AN IMPROVED METHOD FOR THE DETERMINATION OF CRUDE FIBER AND NITROGEN FREE EXTRACT IN FEEDS.[1,2]

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Introduction

The crude fiber method in use today was originated in Germany by Henneberg and Stohmann (1) back in 1863 and is known as the Weende method after the name of the Experiment Station. The method consists of boiling a fat and moisture free sample (about 1 gram) with 200 ml. of a 1.25% sulfuric acid solution for 30 minutes and following that with a 30 minute boiling in 200 ml. of a 1.25% sodium hydroxide solution. The residue is then filtered, dried and the loss in weight on ashing is calculated as the crude fiber content of the material sampled. This is still the official method of the Association of Official Agricultural Chemists (A.O.A.C.) (2).

The method has several drawbacks. One of the most striking is the fact that about 85% of the lignin contained in a feed is not accounted for in the crude fiber fraction. Maynard (3) has noted that the alkali of the Weende method removed the lignin from the crude fiber fraction and Crampton and Maynard (4) in 1938 suggested that the determinations of lignin and cellulose and a fraction known as "other carbohydrates" should replace the customary crude fiber and nitrogen-free extract in digestion trials. The suggested change was considered superior because it provides fractions of biological significance instead of arbitrary chemical significance. Norman (5) in 1939 believed that the failure to include lignin in the crude fiber was the most serious criticism of the usual method of grass analysis. McCall, Clark and Patton (6) of the Montana Experiment Station

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2. Material presented by the senior author in partial fulfillment of the requirements for the degree of Master of Science.

dropped the use of the crude fiber method in 1940 for that reason and used lignin and cellulose determinations for their digestion trials.

Anderson (7) worked on cellulose, lignin and crude fiber changes in maturing western wheatgrass at this station and found that an average amount of about 15% of the lignin and about 80% of the cellulose of the original grass was included in the crude fiber fraction. With these figures in mind, it is easy to understand why some digestion trials have shown the crude fiber fraction to be more digestible than the nitrogen-free extract (NFE) which contains most of the plant lignin.

An ideal method for crude fiber would be one in which all fractions except cellulose and lignin would be removed. Work was undertaken to find a treatment that would not remove the cellulose and the lignin but which would remove most all of the other carbohydrates as well as the proteins.

Experimental

A large sample of native hay from western South Dakota was dried and extracted with ether as in regular feed analysis. One gram samples of this fat-free and moisture-free experimental sample were used in the experiment. Six samples were treated at each time with two being used for cellulose determinations, two for lignin determinations and two for Kjeldahl determinations. Methods for the determinations of lignin and cellulose were those of Crampton and Maynard as modified by Patton (8). The methods were chosen because they are somewhat similar and required less time than other methods and, although the values obtained by these methods are probably not as precise as those obtained by more complicated methods, they are accurate enough for the comparisons to be made in this study.

One of the first trials was set up to determine whether or not the time element was as critical in the crude fiber determination as the procedure for the A.O.A.C. official method (2) would seem to indicate.

Sulfuric acid solution (1.25%) was used for varying lengths of boiling time and the results are shown in Table I.

TABLE I

Effects of various lengths of boiling time on cellulose lignin and protein.

Time	Cellulose %	Lignin %	Protein %
0 minutes	35.84	28.60	7.27
10 minutes	33.00	21.80	4.90
20 minutes	33.27	19.36	4.61
30 minutes	32.96	20.62	4.48
60 minutes	32.40	16.67	3.78

The effect of the acid is shown to be greatest in the first few minutes of boiling and drops off rather rapidly as the time interval is lengthened. Accordingly, small variations in time would not be expected to produce appreciable differences in results. A 30 minute boiling period was used in the remainder of the experiments.

Another of the early trials was to determine the effect of varying the strength of the alkali and the use of a different alkali on the acid treated residue. Various strengths of sodium hydroxide and sodium carbonate solutions were used and the results were as shown in Table II.

TABLE II

Effects of various strengths of alkalies.
Lignin, cellulose and protein values calculated as percentages of the experimental sample.

	Lignin %	Cellulose %	Protein %
1.25% H_2SO_4 treated, only	19.88	32.32	4.25
above plus 30 min. of 1% NaOH	1.83	29.02	.17
above plus 30 min. of .1% NaOH	4.06		1.31
above plus 30 min. of .025% NaOH	10.61		2.47
above plus 30 min. of 1% Na_2CO_3	3.86	31.13	1.29
above plus 30 min. of .05% Na_2CO_3	18.40		2.65

Alkalies, except in very dilute solutions, tended to remove lignin to a considerable degree. These results showed that an improvement of the crude fiber method would result by the elimination of the alkali treatment if a means of removing protein could be developed.

Another trial was run to determine the effect of various acids on the removal of lignin and cellulose from the experimental material. Various concentrations of sulfuric, nitric, acetic and hydrochloric acids were used. Residues were determined by direct weighing of the dried samples after the indicated treatment and the values were used as an indication of the crude fiber values to be expected. The results are shown in Table III.

TABLE III

Treatments of the Experimental Sample

Cellulose, lignin, protein and residue values calculated as percentages of the experimental sample

Experimental sample	Cellulose %	Lignin %	Protein %	Residue %
(no treatment)	35.84	28.60	7.27	100.0
5% HNO_3	30.2	11.4	3.20	45.8
5% HAc	35.8	22.8	5.53	85.5
5% HCl	33.3	13.8	4.74	57.4
2.5% H_2SO_4	32.9	20.6	4.48	76.0
2.5% HAc	34.6	23.3	5.52	81.3
1% H_2SO_4	34.5	16.3	3.78	66.8
1% HCl	34.5	17.9	3.59	54.4
1% HNO_3	33.1	13.5	4.44	56.9
2.5% HCl + 2.5% HNO_3	30.0	9.2	2.96	44.9
2.5% H_2SO_4 + 2.5% HNO_3	31.8	14.6	3.56	48.6
2.5% H_2SO_4 + 2.5% HCl	32.0	21.0	3.47	51.7
2.5% HAc + 2.5% HCl	30.2	18.9	3.87	----
5% HAc + 2.5% HCl	33.6	22.4	3.43	49.9
2.5% H_2SO_4 + 2.5% HAc	32.7	15.2	4.14	----

Nitric acid was found to give the greatest reduction in the cellulose and lignin remaining in the residue both when used alone and when used in combination with other acids. Hydrochloric acid and sulfuric acid also gave considerable reductions in the lignin values of the residues. Acetic acid treatment gave the greatest promise and was used henceforth in varying dilutions and a pretreatment with pepsin solution was tried to reduce the protein content of the residues. The results are shown in Table IV.

TABLE IV

Acetic Acid Treatments

Experimental sample	Cellulose %	Lignin %	Protein %	Residue %
(no treatment)	35.84	28.60	7.27	100.00
2.5% acetic acid	34.60	23.30	5.50	81.30
5% acetic acid	35.80	22.80	5.50	85.50
2% pepsin overnight then 5% HAc	36.60	27.70	1.80	75.40

The pepsin digestion pretreatment followed by a treatment with 5% acetic acid produced the best results in terms of lignin and cellulose remaining in the residue. The residue figure showed that most of the other constituents were removed. The nitrogen was reduced to a low level which approximates the levels found by Bondi and Meyer (9) in their purified lignins.

The method as used in the remainder of the experiments is as follows: Samples of about 1 gram in weight were dried and ether-extracted as in the usual feed analysis. The material was then placed in 50 ml. glass stoppered Erlenmeyer flasks and 40 ml. of a 2% pepsin in 0.1 normal HCl solution were added. The flasks were stoppered, shaken to thoroughly mix the contents and placed in a constant temperature bath at 40° C. for 12-14 hours (overnight). The residue was filtered off by suction, washed with hot water and transferred to the crude fiber flasks. 200 ml. of boiling 5% acetic acid solution were added and the mixture boiled for 30 minutes. The residue was again filtered off by suction and washed with hot water to remove residual acid. The residue was then transferred to a 400 ml. beaker and allowed to stand with 200 ml. of distilled water and about 2 grams of sodium sulfate which speeds up filtration of otherwise slow filtering colloidal residues, especially corn, soybean, and feces residues. After standing at least four hours, the residue was filtered through a Gooch crucible prepared with an asbestos mat as in the AOAC method of analysis for crude fiber, dried for 1 hour at 105° C. and the loss in weight upon ashing at 800° C. was calculated as percentage of "improved" fiber.

In order to test the method on concentrates, this "improved" fiber method was used on oats, corn, soybean oil meal and steer feces from a current digestion trial.[3] The results are shown in Table V.

3. The digestion trial was conducted by George Staples of the Animal Husbandry Department of South Dakota State College.

TABLE V

Comparison of the standard and "improved" methods

	Cellulose	Lignin	Lignin + Cellulose	AOAC crude fiber	"improved" fiber
	%	%	%	%	%
Oats	10.12	7.40	17.52	9.34	24.50
Corn	5.29	2.20	7.49	2.50	12.34
Soybean oil meal	12.68	1.53	14.21	5.46	11.50
Steer feces, dry	26.63	28.87	55.50	24.65	61.62

The "improved" fiber values for the concentrates did not check too well with the combined cellulose and lignin values but here the effects of large amounts of starch and protein in the samples probably had the effect of giving erroneously high values for the lignin and cellulose as has been noted by Norman and Jenkins (10) (11).

An attempt was made to increase the solution of the starches and the proteins by increasing the amount of acid solution from 200 to 400 ml. per gram of sample. The results are compared in Table VI.

TABLE VI

Comparison showing the effects of increasing the volume of acid solution

	AOAC Crude Fiber	"Improved" fiber 200ml/gm.	400 ml/gm.
	%	%	%
Oats	9.34	24.50	23.46
Corn	2.50	12.34	10.98
Soybean oil meal	5.46	11.50	8.57

Much better agreement between duplicate samples was noted when the amount of the acid solution was increased to 400 ml. and the values are probably more accurate with the use of that amount when concentrates and grains are being analyzed.

In order to show the effect of the "improved" fiber method on digestion trials, two sets of samples from the digestion trial mentioned previously were analyzed and the digestion coefficients were calculated using both the standard analysis (AOAC) and the "improved" fiber values and the resulting nitrogen-free extract (NFE) values. The results are shown in Table VII.

TABLE VII

Comparison of the Digestion Coefficients Obtained by the Standard (AOAC) and by the "Improved methods".

	AOAC analysis %	"improved method" %
Steer No. 1.		
—crude fiber	60.83	49.94
—NFE	58.69	94.09
Steer No. 2.		
—crude fiber	65.49	55.97
—NFE	62.90	94.13

The high digestibility of the nitrogen-free extract when the "improved" fiber values are used indicates that it consists mainly of readily digestible plant constituents. The digestibility of the "improved" fiber results from the rumen digestion of the cellulose portion of the fiber. The total-digestible-nutrients of the feed remained the same since the nitrogen-free extract (NFE) values varied inversely with the fiber values as shown in Table VIII.

TABLE VIII

Comparison of the Crude Fiber and Nitrogen-free extract values obtained by the AOAC and by the "improved methods".

	AOAC analysis		"improved" method	
	C. F. %	NFE %	C. F. %	NFE %
Hay	32.12	42.31	63.64	10.79
Oats,	9.34	61.03	24.50	45.87
Soybean oil meal	5.46	29.15	11.50	23.11
Orts, Steer No. 1	21.34	42.40	56.99	6.75
Orts, Steer No. 2	20.28	45.62	54.07	11.83
Feces, Steer No. 1 ,fresh	6.04	9.47	15.02	.49
Feces, Steer No. 2, fresh	6.46	10.36	16.20	.57

Although certain revisions of this method will probably result from further studies, we feel that the values by the "improved" fiber method as shown in the preceding tables gives a more exact and more useful division of the digestion-resistant material from the readily digestible material in a given feed. More work will have to be done with concentrates and feeds other than those included in this report.

Summary

A new method for determining fiber in feeds has been shown to retain both the cellulose and the lignin in a single fraction. The nitrogen-free extract resulting from this determination has been shown to be almost completely digestible. The proposed method makes possible more determinations within a given time since it is shorter and requires less equipment than the AOAC method for crude fiber. The values determined by this method have more meaning with respect to nutritional value of feeds since the division into digestion-resistant and readily-digestible fractions is more accurate and more complete than by other simple methods.

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