Chemical Composition and Digestibility of Ryegrass Straw

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The Lolium ryegrass straw was analyzed for cell soluble matter (CSM), cellulose, hemicellulose, lignin, and ash, and the digestibility of each component was determined. Respective digestibilities were 64.5, 45.6, 42.8, 0, and 0%. Based on data obtained on 12 straws, the presence of higher lignin levels resulted in reduced in vitro rumen digestibility (IVRD) values, and the lignin was not digested. The addition of lignin to ryegrass straw did not influence IVRD. Hemicellulose was necessary for the digestion of straw, but removal of CSM from the straw did not affect its IVRD. Digestibility of the whole straw, CSM-free straw, and undigested residue of rumen-fermented straw was increased by NaOH (2%) treatment, whereas that of hemicellulose-free straw was increased by dioxane treatment. Treating straw with the culture filtrate of lignin-decomposing organisms reduced the digestibility by 27%, whereas that treated with cellulase increased by 14%. Polyphenoloxidase, peroxidase, and chitinase had no apparent effect on the digestibility.

Increased concern for environmental pollution and increased demand for more forage feeds prompted much research for the development of animal feed from grass straw (Anderson and Ralston, 1973; Guggolz et al., 1971; Oh et al., 1971). Even though straw contains enough cellulose to make it an excellent source of energy for the ruminants, it is a poor-quality feed in its natural state. The main shortcomings of straw as animal feed are its low digestibility, low protein content, poor palatability, and bulkiness. Many researchers have tried to improve the digestibility of straw by chemical, physical, and enzymatic treatments (Baker, 1973; Guggolz et al., 1971; Chandra and Jackson, 1971; Waiss et al., 1972; Tarkow and Feist, 1969; Baker et al., 1959; Han and Callihan, 1974). However, the digestibility characteristics of each constituent of straw are not well elucidated. The purpose of this study was to elucidate the role of each constituent in overall digestibility of grass straw.

MATERIALS AND METHODS

Substrate. Annual ryegrass (*Lolium multiflorum Lam.*), sun-dried and ground to pass a 20-mesh screen, was used as a test substrate. In some experiments, 12 samples of various straw produced in the Willamette Valley of Oregon were also used. Acid detergent fiber (ADF) straw was prepared by the method of Van Soest (1963): 100 g of straw was mixed with 1.5 l. of acid detergent (20 g of cetyltrimethylammonium bromide dissolved in 1 l. of 1 *N* H_2SO_4) and 30 ml of decalin (decahydronaphthalene). The mixture was heated to boil in 5 to 10 min. Heat was reduced as boiling began. Boiling was adjusted to an even level, and refluxed 60 min from the onset of boiling. The mixture was filtered through four layers of cheesecloth and washed four times with hot water (90–100°) and twice with acetone. By this treatment, the hemicellulose portion was completely removed. The treated and washed straw was then dried overnight under a draft hood at room temperature. Cell wall component (CWC) straw was prepared exactly in the same manner as described above except using neutral detergent, which removed cell-soluble matter (CSM) from the straw. RR straw was the undigested residue of rumen-fermented straw. About 200 g of straw was fermented with 8 l. of rumen fluid under CO_2 atmosphere at 39° for 7 days. The undigested residue was filtered and washed through four layers of cheesecloth and dried at room temperature.

Enzyme Preparation. Lignolytic and cellulolytic enzymes were prepared as follows. Cultures of *Polyporus versicolor, Polyporus abietnus* (Institute of Fermentation, Osaka, Japan), *Poria sabacida* (New York Botanical Garden, New York, N.Y.), and *Trichoderma viride* (U.S. Army Natick Laboratories, Natick, Mass.) were grown on straw-basal medium or steamed wheat bran. The straw-basal medium consisted of $(NH_4)SO_4$, 6.0 g; KH_2PO_4, 1.0 g; K_2HPO_4, 1.0 g; $MgSO_4$, 0.1 g; $CaCl_2$, 0.1 g; yeast extract, 0.5 g; $FeCl_3 \cdot 6H_2O$, 16.7 mg; $ZnSO_4 \cdot 7H_2O$, 0.18 mg; $CuSO_4 \cdot 5H_2O$, 0.16 mg; $CoCl_2$, 0.18 mg; EDTA, 20.1 mg; and 10–15 g of ground straw per liter of distilled water.

Table I. Chemical Composition and Digestibility (IVRD) of Ryegrass Straw

Constituent	Dry matter, % Composition	Dry matter, % Indigestible[a]	IVRD, %
Whole straw	100.0	52.0	48.0
Cell soluble matter	29.6	10.5	64.5
Cellulose	36.8	20.0	45.6
Hemicellulose	27.1	15.5	42.8
Lignin	5.4	5.4	0
Ash	2.0	2.0	0

[a] Indigestible residue after 3-day in vitro rumen fermentation.

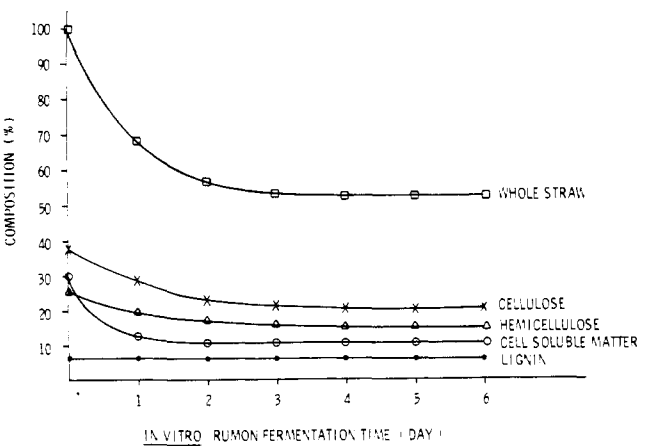

Figure 1. Digestion kinetics of ryegrass straw component by rumen microorganisms.

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Table II. Chemical Composition and Digestibility of 12 Different Straws[a]

Sample[b]	IVRD,[c] %	CSM,[d] %	Hemicellulose, %	Cellulose, %	Lignin, %	Ash, %
Orchardgrass, Pennlate (green)	73.00	21.92	24.69	27.25	4.42	1.72
Bentgrass, Highland (TH6239)	64.26	35.98	24.66	30.87	6.31	2.18
Red fescue, Cascade (green)	63.72	29.59	33.08	31.37	4.44	1.52
Bentgrass, Highland (untreated)	62.96	34.40	29.69	28.08	5.77	2.06
Kentucky bluegrass, Newport	61.97	18.72	35.98	38.58	6.59	0.13
Perennial ryegrass (leaves)	60.90	28.94	29.79	34.96	6.19	0.12
Bentgrass, Highland (CCC)	57.50	34.86	27.74	30.05	4.89	2.46
Red fescue, Cascade (stem)	56.20	15.25	36.97	37.98	8.80	1.00
Perennial ryegrass (stem)	55.40	26.17	26.54	38.81	7.19	1.29
Orchardgrass, Pennlate (stem)	48.24	25.27	19.88	44.83	8.72	1.30
Annual ryegrass	45.36	27.21	24.93	38.87	7.18	1.80
Acid-hydrolyzed annual ryegrass[e]	16.60	14.90	8.10	54.89	18.56	4.55

[a] Values are means of triplicate sample. [b] Parentheses indicate the portions of the straw taken or the chemicals used for the plant. [c] In vitro rumen digestibility. [d] Cell soluble matter. [e] Residue of the straw hydrolyzed with 0.5 N H_2SO_4, 1 hr, at 121°.

Table III. Correlation Coefficients of Straw Components and Digestibility

Item	r
Digestibility: lignin	−0.9308
Digestibility: cellulose	−0.9056
Digestibility: hemicellulose	0.8015
Digestibility: cell soluble matter	0.4866
Digestibility: lignin − cellulose	−0.9380[a]

[a] $y = 97.51 - 1.3580x_1 - 0.6129x_2$, where x_1 = lignin content and x_2 = cellulose content.

Table IV. Effect of Added Lignin on Digestibility of Straw

Lignin	Amount added, %	IVRD,[a] %
Control	0	60.81 ± 2.38
NH_3-lignin sulfonate[b]	1	61.32 ± 1.62
	5	61.92 ± 0.60
	10	66.61 ± 3.06
	20	62.25 ± 0.85
Polyfon O[b]	1	61.32 ± 1.73
	5	58.57 ± 0.63
	10	58.72 ± 1.45
	20	56.48 ± 0.27
Polyfon T[b]	1	63.95 ± 7.57
	5	62.85 ± 1.75
	10	63.10 ± 0.76
	20	60.97 ± 1.92

[a] In vitro rumen digestibility. Values are means of three replicates with standard deviations. [b] Products of West Virginia Pulp and Paper Co.

When the liquid medium was used, the whole culture (10–15 days old) was blended and filtered through Whatman No. 1 filter paper. When the organisms were grown on steamed bran, a part of mycellia–substrate mixture was blended with three parts of distilled water and filtered through Whatman No. 1 filter paper. These filtrates were used as crude enzyme. Polyphenoloxidase (Worthington Biochemical Corp.), peroxidase (Sigma Chemical Co., St. Louis, Mo.), chitinase (Nutritional Biochemical Corp., Cleveland, Ohio), and cellulase (Onozuka SS, Yakult Biochemical Co.) were also used.

Polyphenoloxidase activity was determined by measuring at 400 nm the brown color developed upon reaction of the enzyme with chlorogenic acid. One unit of enzyme activity was defined as that amount of enzyme necessary to oxidize 1 mM of chlorogenic acid per hr. The assay mixture contained 0.1 ml of enzyme solution and 1.0 ml of 10^{-2} M substrate, and 2.0 ml of 0.05 M phosphate buffer (pH 4.8). The color developed by incubating the reaction mixture at 30° for 30 min.

Cellulase activity was determined by measuring the production of reducing sugars from filter paper (FP activity) and from cotton (C_1 activity), according to the formula of Mandels and Weber (1969).

Substrate Treatment. Straw was mixed with 2% NaOH (1:8 w/w), and kept at room temperature for 1 to 2 days. Another portion of straw was soaked in dioxane (1,4-diethylene dioxide) containing 1.2% HCl for 1 hr, and washed 20 times with hot water to remove residual dioxane and other chemicals (e.g., cetyltrimethylammonium bromide in ADF straw). Chemically treated straw was dried at room temperature before subjecting it to in vitro rumen fermentation. Another part of straw was mixed with six parts (by weight) of culture filtrate or enzyme solutions (containing 3 mg of enzyme per ml of distilled water), kept at 39° for 3 days and heated (121°, 15 min) before subjecting it to a digestibility test.

Digestibility. Digestibility of straw was measured by an in vitro rumen fermentation technique that was a modified method of Mellenberger et al. (1970). To 50-ml screw-capped bottles was added 0.5 g of substrate. Rumen fluid was obtained from a Holstein bull and mixed with a mineral and buffer mixture (McDougall, 1948), at a ratio of 1:1. The inoculum was gassed with CO_2 and warmed to 39° before inoculation. Samples were incubated at 39° for 1 to 6 days. The contents of each bottle were filtered through a sintered glass crucible (Pyrex, 30 ml, coarse porosity) and dried overnight at 105°; the weight loss was reported as percent in vitro rumen digestibility (IVRD).

Chemical Analysis. Cellulose, hemicellulose, lignin, CSM, and ash were determined according to the method of Van Soest (1963). Klason lignin was determined according to the method of Brauns (1952).

RESULTS AND DISCUSSION

Chemical Composition and IVRD of Ryegrass Straw. Annual ryegrass straw contained CSM, cellulose, hemicellulose, lignin, and ash at the levels of 29.6, 36.8, 27.1, 5.4, and 2.0%, respectively. Their respective digestibilities were 64.5, 45.6, 42.8, 0, and 0% (Table I). These figures are for the various cell wall components prepared according to the Van Soest analytical scheme. Therefore, they may be different for those components isolated by other methods.

Table V. Chemical Composition and Digestibility of Fractionated Straw[a]

Straw	IVRD, %	Chemical composition, %				
		CSM	Hemicellulose	Cellulose	Lignin	Ash
Whole straw	48.0	31.1	24.5	37.0	5.6	2.0
Dioxane treated	35.2	13.8	29.6	46.7	7.6	2.2
NaOH treated	77.1	36.3	20.3	39.5	1.9	2.4
CWC straw	48.0	0 (9.1)[b]	32.2	52.0	6.2	1.20
Dioxane treated	50.6	0 (10.5)[b]	29.3	52.5	6.6	1.20
NaOH treated	64.5	0 (15.4)[b]	25.2	53.1	6.2	0.31
ADF straw	0	9.5	0	75.5	14.5	3.5
Dioxane treated	27.0	9.0	0	75.0	14.5	3.5
NaOH treated	6.7	11.2	0	75.0	13.0	2.0
RR straw	4.7	15.8	23.1	44.0	13.5	3.6
Dioxane treated	4.7	12.7	25.6	44.5	13.0	3.7
NaOH treated	16.8	14.9	18.9	45.1	13.8	3.3

[a] Values are means of triplicate sample. [b] Weight loss caused by refractionation with neutral detergent.

Table VI. Effect of Glucose on Digestibility of Fractionated Straw

Straw	IVRD, %[a]	
	Control	Glucose added[b]
Whole straw	43.60 ± 0.24	42.31 ± 0.48
Dioxane treated	35.21 ± 0.69	32.78 ± 1.78[c]
ADF straw	2.95 ± 0.29	0.50 ± 0.63[c]
Dioxane treated	25.60 ± 0.10	24.06 ± 2.16
RR straw	2.50 ± 0.47	0.37 ± 0.35[c]
Dioxane treated	4.76 ± 0.44	2.66 ± 0.36[c]

[a] Values are means of three replicates with standard deviation. [b] Glucose (0.5% of rumen fluid) added before in vitro rumen fermentation. [c] The mean is significantly ($P < 0.05$) different from the mean of control.

Table VII. Digestibility of Ryegrass Straw Treated with Culture Filtrate of Lignin and Cellulose Decomposing Organisms

Organism	IVRD, %[a]
Control (untreated)	56.1 ± 0.30
P. versicolor[b]	41.1 ± 1.39
P. abietnus[b]	46.7 ± 1.21
P. sabacida[b]	43.3 ± 3.10
T. viride[c]	55.3 ± 0.69

[a] Values are means of three replicates with standard deviations. [b] Culture filtrate contained 7.5 units of polyphenoloxidase per ml. [c] Culture filtrate contained 1.1 units of filter paper activity and 0.5 unit of C_1 activity per ml.

Figure 1 shows the digestion kinetics for each straw component. Of the straw components, CSM was the most readily digested, and most of its digestible portion solubilized during the first day of the fermentation. The cellulose and hemicellulose were also digested readily, but the lignin and ash were not. About 50% of the dry matter of straw was digested during the first 2 days, and no further digestion could be detected thereafter. The composition of the indigestible portion of the straw remained unchanged, except that the relative concentration of CSM decreased, and that of lignin increased.

Effect of Lignin on IVRD of Grass Straw. To study how lignin content affected the digestibility of the straw, the digestibilities of 12 straws with different lignin levels were compared (Table II). A correlation analysis (Table III) revealed that the level of lignin was significantly related to digestibility ($r = -0.93$). The level of cellulose also correlated highly with digestibility ($r = -0.90$), but multiple correlation analysis showed that in the lignin–cellulose complex, mainly lignin influenced the digestibility of straw (F values of lignin and cellulose were 4.75 ($P < 0.10$) and 1.41 (NS), respectively). Even though CSM was the most readily digested, its correlation coefficient with digestibility was low ($r = 0.48$). The levels of lignin and cellulose were correlated inversely with the digestibility, whereas the hemicellulose and CSM levels were positively correlated with the digestibility.

Although the level of native lignin in the forage influenced the digestibility, addition of isolated lignin to the straw did not affect its digestibility. As shown in Table IV, the addition of 1 to 20% lignin did not significantly ($P > 0.1$) influence digestibility. Thus, the recalcitrant effect of lignin in straw appears to be caused, not by the lignin per se, but by the complex that it forms with cellulose and other straw constituents.

Digestibility of Fractionated Straw. As Table V shows, whole straw and CWC straw were readily digested (about 50%), whereas ADF straw and RR straw were not (0 and 4.7%, respectively). Thus, it appears that the removal of hemicellulose (ADF straw) inhibits the digestion, but the removal of CSM does not affect the digestibility of straw. Several chemical treatments known to improve the digestibility of cellulosic materials were applied on the residue of rumen-digested straw and on the several fractionated straws. Treatment with NaOH increased the IVRD of whole straw, CWC straw, and RR straw, but it did not increase the digestibility of ADF straw. The digestibility of ADF straw, however, could be increased by dioxane treatment (IVRD increased from 0 to 27%). The three former straws contained some hemicellulose (23–32%), whereas ADF straw contained none. Thus, the presence of hemicellulose appears to be essential for the effectiveness of NaOH treatment, whereas dioxane works on the hemicellulose-free straw.

It was speculated that the recalcitrance of ADF straw and RR straw was caused by the lack of initial level of readily digestible carbohydrates, such as CSM and hemicellulose. Addition of 0.5% glucose into rumen fluid, however, did not restore the digestibility of ADF straw or RR straw (Table VI). Instead, the addition of glucose reduced the IVRD of some of the straw.

Effect of Enzymes on the Digestibility of Grass Straw. IVRD of ryegrass straw treated with the culture filtrate of lignin and cellulose decomposing microorganisms are shown in Table VII. The digestibilities of straw treated with *P. versicolor* (41.1%), *P. abietnus* (46.7%), and *P. sa-*

Table VIII. Lignin Content of Ryegrass Straw Treated with Culture Filtrate of Lignin and Cellulose Decomposing Organisms

Organism	Lignin, % dry matter[a]	
	Acid-detergent lignin	Klason lignin
Control (untreated)	8.25 ± 0.40	16.80 ± 0.57
P. versicolor	7.43 ± 0.40[b]	17.36 ± 0.57
P. sabacida	7.10 ± 0.40[b]	17.45 ± 0.57

[a] Values are means of three replicates with standard deviations. [b] The mean is significantly ($P < 0.01$) different from the mean of control.

bacida (43.3%) were all significantly ($P < 0.01$) lower than that of untreated control (56.1%): the digestibility decreased up to 27%. The straw treated with *T. viride*, however, had about the same digestibility as the control. It was speculated that these culture filtrates might contain antimicrobial agents which could inhibit rumen microorganisms. Heating the enzyme-treated straw before rumen fermentation did not affect the digestibility pattern: lignin decomposing enzyme reduced the digestibility of the straw. The culture filtrate of *P. versicolor* and *P. sabacida* significantly ($P < 0.01$) reduced the acid-detergent lignin content of the straw; however, the changes in the Klason lignin were not apparent (Table VIII).

Table IX shows the digestibility of straw treated with commercially prepared cellulase, peroxidase, polyphenoloxidase, and chitinase. The digestibility increased by 14% with the cellulase, but the other enzymes had no significant effect ($P > 0.1$). Application of these enzymes had no apparent effect on the digestibility of the fractionated straws.

Low digestibility of lignocellulosic material is generally believed due to the lignin–cellulose complex in the cell wall which impedes the cellulase action. Many attempts have been made to decompose the lignin and increase the digestibility of ligno-cellulosic material, but the results were inconclusive (Klein et al., 1970; Rockhill et al., 1972; Goering et al., 1973). In nature, lignin is decomposed by the wood rot fungi belonging to Basidiomycetes, and the enzyme responsible is reported to be polyphenoloxidase (Ishakawa et al., 1963). Application of polyphenoloxidase or the culture filtrate of these organisms reduced the lignin content, but did not increase the digestibility of straw. Several hypotheses may be advanced for the inhibitory effect of the polyphenoloxidase: (1) degradation products of lignin may include phenolic compounds which are inhibitory to rumen microflora, (2) polyphenoloxidase may oxidize phenol derivatives to quinones, which aggregate with protein and other cell wall components of the straw to reduce the digestibility, and (3) polyphenoloxidase may inactivate some of the digestive enzymes in rumen.

Table IX. Effect of Enzymes on the Digestibility of Fractionated Straw

Enzyme	IVRD, %[a]		
	Whole straw	ADF straw	RR straw
Control (untreated)	46.5 ± 2.0	0.61 ± 1.44	4.7 ± 0.49
Cellulase	54.4 ± 1.41[b]	3.26 ± 1.72	5.7 ± 0.49
Polyphenol-oxidase	49.5 ± 3.34	0.44 ± 1.78	4.8 ± 0.42
Cellulase + polyphenol-oxidase	52.1 ± 1.16[c]	3.91 ± 2.76	
Peroxidase	48.5 ± 1.36		4.6 ± 0.34
Chitinase	48.0 ± 2.50	0.89 ± 1.50	

[a] Values are means of three replicates with standard deviations. [b] The mean is significantly ($P < 0.01$) different from the mean of control. [c] The mean is significantly ($P < 0.05$) different from the mean of control.

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