Correlations of Phenolic Acids and Xylose Content of Cell Wall with In Vitro Dry Matter Digestibility of Three Maturing Grasses

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ABSTRACT

Maturing reed canarygrass, Russian wildrye, and smooth bromegrass cell walls were analyzed for lignin, phenolic acids, and neutral sugars. Linear correlation between in vitro dry matter digestibility and the p-coumaric acid content was −.86. The correlation between in vitro dry matter digestibility and ratio of p-coumaric to ferulic acid was −.84. Samples with high percentages of lignin also contained high ratios of p-coumaric to ferulic acid. Thus, p-coumaric acid may be more important than ferulic acid in crosslinking lignin to structural carbohydrates. Percent xylose and in vitro dry matter digestibility also were correlated significantly in all three species. A Russian wildrye regrowth sample contained a relatively high percentage of lignin despite its immature appearance. The lower digestibility of that regrowth sample may have resulted both from its high lignin content as well as branched xylans in the hemicellulosic fraction.

INTRODUCTION

Digestibility is an important criterion in defining the value of forages. Over the years researchers have sought to predict accurately forage digestibility by laboratory methods. To date, lignin and acid detergent fiber have given the best correlations with in vivo dry matter digestibility (16). However, neither of these measures yields accurate predictions of digestibility of different forage species from a single regression equation (1, 14). These and many other measures commonly used for describing forage composition are based on gravimetric methods rather than upon analysis of specific chemical constituents. Plant cell walls are highly organized, complex structures, consisting largely of carbohydrate polymers that can be quantitated in terms of constituent monosaccharides (9). To analyze plant cell walls in terms of specific chemical components could be beneficial for predicting digestibility as well as understanding plant-related factors that influence digestibility.

The purpose of this study was to investigate the relationship between certain specific plant cell wall constituents and in vitro dry matter digestibility (IVDMD) in three maturing grass species. Xylose, ferulic acid (FA), and p-coumaric acid (PCA) were the measures used.

Phenolic acids may serve in crosslinking structural carbohydrates and lignin (12) and have been used as indicators of grass digestibility (7). These cross linkages are broken upon treatment with alkali, a process known to increase digestibility of poor quality grasses and straws (10).

The percentage of xylose in grass cell walls also was considered a desirable variable for

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analysis, as xylans constitute the major noncellulosic cell wall component of grasses and have a definitive relationship with forage digestibility (18).

MATERIALS AND METHODS

Reed canarygrass (*Phalaris arundinacea* L.) leaves were collected at six stages of maturity from April 22 to June 4 from the University dairy farm. Maturities ranged from early leaf to milk stage. Russian wildrye (*Elymus juceus* Fisch.) plants were collected at five stages of maturity between April 24 and June 16 from a neighboring USDA plant breeding test plot. The first four samples were collected between immature and dough stages, and the fifth sample was harvested as immature regrowth on June 16, which was 10 days after the plot had been mowed to within 15 cm of the soil. Six smooth bromegrass (*Bromis inermis* L.) samples were collected from April 16 to June 16. Stages of maturity ranged from vegetative to dough stage. Upon collection, samples were immediately frozen on dry ice, freeze-dried, and ground in a cyclone mill equipped with a .5-mm screen.

Approximately 5 g of ground sample was refluxed for 1 h in 150 ml of 80% aqueous ethanol while continuously stirred with a magnetic stirring bar. Samples then were filtered under negative pressure through a buchner funnel with a fritted disc (coarse porosity). The remaining residue was washed with 3 × 50 ml of hot ethanol or until the filtrate was colorless. The alcohol insoluble residue (AIR) was dried in a vacuum oven overnight at 40°C.

The AIR was hydrolyzed in 72% sulfuric acid at 20°C for 30 to 45 min depending on the lignification of the sample. Samples then were diluted to 2 N acid, and hydrolysis was continued for 1 h at 95°C. Aliquots of the acid hydrolysates were analyzed for neutral sugars as described by Bittner et al. (2). Samples were injected into a Tracor MT-220 gas chromatograph equipped with a 120-cm glass column (6 mm i.d.) packed with a 10% SP-2330 on Supelcoport W AW (100/120 mesh). Nitrogen gas carrier flow was 35 ml/min. The column temperature was held at 200°C for 2 min, then programmed at 2°C/min to 240°C, and held at that temperature for 18 min. Detector and injector temperatures were held at 225 and 245°C, respectively.

Phenolic acids were extracted from the AIR with ethyl acetate by the method of Salomonsson et al. (15). The ethyl acetate phase was filtered through sodium sulfate and the volume reduced on a rotary evaporator. Samples were transferred to 16 × 150 mm screw-capped tubes and evaporated to dryness with a stream of nitrogen. Residues were redissolved in .02 *M* phosphorus acid at 60°C. Samples were injected into a Varian Model 5000 high pressure liquid chromatograph equipped with a MicroPak MCH-10 octadecylsilane column and eluted at a flow of 2 ml/min. The eluting solvents were .02 *M* aqueous phosphate buffer and acetonitrile. Phenolic acids were detected with an ultraviolet detector at 280 nm.

Lignin was determined gravimetrically as acid detergent lignin by the method of Goering and Van Soest (5) except the asbestos was omitted.

In vitro dry matter digestibilities were measured by a two-stage in vitro rumen fermentation procedure (11). Rumen fluid was obtained from a fistulated dairy cow maintained on alfalfa hay.

Correlation and regression analyses measured the relationship between phenolic acid content and IVDMD as well as the relationship between percent xylose and IVDMD.

RESULTS AND DISCUSSION

The lignin content and phenolic acid composition of the cell walls of maturing Russian wildrye, smooth bromegrass, and reed canarygrass are in Table 1. Lignin content increased during plant growth in the Russian wildrye and smooth bromegrass samples. In reed canarygrass, lignin did not conform to an expected pattern, although it increased in the most mature samples. An increase in the ratio of PCA:FA and PCA content was observed during growth of all three species. The relationship between the ratio of PCA:FA and lignin content of the grasses was significant ($r=.89$, $P<.01$). An increasing PCA content coincided with increasing lignification of plant cell wall.

Detergent fiber measures and monomeric constituents of plant cell wall polysaccharides for all plant samples along with their respective correlations with in vitro dry matter digestibility are in (4).

The ratio of ferulic to p-coumaric acid was correlated with in vivo dry matter digestibility

TABLE 1. Lignin content and cell wall phenolic acids of maturing Russian wildrye, smooth brome grass, and reed canarygrass.[a]

Sample	Collection date	Lignin	PCA[b]	FA[c]	Ratio PCA:FA
		(%)	—— (μg/100 mg)	——	
Russian wildrye	4–24	2.3	80.6	155.7	.61
	4–30	2.6	60.3	165.6	.39
	5–12	3.1	182.7	237.2	.78
	6–04	5.1	227.7	236.6	.97
	6–16[d]	3.4	98.0	161.4	.61
Smooth bromegrass	4–16	1.8	56.5	210.0	.27
	4–24	1.9	61.4	213.4	.29
	4–30	2.4	99.2	263.4	.38
	5–12	2.2	101.6	265.0	.38
	6–04	3.0	215.0	271.6	.79
	6–16	4.6	218.4	276.1	.79
Reed canarygrass	4–22	2.0	55.2	151.1	.37
	4–28	1.3	39.2	160.4	.24
	5–05	1.1	74.0	326.8	.21
	5–13	1.2	70.9	196.4	.36
	5–22	2.0	119.2	224.3	.53
	6–04	2.6	116.9	179.4	.65

[a]Data expressed on a dry matter basis.

[b]p-Coumaric acid.

[c]Ferulic acid.

[d]Immature regrowth.

in perennial ryegrass (7). Our data demonstrated significant linear relationship between the ratio of PCA:FA and IVDMD ($r=-.84$, $P<.01$, Figure 1). The relationship between the PCA content and IVDMD ($r=-.86$, $P<.01$) was also significant (Figure 2). However, the relationship between the FA content and IVDMD was not significant. Increase of PCA content during growth was larger and more consistent than for FA.

In grasses, FA was bound to cell wall polysaccharides in both unlignified cell types (6) and in lignified cell walls (8); PCA served as the crosslinking agent between lignin and structural carbohydrates (12). In addition, Hartley (7) demonstrated that FA was solubilized more readily by sodium sulfite and also in the alimentary tract of sheep than was PCA. Those findings, along with trends in our study, indicate that FA and PCA may reside in different structural environments within the cell wall.

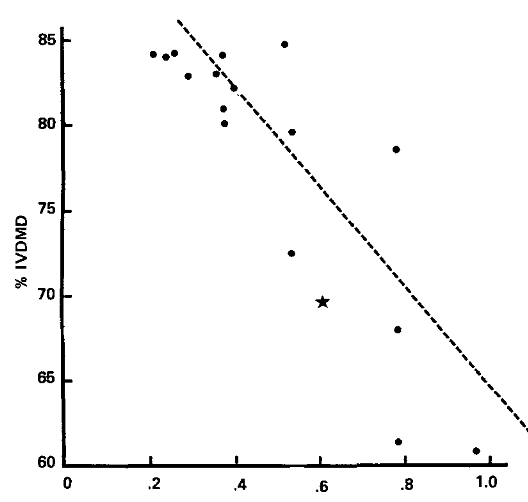

Figure 1. Relationship between ratio of p-coumaric to ferulic acid (PCA:FA) and in vitro dry matter digestibility (IVDMD) in three maturing grasses ($r=-.84$, $P<.01$, SEE = 4.51). ★, Russian wildrye regrowth data point.

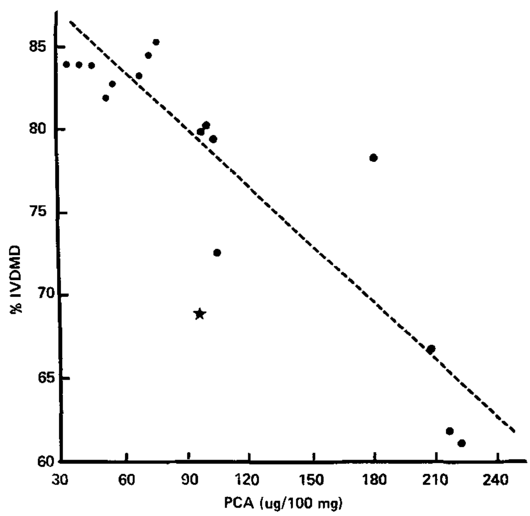

Figure 2. Relationship between p-coumaric acid (PCA) and in vitro dry matter digestibility (IVDMD) in three maturing grasses (r=–.86, *P*<.01, SEE = 4.24). ★, Russian wildrye regrowth data point.

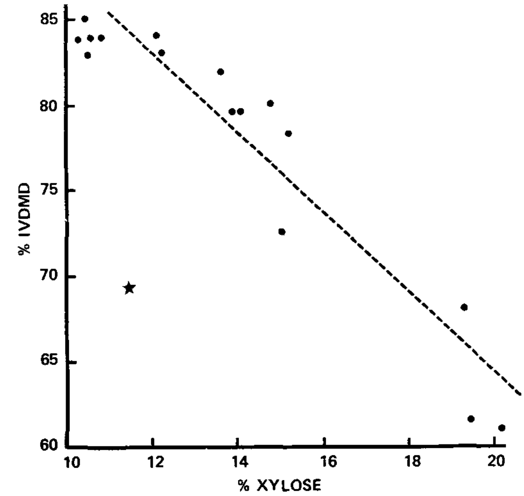

Figure 3. Relationship between percent xylose and in vitro dry matter digestibility (IVDMD) in three maturing grasses (r=–.85, *P*<.01, SEE = 4.38). The correlation coefficient omitting the Russian wildrye regrowth data point was –.94 (*P*<.01, SEE = 2.82). ★, Russian wildrye regrowth data point.

In addition, PCA may be involved more than FA in crosslinking lignin to structural carbohydrates.

The correlation between percent xylose and IVDMD was also significant (r=–.85, *P*<.01). This correlation was increased substantially by omission of the wildrye regrowth data point (r=–.94, *P*<.01, Figure 3). The significant correlation between percent xylose and IVDMD may have reflected the increasing association of lignin with xylans in maturing grass cell walls resulting in reduced digestibility. Waite et al. (18) observed that digestibility of xylans decreased more substantially during plant growth than cellulose or other hemicellulosic polymers. Xylans were associated closely with lignin in lignin-carbohydrate complexes (12).

Both percentage of xylose and the ratio of xylose to arabinose (X:A) increased during plant growth in all three plant species (Table 2). Trends were similar for other grass species (19). Morrison (13) reported that an increase in the ratio of X:A in ryegrass reflected an increase of linear xylan relative to branched xylan in the hemicellulosic fraction. The increase of the ratio of X:A in each of the three plant species also may indicate a relative change with maturity of hemicellulosic polymer type from branched to linear xylan.

TABLE 2. Xylose content and xylose to arabinose ratio of maturing Russian wildrye, smooth bromegrass, and reed canarygrass.[a]

Sample	Collection date	Xylose	Ratio X:A[b]
		(%)	
Russian wildrye	4–24	10.5	6.2
	4–30	13.6	8.4
	5–12	15.2	10.6
	6–04	20.5	11.8
	6–16[c]	11.4	7.8
Smooth bromegrass	4–16	10.1	5.0
	4–24	10.8	5.3
	4–30	13.9	7.1
	5–12	14.7	7.4
	6–04	19.8	13.1
	6–16	19.6	13.2
Reed canarygrass	4–22	10.6	3.4
	4–28	10.8	3.2
	5–05	12.1	3.8
	5–13	12.3	3.9
	5–22	14.1	4.3
	6–04	15.0	4.8

[a]Data expressed on a dry matter basis.

[b]Ratio of xylose to arabinose.

[c]Immature regrowth.

The Russian wildrye regrowth sample from June 16 was similar in appearance to the wildrye sample collected on April 24. Both regrowth sample and most immature wildrye samples had similar ratios of X:A and percentages of xylose in their cell walls. The phenolic acid composition of the wildrye regrowth sample was also comparable to the most immature wildrye samples. However, the regrowth sample was high in lignin despite its immature appearance. Lignification of plant cell wall increased with increasing temperature (17), and the higher mean temperature during the latter season may have contributed to the relatively higher lignin content in the regrowth sample.

Digestibility of the regrowth sample was considerably less than that of the May 12 sample despite its similar lignin content. In addition to the influence of the greater lignin content, the digestibility of the regrowth sample may have been affected by branched arabinoxylans in the cell walls. Branched xylans are more abundant in cell walls of immature grasses than are linear xylans as reflected by lower ratios of X:A (13). Studies using hemicellulose isolates from plant cell walls showed that branched xylans tend to be less digestible than linear xylans (3). As a result, the ratio of arabinose to xylose in conjunction with other cell wall components may be useful factor for predicting IVDMD of grasses (4).

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