AN EVALUATION OF THE LIGNIN-RATIO METHOD AS COMPARED TO THE CONVENTIONAL METHOD FOR DETERMINING THE DIGESTIBILITY OF A MIXED RATION FOR STEERS

by

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INTRODUCTION AND PURPOSE

The accurate determination of the coefficients of digestibility of feedstuffs and the relative feeding values of the ingredients in a ration must be determined if the total digestible nutrients and the digestible protein of a feedstuff are to be known. It is through these data that the nutritionist, feeder, and formula feed manufacturer are able to determine how rations may be compounded for accurate economical feeding.

Several methods for ascertaining digestion coefficients have been devised which have met with varying degrees of success. In general, these coefficients have been determined by calculation using the quantities and the chemical analysis of feed consumed and feces voided or by calculation from the ratio of amounts of specific nutrients in the feed and in the feces to the amount of a relatively, non-digestible reference material in the same feed and feces. This reference material may be endogenous of or added to the feed.

The method for the determination of coefficients of digestibility that is commonly referred to as the conventional method, requires a knowledge of the weights and the chemical analysis of the nutrients in the feeds and in the feces. Knowing these facts, the amount of a nutrient absorbed by the test animal can be determined by difference. From the amount of a nutrient absorbed and the amount of the nutrient ingested, the coefficient of digestibility of the nutrient may be obtained.

While determination of the coefficients of digestibility by the conventional method requires knowledge of the nutrients

apparently absorbed, the ratio techniques do not require this information, since they are based upon the use of a relatively nondigestible material as a reference standard. The ratios of the per cent of each nutrient in the feed and in the feces to the per cent of the reference material in the same feed and feces are used to calculate digestibility. Knowledge of the exact quantities of feeds consumed and feces voided is not required.

During the development of each of the several ratio techniques, studies have been made comparing them to the conventional method. In a review of the literature, reports (1, 2) were found in which the lignin-ratio method and the conventional method were compared on rations composed of roughages (hays and grasses) but the only reports found on comparison of these two methods using a ration composed of two or more ingredients were those of Swift, et al. (3) and Ellis, Matrone, and Maynard (4). Since it was believed additional information on the relative merits of the conventional and the lignin-ratio methods for the determination of the coefficients of digestibility of mixed rations was desirable, the present study was undertaken.

LITERATURE REVIEW

In order more thoroughly to understand the two general techniques for determining the digestibility of feeds (the conventional and the ratio methods), a brief resume of the procedures and their development will be given.

The conventional method for the determination of digestion coefficients largely is the result of the early work of Henneberg and Stohmann (5). During their investigations at the Weende experiment station, they devised a method over 80 years ago which, with few changes, is probably the most widely used of the several methods for these determinations.

Since determination of digestion coefficients by the conventional method requires a knowledge of the composition of the feed and feces, as well as a knowledge of the weight of the feed and feces, a routine feedstuff analysis is made. The analysis consists of the determinations of dry matter, crude protein (obtained by multiplying nitrogen by 6.25) ether extract, crude fiber, and nitrogen free extract, the latter being determined by difference. Ash also sometimes is determined.

There are certain limitations of the procedure. One of these is the extent to which the procedure is applicable. Since the amounts of feeds and feces must be known, the test animal must be in a stanchion or isolated in some other way so that accurate feeding and collecting of feces is possible. This eliminates the use of the procedure for pasture studies. Another difficulty, from the economic standpoint, is that an attendant must be present constantly to collect the feces as eliminated,

or special devices must be employed for this purpose.

In order to overcome some of the difficulties of the conventional method, attempts have been made to determine the coefficients of digestibility of feedstuffs by the inclusion of some substance, or by the use of some fraction of the feed, that is relatively insoluble and nondigestible. This nondigestible substance has been used as a reference material. The nutrient-reference ratios in the feeds and in the feces were used to determine the coefficients of digestibility.

Apparently the first of the ratio methods to be used was that proposed by Bergeim (6), who suggested the use of ferric oxide. Gallup (7), using albino rats, found that for cottonseed products the digestibility was apparently less complete when calculated by the ferric oxide method. He attributed the confusing data obtained in the analysis of fecal iron to interference by substances in greater quantities in the feces than in the feed (8). Knott, Murer, and Hodgson (9) reported that the iron-ratio method was not adaptable to rumen or total digestion studies in cattle. They found that the iron progressed through the rumen irregularly, being passed at some times and retained at others.

When it became apparent that the iron-ratio method was unsatisfactory, at least for ruminant studies, some investigators tried adding other compounds, e.g., silica and chromic oxide to the ration. Skulmouski, Szymanski and Wyszynski (10) included silica in the feeds of sheep and horses and chromic oxide in the diet of horses and found that these reference substances yielded results comparable to those obtained by the conventional method.

Gallup, Hobbs, and Briggs (11) demonstrated that silica in the feeds was recovered practically quantitatively in the feces when steers were in stanchions. This indicates that the method was satisfactory; however, when the steers were in a dry lot and the feces collected in canvas bags, the silica excreted exceeded that ingested. They reported fecal silica was about 36 per cent higher than the calculated intake, presumably due to quantities of dirt eaten with the ration.

In earlier studies, Gallup and Kuhlman (12) investigated the digestibility of mung bean silage by the silica-ratio technique and found it to be unsatisfactory. They reported that the essential requirements for a digestion study, i.e., uniform distribution of the silica through the intestinal tract without stratification and the elimination of the silica in uniform quantities, were not met. In an animal metabolism study, Forbes et al. (13) observed that most of the silica ingested by milk cows was recovered in the feces and that additional amounts were recovered in the urine. The total recovery was over 85 per cent, which represents a loss of sufficient magnitude to make digestibility studies using this method impractical.

It was suggested that since the silica- and iron-ratio techniques often gave results for the coefficients of digestibility that differed significantly from those obtained by the conventional method, a lignin ratio method might be used. Rogozinski and Starzewska (14) found that lignin, prepared according to Beckman[1],

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[1]Lignin was prepared by mixing 500 gm chopped oats with 4 l. of 1.8 per cent sodium hydroxide and allowing to stand 72 hours at room temperature. The brown extract was decanted and neutralized with hydrochloric acid, heated to boiling and placed in an autoclave for two hours. The solution was evaporated and dessicated at 100°C.

when fed to sheep was eliminated almost quantitatively, indicating its indigestibility. Hale, Duncan, and Huffman (15), in their investigations on rumen digestion in cattle, found that lignin passed through the rumen virtually unchanged. Ellis, Matrone, and Maynard (4) made extensive studies on the degree of digestibility of lignin and reported that in cows, sheep, and rabbits, lignin of the feed was recovered practically quantitatively in the feces. It was demonstrated that there was only a small daily variance in the lignin content of the feces from sheep on a diet of timothy hay. The standard error of the values during a nine day collection period from each of three sheep studied was 24.6 ± 0.13, 23.1 ± 0.08 and 23.1 ± 0.18, respectively.

Adolph et al. (1) in their report on the digestibility of morning and evening cuttings of alfalfa, stated there was a high variability in the digestion of lignin by rabbits fed the alfalfa. The mean values obtained on the digestion of lignin by two groups of rabbits was 5.8 and 3.4 per cent. Swift et al. (3), in their study of the effects of different ingredients in the ration, found that the digestion of lignin by sheep on various supplements did not exceed -3 per cent in twelve of the seventeen cases and the extremes were -6.2 and -5.2 per cent. They also found that the concentrations of various nutrients of the ration apparently had no effect on the digestion of lignin. Furthermore, there was a close relationship between the coefficients of digestibility of pasture grasses by steers when calculated by the conventional and by the lignin-ratio techniques. These authors found the

average recovery of lignin in the feces from rations fed steers was 102±7 per cent. Coefficients of digestibility of dry matter, digestible protein and total digestible nutrients were calculated by the conventional and the lignin-ratio methods. The results of the determinations by the two methods were in sufficient agreement to show the practicability of the lignin-ratio method for the determination of coefficients of digestibility of the ration they investigated.

There are numerous factors influencing the apparent recovery in the feces of lignin in the feed. These factors tend to introduce possible errors. It has been suggested (4) that possibly not all the lignin is isolated by the 72 per cent sulphuric acid method. Norman and Jenkins (16) recommended the removal of pentosans by dilute acid hydrolysis previous to the strong acid treatment in order to prevent the formation of insoluble residues. These residues would yield higher results for lignin. Norman and Jenkins (17), in furthering their investigations, found that protein alone gave no precipitation on standing with 72 per cent sulphuric acid but that when added to plant materials it increased the apparent lignin content. The magnitude of the disturbance was quite different when the material was subjected to a hydrolytic pretreatment.

Sherrard and Harris (18) and Ritter, Seborg, and Mitchell (19) have shown that time and temperature during the 72 per cent sulphuric acid treatment effects the lignin yield. MacDougall and DeLong (20) made the important observation that the method and temperature used to dry the sample were important in determining the lignin recovery.

Despite these difficulties, Ellis, Matrone, and Maynard (4) have devised a method for determining lignin which gave satisfactory results in their studies of digestibility of feeds. Other studies (2, 3, 21) have confirmed the value of this procedure in the determination of digestion coefficients by ruminants.

PROCEDURE

Care of the Experimental Animals and the Collection of Samples

Three groups, each composed of four Herford feeder steers, were put in stanchions and fed a daily ration consisting of 419 gm cottonseed meal, 3221 gm milo maize, 45 gm salt, and 23 gm limestone (dry weight). In addition to these ingredients, each steer received varying amounts of Atlas Sorgo silage. The quantities of silage fed were determined during the first five days of the 10-day preliminary period and were adjusted to the amount each steer would consume.

Maize was fed to Group I as the whole grain, to Group II in a coarsely ground form, and to Group III in a finely ground form. During the last five days of the preliminary period, the steers received the same quantity of each ingredient of the ration that they were to receive during the 10-day collection period. There were no weigh-backs of feed.

The feces were caught on shovels and put in large cans. An attendant was present at all times to make the collections. The floor was kept clean to prevent contamination of the samples if the feces were not caught as voided. Aliquots of 1/30 the weight of the feces from each steer were taken daily, placed in a pan marked with the steer's number and dried in a forced draft oven at a temperature between 65° and 85° C. Each day's aliquot was added to the pan containing the previously collected portions.

500 gm samples of silage fed were collected daily and kept in a refrigerator at 3° C. until the feeding trial was finished.

The maize was sacked during the preliminary period, each bag containing enough maize for one daily feeding. Samples for analysis were taken at random as the sacks were filled. The samples of cottonseed meal for analysis were taken daily as the steers' rations were being weighed.

When the collection period was finished, the feces and the silage were dried to constant weight in a forced draft oven at 100° C. (This is not the temperature recommended by Ellis, Matrone and Maynard (4)). The feeds and feces then were ground in a Wiley mill to one mm fineness. Samples of all the feedstuffs and feces were sent to the analytical laboratory for a routine feedstuff analysis.

Method of Analysis

The analysis for lignin was essentially according to the procedure of Ellis, Matrone, and Maynard (4). A one gm sample of the material to be analysed was placed in an alumdum extraction thimble and extracted with an alcohol-benzene mixture (32 parts 95 per cent ethanol to 68 parts by weight of benzene) for four hours. The sample, still in the extraction thimble, was washed with two small portions of 95 per cent ethanol, followed by two small portions of ether. Suction was used to aid washing. The thimble containing the extracted sample was placed in a 45° C. non-sparking oven until all the ether was driven off. The sample was transferred to a 250 ml wide mouth Erlenmeyer flask[1]. Forty ml

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[1]The wide mouth Erlenmeyer flasks used in the analysis were ASTM extraction flasks used for extracting bituminous mastics, grouts, and like mixtures.

of 1 per cent pepsin, USP, in 0.1 N hydrochloric acid was added to the sample and the mixture incubated overnight at 40°C.

The next morning the mixture was filtered using a filter stick on which a layer of Super-Cel had been deposited by application of suction. The residue in the flask was washed with 20-30 ml hot distilled water and filtered again. The washing and filtering procedures were repeated once. The filter stick was not removed and 150 ml of 5 per cent sulphuric acid (by weight) was added. The sample was boiled one hour, during which time sufficient hot, distilled water was added to maintain constant volume. The liquid was filtered through the filter stick using vacuum. The residue was washed, first with hot distilled water, then twice with 15 ml portions of 95 per cent ethanol, and finally twice with 15 ml portions of ether. After the final ether washing and filtration, the vacuum was left on a few minutes to remove as much ether as possible. The sample was placed in the 45°C. oven to complete drying. Twenty ml of 72 per cent sulphuric acid (by weight) was added to the residue, stirring with the filter stick to obtain a homogenous mixture. The mixture was placed in a 20 C. thermostatically controlled water bath for two hours and was occasionally stirred. One hundred twenty-five ml distilled water was added and the acid solution was removed by filtration. The residue was washed once with 15-20 ml of hot distilled water and filtered. One hundred fifty ml of 3 per cent sulphuric acid (by weight) was added and the solution was boiled for two hours, the volume being kept constant with hot distilled water. The solution was removed from the residue by filtration

on a Gooch crucible. The residue was dried at 105°-110°C. The lignin was determined by loss on ignition at 600 C.

It was difficult to wet thoroughly some of the samples, especially maize, with the pepsin solution. This indicated the possibility that the lignin recovered might contain extraneous undigested nitrogenous material. In order to determine whether this were true, a sample was autoclaved with 0.1 N hydrochloric acid for 10 minutes at 10 pounds pressure to facilitate wetting. The autoclaved sample was cooled to room temperature, the pepsin added and the sample incubated overnight. The procedure for the remainder of the analysis was as described in the foregoing section. Better wetting of the sample with the pepsin was obtained, but the final results were no different than those obtained by the original method. This indicates that the pepsin hydrolyzed the protein even though the wetting of the sample by the enzyme solution appeared to be incomplete.

When it was found that autoclaving caused no apparent harm to the sample, it was decided to try to eliminate the enzymatic hydrolysis by using acid hydrolysis. It was believed that this might prove satisfactory since many commercial protein hydrolyzates are prepared by acid treatment. The sample was treated as described for a routine lignin analysis, except autoclaving for 45 minutes at 10 pounds pressure with 5 per cent sulphuric acid (by weight) was substituted for the pepsin hydrolysis. The method was unsatisfactory. Further attempts were made using the same reagent and pressure but increasing the autoclaving time to an hour. These results also were unsatisfactory, as shown in Table 1.

Table 1. Per cent lignin found in sample by various modifications in procedure.

Method used to determine lignin	Per cent lignin found		
Standard method using pepsin hydrolysis	7.41	7.89	8.03
Autoclaving at 10 pounds pressure for 45 minutes	12.9	13.3	
Autoclaving at 10 pounds pressure for 60 minutes	10.4	10.5	13.8

The equation used to calculate the coefficients of digestibility by the conventional method is:

$$y = 100\ x/z$$

where

y = coefficient of digestibility

x = weight nutrient in feed - weight nutrient in feces

z = weight nutrient in feed.

The equation for the calculation of the coefficients of digestibility by the lignin-ratio method follows (Ellis, Matrone, and Maynard (4)):

$$y = 100 - 100 \cdot \frac{x}{z} \cdot \frac{n \text{ feces}}{n \text{ feed}}$$

where

y = coefficient of digestibility

x = per cent lignin in feed

z = per cent lignin in feces

n = per cent nutrient in the feed and feces.

A sample calculation for the coefficients of digestibility by both the lignin-ratio method and the conventional method is shown. (It was necessary to convert all weights to a dry matter basis since the lignin was not determined at the time that the feedstuff analysis was made.) The calculation is shown for

digestibility of crude protein by steer 88.

Crude protein intake	786.8	gm daily
Dry matter intake	6358.	gm daily
Per cent protein intake	12.38	
Per cent lignin intake	4.02	
Crude protein voided	460.8	gm daily
Dry matter voided	3294.	gm daily
Per cent crude protein voided	13.99	
Per cent lignin voided	7.80	

Using the equation for the conventional method:

$$y = 100\ x/z$$

$$= 100\ (786.8 - 460.8)\ /786.8$$

$$= 41.42 \text{ per cent.}$$

Calculating by the lignin-ratio method:

$$y = 100 - 100 \cdot \frac{x}{z} \cdot \frac{n \text{ feces}}{n \text{ feed}}$$

$$= 100 - 100\ (4.02/7.80) \cdot (13.99/12.38)$$

$$= 41.76 \text{ per cent}$$

RESULTS

A feedstuff analysis was made on both feeds and feces. The results are shown in Tables 2 and 3, respectively.

Table 2. Analysis of the feeds used in the ration.

Feedstuff	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Moisture
	Per cent				
Cottonseed meal	41.63	5.10	9.86	29.52	7.67
Milo maize, whole grain	11.25	3.30	1.46	70.93	11.21
Milo maize, coarsely ground	11.38	3.28	1.54	71.37	10.71
Milo maize, finely ground	11.38	3.44	1.59	70.99	10.71
Silage	7.00	2.54	24.70	56.09	2.15

Table 3. Analysis of feces from each steer.

Steer number	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract
	Per cent			
88	13.99	3.17	10.39	65.91
53	14.79	3.08	9.26	65.94
47	15.11	3.28	8.50	67.29
84	14.55	3.24	7.34	68.66
15	14.53	2.56	14.71	60.37
16	14.82	2.27	11.86	64.08
58	15.44	2.54	11.34	63.00
71	14.69	2.57	12.13	64.01
3	14.87	2.35	13.75	61.77
68	15.10	2.36	12.92	61.54
22	16.09	2.55	13.22	60.39
170	15.06	2.40	13.82	60.83

The lignin content of the feeds and feces are presented in Table 4.

Table 4. Lignin content of feeds and feces.

	Per cent lignin*
Feed	
Cottonseed meal	9.1
Milo maize, whole grain**	1.09
Silage	6.88
Steer number	
88	7.80
53	6.98
47	7.18
84	6.53
15	9.03
16	8.30
58	7.88
71	8.65
3	9.66
68	9.66
22	9.67
170	9.19

*Calculated to dry-weight basis.
**The lignin content of all maize samples was assumed to be the same as that of the whole grain.

The coefficients of digestibility of the nutrients calculated by the lignin-ratio method and the conventional method are shown in Table 5. The mean values and the standard errors for the coefficients of digestibility of the rations by each of the groups also are shown. The standard errors indicate that the lignin-ratio method gives results which are less widely distributed than does the conventional method for calculating the digestibility of the rations investigated. Calculations of digestibility of the nutrients of the rations by the individual steers using the two methods, however, agreed within 5 per cent.

Table 5. Coefficients of digestibility of nutrients of total ration calculated by the lignin-ratio and by the conventional methods.

Steer Number	Method used	Dry matter	Crude protien	Ether extract	Crude fiber	Nitrogen-free extract
Group I						
88	conventional	48.2	41.4	48.3	55.4	48.8
88	lignin-ratio	48.5	41.8	52.6	55.6	51.9
53	conventional	48.7	44.8	55.6	46.4	50.9
53	lignin-ratio	52.4	48.4	58.2	50.3	49.7
47	conventional	53.6	48.1	54.8	57.1	54.6
47	lignin-ratio	52.7	47.1	55.6	56.2	53.5
84	conventional	41.6	36.6	43.7	55.1	41.4
84	lignin-ratio	46.9	42.2	50.4	59.1	46.5
Mean	conventional	48.0 ± 2.46	42.7 ± 2.46	50.1 ± 2.57	53.5 ± 2.35	48.9 ± 2.78
Mean	lignin-ratio	50.1 ± 1.45	44.9 ± 1.69	54.2 ± 1.72	55.3 ± 1.84	50.4 ± 1.51
Group II						
15	conventional	52.2	43.8	61.5	48.5	57.7
15	lignin-ratio	52.8	42.5	63.1	47.4	56.6
16	conventional	52.6	48.0	68.2	49.7	56.9
16	lignin-ratio	55.2	48.6	70.1	50.3	57.4
58	conventional	56.1	51.7	68.0	50.1	61.4
58	lignin-ratio	56.0	49.2	67.8	47.6	59.3
71	conventional	48.4	43.8	61.0	53.0	54.2
71	lignin-ratio	53.0	43.8	63.6	53.5	54.6
Mean	conventional	52.3 ± 1.59	46.8 ± 1.91	64.7 ± 1.99	50.3 ± 3.06	57.5 ± 1.48
Mean	lignin-ratio	54.2 ± 0.79	46.0 ± 1.67	66.1 ± 1.69	49.7 ± 1.44	57.0 ± 0.98
Group III						
3	conventional	56.5	50.7	70.1	50.0	61.3
3	lignin-ratio	59.7	52.5	71.8	51.7	62.8
68	conventional	60.8	55.5	73.2	56.3	65.4
68	lignin-ratio	59.8	52.5	72.0	53.3	63.2
22	conventional	65.8	60.7	75.7	54.8	71.0
22	lignin-ratio	64.1	56.8	73.6	50.2	68.3
170	conventional	57.7	52.8	70.9	47.8	63.4
170	lignin-ratio	59.5	52.8	71.43	47.7	63.5
Mean	conventional	60.2 ± 2.06	54.9 ± 2.16	72.5 ± 1.26	52.2 ± 1.98	65.3 ± 2.08
Mean	lignin-ratio	60.8 ± 1.11	53.7 ± 1.07	72.2 ± 0.49	50.7 ± 1.19	64.4 ± 1.28

In order to determine whether the lignin ingested was being voided completely, the lignin recovery was calculated (Table 6). There is some variance in the individual recoveries, but the means of the groups can be included in 101 ± 3 per cent recovery with the mean of all groups 100.5 per cent recovery.

Table 6. Per cent of lignin in feed consumed which was recovered in the feces.

Steer Number	Per cent recovery
88	100.5
53	107.7
47	98.1
84	110.0
Mean	104.1
15	98.1
16	101.6
58	95.9
71	101.5
Mean	99.3
3	104.2
68	95.9
22	91.7
170	100.5
Mean	98.1
Mean of all groups	100.5

To determine whether there were significant differences in the results obtained by the two methods of calculating coefficients of digestibility, the *t*-test (method of individual comparisons (22)) was applied. The results of the statistical analysis are shown in Table 7.

Table 7. Results on test of significance of differences (t) in determination of coefficients of digestion by conventional and lignin-ratio methods.

Group	Dry matter	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract
			t		
I	1.84	2.22	3.34*	2.40	2.75
II	1.82	1.82	3.27*	2.56	2.66
III	3.92*	2.62	4.05*	2.50	3.10

*Significant at 5 per cent level, 3 degrees of freedom

The t-test showed that for 11 of the 15 determinations of coefficients of digestibility of various nutrients by the two methods, there was no significant difference in the results. The results indicate, however, that a difference existed, which was significant, in one determination of digestibility of dry matter, and that there was a significant difference in digestibility of the ether extract.

DISCUSSION

The earlier ratio techniques had disadvantages which prevented their use for determination of coefficients of digestibility. These usually were due to metabolization of the substance used for the reference or to unequal distribution of the reference substance in the feed and feces. The lignin-ratio method appears to be more satisfactory, probably because lignin is an integral part of the feed, although it is not digested.

MacDougall and DeLong (20), investigating the effects of the method used to dry the sample before analysis, observed that samples dried in an oven at 105°C resulted in higher yields of lignin than those dried at lower temperature. The nitrogen and methoxyl content of the lignin was greater than for air dried samples, indicating the inclusion of nitrogenous and carbohydrate materials. The samples were dried a few months before the present investigation was undertaken and were used for the conventional determination. Thus, it was not possible to follow the recommended procedure. The amounts of extraneous nitrogenous and carbohydrate materials in the lignin recovered in the samples investigated was unknown.

Ellis, Matrone, and Maynard (4) have reported a significant difference was found in the two instances in which lignin recovery was high or low (106 per cent and 94 per cent). The average lignin recovery of the groups in the present investigation were 104.1, 99.3, and 98.1 per cent. It seems, then, that at least for the rations used in the present study, there is no definite relationship existing between lignin recovery

and the significance of the observed differences.

It has been reported (4) that the daily variation in the lignin content of feces from sheep fed a ration of timothy hay and concentrate was remarkably small. The averages and standard errors for three individual sheep studied for nine days were 24.6 ± 0.13, 23.1 ± 0.08, and 23.1 ± 0.18 per cent, respectively. No attempt was made to take aliquots of the daily samples, but rather the approximate first 75 gm taken from the collection bag was used for analysis. Similar studies for cattle apparently have not been made. If future studies reveal equally low variability in the daily lignin content of the feces from cattle, a reduction of cost in the determination of coefficients of digestibility of feeds by cattle is possible.

If a relatively small variability in the daily lignin content of random samples of the feces voided by cattle exists, the lignin-ratio technique would be valuable in pasture studies. If in a pasture, only one type of feedstuff were present, random samples of the feed and of the feces might be sufficient for digestibility studies.

A disadvantage to the lignin-ratio technique is that two analyses, a feedstuff analysis and a lignin analysis, must be made on both feeds and feces. The lignin analysis is time consuming and relatively empirical. A faster more accurate method for the determination of lignin would undoubtedly offer impetus to the use of the lignin-ratio technique for digestion studies. However, lignin-ratio technique applied to studies of digestibility of pasture grasses, where the conventional method can not

be used, should yield information of sufficient value to merit the extra analysis. Since the amounts of the different ingredients fed must be known in the case of studies using a mixed ration in order to determine the lignin intake, it would seem that the lignin-ratio method is of limited value in these studies.

SUMMARY

A study has been made comparing the lignin-ratio method and the conventional method for determining the apparent coefficients of digestibility of a ration composed of milo maize, silage, and cottonseed meal.

Analysis for lignin was made using essentially the method of Ellis, Matrone, and Maynard (4).

Statistical analysis has shown that the results by the lignin-ratio method are comparable to those obtained by the conventional method. The only significant differences ($P = 0.05$) found when the *t*-test was applied were for digestibility of dry matter of ration fed group three, and ether extract in rations fed all groups.

During the analysis it was observed that some of the samples were not completely wetted by the pepsin solution. Controlled studies in which the sample was autoclaved prior to the addition of pepsin to achieve better wetting indicated that, even though incomplete wetting apparently occurred, the enzymatic hydrolysis was not increased by the modified sample preparation.

Studies were made to determine whether autoclaving in the presence of the five per cent sulphuric acid (by weight) would hydrolyze the protein in the sample. Controlled studies showed, however, that the lignin yield in the sample treated with acid rather than pepsin was too high.

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