SOLUBILISATION OF LIGNIN IN TROPICAL GRASSES AND LEGUMES: EFFECT ON DIGESTIBILITY

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SUMMARY

The progress of total dry matter fermentation in an *in vitro* system, as indicated by gas pressure, was determined for 4 tropical grasses *(Chloris gayana, Heteropogon contortus, Panicum maximum* and *Sorghum* sp) and 4 tropical legumes *(Clitoria ternatea, Dolichos lablab, Macroptylium atropurpureum* cv. Siratro and *Stylosanthes hamata* cv. Verano). In the case of the legumes virtually all fermentation was complete by 48 h, but the grasses, although initially less fermentable, continued to ferment slowly up to 120 h. Tropical grasses have a high lignin content, which is distributed within a large fraction of non-cellulosic polysaccharides, principally xylans. Any dissolution of this fraction allows some of the lignin to be dispersed in soluble or micellar form. Thus, although lignin acts as a constraint on digestion, this inhibition is not total and digestion can proceed slowly for a prolonged period. In contrast, in legumes the lignin is not distributed through a large xylan fraction and is not easily solubilised. It appears to produce more complete inhibition of fermentation after the more readily accessible carbohydrates have been fermented. In grasses, inhibition of fermentation can come not only from *in situ* matrix lignin, but also from the soluble lignin released into the fermentation medium. When the concentration of soluble lignin was increased by adding that from a separate fermentation, gas production was depressed and the *in vitro* NDF digestibility of spear grass was reduced from 32.3 to 29.6%.

Keywords: hemicellulose, lignin-carbohydrate complex, soluble lignin, acid detergent-dispersible lignin

INTRODUCTION

Lignin has traditionally been regarded as insoluble and inert. Its effect on digestibility has been seen as that of an encrusting barrier, preventing access to cell wall carbohydrates (Van Soest 1982). However studies in Townsville showed that in cattle fed spear grass, almost half the forage lignin was released in the rumen fluid as a soluble lignin-carbohydrate complex (Gaillard and Richards 1975; Neilson and Richards 1982). Subsequently, others have made similar findings (Conchie *et al.* 1988). Recently we have shown that the acid detergent treatment solubilises more than half the lignin in tropical grasses, but relatively little in tropical legumes (Lowry *et al.* 1994). In temperate forages, the content of non-cellulosic polysaccharides in legumes is much less than in grasses (Butler and Bailey 1973). Similar results apply to 136 species of tropical forages (Lowry *et al.* 1992). Indeed, if non-cellulosic polysaccharides are equated with the "hemicellulose" estimated as the difference between neutral detergent fibre and acid detergent fibre, the difference is a general one between monocotyledons and dicotyledons. In cell walls, lignin is associated with the non-cellulosic polysaccharides rather than cellulose (Morrison 1979; Weimer 1992). Direct covalent links between lignin and xylan are now known (Lam *et al.* 1992). We thus attribute the dispersal of lignin by acid detergent to acid hydrolysis of the hemicellulose leaving the lignin as an unsupported open matrix, much of which can be solubilised by hot detergent. A similar argument suggests that enzymatic hydrolysis of xylans would allow some lignin to be solubilised, in this case as a soluble lignin-carbohydrate complex (LCC), and this would occur in grasses more than in legumes.

This leads to the hypothesis that, in addition to differences in plant morphology (Wilson 1993), the effect of lignin on digestibility in grasses is very different from that in legumes. In grasses, removal of matrix lignin as LCCs would allow continuing, if restricted, access to hemicellulose. The smaller hemicellulose fraction in legumes suggests the lignin would occur in more compact form, be less susceptible to formation of soluble LCCs, and thus more likely to act as a direct barrier for further cell wall degradation once accessible polysaccharides had been fermented.

METHODS

The legumes in these experiments were Siratro (*Macroptilium atropurpureum* cv. Siratro), clitoria (*Clitoria tematea),* verano stylo (*Stylosanthes hamata* cv. Verano), and dolichos (*Dolichos lablab*). *The* grasses were black spear grass *(Heteropogon contortus),* Rhodes grass (*Chloris gayana),* green panic

(*Panicum maximum)* and forage sorghum *(Sorghum sp.).* All were grown in North Queensland in bulk lots for feeding trials. Samples were dried at 60° C and ground to pass a l-mm screen.

Fermentation of each species was carried out on triplicate 100-mg samples in 50-ml serum bottles according to the procedure of Pell and Schofield (1993) in which dry matter disappearance is directly related to fermentation gas pressure. The dry sample was pre-wetted with deaerated distilled water, placed in an anaerobic cabinet and 7 ml of buffer added followed by 3 ml of rumen fluid strained through a 50-micron mesh. Rumen fluid was taken from sheep on a Rhodes grass-luceme diet. The samples were capped and placed in an incubator with periodic individual stirring of each bottle. Incubation was continued until there was no increase of pressure, or until 120 h, when it was considered that microbial metabolism would no longer be representative of fresh rumen fluid. Gas pressure was monitored during fermentation by means of a pressure transducer mounted on a probe inserted through the cap, and corrected using that from bottles with buffer and inoculant only. A micro-NDF determination at the end of incubation enabled determination of NDF digestibility, but not dry matter digestibility, for each sample.

Crude LCC was prepared by similar *in vitro* fermentation of spear grass that had been milled to about 50 micron by a Spex ring mill. The product was centrifuged at 1000 g to remove large particles and then at 15000 g to obtain a particle-free supematant. This was then subject to ultrafiltration in an Amicon ultrafiltration cell with a membrane with a 3kD cut-off. Solution was forced through by nitrogen at 200kP until no free liquid remained. The retained product was then resuspended in distilled water.

For investigating the effect of LCC on fermentation , the substrate was spear grass that had been extracted with water at room temperature and dried at 60 °C. Duplicate samples were pre-wetted with 1 ml water in the usual way (controls), or with solution containing LCC from an equivalent amount of spear grass (LCC treatment).

RESULTS

The progressive increase of pressure for several different experiments are shown in Fig. 1. In each case there was a rapid initial increase attributable to the fermentation of simple sugars and other soluble components. Other experiments have shown that this is usually complete by 12 h. In the case of the legumes, there was rapid fermentation of cell wall for about 24 h, and effectively none after 48 h. In contrast the grasses, although less digestible than the legumes initially, continued to undergo fermentation until incubation was terminated at 120 h, and c. 25% of the fermentation occurred after 48h. For the grasses, final pressures had a mean S.D. of ±0.64 mV/g. The initial NDF content (% dry matter) and NDF digestibility at end of incubation (% original NDF ± SD) of each species were as follows: clitoria, 55.8 and 33.7 ± 1.4; dolichos 70.6 and 38.1 ± 1 .1; verano 54.7 and 41 .1± 1.1; green panic, 60.9 and 79.5 ± 0.4; sorghum, 66.5 and 61.4 ± 0.4; spear grass 74 and 43.9 ± 0.3; rhodes grass 79.2 and 61.1 ±.0.9.

Incubation of water-washed spear grass showed that when the LCC fraction of molecular weight greater than 3kD was added to the medium, approximately doubling the concentration of LCC that would eventually be released, there was little initial effect on fermentation but a marked decrease after 8h. (Fig. 2). There was a corresponding reduction in the NDF digestibility at 68 h; from 32.3 -1.2% to 29.6±0.8%.

DISCUSSION

It would have been desirable to carry out this comparison with pure cell wall rather than intact forages. However we have found that preparing cell walls by neutral detergent extraction has a drastic effect on the cell wall itself, extracting some lignin, hydrolysing some cinnamic ester bonds, and causing an increase in digestibility in grasses, but not in legumes (Kennedy and Lowry, unpublished data). In this *in vitro* system we have found that crystalline celluloses, such as cotton thread, are fully fermented (>95% at 120 h). NDF digestion in the species studied here was mainly in the range 40-60%. Thus, it seems likely that the limitation of cell wall digestion with these species was due to lignin. These results then suggest significant differences in the way lignin acts on digestibility in grasses as compared with legumes. Previous comparisons between grasses and legumes do not appear to show the differences following initial fermentation found here. Digestibility measurements carried out at one or two fixed times are unlikely to reveal these trends. To some extent the differences in rate of cell wall digestion are compensated by the relative composition. The nutritional value of legumes lies more in the cell contents, the fibre fraction is usually much less than in grasses, so that its relative digestibility after 24 h is of less consequence. Mature grasses have a much higher fibre fraction, but more of it is digestible eventually. The results also indicate that animals on dry season pastures, with a slow rate of passage from the reticulorumen, may extract more nutrients from grasses than would be apparent from estimates based on 24- or 48h exposure to the rumen.

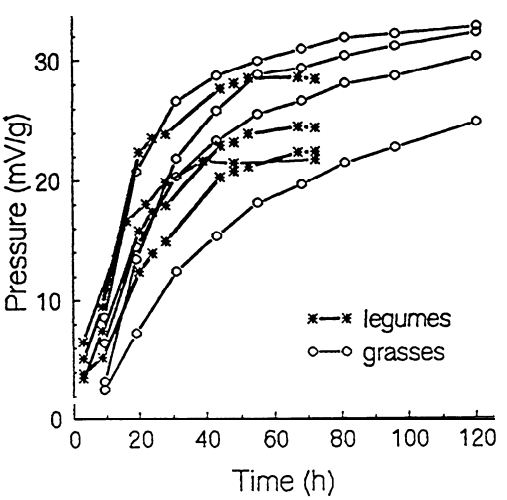

Fig. 1. Gas production from fermentation of grasses and legumes. Grasses (in order of highest final values): green panic, Rhodes grass, sorghum, spear grass. Legumes: verano, clitoria, dolichos, siratro

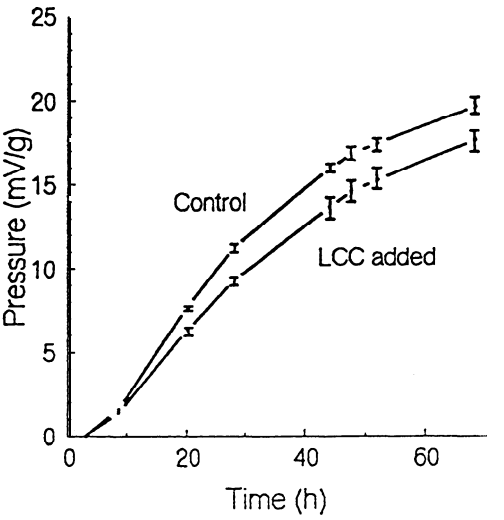

Fig. 2. Effect of added LCC on fermentation of water-washed spear grass. Pressures given as mean and range for duplicate samples, adjusted to zero at 3 h

In comparing the effect of lignin on digestibility in legumes and grasses it has been claimed that grass lignin is more inhibitory (Buxton and Fritz 1985; Buxton and Russell 1988). However this perception is due to the measurement of lignin as acid detergent lignin giving rise to a gross underestimate of total lignin in grasses but not in legumes (Lowry et *al.*1994), and has no relation to the results presented here.

Inhibition of fermentation by the LCCs released by cell wall degradation is in agreement with other results (Chemey et *al.* 1992; Jung 1988), but their effect in the rumen rather than in a closed *in vitro* system remains to be determined. However, the results suggest a possible strategy for increasing digestion of tropical grasses. Enhanced fluid passage through the rumen, whether by increased water intake or salivary flow, should sweep away soluble lignin and reduce its inhibitory effects. However, it should be said that with Bedouin goats, lignin degradation (as distinct from solubilisation) actually increased under water stress (Silanikove and Brosh 1989). It may be significant that buffalo, which have a higher rumen water flux than cattle, also showed higher dry-matter digestibilities *in sacco* (Kennedy *et al.* 1992). Other strategies for decreasing the inhibition due to soluble lignin are under investigation.

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