

**The nutritive value of Italian ryegrass  
(*Lolium multiflorum*)  
selected for high dry matter and nonstructural  
carbohydrate contents**

by  
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## Preface

The experimental work described in this thesis was carried out at the Department of Agriculture and Environmental Affairs, Pietermaritzburg and at Hopewell, Nottingham Road from March 1998 to October 2001 under the supervision of Dr Johan Marais and Professor Clive Dennison.

These studies represent original work by the author and have not been submitted in any other form to another university. Where use was made of the work of others, it has been duly acknowledged in the text.



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## Abstract

In traditional forage breeding programmes, breeders have spent decades improving the agronomic characteristics of grasses, such as herbage yield, persistence and resistance to diseases, without considering the nutrient requirements of the grazing animal. In an attempt to improve the nutritive value of Italian ryegrass, which is widely utilised for intensive dairy, lamb and beef production in South Africa, Enhancer ryegrass was developed from predominantly Italian types of *Lolium multiflorum*, with a minor Westerwolds component, by selecting for a higher concentration of total nonstructural carbohydrate (TNC) and lower moisture content than that currently available in commercial cultivars.

The nutritional value of Enhancer was compared with Midmar ryegrass in a controlled environment study and in a grazing trial with weaned lambs; and with Dargle ryegrass in a grazing trial with Holstein dairy cows. Neutral detergent fibre, acid detergent fibre, lignin, nitrogenous compounds, mineral content and *in vitro* digestibility were also investigated as parameters of nutritive value. The anatomical features of Enhancer and Midmar were studied to determine possible structural differences. Weaned lambs grazed Enhancer and Midmar in an eight-paddock rotational grazing system, with 3.5 days spent in each paddock, allowing a 24.5 day regrowth period for the pastures. Holstein dairy cows grazed Enhancer and Dargle which were established on 16 and 19 hectare pastures, respectively. The *n*-alkane technique was used to estimate dry matter intake (DMI) in both grazing trials.

Results from the controlled environment study suggest that the differences in the dry matter and TNC concentration of Enhancer are not positively linked to anti-quality factors associated with forage species, but can be attributed to genetic differences between the two grasses. Despite the significantly higher ( $P < 0.01$ ) DMI of weaned lambs grazing Midmar compared with Enhancer, the lambs on Enhancer outperformed those on Midmar in terms of liveweight gain and carcass quality. The superior animal performance on Enhancer is likely due to an improvement in the readily digestible energy to protein ratio as a result of its significantly higher ( $P < 0.001$ ) concentration of TNC compared with Midmar. Milk yield for cows grazing Enhancer in period 1 of the cross-over study was significantly higher ( $P < 0.05$ ) than for cows grazing Dargle, despite the significantly lower ( $P < 0.05$ ) DMI of animals on Enhancer. The higher TNC concentration relative to the true protein content of Enhancer would suggest that the protein metabolism in the rumen can be enhanced.

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## List of Abbreviations

ADF	acid detergent fibre
ADG	average daily gain
ADL	acid detergent lignin
CP	crude protein
C	cold
CTAB	cetyltrimethyl ammonium bromide
CW	cell wall
DAP	di-ammonium phosphate
DM	dry matter
DMD	dry matter digestibility
DMI	dry matter intake
EDTA	disodium ethylenediaminetetraacetate
EPI	epidermis
FAA	formalin-acetic acid-alcohol
FMC	field moisture capacity
IVDMD	<i>in vitro</i> dry matter digestibility
LAN	limestone ammonium nitrate
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NDF	neutral detergent fibre
NS	not significant
OM	organic matter
PAS	permissible acid saturation
PBS	parenchyma bundle sheath
PEP	phosphoenolpyruvate
ppm	parts per million
RDP	rumen degradable protein
SCL	sclerenchyma
S.E.M.	standard error of the mean
TNC	total nonstructural carbohydrates
TP	true protein
UDP	undegraded or bypass protein
VI	voluntary intake
W	warm
WSC	water soluble carbohydrates

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## Chapter 1

### Introduction

*Lolium multiflorum* is native to temperate and Mediterranean Europe, to temperate Asia and North Africa and has been introduced in temperate and subtropical regions throughout the world (Rhind, 1974). The species *L. multiflorum* is often subdivided into two distinct types, namely Italian ryegrass and Westerwolds ryegrass. The Italian types require an extended period of winter cold (vernalisation) in order to flower, while the Westerwolds types flower and form seed in response to increasing day length and/or warm temperatures (Aamlid *et al.*, 1997). In South Africa, *L. multiflorum* is an important cool season forage grass, and is grown mainly in high-rainfall areas. It is often used for oversowing perennial pastures to increase winter production, or for undersowing annual summer crops, thereby allowing the land to be utilised more efficiently. In mixed pastures with clover it provides particularly high quality grazing. Commercial farmers grow *L. multiflorum* for intensive dairy, lamb and to a lesser extent for beef production. It is also a valuable forage crop for the small-scale farmer in high-rainfall areas, especially when undersown in maize fields. Production costs in South Africa of *L. multiflorum* are high: currently R 5 792 ha<sup>-1</sup> year<sup>-1</sup> (Müller, 2002). According to the Fourth Draft of the National Water Bill, tabled in the South African Parliament in 1998, the Minister of Water Affairs may, in future, set a price structure for irrigation water to farmers. These measures could further increase the production costs of *L. multiflorum*.

Planted in autumn, *L. multiflorum* pastures provide good autumn growth, reasonable winter growth (depending on the severity of the winter) and excellent spring growth (Bartholomew, 1991). The grass requires high soil fertility and sufficient moisture, due to its shallow root system (Rhind, 1974). The supply of high-quality pasture to the animal can be increased by improving grazing management practices, but there may be occasions when the genetic potential of the grass limits its nutritive value, necessitating the use of relatively expensive feed supplements. There is evidence of relatively poor animal performance on Italian ryegrass cultivars, particularly on those low in DM content, which reduces the DM intake of the animals (Meissner, 1996). Other factors contributing to disappointing animal performance include a high nitrate-N content (De Villiers, 1991) and an imbalance between N and energy (i.e. low total nonstructural carbohydrate content) which could result in decreased organic matter (OM) intake and insufficient microbial protein reaching the small intestine (Aii and Stobbs, 1980).

Herbage feeding value is defined by the production response of an animal. It is a function of the concentrations of nutrients per unit mass of forage (nutritive value) and the amount consumed by the animal (Ulyatt, 1973). The major nutritive traits are well defined and the Delphi survey technique (Wheeler and Corbett, 1989) has been used in ranking them in terms of their relative importance in improving the nutritive value of grasses used in dairy production systems. This technique, based on the anonymous judgements of a group of specialists in dairy nutrition and plant breeding (Smith *et al.*, 1997), ranked the traits in the following order: high dry matter digestibility, low lignin content, optimal

ratio of rumen digestible protein to rumen indigestible protein, increased DM content of herbage, low fibre content, absence of anti-quality factors, high protein content, high magnesium content and high lipid content. In a similar survey, the four most important nutritive value traits in forages for liveweight gain were high digestibility, easy comminution, high nonstructural carbohydrates and high crude protein content (Wheeler and Corbett, 1989). The order of rating for wool production in the same survey was a high digestibility, easy comminution, high sulfur amino acid content and a high nonstructural carbohydrate content.

Preliminary studies conducted at the KwaZulu-Natal Department of Agriculture and Environmental Affairs research station at Cedara showed that two of the prominent factors listed in the Delphi survey, i.e. total nonstructural carbohydrate (TNC) and dry matter (DM) content could be relatively low in early- and late-season regrowth of *L. multiflorum* (Marais *et al.*, 1993) and may have resulted in poor animal performance. In view of the high production costs of *L. multiflorum* and its suspected nutritional inadequacies, a two-phase breeding and selection programme has been introduced in an attempt to improve its nutritive value and to maintain or improve its cost effectiveness.

The first phase was aimed at simultaneously increasing the DM and TNC content of *L. multiflorum*. One thousand five hundred plants derived from the commercial cultivars Exalta, Titania and Lemtal were established in a spaced-plant nursery and the DM and TNC content monitored over the growing season. At the onset of flowering, the top two percent of the plants were re-established in a polycross, to allow cross pollination of the selected plants. These plants provided the seed for the next generation of plants. After four generations the DM and TNC contents of the selected materials were 24% and 78% higher, respectively, than those of the commercial cultivars Exalta, Midmar, Dargle and Hilton (Marais *et al.*, 1997). After the sixth season, seed from the high DM and TNC line, which was subsequently named Enhancer, was bulked up for the second phase of the trial, in which Enhancer was evaluated and compared with the commercial cultivar Midmar, which, since 1975, has been the most widely utilised *L. multiflorum* ryegrass cultivar in South Africa.

This evaluation of the *L. multiflorum* cultivar Enhancer forms the basis of the present dissertation. A study conducted in a controlled growth chamber focused on anatomical and chemical differences between the two cultivars, Enhancer and Midmar. This was aimed at establishing whether traits other than DM and TNC content, which could have an effect on the nutritive value of Enhancer, were altered during selection. The impact of these traits on the intake and digestibility of the ryegrass cultivars was investigated by means of the alkane marker technique, using weaned lambs. Following these preliminary studies, Enhancer was evaluated and compared with a commercial *L. multiflorum* cultivar, Dargle, in a trial with Holstein dairy cows where milk yield and quality and pasture intake were evaluated.

## Chapter 2

### Factors affecting the nutritive value of *Lolium multiflorum*

#### 2.1 Introduction

The chemical composition of *Lolium multiflorum* has been extensively studied (Wilman and Wright, 1978; Gordon *et al.*, 1985; Meissner *et al.*, 1989; Hume, 1991; Marais *et al.*, 1993; Meissner, 1996; Thom and Bryant, 1996; Thom and Prestidge, 1996; Wilman *et al.*, 1996). The major positive and negative traits affecting nutritive value which may have been introduced during selection for a high DM and TNC content are discussed in this review.

#### 2.2 Characteristics of *Lolium multiflorum*

##### 2.2.1 Morphology

*Lolium multiflorum* is an erect annual or short-lived perennial (Figure 2.1). It is loosely tufted, 200-800 mm tall, the leaf blades are 110-220 mm long and approximately 3 mm wide. The expanded leaf blade tapers, is dark green and dull on the upper surface and lighter green and shiny on the lower surface. It has two well-developed clasping auricles (1-4 mm long) at the base of the lamina. The ligules are rounded or truncate and very short (4 mm). Spikes are straight or slightly curved and are usually 8-20 mm long and 2-10 mm wide. Awns are typically present, are usually straight, slender, are attached 0.2-0.7 mm below the apex and are 15 mm long (Rhind, 1974; Gibbs-Russell *et al.*, 1990).

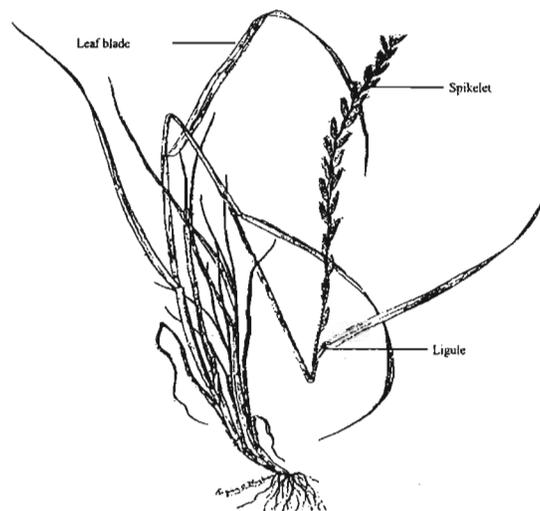


Figure 2.1 The morphology of the *Lolium multiflorum* plant (source: Rhind, 1974).

### 2.2.2 Anatomy

Leaf anatomical features of  $C_3$  (temperate) and  $C_4$  (tropical) grasses have been measured (Dengler *et al.*, 1994). Cross-sectioned areas of all tissues and surface areas of chlorenchymatous tissues have been examined in transverse sections of the leaf blades from 125 grass species. These species, studied by Dengler *et al.* (1994), represented the three biochemical types (NADP-malic enzyme; NAD-malic enzyme and PCK-photosynthetic carbon reduction). The structural features of leaf blade mesophyll and bundle sheath tissue are important in the operation of the photosynthetic pathways in grasses.

The  $C_3$  pathway is characterised by the Calvin cycle. While the  $C_4$  pathway also has the Calvin cycle, it is preceded by a series of reactions involving the carboxylation of PEP (phosphoenolpyruvate) to yield  $C_4$ -dicarboxylic acid. In this pathway ( $C_4$ ) chloroplasts are dispersed between two particular cell types, radially arranged around the vascular bundle, with bundle sheath cells forming the inner layer and mesophyll cells the outer layer. The reactions involved in the Calvin cycle are primarily located in the bundle sheath cells, while those involved in PEP and  $C_4$  dicarboxylic acid formation are found in mesophyll cells (Hatch and Boardman, 1973). The anatomical differences between  $C_3$  and  $C_4$  grass species are illustrated in Figure 2.2.

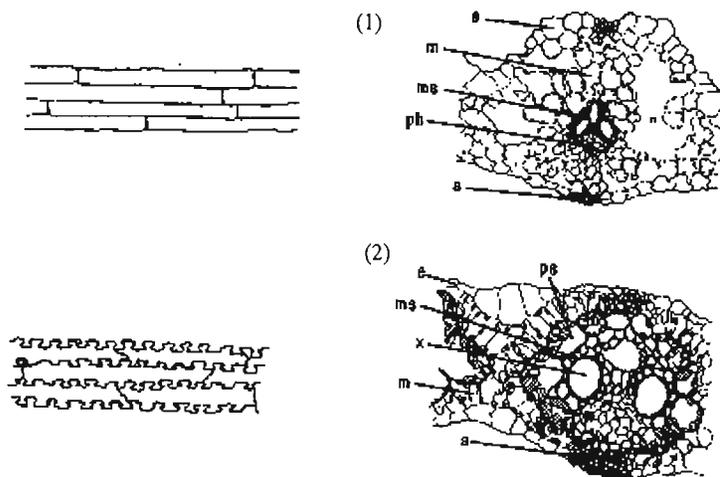


Figure 2.2 Cross-sections of examples of  $C_3$  temperate and  $C_4$  tropical grass species showing differences in the main and paradermal veins.

(1) *Lolium multiflorum* (a  $C_3$  temperate grass),

(2) *Panicum maximum* (a  $C_4$  tropical grass).

e = epidermis, m = mesophyll, ms = mesostome sheath, ph = phloem, s = sclerenchyma, ps = parenchyma bundle sheath, x = xylem (source: Wilson, 1993).

Interveinal distance (used as an estimate of leaf surface area per vein sector) was notably shorter in  $C_4$  grasses than in  $C_3$  grasses. A lower proportion of mesophyll tissue per vein and a higher proportion of bundle sheath tissue were found in  $C_4$  grasses, as opposed to  $C_3$  grasses. In addition, the ratio between mesophyll and bundle sheath tissue was considerably lower for  $C_4$  species than  $C_3$  species. In terms of intercellular spaces, it was reported that the proportion of mesophyll tissue occupied by intercellular space was lower in  $C_4$  than in  $C_3$  grasses. Dengler *et al.* (1994) also demonstrated that the leaf blades of  $C_4$  grasses were thinner than in  $C_3$  grass species. The leaves of  $C_4$  species have a greater proportion of vascular tissue than do  $C_3$  species, but it was found that the ratio of total chlorenchyma tissue to total vascular tissue remains constant across the two photosynthetic types (Dengler *et al.*, 1994).

Wilson (1991) discussed leaf blade anatomy in relation to nutritional aspects. It was found that the leaf blades of  $C_4$  grasses have a greater proportion of thick-walled and less digestible tissues (including parenchyma bundle sheath, vascular tissue and sclerenchyma). These grasses generally have a lower dry matter digestibility (DMD) and higher cell wall (CW) and lignin content than the leaf blades of  $C_3$  grasses. The level of leaf insertion on the tiller can have a marked effect on the proportion of vascular and sclerenchyma tissue. Early-formed leaves at the base of the tiller appear to have a limited requirement for mechanical support and so have less vascular and sclerenchyma tissue, thinner cuticles and a significantly higher DMD than upper leaves (Wilson, 1990).

Leaves of *L. multiflorum* and *L. perenne* have been harvested at different stages of maturity and their compositions of mesophyll, epidermis and fibre cell walls examined (Gordon *et al.*, 1985). Light and electron microscopy examination of these structures has revealed uniformly thin (200 nm) mesophyll cell walls, while epidermis cell walls ranged from 2000-3000 nm at the outer surface, thinning to 300 nm or less at the inner surface. The fibre fraction has been found to consist largely of sclerenchyma, but also contained other vascular cells, detached annular rings and heavily silicified leaf hairs (Gordon *et al.*, 1985).

Wilkins and Sabanci (1990) studied the variation in epidermal cell dimensions among diploid perennial ryegrasses. Differences in the mean epidermal cell length and mean width were found within diploid perennial ryegrass and that the length and width were independent. These differences occurred across a range of cutting frequencies and fertilizer levels. A tetraploid ryegrass cultivar, Tove, was also examined under the same conditions. It was found that the epidermal cells were longer and wider than those of any of the diploid cultivars. The use of such cell dimensions as tools in grass breeding is often difficult, since there is substantial variation in length between individual cells. It is therefore necessary to measure approximately 400 cells to obtain sufficiently accurate mean values for different populations. These dimensions are useful when considering parents for hybridisation and for defining breeding objectives (Wilkins and Sabanci, 1990).

## 2.3 Chemical composition of *Lolium multiflorum*

### 2.3.1 Moisture

Water is essential for plant growth. It is involved in the structural integrity of biological molecules and is directly involved in biochemical processes occurring in the plant. It plays a vital role as a solvent, translocating mineral nutrients and other foodstuffs throughout the plant body (Slatyer, 1967; Bailey, 1973a). Together with carbon dioxide, water is one of the building blocks of many plant constituents. At the plant physiological pH levels it may be regarded as the ultimate source of the  $H^+$  and  $OH^-$  ions for many reactions (Bailey, 1973a).

Plants have developed an efficient system which allows rapid movement of water from the absorbing surfaces to the transpiring surfaces, while simultaneously maintaining some restriction on water loss. The tissue water balance is a dynamic system under continual flux due to climatic conditions, stage of maturity, water supply, and nutritional status (Bailey, 1973a; Minson, 1990a). According to Burton *et al.* (1959) and Deinum (1966) an increase in temperature with no humidity control can lower the water content of ryegrass, while lower light intensity increases water content. Wilman and Wright (1978) reported a 4.8% decline in the DM content of *L. multiflorum* in the early stages of regrowth, following defoliation. As plants grow to maturity, there is a consistent increase in the DM content of the total herbage (Bailey, 1973a). Higher water contents are attained when excessive nitrogen fertilizer is applied. An increase in nitrogen fertilizer from 44 kg to 300 kg  $ha^{-1}$  increased the water content of *L. multiflorum* from 78 to 85% (Bailey, 1973a).

When plants are subjected to water deficits, plant metabolism is affected mainly by a reduction in hydrostatic pressure (turgor) inside the cell which, in turn, has a negative effect on leaf enlargement, photosynthesis and yield (Davies, 1986). Nearly all aspects of plant growth are decreased as the water content falls to stress levels. When the water to dry matter ratio in the tissue is limited, the chemical potential of water decreases, and changes occur in the concentrations of solutes in the cells and the properties of the protoplasm. A decrease in DNA, RNA and protein synthesis causes a breakdown of these polymers which result in an increase in the transport of nitrogen and phosphorus compounds from leaves to stems. While protein and RNA synthesis are susceptible to water stress, amino acid synthesis is less influenced (Barnett and Naylor, 1966). Associated with water stress is a higher nonstructural carbohydrate content and lower starch levels. According to Munns and Weir (1981), unevenness in water application could affect both the DM and TNC contents of individual plants. It is therefore necessary to apply fertilizer and water evenly to trials and in sufficient amounts when comparing DM and chemical composition of individual plants.

The dry matter and chemical composition of various *L. multiflorum* cultivars is presented in Table 2.1. When *L. multiflorum* plants are selected for low moisture content it is possible that the resulting low-moisture selections may be more susceptible to drought stress. However, this seems unlikely, provided that the normal hydrostatic control mechanisms remain intact, since the amount of water normally held in the plant tissue is small compared to the volume of water absorbed and transpired

(Davies, 1986). Under certain circumstances the moisture content of forage has a major effect on the dry matter intake (DMI) by ruminants. According to Arnold (1962) the voluntary intake of fresh herbage is not affected by moisture if the moisture content fluctuates below about 780 g kg<sup>-1</sup> of fresh forage. Vérité and Journet (1970) found that the critical level above which intake is negatively affected is a moisture content of 819 g kg<sup>-1</sup> (or a DM content of below 180 g kg<sup>-1</sup>). John and Ulyatt (1987) found that the negative relation between voluntary intake of dry matter and the moisture content of forage is applicable over a wide range of forage DM contents (120 - 250 g kg<sup>-1</sup>) and at all stages of maturity.

The cause of the decline in intake of high-moisture forage is poorly understood. Chewing during ingestion and rumination is the most important mechanism of reducing food to a smaller particle size. The number of jaw movements that are made is influenced by factors such as the type of forage fed (Church, 1969). Fresh herbage was swallowed at a faster rate than hay and chewing involved fewer jaw movements per bolus than for hay. Gill *et al.* (1966) found that hay was chewed to a mean particle size of 1.3 mm before swallowing, while fresh herbage was only reduced to 2.1 mm. Particle size is an important factor influencing the rate of passage of ingesta from the rumen, which will, in turn, determine the amount of forage that can and will be consumed. Animals spend about 33% of their time in rumination. This period can be extended at the expense of grazing time by the presence of coarse material in the rumen (Church, 1969). Cattle that were fed grass sprayed with water, spent 70% more time ruminating, possibly due to insufficient breakdown of forage particles during ingestion (Burtis and Phillips, 1987). This could further reduce the daily intake of dry matter.

Table 2.1 indicates that the DM content of *L. multiflorum* cultivars ranges from 125 to 250 g kg<sup>-1</sup>. Some of these values are below the critical level, and may be due to past breeding practices and strategies which were based on visual observations and the selection of higher yielding plants which showed lush growth. The DMI of ruminants should be improved if the DM content of *L. multiflorum* pastures can be increased by selection to a value above 180 g kg<sup>-1</sup> DM.

### 2.3.2 Carbohydrates

The most abundant class of compounds found in plants are carbohydrates, which account for 50 to 80% of the dry biomass of forage species (Van Soest, 1982). In the plant, carbohydrates play a major role in intermediary metabolism, energy transport, and energy storage (Moore and Hatfield, 1994). The plant cell wall is comprised of carbohydrates which maintain the structural integrity of individual cells, organs and tissues (Hatfield, 1989). In the plant, carbohydrates can be divided into the nonstructural polysaccharides, such as starch and fructans, and the structural polysaccharides, such as pectin, cellulose and hemicellulose (Moore and Hatfield, 1994).

Table 2.1 Chemical indices of the nutritive value of *L. multiflorum*.

References	DM	CP	NDF	ADF	ADL	N	TNC	Cutting or grazing height (cm)	Age of regrowth (weeks)	Grazed/ ungrazed	Season
Kaltofen & Wojahn (1981)	-	182	-	-	48	-	-	-	-	ungrazed	Spring
Nelson & Rouquette, Jr (1981)	-	126	597	-	-	-	-	5	-	ungrazed	Autumn
Bredon <i>et al.</i> (1987)	200	462	-	-	-	-	-	-	-	-	-
Moseley <i>et al.</i> (1988)	223	-	-	-	-	19.5	-	-	-	-	-
Andrieu <i>et al.</i> (1989)	-	228	-	233	-	-	-	-	-	grazed	-
Berardo <i>et al.</i> (1989)	-	84.4	578	350	45.2	-	-	-	-	ungrazed	Autumn
De Villiers <i>et al.</i> (1993)	125-188	163-260	171-234	250-288	-	-	85-199	-	-	grazed	Autumn/Winter
Du Preez & Meissner (1992)	180	-	485	291	-	29.1	-	15	-	ungrazed	Autumn
Meissner <i>et al.</i> (1992)	140	-	385	204	-	32.4	-	23	-	grazed	Autumn/Winter
Thompson <i>et al.</i> (1992)	-	193	510	342	-	-	-	-	6-8	ungrazed	Various

References	DM	CP	NDF	ADF	ADL	N	TNC	Cutting or grazing height (cm)	Age of regrowth (weeks)	Grazed/ ungrazed	Season
Snyman & Joubert (1993)	-	246	477	306	-	-	-	3-5	-	ungrazed	-
Sulc <i>et al.</i> (1993)	-	166	552	321	-	-	-	-	-	ungrazed	Spring
Flachowsky <i>et al.</i> (1994)	-	-	553	304	32	-	-	-	-	ungrazed	-
Dugmore (1995)	-	231	440	250	-	-	-	-	-	ungrazed	Autumn
Panciera <i>et al.</i> (1995)	-	73	530	300	-	-	-	5	-	ungrazed	Summer
Meissner (1996)	183	174	431	233	36	-	152	-	-	grazed	Autumn
Sulc & Albrecht (1996)	-	186	470	264	-	-	-	7-8	-	ungrazed	Spring
Lippke & Ellis (1997)	-	228	375.1	217.5	-	16.0	-	-	-	ungrazed	Winter
Marais <i>et al.</i> (1997)	150-170	-	-	220-260	41-53	-	120-260	5	-	ungrazed	Autumn

DM = dry matter, CP = crude protein, NDF = neutral detergent fibre, ADF = acid detergent fibre, ADL = acid detergent lignin, N = nitrogen, TNC = total nonstructural carbohydrates. All values expressed as g kg<sup>-1</sup> DM

### 2.3.2.1 Nonstructural carbohydrates

The major nonstructural carbohydrates found in plants are starches and fructans. Starch is the principal storage polysaccharide in most higher plants. Starches are glucose polymers composed of D-glucopyranose units joined through  $\alpha$  1-4 glycosidic links (Davies *et al.*, 1964) and occur in two forms in the plant: amylose and amylopectin. Fructans occur in a number of forages and are the main storage polysaccharides in Festucoid grasses, which account for most of the temperate forage species (Manners, 1985; Pontis and Del Campillo, 1985). Fructans are composed almost entirely of fructose residues. Due to their unique structure, fructans are non-reducing polymers, are highly soluble in hot water, and are easily hydrolysed in weak acid solution (Moore and Hatfield, 1994). In temperate grasses, stem tissue contains a higher concentration of sugars and fructans than leaf tissue, with concentrations in the leaf sheaths being generally higher than in leaf blades. There is an increasing concentration gradient of nonstructural carbohydrates in leaf blades, leaf sheaths and stem internodes from the top to the bottom of the plant, especially during shoot maturation (Smith, 1973).

Various factors have an impact on the concentration of nonstructural carbohydrates in the plant. The concentration of water-soluble carbohydrates shows diurnal fluctuations, increasing from sunrise until the afternoon and then decreasing until daylight the next day. Nonstructural carbohydrate concentrations are influenced by current rates of photosynthesis and growth. Most of the diurnal variation would appear to be due to changes in sucrose concentration. Diurnal changes in fructan content has not been well established (Smith, 1973). Seasonal variations in the concentration of nonstructural carbohydrates are the result of changes in the temperature, the light intensity and the growth stage of the plant. Temperature influences the concentration and molecular size of fructans in temperate grasses, with higher concentrations found at cool, rather than at warm, temperatures (Smith, 1973). Numerous studies have shown that a reduction in light intensity results in a decrease in nonstructural carbohydrate content in the herbage of grasses and legumes (Alberda, 1957, 1965; Mackenzie and Wylam, 1957; Bathurst and Mitchell, 1958). The ratio of stem to leaf tissue is particularly important when studying the percentage of nonstructural carbohydrates in temperate herbage. As grasses mature and the proportion of stem tissue increases, the concentration of nonstructural carbohydrates increases, due primarily to an increase in fructosan percentage (Smith, 1973). A negative correlation was also found between the nonstructural carbohydrates and nitrogen content of the plant (Jones, 1970). When growth is stimulated by nitrogen fertilization, there is a demand for nonstructural carbohydrates and this eventually leads to a decrease in carbohydrate concentration in the plant. This decrease appears to be due to a decrease in fructans rather than total sugars.

The nonstructural carbohydrates in plants serve as important energy stores (Smith, 1973). They are involved in good tiller survival, sward persistency (Thomas and Norris, 1981) and improved regrowth after defoliation. Nonstructural carbohydrates have potential in terms of osmotic regulation and are associated with adaptation to drought (Munns and Weir, 1981), where water stress favours the accumulation of nonstructural carbohydrates. High TNC plants are, however, often low in DM yield.

Marais *et al.* (1993) showed a negative correlation between TNC and DM yield of *L. multiflorum*. It was suggested that the high TNC plants differ from low TNC plants in that they are less efficient in converting carbohydrate reserves into structural components (Marais *et al.*, 1993).

In ruminants, more than 90% of the carbohydrate digestion occurs within the rumen (Bailey, 1973), where rumen microbes rapidly ferment sucrose and other soluble sugars to yield volatile fatty acids. These acids are absorbed through the rumen wall into the blood and serve as the primary substrate for energy metabolism in the ruminant. Studies have shown that by increasing the nonstructural carbohydrate content of *L. multiflorum*, the palatability of the grass and the voluntary DM intake by the ruminant is improved (Bailey, 1964, 1965; Cooper, 1973; Beever *et al.*, 1978). Bailey (1964) studied ryegrass cultivars and found that Italian ryegrass forage particles were more rapidly broken down in the rumen than perennial ryegrass, resulting in a faster rate of passage, and consequently a greater consumption of feed by sheep. Feed rich in readily fermentable carbohydrates (soluble sugars and fructans) favours propionate and butyrate production in the rumen, which animals can more efficiently utilise as energy than acetate, accumulated in cellulose-rich feed.

The TNC content of Italian ryegrass is presented in Table 2.1. In perennial and Italian ryegrass, water-soluble carbohydrates have a high heritability (0.84), which indicates the possibility of rapid change under selection (Cooper, 1961). The improvement of forage grasses to maximise animal performance has been studied extensively (Ulyatt, 1981; Hacker, 1982 and Marten, 1989). Carbohydrates constitute a high percentage of the DM of forage species, and therefore serve as important factors determining the nutritive value of grasses. According to Beever *et al.* (1978), high concentrations of water-soluble carbohydrate in forages are positively correlated to efficient ruminant digestion and are important for breeding high-quality forage. Few selection studies have been conducted to develop Italian ryegrass cultivars high in TNC content and to confirm their beneficial effects on animal performance. Meissner (1996) compared two *L. multiflorum* cultivars (Midmar and Exalta) in terms of their nutritive value for sheep. Compositional differences between the two cultivars were small and not significant. The DM content exceeded 180 g kg<sup>-1</sup> DM and was therefore unlikely to affect the forage intake. Exalta had a higher TNC content than that of Midmar (156, compared with 130 g kg<sup>-1</sup> DM) and appeared to have a more efficient protein metabolism in the rumen than Midmar.

Humphreys (1989a) successfully selected perennial ryegrass cultivars high in water-soluble carbohydrates (WSC). Miller *et al.* (1999) showed that forage bred for increased WSC stimulates higher DM intakes and increased milk production. An Italian ryegrass cultivar, Tribune, with high DMD and WSC content was found to increase milk production significantly (Miller *et al.*, 1999). Increased levels of WSC in a *L. perenne* cultivar were found to improve animal performance. Liveweight gain and animal production were greater on AberDove (high WSC cultivar) than on AberElan (control cultivar) by approximately 12 and 23%, respectively (Lee *et al.* 1999). Dairy cows fed AberDove and AberElan towards the end of their lactation ate more AberDove than the control cultivar and produced

more milk. Animals that were fed AberDove excreted less nitrogen in their urine and faeces and produced more milk protein, which emphasises the importance of high sugar to the ruminant.

### 2.3.2.2 Structural carbohydrates

The cell walls provide mechanical and structural support to plant organs (Varner and Lin, 1989). Plant structural components are comprised mostly of polysaccharides, with lesser amounts of lignin and protein. These structural carbohydrates differ from reserve carbohydrates in that they are not normally remobilised once they are formed. The structural polysaccharides can be divided into two main classes: the matrix and the fibre polysaccharides. The latter compounds, mostly cellulose, are largely crystalline and exist as micro-fibrils held together by various bonds in a cement of amorphous matrix polysaccharides, lignin and protein, to form large fibrils and cell walls (Bailey, 1973b). The matrix polysaccharides are usually separated into two groups, the pectic substances and hemicellulose. In forage, hemicellulose, xylose and arabinose account for most of the neutral sugars.

There is considerable variation in the concentration and composition of structural carbohydrates among plant species. The concentrations of cellulose and hemicellulose in temperate (cool-season) grasses range from 150 - 450 and 120 - 270 g kg<sup>-1</sup> DM, respectively, while the pectin concentration ranges from 10 - 20 g kg<sup>-1</sup> DM. In mature temperate grasses the ratio of hemicellulose:cellulose ranges from 0.57 to 0.70 (Buxton *et al.*, 1987). In warm-season grasses, the cellulose and hemicellulose concentrations are higher than in temperate grasses and range from 200 - 400 and 250 - 400 g kg<sup>-1</sup> DM respectively, while pectin concentrations are similar to those in temperate grasses. Results from different grass species, including ryegrass, cocksfoot and tall fescue, showed that stem tissue has generally higher concentrations of cellulose and hemicellulose than leaf tissue, accounting for the stem's more rigid structure. The stems also have a larger proportion of cellulose relative to hemicellulose (Minson, 1990a).

The concentration of structural carbohydrates in forage is influenced by a number of factors. Seasonal or stage of growth changes can affect the concentration of structural carbohydrates, since the proportion of stem tissue, relative to leaf tissue, would increase upon maturity. According to Jarrige and Minson (1964), hemicellulose and cellulose increased over the growing season from 120 to 200 g kg<sup>-1</sup> DM and from 140 to 240-280 g kg<sup>-1</sup> DM, respectively. Structural carbohydrates are also influenced by fertilizer applications. In ryegrass, the hemicellulose content was lowered more (4%) than the cellulose content (2%) after nitrogen fertilizer was applied (Waite, 1970). In a study of the protein degradability and chemical composition of *L. multiflorum*, Babnik (1995) showed that ageing and an increase in N fertilization resulted in an increased content of cellulose, ADF, NDF and crude fibre. Climatic factors can also affect structural polysaccharides. In temperate climates where growth continues during mild winters, herbage appears to contain lower levels of cellulose, possibly because the maturation processes are delayed. According to Bailey (1964), ryegrass pastures contained significantly lower levels of cellulose in winter (111 - 112 g kg<sup>-1</sup> DM) than in early summer (140 - 150 g kg<sup>-1</sup> DM).

In the ruminant, structural carbohydrates (components of dietary fibre) are important for normal rumen function, and serve as a source of energy. Ruminants are able to digest a large proportion of the plant structural polysaccharides by means of their rumen microflora (Bailey, 1973b). Cellulose and hemicellulose polysaccharides are more slowly and less completely degraded, than pectins (Hatfield, 1989). Fibre stimulates rumination and ensalivation, and its cation exchange properties are essential for ruminal buffering (Van Soest *et al.*, 1991). Forages with a limited amount of fibre tend to decrease the animal's chewing time, saliva secretion, and pH and acetate:propionate ratio of rumen fluid. These factors can have an adverse effect on rumen fermentation, fibre degradation and milk fat percentage (NRC, 1989). A certain quantity of fibre is required by the animal to obtain maximum dry matter and energy intakes (NRC, 1989). However, fibre is also involved in regulating the voluntary intake of forages (Mertens, 1987). Voluntary intake is related to the concentration of neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin in the forage. Fibre decreases voluntary intake due to its effect on the resistance of the forage to chewing during eating and ruminating (Minson, 1990a) and its effect on the rate of passage through the digestive tract.

Two *L. multiflorum* cultivars, Exalta and Midmar, were studied in terms of their nutritive value for sheep (Meissner, 1996). Values for NDF, ADF and cellulose were significantly lower in Exalta than in Midmar. Exalta had NDF, ADF and cellulose values of 414, 210 and 169 g kg<sup>-1</sup> DM, respectively, while Midmar had values of 431, 233 and 198 g kg<sup>-1</sup> DM, respectively (Table 2.1). The NDF and ADF contents of Italian ryegrass ranged from 171 - 570 and 204 - 350 g kg<sup>-1</sup> DM, respectively (Berardo *et al.*, 1989; De Villiers *et al.*, 1993; Du Preez and Meissner, 1992; Meissner *et al.*, 1992; Thompson *et al.*, 1992; Snyman and Joubert, 1993) (Table 2.1).

Having selected Italian ryegrass cultivars for higher DM and TNC contents, Marais *et al.* (1997) investigated whether the selection parameters (DM and TNC) were associated with other traits affecting the nutritive quality of the forage. It was found that the selected F3 plants contained less ADF and ADL (factors which could reduce the nutritive value of Italian ryegrass) than the control varieties and that the DM and TNC contents were not positively related to the main anti-quality factors (fibre and lignin) associated with forage species. Further investigations into the selected lines established in the sward situation will provide more insight into the content of fibre and its effect on the high DM and TNC Italian ryegrass selection.

### 2.3.3 Lignin

Plant cell walls have been characterised as cellulose micro-fibrils embedded in a ligno-hemicellulosic macromolecule to which acetyl and phenolic acid groups are bound (Morrison, 1979). As plant cell development occurs, the primary cell wall is deposited initially and contains cellulose, hemicellulose and pectins. Lignin becomes part of the cell wall during formation and thickening of the secondary cell wall. According to Hartley (1972), lignin can be divided into core and noncore components. Core lignin is described as a highly condensed, high molecular weight polymer with two or more covalent bonds between monomers. It consists mainly of three closely related phenylpropanoid monomers: *p*-

coumaryl, coniferyl and sinapyl alcohols. The noncore lignin is composed mostly of *p*-coumaric and ferulic acids esterified to either core lignin or to hemicellulose.

All forages contain core lignin, but concentrations are higher in legumes than in grasses (Jung, 1989), while the concentration of core lignin is greater in stem than in leaf tissue (Morrison, 1980). The noncore lignins have been found routinely in grasses, but are either not found in legumes or are present in much lower quantities than in grasses (Hartley and Jones, 1977; Jung *et al.*, 1983). The important temperate, festucoid, forage species contain 3 - 5% of lignin in their leaves and 6 - 7% in their stems (Harkin, 1973).

The concentration of core lignin in forages is affected by a number of factors (Jung, 1989). The concentration of total core lignin increased with physiological maturity in both leaf and stem tissue (Morrison, 1980). Most of this increase is the result of the increased proportion of stem tissue, which contains more lignin than the leaves and also undergoes greater lignification during maturation (Harkin, 1973). However, when forage is maintained in a leafy state by grazing and cutting, there is a very marginal change in lignin levels. During maturation the levels of lignin in leaves and stems increase to 5 - 6% and 11 - 14%, respectively. Differences in lignin content between species is not very marked, but ryegrass tends to contain lower levels of lignin than cocksfoot (Harkin, 1973). Environmental effects on core lignin content are variable. Bowman and Law (1964) showed that changes in temperature and day length had no effect on the lignin level of temperate grasses. Physiological maturity also caused an increase in noncore lignin components where the ratio of *p*-coumaric to ferulic acid in noncore lignin of grasses increased with maturity (Jung, 1989; Burritt *et al.*, 1984).

Lignin plays a central role in determining the quality of herbage for animal nutrition (Harkin, 1973). It is the chemical component in forage cell walls which is most commonly associated with reduced digestibility of fibre (Jung, 1989). Both core and noncore lignin concentration of forage cell walls has been found to limit forage digestibility (Jung, 1989). Experiments have shown that in young forages, with relatively low core lignin contents, a small increase in lignification has a large negative effect on digestibility. In contrast, mature forages having large amounts of lignin appear to have small decreases in digestibility with further increases in lignin content (Jung and Vogel, 1986). In noncore lignin, Akin (1986) concluded that *p*-coumaric acid is more toxic to ruminal microbes than other phenolic acids and that the presence of this acid may reduce digestibility. Strong chemical bonds occur between lignin and other plant polysaccharides; this cross-linking constitutes a highly efficient metabolic block to enzymatic hydrolysis of the plant's sugar polymers and consequently renders them inaccessible as energy sources for ruminants (Harkin, 1973). Lignin is therefore deleterious in high-energy feeds, where maximum utilisation of protein, fat and carbohydrate is required (Harkin, 1973). Voluntary intake in ruminants can also be affected by high lignin concentration, since undigested hemicellulose remains in the rumen (Van Soest, 1965). However, lignin may be beneficial in some

forms of roughage, which enhance rumen digestion in a properly balanced ration (Maynard and Loosli, 1969) and is therefore to some extent useful in concentrates for ruminants.

In *L. multiflorum*, the concentration of lignin ranges from 32 - 53 g kg<sup>-1</sup> DM (Table 2.1). Although the negative correlation between forage fibre digestibility and lignin content has been known for many years, knowledge about lignin structure is limited. As a result, the mechanism by which lignins reduce carbohydrate fermentation in the rumen has not been established (Jung, 1989). Lower proportions of syringyl units in core lignin and reduced levels of *p*-coumaric acid in noncore lignin should enhance forage fermentation in ruminants.

Sosulski *et al.* (1960) showed varietal differences in the degree of lignification in some forage species such as cocksfoot. In a *L. multiflorum* selection programme, the ADF and ADL fractions were significantly lower in a high DM and TNC cultivar than in commercial cultivars (Marais *et al.*, 1997), suggesting the need for investigation of the impact of these selection criteria on the voluntary intake and digestibility of grazing animals.

### 2.3.4 Nitrogenous compounds

The major nitrogenous compounds absorbed from the gastrointestinal tract consist of ammonia, amino acids, purines and pyrimidines. Each of these compounds has specific functions in the nutrition of animals. Other nitrogenous compounds include nitrogen, inorganic nitrogen compounds, low molecular weight peptides and other less common nitrogenous constituents such as alkaloids (Lyttleton, 1973).

#### 2.3.4.1 Proteins

Most plant proteins are divided into two major categories, namely seed and leaf proteins. Seed proteins are part of the reserve material and act as a nutrient supply for the developing embryo. Such proteins are typical in legumes, e.g. peas, beans and groundnuts. The seeds of herbage grasses are much smaller and contain insignificant amounts of protein compared to that in leaves. Leaf proteins are almost entirely metabolic and are involved in the growth and biochemical function of the leaf cells (Lyttleton, 1973).

The crude protein (CP) content in temperate grasses is usually higher than in tropical species and varies between 150 and 200 g kg<sup>-1</sup> DM in favourable areas, and 70 and 150 g kg<sup>-1</sup> DM in situations where drought stress or low temperatures prevail. Ryegrass requires temperate, moist conditions and has high CP yields, whereas grasses adapted to cold (e.g. Timothy) or drought conditions (e.g. cocksfoot) may have CP yields substantially lower (Lyttleton, 1973). In all forages, the leaf blades have approximately double the CP concentration of the leaf sheath and stem fractions (Minson, 1990b).

The CP content is affected by a number of factors. As the plant matures the CP content decreases. This may be due to the reduction in leaf proportion and protein content of the leaf itself (Lyttleton, 1973). By increasing the period of regrowth of *L. multiflorum* from 2 to 10 weeks, the concentration of CP decreased from 188 to 69 g kg<sup>-1</sup> DM (Minson, 1990b). Nitrogenous fertilizer increases the CP content in grasses and results in a greater increase in CP yield due to the simultaneous stimulation of dry matter production. In *L. multiflorum* the CP concentration increased by application of N fertilizer, with the largest response occurring in the leaf (Minson, 1990b). In regularly cut and fertilized temperate pastures, the CP concentration is lowest in midsummer (170 g kg<sup>-1</sup> DM) and highest in autumn (230 g kg<sup>-1</sup> DM), due to the increased proportion of leaf in the forage (Minson, 1990b). Seasonal variation in CP concentration may be caused by differences in light intensity. Studies have shown that the CP concentration in forage is decreased by high light intensity (Bathurst and Mitchell, 1958; Burton *et al.*, 1959; Alberda, 1965).

One of the essential functions of forage protein in the diet of ruminants is to supply the animal with  $\alpha$ -amino nitrogen (Broderick, 1994). The protein requirements of ruminants are obtained from two sources: the rumen undegraded or bypass protein (UDP) and the rumen degradable protein (RDP). The UDP escapes rumen fermentation, allowing the amino acids after protein digestion in the small intestine to be absorbed directly from the small intestine into the bloodstream. The RDP is largely deaminated by rumen microbes, which incorporate the liberated ammonia into microbial protein. On reaching the small intestine, the microbial protein is digested and becomes available to the ruminant. Any excess ammonia is absorbed from the rumen and intestine and converted to urea in the liver (Minson, 1990b).

Microbial protein has a different amino acid profile from that of the rumen undegradable protein (Weston and Hogan, 1971; Beever *et al.*, 1981). The quantity of microbial protein produced varies with the amount of N released and the energy available for microbial protein synthesis (ARC, 1984). The average production of microbial protein is 81 g kg<sup>-1</sup> forage DM eaten, with values ranging between 34 and 162 g kg<sup>-1</sup> DM. Microbial protein production is highest in immature, fresh, highly digestible forage, whereas production of microbial protein is low with dried, mature forages.

The concentration of CP in Italian ryegrass cultivars has been found to range between 84.4 and 462 g kg<sup>-1</sup> DM (Table 2.1). Previously, protein content was considered an index of quality because it is often highly correlated with digestibility, but in most intensive grassland systems in temperate environments, the protein level rarely limits animal production (Raymond, 1969). High protein contents are often associated with lower animal production (Asay *et al.*, 1968; Rogers, 1970) and there is an inverse correlation between CP content and soluble carbohydrates (Cooper, 1961; Rogers, 1970). High CP content is also associated with the accumulation of nitrate and other harmful nonprotein nitrogenous compounds (Wright and Davison, 1964). Excessive protein levels should therefore be avoided, particularly since protein levels can be increased by nitrogen fertilization. There

may be a need to select cultivars with lower protein content which will respond to nitrogen by increased dry matter and energy production, rather than by increased protein content (Cooper, 1973).

Proteins play an important role in many tissue processes, including the maintenance and growth of muscle tissues, hair and wool. For a 70 kg lactating ewe suckling a single lamb, the CP requirement is 134 g kg<sup>-1</sup> DM, while for a 30 and 40 kg lamb, CP requirements are 147 and 116 g kg<sup>-1</sup> DM, respectively (NRC, 1985).

#### 2.3.4.2 Nitrate-N

The nitrate-N content of the plant tissue serves as an indication of an adequate supply of N when plants are fertilized with nitrogen (Whitehead, 1966). According to de Wit *et al.* (1963), the critical level (in terms of optimal requirements for growth) of nitrate in plants is 1.4 g kg<sup>-1</sup> nitrate-N. Very high levels of nitrate usually only occur in herbage that has been heavily fertilized with N. Other factors such as rate of growth can influence its accumulation, however.

Species differences in accumulation of nitrate have been noted, with highest quantities generally occurring in the pre-flowering stage (Whitehead, 1966). Nitrate absorption is stimulated in very acidic soil solutions and when P is relatively deficient. Sulfur is involved in the utilisation of N, and a deficiency will result in an increase in nitrate accumulation (Whitehead, 1966). MacLeod (1965) reported that application of potassium reduced herbage nitrate content. By increasing the successive increments of N fertilizer, the nitrate-N content of herbage increased (Griffith, 1960). Goswami and Willcox (1969) used varying doses of nitrogen to determine the variation in composition of the different nitrogenous fractions and free amino acid composition of ryegrass. It was found that an increase in fertilizer N application caused an increase in true-protein, which was accompanied by a sharp rise in the free amino acid N, other organic nitrogenous constituents and nitrate-N content. Relatively low temperatures and low light intensities tend to promote nitrate accumulation by reducing the utilization rather than the uptake of nitrate (Whitehead, 1966).

Wright and Davison (1964) pointed out that the nitrate ion itself is relatively non-toxic to animals, and that toxic effects in animals are produced by nitrite, which is formed by reduction by rumen micro-organisms. Nitrite absorbed into the bloodstream will convert haemoglobin into methaemoglobin due to the oxidation of Fe<sup>2+</sup> to Fe<sup>3+</sup>. The methaemoglobin will prevent the transport and release of oxygen by the blood and the conversion of a substantial proportion of haemoglobin will therefore result in internal asphyxiation (Butler and Jones, 1973). Research has shown that animals can ingest relatively high levels of nitrate without any ill effects (Butler and Jones, 1973), but forages that contain more than 3.4 - 4.5 g nitrate-N kg<sup>-1</sup> DM should be regarded as potentially toxic (Wright and Davison, 1964). Pasture species differ in their ability to accumulate nitrates and varietal differences in nitrate content have also been reported (Murphy and Smith, 1967; Dotzenko and Henderson, 1964). The possibility of selecting cultivars for lower nitrate content is, however, complicated by the fact that nitrate accumulation is strongly influenced by the rate and level of N fertilizer application (Cooper, 1973).

### 2.3.5 Minerals

'Mineral' elements constitute approximately 10% of herbage dry matter. A wide range of elements are detected in herbage and are essential for the growth of higher plants. These include C, H, O, N, P, S, K, Na, Ca, Mg, Fe, Mn, Zn, Cu, Mo, Cl and B (Fleming, 1973). Certain elements are essential for the nutrition of grazing animals, e.g. iodine, cobalt, selenium and sodium (Butler and Jones, 1973), but such elements differ significantly in their essentiality for plants. The levels and availability of elements in forage are clearly of importance. Knowledge of the modes of action and the various states of chemical combination of functional elements in herbage is essential to understanding the metabolic processes in herbage and animal nutrition, particularly since their chemical status influence the availability of elements to the animal (Butler and Jones, 1973). The mineral composition of *L. multiflorum* is reported in Table 2.2.

Plants obtain their minerals from soils by absorption from solutions. These minerals enter the xylem sap of the root and move into the stems and leaves via the transpiration stream (Loneragan, 1973). There are many environmental and metabolic factors which influence the mineral composition of herbage. The supply of a particular nutrient is clearly important, but its availability can be influenced considerably by soil type and weather conditions. The uptake of nutrients by plants is also influenced by stage of maturity, and by seasonal and climatic factors.

#### 2.3.5.1 Calcium (Ca)

According to Nason and McElroy (1963), calcium is a constituent of cell wall middle lamella (calcium pectate) and complements potassium in maintaining cell organisation, hydration and permeability, thereby indirectly affecting many enzyme systems. The Ca level in forage is usually in the range 4 - 10 g kg<sup>-1</sup> DM (Whitehead, 1966), with the critical level for grass about 1 g kg<sup>-1</sup> DM (de Wit *et al.*, 1963).

With frequent defoliation, Walker *et al.* (1953) and Hemingway (1961) found that Ca contents in grasses tend to increase during the growing season to peak in late summer. This increase may be related to soil temperature, since Nielsen and Cunningham (1964) found that higher soil temperatures in the range 11 - 28°C significantly increased the Ca content in Italian ryegrass. In contrast, Fulkerson *et al.* (1998) reported seasonal changes in Ca in *L. multiflorum*, with a significant decrease from October. This change was possibly associated with the reproductive development of the plant. A Ca to P ratio of 1.9:1 was reported for *L. multiflorum*, with even higher ratios for perennial ryegrass/white clover (Grace and Wilson, 1972). According to NRC (1978) a Ca to P ratio below 1:1 can reduce performance. Nitrogen fertilizer applications can influence the Ca content of forage. Nielsen and Cunningham (1964) showed that the Ca content of Italian ryegrass was increased substantially by nitrate-N, and slightly decreased by ammonium-N.

Ca is particularly important in the development and maintenance of the skeletal system of animals. The bones and teeth contain almost 99% of the calcium in the body, the remaining 1% being widely distributed in body fluids and soft tissues (NRC, 1985). The major site of Ca absorption is the small

intestine. The nutritional requirement of the animal influences the absorption of dietary calcium, and on a low-calcium diet the efficiency of absorption is increased. The Ca requirements of a 55 kg lactating ewe are approximately 6 g Ca day<sup>-1</sup>, while for growing lambs at 10 and 20 kg the requirements are 1.9 and 2.9 g Ca day<sup>-1</sup> respectively. For a 40 kg lamb gaining 300 g mass day<sup>-1</sup>, the Ca requirements increased to 5.8 g day<sup>-1</sup> (Grace, 1983). Therefore, provided that the DM intakes are adequate, the Ca requirements for sheep are met by pastures containing 2.9 to 4.4 g Ca kg<sup>-1</sup> DM (Grace, 1983).

Table 2.2 Mineral composition of *L. multiflorum*.

References	P	K	Ca	Mg	Na	S	Cl
Thomas <i>et al.</i> (1977)	2.4-3.2	-	3.4-4.5	1.1-1.3	3.4-5.3	-	-
NRC (1985)	4.1	20	6.5	3.5	0.1	1	-
Bredon <i>et al.</i> (1987)	5	54	7	-	-	-	-
Moseley & Baker (1991)	2.6	21.4	3.85	0.93	1.88	-	-
De Villiers <i>et al.</i> (1993)	2.7-3.2	24.4	29.4	3.8-6.8	2.9-4.4	-	-
Hillard <i>et al.</i> (1992)	1.8	26.3	1.38	0.58	-	-	-
Pires <i>et al.</i> (1992)	-	29.4	1.1	0.9	-	-	-
Flachowsky <i>et al.</i> (1994)	3.4	-	10.9	1.99	-	-	-
Warman & Sampson (1994)	1.4	16.4	15.5	5.8	-	2	-
Thom & Prestidge (1996)	3	35	4.1	1.9	2.7	2.8	-
Fulkerson <i>et al.</i> (1998)	3.1	34	5.9	2.7	3.7	1	14.3
Lippke & Ellis (1997)	-	29	4.9	2.5	1.2	-	-
Miles (1997)	2.7	37	5	2.3	-	-	-

All values are expressed as g kg<sup>-1</sup> DM

### 2.3.5.2 Magnesium (Mg)

Approximately 10% of total leaf magnesium is present in chlorophyll. Magnesium is relatively mobile in herbage, and diurnal magnesium fluctuations in chloroplasts may represent photosynthetic control mechanisms (Nicholas, 1961; Nason and McElroy, 1963). Herbage Mg levels are usually within the range of 0.8 - 3 g kg<sup>-1</sup> DM, with the critical level for grass in the vegetative stage being 0.6 g kg<sup>-1</sup> DM. Mg contents in Italian ryegrass are significantly increased by an increase in soil temperature (Nielsen and Cunningham, 1964). As the growing season advances, Mg levels in forage rise considerably (Hemingway, 1961; Reith, 1963a). Nitrogen fertilization can increase herbage Mg levels. Nielsen and Cunningham (1964) showed that Mg levels in Italian ryegrass increased with nitrate-N but not with ammonium-N applications. The application of K generally results in a decrease in herbage Mg, while P often shows no significant effect on Mg content (Gardner *et al.*, 1960; Reith *et al.*, 1964; Hunt *et al.*, 1964). The application of Mg also results in an increase in Mg content (Wolton, 1960; Reith, 1963).

Magnesium is an important mineral in the nutrition of ruminants because of a disorder called hypomagnesaemic grass tetany (Moseley and Baker, 1991), which usually occurs in recently calved dairy and beef cows and less frequently in ewes before and after lambing. Magnesium is a constituent of bone and is required for skeletal developments in ruminants. It is also involved in many important biochemical and physiological functions, as it is a cofactor for many cellular enzymes which are involved in oxidative phosphorylation and the metabolism of carbohydrates, lipids, proteins and nucleic acids (Grace, 1983; NRC, 1985). The dietary requirement of a 55 kg lactating ewe is about 2.5 g Mg day<sup>-1</sup>. For a 10 and 20 kg growing lamb, the Mg requirements are 0.3 and 0.7 g Mg day<sup>-1</sup>, respectively, while for a 40 kg lamb gaining 300 g day<sup>-1</sup>, the requirements are 1.4 g Mg day<sup>-1</sup>. Therefore, assuming that there is a sufficient DM intake to meet the ruminant's energy requirements, pastures with Mg contents of 1.2 and 1.9 g Mg kg<sup>-1</sup> DM should supply adequate Mg. It has been suggested that a value of 2 g kg<sup>-1</sup> Mg in herbage is critical to avoid the possibility of hypomagnesaemia. The cation ratio K:Ca + Mg should be in the order of 2.2, since higher ratios are associated with grass tetany (Butler, 1963). Essentially it is the total amount of Mg that is ingested and absorbed by the ruminant that is more important than the concentration of Mg in the pasture (Grace, 1983).

### 2.3.5.3 Sodium (Na)

The essentiality of Na in herbage has not been well demonstrated. However, some crops do respond to Na in conditions of slight to moderate K deficiency. Plants show marked genetic variability in the amount of Na absorbed (Whitehead, 1966; Butler and Jones, 1973) and the amount of Na in herbage varies considerably. Values range from 0.02 to 21.2 g kg<sup>-1</sup> DM and values between 0.5 and 10 g kg<sup>-1</sup> DM would not be unusual (Whitehead, 1966). The Na content in Italian ryegrass ranged from trace to 21.2 g kg<sup>-1</sup> DM when sampled at various times and in several seasons (Cunningham, 1964).

The factors influencing the Na content in herbage have been reviewed by Henkens (1965). Na contents in herbage are largely determined by the Na content and K status in the soil, with the

influence of soil Na content being greater when the K status in the soil is low. According to Flowers and Läuchli (1983), sodium is capable of partially substituting potassium in certain plants. This is particularly important in terms of the fertilization of pastures, since a high uptake of K can negatively affect pasture quality (Miles *et al.*, 1986). In Italian and perennial ryegrasses, sodium uptake can result in a decline in potassium requirements without affecting DM yields (Nowakowski *et al.*, 1974; Mundy, 1983; Smith *et al.*, 1980). The Na content of regularly grazed or cut herbage does not appear to be influenced by the season. No consistent seasonal changes were found by Stewart and Holmes (1953), but Reith *et al.* (1964) showed that Na contents increased throughout the season. No consistent trends in Na content in grasses were found with increasing maturity (Whitehead, 1966). The application of N in the absence of K generally increases the Na contents of many species (Stewart and Holmes, 1953; Reith *et al.*, 1964), while K often causes large reductions in the Na content of grasses (McNaught and Karlovsky, 1964). In a study of the response of *L. multiflorum* to sodium, lime and potassium on an acidic Natal soil, Manson (1995) showed that sodium application decreased herbage Ca and Mg where herbage K was low, but had no effect on herbage K. In autumn and winter, *L. multiflorum* may respond positively to sodium fertilizer when soil P and K reserves are marginal.

In ruminants Na is important in the body, in that it maintains osmotic pressure, controls water metabolism and regulates acid-base balance. Sodium is found mostly in the extracellular fluids and bones. A lack of salt in the diet of sheep can result in growth retardation, inefficiency of feed use and increased water consumption (Hagsten *et al.*, 1975; Underwood, 1981). There is a rapid turnover of Na within the body of ruminants, with Na actively absorbed from the gut contents against a concentration gradient (Towers and Smith, 1983). Assuming a Na availability of 91%, a 55 kg lactating ewe requires approximately 1.08 g Na day<sup>-1</sup>. For 20 and 30 kg growing lambs the requirements of Na are 0.34 and 0.40 g day<sup>-1</sup>, respectively, while for a 40 kg lamb gaining 300 g day<sup>-1</sup>, the requirements are 0.67 g Na day<sup>-1</sup>. In a study of lactating ewes with lambs, it was shown that a diet containing 0.87g Na kg<sup>-1</sup> DM giving intakes of 1.9 g Na day<sup>-1</sup> was adequate to maintain full Na status (Morris and Peterson, 1975).

#### 2.3.5.4 Potassium (K)

Potassium is present in plant tissues as a free ion or in readily exchangeable combination and is the most mobile of the elements. In herbage, potassium functions as a univalent cation activator for a wide variety of important enzymes (Evans and Sorger, 1966). The K content of herbage is usually in the range of 10 - 40 g kg<sup>-1</sup> DM, with the dominant factor being the supply of available K in the soil. A deficiency of K often restricts crop yields, but herbage seldom contains insufficient K for animal requirements (Whitehead, 1966).

Under frequent defoliation and an adequate supply of nutrients, seasonal differences in K content are generally small. However, if herbage is allowed to mature, its K content often declines. Fertilizer applications have been found to have large effects on seasonal changes in K content. Wolton (1960) showed that N applications increase herbage K content when there is an adequate supply of K in the

soil and decrease herbage K when soil is K deficient. In pot experiments conducted by Nielsen and Cunningham (1964) the form and level of N had a slight effect on the K content of Italian ryegrass, but all the samples contained high levels ranging from 58 to 68 g kg<sup>-1</sup> DM. Application of P showed little effect on herbage K content (Reith *et al.*, 1964; Saunders *et al.*, 1963; Hemingway, 1961), while K applications caused a significant increase in herbage K content (McNaught and Karlovsky, 1964). Nowakowski *et al.* (1974) studied the interactions of potassium and sodium on the growth of *L. multiflorum* in two pot experiments. It was found that the yields of *L. multiflorum* were increased by both potassium and sodium, when sufficient nitrogen was applied. However, responses to potassium were larger than to sodium. With more nitrogen, the potassium and sodium increased the production of soluble carbohydrates, particularly the fructans. In a study of the response of *L. multiflorum* to sodium, lime and potassium on an acidic Natal soil, Manson (1995), showed that potassium decreased the Ca, Mg and Na concentrations in the herbage. Sodium application also decreased herbage Ca and Mg where herbage K was low, but had no effect on herbage K. In autumn and winter, *L. multiflorum* may respond positively to sodium fertilizer when soil P and K reserves are marginal.

Potassium is one of the most abundant minerals in plants and animals, and with herbage levels always high, the intake of K by grazing animals often greatly exceeds their requirements. This excessive intake is of some importance because it could depress Mg absorption, contributing to the problems of hypomagnesaemia (McNaught *et al.* 1973). Potassium is found mostly in the intracellular fluids (skin and muscle) and is the third most abundant mineral in the body, being roughly 0.3% of the body's dry matter. Potassium affects osmotic pressure, maintains the acid-base balance within the cell and also aids in activating several enzyme systems involved in energy transfer and utilization, protein synthesis and carbohydrate metabolism (Underwood, 1981). Ingested K is readily absorbed, mostly from the small intestine, but with small amounts from the stomach region and hind gut (Grace *et al.* 1974). The livestock requirements for potassium varies with the amounts of protein, phosphorus, calcium and sodium consumed (NRC, 1985). For animal requirements, K concentrations of 2 to 6 g K kg<sup>-1</sup> DM are sufficient. From this data, it is clear that, under normal management systems, a potassium deficiency is not common (Towers, 1983).

### 2.3.5.5 Phosphorus (P)

Plant tissues maintain high inorganic phosphate in their tissues. Phosphorus plays an essential role in energy transfer in both respiration and photosynthesis and is a mobile nutrient (Nason and McElroy, 1963). The contents of P are normally in the range 2 - 5 g kg<sup>-1</sup> DM.

Seasonal variation in P content is not marked in grasses (Reith *et al.*, 1964). Saunders *et al.* (1963), however, reported higher P levels in winter and spring than in summer and autumn. The content of P in herbage decreases with maturity (Fleming and Coulter, 1963). Fulkerson *et al.* (1998) investigated the seasonal variation on the mineral content of pastures. It was found that the P levels in *L. multiflorum* remained high until September and then decreased significantly during the reproductive development of the plant. Fertilizer effects are relatively slight and variable. Stewart and Holmes

(1953) found that N, P and K treatments had little effect on herbage P contents, while other studies revealed that N applications caused definite depressions of herbage P contents (Mortensen *et al.*, 1964). Miles and Eckard (1991) investigated the response of *L. multiflorum* to P on highly-weathered soils. It was found that the DM yield responses to P increased in both soils, with the P effects being greater in the growth phase (autumn). Furthermore, the herbage concentration of P was considerably lower in winter than in autumn or summer. This reduction in yield was attributed to the restricted P uptake. The results from their investigation indicated that the concentration of P in *L. multiflorum* during winter and early spring were well below the requirements of the ruminant animal and consequently established the need for supplemental feeding of P.

In ruminants, phosphorus, like calcium, is involved in the development and maintenance of the skeletal system. Approximately 80% of the body's phosphorus is found in bones and teeth. The 20% of phosphorus not present in the skeletal system is widely distributed in body fluids and soft tissues. It is involved in the blood buffer systems and also forms part of the genetic materials DNA and RNA (NRC, 1985). The small intestine is the major site of P absorption and vitamin D stimulates the absorption of P from the intestine (Grace, 1983). The P requirements of a 55 kg lactating ewe are about 3.5 g P day<sup>-1</sup>. For 10 and 20 kg growing lambs, requirements of P are 1.4 and 1.6 g P day<sup>-1</sup> respectively, while for a 40 kg lamb gaining 300 g day<sup>-1</sup> the requirements are 3.9 g P day<sup>-1</sup>. Thus pastures containing at least 2.5 g P kg<sup>-1</sup> DM will ensure that the requirements of P for sheep will be met, provided that their DM intakes are adequate (Grace, 1983).

Due to the importance of herbage mineral content in animal nutrition, comprehensive studies of most temperate forage species have been made (Whitehead, 1966). The mineral content of herbage is often a reflection of the mineral status in the soil, with the minerals being influenced by environmental and genotypic differences. As a result of genetic variation in mineral content in forage, it should be possible to select varieties that have high or low levels of certain minerals. In *L. multiflorum*, selections for high and low magnesium content have been studied. Chemical analysis revealed a 44% higher Mg, 22% higher Ca and 25% higher DM in the high-Mg selection (Moseley and Griffiths, 1984). Furthermore, the high-Mg selection showed significantly greater Mg intake, apparent availability and retention. In both selections there was no significant difference in the Na and K contents. Moseley and Baker (1991) studied the effect of a high magnesium cultivar of *L. multiflorum* in controlling hypomagnesaemia in grazing animals. The magnesium, calcium and phosphorus contents were significantly higher in the high magnesium cultivar compared to the other variety tested. Significantly higher potassium and nitrogen contents and lower sodium content were found in the high magnesium cultivar.

#### **2.3.5.6 Zinc (Zn), manganese (Mn) and copper (Cu)**

In herbage zinc is an essential constituent of many enzymes, including glutamate dehydrogenase and carbonic anhydrase (Nicholas, 1961). The content of Zn in herbage varies from 15 to 60 ppm. Manganese serves as a metal activator for many enzyme systems and is required in the

photoproduction of oxygen in chloroplasts (Nason and McElroy, 1963). The Mn content in herbage is usually within the range of 25 to 200 ppm (Whitehead, 1966). In herbage, copper serves as a component of plastocyanin, an electron carrier protein in the photochemical system of photosynthesis and a component of enzymes that mediate substrate oxidation by atmospheric oxygen (Mason, 1965; Bishop, 1966). The content of Cu in herbage species usually varies from 2 to 15 ppm (Whitehead, 1966).

Fertilizer applications can influence the content of Zn in herbage. Phosphate applications were found to decrease Zn availability to various crops, while heavy applications of P and K and Mn, Cu, Co and Mo had no effect on the Zn content of herbage (Whitehead, 1966). The uptake of Mn by the plant is influenced by soil factors, especially pH and drainage status. Seasonal changes appear to increase the Mn content in grasses (Hemingway, 1962). Applications of N, P and K had no significant effect on the content of Zn in herbage. Plant species have been found to differ in their Cu content, but these differences are influenced by the available Cu supply. Grasses showed a decrease in Cu content upon increasing maturity of herbage (Fleming, 1973).

Zinc is an essential element in animals. It is involved in nucleic acid metabolism, protein synthesis and carbohydrate metabolism. The Zn requirements of a 55 kg lactating ewe are about 45 mg Zn day<sup>-1</sup> while in 20 and 30 kg growing lambs, 20 and 23 mg Zn day<sup>-1</sup>, respectively, are required for maintenance. Manganese is nutritionally essential for the animal and plays a role in many enzyme systems. It is absorbed from the small and large intestine. Mn requirements for growth are met by a diet containing 10 mg Mn kg<sup>-1</sup> DM. Copper is necessary in connective tissue metabolism and haemoglobin formation (NRC, 1985). In a 55 kg lactating ewe, a Cu content of 10.0 mg Cu day<sup>-1</sup> is required, while for 20 and 30 kg growing lambs, a Cu requirement of 1.2 and 3.7 mg Cu day<sup>-1</sup>, respectively, would be sufficient. Provided that DM intakes are adequate, pastures containing 5 to 6 mg Cu kg<sup>-1</sup> DM should meet the requirements of sheep.

### 2.3.6 Intake

Dry matter intake is one of the most important factors affecting nutritive value and animal performance (Raymond, 1969; Mertens, 1994). Voluntary intake (VI) of forages may be defined as the amount of dry matter eaten each day when animals are offered excess feed (Minson, 1990a). If the voluntary intake is low, then it is likely that the rate of production will be low, making the requirements for maintenance a large proportion of the metabolisable energy in the food and therefore resulting in a poor efficiency of food conversion. If the intake is too high, then excess fat deposition occurs (Forbes, 1995). It is therefore necessary to balance the food consumption with the required level of production. For a measurement of voluntary intake, forage must be available at all times and more forage must be offered than can be eaten. Voluntary intake of forage is affected by the amount of excess feed offered. This variation in intake is caused by two factors. Animals have an increased opportunity to select the more desirable and usually less fibrous parts of forage when excess material is available. The second reason is that the animal's appetite varies from day to day, and sufficient forage must be

available on days when appetite is high (Minson, 1990a). Voluntary intake of forage also varies between animal species, this difference being mainly due to body size (Anderson *et al.*, 1977). Differences in VI between animals are related to metabolic size, which is calculated as body mass (kg) raised to the power of 0.75 (Crampton *et al.*, 1960).

The VI of forage is affected by forage species, cultivar, stage of growth, soil fertility, climatic conditions and various other physical constraints (Minson, 1990a). The amount of forage eaten each day is dependent on the time spent grazing, the rate of biting and the size of each bite. As a sward is grazed down, large changes in bite size occur, but differences in grazing time and biting rate are small. The yield of forage influences the VI by the animal. When the yield of young forage is above 2 000 kg DM ha<sup>-1</sup> and grazing is not restricted, ruminants are not limited to satisfying their appetite and taking in large quantities of forage with each bite (Aldden, 1962; Aldden and Whittaker, 1970). When forage yield falls below 2 000 kg DM ha<sup>-1</sup>, there is a reduction in bite size. The fall in intake is possibly due to a reduction in length of tillers because the bite size of sheep is directly proportional to tiller length over a range of 4 - 37 cm (Aldden and Whittaker, 1970). Differences in intake were found between grass species and within different forage cultivars (Minson, 1990a). Voluntary intake of forage depends on its resistance to breakdown (Balch and Campling, 1962). Before particles can flow from the rumen, digesta particles must be reduced to a size that will pass through a 1-mm screen (Poppi *et al.*, 1980). Chewing during eating and ruminating is the most important way of reducing forage particle size (Balch and Campling, 1962). Temperate forages generally have a higher VI than tropical forages, due to a lower level of fibre and a higher digestibility of dry matter (Minson, 1990a).

In temperate and tropical forages, the leaf fraction appears to be eaten in greater quantity than the stems. This difference in VI is due to the lower resistance of the leaves to chewing and a decrease in energy required to reduce leaf particles to a sufficiently small size. The VI of forage decreases with maturity. This decrease is associated with higher lignin content, grinding energy and the time that the forage is retained in the rumen (Minson, 1990a). The low VI of mature forage could also be due to a protein deficiency. Milford and Minson (1965) reported a rapid decrease in VI when the CP level fell below about 70 g kg<sup>-1</sup> DM. In controlled experiments, application of N fertilizer increased the yield of the forage but had no consistent effect on VI. Seasonal changes in intake have been found even when there are no apparent changes in forage maturity. The lower intake of autumn forage is possibly due to the presence of excreta voided during grazing periods earlier in the season, excess moisture in the forage, fungal infections such as rusts, and soil contamination (Minson, 1990a).

Studies have shown that low dry matter content in young actively-growing pastures may limit intake (Burtis and Phillips, 1987; Osoro and Cebrian, 1989). It is believed that bulkiness of wet feed limits intake. However, John and Ulyatt (1987) showed that any limitation due to bulkiness did not act through negative feedback from distension of the rumen. During chewing of plant material intracellular solutes are released and can be readily absorbed. Placement of a balloon containing water equivalent to one-third of digesta volume in the rumen, to simulate a bulk effect, did not reduce feed intake with

perennial ryegrass feed. In contrast, Lloyd Davies (1962) found that suspending two litres of water within a balloon in the rumen reduced intake by 27%, while introducing larger quantities of water into the rumen via a fistula had no effect on VI. Burtis and Phillips (1987) found that spraying the forage with water, to increase the moisture content from 779 to 854 g kg<sup>-1</sup>, actually reduced the VI by cattle by as much as 22%, and increased the time spent ruminating. Furthermore, distention of the rumen due to the larger forage particles would inhibit the animals' intake and the rumen microbes would need to breakdown the forage particles to a 1-mm size to pass from the rumen. Conversely, Bailey (1973a) suggested that herbage with high water content tended to be softer and was broken down quicker by chewing to give rumen digesta of low dry matter, than herbage of low water content.

Meissner *et al.* (1992) reported low intakes on Midmar ryegrass (*Lolium multiflorum* cv Midmar) and concluded that DM contents should be in the range of 180 - 200 g kg<sup>-1</sup> to prevent a decrease in intake. In ruminants, intake depends on the capacity of the rumen and the rate of flow of digesta through the digestive tract. Forage that is less digestible occupies more volume in the rumen for a longer period, resulting in lower intakes, while more digestible forage moves rapidly through the rumen, resulting in a higher intake (Balch and Campling, 1962). According to Thornton and Minson (1972), DMI is inversely related to the retention time in the rumen, with the fibre component being the principal factor affecting DMI. In other studies, John and Ulyatt (1987) observed that intake of feed DM was positively correlated with forage DM content at all stages of plant maturity. However, Poppi *et al.* (1987) found this to be a difficult area to examine and the influence of pasture water content on intake was therefore unclear. Furthermore, the NRC (2001) found that the relationship between dietary DM content and DMI in published reports were inconsistent and 'no optimum DM content of the diet for maximum DMI is apparent'.

In a study of several temperate forage species, Ingalls *et al.* (1965) showed that 70% of the variation in animal production potential of forages was determined by differences in intake, and only 30% by digestibility. Raymond (1969) postulated that differences in intake were related to the relative proportion of water-soluble or pepsin-soluble material and digestible fibre. Bailey (1964) showed that the greater voluntary intake of Italian compared to perennial ryegrass was due to the higher content of water-soluble carbohydrates and lower cellulose content in the Italian type. Evidence suggests that the VI and palatability of Italian ryegrass can be improved by increasing the nonstructural carbohydrate content of the grass (Bailey, 1964, 1965; Cooper, 1973).

### 2.3.7 Digestibility

The process of digestion involves the degradation of macromolecules in food to simple compounds that can be absorbed from the gastrointestinal tract (Merchen and Bourquin, 1994). Wilkins (1969) defined the potential digestibility of forage "as the maximum digestibility attainable when the conditions and duration of fermentation are not limiting factors." In forage the organic acids and soluble carbohydrates are virtually absent in faeces and therefore have a potential digestibility of 1.00. In contrast, almost all the lignin, silica, and cutin are excreted in the faeces and can be regarded as largely indigestible (Minson, 1990c).

Ruminants have achieved maximum capability for forestomach fermentation and are characterised by the role of fermentation in the reticulo-rumen, although digestion in the abomasum and small intestine are also important processes. For adequate microbial digestion and synthesis of microbial components to occur in the rumen, certain conditions must be provided by the host. These include the retention of digesta and ruminal microbes for extended periods of time, anaerobic conditions, constant temperature of 39°C, a neutral to slightly acidic pH of 5.5 to 7.0 and the removal of end-products (Merchen and Bourquin, 1994). The cell contents of forages are rapidly fermented by the rumen microbes, while the digestion of cell walls is a relatively slow process. The maximum digestion of the fibrous fraction will be achieved only once the forage has been exposed to the action of the microbes for many days or weeks (Wilkins, 1969). Rumination increases the surface area available for microbial action and is thought to increase digestibility. However, it actually reduces the time that feed is retained in the rumen and decreases digestibility (Minson, 1990c). The effect of grinding and pelleting on efficiency of digestion is similar to that of rumination. An increase in the quantity of forage eaten will reduce the digestive efficiency of ruminants for energy (Blaxter and Wainman, 1964) and organic matter (Raymond *et al.*, 1959; Alwash and Thomas, 1971). This decrease is a result of a reduction in the extent of fibre digestion and is associated with a decrease in the mean time that feed is retained in the digestive tract, a decrease in pH, and a lower rate of digestion.

The dry matter digestibility (DMD) of forage is very variable and is affected by a number of factors. Minson and McLeod (1970) found that temperate grasses had a higher mean DMD coefficient (0.13) than tropical grasses. Tropical grasses have a different anatomical structure associated with the different photosynthetic pathways and are normally grown under higher temperatures than the temperate species, which will result in lower digestibilities. The leaf blades of tropical species have more vascular bundles per unit cross-sectional area, and therefore have more sites for lignification. The thick walled bundle sheaths of tropical species have a high resistance to penetration by rumen microbes. The stems of tropical grasses have a higher proportion of vascular bundles than temperate grasses. These anatomical factors lead to the low potential digestibility of leaves and stems of tropical forages (Minson, 1990c). Wilson *et al.* (1983) showed that when tropical and temperate forages were grown under the same conditions, the anatomical differences caused a 0.07 difference in DMD.

At an immature stage of growth the DMD of all plant parts were similarly high, but at maturity large differences in DMD between the plant parts were found (Minson, 1990c). Wilman *et al.* (1976) found similar differences in DMD between plant parts of *L. multiflorum*. In many temperate grasses, including ryegrass, digestibility remains high (> 65-70%) during spring and early summer, but falls rapidly and regularly to (< 50%) following ear emergence (Cooper, 1973). As grasses grow, the proportion of leaf lamina decreases and the proportion of leaf sheath, stem and inflorescence increases. There is a reduction in the proportion of CP and an increase in cellulose, hemicellulose and lignin (Jarrige and Minson, 1964). Although these factors are not necessarily causative, they may result in a rapid fall in DMD.

The application of fertilizer N will increase the DM yield, protein and water content of forage and reduce the proportion of leaf. No consistent findings in the response to fertilizer N were found, and increases and decreases in DMD occurred with both young and mature forage (Minson, 1990c). Seasonal variations in DMD were found, with forages having a low DMD in the middle of summer. These differences were caused by changes in temperature, water and light intensity. Deinum (1966) found that high temperatures increased the concentration of fibre in forage and reduced the DMD. It was found that the lower DMD of forages grown at high temperatures was due to a higher rate of transpiration. This could have been caused by the larger vascular systems developed to transport larger quantities of water passing through the plant (Minson and McLeod, 1970). In studies with both temperate and tropical grasses, high radiation increases the DMD (Garza *et al.*, 1965).

Forage digestibility can be predicted from many different chemical fractions (Minson, 1990c). The neutral detergent fibre (NDF) fraction contains all the hemicellulose, cellulose, lignin and some ash and is negatively correlated with DMD (Van Soest, 1965). The acid detergent fibre (ADF) fraction contains cellulose, lignin and some ash in forages and for a range of grasses and legumes; the ADF and DMD are related (Van Soest, 1963). Lignin content of forages is negatively correlated with DMD (Richards *et al.*, 1958; Sullivan, 1962). As a plant grows, its lignin content increases and this is correlated with decreasing digestibility of the structural carbohydrates encrusted by the lignin (Hartley, 1972). Evidence suggests that the chemical composition of lignin is more important than its quantity in inhibiting digestibility (Gordon, 1975; Reeves, 1985 a, b).

In order to increase the digestibility of forage, temperate species should be sown in preference to tropical species, wherever possible. Since digestibility decreases as forage matures, it is necessary to maintain forage in a young vegetative stage of growth by grazing or regular cutting. According to Hutton (1970) an important breeding aim is to develop varieties that do not decline so rapidly in digestibility. It has been suggested that the digestibility of forages can be increased through breeding and selection, provided that there is a range of DMD within the species and that some variation is of genetic origin (Hacker, 1982). The range in DMD in many species exceeds 0.10 and approximately half of this variation is of genetic origin (Minson, 1990c).

In a study of *Lolium* genotypes, Wilson (1965) examined the variation in leaf tensile strength and cellulose content. High heritabilities (about 0.8) were found for both characteristics with a genetic correlation of 0.93 between them. These characteristics can serve as useful screening parameters for nutritive value in ryegrass and can be related to DMD. In selecting for a low moisture content and high total nonstructural carbohydrate content in *L. multiflorum*, Marais *et al.* (1997) showed that the selected cultivar had lower ADF and ADL contents than the commercial lines of Italian ryegrass, and hence had higher *in vitro* digestibility values.

## Chapter 3

### Analytical procedures

#### 3.1 Introduction

The weaned lamb experiments were conducted at the Cedara Agricultural Research Station (29°32'S 30°16'E, altitude 1067 m), which is approximately 15 km north-west of Pietermaritzburg. The pot experiments were performed in a controlled microclimate growth chamber at the University of Natal, Pietermaritzburg. The anatomical and morphological features of the grass were studied at the Department of Range and Forage Resources, University of Natal, Pietermaritzburg. The Holstein dairy cow experiments were conducted at Hopewell, Nottingham Road (29°25'S 30°01'E, altitude 1410 m), which is approximately 63 km north-west of Pietermaritzburg.

#### 3.1.1 Experimental sites

##### 3.1.1.1 Cedara Agricultural Research Station

The weaned lamb experiments were conducted at Cedara during the winter of 1998. The area receives a long-term (83-year) average annual rainfall of  $875.9 \pm 142$  mm (mean  $\pm$  s.e.). Frost occurs frequently during the winters. The monthly long-term maximum and minimum temperatures and rainfall, and the same data for the period of the study are shown in Figures 3.1 and 3.2. From Figure 3.2 it can be seen that less rainfall was received from March to September of the period of study compared with the long-term data during the same months as illustrated in Figure 3.1. The maximum and minimum temperatures obtained for the period of study were similar to those of the long-term data.

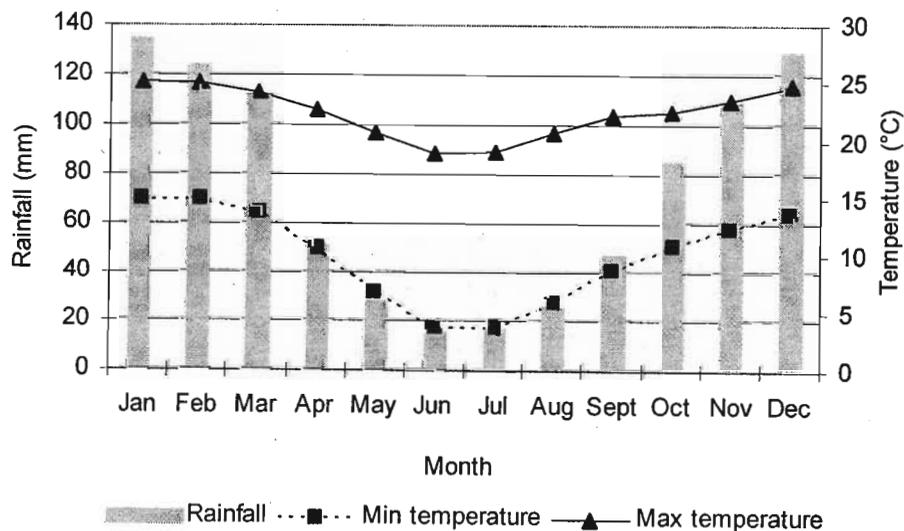


Figure 3.1 Monthly long-term (83 year) climatic data at the Cedara Agricultural Research Station.

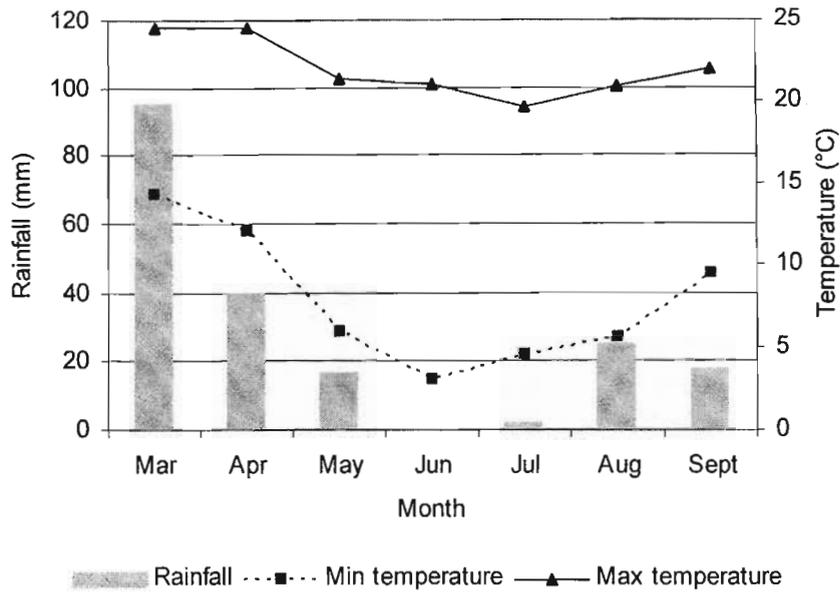


Figure 3.2 Mean monthly rainfall and maximum and minimum temperatures at the Cedara Agricultural Research Station for the period of study (1998).

### 3.1.1.2 Hopewell, Nottingham Road

The Holstein dairy cow experiments were conducted at Hopewell during the summer of 2001. The area receives an approximate average annual rainfall of 1200 mm. Frost occurs frequently during the winters. The mean monthly maximum and minimum temperatures and rainfall for the period of study are shown in Figure 3.3.

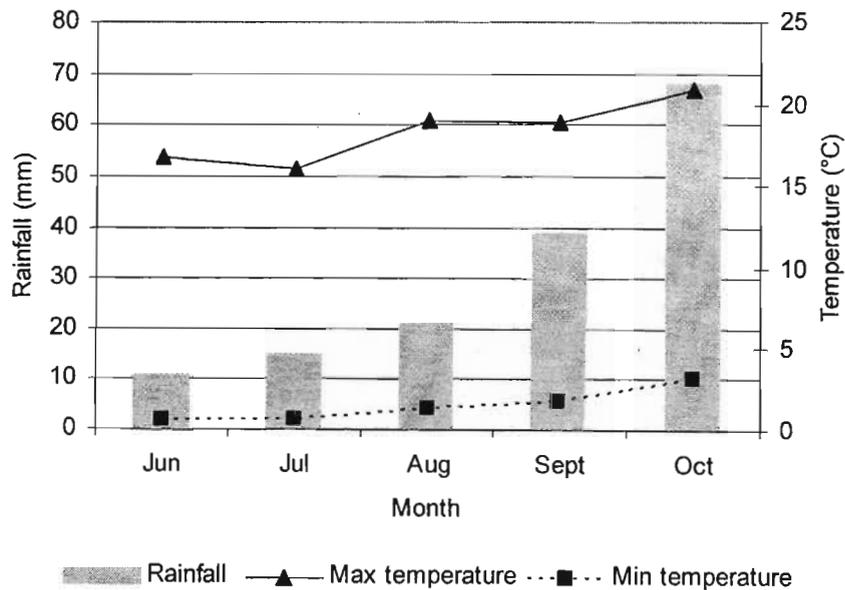


Figure 3.3 Mean monthly rainfall and maximum and minimum temperatures at Hopewell, Nottingham Road for the period of study (2001).

### 3.1.2 Experimental procedures

#### 3.1.2.1 Cedara Agricultural Research Station

The *Lolium multiflorum* cultivars, Midmar (Westerwolds type) and Enhancer (Italian type) were established on two adjacent 0.5 hectare pastures, using a Connor-Shea seed-drill, in March 1998. The two pastures were each divided into eight paddocks of 0.0625 hectares and permanently enclosed with 'Bonnox' fencing. Results of soil analyses on the pastures presented in Table 3.1 were used to determine the fertilizer requirements. The acid saturation for Enhancer and Midmar pasture soils was 14 and 19%, respectively, and pH (KCl) was measured as 4.42 and 4.36, respectively. The permissible acid saturation (PAS) for Italian ryegrass is 20% as determined by the Soil Fertility Unit at Cedara. In this experiment no lime was required because the acid saturation in both pastures was below the PAS. Prior to planting, the site was fertilized with di-ammonium phosphate (DAP) at a rate of 20 kg P ha<sup>-1</sup>. The pastures were fertilized with 250 kg N ha<sup>-1</sup> over the growing season. The applications were split among the 16 paddocks, each receiving 16 kg limestone ammonium nitrate (LAN 28%) after each grazing. Approximately 20-25 mm of irrigation water was applied once a week with an overhead sprinkler system.

Table 3.1 Results of soil analyses performed on Enhancer and Midmar pastures prior to planting for the weaned lamb experiments.

Sample	Minerals (mg L <sup>-1</sup> )						Exch. <sup>1</sup> acidity (cmol L <sup>-1</sup> )	Total cations (cmol L <sup>-1</sup> )	Clay (%)	Organic carbon (%)
	P	K	Ca	Mg	Zn	Mn				
Enhancer	14	187	502	126	2.5	4	0.64	4.66	38	2.2
Midmar	12	160	512	119	2.3	3	0.9	4.84	35	2.1

Note: Clay % and organic carbon % were measured by near infra-red reflectance spectrophotometry

<sup>1</sup> Exchangeable

#### 3.1.2.2 Hopewell, Nottingham Road

The *Lolium multiflorum* cultivars, Dargle and Enhancer (both Italian types) were established on 19 and 16 hectare pastures, respectively, using a Connor-Shea seed-drill, in March 2001. Results of soil analyses on the pastures presented in Table 3.2 were used to determine the fertilizer requirements. The acid saturation for Enhancer and Dargle pasture soils were 16.63 and 10%, respectively, and pH (KCl) was measured as 4.21 and 4.51, respectively. No lime was required because the acid saturation in both pastures was below the PAS. Prior to planting, the site was fertilized with 2:3:4 (30) at a rate of 300 kg ha<sup>-1</sup>. The pastures were fertilized with 250 kg N ha<sup>-1</sup> over the growing season. Fertilizer was applied at 60 kg N as urea after each grazing. Approximately 20-25 mm of irrigation water was applied once a week with an overhead sprinkler system.

Table 3.2 Results of soil analyses performed on Enhancer and Dargle pastures prior to planting for the Holstein dairy cow experiments.

Sample	Minerals (mg L <sup>-1</sup> )						Exch. <sup>1</sup> acidity (cmol L <sup>-1</sup> )	Total cations (cmol L <sup>-1</sup> )	Clay (%)	Organic carbon (%)
	P	K	Ca	Mg	Zn	Mn				
Enhancer	18.9	184.6	796	208.6	3.8	7.3	1.26	7.42	54.5	4.55
Dargle	22.2	133.7	841.8	179.7	4.6	5.4	0.57	6.59	49.37	4.08

Note: Clay % and organic carbon % were measured by near infra-red reflectance spectrophotometry

<sup>1</sup> Exchangeable

### 3.2 Morphology

Morphological studies of ryegrass cultivars were performed on the ryegrass cultivars using a modification of the technique described by Johansen (1940).

#### 3.2.1 Reagents

Fixative solution (FAA). Formalin-acetic acid-alcohol (FAA) was prepared by adding acetic acid (5 ml) and 37% formaldehyde (10 ml) to 96% ethanol (50 ml).

Dehydration series. The graded tertiary-butanol series used to dehydrate the samples is outlined in Table 3.3

Table 3.3 The tertiary-butanol series.

Series	Ratio of water:ethanol:tertiary-butanol	Minimum time (h)	Temperature (°C)
1	45:45:10	1	20
2	30:50:20	12	20
3	15:50:35	1	20
4	0:45:55	1	20
5	0:25:75	1	20
6	0:0:100	2	40
7	0:0:100	18	40

Wax infiltration solutions. The dehydrated tissue was infiltrated with wax using the reagents outlined in Table 3.4.

Table 3.4 Solutions used for infiltrating the dehydrated tissue with wax.

Solution	Minimum time (h)	Temperature (°C)
Tertiary-butanol: liquid paraffin (50:50)	24	40
Liquid paraffin	12	40
Liquid paraffin and a few wax pellets	12	40
Liquid paraffin and wax pellets in an open vial	24	60
Pure molten wax	48	60

Staining solutions. Prior to staining, wax sections were treated with various solutions, as outlined in Table 3.5.

Table 3.5 Solutions required in the staining procedure of wax samples.

Solution	Minimum time (min)
Xylene	10
Xylene	10
Xylene/alcohol	5
Absolute alcohol	5
80% alcohol	5
70% alcohol	5
Safrinin	2-24 h
Wash with water	until clear
Alcohol/picric acid	2-5 s
Ammonia/alcohol	2
Absolute alcohol	10
Fast Green in clove oil	30 s
Clear clove oil	2-5 s
Clove oil/xylene	2-5 s
Xylene	2-5 s
Xylene	leave in xylene until coverslip is applied

### 3.2.2 Procedure

Sections (5 mm) of the fully expanded leaf blades of both ryegrass cultivars were obtained and fixed in formalin-acetic acid-alcohol (FAA) for a minimum of 24 h in order to preserve the cellular and structural elements in as nearly the natural living condition as possible. Following fixation in FAA, samples were thoroughly washed in distilled water (35 ml) and dehydrated in tertiary-butanol, as outlined in Table 3.3. Samples were infiltrated with paraffin and completely impregnated with wax, as described in Table 3.4. Each wax-impregnated specimen was embedded in a block of pure wax and refrigerated for approximately 2 h. The blocks were trimmed to the required thickness and orientation. Sections (15  $\mu$ ) were cut, using a rotary microtome. Sections were mounted on glass microscope slides and coated with Haupt's adhesive (chrome-gelatin-alum). A drop of 3% FAA was placed on each slide. The ribbons of sections were floated onto the FAA, after which they were stretched and dried at 40°C. The sections were dewaxed in two 3 min rinses of xylene and stained with safranin and Fast Green, as described in Table 3.5.

## 3.3 Chemical analyses of cultivars

### 3.3.1 Dry Matter

Dry matter was determined by recording the weight of an empty container, adding fresh material and recording the combined weight. The dish containing the sample was dried in a forced draught oven at 80°C overnight, removed and allowed to cool in a desiccator before recording the combined weight. The dry matter ( $\text{g kg}^{-1}$ ) was calculated as follows:

$$\text{Dry Matter (g kg}^{-1}\text{)} = \frac{\text{Combined Dry Wt} - \text{Container Wt}}{\text{Combined Wet Wt} - \text{Container Wt}} \times 1000$$

### 3.3.2 Total nonstructural carbohydrates (TNC)

Nonstructural carbohydrates were analysed as reducing sugars following quantitative hydrolysis to monosaccharides through a carefully controlled acid hydrolysis procedure (Marais, 1979). The reducing sugars released by hydrolysis were determined quantitatively by a modified Nelson-Somogyi method (Marais *et al.*, 1966).

#### 3.3.2.1 Reagents

0.05 M Sulfuric acid. Concentrated  $\text{H}_2\text{SO}_4$  (2.8 ml) was slowly added to dist.  $\text{H}_2\text{O}$  (500 ml) and further diluted to 1 L.

0.1 M Sulfuric acid. Concentrated  $\text{H}_2\text{SO}_4$  (0.56 ml) was slowly added to dist.  $\text{H}_2\text{O}$  (50 ml) and further diluted to 100 ml.

Copper reagent. Solution I.  $\text{Na}_2\text{CO}_3$  (30 g),  $\text{NaHCO}_3$  (20 g),  $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$  (15 g) and  $\text{Na}_2\text{SO}_4$  (180 g) were dissolved in dist.  $\text{H}_2\text{O}$  (1 L).

Copper reagent Solution II.  $\text{Na}_2\text{SO}_4$  (45 g) and  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (5 g) were dissolved in dist.  $\text{H}_2\text{O}$  (250 ml). Immediately before use, 4 volumes of Solution I were mixed with 1 volume of Solution II.

Arsenomolybdate reagent.  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$  (25 g) was dissolved in dist. $\text{H}_2\text{O}$  (400 ml) and concentrated  $\text{H}_2\text{SO}_4$  (21 ml) was carefully added.  $\text{AsHNa}_2\text{O}_4\cdot 7\text{H}_2\text{O}$  (3 g) was dissolved in dist. $\text{H}_2\text{O}$  (25 ml) and added to the acidic ammonium molybdate solution and made up to 500 ml. The solution was incubated at 39°C for 48 h and stored in a glass-stoppered brown bottle.

0.02 M sugar standard. Glucose (0.7208 g) was dissolved in dist. $\text{H}_2\text{O}$  (200 ml).

### 3.3.2.2 Procedure

Samples of plant material (0.3 g) were weighed into test-tubes and 0.05 M  $\text{H}_2\text{SO}_4$  (10 ml) was added to each test-tube and mixed well. A blank and standard were prepared. The blank contained 0.05 M  $\text{H}_2\text{SO}_4$  (10 ml) and no plant material, while the standard contained 0.1 M  $\text{H}_2\text{SO}_4$  (5 ml) and sugar standard (5 ml). Solutions were heated in a boiling water bath for 30 min, immediately cooled to room temperature, transferred quantitatively to a 250 ml volumetric flask and made up to volume. All solutions were filtered before analysis for reducing sugars. Sample filtrate (1 ml) was diluted to 3 ml with dist. $\text{H}_2\text{O}$ , while the standard and blank test-tubes contained 3 ml aliquots of standard sugar solution and 3 ml of dist. $\text{H}_2\text{O}$ , respectively. Mixed copper reagent (3 ml) was added, mixed in well and the solutions were heated in a boiling water bath for exactly 20 min and cooled to room temperature. Arsenomolybdate reagent (3 ml) was added and the solutions were shaken until bubble formation ceased. The colour was allowed to develop for 1.5 h before the solution was transferred to a 200 ml volumetric flask and made up to volume. Absorbance was read at 750 nm against the blank. TNC were calculated as follows:

$$TNC (g\ kg^{-1}\ DM) = \frac{\text{Absorbance sample}}{\text{Absorbance std}} \times \frac{5555.55}{DM\% \text{ sample}}$$

### 3.3.3 Neutral detergent fibre (NDF)

Neutral detergent solution was used to remove the cell contents from the grass tissue. Cell wall material is recovered in the neutral detergent fibre residue (Van Soest and Robertson, 1979). The insoluble residue consists of cellulose, hemicellulose, lignin, cell wall protein and biogenic silica, while soluble substances such as carbohydrates, pectins and tannins are removed.

#### 3.3.3.1 Reagents

Neutral detergent solution. EDTA (124 g),  $\text{Na}_2\text{B}_4\text{O}_7\cdot 10\text{H}_2\text{O}$  (45.3 g), sodium lauryl sulfate (200 g), ethylene glycol (67 ml) and  $\text{Na}_2\text{HPO}_4$  (30.4 g) (in that order), were dissolved in dist. $\text{H}_2\text{O}$  and made up to 5 L.

#### 3.3.3.2 Procedure

Finely milled oven-dried samples (0.5 g) were weighed into sintered glass crucibles (34 × 2.8 mm) (porosity 2) of known weight. Cold (room temperature) neutral detergent solution (50 ml) was added

to Pyrex crucible holders containing a marble. These were placed into the digestion block until foam ceased to form. The crucibles were covered with rubber plugs and placed in the holders in the digestion block at 110°C for 1 h 10 min. The crucibles in the holders were removed from the block and lifted to allow the neutral detergent solution to drain. Samples were washed with boiling dist.H<sub>2</sub>O (3 times) and finally rinsed with acetone. The crucibles were dried in an oven and the NDF content calculated as follows:

$$NDF (g\ kg^{-1}\ DM) = \frac{\text{residue after drying}}{\text{original sample mass}} \times 1000$$

### 3.3.4 Acid detergent fibre (ADF)

The cell wall material was treated with an acid detergent solution to remove hemicelluloses and cell wall proteins. The acid detergent fibre residue consists mainly of cellulose and lignin (Van Soest, 1963).

#### 3.3.4.1 Reagents

Acid detergent solution. Concentrated H<sub>2</sub>SO<sub>4</sub> (140 ml) was added to dist.H<sub>2</sub>O (3 L). Cetyl trimethylammonium bromide (CTAB) (100 g) was dissolved in the acid solution by gentle heating with stirring and made up to 5 L.

#### 3.3.4.2 Procedure

As for NDF, except that 50 ml aliquots of acid detergent solution were used for the digestion. ADF content was calculated as follows:

$$ADF (g\ kg^{-1}\ DM) = \frac{\text{residue after drying}}{\text{original sample mass}} \times 1000$$

### 3.3.5 Acid detergent lignin (ADL)

In the determination of ADL, the ADF procedure was used as a preparatory step. Treatment of the ADF residue with 72% sulfuric acid removes the cellulose. Ashing of the residue removes the crude lignin fraction, including cutin (Van Soest, 1963).

#### 3.3.5.1 Reagents

72% Sulfuric acid. H<sub>2</sub>SO<sub>4</sub> (734 ml) was slowly added to cold dist.H<sub>2</sub>O (266 ml).

#### 3.3.5.2 Procedure

The crucible containing the ADF was placed in a 50 ml beaker for support. The ADF in the crucible was covered with 72% H<sub>2</sub>SO<sub>4</sub> and stirred with a glass rod to a smooth paste, breaking up all lumps. The crucible was half filled with acid and stirred. As the acid drained away, the crucible was refilled with acid and stirred at hourly intervals for a period of 3 h. After 3 h, the dissolved sample was

removed by vacuum filtration and the contents washed with hot dist.H<sub>2</sub>O until free from acid (tested with indicator paper). The crucible was dried at 100°C and weighed after cooling in a desiccator. It was heated in a muffle furnace at 500°C for 2 h, placed into a desiccator to cool and re-weighed. ADL was calculated as follows:

$$ADL (g\ kg^{-1}\ DM) = \frac{\text{weight loss upon ashing}}{\text{oven-dried sample weight}} \times 1000$$

### 3.3.6 Nitrate-N

The procedure was based on the nitration of salicylic acid under highly acidic conditions and colorimetric determination of the resulting coloured complex, which absorbs maximally at 410 nm in basic solution (Cataldo *et al.*, 1975).

#### 3.3.6.1 Reagents

Salicylic acid - sulfuric acid reagent. Salicylic acid (5 g) was dissolved in concentrated H<sub>2</sub>SO<sub>4</sub> (100 ml) and stored in a brown bottle.

2M Sodium hydroxide. NaOH (980 g) was dissolved in dist.H<sub>2</sub>O and made up to 1 L.

Nitrate standard. KNO<sub>3</sub> (0.1804 g) was dissolved in dist.H<sub>2</sub>O and made up to 1 L.

#### 3.3.6.2 Procedure

Samples of finely milled plant material (0.2 g) were weighed into test-tubes. dist.H<sub>2</sub>O (10 ml) was added, mixed well and the material washed off the sides of the tube with a further volume of dist.H<sub>2</sub>O (10 ml). Samples were incubated for 1 h at 45°C, with occasional shaking. After incubation, samples were mixed well and filtered through Whatman No. 1 filter paper. An aliquot of filtrate (0.2 ml) was transferred to a test-tube and salicylic acid reagent (0.8 ml) added and mixed in well. A blank and standard were prepared. The blank contained filtrate solution (0.2 ml) and concentrated H<sub>2</sub>SO<sub>4</sub> (0.8 ml), while the standard contained nitrate standard (0.2 ml) and salicylic acid reagent (0.8 ml). Samples were left at room temperature for 20 min and 2 M NaOH (19 ml) was added to raise the pH above 12. Samples were cooled to room temperature and the absorbance measured at 410 nm against the blank. Nitrate-N content was calculated as follows:

$$\text{Nitrate - N content (g kg}^{-1}\text{ DM)} = \frac{\text{Absorbance sample}}{\text{Absorbance std}} \times \frac{250}{\text{DM\% sample}}$$

### 3.3.7 Determination of calcium, magnesium, potassium and sodium

Mineral analyses of plant material were conducted using the 'Hunter' system, as described by Farina (1981). After dry ashing, P was determined colorimetrically and cations (Ca, Mg, Na, K, Al, Zn and Mn) by atomic absorption spectrophotometry.

### 3.3.7.1 Reagents

Strontium solution.  $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$  (76 g) was dissolved in de-ionized  $\text{H}_2\text{O}$  and made up to 10 L.

Calcium setting standards. Calcium solutions of 0, 100, 200, 350 and 500  $\text{mg L}^{-1}$  were prepared in 1 M HCl containing a few drops of nitric acid. These were diluted in the same manner as the 'unknowns'.

Potassium setting standards. Oven-dried ( $100^\circ\text{C}$ ) KCl (19.069 g) was dissolved in dist.  $\text{H}_2\text{O}$  and made up to 1 L. Aliquots (0, 20, 40, 70 and 100 ml) were made up to 1 L using 1:9 HCl (1 part HCl with 9 parts water).

Magnesium and Sodium setting standards. Setting standards were obtained as stock solutions of 1000 ppm in 1 M HCl (Merck and Co, Rahway, NJ). A 500 ml solution of 1000  $\text{mg L}^{-1}$  Mg and 200 ml of 1000  $\text{mg L}^{-1}$  Na was made up to 2 L with distilled water as a stock solution. From the stock solution, volumes of Mg (0, 50, 100, 200, 300 ml) and Na (0, 10, 20, 40, 60 ml) were made up to 1 L with 1:9 HCl

### 3.3.7.2 Procedure

Plant samples (1 g) were weighed into 100 ml beakers and ashed overnight in a furnace at  $450^\circ\text{C}$ . The samples were cooled and wet with a few drops of dist.  $\text{H}_2\text{O}$  before adding concentrated HCl (2 ml). The samples in the beakers were placed on a water bath and evaporated slowly to dryness. An aliquot (25 ml) of a 1:9 HCl solution (approximately 1 M HCl) was added to each sample, which was filtered through Whatman No. 41 filter paper. To an aliquot (1 ml) of the filtrate, strontium solution (24 ml) was added and the Ca, Mg, K and Na read on a Varian 40+ Atomic Absorption Spectrophotometer, with the following settings:

	Wavelength (nm)	Current (mA)	Slit width (nm)
Ca	422.7	4	0.5
Mg	285.2	4	0.5
K	766.5	5	1.0
Na	589.0	10	0.5

Mineral concentrations of the samples were calculated from their absorbance values relative to that of the standards.

### 3.3.8 Determination of zinc, copper and manganese

#### 3.3.8.1 Reagents

Setting standards. Setting standards of Zn, Cu and Mn were obtained as solutions of 1000 ppm in 1 M HCl. A stock solution was prepared containing 100  $\text{mg L}^{-1}$  Zn, 100  $\text{mg L}^{-1}$  Cu and 200  $\text{mg L}^{-1}$  Mn in de-ionised water. Stock solution (0, 10, 20, 30 and 40 ml) was made up in 1 M HCl (1 L).

### 3.3.8.2 Procedure

The undiluted filtrate was used for the determination of Zn, Cu and Mn by atomic absorption spectrophotometry. The concentration mode was used, using actual plant concentrations. The atomic absorption instrument settings were as follows:

	Wavelength (nm)	Current (mA)	Slit width (nm)
Zn	213.9	5	1
Cu	324.8	5	1
Mn	279.5	5	1

### 3.3.9 Determination of Phosphorus

#### 3.3.9.1 Reagents

Phosphate colour reagent Solution A - Concentrated Reagent. Distilled water (800 ml) and concentrated H<sub>2</sub>SO<sub>4</sub> (300 ml) were slowly added, while mixing, to antimony potassium tartrate (2 g) in a 2 L Pyrex volumetric flask. This was allowed to cool overnight. (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O (15 g) was dissolved in dist.H<sub>2</sub>O (600 ml). This solution was added to the acid antimony solution and made up to 2 L with dist.H<sub>2</sub>O.

Solution B - Diluted Reagent. Solution A (150 ml) was diluted to 1 L with a solution containing gelatin (1 g) in dist. H<sub>2</sub>O just before use. Ascorbic acid (1 g) was dissolved in the resulting solution.

Standards. A stock solution was prepared by dissolving KH<sub>2</sub>PO<sub>4</sub> (8.780 g) in approximately 800 ml de-ionized water, 7N H<sub>2</sub>SO<sub>4</sub> (25 ml) (i.e. 9.5 ml 98% H<sub>2</sub>SO<sub>4</sub> diluted to 50 ml) was added and the volume made up to 2 L with water. The stock solution contained 1000 mg P L<sup>-1</sup>. Further diluted stock solutions (0, 25, 50 and 100 ml) were made up in 1M HCl (500 ml).

#### 3.3.9.2. Procedure

De-ionized water (8 ml) and phosphate colour reagent B (10 ml) was added to an aliquot of strontium mixture (2 ml). This was allowed to stand for 30-40 min and the concentration of P (% in plant material) read on the colorimeter at a wavelength of 670 nm.

### 3.3.10 Dry matter digestibility *in vitro*

The rumen buffer system used was based on the procedure of Minson and McLeod (1972), while the digestion was done in F57 filter bags in a DAISY<sup>II</sup> Incubator as developed by Ankom Technology. Grass samples were initially digested under conditions simulating rumen fermentation, which was followed by an acid pepsin digestion to solubilise the protein in the sample.

#### 3.3.10.1 Reagents

Salivary buffer solution A. NaHCO<sub>3</sub> (9.8 g), Na<sub>2</sub>HPO<sub>4</sub> (3.7 g), KCl (2.11 g), NaCl (1.74 g) and MgCl<sub>2</sub>·6H<sub>2</sub>O (0.48 g) were dissolved in dist.H<sub>2</sub>O (1L).

Salivary buffer solution B.  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (5.3 g) was dissolved in dist.  $\text{H}_2\text{O}$  (100 ml).

Buffer solution A (1330 ml) was pre-warmed to 39°C and Buffer solution B (266 ml) was added to buffer A. Carbon dioxide was bubbled through the buffer for at least 15 min to obtain a final pH of 6.8 at 39°C.

Rumen fluid inoculum. Two 2 L Thermos flasks were pre-heated by filling with water at 39°C. The heated water was emptied prior to collection of rumen inoculum. At least 2 L of rumen content was removed from rumen fistulated Mutton Merino wethers on a lucerne hay diet and placed in the Thermos flasks. Approximately two 'fistfuls' of the fibrous mat from the rumen were included with the collection in one of the flasks. Rumen inoculum was emptied from the Thermos flasks into a blender, purged with  $\text{CO}_2$  gas and blended at high speed for 30 s. The blending action served to dislodge microbes attached to the fibrous particles and assured a representative microbial population for the *in vitro* fermentation. The blended digesta was filtered through four layers of cheesecloth into a 5 L flask, purged with  $\text{CO}_2$  and maintained at 39°C.

Acid pepsin solution. A 2 L graduated Erlenmeyer flask was half filled with dist.  $\text{H}_2\text{O}$  and concentrated HCl (20 ml) was added and mixed in well. Pepsin (activity, 1:10 000) (4 g) was added and made up to 2 L with dist.  $\text{H}_2\text{O}$ . The pepsin was dissolved by stirring with a magnetic stirrer.

### 3.3.10.2 Procedure

Ankom Technology F57 filter bags were pre-rinsed in acetone for 3 to 5 min, to remove a surfactant that may inhibit microbial digestion, and completely air-dried. Samples (0.25 g) were weighed directly into the filter bags. Each bag was heat sealed and placed in the Ankom Technology - DAISY<sup>II</sup> Incubator digestion jar (up to 25 samples per jar). Samples were evenly distributed on both sides of the digestion jar divider. A weighed and sealed blank bag was included. A volume of buffer (1600 ml) was added to each jar containing the sample bags. The digestion jars with samples and buffer solution were placed into the DAISY<sup>II</sup> Incubator and the heat and agitation switches activated. The temperature of the digestion jars was allowed to equilibrate for 20 to 30 min before adding rumen inoculum. Rumen inoculum (400 ml) was added to each digestion jar, purged with  $\text{CO}_2$  gas for 30 s, and the lid secured. The DAISY<sup>II</sup> Incubator maintained a temperature of  $39.5 \pm 0.5^\circ\text{C}$ . On completion of incubation (48 h), the jars were removed and the fluid drained. Bags were rinsed thoroughly with cold tap water until the effluent was clear. Acid pepsin solution (2 L) was added to each digestion jar containing the bags and incubated at 39°C for 24 h in the DAISY<sup>II</sup> Incubator. Digestibility *in vitro* was calculated as follows:

$$\text{Digestibility (g kg}^{-1} \text{ DM)} = \frac{D - (A - B) + \text{Blank}}{D} \times 1000$$

Where: D = sample weight (0.25 g)

A = weight of residue after digestion

B = weight of bag

### 3.3.11 True protein and total nitrogen

True protein determinations were performed as described by Marais and Evenwell (1983). The method was based on the precipitation of protein with trichloroacetic acid (TCA) and the separation of the insoluble protein from the soluble non-protein fraction by filtration. Nitrogen in the fractions was determined on an Auto Analyser.

#### 3.3.11.1 Reagents

10% Trichloroacetic acid. Trichloroacetic acid (TCA) (50 g) was dissolved in dist. H<sub>2</sub>O (500 ml).

2.5% Trichloroacetic. Trichloroacetic acid (TCA) (25 g) was dissolved in dist.H<sub>2</sub>O (1 L).

Kjeltabs CT catalyst tablets. Kjeltabs CT (Instrulab CC, Midrand, SA). Each tablet contains: potassium sulfate (5 g), copper (II) sulfate (0.15 g) and titanium dioxide (0.15 g).

Scrubber Solution. Sodium carbonate (600 g) was dissolved in dist.H<sub>2</sub>O (2.8 L) and bromophenol blue (0.1 g) added as an indicator.

#### 3.3.11.2 Procedure

Feed samples (1 g) were weighed into 150 mm × 24 mm test-tubes. Distilled water (15 ml) was added and the tubes heated in a boiling water bath for 10 min and allowed to cool to room temperature. Cold 10% TCA (15 ml) was added and mixed well. Samples were left to stand for 2 h, to allow complete precipitation of proteins, and filtered through Whatman No. 541 filter paper. and the precipitated sample washed on the filter paper with cold 2.5% TCA (50 ml) to remove all traces of non-protein nitrogen. Filter papers with precipitated protein were transferred to Kjeldahl flasks. Two catalyst tables and concentrated H<sub>2</sub>SO<sub>4</sub> (24 ml) were added to each flask and digestion was effected on a Büchi B-435 Digestion Unit (Büchi Labortechnik AG, Postfach, CH-9230 Flawil/Schweiz) for 1 h. Tubes were allowed to cool and distilled water (250 ml) added, and mixed in well. The solution was poured into cups for nitrogen reading on a Nitrogen Auto-Analyser (Technicon Auto-Analyser II).

### 3.4 Alkane technique for intake and digestibility estimation

A problem confronting pasture and animal scientists is the accurate estimation of forage intake in grazing animals. Forage intake under field conditions is difficult to measure, even if the sward is composed of a single species. Herbage intake is commonly estimated from faecal dry matter output, calculated from the dilution of orally-administered indigestible markers such as chromium oxide (Cr<sub>2</sub>O<sub>3</sub>), or the total collection of faeces with faecal bags, and the *in vitro* estimation of herbage digestibility (Waite *et al.*, 1964). The use of markers such as chromium oxide could result in diurnal variation in the faecal concentration of the marker. This will result in alternate samples no longer being representative of the mean faecal marker concentration which will, in turn, cause errors in the

estimation of intake (Dove and Mayes, 1991). Furthermore, estimation of digestibility *in vitro* does not account for the effect of different levels of intake on digestibility.

These limitations can be eliminated by the simultaneous use of alkanes as internal and external markers, first investigated by Mayes and Lamb (1984). Alkanes form part of the cuticular wax layer of higher plants, and make up approximately 0.2% of the dry mass in grasses (Daly, 1964). The alkane content of the epicuticular wax in members of the Gramineae family is usually 18% but is reported to vary between 2 and 50% (Tulloch, 1981). The predominant hydrocarbons are odd-chain mixtures of C<sub>25</sub> (pentacosane) to C<sub>35</sub> (pentatriacontane), usually with C<sub>29</sub> (nonacosane), C<sub>31</sub> (hentriacontane) or C<sub>33</sub> (tritriacontane) as major components. Table 3.5 represents the alkane composition of pasture grasses expressed in mg kg<sup>-1</sup> DM. Odd-chain alkanes present in sufficient quantities in the plant can be used as internal markers in forages. Since even-chain alkanes are only present in plants in trace amounts, they can be fed to animals as external markers.

It was reported that the recovery of alkanes in faeces is generally incomplete. However, because alkanes of adjacent chain length have similar faecal recoveries, the errors that arise from incomplete recoveries cancel out in the calculation of intake (Mayes *et al.*, 1986a). This method has a major advantage in that it reflects the digestibility in individual animals and is thus suited to grazing systems (Dove *et al.*, 1989). Mayes *et al.* (1986a) initially dosed alkanes as external markers in filter paper pellets impregnated with the required alkane. This procedure involved the uniform absorption of marker alkane dissolved in hot *n*-heptane by sheets of filter paper in a hot aluminium tray. The paper was shredded after drying and fixed amounts of paper compressed into a pellet, wrapped in tissue paper and sealed with starch paste. A coefficient of variation of pellet alkane content of 2-5% was obtained.

Dove *et al.* (1988) simplified the procedure by suspending the alkane onto powdered cellulose in a No.13 gelatin capsule, which could be administered easily with a dosing gun. They obtained a coefficient of variation of capsule content of 1-2%. In a further modification of the technique, Vulich *et al.* (1991) coated cellulose fibre with alkanes by means of a rotary evaporator. The required amount of alkane coated cellulose was then weighed into gelatin capsules.

An even simpler technique would be to dose alkane markers as solutions by means of a dosing gun or syringe. Marais *et al.* (1996) developed a simple, accurate procedure to dose alkanes in a suspension form. In this procedure, grass samples were dried, milled and coated with *n*-alkanes on a rotary evaporator. The coated grass was suspended in a xanthan gum solution. The coefficients of variation of the alkane content per dose delivered by the dosing gun and syringe were 2.6 and 2.3%, respectively.

Table 3.5 Alkane content in the cuticular wax layer of ryegrass species.

Pasture spp.	C25	C27	C28	C29	C30	C31	C32	C33	C35	References:
<i>L. perenne</i>		19	5	73	9	137	9	116	18	Mayes <i>et al.</i> (1986a)
		29		93		119		79	14	Dove <i>et al.</i> (1990)
		36	6	142	12	250	7	99	9	Malossini <i>et al.</i> (1990)
<i>L. multiflorum</i>		105	8	260	11	250	4	43	0	Malossini <i>et al.</i> (1990)
	11.6	33.7		248.1		350.3	11.6	100.2		Marais (pers. comm.)
	10	40		230	12	242		57	7	Dove & Mayes (1991)
<i>L. rigidum</i>	33	83		196		298		47	0	Dove (1992)
	14	38	11	187	15	263	8	122	1	Dove <i>et al.</i> (unpublished)

All values expressed as mg kg<sup>-1</sup>

There was no significant difference in the faecal alkane ratio of alkanes that were dosed in the suspension form and directly placed into the rumen, suggesting that alkane markers can be quantitatively dosed in suspension form. According to Dove and Mayes (1991), the form in which the alkane is administered has no notable effect on the resultant faecal alkane levels.

### 3.4.1 Preparation of external alkane marker

A milled grass sample which passed a 1 mm sieve but was retained by a 0.5 mm sieve was coated with the external marker alkane C<sub>32</sub>. The delivery of alkanes was based on the procedure described by Marais *et al.* (1996).

#### 3.4.1.1 Reagents

Petroleum spirit. Distillation range 60-80°C.

Xanthan gum. (Keltrol GM, Merck and Co, Rahway, NJ).

Dotriacontane (C<sub>32</sub>). (Sigma Chemical Co, St Louis, MO).

#### 3.4.1.2 Procedure

Dotriacontane (C<sub>32</sub>) (10 g) was dissolved in petroleum spirit (700 ml) in a rotary evaporator flask. Milled kikuyu grass (particle size 0.5 - 1.0 mm) (100 g) was added and the suspension evaporated to dryness on a rotary evaporator at 40°C and 700 mbar vacuum. The coated grass was dried overnight in an oven at 60°C, to remove any petroleum spirit, and sieved (1 mm) to remove any lumps. The grass (49 g) was suspended in water (2.5 kg) containing xanthan gum (0.4%) and dosed to sheep using a plastic 50 ml syringe.

### 3.4.2 Alkane extraction and analysis

This technique is based on a procedure described by Marais *et al.* (1996).

#### 3.4.2.1 Reagents

Petroleum spirit. Distillation range 80-100°C and 60-80°C.

Silica gel. Silica gel 60 for column chromatography (63 to 200 micron).

Hexane. 97 % for column chromatography.

Mini silica gel columns. Varian bond.

C<sub>36</sub> (hexatriacontane) internal standard. C<sub>36</sub> (0.2 g) (Sigma Chemical Co, St Louis, MO) was dissolved in undecane (300 g) (0.2 g of internal standard contained 0.4 mg of C<sub>36</sub>).

### 3.4.2.2 Procedure

The C<sub>36</sub> internal standard (0.6 g) was weighed into 50 ml glass-stoppered tubes. Sample (1 g faeces or 1.5 g herbage) was added. The tubes were filled with petroleum spirit (80-100°C) (30 ml), heated in a boiling water bath for 1 h, removed, shaken and incubated for a further 1 h. Supernatants were decanted into 100 ml beakers and evaporated to dryness at room temperature. The alkane extract was re-dissolved in hot petroleum spirit (60-80°C) (10 ml) and applied to silica gel columns (bed volume 5 ml). Alkanes were eluted with hot petroleum spirit (50 ml), evaporated to dryness, redissolved in hexane (0.7 ml) and transferred to 2 ml screw cap vials, in preparation for analysis by gas chromatography (GC).

A Varian 3400 gas chromatograph with a Varian 8200 autosampler was used. A bonded phase BPX 5 (non-polar) 12 m × 0.32 mm fused silica column was installed. Nitrogen was used as the carrier gas. Two column temperature programmes were used. The initial column temperature was programmed at 220°C for 3 min, followed by 30°C minute<sup>-1</sup> to 240°C in Programme 1 and 35°C minute<sup>-1</sup> to 298°C for 5 min in Programme 2. The injector and detector temperatures were 300 and 320°C, respectively. The injection volume was 1 µl and the computed end time between runs was 10.31 min. Herbage intake was estimated using the C<sub>31</sub> (hentriacontane): C<sub>32</sub> (dotriacontane) and C<sub>32</sub> (dotriacontane): C<sub>33</sub> (tritriacontane) ratios using the following equation:

$$\text{Daily herbage intake (kg DM day}^{-1}\text{)} = \frac{\frac{F_i}{F_j} * D_j}{H_i - \frac{F_i}{F_j} * H_j}$$

Where:

F<sub>i</sub> and H<sub>i</sub> = Faecal and herbage concentrations of the odd-chain alkane

F<sub>j</sub> and H<sub>j</sub> = Faecal and herbage concentrations of the even-chain alkane

D<sub>j</sub> = Daily dose of even-chain alkane

Dry matter digestibility was estimated as follows:

$$\text{Dry Matter Digestibility (g kg}^{-1}\text{)} = 1000 * (1 - (M_h / M_f) * 0.87)$$

Where:

M<sub>h</sub> = Concentration of C<sub>33</sub> in the faeces

M<sub>f</sub> = Concentration of C<sub>33</sub> in the herbage

## Chapter 4

### A comparison, under controlled environmental conditions, of *Lolium multiflorum* cultivars Enhancer and Midmar

#### 4.1 Introduction

In an attempt to improve the nutritive value of Italian ryegrass in South Africa, Enhancer ryegrass was developed from predominantly Italian types of *Lolium multiflorum*, with a minor Westerwolds component, by selecting for a higher concentration of total nonstructural carbohydrate and lower moisture content than that currently available in commercial cultivars.

In selecting for a particular trait, the plant breeder should be aware that other, less favourable characters may be linked on the same chromosomes and might result in a reduction in the nutritive value of the forage. In *L. multiflorum* such a reduction could be caused by an increase in levels of neutral-detergent fibre (NDF) and acid-detergent fibre (ADF), acid-detergent lignin (ADL) or nitrogenous compounds. In this respect differing requirements for reproductive development, and therefore stem formation, in the progenitors of Enhancer ryegrass could be important. Italian ryegrasses have a dual-induction requirement for flowering: low temperatures and/or short daylengths are required for the primary induction of floral primordia; the secondary requirement is long daylengths for inflorescence development and culm elongation. In contrast, Westerwolds ryegrasses have only a single induction requirement, i.e. long daylengths, and readily flower in the year they are sown (Aamlid *et al.*, 1997).

It is therefore essential that new selections are monitored for the presence of undesirable characters. The recognition of genotypic differences by minimising environmental effects may be achieved under controlled environment conditions, also enabling more rapid acquisition of data. Any results obtained in this way from pot experiments should be confirmed in field experiments (Graven, 1978).

The objective of this study was to compare the predominantly Italian ryegrass cultivar, Enhancer, with the commonly used Westerwolds ryegrass cultivar, Midmar, in a controlled environment, in terms of their chemical constituents that affect nutritive value. The controlled environment regimes simulated spring/autumn and winter conditions.

#### 4.2 Materials and methods

##### 4.2.1 Experimental design

Seedlings of Enhancer Italian ryegrass and the Westerwolds ryegrass cultivar Midmar were grown in seedling trays for three to four weeks (two-leaf growth stage) and thereafter transplanted into 4 kg of soil in 5 L undrained pots (19.02 cm diameter,  $\pm 0.045$ ) (mean  $\pm$  s.e.) in a growth chamber. Thirty pots each of Enhancer and Midmar, containing four seedlings per pot, were arranged in a randomized block design on benches in the environment chamber. The soil used was an Orthic topsoil, from a low-lying topography, which was initially air-dried, milled to pass a 1 mm sieve, and analysed for

mineral content (Table 4.1). This was followed by a uniform incorporation of a solution providing supplemental nutrients (Table 4.2). Fertilizer was applied to each pot as a 50 ml solution, with the exception of calcium dihydrogen phosphate which was applied in solid form and thoroughly mixed into the soil. The pots received 0.4 g of nitrogen (N) in the form of ammonium nitrate after each cut.

Table 4.1 Chemical composition of potting soil used in the growth chamber experiment.

Minerals (mg L <sup>-1</sup> )						Exch. Acidity (cmol L <sup>-1</sup> )	Total cations (cmol L <sup>-1</sup> )	Clay (%)	Organic carbon (%)
P	K	Ca	Mg	Zn	Mn				
47	230	1105	190	5.2	42	1.35	9.02	50	4.8

Note: The percentage soil contents of clay and organic carbon were measured by near infra-red reflectance spectrophotometry

Table 4.2 Fertiliser requirements for 1 kg of soil.

Minerals	Fertiliser required
300 mg P	1.22 g Ca (H <sub>2</sub> PO <sub>4</sub> ).H <sub>2</sub> O
100 mg K	0.223 g K <sub>2</sub> SO <sub>4</sub>
100 mg N	0.286 g NH <sub>4</sub> NO <sub>3</sub>
50 mg Mg	0.513 g MgSO <sub>4</sub> .7H <sub>2</sub> O
4 mg Cu	0.016 g CuSO <sub>4</sub> .5H <sub>2</sub> O
1 mg B	0.087 g Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> .10H <sub>2</sub> O
0.5 mg Mo	0.00092 g (NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> .4H <sub>2</sub> O

\*Topdress with 100 mg N kg<sup>-1</sup> after 3-5 weeks of regrowth

The field moisture capacity (FMC) of the soil was determined to ensure sufficient addition of water to each pot during the experiment. A simple procedure in estimating FMC was described by Graven (1978). A glass tube was placed down the centre of a 1000 ml measuring cylinder and soil was lightly packed around it to simulate an undisturbed column of soil. Water was applied to the column of soil and allowed to move down its length to approximately 25 mm from the bottom. The cylinder was sealed with a plastic cover to prevent evaporation and allowed to equilibrate until no further movement of the water front. Samples were taken from the middle of the column, the wet mass determined and then oven-dried at 105°C for 48 h to determine dry mass. The difference between the wet and dry masses of the samples gave the total amount of water held by the soil, that is, the FMC. Polyethylene bags were filled with 4 kg of soil and placed in individual pots to prevent any drainage of water from the soil.

#### 4.2.2 Growth chamber conditions

The air and soil temperature, light intensity, humidity and dew point temperature were monitored using a HOBO<sup>®</sup> H8 Logger (Onset Computer Corporation, Bourne, Massachusetts). The data logger is a battery-operated device equipped with a microprocessor, data storage and sensor. The logger has a capacity of 7 943 measurements, a temperature range of -20°C to 70°C, a relative humidity range of 25 to 95% and a light intensity range of 2 to 600 footcandles (lumens ft<sup>-2</sup>). The logger was placed in the centre of the growth chamber to obtain sufficient measurements. The air temperature, soil temperature, relative humidity, absolute humidity, light intensity and dew point temperature during the growth chamber experiment are illustrated in Figures 4.1 - 4.6, respectively.

In Figure 4.1 the maximum air temperature during the warm (W) regime was 30°C. From the 108th day of the year (18/4) to the 159th day of the year (28/6) the maximum air temperature fluctuated slightly. The minimum air temperature during the same period ranged between 23°C and 12°C. During the cold (C) regime from day 241 (18/9) to day 289 (5/11) the maximum temperature was more constant and ranged between 18 and 20°C, while the minimum temperature remained at about 12°C. The soil temperature (Figure 4.2) is particularly important as it drives many of the chemical reactions taking place in the plant and the soil. The maximum soil temperature during the W regime ranged between 20 and 25°C, while the minimum temperature during the same period ranged between 7 and 23°C. During the C regime, the maximum temperature dropped from 17°C to 10°C where it remained constant throughout the experiment, while the minimum temperature remained constant at 10°C (Figure 4.2).

The soil temperature in both the W and C regimes did not exceed 25°C, while the air temperature during the W regime did so. Soil temperature is buffered against sudden temperature changes because of the soil itself and the amount of water it contains. The maximum and minimum temperature range in the soil is smaller than in the air, where large changes were found. This could be attributed to air circulation, lights and humidity within the chamber.

The maximum relative humidity during the W regime ranged between 40 and 60%, while the minimum relative humidity ranged between 22 and 50% (Figure 4.3). During the C regime, however, the maximum relative humidity reached 100%. This is because of the large decrease in minimum air temperature from the W to the C regime. Minimum air temperature during the W regime was about 20°C, which dropped to 7°C during the C regime. The high humidity during the C regime could have made the plants more susceptible to disease, due to water condensation remaining on the leaves.

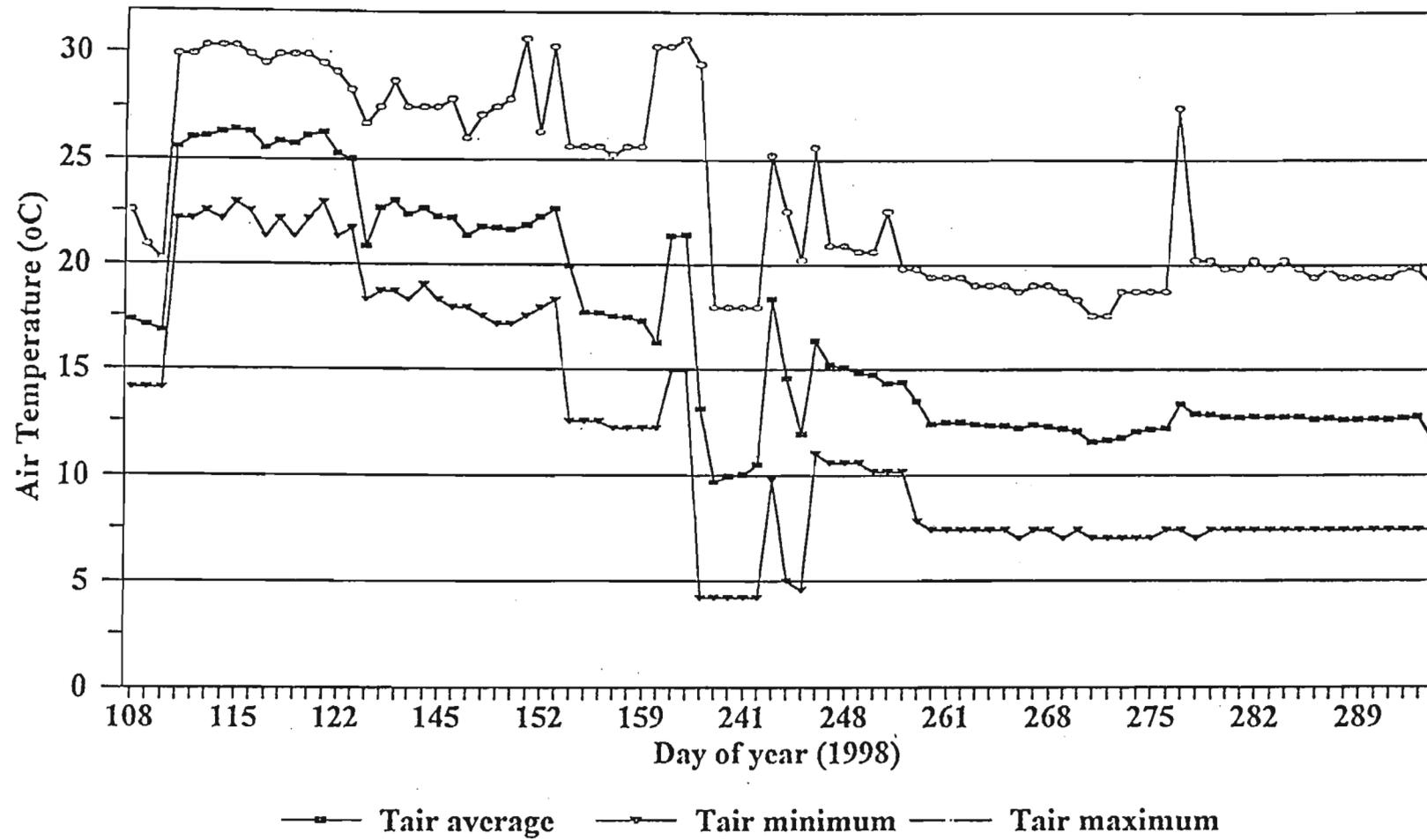


Figure 4.1 Maximum, minimum and average air temperature in the controlled growth chamber during the period of study.

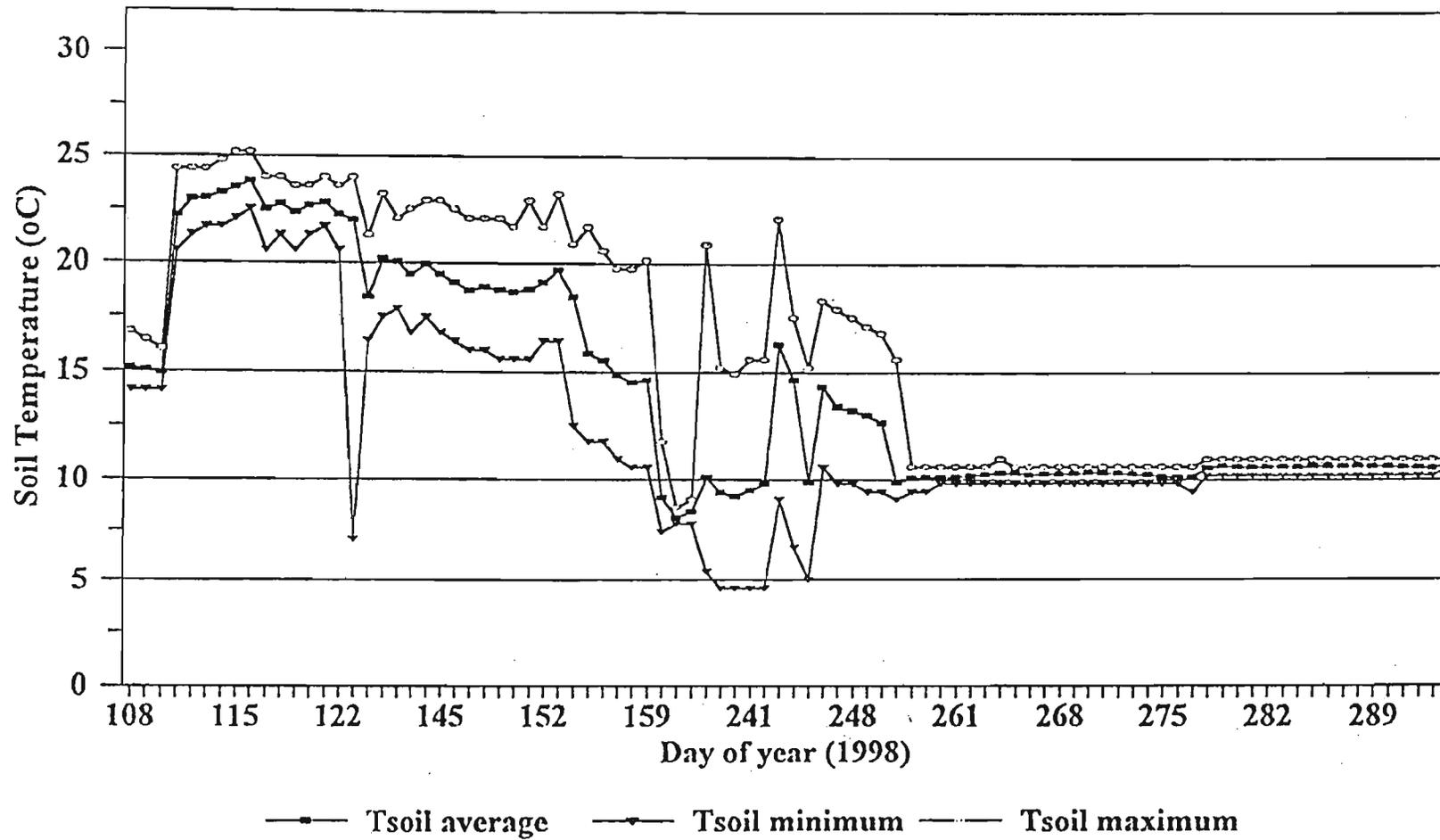


Figure 4.2 Maximum, minimum and average soil temperature in the pots during the period of study.

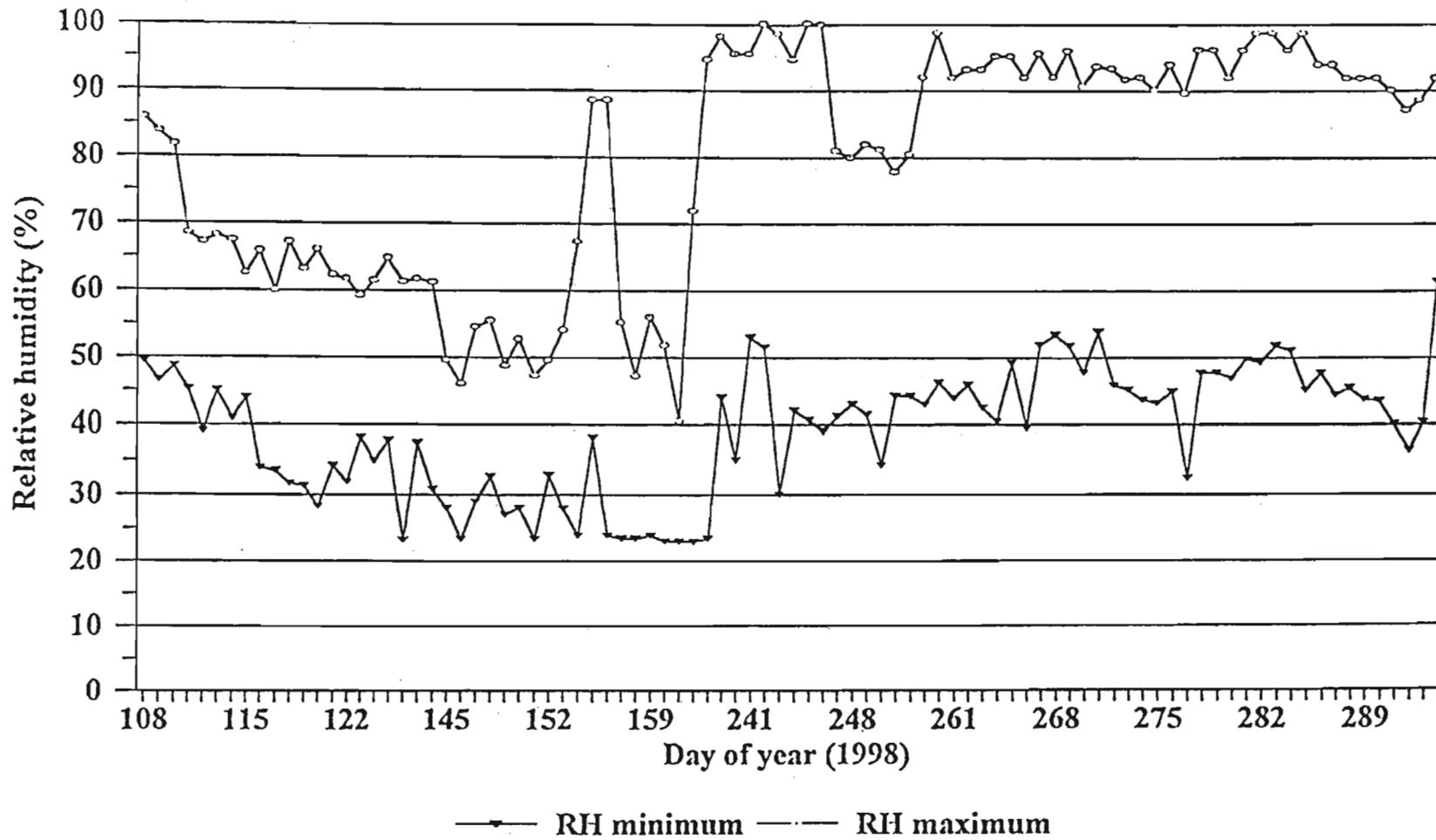


Figure 4.3 Maximum and minimum relative humidity in the controlled growth chamber during the period of study

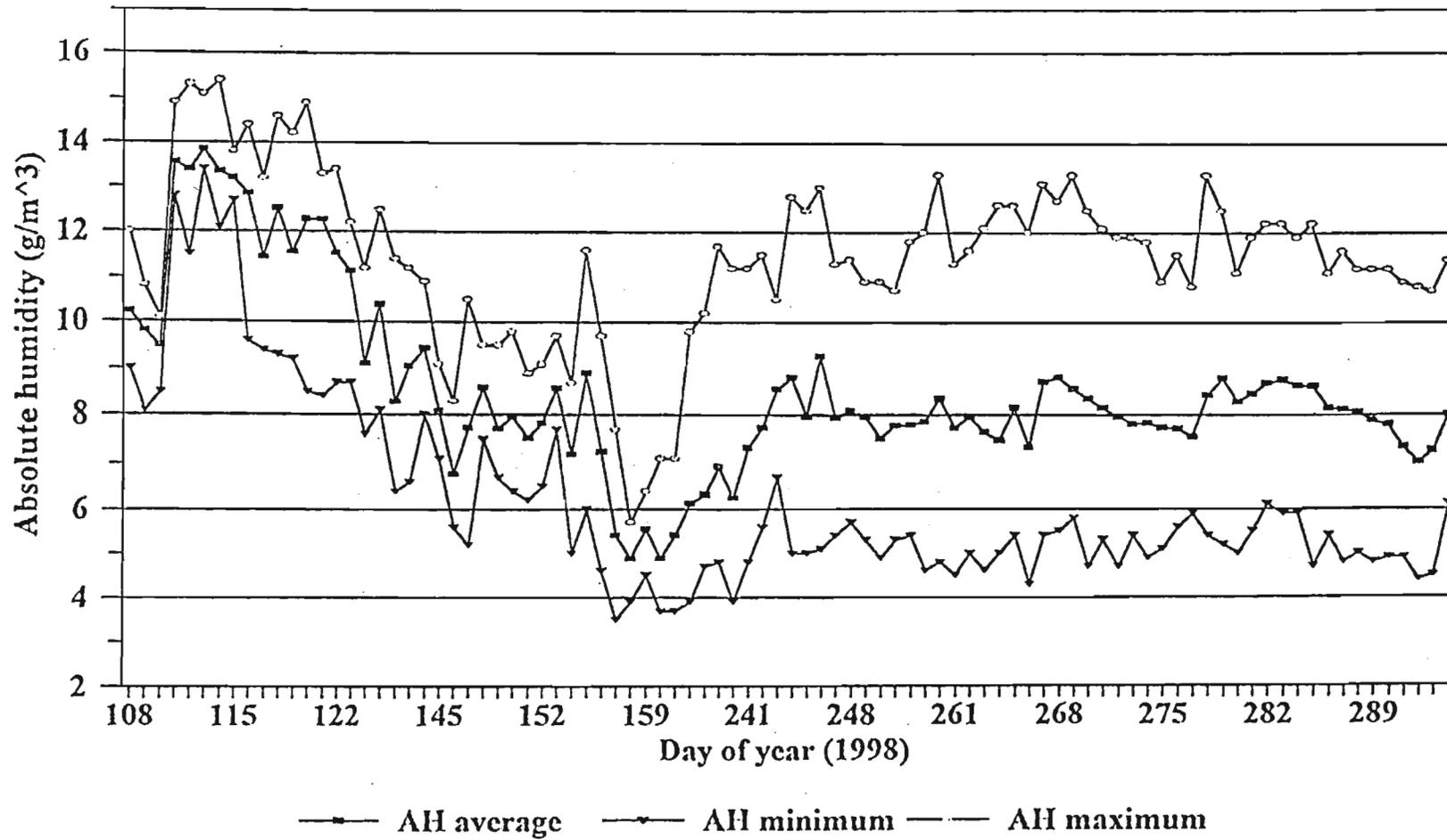


Figure 4.4 Maximum, minimum and average absolute humidity in the controlled growth chamber during the period of study.

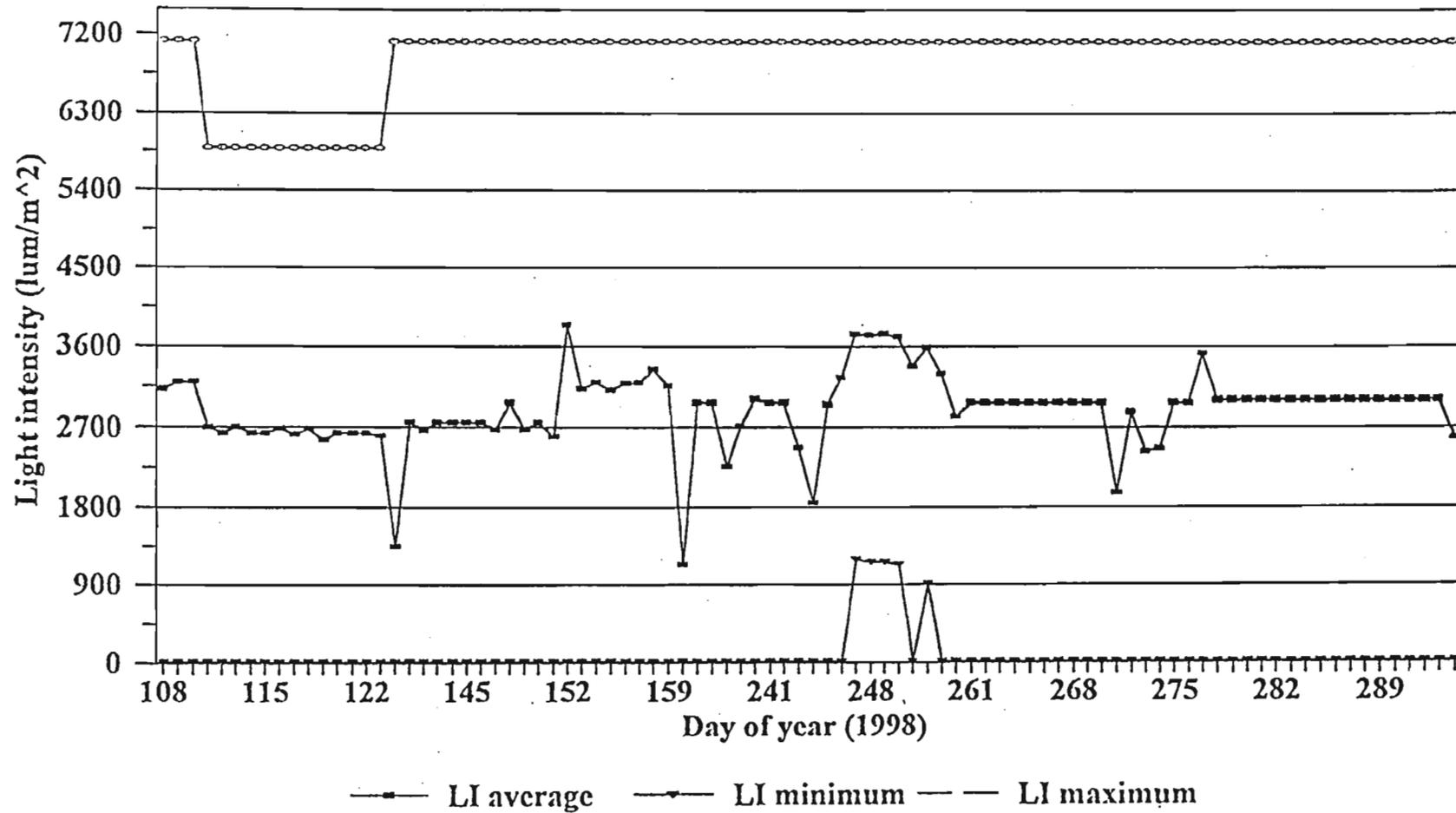


Figure 4.5 Maximum, minimum and average light intensity in the controlled growth chamber during the period of study.

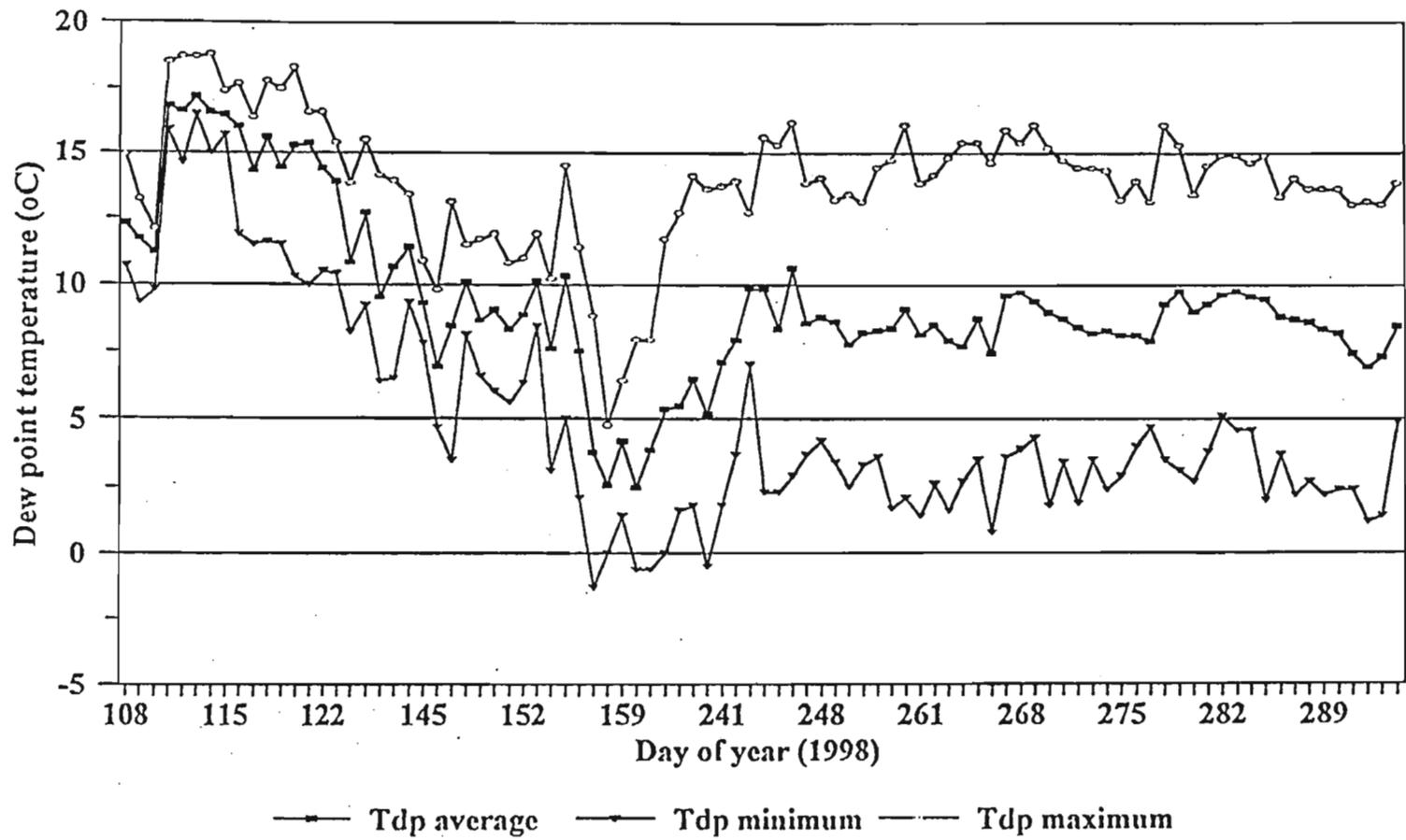


Figure 4.6 Maximum, minimum and average dew point temperature in the controlled growth chamber during the period of study.

The absolute humidity (Figure 4.4) reflects the mass of water (g) per volume of air ( $\text{m}^3$ ). During the W regime, the average absolute humidity decreased from 14 to  $5 \text{ g m}^{-3}$ . This decrease in absolute humidity can be attributed to the large decrease in temperature from the W to the C regime and corresponds to the relative humidity. During the C regime, the average absolute humidity remained constant at  $8 \text{ g m}^{-3}$  as the air temperature remained constant.

The light intensity shown in Figure 4.5 illustrates that the lights went on during the day and were off at night. The change in maximum light intensity from day 108 to day 122 was due to additional lights fitted into the growth chamber. The increased minimum light intensity between day 248 and day 259 is due to an error in the timer on the light switch in the growth chamber. There was an effective daylength of 10.5 h during the W and C regimes achieved using incandescent and fluorescent bulbs which are both necessary for flowering.

The average dew point temperature (Figure 4.6) decreased from 17 to  $2^\circ\text{C}$  during the W regime as the absolute humidity decreased and is due mainly to the decrease in air temperature. During the C regime, dew point temperature remained constant at  $7^\circ\text{C}$  and corresponds to the constant air temperature.

#### **4.2.3 Herbage sampling**

The herbage from four plants per pot was harvested at the 3.5 leaf growth stage (cut 50 mm above the soil level) five hours into the light cycle, three times during the warm regime and twice during the cold regime. Samples were placed in a hot forced-draught oven within 10 min of sampling and dried to constant weight at  $80^\circ\text{C}$  (24 h). During the warm and cold temperature regimes, the second fully expanded leaf blades of Enhancer and Midmar were sampled one-third to one-half of the total blade length above the ligule for morphological studies. Leaf width and number of vascular bundles per leaf were measured.

#### **4.2.4 Statistical analysis**

Analysis of variance of the data was effected using Genstat 5, Release 4.2 (Genstat, 2000).

### **4.3 Results and discussion**

#### **4.3.1 Morphological features of Enhancer and Midmar ryegrass**

Some morphological characteristics of the leaf blade of the two grass cultivars are presented in Table 4.3. The mean leaf width of Italian ryegrass cultivar Enhancer was significantly narrower ( $P < 0.01$ ) than that of Westerwolds ryegrass cultivar Midmar and contained fewer vascular bundles per leaf than Midmar, but the number of vascular bundles per mm leaf width was similar in both grasses. Vascular and sclerenchyma fibre strands are the main contributors to poorly digestible plant particles in the rumen, reducing forage quality (Minson and Wilson, 1994). The proportion of poorly digestible to more digestible tissue in the leaves in both grasses, therefore, appears to be similar.

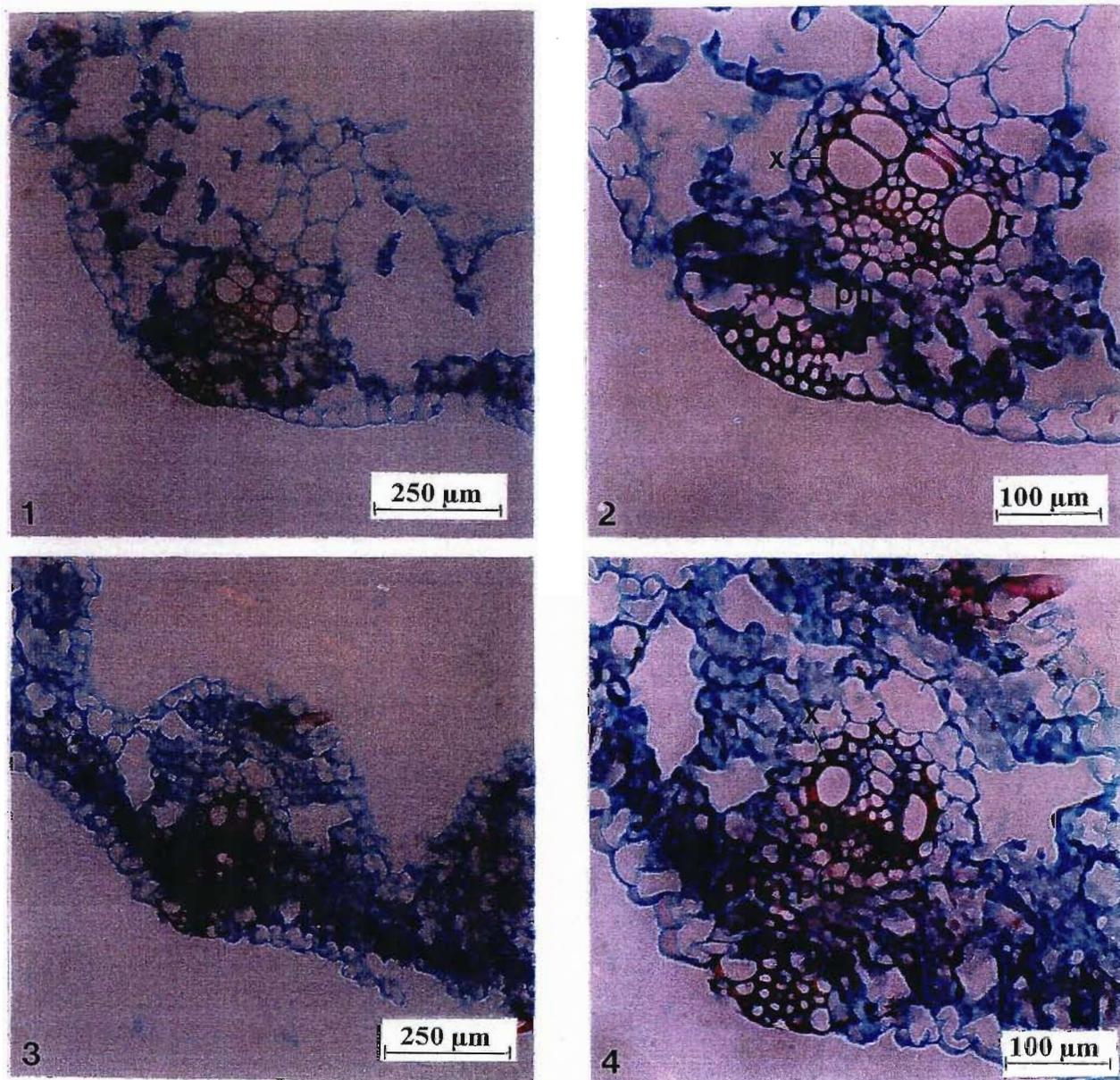


Figure 4.7 Cross sections of a main vein of Midmar (1 and 2) and Enhancer (2 and 4) ryegrasses.

Table 4.3 Comparison of mean leaf width and number of vascular bundles in the second fully expanded leaf blades of Italian ryegrass cultivar Enhancer and Westerwolds ryegrass cultivar Midmar.

	Enhancer	Midmar	S.E.M.	Significance
Leaf width (mm)	4.6	5.7	0.51	**
Vascular bundles (no.per leaf)	16.4	19.7	1.92	**
Vascular bundles (no. mm <sup>-1</sup> leaf width)	3.5	3.5	0.33	NS

NS, not significant; \*\* $P < 0.01$ .

The xylem vessels appeared to be more prominent in Midmar than in Enhancer. Since xylem is the principal water-conducting tissue it is possible that the larger xylem in Midmar could have contributed to the higher moisture content of this cultivar. No other pattern in the vascular tissue of Enhancer ryegrass appears to be linked to changes in nutritive value. The vascular bundles appear to be more rounded in Enhancer than in Midmar which are more oval in shape (Figure 4.7). In Midmar there were 9 vascular bundles on either side of the midrib, while in Enhancer ryegrass, 5 vascular bundles were found. This accounts for the broader leaf blades in Midmar than in Enhancer. It was difficult to section the two ryegrass cultivars, particularly Enhancer ryegrass, due to the fragility of the tissue. The Enhancer ryegrass was therefore sectioned thicker than Midmar ryegrass.

#### 4.3.2 Nutrient composition of ryegrass cultivars

The nutrient composition of the high-TNC, low-moisture cultivar Enhancer and the cultivar Midmar grown in a growth chamber under warm and cold regimes are presented in Table 4.4. The DM content and TNC concentration of Enhancer were significantly higher ( $P < 0.001$ , except TNC, cold regime  $P < 0.05$ ) than those of Midmar. As comparisons were conducted under identical environmental conditions, the recorded differences could be of genetic origin. The design of the experiment does not allow comparisons between the warm and the cold regime, although it is probable that the much higher TNC concentration in both cultivars in the cold regime is due to the reduced demand for carbohydrate reserves during the slower growth at low temperatures (Buxton, 1996). In the warm regime, the light intensity may have been a limiting factor for the accumulation of carbohydrates, which would be higher in field trials. Although significantly different, the DM contents of both grasses were lower than would be expected under field conditions (Marais and Goodenough, 2000). In order not to affect the DM intake of grazing ruminants adversely, the DM content of forages should be at least 18-20% (Meissner *et al.*, 1992). However, a higher TNC concentration (17 – 23%) in Enhancer compared with Midmar suggests a higher nutritive value of Enhancer. A perennial ryegrass (*Lolium perenne*) variety bred for increased levels of water-soluble carbohydrates has been shown to stimulate higher DM intakes and increase milk production in dairy cows (Miller *et al.*, 1999), and to increase liveweight gain from pre-weaned lambs (Lee *et al.*, 1999).

Table 4.4 Dry-matter yield, content and nutrient composition of herbage of Italian ryegrass cultivar Enhancer and Westerwolds ryegrass cultivar Midmar grown in a controlled environment chamber under warm and cold regimes.

Nutrient composition (g kg <sup>-1</sup> DM)	Warm regime					Cold regime				
	Enhancer	Midmar	CV %	S.E.M.	Significance	Enhancer	Midmar	CV %	S.E.M.	Significance
DM yield (g/pot)	10.3	12.8	4.9	0.34	***	14	16.3	5.2	0.53	***
DM (g kg <sup>-1</sup> fresh weight)	148.2	126.7	4.6	0.12	***	154.9	123.5	5.7	0.15	***
TNC	64.9	55.7	13.8	1.52	***	150	122.5	12.4	8.43	*
NDF	501.9	514.2	3.2	3.00	**	510.6	517.8	4.5	4.19	NS
ADF	291.8	295.1	7.0	3.77	NS	262.6	276.4	10.6	5.22	NS
ADL	48.0	49.7	20.4	1.82	NS	39.4	51.7	21.8	21.8	***
Nitrate-N	13.3	16.0	13.7	0.37	***	11.4	12.4	24.0	0.52	NS
<i>In vitro</i> DMD	776.8	770.4	3.5	7.07	NS	788.3	777.3	1.8	3.60	*
True protein	213.9	201.5	4.9	2.61	*	196.7	192	5.6	2.79	NS
Total N	48.9	47.4	3.8	0.48	**	45.8	46.5	8.1	0.96	NS

NS not significant; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$

DM = dry matter; TNC = total nonstructural carbohydrates; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; *in vitro* DMD = *in vitro* dry matter digestibility; Total N = total nitrogen

The high-TNC Italian ryegrass cultivar Enhancer was significantly lower ( $P < 0.001$ ) yielding than Midmar, giving 24% and 16% lower DM yields than Midmar during the warm and cold regimes, respectively. This is in accordance with the findings of Humphreys (1989b) in perennial ryegrass who showed that DM production is often inversely proportional to the concentration of water-soluble carbohydrate in the herbage. However, it is also noteworthy that the plants of Italian ryegrass cultivar Enhancer would have remained in the vegetative phase during both the warm and cold regimes, if the minimum 7°C night temperature had been insufficiently cold to induce the early reproductive phase during the seven-week cold regime. In contrast, the more vigorous growth of the plants of Midmar Westerwolds ryegrass may be attributed to the fact that they do not have a cold requirement for floral induction and would thus be in the early reproductive phase (Aamlid *et al.*, 1997), continuing this process during the entire period of both the seven-week warm and the subsequent seven-week cold regime. Nevertheless, in field experiments over a growing period of nine months, following autumn establishment, Enhancer gave a slightly higher DM yield than Midmar (Marais and Goodenough, 2000).

Table 4.4 shows that for Enhancer, values for the anti-quality characteristics ADF, ADL and nitrate concentrations were either similar to, or significantly lower than in Midmar. The early reproductive state of the Midmar Westerwolds ryegrass plants may account for their higher ADF and other values, as compared with Enhancer Italian ryegrass plants, which were perceived to still be in the vegetative phase for much of the duration of the experiment. The different growth phases of the Midmar and Enhancer plants may have contributed to variable measurements of other characteristics, including the *in vitro* dry matter digestibility of Enhancer, which was slightly higher than that of Midmar during the cold regime but similar to Midmar during the warm regime. During the warm regime true protein and total-N levels were higher in Enhancer than in Midmar, but similar during the cold regime. These results are in contrast to field results which showed a strong negative relation between TNC and the nitrogen concentration in the grass (Marais and Goodenough, 2000).

#### 4.3.3 Mineral composition of ryegrass cultivars

The mean mineral concentrations in Italian ryegrass cultivar Enhancer and Westerwolds cultivar Midmar are presented in Table 4.5. With the exception of the concentration of K during the cold regime, the mineral concentration of Enhancer was lower than that of Midmar. The apparent lower mineral values, with the exception of the Na concentration, could be due mainly to a dilution effect as a result of the higher concentration of nonstructural carbohydrate of Enhancer. During the warm and cold regimes the concentration of Na of Enhancer was 45% and 51% lower than in Midmar. These values are much lower than can be expected from a dilution effect and could be linked to the high K concentration in the plant. High levels of K in the soil will depress Na concentrations in temperate forage (Hemingway, 1961; Reith *et al.*, 1964). A lack of Na has been shown to reduce voluntary intake in calves (Morris and Murphy, 1972). However, the low levels in Italian ryegrass cultivar Enhancer appear to be sufficient for ruminants with the highest requirement for Na.

Table 4.5 Mean mineral concentration of herbage of Italian ryegrass cultivar Enhancer and Westerwolds ryegrass cultivar Midmar grown in a controlled environment chamber under warm and cold regimes.

Mineral concentrations (g kg <sup>-1</sup> DM)	Warm regime (30°C/20°C)					Cold regime (20°C/7°C)				
	Enhancer	Midmar	CV %	S.E.M.	Significance	Enhancer	Midmar	CV %	S.E.M.	Significance
Ca	6.9	7.0	7.7	0.09	NS	6.5	7.9	15.9	0.21	***
Mg	3.2	3.6	7.0	0.04	***	3.5	4.3	11.1	0.08	***
K	57.6	68.3	6.4	0.73	***	44.5	36.8	14.9	1.1	***
Na	1.6	2.9	38.4	0.16	***	3.9	7.9	27.7	0.3	***
P	3.2	3.3	9.1	0.05	NS	3.0	3.3	8.8	0.05	**
Zn (ppm)	54.2	53.5	9.7	0.95	NS	40.0	48.7	13.1	1.06	***
Cu (ppm)	13.5	13.9	7.5	0.19	NS	11.1	11.1	12.1	0.25	NS
Mn (ppm)	242.6	292.2	13.1	6.39	***	268.8	340.3	12.6	7.0	***

NS, not significant; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

The excessively high levels of K in the herbage are due to luxury uptake from the soil by means of an active process, i.e. against the concentration gradient. The K:Ca + Mg ratio, expressed on an equivalents basis, in excess of 2.2, was shown to be linked to an increased incidence of grass tetany (Azevedo and Rendig, 1972). However, in Enhancer and Midmar, grown under both temperature regimes, these ratios were not sufficiently high to be a potential cause of grass tetany.

#### **4.4 Conclusion**

The results of this controlled environment experiment have important implications for pasture breeding programmes. The experiment has improved the accuracy of assessment of genetic differences, the expression of which is sensitive to environmental factors, and has enabled the verification of results obtained in conventional breeding and assessment experiments. In the warm and cold regimes, despite the higher DM yield of Midmar relative to Enhancer, the latter is superior to Midmar in terms of the nutritive quality characteristics, the DM and TNC contents. No undesirable traits have evidently been introduced, confirming the work of Marais *et al.* (1997), who found that the DM content and the concentration of TNC in Italian ryegrass in a spaced-plant experiment did not appear to be positively linked to the main anti-quality factors associated with pasture grasses.

## Chapter 5

### The relative performance of weaned lambs grazing *Lolium multiflorum* cultivars Enhancer and Midmar

#### 5.1 Introduction

*Lolium multiflorum* is widely used in South Africa as autumn, winter and spring pastures. Its seasonal growth pattern is well suited to the traditional autumn lambing period (De Villiers, 1991). In a ewe-lamb-ryegrass system, weaned lambs will continue to graze irrigated ryegrass to attain a target slaughter mass of approximately 45 kg and achieve carcass grades desired by the consumer. The response of an animal to forage intake with respect to muscle growth, fat deposition, fibre growth or milk production depends on the quantity and balance of nutrients absorbed (Poppi *et al.*, 1997). The quantity and balance of nutrients absorbed, in turn, may depend on many quality or anti-quality features of the forage. *Lolium multiflorum* is regarded as a high-quality pasture grass with few anti-quality traits. However, there is increasing evidence of poor performance of weaned lambs on commercially available cultivars (Rutter, 1970; De Villiers, 1991; Meissner, 1996). This could be attributed to the high moisture content of the grass reducing dry matter (DM) intake (John and Ulyatt, 1987) and to poor utilisation of forage protein in the rumen due to insufficient amounts of readily digestible energy in the grass (Miller *et al.*, 1999).

The objective of this study was to compare *L. multiflorum* cultivars, Enhancer (a predominantly Italian type, bred for a high DM content and TNC concentration) and Midmar (a predominantly Westerwolds commercial ryegrass cultivar), which have potentially different quality traits, in terms of herbage intake, liveweight gain, wool growth and carcass quality of weaned lambs.

#### 5.2 Materials and methods

##### 5.2.1 Grazing trial

The area and cultivars of ryegrass used in this experiment are identical to those described in Chapter 3. Prior to the onset of the study, twenty South African Mutton Merino lambs were managed with their dams on the two ryegrass cultivars, Enhancer and Midmar, from 23 April 1998, and were weaned at the 12th week of lactation (29 June 1998). Lambs were allocated to the two ryegrass cultivars according to their weaning weights. At the commencement of the grazing trial, the lambs on Enhancer and Midmar had average weaning weights of 26.20 and  $26.19 \pm 1.45$  (mean  $\pm$  s.e.) kg, respectively. Lambs were drenched with an anthelmintic prior to starting the trial and approximately two weeks before weaning. The lambs remained on the two adjacent ryegrass cultivars Enhancer and Midmar and were stocked at a rate of 20 single weaned lambs ha<sup>-1</sup> to eliminate any competition for herbage. An eight-paddock rotational grazing system with a fixed rotation of 3.5 days in each paddock and a grazing cycle of 24.5 days provided adequate regrowth of the pasture. The lambs were weighed weekly, without prior fasting, to calculate the average liveweight gain of each animal. At the start of the trial the wool in the midrib area of all the lambs was clipped as close as possible to the skin (Oster clippers, no. 40 head). At the end of the trial an area of 100 cm<sup>2</sup> was clipped on the previously shorn

midrib area to determine wool growth. The lambs had free access to fresh water in portable water troughs and to a mineral lick consisting of 34% salt, 33% bone meal and 33% feed lime. The lambs remained on the two pastures until they reached a marketable mass (as determined by liveweight and classification on the hoof) was obtained. They were individually classed and slaughtered at the Cato Ridge Abattoir, at the end of the trial after 77 days.

### 5.2.2 Estimation of intake and digestibility

Dry matter intake and digestibility of the herbage consumed by the weaned lambs was estimated using a modification of the *n*-alkane technique (Marais *et al.*, 1996). The study was conducted from 6 to 22 July 1998 (winter) and included a 7-day adaptation period followed by a 10-day experimental period. Each lamb was dosed twice daily (08:00 and 15:00) with 100 mg of C<sub>32</sub> in the form of a suspension (50 ml) starting on the first week after weaning. During the experimental period rectal faecal samples were collected twice daily after dosing and pooled to form two 5-day composite samples for each animal. These samples were dried at 60°C and milled to pass a 1 mm sieve before being analysed for *n*-alkanes. Relative digestibility of the two grasses was estimated using the naturally-occurring alkane C<sub>33</sub>, as internal marker, assuming a recovery rate from the faeces of 87% (Marais *et al.*, 1996).

### 5.2.3 Herbage sampling

Herbage pluck samples were collected daily from the two pastures during the experimental period, by hand-plucking the pastures to an approximate grazing height, representative of that consumed by the lambs. All samples were placed in a warm forced-draught oven within 15 minutes of sampling, dried at 80°C and milled to pass a 1 mm sieve, in preparation for chemical analysis. Pasture yield (kg DM ha<sup>-1</sup>), apparent intake and regrowth following defoliation, were determined using the pasture disc meter (Bransby and Tainton, 1977) and expressed in cm of disc meter height and pasture yield under the disc (*y*). The linear regression equation used to predict pasture yield was regarded as appropriate for a range of total rainfall and stocking rate (Bartholomew, 1985). The prediction of pasture yield is given by the equation:

$$\text{Pasture yield (kg DM ha}^{-1}\text{)} = 1101 + 156.06 \times \text{disc meter height (cm)} \quad (r^2 = 0.78)$$

### 5.2.4 Statistical analysis

Analysis of variance of the data was effected using Genstat 5, Release 4.2 (Genstat, 2000). Exponential curves were fitted and an analysis of parallelism was done using Fitcurve in Genstat.

## 5.3 Results and discussion

### 5.3.1 Diurnal fluctuations in dry matter and total nonstructural carbohydrate content

The diurnal fluctuations in dry matter (DM) and total nonstructural carbohydrate (TNC) of the two ryegrass cultivars are illustrated in Figures 5.1 and 5.2, respectively.

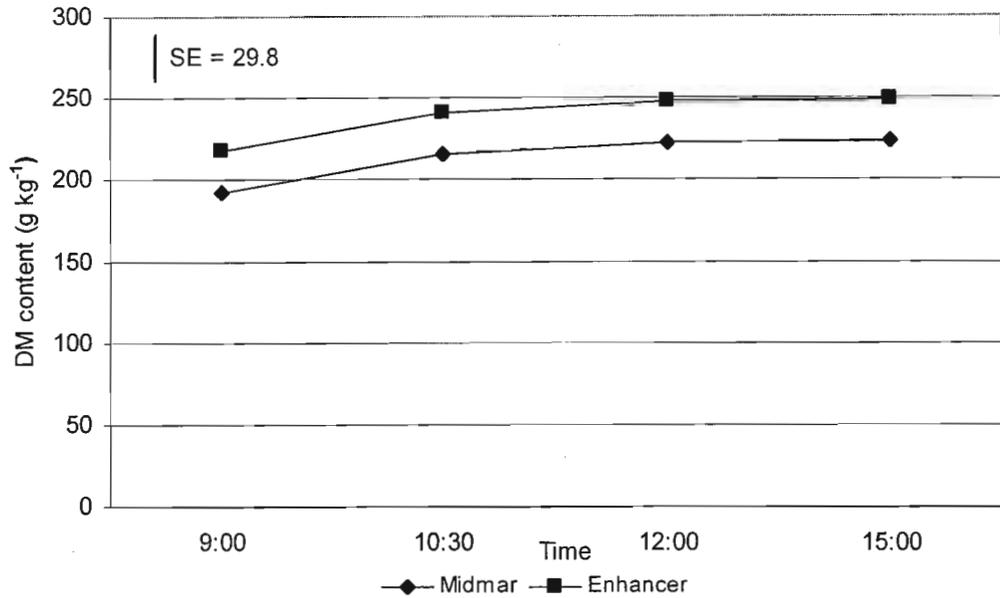


Figure 5.1 Mean diurnal fluctuations in DM for Enhancer and Midmar ryegrass cultivars.

The DM content in both ryegrass cultivars showed significant ( $P < 0.001$ ) diurnal fluctuations (Figure 5.1). The DM content increased in the morning and levelled out in the afternoon. DM content ( $y$ ) showed a significant positive trend ( $P < 0.01$ ) with time ( $x$ ) in both ryegrass cultivars, as described below:

$$\text{Enhancer: } y = 249.0 - 453459 \cdot \exp(-kx) \quad (r^2 = 0.25)$$

$$\text{Midmar: } y = 223.4 - 453459 \cdot \exp(-kx) \quad (r^2 = 0.25)$$

$$\text{where } k = -\log 0.345$$

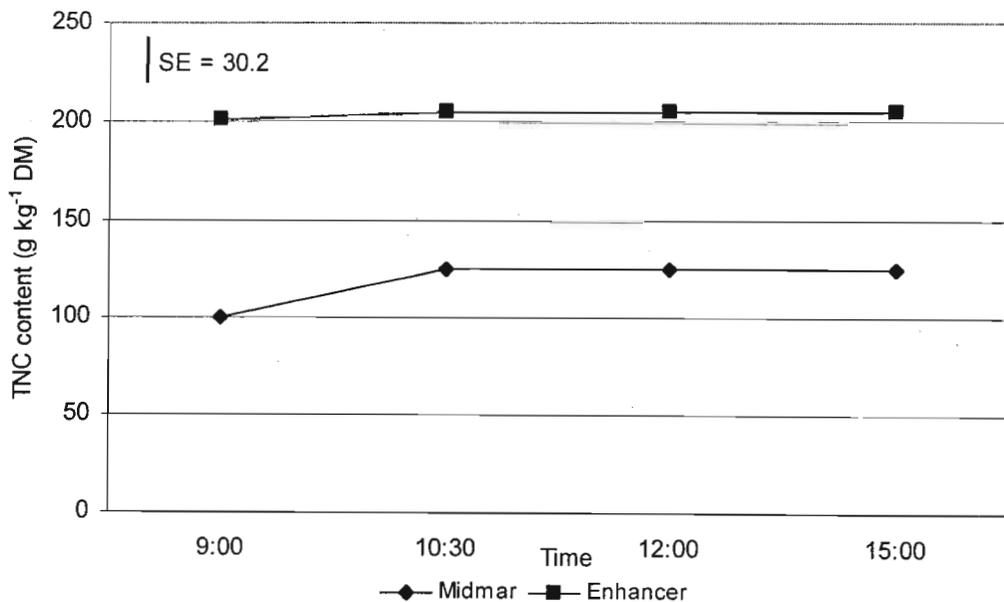


Figure 5.2 Mean diurnal fluctuations in TNC for Enhancer and Midmar ryegrass cultivars.

Over the period measured, no significant differences in diurnal fluctuations in TNC content were found for Midmar and Enhancer ryegrass (Figure 5.2). The TNC content in Midmar ryegrass increased from 09:00 and levelled out from 10:30 to mid afternoon. In Enhancer ryegrass the TNC content remained high throughout the measuring period with no diurnal fluctuations. Weather conditions during the sampling days (13 - 22 July 1998) were generally cold and overcast. Smith (1973) found that daily changes in sugar concentrations were larger on warm, sunny days than on cold, cloudy days. The TNC content (y) showed a significant positive correlation ( $P < 0.01$ ) with time (x) in the two pastures as described below:

$$\text{Enhancer } y = 204.7 - 2.037 \times 10^{21} \exp(-kx)$$

$$\text{Midmar: } y = 125.3 - 1.288 \times 10^{22} \exp(-kx)$$

$$\text{where } k = -\log 0.005 \text{ (} r^2 = 0.67 \text{)}$$

### 5.3.2 Nutrient composition of ryegrass pastures

The mean nutrient content and mineral composition ( $\text{g kg}^{-1}$  DM) of the two ryegrasses (mean of 10 samples) during the period of alkane estimation are outlined in Tables 5.1 and 5.2, respectively.

Table 5.1 Mean nutrient composition of the *L. multiflorum* cultivars Enhancer and Midmar.

Nutrient composition ( $\text{g kg}^{-1}$ DM)	Ryegrass cultivars		CV %	S.E.M.	Significance
	Enhancer	Midmar			
DM ( $\text{g kg}^{-1}$ fresh grass)	216.8	180.6	6.7	4.18	***
TNC	173.2	87.6	30.5	12.57	***
NDF	567.8	593.7	7.2	13.15	NS
ADF	252.0	322.4	9.7	8.79	***
ADL	79.7	109.8	18.5	5.54	**
Nitrate-N	0.71	1.31	42.6	0.14	*
<i>in vitro</i> DMD	738.9	736.5	1.1	2.58	NS
True protein	203.3	224.9	4.4	2.98	***
Total N	44.54	49.78	9.2	1.38	*

NS, not significant; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$

DM = dry matter; TNC = total nonstructural carbohydrates; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; *in vitro* DMD = *in vitro* dry matter digestibility; Total N = total nitrogen

Production responses and the efficiency of nitrogen utilisation from ryegrass pastures by ruminants are low, partly due to a lack of readily available energy (Miller *et al.*, 1999). Highly significant ( $P < 0.001$ ) differences in DM and TNC contents were observed between the two cultivars in this study. Enhancer ryegrass had a mean TNC concentration of  $173.2 \text{ g kg}^{-1}$  DM, which was significantly higher (98%;  $P < 0.001$ ) than that of Midmar and a true protein content significantly lower (10%;  $P < 0.001$ ) than that of Midmar. This gives Enhancer a more favourable readily available energy to protein ratio for ruminant production than Midmar. The relatively low total N content of Enhancer ( $44.54 \text{ g kg}^{-1}$  DM) is still more than adequate for animal production (Poppi *et al.*, 1997).

John and Ulyatt (1987) showed that voluntary intake of fresh forage is limited by a mechanism regulating the intake of wet feed and that DM content may be an important factor limiting nutrient intake. According to Vérité and Journet (1970) voluntary intake of lactating cows decreased by  $0.337 \text{ kg DM}$  for each  $10 \text{ g kg}^{-1}$  rise in water content of the forage above  $820 \text{ g kg}^{-1}$  fresh grass. However, the effect disappears if the moisture content decreases below  $820 \text{ g kg}^{-1}$  fresh grass or the DM content rises above  $180 \text{ g kg}^{-1}$  fresh grass. Although Enhancer had a significantly higher (20%;  $P < 0.001$ ) DM content than Midmar ryegrass, the mean DM content of Midmar was  $180.6 \text{ g kg}^{-1}$  fresh grass, suggesting that moisture content was probably not a factor affecting voluntary intake in this trial. These results are consistent with those of Meissner *et al.* (1992) who found that moisture was not a limiting factor to intake of Midmar ryegrass, provided that the DM content was above 18 – 20%.

The NDF content of forages is usually highly correlated with the average daily gain of ruminants (Paterson *et al.*, 1994). However, differences in NDF content between the two cultivars were not significant; suggesting that total cell wall content did not contribute to differences in animal response on the two grasses. The mean ADF and ADL contents, which have a negative effect on forage quality and animal production (Van Soest, 1963), were significantly lower (22%;  $P < 0.001$  and 27%;  $P < 0.01$ , respectively) in Enhancer than in Midmar. No significant differences in *in vitro* DMD were found between Enhancer and Midmar. Although the TNC concentration of Enhancer was significantly higher ( $P < 0.001$ ) than that of Midmar, it did not appear to influence the *in vitro* DMD as suggested by Grimes *et al.* (1967) who found strong positive correlations between soluble carbohydrate content and digestibility over a range of species.

Annual grass species such as *Lolium multiflorum* have a greater tendency to accumulate nitrate than perennial forage species (Crawford *et al.*, 1961; Darwinkel, 1975). In the rumen of the grazing animal, nitrate is readily reduced to nitrite, a highly toxic compound, which could have a negative effect on animal production (Marais, 1997). Although the nitrate content of Midmar ryegrass was significantly higher (46%;  $P < 0.05$ ) than that of Enhancer, in both cultivars it appears to be too low to affect animal production (Wright and Davison, 1964).

Table 5.2 Mean mineral composition of the *L. multiflorum* cultivars Enhancer and Midmar (mean of 10 replicates).

Mineral composition (g kg <sup>-1</sup> DM)	Ryegrass cultivars		CV %	S.E.M.	Significance
	Enhancer	Midmar			
Ca	3.3	3.6	16.0	0.18	NS
Mg	2.4	2.3	12.8	0.09	NS
K	30.4	34.9	20.4	2.10	NS
Na	3.3	3.5	26.7	0.28	NS
P	2.8	2.9	13.3	0.12	NS
Zn (mg kg <sup>-1</sup> )	33.6	31.3	17.7	1.81	NS
Cu (mg kg <sup>-1</sup> )	17.4	15.6	15.4	0.80	NS
Mn (mg kg <sup>-1</sup> )	88.9	93.4	7.0	2.03	NS

NS, not significant

There were no significant differences in the mineral content of the two cultivars and the amounts present appear to be within the normal range for animal production (Miles, 1998). According to the Committee on Mineral Nutrition (1973) the ratio of Ca:P in pastures has no special significance provided the animal receives enough Ca and P. However, the absorption of Mg is depressed by high levels of K in the diet. The tendency for animals to develop hypomagnesemic tetany is increased if the K: (Ca + Mg) ratio in the forage, expressed in milliequivalents, exceeds 2.2 (Azevedo and Rendig, 1972). The K: (Ca + Mg) ratios of Midmar and Enhancer were calculated at 2.41 and 2.15, respectively, indicating that the likelihood of animals developing a magnesium deficiency is small on both pastures.

### 5.3.3 Pasture availability and growth

Results of the available and residual grass after grazing, as measured by the disc meter over the experimental period (July – September), are presented in Figure 5.3. The relationship between apparent intake and available pasture for the two ryegrasses is illustrated in Figure 5.4.

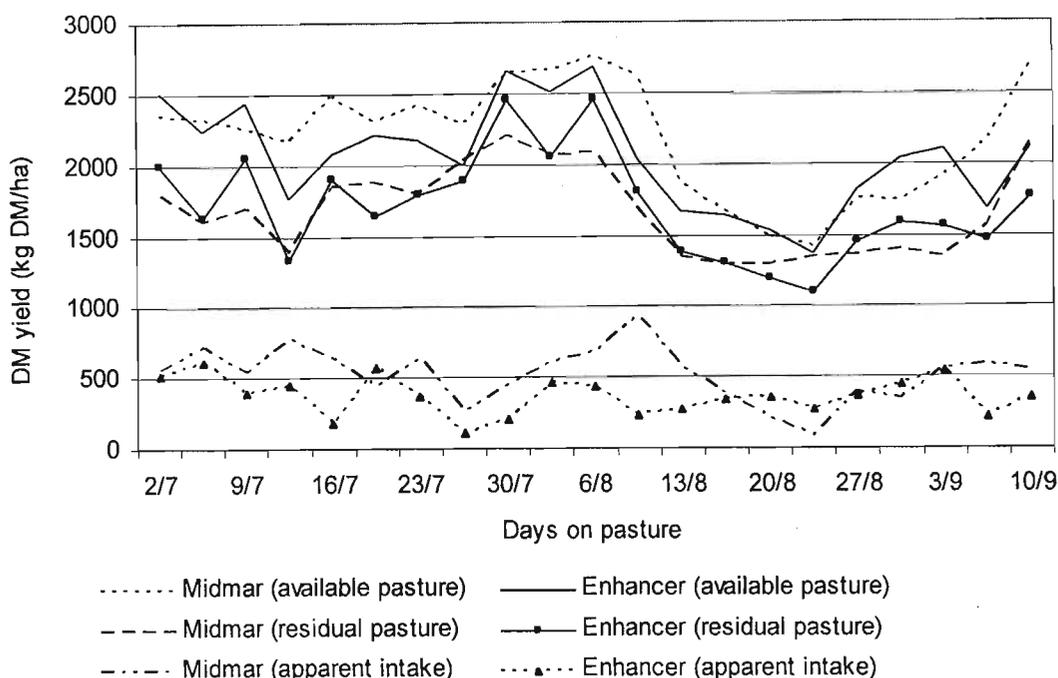


Figure 5.3 Apparent intakes and the available and residual pasture on *L. multiflorum* cultivars Enhancer and Midmar.

According to the NRC (1985), the dry matter intake (DMI) requirement of a 30 kg weaned lamb is  $1.4 \text{ kg day}^{-1}$ . In this study, the average availability of Midmar and Enhancer ryegrass was  $2184$  and  $2067 \text{ kg DM ha}^{-1}$ , respectively, providing the lambs on Midmar and Enhancer with  $2.83$  and  $2.68 \text{ kg DM day}^{-1}$ , respectively. No significant differences in pasture availability between the two ryegrass cultivars were found (Figure 5.3). However, highly significant ( $P < 0.001$ ) differences in availability over the season were observed. In July the average availability for Midmar and Enhancer ryegrass was  $2522$  and  $2318 \text{ kg DM ha}^{-1}$ , respectively, while in August/September availability decreased to  $1745$  and  $1732 \text{ kg DM ha}^{-1}$  for Midmar and Enhancer, respectively. The dramatic decrease in availability in both the ryegrass cultivars from 7 August could be attributed to the decrease in minimum temperatures during this period, with the coldest temperatures measured at  $2.0$ ,  $-0.2$  and  $0.8^\circ\text{C}$  for 15, 16 and 17 August, respectively. Another possibility for the decrease in available pasture was a depletion of tillers upon grazing particularly since Midmar, being a Westerwolds ryegrass cultivar, would have progressed into an early reproductive phase, and similarly for the Westerwolds component of Enhancer ryegrass. From 7 to 11 September the availability on Midmar was higher than on Enhancer, due to Midmar being in the flowering stage. The seed heads are more fibrous which could have resulted in a higher disc meter reading.

No significant differences in residual pasture between the two ryegrass cultivars were found. However, highly significant differences ( $P < 0.001$ ) over the growing season were observed, with a mean residual pasture of  $1971 \text{ kg DM ha}^{-1}$  in August and  $1373 \text{ kg DM ha}^{-1}$  in September. Midmar

had a significantly higher ( $P < 0.01$ ) intake than Enhancer ryegrass. The apparent intake on Midmar was 569, 448 and 366 kg DM ha<sup>-1</sup> for July, August and September, respectively, while on Enhancer the apparent intake for the same period was 489, 321 and 350 kg DM ha<sup>-1</sup>, respectively.

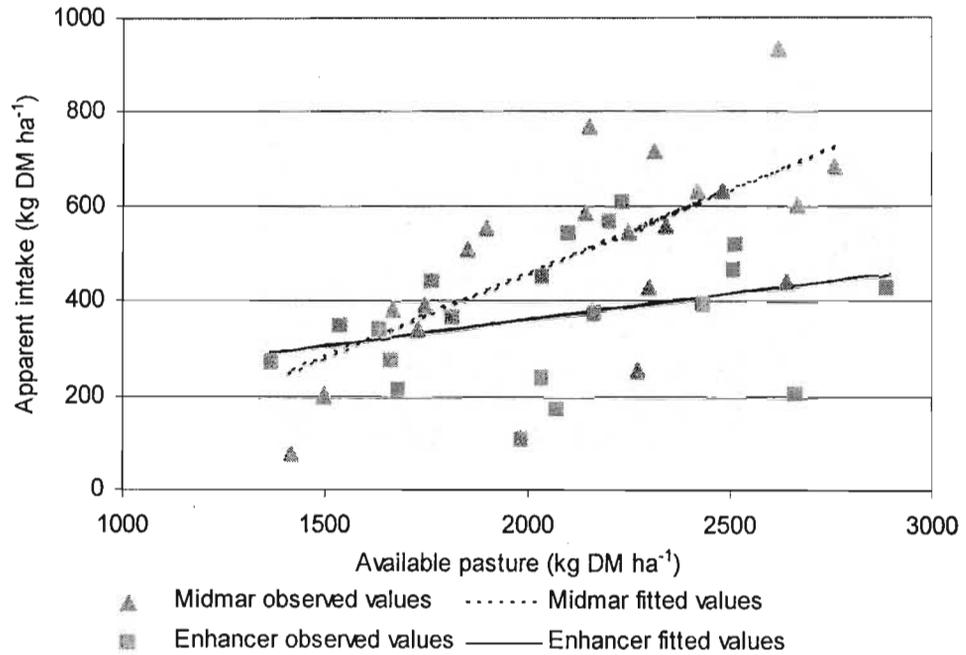


Figure 5.4 Relationship between apparent intake and pasture availability of Enhancer and Midmar ryegrass cultivars.

In Figure 5.4 intake (y) and pasture availability (x) were not significantly correlated in either of the ryegrass cultivars ( $P = 0.178$  and  $P = 0.132$  for Midmar and Enhancer, respectively), suggesting that intake was not restricted in the cultivars. The regression equations are as follows:

$$\text{Enhancer: } y = 138 + 0.1109x \quad (r^2 = 0.48)$$

$$\text{Midmar: } y = -245.26 + 0.3509x \quad (r^2 = 0.10)$$

#### 5.3.4 Estimated dry matter intake

Figures 5.5 and 5.6 illustrate the differences in DMI between the two ryegrass cultivars estimated using C<sub>31</sub>/C<sub>32</sub> and C<sub>32</sub>/C<sub>33</sub> alkane pairs, respectively.

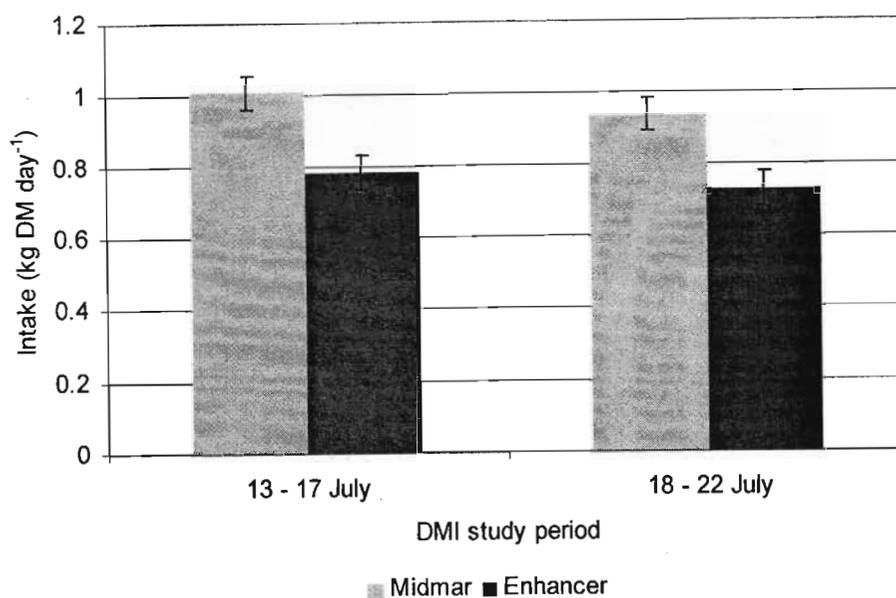


Figure 5.5 DMI of weaned lambs grazing Enhancer and Midmar ryegrass cultivars estimated using the C<sub>31</sub>/C<sub>32</sub> alkane pair.

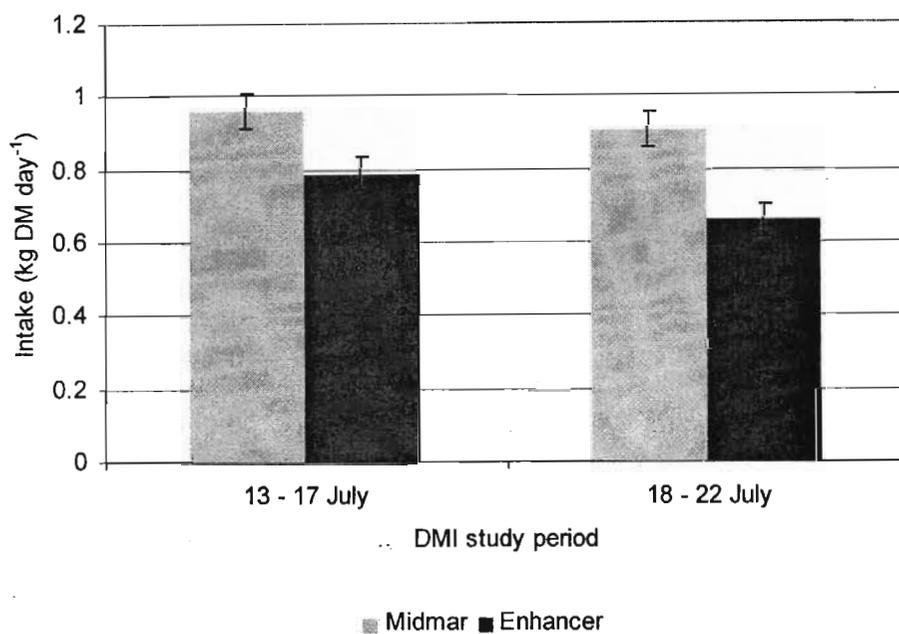


Figure 5.6 DMI of weaned lambs grazing Enhancer and Midmar ryegrass cultivars estimated using the C<sub>32</sub>/C<sub>33</sub> alkane pair.

Although differences between the two cultivars in pasture availability and residual pasture were not statistically significant, lambs on Enhancer had a significantly lower (29%;  $P < 0.01$ ) DMI over the whole season (2 July – 10 September) than those on Midmar, based on disc meter readings (Figure

5.3). This trend was confirmed by intake estimates with the *n*-alkane technique over a 10-day period (13 July – 22 July) using both the C<sub>31</sub>/C<sub>32</sub> and C<sub>32</sub>/C<sub>33</sub> *n*-alkane pairs as markers. The mean values for the two alkane pairs showed a significantly lower (23 %;  $P < 0.001$ ) daily DMI for Enhancer than for Midmar. The lower intake of Enhancer compared with Midmar does not appear to be due to the presence in Enhancer of high concentrations of anti-quality factors such as ADF, ADL, N, Nitrate-N or a high moisture content.

### 5.3.5 Dry matter digestibility

The mean dry matter digestibility (DMD) for lambs grazing the two ryegrass cultivars is compared in Table 5.3. DMD was determined using naturally occurring C<sub>33</sub>.

Table 5.3 A comparison of DMD determined using naturally-occurring C<sub>33</sub> in Enhancer and Midmar cultivars.

Days	DMD (g kg <sup>-1</sup> DM) of lambs grazing the ryegrass cultivars:		CV %	S.E.M.	Significance
	Enhancer	Midmar			
13 - 17 July	816.4	832.8	1.40	3.65	**
18 -22 July	816.9	804.1	2.40	6.14	NS
10 day alkane experimental period	816.7	818.4	2.14	3.91	NS

NS = not significant; \*\*  $P < 0.01$

Midmar ryegrass was significantly higher (2%;  $P < 0.01$ ) in digestibility than Enhancer ryegrass for the first 5-day composite sample (13 - 17 July), but not in the second 5-day composite sample. No significant difference in DMD was found between the two ryegrass cultivars when compared over the entire alkane experimental period.

### 5.3.6 Performance of weaned lambs

#### 5.3.6.1 Average daily gain

Table 5.4 compares the cumulative average daily gain (ADG) of weaned lambs grazing Midmar and Enhancer. Differences in ADG of lambs grazing the ryegrass pastures are illustrated in Figure 5.7. The relationship between ADG (g day<sup>-1</sup>) and pasture height and residual pasture height (cm) is illustrated in Figures 5.8 and 5.9, respectively.

Table 5.4 Cumulative average daily gain of weaned lambs grazing Enhancer and Midmar ryegrass cultivars.

Date	ADG (g day <sup>-1</sup> ) of lambs on ryegrass cultivars:		CV%	S.E.M.	Significance
	Enhancer	Midmar			
	06/7	336			
13/7	275	240	30.1	24.5	NS
20/7	267	241	22.8	18.3	NS
27/7	254	218	20.7	15.4	NS
03/8	280	230	20.5	16.5	*
10/8	261	247	16.3	13.1	NS
17/8	204	205	16.3	10.6	NS
24/8	174	156	19.9	10.4	NS
31/8	203	169	18.8	11.1	*
07/9	209	177	22.1	13.5	NS
14/9	185	164	17.4	9.6	NS

NS = not significant; \*  $P < 0.05$

The results outlined in Table 5.4 show that in the first week after weaning (6 July) the lambs grazing Enhancer had a significantly higher (73%;  $P < 0.05$ ) ADG than lambs grazing Midmar. From 13 to 27 July no significant differences in ADG were found, although the ADG for lambs grazing Enhancer tended to be higher than that of Midmar. From 10 to 24 August there was a decrease in the ADG for lambs on both pastures. This decrease corresponded to the drop in available pasture and consequently a decline in apparent intake (Figure 5.3). With the increase in available pasture from 24 August the ADG of lambs increased. De Villiers *et al.* (1995) investigated the relationship between pre-weaning stocking rate and post-weaning ADG. Their analyses showed that the regression was not significant, but, at a pre-weaning stocking rate of 20 ewes with lambs ha<sup>-1</sup>, the predicted ADG was calculated at  $169 \pm 4.99$  g day<sup>-1</sup>. A positive correlation was found between weaning mass and post-weaning ADG of lambs within a specific stocking rate (De Villiers *et al.*, 1995). In the current study the predicted post-weaning ADG of lambs grazing Midmar and Enhancer at the given pre-weaning stocking rate using the equation of De Villiers *et al.* (1995) was 173.03 and 173.06 g day<sup>-1</sup>, respectively. The total post-weaning ADG outlined in Table 5.4 of lambs grazing Midmar and Enhancer was 164 and 185 g day<sup>-1</sup>, respectively. For lambs grazing Midmar, an ADG of 164 g day<sup>-1</sup>

was below the predicted ADG, while for lambs grazing Enhancer; the ADG was higher than the predicted ADG using both regression equations as given by De Villiers *et al.* (1995).

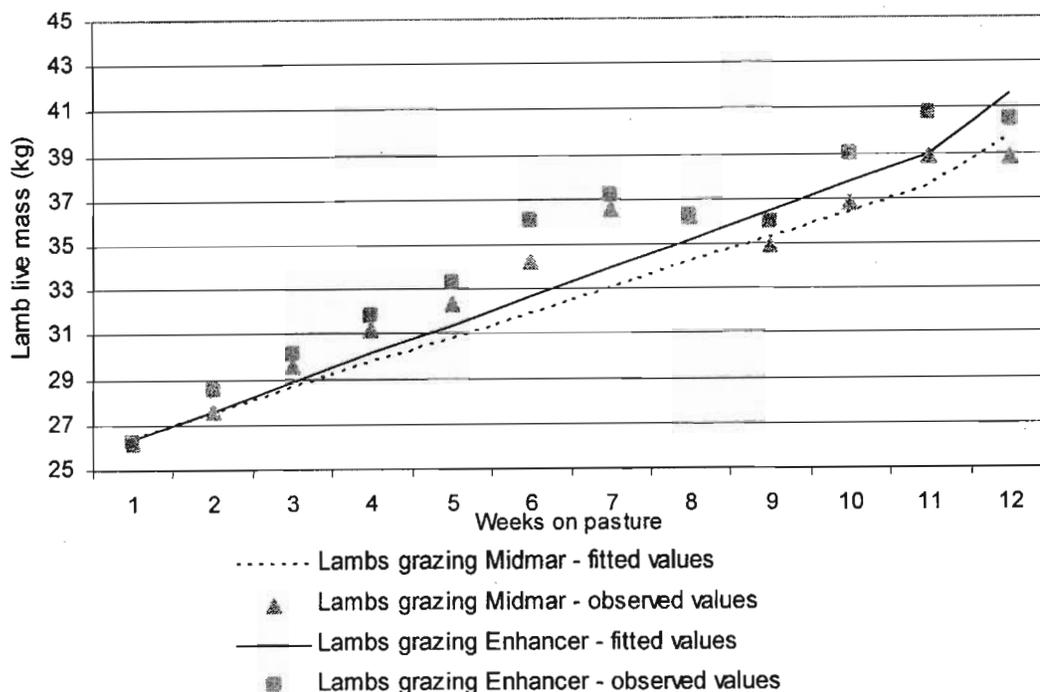


Figure 5.7 Relationship between mass and weeks after weaning of lambs grazing Enhancer and Midmar ryegrass cultivars.

A highly significant positive correlation ( $P < 0.001$ ) was found between lamb mass ( $y$ ) and days after weaning ( $x$ ) for the grazing period (Figure 5.7), as described below:

$$\text{Enhancer: } y = 26.358 + 1.2686x \quad (r^2 = 0.94)$$

$$\text{Midmar: } y = 26.358 + 1.114x \quad (r^2 = 0.92)$$

The positive correlation was observed in both ryegrass cultivars. However, the mass of lambs on Enhancer ryegrass were significantly higher ( $P < 0.05$ ) than lambs on Midmar. After 77 days, the lambs stocked at a rate of 20 lamb ha<sup>-1</sup> on Midmar and Enhancer and weaned at an average mass of  $26.19 \pm 1.45$  and  $26.20 \pm 1.45$  kg, respectively, reached a final mass of  $38.8 \pm 1.78$  and  $40.45 \pm 1.79$  kg, respectively. The lambs on Enhancer gained 14.25 kg post-weaning while lambs on Midmar gained 12.61 kg, despite the fact that the lambs had similar weaning weights. The increased liveweight gain of lambs grazing Enhancer compared with Midmar could be due to an improved utilisation of forage protein in the rumen as a result of the increased TNC levels. Lee *et al.* (2001) showed similar results with an increase in liveweight gain of lambs grazing a high water soluble carbohydrate (WSC) variety compared with a control. The increased lamb performance was related to improvements in the balance of energy and nitrogen supply to the rumen through elevated levels of WSC. Sinclair *et al.* (1995) and Witt *et al.* (1999) showed that diet formulations that improve the

energy and nitrogen balance in the rumen would enhance the efficiency of microbial protein synthesis and increase lamb growth rates.

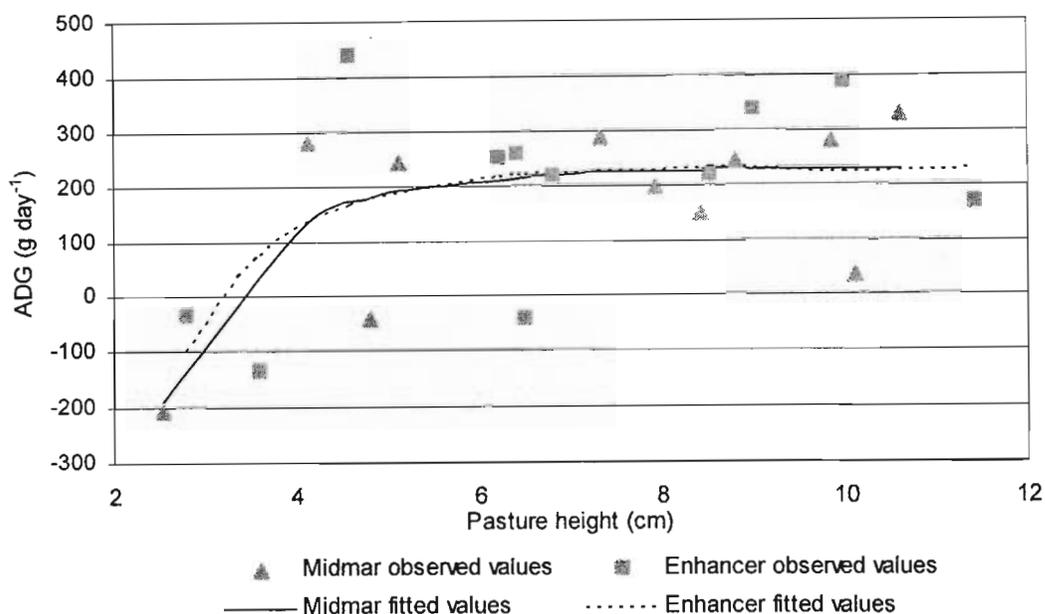


Figure 5.8 Relationship between ADG of weaned lambs and pre-grazing pasture height of Enhancer and Midmar ryegrass cultivars.

A significant relationship ( $P < 0.01$ ) was found between ADG ( $y$ ) and pre-grazing pasture height ( $x$ ) in the two ryegrasses, but no significant differences ( $P = 0.939$ ) were found between Midmar and Enhancer (Figure 5.8). The quadratic equation for pre-grazing pasture height in both pastures and ADG accounts for 35.2% of the variation in lamb growth. The equation is:

$$y = 0.2287 - 4.47 \cdot \exp(-kx) \text{ where } k = -\log 0.393 \text{ (} r^2 = 0.35 \text{)}$$

Figure 5.8 shows that ADG reaches a maximum at a pre-grazing height of approximately 8 cm in both ryegrass cultivars. This height equates to approximately 2 349 kg of DM ha<sup>-1</sup>. This suggests that a pre-grazing pasture height of approximately 7 cm and an available pasture yield between 2 000 and 3 000 kg DM ha<sup>-1</sup> is optimal for lamb growth of 300 g d<sup>-1</sup>. Williams *et al.* (1976) reported similar data, where the growth rates of lambs increased with tiller length. It was found that if weaned lambs are to obtain any growth advantage, more than a critical level of pasture should be available. Lambs therefore need to be moved onto pastures that have a high availability. In this study, the available pasture height on Midmar and Enhancer was measured at 6.9 and 6.2 cm, respectively, while the residual pasture height on Midmar and Enhancer was 3.2 and 3.4 cm, respectively. This data is consistent with results reported by De Villiers *et al.* (1995) who showed that lambs perform better on shorter than on longer ryegrass.

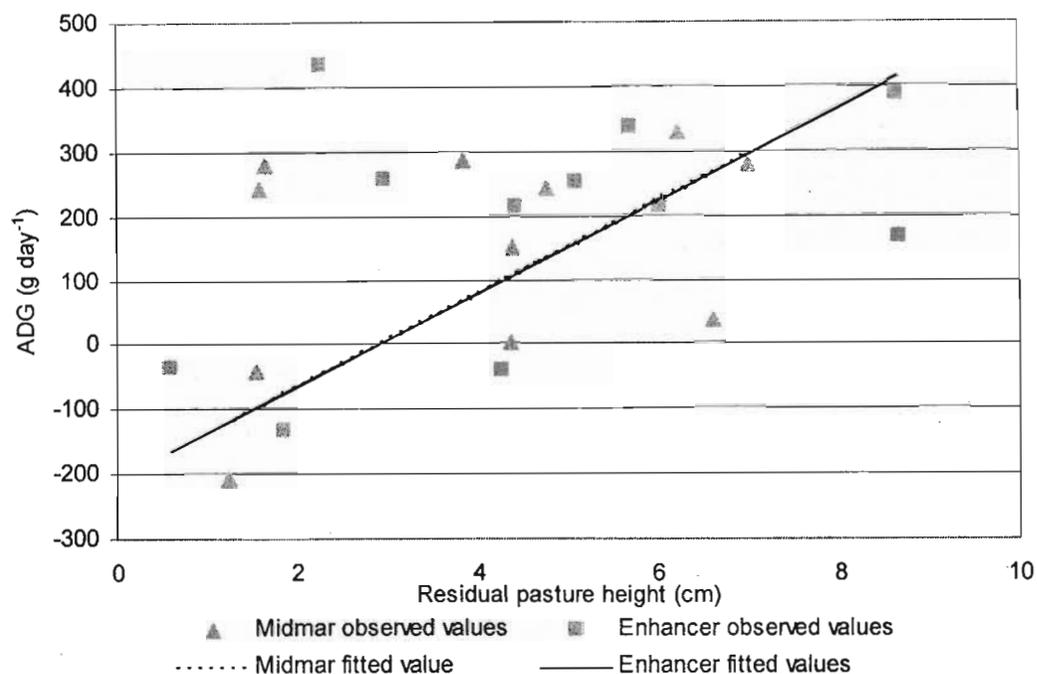


Figure 5.9 Relationship between ADG of weaned lambs and post-grazing pasture height of Enhancer and Midmar ryegrass cultivars.

A significant relationship ( $P < 0.05$ ) between ADG and residual pasture height was found, but no significant differences ( $P = 0.97$ ) were found between the two ryegrass cultivars (Figure 5.9). The equation for ADG ( $y$ ) and residual pasture height ( $x$ ) accounts for only 14.5% of the variation in lamb growth and is:

$$y = 0.0313x + 0.0407 \quad (r^2 = 0.145)$$

### 5.3.6.2 Wool growth and carcass qualities

Once the lambs on the two ryegrass cultivars had attained a marketable weight, determined by liveweight and classification on the hoof, they were taken to the Cato Ridge abattoir where they were slaughtered and the carcasses individually graded. Table 5.5 compares the differences in wool growth and carcass quality between the lambs grazing Enhancer and Midmar.

Table 5.5 Wool growth and carcass quality of lambs on Enhancer and Midmar ryegrass cultivars.

Parameter	Wool growth and carcass quality of lambs on ryegrass pastures:		CV%	S.E.M.	Significance
	Enhancer	Midmar			
	Wool (g 100cm <sup>-2</sup> )	14.58			
Carcass mass: warm (kg)	20.30	18.30	16.9	1.03	NS
Carcass mass: cold (kg)	19.80	18.10	15.6	0.93	NS
Warm carcass dressing %	50.25	46.98	8.2	1.26	NS
Fat coverage (mm)	2.60	2.30	34.0	0.26	NS
Hindfat (mm)	4.51	2.09	44.8	0.47	**
Loinfat (mm)	4.16	3.45	60.7	0.73	NS
Forefat (mm)	1.99	0.97	60.2	0.28	*
Conformation	3.80	3.80	11.1	0.13	NS
Price (Rand/carcass)	296.00	259.00	17.9	15.7	NS

NS = not significant; \*  $P < 0.05$ ; \*\*  $P < 0.01$

No significant differences in the wool growth were found. Similarly, there were no significant differences in the warm and cold carcass mass of lambs grazing Enhancer and Midmar ryegrass. The lambs on Enhancer had a significantly higher ( $P < 0.01$  and  $P < 0.05$ ) hindfat and forefat than the lambs on Midmar, which may have contributed to the difference in price/carcass between Enhancer and Midmar. Although there was no statistically significant difference in price/carcass, the lamb carcasses from Enhancer sold at a mean of R37.00 more than those from Midmar, which would be financially significant for a farmer slaughtering large numbers of weaned lambs (Table 5.5).

#### 5.4 Conclusion

Sufficient pasture was available to the weaned lambs on both Midmar and Enhancer. No significant differences in available and residual pastures were found between the two ryegrass cultivars. However, DMI by lambs was lower on Enhancer compared with Midmar. Despite the lower DMI, the lambs on Enhancer outperformed those grazing Midmar, in terms of liveweight gains and carcass quality. Although Enhancer had a much higher (20%) DM content than Midmar, the DM content of Midmar in the present experiment was probably sufficiently high ( $180.6 \text{ g kg}^{-1}$ ) not to have lowered its intake by the weaned lambs. Observed differences in animal performance between the two ryegrass cultivars could be largely due to the improved readily digestible energy to protein ratio. The potential benefit of these factors to the lambs grazing Enhancer appears to have been partially negated by the reduction in DMI. The fact that animal production was maintained at the relatively low DMI of Enhancer as compared with Midmar, suggests that an important advantage of pasture grasses high in nonstructural carbohydrates is the possibility that the stocking rate could be increased, giving higher animal production per unit area.

## Chapter 6

### The relative performance of Holstein dairy cows grazing *Lolium multiflorum* cultivars Enhancer and Dargle

#### 6.1 Introduction

*Lolium multiflorum* (Italian and Westerwolds ryegrass) is an important forage crop for milk production in many countries. However, in a sub-tropical environment, this forage grass usually does not fulfil the requirement of the dairy animal for readily digestible energy. A lack of readily digestible energy may result in the loss of a large proportion of dietary nitrogen as rumen ammonia, due to the inefficient incorporation of non-protein nitrogen into microbial protein (Beever *et al.*, 1986, Ulyatt *et al.*, 1988). This is partly a result of the high solubility and breakdown of leaf proteins by plant and microbial proteases to release amino acids and ammonia. Although these breakdown products are normal precursors for the synthesis of microbial protein, a source of readily available energy is required to enable the rumen microbes to utilize the available nitrogen to synthesise microbial protein and capture all the ammonia produced (Miller *et al.*, 2001). When there is insufficient energy supplied from carbohydrate fermentation, amino acids are used as an energy source; which leads to an accumulation of ammonia in the rumen. When fermentable carbohydrates are readily available in the rumen, amino acids are taken up by microorganisms and can be incorporated into microbial protein (Miller *et al.*, 2001).

Furthermore, the moisture content of South African *L. multiflorum* cultivars tends to be relatively high (Meissner *et al.*, 1992) and dry matter intake can be reduced if the moisture content of forages is excessively high (John and Ulyatt, 1987). According to Sheaffer *et al.* (1998) forage quality is best evaluated by measuring dairy performance. However, agronomically, forage quality is described in terms of nutritive value, intake potential and anti-quality factors (Marten, 1985) and is in turn influenced by sward characteristics, maturity, management and the environment.

The objective of this study was to compare two *L. multiflorum* cultivars in a grazing trial; Enhancer, which was bred from plants with elevated levels of DM content and nonstructural carbohydrates, and Dargle, a cultivar developed from plants selected for their superior yield potential without consideration for any herbage quality selection criteria. The two cultivars therefore potentially have different quality traits which may influence herbage intake and milk production of Holstein dairy cows.

#### 6.2 Materials and methods

##### 6.2.1 Grazing trial

The area and cultivars of ryegrass used in this experiment are identical to those described in Chapter 3. Fifty Holstein dairy cows were randomly allocated to each of the Enhancer and Dargle Italian ryegrass cultivars in October 2001. The pastures were strip grazed. Of the fifty Holstein dairy cows in each treatment, thirty-two animals in early- to mid lactation,  $148 \pm 10.9$  days in milk (mean  $\pm$  s.e.),

were used in the intake and milk production study which was conducted using a cross-over design from 7 to 16 October and 22 to 31 October 2001. Prior to the onset of the study, the 32 lactating animals produced  $33.4 \pm 0.32$  (mean  $\pm$  s.e) kg milk cow<sup>-1</sup> day<sup>-1</sup> whilst grazing Dargle ryegrass and supplemented with 8 kg concentrates in the form of Meadow Hilak 15% meal (Meadow Feeds Natal, Pietermaritzburg, South Africa). At the onset of the experimental period, milk production was  $31.0 \pm 1.24$  (mean  $\pm$  s.e) kg milk cow<sup>-1</sup> day<sup>-1</sup>. During the study, milk production was recorded daily and samples collected twice weekly for milk composition analysis. Milk samples were preserved with 800 Broad Spectrum Microtabs® II (D & F Control Systems, California, USA) and analysed using a milkoscan S 50 (Type 75600), infrared analyzer (FOSS Electric A/S, Hillerød, Denmark). The cows had free access to fresh water in portable water troughs and received 8 kg of concentrates (Meadow Hilak 15% meal) daily, fed in two equal portions after milking at 06:00 and 15:00. The nutritional composition of the formulated ration is outlined in Table 6.1.

Table 6.1 Nutritional composition of formulated ration (Meadow Hilak 15% meal).

Nutrient (g kg <sup>-1</sup> DM)	Composition
Ash	76.3
Fat	56.7
ADF	74.0
NDF	220.1
Starch	353.8
Protein	153.1
Ca	11.9
Mg	3.2
K	10.8
Na	4.2
P	6.0
Zn (mg kg <sup>-1</sup> )	303
Cu (mg kg <sup>-1</sup> )	52
Mn (mg kg <sup>-1</sup> )	216

### 6.2.2 Estimation of intake and digestibility

Dry matter intake of the dairy cows was estimated using a modification of the *n*-alkane technique (Marais *et al.*, 1996). The two intake studies (7 to 16 October and 22 to 31 October 2001) included a 7-day adaptation period followed by a 10-day experimental period. Each cow was dosed twice daily (06:00 and 15:00) with 1 g of C<sub>32</sub> in the form of a suspension (160 ml). During the experimental period

rectal faecal samples were collected twice daily for each animal and pooled, providing one sample a day for each animal over the 10 day experimental period. These samples were dried at 60°C and milled to pass a 1 mm sieve before being analysed for *n*-alkanes. Herbage intake was estimated using the C<sub>32</sub>/C<sub>33</sub> alkane pair, since the recoveries of C<sub>32</sub> and C<sub>33</sub> in this trial were found to be similar and this alkane combination most accurately estimated known herbage intakes (Mayes *et al.*, 1986c; Dillon and Stakelum, 1989, 1990; Stakelum and Dillon, 1990). The recoveries of C<sub>32</sub> and C<sub>33</sub> in the concentrate samples were too low to be included in the digestibility estimation.

### 6.2.3 Herbage sampling

Herbage pluck samples were obtained throughout the season (June to October 2001) immediately before grazing at 14:00 by hand-plucking to an approximate grazing height, representative of that consumed by the cows. All samples were initially frozen, dried at 80°C and milled to pass a 1 mm sieve in preparation for chemical analysis as described in Chapter 3. Pasture yield (kg DM ha<sup>-1</sup>), apparent intake and regrowth following defoliation, were determined using the rising plate meter (RPM) (Earle and McGowan, 1979). The RPM was calibrated from 50 individual meter readings (disc height in cm and pasture yield under the disc) pre-and post-grazing from the two cultivars and subjected to regression analysis. The prediction of pasture yield is given by the equations:

$$\text{Enhancer: Pasture yield (kg DM ha}^{-1}\text{)} = 682 + 75.88 \times \text{disc meter height (cm)}$$

$$\text{Dargle: Pasture yield (kg DM ha}^{-1}\text{)} = 83 + 75.88 \times \text{disc meter height (cm)} \text{ (} r^2 = 0.54 \text{) (} P < 0.001 \text{)}$$

### 6.2.4 Statistical analysis

Milk production data was analysed by analysis of covariance, with pre-experimental (12 weeks) milk yields as covariates for period 1 and milk yield from period 1 as covariates for period 2, using Genstat 5, Release 4.2 (Genstat, 2000). Dry matter intake (DMI), digestibility, nutritional and mineral composition data were all subjected to analysis of variance in a similar way but without covariate adjustment.

## 6.3 Results and discussion

### 6.3.1 Nutrient composition of ryegrass pastures

The mean nutrient content and mineral composition (g kg<sup>-1</sup> DM) of the two ryegrass cultivars throughout the season (mean of 17 samples) are outlined in Tables 6.2 and 6.3, respectively. The nutrient content of Enhancer and Dargle during the cross-over study showing the two different periods (mean of 10 samples per period) is presented in Table 6.4. The seasonal TNC concentration of Enhancer and Dargle is illustrated in Figure 6.1.

Table 6.2 Mean nutrient composition of the *L. multiflorum* cultivars Enhancer and Dargle during the growing season (June to October 2001).

Nutrient composition (g kg <sup>-1</sup> DM)	Ryegrass pasture		CV %	S.E.M.	Significance
	Enhancer	Dargle			
DM (g kg <sup>-1</sup> fresh grass)	224.3	187.4	6.5	0.33	***
TNC	128.5	69.3	30.6	7.35	***
NDF	440.6	472.2	5.2	0.58	**
ADF	249.4	284.4	14.2	0.92	*
ADL	74.6	84.7	21.1	0.41	NS
Nitrate-N	1.8	3.3	54.3	0.34	**
<i>in vitro</i> DMD	681.4	673.6	6.1	0.99	NS
True protein	175.4	193.8	11.4	0.51	*
Total N	39.5	46.8	5.7	0.6	***

NS, not significant; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$

DM = dry matter; TNC = total nonstructural carbohydrates; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; *in vitro* DMD = *in vitro* dry matter digestibility; Total N = total nitrogen

Since most of the feed is digested by microorganisms in the rumen, a strong interaction exists between carbohydrate and protein (N) metabolism. If there is insufficient carbohydrate to match protein, excess ammonia is produced which is not assimilated into microbial protein and is consequently lost in the form of urea (Nocek and Russell, 1988). Enhancer ryegrass had a mean TNC concentration of 128.5 g kg<sup>-1</sup> DM throughout the growing season, significantly higher (85%;  $P < 0.001$ ) than that of Dargle, and true protein and total nitrogen contents significantly lower ( $P < 0.05$  and  $P < 0.001$ , respectively) (Table 6.1). These values for Enhancer supported the findings of Marais *et al.* (2003) for plants grown in spaced-plant trials. The high TNC concentration of Enhancer suggests that a more effective protein metabolism can be induced in the rumen (Poppi *et al.*, 1997). These results are consistent with results of Nowakowski and Byers (1972) who found that a decrease in the non protein-N fraction will improve the nutritive value of herbage. The higher TNC concentration relative to the true protein content of Enhancer can also result in more efficient rumen fermentation with a higher yield of volatile fatty acids, particularly propionic acid (Beever *et al.*, 1978), although this can only be inferred as no direct measurements were obtained. The total-N content of Enhancer was significantly lower (18%;  $P < 0.001$ ) than that of Dargle. This is in accordance with Humphreys (1989b) who reported a reduction in total-N in grasses of high WSC concentration. A significantly lower (89%;  $P < 0.01$ ) Nitrate-N content was found in Enhancer compared with Dargle. This reduced accumulation of toxic levels of nitrate is a further benefit of the high TNC and low total-N content of Enhancer, since

low nitrogen content is often associated with low nitrate levels (Marais *et al.*, 2003). Dargle had a significantly higher (7%;  $P < 0.01$  and 14%;  $P < 0.05$ , respectively) NDF and ADF content than Enhancer.

Table 6.3 Mean mineral composition of the *L. multiflorum* cultivars Enhancer and Dargle during the growing season (June to October 2001).

Mineral composition (g kg <sup>-1</sup> DM)	Ryegrass pasture		CV %	S.E.M.	Significance
	Enhancer	Dargle			
Ca	4.1	4.4	11.4	0.12	NS
Mg	2.5	2.8	15.7	0.09	*
K	33.0	44.0	15.6	1.46	***
Na	2.5	2.6	35.6	0.22	NS
P	3.3	3.7	10.4	0.09	**
Zn (ppm)	36.3	35.9	11.2	0.98	NS
Cu (ppm)	9.6	14.8	56.2	1.67	*
Mn (ppm)	119.4	85.1	17.1	4.24	***

NS, not significant; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$

A critical factor in ryegrass pastures is the negative effect of K uptake on the uptake of Ca and Mg (Miles, 1986). Furthermore, a deficiency of Mg and Ca in the blood serum, together with a high K level, can result in grass tetany (hypomagnesaemia) in the grazing animal (Allcroft and Burns, 1968). The Mg and K levels in Dargle were significantly higher respectively (13%;  $P < 0.05$  and 25%;  $P < 0.001$ ), than those of Enhancer. The higher K content of Dargle is associated with its higher Total N content. These results confirm the findings of Nowakowski and Byers (1972) who reported the effect of potassium on both carbohydrate and nitrogen metabolism. Azevedo and Rendig (1972) showed that a K:Ca+Mg ratio (where ion concentrations are expressed on an equivalents basis) in excess of 2.2 was linked to an increased incidence of grass tetany. In Enhancer and Dargle, the K:Ca+Mg ratios were 1.95 and 2.5, respectively, both of which are not regarded as critically high. The Na levels in Table 6.2 were similar to the value of 3.7 g Na kg<sup>-1</sup> DM reported by Fulkerson *et al.* (1998). The K:Na ratio was measured at 13.3 and 16.7 for Enhancer and Dargle, respectively. In this study, the cows on both cultivars received a nutritionally balanced concentrate with recommended mineral allowances (see Table 6.1), therefore, any differences in the performance of the cows is not likely due to the differences in mineral content between Enhancer and Dargle.

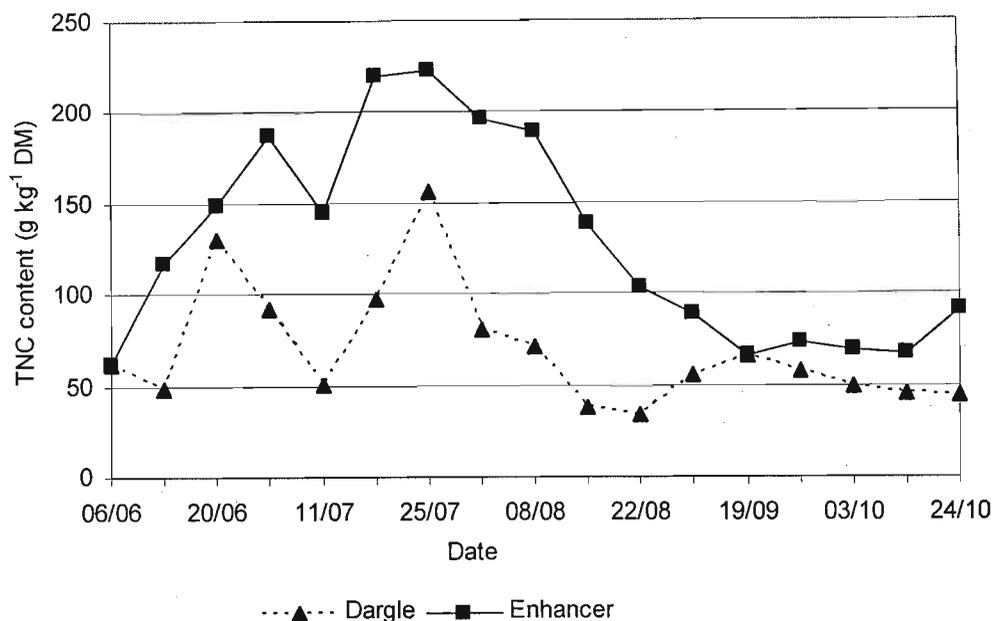


Figure 6.1 Mean seasonal TNC concentration of Enhancer and Dargle ryegrass cultivars.

Seasonal variation in the TNC concentration of the two ryegrass cultivars in the current study (Figure 6.1) followed similar trends to those previously observed in perennial ryegrass (Waite and Boyd, 1953; Humphreys, 1989b; Radojevic *et al.* 1994). From the beginning of June, the TNC concentration of Enhancer was always higher than that of Dargle and peaked at 223 g kg<sup>-1</sup> DM towards the end of July, while the concentration of TNC in Dargle peaked at 156 g kg<sup>-1</sup> DM. The TNC concentration in both cultivars dropped towards the end of the season, possibly due to the simultaneous increase in fibre content and from the end of September the differences in TNC concentration between the two cultivars was small. The fluctuations in TNC concentration towards the end of the season would appear to be associated with floral initiation and carbohydrate formation in the seed. The TNC concentration of ryegrass is negatively correlated with the nitrogen content of the plant (Jones, 1970). Therefore, as nitrogen stimulates growth in the plant, the demand for nonstructural carbohydrates increases, thereby reducing the TNC concentration. Conversely, in conditions that limit growth but not photosynthesis, the TNC concentration increases. These effects could be environmentally induced and may not be relevant to heritable differences in the plant (Humphreys, 1989b).

Table 6.4 Mean nutrient composition of the *L. multiflorum* cultivars Enhancer and Dargle during the cross-over study (7 to 16 October 2001 for period 1, and 22 to 31 October 2001 for period 2).

Nutrient composition (g kg <sup>-1</sup> DM)	Period 1					Period 2				
	Enhancer	Dargle	CV %	S.E.M	Significance	Enhancer	Dargle	CV %	S.E.M	Significance
DM (g kg <sup>-1</sup> fresh grass)	201.3	179.8	6.3	0.38	**	202.1	160.4	6.1	0.4	***
TNC	79.4	55.7	13.4	2.86	**	75.2	44.4	20.8	3.93	**
NDF	460.8	466.7	3.6	0.53	NS	463.7	471.2	3.4	0.56	NS
ADF	273.9	260.5	7.3	0.62	NS	288.1	268.3	9.7	0.95	NS
ADL	63.4	62.5	21.8	0.43	NS	79.8	62.4	21.1	0.47	NS
Nitrate-N	1.7	2.2	39.6	0.25	NS	1.89	2.59	50.9	0.36	NS
<i>in vitro</i> DMD	727.9	642.6	3.5	0.75	**	638.5	624.2	3.7	0.73	NS
True protein	177.4	193.0	10.5	0.62	NS	179.8	186.8	14.6	0.85	NS
Total N	38.8	45.7	5.9	0.79	***	41.3	45.4	10.4	1.59	NS

NS, not significant; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$

DM = Dry matter; TNC = total nonstructural carbohydrates; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; *in vitro* DMD = *in vitro* dry matter digestibility; Total N = total nitrogen

Enhancer had mean DM and TNC contents 12% and 43% higher, respectively, in period 1 and 26% and 70% higher in period 2 than the DM and TNC contents of Dargle (Table 6.3). These values for Enhancer were significantly higher ( $P < 0.01$  and  $P < 0.001$ ) than for Dargle and were consistent with results reported by Marais *et al.* (1997) for plants grown in spaced-plant trials. The *in vitro* DMD (IVDMD) values were significantly higher ( $P < 0.01$ ) for Enhancer compared with Dargle in period 1, while no significant differences were found in period 2. The higher IVDMD values of Enhancer compared with Dargle are largely due to the significantly ( $P < 0.01$ ) higher TNC concentration of Enhancer. The higher IVDMD values of Enhancer observed in this experiment are consistent with results reported by Grimes *et al.* (1967) who found strong positive correlations between soluble carbohydrate content and digestibility over a range of species.

### 6.3.2 Pasture availability and growth

Results of the available and residual grass after grazing, as measured by the rising plate metre over the two experimental periods (7 to 16 October and 22 to 31 October), are presented in Figures 6.2 and 6.3 respectively.

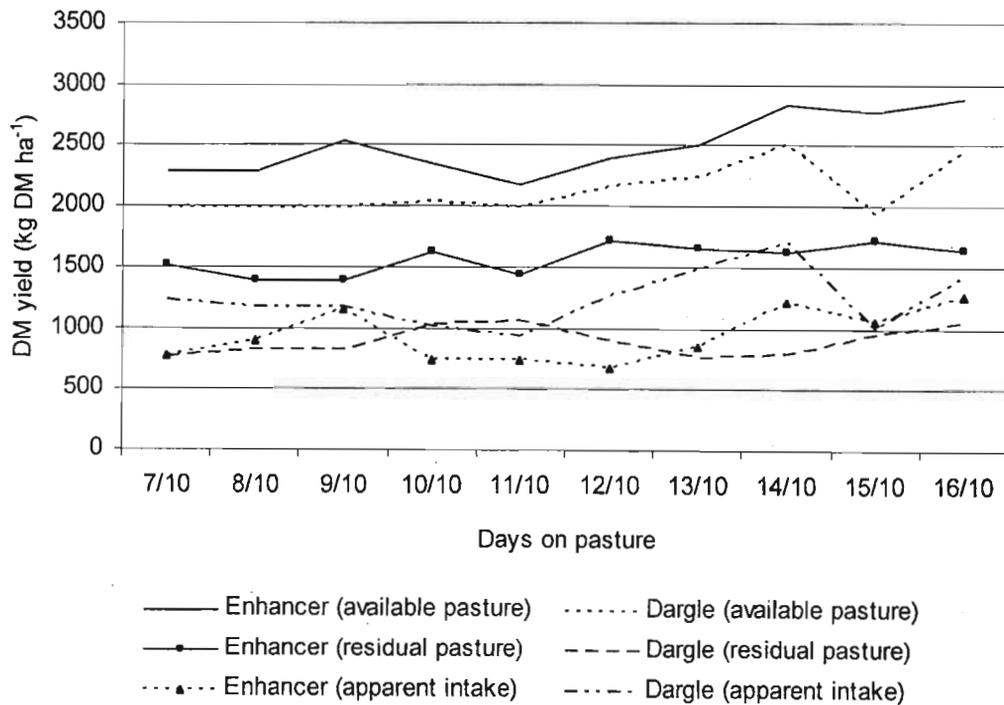


Figure 6.2 Apparent intakes and the available and residual pasture of Enhancer and Dargle ryegrass cultivars for period 1 (7 to 16 October 2001).

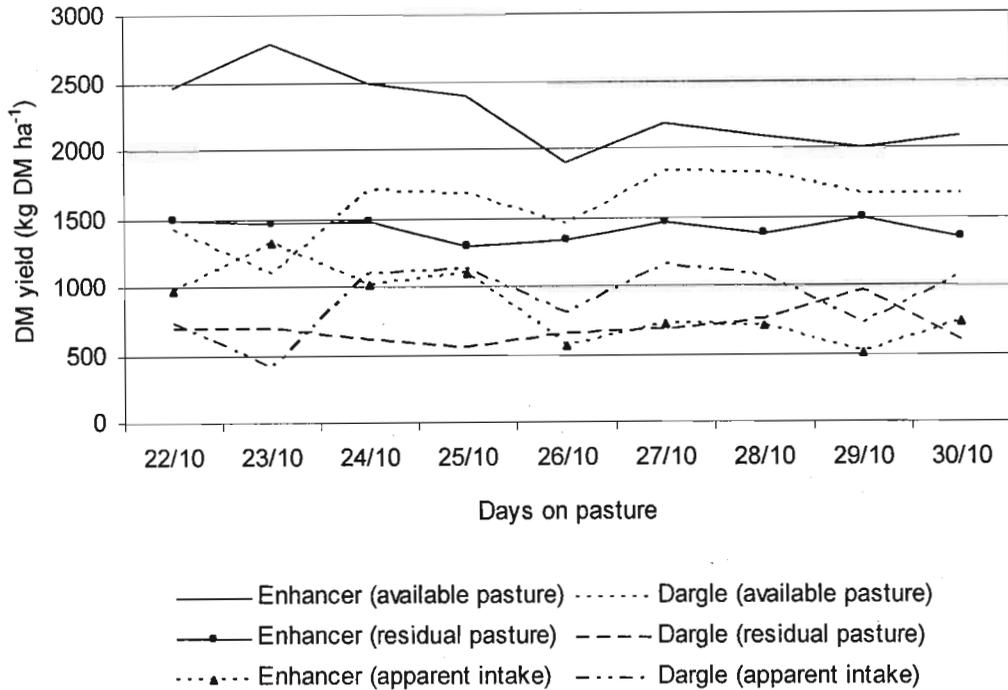


Figure 6.3 Apparent intakes and the available and residual pasture on Enhancer and Dargle ryegrass cultivars for period 2 (22 to 31 October 2001).

Enhancer had a significantly higher ( $P < 0.001$ ) available and residual pasture, respectively, than Dargle during period 1. However, the apparent intake on Dargle was significantly higher ( $P < 0.01$ ) than that of Enhancer in this period (Figure 6.2). Similarly in period 2, Enhancer had a significantly higher ( $P < 0.01$ ) available and residual pasture than Dargle, however, no significant differences in apparent intake were found between the two cultivars in the second period (Figure 6.3).

### 6.3.3 Performance of Holstein dairy cows.

The performance of dairy cows grazing Enhancer and Dargle during the period of study is presented in Table 6.5. Figure 6.4 shows the milk yield (kg cow<sup>-1</sup>day<sup>-1</sup>) of cows grazing Enhancer and Dargle during the cross-over study.

Table 6.5 Performance of Holstein dairy cows grazing ryegrass pastures during the cross-over study (7 to 16 October 2001 for period 1, and 22 to 31 October 2001 for period 2).

	Period 1					Period 2				
	Enhancer	Dargle	CV %	S.E.M	Significance	Enhancer	Dargle	CV %	S.E.M	Significance
Milk yield (kg cow <sup>-1</sup> day <sup>-1</sup> )	32.24	30.74	6.2	0.49	*	28.86	29.48	8.6	0.67	NS
Milk constituents (%)										
Butterfat	3.14	3.03	18.3	0.14	NS	3.30	3.26	18.9	0.16	NS
Protein	3.12	3.09	7.9	0.06	NS	3.04	3.08	8.5	0.06	NS
Lactose	4.86	4.84	3.6	0.04	NS	4.84	4.83	3.3	0.04	NS
DM intake using C <sub>32</sub> /C <sub>33</sub> alkane pair (kg DM day <sup>-1</sup> )	10.05	10.79	10.3	0.267	*	14.25	14.14	18.2	0.646	NS

NS, not significant; \**P* < 0.05

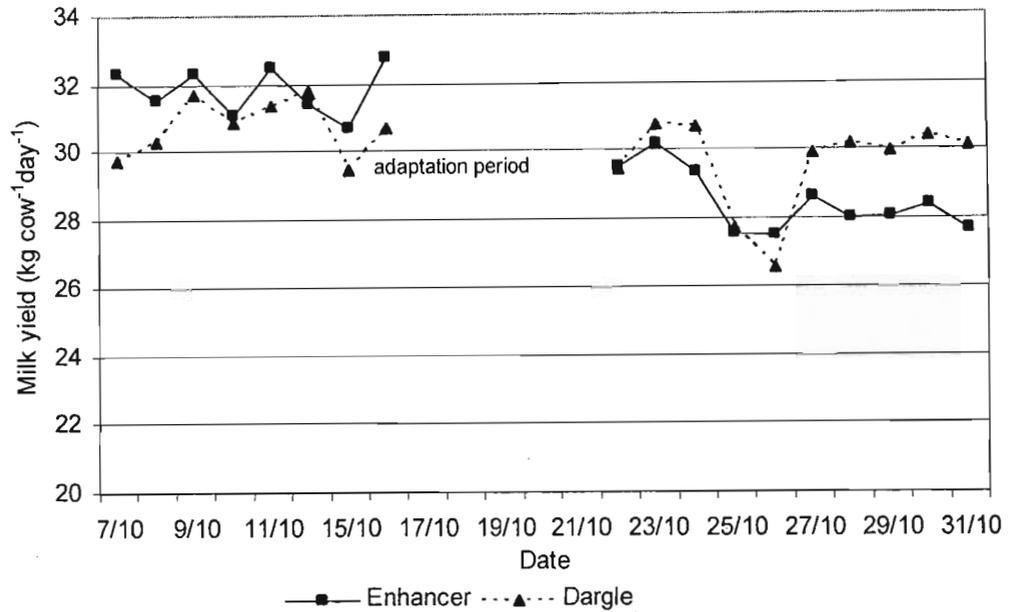


Figure 6.4 Milk yields of cows grazing Enhancer and Dargle ryegrass cultivars during the cross-over study.

In period 1, milk yields from cows grazing Enhancer were significantly higher ( $1.5 \text{ kg milk cow}^{-1} \text{ day}^{-1}$ ;  $P < 0.05$ ) than for cows grazing Dargle. No significant differences in milk yield were found in period 2. Notwithstanding the fact that the milk yields for cows grazing Enhancer and Dargle were similar ( $29.46$  and  $29.54 \text{ kg cow}^{-1} \text{ day}^{-1}$ , respectively) at the onset of period 2, it would appear from Figure 6.4 that the cows grazing Dargle (having previously grazed Enhancer in period 1) had a slightly higher milk yield than cows on Enhancer. This may have been due to carry-over effects (Broster and Broster, 1984) as a result of a limited adaptation period. Furthermore, despite the fact that the cows grazing Enhancer had more pasture available than cows on Dargle (Figure 6.3), it is possible that the Enhancer material may have been in an earlier stage of reproduction than Dargle, since the ADF and ADL content of Enhancer was higher (although not statistically significant) than that of Dargle; and consequently Enhancer was of a lower nutrient quality. The increase in milk yield on Dargle from 27/10 corresponds with the increase in intake of Dargle compared with Enhancer during the same period (Figure 6.3).

In period 1, the DMI of cows on Dargle ryegrass was significantly higher ( $P < 0.05$ ) than for cows on Enhancer, although the DM content of Enhancer was 12% higher than that of Dargle. The higher milk yield for cows grazing Enhancer may have resulted from the significantly higher ( $P < 0.01$ ) IVDMD during the same period, even though DMI did not increase with the improved IVDMD of Enhancer compared with Dargle. These results are similar to those reported by Holmes (1987). No significant differences in DMI were found in period 2.

#### **6.4 Conclusion**

In period 1, despite the lower DMI of cows on Enhancer compared with those on Dargle, the cows grazing Enhancer outperformed those grazing Dargle in terms of milk yields. This increase in milk yield may be due to an improvement in the efficiency of utilisation of dietary nitrogen as a result of the significantly higher ( $P < 0.001$ ) TNC concentration of Enhancer. The higher TNC concentration relative to the protein content of Enhancer suggests a more favourable readily available energy to protein ratio for milk production.

## Chapter 7

### General discussion

Formal selection and breeding programmes for improved cultivar development began in the late 19<sup>th</sup> century in Europe and North America. These early efforts focused on forage and seed yield, growth habit, timing of reproductive maturity, disease resistance and persistence. The documented reports on high quality forages were vague in their definition of 'high quality', which referred to plants and strains that were free from visual blemishes, insect damage, or early senescence. The modern meaning of forage quality can be regarded as the 'ability of forage to support animal functions such as maintenance, growth, reproduction and lactation' (Casler, 1997). The move towards improved forage quality has shown consistent and sustained improvements through at least three selection cycles in different species. Forage quality and yield have been increased simultaneously in some species, while in others; forage yield has decreased or remained constant. Despite some species having a decreased yield, the increased forage quality of some cultivars is enough to compensate for the yield reduction, giving higher animal gains (profit) per hectare (Casler, 1997).

Following the results from a survey of important nutritive value traits in forage crops (Wheeler and Corbett, 1989), and the relatively low dry matter (DM) and total nonstructural carbohydrate (TNC) concentration in early- and late-season regrowth of *L. multiflorum* (Marais *et al.*, 1993), local breeding programmes focused on developing cultivars with high DM and TNC concentrations to improve animal performance. Improved animal production can be expected from *L. multiflorum* if the DM content is raised to above 180 g kg<sup>-1</sup> fresh material, due to an expected more rapid breakdown of feed particles during ingestion, a faster rate of passage of digesta and possibly an extension of the total daily grazing period of the high-DM grass. Since leaf proteins from green herbage are highly soluble and readily de-aminated by plant and microbial proteases, up to 40% of dietary nitrogen can be lost from the rumen if the rumen microbes do not have access to a source of readily available energy (Miller *et al.*, 2001). Microbial protein synthesis and subsequent animal production should therefore be improved by increasing the nonstructural carbohydrate concentration of *L. multiflorum* by breeding and selection, provided no anti-quality factors are introduced.

During the first study (Chapter 4), in both the warm and cold regimes under strictly controlled environmental conditions, the DM and TNC concentration of the predominantly Italian ryegrass cultivar Enhancer were noticeably higher than those of the predominantly Westerwolds ryegrass cultivar Midmar and do not appear to be associated with any of the known anti-quality factors such as a high ADF and ADL content. The concentration of these factors was lower in Enhancer than in Midmar ryegrass. The excessively high nitrate-N content in both ryegrass cultivars could be attributed to the high organic carbon content in the soil and the undrained pots which prevented leaching of nitrate-N which should not occur in the field. Enhancer had significantly lower nitrate-N contents than Midmar which could be attributed to cultivar differences. The mineral contents of both cultivars were above the

critical range reported by Miles (1998). However, Midmar had a considerably higher mineral content than Enhancer in both temperature regimes which is possibly related to the higher nitrate-N content of Midmar or to a dilution effect due to the higher TNC content.

The DM and TNC concentrations of Enhancer were significantly higher than those of Midmar in the weaned lamb grazing trial (Chapter 5) in winter. The main anti-quality factors ADF, ADL and the nitrate-N content were lower in Enhancer than in Midmar. These results were similar to the data obtained in the controlled growth chamber during the warm and cold regimes, which simulated autumn and winter conditions, respectively. It would appear from the controlled environment study and grazing trial that the differences in nutritional parameters between the two ryegrass cultivars were mainly of genetic origin, particularly since environmental effects were reduced in the growth chamber, and in the grazing trial both ryegrass cultivars had identical fertilizer and irrigation schedules and were sampled at the same time.

The vast amount of information relating to forage intake and the factors that influence it are often inconsistent (Balch and Campling, 1962; Bailey, 1964, 1965; Ingalls *et al.*, 1965; Raymond, 1969; Vérité and Journet, 1970; Thornton and Minson, 1972; Cooper, 1973; John and Ulyatt, 1987; Poppi *et al.*, 1987; NRC, 2001). Although Enhancer was selected for improved DM content with the potential to increase DMI by the grazing ruminant, in the current study, the DM contents of Midmar and Dargle were sufficiently high not to affect intake. Bailey (1964, 1965) and Cooper (1973) suggested that voluntary intake and palatability of Italian ryegrass can be improved by increasing the nonstructural carbohydrate content. In both grazing trials in the current study, the higher TNC concentration of Enhancer did not result in improved DMI. According to Poppi *et al.* (1987) there is no one factor that can explain intake regulation, rather a combination of factors, both physical (e.g. rate of disappearance of digesta from the rumen and rumen fill) and metabolic (e.g. related to the rate at which nutrients are supplied to and utilized by the tissues) are integrated to control intake. Another significant advance in the intake regulation by a mechanism which integrates physical and metabolic factors is the influence of the physiological state of the animal. In order to explain the lower DMI of both weaned lambs and Holstein dairy cows grazing Enhancer in the current study and to form a hypothesis, further investigations measuring metabolic and physiological parameters would be required. These include measuring substances from the digestive tract, outflow of end products such as microbial protein, rumen fill (volume effects) in relation to rumen fermentation rate and passage rate and other pasture management factors.

There were no significant differences in available and residual pasture on Midmar and Enhancer in the trial with weaned lambs. Despite the DMI being significantly higher on Midmar than on Enhancer, the weaned lambs grazing Enhancer outperformed the animals on Midmar in terms of liveweight gain and carcass quality. These differences can be attributed to the 98% higher TNC concentration of Enhancer relative to Midmar. In the trial with Holstein dairy cows grazing Enhancer and Dargle (Chapter 6), similar trends were found. Cows grazing Enhancer had notably higher milk yields in the

first period of the cross-over study, despite a lower DMI by these animals compared with those on Dargle. These differences were possibly due to the 43% higher TNC concentration in Enhancer relative to Dargle.

The superior animal performance in the current studies may be due to improvements in the balance of energy and nitrogen supply to the rumen as a result of the increased TNC concentration, which provides a source of energy to the micro-organisms. Since the TNC concentration is naturally present in the grass and provides readily available energy, it is possible that the supplemental feeding of concentrates to dairy cows can be reduced; however, further experimentation is required to investigate this aspect. Furthermore, the lower nitrogen levels in Enhancer throughout the season due to the higher TNC concentration may also result in a more effective protein metabolism in the rumen. In intensive forage systems, nitrogen fertiliser application is not only a key factor in determining DM yield but is positively correlated to the nitrogen and nitrate-N content of the grass (De Villiers and van Ryssen, 2001). Since only a moderate nitrogen level in the grass is required for optimal animal production, this may require a reduction in fertiliser application in existing *L. multiflorum* cultivars, which could adversely affect DM production (Marais *et al.*, 2003). However, Casler (1998) found that traits other than forage yield are important for dairy production. In a study by Clark and Wilson (1993) it was predicted that a 5% increase in pasture digestibility, combined with a 5% decrease in forage yield, would still increase profits for dairy farming. Although it is possible that a reduction in nitrogen fertiliser could compromise the DM yield, it is likely that the improved forage quality of cultivars such as Enhancer would override the effects of reduced DM production and still enhance animal performance; however, further investigations are required to confirm this.

The results of the present study endorse the hypothesis that feeding forage high in TNC concentrations can improve the utilization of dietary nitrogen for microbial protein synthesis which, in turn, can contribute to increased growth rates of weaned lambs and milk yields of dairy cows. These results support the observations by Poppi *et al.* (1997) who found that increasing the efficiency of microbial protein production is one of the most important strategies in improving the forage quality of temperate grasses.

Further experiments with longer periods of assessment, investigating the mechanisms involved in Enhancer, with its high DM and TNC concentration and the expected improved microbial protein synthesis in the rumen are required. Should further investigations confirm the results of the current study, considerable annual financial benefits of utilizing Enhancer Italian ryegrass could be achieved. There are an estimated 100 000 dairy cows producing milk off pastures on the eastern seaboard of South Africa (Dugmore, 2003). The vast majority of these dairy cows utilize irrigated ryegrass pastures for a minimum of 180 days during the period mid-autumn to mid-spring. Assuming that factors such as poor management do not play a role, farmers in South Africa could gain substantial financial benefit by utilizing Enhancer Italian ryegrass, with concomitant increased milk yields, rather

than ryegrass cultivars with relatively lower herbage quality. The estimated potential value increase of utilizing Enhancer Italian ryegrass is calculated as follows:

$$100\ 000\ \text{cows} \times 1\ \text{L} \times 180\ \text{days} \times \text{R2/L} = \text{R36 million}$$

Regarding future endeavours to further improve the forage quality of ryegrass pastures, it may be possible to breed for higher TNC levels, as well as other factors such as improved digestibility. Tetraploid ryegrasses are known to be generally higher in carbohydrates and more palatable than diploid ryegrasses (Castle and Watson, 1971). By doubling the chromosome number, by colchicine induction, of Enhancer and other diploid varieties with improved forage quality, the TNC levels may be further increased. Obviously, any new cultivar with TNC and DM levels and milk yields higher than Enhancer, would undoubtedly be commercialized and find immediate acceptance by the farming community.

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## **Publications**

Short paper and poster abstracts: 38<sup>th</sup> Congress of the South African Society of Animal Science

## The performance of weaned lambs grazing a high dry matter and nonstructural carbohydrate selection of *Lolium multiflorum*

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### Introduction

*Lolium multiflorum* cultivars are widely utilised in South Africa as autumn and late-winter/spring pastures, and their seasonal growth pattern during the year is well suited to the traditional lambing period (De Villiers, 1991). However, since there is increasing evidence of poor performance of weaned lambs on *L. multiflorum* (Rutter, 1970; De Villiers, 1991; Meissner, 1996), the actual herbage intake, liveweight gain, wool growth and carcass quality of weaned lambs on selection 121-A ryegrass was determined and compared with these indices on Midmar ryegrass.

### Materials and Methods

Lambs were weaned at the 12<sup>th</sup> week of lactation and remained on the two ryegrass pastures at a stocking rate of 20 weaned lambs/ha. The lambs on Midmar and 121-A had average weaning weights of 26.19 and 26.20 ± 1.45 kg, respectively. An eight-paddock rotational grazing system, with 3.5 days spent in each paddock, was used. This allowed a 24.5 day re-growth period. The lambs were weighed weekly in order to determine the liveweight gain of each animal. Dry matter (DM) intake and digestibility of the weaned lambs were measured using *n*-alkanes as indigestible markers. The lambs remained on the two pastures until a marketable mass was obtained and were individually classified and slaughtered at Cato Ridge abattoir at the end of the trial.

### Results and Discussion

The DM intake of weaned lambs was significantly higher (29 %;  $P < 0.01$  and 21 %;  $P < 0.05$ ) on Midmar than 121-A using the C<sub>31</sub>/C<sub>32</sub> and C<sub>32</sub>/C<sub>33</sub> alkane pairs respectively. No significant differences in digestibility were found between Midmar and 121-A ryegrass. In the first week after weaning, lambs grazing 121-A had a higher (73 %;  $P < 0.05$ ) ADG (g/day) than lambs grazing Midmar. The post-weaning ADG of lambs grazing Midmar and 121-A was 164 and 185 g/day respectively. The lambs on 121-A ryegrass were significantly ( $P < 0.05$ ) higher in mass than lambs on Midmar. After 77 days on the pasture, the lambs stocked at a rate of 20 lambs/ha on Midmar and 121-A and weaned at an average mass of 26.19 and 26.20 ± 1.45 kg, respectively reached a final mass of 38.8 ± 1.78 and 40.45 ± 1.79 kg, respectively. The lambs on 121-A gained 14.25 kg post-weaning while lambs on Midmar gained 12.61 kg, despite the fact that the lambs had similar weaning weights. No significant differences were found for wool growth and warm or cold carcass mass. The lambs on 121-A had a significantly higher ( $P < 0.01$  and  $P < 0.05$ ) hindfat and forefat than the lambs on Midmar. The Rand/carcass value for Midmar and 121-A was R259.00 and R296.00, respectively but there was no statistically significant difference.

### Conclusion

Despite the lower DM intake on 121-A, the lambs outperformed those grazing Midmar, in terms of liveweight gains and carcass mass (although not statistically different). Although selection 121-A had a much higher (20%) DM content than Midmar, the DM content of Midmar in this experiment was probably high enough (180.6 g/kg) not to have resulted in lowered intake by the weaned lambs. Observed differences in animal performance between the two ryegrasses were therefore possibly largely due to the 98% higher TNC content of selection 121-A.

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## The development of a *Lolium multiflorum* cultivar with a low moisture content and an increased readily digestible energy to protein ratio

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**Abstract.** A breeding program was conducted to improve the forage quality of *Lolium multiflorum*. Fifteen cultivars grown in spaced-plant trials were subjected to selection for a low moisture and a high non-structural carbohydrate content. Results showed consistent positive correlations between non-structural carbohydrate content, dry matter content, and digestibility *in vitro* of the grass. In a small plot trial comparing F<sub>6</sub> plants (Selection 121) with 11 commercial *L. multiflorum* cultivars, Selection 121 gave the second-highest dry matter yield (difference not significant,  $P > 0.05$ ) of the cultivars tested. The dry matter content of Selection 121 was significantly higher and the acid detergent fibre content significantly lower than that of the commercial cultivars. Furthermore, the total non-structural carbohydrate content was significantly higher and the nitrogen content significantly lower than that of the other *L. multiflorum* cultivars, giving Selection 121 nutritionally a much more favourable readily digestible energy to protein ratio than the commercial cultivars. Selection 121 was subsequently named 'NCD Enhancer' and is inscribed on the South African Variety List.

**Additional keywords:** nitrogen, nitrate, acid detergent fibre, acid detergent lignin, disease resistance, nutritive value.

### Introduction

Italian and Westerwolds ryegrasses (*Lolium multiflorum*) are important winter forages for milk production in Australia, South Africa, New Zealand, Great Britain, Europe, and parts of the United States of America and South America. They are highly valued for forage/livestock systems due to their high palatability and digestibility. However, animal performance on ryegrasses in South Africa is often disappointing, possibly as a result of factors such as a high moisture content and a relatively low readily digestible energy to protein ratio. The moisture content of *L. multiflorum* in South Africa is usually above 820 g/kg fresh herbage (de Villiers 1991). Above this moisture level, forage intake by the grazing ruminant decreases with increasing moisture content (Vérité and Journet 1970; Burtis and Phillips 1987; John and Ulyatt 1987). Meissner *et al.* (1992) concluded that if the dry matter (DM) intake of Midmar Westerwolds ryegrass is to be maximised, the moisture content of the pasture should be below 800–820 g/kg fresh herbage.

Due to the high solubility of forage leaf protein it is readily deaminated in the rumen to form ammonia, much of which is absorbed into the bloodstream and subsequently excreted as urea. An important prerequisite for satisfactory protein metabolism is the presence in the forage of an adequate supply

of readily digestible carbohydrates (Poppi *et al.* 1997). Furthermore, an increase in the level of non-structural carbohydrates in herbage appears to result in increased consumption of grass by sheep (Michell 1973; Jones and Roberts 1991). A *Lolium perenne* cultivar selected for a high water-soluble carbohydrate content has recently been shown to improve liveweight gain in pre-weaned lambs (Lee *et al.* 1999) and milk production in dairy cows (Miller *et al.* 1999).

A breeding program was undertaken to improve the forage quality of *L. multiflorum* by selecting for both low moisture and high non-structural carbohydrate contents. The chemical composition of the selected line (Selection 121) was compared with that of available commercial cultivars.

### Materials and methods

When conducting a breeding and selection trial involving genetic characters such as total non-structural carbohydrate (TNC) and DM contents, which are also affected by environmental conditions, special precaution should be taken to minimise environmental effects.

#### Expt 1

The experiment was conducted on a Hutton soil type at the Cedara Research Station in the Natal Mistbelt, South Africa (29° 32' S 30° 16' E), at an altitude of 1075 m and with a mean annual rainfall of 885 mm. The long-term mean annual temperature is 16.2°C, and the long-term mean minimum temperature for the coldest months of the year (June and July) is 3.7°C.

Seed of 15 *L. multiflorum* cultivars or crosses (Table 1), including both diploid (D) and tetraploid (T) Italian (I) and Westerwolds (W) types, was germinated in Petri dishes in a controlled environment. Seedlings were transferred to seedling trays in a plastic tunnel. Six seedlings of each cultivar were planted in a spaced-plant nursery at 0.5-m centres. Prior to planting, basal P and K levels in the soil were restored to 20 and 150 mg/kg, respectively. Nitrogen was applied as limestone ammonium nitrate at a rate of 50 kg N/ha after each cut, and K was applied as potassium chloride at a rate of 50 kg K/ha after each alternate cut. Plants were irrigated at weekly intervals to an equivalent of 25 mm. Plants were well irrigated the day before sampling to eliminate local conditions of drought stress that would affect DM content. Cutting commenced at 1200 hours on sampling days, which corresponds with the time during the diurnal cycle when TNC content of pasture grasses starts levelling out after the rapid early morning rise (Marais and Figschou 1990). Plants were cut at a height of 50 mm above ground level every 4 weeks for DM determination and subsequent chemical analysis of individual plants. Just before flower emergence, the rust-free, 20 top-ranking plants in terms of their seasonal DM content, TNC content, and DM yield were re-established in an isolated polycross. Seed harvested from the polycross gave rise to the next generation of about 700 spaced plants subjected to selection. Seed from F<sub>6</sub> plants (Selection 121) was bulked up for further evaluation.

#### Expt 2

The chemical composition of F<sub>6</sub> plants (Selection 121) was compared with that of 11 commercially available *L. multiflorum* cultivars, all diploids, in a small-plot cutting trial. Three replications of the selected line and the 11 commercial cultivars were planted in 2 by 6 m plots, using a randomised block design. The sowing rate was 25 kg/ha, drilled in rows 150 mm apart. Each plot received N at a rate of 50 kg/ha after each cut and K at a rate of 50 kg/ha after each second cut. Plots were harvested 9 times at 4-weekly intervals over the growing season. Plots were irrigated on the day before harvesting and harvesting commenced at 1200 hours the following day by means of a reciprocating mower set at 50 mm above ground level. Net plot size after border removal was 1.4 by 4.6 m. The fresh herbage was weighed within 10–15 min of sampling and dried to constant weight in a forced-draught oven at 80°C.

The samples were milled to pass a 1-mm screen for subsequent chemical analysis.

#### Analytical procedures

Dry matter was calculated after oven-drying at 80°C to constant weight. Total nitrogen (N) was assayed by means of a Kjeldahl digestion, followed by measurement of the liberated ammonia using an autoanalyser procedure. Total non-structural carbohydrates were analysed by means of a mild acid hydrolysis procedure described by Marais (1979). The N and TNC analyses were used to develop calibration equations for near-infrared spectroscopy (NIRS), which were used to analyse F<sub>6</sub> and commercial cultivar samples (Table 2). The N values were predicted by the calibration equation with an 89.5% variance and the TNC values with a 94.7% variance. Digestibility *in vitro* (IVDMD) was determined by the procedure described by Minson and McLeod (1972). Acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined by the procedures of Van Soest (1963).

#### Statistical analysis

Results were subjected to analysis of variance, using GENSTAT 5, Release 3.2, Lawes Agricultural Trust (Rothamsted Experimental Station). Inter-relations between parameters measured were investigated using regression analysis. A randomised block in 3 replicates was used on the results of the small-plot trial, testing the variables DM yield, DM content, ADF, TNC, N, and TNC/N (d.f. = 30).

#### Results

The seasonal mean DM, TNC, and N contents of the initial 15 *L. multiflorum* cultivars or crosses on which the new selection (Selection 121) was based are presented in Table 1. The DM content ranged from 193 g/kg fresh material in Exalta × Lemtal to 150 g/kg fresh material in Amenda × unknown source. The TNC concentrations varied from 194 g/kg DM in Exalta × Lemtal to 136 g/kg DM in line LM87/19. Plants derived from Exalta, Lemtal, Midmar, Titania, Moata, Caramba, and Amenda ranked highest in

Table 1. Chemical composition of *Lolium multiflorum* cultivars grown in a spaced-plant trial, the diploid Italian (DI) component of which was subsequently used to develop Selection 121

Results are mean values of 6 cuts over the growing season of 6 plants per cultivar. DM, dry matter content; TNC, total non-structural carbohydrate content; N, nitrogen content; CV, coefficient of variation (%). DI, diploid Italian; TI, tetraploid Italian; DW, diploid Westerwolds; TW, tetraploid Westerwolds

Cultivar	DM		TNC		N	
	(g/kg fresh)	CV	(g/kg DM)	CV	(g/kg DM)	CV
Exalta (DI)	184	3.9	164	10.0	42.6	3.7
Exalta × Lemtal (DI)	193	3.6	194	6.7	42.2	2.9
Matador (DI)	176	5.0	162	13.9	42.7	7.5
Moritz (DI)	177	3.9	161	8.8	43.6	3.3
Midmar × Titania (DI)	178	6.1	183	20.8	41.5	6.9
LM87/19 (DI)	159	8.7	136	9.6	44.4	2.9
Amenda × unknown (TI)	150	5.9	166	11.2	42.6	4.4
Tetrone (TI)	166	3.4	153	8.3	45.1	3.1
Moata (TI)	156	4.0	180	6.7	42.7	2.9
Midmar (DW)	162	6.7	155	8.7	43.0	8.2
Progrow (DW)	169	4.3	145	16.5	45.2	3.5
Vitesse (DW)	178	4.8	140	12.5	43.6	2.7
Caramba (TW)	164	5.3	176	8.6	44.9	3.3
Energa (TW)	159	3.0	158	7.9	44.8	2.7
Billion (TW)	156	4.9	158	8.9	46.2	2.2

**Table 2. Prediction of total non-structural carbohydrate (TNC) and nitrogen (N) contents using near-infrared reflectance spectroscopy (NIRS)**

*A*, absorbance at subscript wavelengths (nm)

Parameter	NIRS prediction equation	d.f.	r <sup>2</sup>	r.s.d.
TNC	$y = 37.599 - (263.77 \times A_{2345}) + (8898 \times A_{1759}) - (4447.5 \times A_{1722}) - (4226.9 \times A_{1840})$	35	0.947	2.433
N	$y = 4.5942 + (181.81 \times A_{2180}) - (161.47 \times A_{2139}) - (34.673 \times A_{1709})$	34	0.895	0.379

**Table 3. Correlation coefficients from linear equations for quality parameters measured in F<sub>3</sub> spaced plants**  
DM, dry matter content; TNC, total non-structural carbohydrates; IVDMD, dry matter digestibility *in vitro*; ADF, acid detergent fibre; ADL, acid detergent lignin; d.f. = 32

	DM	TNC	IVDMD	ADF	ADL
DM	1.000				
TNC	0.619**	1.000			
IVDMD	0.389**	0.461**	1.000		
ADF	0.105	0.251	-0.077	1.000	
ADL	-0.161	-0.204	-0.361*	0.202	1.000

\**P* < 0.005; \*\**P* < 0.01.

non-structural carbohydrates and lowest in nitrogen. The N concentration of the cultivars ranged from 46.2 g/kg DM in Billion to 41.5 g/kg DM in Midmar × Titania. The high-TNC cultivars Exalta × Lemtal, Exalta, and Midmar × Titania were also high in DM content.

Correlation coefficients for linear equations for the quality parameters TNC, DM content, ADF, ADL, and IVDMD, measured in the F<sub>3</sub> plants selected for polycross (selection based on the 2 cuts prior to the boot stage of flower development), are given in Table 3. Results show a significant positive relation (*r* = 0.62, *P* < 0.01) between DM content and

TNC content. Furthermore, results show significant positive trends (*r* = 0.39 and *r* = 0.46, *P* < 0.05) between IVDMD and DM content and between IVDMD content and TNC content, respectively. The DM and TNC contents were poorly correlated with the ADF content and showed non-significant negative trends with the ADL content.

A comparison of the total dry matter yield and mean chemical composition of 9 cuts of the F<sub>6</sub> generation of plants (Selection 121), and 11 commercially available *L. multiflorum* cultivars, is given in Table 4. Selection 121 (14.8) was similar (*P* > 0.05) in dry matter yield to Agriton (14.4), Caversham (13.5), Concord (14.2), Energyl (13.3), Exalta (15.6), Flanker (13.6), and Mispah (14.2). Agri-Hilton had the lowest annual DM yield (11.6 t/ha). The cultivars generally gave similar trends at each of the 9 cuts (Fig. 1a), giving the highest yield in October. However, Selection 121 grew relatively well late in the season (late Oct.–Jan.) compared with cultivars such as Agri-Hilton (Fig. 1a).

The mean DM content over the season of Selection 121 (195 g/kg fresh grass) was significantly (*P* < 0.05) higher (10%) than that of the second-highest cultivar, Energyl, and 20% higher than the lowest, Dargle (Table 4). All cultivars gave similar trends in DM content at each of the 9 cuts (Fig. 1b). Dry matter contents peaked during midwinter (July–Aug.) and again at the end of the growing season (Dec.–Jan.) when the plants were in the reproductive stage.

The ADF content of Selection 121 (249 g/kg DM) was significantly (*P* < 0.05) lower than that of all the commercial cultivars tested (Table 4). The cultivar Agri-Hilton had the second lowest mean ADF content (267 g/kg DM) over the 9 cuts and Flanker had the highest (284 g/kg DM) (Table 4). The ADF values (Fig. 1c) were lowest during midwinter (July–Aug.) and highest at the end of the growing season.

Results presented in Table 4 show that the mean TNC content over the 9 cuts of Selection 121 (214 g/kg DM) was significantly (*P* < 0.05) higher (22%) than that of Exalta,

**Table 4. Total dry matter yield and mean chemical composition of F<sub>6</sub> diploid plants (Selection 121) over the growing season (9 cuts) compared with that of commercially available *Lolium multiflorum* diploid cultivars grown in small plots**  
I, Italian ryegrass cultivars; W, Westerwolds ryegrass cultivars

Cultivar	DM yield (t/ha)	DM cont. (g/kg fresh)	ADF (g/kg DM)	TNC (g/kg DM)	N (g/kg DM)	TNC:N
Select. 121(I)	14.8	195	249	214	31	7.9
Concord (I)	14.2	164	271	154	34	5.0
Dargle (I)	13.0	162	271	136	36	4.1
Flanker (I)	13.6	169	284	139	36	4.2
Caversham (I)	13.5	170	270	152	34	5.1
Exalta (I)	15.6	174	272	175	33	5.8
Energyl (I)	13.3	177	273	143	37	4.1
Agriton (I)	14.4	168	269	142	35	4.5
Midmar (W)	11.9	163	270	132	37	3.9
Burgundy (W)	13.1	166	272	139	35	4.1
Mispah (W)	14.2	167	269	145	34	4.6
Agri-Hilton (W)	11.6	169	267	139	34	4.3
I.s.d. ( <i>P</i> = 0.05)	1.70	6.2	10.0	20.3	2.4	1.08

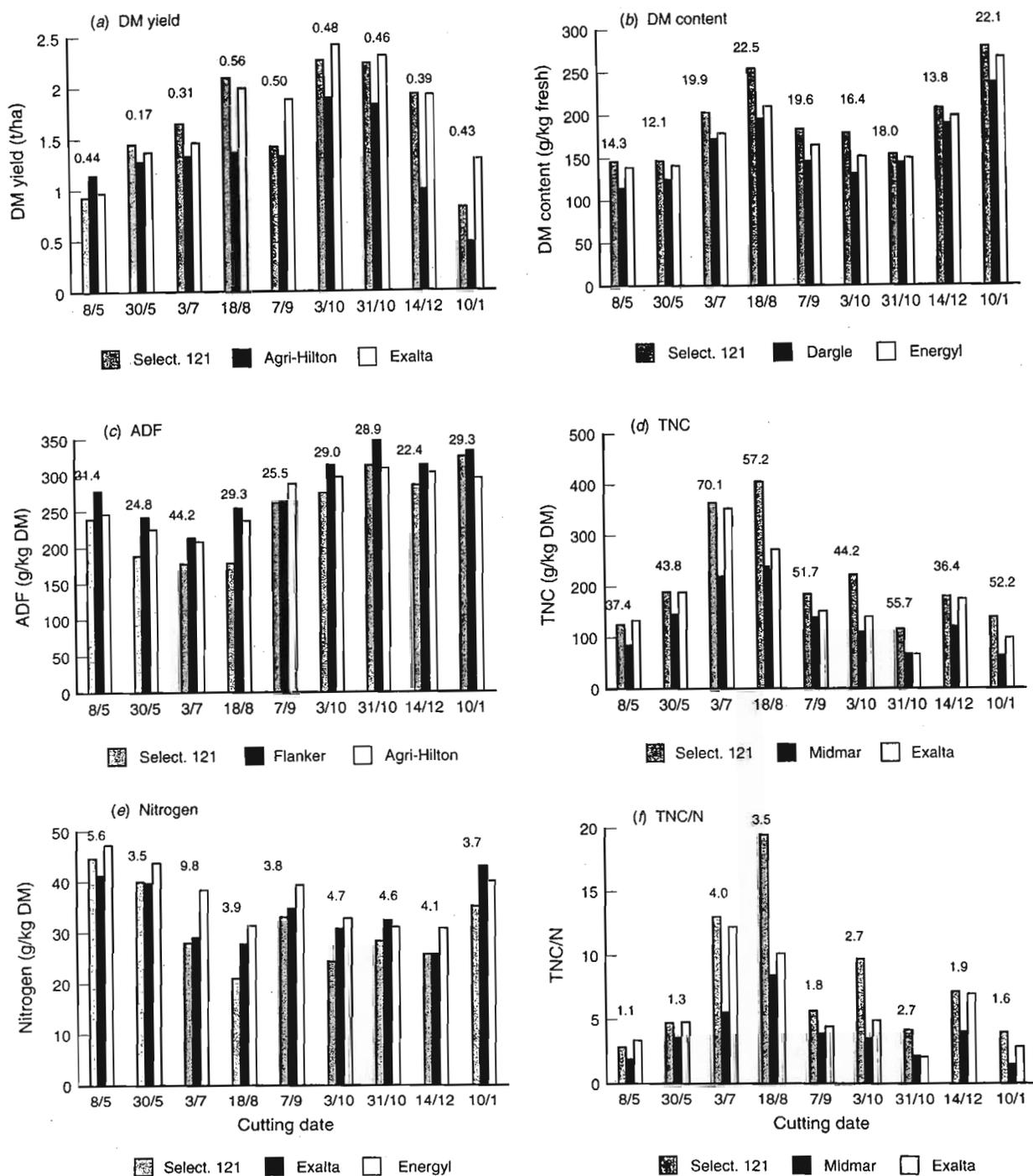


Fig. 1. Dry matter yield and chemical composition of *Lolium multiflorum* Selection 121 compared with that of commercial *Lolium multiflorum* cultivars over 9 cuts. Plants were grown in small plots. (a) Dry matter yield, (b) dry matter content, (c) acid detergent fibre, (d) total non-structural carbohydrates, (e) nitrogen, (f) total non-structural carbohydrate to nitrogen ratio. Values above bars indicate 1 s.d. ( $P = 0.5$ ).

which is generally regarded as a grass with high sugar content (Meissner 1996), and 62% higher than that of Midmar, which had the lowest TNC content of the cultivars tested. The seasonal TNC pattern of Selection 121, Exalta, and Midmar (Fig. 1d) shows peak values during midwinter (July–Aug.), probably due to lower respiration losses.

The mean seasonal nitrogen content of Selection 121 (31 g/kg DM) was the lowest of all the cultivars tested, followed by Exalta (33 g/kg DM) (Table 4). Midmar and Energyl had the highest nitrogen content (both 37 g/kg DM). Over most of the growing season the N content of Selection 121 fluctuated between 20 and 30 g/kg DM (Fig. 1e), which

is a desirable level for efficient transfer of ingested protein to the intestines in the form of microbial protein (Poppi and McLennan 1995). The mean seasonal TNC:N ratios (Table 4) suggest that Selection 121 has a much more favourable readily digestible energy to protein content than all the cultivars tested. Highest ratios were obtained during the coldest winter months (July–Aug.), when non-structural carbohydrate levels peaked (Fig. 1f).

### Discussion

Recurrent selection for both a high TNC and DM content resulted in a marked increase in the concentration of both factors and led to the development of a significantly improved annual ryegrass. Analysis of  $F_6$  plants of Selection 121 (Fig. 1b) showed that the DM content over most of the growing season was on or above the threshold value of 180 g/kg fresh grass, below which DM intake by ruminants may be reduced (John and Ulyatt 1987).

Humphreys (1989) showed that correlations between water-soluble carbohydrate content and DM yield in *L. perenne* were virtually absent. In the present investigation, poorly growing plants often had high TNC contents. However, by also taking DM yield into consideration when selecting for high TNC and DM contents, newly selected lines maintained a high DM production.

De Villiers (1991) reported moisture contents in Midmar ryegrass ranging from 812 to 891 g/kg fresh herbage (11–19% DM). Of the 6 *L. multiflorum* diploid Italian (DI) cultivars on which Selection 121 was based ( $F_1$  plants) (Table 1), only Exalta and Exalta  $\times$  Lemtal had a DM content higher than 180 g/kg fresh herbage (Moisture content <820 g/kg fresh). The initial cultivars used in the selection program therefore did not appear to have much potential as a starting base for improving the DM content of the cultivar. However, the TNC content, which appears to be a highly heritable trait (Radojevic *et al.* 1994), differed considerably between the 15 cultivars. Since both water and non-structural carbohydrates are stored in cell vacuoles and a negative association was observed between TNC and moisture contents of the cultivars, it was envisaged that an increase in DM content could be effected by non-structural carbohydrates replacing water in cell vacuoles.

Although the present investigation showed only a non-significant negative association between ADF content and digestibility, the significantly lower ADF value of Selection 121 suggests a higher digestibility for the selected line than for the commercial cultivars. In many grasses, ADF, which consists of cellulose lignin and some ash, is strongly related to the digestibility of the grass (Van Soest 1963). The ADF values for all grasses rose steadily towards the end of the growing season as stem tissue increased during the reproductive phase. The higher TNC contents of the selected plants could also have resulted in higher IVDMD values compared with the commercial cultivars. Selection 121,

which consists of a predominantly Italian ryegrass component, yielded well late in the season (late Oct.–Jan.) compared with Midmar, a predominantly Westerwolds ryegrass cultivar.

In the present investigation, plants were visually assessed and diseased plants rejected annually when polycross selections were made. However, there was no indication that high-sugar plants were more prone to fungal diseases than low-sugar plants. Breese and Davies (1970) showed that by increasing the water-soluble carbohydrates in *Lolium perenne* by 37%, through selection, the incidence of crown rust (*Puccinia coronata*) increased by 128%.

Selection 121, which has a significantly higher TNC content than Exalta (generally regarded as a high-sugar-content grass), could induce a more effective protein metabolism in the rumen than the other grasses studied. This is particularly likely to occur during midwinter when sugar levels in the grass peak, probably as a result of reduced plant respiration. For optimal animal production, 1 kg of feed should contain 10–11 MJ of metabolisable energy (ME) and not more than 22–26 g of nitrogen (Poppi *et al.* 1997). If this ratio is exceeded, milk production is reduced and the animal is adversely affected due to the loss of energy used in the liberation of excess ammonia in the rumen and its detoxification in the liver. Poppi *et al.* (1997) regards increasing the efficiency of microbial protein production as one of the most important strategies to improve the forage quality of temperate grasses. The nitrogen content of *L. multiflorum* can be in excess of 40 g/kg DM, depending on the level of nitrogen fertilisation, and the ME content usually drops to below 10 MJ/kg DM during the reproductive phase of the grass (Fulkerson *et al.* 1998). Due to the high solubility of forage leaf protein, it is readily deaminated in the rumen and nitrogen losses of 30–40% may occur in the absence of sufficient energy for microbial protein synthesis (Ulyatt *et al.* 1988). Animal production off *L. multiflorum* pastures is therefore likely to improve if the ME content and, in particular, the readily digestible energy in the form of non-structural carbohydrates in the grass can be increased.

The moderate nitrogen levels in Selection 121 during most of the growing season could also favour effective protein metabolism in the rumen. In intensive forage systems, nitrogen fertiliser application is a key factor determining DM yield, but is also positively related to the nitrogen content of the grass. Since only a moderate nitrogen level in the grass is necessary for optimal animal production, this may require a reduction in fertiliser application in existing *L. multiflorum* cultivars, which could adversely affect DM production. Cooper (1973) suggested that there may be a need to select for cultivars lower in protein content, which respond to nitrogen by increased DM and energy production, rather than by increased protein content. The high non-structural carbohydrate to nitrogen ratio of Selection 121, compared with that of the other cultivars tested, suggests that this goal has largely been achieved in

*L. multiflorum*. Similar improvements have been made in *L. perenne* (Radojevic et al. 1994).

A further benefit of the high sugar and low nitrogen contents of Selection 121 could be a reduced tendency to accumulate toxic levels of nitrate, since a low nitrogen content is usually associated with low nitrate levels. Annual species such as *L. multiflorum* have a much greater tendency to accumulate nitrate than perennial forage species (Crawford et al. 1961; Wright and Davison 1964). Nitrate-N concentrations as high as 16.6 g/kg DM have been reported in *L. multiflorum* (Darwinkel 1975), and would be lethal to unadapted animals.

In terms of DM yield and quality parameters such as ADF content, Selection 121 compares well with that of the best commercial cultivars tested, and it also has a much more favourable DM content and readily digestible energy to nitrogen ratio than the commercial cultivars. Grazing trials with sheep and dairy cattle will be used to establish to what extent improved forage quality parameters will result in improved animal production.

Selection 121 was subsequently named 'NCD Enhancer' and granted plant breeder's rights. It is inscribed on the South African Variety List.

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# A comparison, under controlled environmental conditions, of a *Lolium multiflorum* selection bred for high dry-matter content and non-structural carbohydrate concentration with a commercial cultivar

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## Abstract

The nutritional value of Italian ryegrass (*Lolium multiflorum*) selection 121, bred for a high dry-matter content and a high concentration of total non-structural carbohydrate (TNC), was compared with that of Westerwolds ryegrass (*L. multiflorum* ssp. *Westerwoldicum*) cv. Midmar in a controlled environment. The concentration of neutral-detergent fibre (NDF), acid-detergent fibre (ADF), acid-detergent lignin (ADL), nitrogenous compounds, minerals and *in vitro* digestibility were investigated as characteristics of nutritive value. The anatomical features of selection 121 and the Midmar cultivar were studied to determine possible structural differences. Thirty pots each of selection 121 and Midmar containing four plants per pot were arranged in a randomized block design in a controlled environment chamber. There were two temperature regimes during the study, the first being a warm regime (30 °C/20 °C) for 7 weeks followed by a cold regime (20 °C/7 °C) of a further 7 weeks. In the warm regime, the dry-matter (DM) content and the TNC concentration of selection 121 were 0.17 and 0.16 higher, respectively, than Midmar. The NDF concentration was significantly ( $P < 0.01$ ) higher in Midmar than in selection 121. When grown under warm conditions, Midmar had significantly ( $P < 0.001$ ) higher concentrations of Mg, K, Na and Mn than selection 121. In the cold regime, the DM content and TNC concen-

tration of selection 121 were 0.25 and 0.22 higher, respectively, than Midmar. No significant differences in the anti-quality factors investigated were found between the two ryegrasses. In the cold regime, Midmar had significantly ( $P < 0.001$  and  $P < 0.01$ ) higher Ca, Mg, K, Na, Zn, Mn and P concentrations than selection 121. The results from this controlled environment study suggest that selection 121 is superior to Midmar in terms of the quality characteristics DM and TNC, and that these characteristics are not positively linked to anti-quality factors associated with forage species.

**Keywords:** neutral-detergent fibre, acid-detergent fibre, acid-detergent lignin, nitrogenous compounds, minerals, *in vitro* digestibility, anatomical features

## Introduction

*Lolium multiflorum* is an important forage crop as an animal feed for milk production in many countries. However, forage grasses usually do not completely fulfil the requirement of the dairy cow for metabolizable energy. Furthermore, South African cultivars of *L. multiflorum* tend to have relatively high moisture contents (Meissner *et al.*, 1992). John and Ulyatt (1987) have shown that dry-matter (DM) intake is reduced if the moisture content of forages is excessively high. In an attempt to increase the nutritive value of Italian ryegrass in South Africa, selection 121 was developed from predominantly Italian types of *Lolium multiflorum*, with a minor Westerwolds component, by selecting for a higher concentration of total non-structural carbohydrate (TNC) and a lower moisture content than that currently available in commercial cultivars.

In selecting for a particular favourable trait, the plant breeder should be aware that other, less favourable

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characters may be linked on the same chromosomes and might result in a reduction in the nutritive value of the forage. In *L. multiflorum*, such a reduction could be caused by an increase in levels of neutral-detergent fibre (NDF), acid-detergent fibre (ADF), acid-detergent lignin (ADL) or nitrogenous compounds. In this respect, differing requirements for reproductive development and, therefore, stem formation in the progenitors of selection 121 could be important. Italian ryegrasses have a dual-induction requirement for flowering: low temperatures and/or short daylengths are required for the primary induction of floral primordia; the secondary requirement is long daylengths for inflorescence development and culm elongation. In contrast, Westerwolds ryegrasses have only a single induction requirement, i.e. long daylengths, and flower readily in the year in which they are sown (Aamlid *et al.*, 1997).

It is therefore essential that new selections are monitored for the presence of undesirable characters. The recognition of genotypic differences by minimizing environmental effects may be achieved under controlled environment conditions, also enabling more rapid acquisition of data. Any results obtained in this way from pot experiments should be confirmed in field experiments (Graven, 1978).

The objective of this study was to compare the predominantly Italian ryegrass selection 121 with the commonly used Westerwolds ryegrass cultivar, Midmar, in a controlled environment, in terms of their chemical constituents that affect nutritive value. The controlled environment regimes simulated spring/autumn and winter conditions in South Africa.

## Materials and methods

### Experimental design

Seedlings of Italian ryegrass selection 121 and the Westerwolds ryegrass cultivar Midmar were grown in seedling trays for 3–4 weeks (two-leaf growth stage) and thereafter transplanted into 4 kg of soil in 5-l undrained pots (19 cm diameter) in a growth chamber. Thirty pots each of selection 121 and Midmar, containing four seedlings per pot, were arranged in a randomized block design on benches in the environment chamber. The soil used was an orthic topsoil

from a low-lying topography, which was initially air-dried, milled to pass a 1-mm sieve and analysed for mineral content (Table 1). This was followed by a single uniform incorporation of a solution providing supplemental nutrients (300 mg P kg<sup>-1</sup> soil as Ca(H<sub>2</sub>PO<sub>4</sub>)·H<sub>2</sub>O, 100 mg K kg<sup>-1</sup> soil as K<sub>2</sub>SO<sub>4</sub>, 100 mg N kg<sup>-1</sup> soil as NH<sub>4</sub>NO<sub>3</sub>, 50 mg Mg kg<sup>-1</sup> soil as MgSO<sub>4</sub>·7H<sub>2</sub>O, 4 mg Cu kg<sup>-1</sup> soil as CuSO<sub>4</sub>·5H<sub>2</sub>O, 1 mg B kg<sup>-1</sup> soil as Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O, 0.5 mg Mo kg<sup>-1</sup> soil as (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O). The field moisture capacity (FMC) of the soil was determined as described by Graven (1978) to ensure sufficient addition of water to each pot during the experiment. The pots received 0.4 g of nitrogen (N) in the form of ammonium nitrate after each cut.

### Growth chamber conditions

The air and soil temperature, light intensity and humidity were monitored using a HOBO<sup>®</sup> H8 Logger (Onset Computer Corporation, Bourne, MA, USA) placed in the centre of the growth chamber. The growth chamber temperature was adjusted to a day/night temperature regime of 30 °C/20 °C for a 7-week warm period, followed by a 7-week cold period at a day/night temperature of 20 °C/7 °C. During the warm period, the absolute humidity fluctuated between 14 and 5 g m<sup>-3</sup>, whereas it remained constant at 8 g m<sup>-3</sup> during the cold regime. Plants were grown at a daylength of 10.5 h; the temperature alternation was in step with the change in daylength. Lighting consisted of a bank of fluorescent tubes alternating with incandescent lights giving a light intensity at the leaf canopy of 7200 lumens m<sup>-2</sup>. Plants were regularly watered to FMC.

### Herbage sampling

The herbage from four plants per pot was harvested at the 3.5-leaf growth stage (cut 50 mm above the soil level) 5 h into the light cycle, three times during the warm regime (every 16 d) and twice during the cold regime (every 24 d). Samples were placed in a forced-draught oven at 80 °C within 10 min of sampling and dried to constant weight (24 h). For each replicate, the dried samples from all harvests were bulked and milled

**Table 1** Chemical composition of potting soil used in the growth chamber experiment.

Minerals (mg l <sup>-1</sup> )						Exchange acidity (mmol l <sup>-1</sup> )	Total cations (mmol l <sup>-1</sup> )	Clay (%)	Organic carbon (%)
P	K	Ca	Mg	Zn	Mn				
47	230	1105	190	5.2	42	13.5	90.2	50	4.8

The percentage soil contents of clay and organic carbon were measured by near-infrared reflectance spectrophotometry.

to pass a 1-mm sieve for subsequent chemical analysis. Before harvesting during the warm and cold temperature regimes, the second fully expanded leaf blades of selection 121 and Midmar were sampled one-third to one-half of the total blade length above the ligule for morphological studies. Leaf width and number of vascular bundles per leaf were measured on 30 leaves.

### Chemical analyses

Total non-structural carbohydrates were analysed as reducing sugars after quantitative hydrolysis to monosaccharides through a carefully controlled acid hydrolysis procedure (Marais, 1979). The reducing sugars formed during hydrolysis were determined quantitatively by a modified Nelson–Somogyi method (Marais *et al.*, 1966). NDF, ADF and ADL concentrations were determined according to the procedures of Van Soest (1963). Nitrate-N analyses were based on the nitration of salicylic acid under highly acidic conditions and the colorimetric determination of the resulting coloured complex, which absorbs at 410 nm maximally in basic solution (Cataldo *et al.*, 1975). *In vitro* DM digestibility was determined by the procedure described by Minson and McLeod (1972). The true protein and total-N analyses were based on the precipitation of protein with trichloroacetic acid and the separation of the insoluble protein from the soluble non-protein fraction by means of filtration (Marais and Evenwell, 1983). Mineral analyses were conducted using the 'Hunter' system, as described by Farina (1981). After dry ashing, P was determined colorimetrically and cations (Ca, Mg, K, Al, Zn and Mn) by atomic absorption spectrophotometry.

### Statistical analysis

Results were analysed by analysis of variance using Genstat 5, Release 4.2 (Genstat, 2000).

## Results and discussion

### Morphological features

Some morphological characteristics of the leaf blade of the two grass cultivars are presented in Table 2. The mean leaf width of Italian ryegrass selection 121 was significantly ( $P < 0.01$ ) narrower than that of Westerwolds ryegrass cultivar Midmar and contained fewer vascular bundles per leaf than Midmar, but the number of vascular bundles per mm leaf width was similar in both grasses. Vascular and sclerenchyma fibre strands are the main contributors to low digestible plant particles in the rumen, reducing forage quality (Minson and Wilson, 1994). The proportion of poorly digestible

**Table 2** Comparison of mean leaf width and number of vascular bundles in the second fully expanded leaf blades of Italian ryegrass selection 121 and Westerwolds ryegrass cultivar Midmar.

	121	Midmar	s.e.m.	Significance
Leaf width (mm)	4.6	5.7	0.51	**
Vascular bundles (no. per leaf)	16.4	19.7	1.92	**
Vascular bundles (no. mm <sup>-1</sup> leaf width)	3.5	3.5	0.33	NS

NS, not significant; \*\* $P < 0.01$ .

to more digestible tissue in the leaves in both grasses therefore appears to be similar.

### Nutrient composition

The nutrient composition of the high-TNC, low-moisture selection 121 of Italian ryegrass and the Midmar cultivar of Westerwolds ryegrass grown in a growth chamber under warm and cold regimes is presented in Table 3. The DM content and TNC concentration of selection 121 were significantly higher ( $P < 0.001$ , except TNC, cold regime  $P < 0.05$ ) than those of Midmar. As comparisons were conducted under identical environmental conditions, the recorded differences would be of genetic origin. The design of the experiment does not allow comparisons between the warm and the cold regime, although it is probable that the much higher TNC concentration in both cultivars in the cold regime is because of the reduced demand for carbohydrate reserves during the slower growth at low temperatures (Buxton, 1996). Although significantly different, the DM contents of both grasses were lower than would be expected under field conditions (Marais and Goodenough, 2000). In order not to affect the DM intake of grazing ruminants adversely, the DM content of forages should be at least 18–20% (Meissner *et al.*, 1992). However, the 0.17–0.23 higher TNC concentration in selection 121 compared with Midmar indicates a higher nutritive value of selection 121. A perennial ryegrass (*Lolium perenne*) variety bred for increased levels of water-soluble carbohydrates (WSC) has been shown to stimulate higher DM intakes and increase milk production in dairy cows (Miller *et al.*, 1999), and to increase liveweight gain from preweaned lambs (Lee *et al.*, 1999).

The high-TNC Italian ryegrass selection 121 was significantly ( $P < 0.001$ ) lower yielding than Midmar, giving DM yields 0.2 and 0.14 lower than Midmar during the warm and cold regimes respectively. This is in accordance with the findings of Humphreys (1989) in perennial ryegrass, who showed that DM production is

**Table 3** Dry-matter yield, content and nutrient concentrations in herbage of Italian ryegrass selection 121 and Westerwolds ryegrass cultivar Midmar grown in a controlled environment chamber under warm and cold regimes.

Nutrient composition†	Warm regime				Cold regime			
	121	Midmar	s.e.m.	Significance	121	Midmar	Significance	s.e.m.
DM yield (g per pot)	10.3	12.8	0.34	***	14	16.3	0.53	***
DM content (g kg <sup>-1</sup> fresh weight)	148.2	126.7	0.12	***	154.9	123.5	0.15	***
TNC	64.9	55.7	1.52	***	150	122.5	8.43	*
NDF	501.9	514.2	3.0	**	510.6	517.8	4.19	NS
ADF	291.8	295.1	3.77	NS	262.6	276.4	5.22	NS
ADL	48	49.7	1.82	NS	39.4	51.7	21.8	***
Nitrate-N	13.27	16.02	0.365	***	11.4	12.4	0.52	NS
<i>In vitro</i> DMD	0.777	0.770	0.007	NS	0.788	0.777	0.004	*
True protein	213.9	201.5	2.61	*	196.7	192.0	2.79	NS
Total N	48.9	47.4	0.48	**	45.8	46.5	0.96	NS

TNC, total non-structural carbohydrate; NDF, neutral-detergent fibre; ADF, acid-detergent fibre; ADL, acid-detergent lignin.

†Given as concentrations (g kg<sup>-1</sup> DM) unless stated otherwise.

*In vitro* DMD, *in vitro* dry-matter digestibility (as a decimal fraction).

NS, not significant; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

often inversely proportional to the concentration of WSC in the herbage. The more vigorous growth of the plants of Midmar Westerwolds ryegrass may be attributed to the fact that they do not have a cold requirement for floral induction, but respond to long days and would thus be in the early reproductive phase (Aamlid *et al.*, 1997). In the case of the South African-bred Midmar Westerwolds ryegrass cultivar, the 10.5-h daylength in which they were grown was sufficient to induce flowering, evidenced by the elongation of internodes. In contrast, conditions were not sufficient for floral induction of the major Italian ryegrass component in selection 121 Italian ryegrass, which remained in the vegetative phase under the daylength conditions of 10.5 h, during both warm and cold regimes and the minimum 7 °C night temperature. Nevertheless, in field experiments over a growing period of 9 months, after autumn establishment, selection 121 gave a slightly higher DM yield than Midmar (Marais and Goodenough, 2000). Table 3 shows that, for selection 121, values for the anti-quality characteristics NDF, ADF, ADL and nitrate concentrations were either similar to, or significantly lower than, those for Midmar. The early reproductive state of the Midmar cultivar of Westerwolds ryegrass plants may account for its higher ADF and other values compared with selection 121 Italian ryegrass plants. The different growth phases of the Midmar and selection 121 plants may have contributed to variable measurements of other characteristics, including the *in vitro* DM digestibility of selection 121, which was slightly higher than that of Midmar during the cold regime but similar to Midmar

during the warm regime. During the warm regime, true protein and total-N levels were higher in selection 121 than in Midmar, but similar during the cold regime. These results are in contrast to field results, which showed a strong negative relationship between TNC and nitrogen concentrations in the grass (Marais and Goodenough, 2000).

#### Mineral composition of ryegrass cultivars

The mean mineral concentrations in Italian ryegrass selection 121 and Westerwolds cultivar Midmar are presented in Table 4. With the exception of the concentration of K during the cold regime, the mineral concentration of selection 121 was lower than that of Midmar. The apparent lower mineral values, with the exception of the Na concentration, could result mainly from a dilution effect as a result of the higher concentration of non-structural carbohydrate in selection 121. During the warm and cold regimes, the concentrations of Na of selection 121 were 0.45 and 0.51 lower than in Midmar respectively. These values are much lower than can be expected from a dilution effect and could be linked to the high K concentration in the plant. High levels of K in the soil will depress Na concentrations in temperate forages (Hemingway, 1961; Reith *et al.*, 1964). A lack of Na has been shown to reduce voluntary intake in calves (Morris and Murphy, 1972). However, the low levels in Italian ryegrass selection 121 appear to be sufficient for ruminants with the highest requirement for Na (Minson, 1990). The excessively high levels of K in the herbage result from luxury uptake from the

**Table 4** Mean mineral concentrations in herbage of Italian ryegrass selection 121 and Westerwolds ryegrass cultivar Midmar grown in a controlled environment chamber under warm and cold regimes.

Mineral concentrations (g kg <sup>-1</sup> DM)	Warm regime				Cold regime			
	121	Midmar	s.e.m.	Significance	121	Midmar	Significance	s.e.m.
Ca	6.9	7	0.09	NS	6.5	7.9	0.21	***
Mg	3.2	3.6	0.04	***	3.5	4.3	0.08	***
K	57.6	68.3	0.73	***	44.5	36.8	1.1	***
Na	1.6	2.9	0.16	***	3.9	7.9	0.3	***
P	3.2	3.3	0.05	NS	3	3.3	0.05	**
Zn (p.p.m.)	54.2	53.5	0.95	NS	40	48.7	1.06	***
Cu (p.p.m.)	13.5	13.9	0.19	NS	11.1	11.1	0.25	NS
Mn (p.p.m.)	242.6	292.2	6.39	***	268.8	340.3	7	***

NS, not significant; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

soil by means of an active process, i.e. against the concentration gradient. The K:Ca + Mg ratio, expressed on an equivalents basis, in excess of 2.2 was shown to be linked to an increased incidence of grass tetany (Azevedo and Rendig, 1972). However, in selection 121 and Midmar, grown under both temperature regimes, these ratios were not sufficiently high to be a potential cause of grass tetany.

## Conclusion

The results of this controlled environment experiment have important implications for pasture breeding programmes. The experiment has improved the accuracy of assessment of genetic differences, the expression of which is sensitive to environmental factors, and has enabled the verification of results obtained in conventional breeding and assessment experiments. In the warm and cold regimes, despite the higher DM yield of Midmar relative to selection 121, the latter is superior to Midmar in terms of nutritive quality characteristics, DM content and TNC concentration. No undesirable traits have evidently been introduced, confirming the work of Marais *et al.* (1997), who found that the DM content and the concentration of TNC in Italian ryegrass in a spaced-plant experiment did not appear to be positively linked to the main anti-quality factors associated with pasture grasses.

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