
THE PROCEEDINGS OF THE
NEW ZEALAND SOCIETY
OF
ANIMAL PRODUCTION
VOLUME 62 2002

Effects of selection for shear strength on structure and rumen digestion of perennial ryegrass

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ABSTRACT

Low voluntary intake and animal performance with perennial ryegrass has been attributed to its high leaf strength. Perennial ryegrass populations were selected for high or low shear strength and individual plants of low (LS) and high (HS) selections grown in a controlled environment. Shear strength of LS leaves (290 g) was half that of HS leaves. LS leaves were shorter, narrower and lighter. However, shear strength of LS remained significantly lower after adjustment for differences in leaf width. Shear strength in both selections declined from leaf base to leaf tip. LS leaves contained less sclerenchyma but had similar vascular tissue content. Sclerenchyma was significantly correlated with shear strength ($r = 0.82$).

Chopped leaves (5 cm) from both selections, placed in polyester bags were subjected to rumen microbial digestion. The higher shear strength of HS selected plants was maintained for at least 24 h rumen digestion.

It is concluded that lower shear strength was a result of decreased sclerenchyma content and that potential improvements arising from easier breakdown would be maintained during rumen digestion in vivo.

Keywords Leaf sheaf strength; leaf structure; ryegrass; digestion

INTRODUCTION

Resistance to physical breakdown by chewing and consequent slow clearance from the rumen are believed to contribute to the low voluntary intake and low performance of animals consuming perennial ryegrass (Waghorn and Barry, 1987). Easton (1989) used the genetic variability of leaf shear strength in perennial ryegrass to select grass lines with high (HS) or low (LS) strengths. Shear strength was chosen because rupture by shear may correlate with the mode of chewing action during eating and ruminating. MacKinnon *et al.* (1989) reported that a LS ryegrass selection had slightly lower cellulose and hemicellulose contents and was consumed more rapidly when fed to sheep. Previous selection for low tensile strength in perennial ryegrass failed to improve weight gains in grazing sheep (Lancashire and Ulyatt, 1975). However, that comparison was most likely confounded by different levels of Lolium endophyte in the ryegrass (Lancashire *et al.*, 1977).

This paper presents a progress report of studies of the morphology, anatomy and rumen digestion of perennial ryegrass populations selected over two generations for high and low leaf shear strength. Responses of sheep fed LS and HS forage are presented by Inoue *et al.* (1989).

METHODS

Controlled Environment Plant Growth

Sixty LS and sixty HS plants were grown in a controlled environment (20°C, 79% relative humidity, 12 h photoperiod at 150 watts/m^2) at the Climate Laboratory, Plant Physiology Division, DSIR. Individual tillers were identified and the second, fourth and sixth emergent leaves sampled at maximum leaf size (ligule appearance) and at maximum size + 10 days. Results for leaf 2 only are presented here. Length (L) and weight were recorded on individual leaves and leaf width at three leaf positions: 0.25 L, 0.5 L and 0.75 L was recorded. Shear strength was measured at each of the three leaf positions on groups of 10 leaves using a Warner-Bratzler shear machine (Easton, 1989).

Light photomicrographs were used to estimate proportions of vascular bundle and sclerenchyma tissues in a cross section at the 0.5 L position as an indicator of internal leaf anatomy.

Rumen Digestion

Leaves from HS and LS pastures were cut into 5 cm lengths
and samples of about 4 g DM in polyester bags (62 micron pore size) were placed in the rumen of two cows offered ryegrass-white clover pasture (8 kg DM/day offered hourly). Bags were removed at 3, 6, 12, 19, 24 and 36 h to determine in duplicate shear strength and DM remaining. Shear strength was determined with an Instron 1140 machine (crosshead speed = 80 mm/min) on groups of 10 leaf pieces (n = 8 groups per bag per sampling time). Shear strength was calculated as N/leaf divided by proportion of initial DM remaining.

Statistical differences were determined by a generalised linear model analysis of variance. Effect of leaf width on shear strength was determined by covariate analysis.

RESULTS AND DISCUSSION

Morphology and Shear Strength

Shear strengths and morphology of LS and HS leaves are presented in Table 1. LS leaves had approximately one-half the shear strength of HS leaves. LS leaves were also shorter, narrower and lighter. Shear strengths of LS and HS leaves predicted when leaf width was fitted as a covariate were 394 and 627 g ($P<0.001$), respectively. Thus LS and HS leaves of the same width still had a large difference in breaking strength.

TABLE 1 Shear breaking strength and morphology of LS and HS leaves (HS>LS, $P<0.001$).

	LS	HS
Shear strength (g)[1]	289	592
Length (mm)	189	239
Width (mm)[1]	2.9	3.5
Wet	90	150
Dry	20	36

[1] Mean for 3 leaf positions.

Shear strength and width of both selections decreased from leaf base to leaf tip. Leaf widths at 0.5 L and 0.75 L positions were 6% and 21% less than 0.25 L while shear strengths were 19% and 48% lower, respectively. After correction for leaf width, shear strengths at 0.5 L and 0.75 L positions remained lower ($P<0.001$) than at 0.25 L. These results suggest that with both selections, leaf anatomy changes between leaf base and leaf tip in such a way as to reduce shear strength. Tensile strength has also been reported to decrease between leaf base and tip (Evans, 1967; Betteridge *et al.*, 1986). There were no significant differences between shear strength of leaves at maximum size and 10 days later.

TABLE 2 Vascular and sclerenchyma tissue content of LS and HS leaves (% of total).

	LS	HS	Significance[1]
Vascular tissue	5.9	5.6	NS
Sclerenchyma:			
inner	2.5	4.2	***
outer	1.3	4.1	***

[1] LSD at $P<0.05$ = 1.44 for comparison of tissues

Leaf Anatomy

Ryegrass leaves in cross section show a corrugated inner leaf surface and smooth outer surface. Vascular bundles, containing xylem and phloem elements, are located at about the centre of each corrugation. Sclerenchyma tissue, which has heavily thickened cell walls and strengthens the leaf, is found at the apex (inner surface) and base (outer surface) of the corrugations. The number of vascular bundles at 0.5 L position in LS leaves (12 ±1.5) was smaller than in HS leaves (14.2 ±2.1).

This corresponds with the smaller width of LS (Table 1). The proportions of vascular and sclerenchyma tissue at 0.5 L position are shown in Table 2. There were no differences in proportions of vascular tissue but there were major differences in schlerenchyma (Table 2). LS leaves contained less total sclerenchyma (3.2 ±0.44% *v* 7.1 ±1.6%) and this difference was more marked with sclerenchyma on the outer surface (Table 2).

Leaf shear strength was correlated with the proportion of sclerenchyma (r = 0.82 $P<0.001$) but poorly correlated with proportion of vascular tissue (r = -0.10, $P<0.72$).

These results suggest that lower leaf shear strength is associated with lower sclerenchyma content. However, both vascular bundle and sclerenchyma contain cells with secondary thickened walls and thus may contribute to leaf shear strength. There have been no measurements of shear characteristics of these tissues but measurements of tensile characteristics have been made on grass vascular bundles and sclerenchyma fibres isolated by microscopic dissection of the mid-rib region. Vincent (1982) reported tensile breaking stresses for ryegrass sclerenchyma and vascular bundles of 22.6 $\pm 9.28 \times 10^9$ N/m^2 and 0.84 $\pm 0.46 \times 10^9$ N/m^2 respectively. Betteridge *et al.* (1986) determined breaking stresses for four grass species, mean values for vascular bundles and sclerenchyma were 2.5 $\pm 0.8 \times 10^9$ N/m^2 and 0.38 $\pm 0.15 \times 10^9$ N/m^2, respectively while breaking strains (fractional change in length at break) were 4.3 ±1.27 and 16.7 ±4.0%, respectively. Both studies show sclerenchyma fibres are harder to break than vascular bundles and the Betteridge *et al.* study indicates sclerenchyma fibres deform less.

If these differences in tensile characteristics are reflected in shear strength then it may be calculated from data in Table 2 that sclerenchyma fibres provide 90% or more of the strength of the leaf. A lower sclerenchyma content could thus account for the lower shear strength of LS leaves. Tensile breaking strength of LS and HS leaves was 2.5 and 4.3 N/leaf, respectively (Betteridge *et al.*, 1986).

Shear Strength and Microbial Digestion

After incubation in the rumen for up to 24 h, shear strength of HS leaf residues was about 60% higher than LS residues (Fig. 1). Individual leaves were too difficult to identify and handle for shear measurement at 36 h. Shear strength of both cultivars increased up to 19 h and then decreased. The increased strength is probably due to the more rapid loss of DM in the relatively weak mesophyll tissue and consequent increase in proportion of fibrous tissues. The decreased strength at 24 h may reflect weakening of fibres by microbial fermentation.

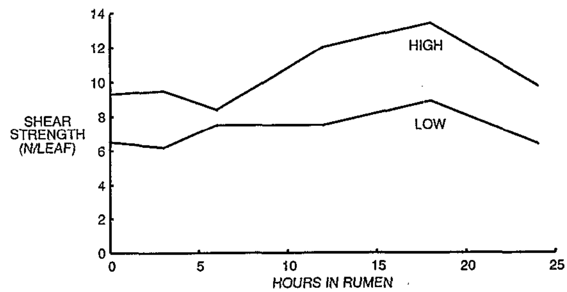

FIG. 1 Shear strength (N/leaf/g DM present) during rumen digestion of ryegrass leaves from HS (HIGH) and LS (LOW) selections.

Rumination chewing is important for reduction of digesta particle size (John *et al.*, 1988) and for clearance of the rumen. These results indicate that the lower leaf shear strength of LS ryegrass is maintained during ruminal digestion and may result in easier breakdown by chewing during rumination. Sheep fed LS forage (Inoue *et al.*, 1989) had higher feed intakes and lower rumination times.

ACKNOWLEDGEMENTS

The authors acknowledge the assistance of Elizabeth A. Halligan and the Technical Services Group at P.P.D., and John J.A.M. Kook, Biotechnology Division, DSIR.

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