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Cellulose Analysis for Digestion Trials

Effect of Two Cellulose Methods on Cellulose Content of Feed and Feces and the Coefficients of Digestibility of Cellulose

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During investigations (Brooks *et al.*, 1954, Pfander *et al.*, 1957 a, b,) of factors which influence roughage utilization by ruminants, it became apparent that the cellulose fraction of feedstuffs had greater variability in digestion coefficients than any other ration component. It was thought that the cellulose method might be responsible for the variation. The Missouri Station Laboratory was using the Crampton-Maynard (1938) method based on digestion with acetic and nitric acids for routine cellulose determinations. However, other investigators have questioned the validity of this method and the Matrone-Ellis-Maynard (1947) modification of the Norman-Jenkins method, developed as a more specific cellulose determination, has replaced the Crampton-Maynard method in some laboratories. The review of Hansen, Forbes, and Carlson (1958) should be consulted for details and background.

This bulletin reports studies of the suitability of the two methods for determining cellulose in alfalfa hay, corn, and the feces produced by wethers fed rations of alfalfa hay and corn. The variation due to analysts is also considered with 12 samples in which cellulose was determined by the Crampton-Maynard technique.

MATERIALS AND METHODS

Twelve mature western wethers were fistulated and housed in concrete-floored pens. They were fed twice daily in individual stalls. The ration, fed in equal parts at 7:00 a.m. and 4:00 p.m., consisted of 800 gm. of U. S. Number 3 chopped alfalfa hay and 400 grams of shelled corn per day. Two trials were run. During each trial, three sheep received only the basal ration. The remaining sheep were divided equally into three lots and received either 10 mg. testosterone, 1.5 mg. hexestrol or 20 mg. cortisone, as previously described (Pfander, *et al.*, 1957).

During each trial the 14-day preliminary period was followed by a four-day total collection period. Fecal bags were removed daily and the feces were mixed

carefully. Twenty-five percent of the daily collection was transferred to Pyrex[1] dishes, dried in an oven for 18 hours at 82° C, and stored in Kraft bags until it adjusted to atmospheric moisture. The combined samples were ground through a 40 mesh screen in a Wiley Mill, mixed, quartered, and stored in air-tight jars until ready for analysis.

One analyst determined cellulose in duplicate or triplicate with modifications of the Crampton-Maynard (1938) and the Matrone-Ellis-Maynard (1947) methods. Duplicates were processed on different days. Two analysts ran duplicate determinations and a third analyst ran a single determination on the 12 samples used to study the variation due to analyst on the values obtained with the Crampton-Maynard method.

Modified Crampton-Maynard Procedure

Weigh a 2-gm. sample of air-dry feed or feces into an extraction thimble and extract on a Goldfisch apparatus with ether for four hours. Dry overnight under a forced-draft hood and transfer from the thimble to a 600-ml. crude fiber beaker. Add 30 ml. of digestion solution (10 parts 80 percent acetic acid and 1 part concentrated nitric acid, by volume). Place the beaker on the crude fiber apparatus, and reflux for 20 minutes. Tranfer the beaker to an ice-water bath. When the beaker is cool, transfer the contents with 70° C alcohol to a shimer filter funnel containing an ignited asbestos pad. Wash the residue four successive times with approximately 10-ml. portions of hot alcohol, four 10-ml. portions of hot benzene, and four 10-ml. portions of room temperature anhydrous ether. Remove the asbestos pad and residue and place in a previously ignited crucible and dry at 110° C overnight. Remove the crucible and place in a desiccator until cool and weigh to 0.5 mg.; place in a cool muffle furnace and bring to 580° C overnight. Remove the crucible from the muffle furnace and cool in a desiccator. Weigh and calculate cellulose as the loss in weight upon ashing.

Modified Matrone-Ellis-Maynard Procedure

Weigh 1 gram of air-dried feed or feces into an extraction thimble, and extract with alcohol-benzene (3.4 volumes 95% ethyl alcohol and 6.6 volumes benzene) on a Goldfisch apparatus for four hours. After the sample is dry, remove from the thimble and place in a 100-ml. Pyrex[1] beaker. Add 50 ml. of distilled water and bring the contents to boiling on a hot plate. Cool the solution to 30° C, and add 3 ml. of Mylase-buffer solution (1 gm. Mylase P in 25 ml. buffer prepared from 164 gm. anhydrous sodium acetate and 125 ml. glacial acetic acid made to 1 liter with distilled water). Incubate at 30° C for 1 hour and 45 minutes. After incubation filter off the supernate and wash the residue three times with approximately 20 ml. of hot distilled water. Add 50 ml. of 3 percent sodium sulfite solution to the residue and bring to boiling on the hot plate. Filter.

[1]Trade mark of Corning Glass Works, Corning, New York.

Add 10 ml. of ethanolic NaOH (.25 N in 60% ethanol) to the residue, stir thoroughly and neutralize with 1 ml. of 2.5 N H_2SO_4 and allow to stand 10 minutes before filtering. Wash the residue once with 30 ml. hot distilled water and then add 43 ml. cold distilled water and 7 ml. Clorox (2.25% available chlorine).[2] Allow to stand ten minutes with occasional stirring, and then filter.

Repeat the sodium sulfite treatment but allow the solution to boil 10 minutes before filtering. Repeat the ethanolic NaOH treatment.

Mix the residue with 50 ml. distilled water, 1.5 ml. Clorox[2] and 1.0 ml. of 20 percent H_2SO_4, and allow to stand for 10 minutes, away from direct sunlight. After filtering, add 50 ml. of sodium sulfite solution. If lignin is present (an intense purple color develops), repeat the treatments starting with the first ethanolic washing, until no lignin is present.

After the final treatment with sodium sulfite, filter while hot and wash three times with 50 ml. hot distilled water. When the sample is in the final washing, transfer it to the shimer filter, equipped with an ignited asbestos pad. After the water has filtered off, transfer the asbestos pad and residue to a previously-ignited crucible and dry overnight in a 110° C oven.

Remove the crucible from the oven, cool in a desiccator, weigh to 0.5 mg. and then place in a cool muffle furnace and bring to 580° C overnight.

Statistical Analysis

Results were analyzed for statistical significance by Snedecor (1948) methods.

RESULTS

The cellulose composition obtained by the first analyst, using the two methods for corn and hay, is presented in Table 1. The Matrone-Ellis-Maynard method gave a higher value than the Crampton-Maynard method for cellulose in the corn ($P<.05$), probably due to the inclusion of certain hemicelluloses found in the cob fragments and tip caps present in the shelled corn. The fractions have been called cellulosans by Hawley and Norman ('32).

The Crampton-Maynard method gave a higher percentage of cellulose in the hay ($P<.01$) than the Matrone-Ellis-Maynard method. The Crampton-Maynard method did not remove all of the residual lignin from the hay particles whereas the acid hypochlorite and sodium sulfite treatments in the Matrone-Ellis-Maynard method were repeated until there was no residual lignin.

The cellulose content of feces is shown in Table 2. Fecal samples contained 2% more cellulose when analyzed by the Crampton-Maynard method than by the Matrone-Ellis-Maynard method ($P<.01$).

Evaluation of the two methods was continued by calculating the coefficients of digestibility of cellulose; results are shown in Table 3. The Crampton-Maynard technique gave higher coefficients of digestibility.

[2]Clorox Chemical Company, Oakland, California.

TABLE 1-CELLULOSE CONTENT OF SHELLED CORN AND HAY AS DETERMINED BY TWO METHODS

	Percent Cellulose	
	Crampton-Maynard Method	Matrone-Ellis-Maynard Method
Corn Sample 1	3.00	3.13
	2.40	5.05
	2.53	4.23
Mean	2.97	4.13
Corn Sample 2	2.25	4.98
	2.33	4.30
	2.48	4.25
Mean	2.52	4.84
Hay Sample 1	34.4	30.0
	34.5	29.6
Mean	34.4	29.8
Hay Sample 2	35.8	29.4
	35.5	29.6
Mean	35.6	29.5

TABLE 2-COMPARISON OF CELLULOSE CONTENT OF FECES AS DETERMINED BY TWO METHODS

Sheep No.	Percent Cellulose*	
	Matrone-Ellis-Maynard Method	Crampton-Maynard Method
TRIAL I		
1	30.45	30.85
6	31.64	31.92
9	31.62	31.42
11	30.48	31.08
00	30.30	31.83
12	31.75	31.13
13	30.38	31.00
15	31.35	31.10
17	30.43	31.19
18	29.90	30.99
19	29.13	30.39
20	29.74	29.77
Mean	30.60	31.06
TRIAL II		
1	30.20	30.64
6	29.79	30.85
9	29.60	31.29
11	30.33	31.60
00	30.62	31.55
12	29.87	31.23
13	30.34	31.04
15	29.57	30.92
17	29.63	30.80
18	28.64	30.27
19	29.49	30.25
20	30.10	30.72
Mean	29.85	30.93

*Each value is a mean of two independent determinations.

TABLE 3-COEFFICIENTS OF DIGESTIBILITY OF CELLULOSE OF AN ALFALFA HAY-CORN RATION AS DETERMINED IN MATURE WETHERS

Sheep No.	Coefficients of Digestibility: Crampton-Maynard Method	Coefficients of Digestibility: Matrone-Ellis-Maynard Method
TRIAL I		
1	63.6	59.9
6	60.8	56.1
9	63.3	58.9
11	63.8	60.1
00	68.6	66.2
12	72.4	66.8
13	65.3	62.2
15	63.5	59.2
17	77.6	75.7
18	77.5	75.8
19	72.5	68.8
20	66.7	63.4
Mean	68.0 ± 5.5	64.4 ± 6.2
TRIAL II		
1	70.0	67.9
6	66.8	66.8
9	63.6	62.8
11	61.7	60.3
00	59.4	57.3
12	65.3	64.4
13	65.4	63.7
15	62.3	62.4
17	62.3	60.8
18	72.8	72.0
19	70.5	68.7
20	67.1	64.3
Mean	65.6 ± 3.9	64.3 ± 3.9
Mean of both trials	66.8 ± 4.7	64.4 ± 5.2
$\sigma / \bar{x}$	0.07	0.08

"Students T": Between Trials 1.66 ($P < .05$)
Between Methods 1.87 ($P < .01$)

The coefficients of variation of the determinations within a trial indicated that the Matrone-Ellis-Maynard method is no more reliable than the Crampton-Maynard method (Table 3). Although neither method was satisfactory from the standpoint of variation, the Crampton-Maynard method should give representative values, and since it requires considerably less time than the Matrone-Ellis-Maynard method, it can be recommended for routine determinations.

The large deviation associated with the Crampton-Maynard method was studied in detail as shown in Table 4. The coefficients of variation for corn are prohibitive and indicate that considerably larger samples are necessary for substances relatively low in cellulose.

TABLE 4-SOURCES OF VARIATION IN THE CRAMPTON-MAYNARD METHOD

Sample No.	Nature of Sample	Crampton-Maynard Cellulose as determined by: Analyst 1 Run 1	Analyst 1 Run 2	Analyst 2 Run 1	Analyst 3 Run 1	Analyst 3 Run 2	Mean
73	feces	30.8	30.9	30.7	30.5	31.8	30.9 ± .4
74	feces	31.5	32.3	31.5	31.5	31.9	31.7 ± .3
75	feces	31.3	31.6	31.6	32.1	31.2	31.6 ± .3
76	feces	30.6	31.5	31.6	31.9	31.8	31.4 ± .5
77	feces	31.7	32.2	31.3	31.2	31.2	31.6 ± .4
78	feces	30.8	31.4	31.8	32.3	31.3	31.5 ± .5
79	feces	31.0	31.0	31.4	31.4	32.2	31.4 ± .4
80	feces	31.0	31.1	31.7	31.9	31.7	31.5 ± .3
81	feces	31.0	31.4	31.6	31.3	32.0	31.5 ± .3
82	feces	31.2	30.8	30.8	31.2	30.6	30.9 ± .2
Mean		31.1 ± .3	31.4 ± .5	31.3 ± .4	31.5 ± .5	31.6 ± .4	
85	hay	34.4	34.5	34.0	35.3	33.4	34.3 ± .2
86	corn	2.4	2.5	2.6	2.8	2.7	2.6 ± .1

Analysis of Variance of feces samples

Source	Degrees of freedom	Variance	Mean square	F
Total	49	10.78	- - - -	- - - -
Samples	9	3.18	.3533	3.02**
Rations	3	.69	.2300	1.81
Runs	4	1.88	.4700	3.71*
Operators	2	1.20	.6000	4.73*
Error	31	3.93	.1268	- - - -

* ($P<.05$)
** ($P<.01$)

There is greater variation in the feces cellulose values than in hay cellulose, suggesting that events occurring in the sheep's digestive tract have altered ingesta in some manner which makes it difficult to obtain a relatively low error under routine conditions; similar variations did not exist for nitrogen digestibility. This conclusion is substantiated by the analysis of variance which indicates that the feces sample ($P<.01$), the analyst ($P<.05$), and the time of making the run ($P<.05$) all contributed to the variation in fecal cellulose values. Part of the variation between run and time may have resulted from changes in samples under storage. It can be seen that cellulose values, on the average, increased slightly with time.

If the Crampton-Maynard method is to be used routinely it seems essential that all samples from a given digestion trial be run by one analyst in as short a time as possible. Strict attention should be given to insure that comparable conditions are employed throughout. This may necessitate randomization of samples and analytical equipment.

CONCLUSIONS

The Crampton-Maynard method appears to be as suitable for the routine determination of cellulose in feeds and feces obtained from digestion trials as the more involved Matrone-Ellis-Maynard method. The coefficients of variation associated with cellulose digestibility determinations were large and indicated that the method as now used is not satisfactory for detecting a 5% difference in digestibility between animals on two treatments. More care on the part of the analyst could reduce variation but a considerable part of the variation is associated with fecal samples. This may be related to changes, as yet unexplained, which occur in the gastro-intestinal tract.

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