

The Use of Chromium and Acid-Detergent Lignin in Complete Rations as Indicators of the Fecal Excretion Rate in the Ruminant Animal¹

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INTRODUCTION

For many years there has been an increasing interest in utilizing inert, nonabsorbed materials to determine the digestibility of nutrients in the feeds of nonruminant and ruminant animals. These index substances, intended to be nontoxic and indigestible to the animal, are being used as indicators of digestibility by means of ratio techniques. Chromic oxide (23, 27)³ and lignin (13, 21) are two of the most used markers in digestibility studies.

Chromic oxide, often administered in capsule form and used as an external indicator (31), has been observed to present a wide range of concentrations in its excretion pattern during a 24-hour interval (16). The close agreement between the total collection and the chromic oxide method indicated that accurate coefficients of digestibility may be obtained by using this indicator mixed uniformly into a complete ration for ruminant animals (13, 23, 26, 30).

Lignin, on the other hand, is a natural constituent of the ration utilized frequently as an internal marker by the ratio technique (1, 21, 22). At times the low recovery in the feces (12) makes its use questionable. Porter (29) suggested that lignin as measured by the acid-pepsin technique undergoes more extensive changes than the acid-detergent lignin (L), introduced by Van Soest (35). As a result, L may act as a more valid marker than other lignin forms and chromium (Cr).

Feed dry matter has been divided by Van Soest (36) on the basis of solubility into two fractions: cellular contents or neutral-detergent solubles

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³ Italic numbers in parentheses refer to Literature Cited, pp. 673.

(NDS) and cell-wall constituents (CWC). The NDS fraction is completely available and is composed of soluble carbohydrates, lipids, most proteins and other water-soluble matter. The CWC or total fiber fraction (37) is partially and nonuniformly available and consists of hemicellulose (HCD), cellulose (C) and L (35).

This study reports the evaluation of Cr and L as index substances for determining digestibility of ration nutrients using growing steers consuming complete, low-fiber rations.

PROCEDURE

Intake and fecal data, collection and processing of samples, and weighing of animals were conducted as outlined (3). Growing Jersey steers were fed once daily. The rations were formulated from corn cobs, beet pulp, corn-starch, soybean oil, molasses, minerals, vitamins, choline chloride, several nitrogen (N) sources, and with or without Cr as chromic oxide (3).

Chemical analyses were made on all feed, fecal and fecal grab samples by the methods in the indicated references: Cr, calcium (Ca) and magnesium (Mg) (2), dry matter and Kjeldahl nitrogen (4) and CWC (37). Acid-detergent fiber (ADF) and L were determined essentially as outlined by Van Soest (35), except that 75 ml. of the detergent solution were added to a 0.5-g. sample. For the determination of C in feed and fecal samples, a slight modification of the Crampton and Maynard (10) method was used. Regression equations to predict C (3) were employed, and HCD was represented by CWC minus ADF.

Statistical analyses were performed according to Snedecor and Cochran (32).

TRIAL I

The objective was to study the rate of recovery in the feces of ration L and Cr during depletion and repletion with Cr in steers consuming complete low-fiber feeds.

During a 10-week period, four steers, ranging in weight from 223 to 400 kg., were fed a mixture of rations 11 and 14 (3). For the first 3 weeks and the last 4 weeks of the 10-week period, the total ration contained 0.58 to 0.62 percent Cr. The L content for the 10-week period was 3.5 to 4.9 percent. Chromium was not fed during the second 3-week interval. Beginning with the third week, total fecal collections were made on days 6 and 7 of each subsequent week until the termination of the trial for a total of eight collections.

During a 4-week period, three steers, ranging in weight from 244 to 336 kg., were fed a chromium-free mixture of rations 11 and 14 (3). However, for the first day of the last week, the total ration contained 0.63 percent

Cr, and the L ranged from 3.7 to 4.2 percent. During the last week total fecal collections were carried out daily.

TRIAL II

The objective was to study the rate of recovery of Cr and L in complete feeds consumed by growing ruminant animals.

Digestibility trials totaling 140 collections using steers, ranging in weight from 134 to 643 kg., in previous trials I, II and III (3) were conducted. Trials I and II consisted of four consecutive 21-day feeding periods, with total collection of feces during the last 3 days of each period. In trial III the 3-day collections started on the 7th, 23d and 39th day of each of the five 42-day periods. One hundred twenty eight of the 140 total collections were used in Cr recovery determinations, and 39 of the 60 total collections in trial III (3) were used in L recovery determinations. The rations contained 0.19 to 0.54 percent Cr and 1.3 to 5.2 percent L.

TRIAL III

This trial was conducted to determine the excretion patterns of nutrients, L and Cr during a 24-hour period for complete feeds by growing steers.

During previous trials I and II, after the animals were adjusted to their rations for a minimum of 42 days, 80 fecal grab samples (16 steers for 5 times each within 24 hours) were collected from steers ranging in weight from 152 to 626 kg. These rations contained 0.19 to 0.21 percent Cr and 1.6 to 3.1 percent L.

The steers were fed once daily at 11 a.m., with the five fecal grab samples taken at 4.5-hour intervals after feeding. The grab samples were taken either directly from the rectum or from the last defecation, if feces was voided voluntarily at collection time.

RESULTS, DISCUSSION AND, CONCLUSION

TRIAL I

The rates of recovery of Cr and L are presented graphically in figures 1 and 2.

Depletion and Repletion of Cr in the Feces

Chromium was recovered in the feces after a 7-day depletion period to the extent of 1.9 ± 0.3 percent, and recovery continued to decrease to 0.54 ± 0.02 percent after a 21-day depletion period (fig. 1).

When Cr was supplied in the diet for only one day, the amount recovered in the feces on the seventh day was 1.1 ± 0.2 percent. A maximum concentration of 29.8 ± 0.9 percent Cr was recovered on the second day.

Kallai *et al.* (19) reported that, after chromic oxide was fed to wethers, 15 to 20 percent appeared in the feces in 24 hours and nearly all after 8 days (98.2 percent). The accumulation of the Cr recovered after the 7- and the 21-day depletions totaled 85.5 ± 7.2 percent and 90.5 ± 2.4 percent.

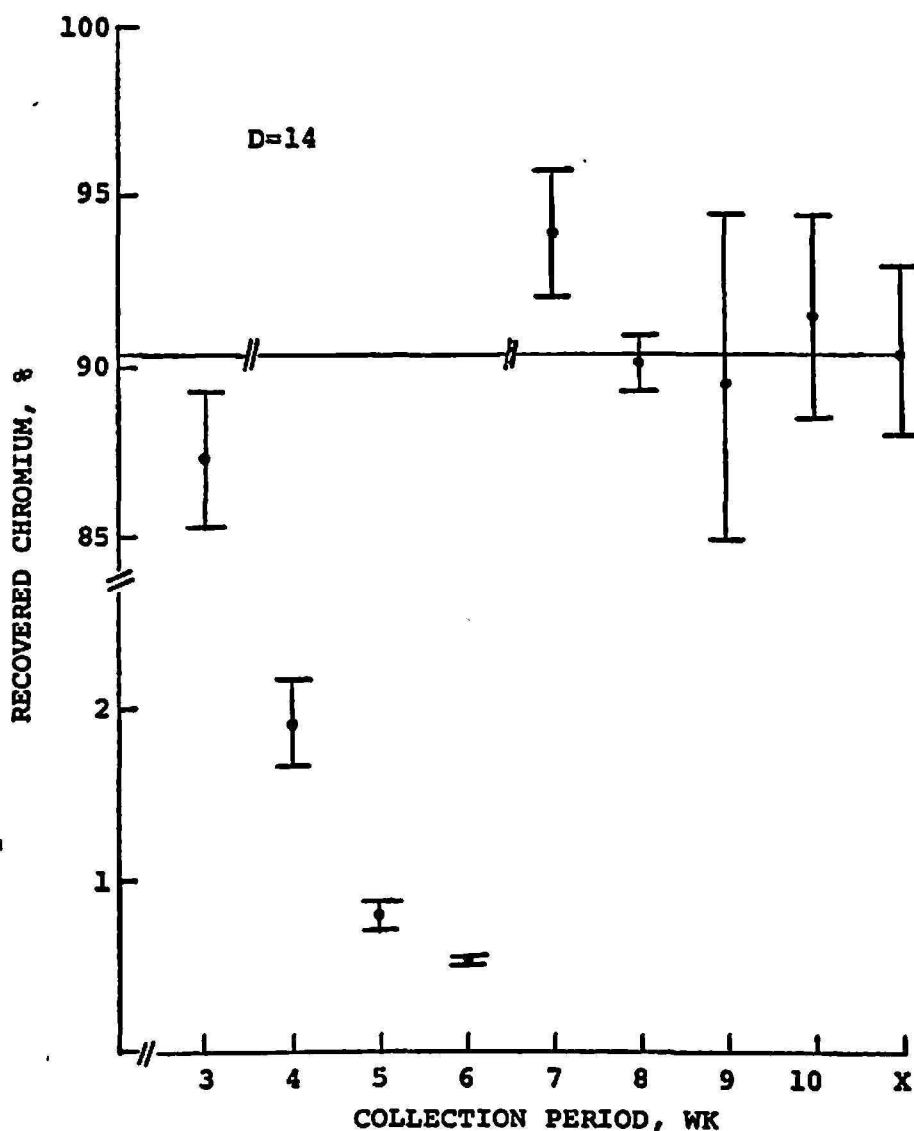


FIG. 1.—Recovery rate (mean \pm SE) of chromium during depletion and repletion periods, with Tukey's D for $P < 0.05$. X is mean for repletion samples.

From the 8th to the 21st day, the amount of Cr recovered in the feces was small (5.0 percent), thus showing that, within a 7-day period 85.5 percent of the total Cr in complete feeds was eliminated. Johnson *et al.* (18) indicated that chromic oxide concentration in the feces of wethers fed pelleted alfalfa rations containing chromic oxide impregnated in paper and chromic oxide powder appeared to reach near maximum levels 72 hours after its administration. The same trend was observed in these trials, where

most of the ration Cr was recovered in the feces during the first 5 days (82.3 ± 6.8 percent).

Hardison *et al.* (17), working with forages, reported that 3 to 7 days may be required for chromic oxide to reach a stable level in the feces. Crampton and Lloyd (9) observed that, when chromic oxide was mixed with some portion of the ration, a minimum period of 5 days was required after the initial administration before the concentration of the indicator reached a constant or maximum level in the feces. They also suggested that, when

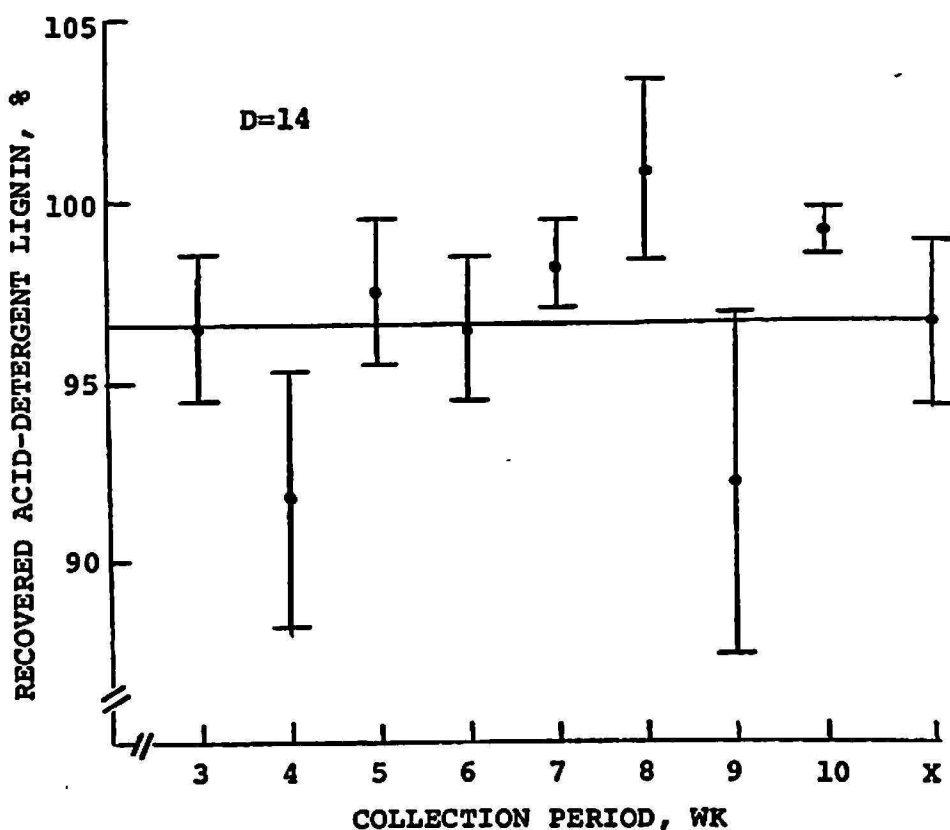


FIG. 2.—Recovery rate (mean \pm SE) of acid-detergent lignin during a 10-week period, with Tukey's D for $P < 0.05$. X is mean for weekly samples.

chromic oxide was fed in a concentrated form, the rate of excretion of chromic oxide was slower and a minimum period of 9 days seemed necessary to bring the fecal indicator level to a maximum.

Chromium was recovered to the extent of 93.9 ± 1.9 percent (fig. 1) after a diet containing 0.6 percent Cr was fed for a 7-day period, with a mean of 90.5 ± 2.5 percent for a 4-week repletion period. The value, 93.9 ± 1.9 percent, was the maximum Cr recovery value obtained during the 4-week Cr repletion period. No statistical differences ($P < 0.05$) were found among the weekly means for recovered Cr, indicating that a steady state had been reached during the first week of repletion. However, at

the beginning of repletion, a residual of 0.54 ± 0.02 percent Cr in the feces was present. Anderson *et al.* (1), Barnicoat (5), Clanton (6), Hardison *et al.* (17), Johnson *et al.* (18), and Moore (28) have reported from 80 to 95 percent recovery of dietary chromic oxide in feces.

The Cr, not eliminated in the feces, may have been absorbed from the digestive tract or may have been adsorbed to the mucosal walls of the digestive tract. Deinum *et al.* (11) concluded that the small nonexcreted part of the chromic oxide was absorbed. McGuire *et al.* (27) could offer no explanation for the low chromic oxide recovery value, 94.2 percent, obtained when feeding a 0.5-percent chromic oxide complete ration to growing steers. MacKenzie *et al.* (25) administered a dose of radioactive chromate to rats similar to the amount that they would receive per day while drinking water containing 2 p.p.m. Cr. They determined that, after feeding radioactive chromate for 1 day, the liver showed a maximal uptake of about 1 percent whereas kidney and blood contained 0.1 to 0.2 percent. However, a decreased concentration of Cr with time was observed. Deinum *et al.* (11) indicated that traces of chromic oxide were absorbed in parts of the digestive tract and could be found in liver, lymph glands and kidneys. Observations made in this laboratory of characteristic dark coloration of the rumen wall of slaughtered steers consuming rations containing chromic oxide for extended periods suggested that the indicator was adsorbed.

Rate of Recovery of L in the Feces

It has been suggested that L (35) may be a more valid marker because it undergoes less extensive change than the lignin prepared by the acid pepsin technique (29).

Lignin is a naturally-occurring constituent; however, it has not been identified as a chemical entity of definite composition. Furthermore, Sullivan (33) suggested that the methods of lignin analysis were empirical and not standardized in an acceptable form. As a result, use of lignin as an indicator in digestibility studies with forage has been considered unreliable. Nonetheless, Forbes and Garrigus (15), Kane *et al.* (21) and Swift *et al.* (34) utilized the lignin ratio techniques in feed studies with apparent success.

In complete pelleted rations, the crude lignin method of Ellis *et al.* (14) resulted in low coefficients of digestibility. Kane *et al.* (20) also obtained low coefficients calculated by the crude lignin technique. Elam and Davis (12) showed that an average of only 87.1 percent of the crude lignin fed was recovered in the feces.

When L was used as an indicator over a 10-week period, 96.6 ± 2.3 percent L was recovered (fig. 2) in contrast to a value of 104 percent obtained with all forage rations (8). Similarly, during a 7-day period, $96.7 \pm$

0.6 percent L was recovered, ranging from 94.3 ± 0.5 percent to 98.3 ± 0.3 percent. Anderson *et al.* (1) also obtained a recovery rate for L of 96.2 ± 2.2 percent in concentrate mixtures during a 17-day period. No statistical differences ($P < 0.05$) were obtained among weekly means, thus showing (fig. 2) a steady state of L excretion.

As indicated by Colburn and Evans (7), ADF represents essentially the sum of C and L. Van Soest (36) reported that L itself, in contrast to the structural carbohydrates, appears uniform in the Lucas (24) test, indicating an availability not significantly different from zero. As a result, L may be considered a suitable indicator for the ratio techniques in complete feeds, especially when it may be essentially recovered by utilizing the techniques of Van Soest (35).

TRIAL II

Digestibility trials were conducted for extended periods as indicated (3), and the rates of recovery of Cr and L were determined.

Rate of Recovery of Cr in the Feces

It was determined that 96.0 ± 2.1 percent of the Cr was recovered at the end of the first 21-day period. Slightly lower values of 91.8 ± 1.6 , 95.6 ± 1.8 and 95.2 ± 1.6 percent were obtained at the end of the next three consecutive 21-day periods, respectively. However, no statistical differences ($P < 0.05$) were found among period means, thus showing that a steady state existed at the end of period 1, with more uniformity developing during periods 3 and 4.

Results were similar in a second trial. Recovery rates of Cr were 92.6 ± 1.8 , 94.4 ± 1.4 , 97.6 ± 1.7 and 96.6 ± 1.4 percent in successive 21-day periods. No statistical differences ($P < 0.05$) were found among period means. A mean Cr recovery value of 95.4 ± 0.6 percent was obtained for both trials.

Similar results were again obtained per period in a third trial (3) (89.6 ± 0.9 , 93.1 ± 2.0 , 88.8 ± 1.9 , 94.4 ± 1.8 and 95.4 ± 1.3 percent), with no statistical differences ($P < 0.05$) among period means. A mean Cr recovery value of 92.3 ± 0.9 percent was obtained. An overall Cr recovery of 94.0 ± 0.5 percent was obtained for the three trials, in contrast to recovery rates of 90.5 ± 2.5 percent (fig. 1) and 85.5 ± 5.9 percent in short-term feeding trials. Anderson *et al.* (1) obtained Cr recovery values of 84.8 ± 1.4 percent and 83.8 ± 8.0 percent for 17-day periods. Similar results were obtained by Lassiter *et al.* (23) and McGuire *et al.* (27). However, Kane *et al.* (21) recovered chromic oxide in the feces at the rate of 99.9 percent during a 9-day period, with a 10-day preliminary period. Although it has been indicated by this study and by others (9, 17) that a level of 85 percent

or more of Cr excretion in the feces was reached after 3 to 7 days, the saturation of the adsorption sites may take longer than 3 weeks.

Rate of Recovery of L in the Feces

A total of 39 entire collections out of 60 were used to calculate L recovery in growing steers consuming complete feeds. The remaining fecal collections were not utilized because wood shavings bedding was consumed by some steers. A grand mean recovery for L of 94.1 ± 1.0 percent was obtained, comparing favorably with means of 96.6 ± 2.3 percent (fig. 2) and 96.7 ± 0.6 percent recovered L obtained in 10-week and 7-day periods. Although similar recoveries were obtained under varying conditions of L intake, the level of L intake and the length of the pre-collection feeding period are the most important considerations when the indicator, L, is used. Yang and Thomas (38) reported that the coefficients of digestibility obtained by using chromic oxide and L as indicators were very similar, except in the rumen where chromic oxide reduced values.

TRIAL III

The patterns of excretion of nutrients, Cr and L per unit of indicator at 4.5-hour intervals during a 24-hour period, are presented graphically in figures 3 and 4. No statistical differences ($P < 0.05$) were found among grab-sampling times in rates of excretion of nutrients per unit of indicator. Similar results were obtained by McGuire *et al.* (27) with chromic oxide and protein excretion rates.

Indicator-to-indicator Ratio

The rate of excretion of Cr (fig. 3) and L (fig. 4) was not affected significantly ($P < 0.05$) by sampling period in relation to feeding time, though L-to-Cr ratio showed a decreasing but not a significant ($P < 0.05$) trend as sampling time progressed in a 24-hour period.

An increasing trend in Cr-to-L ratio was observed as sampling time progressed during a 24-hour interval. Johnson *et al.* (18) suggested that, in relation to lignin, chromic oxide is passed out more rapidly from the anterior portion and less rapidly from the posterior portion of the tract, effecting a build-up or concentration of the indicator in the latter portion. McGuire *et al.* (27) obtained no significant differences due to sampling time or feeding frequency in chromic oxide concentration in the feces.

These data indicate that the difference in grab sampling times was minimal when the Cr and L ratio techniques were used to determine digestibility. Utilizing the Ellis *et al.* (14) method for lignin, Elam and Davis (12) arrived at the same conclusions. In this study, the hourly variation in Cr and L excretion patterns tended to be minimal in complete feeds.

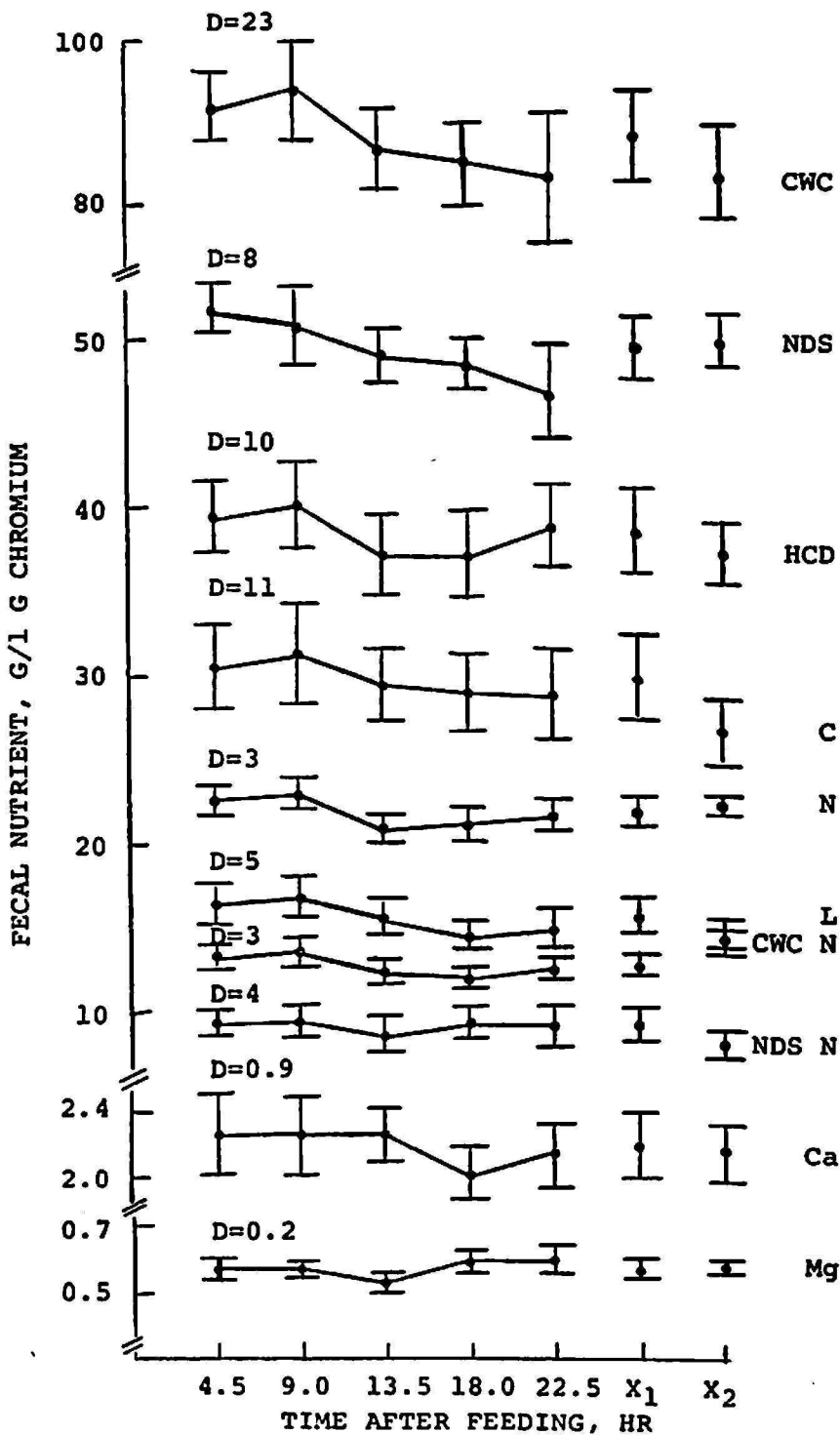


FIG. 3.—Rate of excretion of nutrient (mean ± SE) per 1 g. of chromium during a 24-hour interval, with Tukey's D for $P < 0.05$. X_1 is mean for five grab samples; X_2 , total collections.

Nutrient-to-indicator Ratio

The fibrous fractions (CWC, HCD, C and CWC N) -to-Cr ratios (fig. 3) tended to decrease as time-after-feeding sampling increased, indicating that the greater part of the nutrients were eliminated more quickly.

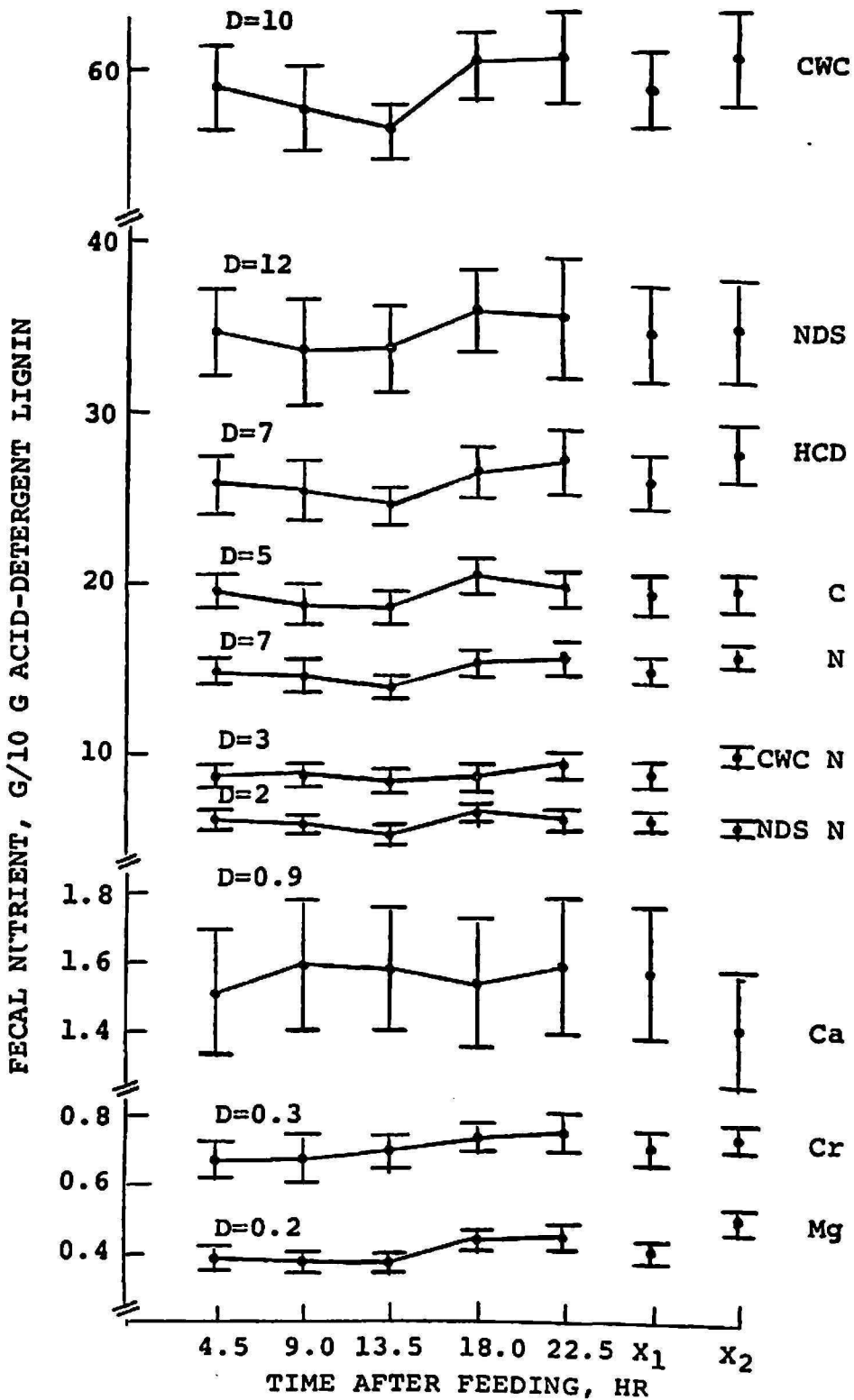


FIG. 4.—Rate of excretion of nutrient (mean ± SE) per 10 g. of acid-detergent lignin during a 24-hour interval, with Tukey's D for $P < 0.05$. X_1 is mean of five grab samples; X_2 , total collections.

The mean values for the grab samples (X_1) were closer to sampling period 3 (13.5 hours after feeding). However, the excretion values from the total collection trial (X_2) corresponding to the grab sampling period were in all cases, except for CWC N, lower than the fecal grab sample mean. The total collections agreed more closely with sampling periods 4 or 5 (18 or 22.5 hours after feeding) in the CWC and HCD fractions.

The nonfibrous fractions (NDS, N, NDS N, Ca and Mg) -to-Cr ratios (fig. 3) followed the same pattern as the fibrous fractions. There seemed to be a tendency for a larger nutrient fraction to be excreted sooner with a uniform quantity of Cr. In all nonfibrous fractions, except NDS, a tendency of the nutrients to increase at sampling periods 3 or 4 (13.5 or 18 hours after feeding) was evident.

The excretion of the fibrous fractions-to-L ratio (fig. 4) followed a somewhat reverse pattern from the Cr ratio, possibly demonstrating the slower excretion of nutrients per L. The mean value tended to be related to sampling periods 3 or 4. The total collection value was higher than the mean value and agreed better with sampling period 5.

The excretion of the nonfibrous fractions-to-L ratios (fig. 4) followed a similar pattern to that of the fibrous fractions. The total collection values were either equal to or higher than the mean values, except for Ca and NDS N excretion. In a similar study, Johnson *et al.* (18) obtained a very similar rate of flow of Ca and chromic oxide, indicating that the flow of Ca through the various sections of the tract paralleled the flow of chromic oxide.

Estimation of Digestion Coefficients from the Ratio Techniques and from Total Collections

Digestion coefficients were calculated from the Cr and L indicator methods and from the total collection data (tables 1 and 2). No significant differences ($P < 0.05$) among digestion coefficients were obtained between means of grab samples and total collections, indicating that Cr and L ratio techniques may be utilized in digestibility determinations. Longer periods of Cr intake may provide a better estimation of recovery, resulting in more reliable results.

Recovery data tends to show that L may be a more valid indicator than Cr. Recovered Cr appeared lower than recovered L and a larger variation in digestion coefficients was found for Cr.

SUMMARY

Chromium (Cr) and acid-detergent lignin (L) were evaluated as index substances for determining digestibility of complete, low-fiber rations with

TABLE 1.—*Mean intake and fecal excretion data of nutrients per unit of indicator*

Chemical component	Feed intake ¹		Fecal output			
	G.N/1 g. Cr	G.N/10 g.L	G.N/1 g. Cr	G.N/10 g. L	G.N/1 g. Cr	G.N/10 g. L
Nitrogen X 6.25						
Total	85.4 ± 3.5	66.2 ± 4.9	21.8 ± 0.9 ²	14.8 ± 0.8 ²	22.0 ± 0.7 ³	15.6 ± 0.7 ³
NDS	58.9 ± 3.6	45.5 ± 3.8	9.1 ± 1.0	5.9 ± 0.6	7.9 ± 0.8	5.7 ± 0.5
CWC	26.5 ± 1.2	20.7 ± 1.8	12.7 ± 0.7	8.9 ± 0.7	14.1 ± 0.4	9.9 ± 0.6
NDS	360.4 ± 5.3	272.5 ± 16.5	49.4 ± 1.9	34.6 ± 2.8	49.7 ± 1.6	34.9 ± 3.1
CWC	183.9 ± 5.0	139.4 ± 5.9	88.0 ± 5.7	58.9 ± 2.2	82.6 ± 4.7	60.8 ± 2.8
Hemicellulose	118.3 ± 3.3	90.7 ± 5.1	38.5 ± 2.4	25.9 ± 1.6	37.2 ± 1.8	27.7 ± 1.7
Cellulose	47.3 ± 2.2	35.2 ± 1.1	29.8 ± 2.5	19.4 ± 1.1	26.4 ± 2.0	19.5 ± 1.2
Lignin	14.7 ± 0.9	—	15.5 ± 1.1	—	14.2 ± 1.0	—
Chromium	—	0.78 ± 0.05	—	0.70 ± 0.05	—	0.73 ± 0.04
Calcium	3.09 ± 0.19	2.38 ± 0.20	2.19 ± 0.20	1.56 ± 0.19	2.16 ± 0.20	1.40 ± 0.17
Magnesium	0.88 ± 0.04	0.67 ± 0.05	0.57 ± 0.03	0.40 ± 0.03	0.58 ± 0.01	0.49 ± 0.04

¹ Data are included for 16 steers. Grams nutrient per one gram chromium expressed as G. N/1 g. Cr.

² Mean of 80 fecal grab samples (5 per total collection).

³ Mean of 16 total collections corresponding to fecal grab sampling.

growing steers. The rate of recovery of ration Cr was studied during depletion and repletion of the indicator.

Total fecal collections were carried out for 7 days after a 1-day period of Cr intake. Recovered Cr for the 7-day period amounted to 85.5 ± 7.2 percent with a value of 82.3 ± 6.8 percent for the first 5 days, with a maximum amount of 30 percent on the second day. During the 21-day depletion following a 21-day repletion, fecal Cr decreased rapidly for the

TABLE 2.—Mean digestion coefficients calculated from grab sample and total collection data

Chemical component	Digestion coefficients ¹				
	Mean ²		Mean ³		Mean ⁴
	Percent	Percent	Percent	Percent	Percent
Nitrogen					
Total	74.5 ⁵	77.7 ⁶	74.2 ⁵	76.5 ⁶	74.8 \pm 1.2
NDS	84.5	86.9	86.5	87.4	84.7 \pm 1.9
CWC	52.3	57.8	47.1	52.3	48.8 \pm 3.2
NDS	86.3	87.3	86.2	87.2	87.0 \pm 0.4
CWC	52.2	57.7	55.1	56.3	56.1 \pm 2.1
Hemicellulose	67.4	71.5	68.6	69.4	68.9 \pm 1.9
Cellulose	37.1	45.0	44.1	44.6	45.0 \pm 3.1
Lignin	105.4 ⁷	—	96.6 ⁷	—	—
Chromium	—	90.0 ⁷	—	94.0 ⁷	88.8 \pm 1.1 ⁷
Calcium	29.1	34.5	30.1	41.2	33.0 \pm 3.5
Magnesium	35.2	40.3	34.1	26.9	37.0 \pm 2.2

¹ No significant difference ($P < 0.05$) within nutrients.

² Data included for 80 fecal grab samples (5 per total collection) using the indicator ratio technique.

³ Data included for 16 total collections corresponding to fecal grab samples using the indicator ratio technique.

⁴ Means for digestibility and recovery data calculated directly from total intake and output data.

⁵ Calculated from g. nutrients/1 g. Cr.

⁶ Calculated from g. nutrients/10 g. L.

⁷ Recovery data.

first 7 days and continued to decrease to 0.54 ± 0.02 percent at the end of the depletion. During 7-day and 10-week periods, L was recovered to the extent of 96.7 ± 0.5 and 96.6 ± 2.3 percent, respectively.

Recovered Cr amounted to 94.4 ± 0.5 percent in long-term trials and to 90.5 ± 2.5 percent in short-term trials. In long-term trials, L was recovered to the extent of 94.1 ± 1.0 percent. No statistical differences ($P < 0.05$) were found among grab-sampling times or between grab samples and the total collection samples in rates of excretion of nutrients per unit of indicator.

It was suggested that the differences in grab-sampling times were minimal when either Cr or L indicator techniques were used to determine digestibility. Recovery data tend to show that L is a valid marker and has the desirable characteristic of being a constituent of ruminant feedstuffs.

RESUMEN

El cromo (2) y la lignina (35) se evaluaron como indicadores en novillos castrados para determinar la digestibilidad de raciones completas bajas en fibra. Durante los períodos de agotamiento y repleción del cromo, que fue incorporado en la ración en forma de óxido crómico, se estudió el porcentaje de recuperación en las heces. Las heces se recogieron durante 7 días consecutivos, después de suministrársele cromo por 1 día a los novillos.

El cromo recuperado en los 7 días ascendió a 85.5 ± 7.2 por ciento, con un valor de 82.3 ± 6.8 por ciento en los primeros 5 días. Durante el período de agotamiento de 21 días, que siguió a un período de repleción de 21 días, el cromo fecal se redujo rápidamente durante los primeros 7 días y luego gradualmente hasta bajar a 0.54 ± 0.02 por ciento a los 21 días. La recuperación de la lignina fue de 96.7 ± 0.6 por ciento y 96.6 ± 2.3 por ciento durante períodos de 7 días y 10 semanas, respectivamente.

La recuperación del cromo fue de 94.0 ± 0.5 por ciento y 90.5 ± 2.5 por ciento durante períodos largos y cortos, respectivamente. Durante los períodos largos, la recuperación de la lignina fue de 94.1 ± 1.0 por ciento. No se encontraron diferencias significativas ($P < 0.05$) entre las muestras de heces tomadas cada 4.5 horas durante 24 horas o entre éstas y las muestras de la colección total usándose como criterio las unidades de nutrientes excretados por unidad del indicador (figuras 2 y 3).

Se concluyó que las diferencias en digestibilidad durante el ciclo de excreción fecal fueron mínimas al usarse el cromo o la lignina como indicadores. El porcentaje de recuperación de los indicadores tiende a indicar que la lignina es un indicador más ventajoso y posee la buena característica de ser un componente de los alimentos.

LITERATURE CITED

1. Anderson, M. J., Oleson, W. H., and Stoddard, G. E., The influence of concentrate: forage ratios on digestibility using indicator techniques, abs., *J. Dairy Sci.* 50: 989, 1967.
2. Anonymous, Analytical Methods for Atomic Absorption Spectrophotometry, Perkin-Elmer Corp., Norwalk, Conn., 1966.
3. Arroyo-Aguilú, J. A., and Evans, J. L., Nutrient digestibility of complete, low-fiber rations in the ruminant animal, *J. Dairy Sci.* (In press.)
4. Association of Official Agricultural Chemists, Official Methods of Analysis, 10th ed., Washington, D. C., 1965.
5. Barnicoat, C. R., Estimation of apparent digestibility coefficients by means of an inert "reference-substance", *N. Z. J. Sci. Technol.* 27: 202-12, 1945.

6. Clanton, D. C., Variation in chromic oxide methods of determining digestibility of hand-fed beef cattle rations, *J. Anim. Sci.* 21: 214-8, 1962.
7. Colburn, M. W., and Evans, J. L., Chemical composition of the acid-detergent fraction of orchardgrass, alfalfa and mixed forages, Abstr., *J. Dairy Sci.* 48: 1557-8, 1965.
8. Colburn, M. W., Evans, J. L., and Ramage, C. H., Apparent and true digestibility of forage nutrients by ruminant animals, *J. Dairy Sci.* 51: 1450-7, 1968.
9. Crampton, E. W., and Lloyd, L. E., Studies with sheep on the use of chromic oxide as an index of digestibility of ruminant rations, *J. Nutr.* 45: 319-27, 1951.
10. Crampton, E. W., and Maynard, L. A., The relation of cellulose and ligning content to the nutritive value of animal feeds, *J. Nutr.* 15: 383-95, 1938.
11. Deinum, B., Immink, H. J., and Deijis, W. B., The excretion of chromium sesquioxide in faeces by cows after administration of Cr₂O₃-containing paper, *Jaarb. Inst. Biol. Scheik. Onderz. Landbgewass* 188: 123-9, 1962.
12. Elam, C. J., and Davis, R. E., Lignin excretion by cattle fed a mixed ration, *J. Anim. Sci.* 20: 484-6, 1961.
13. Elam, C. J., Reynolds, P. J., Davis, R. E., and Everson, D. O., Digestibility studies by means of chromic oxide, lignin, and total collection techniques with sheep, *J. Anim. Sci.* 21: 189-92, 1962.
14. Ellis, G. H., Matrone, G., and Maynard, L. A., A 72 percent H₂SO₄ method for the determination of lignin and its use in animal nutrition studies, *J. Anim. Sci.* 5: 285-97, 1946.
15. Forbes, R. M., and Garrigus, W. P., Application of a lignin ratio technique to the determination of the nutrient intake of grazing animals, *J. Anim. Sci.* 7: 373-82, 1948.
16. Hardison, W. A., Engel, R. W., Linkous, W. N., Sweeney, H. C., and Graf, G. C., Fecal chromic oxide concentration in 12 dairy cows as related to time and frequency of administration and to feeding schedule, *J. Nutr.* 58: 11-7, 1956.
17. Hardison, W. A., Linkous, W. N., Engel, R. W., and Graf, G. C., Observations on the use of chromic oxide for estimating the fecal output of dairy animals, *J. Dairy Sci.* 42: 346-52, 1959.
18. Johnson, D. E., Dinusson, W. E., and Bolin, D. W., Rate of passage of chromic oxide and composition of digesta along the alimentary tract of wethers, *J. Anim. Sci.* 23: 499-505, 1964.
19. Kállai, L., Till, F., Keresztes, M., and Tangl, H., Digestibility trials with wethers: barium sulfate and chromic oxide as markers, *Kiserletuyi Koslemenyek* 54B: 15-39, 1961 (from *Nut. Abs. Revs.* 33: 848, 1963).
20. Kane, E. A., Ely, R. E., Jacobson, W. C., and Moore, L. A., A comparison of various digestion trial techniques with dairy cattle, *J. Dairy Sci.* 36: 325-33, 1953.
21. Kane, E. A., Jacobson, W. C., and Moore, L. A., A comparison of techniques used in digestibility studies with dairy cattle, *J. Nutr.* 41: 583-96, 1950.
22. Kane, E. A., Jacobson, W. C., and Moore, L. A., Diurnal variation in the excretion of chromium oxide and lignin, *J. Nutr.* 47: 263-73, 1952.
23. Lassiter, J. W., Alligood, V., and McGaughey, C. H., Chromic oxide as an index of digestibility of all-concentrate rations for sheep, *J. Anim. Sci.* 25: 44-7, 1966.
24. Lucas, Jr., H. L., Smart, Jr., W. W. G., Cipolloni, M. A., and Gross, F. D., Relations between digestibility and composition of feeds and foods, S-45 Report, N. C. St. Coll. Raleigh, N. C., 1961.

25. MaKenzie, R. D., Anwar, R. A., Byerrum, R. U., and Hoppert, C. A., Absorption and distribution of Cr⁵¹ in the albino rat, *Arch. Biochem. Biophys.* 79: 200-5, 1959.
26. McCoy, G. C., Olson, H. H., and Reed, A., Diurnal excretion pattern and digestibility of three complete feeds, *J. Dairy Sci.* 49: 211-4, 1966.
27. McGuire, R. L., Bradley, N. W., and Little, C. O., Effects of frequency of feeding on excretion of chromic oxide, crude protein and gross energy and on nutrient digestibility by steers, *J. Anim. Sci.* 25: 185-91, 1966.
28. Moore, J. H., Diurnal variations in the composition of the faeces of pigs on diets containing chromic oxide, *Brit. J. Nutr.* 11: 273-88, 1957.
29. Porter, P., Lignin as an inert marker in studies of ruminant digestion, Abstr., *Proc. Nutr. Soc.* 24: VI-VII, 1965.
30. Putnam, P. A., Elam, C. J., and Everson, D., Comparison of chromic oxide and conventional methods in digestion trials using steers fed pelleted rations, USDA, ARS., Tech. Bull. 1312, 1964.
31. Smith, A. M., and Reid, J. T., Use of chromic oxide as an indicator of fecal output for the purpose of determining the intake of pasture herbage by grazing cows, *J. Dairy Sci.* 38: 515-24, 1955.
32. Snedecor, G. W., and Cochran, W. G., *Statistical Methods*, 6th. ed., Ia. St. Coll. Press, Ames, Iowa, 1962.
33. Sullivan, J. T., Evaluation of forage crops by chemical analysis, A Review, *Agron. J.* 54: 511-5, 1962.
34. Swift, R. W., Thacker, E. J., Black, A., Bratzler, J. W., and James, W. H., Digestibility of rations for ruminants as affected by proportion of nutrients, *J. Anim. Sci.* 6: 432-44, 1947.
35. Van Soest, P. J., Use of detergents in the analysis of fibrous feeds, II, A rapid method for determination of fiber and lignin, *J. Assoc. Off. Agr. Chem.* 46: 829-35, 1963.
36. Van Soest, P. J., Development of a comprehensive system of feed analysis and its application to forages, *J. Anim. Sci.* 26: 119-28, 1967.
37. Van Soest, P. J., and Wine, R. H., Use of detergents in the analysis of fibrous feeds, IV, Determination of plant cell-wall constituents, *J. Assoc. Off. Anal. Chem.* 50: 50-5, 1967.
38. Yang, M. G., and Thomas, J. W., Absorption and secretion of some organic and inorganic constituents and the distribution of these constituents throughout the alimentary tract of young calves, *J. Nutr.* 87: 444-58, 1965.