Composition and Susceptibility to Rumen Microbial Degradation of Nonmesophyll Cell Walls Isolated from Caucasian Bluestem [*Bothriochloa caucasica* (Trin)] Leaf Tissue†

Edward J. Piwonka, Jennifer W. MacAdam, Monty S. Kerley,* and John A. Paterson

Department of Animal Sciences, University of Missouri—Columbia, Columbia, Missouri 65211

This experiment was conducted to isolate sufficient quantities of nonmesophyll (NM) cell wall (CW) from caucasian bluestem [*Bothriochloa caucasica* (Trin.) C. E. Hubbard] leaf tissue such that NM CW composition and in vitro degradability could be studied as the plant matured. The mesophyll (M) fraction was also collected and subjected to similar analyses. Fresh leaf material was harvested at the vegetative (V), jointing, and early and late reproductive (LR) stages of development. Leaf tissue was ball milled (ca. 15 g of wet tissue/L of water) for 24 h to macerate the tissue, and NM cells were collected on a 75-μm sieve. Cells not retained by the 75-μm screen were collected as the M fraction. Cell wall of NM, M, and whole leaf (WL) tissue was isolated by ethanol (80% v/v) and chloroform extraction followed by treatment with an α-amylase. The ratio of M to NM CW tissue varied from 1.45:1 to 0.87:1 (V and LR stages, respectively). Crude protein content (% N × 6.25) was lower ($P < 0.05$) in NM than WL. Klason and acid detergent lignin concentrations were highest ($P < 0.05$) for M and similar ($P > 0.05$) between NM and WL, with no effect due to maturity. The CW and NM contained a higher concentration of glucose ($P < 0.01$) and total monosaccharides ($P < 0.05$) compared to M and WL, although the concentration of glucose and total monosaccharides did not change as the grass matured. In vitro CW digestibility was greatest ($P < 0.01$) for NM and lowest ($P < 0.01$) for M compared to WL and was not affected by maturity. In vitro digestibility of arabinose, xylose, and glucose did not differ among tissue types, and xylose digestibility decreased linearly ($P < 0.01$) as the grass matured. On the basis of these data, we concluded that isolation of a NM CW fraction was possible, although it did not appear to concentrate factors suspected to limit CW degradation by ruminal microbes.

INTRODUCTION

To maintain desired levels of ruminant animal production, it is essential that forages of high quality are continually available. Due to the growth pattern of cool-season grasses, insufficient forage quantity and nutrient availability occurs during midsummer. Warm-season grasses have application in grazing systems due to their primary (vegetative) growth occurring during the midsummer. A major disadvantage of using warm-season grass pastures for cattle is their rapid decline in dry matter digestibility during late summer as the grass matures, which substantially limits the production potential of the animal.

Cell wall (CW) digestibility decreases with advancing maturity (deRuiter and Burns, 1987) and is closely related to dry matter digestibility of the grass (Duble et al., 1971). Cell wall content of grasses increases with advancing plant maturity and comprises the largest proportion of dry matter. The decline in CW digestibility is believed to be due to the limited susceptibility of structural polysaccharides to microbial degradation in the rumen. To achieve more efficient utilization of warm-season grasses, it is imperative to have a more accurate understanding of factors that limit CW degradation by ruminal microorganisms, especially during the later stages of grass maturity. Efforts could then be employed through pasture management and/or genetic manipulation to prevent declining forage quality.

Cell wall polysaccharide degradation in warm-season grasses has been proposed to be limited by either the close spatial arrangement of the plant cells (Hanna et al., 1973) or the composition and structural architecture inherent to the CW (Hatfield, 1989). It is difficult to define limitations of CW degradation due to the heterogeneity of CW tissue present in grass. Microscopic and histochemical examinations of forages before and after digestion have demonstrated the diversity and degree of susceptibility of cell types to microbial degradation. Primary CW, such as mesophyll and phloem, have been found to be degraded more rapidly than secondary CW, which comprises the vascular tissue (Akin, 1979; Hastert et al., 1983). Akin et al. (1977) found that the percentage of tissue types staining positive for lignin increased with maturity and had a corresponding decreased susceptibility to microbial degradation. On the basis of previous studies, it appeared that advances in our knowledge of limitations to CW degradation could be achieved by studying homogeneous, intact CW material.

Gordon et al. (1977) and Harris and Lowry (1979) were able to isolate homogeneous, intact cell types using a mechanical maceration and sieve pore size separation procedure. Gordon et al. (1985) used this isolation procedure to study compositional differences among cell types at different maturity stages in two cool-season grasses. Large differences in the concentration of monosaccharides, alkali-labile phenolic acids, and crude protein were found among the cell types and maturity stages. They also found that the proportion of fiber cells (mainly vascular tissue) increased with maturity of the grass. Chesson et al. (1986) indicated that isolated mesophyll (M) and epidermal cell walls were more rapidly and extensively degraded than fiber cell walls. This type of isolation and degradation study could aid our understanding of limitations to degradation of warm-season grass CW by ruminal microbes as influenced by maturity, providing that

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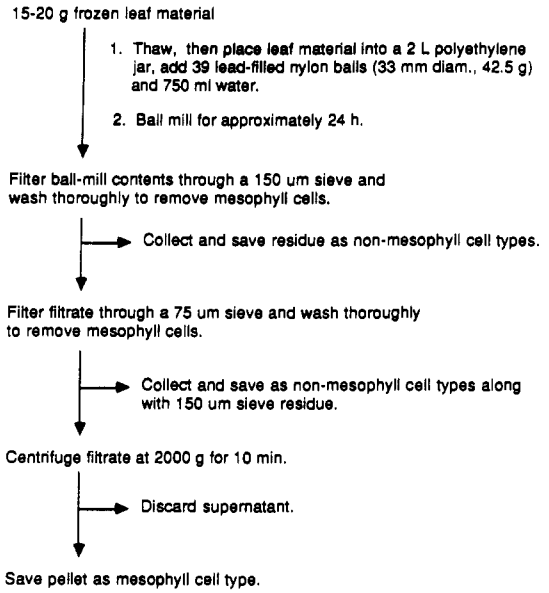

Figure 1. Procedure used to separate mesophyll from nonmesophyll cells in Caucasian bluestem leaf blade tissue.

cell fractions could be harvested in sufficient quantities to allow extensive in vitro studies to be conducted.

The objectives of this study were to isolate sufficient amounts of homogeneous, nonmesophyll (NM) CW tissue from Caucasian bluestem leaves at different stages of maturity to determine if the proportion of NM to M CW changed and if compositional and degradational changes of these two CW fractions occurred as the grass maturity stage advanced. Leaf tissue was studied to best approximate what the animal would consume in a grazing environment.

MATERIALS AND METHODS

Grass Sample Collection. Caucasian bluestem [*Bothriochloa caucasica* (Trin.) C. E. Hubbard] was established in 1981 on 2 m × 4 m plots (Mexico silt loam soil) at the University of Missouri Agronomy Farm, Columbia, MO. Grass samples were collected at vegetative, jointing, early reproductive, and late reproductive stages on the following dates: June 7, June 29, July 29, and September 3, 1988, respectively. Each sample represented the cumulative growth for the entire season up to the time the sample was harvested. Grass was clipped 10 cm above the ground surface, immediately placed in a plastic bag, and frozen by immersing the bag in an acetone–dry ice bath. The grass was transported to a cold room (5 °C) where leaves were removed from stems (ca. 1 cm above the ligule). Thirty percent of the total leaf sample was saved for analyses, and the remaining leaf sample was separated into M and NM cell types. Weights of fresh leaf samples were recorded for later comparisons. Leaf samples were placed in sealed freezer bags, and the bags were filled with water, frozen, and stored (−20 °C) until chemical analyses could be performed.

Separation of Cell Types. Figure 1 depicts the scheme for the separation of cell types. Frozen leaf samples were thawed and placed in a polyethylene ball mill jar (15 g of wet tissue/jar). Thirty-nine lead-filled nylon grinding balls (33-cm diameter, 42.5 g) and 750 mL of distilled water were added to the jar. Grass material was ball milled for approximately 24 h or until most of the green coloration or mesophyll, as viewed microscopically, was removed from the 150-μm sieve residue.

After the material was ball milled, contents of the jar were filtered through a 150-μm-pore sieve with the retained residue collected as NM cells. The filtrate was further filtered through a 75-μm sieve. The retained residue was collected and combined with the NM. The 75-μm-sieve filtrate was centrifuged at ca. 2000*g* and the supernatant discarded. The pellet was collected as M cells. Both cell type fractions were frozen and stored at −20 °C until laboratory analyses were performed.

Microscopic Examination. Mesophyll and NM cell fractions were viewed under the light microscope after isolation. Observations were made at 400× on wet mount slides. Isolated cell preparations were stained with an aqueous solution containing 0.05% *o*-toluidine blue in a 0.1 M phosphate buffer (pH 6.8) according to the procedures of O'Brien et al. (1964). Wet mounts of both stained cell fractions were observed under a light microscope.

Extraction of Cell Wall Tissue. Cell fractions and whole leaf (WL) samples were lyophilized and extracted according to the method of Theander (1979). From preliminary work, our laboratory validated that large quantities of material (20–30 g) could be extracted with 300–500 mL of 80% ethanol and 200–300 mL of chloroform. The volume of 80% ethanol and chloroform used depended upon sample size. These were added at a concentration of approximately 150 mL of 80% ethanol and 100 mL of chloroform per 10 g of dry matter. The material was refluxed with 80% ethanol for 45 min, filtered, refluxed with chloroform for 30 min, and then filtered and dried at 55 °C.

After extraction with ethanol and chloroform, starch was hydrolyzed by using heat-stable α-amylase (Sigma, A3403 α-amylase, St. Louis, MO). Extracted material was placed in a Berzelius beaker, 300–400 mL of a 0.02 M phosphate buffer (pH 6.9) was added, and the mixture was heated in a water bath at 85 °C for 30 min. The sample was cooled to 55 °C, and then α-amylase (7500 units of activity) was added to the buffer. Enzymatic hydrolysis was allowed to occur for 45 min, and then the solution was thoroughly rinsed with boiling water and filtered to collect the CW material. The CW material was dried at 55 °C for 48 h and weighed to determine the CW ratio of M and NM cell types that existed in the leaf tissue.

In Vitro Digestion. Cell wall material of M, NM, and WL was digested in vitro (Galyean, 1987) to measure the rate and extent of CW digestion. Ruminal fluid used as the inoculation source was collected from an area immediately beneath the rumen mat of a steer consuming a Caucasian bluestem hay diet. Ruminal fluid was kept under anaerobic conditions with CO_2 and mixed with McDougall's buffer (1:2, ruminal fluid to buffer ratio). Ammonium sulfate was added to make a 0.2 M NH_3 solution. Cell wall samples (0.5 g) were diluted with 30 mL of the ruminal fluid–buffer mixture and incubated at 39 °C for 12, 24, and 48 h. In vitro tubes were removed from the incubator at the predetermined times and placed in an ice bath for 1 h and then frozen and stored in a −20 °C freezer until laboratory analyses on the digested residue could be performed. The in vitro digestion was performed two times.

Residue remaining after in vitro digestion was refluxed with 100 mL of 80% ethanol for 45 min. The ethanol-extracted residue was centrifuged at 10000*g* for 10 min. The supernatant was removed, 100 mL of 80% ethanol was added, and the mixture was centrifuged at 10000*g* for 10 min. The supernatant was removed, and the insoluble material was extracted with 100 mL of chloroform for 30 min. The extracted residue was collected on filter paper and rinsed with chloroform. Extracted residue was dried at 55 °C for 48 h and weighed to calculate the percentage of CW digested.

Acid Hydrolysis. Undigested and digested M, NM, and WL CW was hydrolyzed with acid to determine monosaccharide composition. Hydrolysis was performed according to the method of Blakeney et al. (1983) by hydrolyzing 200 mg of CW with 1.25 mL of 12 M H_2SO_4 for 1 h. A secondary hydrolysis was performed by diluting the solution from the primary hydrolysis to M H_2SO_4 and heating in an autoclave (100 °C for 2 h). The hydrolysate was neutralized with 3.2 mL of 14.8 M NH_4OH.

Uronic Acids. Uronic acid concentration in the undigested and digested M, NM, and WL CW was measured according to the method of Blumenkrantz and Asboe-Hansen (1973). Acid hydrolysate (80 μL) was diluted with 320 L of deionized water and treated with a solution of $Na_2B_4O_7$/36 N H_2SO_4 (0.25% w/v). *m*-Hydroxydiphenyl was added as the coloring agent, and the uronic acid concentration was measured colorimetrically at 540 nm.

Monosaccharide Quantification. Monosaccharides were measured as their derivatized alditol acetates according to the method of Blakeney et al. (1983). *myo*-Inositol was used as an internal standard. Acid hydrolysate (0.2 mL) was reduced with

1 mL of a $NaBH_4$/DMSO solution (2% w/v) at 40 °C for 90 min. Excess $NaBH_4$ was destroyed with 0.1 mL of 17.4 M acetic acid. The reduced monosaccharides were derivatized with 2 mL of acetic anhydride by using 0.2 mL of 1-methylimidazole as a catalyst. Excess acetic anhydride was destroyed with 10 mL of deionized water. Derivatized monosaccharides, extracted with 1 mL of methylene chloride, were placed in septum-capped vials and stored at −20 °C until samples could be analyzed by gas–liquid chromatography. Derivatized monosaccharides were separated and quantified by using a Perkin-Elmer Model 8500 gas–liquid chromatograph fitted with a flame ionization detector. A 2.83 m × 2 mm i.d. glass column packed with 3% SP-2330 on 100/120 Supelcort (Supelco, Bellefonte, PA) was used to separate derivatized monosaccharides. Flow rate of the nitrogen carrier gas was 18 cm^3 min^{-1}. Oven, inlet, and flame ionization temperatures were 228, 300, and 275 °C, respectively.

Alkali-Labile Phenolic Acid Quantification. Alkali-labile phenolic acids were extracted from M, NM, and WL CW according to the method of Jung et al. (1983), with the exception that 2 N NaOH was used. Phenolic monomers were extracted in ethyl ether, with the ether evaporated off and the phenolic acids then reconstituted in 5 mL of methanol and quantified by high-performance liquid chromatography. Twenty microliters of the phenolic acid sample was injected onto an Altex ODS column (C-18 stationary phase: 25 cm × 0.45 mm i.d.) fitted on a Beckman System Gold HPLC. The mobile phase consisted of water, 1-butanol, and glacial acetic acid (350:7:1 v/v). The mobile phase was pumped isocratically at 1 mL/min, and the column was maintained at 35 °C. Phenolic acids were quantified by integrating the area under the curve generated by reading samples at 275 nm.

Lignin Content. Lignin was measured as Klason lignin and acid detergent lignin (Goering and Van Soest, 1970). Klason lignin was determined gravimetrically as the acid-insoluble residue from the acid hydrolysis of CW material, correcting for ash content. Acid detergent lignin was determined gravimetrically, as the 12 M sulfuric acid insoluble residue from acid detergent extracted material, corrected for ash content.

Crude Protein. Cell walls of M, NM, and WL were analyzed for their crude protein content (% N × 6.25) by Kjeldahl N determination (AOAC, 1984).

Statistical Analyses. Data were analyzed as a split-plot design, with maturity stages as main effects and tissue types as subplots. Analysis of variance was performed by using General Linear Model procedures of the Statistical Analysis System (SAS, 1984). All means were separated by least significant difference when a significant ($P < 0.05$) F-test was detected. Orthogonal contrast of linear (−3, −1, 1, 3), quadratic (1, −1, −1, 1), and cubic (−1, 3, −3, 1) effects was tested to identify differences attributable to maturity stage.

RESULTS

Light Microscopy Examination. The isolated M fraction was comprised of M cells contaminated by fragmented vascular and epidermal tissue. When the M fraction was stained with *o*-toluidine blue, the M cells stained purple (evidence of no lignin presence), while the epidermal and vascular tissue stained green (evidence of lignin presence). The NM fraction appeared to be void of contamination by M cells. Some of the epidermal cell walls stained purple, yet others stained green. Most of the vascular cell walls stained bright green, illustrating the presence of heavily lignified cell wall. Thus, we concluded that the NM fraction consisted primarily of cell types with secondary cell wall lignification.

Cell Wall Composition of Nondigested Tissue. When the M and NM CW weight was compared to WL CW weight, approximately 87% of the CW material was recovered. The ratio of M to NM CW was 1.45:1 for vegetative, 1.35:1 for jointing, 1.28:1 for early reproductive, and 0.88:1 for late reproductive maturity stages, respectively, but was not statistically different ($P > 0.05$) among maturity stages (data not shown).

Table I. Crude Protein, Uronic Acid, Klason Lignin, and Acid Detergent Lignin Composition of Mesophyll, Nonmesophyll, and Whole Leaf Cell Walls Isolated from Caucasian Bluestem

cell wall component	mesophyll	non-mesophyll	whole leaf	SEM[a]
crude protein,[b] % of CW	15.92[c]	1.95[d]	6.14[e]	0.74
uronic acid, % of CW	1.56	1.70	1.72	0.06
Klason lignin, % of CW	24.25[c]	10.17[d]	13.67[d]	1.00
acid detergent lignin, % of CW	11.75[c]	3.67[d]	4.37[d]	0.92

[a] Standard error of the mean. [b] Crude protein was calculated as % N × 6.25. [c–e] Means within the same row with different superscripts are different ($P < 0.05$).

Table II. Alkali-Labile Phenolic Acid Composition of Caucasian Bluestem Cell Wall at Four Different Maturity Stages

	maturity stage				
phenolic acid	vegetative	jointing	early reprod	late reprod	SEM[a]
p-coumaric acid,[b] mg g^{-1} CW at	2.72	2.48	1.76	1.33	0.183
ferulic acid,[b] mg g^{-1} CW at	6.52	7.13	4.70	3.71	0.484

[a] Standard error of the mean. [b] Linear effect due to maturity stage ($P < 0.05$). [c,d] Means in the same row with different superscripts are different ($P < 0.05$).

Table III. Monosaccharide Composition of Mesophyll, Nonmesophyll, and Whole Leaf Cell Walls Isolated from Caucasian Bluestem

monosaccharide	mesophyll	nonmesophyll	whole leaf	SEM[a]
arabinose, mg g^{-1} CW	148.4	154.3	147.2	3.7
xylose, mg g^{-1} CW	292.7	383.0	318.6	19.7
glucose, mg g^{-1} CW	296.1[b]	408.5[c]	373.3[d]	5.8
total, mg g^{-1} CW	737.1[b]	945.8[c]	839.0[c]	26.8

[a] Standard error of the mean. [b–d] Means within the same row with different superscripts are different ($P < 0.05$).

A tissue type by maturity stage interaction ($P < 0.01$) occurred for CW crude protein concentration. Mesophyll CW contained more ($P < 0.01$) crude protein than either NM or WL CW at (Table I) all stages of maturity except the vegetative stage, when its crude protein content was not different from that of WL CW (data not shown). Nonmesophyll CW had the lowest ($P < 0.05$) crude protein content of the tissue types at all stages of maturity except at the jointing and late reproductive stages, when its crude protein content did not differ from that of WL CW. Uronic acid concentration did not differ ($P > 0.05$) among tissue types (Table I) or maturity stages. Mesophyll CW contained the highest ($P < 0.01$) concentration of Klason and acid detergent lignin compared to NM and WL CW (Table I). However, no differences ($P > 0.05$) in either Klason or acid detergent lignin concentrations occurred among maturity stages.

Tissue types did not differ in ferulic or *p*-coumaric acid concentration, but the concentration of both phenolic acids decreased linearly ($P < 0.01$) as the grass matured (Table II). There was no difference in the ratio of *p*-coumaric to ferulic acid among tissue types, although this ratio decreased linearly ($P < 0.01$) from 0.42:1 to 0.36:1 as grass maturity stage advanced (data not shown).

Arabinose and xylose concentrations did not differ among tissue types (Table III) or maturity stages. Nonmesophyll CW had the highest ($P < 0.01$) concentration of glucose and total CW monosaccharides compared to mesophyll and WL. No difference due to maturity stage

Table IV. In Vitro Extent of Cell Wall and Cell Wall Monosaccharide and Uronic Acid Digestion from Mesophyll, Nonmesophyll, and Whole Leaf Cell Walls Isolated from Caucasian Bluestem

component	mesophyll	nonmesophyll	whole leaf	SEM[a]
cell wall, % digested	37.9[b]	63.5[c]	50.3[d]	2.20
monosaccharides				
arabinose, % of CW monosaccharide	77.2	87.7	82.5	3.09
xylose, % of CW monosaccharide	70.4	72.0	65.8	3.94
glucose, % of CW monosaccharide	70.8	81.0	73.1	4.36
total, % of CW monosaccharide	72.1	78.4	72.0	3.71
uronic acids % of CW monosaccharide	66.5	79.1	71.9	3.80

[a] Standard error of the mean. [b-d] Means in the same row with different superscripts are different ($P < 0.05$).

Table V. In Vitro Cell Wall Digestion Rate and Monosaccharide Composition of in Vitro Digested Cell Wall Residue of Caucasian Bluestem Cell Wall Harvested at Different Stages of Maturity

	maturity stage				
monosaccharide	vegetative	jointing	early reprod	late reprod	SEM[a]
arabinose, mg g^{-1} CW	41.8	46.0	55.4	67.8	11.48
xylose,[b] mg g^{-1} CW	161.8	185.5	217.4	232.8	17.57
glucose, mg g^{-1} CW	131.5	114.8	131.2	147.6	16.93
total, mg g^{-1} CW	335.0	346.3	404.1	448.1	44.08
CW digestion rate,[b] mg g^{-1} CW	2.49	2.20	1.75	1.53	0.20

[a] Standard error of the mean. [b] Linear effect due to maturity stage ($P < 0.05$).

occurred. The ratio of arabinose to xylose in M and WL CW was similar ($P > 0.05$) and higher ($P < 0.05$) than NM CW.

In Vitro CW Digestibility. Nonmesophyll had the highest ($P < 0.01$) extent of in vitro CW digestibility (63.5%) compared to M (37.9%) and WL (50.3%) (Table IV). No difference ($P > 0.05$) in in vitro CW digestibility occurred among maturity stages in this experiment. Although there was no difference detected in the rate of in vitro CW digestibility among tissue types, there was a linear decrease in the rate of digestion as the grass matured (Table V). The percentage of arabinose, glucose, and total monosaccharides digested did not differ ($P > 0.05$) among tissue types or maturity stages. Xylose digestibility did not differ among tissue types, but decreased linearly ($P < 0.05$) as the grass matured. Similarly, the type of tissue or grass maturity stage did not affect uronic acid digestibility.

Cell Wall Composition of Digested Residue. The concentration of arabinose, xylose, glucose, or total monosaccharides in the digested CW residue was not different ($P > 0.05$) among tissue types (data not shown). However, there was a linear ($P < 0.01$) increase in the concentration of xylose in digested residues as grass matured, with no differences ($P > 0.05$) in arabinose or glucose concentration due to maturity (Table V). Digested M CW residue also contained a higher ($P < 0.01$) content of Klason lignin than did NM and WL CW (36.3, 22.6, and 21.5% of digested CW residue, respectively). The concentration of lignin in the digested CW residue increased linearly ($P < 0.01$) from 23.5 to 28.5% as grass matured, with no differences among tissue type.

DISCUSSION

Recovery of WL CW in M and NM fractions was approximately 87%, similar to previous studies conducted with cool-season grasses. The ratio of M to NM CW found in this study was also similar to results reported by Gordon et al. (1985). However, in their research information was reported on a cell basis, which may be different from on a cell wall basis as expressed in our research. Mesophyll cells were reported to comprise approximately 66% of cool-season grass leaf tissue, compared to 60% of warm-season grass leaf tissue found in our study. The ratio of M to NM CW found in this study may be overestimated due to contamination of epidermal and vascular CW tissue fragments in the M fraction. However, warm-season grasses would be expected to have a lower ratio of M to NM than cool-season grasses (Akin and Burdick, 1975). Furthermore, similar to Gordon et al. (1985), the NM proportion increased numerically at the most mature stage of grass.

Light microscopy observations of lignin-stained cells indicated that little contamination of NM cells by M cells occurred. Therefore, we inferred that the ball-milling procedure achieved separation of M cells from NM cells similar to that previously reported for a mortar and pestle procedure but in larger quantities, allowing larger scale studies to be conducted.

In our study, the high crude protein content of M CW and low crude protein content of NM CW were similar to the results reported by Gordon et al. (1985). The high crude protein content of M CW appears to coincide in part with the literature reported for primary cell walls. The crude protein was most likely composed of extensin, a glycoprotein of primary (Fry, 1982) CW, and cell wall associated proteins not successfully removed by ethanol extraction. The acid-insoluble residue, reported as Klason acid detergent lignin, of M CW contained a high concentration of crude protein (42.7 ± 6.7‰ of the Klason-insoluble residue). The crude protein measured in the acid-insoluble residue was most likely extensin and CW-associated proteins resistant to hydrolysis, in part due to isodityrosine linkages (Fry, 1986). If the high level of crude protein in the M fraction was primarily extensin and resistant to degradation by ruminal microorganisms, then the low in vitro digestibility of M CW could be explained by its high concentration of indigestible nonpolysaccharide components. Because the M fraction was contaminated with NM fragments, it should not be deduced that only M CW contained extensin, but only that extensin (CW-associated protein) may be an important factor limiting CW degradation of Caucasian bluestem by ruminal microbes.

Contrary to research by Harris et al. (1980) and Gordon et al. (1985) and the differences measured in lignin content between cell fractions in this study, the M, NM, and WL CW contained similar concentrations of *p*-coumaric and ferulic acid. Some of the *p*-coumaric acid measured in the M CW fraction could have been contributed by contaminating NM CW fragments. Similar concentrations of *p*-coumaric and ferulic acid between the M and NM CW indicated that alkali-labile phenolic acid concentration had little effect on susceptibility of Caucasian bluestem CW to microbial degradation. Furthermore, the linear

decrease in CW phenolic acid composition as the plant matured did not parallel CW digestibility.

Although no differences were found in the concentrations of arabinose and xylose among cell types, the higher ratio of arabinose to xylose for M CW compared to NM CW was similar to results reported by Gordon et al. (1985). Also similar to our results, Gordon et al. (1977) found NM CW contained a higher concentration of glucose than M CW. The greater concentration of monosaccharides in the NM CW was expected since NM tissue should contain a higher proportion of secondary CW.

The greater in vitro digestibility of NM compared to M CW found in this study was unexpected and contradicted results reported by Chesson et al. (1986) and Akin (1979). This was most likely due to contamination of the M fraction by NM cells, which was not readily apparent from visual observations. In addition, the relatively high in vitro CW digestibility of NM CW, which was apparently free of M CW, was equally unexpected. This probably occurred due to the most poorly degraded NM CW tissue being erroneously recovered in the M fraction. When the digestibility of individual or total monosaccharides was compared among CW fractions, no differences occurred. This would further indicate that poor digestibility of Caucasian bluestem CW may be due to nonpolysaccharide constituents of the CW, such as extensin.

We further concluded that the close spatial arrangement of cells in warm-season grasses, such as Caucasian bluestem, had little effect on limiting structural polysaccharide degradation by ruminal microbes as had been previously suggested (Hanna et al., 1973). The summative CW digestibility of M and NM (49.4%) was equal to CW digestibility of the WL CW (50.3%), indicating that no additive effect occurred due to separation of cells by ball milling. This inference was supported by the M fraction, having passed through a 75-μm screen and consisting of single cells, being the most poorly digested.

The linear increase in xylose concentration of digested residue as the grass matured agreed with findings in other grass species that xylan is the most slowly degraded polymer in the plant cell wall (Hespell, 1988). The linear ($P < 0.05$) increase in xylose concentration of indigestible residue was the primary reason for the increasing ($P = 0.18$) concentration of total monosaccharides in undigested residue as the plant matured. These data were interpreted as xylose hydrolysis being the most influential on CW polysaccharide degradation of Caucasian bluestem.

In conclusion, it appeared that isolation of a NM CW fraction free of M cells was possible. Nonpolysaccharide CW components, such as extensin and lignin, were poorly degraded by ruminal microbes and are the primary contributors to the limited digestibility of Caucasian bluestem. Alkali-labile phenolic acids cannot be used to assess the digestibility of Caucasian bluestem, nor does the close spatial arrangement of cells play a role in limiting CW digestion of Caucasian bluestem. Xylose is the most poorly digested monosaccharide in Caucasian bluestem CW.

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