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Structural modifications of timothy lignin by *in vitro* rumen fermentation

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Lignin in forages is accepted as one of the most important chemical components due to its key role in ruminal degradation of forage fibers. The nutritional effect of forage lignin is closely related to its structure (Besle *et al,* 1994, J Sci Food Agric, 64, 171-190) and thus probably affected by changes that forage lignin undergoes in the digestive tract. Rumen fermentation has been reported to cause decomposition of a lignin model dimmer into monoaromatic compounds (Chen *et al,* 1985, Appl Environ Microbiol, 50, 1451-1456) and demethylation of the syringyl units of grass lignin (Mossoni *et al,* 1994, J Sci Food Agric, 64, 379-387), but no further evidence is presented showing structural degradation of forage lignin by rumen fermentation. The objective of the present study was to investigate structural modifications of forage grass lignin occurring in the rumen.

A lignin fraction (FHL) was isolated with 90 % dioxane from the ball-milled and *in vitro* rumen fermented residue of timothy hay harvested at the seed setting stage. The lignin fraction was compared with 90 % dioxane-soluble lignins isolated from the ball-milled and cellulase-treated residue of the timothy hay (EHL) and from the faeces of a heifer fed on the timothy hay (FSL).

FHL consisted of more lignin components, less carbohydrate and less bound phenolic acids than EHL and had a compositional resemblance to FSL (Table). Alkaline nitrobenzene oxidation and IR spectra showed all the lignins to be guaiacyl-syringyl lignins. In gel permeation chromatograms, FHL and FSL were distributed in lower molecular size regions than EHL.

Lignin modifications occurring in the rumen have been often reported and the observed major modification is solubilisation of lignin. Release of lignin due to solubilisation was considered as a consequence of degradation of surrounding polysaccharides in the rumen (Conchie *et al,* 1988, Carbohydr Res, 177, 127-151). The release of EHL from the ball-milled timothy is also based on the enzymatic degradation of associated polysaccharides. FHL, produced by the *in vitro* rumen fermentation of the ball-milled timothy, differed in chemical and physical properties from EHL, suggesting removal or degradation of the phenolic acids bound to timothy lignin and depolymerisation of timothy lignin by rumen fermentation.

Chemical components	EHL	FHL	FSL
		(g/kg lignin fraction)	
Klason lignin	716	887	890
Total carbohydrate	189	50	37
Esterified *p*-coumaric acid	15.6	3.7	3.5
Esterified ferulic acid	2.1	0.4	0.5