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J. O. Fritz
USDA-ARS

K. J. Moore
USDA-ARS

Kenneth P. Vogel
University of Nebraska-Lincoln, kvogel1@unl.edu

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J. O. Fritz, K. J. Moore,* and K. P. Vogel

ABSTRACT

Improvement in the forage quality of switchgrass (*Panicum virgatum* L.) through phenotypic selection for increased in vitro dry matter digestibility (IVDMD) has been demonstrated. This study tested the hypothesis that genetic improvement of fiber digestibility in switchgrass has been achieved by selection for a strain with a decreased quantity of ammonia-labile bonds. Tissue samples of a high-digestibility (high-IVDMD) and a low-digestibility strain (low-IVDMD) of switchgrass were ammoniated at rates of 0, 10, 20, and 40 g kg^{-1} dry matter. Fiber composition and in vitro rate and extent of neutral-detergent fiber (NDF) digestion were determined on control and ammoniated samples. The high-IVDMD strain had lower ($P < 0.05$) concentrations of NDF and acid-detergent lignin (ADL) than the low-IVDMD strain. Lignin concentrations averaged 53 and 71 g kg^{-1} for the high- and low-IVDMD strains, respectively. The high-IVDMD strain had a greater ($P < 0.05$) extent of NDF digestion when compared with the low strain; however, the rate of NDF digestion did not differ ($P > 0.05$) between strains. Increased digestibility of the high-IVDMD strain was primarily attributed to increased cell-wall (NDF) digestibility. Ammoniation at 20 and 40-g kg^{-1} resulted in small decreases ($P < 0.05$) in NDF concentrations when compared with the control; however, ammoniation had no effect on hemicellulose, cellulose, or ADL concentrations. Ammoniation increased ($P < 0.05$) both the rate and extent of NDF digestion. Extent of NDF digestion averaged 0.395 for the control and 0.465, 0.498, and 0.493 for the 10, 20, and 40-g kg^{-1} treatments, respectively. Strain × ammoniation rate interaction was not significant for rate and extent of digestion, suggesting that genetic improvement in digestibility of switchgrass was not related to the number of ammonia-labile bonds.

Switchgrass is a perennial, warm-season grass with the potential to support ruminant animal production during the summer, when forage production from cool-season grasses normally is inadequate. Significant improvement in the forage quality of switchgrass has been achieved through selection for IVDMD. Vogel et al. (1981) reported an average difference in IVDMD of 34 g kg^{-1} DM between high- and low-IVDMD polycross (PC) strains after a single cycle of selection from a Nebraska experimental switchgrass population. Other studies have similarly reported a consistent difference between these strains in IVDMD (Vogel et al., 1984; Anderson et al., 1988). In a 3-yr pasture study, Anderson et al. (1988) reported greater ($P < 0.05$) average daily gains and gain per hectare for yearling beef cattle (*Bos taurus*) grazing the high-IVDMD PC ('Trailblazer') vs. the low-IVDMD PC strain.

The relationship between composition and digestibility is not fully understood for these divergently selected switchgrass strains. No substantial differences have been reported between these strains in detergent fiber composition (Anderson et al., 1988; Gabrielsen et al., 1990). Gabrielsen et al. (1990) recently reported greater ($P < 0.09$) concentrations of *p*-coumaric acid (PCA) in the low-IVDMD PC strain when compared with the high-IVDMD PC strain. *p*-Coumaric acid concentration is negatively correlated with fiber digestibility (Fritz et al., 1990) and differences between strains in PCA concentration may, at least in part, explain the observed differences between strains in IVDMD and animal performance.

Ammoniation has been shown to improve fiber digestibility of mature cool- and warm-season grass hays (Moore et al., 1985; Brown, 1988). Increased digestibility of ammoniated forage has been attributed to the breaking of ester linkages between lignin and cell-wall carbohydrates (Buettner et al., 1982; Tarkow and Feist, 1969). Current evidence suggests that alkali-labile linkages, such as ester bonds, function as cross-links between lignin and cell-wall carbohydrates (Gaillard and Richards, 1975; Scalbert et al., 1985) with phenolic acids serving as the bridging or cross-linking units (Morrison, 1974). The extent to which fiber digestibility can be improved through genetic selection for reduced lignin–carbohydrate bonding has not been examined.

The objective of this study was to test the hypothesis that the genetic improvement in digestibility achieved in switchgrass was accomplished by selection of strains with a reduced content of ammonia-labile linkages, particularly ester linkages, between lignin and cell-wall carbohydrates. The hypothesis was tested by ammoniating herbage from high- and low-IVDMD switchgrass strains and comparing the relative increase in the extent of fiber digestion between the strains. A similar amount of improvement in fiber digestibility due to ammoniation for the two strains would indicate that the genetic improvement in switchgrass digestibility was not due to genetic alteration of the quantity of ammonia-labile linkages.

MATERIALS AND METHODS

Switchgrass samples of two strains differing in IVDMD were harvested from a 3-yr-old stand established near Lincoln, NE. The strains will be referred to as high-IVDMD and low-IVDMD. The high-IVDMD strain was developed from seed collected from one of the original 25 parent clones of the high-IVDMD PC strain described by Vogel et al. (1981). Similarly, the low-IVDMD strain was developed from PC seed collected from the parent clone of the low-IVDMD PC strain determined to have the lowest IVDMD. Plants for each strain were established in single-row plots containing 12 plants. Distance between and within rows was 1.1 m. Plots were arranged in a randomized complete-block

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Abbreviations: ADF, acid-detergent fiber; ADL, acid-detergent lignin; DM, dry matter; DNDF, digestible neutral-detergent fiber; INDF, indigestible neutral-detergent fiber; IVDMD, in vitro dry matter digestibility; NDF, neutral-detergent fiber; PC, polycross; PCA, *p*-coumaric acid; *R*, reflectance.

design with four replicates. Randomly selected plants from each plot were cut to a stubble height of ≈10 cm using hand clippers, dried at 60 °C, and ground in a shear mill to pass a 1-mm screen. Both genotypes were at the head stage of maturity at time of harvest (10 August).

Samples of ground plant tissue were ammoniated at 0, 10, 20, and 40 g kg^{-1} DM. A 1.26-mL aliquot of a 0, 3.75, 7.5 and 15 *M* ammonia (NH_3) solution was added to 120-mL plastic containers containing 8 g of sample dry matter. Dry matter of plant material before ammoniation treatment averaged 970 g kg^{-1}. Ammonia solutions were made using commercially obtained ammonium hydroxide (15 *M* NH_3). The control solution contained deionized water. The containers were sealed and the treated samples incubated for 14 d at 39 °C. Samples were reground in a cyclone mill to pass a 1-mm screen and thoroughly mixed after ammoniation.

Samples of the treated plant tissue were analyzed for NDF, ADF, cellulose, and ADL using the procedures of Goering and Van Soest (1970). Hemicellulose was calculated as the difference between NDF and ADF. Neutral sugars in NDF were hydrolyzed and derivatized using the procedures described by Blakeney et al. (1983). Alditol acetate derivatives of the neutral sugars were separated and quantified by gas chromatography (Fritz et al., 1990). Total N concentration was determined for treated plant tissue and NDF (NDF-N) using procedures described by Carlson (1978).

Samples (250 mg) of the treated plant tissue were incubated in buffered rumen fluid for 6, 12, 18, 24, 36, 48, and 72 h using the inoculation procedures described by Marten and Barnes (1980). Rumen fluid was collected from two fistulated crossbred steers, one fed a diet of alfalfa (*Medicago sativa* L.) and the other a diet of coarsely ground corn (*Zea mays* L.) cobs, and blended (1:1 v/v). Fermentation was halted by rapid cooling (−10 °C) and residues were analyzed for NDF concentration within 48 h. Digestion kinetics for NDF digestion were calculated using the ln-linear least squares regression procedure described by Moore and Cherney (1986).

A detailed analysis of the interaction between the extent of NDF digestion and ammoniation rate was accomplished by fitting the nonlinear regression model $Y = \alpha - \beta e^{-kx}$, where α = asymptotic maximal extent of fiber digestion, β = increase in concentration of digestible fiber due to ammoniation treatment, and the expression e^{-kx} = fractional proportion of β made available at a given ammoniation rate. Estimates of model parameters were determined using a multivariate secant nonlinear least squares procedure (Ralston and Jennrich, 1979) and calculated using the NLIN procedure of SAS Institute (1985). In a separate fermentation, samples (1 g) of NDF extracted from ammonia-treated plant tissue were incubated with buffered rumen fluid for 96 h as previously described with the exception that urea was added to the buffer solution (0.5 g L^{-1}). Neutral-detergent fiber remaining after this fermentation was considered to be indigestible (INDF). Samples of INDF were analyzed for total N (INDF-N) as previously described.

Neutral-detergent fiber from control and ammoniated samples was analyzed spectrally using a Technicon InfraAlyzer 500 (Bran+Luebbe Analyzing Technologies, Elmsford, NY) near infrared reflectance scanning monochromator. Reflectance spectra were generated for wavelengths between 1100 and 2500 nm at 2-nm intervals. Reflectance (*R*) data were recorded as log 1/*R*. Spectra for all samples were regressed against known N values to determine which wavelengths were most strongly associated with changes in N concentration due to ammoniation.

Statistical significance of main and interaction effects were determined using conventional analysis of variance techniques. Given a significant *F*-test ($P < 0.05$), differences between treatment rate means were determined using Fisher's least significant difference (SAS Institute, 1985).

RESULTS AND DISCUSSION

Genetic Effect

The high-IVDMD strain had lower ($P < 0.05$) concentrations of NDF and ADL when compared with the low-IVDMD strain (Table 1). There were no differences ($P > 0.05$) between strains in the concentration of cellulose, hemicellulose, and N. Vogel et al. (1984) reported small differences between high- and low-IVDMD PC strains in NDF concentration; however, they found no significant differences between strains in the concentration of permanganate lignin. Anderson et al. (1988) and Gabrielsen et al. (1990) reported no biologically significant differences between the high- and low-IVDMD PC strains in either NDF or lignin concentration. In agreement with results for this study, other studies have reported similar crude protein concentrations in the high- and low-IVDMD PC strains (Vogel et al., 1984; Anderson et al., 1988).

Strains did not differ ($P > 0.05$) in the concentration of any cell-wall neutral sugar (Table 2). These results are consistent with the detergent-fiber data and indicate that decreased NDF concentration of the high-IVDMD strain may primarily be attributed to a lower ADL concentration.

Strains did not differ ($P > 0.05$) in the rate of in vitro NDF digestion or digestion lag time (Table 3). The high-IVDMD strain, however, had a greater ($P < 0.05$) 72-h extent of in vitro NDF digestion and concentration of digestible NDF (DNDF) when compared with the low-IVDMD strain. In vitro 72-h true digestibility averaged 660 and 565 g kg^{-1} DM ($P < 0.05$) for the high- and low-IVDMD strains, respectively.

Previous studies evaluating the divergently selected switchgrass strains described by Vogel et al. (1981) have tried to explain differences among strains in IVDMD based on differences in chemical composi-

Table 1. Concentrations of neutral-detergent fiber (NDF), cellulose, hemicellulose, acid-detergent lignin (ADL), and N in ammoniated switchgrass herbage as affected by genetic strain and ammoniation rate.

		NDF	Cellulose	Hemicellulose	ADL	N
		g kg^{-1} dry matter				
Strain†						
High-IVDMD		706	376	259	53	15.2
Low-IVDMD		730	388	249	71	15.0
SE‡		9.7	8.5	6.4	2.6	0.30
Ammoniation rate, g kg^{-1}						
0		725	383	254	67	10.0
10		719	381	259	58	14.6
20		713	383	249	59	17.6
40		715	380	247	64	18.3
SE		2.3	7.0	6.3	4.1	0.40
		ANOVA				
Source	df					
Strain (S)	1	*	NS	NS	**	NS
Rate (R)	3	*	NS	NS	NS	**
S × R	3	NS	NS	NS	NS	NS

**, *Significant at the 0.01 and 0.05 levels of probability, respectively. NS = not significant ($P > 0.05$).

† IVDMD = in vitro dry matter digestibility.

‡ Standard error of mean.

Table 2. Concentrations of neutral sugars in cell-wall hydrolysates from ammoniated switchgrass herbage as affected by genetic strain and ammoniation rate.

		Arabinose	Xylose	Galactose	Glucose
		g kg^{-1} hydrolysate			
Strain†					
High-IVDMD		39.5	430	15.2	515
Low-IVDMD		36.3	419	10.6	534
SE‡		3.01	8.6	0.99	3.4
Ammoniation rate, g kg^{-1} dry matter					
0		34.8	413	9.4	542
10		36.8	429	11.2	523
20		41.7	420	10.1	519
40		38.3	436	10.6	515
SE		2.41	8.5	0.54	5.0
		ANOVA			
Source	df				
Strain (S)	1	NS§	NS	NS	NS
Rate (R)	3	NS	NS	NS	NS
S × R	3	NS	NS	NS	NS

† IVDMD = in vitro dry matter digestibility.
‡ Standard error of the mean.
§ $P > 0.05$.

Table 3. ***In vitro*** **neutral-detergent fiber (NDF) digestion rate constant, 72-h extent of NDF digestion, digestion lag time, and concentration of digestible NDF (DNDF) in ammoniated switchgrass herbage as affected by genetic strain and rate of ammoniation.**

		Rate constant	Extent	Lag time	DNDF
		h^{-1}		h	g kg^{-1}
Strain†					
High-IVDMD		0.057	0.520	10.3	368
Low-IVDMD		0.061	0.406	10.4	296
SE†		0.0049	0.0045	0.64	5.9
Ammoniation rate, g kg^{-1} dry mater					
0		0.043	0.395	8.6	289
10		0.060	0.465	10.6	333
20		0.064	0.498	10.8	354
40		0.068	0.493	11.5	351
SE		0.0036	0.0116	0.99	7.8
		ANOVA			
Source	df				
Strain (S)	1	NS	**	NS	**
Rate (R)	3	**	**	NS	**
linear		**	**	NS	**
quadratic		*	**	NS	**
S × R	3	NS	NS	NS	NS

**,* Significance at the 0.01 and 0.05 levels of probability, respectively. NS = not significant ($P > 0.05$).
† IVDMD = in vitro dry matter digestibility.
‡ Standard error of the mean.

tion. Vogel et al. (1984) speculated that increased IVDMD in the high-IVDMD PC strain may, at least in part, be attributed to a decreased cell-wall (NDF) concentration. Gabrielson et al. (1990), however, concluded that differences between these strains in IVDMD could not be explained based on results of detergent-fiber analysis. Instead, they suggested that increased IVDMD in the high-IVDMD PC strain may be related to a decreased concentration of noncore lignin components, particularly PCA.

In this study, increased digestibility reported for the high-IVDMD strain could be attributed, in part, to an increase in cell-solubles content (decreased NDF) and a decreased core lignin concentration. Partitioning of the increase in true digestibility into the relative contributions from increased cell solubles and increased DNDF indicated that 75.6% of the increase in true digestibility was due to increased DNDF. It appears from these results that improvement in digestibility was largely due to selection for strains with increased cell-wall digestibility. The increased extent of fiber digestion observed for the high-IVDMD strain is likely attributable to a decreased lignin content. Rate of NDF digestion was not affected by the differences between strains in lignin concentration. These results are similar to those reported by Fritz et al. (1990) for brown-midrib lignin mutants of sorghum [Sorghum bicolor (L.) Moench] × sudangrass [*Sorghum* × *drummondii* (Steudel) Millsp. & Chase] hybrids and support the general hypothesis that lignin content affects the extent but not the rate of fiber digestion.

Ammoniation Effect

Neutral-detergent fiber concentrations were slightly lower ($P < 0.05$) for switchgrass herbage ammoniated at 20 and 40 g kg^{-1} DM when compared with the unammoniated control (Table 1). Ammoniation at these rates reduced NDF concentration by an average of only 1.5%. Hemicellulose, cellulose, and ADL concentrations were not affected ($P > 0.05$) by ammoniation treatment. The strain × ammonia treatment rate interaction was not significant for NDF or any fiber constituent. Ammoniation has consistently been shown to reduce the NDF concentration of mature grass hays (Moore et al., 1985; Gates et al., 1987). The reduction in fiber content following alkaline chemical treatment has largely been attributed to solubilization and loss of hemicellulose (Klopfenstein, 1978). Moore et al. (1985) determined that the amount of hemicellulose solubilized from orchardgrass (*Dactylis glomerata* L.) hay following ammoniation increased with increasing moisture concentration of the hay prior to treatment. In this study, switchgrass herbage was purposely ammoniated at a moisture concentration <5 g kg^{-1} in an attempt to minimize loss of hemicellulose. As a result, solubilization of hemicellulose was not apparent. In support of this, ammoniation had no affect ($P > 0.05$) on the concentration of any cell-wall neutral sugar (Table 2). The reduction in NDF concentration observed for this study is relatively small compared with reductions reported for other studies evaluating ammoniated switchgrass (Ward and Ward, 1987; Gates et al., 1987) and is not consistent with results observed for the individual fiber constituents (Table 1). Although statistically significant, it is doubtful that this reduction constitutes a real decrease in NDF due to chemical treatment and probably reflects a slight dilution due to increased N added by ammoniation. In agreement with results from this study, cellulose and lignin are generally considered to be unaffected by ammoniation and other alkaline treatments (Klopfenstein, 1978).

Total N concentration of switchgrass herbage increased ($P < 0.05$) nonlinearly with increasing ammoniation rate (Table 1). A similar relationship for increases in N with respect to ammoniation rate have been reported by Moore et al. (1985) for orchardgrass hay and by Brown (1988) for stargrass (*Cynodon nlem-*

Table 4. Concentrations of total nitrogen in neutral-detergent fiber (NDF-N) and 96-h indigestible neutral-detergent fiber (INDF-N) from ammoniated switchgrass herbage.

Ammoniation rate	NDF-N	INDF-N
g kg⁻¹ dry matter	g kg⁻¹	
0	0.57	0.33
10	0.71	0.36
20	0.74	0.42
40	0.71	0.43
SE†	0.023	0.012

† Standard error of the mean.

fuensis Vanderyst) hay. In this study, ammoniation at 40 g kg^{-1} resulted in an 83% increase in total N concentration. This increase is lower than the 112% increase reported by Ward and Ward (1987) and the 100% increase reported by Gates et al. (1987) for switchgrass hay ammoniated at 30 g kg^{-1} and most likely resulted from the lower pretreatment moisture content used in this study. Moore and Lechtenberg (1987) determined that moisture content was critical in ammonia absorption and retention in ammoniated hays. Ammoniation increased ($P < 0.05$) NDF-N concentration by an average of 26% (Table 4). There was no effect ($P < 0.05$) of strain or interaction of strain with ammoniation rate on NDF-N concentration. Increased concentration of NDF-N probably reflects amide formation resulting from ammonolysis of ester linkages in the cell-wall matrix. Nelson et al. (1984) reported small increases in cell-wall N (NDF-N and ADF-N) of corn cobs following ammonia treatment. All levels of ammonia treatment also increased ($P < 0.05$) the concentration of INDF-N when compared with control (Table 4).

Ammoniation increased ($P > 0.05$) the rate and 72-h extent of in vitro NDF digestion as well as the concentration of DNDF (Table 3). Increases in rate and extent of digestion and in DNDF concentration with respect to treatment rate occurred nonlinearly. Digestion lag time was only slightly increased ($P = 0.09$) with ammonia treatment. The strain × ammonia treatment rate interaction was not significant for any of the digestion kinetic parameters or for DNDF concentration. Increases in both the rate and extent of NDF digestion have been reported by Moore et al. (1985) for ammoniated orchardgrass silage and by Chestnut et al. (1984) for ammoniated tall fescue (*Festuca arundinacea* Schreb.). Gates et al. (1987) reported a significant increase in the in vivo rate of NDF digestion of ammoniated switchgrass. In contrast, Brown (1988) reported that the rate of NDF digestion of stargrass hay was not affected by ammoniation; however, 96-h extent of NDF digestion increased linearly with ammoniation level.

The increase in fiber digestibility of ammoniated forages is attributed to ammonolysis of ester linkages between components of the cell-wall matrix. Presumably, removal of the chemical and/or physical barriers imposed by these linkages would increase the susceptibility of polysaccharide components of the cell-wall matrix to enzymatic hydrolysis by ruminal microorganisms. The lack of a significant strain × rate interaction for extent of digestion (Table 3) implies that the increased fiber digestibility observed for the high-IVDMD strain was not due to selection for a cell-wall matrix with a lower ammonia-labile bond content. The relationship between the two strains for the effect of ammoniation on the extent of NDF digestion is illustrated in Fig. 1. The greater theoretical maximum extent of digestion (α) calculated for the high-IVDMD strain (0.569 for the high-IVDMD vs. 0.452 for the low-IVDMD strain) is consistent with the previously discussed in vitro kinetic data (Table 3). Increases in the concentration or pool of digestible fiber due to ammoniation (β), however, were similar for both strains (125 and 107 g kg^{-1} for the high- and low-IVDMD strains, respectively). Had the improvement in digestibility been due to selection for a strain with a lower concentration of ammonia-labile bonds, the increase in the digestible fiber concentration due to ammoniation would have been smaller for the high-IVDMD strain than for the low-IVDMD strain.

Average near infrared reflectance spectra for NDF from control and ammoniated switchgrass herbage are shown in Fig. 2. The most prominent differences be-

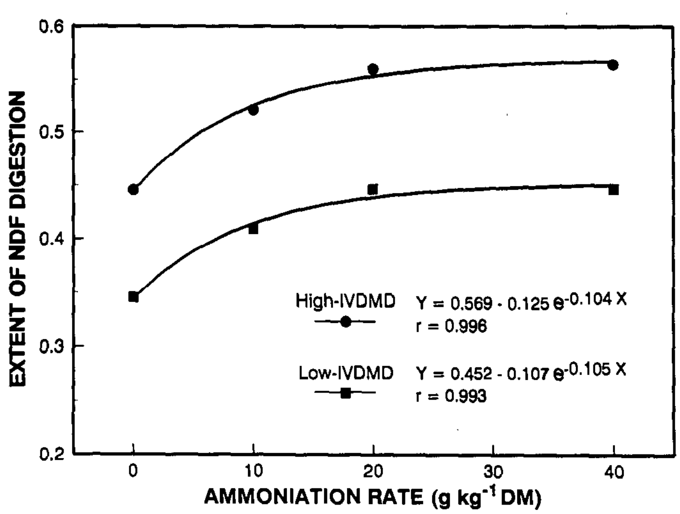

Fig. 1. Effect of ammoniation rate on the extent of neutral-detergent fiber (NDF) digestion for two switchgrass strains differing in in vitro dry matter digestibility (IVDMD).

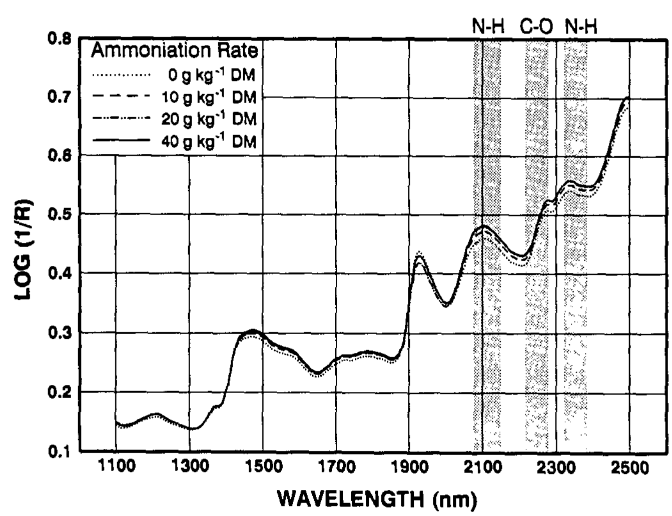

Fig. 2. Average near infrared reflectance (R) spectra for neutral-detergent fiber of control and switchgrass herbage ammoniated at different rates.

tween spectra for control and ammoniated samples occurred at wavelengths near 2100, 2280, and 2350 nm. These wavelengths lie within near infrared absorption bands which have been assigned to amide (2100 nm: N–H stretch; 2350 nm: N–H bend) and primary alcohol (2280 nm: C–O stretch) groups (Osborne and Fearn, 1986; Murray and Williams, 1987). Formation of these functional groups would be expected with ammonolysis of ester linkages within the cell wall. Buettner et al. (1982) reported a decreased infrared ester absorbance index (1730 cm^{-1}/2920 cm^{-1}) and an increased infrared amide absorbance index (1664 cm^{-1}/2920 cm^{-1}) for ammoniated tall fescue hay. Results of this study clearly support the hypothesis that the improvement in fiber digestibility of ammoniated forages occurs via a mechanism which involves the breaking of ester linkages within the cell-wall matrix. Wavelengths selected from regression of spectra for NDF of control and ammoniated samples against N concentrations were, with one exception, contained within the limits of the first near infrared band (near 2100 nm) illustrated in Fig. 2.

CONCLUSIONS

The genetic improvement in digestibility of the high-IVDMD strain of switchgrass evaluated in this study can be attributed largely to an increase in cell-wall (NDF) digestibility. This increase in cell-wall digestibility is probably due to selection for a strain with a lower lignin content and possibly altered lignin composition (Gabrielson, 1990). Genetic improvement in fiber digestibility did not occur as a result of selection for a decreased content of ammonia-labile bonds between lignin and cell-wall carbohydrates.

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