INHIBITION OF ARABINOZYLAN FERMENTATION BY VARIOUS CELL WALL PHENOLICS

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Introduction

Lignin content of forages has long been known to be negatively related to fiber digestibility by ruminants. In recent years both composition of lignin and esterified phenolics of plant cell walls have been implicated as influencing ruminal fiber fermentation (Jung, 1989). Assessment of the relative importance of these cell wall phenolic fractions as limitors of degradability has been difficult. Chesson (1988) suggests that esterified cell wall phenolic acids do not limit polysaccharide digestibility. This point must be resolved as plant breeders are beginning to view esterified phenolic acids as selection criteria in forage quality improvement programs (Gabrielson et al., 1987). In this study we have attempted to partition the effects of various cell wall phenolic fractions on ruminal arabinoxylan fermentability.

Materials and Methods

Stem material of alfalfa (*Medicago sativa*), smooth bromegrass (*Bromus inermis*) and corn (*Zea mays*) was pre-treated, in triplicate, with amylase and amyloglucosidase, and extracted with 80 % ethanol to remove starch and other cytoplasmic components. Samples were chemically delignified by treatment with 1 M NaOH, 2 M NaOH and nitrobenzene, NaOH and H_2O_2, $NaClO_2$ or $KMnO_4$. *Phanerochaete chrysosporium* also was used to biologically delignify the forage samples. Sufficient ethanol was added to all treatment reaction mixtures to reach 80 % ethanol in order to prevent solubilization of arabinoxylan. Delignified samples were fermented *in vitro* with a mixed ruminal inoculum collected from an alfalfa fed cow. Samples were incubatd at 39 °C for 72 h. All forage samples and fermented residues were analyzed for arabinose and xylose content. Control and delignified forage samples were analyzed for Klason lignin content, lignin composition by nitrobenzene oxidation, and esterified phenolics by alkaline extraction. The data were analyzed as a 3 x 7 factorial. Influence of the various lignin fractions on arabinoxylan digestibility was determined by linear regression.

Results

The chemical delignification procedures all resulted in a reduction ($P < .05$) in Klason lignin and esterified phenolic content of forage cell walls (table 1). The fungal treatment also removed esterified phenolics, but not Klason lignin. The nitrobenzene, $NaClO_2$, and $KMnO_4$ treatments removed the most lignin. The NaOH and nitrobenzene reduced esterified phenolics to the greatest extent. Forage species did show a significant interaction with delignification method because less lignin was generally removed from alfalfa than the grasses. Composition and/or structure of the lignin was altered by the nitrobenzene and $NaClO_2$ treatments as illustrated by the increase in molar ratio of vanillin to syringaldehyde (table 1). Esterified phenolic acid content of the cell walls was changed by all treatments except for $KMnO_4$. Nitrobenzene and NaOH-H_2O_2 both caused an increasing proportion of *p*-coumaric to ferulic acids. In contrast NaOH, $NaClO_2$ and the fungi shifted the molar ratio to more ferulic acid. The data clearly indicated that the delingnification procedures produced differential variation in both quantity of lignin fractions and their composition.

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TABLE 1. CHEMICAL COMPOSITION OF DELIGNIFIED FORAGE CELL WALLS

Delignification method	Cell wall (%)		Molar ratios	
	Klason lignin	Esterified phenolics	Vanillin/ syringaldehyde	*p*-Coumaric/ ferulic
Control	20.1[a]	1.56[a]	1.32[a]	4.71[a]
1 M NaOH	12.0[b]	.03[b]	1.04[a]	3.00[b]
Nitrobenzene	6.1[c]	.01[b]	all vanillin[b]	all *p* coumaric[c]
$NaOH-H_2O_2$	12.2[b]	.44[c]	1.10[a]	23.09[d]
$NaClO_2$	7.7[c]	.13[b]	2.34[c]	.75[e]
$KMnO_4$	9.1[bc]	.47[c]	1.23[a]	5.38[a]
Fungi	26.4[d]	.53[c]	1.69[ac]	1.10[e]
SEM	1.1	.06	.27	.39

[abcde]Means in the same column not sharing a common superscript are different ($p < .05$).

Fermentability of arabinoxylan in these forages was affected ($P < .05$) by delignification (table 2). All the chemical delignification methods resulted in an increase in fermentability of arabinose and xylose. The fungal treatment did not improve degradation of arabinose or xylose. The increase in digestibility by delignification was not related to forage species. The NaOH, nitrobenzene, $NaOH-H_2O_2$ and $NaClO_2$ treatments were equally effective in improving arabinose fermentability. Xylose degradation was most improved by NaOH, nitrobenzene and $NaOH-H_2O_2$.

Although not shown, the delignification procedures resulted in small, but significant, changes in molar ratios of the cell wall polysaccharide neutral sugars which were related to forage species. Therefore, the effects of treatment and forage were accounted for in the linear regression of lignin fractions with arabinoxylan fermentability. The ferulic acid content of the cell walls accounted for the greatest amount of variation ($r^2 = .78$) in arabinose degradation. Addition of Klason lignin content to the model was significant and increased the coefficient of determination ($r^2 = .84$). Xylose fermentability was related ($r^2 = .84$) most strongly to Klason lignin concentration in the cell wall. Total esterified phenolics concentration (p-coumaric and ferulic acids, plus several minor phenolic acids and aldehydes) addition to the xylose model was significant ($r^2 = .86$). Composition of the lignin and esterified phenolic fractions did not contribute significantly to explaining the variation in arabinose or xylose fermentability.

TABLE 2. IN VITRO RUMINAL FERMENTABILITY OF ARABINOXYLAN

Delignification method	Degradation (%)	
	Arabinose	Xylose
Control	72.2[a]	43.8[a]
1 M NaOH	94.7[b]	87.5[bcd]
Nitrobenzene	97.5[b]	96.8[d]
$NaOH-H_2O_2$	97.4[b]	88.9[cd]
$NaClO_2$	87.1[bc]	77.4[bc]
$KMnO_4$	81.7[bc]	75.8[b]
Fungi	63.9[a]	48.5[a]
SEM	3.8	4.5

[abcd]Means in the same column not sharing a common superscript are different ($p < .05$).

Discussion

The negative relationship observed between forage digestibility and *p* coumaric acid concentration of grasses (Burritt et al., 1984) could result from the increasing concentration of *p*-coumaric acid associated with lignification during forage maturation (Chesson, 1988; Jung, 1989). The data presented here suggest *p*-coumaric acid has little, if any, role in arabinoxylan fermentability. Esterified ferulic acid was negatively associated with arabinose fermentability. Klason lignin content also was negatively related to arabinose

degradation, but to a lessor extent. This relationship between arabinose fermentability and ferulic acid is not surprising as ferulic acid has been shown to be linked to arabinose in plant cell walls (Fry, 1982). Lignin is apparently linked to xylose directly and via arabinose bridges, therefore, the effect of lignin on xylose fermentation is expected. The data indicate that lignin concentration is the most important cell wall phenolic limiting arabinoxylan fermentation, but the degradability of arabinose is more directly affected by esterified ferulic acid.

Literature Cited

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