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LITERATURE CITED

AOAC *Official Methods of Analysis*, 11th ed.; Association of Official Analytical Chemists: Washington, DC, 1970.
AOAC *Official Methods of Analysis*, 12th ed.; Association of Official Analytical Chemists: Washington, DC, 1975.
Apostolatos, G.; Hoff, J. E. *Anal. Biochem.* **1981**, *112*, 126.
Becker, H. C.; Milner, R. T.; Naget, R. H. *Cereal Chem.* **1940**, *17*, 447.
Beckman Instruments *Procedures Manual* 120-PM-1; Spinco Division: Palo Alto, CA, 1966.
Bressani, R.; Elias, L. G.; Navarete, D. A. *J. Food Sci.* **1961**, *26*, 525.
Bressani, R.; Braham, J. E.; Elias, L. G. 5th Congreso Latinoamericano de Nutricion, Puebla, Mexico, 1980; p 44.
Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. *Anal. Chem.* **1956**, *28*, 350.
Elias, L. G.; Fernandez, D. G.; Bressani, R. *J. Food Sci.* **1979**, *44* 524.
Evans, R. J.; Bandemer, S. L. *J. Agric. Food Chem.* **1967**, *15*, 439.
Evans, R. J.; Bauer, D. H. *J. Agric. Food Chem.* **1978**, *26*, 779.
Irrman, M. A. K. *J. Chromatogr.* **1974**, *97*, 175.
Jaffé, W. G. *Arch. Venez. Nutr.* **1950a**, *1*, 107.
Jaffé, W. G. *Proc. Soc. Exp. Biol. Med.* **1950b**, *75*, 219.
Jaffé, W. G.; Vega Lette, C. L. *J. Nutr.* **1968**, *94*, 203.
Jaffé, W. G.; Brücher, O. *Arch. Latinoam. Nutr.* **1972**, *22*, 267.
Joslyn, M. A. *Methods in Food Analysis: Physical, Chemical and Instrumental Methods of Analysis*, 2nd ed.; Academic: New York, 1970.
Kanesiro, M. A. B.; Faleiros, R. R. S.; Bellingieri, P. A.; Albertini, P. E. G. *Cietifica* **1977**, *5*, 276.
Martin-Tanguy, J.; Guillaume, J.; Kossa, A. *J. Sci. Food Agric.* **1977**, *28*, 757.
McIntosh, A.; Ellinger, G. M. *Proc. Nutr. Soc.* **1976**, *35*, 148A.
NBC *Diet Catalog of the INC*; Nutritional Biochemicals Corp.: Cleveland, OH, 1977/1978.
Oliveira, A. C.; Sgarbieri, V. C. *J. Nutr. Sci. Vitaminol.* **1986**, *32*, 425.
Pusztai, A.; Palmer, R. *J. Sci. Food Agric.* **1977**, *28*, 620.
Rogers, Q. R.; Harper, A. E. *J. Nutr.* **1965**, *87*, 267.
Sgarbieri, V. C.; Whitaker, J. R. In *Advances in Food Research*; Chichester, C. O., Mrak, E. M., Schweigert, B. S., Eds.; Academic: New York, 1982; Vol. 28, p 93.
Sgarbieri, V. C.; Garruti, R. S. *Can. Inst. Food Technol. J.* **1986**, *19*, 202.
Sgarbieri, V. C.; Antunes, P. L.; Almeida, L. D. *J. Food Sci.* **1979**, *44*, 1306.
Sgarbieri, V. C.; Antunes, P. L.; Junqueira, R. G. *Cienc. Tecnol. Aliment.* **1982**, *2*, 1.
Spies, J. R. *Anal. Chem.* **1967**, *39*, 1412.
Tabatabai, M. A.; Bremmer, J. M. *Agron. J.* **1970**, *62*, 805.

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Hemicellulose Digestibility by Steers Fed Sun-Cured Hay and Drum-Dehydrated Alfalfa and Coastal Bermuda Grass

William R. Windham,* Henry E. Amos, and John J. Evans

Four ruminally and abomasally cannulated steers were used in a 4 × 4 Latin square design experiment to determine the digestibility of hemicellulose (HC) in coastal Bermuda grass (CBG) and alfalfa (Alf) and to assess the effect of analytical method on the determination and digestibility of HC. Forages: (a) CBG-hay (CBG-H); (b) CBG-dehydrated (CBG-D); (c) Alf-hay (Alf-H); (d) Alf-dehydrated (Alf-D). Hemicellulose was determined by three methods: (1) conventional (HC-C), as the difference between neutral detergent fiber (NDF) and acid detergent fiber (ADF) determined on separate aliquots; (2) sequential (HC-S) as the material solubilized by acid detergent treatment of NDF; (3) trifluoroacetic acid soluble (HC-TFA), as the material solubilized by TFA treatment of NDF. Xylose and arabinose were the major components of HC-TFA. Apparent digestibility (AD) of HC was higher ($P < 0.05$) in CBG than in Alf and appeared to be related to differences in lignin content and hemicellulosic monosaccharides. Xylose was less digested than arabinose in all forages. Across all forages, analytical methods ranked HC content and AD differently reflecting a method × diet interaction ($P < 0.002$). Results indicate that grass and legume HC differ in digestibility and that the choice of analytical methods may significantly affect the interpretation of HC degradation in the ruminant.

The plant cell wall has been envisioned to be composed of cellulose fibers embedded in an amorphous mixture of hemicellulose (HC), pectin, glycoprotein, and lignin. The HC fraction is a group of cell wall polysaccharides that comprise a large portion of the dry matter of many tropical and temperate grasses with lesser amounts in legumes. Hemicellulose polysaccharides have the ability to bind noncovalently through hydrogen bonding to cellulose and to bind covalently to the pectin polysaccharide (Albersheim, 1976). The HC polysaccharides may therefore serve to interconnect the cellulose fibrils and the pectin polysaccharides of the cell wall. Considerable research has subsequently been focused on the carbohydrate composition and digestion of HC (Gaillard, 1962; Bailey and Ulyatt, 1970; Bailey et al., 1976; Daughtry et al., 1978; Bacon, 1979; Windham et al., 1983; Bittner and Street, 1983; Pitman and Moore, 1985). However, because of a wide variety of analytical protocols and nomenclature, results are difficult to compare. Often plant polysaccharides are classified according to the analytical procedure used to separate given components. These procedures generally fall into two categories: (a) those involving hydrolysis of the cell wall; (b) those involving extraction

Richard B. Russell Agricultural Research Center, Plant Structure and Composition Research Unit, USDA—ARS, Athens, Georgia 30613 (W.R.W., J.J.E.), and Department of Animal and Dairy Science, University of Georgia, Athens, Georgia 30602 (H.E.A.).

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with various solvents. The difficulty in comparing results is caused by the fact that HC is not a uniform substance but a mixture of different polysaccharides with different solubilities (Blake and Richards, 1970).

The objectives of this research were to determine the digestibility of the HC and constituent monosaccharides in coastal Bermuda grass and alfalfa fed to growing steers and to assess the effect of analytical method on the determination of HC.

MATERIALS AND METHODS

Feeding Trial. Four Holstein steers were fitted with permanent ruminal and abomasal latex rubber cannulae. After a 3-week postoperative recovery period, the steers were placed in individual pens with free stalls on a concrete flush floor and assigned to treatments in a 4 × 4 Latin square. Treatments: (a) coastal Bermuda grass hay (CBG-H); (b) dehydrated coastal Bermuda grass (CBG-D); (c) alfalfa hay (Alf-H); (d) dehydrated alfalfa hay (Alf-D). The steers were fed 2.02 and 1.98% of their body weight in dry matter (DM) from CBG and Alf, respectively, daily as single sources of protein and energy. Trace mineralized salt and water were available at all times. The CBG-H was second cutting, fertilized with 252 kg of N/ha and harvested at 23 days. The CBG-D was obtained from a commerical dehydrator and harvested at 23 days. Alfalfa hay was grown in Minnesota, and Alf-D was obtained from a local feed broker; age at harvest was unavailable for the alfalfa.

Each period of the Latin square consisted of a 10-day preliminary period and a 5-day sample collective period. Abomasal digest samples were collected at 2-h intervals (0800, 1000, 1200, 1400, 1600 h) on days 1–5 of the collection period. Composites of individual abomasal samples were made within treatment by period by combining 100 mL from each sampling time and were separated into solid and liquid digest phases by centrifuging at 1000*g* for 30 min. The particulate digest was washed two times with 150 mL of 1 N HCl and centrifuged as above. Fecal grab samples were taken at 0600, 0800, 1000, and 1200 h from each steer on the third day of each collection period. The washed abomasal particulate digests and composite fecal samples were lyophilized and chemically analyzed as described below. Further details of the feeding trial were described by Amos et al. (1983).

Chemical Analyses. Diets and lyophilized abomasal particulate digests and feces were analyzed for neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (72% H_2SO_4), and ash; cellulose content was calculated as described by Goering and Van Soest (1970). Hemicellulose was calculated by one method—conventional (HC-C), as the difference between ash-free NDF and ash-free ADF determined on separate aliquots (Goering and Van Soest, 1970)—and determined by two methods—(1) sequential (HC-S), as the material solubilized by acid detergent treatment of NDF (Van Soest and Robertson, 1979), and (2) trifluoroacetic acid soluble (TFA, HC-TFA), as the material solubilized by 2 N TFA treatment of NDF (Windham et al., 1983).

Hydrolysis and Fractionation of NDF. The flow diagram for the fractionation of NDF by TFA hydrolysis is shown in Figure 1. Isolated NDF of all samples was prepared for constituent monosaccharide analysis as outlined by Windham et al. (1983). Monosaccharide analysis of isolated NDF was conducted by weighing duplicate 100-mg samples into 25-mL reaction flasks, adding 20 mL of 2 N TFA, and hydrolyzing at 121 °C for various times. The NDF from the CBG diets was hydrolzyed for 60 min (Barton et al., 1982), whereas for the Alf diets,

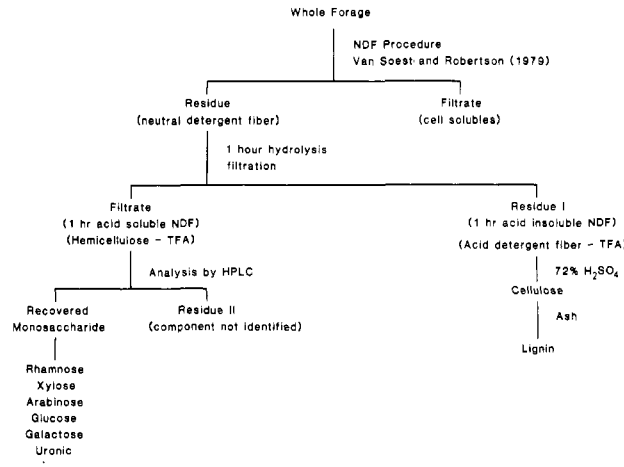

Figure 1. Flow diagram for the fractionation of forage cell walls by TFA hydrolysis.

abomasal particulate digests and feces for all forages were hydrolyzed for 15, 30, 45, 60, and 120 min to determine the maximum time of hydrolysis for optimum-recovery component sugars. The hydrolysate was filtered through a 15-mL Buchner funnel and washed with 35 mL of distilled water to yield a filtrate [i.e., hemicellulose determined by TFA hydrolysis (HC-TFA)] and residue I [i.e., estimate of ADF (ADF-TFA)]. The HC-TFA discussed in this paper refers to the fraction designated acid-soluble NDF (ASNDF), by Windham et al. (1983). Residue I was lyophilized to determine the amount of cell wall material not hydrolyzed, and for cellulose, ADL, and ash determinations as previously described (Goering and Van Soest, 1970).

The filtrate (HC-TFA) was evaporated to dryness on a rotary evaporator at 40 °C under reduced pressure (30 mmHg) and washed three times with distilled water (35 mL). The dried hydrolysate was then diluted to 6 mL with distilled water and the resultant solution passed through a Waters C_{18} Sep-Pak cartridge charged with 6 mL of acetonitrile followed by a 6 mL of water wash. The hydrolysate was eluted through the cartridge followed by a 4-mL water wash and evaporated to dryness. The hydrolysate was subsequently dissolved in 2 mL of acetonitrile–water (75:25, v/v) containing 2.5 mg/mL of inositol as an internal standard. A 1-mL aliquot was used for component sugar analysis by high-performance liquid chromatography (HPLC) with a Waters Associates ALC 201 chromatograph as described by Windham et al. (1983). After determination of component sugars, the remaining hydrolysate (1 mL) was evaporated to dryness and dissolved in 90 mL of distilled water for the determination of uronic acids as outlined by Blumenkrantz and Asboe-Hansen (1973). Total recovery of component sugars (i.e., recovered monosaccharides; Figure 1) was calculated and expressed as a percentage of the HC-TFA. Residue II was calculated as the difference between HC-TFA and recovered monosaccharides.

Sugar Standards. Working standards in the range of 1–4 mg/mL were prepared by dilution in acetonitrile–water (75:25, v/v) solution. A concentration of 3.75 mg/mL for rhamnose, xylose, arabinose, and galactose and 1.75 mg/mL for glucose yielded optimum resolution and was used to determine response factors for the individual monosaccharides.

Digestibility Calculations. Total daily DM recovered in the abomasal particulate digests and feces was estimated by using lignin (72% H_2SO_4) from the conventional detergent system (Goering and Van Soest, 1970) as a marker. Apparent digestibility coefficients (ADC) for DM, NDF,

Table I. Effect of Analytical Method on the Estimation of Forage Cell Wall Constituents (Percent Dry Matter Basis) in Alfalfa and Coastal Bermuda Grass Diets[f]

forage	method[a]	NDF[b]	hemicellulose	ADF[c]	cellulose	ADL[d]
CBG[e]-hay	conventional	70.2	36.6[f]	33.6[f]	29.9[f]	3.7[f]
	sequential	69.6	37.8[g]	31.8[g]	28.8[g]	3.0[g]
	TFA hydrolysis	69.5	38.5[g]	31.0[g]	27.9[g]	3.1[g]
CBG-dehydrated	conventional	66.5	33.8[f]	32.7[f]	28.3[f]	4.4[f]
	sequential	65.5	35.3[g]	30.2[g]	26.2[g]	4.0[g]
	TFA hydrolysis	66.0	36.5[g]	29.5[g]	25.3[g]	4.2[f,g]
alfalfa-hay	conventional	48.8	12.1[f]	36.6[f]	27.9[f]	8.7[f]
	sequential	47.3	15.2[g]	32.1[g]	24.0[g]	8.0[g]
	TFA hydrolysis	48.0	17.8[h]	32.2[g]	23.0[h]	7.2[h]
alfalfa-dehydrated	conventional	41.8	9.7[f]	32.1[f]	24.5[f]	7.6[f]
	sequential	41.4	12.5[g]	28.9[g]	22.6[g]	6.4[g]
	TFA hydrolysis	41.6	15.2[h]	26.4[h]	19.9[h]	6.5[g]
SEM[g]			0.2	0.4	0.2	0.3

[a] Conventional detergent analysis, Georing and Van Soest (1970); sequential detergent analysis, Van Soest and Robertson (1979); trifluoroacetic acid hydrolysis, Windham et al. (1983). [b] Neutral detergent fiber. [c] Acid detergent fiber. [d] Acid detergent lignin. [e] Coastal Bermuda grass. [f] Means within columns within forage with unlike superscripts differ ($P < 0.05$). [g] Standard error of mean.

ADF, cell content (CC), cellulose, HC-C, HC-S, HC-TFA, and component sugars of HC were calculated from the dietrary intake compared to amounts recovered in the abomasal particulate digesta and feces.

Statistical Analysis. Data were treated by analysis of variance for a 2 × 2 factorial design within a 4 × 4 Latin square by using the statistical analysis system described by SAS (1985). Data for HC content, digestibility of HC, and fiber content of forages, abomasal digests, and feces due to analytical methods were treated by analysis of variance within the Latin square (SAS, 1985). Error term to test main effects and interactions was the residual sum of squares from period × analytical methods, animal × analytical methods, and period × animal × analytical methods with 24 degrees of freedom. Time of hydrolysis to establish optimum recovery of component sugar monomers in Alf-NDF and abomasal particulate digests and feces from all forages was analyzed by orthogonal polynomials for unequally spaced treatments (Steel and Torrie, 1960). Difference between means were determined by using Scheffe's multiple-comparison procedure as described by Kleinbaum and Kupper (1978).

RESULTS AND DISCUSSION

The effects of analytical methods on the forage cell wall composition are shown in Table I. When averaged across all methods, the two Alf samples were lower in percentage NDF (44.8 vs. 67.9), HC (13.8 vs. 36.4), and cellulose (23.7 vs. 27.7), equal in ADF (31.4 vs. 31.5), but higher in ADL (7.4 vs. 3.7) than the two CBG diets samples. The ADF content by conventional analysis was greater ($P < 0.05$) than that obtained from acid detergent (AD) treatment of NDF for all forages. As a result, the estimate of HC as the difference between NDF and ADF from conventional analysis was less than ($P < 0.05$) the difference from sequential analysis. As pectic substances are not as fully extracted by AD reagent as by ND reagent (Bailey and Ulyatt, 1970), ADF is greater due to pectin residues in the HC-C than the HC-S method. As HC is calculated by difference, HC-S exceeds HC-C for feedstuffs containing pectic substances like alfalfa. The presence of noncellulosic sugars in the AD residues (Theander and Aman, 1980; Bittner and Street, 1983; Pitman and Moore, 1985) and the apparent solubility of noncellulosic polysaccharides in the ND reagent (Bittner and Street, 1983) contributed to the underestimation of calculated HC. In addition, Bailey and Ulyatt (1970) reported that at least half of the pectic substances is not removed and much of the HC not extracted with 1-h AD treatment, which would result in a higher estimate of ADF and a lower estimate of HC. Van Soest and Robertson (1979) reported that preextraction with ND followed by AD (i.e., sequential method) would allow a more accurate determination of HC.

Direct estimates of HC from hydrolysis of plant cell walls with TFA have been reported by numerous researchers (Collings and Yokoyama, 1979; Barton et al., 1982; Windham et al., 1983). Upon hydrolysis of plant cell walls or NDF by TFA, HC was assumed to be the acid-soluble portion of NDF (HC-TFA) and the acid-insoluble portion was cellulose plus lignin (i.e., ADF-TFA) (Figure 1). Hydrolysis of NDF by TFA for the estimate of HC-TFA and ADF-TFA and comparison of these data with convential and sequential methods resulted in an analytical method × forage interaction ($P < 0.03$).

This interaction, or lack of consistent extraction across forages, is due to the greater ($P < 0.05$) extraction of HC by TFA in Alf compared with CBG. In CBG, sequential and TFA values for HC and ADF were equal; however, in Alf the TFA values for HC were higher ($P < 0.05$) and those of ADF were lower ($P < 0.05$) than in the sequential method. These differences in HC-TFA and ADF-TFA are due to the hydrolysis of cellulose from Alf-NDF as reflected by the cellulose values [Table I; analytical method × forage interaction ($P < 0.005$)]. One hour hydrolysis was generally optimum for the majority of component sugars in HC (or NDF) but may be in excess for glucose since 1-h TFA hydrolysis may hydrolyze glucose from cellulose as well as HC.

The lignin values obtained from Alf and CBG differed due to forage ($P < 0.001$) and analytical method ($P < 0.04$). The effect of analytical method is due to conventional ADL being higher ($P < 0.05$) than sequential or ADL-tFA. The lower lignin values from sequential detergent analysis are possibly due to the use of sodium sulfite in the ND procedure, which unfortunately degrades lignin as well as reduces fiber nitrogen (Van Soest and Robertson, 1979). The trend of a further decrease in lignin content from the ADF-TFA residue is possibly due to the combination of sodium sulfite and the hydrolysis with TFA. Barton et al. (1982) and Windham et al. (1983) reported the presence of an acid-resistant macromolecule, possibly a lignin–carbohydrate complex during TFA hydrolysis and fractionation of forage cell walls. The decrease in lignin could possibly be due to the solubilization of this macromolecule upon hydrolysis with TFA.

Windham et al. (1983) found that hydrolysis of NDF from CBG with 2 N TFA at 121 °C was optimum at 1 h on the basis of the recovery of component sugars. This time of hydrolysis is in agreement with Barton et al. (1982) for NDF from several tropical grasses. After 1 h the re-

Table II. Monosaccharide Composition of Hemicellulose Hydrolyzed from Neutral Detergent Fiber by Trifluoroacetic Acid (Percent of Whole Forage, Dry Matter)[a]

forage	rhamnose	xylose	arabinose	glucose	galactose	uronic acid	rec monosaccharide
GBC[b]-hay	0.15[c]	15.2[c]	4.2[c]	4.9[c]	1.7[c]	1.5[c]	27.6[c]
CBG-dehydrated	0.14[c]	13.3[d]	3.6[d]	5.0[c]	1.1[d]	1.5[c]	24.7[d]
alfalfa-hay	0.33[d]	6.2[e]	1.7[e]	1.3[d]	1.3[d]	2.0[d]	12.8[e]
alfalfa-dehydrated	0.31[d]	4.7[f]	1.2[f]	0.9[d]	.9[d]	1.7[d,c]	9.9[f]

[a] Each value is the mean of six observations (i.e., three injections of duplicate hydrolysates). Means within columns with unlike superscripts differ ($P < 0.05$). [b] Coastal Bermuda grass.

Table III. Effect of Analytical Method on Estimation of Forage Cell Wall Constituents (Percent Dry Matter Basis) in Abomasal Particulate Digests of Steers Fed Coastal Bermuda Grass and Alfalfa Forages[a]

forage	method[b]	hemicellulose	acid detergent fiber	cellulose	acid detergent lignin
CBG-H	conventional	26.1[b]	34.6[b]	24.4[b]	10.2[b]
	sequential	32.1[c]	31.0[c]	23.7[b,c]	7.3[c]
	TFA hydrolysis	31.3[c]	28.9[c]	20.9[c]	8.0[c]
CBG-D	conventional	24.1[b]	36.4[b]	24.1[b]	12.3[b]
	sequential	27.4[b,c]	32.7[c]	24.3[b]	8.4[c]
	TFA hydrolysis	29.6[c]	31.0[c]	22.0[b]	9.0[c]
Alf-H	conventional	12.8[b]	50.0[b]	32.2[b]	17.8[b]
	sequential	17.5[c]	45.4[c]	31.8[b]	13.6[c]
	TFA hydrolysis	24.1[d]	37.6[d]	25.8[c]	11.8[d]
Alf-D	conventional	13.7[b]	45.4[b]	29.9[b]	15.5[b]
	sequential	17.5[c]	41.4[c]	28.8[b]	12.6[c]
	TFA hydrolysis	22.8[d]	33.7[d]	23.5[c]	10.2[d]
SEM[c]		0.4	0.4	0.3	0.2

[a] Means within columns within forage with unlike superscripts differ ($P < 0.05$). [b] Conventional detergent analysis, Georing and Van Soest (1970); sequential detergent analysis, Van Soest and Robertson (1979); trifluoroacetic acid hydrolysis, Windham et al. (1983). [c] Standard error of mean.

covery of each sugar except glucose began to decrease. The Alf diets, abomasal particulate digests, and feces from CBG and Alf were hydrolyzed for various times to determine the maximum time for optimum recovery of component sugars. Analysis of variance indicated a difference ($P < 0.01$) in the amount of carbohydrate recovered due to the time of hydrolysis. In Alf diets, recovery of all carbohydrates except glucose indicated a quadratic effect ($P < 0.01$) with increasing time of hydrolysis, with 45 min being the maximum for optimum recovery component sugars. There was a linear increase ($P < 0.05$) in the recovery of glucose, which was due to the hydrolysis of cellulose from the cell wall (Table I). The recovery of component sugars with increasing time of hydrolysis of NDF from abomasal particulate digests and feces from all forage also indicated a quadratic response ($P < 0.01$) and a linear increase ($P < 0.05$) with glucose. On the basis of recovery of component sugars, 1 h was chosen as the optimimum time of hydrolysis for abomasal particulate digests and feces from all diets.

Monosaccharides in HC-TFA, expressed as a percentage of whole forage DM, are given in Table II. Xylose, arabinose, and glucose are the major components of the HC-TFA in CBG compared to predominance of xylose in Alf. Coastal Bermuda grass contained 2.6, 2.7, and 4.5 times more xylose, arabinose, and glucose, respectively, than Alf. The amounts of rhamnose and uronic acid were greater ($P < 0.05$) in Alf than CBG and varied only slightly in the percentage of galactose. The xylose and arabinose contents of the dehydrated forages were less ($P < 0.05$) than that in the hay. This difference was also reflected in the recovered monosaccharides based on HPLC and UV analysis. The recovery of total monomers was 71.6, 67.7, 71.9, and 65.3% for CBG-H, CBG-D, Alf-H, and Alf-D, respectively. Goering (1976) reported that drying of forages at 40, 60, 80, and 100 °C for 24 h resulted in an inverse relationship between HC and acid detergent insoluble nitrogen (ADIN) content with increasing temperatures. Amos et al. (1983) using these same forages reported ADIN values of 0.12, 0.29, 0.17, and 0.18% of the DM for CBG-H, CBG-D, Alf-H, and Alf-D, respectively. Goering (1976) reported that when ADIN exceeded 0.29% of Alf DM, the protein was heat damaged. Thus, the CBG-D used in this study is borderline for heat-damaged protein. The ADIN of Alf-D does not indicate extensive heat damage during dehydration, but on the basis of the lower ($P < 0.05$) recovered monomer composition it appears that dehydration resulted in the formation of protein–carbohydrate bonds. The lower recovery of monomers from NDF of CBG-D and Alf-D could reflect the process of dehydration that affected the recovery of monomers from the TFA filtrate.

Estimations of forage cell wall constituents in abomasal particulate digests and feces of steers fed CBG and Alf are shown in Tables III and IV, respectively. The estimates for HC and ADF from abomasal particulate digests and fecal residue indicated an analytical method × forage interaction ($P < 0.02$). This interaction is due to the higher ($P < 0.05$) hemicellulose and lower ($P < 0.05$) ADF values by TFA treatment of the Alf-NDF residues. The ADF content in digest residues from all forages by conventional detergent analysis was greater ($P < 0.05$) than that obtained from AD treatment of NDF. Therefore, the estimates of HC as the difference between NDF and ADF from sequential analysis were greater than ($P < 0.05$) the difference from conventional analysis. The further increase ($P < 0.05$) in HC-TFA and decrease ($P < 0.05$) in ADF-TFA of Alf with TFA hydrolysis was due to the time of hydrolysis. The effect of hydrolysis time is reflected in the Alf cellulose value obtained from 72% H_2SO_4 treatment of the ADF-TFA residue. This treatment resulted in lower ($P < 0.05$) cellulose values than those obtained with 72% H_2SO_4 treatment of the conventional and (or) sequential AD residues.

The determination of ADL in the abomasal particulate digests and feces also exhibited an analytical method × forage interaction ($P < 0.02$). The lower ($P < 0.05$) ADL values from 72% H_2SO_4 treatment of the ND preextracted ADF residue was due to the use of sodium sulfite in the

Table IV. Effect of Analytical Method on Estimation of Forage Cell Wall Constituents (Percent Dry Matter Basis) in Feces of Steers Fed Coastal Bermuda Grass and Alfalfa Forages[a]

forage	method[b]	hemicellulose	acid detergent fiber	cellulose	acid detergent lignin
CBG-H	conventional	25.2[b]	35.7[b]	24.5[b]	11.2[b]
	sequential	29.8[c]	30.8[c]	23.9[b,c]	7.0[c]
	TFA hydrolysis	30.9[c]	29.0[c]	20.8[c]	8.2[c]
CBG-D	conventional	24.1[b]	35.6[b]	24.3[b]	11.2[b]
	sequential	28.4[c]	31.1[c]	23.5[b]	7.6[c]
	TFA hydrolysis	30.1[c]	28.7[c]	20.5[c]	8.2[c]
Alf-H	conventional	12.6[b]	49.3[b]	31.7[b]	17.6[b]
	sequential	19.1[c]	43.0[c]	29.6[c]	13.4[c]
	TFA hydrolysis	24.8[d]	36.7[d]	24.8[c]	11.9[d]
Alf-D	conventional	10.7[b]	45.5[b]	29.0[b]	16.5[b]
	sequential	16.4[c]	39.7[c]	28.3[c]	12.1[c]
	TFA hydrolysis	21.8[d]	34.4[d]	23.9[c]	10.5[d]
SEM[c]		0.7	0.4	0.4	0.2

[a] Means within columns within forage with unlike superscripts differ ($P < 0.05$). [b] Conventional detergent analysis, Goering and Van Soest (1970); sequential detergent analysis, Van Soest and Robertson (1979); trifluoroacetic acid hydrolysis, Windham et al. (1983). [c] Standard error of mean.

ND procedure to eliminate keratinaceous tissues in the abomasal particulate digests and fecal residue. The further decrease ($P < 0.05$) in ADL values in Alf digest residues obtained from the 72% H_2SO_4 treatment of the ADF-TFA residue is due to combination of ND extraction with sodium sulfite followed by TFA hydrolysis. Barton et al. (1982) reported the presence of a lignin-carbohydrate complex (LCC) from the TFA hydrolysis of CBG-NDF. This LCC fraction is yellow to light brown in color and precipitates on the wall of the flasks during evaporation. The amount of the LCC fraction accounts for the remaining DM (Figure 1, residue II) that was hydrolyzed by TFA from NDF but not recovered as free monosaccharides through the HPLC. The LCC fraction analyzed by Barton et al. (1982) with ^{13}C nuclear magnetic resonance spectroscopy is equivalent to residue II (Figure 1) in this study. The LCC fraction contained *p*-coumaric and ferulic type lignin groups in both the A and B forms. The carbohydrates were bound to the "lignin" moieties and, while solubilized by TFA, were not hydrolyzed. However, no interpretation was made as to how the lignin and carbohydrates are bound. Therefore, the lower ($P < 0.05$) Alf ADL values in the ADF-TFA residue are due to the combination of sodium sulfite in the ND procedures and solubilization of the LCC fraction by TFA hydrolysis. The data presented in Tables III and IV, as well as Table I, "illustrate the difficulty of designing a single system of analysis for all conditions" (Van Soest and Robertson, 1979).

The apparent digestibility coefficients (ADC) and the effect of analytical methods on ADC of HC are shown in Table V. The differences in the estimates of HC in the forages and particulate digests resulted in an analytical method by forage interaction ($P < 0.002$) for ADC. Hemicellulose digestibilities were higher ($P < 0.05$) from conventional analysis in all forages compared to the other analyses. Estimates of HC digestibility from sequential analysis of CBG were not different from that obtained by TFA hydrolysis and HPLC analysis with the exception of the reticulorumen values of CBG-H and CBG-D from HPLC analysis. However, ADC values of Alf-HC from TFA hydrolysis and HPLC analysis were lower ($P < 0.05$) than that obtained from either detergent system analysis.

Apparent digestibility coefficients were higher ($P < 0.05$) in the reticulorumen and whole gastrointestinal tract for CBG-H and CBG-D than for either Alf forage. These data are in agreement with Gaillard (1962) who reported that the ADC of the HC of legumes was lower than that of grasses. Gaillard (1966), Sullivan (1966), and Balwani et al. (1969) have also reported that grass and legume HC

Table V. Effect of Analytical Method on Apparent Digestibility Coefficients for Hemicellulose in Coastal Bermuda Grass and Alfalfa Forages[d]

	analytical method,[a] %			
forage	conventional	sequential	TFA hydrolysis	HPLC
coastal Bermuda grass hay				
RR[b]	73.3[d]	66.1[e]	69.1[f]	68.6[f]
WT[c]	76.6[d]	73.3[e]	72.8[e]	71.9[e]
coastal Bermuda grass dehydrated				
RR	74.1[d]	72.8[d,e]	70.1[e]	65.6[f]
WT	72.1[d]	67.6[e]	66.3[e]	65.5[e]
alfalfa-hay				
RR	47.9[d]	43.2[e]	31.6[f]	34.5[f]
WT	61.9[d]	48.3[e]	36.0[f]	33.7[f]
alfalfa-dehydrated				
RR	30.5[d]	31.4[d]	19.2[e]	20.8[e]
WT	48.5[d]	39.7[e]	32.3[f]	33.8[f]

[a] Conventional detergent analysis, NDF-ADF = hemicellulose; sequential detergent analysis, NDF-ADF = hemicellulose; trifluoroacetic acid hydrolysis, % hydrolyzed × % NDF = ASNDF; recovered "hemicellulosic" monosaccharides based on high-performance liquid chromatography analysis and colorimetric determination of uronic acids. [b] Reticulorumen. [c] Whole gastrointestinal tract. [d] Means on the same row with unlike superscripts differ ($P < 0.05$).

differed in digestibility and suggested that this may be due to a difference in the chemical heterogeneity of the xylans, which are the principal hemicellulosic components in the forages (Table II). Furthermore, Waite et al. (1964) and Gaillard (1966) have shown that the ADC of HC decreases as the plant matures, and this trend has been shown to correlate with the increased lignification of the plant. Therefore, the higher lignin value of Alf (Table I) compared to CBG might explain the lower ADC of HC due to lignin impeding polysaccharide digestion by its linkage to specific points in the polysaccharide chain.

The majority of forage HC digestion occurred in the reticulorumen (RR) as shown in Table V. As the undigested HC passes from the RR to the lower digestive tract, there is the possibility of further degradation by hemicellulolytic cecal microorganisms. However, with the exception of Alf-D, results obtained in this study indicated little postruminal digestion of HC. The data reported herein are in agreement with MacRae and Armstrong (1969) who found that when all or a major part of the ration fed to sheep was hay, 93–97% of the digestible HC was digested in the reticulorumen.

Table VI. Apparent Digestibility Coefficients (%) of Hemicellulose Monosaccharides Hydrolyzed from Neutral Detergent Fiber by Trifluoroacetic Acid[c]

item	CBG-H	CBG-D	Alf-H	Alf-D	SEM
rhamnose					
RR[a]	56.5[c]	48.2[d]	45.1[d]	47.9[d]	1.3
WT[b]	61.8[c]	45.4[d]	47.2[d]	56.2[c]	2.3
xylose					
RR	62.7[c]	59.3[c]	25.0[d]	3.3[d]	1.9
WT	66.0[c]	52.7[d]	22.6[e]	12.7[f]	2.2
arabinose					
RR	71.1[c]	71.5[c]	58.7[d]	45.2[e]	0.9
WT	74.9[c]	67.2[d]	52.6[e]	53.8[e]	0.7
glucose					
RR	86.4[c]	85.2[c]	55.1[d]	50.4[d]	0.9
WT	87.1[c]	84.4[c]	59.7[d]	57.2[d]	1.8
galactose					
RR	72.7[c]	70.3[c]	54.9[d]	58.6[d]	2.0
WT	77.5[c]	64.1[d]	49.5[e]	64.5[e]	1.1
uronic acids					
RR	69.9[c]	69.3[c]	32.4[d]	27.5[d]	1.9
WT	70.4[c]	68.1[c]	34.5[d]	43.8[e]	1.5

[a] Reticulorumen. [b] Whole gastrointestinal tract. [c] Means on the same row with unlike superscripts differ ($P < 0.05$).

Apparent digestibility coefficients of HC monosaccharides hydrolyzed from NDF by TFA indicate that the HC components were degraded to a greater ($P < 0.01$) extent in CBG than Alf and were not fermented equally in all diets (Table VI). The ADC of xylose, arabinose, and galactose was greater ($P < 0.05$) in CBG-H than CBG-D in whole gastrointestinal tract. These differences could be due to excessive heating during dehydration. Gaillard (1962) found that the ADC of xylose, arabinose, and galactose decreased in heated hay compared to fresh grass by 24, 72, and 47%, respectively. The ADC of arabinose was greater than for xylose, which is consistent with the labile nature of the arabinose (1→3) linkage to the xylan chain (Waite and Gorrod, 1959). Daughtry et al. (1978) reported in vigro disappearance of arabinose was greater than of xylose in the HC of Kentucky-31 and orchardgrass leaves. Daughtry et al. (1978) also reported that the xylose:arabinose (X:A) ratio increased from 1.6 in the original leaf tissue to 3.0 after rumen fermentation. As an indicator of plant maturity, the X:A ratio has been used; i.e., the ratio increases with age of the plant (Buchala and Wilkie, 1973). The X:A ratio was 3.6, 3.7, 3.6, and 3.9 for CBG-H, CBG-D, Alf-H, and Alf-D, respectively. The X:A ratio in this study increased after digestion to 5.1, 4.7, 7.4, and 7.2 for CBG-H, CBG-D, Alf-H, and Alf-D, respectively. Therefore, these data as well as those of Daughtry et al. (1978) and Collings and Yokoyama (1979) suggest that the X:A ratio may provide a selection criteria for improving forage utilization by ruminants.

There was no difference ($P > 0.05$) in the ADC of glucose and uronics in the hay compared to the dehydrated forages; however, the ADC values for glucose and uronics were 32 and 43.5% greater, respectively, in CBG than Alf. In Alf the main factor limiting the digestion of xylose and its associated uronic acid units is the lignin that is interlinked with the xylan chains, probably via glucuronic acid units. Uronic acis are proposed to be the primary monosaccharide constituents linking heteroxylans to phenolic components of lignin (Wilkie, 1979). Support for this explanation comes from the fact that both xylose and the uronic acid were the least digestible among the hemicellulosic sugars.

Apparent digestibility coefficients were higher ($P < 0.05$) for DM, NDF, ADF, and cellulose (Table VII) in the reticulorumen for CBG-D and CBG-H than for either Alf. The total-tract ADC values for DM, NDF, ADF, and cellulose were also higher ($P < 0.05$) for CBG than Alf; however, ADC values for NDF, ADF, and cellulose were lower in CBG-D than in CBG-H ($P < 0.05$). The ADC for cell content (CC) was higher ($P < 0.05$) for CBG than alfalfa. Digestion of DM and cellulose for Alf was lower in the present study than would normally be expected; however, due to its higher lignin, the lower digestibility for Alf than CBG seems reasonable (Barton et al., 1976). Moreover, the summative equation of Goering and Van Soest (1970) predicts a DM digestibility of 56.1 and 58.3% for Alf-H and Alf-D, respectively, and 62.0 and 57.7% for CBG-H and CBG-D, respectively. The lower digestibility of NDF and ADF from Alf (reticulorumen and whole tract, $P < 0.05$) than from either CBG also appears reasonable, because Alf contains less NDF and more lignin than CBG, but both forages contained similar amounts of ADF. Therefore, the cell walls of Alf are more highly lignified, resulting in lower HC, NDF, and ADF digestibility. Conversely, digestibility of CC in Alf was greater ($P < 0.05$) than CBG, and the cell walls of CBG were more digestible than cell content. The recovery values of fecal DM corrected for CC content and expressed as a percent of total fecal DM were 60.1 and 59.3% for CBG and Alf, respectively; as such, the greater digestibility of CC in Alf was due to the higher cell content. The lower digestibility of cell walls in CBG compared to CC could be due to the unavailability of starch grains and other nutrients to the rumen microorganisms in the leaf parenchyma bundle sheath cells (Akin and Burdick, 1977).

Table VII. Apparent Digestibility Coefficients (%) for Coastal Bermuda Grass and Alfalfa Forages[d]

item	CBG-H	CBG-D	Alf-H	Alf-D	SEM[a]
dry matter					
RR[b]	62.9[d]	60.7[d]	50.5[e]	50.7[e]	1.6
WT[c]	70.8[d]	59.9[d]	50.4[e]	53.6[e]	1.7
cell content					
RR	48.0[d]	51.9[d]	63.3[e]	65.0[e]	2.7
WT	55.6[d]	52.4[d]	63.6[e]	65.4[e]	2.8
NDF					
RR	67.1[d]	67.3[d]	36.8[e]	30.3[e]	2.3
WT	70.8[d]	64.0[e]	37.1[f]	37.6[f]	2.0
ADF					
RR	62.0[d]	59.8[d]	33.2[e]	34.9[e]	2.8
WT	64.5[d]	56.6[e]	33.7[f]	34.3[f]	1.7
cellulose					
RR	69.6[d]	69.2[d]	43.6[e]	43.3[e]	2.9
WT	72.4[d]	65.5[e]	44.1[f]	45.0[f]	2.0

[a] Standard error of mean. [b] Reticulorumen. [c] Whole gastrointestinal tract. [d] Means on the same row with unlike superscripts differ ($P < 0.05$).

These data illustrate the effect of different analytical methods on the determination of plant cell wall constituents and their digestibility. In addition, these differences reflect the difficulty of using a single system of analysis for the determination of plant cell wall constituents and digestibility. Although plant cell walls can be degraded and utilized to a considerable extent during their passage through the ruminant, a large proportion remains undegraded and is voided in the feces. Many factors, including lignification, crystallinity, hemicellulose–cellulose interactions, *O*-acetyl groups, phenolic acids, and species differences between plants have been implicated. However, it appears that lignin concentration is the overriding factor determining the extent of cell wall degradation.

Registry No. HC, 9034-32-6; cellulose, 9004-34-6; arabinose, 147-81-9; xylose, 58-86-6; rhamnose, 3615-41-6; glucose, 50-99-7; galactose, 59-23-4; lignin, 9005-53-2.

LITERATURE CITED

Akin, D. E.; Burdick, D. *Crop Sci.* **1977**, *17*, 529.

Amos, H. E.; Windham, W. R.; Evans, J. J. *J. Anim. Sci.* **1983**, *58*, 987.
Albersheim, P. In *Plant Biochemistry*; Bonner, J., Varner, J. E., Eds.; Academic: New York, 1976; pp 225–274.
Bacon, J. S. O. *Annu. Rep. Anim. Sci. Allied Sci.* **1979**, *35*, 99.
Bailey, R. W.; Ulyatt, M. J. *N.Z. J. Agric. Res.* **1970**, *13*, 591.
Bailey, R. W.; Monro, J. A.; Pickmere, S. E.; Chessen, J. *Misc. Pap—Landbouwhogesch Wageningen* **1976**, *12*, 1.
Balwani, J. L.; Johnson, R. R.; Dehority, B. A. *J. Dairy Sci.* **1969**, *52*, 1290.
Barton, F. E. II; Amos, H. E.; Burdick, D.; Wilson, R. L. *J. Anim. Sci.* **1976**, *43*, 504.
Barton, F. E. II; Windham, W. R.; Himmelsbach, D. S. *J. Agric. Food Chem.* **1982**, *30*, 1119.
Bittner, A. S.; Street, J. C. *J. Agric. Food Chem.* **1983**, *31*, 7.
Blake, J. D.; Richards, G. W. *Aust. J. Chem.* **1970**, *23*, 2353.
Blumenkrantz, W.; Asboe-Hansen, G. *Anal. Biochem.* **1973**, *54*, 484.
Buchala, A. J.; Wilkie, K. C. B. *Phytochemistry* **1973**, *12*, 655.
Collings, G. F.; Yokoyama, M. T. *J. Agric. Food Chem.* **1979**, *27*, 373.
Daughtry, C. S. T.; Holt, O. A.; Lechtenberg, V. L. *Agron. J.* **1978**, *70*, 550.
Gaillard, B. D. E. *J. Agric. Sci.* **1962**, *59*, 369.
Gaillard, B. D. E. *Neth. J. Agric. Sci.* **1966**, *14*, 205.
Goering, H. K. *J. Anim. Sci.* **1976**, *43*, 869.
Goering, H. K.; Van Soest, P. J. *Forage Fiber Analyses (Apparatus, Reagents, Procedures and some Applications)*; USDA, U.S. Government Printing Office: Washington, DC, 1970; Handbook 379.
Kleinbaum, D. G.; Kupper, L. L. *Applied Regression Analysis and Other Multivariable Methods*; Duxburg: North Scituate, MA, 1978.
MacRae, J. C.; Armstrong, D. G. *Br. J. Nutr.* **1969**, *23*, 377.
Pitman, W. D.; Moore, J. E. *Crop Sci.* **1985**, *25*, 790.
SAS Institute Inc. *SAS User's Guide: Statistics*; SAS Institute Inc.: Carry, NC, 1985.
Steel, R. G. D.; Torrie, J. H. *Principles and Procedures of Statistics*; McGraw-Hill: New York, 1960.
Sullivan, J. T. *J. Anim. Sci.* **1966**, *25*, 83.
Theander, O.; Aman, P. J. *J. Sci. Food Chem.* **1980**, *31*, 31.
Van Soest, P. J. *J. Anim. Sci.* **1964**, *24*, 838.
Van Soest, P. J.; Robertson, J. B. In *Standardization of Analytical Methodology of Feeds*; Pigden, W. J., Balch, C. C., Graham, M., Eds.; International Development Research Center: Ottawa, Canada, 1979; pp 49–71.
Waite, R.; Gorrod, A. R. W. *J. Sci. Food Agric.* **1959**, *10*, 308.
Waite, R.; Johnston, M.; Armstrong, D. G. *J. Agric. Sci.* **1964**, *62*, 391.
Wilkie, K. C. B. *Adv. Carbohydr. Chem. Biochem.* **1979**, *36*, 215.
Windham, W. R.; Barton, F. E. II; Himmelsbach, D. S. *J. Agric. Food Chem.* **1983**, *31*, 471.

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Analysis of Total and Insoluble Mixed-Linked (1→3),(1→4)-β-D-Glucans in Barley and Oats

Per Åman* and Hadden Graham

An enzymatic method for analysis of total mixed-linked (1→3),(1→4)-β-D-glucans (β-glucans), and insoluble β-glucans after extraction of soluble β-glucans with water for 2 h at 38 °C, has been developed. The method involves complete removal of starch, hydrolysis of β-glucans to glucose with a technical β-glucanase preparation, and analysis of formed glucose by the glucose oxidase method. Soluble β-glucans are calculated as the difference between total and insoluble β-glucans. Total and insoluble β-glucans were analyzed in barley samples from Montana and Scandinavia and in Swedish oat samples. The average content of total β-glucans in barley was 4.5%, with a range from 3.0 to 6.9%, and that in oats was 3.2%, with a range from 2.2 to 4.2%. In barley, on average, 54% of the β-glucans was soluble and in oats 80%.

Mixed-linked (1→3),(1→4)-β-D-glucans, referred to hereafter as β-glucans, are frequently present in endosperm cell walls of cereals (Anderson et al., 1978; Bacic and Stone, 1981). Compared to other cereals, barley and oats have relatively high contents of total β-glucans and figures between 2 and 10% for barley (Bamforth, 1982) and 2 and 4% for oats (Wood and Weisz, 1984) have been reported. The total content of β-glucans varies with both genetic and environmental factors (Hesselman, 1983; Henry, 1986). Both soluble and insoluble β-glucans are present in cereals with factors such as particle size, β-glucanase activity of the flour, and temperature, pH, and ionic strength of the extraction media affecting solubility (Wood et al., 1978; Ahluwalia and Ellis, 1985).

In the brewing industry a high content of β-glucans in the barley may lead to problems such as diminished rate of wort filtration, haze formation in the beer, and possibly reduced extraction efficiency (Bamforth, 1985; McCleary and Glennie-Holmes, 1985). β-Glucans can also have antinutritional properties, particularly in chicken diets where they may give sticky droppings and affect food intake, growth rate, and feed-conversion efficiency (Hesselman, 1983; Hesselman and Åman, 1986). Oat bran diets, rich in soluble fibers (β-glucans), may have distinct hypocholesterolemic effects in humans, with decreased serum and low-density lipoprotein cholesterol and increased fecal bile acid excretion (Kirkby et al., 1981; Anderson et al., 1984). Porridge oats have been shown to have a lowering effect on the glycemic index, indicating that β-glucan-rich products may be useful food ingredients for people with low blood glucose tolerance (Jenkins et al., 1981).

Insoluble β-glucans in grain cell walls encapsulate easily available nutrients such as starch, intracellular protein, and fat and act as a physical hindrance to nutrient hydrolysis and utilization, while soluble or solubilized β-glucans give rise to viscous solutions, which also may interfere with nutrient availability (Hesselman and Åman, 1986). In order to further evaluate the technical and nutritional effects of β-glucans in cereals, reliable methods for the

Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden.

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