fractions are required if the fibre content of the diet is low enough to be of concern for adequate rumination. These data indicate that the N fraction is of far more concern and that CP in itself is insufficient to draw adequate conclusions on the adequacy of N in the diet. The NPN and even nitrate contents of the diet need to be evaluated due to the strong effect they exert on digestibility and intake. Similar conclusions have been drawn on evaluating temperate forages with high N concentrations in Europe (Orosz, personnel communication). The use of water front detectors, where the moisture in the soil can be sampled for NO_3-N analysis, after rain or irrigation, and consequently the need for N fertilization offers a new tool in improving the N management of kikuyu. Discussions with dairy farmers on these issues has led to debate on the use of small hand held nitrate meters, to measure the relative level of nitrates in vegetables and fruit, similar to those used for determining water soluble carbohydrate concentrations in herbage prior to ensiling. These questions stimulate debate on the problem and potentially offer a practical solution to N management in kikuyu.

CONCLUSION

Future research on kikuyu could concentrate on the effect of soil water (wet and dry) on the composition of kikuyu differentially fertilized with N under controlled environmental conditions in a glass house. The effects on the N fractions could be monitored over time to determine the response under different soil moisture and fertilization regimes to determine optimal levels of N and NO_3-N in the soil and consequently the herbage for optimal ruminal function. Feed additives which modify or improve ruminal fermentation, such as yeast for these data, or absorb surplus ammonia in the rumen need to be further evaluated in animal production trials under field conditions.

In terms of reducing the heat liberated in the digestion process of feeds, the Effective energy system proposed by Emmans (1994) which has resulted in greater efficiencies of production under high environmental temperatures in poultry (Young, 1999) may be appropriate for formulating diets for dairy cows to reduce the heat generated by the digestive process on the cow as environmental temperatures continue to increase.

In practice many dairy farmers accept the limitations of kikuyu and that kikuyu will not produce high milk yields in dairy cows. However its soil conservation attributes, its ability to build up soil organic matter levels with a consequent improvement in soil moisture status and its high DM yields, enabling it to sustain high stocking rates with N fertilization, make it an ideal grass to compliment ryegrass and the role of kikuyu on many dairy farms is as a base perennial pasture into which ryegrass is seeded and then managed as a ryegrass pasture, with reseeding occurring on a regular basis. Pure kikuyu stands tend to be utilized with later lactation, lower producing cows or with growing heifers. Unfortunately, rule of thumb strategies for fertilization such as 50 kg N/ha after each grazing can contribute to high N fertilization levels, especially in warmer areas with longer growing seasons. Some measure of soil nitrate levels, or plant nitrate levels, to indicate if nitrate levels in the soil or plant are low or high and N fertilization strategies based on a minimal level could improve both the efficiency of N fertilizer utilization, reduce the negative effects of herbage N on digestibility and feed intake, reduce the amount of ammonia in the blood that requires detoxification by the liver at an energy cost (energy which could be used for milk production) and ultimately result in improved animal production at a lower input cost.

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Appendix Tables

Appendix Table 1 The main characteristics of kikuyu for animal production.

Attributes	**Reference**
The optimum temperature for growth to be 16 to 21^{o} C, with poor adaptation to high temperatures.	Russel & Webb, 1976
Kikuyu is more tolerant of lower temperatures than most tropical species	Colman, 1971
Kikuyu is markedly more frost tolerant than other commonly grown tropical grasses in the sub-tropics	Quinlan, *et al.*, 1975
Kikuyu can withstand moderate frost, but heavy frosts will kill top growth. High nitrogen fertilization and an adequate water supply increased kikuyu's frost tolerance.	Ballinger, 1962; Drummond, 1975 Gartner & Everett, 1970; Wetherall, 1970
Kikuyu has an advantage over most tropical grasses in its ability to grow well during autumn, with little deterioration in forage quality until frosts occur.	Murtagh, 1975
Kikuyu is considered a high quality grass for dairying and cattle finishing in high altitude areas of the tropical and sub-tropical world.	Skerman & Riveros, 1990 Hacker & Jank, 1998
Kikuyu can utilize moisture to a considerable depth, roots having been observed at a depth of 5.5 m and in a long dry season a kikuyu sward exploited soil moisture to this depth. However, 95 % of the roots are found in the top 70 cm below the ground surface	Hosegood, 1963 Quinlan *et al.*, 1975
The application of nitrogen has considerable impact on the length of the growing season (with growth greater than 5 kg DM/ha/day) of kikuyu.	Colman, 1971
Kikuyu fertilized with nitrogen is one of the most productive pastures in regions with a humid mesthermal climate, with a longer growing season at high N fertilization.	Colman, 1970
Kikuyu is highly responsive to N fertilization, yield varying from 13 to 43 kg DM/kg N applied. Ceiling yields of 30.9 ton DM/ha were recorded in northern new South Wales at 1120 kg N/ha and 35.3 ton DM/ha in Hawaii at 874 kg n/ha. However, at low levels of nitrogen it becomes uncompetitive.	Morrison, 1966; Gartner, 1966; Colman, 1971; Mears, 1974; Whitney, 1974; Ivory & Jacobsen, 1977; Miles, 1998 Theron, *et al.* 1960
Kikuyu shows substantive responsive to early spring N dressings, thus increasing the growing season.	Colman. 1971
The major factor influencing the level of animal production per ha from kikuyu pastures is the rate of nitrogen applied, although increases in stocking rate are necessary to allow the nitrogen effects to be expressed.	Mears & Humphreys, 1974; Quinlan *et al.*, 1975.

An advantage of kikuyu over most other tropical grasses is its ability to grow well during autumn with little deterioration in forage quality until frosts occur, or until June-July (mid-winter) in frost free areas. Kikuyu maintains its nutritive levels in the dry season. Kikuyu produces good foggage (deferred grazing) for overwintering dry stock.	Murtagh, 1975. Wetherall, 1970; Minson, 1972; Mears & Humphreys, 1974; Cross, 1979; Gertenbach, 1998.
Few grasses maintain high protein levels throughout growth, however, kikuyu is a notable exception in the protein levels remain at high levels throughout growth and at maturity.	Norten, 1982
Protein levels in kikuyu are not limiting to animal production	Dale & Holder, 1968; Mears, 1970; Royal & Jeffrey, 1972; Pienaar *et al*., 1993; Reeves *et al*., 1996

Animal Production	
Low production per cow, and animal disorders in cattle grazing kikuyu have been recorded.	Mears, 1970; Colman & Kaiser, 1974
Low dry matter intake and low digestibility are considered the reasons for low animal production	Colman & Kaiser, 1974
Animal intake from kikuyu has also been frequently lower that from other grasses, although the level of intake changes little with increasing age.	Colman, 1975
Low animal production is attributed to inadequate energy intake	Royal & Jeffrey, 1972; Colman, 1975; Moir *et al.*, 1979; Reeves *et al.*, 1996
Animal performance on well fertilized kikuyu is lower than expected and it appears that low intake of kikuyu is the problem	Milford, 1960; Milford & Minson, 1965; Joyce, 1974; Kemp, 1976
Depressed intake has been observed on young pasture heavily fertilized with nitrogen and this may be due to excess CP.	Milford & Minson, 1965
Individual animal performance on kikuyu pasture negatively related to nitrogen fertilization, with marginal returns favouring the lowest N treatment.	Tainton *et al.*, 1982, Karnezos, 1986; Karnezos *et al.*, 1986
Atypical negative relationships between N and digestibility recorded. Similarly a positive relationship recorded between ADF and digestibility. Nitrate levels in kikuyu negatively correlated to digestibility.	Dugmore *et al.*, 1986

Nitrogen fraction of kikuyu negatively associated with acceptability, ADF not significantly correlated to acceptability. Nitrate levels of kikuyu negatively associated with acceptabiliy.	Austin, 1980
Selection against kikuyu with higher CP contents and for herbage with a higher ADF content	Pattinson, 1981
Dry matter disappearance of oesophageal fistula samples from nylon bags positively associated with ADF levels	Pattinson, 1981
The nitrogen content of the leaves of kikuyu negatively related to voluntary feed intakes. Rumen ammonia levels negatively associated with feed intake.	Pienaar *et al*., 1993
IVDMD in kikuyu not significantly different for re-growths of 15, 30 and 60 days	Henning, 1993
Poorest dry matter intakes and digestibility recorded at youngest cuts of kikuyu, 23 days	Jeffrey, 1971
Voluntary feed intake of sheep increased with increasing herbage maturity of kikuyu for 39, 50 & 78 day re-growths	Soto, *et al*., 1980
Voluntary intake of kikuyu by dairy cows 20 % lower for 3 week re-growth relative to 6 week re-growth in kikuyu.	Brand, 1990
Voluntary intake of sheep consuming kikuyu reduced if the kikuyu was fertilized with N	Holder, 1967
Digestibilities of organic matter, CF & NFE depressed at N fertilizer levels of 336 kg/ha relative to lower rates of N fertilization (0, 112 & 224 kg/ha)	Campbell,1971
Digestibility and intake depressed in mid-season, relative to early and late season, for kikuyu highly fertilized with N relative to low N fertilization levels	Minson, 1973
A satisfactory balance between quantity and quality could be obtained, with approx. 200 g CP/kg DM and 700 g DDM/kg DM, by grazing the re-growth of kikuyu between 5 & 6 weeks after re-growth has commenced.	Drummond, 1975
The growth rate of yearling Holstein-Friesian heifers on kikuyu is very poor relative to beef animals.	Du Plessis, 1992; Allwood, 1994; Horne, 1996; Fushai, 1997

Mineral Balances	
Inverse Ca:P ratios recorded, particularly in mid to late season	Taylor, 1941; Awad, *et al*, 1979; Miles, *et al.*, 1995; Fulkerson *et al.*, 1998
Mineral (Na Ca & P) supplementation increased liveweight gains by 27 %	Kaiser, 1975
Ca the only plant fraction that significantly influenced live weight gain in steers	Karnezos, 1986
Oxalates in kikuyu compound the low Ca problems in kikuyu by reducing its availability.	Marais, 1990
Ca deficiency resulted in poor P absorption, poor P status in the animal and reproductive problems	Reason *et al.*, 1989
Low Na levels in kikuyu a implicated with bloat on kikuyu	Reason *et al.*, 1989
Supplementing cows on kikuyu pasture with NaCl increased milk production by 1.2 kg per cow per day.	Davison *et al.*, 1980
Excessive K/(Ca + Mg) ratios in kikuyu could enhance milk fever and grass tetany problems	Miles *et al.*, 1995; Fulkerson *et al.*, 1998

Animal Disorders	
Animal disorders recorded in animals grazing kikuyu pasture fertilized with nitrogen. Animals affected by a metabolic like disorder with symptoms similar to grass tetany.	Colman & Kaiser, 1974; Awad *et al.*, 1979.
Outbreaks of “kikuyu poisoning” recorded. Rapid growth of kikuyu following drought or over grazing produced toxic nitrogen compounds.	Last, 1993; MacFarlane, undated.
A common theme of lush fast growing pastures following drought breaking rains or other stress, often with N fertilization, high levels of herbage N & K are associated with animal disorders on kikuyu.	Cordes *et al.*, 1969; Colman & Kaiser, 1974; Last, 1993; MacFarlane, undated.

Appendix Table 2 Milk production from dairy cows grazing kikuyu pastures.

Milk yield	**BF**	**Concentrates**	**Breed**	**Comments**	**Reference**
2962 kg FCM/lact 2769 kg FCM/lact 2485 kg FCM/lact	118 kg 110 kg 99 kg	0	Guernsey & Jersey - *c.* 310 kg - primiparous	SR : 1.65 cows/ha SR : 2.27 cows/ha SR : 3.29 cows/ha	Holder, 1967.
12.5 kg FCM/d 9.0 kg FCM/d		0	Guernsey & Jersey	early lactation yield ave. lactation yield	Colman & Holder, 1968
7.5 kg/d 8.4 kg/d 8.8 kd/d 9.4 kg/d	5.09 % 5.09 % 4.82 % 4.86 %	0 1.1 kg/d 2.67 kg/d 3.77 kg/d	Jersey	mid-lactation late-winter grazing energy - not protein considered limiting factor	Royal & Jeffrey, 1972.
2467 ℓ FCM/lact 2068 ℓ FCM/lact 2490 ℓ FCM/lact		0 0 390 kg oats/lact	Guernsey 350 - 400 kg	SR: 2.47 cows/ha SR: 4.94 cows/ha SR: 4.94 cows/ha	Colman & Kaiser (1974).
3500 kg/cow/yr 2800 kg/cow/yr 2600 kg/cow/yr 2600 kg/cow/yr	3.9 % 4.2 % 4.0 % 4.0 %	2.3 kg/d 3.4 kg/d 2.6 kg/d 4.1 kg/d	Friesian Friesian Friesian Illawara Shorthorn	SR: 2.0 cows/ha SR: 3.6 cows/ha SR: 2.5 cows/ha SR: 3.5 cows/ha	Moir *et al*, 1979.
7.2 to 11.7 kg FCM/d		0	Guernsey & Jersey - 364 kg liveweight	measured monthly over season - lowest yields in Feb - Apr	Murtagh et al., 1980.
9.11 kg SCM/d 11.53 kg SCM/d 6.01 kg SCM/d 10.47 kg SCM/d	0.38 kg/d 0.46 kg/d 0.25 kg/d 0.42 kg/d	0 4 kg barley 0 4 kg barley	Australian Friesian - Multiparous	SR: 5 cows/ha SR: 5 cows /ha SR: 7 cows/ha SR: 7 cows ha	Olney & Albertssen, 1984.
6.5 ℓ cow/d 8.7 ℓ cow/d	4.30 % 4.36 %	-	Friesian & Guernsey	short kikuyu *vs* long kikuyu	Hughes *et al.*, 1988.

Appendix Table 2 cont'd

Milk yield	**BF**	**Concentrates**	**Breed**	**Comments**	**Reference**
18.0 kg/d 18.6 kg/d	3.37 % 3.38 %	3 kg sorghum 3 kg SF sorghum		early lactation - cracked vs steam flaked sorghum	Hamilton, 1989 - cited in Kellaway & Porta, 1993.
14.7 ℓ/d 17.9 ℓ/d 18.9 ℓ/d	3.62 % 3.41 % 3.41 %	0 3 kg barley 3 kg barley + SFOC	Friesian early lactation multiparous	SFOC: formaldehyde (0.5%) treated	Hamilton *et al.*, 1992.
5695 kg/lact 3681 kg/lact		6.1 kg/d	Holstein:550 kg Jersey: 350 kg	305 d/lact	van der Grinten *et al.*, 1992
14.5 kg/d 10.8 kg/d 9.5 kg/d	*c*. 3.55 %	no concentrates no minerals	Holstein-Friesland *c*. 550 kg	early summer late summer autumn	Henning, 1993.
9.5 kg/d 10.9 kg/d 8.9 kg/d	3.7 % 3.6 % 3.4 %	no concentrates no minerals	Holstein-Friesland *c*. 550 kg	15 day grazing cycle 30 day grazing cycle 60 day grazing cycle	Henning *et al.*, 1995.
17.3 ℓ/d 14.2 ℓ/d 12.5 ℓ/d	3.41 % 3.77 % 3.93 %	0 0 0	Friesian	early lactation mid-lactation late lactation	Reeves *et al.*, 1996.
14.2 ℓ/d 18.3 ℓ/d 18.0 ℓ/d	3.77 % 3.51 % 3.26 %	0 kg barley/d 3 kg barley/d 6 kg barley/d	Friesian	mid-lactation	Reeves *et al.*, 1996.
12.5 ℓ/d 18.5 ℓ/d 17.4 ℓ/d	3.93 % 3.89 % 3.26 %	0 kg/d 3 kg/d 6 kg/d	Friesian	late lactation	Reeves *et al.*, 1996.
19.5 kg/d	3.91%	6 kg/d	Jersey	Early- to mid-lactation	Malleson *et al.*, 2009
15.0 kg/d 14.4 kg/d 12.1 kg/d	5.05% 4.41% 4.95%	4 kg/d 4 kg/d 4 kg/d	Jersey	-	Botha *et al.*, 2008

Appendix Table 3 The chemical composition and digestibility of kikuyu (DM basis) from various sources

Chemical component (g/kg DM)					DMD (g/kg)	OMD g/kg	TDN g/kg	$\mathrm{ME}^{\#}$ MJ/kg	Notes	Reference
CP	ADF	NDF	CF	NSC						
208			228				620	9.7	July growth - Kenya	Dougall, 1960.
169			241				610	9.5	August growth	
116			240				620	9.7	September growth	
122			250				600	9.3	October growth	
109			223				630	9.8	December growth	
112			228				630	9.8	February growth	
193			258				600	9.3	March growth	
179			258				590	9.1	April growth	
167			222				620	9.7	May growth	
139			219				630	9.8	June growth	
242			204		-		-	-	8 week re-growth-India	Katiyar & Ranjhan, 1969.
143			378		633		616	9.6	12 week re-growth	
					740 (36) *	-		10.5	Max value - New South	Mears, 1970
					530 (180)			7.0	Min value - Wales	
					600 (42)	-		8.2	Max value - Hawaii	Mears, 1970
					400 (168)			4.8	Min value	
					630 (20)	-		8.7	Max value - Queensland	Mears, 1970
					530 (150)			7.0	Min value	
117					646	654		8.9	With N fertilizer	Jeffrey, 1971.
169					612	628		8.4	Without N fertilizer	
88			321			620	656	10.3	0 kg N/ha	Campbell & Ho-a, 1971.
84			323			628	594	9.2	112 kg n/ha	
91			333			604	565	8.6	224 kg N/ha	
107			319			773	538	8.1	336 kg N/ha	

* ( ) days regrowth; # estimated using: ME = 0.16 OMD% - 1.8 or ME = 0.17 DMD% - 2.0 (AAC, 1990); ME = -0.45 + 1.01.DE where DE = 4.409 Mcal DE).

Appendix Table 3 cont'd

Chemical component (g/kg DM)					DMD (g/kg)	OMD g/kg	TDN g/kg	ME$^{\#}$ MJ/kg	Notes	Reference
CP	ADF	NDF	CF	NSC						
156	307	612			606	602		8.3	15-28 day re-growth	AFIC-CSIRO, 1987
126	-	-			567	-		7.6	29-42 day re-growth	
126	329	659			570	-		7.7	43-56 day re-growth	
76	340	683			485	495		6.2	71-84 day re-growth	
74	370	708			473	480		6.0	85-98 day re-growth	
207	231	602		54		734		9.9		Reeves *et al.*, 1996
200				59		645		8.5	summer growth	Fulkerson *et al.*, 1998
					in vitro				Fertilizer application - cut at:	De Figueiredo, 1987
177				48	410				300 kg N - 3 week re-growth	
205				45	434				400 kg N - 3 week re-growth	
226				45	428				500 kg N - 3 week re-growth	
152				49	396				300 kg N - 6 week re-growth	
175				49	400				400 kg N - 6 week re-growth	
198				49	419				500 kg N - 6 week re-growth	
130				59	391				300 kg N - 9 week re-growth	
						IVDOM				Köster , 1991
237	244	604							Spring	
232	327	646				625			Early summer	
267	304	593				624			Mid summer	
272	308	658				657			Late summer	
249	318	639				662			Autumn	
						671				
218	263	550				665		9.3	Summer	Granzin, 2003
221	305	603				699		10.0	Summer	Malleson *et al.*, 2009
237		647						8.92	Summer	Botha *et al.*, 2008
231		626						8.13	Autumn	

$^{\#}$ estimated using: ME = 0.16 OMD% - 1.8 or ME = 0.17 DMD% - 2.0 (AAC, 1990); ME = -0.45 + 1.01.DE where DE = 4.409 Mcal DE).

Appendix Table 4. The mineral composition of kikuyu herbage from various sources

Mineral (g/kg DM)						Notes	Reference
Ca	P	K	Mg	Na	Ca:P		
2.7		46	-	-	0.71	Fertilized NPK	Taylor, 1941
4.1	2.9	27.3	-	3.0	1.41	Fertilized	Weinmann, 1955
4.9	3.8	19.0	3.2	4.7	1.29	NPK; cut 20-30 cm	Joyce, 1974
4.4	3.6	21.8	2.5	3.5	1.22	NPK; cut 8-13 cm	Joyce, 1974
3.5	3.1	36.6	4.0	0.3	1.13	Fertilized; NP	Kaiser, 1975
3.4	3.2	34.6	0.8	0.8	1.06	NPK; cut every 12 days	Hennessy & Williamson, 1976
2.5	2.5	21.3	2.7		1.00	0 kg N/ha	Campbell & Ho-a, 1971.
3.1	2.6	21.4	3.5		1.19	112 kg n/ha	
2.8	2.8	20.1	4.0		1.90	224 kg N/ha	
2.4	2.8	19.2	2.1		0.86	336 kg N/ha	
3.0	4.9	41.8	3.4		0.61	27 Oct - Highveld	Williams, 1983.
3.3	3.8	-	3.9		0.87	10 Nov	
2.7	6.3	20.4	2.3		0.43	01 Dec	
3.1	4.0	12.1	2.4		0.77	20 Dec	
2.4	5.0	20.5	1.7		0.48	12 Jan	
2.6	4.5	41.1	2.2		0.58	02 Feb	
3.5	4.1	46.2	2.8		0.85	21 Feb	
2.8	3.9	38.8	3.0		0.72	16 Mar	
1.9	2.0	17.5	1.9	0.08	0.95	minimum value	AFIC-CSIRO,1987.
3.0	3.4	32.4	3.6	0.34	0.88	mean value (n = 50)	
7.0	5.0	49.3	7.7	0.90	1.40	maximum value	
2.8	3.5	38.7	2.3	0.2	0.80		Dugmore *et al.*, 1987.
3.14	3.90	20.52	3.10	0.27	0.81	Nov-Dec	Reeves *et al.*, 1996
2.98	3.89	15.82	3.14	0.47	0.77	Jan-Feb	
4.21	3.40	11.79	5.28	0.32	1.24	Mar-Apr	
4.2	2.8	29.0	2.9	0.97	1.50	5 cm stubble height	Fulkerson *et al.*, 1998
5.2	4.0	34.0	3.6	2.4	1.30	Non acidic soils within 3 km of sea	Miles *et al.*, 2000.
2.9	3.8	23.8	2.3	0.5	0.76	Summer	Granzin, 2003
4.0	3.0	39.0	3.0	0.0	1.33	Autumn	Granzin, 2005
3.7	3.5				1.08	Summer	Malleson *et al.*, 2009
3.2	5.1				0.63	Summer	Botha *et al.*, 2008
3.6	5.8				0.62	Autumn	

Appendix Table 5 Summary of the main results on digestibility for the digestion trials conducted over the experimental period

Year	Season	Fertilization (treatment)	Digestibility at re-growth stage			AOV for main effects				
			20 days	30 Days	40 days	N effects	Stage of re-growth		N X Stage interaction	
							Linear	Quadratic	linear	Quadratic
1	Spring	low N high N	627 643	702 639	**773** **786**	ns	P<0.001	P=0.011	ns	P=0.008
	Summer	low N high N	635 608	541 547	**696** **687**	ns	P=0.002	P<0.001	ns	Ns
	Autumn	low N high N	701 700	**745** 689	529 634	ns	P<0.001	P=0.001	P=0.036	P=0.016
2	Spring	low N high N	746 730	725 **765**	740 **770**	positive P=0.025	Ns	Ns	P<0.005	
	Summer	low N high N	684 701	**743** **764**	735 688	ns	Ns	P<0.001	P=0.019	Ns
	Autumn	low N high N	**721** **758**	666 692	642 668	ns P=0.057	P<0.001	Ns	ns	Ns
3	Spring	low N high N	615 593	**712** 640	654 601	negative P < 0.001	Ns	sig P<0.001	ns	Ns
	Summer	low N high N	- -	645 578	650 646	ns	Ns	Ns	ns	Ns
	Autumn	low N high N	671 653	**726** **695**	668 616	negative P<0.009	Ns	sig P<0.001	ns	Ns

Appendix Table 5 Continued

Year	Season	Fertilization (treatment)	Digestibility at re-growth stage			AOV for main effects				
			20 days	30 days	40 days	liming effects	Stage of re-growth		Lime X Stage interaction	
							Linear	Quadratic	linear	Quadratic
4	Spring	limed unlimed	694 674	621 580	**774** 672	positive P<0.001	P<0.011	P<0.001	P<0.023	P<0.008
	Autumn	limed unlimed	**553** **545**	522 521	522 411	positive P<0.001	P<0.001	-	P=0.001	-
5	Spring	limed unlimed	682 659	664 650	584 598	ns	P<0.001	P<0.010	ns	Ns
	Summer	limed unlimed	573 **593**	549 525	488 535	ns	P<0.001	Ns	ns	Ns
	Autumn	limed unlimed	**593** 569	519 540	557 578	ns	P = 0.038	-	ns	-