THE EFFECTS OF LEAF SHEAR BREAKING LOAD ON
THE FEEDING VALUE OF
PERENNIAL RYEGRASS FOR
SHEEP

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Preface

The contents of this thesis represent original work conducted by the author under the supervision of Dr Ian Brookes and Professor Tom Barry of Department of Animal Science, Massey University and Dr Andrew John of Applied Biotechnology Division, DSIR and Dr Warren Hunt of Grasslands Division, DSIR.

Abstract

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Reducing physical resistance has been thought to be a key factor to increase efficiency of masticatory breakdown of forage, which may lead to faster rumen fractional outflow rates (FOR) and consequently to increased voluntary feed intake and hence improved feeding value (FV). Two selections of perennial ryegrass (PRG) were selected for low (LS) and high leaf shear breaking load (HS) based on the maximum load required to shear across the leaf, i.e. leaf shear breaking load (LSBL). The series of experiments were conducted to investigate the effects of LSBL on the FV of PRG for sheep.

LSBL, morphological and anatomical parameters were measured on the LS and HS PRG selections grown under the optimum climatic conditions. LSBL for the LS PRG selection was approximately 41 % lower the HS PRG selection. However, the LS selection had shorter leaf lengths, narrower leaf widths and narrower leaf cross-sectional area (CSA) than the HS selection. Therefore, in leaf shear strength (LSS), the LS selection was estimated to be approximately 27 % less resistant to shear than the HS selection per unit of CSA. The lower LSS in the LS selection is due to the lower concentration of sclerenchyma tissues in leaf CSA compared with the HS selection. However, the differences in the total shear load required to breakdown a unit dry weight of leaves to 1 x 1 mm particle size, namely, leaf index of masticatory load (IML) between the selections were influenced by the differences in morphological characteristics of leaves between the two PRG selections.

Comparisons were made between the LS and HS PRG selections in the efficiency of mastication by sheep on particle breakdown. There were no major effects of reduced LSBL in PRG on the efficiency of mastication during eating and during

rumination. Although the LS PRG selection was approximately 29 % lower in LSBL than the HS PRG selection, the difference for the two PRG selections in IML was almost nil.

Effects of LSBL in PRG on rumen fractional outflow rate (FOR) and apparent digestibilities were investigated in sheep fed at restricted feed intake levels. There were no effects of reduced LSBL on FOR, although the LS PRG selection was approximately 39 and 12 % lower than the HS PRG selection in LSBL and IML, respectively. The digestibility of the cellulose fractions was approximately 16 % lower in the LS PRG selection than the HS PRG selection. The leaf morphology in PRG may affect the efficiency of fibre digestibility.

Two field trials were conducted to test the hypothesis that LSBL in PRG improves FOR and leads to higher voluntary feed intake, and hence achieves improved live weight gain and wool production, namely FV. Although the LS PRG selection had 25-30 % lower LSBL than the HS PRG selection, live weight gain and wool production of sheep were not improved by reduced LSBL. FOR in sheep showed no indications of difference and voluntary feed intake was similar between the animals grazing the LS and HS PRG selections. The lack of difference in IML between the LS and HS PRG selection can be considered as a main reason for this. The hypothesis, that reduced LSBL in PRG would improve its FV, was therefore rejected.

In conclusion, there were no major effects of reduced LSBL in PRG on efficiency of masticatory particle breakdown, and consequently, FOR, feed intake and hence FV in sheep. This is due to the lack of selection effect of PRG in IML. IML is a determining factor for the efficiency of mastication both during eating and rumination. The selection of PRG for a lower IML will, therefore, be necessary in order to increase efficiency of masticatory particle breakdown, FOR and hence FV of PRG.

Acknowledgements

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List of Abbreviations

General

ADF	acid detergent fibre
CAC	controlled atmosphere cabinet
<C.EAT>	efficiency of chewing during eating in reducing particles to < 1.0 mm
Co	cobalt
Co(III)-EDTA	cobalt ethylenediaminetetraacetic acid
Cr	chromium
<C.RUM>	efficiency of chewing during rumination in reducing particles to < 1.0 mm
Cr_2O_3	chromium sesquioxide
Cr-EDTA	chromium ethylenediaminetetraacetic acid
CSA	cross-sectional area
d	day
DM	dry matter
DOMI	voluntary intake of digestible organic matter
DSIR	Department of Scientific and Industrial Research
ep	epidermis
EPM	Ellinbank pasture meter
Fig.	figure
FOR	fractional outflow rate
FV	feeding value
GLM	general linear model
HS	high leaf shear breaking load
IML	index of masticatory load
IVDMD	in vitro dry matter digestibility
IVOMD	*in vitro* organic matter digestibility
L.	*Linnaeus*

LS	low leaf shear breaking load
LSBL	leaf shear breaking load
LSS	leaf shear strength
LWG	live weight gain
LWR	leaf length:dry weight ratio
M.	*Musculi*
M00	maturation
M10	10 days after full maturation
M20	20 days after full maturation
ms	mesophyll
N	nitrogen
na	not available
NDF	neutral detergent fibre
NH_3-N	ammonia
NV	nutritive value
OM	organic matter
OE	oesophageal extrusa
PRG	perennial ryegrass
RTOM	apparent retention time of organic matter in the rumen
Ru	ruthenium
Ru-Phen	ruthenium tris (1,10-phenanthroline) ruthenium (II) chloride
sc	sclerenchyma
<TEC>	theoretical efficiency of chewing
TSL	total shear load
VFA	volatile fatty acid
VOMI	voluntary organic matter intake
vs	vascular bundle
v/v	volume by volume
WB	Warner-Bratzler
WC	white clover

Units

a	are
c	centi-
C°	degree centigrade
g	gram
G	Newtonian constant of gravitation
h	hecto-
hr	hour
in	inch
J	joule
k	kilo-
l	litre
m	milli-
m	metre
M	mega-
M	mole
μ	micro-
min.	minute
%	per cent
W	watt

Statistical

df	degrees of freedom
n	number of observations
r	correlation
RSE	root square of error
s.e.	standard error
S.E.D.	standard error of difference
ns	not significant
*	significant at $p < 0.05$

**	significant at p<0.01
***	significant at p<0.001
I	indicating ranges of standard error in figures

Introduction

In New Zealand, pasture is the source of most of the nutrients consumed by grazing livestock, and perennial ryegrass (*Lolium perenne L.*) is the most widely established pasture grass. Although the application of agronomic knowledge and the continuous efforts of plant breeders have achieved high levels of pasture productivity and adaptability, the efficiency of conversion of perennial ryegrass to animal products is quite low in comparison with other temperate forages.

Feeding value is a parameter used to quantify the 'efficiency of animal production' and is described by the formula :

$$\text{Feeding value} = \text{Intake} \times \text{Nutritive Value.}$$

Over the years, plant breeders have concentrated more on improving 'nutritive value', but other attributes which may improve 'intake' have been almost neglected.

Recently, our understanding of ruminant nutrition has increased to the stage where the constraints on voluntary feed intake can be identified. Digesta passage from the rumen is of great importance in determining feed intake. The faster the rate of passage, the faster the digesta load is reduced, and the greater the amount the animal is able to consume. The rate of clearance depends on two roles of the rumen, particle breakdown and actual passage from the rumen. Mastication contributes most effectively to particle breakdown, and hence to passage from the rumen.

These facts have led to a new concept amongst plant breeders -reducing physical resistance of the grass against breakdown when the plant is masticated. Leaf shear breaking load has been chosen as a parameter of the resistance to breakdown, and two types of perennial ryegrass have been selected by the DSIR's Grassland

Division for either low leaf shear breaking load or high leaf shear breaking load.

This thesis investigates whether reduced leaf shear breaking load in perennial ryegrass demonstrates advantages in feeding value.

Chapter One

A REVIEW OF FACTORS LIMITING THE FEEDING VALUE OF PERENNIAL RYEGRASS

1.1 Introduction

This chapter, based on previously published studies, describes the current status of and factors limiting the feeding value (FV) of perennial ryegrass (PRG) in New Zealand pastoral agriculture. Numerous studies examining the factors limiting the FV of PRG led plant breeders to a new concept - reducing physical resistance to breakdown by mastication. Using this new concept, attempts to improve FV of PRG are also reviewed and the hypotheses for the present investigations are developed in this chapter.

1.2 The role of perennial ryegrass in the New Zealand pasture

1.2.1 Agronomic characteristics of perennial ryegrass

PRG is the most widely used pasture grass in New Zealand. Approximately 69 % of grass seed dressed in New Zealand is PRG (New Zealand Department of Statistics, 1983).

The advantages of PRG in New Zealand pasture, e.g. ease of establishment and the ability to support grazing animals over a wide range of climatic conditions, the ability to withstand poor soil fertility, and good winter growth etc. are well recognized.

White clover (WC) is usually sown together with PRG to improve overall pasture quality, to fix atmospheric nitrogen and return this to the soil for subsequent

utilization by the PRG. Normally, New Zealand PRG pastures contain PRG:WC in the approximate ratio of 80:20.

The suitability of PRG to the range of New Zealand farm ecosystems, and further improvement, is being continually assessed amongst other grasses by the Plant Breeding section of DSIR Grasslands Division. Recently, the role of PRG in certain grazing situations has been challenged by the introduction of new pasture grass cultivars, such as an improved prairie grass (*Bromus catharticus*) - Grasslands Matua, and also Grasslands Roa, an improved tall fescue (*Festuca arundinacea*) variety, which are more tolerant to the wide range of agronomic conditions found in New Zealand. Recent studies show an association between the fungal endophyte that infects PRG and the neurotoxic disorder ryegrass staggers (Fletcher *et al.*, 1981; Fletcher, 1982, 1986). Also, presence of the endophyte in PRG has been associated with increased resistance of the plant to attack by Argentine stem weevil (*Listronotus bonariensis*) (Prestidge *et al.*, 1982).

In spite of the fact that the extensive use of PRG in New Zealand pastures is being reconsidered with regard to new species, it will remain an integral part of the grassland system for sometime because of its wide adaptability and relatively high productivity.

1.2.2 Animal production response to perennial ryegrass

1.2.2.1 Definition of feeding value

The ultimate criterion of pasture quality under New Zealand conditions must be the productivity of the grazing ruminant. Numerous methods of forage evaluation have been devised (Raymond, 1969). However, the preferred method for a grazing ecosystem is to measure animal production while the pasture is being grazed. Under these conditions, it is important that intake is not limited by herbage availability.

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Animal production depends on the amount of nutrients that the animal can utilize from the food consumed. Ulyatt (1970) suggested use of FV of a forage to evaluate pasture quality, which is defined and measured by an animal response to the total amount of herbage consumed.

$$FV = \text{Nutritive Value (NV)} \times \text{Intake}$$

NV is defined as the concentration of nutrients in a feed or animal response per unit of feed intake. Thus, FV is a function of both NV and intake (Ulyatt, 1970, 1973). The nutrients utilized by an animal can be partitioned into those required for maintenance and those required for the production of meat, wool or milk. It is difficult to make an accurate estimate of maintenance with grazing animals but production can be measured in terms of liveweight gain, wool growth or milk yield under the assumption that maintenance requirements are not affected by the pasture type fed, so that pasture can be assessed in terms of animal production.

1.2.2.2 Feeding value of perennial ryegrass

The comparative FV's of several temperate herbage species for sheep liveweight gain are summarized in Table 1.1. The data are expressed relative to PRG and the range in liveweight gains has also been included. PRG, which is the major improved species used in the New Zealand pastoral farming, is of lower FV than the other species tested. The more annual ryegrasses (e.g. Italian and Manawa) are of higher FV than the more perennial varieties (e.g. Ariki). The legumes, white clover and lucerne, are of higher FV than any of the grasses studied. Differences between pasture species have also been observed in milk production (Wilson *et al.*, 1966; Wilson *et al.*, 1967; Greenhalgh *et al.*, 1969) with similar ranking to those observed for liveweight gain in lambs. However, differences in wool growth response between pasture species have not been clearly demonstrated (Gallagher *et al.*, 1967). Within the annual and perennial grasses, there is generally an inverse relationship between FV to ruminant livestock and persistency.

Table 1.1   The relative feeding value of New Zealand pasture species under grazing conditions for young sheep (Ulyatt, 1970).

	No.of experiments	Relative liveweight gain	Range in liveweight gain (g/day)
Perennial ryegrass[1]	12	100	23 - 227
Manawa ryegrass[2]	11	148	45 - 270
Ariki ryegrass[3]	2	111	86 - 150
Italian ryegrass[4]	1	160	141
Timothy	5	129	127 - 168
White clover	7	186	168 - 331
Lucerne	5	170	154 - 291

1: *L. perenne.*

2: *L. perenne x L. multiflorum.*

3: *L. perenne x (L. perenne x L. multiflorum).*

4: *L. multiflorum.*

1.2.3 Conclusion

PRG is the most important resource of nutrients for ruminant livestock in New Zealand. Plant breeders have largely concentrated on improving PRG in agronomic factors. However, PRG has the lowest FV amongst temperate forages in New Zealand. Little attention has been paid by plant breeders to the efficiency of conversion of PRG to animal production. Large differences in FV exist among herbage species and there must be a considerable potential for improvement for PRG in FV.

1.3 Factors limiting the feeding value of perennial ryegrass

1.3.1 Factors limiting nutritive value of perennial ryegrass

Many variables can influence NV. Ulyatt (1973) suggests two major factors contributing to NV are ;

(1) apparent digestibility,

(2) the efficiency with which digested nutrients are converted into products within the animal tissues.

1.3.1.1 Apparent digestibility of perennial ryegrass

The factors that affect the apparent digestibility of temperate pasture species have been examined in great detail (Raymond, 1969). Apparent digestibility is clearly related to herbage maturity and there is a general pattern for all species; a high apparent digestibility is maintained in the spring and this declines as the plant matures over the summer. These changes in apparent digestibility with maturity can be explained in terms of changes in plant structure and chemical composition (Table 1.2). As a plant matures, the proportion of stem increases, the proportions

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Table 1.2 **Composition, decline in apparent *in vivo* digestibility and utilisation of metabolisable energy (ME) of dried S23 perennial ryegrass harvested at 4 stages of maturity and fed to sheep at maintenance level of intake** (adapted from Waghorn *et al.*, 1987).

	Young leafy	Late leafy	Head emergence	Seed setting	Decline in digestibility (%)
Composition (% DM):					
Water soluble sugars	14	12	11	10	100-100
Cellulose	21	22	24	27	92- 73
Hemi-cellulose	16	19	19	26	93- 56
Lignin	3	4	4	7	23- 0
Ash	8	8	7	6	64- 52
Lipid	9	8	7	5	65- 43
Cell wall[1]	40	45	47	60	
Digestibility of DM (%)	86	83	79	62	
ME/gross energy (%)	66	61	62	52	
Efficiency of ME utilisation for:					
Maintenance (%)	78	76	75	74	
Growth (%)	53	54	47	34	

1: Cellulose, hemi-cellulose plus lignin.

of slowly digested and indigestible chemical constituents (cellulose, hemi-cellulose and lignin) in the stem also increase, and as a result apparent digestibility declines. Differences in apparent digestibility between and within species can be defined in terms of differences in leaf to stem ratio and in chemical composition.

Plant breeders of temperate forage plants have selected to improve apparent dry matter digestibility (Dennis *et al.*, 1986), and these are considerably high (70 - 80 %) in their vegetative state. However, improved apparent dry matter digestibility may not necessarily lead to improved animal performance because ruminants utilize the major dietary nutrients with different efficiencies (Ulyatt, 1973, 1980).

1.3.1.2 Efficiency of utilisation of perennial ryegrass

Of further importance is the site at which digestion takes place (Ulyatt, 1981). A good example, the comparison between PRG and WC is shown in Table 1.3. There are large differences between the species in live weight gain. There are also differences in chemical composition between the species, indicated by the higher ratio of structural to readily fermentable carbohydrate in PRG. However, there are no significant differences in apparent digestibility. Apparent digestibility of a feed only measures the total amount of nutrients digested and indicates nothing of the processes of digestion or end products of digestion. Digestion in the rumen and caecum is by microbial fermentation and while this process is beneficial, in that it enables ruminants to digest structural carbohydrates, it occurs with a loss of approximately 25 % of digestible energy as methane and heat (Ulyatt, 1981). The results also emphasize the importance of a specific nutrient, - protein, rather than just energy in influencing growth of animals, a physiological function which requires a high amount of protein. The fermentation of protein in the rumen or caecum causes loss of nitrogen as ammonia. When protein is digested in the small intestine there is little loss and the animal benefits by the absorption of amino acids.

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Table 1.3 Data on the digestion of Ruanui perennial ryegrass and Huia white clover by sheep (Ulyatt, 1981).

	Perennial ryegrass	White clover
Field experiments:		
Liveweight gain (g/d)	227	331
OM intake (g/d)	1086	1243
Liveweight gain per 100g digestible OM intake (g)	26.0	32.6
OM retention time in rumen (h)	10.4	6.3
Structural/readily fermentable carbohydrate in diet	1.7	0.8
Indoor experiments:		
OM intake (g/d)	800	800
OM digestibility (%)[1]	80.4	81.6
OM digested in stomach (% OM intake)	54.6	53.9
Rumen VFA production (moles/d)	4.7	4.8
Nitrogen intake (g/d)	37.8	35.2
Nitrogen digested in stomach (% N intake)	26.5	18.0
Protein-N digested in small intestine (% N intake)	40.0	49.4
Protein-N digested in small intestine at field intakes (g/d)	119	188
Absorbed protein/VFA energy (g/MJ)	11.7	19.0

1: Apparent digestibility.
OM: organic matter.
VFA: volatile fatty acid

However, the main cause of the WC's higher nutritional efficiency is the faster passage rate of WC due to the low ratio of structural to readily fermentable carbohydrate. This resulted in more nitrogen in WC available for digestion in small intestine.

Ulyatt (1981) suggested that the legume diets provide the animals' tissues with a higher ratio of absorbed protein to energy and that in case of PRG this can be achieved if rumen retention time is reduced.

1.3.1.3 Conclusion

Nutritive value of PRG depends on its digestibility and its efficiency as a nutrient source in animal metabolism. Apparent digestibility of PRG is considerably high (70-80 %) in the vegetative stage. Nutrients supplied from PRG are less effectively used by animals mainly because of slow passage from the rumen and of low absorption of protein relative to energy.

1.3.2 Factors limiting intake of perennial ryegrass

When animals are grazing pasture, intake is determined by the opportunity for animals to harvest pasture. When pasture is offered to an animal in increasing quantities, intake increases curvilinearly (Fig. 1.1). In the ascending part of the curve (non-nutritional), the ability of the animal to harvest pasture, is influenced by non-nutritional factors such as pasture structure and the grazing behaviour of the animal. At the plateau section of the curve (nutritional), nutritional factors such as digestibility, feed retention time in the rumen and concentration of metabolic products are important in controlling intake.
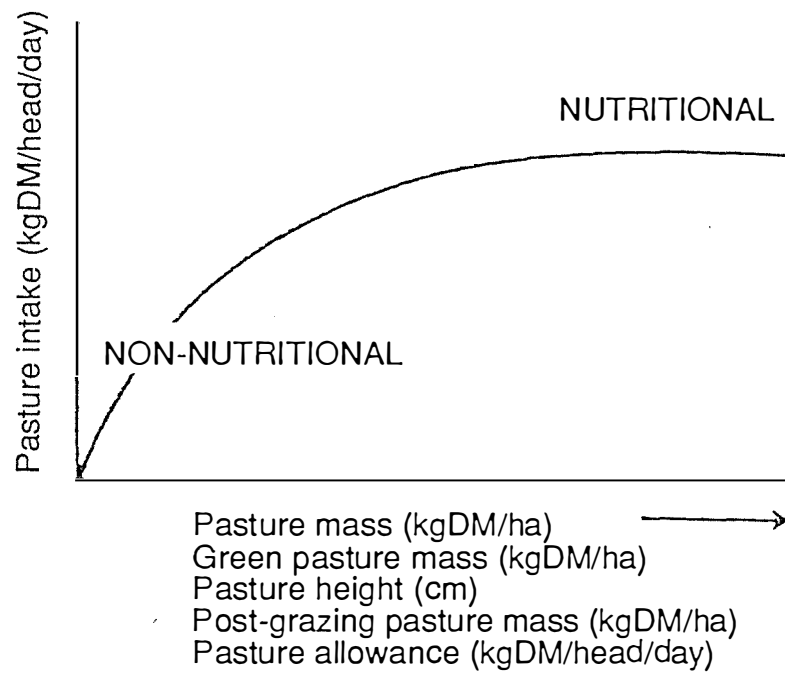
Figure 1.1 The relationship of pasture intake to various pasture characteristics and methods of pasture allocation (Poppi *et al.*, 1987).

1.3.2.1 Non-nutritional factors

1.3.2.1.1 Sward condition and prehending behaviour

Grazing animals generally select leaf in preference to stem and reject dead material where possible (Barthram *et al.*, 1984; Rattray *et al.*, 1984; L'Huillier *et al.*, 1986).

In place of sheep's upper incisor teeth there is a thick pad of connective tissue against which the chisel-like lower incisors close to grip each mouthful of herbage and it is severed by a quick jerk of the head (Hodgson, 1990). Prehending intake can be expressed as a physical process with the following components;

Intake = bite size (g/bite) x rate of biting (bites/min) x grazing time (hr).

Pasture sward height is a major factor affecting all these components and hence intake (Fig. 1.2). Bite size has been shown to have the most influence on intake and represents the major component of the non-nutritional constraint (Hodgson, 1985). Rate of biting changes in relation to sward structure, but is not as responsive as bite size (Kenney *et al.*, 1984; Black *et al.*, 1984). Grazing time has a large influence on intake. Animals can graze up to 12 - 13 hours a day when pasture of an adequate herbage mass is presented.

Pasture mass is defined as the amount of pasture per unit area. Therefore, pasture mass influences intake through both pasture height and/or pasture density, which are components of pasture structure (Fig. 1.3). Black *et al.* (1984) reported the rate of intake by sheep grazing pasture increased with both the height and density of the pasture and was best described by herbage mass per unit area effectively covered by one bite.
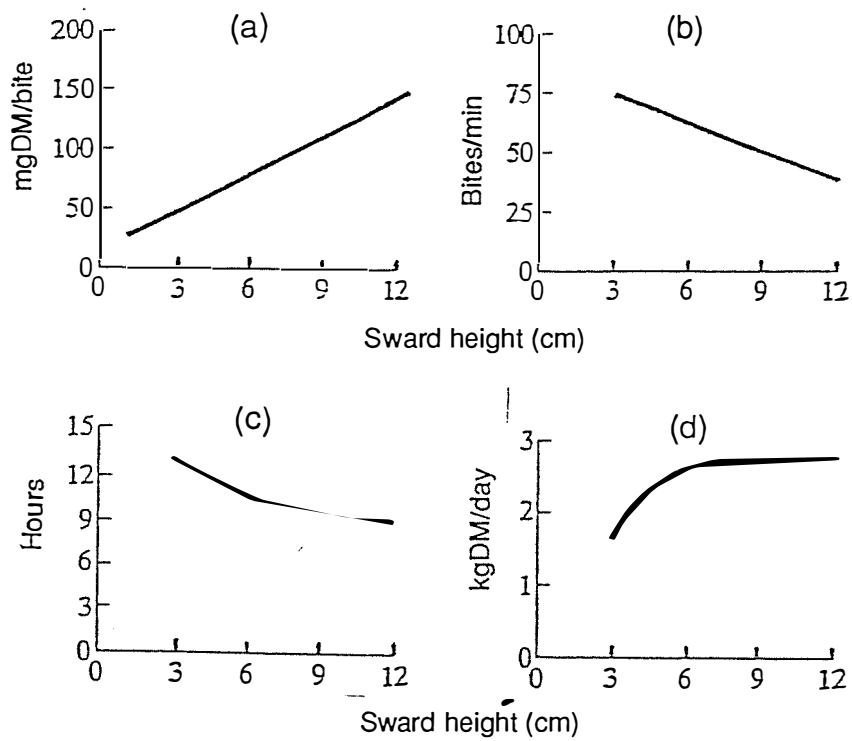
Figure 1.2 The influence of sward height on the components of ingestive behaviour (Penning, 1985).

(a) bite size (b) rate of biting (c) grazing time (d) daily herbage intake.

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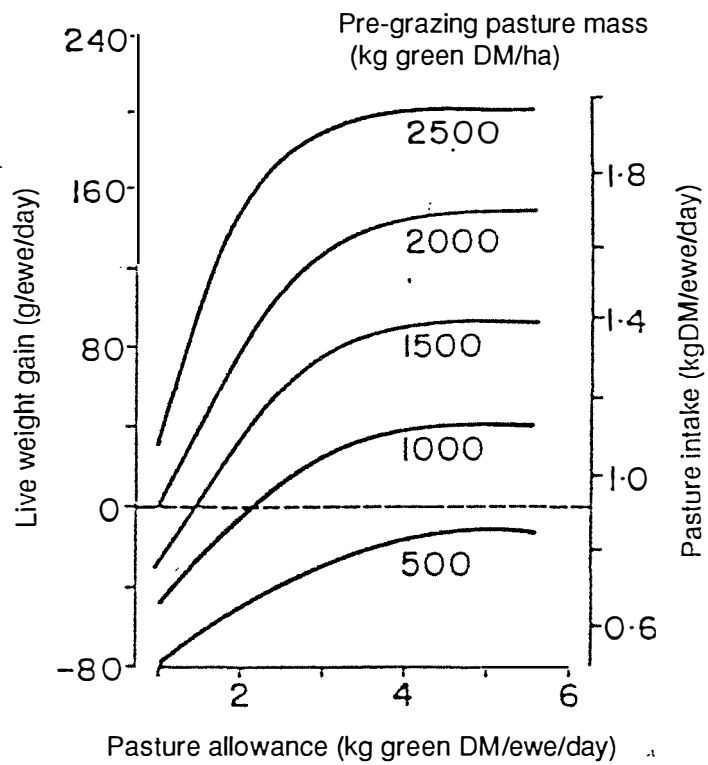
Figure 1.3 **The influence of pasture mass on the liveweight gain of ewes offered pasture at various levels of pasture allowance** (Rattray *et al.*, 1984).

1.3.2.2 Nutritional factors

Nutritional constraints on intake occur when non-nutritional factors are not restrained. Level of intake is regulated by the amount of pasture which can be accommodated in the rumen and the rate at which it disappears by digestion and passage.

The major limitation to intake of herbage has been generally considered to be physical capacity of the rumen (Campling *et al.*, 1961; Jones, *et al.*, 1972; Thornton *et al.*, 1973). However, the final intake level achieved by an animal in any particular physiological state is influenced by;

   (1) the nutrient supply (i.e. metabolic factors),

   (2) the extent to which the animal is prepared to distend its rumen and the rate of disappearance of digesta from the rumen (i.e. physical factors) (Weston *et al.*, 1987; Cruickshank, 1986).

1.3.2.2.1 Metabolic factors

Metabolic factors play a role in the regulation of intake of high quality pasture (Thomson *et al.*, 1985; Cruickshank *et al.*, 1987). Metabolic factors are those metabolites that influence intake and are generally associated with energy-yielding substrates, such as VFAs, lipids, and amino acids. Intake regulation relates to the amount of energy the animals can metabolize (Thompson *et al.*, 1990). Sheep grazing legumes tend to have approximately 25 % lower rumen fill than sheep grazing grasses despite similar or higher intakes (McLean *et al.*, 1965; Ulyatt, 1971). This suggests that intake for the animals on legume pastures appears to be regulated by both the nutrient supply, i.e. metabolic factors, and physical factors (Weston *et al.*, 1987). Cruickshank (1986) considers that metabolic regulation of intake is less important in sheep grazing grasses than in sheep grazing legumes.

1.3.2.2.2 Physical factors

The feed intake required to satisfy total energy demand of grazing ruminants commonly may not be reached because of limitations due to gastro-intestinal tract capacity (Black, 1990). Grovum (1987) considers capacity of the rumen and reticulum and not the intestines or abomasum is the major limiting factor. Thus, the amount of material that can accumulate in the rumen and its rate of disappearance from the rumen affect voluntary feed intake.

From computer simulation studies Black *et al.*, (1982) deduced that slow degradation and outflow rates from the rumen were the major factors causing long rumen retention time and reduced voluntary intake in sheep fed PRG, although rate of digestion might contribute approximately 5 % of the difference in feed intake between sheep eating WC and PRG.

Figure 1.4 shows the relationship between the apparent retention time of digesta in the rumen and voluntary intake. The rate at which particles reduce in size is a major factor which influences overall retention time of material in the rumen. Large particles have a low probability of leaving the rumen and must be therefore broken down physically before they can pass out of the rumen (Ulyatt *et al.*, 1986; Poppi *et al.*, 1987).

1.3.2.3 Conclusion

Voluntary intake of animals is affected by non-nutritional factors and nutritional factors. Non-nutritional factors depend substantially on grazing management. Of the nutritional factors, metabolic factors appear to be less significant than physical factors in the case of PRG. Slow degradation and hence long rumen retention times of PRG are major factors reducing intake. Rate of passage of digesta out of the rumen is influenced by the rate of breakdown of large feed particles to small ones and their rate of outflow from the rumen.
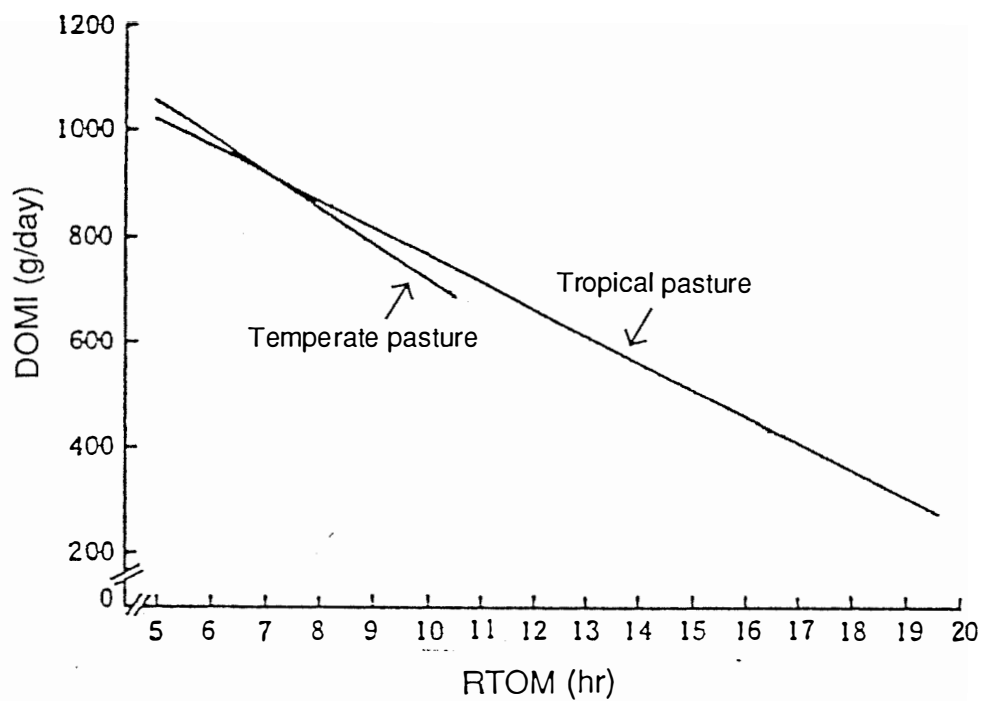
Figure 1.4 Relationship between the voluntary intake of digestible organic matter (DOMI) and the apparent retention time of organic matter in the rumen (RTOM) for temperate (from Ulyatt, 1971) **and tropical pasture species** (Thornton *et al.*, 1973).

1.3.3 Digesta passage from the rumen

The swallowed boli of feed are delivered into the reticulo-rumen in which approximately 60 % of organic matter digestion occurs (Ulyatt, 1983). Clearance of feed residues from the reticulo-rumen depends on two interacting processes:

(1) physical reduction in particle size plus microbial digestion,

(2) passage through the reticulo-omasal orifice.

1.3.3.1 Physical reduction in particle size and microbial digestion

Ingested herbage must be reduced to a critical particle size which is able to pass out from reticulo-omasal orifice. For sheep this threshold particle size appears to be 1 - 2 mm and 2 - 4 mm for cattle (Poppi *et al.*, 1980; Ulyatt, 1983; Ulyatt *et al.*, 1986). This threshold appears to be well regulated and not influenced by a range of dietary and physiological variables.

Particle size reduction is achieved by three major processes:

(1) chewing during eating,

(2) chewing during rumination,

(3) microbial attack in the rumen.

1.3.3.1.1 Chewing during eating

By chewing during eating, long forages are reduced to a size that can be incorporated into a bolus and swallowed, soluble nutrients are released for fermentation and inner structures of forage material are exposed to enable effective

invasion of the reticulo-rumen microbes. Ulyatt *et al.* (1986) reported a relationship between chewing behaviour and diet particle breakdown of sheep (Table 1.4). The chews/min is reasonably constant within species of forage. PRG resulted in a high number of chews/g DM, a low rate of eating, and a high proportion of particulate matter remained on a 4.0 mm sieve. Ulyatt (1983) reported that, in sheep, effectiveness of chewing during eating in reducing particles to < 1.0 mm (<C.EAT>) varied from 46 - 53 % between three fresh herbages and a hay, whereas John *et al.* (unpublished) have shown that <C.EAT> was 49.1, 40.9 and 46.0 % for two fresh PRG cultivars and a tall fescue, respectively (Ulyatt, 1986).

1.3.3.1.2 Chewing during rumination

Rumination serves two purposes; it reduces the particle size of refractory material, and it damages regurgitated digesta to further expose internal plant structure for microbial attack.

Table 1.5 shows effect of chewing during rumination on particle breakdown. PRG shows a high number of chews/gDM, longer rumination time/DM intake, and a high proportion of particles larger than 1 mm in rumen contents. These are similar results to effect of chewing during eating on particle breakdown.

The efficiency of chewing during rumination in reducing particles to < 1 mm (<C.RUM>) in sheep had been measured as 60 % (Ulyatt, 1983), 59 % (Domingue *et al.*, 1991b) and 39 - 65 % (Table 1.5). Chai *et al.* (1984) reported <C.RUM> as 58 - 75 % in cattle, and similarly Kennedy (1985) reported that approximately 70 % of large particles in the mouth were comminuted to small particles during one cycle of rumination and rumination accounted for approximately 85 % of comminution of large particles which entered the rumen. Ulyatt *et al.* (1986) consider that the major role of rumination is to reduce the particle size of refractory material so that it can be cleared from the reticulo-rumen and that chewing during rumination is the more important process than chewing during eating in reducing feed particle size.

Table 1.4 The effect of chewing during eating (C.EAT) on feeding behaviour and particle size reduction in sheep (n=6) fed three fresh herbages and two chaffed hays (adapted from Ulyatt *et al.*, 1986).

	Perennial ryegrass	Red clover	Lucerne	Lucerne hay	Meadow hay	RSE
Intake (gDM/d)	861	918	952	946	943	
Intake rate (gDM/min)	4.1	11.1	13.6	7.7	10.7	0.5
Chewing rate:						
Chews/min	150	145	143	142	125	2.6
Chews/gDM	36.6	13.1	10.5	18.5	11.7	1.59
% large particle DM reduced to < 1.0 mm by C.EAT	48.6	51.6	45.4	37.1	34.6	

For the fresh diets feed particle size >1.0 mm was 100 %, while the values for lucerne and meadow hays were 96.4 and 97.8 %, respectively.

Table 1.5 **Effect of chewing during rumination (C.RUM) on particle size distributions in sheep (n=6) fed three fresh herbages and two chaffed hays** (adapted from Ulyatt et al., 1986).

	Perennial ryegrass	Red clover	Lucerne	Lucerne hay	Meadow hay	RSE
DM intake (g/d)	861	918	952	946	943	
Rumination time (min/d)	540	436	317	570	547	17.4
Chewing rate:						
Chews/min	108	107	108	86	86	0.1
Chews/gDM[1]	30.7	23.3	21.1	20.0	18.5	1.06
% particle DM > 1.0mm in rumen contents	31.5	23.2	16.6	34.9	34.7	1.81
% large particle DM reduced to < 1.0 mm by C.RUM	41.9	39.2	62.6	59.5	64.6	

1: In bolus retained in the animal's mouth at the rumination.

1.3.3.1.3 Role of microbial attack in the rumen

The rumen microorganisms enter the chewed plant tissue, *via* cut ends and damaged surfaces, in the general order of mesophyll, phloem > epidermis, parenchyma bundle sheath > sclerenchyma > lignified vascular tissue (Akin, 1979). As a result, the spatial architecture of the plant tissue remains comparatively intact (Van Soest, 1975). Akin *et al.* (1989) reported that ruminal fungi weakened plant residue more amenable to physical degradation, thus, allowing the plant digesta to be more easily broken apart during rumination. Although it contributes to clearance of dry matter from the reticulo-rumen by weakening the cell wall structure internally, microbial digestion *per se* has relatively little effect on particle size reduction (Poppi *et al.*, 1980; Udén *et al.*, 1982; Ulyatt, 1983).

1.3.3.2 Passage through the reticulo-omasal orifice

Digesta flow from the rumen depends on the frequency and amplitude of reticulo-rumen contractions and the size of particles presented in the reticulo-rumen. The regular contraction of the reticulo-rumen provide the propulsive force for passage. Although particle size reduction is a prerequisite, the material present in the reticulo-rumen at any time is predominantly below the threshold size (Poppi *et al.*, 1981; Ulyatt, 1983).

Evans *et al.* (1973) and Kennedy *et al.* (1988) suggest that density of particles within the rumen also affects the passage. Welch (1987) and Reid (1984) reported that light particles were more likely to be returned to the reticulum from the omasum than heavier particles during the reticulo-rumen contraction. Reduction of feed particle size therefore only increases the probability of passage (Poppi *et al.*, 1980, 1985). The rate of outflow of the material presented in the reticulo-rumen is determined primarily by the rate of breakdown of particles to a size which is small enough to have high probability of passing through the reticulo-rumen orifice (Egan *et al.*, 1984; Poppi *et al.*, 1985).

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1.3.3.3 Conclusion

There is a threshold particle size above which material has low probability of passing from the reticulum to the omasum. The threshold size in sheep appears to be between 1.0 mm and 2.0 mm. Feed particles are reduced in size predominantly by chewing both during eating and ruminating. Chewing during rumination is more important than chewing during eating in reducing the particle size of refractory material and no further particle size reduction occurs after the rumen. PRG requires a higher number of chews both during eating and ruminating to reduce the ingesta particle size compared with legumes. Microbial digestion *per se* is considered to have little effect in reducing particle size. Although the material present in the reticulo-rumen at any time is predominantly less than the threshold size, particle size reduction is a prerequisite for passage.

1.4 Attempts to improve feeding value of perennial ryegrass by reducing physical resistance to breakdown

PRG requires a longer chewing time during both eating and ruminating, which is considered to be a major cause of slow passage from the rumen, thus resulting in lower voluntary intake and less nutrients available for the animals in their small intestine. This hypothesis has led to a new concept for plant breeders - to reduce physical resistance so that plant material can be easily broken down in size by mastication.

1.4.1 Physical resistance of perennial ryegrass to fracture

1.4.1.1 Anatomy of perennial ryegrass

Figure 1.5 is a drawing of a young plant grown outside in full daylight, presented to show the relative positions and proportions of organs of a young PRG.
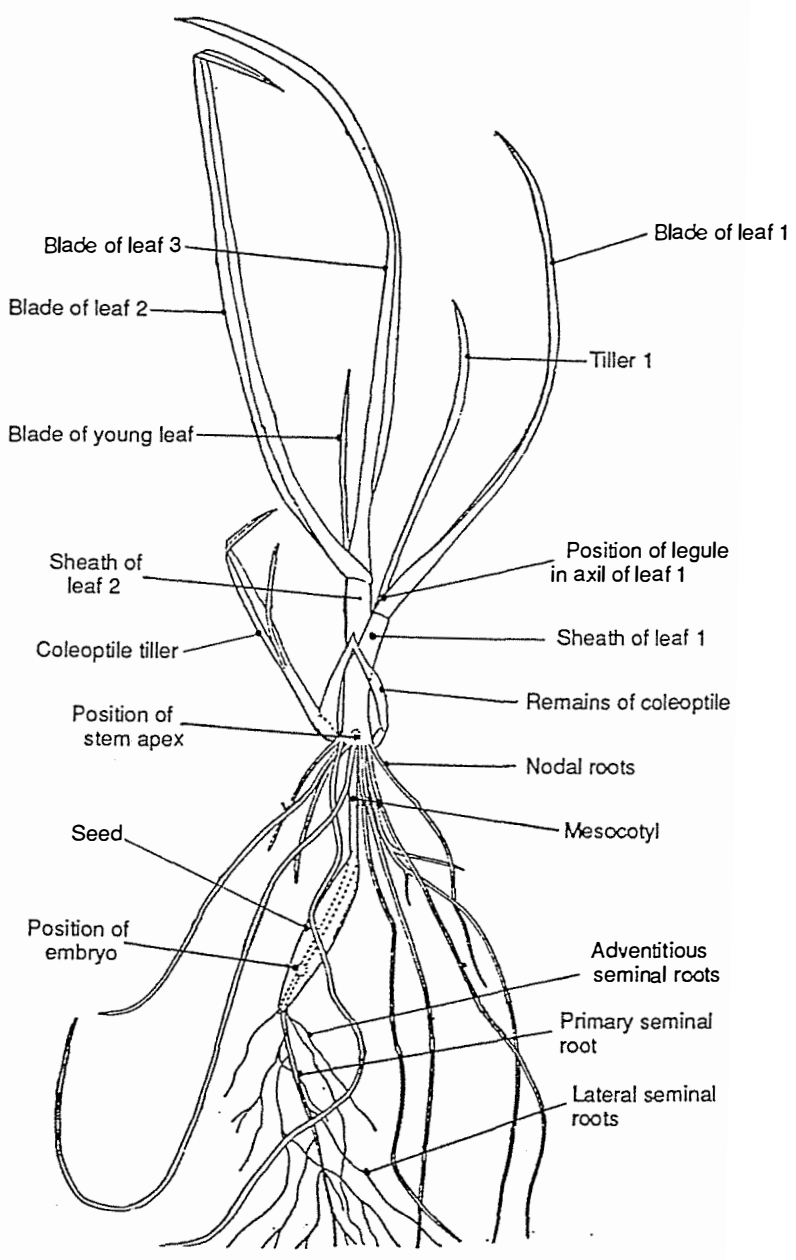
Figure 1.5 Young plant of perennial ryegrass (x 1.75 Soper *et al.*, 1956).

Blade of leaf 3

Blade of leaf 2

Blade of young leaf

Sheath of leaf 2

Coleoptile tiller

Position of stem apex

Seed

Position of embryo

Blade of leaf 1

Tiller 1

Position of legule in axil of leaf 1

Sheath of leaf 1

Remains of coleoptile

Nodal roots

Mesocotyl

Adventitious seminal roots

Primary seminal root

Lateral seminal roots

In terms of animal nutrition, leaf is the most important part of the plant as a nutrient source. Figure 1.6 shows the structure of the leaf of PRG.

The blade consists of a skeleton of parallel vascular bundles which together with the epidermis and sclerenchyma fibre support the mesophyll.

The vascular bundles are effectively continuous cylindrical sheaths that run the length of the leaf. The nutrient supply for the leaf is carried within the bundles. The single large vascular bundle is associated with the mid-rib. The remaining vascular bundles are quite regularly spaced across the leaf, with the medium sized bundles separated by two or three small bundles, depending on the width of leaf.

The sclerenchyma fibres are located immediately above the vascular bundles and a few below the vascular bundles and also along each edge of the leaf. The term sclerenchyma refers to complexes of thick-walled cells, often lignified, whose principal function is mechanical. These cells are supposed to enable plant organs to withstand various strains, such as these resulting from stretching, bending, weight and pressure, without causing damage to the thin-walled softer cells (Esau, 1953). The degree of development of these small continuous fibres varies across the leaf, and from leaf to leaf in the same manner as the vascular bundles (Betteridge *et al.*, 1986).

Surrounding the vascular bundles are two layers of cells. The inner sheath is of thick-walled, more or less lignified cells with the thickening heaviest on the radial and inner tangential walls. This layer probably serves as protection against crushing of the phloem cells and adds rigidity to the leaf as a whole. The outer sheath is of thin-walled parenchyma cells which contain small numbers of chloroplasts (Soper *et al.*, 1956).
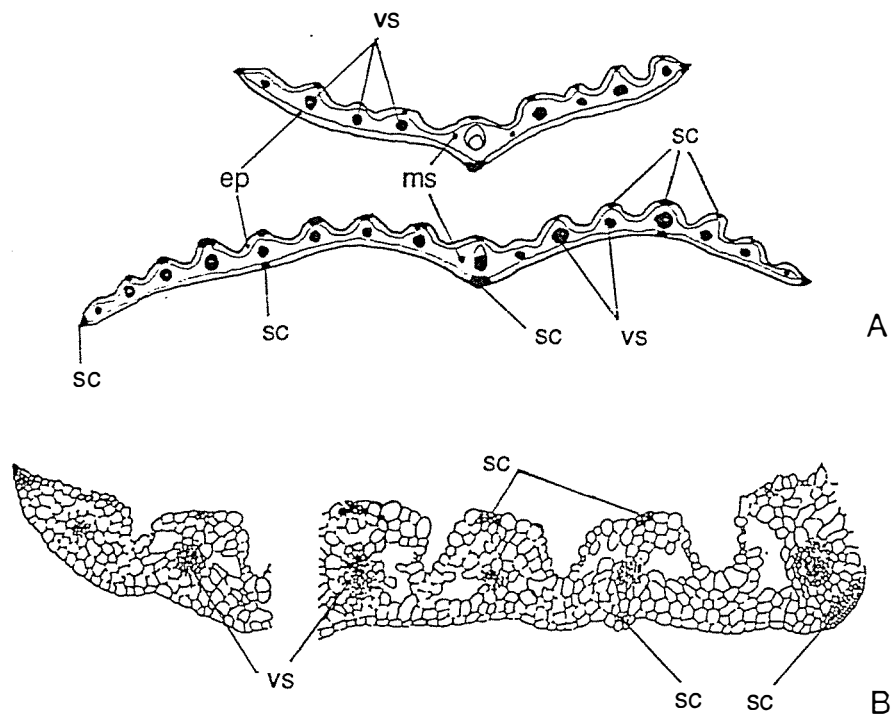
Figure 1.6 Transections of the blade of the mature leaf of perennial ryegrass (after Soper *et al.*, 1956).

A: outline diagrams of leaf two (below) and leaf six (above) on the main

stem showing differences in size between the two leaves (x30).

B: Portions of the leaf on a larger scale including the median vascular

bundle, lateral bundles of varying sizes and the small bundle at the edge of the leaf (x80).

(vs; vascular bundles, ep; epidermis, sc; sclerenchyma,

ms; mesophyll)

1.4.1.2 Forces applied at fracture

Figure 1.7 shows three major different forces that can be applied to the material. Direct tension occurs in a two-force member when the forces are pulling on it in the axial direction, i.e. direct tensile force. When a force acts on a member in a manner that it tends to cause the member to separate along a plane parallel to the applied force, it is called a direct shearing force. Direct compression occurs in a two-force member when the forces are pushing on it in the axial direction. These stresses on the material can be measured at fracture as a criteria of the physical resistance to fracture, i.e. physical strength of the material.

The physical strength of the material for these types of stress is defined as the stress at fracture, Sf;

$$Sf = \frac{\text{Breaking load}}{A}$$

where A is the cross-sectional area at fracture providing no deformation of the material occurs (static).

Young's modulus is often used to express stiffness of material which is the ratio of stress to strain (dynamic). Hooke's solid is a model of a material which is elastic, homogeneous and isotropic. When the Hooke's solid is under tensile or compressive stress (see Fig. 1.8), Young's modulus, E, is given by;

$$E = \frac{\text{tensile or compressive stress, } \sigma}{\text{tensile or compressive strain, } \tau}$$

where
$$\sigma = \frac{\text{force, } f}{\text{area, } A} \quad \text{and} \quad \tau = \frac{\delta l}{l}.$$

where l in the length of the solid.
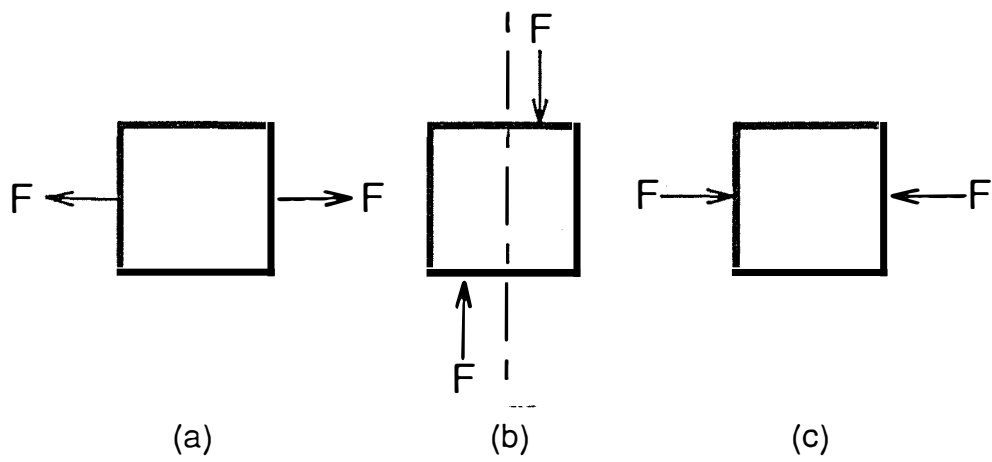
Figure 1.7 Types of force to apply to the material.

(a) direct tension. (b) direct shear. (c) direct compression.

F: force.

no forces applied

(a)

(b)

(c)
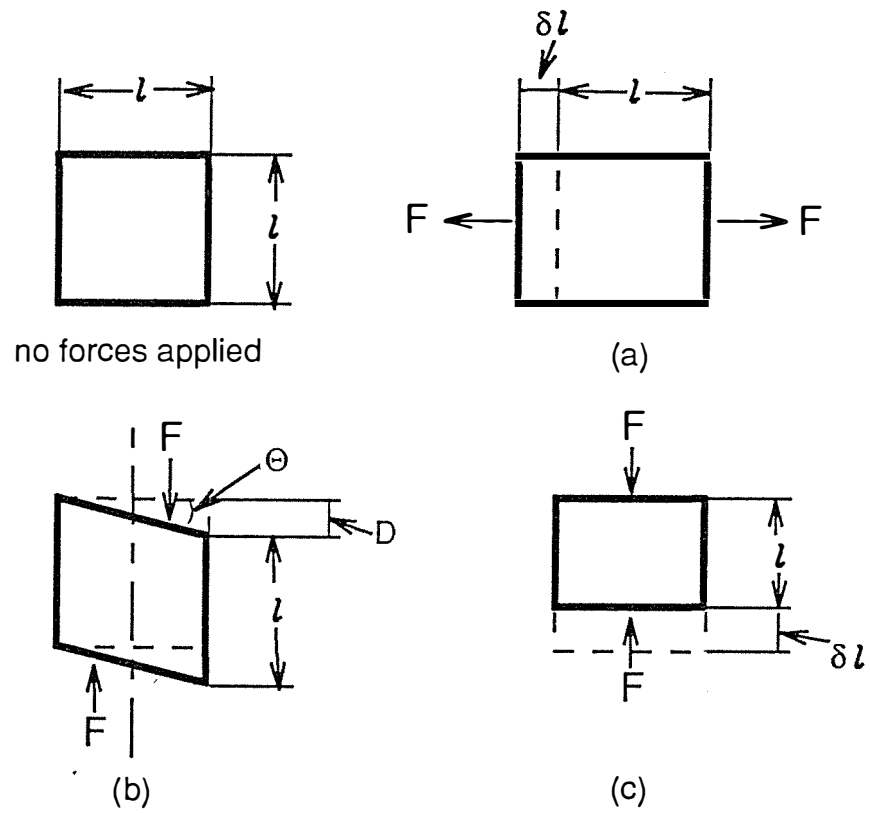
Figure 1.8 Hooke's elasticity in (a) tension, (b) shear and (c) compression. F: force.

When the Hook's solid is subjected to distortion by shear stress, the shear modulus, G, is given by;

$$G = \frac{\text{shear stress, } \mu}{\text{shear strain, } \delta}$$

where $\mu = \dfrac{\text{force, f}}{\text{area, A}}$ and $\delta = \dfrac{D}{l} = \tan \Theta,$

where D is an angle of the strain.

When the material is reinforced with fibres (fibre-reinforced composite) the Voigt model can be applied to give the overall modulus, EL, as;

$$EL = \sum_{i} E_i V_i$$

where Ei and Vi are the modulus and volume fractions of the components (Kelly, 1966).

1.4.1.2.1 Perennial ryegrass in tensile stress

Apart from some early works, there have been very few published studies of the properties of PRG in tensile stress.

In some early studies, Evans (1964a, 1967a, 1967b) used a specially constructed apparatus to compare the tensile strength of leaf in four ryegrass varieties under grazing pasture conditions over a period of one year. He reported seasonal change in the tensile strength and that the varieties with high tensile strength of leaf had high cellulose contents and sclerenchyma wall percentages. These results were consistent with the animal weight gains recorded on the same trial (Rae *et al.*, 1964)(Table 1.6).

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Table 1.6 Mean leaf strengths, sclerenchyma wall percentages and cellulose percentages of perennial, Ariki, Italian and Short-rotation ryegrass and mean liveweight gain of sheep (adapted from Evans, 1964b, and Rae *et al.*, 1964).

Variety	Strength (g/mg of 5 cm length)[1]	Cellulose (%)[1]	Sclerenchyma wall (%)[2]	Mean liveweight gain (g/d)[3]
Perennial	97.3	12.8	2.10	140
Ariki	90.6	12.1	1.60	135
Italian	69.9	10.1	0.87	223
Short-rotation	70.9	10.4	0.98	na

1: Measured on 8 dates during the period of 24/6/63 - 9/10/63.

2: Percentage of total cross-section of the leaf measured on 4 dates during the period of 10/7/63 - 9/10/63.

3: Mean liveweight gains during the period of 5/7/63 - 26/11/63.

Bailey (1964) pointed out the relationship between leaf tensile strength and physical breakdown of digesta particles. However, in these early studies, Evans (1967a, 1967b) and Wilson (1965) suggested the use of leaf strength as a indicator of cellulose content in leaf, which was believed to be a criterion of nutritive value affecting animal performance (Johns, 1962, Rae *et al.*, 1963; Rae *et al.*, 1964).

More recently, Vincent (1982) and Betteridge *et al.*, (1986) criticized these pioneers' studies for using inappropriate measurement methods. In Evans' (1964a, 1967a, 1967b) and Wilson's (1965) studies, their measurement of "tensile strength of leaf" was defined as;

$$\text{tensile strength of leaf} = \frac{\text{tensile breaking load of leaf}}{\text{dry weight of a 5 cm-length-cut leaf.}}$$

The more appropriate parameter for the tensile strength is, as mentioned in the section 1.4.1.2, the stress at fracture, Sf;

$$Sf = \frac{\text{Tensile breaking load}}{\text{Cross-sectional area of leaf at fracture.}}$$

There is no simple relationship between Sf and the tensile strength defined by Evans and Wilson (Betteridge *et al.*, 1986).

Vincent (1982) and Betteridge *et al.* (1986) measured tensile strength of leaf of PRG and estimated cross-sectional area using width and thickness of leaves, although Vincent (1982) reported that this method overestimates the true cross-sectional area by about 25 %. The results of both studies are summarised in Table 1.7. Although values in the Vincent study (1982) were estimated as being higher than those in the study by Betteridge *et al.* (1986), sclerenchyma fibres in both studies are much stronger than leaves or vascular bundles in PRG. The transverse strength of leaf was also reported as less than 0.142×10^7 kg/m^2(Young's modulus)

Table 1.7 Young's modulus of tensile strength and volume-fraction of leaf of perennial ryegrass (adapted from Vincent, 1982, and Betteridge *et al.*, 1986).

Measured by	Vincent		Betteridge *et al.*	
	Modulus (kg/m^2)	Volume fraction (%)[1]	Modulus (kg/m^2)	Volume fraction (%)[1]
Leaf	5.63×10^7		0.82×10^7	
Sclerenchyma fibre	230.4×10^7	4.24 - 1.88	29.6×10^7	2.93
Vascular bundle	8.54×10^7	4.12	4.4×10^7	3.52

1: Percentage of total cross-sectional area.

which could be accounted for by the cuticle covering the leaf and leaf matrix (Vincent, 1982). Regarding the transverse modulus as equivalent to the matrix modulus in the Voigt model (see section 1.4.1.2), Vincent (1982) estimated the sclerenchyma fibres account for between 90 and 95 % of the tensile strength of the leaf. In Betteridge *et al.*'s study (1986) it can be calculated as being approximately 85%.

Vincent (1982) related tensile strength to the animals' behaviour of prehending grass from the ground, which is one of the non-nutritional factors affecting intake. There are very few published studies of the relationship between the mastication of ruminants and the forces applying to the feed material during breakdown by mastication.

1.4.1.2.2 Forces applied to the feed material during mastication by ruminants

1.4.1.2.2.1 Masticatory movements of ruminants

Although Troelsen *et al.* (1964) invented a artificial mechanical masticator, which was constructed from a simple double gear pump, to measure the rate of breakdown of forages by ruminants, there have been very limited published studies of masticatory movements of ruminants.

Figure 1.9 shows the masticatory musculature of sheep. In herbivores like goats, *Musculi masseter* mainly produces the forces caused by sideward tension which moves the lower jaw laterally (Schumacher, 1985). In functional analyses of the mammalian masticatory apparatus, Turnbull (1970) calculated in sheep *Musculi masseter* accounted for approximately 64 % of total jaw closing power compared with 9.7 % and 13.1 % in opposums and cats, respectively.

From a study of the grinding surface of the teeth and articular surfaces of the skull and the jaw bone, Murphy (1959) has determined the axis of the chewing stroke in
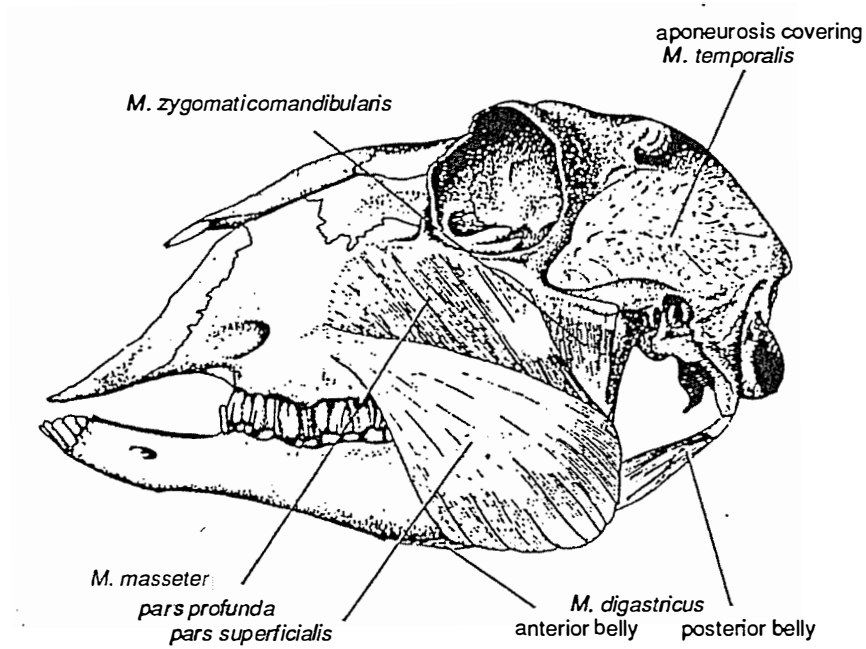
Figure 1.9 Masticatory musculature of sheep (Turnbull, 1970).

Superficial dissection in lateral aspect showing the aponeurosis of *M. temporalis* and showing *M. masseter* and *M. zygomaticomandibularis*.

(M; Musculi).

adult sheep. The masticatory movements as sliding movement of the lower teeth of sheep can be simulated by defining these axes .

Hiiemae (1978), in a comparison of the lower jaw movements in some mammals, noticed that the degree of lateral movement is the greatest in goat and the least in cat (Fig. 1.10). The profiles are shown in frontal view with the working side in the right side of the jaws. (The horizontal line indicates the transverse plane of centric occlusion and the vertical line is perpendicular to it at the point of centric occlusion. The hatched vertical line corresponds to the anatomical midline.)

1.4.1.2.2.2 Forces applying to the feed material by teeth

When feed material is between the upper and lower cheek teeth during lateral masticatory movements, the masticatory force applying to the feed material is the shear force (Fig. 1.11). Rensberger (1973), in his simulation study of mastication and dental wear in herbivorous mammals, showed that the grinding masticatory stroke actually consisted of shear when the tops of the tooth crests are in contact. The function of mastication, however, also occurs on a microscopic scale because each cheek tooth of ruminants has multiple cusps whose individual shapes are irregular or complex. Thus, the major force applying to the feed material by teeth is probably shear, but tensile force may be involved to a certain extent.

1.4.1.2.3 Conclusion

Although in early studies tensile breaking load of PRG was measured, interpretation of data from these studies should remain only as a guide due to the inappropriate definition of strength. More recent works reported that sclerenchyma tissues account for approximately 90 % of tensile strength. However, in actual mastication of sheep, shear appears to be a major force applying to the breaking down of the feed material rather than tensile force.
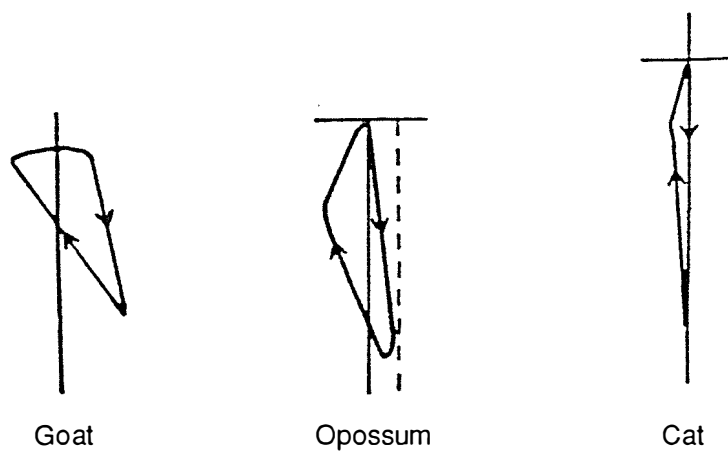
Figure 1.10 The movements of the lower jaw in a "typical" chewing cycle in goat, in opossum and in cat (adapted from Hiiemae, 1978).

(The horizontal line indicates the transverse plane of centric occlusion and the vertical line is perpendicular to it at the point of centric occlusion. The hatched vertical line corresponds to the anatomical midline.)

upper teeth

feed material

F

lower teeth
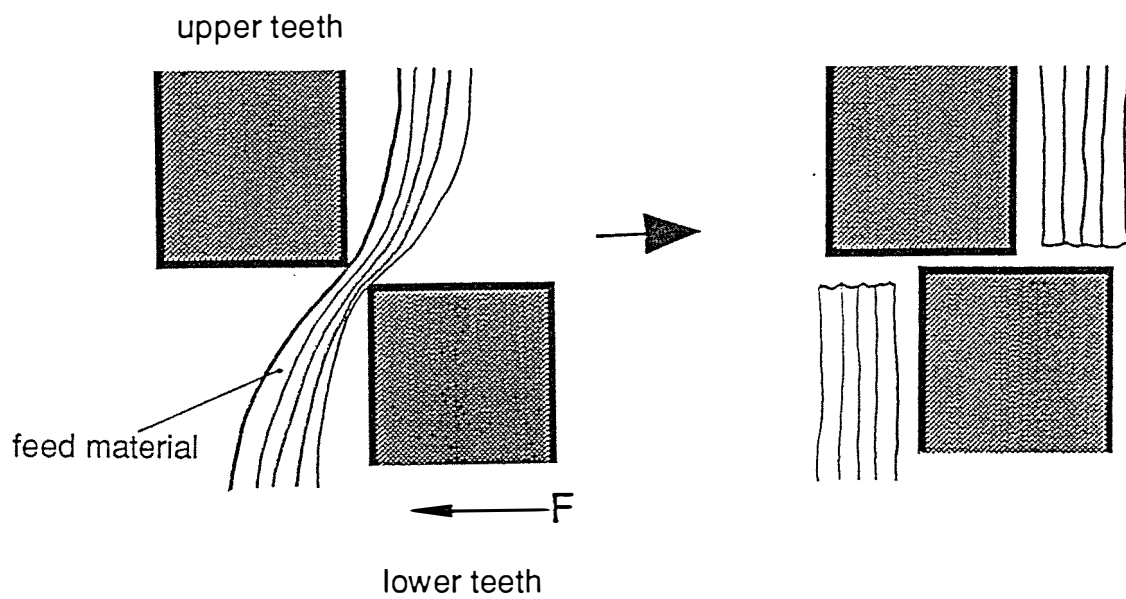
Figure 1.11 Relationship of feed material in lateral masticatory movements of opposing occlusal surfaces.

(F; forces applying)

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1.4.2 Contribution of reduced leaf shear breaking load in perennial ryegrass towards improved intake and feeding value - Background studies

1.4.2.1 Selection for low and high leaf shear breaking load perennial ryegrass

Easton (1989) adopted the leaf shear breaking load (LSBL) as a parameter of the physical resistance to breakdown of the PRG. LSBL was measured as shearing across the leaf at 3 and 6 cm below the ligule by a Warner-Bratzler meat shear apparatus. The maximum load required to shear a leaf was defined as LSBL. In 1984, based on LSBL, six plants of the low leaf shear breaking load (LS) selection and six plants of the high leaf shear breaking load (HS) selection were taken from a population of 155 'Grasslands Nui' *Lolium perenne* plants and 216 (*L. perenne* x *L. multiflorum*) x *L. perenne* plants. Four plants for the HS selection, and two plants for the LS selection were taken from the 'Nui' population, the remainder were from the pair-cross population. The LS and HS selection groups were isolated separately in pollen-proof glasshouses and allowed to interpollinate. Seed from these two selection lines were harvested from individual plants in January 1985, and sown in the glasshouse. These are referred to as progeny.

Mean LSBL of these parent plants and progeny plants are shown in Table 1.8 and Table 1.9, respectively. Predicted heritability within a selection was calculated as 0.36 (Easton, 1989). The LS and HS selections in 1984 had mean LSBL 32.4 % lower and 36.8 % higher, respectively, than the mean of the original population. The large difference in LSBL of the parent lines was still evident in March 1986 ($p < 0.001$).

Table 1.8 Mean leaf shear breaking load (LSBL) of parent plants (n=6) selected for low (LS) and high (HS) leaf shear breaking load at the time of selection (1984) and in January 1986, and of the original population (adapted from MacKinnon et al., 1988).

	Selected parents		Original population	Effect of selection
	LS	HS		
LSBL (kg/leaf)				
Time of selection (1984)	0.46	0.93	0.68	***
January 1988	0.56	0.88	--	*

Table 1.9 Mean leaf shear breaking load (LSBL) of the progeny population for low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections in December 1985 (n=120) and March 1986 (n=240) (adapted from Mackinnon et al., 1988).

	Progeny		Effect of selection
	LS	HS	
LSBL (kg/leaf)			
December 1985	0.48	0.66	***
March 1986	0.66	0.86	***

1.4.2.2. Rates of dry matter consumption, digestibilities and liveweight gains of sheep grazing two perennial ryegrass selections selected for low and high leaf shear breaking load

MacKinnon *et al.*, (1988) also determined rates of dry matter consumption and ruminal degradation of dry matter using the polyester bag technique with the two progeny selections, and also WC using sheep fed a basal diet of fresh PRG/WC pasture (Table 1.10). The LS selection of PRG increased the rate of dry matter consumption by 17 % ($p<0.05$) but this was still lower than the rate of WC consumption by sheep ($p<0.001$). The rate at which the insoluble component was degraded in the rumen (i.e. constant c) was greater for WC than for either PRG selection ($p<0.001$). Selecting PRG for LS had no effect at all upon rate of dry matter degradation (c) or upon potential degradability (a + b). From these results, MacKinnon *et al.* (1988) considered that the plant selection may have resulted in a faster rate of breakdown during chewing, resulting in a faster rate of dry matter consumption.

Kolver (1989) measured live weight gains of 8-month-old lambs grazing either LS or HS PRG swards at generous pasture allowances and reported that the animals grazing the LS selection grew faster than the animals grazing the HS selection ($p<0.01$) (Table 1.11).

1.4.2.3 Conclusion

The HS and LS selections were selected from large numbers of PRG. The heritability of LSBL was estimated to be 0.36. Measurements of LSBL made on the progeny of both selections confirmed this estimate.

The rate of dry matter consumption by sheep was higher in the LS selection than the HS selection, but rumen degradation rate was not different. In a grazing trial, 8-month-old wether lambs grazing the LS selection grew faster than the animals on the HS selection.

Table 1.10 Means of rate of dry matter (DM) eaten and disappearance in the rumen of sheep (n=6), **of vegetative perennial ryegrass (PRG) selected for low (LS) and high (HS) leaf shear breaking load, and of white clover** (adapted from MacKinnon et al., 1988).

	PRG selection		White clover	Effect of selection
	LS	HS		
Rate of eating (gDM/min)	7.6	6.5	9.4	*
Rate of DM disappearance[1]:				
Constant a (%)	36.6	35.9	35.1	ns
Constant b (%)	51.8	52.8	57.8	ns
Constant c (%/h)	9.8	10.0	22.7	ns
Potential degradability (a + b)	88.4	88.7	92.9	ns

1: DM disappearance from polyester bags (y) expressed as a percentage of that present at t=0, was fitted to the equation; $y = a + b \ (1-e^{-et})$, where the instantly soluble fraction (a), the proportion degraded in time t (b) (i.e. the insoluble component) and the degradation rate of the 'b' fraction (c).

Table 1.11 Live weight gains of 8-month-old lambs grazing low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections and means of leaf shear breaking load (LSBL) of the two selections during the trial (adapted from Kolver, 1989).

	Selection		Effect of selection
	LS	HS	
Live weight gain (g/day)	125	108	**
LSBL (g/leaf)	567	751	**

1.4.3 Objectives of investigations in this thesis

As discussed in section 1.4.1.2, physical resistance of leaf to shear, namely leaf shear strength, is a function of LSBL and cross-sectional area of the leaf and is defined as;

$$\text{Leaf shear strength} = \frac{\text{LSBL}}{\text{Cross-sectional area of leaf at fracture.}}$$

The LS and HS PRG were, however, selected and established based on only LSBL (see section 1.4.2.1). Thus, the effect of selection for the LS and HS PRG in the true sense of physical resistance is yet to be investigated. Since leaves of PRG are fibre-reinforced composites having many parallel fibres along the leaf, the strength of leaf to breakdown may differ between when the force applied across the leaf and when the force applied along the leaf. This means that morphology of the leaf may influence the efficiency of breakdown by mastication. In addition, no demonstrations have been made, in comparison between the HS and LS selections, that the LS selection physically degrades more efficiently by mastication of ruminants, and hence, improves rumen digesta outflow rate and consequently leads to higher voluntary intake associated with increased efficiency of utilisation of both protein and the whole diet and, as a result, achieves improved FV.

Therefore, in this thesis, Chapter 2 and Chapter 3 compare the two PRG selections in the physical and morphological properties of leaves and in efficiency of breakdown by mastication. Chapter 4 and Chapter 5 investigate the effect of reduced LSBL in PRG on digesta outflow rate, voluntary intake and FV;

Chapter two compares leaf anatomy, leaf morphology and leaf shear strength between the two PRG selections selected for LS and HS,

Chapter three examines the effect of reduced LSBL in PRG upon particle breakdown during the mastication in sheep

Chapter four examines the effect of reduced LSBL in PRG upon rumen digesta outflow rate and apparent digestibility in sheep

Chapter five examines the effect of reduced LSBL in PRG upon voluntary feed intake, live weight gain and wool production in grazing sheep.

Chapter Two

COMPARISONS OF LEAF ANATOMY, LEAF MORPHOLOGY AND LEAF SHEAR STRENGTH BETWEEN TWO PERENNIAL RYEGRASS SELECTIONS SELECTED FOR LOW AND HIGH LEAF SHEAR BREAKING LOAD

2.1 Introduction

Two selections of perennial ryegrass (PRG) were selected for low (LS) and high leaf shear breaking load (HS) based on the maximum load required to shear across the leaf, i.e. leaf shear breaking load (LSBL) (Easton, 1989). However, the true measure of physical resistance of the leaf to shear, namely, leaf shear strength (LSS) is a function of LSBL and cross-sectional area (CSA) at fracture (see section 1.4.1.2). Leaves of PRG are fibre-reinforced composites that have many sclerenchyma fibres along the leaves. These sclerenchyma fibres account for more than 85 % of the total strength when the leaves are in   tensile stress (Vincent, 1982; Betteridge *et al.*, 1986). This suggests that the morphological and anatomical characteristics of PRG leaf are important factors in understanding its physical resistance to breakdown.

Therefore, this chapter further investigates the differences in LSBL between the two selections in relation to the leaf cross-section, anatomical structure and chemical composition. Furthermore, the relationship between LSBL and leaf morphology, when the two selections are subject to breakdown, is discussed.

2.2 Materials and Methods

LS and HS PRG selections were grown under a controlled atmosphere. Anatomy of the leaf CSA for the two PRG selections was examined using a light

microscope. LSBL and cross-sectional areas of three successively emerged leaves at three aging levels for the two PRG selections were examined to estimate the LSS. Morphological measurements were also made on each leaf. Chemical analyses were performed to determine concentrations of cell-wall constituents.

2.2.1 Low and high leaf shear breaking load perennial ryegrass selections

In May 1987, approximately 150 seeds each of LS and HS PRG selections, harvested in 1985 (See section 1.4.2.1), were sown in a glasshouse of the Plant Physiology Division DSIR, Palmerston North for germination.

In June 1987, 60 young germinated shoots were randomly selected from each selection and transplanted into individual plastic pots. The soil used consisted of 70 % fine gravel, 15 % peat and 15 % vermiculite. The pots remained in the glass house and were watered regularly.

In August 1987, all the pots were moved to a controlled atmosphere cabinet (CAC) of the Plant Physiology Division's Climate Laboratory. All the tillers already emerged were clipped so that sampling could be made on leaves on the newly formed tillers. In the CAC, climatic conditions were set for optimum growth conditions determined for PRG (Hunt *et al.*, 1981; Hunt *et al.*, 1985) i.e. constant temperature of 20 C$^\circ$, 150 W/m^2 irradiance for a 12-h light period and 5 mbar pressure deficit. Each pot received 250 ml of nutrient solution (see appendix 2.1) twice a day supplied through a polyethylene tube inserted into the soil.

2.2.2 Experimental design

Plant and leaf allocations were made as shown schematically in Figure 2.1.

```
Total          Selection      Emergence level                              Aging level

                                                                        ┌── M00 (10 leaves)

                                         ┌── 2nd (20 pots) ── ──  ┌─────────┐
                                         │                        │ within  │ ── ┬── M10 (10 leaves)
                                         │                        │  each   │
                     ┌── LS (60 pots) ───┼── 4th (20 pots)        │  pot    │
                     │                   │                        └─────────┘
                     │                   │                               └── M20 (10 leaves)
    120 pots ────────┤                   └── 6th (20 pots)
                     │
                     └── HS (60 pots)
```
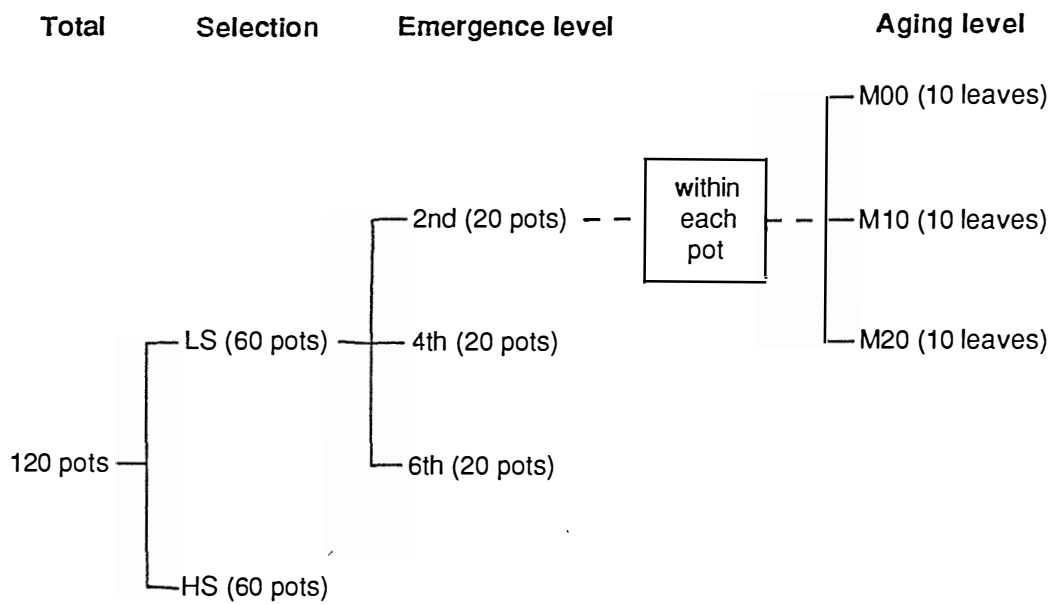
Figure 2.1 Schematic representation of the plant and leaf allocation for the controlled atmosphere trial.

(M00: maturation, M10: 10 days after maturation, M20: 20 days after maturation)

2.2.2.1 Plant allocation

Sixty pots each of both PRG selections were randomly allocated into three 20-pot groups for leaf collections at three emergence levels i.e. the second-emerged (2nd), the fourth-emerged (4th) and the sixth-emerged (6th) leaves to examine effects of the leaf emergence on the physical and chemical parameters of the leaves.

2.2.2.2 Identification and allocation of leaves

In each pot, 30 individual tillers were randomly selected. In each newly emerged tiller, each leaf emergence level was identified by counting leaves from the first newly emerged leaf. All the identified leaves were marked by sticking self-adhesive labels on both their sides. In each pot, the 30 tillers were then randomly allocated into 10 tillers each of three leaf aging levels, i.e. maturation (M00), 10 days (M10) or 20 days (M20) after full maturation, respectively, to examine the aging effects on the physical and chemical parameters of leaf. The maturation of the leaf was defined as appearance of a ligule on each leaf.

2.2.3 Collection of leaves

Leaves of 2nd-M00, 4th-M00 and 6th-M00 were collected over two-day periods, three weeks, five weeks and eight weeks after the clipping, respectively. The sampling dates were decided when ligules had appeared on more than 90 % of the identified leaves. Collections of leaves of M10 and M20 were made over two-day periods, 10 days and 20 days after the collections of leaf M00, respectively.

The leaves were carefully cut off from the tillers immediately below the position of the ligule using a pair of scissors. The leaves collected from each pot were placed into individual plastic bags and immediately stored under refrigeration (approximately 4 C°) for up to 18 hours before taking any measurements. Leaves which had partially or entirely died were omitted from the morphological and

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LSBL analyses. Most of the leaves collected for aging level M20 were found in this category and therefore, only chemical analyses were performed on them.

2.2.4 Measurements of leaf morphology

The morphological measurements were made for length, width and weight of each collected leaf. The length of leaf was measured on each leaf as the distance between the apex of the leaf and the position of the ligule on the leaf. The widths, the distance between both edges of the leaf, were measured on each leaf at three leaf positions i.e. 1/4 (0.25L), 1/2 (0.50L) and 3/4 (0.75L) of the leaf length from the position of the ligule to the apex using a pair of vernier calipers. Fresh weight of each leaf was also recorded.

2.2.5 Determination of leaf shear breaking load

2.2.5.1 The position of shear on the leaf

After the completion of morphological measurements, 10 leaves at each aging level collected from each pot at each emergence level were gathered and aligned along the 0.50L position and were then shorn at that position.

2.2.5.2 Shear apparatus

A Warner-Bratzler (WB) meat shear apparatus (manufactured by G and R Electric Co., Kansas, USA) was used to measure leaf shear breaking load. Originally, the apparatus was designed to simulate the act of mastication of meat in the human mouth, to measure the physical resistance of meat (Warner, 1928). The apparatus was used for selecting the LS and HS PRG selections (see section 1.4.2.1). A steel blade 1.016 mm (0.04 in) thick was moved through a slot that cleared it by 0.127 mm (0.005 in) at a constant speed of 22.86 cm/min (9.0 in/min) by a motor driven

screw. A bundle of 10 leaves was placed, so that the direction of the fibre in the leaf lay across the blade, in a triangular hole in the blade that circumscribed a 25.4 mm (1.0 in) diameter circle. The edges of the hole formed a semicircular edge of 0.508 mm (0.02 in) radius that contacted the sample. These specifications were established by Bratzler (Bratzler, 1949). As the blade was pulled through the slot, the leaves were compressed to a triangular cross-section and finally shorn. The maximum load required to pull the blade through the slot was indicated by a spring scale with a pointer held at maximum load. Movements of the blade and position of the slot of WB in shearing action are shown in Appendix 2.2.

2.2.5.3 Determination of leaf shear breaking load

Although the reading from the WB apparatus was actually a combination of forces of tension, compression and shear (Pool *et al.*, 1969, Larmond *et al.*, 1972, Voisey *et al.*, 1974 and Voisey, 1976), because the material in contact with the cutting edges was subject to complex stress patterns, the direct reading from the apparatus divided by number of the leaves shorn was determined as LSBL. The fragments of leaves were collected each time after the shear for the chemical analyses. The leaf fragments within each aging level were pooled, freeze-dried and ground through a 1 mm mesh sieve to conduct the chemical analyses.

2.2.6 Estimation of leaf cross-sectional area and anatomical examination of leaf cross-section

It was impractical to measure the CSA at fracture (i.e. 0.50L position) directly for each collected leaf due to a large number of samples (total 3600 leaves). Alternatively, regressions between the leaf CSA and the leaf width at 0.50L were determined by the following procedure in 11 plants each selected from the two PRG selections. CSA for the collected leaves was then mathematically estimated from the regressions. Anatomical examinations of leaf cross-sections were also made.

2.2.6.1 Collection of leaf cross-sections

After the above leaf collections were completed, 11 pots were randomly selected from each PRG selection. The tillers were again cut back and allowed to regrow. The 2nd-M00 leaves were then collected, three weeks after the clipping, when ligules appeared on more than 90 % of the leaves. For each collected leaf, an approximately 3 mm length of transverse leaf section was cut out at 0.50L using a safety razor.

2.2.6.2 Preparation of specimens for microscopy

The specimens were fixed in 3 % glutaraldehyde and 2 % formaldehyde in 0.1 M phosphate buffer at pH 7.2 for 12 hours, followed by dehydration with an ethanol series 20 - 100 % (v/v). The specimens were then infiltrated and embedded in Polysciences' JB-4 plastic medium. Transverse sections (1 μm thick) were cut with an LKB-ultramicrotome and transferred to a drop of water on each microscope slide. The slides were then placed on a hot plate and allowed to dry. Sections were stained on the microscope slides by applying several drops of 0.05 % toluidine blue in 0.1 M phosphate buffer. After staining, the toluidine blue solution was rinsed off the sections with distilled water and the sections allowed to dry before light microscope examination.

2.2.6.3 Microscope examination

Each specimen was examined for anatomical structure and photographed through a light microscope with a camera attached (manufactured by Olympus Optical ltd., Japan).

2.2.6.4 Determination of leaf cross-sectional area

The photographs of each specimen were projected and enlarged (x 140) on drawing paper. Leaf width was measured and outlines of cross-section, sclerenchyma tissues and vascular bundles were carefully traced on to the paper for each specimen. Each tissue outline was then cut out and weighed for each specimen. Areas for each tissue segment and total cross-section were determined based on proportional weights of the cutouts to the paper weight of a known area for each specimen.

2.2.6.5 Mathematical estimation of leaf cross-sectional area from width

Regression analyses were performed between the leaf CSA and leaf width in the 11 specimens for each PRG selection. The obtained regression equations were used to estimate CSA from the width at 0.50L for the leaves measured LSBL. The equations were assumed to be effective for all the leaf emergence levels and aging levels.

2.2.7 Determination of leaf shear strength

The (static) LSS for each leaf was determined as LSBL divided by the leaf cross-sectional area estimated by the regressions. A modulus (dynamic) based on the ratio of the shear breaking stress to the shear breaking strain was not estimated due to technical difficulties in determining the strain for the leaf.

2.2.8 Chemical analyses

The chemical analyses were performed for acid detergent fibre (ADF), neutral detergent fibre (NDF) and lignin concentrations (Robertson et al., 1981) and organic matter was determined by ashing overnight at 550 C°.

Cellulose and hemi-cellulose concentrations were determined by subtracting the concentration of lignin from ADF concentration and ADF concentration from NDF concentration, respectively.

2.2.9 Statistical analyses

Statistical analyses were performed by the analysis of variance for a fixed effect completely randomized model with five hierarchies, i.e. two selections, three leaf emergence levels nested within each selection, three aging levels nested within each leaf emergence level, 20 pots nested within each aging level and 10 leaves nested within each pot. Thus, interactions among the hierarchies were not able to be analysed. The Type II test in GLM procedure of SAS (SAS Inst., Inc., NC., USA) was used for the analyses. An example table of the analysis of variance is shown in the appendix 2.3.

2.3 Results

2.3.1 Cross-sections of the leaves of the low and high leaf shear breaking load perennial ryegrass selections

Examples of leaf cross-sections of the LS and HS PRG selections are shown in Plate 2.1. In leaves of the LS selection, sclerenchyma tissues were observed as being less developed than those in the leaves of the HS selection. Sclerenchyma tissues in the inner epidermis were associated with each vascular bundle in the leaves of both PRG selections. In the leaves of the LS selection, however, sclerenchyma tissues in the outer epidermis were only associated with the major vascular bundles, whereas in the leaves of the HS selection, they were well developed and associated with each vascular bundle.
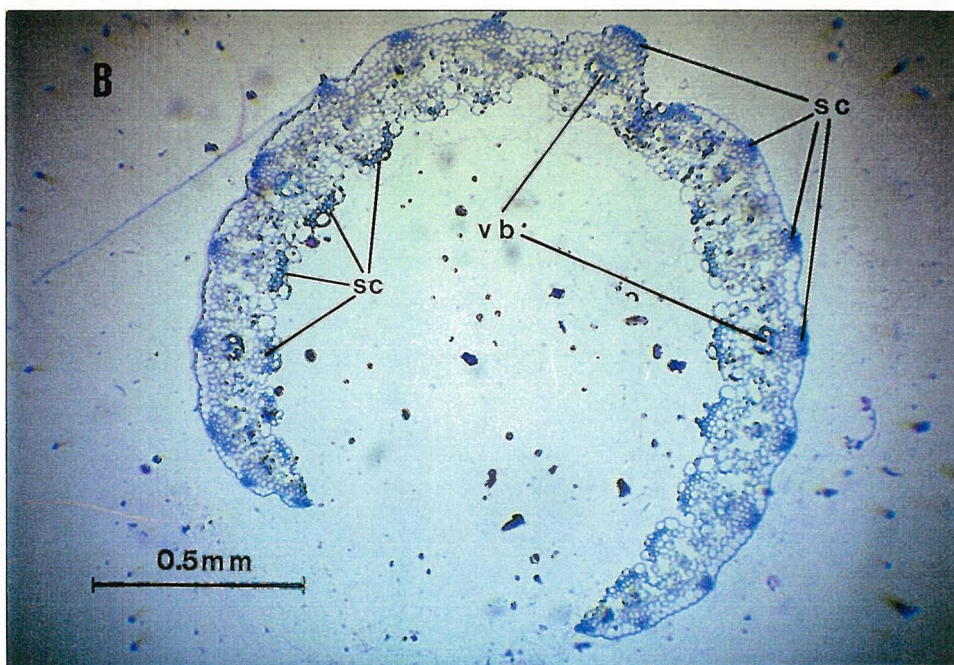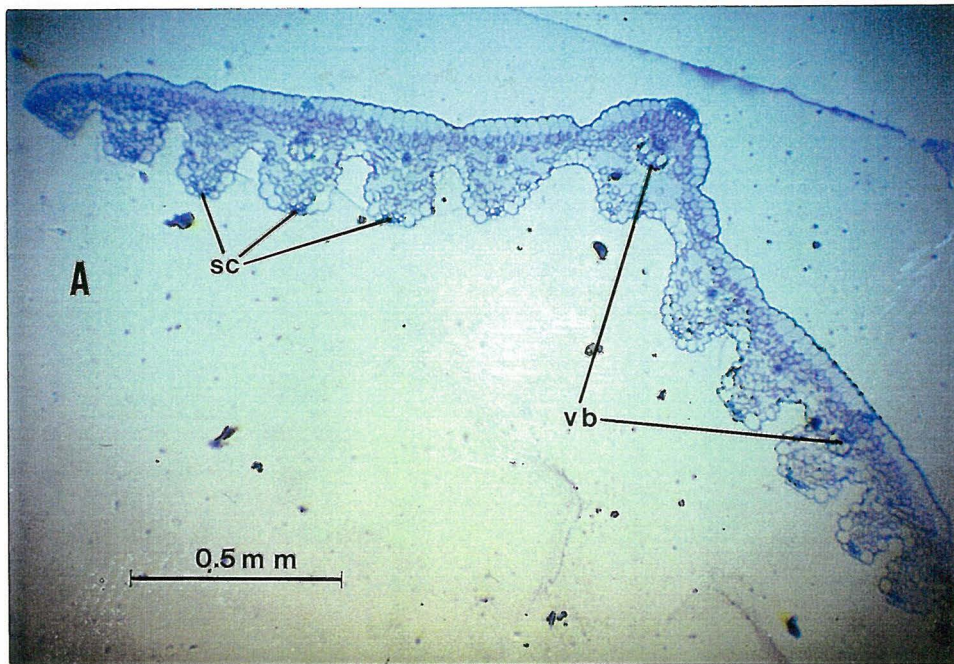
Plate 2.1 Cross-sections of the low and high leaf shear breaking load perennial ryegrass selections cut at the midway point along the leaf.
A: low leaf shear breaking load selection.   B: high leaf shear breaking load selection.
(sc; sclerenchyma tissues. vb; vascular bundles.)

Table 2.1 shows means of anatomical measurements in the leaf cross-section at 0.50L for the LS and HS PRG selections. The leaves of the LS selection were approximately 13 % narrower (p<0.01) and 16 % smaller in total CSA (p<0.05) than the leaves of the HS selection. Major significant differences appeared in the proportion of sclerenchyma tissues in total leaf CSA between the two selections. The proportion of outer sclerenchyma in the leaf CSA of the LS selection was approximately 66 % less (p<0.01) and of inner sclerenchyma was approximately 49 % less (p<0.01) than those present in the leaf CSA of the HS selection. For vascular tissues (including protoxylem lacunae) in the leaf CSA, the LS selection contained approximately 24 % less (p<0.05) than the HS selection.

In both selections, the leaf widths were significantly correlated with the leaf CSA at 0.50L. The relationships between the leaf width and leaf CSA at 0.50L are shown in Table 2.2. The linear regression relating leaf width and CSA was significant for both PRG selections (p<0.01 for the LS and p<0.001 for the HS selections). Since there were no significant differences in linear regression between the selections for either slopes and intercepts (Neter *et al.*,1974), the pooled linear regression was also estimated.

2.3.2 Morphology of the leaves of the low and high leaf shear breaking load perennial ryegrass selections

Least square means of morphological measurements for the leaves of the LS and HS PRG selections are shown in Table 2.3. These values for the 2nd, 4th and 6th leaves in each PRG selection are shown in Table 2.4. No significant (p>0.10) aging effects were observed on these parameters and, therefore, the values are pooled over the aging levels.

The leaves of the LS selection, in comparison with the HS selection, were approximately 18 % shorter, 14 % narrower and 34 % lighter (Table 2.3). In both selections, the leaf widths at 0.75L were approximately 21 % narrower than the leaf widths at 0.25L.

Table 2.1   Means of anatomical measurements and proportions of sclerenchyma and vascular tissue in the cross-sectional area at the midway point along the leaves (n=11) **of the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections.**

	Selection		Effect of selection	
	LS	HS		S.E.D.
Number of veins/leaf	13.5	14.7	ns	0.68
Width (mm)	2.91	3.33	**	0.142
Cross-sectional area (mm^2)	0.54	0.64	*	0.047
Sclerenchyma tissue (%)[1]:				
Outer	0.6	1.8	**	0.39
Inner	2.2	4.3	**	0.65
Total	2.9	6.1	**	1.02
Vascular tissue (%)[1]	6.5	8.5	*	0.88

1: Percentage of total cross-sectional area.

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Table 2.2 Relationships between width and cross-sectional area at the midway point along the leaves (n=11) of low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections.

Selection	Correlation coefficient	Significance	Relationship between width, X (mm) and cross-sectional area Y (mm^2)[1,2]	Significance
LS	0.80	**	Y = 0.339X - 0.447 (±0.0833)(±0.2429)	**
HS	0.87	***	Y = 0.256X - 0.215 (±0.0489)(±0.1639)	***
Pooled	0.86	***	Y = 0.265X - 0.239 (±0.0346)(±0.1085)	***

1: Intercepts for LS and HS are not significantly different from 0, but intercept for pooled significantly differs from 0 at $p < 0.05$.
2: (S.E.).

Table 2.3   Least square means of length, widths at 1/4 (0.25L), 1/2 (0.50L) and 3/4 (0.75L) of the leaf length from the position of the ligule to the apex of the leaf, and fresh weight of the leaves of the low (LS) (n=991) **and high (HS)** (n=998) **leaf shear breaking load perennial ryegrass selections.**

	Selection		Effect of selection	
	LS	HS		S.E.D.
Length (mm)	258.0	316.3	***	4.83
Width (mm):				
0.25L	3.77	4.45	***	0.052
0.50L	3.43	4.10	***	0.046
0.75L	3.00	3.43	***	0.038
Mean	3.42	3.99	***	0.043
Fresh weight (mg)	149.0	225.3	***	5.22

Table 2.4 Least square means of length, widths at 1/4 (0.25L), 1/2 (0.50L) and 3/4 (0.75L) of the leaf length from the fresh weight (mg) position of the ligule to the apex of the leaf, and fresh weight of the second-emerged (2nd), fourth-emerged (4th) and sixth-emerged (6th) leaves of the low (LS) (n=991) and high (HS) (n=998) leaf shear breaking load perennial ryegrass selections.

Selection	LS			Effect of emergence[1]	HS			Effect of emergence[1]	
Emergence	2nd	4th	6th		2nd	4th	6th		S.E.D.
Length (mm)	189.3^a	261.0^b	323.7^c	***	241.0^a	314.1^b	393.7^c	***	7.27
Width (mm):									
0.25L	3.22^a	3.92^b	4.17^c	*	3.96^a	4.55^b	4.84^c	**	0.078
0.50L	3.00^a	3.58^b	3.84^c	**	3.68^a	4.14^b	4.49^c	**	0.069
0.75L	2.61^a	3.09^b	3.31^b	**	3.05^a	3.39^b	3.85^c	**	0.057
Mean	2.95^a	3.53^b	3.77^c	**	3.56^a	4.02^b	4.39^c	**	0.064
Fresh weight (mg)	90.9^a	161.2^b	194.8^c	***	152.2^a	224.2^b	299.4^c	***	7.86

1: Leaves with different superscript differ significantly within a selection.

Leaf size increased with the leaf emergence level (Table 2.4). The 4th leaves were approximately 37 and 30 % longer, 20 and 13 % wider and 77 and 47 % heavier than the 2nd leaves for the LS and HS selections, respectively. Compared to the 4th leaves, the 6th leaves were approximately 24 and 25 % longer, 7 and 9 % wider and 21 and 34 % heavier for the LS and HS selections, respectively.

2.3.3 Shear breaking loads and shear strengths of the leaves of the low and high leaf shear breaking load perennial ryegrass selections

Cross-sectional areas of leaves of the LS and HS PRG selections were estimated by the pooled regression equation, since there were no significant differences in linear regression between the selections. Table 2.5 shows least square means of leaf CSA, LSBL and LSS for the LS and HS PRG selections. These values for the 2nd, 4th and 6th leaves in each PRG selection are shown in Table 2.6. No significant ($p > 0.10$) aging effects were observed on these parameters and therefore, the values are pooled over the aging levels.

The LS PRG selection was approximately 20 % smaller in estimated leaf CSA ($p < 0.001$) and were approximately 41 % lower ($p < 0.001$) in LSBL than the HS PRG selection (Table 2.5). In both of the selections, the estimated leaf CSA increased with the leaf emergence level but LSBL were similar among the leaf emergence levels except for the 2nd leaves of the LS selection, which was lower ($p < 0.05$) than other leaves of the LS selection (Table 2.6). Estimated LSS declined with emergence level in both of the selections. In overall means, estimated LSS for the LS selection was approximately 27 % lower ($p < 0.001$) than the HS selection.

Table 2.5 Least square means of estimated cross-sectional area, shear breaking load and shear strength at the midway point along the leaves of the low (LS) (n=117) and high (HS) (n=120) leaf shear breaking load perennial ryegrass selections.

| | Selection | | Effect of selection | |
	LS	HS		S.E.D.
Estimated cross-sectional area (mm^2)	0.68	0.85	***	0.012
Shear breaking load (g/leaf)	337.5	573.9	***	14.15
Estimated shear strength (g/mm^2)	500.7	689.5	***	17.78

Table 2.6 Least square means of estimated cross-sectional area, shear breaking load and shear strength at midway along the second-emerged (2nd), fourth-emerged (4th) and sixth-emerged (6th) leaves of the low (LS) (n=117) and high (HS) (n=120) leaf shear breaking load perennial ryegrass selections.

Selection	LS			Effect of emergence[1]	HS			Effect of emergence[1]	
Emergence	2nd	4th	6th		2nd	4th	6th		S.E.D.
Estimated cross-sectional area (mm^2)	0.56^a	0.71^b	0.78^c	**	0.74^a	0.86^b	0.95^c	***	0.019
Shear breaking load (g/leaf)	284.2^a	384.3^b	344.1^b	*	597.7	564.5	559.5	ns	21.66
Estimated shear strength (g/mm^2 cross-sectional area)	515.6^a	543.8^a	442.6^b	*	813.5^a	663.0^b	592.0^c	*	27.21

1: Leaves with different superscript differ significantly within a selection.

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2.3.4 Concentrations of dry matter, organic matter, lignin and cell-wall constituents in the leaves of the low and high leaf shear breaking load perennial ryegrass selections

The concentrations of dry matter, organic matter, lignin and cell-wall constituents in the leaves of the LS and HS PRG selections are shown in Table 2.7. These values for each leaf emergence level in each selection are shown in Table 2.8 and for each aging level of each leaf emergence level in each selection are shown in Table 2.9. The values in the tables are expressed as a percentage of fresh weight in order to relate to the leaf CSA which includes the areas filled with cell-water. Because the most of the M20 leaves were entirely or partially dead, the values for the M20 leaves have been excluded from the analyses but they are shown in Appendix 2.4 on a dry matter basis.

The leaves of the LS PRG selection had approximately 45 % more lignin but 10 % less cellulose than the leaves of the HS selection. Concentrations of dry matter, organic matter and hemi-cellulose were similar for both PRG selections (Table 2.7). Dry matter, organic matter and hemi-cellulose concentrations were significantly higher in 2nd leaves than in 4th or 6th leaves for both PRG selections (Table 2.8). These differences were more evident at M00 than at M10 (Table 2.9). Concentrations of dry matter, organic matter and cell-wall constituents were not affected by maturity in 2nd leaves but increased significantly for M10 in the 6th leaves.

Table 2.7 Concentrations (fresh %) of dry matter, organic matter, lignin, cellulose and hemi-cellulose in the leaves (n=12) of the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections.

	Selection		Effect of selection	
	LS	HS		S.E.D.
Dry matter	21.3	21.3	ns	0.65
Organic matter	18.5	18.9	ns	0.57
Lignin	0.61	0.42	**	0.061
Cellulose	4.14	4.61	**	0.150
Hemi-cellulose	5.07	4.88	ns	0.148

Table 2.8 Concentrations (fresh %) of dry matter, organic matter, lignin, cellulose and hemi-cellulose in the second-emerged (2nd), fourth-emerged (4th) and sixth-emerged (6th) leaves (n=12) of the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections.

Selection	LS			Effect of emergence[1]	HS			Effect of emergence[1]	
Emergence	2nd	4th	6th		2nd	4th	6th		S.E.D.
Dry matter	23.3^a	20.1^b	20.1^b	*	24.1^a	19.9^b	20.0^b	**	1.13
Organic matter	20.4^a	17.9^b	17.2^b	*	21.4^a	17.6^b	17.5^b	**	0.99
Lignin	0.68	0.52	0.64	ns	0.42	0.46	0.39	ns	0.105
Cellulose	4.13	4.25	4.03	ns	4.88	4.39	4.57	ns	0.260
Hemi-cellulose	5.60^a	4.93^b	4.68^b	*	5.39^a	4.64^b	4.60^b	*	0.256

1: Leaves with different superscript differ significantly within a selection.

Table 2.9   Concentrations (fresh %) of dry matter, organic matter, lignin, cellulose and hemi-cellulose in the second-emerged (2nd), fourth-emerged (4th) and sixth-emerged (6th) leaves (n=12) of the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections at maturation (M00) and at 10 days after the maturation.

Selection	LS									
Emergence	2nd		Effect of aging	4th		Effect of aging	6th		Effect of aging	
Age	M00	M10		M00	M10		M00	M10		S.E.D.
Dry matter	23.9	22.6	ns	19.0	21.9	ns	16.3	23.9	***	1.75
Organic matter	21.1	19.6	ns	16.8	19.1	ns	14.1	20.4	***	1.40
Lignin	0.69	0.68	ns	0.56	0.47	ns	0.43	0.85	*	0.148
Cellulose	4.18	4.08	ns	3.60	4.89	*	3.31	4.75	**	0.347
Hemi-cellulose	5.69	5.51	ns	4.40	5.45	*	3.79	5.57	***	0.362

Selection	HS									
Emergence	2nd		Effect of aging	4th		Effect of aging	6th		Effect of aging	
Age	M00	M10		M00	M10		M00	M10		S.E.D.
Dry matter	23.5	24.6	ns	19.4	20.3	ns	17.1	22.9	***	1.75
Organic matter	21.1	21.7	ns	17.3	17.9	ns	20.0	17.5	**	1.40
Lignin	0.39	0.45	ns	0.39	0.53	ns	0.31	0.47	ns	0.148
Cellulose	4.82	4.94	ns	4.19	4.58	ns	3.80	5.33	**	0.367
Hemi-cellulose	5.16	5.62	ns	4.42	4.86	ns	3.99	5.21	**	0.362

2.4 Discussion

2.4.1 Effects of selection

The LS PRG selection had approximately 41 % lower (p<0.001) LSBL than the HS PRG selection. The LS selection, however, also had approximately 21 % smaller (p<0.001) leaf CSA than the HS selection. Therefore, in LSS (i.e. LSBL/CSA) the LS PRG selection was approximately 27 % lower (p<0.001) than the HS PRG selection (5.00 x 10^5 vs 6.90 x 10^5 kg/m^2). Pool *et al.* (1969) pointed out that when meat was shorn by the WB meat shear apparatus, tensile stress was generated predominantly in the meat fibres. Voisey (1974, 1976), using wiener sausages as the test material, confirmed that the samples in a WB meat shear apparatus failed in tension not shear. These observations suggest that the LSBL measured in the present study probably included considerable extent of tensile load. Betteridge *et al.* (1986) estimated leaf tensile strength of PRG grown in indoors as 6.52 x 10^5 kg/m^2, which is within the range of two PRG selections measured in the present study. These facts confirm the two PRG selections have been selected for low and high physical resistance to breakdown in the true sense and not only selected for low and high LSBL.

2.4.2 Leaf anatomy

The most remarkable difference in leaf anatomy between the two PRG selections was that the LS selection had approximately 52 % less (p<0.001) sclerenchyma tissues occurring in the total leaf CSA (2.9 vs 6.1 %) than the HS selection. In the leaves of the LS selection the development of sclerenchyma strands in the outer epidermis was poor (Plate 2.1a) whereas the leaves of the HS selection often had sclerenchyma strands in both the inner and outer epidermis of each vein (Plate 2.1b). Sant (1969) reported mean number of sclerenchyma fibres in sixth emerged PRG leaves as 2.5 and 13.0 for the outer and inner epidermis, respectively and mean number of vascular bundles was 13.2 and the proportion of sclerenchyma tissues to total cross-sectional area of lamina was 1.9 %.

Although sclerenchyma tissues occupy only small areas in the leaf CSA, the sclerenchyma tissues are believed to provide up to 95 % of the reinforcement to the leaf structure (Vincent, 1982; Betteridge et $al.$, 1986). Assuming the entire resistance to shear stress is provided by sclerenchyma fibres only, the application of the Voigt model (see section 1.4.1.2) estimates the static shear strength for sclerenchyma fibres in the present study as 1.76×10^7 and 1.40×10^7 kg/m^2 ($\pm$ 0.148×10^7 S.E.D., p<0.05) for the LS and HS selections, respectively. This indicates that sclerenchyma tissue for the leaves of the LS selection is approximately 26 % stronger than for the leaves of the HS selection. This may be related to the higher concentration of lignin in the leaves for the LS selection than the HS selection. However, relationships between the magnitude of lignification and sclerenchyma tissues in leaves of PRG are not well understood (Soper et $al.$, 1956; Wilkins, 1972). Betteridge et $al.$ (1986) dissected sclerenchyma fibres from the mid-rib area of PRG leaves and measured their tensile strengths as 1.6×10^7 kg/m^2, which is approximately the intermediate value of the two selections studied here. Vincent (1982) reported Young's moduli as 5.63×10^7 and 2.30×10^9 kg/m^2 of cross-sectional area of sclerenchyma tissue for tensile stiffness of the leaves and the sclerenchyma fibres of PRG, respectively. However, Vincent's data can not directly be compared with the data from the present study, because only static shear strength was estimated in the present study. These suggest that the difference in proportion of sclerenchyma tissues in leaf CSA mostly accounts for the difference in LSS between the two PRG selections.

2.4.3 Effects of leaf emergence

In the present study, LSBL of PRG was similar for the leaf emergence levels and moreover, LSS decreased with the leaf emergence level. Sclerenchyma tissues are thick-walled cells and their structural organisation is based on cellulose. Inside of these cells are the lacunae (Esau, 1953). Thus, sclerenchyma walls account for the entire strength of sclerenchyma tissues to physical stresses. If the physical strength of sclerenchyma tissues are as high as estimated above, the strength of sclerenchyma walls can be estimated as being far higher than the figures for the

sclerenchyma tissues. Vincent (1991) estimated it as approximately 7.1×10^7 kg/m^2. Evans (1964a) reported the proportion of the sclerenchyma wall to leaf CSA of PRG as 2.10 %. In leaves of orchardgrass, Kawamura et al. (1976, 1981) estimated that the mechanical tissue fractions consisting of sclerenchyma, vascular bundle and epidermis contained 39.3 - 52.7 % of cellulose. These indicate that cellulose is highly concentrated in the cell walls of sclerenchyma probably to generate such high mechanical strength. In the present study, the leaves of the LS PRG selection, which had less sclerenchyma tissues, had lower cellulose concentration than the leaves of the HS PRG selection. The lack of change in LSBL of PRG with the leaf emergence level, therefore, indicates that the amount of sclerenchyma wall in leaf CSA was constant at any leaf emergence levels. In the present study, the leaf CSA enlarged but dry matter concentration declined with leaf emergence level. The latter is more obvious at aging level of M00 and there was no aging effect on the leaf CSA nor LSBL. Thus, the enlargements in the leaf CSA with the leaf emergence level are probably due to the increased amount of water, which is filling the inside of the cells and therefore, the LSS declined with leaf emergence level.

However, anatomical studies of PRG have confirmed that in proportion to the total leaf CSA, sclerenchyma tissues increased with successive leaf emergence (Soper et al., 1956; Evans 1964b; Sant, 1969; Wilkins, 1972). Evans (1964a) found a positive relationship between the cellulose concentration (% freeze-dry weight) and the proportion of the sclerenchyma wall in leaf CSA in the four different grass species. However, in his study, the cellulose concentrations on a fresh basis should have been compared to the proportion of sclerenchyma tissue to total leaf CSA because the tissue cells in the cross-section were expanded with the liquid filling inside of the cells. Some cell walls of fibres may swell and have a great capacity for absorption of water (Esau, 1953). The relationships among water concentration, ability to accommodate water in cells and size of cells in the various plant tissues are perhaps complex. This implies that a comparison of proportions of sclerenchyma tissue in the leaf CSA for the leaves having different water concentrations may not be accurate. In the present study, the dry matter concentrations in leaves for each emergence level between the two selections were

very similar.

The proportions of sclerenchyma tissues in cross-sections of PRG have been reported as varying between 0.7 - 1.7 % (Soper *et al.*, 1956) 1.01 - 2.16 % (Evans, 1964b), 2.72 % (Evans, 1967), 0.9 - 1.9 % (Sant, 1969), 4.49 % (Wilkins, 1972), 4.24 % (Vincent, 1982) and 2.93 % (Betteridge *et al.*, 1986). This variation may partially be due to differences in location on the leaf where the dimensions were taken and in analytical method adopted (Harris *et al.*, 1981) but also the amount of water which is filling cells may have a large influence. Vincent (1983) studied the influence of water concentration on the stiffness and fracture properties of PRG. He demonstrated that work to fracture across the leaf veins was almost independent of water concentration which ranged 0 - 300 % of dry weight. However, the magnitude of changes of water concentration among leaves in the present study are probably too small to make major influences on the resistance of the leaves to the shear.

2.4.4 Effects of aging

Although aging at least up to 10 days after the maturation did not alter the LSBL and LSS, leaves at aging level of M10, relative to M00, tended to have higher concentrations of dry matter, organic matter and cell-wall constituents. This is due probably to the secondary thickening of cell walls in the plant tissues which include sclerenchyma tissues. However, because sclerenchyma tissue may require large amount of cellulose to generate its strength, the extent of the increase of cellulose concentration for 10 days was probably still too small to influence the LSS. Although the M20 leaves increased considerably the concentrations of the cell-wall constituents and lignin, which may be affecting the leaf strength, death of the leaves appears to occur between 10 days and 20 days after appearance of ligules on the leaves under the conditions for optimum growth of PRG.

2.4.5 Interaction between leaf shear breaking load and leaf morphology

As mentioned above, when comparisons are made in the LSS between the two PRG selections, the LS selection is approximately 27 % weaker than the HS selection. However, during actual mastication by animals, each leaf has to be broken down entirely to particles of less than 1 mm length and/or less than 1 mm width in order to pass out the reticulo-rumen of sheep (Ulyatt *et al.*, 1986; Domingue *et al.*, 1991a). Therefore, the total load required to breakdown an entire leaf has to be considered as a masticatory resistance to breakdown. Leaves of PRG are fibre-reinforced composites in which sclerenchyma strands lay along the leaves. This means the leaf strengths vary depending on the directions of forces applying to the leaf. Stiffness of non-fibrous components such as mesophyll and epidermis tissues of PRG appear to be negligible compared to strength of sclerenchyma fibres (2.31×10^9 vs 1.42×10^6 kg/m^2 in Young's moduli of tension for the sclerenchyma and the non-fibrous components, respectively (Vincent, 1982)). Thus, only the strength which applies across the direction of the sclerenchyma fibres will account for the most of resistance to breakdown the leaves. Therefore, the estimation of the total shear load (TSL) required to breakdown an entire leaf to 1 x 1 mm size particles, which is a function of the LSBL and the leaf morphology, is given by;

$$TSL = \sum_{i=1}^{l} (LSS_i \times CSA_i), \quad \text{where } l = \text{leaf length in mm}$$

and because
$$LSS = \frac{LSBL}{CSA,}$$

$$TSL = \sum_{i=1}^{l} LSBL_i.$$

Assuming LSBL at 0.50L equals to the mean LSBL over the leaf;

$$\text{TSL} = \text{LSBL x leaf length in mm.}$$

When the TSL is expressed for the unit mass intake;

$$\text{TSL} = \frac{\text{LSBL x leaf length in mm}}{\text{leaf weight}}$$

An index of masticatory load (IML) of leaf may then be given by;

$$\text{IML (kg/mgDM)} = \frac{\text{LSBL (kg/leaf) x leaf length (mm)}}{\text{leaf dry weight (mg).}}$$

or

$$\text{IML} = \text{LSBL (kg/leaf) x leaf length:dry weight ratio (mm/mgDM).}$$

Thus, IML is a measure of a total shear load (kg) required to break a unit dry weight (mgDM) of leaf into particles small enough to have a high probability of leaving the rumen.

The values of leaf IML for the LS and HS PRG selections are shown in Table 2.10. The LS PRG selection had approximately 26 % higher ($p<0.001$) leaf length:dry weight ratio than the HS PRG selection. In leaf IML, the LS selection was still approximately 27 % lower than the HS selection. However, this difference in leaf IML can be altered by changes in the dry matter concentration of the leaf, the leaf length and the LSBL. In the present study, the two PRG selections were grown under the optimum climatic conditions for PRG. Mitchell *et al.* (1958) and Evans (1964b) reported changes of morphological characteristics with climatic conditions. For the selections growing outdoors where climatic conditions are not constant, variations of IML can be anticipated.

Table 2.10 Length:dry weight ratio, and index of masticatory load (IML) of the leaves of the low (LS) (n=117) and high (n=120) leaf shear breaking load perennial ryegrass selections.

	Selection		Effect of selection	
	LS	HS		S.E.D.
Length:dry weight ratio (mm/mgDM)	8.61	6.86	***	0.141
IML (kg/mgDM)	2.84	3.91	***	0.102

2.5 Conclusion

The LSBL for the LS PRG selection was approximately 41 % lower the HS PRG selection when the leaves were shorn by a WB meat shear apparatus. However, in morphological and anatomical comparison, the LS selection had shorter leaf lengths, narrower leaf widths and narrower leaf CSA than the HS selection. Therefore, in LSS, the LS selection was estimated to be approximately 27 % less resistant to shear than the HS selection per unit of cross-sectional area. The lower LSS in the LS selection is due to the lower concentration of sclerenchyma tissues in leaf CSA compared with the HS selection. The LSS declined as the leaf emergence proceeded and this was associated with the changes in water concentrations, although LSBL was not affected by the leaf emergence level nor by aging level.

However, when the same dry weight of leaves of both selections are masticated by animals, the difference in the total shear load required to breakdown the leaves to 1 x 1 mm particle size, namely, leaf IML between the selections will be influenced by the differences in morphological characteristics of leaves between the two PRG selections. In IML, the leaves of the LS selection were estimated lower than the leaves of the HS selection by approximately 27 %, when both were grown under the optimum climatic conditions.

The effects of reduced LSBL in PRG on actual breakdown during mastication in sheep will be examined in the next chapter.

Chapter Three

THE EFFECTS OF REDUCED LEAF SHEAR BREAKING LOAD IN PERENNIAL RYEGRASS UPON PARTICLE BREAKDOWN DURING MASTICATION IN SHEEP

3.1 Introduction

Reduction of dietary particles to less than 1 mm in size is a prerequisite to passage. Mastication, especially during rumination, is considered the most important process in dietary particle breakdown (Ulyatt, 1983; Ulyatt *et al.*, 1986; Domingue *et al.*, 1991a, 1991b). The high physical strength of perennial ryegrass (PRG) is believed to be as a major limiting factor to breakdown (Bailey, 1964; Troelsen *et al.*, 1964; Evans *et al.*, 1973; Betteridge, *et al.*, 1986; Poppi *et al.*, 1987; MacKinnon *et al.*, 1988; Easton, 1989; Waghorn *et al.*, 1989; Hodgson, 1990).

This chapter, based on an experiment conducted in April and May 1990, compares the actual breakdown during mastication in sheep fed two PRG selections selected for low (LS) and high leaf shear breaking load (HS). Furthermore, the factors which may influence breakdown of PRG during mastication are discussed.

3.2 Materials and Methods

Comparisons were made between the LS and HS PRG selections in efficiency of mastication by sheep on particle breakdown in two trials. The efficiencies of chewing during eating and chewing during rumination on particle breakdown for the two PRG selections were measured in trial 1 and trial 2, respectively. LS or HS PRG selections were offered to eight rumen-fistulated wethers in a cross-over design. Masticated materials were recovered from the rumen and particle analyses were conducted. Jaw movements of the animals were recorded during mastication.

3.2.1 Low and high leaf shear breaking load perennial ryegrass selections

The LS and HS PRG selections harvested in 1985 (see section 1.4.2.1) were sown into separate 1.5 ha halves of a 3.0 ha paddock located at DSIR's Tiritea Research Area, Palmerston North in April 1987. The paddocks were maintained as a pure ryegrass sward . The two PRG selections were harvested from the paddocks at 09:00 hours during the sampling periods. Dry matter concentrations were determined daily by oven drying (105 °C) for 24 hours.

3.2.2 Experimental animals

Eight mature rumen-fistulated Romney wethers (mean live weight 53.4 kg ± 0.74 s.e.) were used. The animals were fitted with permanent rubber cannulae (65 mm i.d.). All the animals were housed individually in metabolism crates with an automatic feeder attached.

3.2.3 Experimental design

3.2.3.1 Adjustment period

Chaffed lucerne hay was fed *ad libitum* to all the animals for five days to adjust them to indoor conditions. The feed was placed upon the belts of the automatic feeders which were set for continuous hourly feeding. Jaw harnesses, which contained jaw movement sensors (see section 3.2.5), were fitted on the animals and left on for increasing periods of time until the animals became used to the harnesses.

3.2.3.2 Voluntary feeding period

Following the adjustment period, freshly cut PRG, containing approximately 20 %

white clover (WC), was fed *ad libitum* to the animals to measure voluntary intake for seven days. The PRG-WC was harvested in the mornings from grazing paddocks at the Dairy Cattle Research Unit, Massey University. The PRG-WC was placed upon the automatic feeders three times a day following storage under refrigeration (approx. 4 °C). Refusals were collected and weighed for each animal and dry matter contents of the fresh forages and refusals were determined daily by oven drying (105 °C) for 24 hours. Daily dry matter intake for each animal was recorded during the seven-day period.

3.2.3.3 Trial periods

The animals were allocated into two groups so that the mean voluntary intake levels were similar for the two groups. The animals received either the LS or HS PRG selections in a cross-over design.

3.2.3.3.1 Trial 1: Efficiency of chewing during eating upon particle breakdown

Feeding level was restricted for each animal to 90 % of voluntary intake on dry matter basis. The two PRG selections were placed upon the automatic feeders three times a day and were fed to the animals for at least three days prior to the sampling. The sampling was made daily on two animals in each treatment. The animals were fitted with the jaw harnesses one day prior to the sampling day.

On each sampling day, the automatic feeders were stopped at 10:00 hours and drinking water and mineral salt blocks were removed from the animals. The rumen contents were removed from the animals through the cannulae by manual bailing. After most of the contents were removed from the rumen, warm water was poured into the rumen using a funnel and fine residues of rumen contents were flushed out of the rumen several times until effluent water became clear. Test meals of 2 kg of either freshly cut LS or HS PRG selections were then placed in the feed bins fitted in front of the animals. The animals were allowed to ingest the test meals for a

period of 30 minutes. At the end of the 30-minute period, residues of the test meals were removed from the feed bins and weights of the consumed test meals were recorded. The boli present in the rumen were collected from the animals through the cannulae. Subsamples of the boli recovered from the rumen were taken for determination of particle size distribution. The rumen contents previously removed were then returned to the animals. When the collections of boli from the rumen were completed for all the animals, the feed treatments were crossed over so that the animals received the other selection of PRG. The animals were then given at least 3 days to adjust to the new treatments prior to another sampling, and the sampling procedure was repeated.

An index of efficiency of chewing during eating (<C.EAT>) (Ulyatt *et al.*, 1986; Domingue *et al.*,1991b) in reducing particle size to less than 1.0 mm for each animals was calculated as;

$$\text{<C.EAT> (\%)} = \frac{\text{g DM <1.0 mm in boli}}{\text{g DM test meal consumed.}} \times 100$$

3.2.3.3.2 Trial 2: Efficiency of chewing during rumination upon particle breakdown

Following the completion of the above sampling period, the animals received either LS or HS PRG selections for five days. Feeding level was restricted for each animal to 90 % of voluntary intake on a dry matter basis. The sampling was made on two animals in each treatment daily. The animals were fitted with the jaw harnesses one day prior to the sampling day.

On each sampling day, the automatic feeders were stopped at 07:30 hours. Test meals of 4 kg of either freshly cut LS or HS PRG selections were placed into the feed bins. The animals were allowed to ingest these for 3 hours until 10:30 hours. At the end of the ingestion period, drinking water and mineral salt blocks were removed from the animals and residues of the test meals were collected from the

feed bins and weights of the consumed test meals were recorded. The rumen contents were then sampled, and again at 17:30 hours after the animals had been left to ruminate for 7 hours. At each of the samplings, the rumen contents were entirely removed from the rumen by manual bailing through the cannulae, weighed, mixed thoroughly, subsampled for 1 kg fresh weight and the remainder returned to the animals. The subsamples were analysed for dry matter concentration by oven drying (105 °C for 72 hours) and particle size distribution. When the collections of the ruminated rumen contents were completed for all the animals, the feed treatment was crossed over. After a four-day of feeding period, the sampling procedure was repeated.

An index of efficiency of chewing during rumination (<C.RUM>) (Ulyatt *et al.*, 1986; Domingue *et al.*,1991b) in reducing particle size to less than 1.0 mm for each animal was calculated as;

$$\text{<C.RUM> (\%)} = \frac{A - B}{A} \times 100$$

where,

A; g DM > 1.0 mm in rumen contents at 10:30 hours (rumen contents at 10:30 refers to the amount actually returned to the animals for rumination after removal of the 1 kg fresh sample),

B; g DM > 1.0 mm in rumen contents at 17:30 hours.

The calculation is based on the assumption that feed particles larger than 1.0 mm cannot leave the rumen (Poppi *et al.*, 1980 Ulyatt 1983, Ulyatt *et al.*, 1986; Domingue *et al.*, 1991a, Domingue *et al.*, 1991c). Hence, it was assumed that the reduction of particles larger than 1 mm in rumen contents during the period of 10:30 - 17:30 hours was due only to rumination, without any loss of particles larger than 1.0 mm flowing out of the rumen.

3.2.4 Recording of jaw movements

Jaw movements were recorded while the animals were ingesting (Trial 1) or ruminating (Trial 2) the test meals. Jaw activities were obtained by sensing the compression of a small rubber balloon held under the lower jaw by the harness when the animal opened its jaw. The air pressure caused by compressing the balloon was transmitted to a pressure transducer connected to a multi-channel amplifying and recording unit (manufactured by Graphtech Corp., Japan). On the recording unit, each time the balloon was compressed by the jaw a spike was drawn on a chart paper travelling at constant speed of 1 mm/second. With this system, any open-close types of jaw movements were recorded, regardless whether the jaw movement was for actual mastication or not and regardless of the extent of masticatory force applied. However, in the present study, each spike recorded on the chart paper was defined as a jaw movement for actual mastication of the test meals. Time spent for mastication was determined from the recorded chart and the mastication rates were determined by counting 10 periods of 60 seconds for each animal in each treatment.

3.2.5 Particle size analyses of rumen contents

This analysis was conducted using a wet sieving machine (manufactured by Turner and Newall Ltd., England) consisting of a cascade of rotating sieves of decreasing square mesh sizes of 8.0, 4.0, 2.0, 1.0 and 0.5 mm for the rumen content samples collected in Trial 1 and mesh sizes of 4.0, 2.0, 1.0, 0.5 and 0.25 mm for the rumen content samples collected in Trial 2. While in operation for 4 minutes, approximately 30 g wet weight of rumen contents were washed by recirculating water (1600 ml, 4 l/min) through the sieves and additional agitation was provided by a stirrer for each sieve. Materials retained on the sieves were washed on to tared filter paper and oven dried (105 °C for 24 hours) to determine the dry weight of each particle size fraction. Particles retained on each sieve were assumed to be larger than each sieve mesh size. Material that passed through all the sieves was mixed and a 1000 ml aliquot taken, centrifuged at 850 G for 20 minutes and the

residue transferred to filter papers and dried as above. The quantity of material not retained on sieves nor as residues, i.e. defined as solubles, was determined by difference from the initial dry sample weight and the sum of recovered particulate dry matter fractions.

3.2.6 Determination of the index of masticatory load of leaves

3.2.6.1 Measurements of leaf morphology

During the two trial periods, fresh LS and HS PRG selections were sampled daily, pooled over the periods and stored in the deep freeze before taking any measurements. Three hundred pieces of green leaf were then randomly subsampled, regardless whether entire or part of a leaf, from each PRG selection. Length and width at the midway point along the leaf piece were recorded on each leaf. The 300 pieces of leaf were then oven-dried (105 °C for 24 hours) and weighed to determine the average dry weight of a leaf piece.

3.2.6.2 Measurement of leaf shear breaking load and determination of index of masticatory load

An Instron Food Testing Instrument (model 1140, manufactured by Instron Ltd., England) fitted with Warner-Bratzler Meat Shear type blades (no. 2830-013, manufactured by Instron Ltd., England) was used to measure the leaf shear breaking load (LSBL). Drive speed was set for 80 mm/min. This assembly provided graphic analog records of the translation of the force applied to the blades. One hundred pieces of green leaf were randomly sampled, regardless whether entire or part of a leaf, from each PRG selection on a day between Trial 1 and Trial 2. The leaves were gathered in bundles of 10 leaves each, aligned along the midway point of the leaf piece length. Ten replicates of 10-leaf bundles for each PRG selection were then sheared. The maximum force exerted divided by number of leaf pieces sheared was determined as LSBL. The index of masticatory

load (IML) of leaf was then estimated for the two PRG selections as defined in section 2.4.5.

3.2.7 Chemical analyses

The chemical analyses were performed for *in vitro* dry matter digestibility and total nitrogen on each PRG selections pooled over Trial 2. The samples of both PRG selections were freeze-dried and ground through a 1 mm mesh sieve. *In vitro* dry matter digestibility (IVDMD) was determined by the procedure described by Roughan *et al.* (1977) and total nitrogen (N) was determined by the Kjeldahal procedure using a Kjeltec Auto 1030 Analyzer (manufactured by Tecator Ltd., Sweden).

3.2.8 Statistical analysis

Statistical analyses were performed by analysis of variance for a cross-over design. GLM procedure of SAS (SAS inst., Inc., NC., USA) was used for the analyses. An example table of the analysis of variance is shown in Appendix 3.1.

3.3 Results

3.3.1 Leaf morphology, leaf shear breaking loads and indices of masticatory load of the low and high leaf shear breaking load perennial ryegrass selections

Means of the leaf morphology, LSBL, leaf IML, IVDMD and concentration of total N of the LS and HS PRG selections are shown in Table 3.1. The LS PRG selection had approximately 19 % shorter (p<0.001, ±4.02 s.e.d.) , 20 % narrower (p<0.001, ±0.05 s.e.d.) and 42 % lighter leaves than the leaves of the HS PRG selection. Thus, in leaf length:dry weight ratio, the LS selection was

Table 3.1 Means of morphology, shear breaking load, index of masticatory load (IML), in vitro dry matter digestibility (IVDMD) and concentration of total nitrogen (N) of the leaves of the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections.

	Selection	
	LS	HS
Morphology:		
Length (mm)	131.1	161.7
Width (mm)	2.18	2.70
Dry weight (mg/leaf)	8.0	13.7
Length:dry weight ratio (mm/mgDM)	16.4	11.8
Shear breaking load(g/leaf)	507.6	710.5
IML (kg/mgDM)	8.32	8.39
IVDMD (%)	76.1	74.7
Total N (%)	3.74	3.75

approximately 39 % greater than the HS selection. The LS selection had approximately 29 % lower LSBL than the HS selection and thus, leaf IML was estimated as 8.32 and 8.39 kg/mgDM for the LS and HS PRG selections, respectively. IVDMD and concentration of total N were similar for the two PRG selections. Statistical analyses were not carried out for leaf weight, leaf length:dry weight ratio, LSBL, leaf IML, IVDMD and total N concentration because leaves were pooled to determine those parameters.

3.3.2 Trial 1

3.3.2.1 Masticatory behaviour and efficiency of chewing in wethers during eating the low and high breaking load perennial ryegrass selections

Table 3.2 shows the masticatory behaviour and <C.EAT> in wethers during eating the test meals of the LS and HS selections. The amount of dry matter consumed was similar for the two PRG selections, although the total number of chews for the LS selection was approximately 18 % less (p<0.01) than for the HS selection. The animals spent approximately 13 % less time chewing for the LS selection than for the HS selection, but this difference was not statistically significant (p=0.107). Both PRG selections were similar in the chewing rate for time (no. chews/min. eating), but in the chewing rate for ingestion (no. chews/gDM ingested), the LS selections were approximately 18 % lower than the HS selection. The LS selection was approximately 25 (ns) and 34 % (p=0.135) higher than the HS selection in the ingestion rate for time (gDM ingested/min. eating) and in the ingestion rate for chewing (mgDM ingested/chew), respectively. There were no major differences between the two PRG selections in <C.EAT> although <C.EAT> (%/chew) tended to be higher for the LS PRG selection than the HS PRG selection (p=0.106). Approximately 40 % of PRG was broken down to a size less than 1 mm during eating for both PRG selections. Figure 3.1 shows decline of chews/min.eating in the wethers for the LS and HS PRG selections. The time progression is expressed in percentage of the total time spent eating because the time spent for chewing varied for each animal.

Table 3.2 Masticatory behaviour and index of efficiency of chewing (<C.EAT>) in wethers (n=8) **during eating the low (LS) and high (HS) shear breaking load perennial ryegrass for a period of 30 minutes.**

	Selection		Effect of selection	
	LS	HS		S.E.D.
Total dry matter consumed (g)	93.9	91.8	ns	8.99
Time spent for chewing (min)	23.0	26.4	ns (p=0.07)	1.60
Recorded total number of chews	3245	3948	**	112.3
Chewing rate:				
(no. chews/min eating)	140.3	148.9	ns	5.25
(no. chews/gDM ingested)	39.6	48.6	ns	8.47
Ingestion rate:				
(gDM ingested/min eating)	4.27	3.41	ns	0.70
(mgDM ingested/chew)	31.3	23.3	ns (p=0.135)	4.31
<C.EAT>[1]				
(%)	40.7	40.7	ns	2.51
(%/min eating)	1.92	1.60	ns	0.236
(%/chew)	0.014	0.011	ns (p=0.106)	0.0014

1: (g DM < 1.0 mm / g DM ingested) x 100.
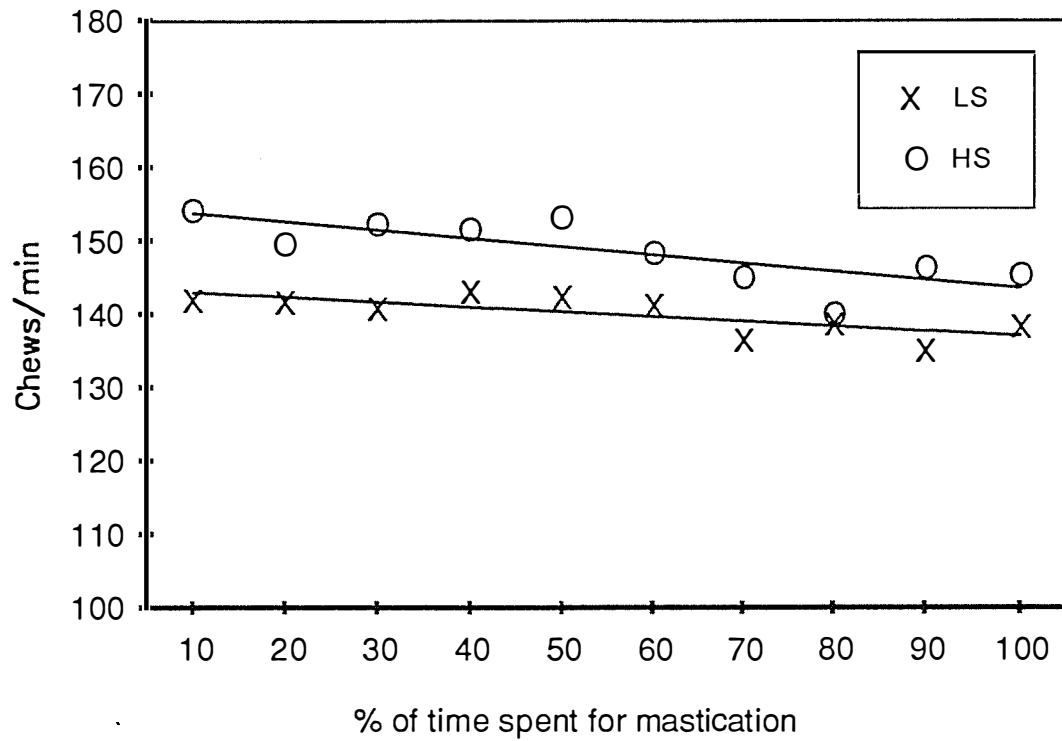
Figure 3.1 Decline of mean chewing rate of the wethers (n=8) **during eating the low (LS) and high (HS) shear breaking load perennial ryegrass selections over a period of 30 minutes.**

(The time progression is expressed in percentage of the total time spent for eating.)

The number of chews/min.eating slightly declined with time for both PRG selections (-0.067 and -0.114 chews/% of total time spent p>0.10 ±0.070 S.E.D. for the LS and HS selections, respectively).

3.3.2.2 Particle size distributions in the rumen contents recovered from the wethers immediately after ingesting the low and high leaf shear breaking load perennial ryegrass selections

The particle size distributions in the rumen contents recovered from the wethers immediately after ingesting the LS and HS PRG selections are shown in Figure 3.2.

There were no major differences in the particle size distribution in the rumen contents for the two PRG selections. The proportion of particles larger than 1 mm was the same for the two PRG selections (59.3 vs 59.3 % for the LS and HS PRG selections, respectively).

3.3.3 Trial 2

3.3.3.1 Rumen pool sizes of the wethers ruminating the low and high breaking load perennial ryegrass selections

During the three-hour ingestion period (07:30-10:30 hours) the animals ingested 363 and 416 g of dry matter (p>0.10 ±31.3 S.E.D.) for the LS and HS PRG selections, respectively.

Table 3.3 shows the rumen pool size at 10:30 and 17:30 hours and the average rate of dry matter disappearance in the wethers ruminating the two PRG selections. The values shown in the table for the rumen pool sizes at 10:30 hours are the amounts actually returned to the rumen for rumination after removal of the 1 kg fresh sample. The dry matter rumen pool size in wethers for the LS PRG selection was approximately 10 % less than for the HS PRG selection at both times
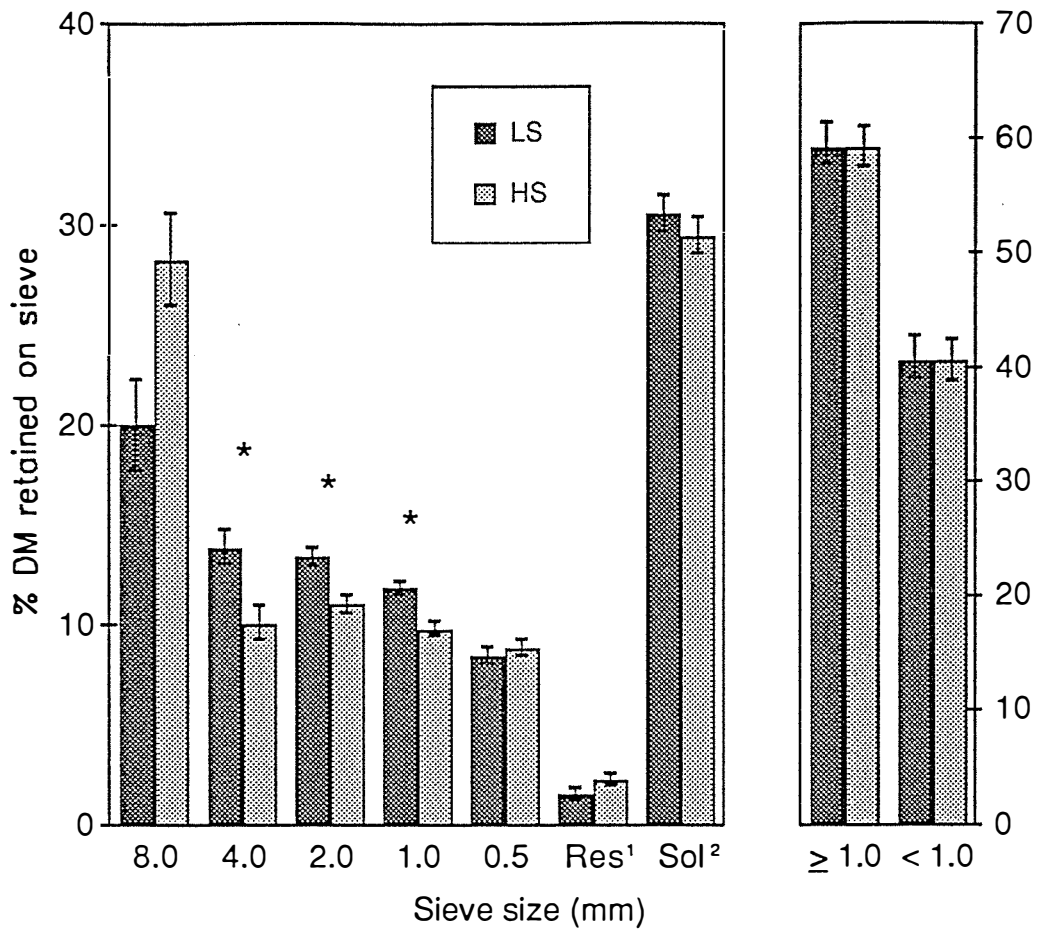
Figure 3.2 Particle size distributions in the rumen contents recovered from the wethers (n=8) immediately after ingesting the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections.

1: Residues.

2: Soluble DM defined as the DM remaining in solution after centrifugation at 850 G for 20 minutes.

Table 3.3 Rumen pool sizes at 10:30 and 17:30 hours and rate of dry matter disappearance in the wethers (n=8) ruminating low (LS) and high (HS) leaf shear breaking load perennial ryegrass and their *in vitro* dry matter digestibilities (IVDMD) and concentrations of total nitrogen (N).

| | Selection | | Effect of selection | |
	LS	HS		S.E.D.
Rumen pool size (g)[1]:				
At 10:30;				
Fresh	4466	4740	ns	141.8
Dry matter	432	478	ns	20.0
DM particles>1.0 mm[2]	155	179	ns	13.8
At 17:30;				
Fresh	2925	3068	ns	202.2
Dry matter	202	223	ns	14.5
DM particles>1.0 mm[2]	45	48	ns	6.7
Rate of dry matter disappearance from the rumen (%/h)[3]	7.7	7.6	ns	0.44

1: The amount actually returned for rumination after removal of the 1 kg fresh sample.

2: DM particles retained on sieves > 1.0 mm mesh.

3: Determined for the period of 10:30 - 17:30.

measured, but the difference was not statistically significant (p>0.10). For both PRG selections, dry matter pool size was reduced by approximately 53 % from 10:30 to 17:30 hours. Thus, the rate of dry matter disappearance from the rumen was similar for the two PRG selections.

3.3.3.2 Masticatory behaviour and efficiency of chewing in wethers during ruminating the low and high breaking load perennial ryegrass selections

Table 3.4 shows the masticatory behaviour and <C.RUM> in wethers during ruminating the test meals of the LS and HS selections for a period of seven hours (10:30-17:30). There were no major differences between the two PRG selections in the masticatory behaviour and in <C.RUM>. Approximately 70 % of PRG was broken down to the size less than 1 mm during rumination in a period of seven hours for both PRG selections.

3.3.3.3 Particle size distributions in the rumen contents recovered at 10:30 and 17:30 hours from the wethers ruminating the low and high leaf shear breaking load perennial ryegrass selections

Particle size distributions in the rumen contents recovered at the two sampling times from the wethers ruminating the LS and HS PRG selections are shown in Figure 3.3. There were no major significant differences in the particle size distribution in the rumen contents for the two PRG selections at both sampling times. The proportion of particles larger than 1 mm were 36.1 and 36.6 % at 10:30 hours and 22.6 and 21.9 % at 17:30 hours for the LS and HS PRG selections, respectively. The proportion of particles larger than 4 mm were reduced approximately 50 % for both PRG selections (36.1 to 18.9 % and 36.6 to 21.9 % for the LS and HS PRG selections, respectively) however the proportion of particles smaller than 0.5 mm but larger than 0.25 mm increased approximately 70 % (11.3 to 18.9 % and 11.9 to 20.4 % for the LS and HS PRG selections, respectively) from 10:30 to 17:30 hours.

Table 3.4 **Masticatory behaviour and index of efficiency of chewing (<C.RUM>) in wethers** (n=8) **during ruminating the low (LS) and high (HS) shear breaking load perennial ryegrass for a period of 7 hours.**[1]

	Selection		Effect of selection	
	LS	HS		S.E.D.
Rumen dry matter pool(g)	432	478	ns	19.9
Dry matter ruminated (g)[2]	110	131	ns	10.3
Time spent for rumination (min)	131.4	139.3	ns	8.73
Recorded total numbers of chews	15594	16274	ns	1039.1
Chewing rate;				
(no. chews/min rumination)	120.3	117.6	ns	2.23
(no. chews/gDM ruminated)[2]	143.9	138.3	ns	6.62
Rumination rate;				
(gDM ruminated/min rumination)[2]	0.87	0.91	ns	0.197
(mgDM ruminated/chew)[2]	7.28	7.74	ns	0.483
<C.RUM>[3]				
(%)	70.7	71.5	ns	3.33
(%/min rumination)	0.57	0.53	ns	0.035
(%/chew)	0.0048	0.0046	ns	0.00030

1: 10:30-17:30 hours.

2: DM ruminated (g) = gDM>1.0 mm at 10:30 hours - gDM>1.0 mm at 17:30 hours.

3: (g DM>1.0 mm at 10:30 hours - g DM>1.0 mm at 17:30 hours)/ g DM>1.0 mm at 10:30 hours.
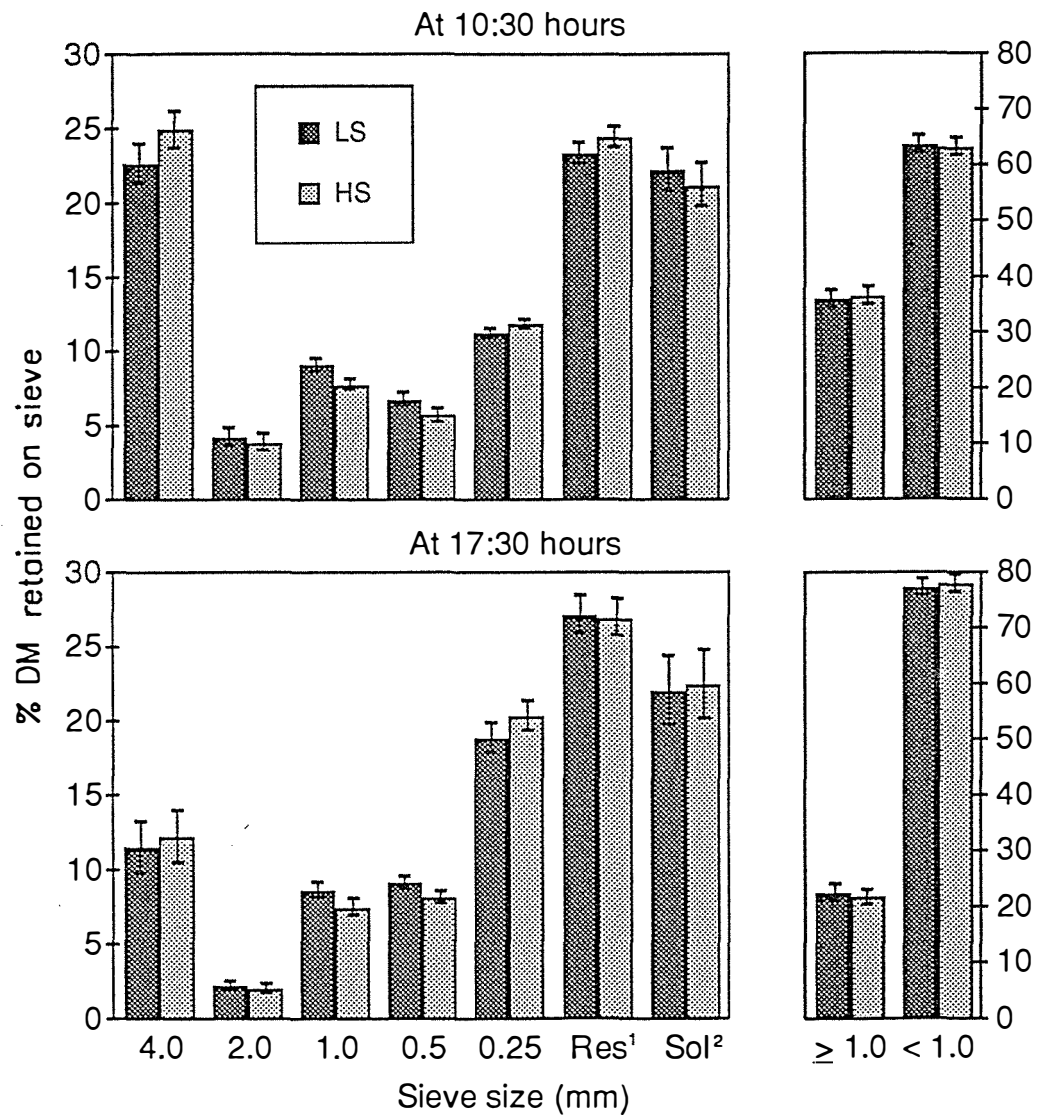
Figure 3.3 Particle size distributions in the rumen contents recovered at 10:30 and 17:30 hours from the wethers (n=8) ruminating the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections.

1: Residues.

2: Soluble DM defined as the DM remaining in solution after centrifugation at 850 G for 20 minutes.

3.4 Discussion

3.4.1 Effect of reduced leaf shear breaking load on the index of masticatory load of leaves

Both PRG selections appeared to consist of mostly green leaves although determination of pasture composition by manual dissection was not conducted. The effect of the selection for LS and HS on LSBL in the present study (507.6 and 710.5 g/leaf for the LS and HS selections, respectively) was not as large as those in the previous chapter, where the PRG selections were grown under the optimum conditions (337.5 and 573.9 g/leaf for the LS and HS selections, respectively). Additionally, there was a large difference in the leaf length:dry weight ratio between the two PRG selection i.e. 16.4 and 11.8 mm/mgDM for the LS and HS selections, respectively and thus, the effect of selection on the leaf IML was almost nil in the present study. Because the Instron Food Testing Instrument had slightly different shear blades and operating conditions from the Warner-Bratzler specifications and also the samples of leaf were not necessarily intact in the present study, comparisons of other corresponding morphological parameters from the previous chapter are not appropriate.

3.4.2 Effects of reduced leaf shear breaking load on particle size distribution in the rumen contents

There were no major effects of reduced LSBL in PRG on the <C.EAT> (40.7 vs 40.7 % for the LS and HS PRG selections, respectively) and on <C.RUM> (70.7 vs 71.5 % for the LS and HS PRG selections, respectively). The particle size distributions in the rumen contents were similar for the two PRG selections in both trials. The ingestion rates during eating tended to be higher for the LS PRG selection than for the HS PRG selection (4.3 vs 3.4 gDM/min eating and 31.3 vs 23.3 mgDM ingested/chew for LS and HS selections, respectively). Total eating and ruminating times during a complete 24-hour period were not measured in the present trial. However, in a previous study (Inoue et al., 1989), when feed intake was high, sheep consumed significantly more the LS PRG selection than the HS PRG selection (102 vs 86 g/kg$^{0.75}$/d). Although ingestion rates were similar (259 vs 225 min/kgDM, for the LS and HS PRG selection, respectively) sheep fed the LS PRG selection spent more time eating during the day than for HS PRG selection (381 vs 279 min/d). Sheep fed LS PRG selection ruminated at a lower rumination rate than for HS PRG selection (426 vs 542 min/kgDM/d). Total time spent for rumination during a day was similar for both PRG selections (626 vs 672 min, for LS and HS PRG selections, respectively). In the present study, however, masticatory behaviour during rumination was similar for the two PRG selections. Ulyatt et al. (1986) reported similar observations where ingestion rates in sheep were 4.1 and 11.1 gDM/min eating respectively for PRG and red clover and proportions of particles less than 1 mm in boli were 48.6 and 51.6 for PRG and red clover, respectively.

Kennedy (1985) also observed in sheep proportions of large particles in chewed boli collected from oesophageal fistulas were not greatly different among four different chopped forage diets. This indicates that animals continue to chew the feeds regardless of their physical and morphological properties until a certain particle size distribution is reached. Thus, the physical and morphological properties of feeds will not alter particle size distribution in masticated rumen contents but will alter ingestion rate. Perhaps, tactile stimulation by dietary particles are largely involved in masticatory behaviour.

3.4.3 Influence of index of masticatory load on masticatory efficiency

The masticatory mechanism is a complex system, which has perceptual skills i. e. the ability of subjects to detect the size of objects and the forces applied to the teeth (Kawamura *et al.*, 1960; Møller, 1966; Bowman *et al.*, 1968). Bigland *et al.* (1954) and Podolsky *et al.* (1980) demonstrated an inverse relationship between force and velocity in muscle contraction. This implies that when a greater bite force is required the masticatory muscles must contract more strongly in each chewing and this may result in slow jaw movement and thus, lower chewing rate. If this is true, the relationship between physical resistance of PRG leaves to breakdown and theoretical efficiency of chewing <TEC> is given by;

$$\text{<TEC> (mgDM broken into 1 mm particles /chew)} = f \times \frac{1}{\text{IML}}$$

and,

$$\text{<TEC> (mgDM broken into 1 mm particles / min)} = f \times k \times \frac{1}{\text{IML}}$$

where,

f (kg) = mean bite force per chew at chewing rate of k (chews/min).

Therefore, IML is a determining factor of the efficiency of mastication. A previous study (John *et al.*, 1989) showed that a difference in LSBL between the LS and HS PRG selection was maintained after incubation in the rumen of cows for at least 24 hours. This suggests that IML influences masticatory efficiency during rumination as well as during ingestion for at least up 24 hours after ingesting PRG.

Rhythmical contractions of the masticatory muscles during chewing are inherently controlled by an oscillating system located within the animal's brain stem (Dellow *et al.*, 1971; Phyllips *et al.*, 1973) and chewing rates are constant within each animal species (Hiiemae, 1978). Ulyatt *et al.* (1986) reported that chewing rates did not greatly differ among animals eating three different fresh forages and two different hays. This suggests that animals chew at a certain chewing rate regardless the properties of the feeds. Similar chewing rate for the two PRG selections in the present study supports this. It is possible to speculate that animals may regulate amount of feed to chew (i.e. ingestion and rumination rates) in each chewing stroke to equalize the breaking load of feed to a constant bite force exerted, so that the animals maintain a certain chewing rate. Figure 3.4 shows a model of a relationship between efficiency of particle breakdown and IML of PRG.

In the present study, number of chews/gDM tended to be lower for the LS PRG selection than for the HS PRG selection although IML and chewing rate during eating (no. chews/min) were similar for the two PRG selections. This is probably due to narrower (20 %, p<0.001) leaf width for the LS PRG selection thus, fewer chews were required to cut the leaf parallel to the sclerenchyma fibres. Although the load to break the leaf in this direction is estimated to be small (0.142 x 10^7 kg/m^2, Young's modulus in tension; Vincent, 1982), IML might have been altered if this breaking load could have been taken into account.

3.4.4 Particle breakdown during rumination

Consumption and dry matter pool size for the LS PRG selection were approximately 13 and 10 %, respectively, lower than for the HS PRG selection at
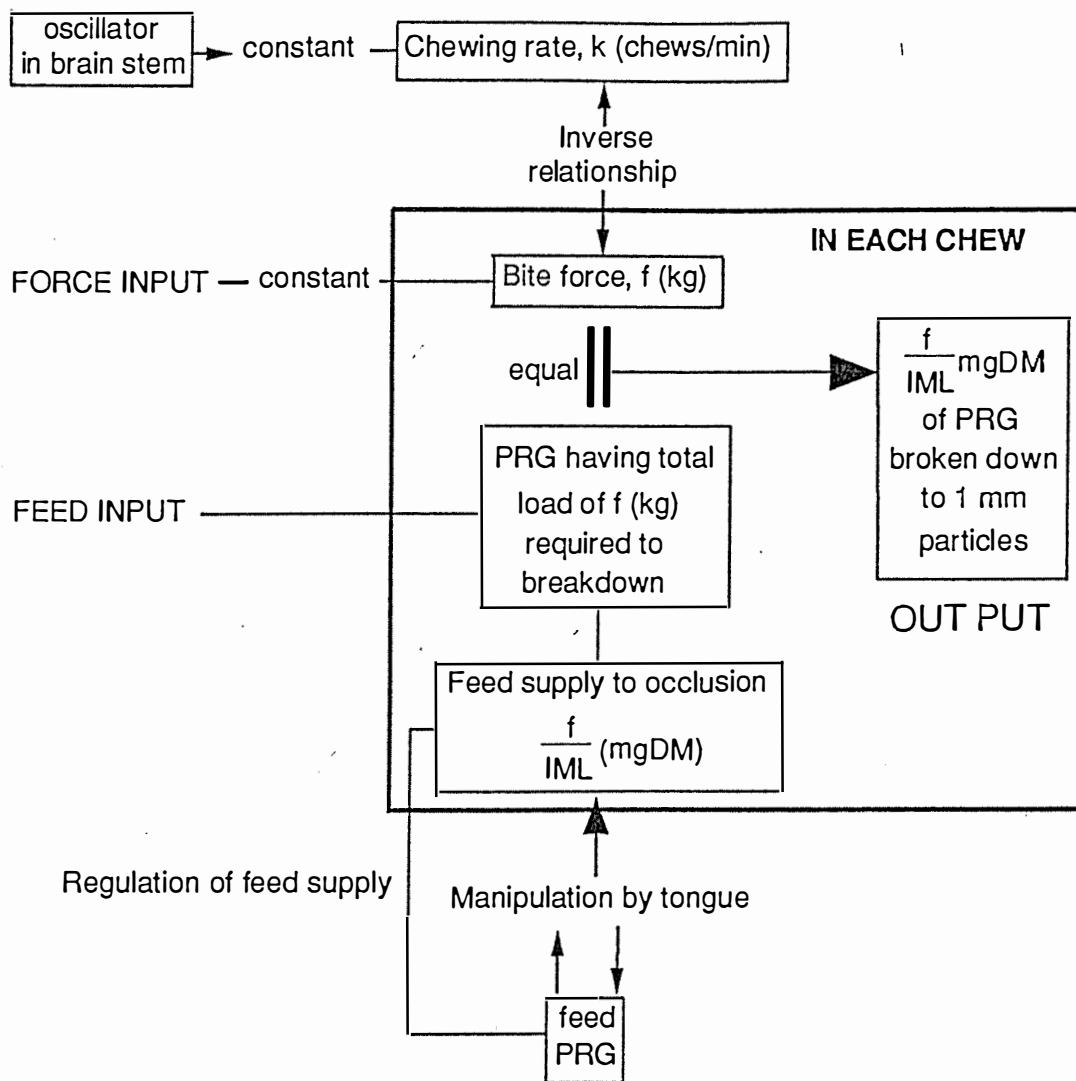
Figure 3.4 A model of a relationship between efficiency of particle breakdown and index of masticatory load (IML) of perennial ryegrass.

both 10:30 and 17:30. IVDMD for the LS PRG selection was slightly higher than HS PRG selection but rate of dry matter disappearance from the rumen was almost the same for the two PRG selections. There were no major differences in the masticatory behaviour during rumination, because the IML was the same for the two PRG selections and morphological differences were lost for both PRG selections after the wethers chewed the PRG selections during eating.

3.5 Conclusion

The LS PRG selection was approximately 29 % lower in LSBL than the HS PRG selection. However, in IML the difference for the two PRG selections was almost nil because the LS selection had an approximately 39 % larger leaf length:dry weight ratio. There were no major effects of reduced LSBL in PRG on the <C.EAT> (40.7 vs 40.7 % for the LS and HS PRG selections, respectively) and on <C.RUM> (70.7 vs 71.5 % for the LS and HS PRG selections, respectively). The particle size distributions in the rumen contents were similar for the two PRG selections in both trials. The ingestion rates during eating tended to be higher for the LS PRG selection than for the HS PRG selection (4.3 vs 3.4 gDM/min eating and 31.3 vs 23.3 mgDM ingested/chew for LS and HS selections, respectively) but masticatory behaviour during rumination was similar for the two PRG selections. This is because the leaves of the LS PRG selection were narrower than leaves of the HS PRG selection, and therefore, the LS PRG selection required fewer chews in the direction parallel to the sclerenchyma fibres. There were no major differences in the masticatory behaviour during rumination due to the lack of difference for the two PRG selection in IML and that morphological difference were lost for both PRG selections after the wethers chewed the PRG selections during eating.

The effect of reduced LSBL in PRG on rumen digesta outflow rate will be examined in the next chapter.

Chapter Four

THE EFFECTS OF REDUCED LEAF SHEAR BREAKING LOAD IN PERENNIAL RYEGRASS UPON RUMEN DIGESTA OUTFLOW RATE AND APPARENT DIGESTIBILITY IN SHEEP

4.1 Introduction

Prolonged retention of digesta in the rumen of forage-fed animals can reduce voluntary intake and feeding value (Thornton *et al.*, 1973). Black *et al.* (1982), using computer simulations, suggested that slow degradation and rumen digesta fractional outflow rates (FOR) were principal factors causing long rumen retention times and reduced voluntary intakes in sheep fed perennial ryegrass (PRG). FOR is likely to be influenced by the rates of particle size reduction through chewing and rumination (Black *et al.*, 1982; Ulyatt *et al*, 1986; Poppi *et al.*, 1987). This chapter, based on an experiment conducted in April and May 1988, investigates the effects of reduced leaf shear breaking load (LSBL) in PRG on rumen FOR and apparent digestibilities in sheep fed at restricted feed intake levels.

4.2 Materials and Methods

Two PRG selections selected for low (LS) and high leaf shear breaking load (HS) were fed to a total of eight rumen-fistulated wethers in a cross-over design. The feeding level was restricted to 90 % of dry matter voluntary intake. FOR was measured by the inert ruthenium-phenanthroline/chromium-ethylenediaminetetraacetic acid dual-phase marker infusion technique and also by lignin as a particulate marker. Total faecal collections were made to determine the apparent digestibilities for the two PRG selections.

4.2.1 Low and high leaf shear breaking load perennial ryegrass selections

The LS and HS PRG selections harvested in 1985 (see section 1.4.2.1) were sown into separate 0.25 ha halves of a 0.5 ha paddock located at DSIR, Palmerston North in April 1987. The paddocks were maintained as a pure ryegrass sward . The two PRG selections were harvested from the paddocks at 09:00 hours daily during the trial period. Dry matter concentrations were determined daily by oven drying (105 C°) for 24 hours.

4.2.2 Experimental animals

Eight mature rumen-fistulated Romney wethers (mean live weight 71.0 kg $\pm$ 0.23 s.e.) were used. The animals were fitted with permanent rubber cannulae (65 mm i.d.). All the animals were housed individually in metabolism crates with an automatic feeder attached.

4.2.3 Experimental design

4.2.3.1 Adjustment period

Chaffed lucerne hay was fed to all the animals for seven days to adjust them to the indoor conditions. The feed was placed upon the belts of the automatic feeders which were set for continuous hourly feeding.

4.2.3.2 Voluntary feeding period

Following the adjustment period, freshly cut PRG, containing approximately 20 % white clover (WC), was fed to the animals to measure voluntary intakes for seven days. The PRG-WC was harvested at 09:00 hours from the grazing paddocks at DSIR, Palmerston North. PRG-WC was placed upon the automatic feeder two

times a day following storage under refrigeration (approx, 4 C°). Refusals were weighed daily before the new forage was fed to the animals. Dry matter concentrations of the fresh forage and refusals were determined daily by oven-drying (105 °C for 24 hours). Daily dry matter intakes were recorded for each animal during the seven-day period.

4.2.3.3 Trial period

The animals were allocated into two groups so that the mean voluntary intake levels were similar for the two groups. The animals received either freshly cut LS or HS PRG selection during the two 12-day feeding periods in a cross-over design. Feeding level for each animal was restricted to 90 % of voluntary dry matter intake measured during the voluntary feeding period. The PRG selections were placed upon the automatic feeders two times a day (11:00 and 23:00 hours) following storage under refrigeration (approx. 4 C°).

4.2.3.3.1 Determination of rumen digesta outflow rate

The inert particulate-phase marker ruthenium tris (1, 10-phenanthroline) ruthenium (II) chloride (Ru-phen) (MacRae *et al.*, 1974; Evans *et al.*, 1977) was prepared by the procedure described by Tan *et al.* (1971) with a concentration of 99.6 mg Ru/kg of marker solution. The inert liquid-phase marker, chromium ethylenediaminetetraacetic acid (Cr-EDTA) (Faichney, 1975) was prepared by the procedure described by Binnerts *et al.* (1968) with a concentration of 4000 mg Cr/kg of marker solution. The Ru-Phen and Cr-EDTA markers were then combined in equal volumes to make a dual-phase (Ru-Cr) marker solution.

During the last 6 days of each 12-day feeding period, the Ru-Cr marker was infused into the rumen at a mean rate of 13 and 537 mg/day, for Ru and Cr respectively. At the end of each feeding period, entire rumen contents were removed, weighed, mixed thoroughly, samples taken for dry matter concentration,

particle size and marker analyses and the contents returned. Dry matter concentrations of rumen contents were determined by oven-drying (105 °C for 72 hours).

FOR for the particulate phase and liquid-phase were estimated as the proportion of the total rumen contents leaving from the rumen per unit time (Faichney, 1975) i.e.

$$\text{FOR for liquid-phase } (\%/h) = \frac{\text{Infusion rate of Cr (mg/h)}}{\text{Total Cr (mg) in rumen}} \times 100$$

$$\text{FOR for particulate-phase } (\%/h) = \frac{\text{Infusion rate of Ru (mg/h)}}{\text{Total Ru (mg) in rumen}} \times 100$$

and

$$= \frac{\text{Faeces lignin output (mg/h)}}{\text{Rumen lignin pool size.}} \times 100$$

Mean rumen digesta retention times (MRT) were calculated as the reciprocal of their respective FOR. Particle size analyses was conducted as described in section 3.2.6 using sieves of 4.0, 2.0, 1.0, 0.5 and 0.25 mm mesh sizes.

4.2.3.3.2 Determination of apparent digestibilities

Total faecal collections were made on the last 6 days of each feeding period using faecal collection bags fitted to the animals. Prior to the faecal collections, the faecal collection bags were fitted to the animals and left on for increasing periods of time so that the animals became used to the bags. Faeces from each animal were weighed daily, pooled over each feeding period and stored in the deep freeze (approx. -20 C°). They were later thoroughly mixed, subsampled, freeze-dried and

ground through a 1 mm sieve for chemical analyses. During the faecal collection period, feed refusals in feeding bins and under-crate residues were collected and weighed daily for each animal. Determinations of dry matter concentrations were made daily by oven-drying (105 °C for 24 hours) for the PRG offered, refusals in feeding bins and under crates residues. Samples of the two PRG selections and refusals were pooled over each digestion trial period and stored in the deep freeze (approx. -20 C°). They were later thoroughly mixed, subsampled, freeze-dried and ground through a 1 mm mesh sieve for chemical analyses. On the last day of the each feeding period, rumen fluid was sampled through the fistulae for pH, ammonia and volatile fatty acid (VFA) analyses.

4.2.4 Determination of the index of masticatory load of leaves

4.2.4.1 Measurements of leaf morphology

During the last 6 days of each feeding period the fresh LS and HS PRG selection were sampled, pooled over each period and stored in the deep freeze (-20 C°) before taking any measurements. One hundred and fifty pieces of green leaf were randomly subsampled from each PRG selection, regardless whether entire or part of a leaf. Length and width at the midway point along the leaf length were recorded on each piece of leaf. The 150 pieces of leaf were then pooled, oven-dried (105 °C for 24 hours) and weighed to determine the average dry weight of a leaf piece. Approximately 150 g of fresh subsample of each PRG selection was dissected manually into green grass and dead material fractions. These dissected fractions were oven-dried (105 C° for 24 hours) and then weighed to determine the composition of each PRG selection.

4.2.4.2 Measurement of leaf shear breaking load of leaf

An Instron Food Testing Instrument (model 1140, manufactured by Instron Ltd., England) fitted with Warner-Bratzler Meat Shear type blades (no. 2830-013,

manufactured by Instron Ltd., England) was used to measure the LSBL. Drive speed was set for 80 mm/min. One hundred pieces of green leaf were randomly sampled from both PRG selections on a day between the two 12-day feeding periods regardless whether entire or part of a leaf. The leaves were gathered in bundles of 10 leaves each, aligned along the midway of the leaf piece length. Ten replicates of 10-leaf bundles for each PRG selection were then sheared. The maximum force exerted divided by the number of leaf pieces sheared was determined as LSBL. The index of masticatory load (IML) of leaf was then estimated for the two PRG selections as defined in section 2.4.5.

4.2.5 Chemical analyses

For the marker analyses, rumen contents were freeze-dried, and ground through a 0.5 mm mesh sieve. Five grams of the ground samples were compressed into self-supporting disks. The disks were analysed for concentrations of Ru and Cr by X-ray fluorescence spectrometry at Victoria University, New Zealand using a Philips RW1404 Automatic Sequential X-ray fluorescence Spectrometer (manufactured by Philips Ltd., Netherlands) (Evans *et al.*, 1977). Chemical analyses were performed on faeces, PRG offered and refusals, for total nitrogen by the Kjeldahal procedure using a Kjeltec Auto 1030 Analyzer (manufactured by Tecator Ltd., Sweden), concentrations of acid detergent fibre (ADF), neutral detergent fibre (NDF), lignin (Robertson *et al.*, 1981) and organic matter by ashing overnight at 550 °C. Cellulose and hemi-cellulose concentrations were determined by subtracting the concentration of lignin from ADF concentration and ADF concentration from NDF concentration, respectively. The pH of the rumen fluid was determined immediately after the sampling on a pH meter (PHM61, manufactured by Radiometer Ltd., Denmark). The samples of rumen fluid were deproteinised and analysed for ammonia-N (NH_3-N) by steam distillation on the Kjeltec Auto 1030 Analyzer (manufactured by Tecator Ltd., Sweden). VFA in the samples of rumen fluid were analysed by gas-liquid chromatography (Shimadzu Gas Chromatograph GC-8A, Shimadzu Ltd., Japan).

4.2.6 Statistical analyses

Statistical analyses were performed by the analysis of variance for a cross-over design. GLM procedure of SAS (SAS Inst., Inc., NC., USA) was used for the analyses.

4.3 Results

4.3.1   Leaf morphology, leaf shear breaking load, index of masticatory load and chemical composition of the low and high shear breaking load perennial ryegrass selections

Means of leaf morphology, LSBL and IML for the two PRG selections are shown in Table 4.1. The leaves of the LS PRG selection were approximately 13 % shorter (p<0.001, ±7.83 s.e.d.), 19 % narrower (p<0.001, ±0.10 s.e.d.) and were 40 % lighter than the leaves of the HS PRG selection. Thus, in leaf length:dry weight ratio, the LS selection was approximately 46 % higher than the HS selection. LSBL for the LS PRG selection was approximately 39 % lower than that for the HS PRG selection and thus, IML for the LS PRG selection was estimated as approximately 12 % lower than for the HS PRG selection. Statistical analyses were not carried out for dry weight, breaking load and estimated IML of the leaves because the leaves were pooled to determined those parameters.

Concentrations of dry matter, organic matter, total nitrogen and cell-wall constituents are shown in Table 4.2. The LS and HS PRG selections offered to the animals contained approximately 25.9 and 20.0 % of dead matter, respectively. The concentrations of dry matter, organic matter, total nitrogen, lignin and hemi-cellulose were all similar for both PRG selections except cellulose concentration, which was approximately 12 % lower for the LS PGR selection than the HS PRG selection.

Table 4.1 Means of morphology, shear breaking load and index of masticatory load (IML) of the leaves of the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections.

	Selection	
	LS	HS
Morphology:		
Length (mm)	238.0	273.6
Width (mm)	2.81	3.51
Dry weight (mg/leaf)	20.7	34.5
Length:dry weight ratio (mm/mgDM)	11.5	7.9
Shear breaking load (g/leaf)	693.2	1141.7
IML (kg/mgDM)	7.98	9.05

Table 4.2. Concentrations of dry matter, organic matter, total nitrogen and cell-wall constituents of the low (LS) and high (HS) leaf shear breaking load perennial ryegrass offered to the animals.

	Selection	
	LS	HS
Dry matter (%)	18.7	19.0
Organic matter (% DM)	87.9	88.1
Total nitrogen (% DM)	3.36	3.35
Lignin (% DM)	1.76	1.74
Cellulose (% DM)	17.8	20.2
Hemi-cellulose (%DM)	26.0	23.9

4.3.2 Rumen pool size, rumen digesta fractional outflow rates, rumen digesta retention times and particle size distribution in rumen contents in wethers fed on the low and high leaf shear breaking load perennial ryegrass selections

Mean daily dry matter intake in wethers during the last 6 days of feeding period was 1114 and 1118 g for the LS and HS PRG selections, respectively. Rumen pool sizes in wethers fed on the LS and HS PRG selections are shown in Table 4.3. The LS PRG were approximately 16 ($p<0.05$), 16 ($p<0.10$) and 13 % higher than the HS PRG in liquid pool size, total rumen pool size and in dry matter pool size, respectively.

The FOR and MRT for liquid-phase and particulate-phase of rumen digesta in wethers fed on the LS and HS PRG selections are shown in Table 4.4. No major differences occurred in FOR and MRT for the two PRG selections.

Particle size distributions in rumen contents in wethers fed on the LS and HS PRG selections are shown in Figure 4.1. There were no major differences in particle size distribution in rumen contents between the two PRG selections. The proportion of particles smaller than 1 mm was almost 80 % for both PRG selections.

4.3.3 Apparent digestibilities, concentrations of VFA and NH_3-N in rumen contents in wethers fed on the low and high shear breaking load perennial ryegrass selections

Table 4.5 shows apparent digestibilities of dry matter, organic matter, total nitrogen and cell-wall constituents in wethers fed on the LS and HS PRG selections. The apparent digestibilities of dry matter, organic matter, total nitrogen and hemi-cellulose were similar for the LS and HS PRG selections. Apparent cellulose digestibility was approximately 16 % ($p<0.01$) lower for the LS PRG selections than for the HS PRG selections. Although the apparent digestibility of lignin showed negative values for both PRG selections due to artifact lignin in the

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faeces analysed, it was also lower (p<0.05) for the LS PRG selection than for the HS PRG selection.

Concentrations and proportions of VFA, concentration of NH_3-N and pH in the rumen fluid in wethers fed on the LS and HS PRG selections are shown in Table 4.6. There were no major differences in those parameters for the two PRG selections.

Table 4.3 Total and dry matter rumen pool sizes in wethers (n=8) fed on the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections.

	Selection		Effect of selection	
	LS	HS		S.E.D.
Total rumen pool size (g)	5809	5005	ns	330.7
Dry matter rumen pool size (g)	567.2	504.2	ns	34.68
Liquid pool size (g)	5241	4501	*	300.9

Table 4.4 Rumen digesta fractional outflow rates (FOR) and rumen digesta mean retention times (MRT) for liquid-phase and particulate-phase in wethers (n=8) fed on the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections.

	Selection		Effect of selection	S.E.D.
	LS	HS		
FOR (%/h):				
Liquid-phase	11.2	11.8	ns	0.62
Particulate-phase				
(based on Ru)	6.4	6.6	ns	0.38
(based on lignin)	5.2	6.1	ns	0.68
MRT (h):				
Liquid-phase	9.1	8.7	ns	0.45
Particulate-phase				
(based on Ru)	16.4	16.2	ns	1.15
(based on lignin)	19.6	20.2	ns	2.76
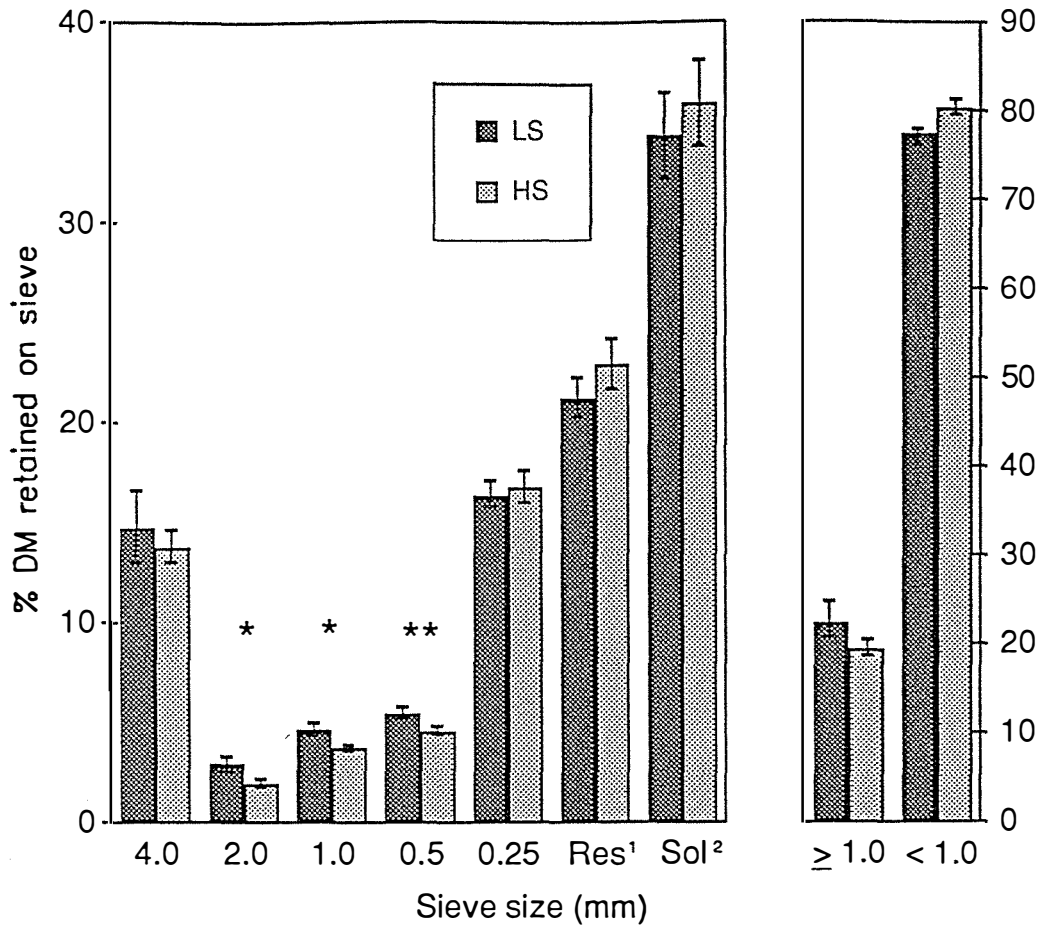
Figure 4.1   Particle size distributions in rumen contents in wethers (n=8) fed on the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections.

1: Residues.
2: Soluble DM defined as the DM remaining in solution after centrifugation at 850 G for 20 minutes.

Table 4.5 Apparent digestibilities (%) of dry matter, organic matter, total nitrogen and cell-wall constituents in wethers (n=8) fed on the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections.

	Selection		Effect of selection	
	LS	HS		S.E.D.
Dry matter	68.8	70.3	ns	0.84
Organic matter	70.3	71.8	ns	0.86
Total nitrogen	74.9	75.6	ns	0.73
Lignin	-17.9	-7.2	*	0.65
Cellulose	66.3	78.5	**	2.62
Hemi-cellulose	68.2	65.6	ns	2.74

Table 4.6 Concentrations and proportions of volatile fatty acids (VFA) and concentration of NH_3-N in the rumen fluid in wethers (n=8) fed on the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections.

	Selection		Effect of selection	S.E.D.
	LS	HS		
Total VFA (mmol/l)	12 8	12 7	ns	0.54
Proportion of VFA (molar %):				
Acetate	68.5	68.6	ns	0.48
Propionate	19.0	18.9	ns	0.37
Iso-butyrate	0.9	0.8	*	0.05
N-butyrate	9.2	9.2	ns	0.34
Iso-valerate	1.5	1.5	ns	0.06
NH_3-N (ppm)	288.0	282.8	ns	8.04

4.4 Discussion

The LS PRG selection was considerably (39 %) lower in LSBL than the HS PRG selection. However this selection effect was somewhat decreased in IML (12 %) because the LS PRG selection was approximately 46 % higher in leaf length:dry weight ratio than the HS PRG selection. The lack of major effects of reduced LSBL in PRG on FOR, rumen pool sizes and particle size distribution in the rumen contents may be explained by this relatively small effect of PRG selection in IML and the considerably low feed intake level (approx. 50 $gDM/kg^{0.75}$) in the present study.

The FOR's based on lignin were slightly lower than FOR's based on Ru. Domingue et al. (1991c) observed a similar relationship between FOR's based on lignin and on Ru-Phen in goats, sheep and deer. FOR based on lignin is believed to be more accurate on the assumption that there was minimal post-ruminal degradation of lignin (Faichney, 1980).

The LS PRG selection had a higher concentration of hemi-cellulose but lower concentration of cellulose than the HS PRG selection. This agrees with the results in chapter two, except for the lignin concentration, in which the LS PRG was similar to the HS PRG selection in the present study. The apparent digestibility of cellulose for the LS PRG selection was approximately 16 % lower than the HS PRG selection. Factors which could influence fibre digestive efficiency include rumen pool size, FOR, particle size distribution and rumen environment (Domingue *et al.*, 1991b). In the present study, however, there were no differences for the two PRG selections in these factors. The rumen microorganisms enter the chewd plant tissues, via cut and damaged surfaces (Akin, 1976, 1979; Elliott *et al.*, 1985). The LS PRG leaves were approximately 19 % narrower and 13 % shorter than these of the HS PRG selection. After both PRG leaves are broken down to the same particle size distribution, particles of the LS PRG leaves must have fewer chewed surfaces than the particles of the HS PRG leaves. Therefore, less damaged surfaces were available for microorganisms to enter for the LS PRG selection than for the HS selection and thus, lower cellulose digestibility might occur. This

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assumption agrees with the observation in chapter three in which a fewer number of chews were required for the LS PRG than for the HS PRG selection. This implies that leaf morphology in PRG may affect the efficiency of fibre digestibility.

Lignin also appeared less digestible for the LS PRG selection. The greater concentration of this lignin, if associated with the cellulose component of the sclerenchyma, could account for the reduced cellulose digestibility of the LS PRG selection.

4.5 Conclusion

The LS PRG selection was approximately 39 % lower than the HS PRG selection in LSBL. When expressed as IML, the LS PRG selection remained approximately 12 % lower than the HS PRG selection. However, there were no effects of reduced LSBL on FOR, rumen pool sizes and particle size distribution. This may be associated with relatively small selection effect of PRG in IML and low feed intake level. The cellulose fraction for the LS PRG selection was digested approximately 16 % less than that for the HS selection. The LS PRG leaves were approximately 19 % narrower than the HS PRG leaves. When the forages were broken down to the same particle size distribution, the LS PRG leaves would have less damaged surfaces available for the rumen microorganisms to enter. The leaf morphology in PRG may affect the efficiency of fibre digestibility.

Chapter Five

THE EFFECTS OF REDUCED LEAF BREAKING LOAD IN PERENNIAL RYEGRASS UPON RUMEN DIGESTA OUTFLOW RATES, VOLUNTARY FEED INTAKE, LIVE WEIGHT GAIN AND WOOL PRODUCTION IN GRAZING SHEEP

5.1 Introduction

Slow rumen fractional outflow rate (FOR) is a major factor causing long mean rumen digesta retention time (MRT) and reduced feed intake, thus, reduced feeding value (FV) of perennial ryegrass (PRG) (Black *et al.*, 1982). The rate at which dietary particles reduce in size influences MRT (Black *et al.*, 1982; Ulyatt *et al.*, 1986; Poppi *et al.*, 1987). Reduction of the dietary particle size is achieved predominantly by mastication. Reducing the physical resistance is believed to be a key factor to increase efficiency of breaking down the ingested forage by mastication (Bailey, 1964; Waghorn *et al.*, 1989; McLeod *et al.*,1989; Hodgson 1990) Increased efficiency of masticatory break down of forage can lead to faster rumen fractional outflow rates (FOR). Improved FOR can achieve increased feed intake and increased nutritive efficiency in the small intestine, thus, improved FV. Two trials were conducted in summer 1988 (Trial 1) and in summer 1989 (Trial 2) to test the hypothesis that reduced leaf shear breaking load (LSBL) in PRG improves FOR and leads to higher voluntary feed intake, and hence achieves improved live weight gain and wool production.

5.2 Materials and Methods

Trial 1 measured FOR, live weight gain and wool production in sheep grazing either the LS or HS PRG selections. FOR was measured in rumen-fistulated

wethers by the inert ruthenium-phenanthroline/chromium-ethylenediaminetetraacetic acid (Ru-Cr) dual-phase marker infusion technique. Live weight gain and wool production were measured in autumn-born lambs grazing the two types of PRG selections in spring. The schematic presentation of the experimental design of this trial is shown in Table 5.1 (p. 125). Trial 2 also measured FOR, live weight gain and voluntary organic matter intake (VOMI) in sheep grazing either the LS and HS PRG selections. FOR and VOMI in rumen-fistulated wethers were measured by the ruthenium-phenanthroline/cobalt-ethylenediaminetetraacetic acid (Ru-Co) dual-phase marker infusion technique. Measurements of live weight gain were made in one-year-old ewe-hoggets. The chromium sesquioxide (Cr_2O_3) marker was also administered to the rumen-fistulated wethers and the ewe-hoggets to measure VOMI. VOMI was cross-compared between the rumen-fistulated wethers and ewe-hoggets. The schematic presentation of the experimental design of this trial is shown in Table 5.2 (p. 126).

5.2.1 Pasture

The LS and HS PRG selections harvested in 1985 (see section 1.4.2.1) were sown into separate 1.5 ha halves of a 3 ha paddock located at DSIR's Tiritea Research Area, Palmerston North in April 1987. The paddocks were maintained as a pure ryegrass sward by applications of urea and herbicide for annual weed control.

5.2.2 Trial 1

5.2.2.1 Determination of rumen digesta fractional outflow rates

On November 6th 1988, 12 rumen-fistulated Romney wethers (mean live weight 36.5 kg ± 1.37 s.e.) fitted with a permanent rubber cannulae were allocated into two groups so that the mean live weight was similar between the two groups. The animals were grazed on either the LS or HS PRG selections in a cross-over design. A backpack, which consisted of a battery operated portable pump and a infusate

reservoir, was constructed (see Appendix 5.1) and fitted on each animal one week prior to the first infusion period. The Ru-Cr dual-phase marker solution was prepared as described in Section 4.2.3.3.1. On November 30th, the Ru-Cr dual-phase marker solution was infused into the rumen for 9 days at an average rate of 542 and 13 mg/day for Cr and Ru, respectively. On December 9th, the animals were rumen-emptied by manual bailing through the cannulae. The bailing was made at midday because preliminary measurements of the diurnal pattern of the rumen pool size showed that around midday the rumen pool size was close to the mean in 8 rumen-fistulated wethers grazing PRG (see appendix 5.2). The bailed rumen contents were weighed, mixed thoroughly, samples were taken for dry matter concentration, particle size and marker analyses and the contents returned. The animals were then changed over between the treatments and left to adjust themselves to the new treatments for 2 days. Another infusion period was started on December 11th for 9 days and the sampling procedure repeated on December 20th. FOR and rumen digesta mean retention time (MRT) of the liquid-phase and particulate phase were estimated as described in Section 4.2.3.3.1. During the trial, however, the marker infusion was accidentally interrupted in one of the rumen-fistulated wethers, therefore, all the data of this animal were excluded from the statistical analyses.

5.2.2.2 Measurements of live weight gain and wool production in autumn-born lambs

5.2.2.2.1 Live weight gain

On November 11th 1988, 30 female and 16 male Romney autumn-born lambs were allocated into two groups so that both groups were balanced for sex. Within each sex, the allocation was made randomly. The two groups were grazed on either the LS or HS PRG selections. Live weight of each animal was recorded weekly from November 18th for a period of 33 days. The animals were not fasted prior to the weighing but recording of live weights was made at 0900 hours each time.

5.2.2.2.2 Wool production

A mid-side patch of wool measuring approximately 100 cm^2 was taken from the left side of each animal by clipping to the skin surface on November 11th and on December 21st. The greasy weight of each wool sample was measured and, after scouring, the clean weight was also determined. On December 23rd, the animals were completely shorn and the greasy whole fleece weight was measured for each animal. A sample of greasy wool was taken from the right midside of each animal and the greasy wool weight-clean wool weight ratio was determined by weighing before and after scouring. The clean whole fleece weight was then estimated. The total daily fleece wool growth rate during the grazing period for each animal was calculated as;

$$\text{Total fleece wool growth, (g/day)} = \frac{C}{\text{no. of days}} \times \frac{B}{A + B}$$

where

A = initial midside wool (g/100cm^2),

B = final midside wool (g/100cm^2),

C = total fleece (g).

5.2.3 Trial 2

5.2.3.1 Determination of rumen digesta fractional outflow rates

On November 18th 1988, 8 rumen-fistulated Romney wethers, fitted with permanent rubber cannulae (mean live weight 50.8 kg $\pm$ 1.64 s.e.) were allocated into two groups so that the mean live weight was similar between the two groups. The animals were grazed on either the LS or HS PRG selections in a cross-over design. A backpack (see section 5.2.2.1) was fitted on each animal one week prior to the first-infusion period. A dual-phase marker was prepared as described in section

4.2.3.3.1 except that Co(III)-EDTA (for preparation see Appendix 5.3) was substituted for Cr-EDTA, because the Cr_2O_3 marker was also administrated to each rumen-fistulated animal (see section 5.2.3.3). On November 29th, the Ru-Co dual-phase marker solution was infused into the animals consecutively for 9 days at an average rate of 581 and 15 mg/day for Co and Ru, respectively. At noon on December 8th, the animals were rumen-emptied and samples were taken as described in section 5.2.2.1. The animals were then changed over between the treatments and left to adjust themselves to the new treatments for 4 days. Another infusion period was started on December 13th, for 9 days and the sampling procedure repeated on December 22nd. FOR and MRT for the liquid-phase and particulate phase were estimated as described in section 4.2.3.3.1 but Co was substituted for Cr in the equation.

5.2.3.2 Measurements of live weight gain in ewe-hoggets

On November 9th, 40 one-year-old Romney ewe-hoggets were randomly allocated into two groups to graze either the LS or HS PRG selections. The animals' live weights were recorded weekly from November 15th for 6 weeks at 0900 hours without fasting. 24 hour-fasted weights of the animals were also measured on November 10th and on December 9th and 23rd. Due to a shortage of the HS PRG selection, however, 4 animals were randomly selected from the HS paddock and were withdrawn from the trial on November 29th. All the data pertaining those animals were, therefore, excluded from statistical analyses.

5.2.3.3 Measurement of voluntary organic matter intake in ewe-hoggets and rumen-fistulated wethers

VOMI of the animals was estimated from the faecal OM output and the *in vitro* organic matter digestibility (IVOMD) of the ingested material from the animals. Faecal OM output for the ewe-hoggets was estimated based on the Cr_2O_3 marker. Faecal OM output for the rumen-fistulated wethers was estimated based on the

Cr_2O_3 marker and also the Ru-Co dual-phase marker. An intra-ruminal Cr_2O_3 controlled release capsule (Captec Chrome for Sheep, manufactured by Captec Pty. Ltd., Australia) was administered to 12 of the ewe-hoggets randomly selected from each paddock and to each rumen-fistulated wether on November 15th and 26th, respectively. The capsules were designed to release Cr sesquioxide faecal marker at a constant rate for approximately 25 days. Faecal collections were made per rectum on each animal. Faecal samples of each ewe-hogget were collected during the total three periods of five consecutive days, namely, from November 23rd to 27th (PI), December 4th to 8th (PII) and after the 2nd administration of the capsules on December 11th, from December 18th to 22nd (PIII). Faecal samples of each rumen-fistulated wether were collected during periods PII and PIII. PII and PIII were synchronized with the last five days of each dual-phase ruminal marker infusion period so that the data measured in the ewe-hoggets were comparable with the data measured in rumen-fistulated wethers. The collected faeces were then pooled within each period for each animal for marker analyses. At the completion of the faecal collection, the capsules were recovered from the rumen-fistulated wethers through the cannulae to determine the actual Cr release rate. The actual Cr release rate was estimated by the rate of disappearance of mass of the Cr-tablets in the capsules and actual concentration of chromium in the tablets (see Appendix 5.4). Daily VOMI was then estimated from the faecal OM output and the IVOMD of ingested material estimated from oesophageal extrusa (OE) recovered from the oesophageal fistulated animals (see section 5.2.4.3) by following;

$$\text{VOMI (g/day)} = \frac{\text{Faecal OM output (g/day)}}{1 - \text{IVOMD of OE.}}$$

Daily faecal OM output was calculated as;

$$\text{Faecal OM output (g/day)} = \frac{\text{Cr, Ru, Co release/infusion rate (mg/day)}}{\text{Cr, Ru, Co in OM of faeces (mg/g).}}$$

IVOMD of OE collected on November 25th, December 4th and 21st were used to estimate intakes for the periods of PI, PII and PIII, respectively.

	Nov 10	12	14	16	18	20	22	24	26	28	30	Dec 2	4	6	8	10	12	14	16	18	20	22	24
Pasture mass					T O			O			O T	O				O		O		C			
Herbage sample								O			O					O		O		C			
OE sample								C	O		O	C		O		O		C					
LSBL/IML						O					O				C								
Marker infusion									O C O O O O O O B	O C O O O O C O O O B									
Live weight				O			O			O		C			C			O					
Wool sample	M																			M	S		

Nov ← → Dec

T: topping   B: bailing   M: midside   S: shearing

Table 5.1 **The schematic presentation of the experimental design of Trial 1.**

o: measured and/or sampled.

	10	12	14	16	18	20	22	24	26	28	30	2	4	6	8	10	12	14	16	18	20	22	24
Pasture mass	O	O		O	T	O		O			C				O				O				
Herbage sample								O							O				O				
OE sample						O				O					O					O			
LSBL/IML		O						O											O				
Marker infusion								O O O O O O O O B						O O O O O O O C O B						
Faecal sample RFW								C			O O O O O						O O O O O		
Faecal sample Ewe			C			O O O O O				O O O O O	C				O O O O O		
Live weight	F	O			O				O				O	F		O				O		F	

                10 12 14 16 18 20 22 24 26 28 30 2   4   6   8   10 12 14 16 18 20 22 24
                              Nov   Dec

T: topping   B: bailing   RFW: wethers   C: marker in   F: fasted

Table 5.2 The schematic presentation of the experimental design of Trial 2.

o: measured and/or sampled.

5.2.4 Herbage analysis

5.2.4.1 Pasture mass

During the trials, pasture mass was calibrated from pasture height measured by a rising plate meter (Earle *et al.*, 1979). An Ellinbank Pasture Meter (EPM) attached with a rising plate of $0.1m^2$, 5.0 kg/m^2 (designed by Dairy Research Institute, New Zealand) was used to measure pasture height at which the plate was held. The EPM reading depends on both height and density and can be used as an indirect measure of pasture mass. Calibration of the pasture height to pasture mass was made by measuring the height of the pasture and dry matter yield of a 0.1 m^2 quadrat. The dry matter yield of a quadrat was determined by cutting the herbage to ground level, then removing, washing and oven-drying the herbage (105 °C for 24 hours). Pasture height and dry matter yield of ten quadrats were taken from each paddock for the calibration. The weight of the dry matter was then calculated as a pasture mass (kgDM/ha) and related to the pasture height by a linear model. Calibrations were made on November 24th and on December 9th for Trial 1 and Trial 2, respectively. Pasture height was determined by taking an average of 100 pasture height measurements randomly from each paddock. Pasture height was determined on November 18th, 24th, 30th, December 5th, 13th, 18th in Trial 1 (see Table 5.1) and on November 10th, 14th, 18th, 23th, 28th, December 3rd, 11th and 19th in Trial 2 (see Table 5.2). During the trials, the LS and HS paddocks were maintained at equal pasture mass in the two paddocks having pasture height within the range of 8 - 12 cm by introducing non-experimental sheep to the paddocks. Emerged seed heads were topped by a rotary mower set at a cutting height of 7.5 cm on November 17th and December 2nd in Trial 1 and November 20th in Trial 2.

5.2.4.2 In vitro digestibility of herbage

Herbage samples were randomly taken from each paddock by clipping to ground level on November 27th, December 5th, 13th and 18th in Trial 1 (Table 5.1) and November 28th, December 13th and 20th in Trial 2 (Table 5.2). The herbage

samples were washed with water, freeze-dried, ground through a 1 mm mesh sieve and analysed for in vitro organic matter digestibility (IVOMD).

5.2.4.3 Composition and in vitro digestibility of material ingested by the sheep

To estimate the digestibility and composition of the material ingested by the experimental animals, four mature oesophageal-fistulated wethers (mean live weight 75.7 kg $\pm$ 2.85 s.e.) were used to collect OE. It was assumed that the OE represented the material ingested by the experimental animals. The oesophageal fistulated wethers were randomly paired and allocated to graze each PRG selection. An OE collection bag was attached on each animal and the animals were allowed to graze for approximately 20 minutes. The bag was removed from the animal before rumination occurred. The OE was collected and pooled within each pair. After completion of the OE collection, the pairs were changed over between the paddocks each time. The collection of OE was made on November 28th, December 1st, 5th, 8th, 12th, 15th and 19th in Trial 1 (Table 5.1) and November 25th, December 4th, 13th, and 21st in Trial 2 (Table 5.2). Approximately 150 g of fresh subsample of each collected OE was dissected manually into green grass and seed heads or dead material fractions. These dissected fractions were oven-dried (105 °C for 24 hours) and then weighed to determine the composition of OE. A subsample of each collected OE was also freeze-dried, ground through a 1 mm mesh sieve and analysed for IVOMD.

5.2.4.4 Determination of leaf shear breaking load and index of masticatory load

Ten 0.1 m^2 quadrat samples of herbage were randomly collected by clipping to ground level from each paddock on November 25th, December 5th and 14th in Trial 1 (Table 5.1) and November 15th, 30th and December 19th in Trial 2 (Table 5.2). The quadrat samples taken were pooled and thoroughly mixed. Subsamples of 100 to 150 green fresh leaf pieces, regardless whether entire or part of a leaf, were randomly selected for measuring morphology and shear breaking load.

Length (longitudal length) of the leaf piece was recorded for each leaf piece and the leaf pieces were then pooled, oven-dried (105 °C for 24 hours) and weighed to determine the average dry weight of a leaf piece. An Instron Food Testing Instrument (model 1140, manufactured by Instron Ltd., England) fitted with Warner-Bratzler Meat Shear type blades (no. 2830-013, manufactured by Instron Ltd., England) was used for measuring the LSBL. Drive speed was set at 80 mm/min. Ten replicates of 10 fresh leaf pieces, which were gathered and aligned along the midway of the length, were sheared and the maximum forces exerted were recorded. The maximum force exerted divided by the number of leaf pieces sheared was determined as the shear breaking load of a leaf piece. The IML of a leaf piece was then estimated as defined in section 2.4.5.

5.2.5 Chemical analyses

5.2.5.1 Rumen contents

Dry matter concentrations of rumen contents samples were determined by oven-drying (105 °C for 72 hours). For the marker analyses, samples of rumen contents were freeze-dried and ground through a 0.5 mm mesh sieve. Concentrations of Ru and Cr (Trial 1), Ru and Co (Trial 2) in the samples were determined by X-ray fluorescence spectrometry as described in section 4.2.5. Particle size analyses were made as described in section 3.2.6 using sieves of 4.0, 2.0, 1.0, 0.5 and 0.25 mm mesh.

5.2.5.2 Faeces

Faecal samples collected in Trial 2 were oven-dried (105 °C for 24 hours) and ground through a 0.5 mm mesh sieve. Concentrations of Ru and Co were determined by X-ray fluorescence spectrometry as described in section 4.2.5. Concentration of Cr was determined by atomic absorption spectrophotometry (1982 IL 457 AA, manufactured by Instrumentation Laboratory Inc., USA)

(Williams *et al.*, 1962; Costigan *et al.*, 1987; Parker, 1990).

5.2.5.3 Herbage and oesophageal extrusa

Analyses of IVOMD for herbage samples and oesophageal extrusa were made using the procedure described by Roughan *et al.* (1977).

5.2.6 Statistical analyses

For the data collected from the rumen-fistulated wethers, statistical analyses were performed by analysis of variance for a cross-over design. For the data collected from lambs and ewe-hoggets, statistical analyses were performed by the analysis of variance for a completely randomized design. GLM procedure of SAS (SAS Inst., Inc., NC., USA) was used for the analyses.

5.3 Results

5.3.1 Trial 1

5.3.1.1 Pasture

Changes in pasture mass and IVOMD of the LS and HS PRG selections during the trial are shown in Figure 5.1. Changes in LSBL and leaf length:dry weight ratio of the two types of PRG selections during Trial 1 are shown in Figure 5.2. Changes in IML of the two types of PRG selections during the trial are shown in Figure 5.3. The mean values of above measures are shown in Table 5.3. There was no significant difference between LS and HS PRG selections in mean pasture mass and IVOMD during the trial. The LS PRG selection had approximately 25 % ($p<0.05$) lower mean LSBL and approximately 30 % higher ($p<0.10$) mean leaf length:dry weight ratio than the HS PRG selection during the trial. However, there
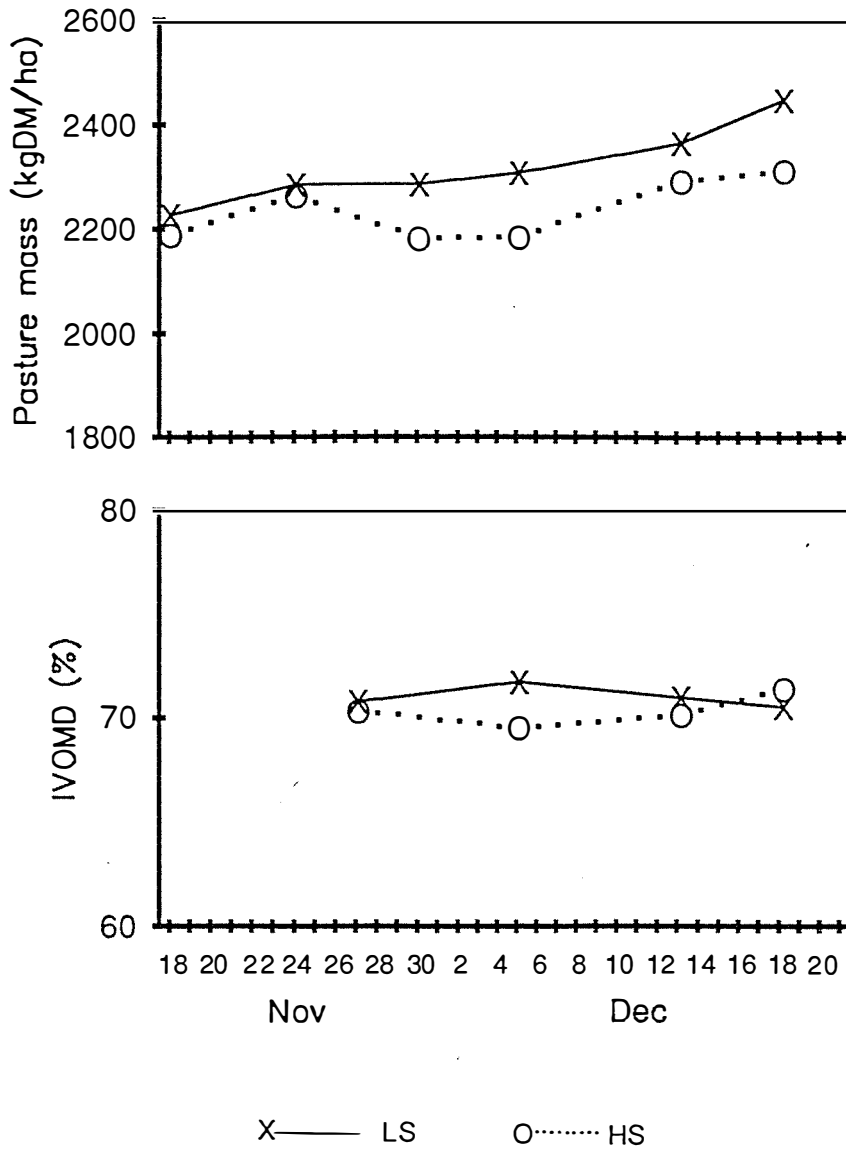
Figure 5.1 Changes in pasture mass and in vitro organic matter digestibility (IVOMD) of the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections during Trial 1.

Figure 5.2 Changes in leaf shear breaking load (LSBL) and leaf length:dry weight ratio (LWR) of low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections during Trial 1.

Figure 5.3 Changes in index of masticatory load (IML) of low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections during Trial 1.

Table 5.3 Mean values of pasture mass (n=6), in vitro organic matter digestibility (IVOMD) (n=4), leaf shear breaking load (n=3), leaf length:dry weight ratio (n=3) and index of masticatory load of leaf (n=3) of low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections on offer during Trial 1.

	Selection		Effect of selection	
	LS	HS		S.E.D.
Pasture mass (kgDM/ha)	2324	2241	ns	39.6
IVOMD (%)	71.1	70.4	ns	0.47
Leaf shear breaking load (g/leaf)	554.7	737.0	*	64.70
Leaf length:dry weight ratio (mm/mgDM)	13.5	10.4	ns (n=0.07)	1.24
Index of masticatory load (g/mgDM leaf)	7.34	7.67	ns	0.619

were no significant differences between the two types of PRG selections in mean IML during the trial.

Changes in composition and IVOMD of OE of animals grazing the LS and HS PRG selections during the trial are shown in Figure 5.4 and mean values are shown in Table 5.4. There were no significant differences between the OE of animals grazing the two types of PRG selections in composition and in IVOMD. Although both OE contained approximately 11-15 % of seed heads and dead material, the IVOMD was approximately 80 % for both PRG selections.

5.3.1.2 Live weight gain and wool production in autumn-born lambs grazing the low and high leaf shear breaking load perennial ryegrass selections

Changes in live weight of lambs grazing LS and HS PRG selections during the trial are shown in Figure 5.5. Mean values of live weight gain and total fleece wool growth are shown in Table 5.5. There were no significant differences in live weight between the two groups of animals grazing LS and HS PRG selections at any time during the trial period (Fig. 5.5). There were no significant differences between the two groups of animals in mean daily live weight gain and in mean daily total fleece wool growth (Table 5.5).

5.3.1.3 Rumen pool size, digesta fractional outflow rates, digesta mean retention times and particle size distributions in rumen contents in wethers grazing the low and high leaf shear breaking load perennial ryegrass selections

Rumen pool size, FOR and MRT of liquid-phase and particulate-phase of wethers grazing the LS and HS PRG selections are shown in Table 5.6. The animals grazing the LS PRG selection had approximately 16 % higher (p=0.07) dry matter rumen pool size and 10 % lower (p<0.05) FOR for the liquid-phase than the animals grazing the HS PRG selection. However, there were no other major differences occurring between the animals grazing the two types of PRG

selections.

Figure 5.6 shows the particle size distributions of the rumen contents of the animals grazing the LS and HS PRG selections. There were no major differences in particle size distribution of the rumen contents between the two groups of animals.
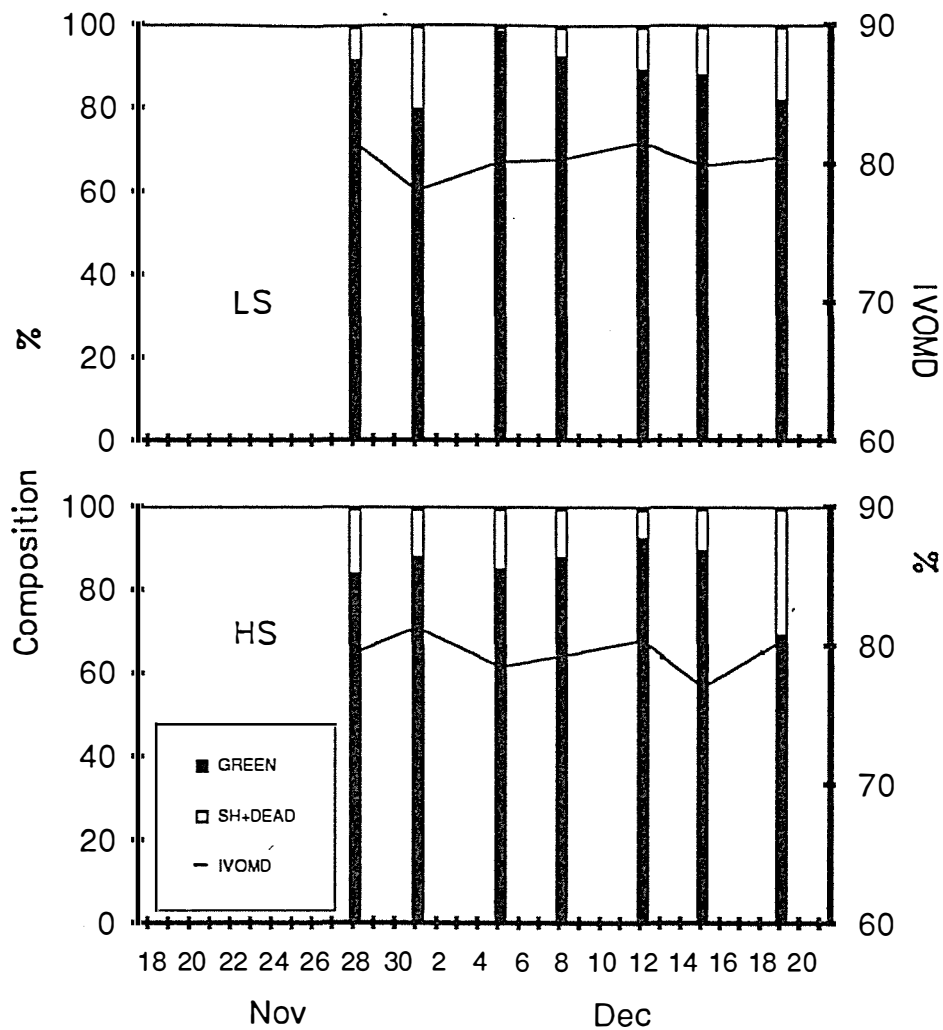
Figure 5.4 Changes in composition and in vitro organic matter digestibility (IVOMD) of the oesophageal extrusa of the animals grazing the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections during Trial 1.

(Composition; green grass (GREEN), seed head and dead material (SH+DEAD)

Table 5.4 Mean values of composition and in vitro organic matter digestibility (IVOMD) of the oesophageal extrusa (n=7) of animals grazing the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections during Trial 1.

	Selection		Effect of selection	
	LS	HS		S.E.D.
Composition (DM%):				
Green material	88.9	85.3	ns	3.70
Seed head and dead material	11.1	14.7	ns	3.70
IVOMD (%)	80.3	79.7	ns	0.75
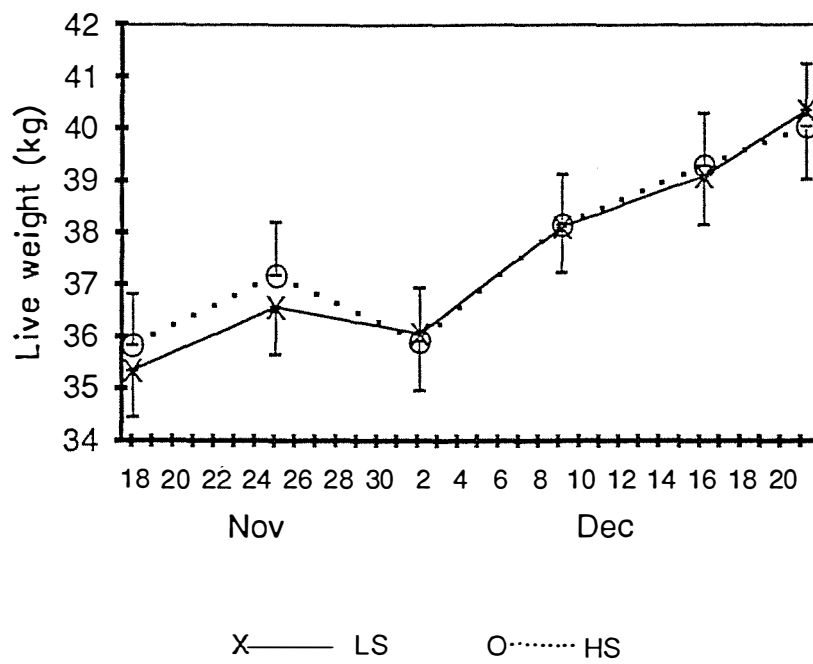
Figure 5.5 Changes in live weight of the lambs (n=23) grazing the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections during Trial 1.

Table 5.5 Mean values of live weight gain and total fleece wool growth of lambs (n=23) grazing the low (LS)and high (HS) leaf shear breaking load perennial ryegrass selections during Trial 1.

	Selection		Effect of selection	
	LS	HS		S.E.D.
Live weight gain (g/day)	138	127	ns	10.0
Total fleece wool growth (g/day):				
Greasy	14.2	13.7	ns	0.67
Scoured	10.3	10.6	ns	0.53

Table 5.6 Rumen pool size, rumen digesta fractional outflow rate (FOR) and rumen digesta mean retention time (MRT) for liquid-phase and particulate-phase in wethers (n=11) grazing the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections.

	Selection		Effect of selection	
	LS	HS		S.E.D.
Rumen pool size (g):				
Total	3978	3805	ns	99.4
Dry matter	413.0	365.6	ns (p=0.07)	19.85
FOR (%/h):				
Liquid-phase	12.3	13.7	*	0.50
Particulate-phase	8.1	7.9	ns	0.51
MRT (h):				
Liquid-phase	8.4	7.6	ns	0.36
Particulate-phase	12.9	13.0	ns	0.90
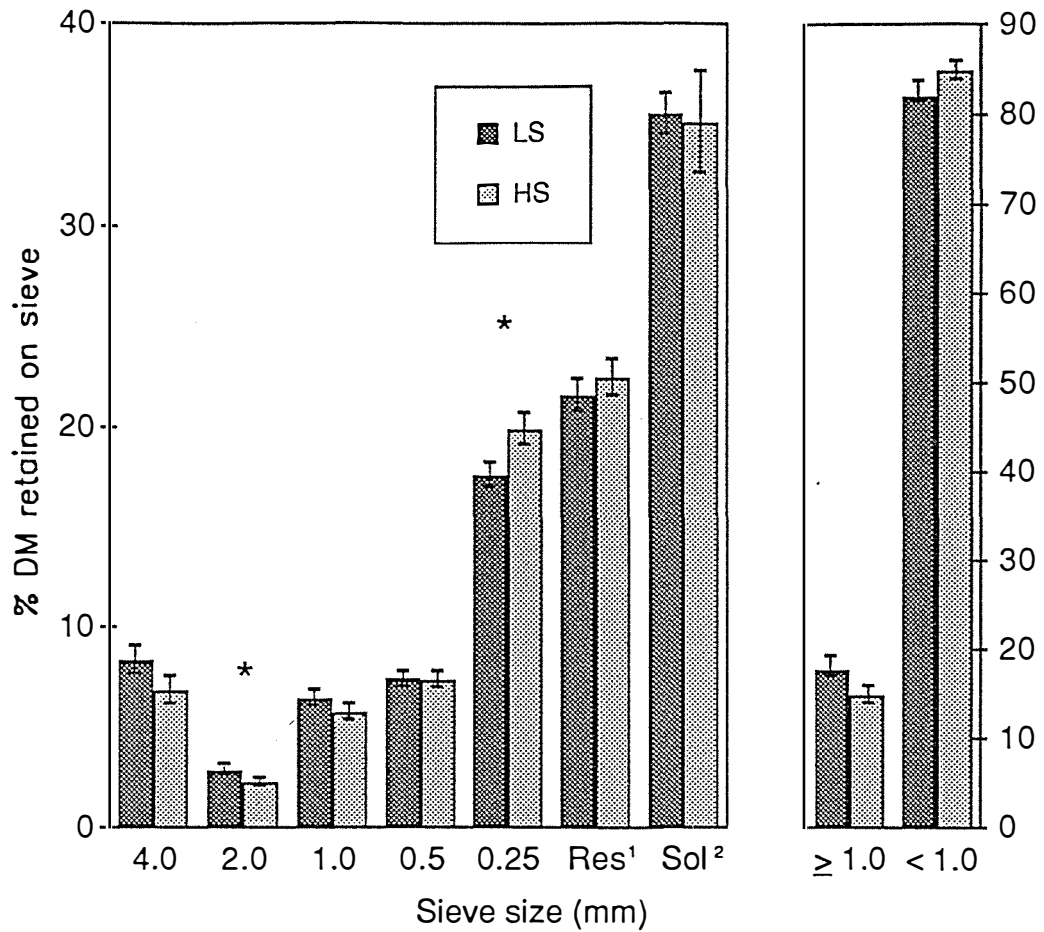
Figure 5.6 Particle size distribution of rumen contents of wethers (n=11) grazing the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections.

1: Residues.

2: Soluble DM defined as the DM remaining in solution after centrifugation at 850 G for 20 minutes.

5.3.2 Trial 2

5.3.2.1 Pasture

Changes in pasture mass and IVOMD of the LS and HS PRG selections during the trial are shown in Figure 5.7. Changes in LSBL and leaf length:dry weight ratio of the two types of PRG selections during the trial are shown in Figure 5.8. Changes in IML of the two types of PRG selections during the trial are shown in Figure 5.9. The mean values of above measures are shown in Table 5.7. There was no significant difference between the LS and HS PRG selections in mean pasture mass and IVOMD during the trial. The LS PRG selection had approximately 31 % lower (p<0.05) LSBL and approximately 37 % higher (p<0.05) leaf length:dry weight ratio than the HS herbage. However, there were no significant differences between the two types of PRG selections in mean IML during the trial. Changes in composition and IVOMD of OE of animals grazing LS and HS PRG selections during the trial are shown in Figure 5.10 and mean values are shown in Table 5.8. There were no significant differences between the OE of the animals grazing the two types PRG selections in composition and in IVOMD. Although both OE contained higher proportion of seed head and dead material than in Trial 1, the IVOMD was over 80 % for both PRG selections.

5.3.2.2 Live weight gain and voluntary organic matter intake in ewe-hoggets grazing the low and high leaf shear breaking load perennial ryegrass selections

Changes in live weight, 24-hour fasted weight and daily VOMI of the ewe-hoggets grazing the LS and HS PRG selections during the trial are shown in Figure 5.11. Mean values of live weight gain based on 24-fasted weight and VOMI estimated based on the Cr_2O_3 of the ewe-hoggets grazing the LS and HS PRG selections during the trial are shown in Table 5.9. There were no significant differences in live weight and in 24-fasted weight between the two groups of ewe-hoggets grazing the LS and HS PRG selections at any time of the trial period (Fig 5.11).

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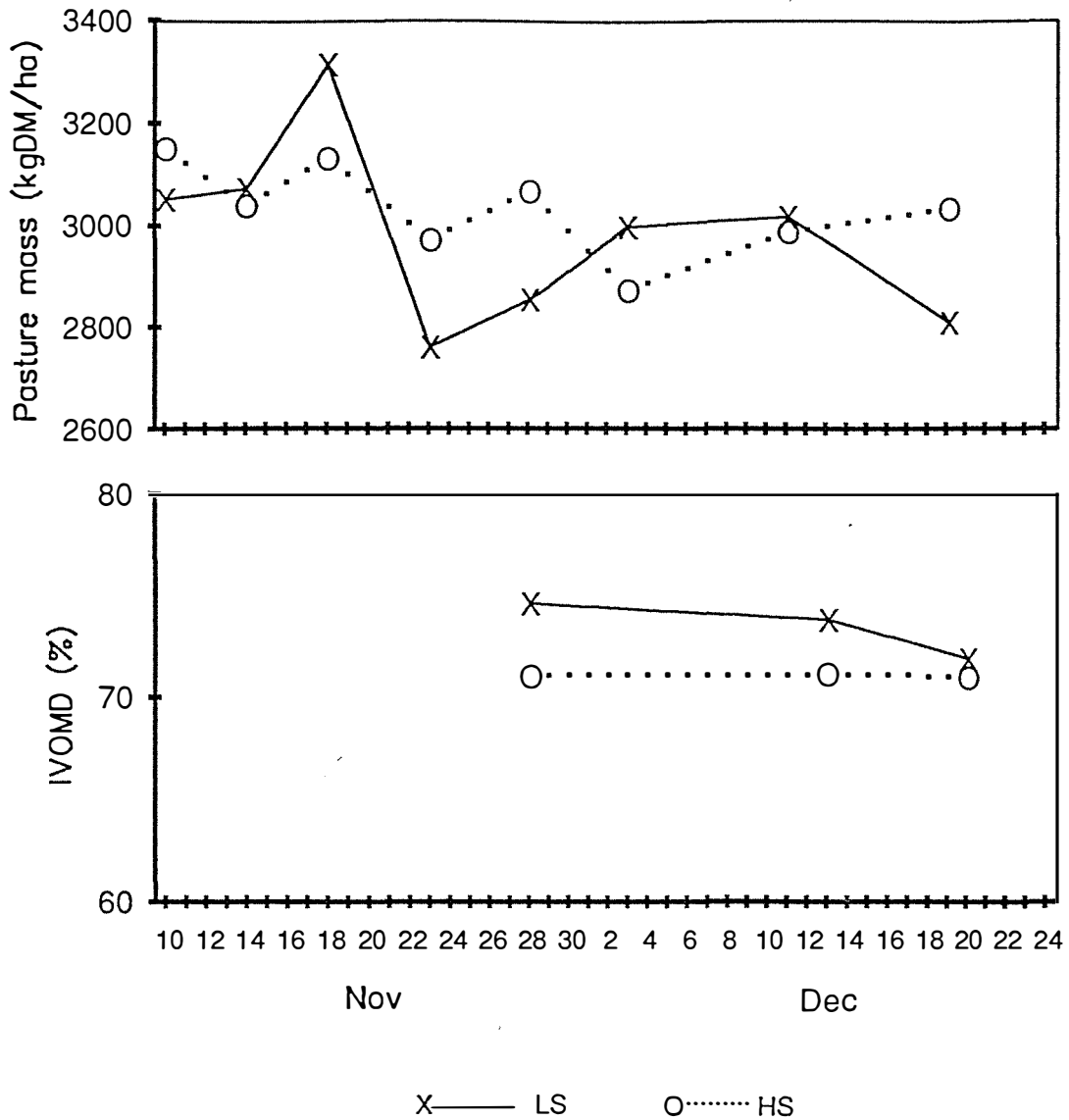
Figure 5.7 Changes in pasture mass and in vitro organic matter digestibility (IVOMD) of the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections during Trial 2.
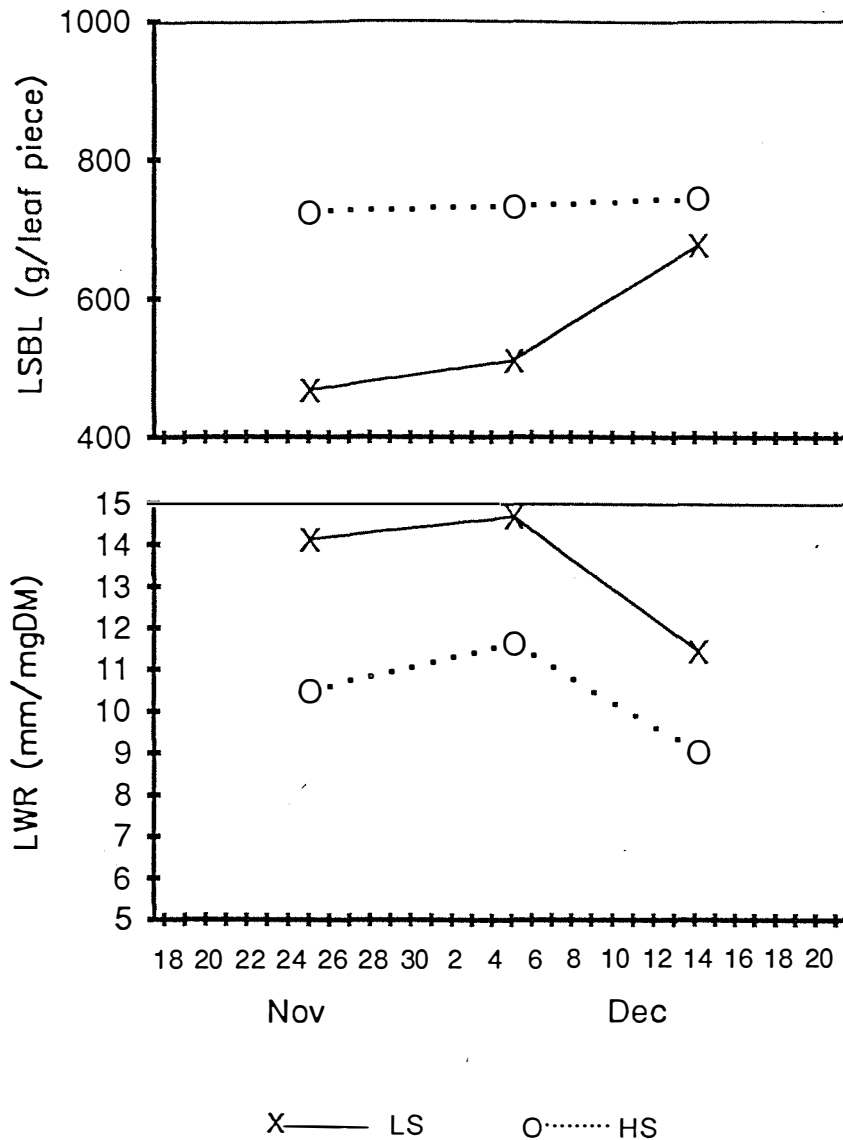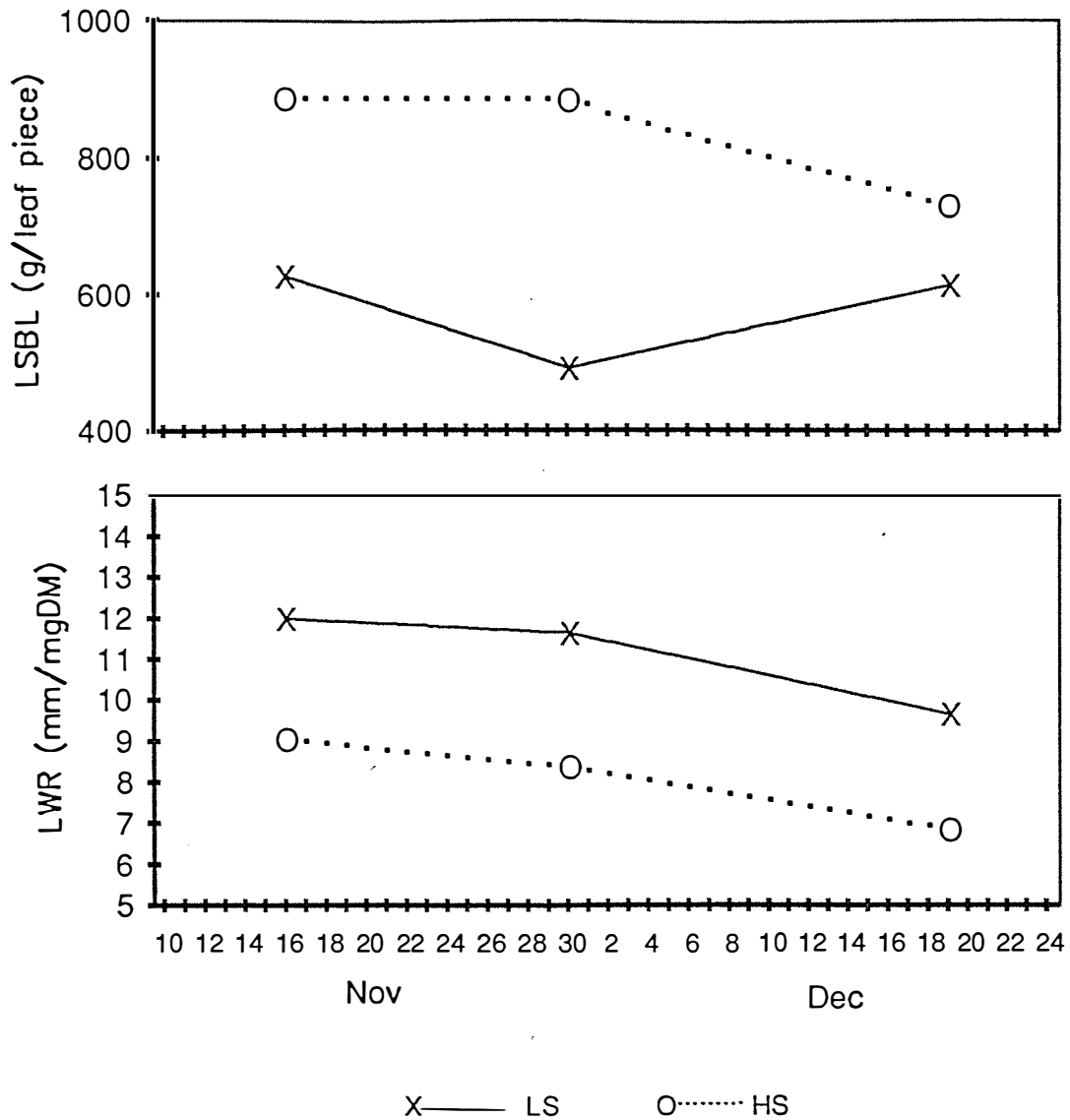
Figure 5.8 Changes in leaf shear breaking load (LSBL) and leaf length:dry weight ratio (LWR) of the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections during Trial 2.
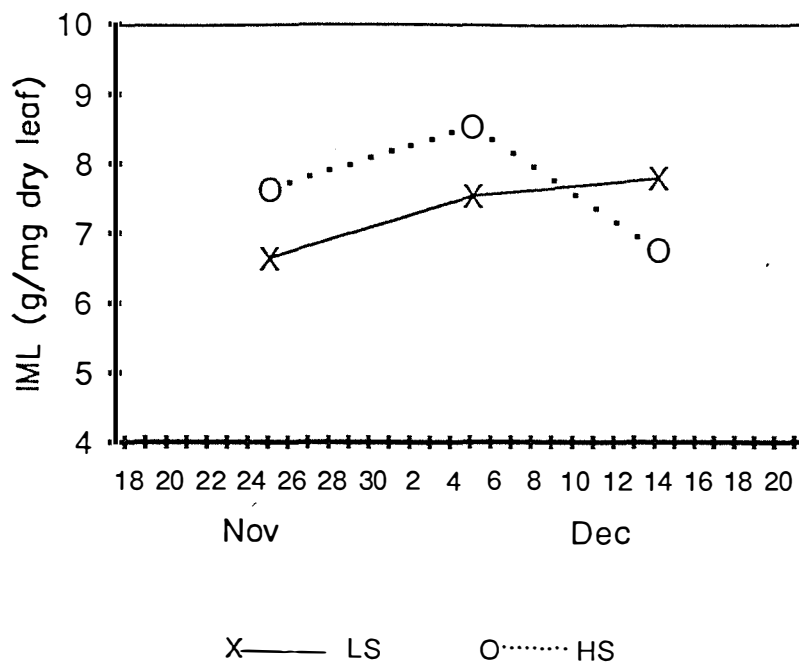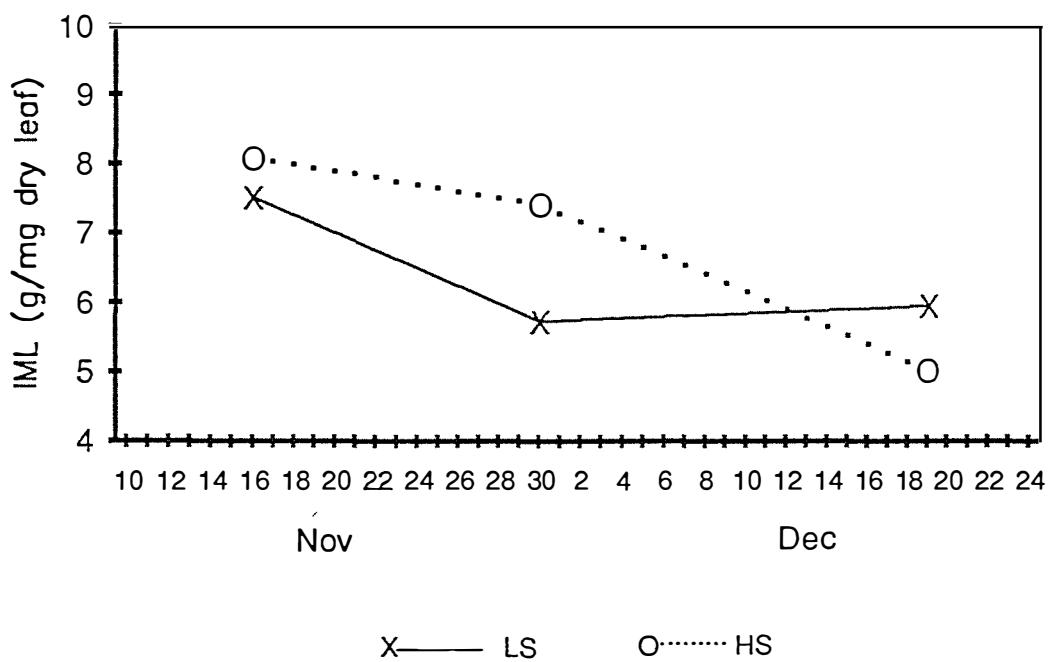
Figure 5.9 Changes in index of masticatory load (IML) of the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections during Trial 2.

Table 5.7 Mean values of pasture mass (n=8), in vitro organic matter digestibility (IVOMD) (n=3), leaf shear breaking load (n=3), leaf length:dry weight ratio (n=3) and index of masticatory load of leaf (n=3) of the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections on offer during Trial 2.

|	Selection		Effect of selection	S.E.D.
	LS	HS		
Pasture mass (kgDM/ha)	2987	3034	ns	70.0
IVDMD (%)	71.2	73.5	ns	0.81
Leaf shear breaking load (g/leaf)	578.7	837.3	*	67.62
Leaf length:dry weight ratio (mm/mgDM)	11.1	8.1	*	0.97
Index of masticatory load (g/mgDM leaf)	6.42	6.86	ns	1.087
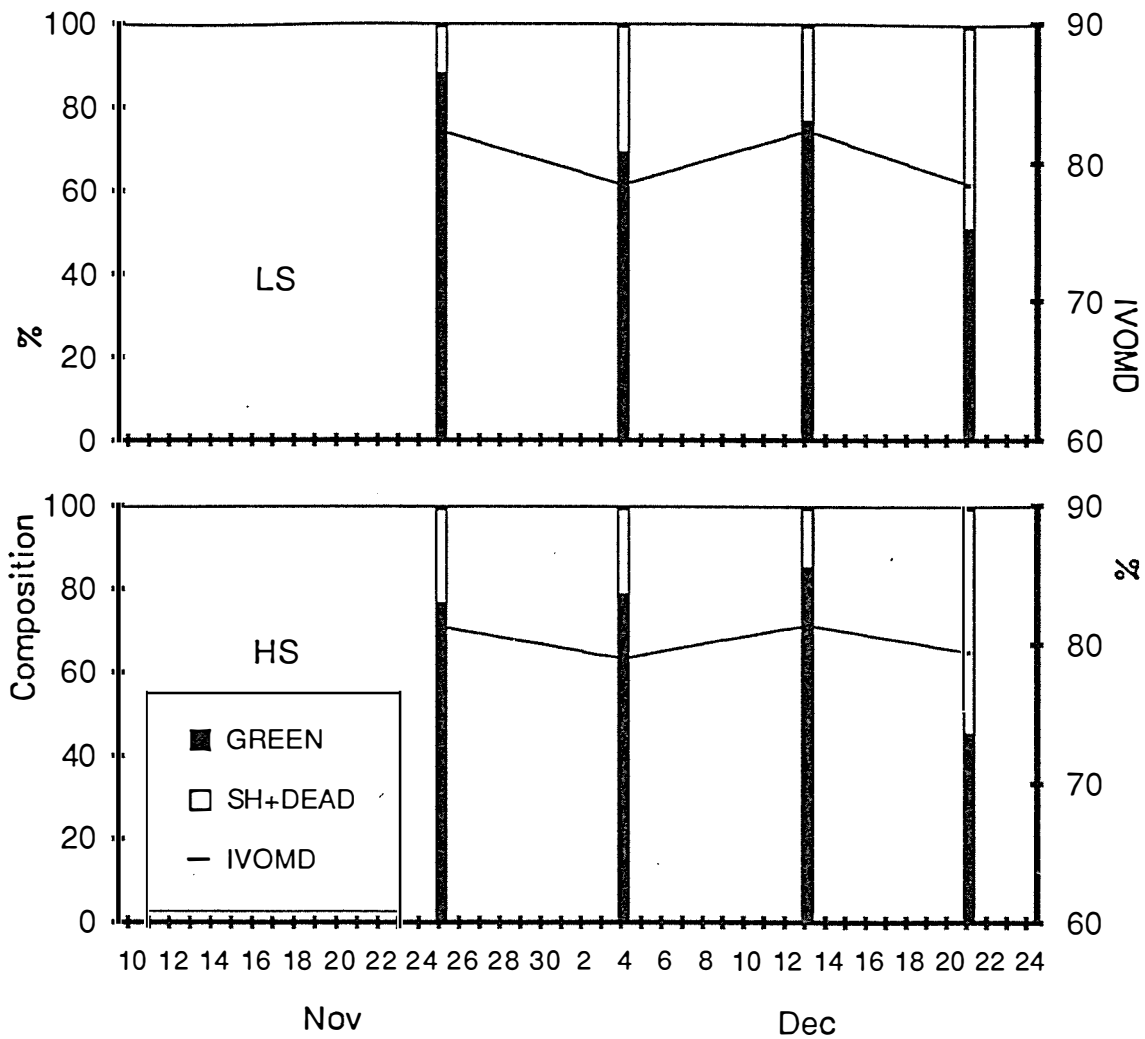
Figure 5.10 Changes in composition and in vitro organic matter digestibility (IVOMD) of the oesophageal extrusa of the animals grazing the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections during Trial 2.

(Composition; green grass (GREEN), seed head and dead material (SH+DEAD)

Table 5.8 Mean values of composition and in vitro organic matter digestibility (IVOMD) of the oesophageal extrusa (n=4) of the animals grazing the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections during Trial 2.

	Selection		Effect of selection	
	LS	HS		S.E.D.
Composition (DM%):				
Green material	71.4	71.7	ns	11.84
Seed head and dead material	28.6	28.3	ns	11.84
IVOMD (%)	80.5	80.4	ns	1.28
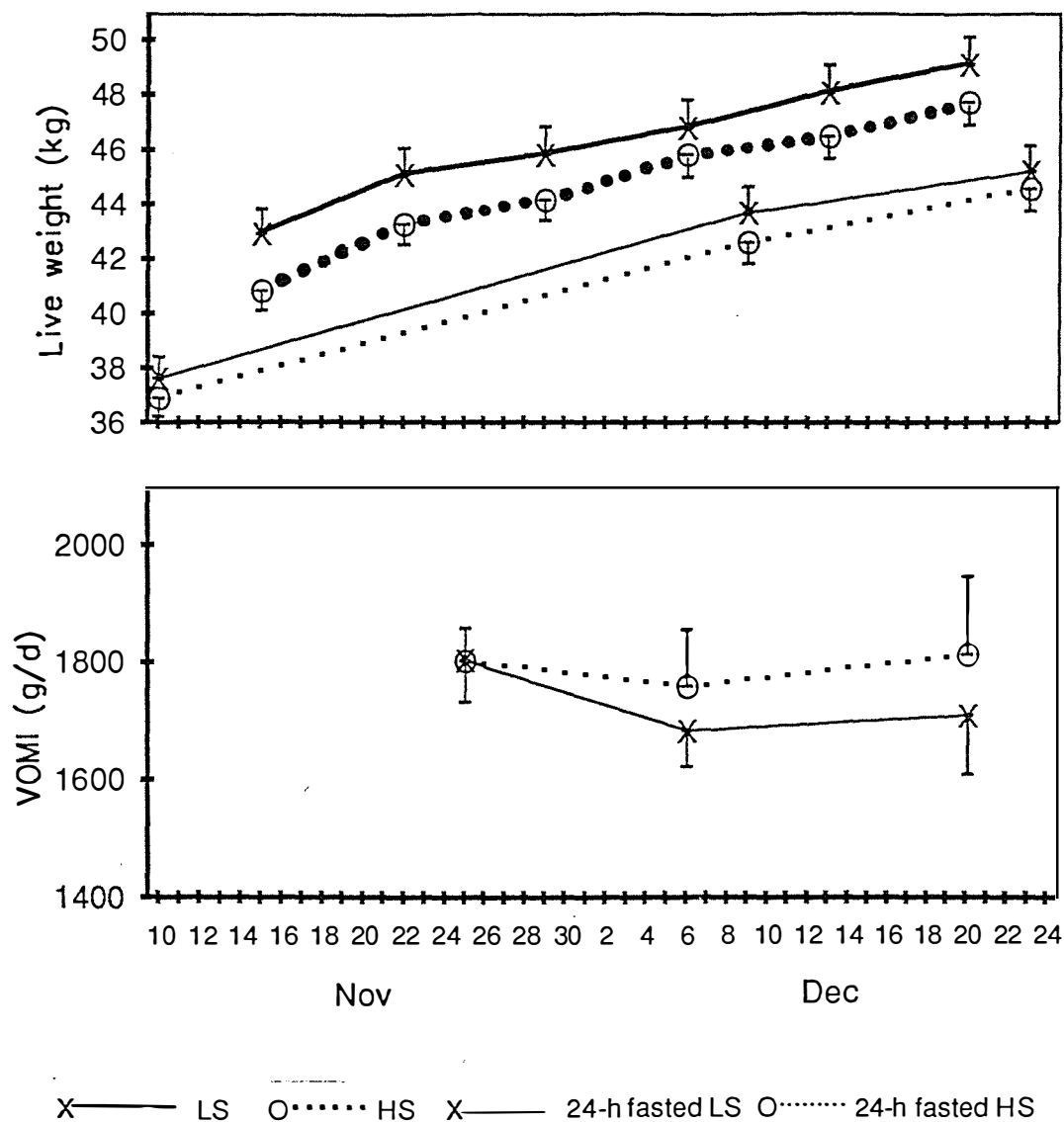
Figure 5.11 Changes in live weight, 24-hour fasted weight and voluntary organic matter intake (VOMI) of the ewe-hoggets grazing the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections during Trial 2.

(n=20 for LS and n=16 for HS in live weight, n=12 in VOMI).

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Table 5.9 Mean values of live weight gain and voluntary organic matter intake (VOMI) of ewe-hoggets grazing the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections during Trial 2.

	Selection		Effect of selection	S.E.D.
	LS	HS		
Live weight gain[1] (g/day)	176 (n=20)	178 (n=16)	ns	11.55
VOMI[2] (g/d)	1735 (n=12)	1795 (n=12)	ns	81.8

1: Based on 24-hour starved weight.
2: Estimated based on Cr_2O_3.

Mean daily live weight gain based on 24-hour fasted weight was similar between the two groups of animals (Table 5.9). Mean daily live weight gain based on non-fasted weight was 176 and 196 g (s.e.d. ±15.2, ns) for the animals grazing the LS and HS PRG selections, respectively. In VOMI, no significant differences occurred at any time during the trial between animals grazing the LS and HS PRG selections (Fig. 5.11) and mean daily VOMI was similar between the two groups of animals (Table 5.9).

5.3.2.3 Rumen pool size, digesta fractional outflow rates, digesta mean retention times and particle size distributions in rumen contents in wethers grazing the low and high leaf shear breaking load perennial ryegrass selections

Rumen pool size, FOR and MRT of liquid-phase and particulate-phase of wethers grazing the LS and HS PRG selections are shown in Table 5.10. The wethers grazing the LS PRG selection had approximately 14 and 16 % lower total rumen pool size and dry matter rumen pool size, respectively than the wethers grazing the HS PRG selection. These differences, however, were not statistically significant ($p>0.10$). There were no significant differences in FOR and MRT between the two groups of wethers grazing the LS and HS PRG selections.

Table 5.11 shows VOMI based on the Cr_2O_3 and Ru-Co dual-phase marker. There were no significant differences in VOMI between the animals grazing the LS and HS PRG selections based on any marker used in the trial. The estimation of VOMI was similar for all the markers used. Correlation coefficients among the VOMI based on three markers were 0.97 ($p<0.001$), 0.70 ($p<0.01$) and 0.62 ($p<0.05$) for VOMI based on Ru and Co, on Ru and Cr and on Co and Cr, respectively. The variability of estimate of VOMI based on Ru marker was the least among the three types of markers.

Particle size distribution of rumen contents of wethers grazing the LS and HS PRG selections is shown in Figure 5.12. There were no significant differences in particle size distribution of the rumen contents between the two groups of animals.

Table 5.10 Rumen pool size, rumen digesta fractional outflow rate (FOR) and rumen digesta mean retention time (MRT) for liquid-phase and particulate-phase in wethers (n=8) grazing the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections.

	Selection		Effect of selection	
	LS	HS		S.E.D.
Rumen pool size (g):				
Total	4330	5053	ns	386.2
Dry matter	453.7	542.2	ns	47.87
FOR (%/h):				
Liquid-phase	9.7	10.4	ns	0.63
Particulate-phase	8.6	7.7	ns	0.74
MRT (h):				
Liquid-phase	11.9	11.3	ns	1.11
Particulate-phase	13.4	15.0	ns	1.61

Table 5.11 Voluntary organic matter intake (VOMI) estimated based the Cr faecal marker and Ru-Co dual-phase ruminal marker of wethers (n=8) grazing the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections.

	Selection		Effect of selection	
	LS	HS		S.E.D.
VOMI (g/day) based on:				
Cr	1840	1862	ns	156.8
Ru	1777	1860	ns	94.1
Co	1772	1852	ns	154.5
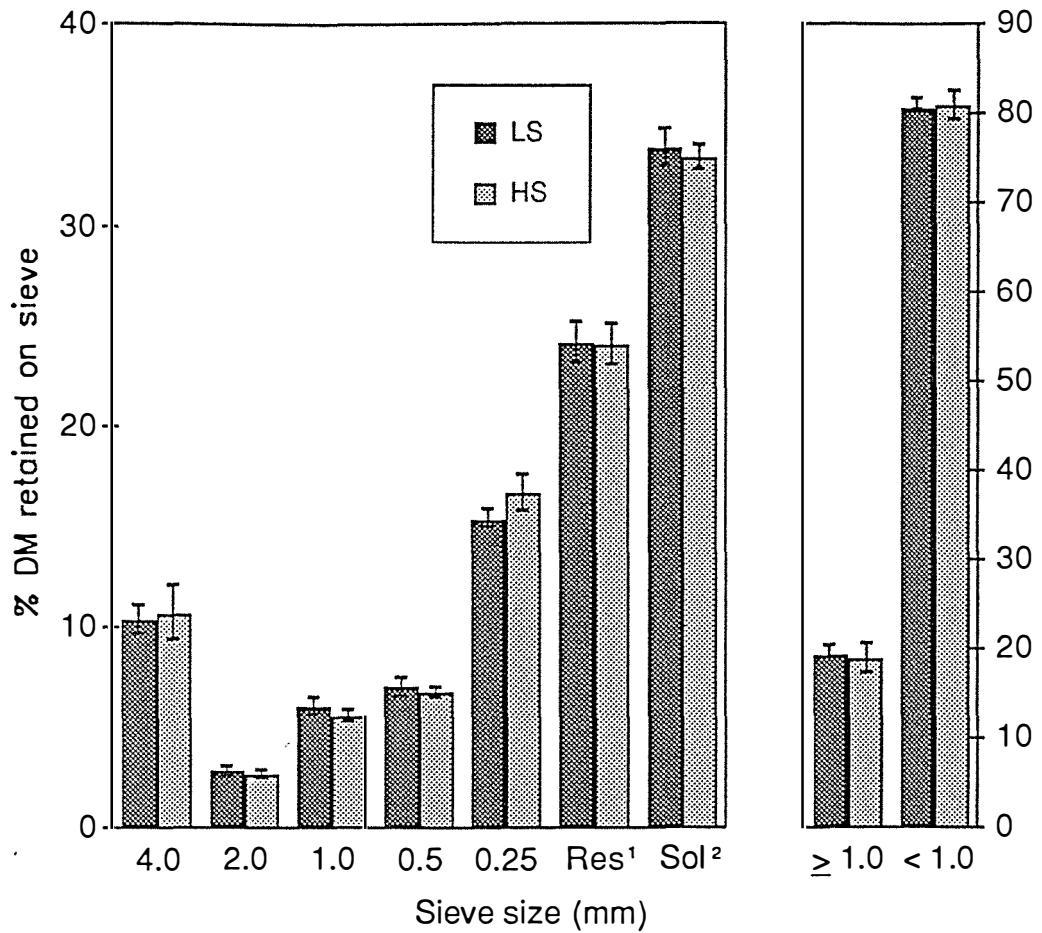
Figure 5.12 Particle size distribution of rumen contents of wethers (n=8) grazing the low (LS) and high (HS) leaf shear breaking load perennial ryegrass selections.

1: Residues.

2: Soluble DM defined as the DM remaining in solution after centrifugation at 850 G for 20 minutes.

5.4 Discussion

In the present study, there was no evidence of improved FV of PRG due to reduced LSBL. Live weight gain of lambs and ewe-hoggets were similar between the groups of animals grazing the LS and HS PRG selections, although the LS PRG selection had 24 - 31% lower LSBL than the HS PRG selection. There were no significant differences in wool growth between the two groups of animals. Table 5.12 compares FVs as live weight gain of animals grazing the LS and HS PGR selections in the previous studies (Kolver, 1989; Brookes *et al.*, personal communication) and in the present study. Kolver (1989) found that animals grazing the LS PRG selection grew at a significantly (p<0.01) faster rate than animals grazing the HS PRG selection, when the LS PRG selection had 24 % lower LSBL than the HS PRG selection (Table 5.12). He suggested that increased live weight gain produced from the LS PRG selection was due to increased voluntary feed intake, although he did not measure the voluntary feed intake. However, there was no significant difference in VOMI estimated in the ewe-hoggets in Trial 2. The VOMI estimated in the rumen-fistulated wethers in Trial 2 represented the mean VOMI of the wethers over the period of the PII and PIII (see 5.2.3.3). The mean VOMI of the ewe-hoggets over the period of the PII and PIII was calculated as 1692 and 1800 g/day, respectively, for the ewe-hoggets grazing the LS and HS PRG selections and difference between the treatments was not significant ($\pm$117 s.e.d.). This is consistent with the lack of difference in VOMI estimated in the rumen-fistulated wethers based on the same marker (Cr_2O_3). The estimation of VOMI for the rumen-fistulated wethers was similar for all the markers used. Therefore, it can be assumed that the FOR estimated in wethers can be applied to the ewe-hoggets. This suggests that reduced LSBL in PRG did not improve FOR, and, consequently, did not increase voluntary feed intake and, thus, did not improve live weight gain. The lack of significant difference in live weight gain, wool production and FOR in Trial 1 corresponds with the results in Trial 2 and support the rejection of the hypothesis (see section 5.1).

Table 5.12 Feeding value in live weight gain (LWG) and leaf shear breaking load (LSBL) for low (LS) and high (HS) perennial ryegrass selections in previous studies and in the present study.

Study	Year	Type of stock	LWG (g/day)		Effect of selection	LSBL (g/leaf)	
			LS	HS		LS	HS
Kolver (1989):							
	1989	Lamb	125	108	**	567	751
Present study:							
	1988	Lamb	138	127	ns	553	727
	1989	Ewe-hogget	176	178	ns	579	837
Brookes et al.[1]:							
	1991	Lactating ewe	-105	-81	ns	647	838
	(Winter)	Lamb	174	181	ns		
	1991	Lactating ewe	166	144	ns	747	809
	(Spring)	Lamb	199	226	ns		

1: unpublished data.

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Although IVOMD of OE was as high as 80 % for both PRG selections, considerable amounts of dead material and seed head were contained in the OE. In September 1989, Atrazine herbicide (Gesaprim 500FW, manufactured by Ciba-Geigy, New Zealand Ltd.) was applied to the paddocks for the annual weed control. The application of Atrazine herbicide suppressed the annual weed successfully but also damaged the ryegrass sward. This resulted in the increased proportion of the dead material and seed head in OE in Trial 2. Application of Tramat herbicide (manufactured by Schering AG., Germany) would be a better alternative for annual weed control in future trials, since this herbicide is very selective to weeds in ryegrass (O'Connor, 1990).

The lack of differences between the animals grazing the LS and HS herbage in the present study may be attributed to the lack of difference in IML between the two type of PRG selections. Although the difference between the LS and HS PRG selections in LSBL in the present study was similar to that in the Kolver's study (1989), there were no differences in IML between the two types of PRG selections in the present study. In Kolver's study (1989), IML was not available. The most recent study included two growth trials conducted in winter and spring 1991 (Table 5.12, Brookes *et al.*, unpublished). In the winter trial , there were no significant differences between the animals grazing the LS and HS PRG selections, although the LS PRG selection had approximately 23 % lower LSBL than the HS PRG selection (IML was not available). No differences between the animals grazing the two types of PRG selections were found during the spring trial again, but the LS PRG selection was only approximately 8 and 3 % lower than the HS PRG selection in LSBL and in IML, respectively. LSBL is one of the main factors to determine IML, however other attributes i.e. leaf morphology also affect IML. These other studies confirm that reduced LSBL *per se* will not improve FV of PRG.

5.5 Conclusion

Although the LS PRG selection had 25-31 % lower LSBL than the HS PRG selection, FV measured as live weight gain and wool production of sheep in the

two separate trials was not improved by reduced LSBL. FOR in rumen-fistulated wethers showed no indications of difference and VOMI was similar between the animals grazing the LS and HS PRG selections. The lack of difference in IML between the LS and HS PRG selection can be considered as a main reason for this. The recent study by Brookes *et al.*(unpublished) supports this clearly. LSBL is one of factors to determine IML, however, IML is not proportional to the LSBL because leaf morphology also affects IML. Therefore, the hypothesis, that reduced LSBL in PRG would improve its FV, was rejected. The next chapter summarizes the results discussed so far and also discusses the prospects for further trials aimed at increasing efficiency of breakdown by mastication, hence, increasing FV of PRG.

Chapter Six

GENERAL DISCUSSION AND CONCLUSION

6.1 Introduction

Reducing physical resistance has been proposed as a key factor to increase efficiency of masticatory breakdown of forage (Bailey, 1964; Black *et al.*, 1982; Waghorn *et al.*, 1989; McLeod *et al.*, 1989; Hodgson 1990), which may lead to faster rumen fractional outflow rates (FOR) and consequently to increased voluntary feed intake and hence improved feeding value (FV). However, the series of experiments conducted in the present investigation did not demonstrate improved FV in sheep by reducing leaf shear breaking load (LSBL) in perennial ryegrass (PRG). In this chapter, the relationships between LSBL and index of masticatory load (IML), limitations of effect of reduced LSBL in PRG on improving FV and influence of IML on particle breakdown are summarized and the directions for further research are discussed.

6.2 Relationships between leaf shear breaking load and index of masticatory load of leaf in perennial ryegrass

Changes in LSBL for the low leaf shear breaking load (LS) and the high leaf shear breaking load (HS) PRG selections in the field during a complete year (April 1989 - March 1990) have been observed and are shown in Figure 6.1. The LS PRG selection had a consistently lower LSBL than the HS PRG selection throughout the year. LSBL for both PRG selections tended to be higher in spring and lower in winter. Similar changes in leaf tensile strength in PRG were observed by Evans (1964a). Averaged over the year, the LS PRG selection was approximately 21 % lower (P<0.01) than the HS PRG selection (586.2 vs 741.2 g/leaf, ±41.21 s.e.d. in LSBL). Differences between the PRG selection lines in physical properties of the leaves did not result in any apparent differences in sward growth characteristics or persistency throughout the year.
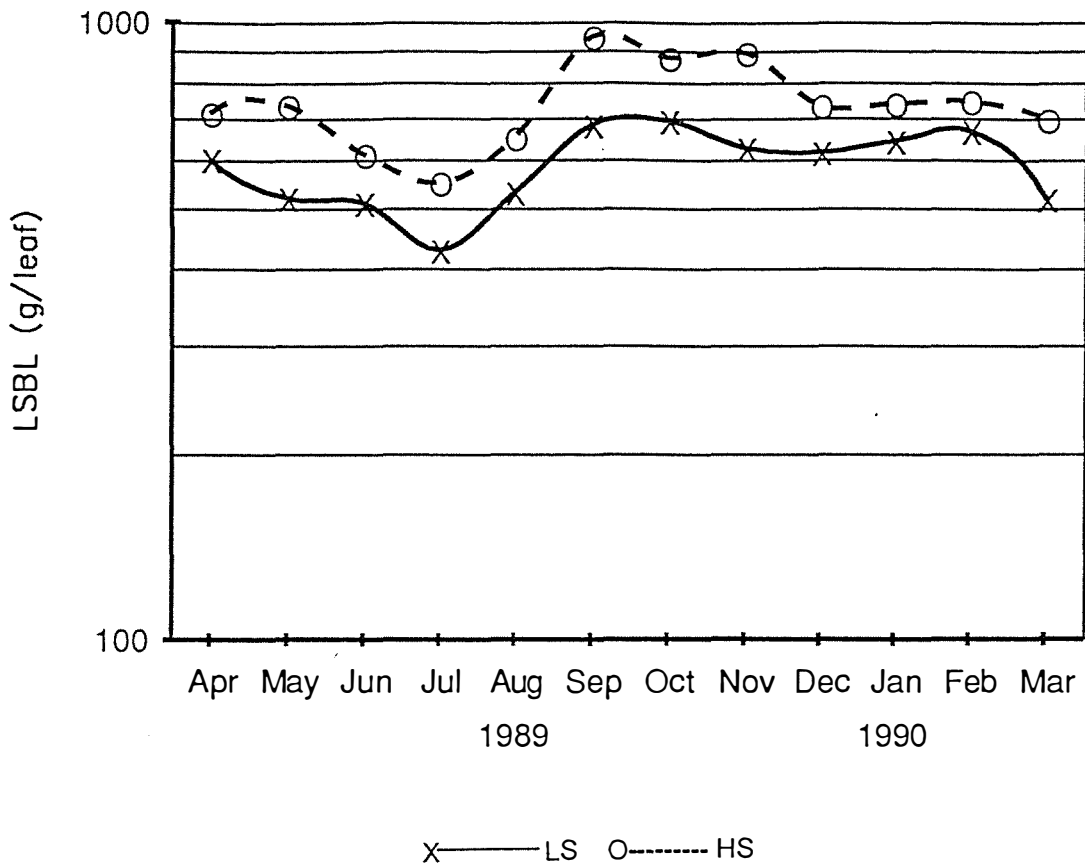
Figure 6.1 Changes in leaf shear breaking load (LSBL) of low leaf shear breaking load (LS) and high leaf shear breaking load (HS) perennial ryegrass selections in the field during a year (April 1989 - March 1990).

(LSBL is expressed in logarithmic scale)

IML is a measure of the total load to break a unit dry weight of PRG leaves into particles small enough to have a high probability of leaving the rumen. IML is a function of LSBL and the leaf length:dry weight ratio (LWR). Table 6.1 summarizes those values in the present investigation. The LS PRG selection had approximately 41 % lower LSBL, 26 % higher leaf length:dry weight ratio (LWR) thus, 27 % lower IML than the HS PRG selection when grown under optimum climatic conditions. However, this selection effect on IML decreased under field conditions, as the LS PRG selection had only 1 - 12 % lower IML than the HS PRG selection. This is due to the decreased selection effect on LSBL and the increased effect on LWR. Reasons for these alterations of selection effect in PRG under field conditions are unknown, although cross-pollination between the two PRG selections may account for part of the effect.

6.3   Limitation of reducing leaf shear breaking load in perennial ryegrass on improving feeding value to sheep

The effect of reduced LSBL in PRG on masticatory behaviour, rumen pool size, rumen FOR and FV are summarized in Table 6.2 and Table 6.3. Efficiency of particle breakdown during eating for the LS PRG selection tended to be higher than for the HS PRG selection. This may lead to the higher ingestion rate for the LS PRG selection than for the HS PRG selection because mastication during eating appears to be continued until similar particle size distribution was achieved for the two PRG selections. Higher ingestion rate for the LS PRG selection than for the HS PRG selection has been observed by MacKinnon *et al.* (1988). However, the efficiency of particle breakdown during rumination was similar for the two PRG selections. Thus, even if the LS PRG selection was ingested faster than the HS PRG selection by animals, ruminal particle breakdown was similar for the two PRG selections (Table 6.2). Consequently, FOR, feed intake and FV were also similar (Table 6.3).

Table 6.1 Leaf shear breaking load (LSBL), index of masticatory load (IML) of leaf and leaf length-width ratio (LWR) of the low leaf shear breaking load perennial ryegrass selection expressed relative to the high leaf shear breaking load perennial ryegrass selection (as 100) in the present investigation.

	LSBL	LWR	IML
Grown under optimum indoor conditions (Chapter 2)	59	126	73
Mastication trial (Chapter 3)	71	139	99
Indoor feeding trial (Chapter 4)	61	146	88
Grazing trial: (Chapter 5)			
1988	75	130	96
1989	69	137	94

Table 6.2 Summary of effect of the reduced leaf shear breaking load (LSBL) in perennial ryegrass on masticatory behaviour during eating in sheep.

	LSBL	
	Low	High
(Chapter 3):		
Chewing rate:		
(chews/min)	140.3	148.9
(chews/gDM ingested)	39.6	48.6
Ingestion rate:		
(gDM/min)	4.27	3.41
(mgDM/chew)	31.3	23.3
MacKinnon *et al.*, (1988):		
Ingestion rate (gDM/min)	7.6	6.5

Table 6.3 Summary of effect of reduced leaf shear breaking load (LSBL) on rumen pool sizes, fractional outflow rates (FOR), voluntary organic matter intake (VOMI) and feeding values in sheep.

	LSBL	
	Low	High
Rumen pool size (g):		
Indoor feeding trial (Chapter 4):		
Total	5805	5005
DM	567.2	504.2
Grazing trial (1988) (Chapter 5):		
Total	3978	3805
DM	413.0	365.6
Grazing trial (1989) (Chapter 5):		
Total	4330	5053
DM	453.7	542.2
FOR (%/h):		
Indoor feeding trial (Chapter 4):		
Liquid	11.2	11.8
Particulate	6.4	6.6
Grazing trial (1988) (Chapter 5):		
Liquid	12.3	13.7
Particulate	8.1	7.9
Grazing trial (1989) (Chapter 5):		
Liquid	9.7	10.4
Particulate	8.6	7.7
VOMI (g/day) (Chapter 5):		
Grazing trial (1989)	1735	1795
Feeding value (Chapter 5):		
Live weight gain (g/day):		
Grazing trial (1988)[1]	138	127
Grazing trial (1989)[2]	176	178
Fleece wool growth (g/day)[1]:		
Greasy	14.2	13.7
Scoured	10.3	10.6

1: Lambs.
2: Ewe-hoggets.

6.4 Influence of index of masticatory load on particle breakdown and masticatory behaviour in sheep

The lack of selection effect of the two PRG selections on IML may account for the lack of significant differences between the two PRG selections in efficiency of particle breakdown. Although a relatively large selection effect on IML (12 %) was recorded in the indoor feeding trial (Chapter 4), there was no difference in FOR between the two PRG selections. However, this could mainly be attributed to the considerably low feed intake. The relationship between physical resistance of PRG leaves to breakdown and theoretical efficiency of chewing <TEC> is given by;

$$\text{<TEC> (mgDM broken into 1 mm particles /chew)} = f \times \frac{1}{\text{IML}}$$

and,

$$\text{<TEC> (mgDM broken into 1 mm particles / min)} = f \times k \times \frac{1}{\text{IML}}$$

where,

f (kg) = mean bite force per chew at chewing rate of k (chews/min).

Therefore, IML is a determining factor of the efficiency of particle breakdown. A previous study (John et al., 1989) showed that a difference in LSBL between the LS and HS PRG selection was maintained after incubation in the rumen of cows for at least 24 hours. This suggests that IML influences masticatory efficiency during rumination as well as during ingestion for at least 24 hours after ingested PRG. Thus, the higher ingestion rates for LS PRG selection than the HS PRG selection (Mastication trial; Chapter 3) may be due to narrower leaf width, which required fewer chews to cut in a direction parallel to the sclerenchyma fibres. There was similar masticatory behaviour for the two PRG selections during

rumination, because both PRG selections became similar in morphology after the mastication during eating. Lower digestibilities for the LS PRG selection than for the HS PRG selection (indoor feeding trial; Chapter 4) supports this interpretation because the LS PRG leaves must have had fewer chewed areas which allow microorganisms to enter into the plant tissues.

6.5 Direction of further studies

IML appears to be a determining factor of efficiency of particle breakdown. IML is determined by the two factors, namely, LSBL and LWR. Thus, selection for lower IML can be approached by either selecting for lower LSBL whilst keeping LWR constant, or for lower LWR whilst keeping LSBL constant. Selection for the LS and HS PRG was originally made based on the LSBL, however, LWR was not held constant. The lower LSBL can be achieved by reducing sclerenchyma tissues in leaf cross-section since sclerenchyma fibres account for the most of physical resistance to breakdown (Vincent, 1982; Betteridge *et al.*, 1986). On the other hand, the lower LWR can be achieved by selecting for the wider and/or thicker leaves. This suggests that the lower IML PRG leaves will be thicker and/or wider but having less sclerenchyma tissues in leaf cross-section. Wider leaves may have increased apparent digestibility but may have decreased ingestion rate as chews/gDM may increase.

IML, a model of work load, developed in the present investigation does not account for the leaf breaking load parallel to the sclerenchyma fibres. Thus, it is desirable to develop a further model to describe the work load, since the lower IML PRG leaves are expected to have wider/thicker leaves. It is also necessary to investigate the relationship between bite force and chewing rate. Electromyography may be a useful method in this future investigation.

In the present investigation, shear was used as a physical parameter of force under the assumption that sheep mainly exert this type of force to break grass. However, further studies are needed to investigate the sheep's chewing pattern in detail to

describe more accurate models of chewing. Also, Warner-Bratzler (WB) Meat Shear Apparatus and Instron Food Testing Instrument fitted with WB type blades were used to measure LSBL in the present investigation. LSBL measured by these instruments probably confound shear and tension (Pool *et al.*, 1969; Voisey, 1974, 1976). This may need to be modified according to the chewing model.

6.7 Conclusion

The primary hypothesis was that reducing LSBL in PRG should increase the efficiency of masticatory particle breakdown both during eating and during ruminating, and hence, improve rumen FOR and consequently lead to higher voluntary intake and as a result, achieve improved FV. The LS PRG selection was 25 - 41 % lower than the HS PRG selection in LSBL. However, in IML the selection effect decreased to 1 - 27 % due to the morphological differences between the two PRG selections. In the present investigation, there were no obvious effects of reduced LSBL in PRG on efficiency of masticatory particle breakdown, and consequently, FOR, feed intake and hence FV in sheep. This is due to the lack of selection effect of PRG in IML. IML is a determining factor for the efficiency of mastication both during eating and ruminating. Therefore, selection of PRG for a lower IML is necessary in order to increase efficiency of masticatory particle breakdown, FOR and hence FV of PRG. Lower IML can be achieved by selecting for lower LSBL whilst keeping LWR constant or vice-versa in future selections of PRG.

Appendix 2.1

Newcastle State University Phytotron Nutrient Solution

Name	g/l Stock sol.	Working sol.	
Solution A:			
Ammonium nitrate (NH_4NO_3)	80.05	NH_4-N N	28.0 28.0
Calcium nitrate ($Ca(NO_3)_2H_2O$)	159.25	Ca N	54.8 37.6
Sequestrene 330 (NaFe (7 %))	29.8	Fe	3.0
Solution B:			
Potassium phosphate (KH_2PO_4)	12.5	K P	7.2 5.6
Potassium phosphate (K_2HPO_4)	5.5	K P	5.0 2.0
Potassium nitrate (KNO_3)	63.9	K N	48.8 17.8
Magnesium sulphate ($MgSO_4 7H_2O$)	30.81	Mg S	6.2 8.2
Sodium sulphate (Na_2SO_4)	35.50	Na S	13.8 19.2
Zinc sulphate ($ZnSO_4 7H_2O$)	0.025	Zn S	0.012 0.070
Manganous chloride ($MnCl_2 4H_2O$)	0.26	Mn Cl	0.113 0.146
Copper sulphate ($CuSO_4 5H_2O$)	0.01	Cu S	0.005 0.003
Boric acid (H_3BO_3)	0.35	B	0.127
Molybdic acid ($MoO_3 2H_2O$)	0.002	Mo	0.002

Notes: Ammonium nitrate and sequestrene are combined in solution A, 200 g of potassium hydroxide is added to bring the pH to 8, then calcium nitrate is added dropping the pH to 7. The major salts of solution B are dissolved, then the minor salts in solution are added slowly with stirring, the final pH is 5.2. Stock solutions A and B are used 1 to 500 or 200 ml stock solutions per 50 l of water.

Front view   Side view
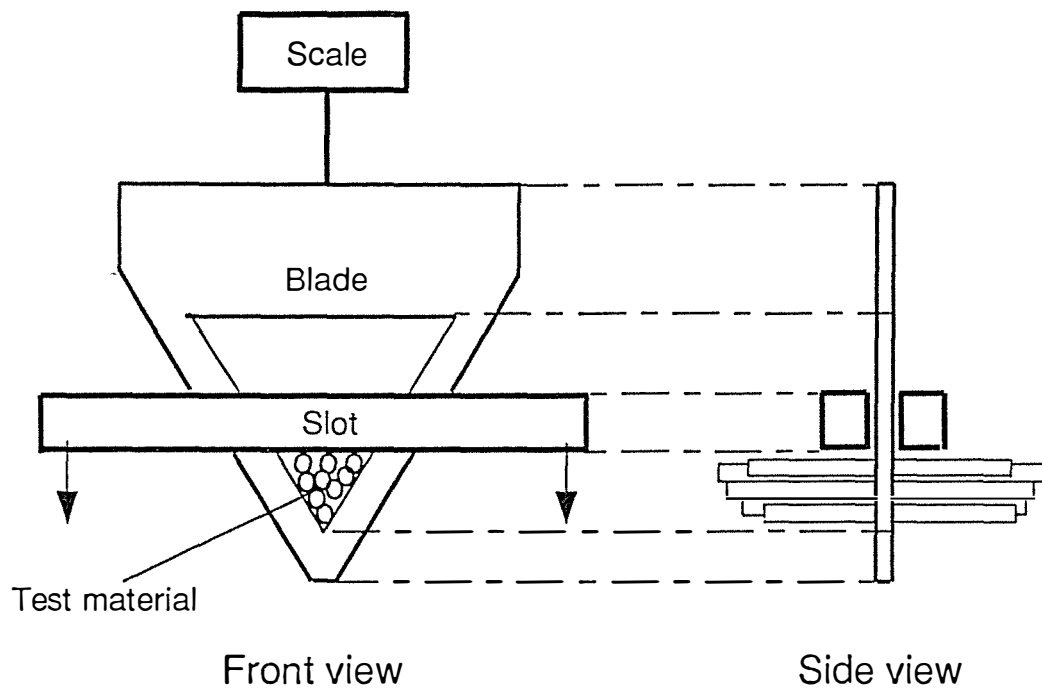
Movements of the blade and position of the slot of a Warner-Bratzler meat shear apparatus in shearing action.

(Arrows indicate directions of the movement)

Appendix 2.3

An example of table of analysis of variance for fresh weight of the leaves of the low and high shear breaking load perennial ryegrass selections.

Source	df	Sum of squares	Mean squares	F value	Probability
Selection	1	3.1333	3.1333	231.19	0.0001
Emergence nested in selection	4	5.5829	1.3957	102.99	0.0001
Aging nested in emergence level	6	0.0140	0.0023	0.17	0.9840
Replicates nested in aging level	225	3.0493	0.0136	Error term	
Tiller nested in replicate	1752	4.6646	0.0027		
Total	1988	16.4441			

Appendix 2.4

Concentrations (%DM) of organic matter, lignin and cell-wall constituents of the second-emerged (2nd), fourth-emerged (4th) and sixth-emerged (6th) leaves of the low (n=18) and high (n=18) leaf shear breaking load perennial ryegrass selections at maturation (M00), 10 days (M10) and 20 days (M20) after the maturation.

Selection	Leaf emergence	Aging level	Organic matter	Lignin	Cellulose	Hemi-cellulose
		M00	88.2[a]	2.83	17.4[a]	22.0[a]
	2nd	M10	86.7[b]	3.00	18.1[a]	22.8[a]
		M20	90.4[c]	3.53	25.3[b]	30.6[b]
		Aging effect[1]	*	ns	***	***
		M00	88.1[a]	2.97[ab]	18.9[a]	22.8[a]
LS	4th	M10	87.4[a]	2.13[b]	22.3[b]	24.0[a]
		M20	91.6[b]	3.45[ca]	28.5[c]	27.9[b]
		Aging effect[1]	***	*	**	***
		M00	86.5[a]	2.63	20.3[a]	23.3[a]
	6th	M10	85.4[a]	3.60	19.9[a]	22.7[a]
		M20	90.8[b]	3.11	27.7[b]	28.2[b]
		Aging effect[1]	***	ns	***	***
		M00	90.0[a]	1.65[a]	20.5[a]	23.9[a]
	2nd	M10	88.2[b]	1.83[ab]	20.0[a]	24.4[a]
		M20	89.2[ab]	2.88[b]	24.6[b]	30.6[b]
		Aging effect[1]	**	*	**	***
		M00	89.1[a]	1.99	21.6[a]	23.1[a]
HS	4th	M10	88.4[a]	2.55	22.6[a]	24.9[a]
		M20	90.5[b]	2.47	28.9[b]	31.2[b]
		Aging effect[1]	*	ns	***	*
		M00	88.2[a]	1.80[a]	22.2[a]	23.2[a]
	6th	M10	87.2[a]	2.04[a]	23.3[ab]	23.3[a]
		M20	90.3[b]	3.17[b]	25.5[b]	30.7[b]
		Aging effect[1]	**	*	*	**
		S.E.D.[2]	0.61	0.52	1.16	1.51
LS	pooled	pooled	88.3	3.03	22.1	26.1
HS	pooled	pooled	89.0	2.61	23.2	24.9
selection effect[3]			**	***	**	*
S.E.D.[3]			0.20	0.17	0.39	0.50

1: Leaves with different superscripts differ significantly within a leaf emergence.
2: Standard error of difference within a leaf emergence.
3: Standard error of difference between selections.

Appendix 3.1

An example of a table of analysis of variance for the proportion of particles larger than 1 mm in rumen contents recovered from wethers immediately after ingesting the low (LS) and high (HS) shear breaking load perennial ryegrass selections.

Sampling scheme;

	Block 1			Block 2	
	LS	HS		LS	HS
Day 1	A B seq1	C D seq2	Day 3	C D seq2	A B seq1
Day 2	E F seq2	G H seq1	Day 4	G H seq1	E F seq2

A.B.C.D.E.F.G.H: sheep

seq: sequence

Source	df	Sum of squares	Mean squares	F value	Probability
Block	1	75.55	75.55	3.00	0.1581
Sequence	1	10.26	10.26	0.41	0.5576
Date	2	12.13	6.07	0.24	0.7963
Selection	1	<0.00	<0.00	<0.00	0.9967
Selection x block	1	0.58	0.58	0.02	0.8862
Sheep	5	32.33	6.47	0.26	0.9156
Error	4	100.57	25.14		
Total	15	231.43			

Portable marker infusion backpack set
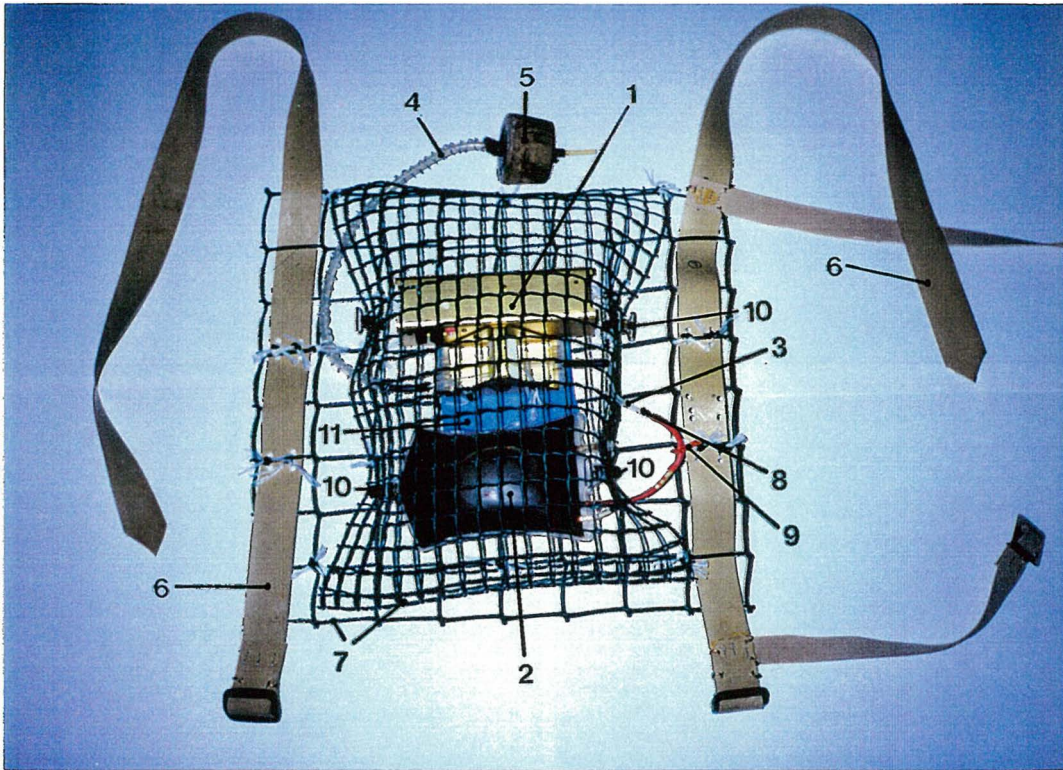
1: Peristaltic Universal Micro-metering Pump (manufactured by Everest Electronics Ltd., Australia).
2: Infusate in IV pack.
3: Silicone rubber tubing (3.35 mm id).
4: Protection silicone rubber tubing (4.80 mm id) and wire coil.
5: Cannulae plug.
6: Rubber webbing.
7: Garden mesh.
8: Joint (IV pack to the silicon rubber tubing).
9: Pinchcock.
10: Suspender clip.
11: Sponge pad.

Portable marker infusion set in operation
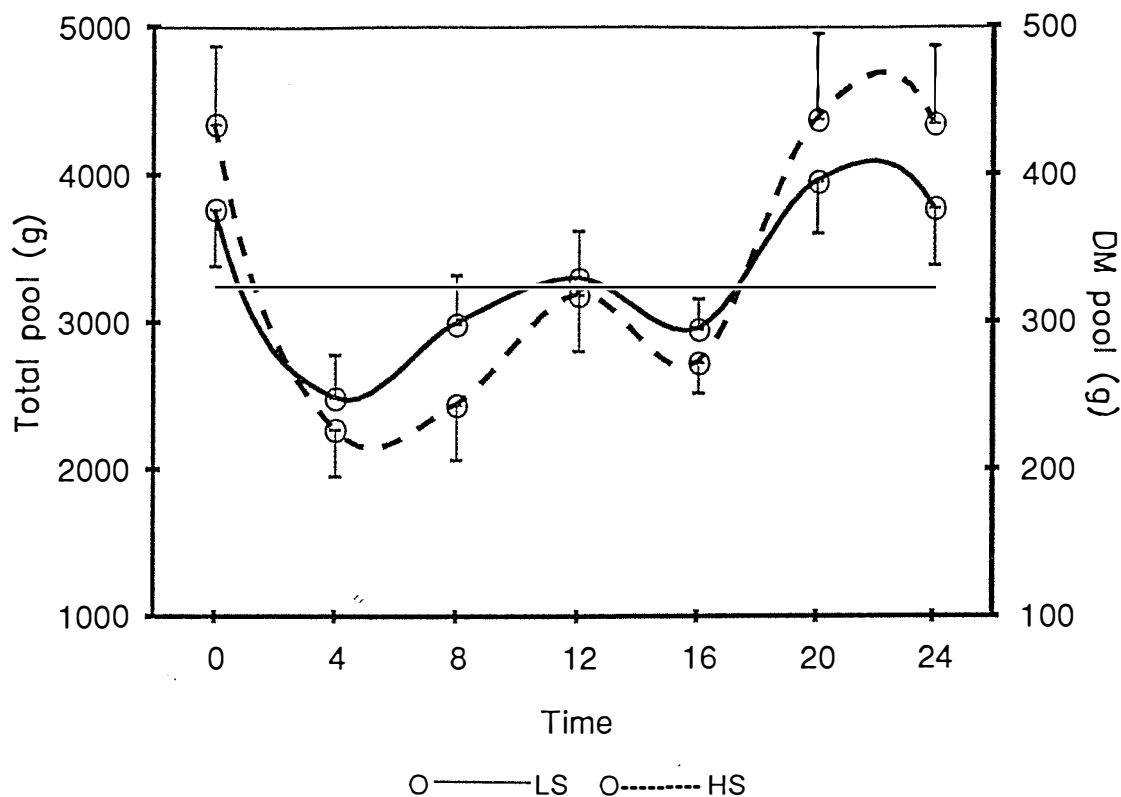
Diurnal pattern of total rumen pool (total pool) and dry matter rumen pool (DM pool) of rumen-fistulated wethers (n=8) **grazing perennial ryegrass.**

Diurnal pattern of rumen pool size was determined by total bailing at 0000, 0400, 0800, 1200, 1600, and 2000 hours in 8 rumen-fistulated wethers grazing PRG. Each animal was rumen-emptied only twice a day, namely, at 0000 and 1200 (day1), 0400 and 1600 (day 2) and 0800 and 2000 (day 3) to minimize the stress on the animals.

Preparation of Co(III)-EDTA.

The Co(III)-EDTA solution (4000 mgCo/L) was prepared as follows.

Equi-molar quantities of cobalt dichloride (Co(II)Cl$_2$•6H$_2$O) and disodium salt of ethylenediaminetetraacetic acid (Na$_2$EDTA) were dissolved in distilled water and heated to form Co(II)-EDTA complex. 30% hydrogen peroxide (H$_2$O$_2$) was added to oxidise Co(II)-EDTA to Co(III)-EDTA until colour changed from red to purple. Excess of H$_2$O$_2$ was then driven off by heating for approximately 4 hours.

Appendix 5.4

Estimation of Cr release rate.

Cr release rate was estimated based on the Cr_2O_3 tablets (n=12) in the recovered capsules from the rumen-fistulated wethers as following;

Cr release rate mgCr/day = Π x r^2 x d x l x (p/100) x mw

where

    r = radius of the tablet (mm) <7>,

    d = density of Cr (g/cm^3) <2.08>,

    l = rate of disappearing of the tablet (in length) (mm/d) <0.915±0.018 s.e.>,

    p = concentration of Cr sesquioxide in the tablet (%) <74.5±1.7 s.e.>,

    mw = proportional molecular weight of Cr in Cr sesquioxide <0.6843>.

  (<values used in the present thesis>)

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