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## Comparison of Laboratory Methods for the Prediction of in Vitro Dry Matter Digestibility in Three Maturing Grasses

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Maturing reed canary grass, Russian wild rye, and smooth brome grass were freeze-dried and ground. Samples were analyzed gravimetrically for detergent fiber components and cell wall constituents by using spectrophotometric and gas-liquid chromatographic assays. The relationship between cell wall composition and in vitro dry matter digestibility (IVDMD) was investigated by using regression methods. Acid detergent fiber and acid detergent lignin were the best single parameters for predicting IVDMD. However, multiple linear regression equations utilizing the monomeric constituents of plant cell wall polysaccharides, lignin and silica, provided the best estimates of IVDMD. The arabinose:xylose ratio and galactose content may reflect the importance of hemicellulosic polymer branching on the digestibility of forages.

Digestibility is one of the major factors determining the feeding values of forages. Since cell contents are considered readily available, dry matter digestibility becomes largely a function of cell wall digestibility (Van Soest, 1975). Presently, almost all descriptions of forage composition use gravimetric methods that fractionate cell walls on the basis of their solubility in a particular solvent system (Goering and Van Soest, 1970). These methods fail to accurately estimate digestibility over a variety of conditions (Oh et al., 1966; Barton et al., 1976). Inglett and Falkenhag (1979) reported that plant cell walls may be quantified by their monosaccharide constituents. Separation of plant cell walls into their constituent monosaccharides may allow for more accurate predictions of digestibility, as well as lead to a better understanding of the plant related factors that influence digestibility.

The purpose of this study was to compare two chemical schemes of analysis as predictors of in vitro dry matter digestibility (IVDMD) in three maturing grass species. The detergent fiber system of analysis and the monomeric constituents of plant cell wall polysaccharides were utilized.

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## MATERIALS AND METHODS

Reed canary grass (*Phalaris arundinacea* L.) leaf blades were collected at six stages of maturity from April 22 to June 4 from the Utah State University dairy farm. Maturities ranged from early leaf to milk stage. Smooth brome grass (*Bromis inermis* L.) whole plants were harvested at six stages of maturity from April 16 to June 16. Stages of maturity ranged from early leaf to dough stage. Russian wild rye (*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 Russian wild rye samples were harvested between the immature and dough stages, while the fifth was harvested as immature regrowth on June 16, ten days after the plot had been mowed to a 15-cm stubble height. Upon collection, samples were immediately frozen on dry ice, freeze-dried, and ground in a Wiley mill equipped with a 1-mm screen. Half of each ground sample was reground through a cyclone mill equipped with a 0.5-mm screen.

Coarsely ground plant samples were analyzed gravimetrically for neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) as outlined by Goering and Van Soest (1970), except that asbestos was not used in the ADL determinations. Acid insoluble ash (AIA) was determined by the method of Fonnesbeck (1976).

Approximately 5 g of finely ground plant material was refluxed for 1 h in 150 mL of 80% aqueous ethanol, while being stirred continuously with a magnetic stirring bar.

**Table I. Detergent Fiber Parameters of Three Grasses Harvested at Different Stages of Maturity<sup>a,b</sup>**

sample	collection date	NDF, %	ADF, %	ADL, %	AIA, %	hemicellulose, <sup>c</sup> %	cellulose, <sup>d</sup> %
Russian wild rye	4-24	48.8 ± 0.1	25.9 ± 0.4	2.3 ± 0.1	0.9 ± 0.0	22.8 ± 0.4	22.8 ± 0.3
	4-30	50.9 ± 0.1	27.9 ± 0.1	2.6 ± 0.1	0.8 ± 0.0	23.0 ± 0.1	24.4 ± 0.4
	5-12	51.7 ± 0.2	29.7 ± 0.3	3.1 ± 0.1	1.2 ± 0.1	22.0 ± 0.3	25.4 ± 0.4
	6-04	64.2 ± 0.3	39.6 ± 0.6	5.1 ± 0.1	1.9 ± 0.1	25.0 ± 0.5	32.6 ± 0.6
smooth bromegrass	6-16	53.7 ± 0.2	32.2 ± 0.1	3.4 ± 0.1	4.0 ± 0.2	21.5 ± 0.2	24.8 ± 0.2
	4-16	43.0 ± 0.2	22.9 ± 0.1	1.8 ± 0.1	2.1 ± 0.1	20.1 ± 0.2	19.0 ± 0.2
	4-24	43.4 ± 0.5	25.0 ± 0.2	1.9 ± 0.1	1.8 ± 0.1	18.4 ± 0.4	21.3 ± 0.1
	4-30	47.7 ± 0.7	28.2 ± 0.1	2.4 ± 0.1	2.0 ± 0.1	19.5 ± 0.7	23.8 ± 0.1
	5-12	48.8 ± 0.1	28.2 ± 0.6	2.2 ± 0.1	1.8 ± 0.1	20.6 ± 0.6	24.2 ± 0.2
	6-04	58.4 ± 0.3	36.3 ± 0.1	3.0 ± 0.1	2.9 ± 0.2	22.0 ± 0.3	30.3 ± 0.4
	6-16	63.1 ± 0.2	40.6 ± 0.1	4.6 ± 0.1	2.8 ± 0.1	22.5 ± 0.2	33.1 ± 0.3
	4-22	35.8 ± 0.2	19.5 ± 0.2	1.9 ± 0.1	2.3 ± 0.1	16.3 ± 0.2	15.2 ± 0.1
reed canary grass	4-28	35.4 ± 0.1	19.2 ± 0.5	1.3 ± 0.1	1.8 ± 0.1	16.2 ± 0.5	16.2 ± 0.3
	5-05	37.8 ± 0.1	22.7 ± 0.1	1.2 ± 0.2	2.7 ± 0.2	15.1 ± 0.1	18.8 ± 0.3
	5-13	36.0 ± 0.4	22.2 ± 0.7	1.2 ± 0.1	2.7 ± 0.1	13.8 ± 0.6	18.4 ± 0.1
	5-22	41.9 ± 0.6	26.4 ± 0.2	2.0 ± 0.1	3.4 ± 0.1	15.5 ± 0.5	21.0 ± 0.9
	6-04	43.1 ± 0.3	27.8 ± 0.3	2.6 ± 0.3	3.4 ± 0.2	15.3 ± 0.3	21.8 ± 0.5

<sup>a</sup>Data expressed on a dry matter basis. <sup>b</sup>Mean values ± s.e. based on duplicate samples. <sup>c</sup>NDF - ADF. <sup>d</sup>ADF - (ADL + AIA).

Samples were filtered through a buchner funnel with a fritted disk (coarse porosity) and the remaining residues were washed with 3 × 50 mL of hot ethanol or until filtrates were colorless. The alcohol insoluble residues (AIR) were dried in a vacuum oven overnight at 40 °C.

A 25–30-mg portion of the AIR was hydrolyzed in 1 mL of 72% sulfuric acid at 25 °C for 30–45 min depending on the degree of lignification of the sample. Samples were diluted to 2 N and hydrolysis was continued for 1 h at 95 °C. Following hydrolysis, a 200 µL aliquot of each acid hydrolyzate was analyzed for neutral sugars (Bittner et al., 1980). The procedure was modified in the following manner: prior to the addition of *N*-methylimidazole, 20 µL of deionized water was added to each tube (Bittner, 1982). Samples were injected into a MT-220 (Tracor) 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. Column temperature was held at 200 °C for 2 min, programmed at 2 °C/min to 240 °C, and held at this temperature for 18 min. Detector and injector temperatures were held at 225 and 245 °C, respectively. A 200-µL aliquot of the 1 h acid hydrolyzates was analyzed colorimetrically for uronic acids (Blumenkrantz and Asboe-Hansen, 1973).

Cellulosic glucose was estimated by extracting finely ground plant material with acid detergent solution (Heller et al., 1977). ADF residues were dried overnight in a 60 °C vacuum oven. A 15–20-mg portion of the ADF residue was hydrolyzed in 1 mL of 72% sulfuric acid for 45 min at 25 °C while being stirred intermittently with a glass rod. Samples were then diluted to 2 N acid, and hydrolysis was allowed to continue for 3 additional h at 95 °C (Bittner and Street, 1983). A 1-mL aliquot of the acid solution was diluted and analyzed for glucose by using the glucose oxidase procedure (Fleming and Pegler, 1963). All the glucose present in the ADF residue was considered to be of cellulosic origin (Morrison, 1980).

Starch content of the AIR was quantitated as the amount of glucose released upon treatment of the AIR with a purified amyloglucosidase enzyme (McRae, 1971).

Total glucan content of the cell wall was estimated as the amount of glucose released from the AIR after a two-step acid hydrolysis. A 25–30-mg sample of the AIR was hydrolyzed in 1 mL of 72% sulfuric acid for 30–45 min. Samples were diluted to 2 N acid and hydrolysis was continued at 95 °C for 3 h. A 1-mL aliquot of the acid hydrolyzates was diluted and analyzed for glucose with the

glucose oxidase procedure (Fleming and Pegler, 1963). Hemicellulosic glucose was determined by subtraction [total glucose in the AIR - (cellulosic glucose + glucose from starch)].

IVDMD values were determined by using a two-stage *in vitro* rumen fermentation technique (Marten and Barnes, 1979). Rumen fluid was obtained from a ruminally fistulated dairy cow maintained on alfalfa hay.

Relationships between plant cell wall constituents and percent IVDMD were determined by using correlation and linear and step-wise linear multiple regression analyses. Standard errors of estimate (SEE) were also computed (Neter and Wasserman, 1974).

## RESULTS AND DISCUSSION

Means and standard errors for detergent fiber parameters are presented in Table I. Monomeric constituents of plant cell wall polysaccharides and IVDMD values are presented in Table II.

In comparing the estimates of hemicellulose by using detergent fiber parameters (NDF-ADF) vs. summing the individual hemicellulosic monomers, differences are apparent. Detergent fiber hemicellulose (NDF-ADF) was probably overestimated in immature samples as a result of protein contamination of the NDF residues (Theander and Aman, 1980). Whereas, in the more mature samples NDF-ADF resulted in lower hemicellulosic values probably due to increasing concentrates of hemicellulosic residues remaining in the ADF fraction (Bittner and Street, 1983) and an increasing AIA concentration (Van Soest et al., 1978).

Correlation coefficients (*r*) and SEE values relating IVDMD to cell wall parameters are reported in Table III. Correlations between detergent fiber parameters and IVDMD are consistent with previous findings (Oh et al., 1966; Mowat et al., 1969). NDF was a less reliable predictor of digestibility than either ADF or ADL. Van Soest (1975) reported that NDF tended to be a better indicator of intake than digestibility. The highly significant correlations between IVDMD and detergent fiber parameters are plausible since NDF, ADF, and ADL all contain lignin, a major factor limiting the digestibility of forages (Barton et al., 1976).

Detergent fiber hemicellulose (NDF-ADF) was a poor indicator of digestibility. The correlation between detergent fiber cellulose [ADF - (ADL + AIA)] and IVDMD was highly significant, although it was lower than correlations for either ADF or ADL.

Table II. Cell Wall Monosaccharide Constituents and in Vitro Dry Matter Digestibilities of Three Maturing Grasses<sup>a,b</sup>

sample	collection date	arabinose, %	xylose, %	galactose, %	hemicellulosic, %	uronic acids, %	cellulosic glucose, %	IVDMD, %
Russian wild rye	4-24	1.7 ± 0.1	10.8 ± 0.3	0.5 ± 0.05	3.0 ± 0.1	3.3 ± 0.3	17.3 ± 0.1	84.8 ± 0.6
	4-30	1.7 ± 0.1	14.0 ± 0.2	0.5 ± 0.01	2.8 ± 0.3	3.3 ± 0.3	20.2 ± 0.3	81.9 ± 0.3
	5-12	1.4 ± 0.1	15.2 ± 0.1	0.5 ± 0.02	1.3 ± 0.2	3.0 ± 0.2	21.8 ± 0.2	78.6 ± 0.8
	6-04	1.8 ± 0.1	21.0 ± 0.2	0.6 ± 0.01	1.0 ± 0.1	3.0 ± 0.3	27.6 ± 0.6	60.9 ± 0.9
smooth bromegrass	6-16	1.5 ± 0.1	11.8 ± 0.4	0.6 ± 0.03	c	3.0 ± 0.1	21.4 ± 1.3	69.2 ± 0.6
	4-16	2.0 ± 0.1	10.1 ± 0.3	0.8 ± 0.04	0.8 ± 0.1	3.2 ± 0.1	16.1 ± 0.4	84.1 ± 1.4
	4-24	2.0 ± 0.1	10.8 ± 0.2	0.7 ± 0.01	0.3 ± 0.07	3.4 ± 0.1	18.3 ± 0.2	83.1 ± 0.7
	4-30	2.0 ± 0.1	13.9 ± 0.8	0.8 ± 0.01	3.1 ± 0.3	3.4 ± 0.3	20.3 ± 0.4	80.0 ± 1.0
reed canary grass	5-12	2.0 ± 0.1	14.7 ± 0.5	0.6 ± 0.01	3.4 ± 0.1	3.4 ± 0.1	19.3 ± 0.6	80.3 ± 0.7
	6-04	1.5 ± 0.1	19.8 ± 0.1	0.6 ± 0.01	4.3 ± 0.3	2.7 ± 0.2	26.5 ± 0.7	68.0 ± 1.1
	6-16	1.5 ± 0.2	19.6 ± 1.3	0.6 ± 0.06	2.3 ± 0.2	2.7 ± 0.1	28.1 ± 0.3	61.5 ± 0.7
	4-22	3.2 ± 0.1	10.5 ± 0.3	1.7 ± 0.09	1.1 ± 0.1	3.6 ± 0.2	13.6 ± 0.2	84.0 ± 2.4
	4-28	3.4 ± 0.2	10.9 ± 0.4	1.8 ± 0.08	2.7 ± 0.1	3.5 ± 0.4	13.4 ± 0.4	84.0 ± 2.1
	5-05	3.2 ± 0.1	12.0 ± 0.3	1.8 ± 0.01	1.6 ± 0.1	3.8 ± 0.3	15.7 ± 0.5	84.3 ± 0.9
	5-13	3.2 ± 0.1	12.2 ± 0.5	1.9 ± 0.05	3.0 ± 0.3	3.6 ± 0.2	15.1 ± 0.6	82.9 ± 1.0
	5-22	3.3 ± 0.2	13.9 ± 0.1	1.3 ± 0.04	1.3 ± 0.2	3.2 ± 0.2	18.1 ± 0.2	79.5 ± 0.7
6-04	3.0 ± 0.1	14.1 ± 0.4	1.5 ± 0.05	0.4 ± 0.05	3.1 ± 0.1	19.0 ± 0.3	72.7 ± 1.1	

<sup>a</sup>Data expressed on a dry matter basis. <sup>b</sup>Mean values ± s.e. based on duplicate samples for monomeric constituents and triplicate samples for IVDMD determination. <sup>c</sup>None detected.

Table III. Simple Correlation Coefficients between in Vitro Dry Matter Digestibility and Plant Cell Wall Constituents

independent variable	r	SEE	independent variable	r	SEE
NDF	-0.84 <sup>b</sup>	4.51	arabinose	-0.47 <sup>a</sup>	7.34
ADF	-0.93 <sup>b</sup>	3.06	xylose	-0.85 <sup>b</sup>	4.38
ADL	-0.91 <sup>b</sup>	3.45	galactose	-0.40	7.62
AIA	-0.41 <sup>a</sup>	7.58	uronic acids	-0.80 <sup>b</sup>	4.99
NDF - ADF	-0.51 <sup>a</sup>	7.15	hemicellulosic glucose	0.08	8.28
ADF - (ADL + AIA)	-0.86 <sup>b</sup>	4.24	cellulosic glucose	-0.90 <sup>b</sup>	3.62

<sup>a</sup>P < 0.05. <sup>b</sup>P < 0.01.

Cell wall monosaccharide constituents were poorer indicators of digestibility than either ADF or ADL. In the forages studied, it appears that lignification of plant cell wall was more important in governing digestibility than was the monomeric composition of plant cell wall polysaccharides (Brice and Morrison, 1982). The correlation coefficients for galactose and hemicellulosic glucose were both nonsignificant (Table III). The correlation between arabinose and IVDMD was significant but low, indicating little predictive value. Xylose was highly correlated with IVDMD, perhaps indicating the increasing association of lignin with xylans in maturing grasses (Burrill et al., 1984). The highly significant correlation between uronic acids and IVDMD probably resulted from the small variations noted in uronic acid contents of the different forage species at different stages of growth.

Cellulose was highly correlated to IVDMD regardless of the system of analyses employed (Table III). The SEE for cellulose was improved when cellulosic glucose was correlated with IVDMD rather than detergent fiber cellulose [ADF - (ADL + AIA)]. Chesson (1981) reported that barley cellulose showed more disorder and degraded at a

faster rate than wheat cellulose. Therefore, the cellulose content of a forage might be a useful indicator of digestibility only if the crystallinities of cellulose polymers were similar between grasses. In addition, Van Soest et al. (1978) reported that lignin and cellulose were highly associated in first cutting forages. The effect of cellulose content on digestibility may be related both to the crystalline nature of the cellulose polymer and their association with lignin.

A summary of the step-wise multiple regression for detergent fiber parameters and monomers of plant cell wall polysaccharides is presented in Table IV. All equations had highly ( $P < 0.01$ ) significant coefficients of determination ( $R^2$ ). Equation 1 used detergent fiber cellulose, hemicellulose, ADL, and AIA to predict IVDMD. Equation 2 used the monomeric constituents of plant cell wall polymers plus ADL and AIA to predict IVDMD and resulted in the highest  $R^2$  with a lower SEE than eq 1. These results suggest that separating plant cell wall polysaccharides into their monosaccharide constituents may be advantageous for the purposes of predicting digestibility.

Two equations (eq 3 and 4), selected by step-wise multiple regression, had higher  $R^2$  and lower SEE values than eq 1 (Table IV). Both of these equations implemented ADL, AIA, and cellulosic glucose for the prediction of IVDMD. The fourth term, the arabinose:xylose ratio (A:X) used in eq 3 and the galactose content used in eq 4, is believed to depict the importance of hemicellulosic polymer branching on forage digestibility.

The A:X ratio used in eq 3 is believed to indicate the extent of hemicellulosic polymer branching in plant cell walls (Morrison, 1974). An increasing A:X ratio reflects an increase in the amount of branched xylan relative to linear xylan in maturing grasses. Brice and Morrison (1982) reported that a positive relationship existed between

Table IV. Summary of Multiple-Linear Regression Analysis for Relating in Vitro Dry Matter Digestibility to Plant Cell Wall Constituents

regression equations <sup>a</sup>	R <sup>2</sup>	SEE
(1) $Y = 100.29 - 5.05(\text{ADL}) - 2.43(\text{AIA}) + 0.46(\text{NDF} - \text{ADF}) - 0.58[\text{ADF} - (\text{ADL} + \text{AIA})]$	0.967 <sup>b</sup>	1.69
(2) $Y = 108.04 - 4.61(\text{ADL}) - 2.36(\text{AIA}) + 2.52(\text{A}) - 0.29(\text{X}) - 3.28(\text{Gal}) - 0.29(\text{HGlu}) + 1.82(\text{UA}) - 29.62(\text{A/X}) - 0.62(\text{Cell})$	0.984 <sup>b</sup>	1.54
(3) $Y = 122.55 - 3.96(\text{ADL}) - 2.21(\text{AIA}) - 35.56(\text{A/X}) - 1.22(\text{Cell})$	0.975 <sup>b</sup>	1.47
(4) $Y = 113.26 - 4.39(\text{ADL}) - 2.31(\text{AIA}) - 3.43(\text{Gal}) - 0.84(\text{Cell})$	0.978 <sup>b</sup>	1.38

<sup>a</sup>ADL = acid detergent lignin; AIA = acid insoluble ash; (NDF - ADF) = hemicellulose; [ADF - (ADL + AIA)] = cellulose; A = arabinose; X = xylose; Gal = galactose; HGlu = hemicellulosic glucose; UA = uronic acids; A/X = arabinose:xylose ratio; Cell = cellulosic glucose. <sup>b</sup>P < 0.01.

an increasing X:A ratio and cell wall degradability. The negative relationship that exists between the A:X ratio and IVDMD is believed to reflect the negative effect that an increase in xylan polymer branching in the hemicellulosic fraction has on forage digestibility.

The percentage of galactose in the cell wall (eq 4) may also be an indicator of polymer branching, since galactose exists predominately in the hemicellulosic fraction of cell wall as a constituent of the side chains of galactarabinoxylans (Reid and Wilkie, 1969). Reed canary grass samples had the highest A:X ratios as well as the highest galactose contents of the grasses studied. A negative relationship exists between galactose and IVDMD in eq 4.

Research relating the monomeric constituents of plant cell polysaccharides to digestibility is limited. Results presented in this study indicate possible advantages in using these types of parameters as estimators of digestibility and as a way to gain a better understanding of plant-related factors influencing digestibility.

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**Registry No.** Lignin, 9005-53-2; arabinose, 147-81-9; xylose, 58-86-6; galactose, 59-23-4; silica, 7631-86-9; hemicellulose, 9034-32-6; cellulose, 9004-34-6.

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## Composition of the Mucilaginous Spore Matrix of *Colletotrichum graminicola*, a Pathogen of Corn, Sorghum, and Other Grasses

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A mucilaginous matrix produced by the fungus *Colletotrichum graminicola* has several properties which enhance survival of fungal spores. The purpose of this research was to resolve and characterize the compounds of the matrix to facilitate investigation of their function. The major component is a group of high molecular weight glycoproteins composed of oxygen-linked oligomers of rhamnose and mannose and high levels of hydrophobic and hydroxylic amino acids. The amino acid composition and percentage carbohydrate of this viscous material are similar to that of mucins, which may account for the antidesiccant property of the matrix. In addition to the previously reported invertase and esterase, a specific  $\beta$ -glucosidase was identified as a matrix component. Three nonprotein, UV-absorbing components were resolved from the matrix; one of these was identified as uracil.

The anthracnose disease of corn and sorghum is a limiting factor in the production of these crops in the developing nations of the humid and semiarid tropics

(Hooker, 1977; Pastor-Corrales and Frederiksen, 1980), and anthracnose has now become one of the most important diseases of corn in the United States where it often reaches epiphytotic proportions resulting in substantial crop loss in localized areas (Hooker, 1977; Lipps, 1983). The potential threat of this disease is further emphasized by the fact that, unlike other fungal pathogens of corn, *Colletotrichum graminicola* (Ces.) Wils. (*Glomerella graminicola* Politis) is capable of attacking all parts of the plant and at any time during the growing season.

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The repeated occurrence of the disease in a localized area is associated with the practice of soil conservation through minimum tillage where the fungus survives the winter in infested plant debris on the soil surface (Lipps, 1983).