CELL WALLS DIGESTION OF RYEGRASS AND LUCERNE BY CATTLE

Nazir Ahmad*, Muhammad Amjed*, Abdur Rehman**, and Altaf ur Rehman*

ABSTRACT

In order to learn more about the extent to which the walls of different cell types are digested in vivo, lucerne (Medicago sativa) and Italian ryegrass (Lolium multiflorum) were fed to cattle. The animals were two 9-month old Limousin × Holstein/Friesian heifers of 180 kg live weight were fed from each of the 6 replicated plots for 3 days per replicate plot, following an introductory period of 2 days. The feed samples were partially dried in field and then in an oven at 85 °C for 48 h for animal feeding. The same heifers were subsequently fed dried Italian ryegrass (Lolium multiflorum), following the same procedure as adopted for lucerne feeding. Intake and digestibility of DM and neutral detergent fibre (NDF) and the output of NDF in the faeces for both in vitro digestible, forage-based diets will be enhanced.

In order to learn more about what actually happens when animals eat different forages, we fed a grass walls of legumes, on the other hand, may be so heavily lignified as to be largely indigestible (Wilson and Mertens, 1995., Hatfield, 1992., Wilson and Hatfield, 1997). Giger-Reverdin (1995) and Wilson (1997) stated that cell wall thickness, surface area available to rumen microorganisms, tightness and packing of cells, all these influence cell wall degradability by ruminants. Wilson (1997) suggested that these arrangements cement adjoining cells together and therefore, bacteria in the rumen are unable to access the outer wall surface of cells within a particle and thus hinder digestibility.

On the basis of Wilson and Mertens (1995), Wilson (1997) and Wilson and Hatfield (1997) one might conclude that thin cell walls in both grasses and legumes would typically be fully degraded in the rumen, that thick cell walls of grasses would be partially degraded in the rumen and that at least some of the thick cell walls of legumes would largely escape degradation. A further inference from Wilson and Mertens (1995) and Wilson and Hatfield (1997) is that some of the thick-walled cells, especially sclerenchyma, are so tightly packed together that rumen microorganisms have very restricted access, so that many of these cells, even in grasses, will largely escape digestion. If cell wall degradation in vivo is to be satisfactorily understood, it is necessary to examine the cell wall material which is swallowed by the animal and that which is excreted in the faeces.

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INTRODUCTION

Incomplete degradation of cell walls is a major factor limiting the value of forages and straws to animals. If the reasons for incomplete digestion or degradation of forages can be more fully understood, the prospects for providing herbivores with more-digestible, forage-based diets will be enhanced. In vitro studies such as those described by Chesson et al. (1986), Akin (1989) and Wilson and Mertens (1995) can provide useful information about the potential degradation of the walls of different types of cell in different plant parts of different species. What might happen to such cell walls when forages and straws are eaten by animals is an area of considerable interest.

It is assumed that the cell content portion of forages (commonly estimated as the fraction soluble in neutral detergent solution) is almost completely digestible (Van Soest, 1994), so that the problem lies with the cell wall fraction. Is it correct to assume that thin, un lignified cell walls are fully digestible and fully digested in practice? If so, the problem would lie with thick cell walls, a high proportion of which are probably lignified. According to Wilson and Mertens (1995), the thick cell walls of grasses may be degraded to the extent of, say, approx. 70%, on slides incubated in rumen fluid, but when grasses are eaten by ruminants the plant particles are not normally in the rumen long enough for the thick walls to be fully degraded. Some of the thick cell walls of legumes, on the other hand, may be so heavily lignified as to be largely indigestible (Wilson and Mertens, 1995., Hatfield, 1992., Wilson and Hatfield, 1997). Giger-Reverdin (1995) and Wilson (1997) stated that cell wall thickness, surface area available to rumen microorganisms, tightness and packing of cells, all these influence cell wall degradability by ruminants. Wilson (1997) suggested that these arrangements cement adjoining cells together and therefore, bacteria in the rumen are unable to access the outer wall surface of cells within a particle and thus hinder digestibility.

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and a legume to cattle and recorded, so far as feasible, what went into cattle, in cell wall terms, and what came out in the faeces, again in cell wall terms.

MATERIALS AND METHODS
A. Animals Feeding experiment:
Two 9-month old Limousin × Holstein/Friesian heifers of 180 kg live weight were fed dried lucerne (Medicago sativa) (c. 90% dry matter (DM) from each of the 6 replicated plots for 3 days per replicate plot, following an introductory period of 2 days. The lucerne was fed at a little below voluntary intake, as the sole diet other than access to a molasses mineral lick and water. The lucerne at the time of harvest comprised 40% leaf and 60% stem, on a DM basis. The feed samples were partially dried in field and then in an oven at 85°C for 48 h for animal feeding. After achieving the required dry matter content the feed samples were transferred to paper sacks and stored for future use. The experimental site was in Cae Plas, Penglais Farm, Aberystwyth, on a gleyed brown earth soil of Sannan series (Rudeforth, 1970), 110 m above the sea level. The area was ploughed and a fine seed bed prepared. The seed was sown by hand in rows 20 cm apart. The crop varieties were European Lucerne and Tribune Italian ryegrass, NIAB 1997. The seed rates were 20 kg ha⁻¹ for lucerne and 30 kg ha⁻¹ for ryegrass.

The same heifers were subsequently fed dried Italian ryegrass (Lolium multiflorum), following the same procedure as adopted for lucerne feeding. The ryegrass at the time of harvest comprised 39% leaf blade, 22% leaf sheath and 39% stem with inflorescence. Intake and digestibility of DM and neutral detergent fibre (NDF) and the output of NDF in the faeces for both lucerne and Italian ryegrass were recorded.

B. Slide preparation
For microscopic studies a cm length of each plant part was cut from the mid portion with razor blade and transferred immediately to formalin acetic acid solution (F.A.A). The rest of the techniques were followed as that used by Moghaddam and Wilman (1998). The volume of thick-walled, thin-walled and epidermal cells in the diets and in the plant particles (in the faeces) were estimated from their percentage in cross sectional area on slides of tissues from different parts of the plants and on slides prepared from the faeces. The thickness of the walls of thick-walled and thin-walled cells and the thickness of the inner and outer walls of epidermal cells were examined microscopically.

The standard errors cited for the present crop species, lucerne and Italian ryegrass fed to cattle were derived from the whole experiment, consist of six field replicates of the crops (Italian ryegrass, lucerne, and wheat (for straw) and all nine combinations of three crop species and three animal species (cattle, sheep and rabbits, with two animals per species). The Excel work sheet was used for computing ANOVA with the following models

\[ Y_{ij}(k) = \mu + \alpha_i + \beta_j + t(k) + e_{ij}(k) \]

where

- \( \mu \) = Average effect
- \( \alpha_i \) = Effect of periods
- \( \beta_j \) = Effect of animals
- \( t(k) \) = Effect of diets
- \( e_{ij}(k) \) = Random error

RESULTS
Dry matter (DM) intake was slightly higher with lucerne than with Italian ryegrass (Table 1). NDF intake was lower in lucerne than the ryegrass, but the reverse was the case for NDF output in the faeces. DM digestibility was lower and NDF digestibility much lower, in lucerne than ryegrass. The proportion of plant tissue volume occupied by thick-walled cells was much higher in faeces than in the forage and the proportion of thin-walled cells and epidermal cells were much lower in faeces than in forage (Table 2). A significant proportion of the thin-walled cells partially or entirely escaped digestion, particularly in ryegrass. The cell walls measured in the faeces samples were thinner, on average, than equivalent cell walls in the forage (Table 2). The difference in cell wall thickness between faeces and forage samples was generally greater with lucerne than ryegrass.

DISCUSSION
The dry matter and neutral detergent fibre intake of the diets are shown in Table 1. The rather higher intake and lower digestibility of lucerne compared with Italian ryegrass is in accord with expectation of Minson (1990). The lower digestibility of cell wall in lucerne than ryegrass might also be expected (Wilson et al. 1991). A reason for this lower digestibility of cell wall may have been the higher average concentration of lignin in cell wall of lucerne compared with ryegrass (11.3 v. 4.5% in leaf blades, S. E. ±0.24, and 13.6 v. 10.3% in stems, S.E. ±0.27). DM soluble in neutral detergent (i.e. cell contents) comprised 54% of the intake in the case of lucerne, compared with 46% in ryegrass. The relative
The contribution of cell contents to digestibility was therefore, greater in lucerne than in ryegrass, as might be expected (Wilson et al. 1991; Ahmad & Wilman 2001). The much lower proportion of thin-walled cells in the faeces than in the diet is as expected, on the basis that thin walls are likely to be readily and rapidly digested. However, it appears that a significant proportion of thin-walled escaped digestion, particularly with ryegrass diet. This suggests that some plant particles passed out of the rumen very quickly, before there was time for even thin walls to be fully degraded. The inaccessibility of microorganisms to secondary walls is a major limitation to wall digestion in forages due to time constrains for microbes to access walls for digestion and thus much potentially digestible cell wall in grass feeds escapes in the faecal particles (Wilson & Mertens 1995; Wilson 1997; Moghaddam & Wilman 1998; Wilman & Ahmad 1999). The forage fiber content and ease of forage breakdown have also a major influence on voluntary feed intake and digestibility (Wilson et al. 1989b; Wilson and Kennedy 1996); which may be affected by the activities of rumen microorganisms, by the handling and fate of plant particles in the rumen, by the load (physical bulk) of plant residues and the contraction of the organ responsible for digesta movement (Allen 1996). The basic requirement for the forage to pass from the rumen is the breakdown of feed from large particles to small particles (McLeod & Minson 1988; Wilson et al. 1989b). These authors suggested that the particles, which are held on a 1.2 mm screen during wet sieving, have a high resistance to passage from the rumen. Chewing during eating and during rumination is considered to be virtually the only factor responsible for reducing the size of particles in the rumen (Ulyatt et al. 1986; Wilson 1994); which in turn enhances the access of rumen microorganisms to the forage cell walls and cell content (Mtengeti et al. 1996; Wilman & Moghaddam 1998b; Hatfield et al. 1999) and hence high digestibility. A proportion of the diet may have been very quickly reduced to particles small enough to pass easily from the rumen (able to pass through a sieve with apertures of 1mm or a little larger (Pippi et al. 1985; Faichney et al. 2004), this may have been particularly the case because the crops had been to a low moisture content and were rather brittle. The proportion of epidermal cells identified in the faeces was low, which is perhaps slightly surprising as the walls of the epidermal cells were not thin. The position of the epidermal cells on the outside of the plant particles presumably increases their accessibility to rumen microorganisms and so increases the likelihood that their walls will be at least partially degraded. Examination of the remains of plant particles in faeces is a difficult technique and it may be difficult to avoid some bias towards selecting the less degraded particles. This may be a reason for the generally modest difference in wall thickness, within a cell wall category, between diet and faeces samples.

**CONCLUSION**

The higher proportion of NDF in the faeces particles appears to be associated with incomplete chewing and limited access of microorganisms to the tightly packed cells. The lower digestibility of lucerne than ryegrass appears to be associated with the higher proportions of thick-walled cells, NDF and lignin content in the lucerne stems. The higher proportion of thick-walled cells in faeces than in the diet appears to be associated with incomplete access of microorganism to the tightly lignified thick-walled tissue. The presence of thin-walled cells in the faeces, especially in the case of ryegrass, appears to be associated with an early escape of particles from the rumen.

**Table I.** Dry matter and neutral detergent fibre intake and digestibility of lucerne and Italian ryegrass fed to cattle

<table>
<thead>
<tr>
<th></th>
<th>lucerne</th>
<th>Italian ryegrass</th>
<th>*S.E (C×A)</th>
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<tbody>
<tr>
<td>Dry matter intake (g/day)</td>
<td>4953</td>
<td>4709</td>
<td>±68.5</td>
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<td>Dry matter intake (g/day W&lt;sup&gt;0.75&lt;/sup&gt;)</td>
<td>98.8</td>
<td>94.0</td>
<td>±2.20</td>
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<td>Dry matter digestibility (%)</td>
<td>64.5</td>
<td>71.3</td>
<td>±1.12</td>
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<tr>
<td>Neutral detergent fibre intake (g/day)</td>
<td>2254</td>
<td>2534</td>
<td>±40.60</td>
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<tr>
<td>Neutral detergent fibre in faeces (g/day)</td>
<td>1130</td>
<td>713</td>
<td>±20.20</td>
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<tr>
<td>Neutral detergent fibre digestibility (%)</td>
<td>49.8</td>
<td>71.8</td>
<td>±1.65</td>
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</table>

*S.E. for comparing crop × animal means (20 D.F.).
Table II. Percentage in cross-sectional area and wall thickness (µm) of different cell type in lucerne and Italian ryegrass fed to cattle and in the faeces.

<table>
<thead>
<tr>
<th></th>
<th>Lucerne</th>
<th>Faeces</th>
<th>Italian Ryegrass</th>
<th>Faeces</th>
<th>Mean±S.E</th>
<th>Mean±S.E</th>
<th>Mean±S.E</th>
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<tr>
<td>Percentage in cross-sectional area of</td>
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<tr>
<td>thick-walled cells (%)</td>
<td>24.2±0.36</td>
<td>22.0±0.36</td>
<td>69.8±1.56</td>
<td>69.8±1.56</td>
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<tr>
<td>thin-walled cells (%)</td>
<td>66.5±0.44</td>
<td>64.5±0.44</td>
<td>27.1±1.55</td>
<td>27.1±1.55</td>
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<tr>
<td>epidermal cells (%)</td>
<td>9.3±0.40</td>
<td>13.5±0.40</td>
<td>3.0±0.37</td>
<td>3.0±0.37</td>
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<tr>
<td>Thickness of cell walls (µm)</td>
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<tr>
<td>thick-walled cells</td>
<td>1.45±0.016</td>
<td>0.92±0.016</td>
<td>0.80±0.057</td>
<td>0.80±0.057</td>
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<tr>
<td>thin-walled cells</td>
<td>0.46±0.009</td>
<td>0.42±0.009</td>
<td>0.38±0.021</td>
<td>0.38±0.021</td>
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<tr>
<td>epidermal outer wall</td>
<td>2.84±0.065</td>
<td>2.02±0.065</td>
<td>1.68±0.056</td>
<td>1.68±0.056</td>
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<tr>
<td>epidermal inner wall</td>
<td>0.91±0.042</td>
<td>0.78±0.042</td>
<td>0.66±0.059</td>
<td>0.66±0.059</td>
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REFERENCES

Allen, M.s. 1996. Physical constraints on voluntary intake of forages by ruminants. J.


