SECTION C, PHYSICAL SCIENCES

Notes on the Determination of Cellulose and Hemicelluloses in Grasses[1]

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In recent years a great deal of attention has been given to improving the grass crops of the world. Also much attention has been focused upon an evaluation of the various components, particularly carbohydrates, as sources of energy for ruminants (Intersociety Symposium, 1959). The Oklahoma Agricultural Experiment Station has been studying the carbohydrates of grasses for several years.

Proximate analysis for ash, protein, ether extract, crude fiber and nitrogen-free extract has provided much valuable information, but these values are not now considered adequate to evaluate fully the nutritive qualities of range forages (Hansen *et al.*, 1958). Some detailed analyses (Waite and Gorrod, 1959; Binger and Sullivan, 1954) have provided useful information on the composition of grasses; however, their methods are too complex and time consuming to permit routine use. These procedures are being shortened and adapted to provide the needed information. Some of these modifications and their application are reported in this paper.

One of the carbohydrate constituents of greatest interest is cellulose. The method described by Patton (1943), which involves the use of nitric and acetic acids to dissolve components other than cellulose, is being used for this determination. The major question to be decided was what type of preliminary treatment should be used on the samples before the actual determination. Three types of sample preparation were available: whole grass samples (40 or 80 mesh); ethanol-extracted residues ground in a ball mill; and holocellulose prepared as described by Binger and Sullivan (1954). Holocellulose is composed chiefly of cellulose and hemicelluloses and is prepared by treating an ethanol-benzene extracted sample with ammonium oxalate followed by a treatment with sodium chloride.

The data in Table I are results obtained with samples of blue grama grass collected at various seasons of the year. Large differences in the cellulose percentages are apparent and the problem becomes one of deciding which method of preparing the samples yields the most nearly correct values. The differences are much greater than those found for bermuda grass (Table II). It would seem that data for cellulose, to be anything more than comparative, must be accompanied by details of how the determination was made. It is not possible to say with finality that results by one method are better than those by another method and that one method truly measures the cellulose content of the samples. Results however indicate that the most reasonable values are those for cellulose determined on the holocellulose preparation (column II, Table I). Cellu-

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TABLE I PERCENTAGE OF CRUDE CELLULOSE IN BLUE GRAMA *(Bouteloua gracilis)* AS INFLUENCED BY THE METHOD OF SAMPLE PREPARATION. DRY WEIGHT PERCENTAGES

Sample	Date	I whole grass 80 mesh	II holocellulose 80 mesh	III extracted ball mill residues
107	5/27/58	38.7	29.2	23.0
108	6/19/58	38.5	29.7	29.9
109	9/15/58	42.0	34.1	29.7
110	10/20/58	41.6	33.6	31.7
111	1/28/59	42.2	34.9	34.4
112	4/21/59	40.7	34.5	27.3
113	5/18/59	38.5	28.5	23.7

TABLE II PERCENTAGE OF CRUDE CELLULOSE IN BERMUDA GRASS *(Cynodon dactylon)* HAY AS INFLUENCED BY THE METHOD OF SAMPLE PREPARATION. DRY WEIGHT PERCENTAGES

sample	I whole sample 40 mesh	II residue from hemicellulose determination	III residue from hemicellulose corrected for ash and protein	IV holocellulose 40 mesh
1	25.6	26.7	23.3	23.7
2	26.6	27.5	24.0	24.8
3	24.6	22.0	21.4	21.8

lose percentages determined on the whole ground samples are the highest, probably because the dissolving agents did not fully penetrate the cellulose and remove all the interspersed lipids, lignin, and proteins. Removal of fatty materials is recommended in practically all schemes for determining holocellulose and, if necessary in that procedure, it likely would be desirable in cellulose determinations as well. The low cellulose percentages found in the extracted ball-milled samples are somewhat difficult to explain for in these samples ethanol-soluble proteins and most fat and waxes have been removed. Two theories might be advanced to explain low results; first, that because of the extreme fineness of the particles (literally dust-like) there is a fairly large mechanical loss in the manipulations; and secondly, that the prolonged processing of the samples (48 hours) mechanically disrupted some of the cellulose structures and rendered them more soluble. Losses due to mechanical treatments have been reported for certain other structural plant constituents. The decision to use the holocellulose samples is also strengthened by the results for bermuda grass (Table II). Included in this table are results from another method of determining cellulose, namely the residue from holocellulose after hemicelluloses have been determined. This is usually reported as alpha cellulose (Paech *et al.*, 1955).

The results (Table II) are for cellulose determinations in samples subjected to four methods of preparation. The data in column I are from unextracted samples, while the data in column II represent the residue from holocellulose determinations after hemicelluloses have been extracted with 24 per cent potassium hydroxide. The data in column III represent the values in II corrected for ash and protein contaminants and column IV gives values secured by determining cellulose on holocellulose preparations. The close agreement between the values in columns III and IV suggests that either method of sample preparation might be used. However, extraction of the hemicelluloses is a costly and time-consuming pro-

cedure, which makes the method impractical when a large number of determinations are to be run.

The ill-defined class of materials known as hemicelluloses is another carbohydrate fraction that may be of interest in nutrition. As indicated in the previous paragraph, this fraction can be determined by extraction with potassium hydroxide but this procedure is long and costly. The data of Table III suggests that this fraction might be estimated as the difference between true holocellulose and cellulose content. These calculated values are given in column III.

TABLE III. PERCENTAGE OF HEMICELLULOSE IN BERMUDA GRASS HAY DETERMINED BY VARIOUS METHODS

Sample	I extracted with 24% KOH	II extracted with 24% KOH corrected for ash and protein	III calcd. by difference (true holocellulose minus cellulose)
1	33.2	27.3	29.8
2	34.7	28.4	30.2
3	28.7	24.9	26.4

The results shown in column I are precentages of hemicellulose extracted from bermuda grass samples using 24 per cent KOH (Hansen *et al.*, 1958; Paech *et al.*, 1955), which is the maximum concentration suggested and the one preferred if only one concentration is to be used. Column III shows the same values corrected for ash and protein contaminants. The values of column III are somewhat higher than those of column II, however the extracted values are always somewhat low due to incomplete extraction and mechanical losses in the process.

The data presented in this paper show that the application of Patton's procedure (Patton, 1943) to holocellulose samples (Binger and Sullivan, 1954) appears to give consistent and acceptable values for the cellulose content. Furthermore, the hemicellulose content can be estimated by difference if proper corrections are made.

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