Fiber Composition and *in Vitro* Digestibility of Corn Stover Fractions in Response to Ammonia Treatment

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The composition and digestibility of corn stover leaves and stems after prolonged NH_3 treatment was compared with that after isonitrogenous NH_3 addition (NH_3 control), represented by samples immediately frozen after initial NH_3 application. Ammonia treatment decreased concentration of hemicellulose in leaves, particularly arabinose residues. Concentration of saponifiable hydroxycinnamic acids was reduced by NH_3 treatment in upper stems only. Ammonia addition, compared to a H_2O control, increased the extent of *in vitro* fiber degradation of all plant fractions but the rate of fiber degradation for leaves only. Ammonia treatment increased the extent of fiber degradation over NH_3 addition in leaves but not in stems. The variable response to NH_3 treatment among stover fractions of drought-stressed corn may be related to the high concentration of water-soluble carbohydrates in the stalks and immobilization of NH_3-N with lignin.

Keywords: *Ammoniation; Zea mays; lignin; hemicellulose; hydroxycinnamic acids; NPN immobilization; digestibility*

INTRODUCTION

Corn stover is characterized by low concentration of crude protein (CP) and low digestibility (Russell, 1986). Anatomical fractions of forages differ in nutritive quality, leaves generally being higher in CP and lower in lignin (Morrison, 1980). Lignin reduces fiber digestibility, presumably by interlinkages with carbohydrates (Jung, 1989, 1990). Recent evidence suggests that esterified ferulic acid acts as nucleation site for lignification by radical dependent dimerization and polymerization with monolignols, thereby providing crosslinks between lignin and hemicellulose (Ralph *et al.*, 1995; Grabber *et al.*, 1995; Jacquet *et al.*, 1995). Furthermore, the lignin monomeric composition (*i.e.*, syringyl/guaiacyl ratio (S/G ratio) or, alternatively, methoxyl content) affects the formation of intra- and intermolecular linkages via quinone methides during lignification and lignin aging (Leary, 1980). The reported negative correlation of S/G ratio (Buxton and Russell, 1988; Reeves, 1985) and methoxyl content (Quicke and Bentley, 1959; Sewalt *et al.*, 1993) with forage digestibility may be explained by the higher likelihood of quinone methide formation and regeneration from syringyl lignin than from guaiacyl lignin (Glasser and Kelley, 1987).

Alkali treatment results in saponification of ester linkages between cell wall components (Chesson, 1988). After treatment of low-protein crop residues with strong alkali, NPN supplementation is required to match the increase in potentially available fermentable energy, if voluntary feed intake is to be maximized (Ørskov and Grubb, 1978). The effectiveness of ammoniation in hydrolyzing ester linkages has been questioned in relation to the buffering capacity of many residues (Van Soest *et al.*, 1984). With ammoniation, the added NPN is likely to be partly responsible for the observed increase in digestibility (Ibrahim *et al.*, 1989).

The objectives of this research were to determine the response in cell wall composition and digestibility of different fractions of drought-stressed corn stover to ammonia treatment and to separate the response to ammoniation into NPN and chemical treatment effects.

MATERIALS AND METHODS

Plant Material and Processing. Stover (approximately 50% DM, 4 weeks after physiological maturity) from mildly drought-stressed corn (*Zea mays* L.) was harvested manually on October 22, 1991, after a dry summer and early fall (average monthly precipitation, June to October: 34 mm) from six replicate field plots near Blacksburg, VA. A 3 × 3 factorial arrangement of treatments was used, consisting of three plant fractions, leaf, upper stem, and lower stem, and three processing methods, H_2O control, NH_3-added control, and NH_3 treatment. The second processing method was used to separate the response to NH_3 treatment into chemical treatment and NPN addition. Field plot replication was maintained throughout the sampling and treatment procedure.

Plant material was separated into leaf blades and stems (including leaf sheaths). Stems were further divided in upper (above ear) and lower (below ear) fractions. Replicate fractions were chopped through a 1-cm screen and subdivided into two batches each. To one batch, NH_4OH was added to provide 3% NH_3, DM basis, for all fractions, and H_2O was added to achieve 45% DM for leaf and upper stem. Initial DM content of lower stem was already lower than desired (35%). Therefore, moisture addition to lower stem was minimized by application of undiluted (30%) NH_4OH. After thorough mixing of the NH_4OH with the plant material, initial samples were taken, which represented the treatment designated as NH_3-added control. For NH_3 treatment, the ammoniated material was packed manually into 4-L cardboard containers double-lined with poly(ethylene) bags (sealed individually), which were stored for 30 days at room temperature (approximately 23 °C). Distilled H_2O was added to the second, untreated batch of each replicate

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plant material to achieve DM levels similar to those for NH_3-added controls. The H_2O and NH_3-added control samples were frozen at −20 °C immediately after mixing. At sampling of the NH_3-treated fractions, molded material, if present, was removed, and samples were frozen at −20 °C.

Chemical Analyses and *in Vitro* Degradation. Dry matter, CP (AOAC, 1990), and water-soluble carbohydrates (WSC) (Dubois *et al.*, 1956; modified by Johnson *et al.*, 1966) were determined after thawing. Subsamples were freeze-dried, ground to pass a 1-mm screen, and analyzed for ash (AOAC, 1990), silica, neutral detergent fiber (NDF), nonsequential acid detergent fiber (ADF), and permanganate lignin (PL) according to Robertson and Van Soest (1981). *In vitro* dry matter digestibility (IVDMD) and NDF disappearance after 12, 24, 48, 72, and 96 h were determined by incubation in 20% (v/v) ruminal fluid in McDougall's buffer (McDougall, 1948) followed by extraction with neutral detergent (Goering and Van Soest, 1970). The ruminal fluid was obtained from a fistulated steer fed a diet of 50% corn silage and 50% corn stover (w/w, DM basis), supplemented with a protein/mineral mixture (0.9 kg/day), consisting of 90% soybean meal, 6% common salt, and 4% dicalcium phosphate. In the preparation of the buffer, $(NH_4)_2SO_4$ was excluded to obtain N-limiting conditions.

Klason lignin, acid-soluble lignin (ASL), and neutral cell wall-derived monosaccharides (glucose, xylose, arabinose, and galactose) were determined by two-stage acid hydrolysis, modified from Kaar *et al.* (1991) using 100−110 mg of NDF as starting material (Sewalt *et al.*, 1996). After filtering (Whatman grade 934 AH), the residual Klason lignin was oven-dried (105 °C), weighed, and retained for ash, nitrogen, and methoxyl group analysis. Acid-soluble lignin was quantified spectroscopically by UV absorption at 205 nm using an absorptivity of 110 L/g·cm (TAPPI, 1989) using nonbuffered filtrate. The UV absorption was adjusted for furfurals as determined by HPLC using an absorptivity of 19.14 L/g·cm (Kaar and Brink, 1991). Lignin methoxyl groups were determined by reaction of Klason lignin with 57% hydriodic acid at 145−150 °C in a methoxyl apparatus and titration with sodium thiosulfate, according to TAPPI standard T 209 su-72 (TAPPI, 1972).

Neutral monosaccharides (glucose, xylose, arabinose, and galactose) after acid hydrolysis were determined by HPLC after volume adjustment of the filtrate, buffering of an aliquot to pH 5.3 with $Ba(OH)_2$, and addition of erythritol as internal standard. The buffered solution was centrifuged at 1175*g* for 10 min; the supernatant was evaporated under vacuum to about 2 mL and passed through an anion exchange column (Kaar *et al.*, 1991) before analysis. Neutral monosaccharides were separated using a Biorad Polypor Aminex HPX-87P ion partition column, 300 × 7.8 mm, kept isothermal at 85 °C. A Biorad Carbo-P guard column, 30 × 4.6 mm, was used before the main column. The sugar samples were applied to the column using a 20-mL sample loop injection valve. Degassed, distilled water was used as eluant at a flow rate of 1.0 mL/min; operating pressure was $8.0-8.5 \times 10^5$ kg/m^2. Peaks were detected by a refractive index (RI) detector.

Furfural degradation products of glucose ((hydroxymethyl)-furfural, HMF) and xylose (2-furaldehyde, 2-F) were determined utilizing HPLC according to Kaar *et al.* (1991). Nonbuffered filtrate was directly injected from a 20-mL sample loop injection valve onto a Biorad Carbo-H guard cartridge (30 × 4.6 mm) without internal standard. The eluant was 0.01 M H_2SO_4, used at a flow rate of 0.8 mL/min; operating pressure was 2.8×10^5 kg/m^2. A UV detector set was used set at 278 nm. An external standard mixture of HMF and 2-F was used for calibration. The determined HMF and 2-F values were used for correction of glucose and xylose contents using the stoichiometric factor 180/126 to convert HMF to glucose and 150/96 to convert 2-F to xylose (Kaar *et al.*, 1991).

Free and saponifiable *p*-CA and FA were determined by GLC after sequential treatments. Starting material was NDF, prepared by 1 h extraction in boiling neutral detergent followed by three washings with hot water. Subsequent rinses with acetone as in the analytical NDF procedure were omitted to avoid solubilization of low molecular weight phenolics. To determine free hydroxycinnamic acids, 50−60 mg of NDF was extracted with 6 mL of diethyl ether overnight at room temperature. The ether extract and two subsequent washings were combined, evaporated under N_2, and derivatized with 200 mL of *N*,*O*-bis(trimethylsilyl)acetamide (BSA) at 100 °C for 10 min.

Esterified *p*-CA and FA in ether-extracted cell walls were saponified under N_2 in 1 M NaOH at 20 °C for 24 h. The extract and two subsequent washings were acidified with 6 M HCl to pH 1, extracted four times with diethyl ether, and evaporated and derivatized as above.

Trimethylsilyl-derivatized hydroxycinnamic acids were separated on a wall-coated open-tubular bonded phase fused silica gel capillary column (30 m × 0.53 mm i.d.) in a gas chromatograph (Perkin Elmer) equipped with a flame ionization detector. Conditions applied were as follows: injection temperature, 280 °C; oven temperature, 180 °C for 5 min, programmed at 5 °C/min to 230 °C, held for 5 min; linear flow rate of He, 30 mL/min. Standards used for quantification (*trans*-*p*-CA and *trans*-FA) were subjected to the respective treatments.

Statistical Analysis. The data were analyzed as a split-plot design using the GLM procedures of SAS (1989) with plant fraction as the main plot tested against replicate within plant fraction and the effect of ammoniation and fraction × ammoniation interaction as the subplot tested against the residual error term. Orthogonal contrasts tested were leaf vs stems (upper and lower) and upper vs lower stem. Nonorthogonal (Bonferroni) contrasts (Lowry, 1992) tested were H_2O vs NH_3 control and NH_3 treatment vs NH_3 control. In case of a trend for difference between NH_3 control and both other treatments ($p < 0.15$), single degree of freedom comparison was used to detect differences between NH_3 treated and H_2O control. In the case of fraction × ammoniation interaction ($p < 0.15$), the contrasts for processing method were tested within fractions by one-way ANOVA.

Fiber digestion kinetics were calculated according to a nonlinear model for degradation of NDF (adapted from Mertens and Loften, 1980) using nonlinear regression procedures (SAS, 1989).

Table 1. Composition of Corn Stover Fractions As Affected by Ammoniation

treatment and contrast		component (%, DM basis)					
fraction	processing	ash	silica	CP[a]	NDF[a]	ADF	PL
leaf	H_2O control	6.0	1.5	6.6^x	74.8^y	40.0	5.50^x
	NH_3 added	6.0	1.5	10.7^y	73.6^y	40.5	7.44^y
	NH_3 treated	6.2	1.5	12.0^y	67.7^x	40.2	5.40^x
upper stem	H_2O control	3.6	0.2	4.2^x	70.3	38.2	6.47^x
	NH_3 added	3.7	0.8	11.3^y	70.6	37.9	7.64^y
	NH_3 treated	3.8	1.0	13.7^z	69.7	39.9	5.97^x
lower stem	H_2O control	3.4	0.5	4.7^x	62.0	38.0	9.04^y
	NH_3 added	3.5	0.5	12.1^y	64.4	38.5	8.77^y
	NH_3 treated	3.7	0.8	13.3^y	64.8	40.4	7.15^x
SE		0.1	0.3	0.6	1.0	0.8	0.33
leaf vs stems		0.01	0.01	0.14	0.01	0.01	0.01
upper vs lower stem		0.15	0.78	0.99	0.01	0.67	0.01
NH_3 added vs H_2O control		0.93	0.50	0.01	0.54	0.66	0.01
NH_3 treated vs NH_3 control		0.09	0.50	0.01	0.01	0.06	0.01
interaction		0.96	0.83	0.01	0.01	0.49	0.07

[a] CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; PL, permanganate lignin. Fraction × ammoniation interaction ($p < 0.15$); means with dissimilar superscripts indicate significant ammoniation contrasts ($p < 0.05$) within plant fractions.

RESULTS AND DISCUSSION

Composition. Dry matter content of leaf, upper stem, and lower stem at harvest were 81, 59, and 35%, respectively, and 47, 41, and 33% after addition of NH_3. Crude protein increased ($p < 0.05$) for NH_3-added control (Table 1), with an additional increase ($p < 0.05$) with NH_3 treatment. Assuming negligible dry matter losses, N recovery of applied NH_3 was calculated to be

Table 2. Concentration of Neutral Cell Wall Sugars, Lignin, and Lignin Methoxyl Groups in NDF of Corn Stover Fractions As Affected by Ammoniation

treatment and contrast		cell wall component (% of NDF)						components in lignin (% of Klason lignin)	
fraction	processing	Glc[a]	Xyl[a]	Ara[a]	Gal[a]	Klason lignin[b]	ASL[a]	N	methoxyl groups[b]
leaf	H_2O control	47.6	27.5	3.93	1.65	11.7^{x}	1.84	0.64	11.9^{y}
	NH_3 added	47.2	26.9	3.81	1.20	13.3^{y}	1.53	0.67	10.4^{x}
	NH_3 treated	51.7	26.7	3.34	1.12	13.3^{y}	1.52	0.71	11.1^{xy}
upper stem	H_2O control	47.9	26.3	3.85	1.35	13.4^{xy}	1.62	0.66	14.4
	NH_3 added	47.8	25.4	3.97	1.28	12.7^{x}	1.56	0.73	14.5
	NH_3 treated	50.8	25.1	2.93	0.91	13.9^{y}	1.44	0.74	14.4
lower stem	H_2O control	51.7	24.9	2.82	1.10	13.8^{x}	1.43	0.70^{x}	14.3
	NH_3 added	51.2	25.3	2.64	0.68	16.6^{y}	1.39	1.01^{y}	14.3
	NH_3 treated	53.5	23.6	1.81	0.69	15.8^{y}	1.30	0.98^{y}	14.7
SE		1.4	0.7	0.31	0.06	0.2	0.06	0.07	0.3
leaf vs stems		0.08	0.01	0.01	0.01	0.01	0.01	0.01	0.01
upper vs lower stem		0.01	0.08	0.01	0.01	0.01	0.01	0.01	0.48
NH_3 added vs H_2O control		0.98	0.56	0.77	0.01	0.01	0.01	0.01	0.99
NH_3 treated vs NH_3 control		0.02	0.22	0.01	0.18	0.63	0.03	0.50	0.25
interaction		0.87	0.85	0.74	0.48	0.01	0.25	0.01	0.01

[a] Glc, glucose; Xyl, xylose; Ara, arabinose; Gal, galactose, ASL, acid-soluble lignin. [b] Fraction × ammoniation interaction ($p < 0.15$); means with dissimilar superscripts indicate significant ammoniation contrasts ($p < 0.05$) within plant fractions.

35, 62, and 56% for NH_3-treated leaf, upper stem, and lower stem fractions, respectively. The N recoveries for NH_3-added control fractions were somewhat lower (27, 46, and 48%, respectively). The higher recovery of N for NH_3-treated vs NH_3-added control samples may be due to CP concentration after fermentative losses during NH_3 treatment or to less extensive volatilization of NH_3 from NH_3-treated than NH_3-added control samples in the Kjeldahl procedure or during sample thawing and weighing. Immobilization of NH_3-N tightly bound to fiber constituents has been reported (*e.g.*, Solaiman *et al.*, 1979; Van Soest and Mason, 1991).

Leaf had a higher ($p < 0.05$) NDF content than stem fractions. The unusual NDF distribution in stover of drought-stressed corn was associated with accumulation of WSC in lower stem (23.5%, DM basis) and, to a lesser extent, in upper stem (13.2%) in contrast to leaf (3.9%). Neutral detergent fiber of leaf but not stems was reduced ($p < 0.05$) by NH_3 treatment (fraction × ammoniation interaction, $p < 0.05$). Ammonia treatment tended to increase ($p = 0.06$) ADF.

Apparently, NH_3 treatment resulted in partial solubilization of hemicellulose, which has been observed previously (Kiangi *et al.*, 1981; Givens *et al.*, 1988; Mason *et al.*, 1988). The decrease in hemicellulose calculated as the difference between NDF and ADF is caused in part by alkali-induced "peeling" reactions. In these reactions, degradation of sugar moieties occurs at the reducing end of hemicellulose chains (Wilkie, 1979), resulting in formation of saccharinic acids (Williams and Morrison, 1982).

In agreement with the relative changes in NDF and ADF, NH_3 treatment increased ($p < 0.05$) the concentration of glucose in NDF but did not affect xylose content (Table 2). Arabinose content of NDF was reduced ($p < 0.05$) by NH_3 treatment, and galactose was reduced ($p < 0.05$) by mere NH_3 addition. Apparently, NH_3 treatment rendered arabinose and galactose substituents more soluble in neutral detergent solution than xylan and total NDF. A reduction in substitution of the xylan backbone with arabinose side chains has been suggested to facilitate degradation of hemicellulose by microbial enzymes (Brice and Morrison, 1982) and may thus contribute to the improvement in nutritive value of corn stover by NH_3 treatment.

Klason lignin increased ($p < 0.05$) with NH_3 addition and was not further affected by NH_3 treatment (Table 2). The pattern for acid-soluble lignin (ASL) was the opposite of that for Klason lignin. Nitrogen content of Klason lignin increased ($p < 0.05$) with NH_3 addition but mostly in the lower stem (fraction × ammoniation interaction, $p < 0.05$). Methoxyl group content, as percentage of Klason lignin, was lower ($p < 0.05$) in leaf than in stems but was not affected by ammoniation.

Concentrations of saponifiable *p*-CA and FA were lower ($p < 0.01$) in leaf than in stems (Table 3). The lower portion of the stem contained more ($p < 0.01$) *p*-CA than the upper portion. Overall, saponifiable *p*-CA was not affected ($p > 0.15$) by NH_3 addition or treatment. However, within upper stem, concentration of saponifiable *p*-CA in NDF tended to decline with NH_3 addition, with further marginal decline after NH_3 treatment, resulting in overall lower ($p < 0.05$) *p*-CA for NH_3 treatment versus untreated as per single degree of freedom comparison. Ammonia treatment reduced ($p < 0.05$) saponifiable FA in upper stem only (fraction × ammoniation interaction, $p < 0.01$). Proportionately more FA than *p*-CA was released from the upper stem as previously reported for cereal straws (Mason *et al.*, 1988).

The amounts of esterified hydroxycinnamic acids released in this study by alkali saponification of corn stover cell walls were lower than in previous research (Hartley and Jones, 1978), presumably due to the preextraction of "unbound" hydroxycinnamic acids in diethyl ether. A considerable portion of the *p*-CA appeared not to be covalently linked to any other cell wall components, as reflected in the relatively high content of ether-extractable *p*-CA (up to 43% of total *p*-CA in leaf). Free hydroxycinnamic acids extractable in water have been reported earlier in wheat and rice plants (Lam *et al.*, 1990), but the high concentration of ether-extractable hydroxycinnamic acids in NDF was unexpected. Extraction in boiling neutral detergent solution followed by extensive washing with hot water and acetone has been shown to solubilize and remove free and some esterified hydroxycinnamic acids from grass cell walls (Bohn and Fales, 1991). It is conceivable that the subsequent washings with hot water alone as employed in this study were inadequate to completely remove the low molecular weight phenolics dispersed in neutral detergent and that these low molecular weight phenolics (including free and/or originally esterified *p*-CA and FA) were subsequently released in the

Table 3. Hydroxycinnamic Acids Released from Corn Stover NDF by Ether Extraction/Saponification As Affected by Ammoniation

treatment and contrast		*p*-coumaric acid (mg/g NDF)		ferulic acid (mg/g NDF)	
fraction	processing	ether[a] extract	saponifiable	ether extract	saponifiable[a]
leaf	H_2O control	2.56	2.6^{x}	0.21	1.32
	NH_3 added	2.76	4.8^{y}	0.03	2.09
	NH_3 treated	4.41	5.3^{y}	0.06	2.25
upper stem	H_2O control	5.56^{y}	17.1^{y}	0.21	6.52^{y}
	NH_3 added	1.60^{x}	14.3^{xy}	0.01	4.93^{y}
	NH_3 treated	1.45^{x}	11.7^{x}	0.10	2.73^{x}
lower stem	H_2O control	5.82^{y}	20.8	0.21	4.06
	NH_3 added	3.50^{xy}	22.2	0.08	3.11
	NH_3 treated	1.80^{x}	22.5	0.12	3.61
SE		0.89	1.8	0.03	0.49
leaf vs stems		0.94	0.01	0.25	0.01
upper vs lower stem		0.26	0.01	0.18	0.01
NH_3 added vs H_2O control		0.01	0.85	0.01	0.15
NH_3 treated vs NH_3 control		0.93	0.68	0.01	0.21
interaction		0.01	0.12	0.45	0.01

[a] Fraction × ammoniation interaction ($p < 0.15$); means with dissimilar superscripts indicate significant ammoniation contrasts ($p < 0.05$) within plant fractions.

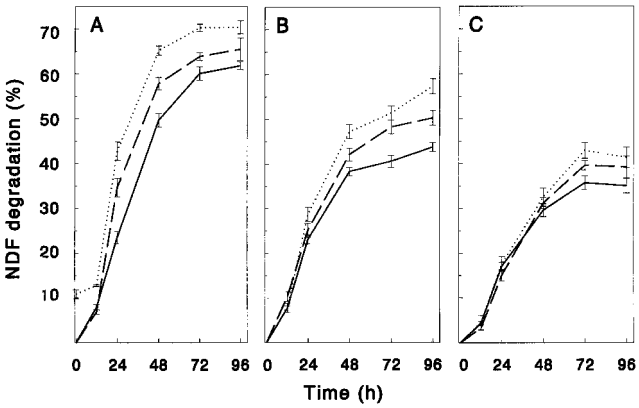

Figure 1. *In vitro* NDF degradation of (a) corn stover leaf, (b) upper stem, and (c) lower stem for control (—), NH_3 added (– – –), and NH_3 treated (···). Contrasts described in the Materials and Methods section were tested for NDF degradation at 24, 48, and 72 h. Leaf differed ($p < 0.001$) from stems, and upper stem differed ($p < 0.001$) from lower stem at all times. Ammonia-added control differed ($p < 0.001$) from H_2O control at all times. Trend for difference ($p = 0.09$) between NH_3-added control and NH_3 treatment at 48 h. Fraction × ammoniation interaction at 24 ($p < 0.001$) and 48 ($p < 0.05$) h. Disappearance of original cell wall differs ($p < 0.05$) between NH_3 treated and NH_3 control for leaf at 24 and 48 h.

overnight extraction in diethyl ether. Given the uncertainty about the extent to which free and/or esterified hydroxycinnamic acids are solubilized by neutral detergent, the isolation of plant cell walls as NDF as a preparatory step for hydroxycinnamic acid analysis is not recommended for future research. If the separate determination of "free" hydroxycinnamic acids in NDF is regarded as erroneous, addition of the amounts of "free" and esterified *p*-CA and FA should give a more accurate estimate of the total concentration of saponifiable hydroxycinnamic acids.

Digestion Kinetics. Extent of NDF degradation determined after 24, 48, and 72 h was enhanced ($p < 0.01$) by NH_3 addition (Figure 1). Within fractions, NDF degradation was enhanced ($p < 0.01$) by NH_3 addition after 24 and 48 h only for leaf (fraction × ammoniation interaction, $p < 0.01$). Ammonia addition was effective in increasing NDF degradation of upper and lower stems ($p < 0.05$) only after 72 h, with no difference ($p > 0.15$) between the NH_3-added control and NH_3 treatment. Leaf NDF degradation after 48 and 72 h tended to be higher ($p < 0.10$) for NH_3 treatment, compared to NH_3 added. Disappearance of original leaf cell wall, calculated as a percentage of NDF of H_2O control leaves, was increased ($p < 0.05$) by NH_3 treatment after 24 and 48 h.

These trends resulted in a higher potential cell wall degradability due to ammoniation for all fractions (Table 4). Ammoniation of leaf improved both rate and extent of degradation, with partial response to NH_3 addition compared with NH_3 treatment. Only leaf 48-h IVDMD was increased by NH_3 addition ($p < 0.01$), with an additional response ($p < 0.01$) to NH_3 treatment for leaves and upper stems (fraction × ammoniation interaction, $p < 0.01$). The improvement in leaf IVDMD (48 h) due to NH_3 addition amounts to 59% of the improvement due to NH_3 treatment, whereas the improvement in extent of leaf NDF degradation (72 h) after NH_3 addition is equivalent to 57% of the improvement by NH_3 treatment. These trends compare well with those for *in vivo* digestibility of urea–ammonia-treated vs urea-supplemented straw (Djajanegara and Doyle, 1989).

In vitro disappearance of all neutral cell wall sugars and Klason lignin (Table 5) was higher ($p < 0.05$) for leaf than for stems. Disappearance of xylose and arabinose was greater ($p < 0.05$) for upper than for lower stems. The relatively low xylan degradation in stems is in agreement with findings for Caucasian bluestem (*Bothriochloa caucasica*) (Piwonka *et al.*, 1991) and alfalfa (*Medicago sativa*) (Albrecht *et al.*, 1987; Titgemeyer *et al.*, 1992). Disappearance of NDF glucose was not affected ($p > 0.15$) by NH_3 addition or treatment, whereas dissappearance of xylose tended to be enhanced ($p = 0.07$, single degree of freedom comparison) by NH_3 treatment, compared to H_2O control. Disappearance of arabinose tended to be higher ($p = 0.08$) after NH_3 addition and was significantly ($p < 0.05$) higher after NH_3 treatment, compared to H_2O control. The largest improvement in arabinose disappearance was obtained for the upper stem (18% points), coinciding with the most extensive saponification of hydroxycinnamic acids among the three fractions. These observations provide an indication that esterification of hydroxycinnamic acids to arabinose is a limiting factor to the hydrolysis of arabinose substituents but less to that of the xylan backbone and not to that of cellulose.

Extent of NDF degradation (72 h) was well correlated with lignin methoxyl content ($r = -0.87$) and esterified *p*-CA ($r = -0.93$). Overall, NDF content and IVDMD

Table 4. *In Vitro* Dry Matter Digestibility and Fiber Degradation Kinetic Parameters As Affected by Ammoniation

treatment and contrast			fiber digestion kinetics[b]			
fraction	treatment	IVDMD[a] (%)	rapidly digested fraction (%)	potential digestibility (%)	rate constant (h^{-1})	lag time (h)
leaf	H_2O control	62.4[x]	0	67.7	0.032	8.7
	NH_3 added	69.1[y]	0	66.3	0.053	10.0
	NH_3 treated	73.7[z]	0	68.4	0.054	10.6
	NH_3 treated[c]	73.7	9.6	70.5	0.068	12.8
upper stem	H_2O control	56.6[x]	0	43.8	0.048	8.1
	NH_3 added	58.1[xy]	0	50.8	0.040	7.9
	NH_3 treated	60.9[y]	0	54.3	0.044	11.1
lower stem	H_2O control	56.4	0	35.8	0.044	9.1
	NH_3 added	56.5	0	42.8	0.034	8.7
	NH_3 treated	55.9	0	43.0	0.035	9.6

[a] IVDMD, *in vitro* dry matter digestibility. All contrasts and interactions significant ($p < 0.05$); means with dissimilar superscripts indicate significant ammoniation contrasts ($p < 0.05$) within plant fractions. [b] Model (after Mertens and Loften, 1980): $Y = A + B \times (1 - e^{-c(t-L)})$, in which Y = NDF degradation (%), A = rapidly digested fraction, B = potentially digestible fraction, c = first-order digestion rate constant, and L = lag time. [c] Fiber degradation calculated as percentage of original "cell wall" (control NDF).

Table 5. *In Vitro* Degradation (72 h) of Fiber Components in Corn Stover Fractions As Affected by Ammoniation

treatment and contrast		disappearance[a] (%)				
fraction	treatment	NDF	Glc	Xyl	Ara[b]	Klason lignin
leaf	H_2O control	60.2	64.9	61.0	66.1	35.3
	NH_3 added	63.9	62.7	60.7	67.1	53.1
	NH_3 treated	66.7	64.3	62.8	76.1	55.1
upper stem	H_2O control	40.6	48.6	37.2	55.7[x]	4.2
	NH_3 added	48.8	51.6	44.8	70.2[xy]	26.4
	NH_3 treated	48.2	44.6	40.8	74.0[y]	28.3
lower stem	H_2O control	35.7	52.3	31.4	38.4	-3.8
	NH_3 added	40.6	45.5	31.8	46.9	29.4
	NH_3 treated	42.2	43.9	38.4	46.6	23.4
SE		1.8	1.9	2.3	5.5	3.8
leaf vs stems		0.01	0.01	0.01	0.01	0.01
upper vs lower stem		0.01	0.51	0.01	0.01	0.32
NH_3 added vs H_2O control		0.01	0.24	0.21	0.11	0.01
NH_3 treated vs NH_3 control		0.34	0.18	0.43	0.38	0.83
interaction		0.76	0.16	0.24	0.72	0.42

[a] NDF degradation determined using six replicates ($n = 6$); disappearance of neutral polysaccharides determined in duplicate using pooled samples ($n = 2$). [b] Trend for difference between NH_3 added and other treatments; means with dissimilar superscripts indicate significant ammoniation contrasts ($p < 0.05$) within plant fractions.

were not correlated, but when contrasting the processing methods within fractions, NDF content and IVDMD appeared well correlated ($r = -0.81$, -0.65, and -0.71, within leaf, upper stem, and lower stem, respectively). Although ammoniation had no direct effect on methoxyl groups in Klason lignin, the difference between leaf and stem lignins in methoxyl content is of potential interest in relation to ruminal fiber degradation. Lignin of a high methoxyl content (*i.e.*, of a high S/G ratio) is relatively rich in β-O-4 ether linkages (Glasser and Kelley, 1987). Quinone methide intermediates, formed in coupling reactions involving the β-carbon, can be regenerated during lignin aging (Leary, 1980) and degradation (Glasser, 1981). These intermediates readily react with nucleophilic cell wall components (carboxyl or hydroxyl groups), thereby establishing ester or ether linkages (Leary, 1980), or with sulfhydryl groups, as is observed in wood pulping reactions (Glasser, 1981). The possibility of quinone methide formation during ruminal degradation of plant cell walls has been suggested as a mechanism by which lignin influences forage digestion after the *in vitro* observation that addition of reducing agents (cysteine-HCl and disodium sulfide) to the *in vitro* medium resulted in S incorporation into NDF of corn stover (Sewalt *et al.*, 1996). Quinone methide regeneration from lignocellulose during ruminal digestion would result in repolymerization of lignin structures or copolymerization with polysaccharides or other digesta components. The interrelationship between extent of quinone methide regeneration with lignin S/G ratio, or lignin methoxyl content (Glasser and Kelley, 1987), provides an explanation for the reported negative correlation between S/G ratio (Buxton and Russell, 1988; Reeves, 1985) and lignin methoxyl content (Quicke and Bentley, 1959; Sewalt *et al.*, 1993) and digestibility.

The increase with ammoniation in concentration of Klason lignin in NDF and in N content of lignin may be due to condensation reactions occurring between NH_3-N and carbonyl groups in lignin as reported by Van Soest and Mason (1991). However, Maillard reaction products are particularly formed during ammoniation at elevated temperature. We hypothesize that during ammoniation at ambient temperature, NH_3-N immobilization through quinone methide addition reactions occurs, which would be an additional mechanism by which the influence of lignin on cell wall degradation is reduced, *i.e.*, by eliminating reactive sites on the lignin polymer. We observed a substantial increase ($p < 0.001$), due to NH_3 addition, in the solubilization of lignin during *in vitro* digestion (Table 5), by 18 (leaf)−28% (stems) points, with no further increase resulting from NH_3 treatment. A recent model study, in which ammonia and lignin were found to interact in covalent linkage dependent on available free phenolic sites, suggested the involvement of quinone methides in this interaction (Sewalt and Glasser, unpublished). Our current observation that lignin solubilization from corn stover stems (high methoxyl content) during *in vitro* digestion is enhanced more by ammoniation than lignin solubilization from corn stover leaves (low methoxyl content) further supports our hypothesis.

In general, the response in digestibility to ammoniation of crop residues has been inversely related to initial quality (digestibility) of the plant material (Kiangi *et al.*, 1981; Givens *et al.*, 1988; Ibrahim *et al.*, 1989; Goto *et al.*, 1991). In contrast, the differential response in digestibility to NH_3 treatment among the corn stover fractions in this experiment was not inversely related to initial quality. However, it could be related, in part, to initial differences in composition. The contribution of NPN addition to the response to NH_3 treatment was higher for stems than for leaves, possibly related to the inherently lower CP content of stems or to the higher N immobilization in stems. In addition, WSC concentration differed substantially among leaf, upper stem, and lower stem. The high concentration of WSC in the

lower stem fraction is a likely reason for the reduced response to chemical treatment, presumably due to neutralization of alkali by the excess of sugars. The effectiveness of NH_3 in hydrolyzing ester linkages has been challenged before, in relation to the high buffering capacity of plant fibers (Van Soest *et al.*, 1984).

To verify the neutralizing effect of sugars, the pH of 3% aqueous NH_3 with increasing concentrations (0, 5, and 10%, w/v) of corn sugar (dextrose) was determined. The pH progressively declined from 12.2 to 11.4, with a large initial drop in pH with 5% dextrose (to pH 11.7). Similarly, the pH of 0.5% NH_3 declined from 11.4 (0% dextrose) to 11.0 (with 5% dextrose) and 10.8 (with 10% dextrose). A similar drop in pH of NH_3 is likely to occur in drought-stressed corn stalks containing high concentrations of WSC. Taking into account the rather low N recovery (indicating a much lower effective NH_3 concentration than 3%), the reported N immobilization in lignin (Van Soest and Mason, 1991), and the buffering capacity of cell wall constituents (Van Soest *et al.*, 1984), it is conceivable that the pH obtained after application of 3% NH_3 is suboptimal for effective saponification, *i.e.*, below pH 10.

Although NH_3 treatment did not lead to appreciable saponification of hydroxycinnamic acids in the leaf fraction, fiber degradation and IVDMD of leaf were enhanced beyond the effect of NH_3 addition. It appears that the improvement in digestibility of the leaf fraction is mainly due to an initial solubilization of hemicellulose. However, even the *in vitro* degradation of residual leaf NDF after NH_3 treatment was enhanced, indicating an additional factor such as the reduced substitution of xylan with arabinose residues or the occurrence of addition reactions of NH_3 with lignin intermediates. More definite evidence on the chemical nature and nutritional availability of immobilized N may help explain the benefits of ammoniation of crop residues.

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