

# Precision of Indirect Methods for Estimating Digestibility of Forage Consumed by Grazing Cattle<sup>1</sup>

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## Highlight

Comparisons were made of the lignin ratio and the fecal nitrogen index methods of estimating digestibility of diets of grazing animals. Special attention was given to sources of error and variability in these estimates. Evaluation of indirect methods of estimating digestibility of grazed forage were made by sampling forage from the range with esophageally-fistulated steers and later feeding it to sheep in conventional digestion trials. Regression equations for predicting diet digestibility from fecal nitrogen and factors for correcting for lignin digestibility were obtained from the digestion trials with sheep. These equations and correction factors were used with composition data for fecal and forage samples from steers on the range to calculate digestibility under grazing conditions.

The procedures most commonly used for estimating digestibility of the diet selected by grazing cattle are the fecal nitrogen index (fecal N) method in Australia and Great Britain (Langlands, 1967a, b; Holmes et al., 1961) and the lignin ratio method in the United States (Van Dyne, 1969). In the present study, results obtained by the above methods were compared to those obtained when the diet chosen by cattle grazing eastern Colorado sandhill range was collected via esophageal fistulae and fed to

sheep in conventional digestion trials. The main emphasis of the study was to evaluate sources of error in indirect methods of determining digestibility of forage consumed by grazing animals. Results reported in the present paper are part of a broad study in range nutrition; other phases of the work are reported by Wallace and Denham (1970) and Wallace et al. (1970) while details on procedures are given by Free (1969) and Wallace (1969).

## Consideration of Methods

Two recent reviews are available concerning methods of estimating digestibility of grazed forage (Van Dyne, 1969; Streeter, 1969) so we will consider here only aspects specifically related to this experiment.

## Fecal Nitrogen Index Method

The fecal N method entails harvesting herbage for conventional digestion trials. A regression is established between digestibility and percent N in the feces. Fecal samples from animals grazing on the pasture in question are analyzed for N and the resultant percentage fit into the equation established in the conventional trial. The method does not require sampling of the grazed forage (an obvious advantage over ratio procedures), but it serves as an estimate of digestibility by groups of animals rather than individuals (Van Dyne and Meyer, 1964). A simplified, hypothetical example of how the method is utilized is shown below:

- (i) A regression analysis relating dry matter digestibility (Y) to percent fecal N (X) is conducted on results of feeding harvested pasture herbage in conventional trials which yields the equation

$$Y = bX + a \quad [1]$$

- (ii) If we assume, for example,  $b = 17$  and  $a = 35$  and N is 1.3% for feces of animals grazing on the pasture we use equation [1] to get

$$Y = 17 (1.3) + 35$$

and dry matter digestibility by the animals at pasture is found to be 57%.

The equations (e.g., as in (i) above) usually are developed on a rather broad base of feeds (e.g., Raymond et al. (1954) used 40 different herbage representing a wide range of feeds). For this reason such equations often have only limited value for a specific herbage at a particular stage of maturity. The assumptions behind the fecal nitrogen index

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Table 1. Apparent digestibility of lignin in various forages, by different classes of animals, and as determined by different methods of lignin analysis.

Reference	Diet	Description or Stage of Maturity	Class of Animal	Method of Lignin Analysis <sup>1/</sup>	Apparent Digestibility of Lignin <sup>2/</sup>
Archibald et al. (1961)	Alfalfa	2nd cutting	Cattle	F	14
	Timothy	1st crop	Cattle	F	10
Bondi and Meyer (1943)	Various grasses	Immature	Sheep	D	64 to 35
Davis et al. (1947)	Bean and pea vines	Dehydrated	Sheep	B	16 to 11
Ely et al. (1953)	Orchardgrass	Immature to mature	Cattle	C	16 to .4
Forbes and Garrigus (1950)	Different forages	Mostly introd. grasses	Cattle & sheep	C	11 to -27
Guibert and Goss (1944)	Annual range species	Mature	Sheep	A	24 to -20
Hale et al. (1940)	Alfalfa	Unspecified	Cattle	A	17
Lancaster (1943)	Various feedstuffs	Varied	Sheep	E	32 to -40
Louw (1941)	Veld grass mixture	1 to 4 months growth	Sheep	E	24 to 12
McCann (1967)	Alfalfa	No. 1 grade	Cattle	G	12
Miller et al. (1954)	Ladino clover	Immature	Rabbits	C	26
	Tall fescue	Immature	Rabbits	C	6
Smith et al. (1956)	Several browse species	Varied	Deer	C	42 to -7
Sullivan (1955)	Different grasses	Immature to mature	Sheep	C	17 to -3
Troelsen and Campbell (1959)	Different introd. species	Late bloom to early seed	Sheep	C	9 to -2
Waite et al. (1964)	Ryegrass	Immature to mature	Sheep	C	42 to 0
Watkins (1955)	Different native & introd. spp.	Immature to weathered	Sheep	C	24 to -17
Van Dyne (1963)	Alfalfa	Pelleted	Cattle	C	18
	Alfalfa	Pelleted	Sheep	C	8

<sup>1/</sup> A = Crampton and Maynard (1938), B = Davis and Miller (1939), C = Ellis et al. (1946), D = Kalb (1932), E = Norman and Jenkins (1934), F = Sullivan (1959), and G = Van Soest (1963).

<sup>2/</sup> Ranges given with varied stages of maturity reflect digestibility values in youngest and oldest stages of maturity, respectively.

method are (1) the herbage fed to the animal is similar to that grazed by the animal, and (2) the penned and grazing animals digest herbage to the same degree. The regressions have been found to vary between first growth and aftermath herbage (Greenhalgh and Corbett, 1960), for leaf and stem components of the herbage (Lambourne and Reardon, 1962), from year to year (Topps, 1962), and for different varieties of the same herbage species (Minson and Milford, 1967). Still the technique has given useful results even when a general rather than specific regression was used (Holmes et al., 1961; Langlands, 1967b) and when cattle and sheep are used interchangeably in the hand-fed and pasture trials (Holmes et al., 1961; Topps, 1962; Langlands et al., 1963).

Australians use the fecal N method under pasture conditions (Arnold and Dudzinski, 1967a,b; Langlands, 1967a,b; McManus et al., 1967) much more than do North American workers.

### Lignin Ratio Technique

If one assumes lignin, a naturally-occurring plant constituent, to be indigestible, it may be used as an indicator:

% digestibility of a nutrient =

$$100 - 100 \left[ \frac{\% \text{ indicator in food consumed}}{\% \text{ indicator in feces}} \cdot \frac{\% \text{ nutrient in feces}}{\% \text{ nutrient in food consumed}} \right] \quad [2]$$

If the indicator is digestible the equation must be modified for incomplete recovery (Harris, 1968). Although the degree of digestibility of lignin may vary widely (Table 1), it has been used as an indigestible indicator in many North American range and pasture studies, especially by Utah workers (Cook and Harris, 1951, 1968 a,b). It is generally agreed lignin is more digestible in young plants (Table 1 and Bondi

and Meyer, 1943). Lignin is not a distinct chemical entity; it presents numerous analytical problems (Van Soest, 1963); it varies in chemical nature among plant groups (Towers and Gibbs, 1953), and among parts of the same plant (Stafford, 1962). Protein in plant tissue may interfere with lignin determinations (Hungate, 1966), and lignin may be altered in chemical composition as it passes down the alimentary tract (Bondi and Meyer, 1943; Kane et al., 1953), after ingesta leaves the rumen (Hale et al., 1940), or it may be altered by rumen microbial attack (Pazur and Delong, 1948). Lignin may even undergo changes during the mastication-ensalivation process (Heinemann and Evans, 1966; Connor et al., 1963).

### Experimental Procedure

Forage samples were collected morning and evening from 8 esophageal-fistulated steers grazing eastern Colorado sand-hill range over 5-day periods in June, July, September, and December of 1967. About 55 kg of forage collected each period was later fed to 4 sheep in conventional digestion trials. Fecal grab samples were taken once daily from each steer and composited into 24 steer  $\times$  period groups; forage samples were composited over steers by days. Steers were fed a protein supplement in December and no fecal samples were collected. Lignin and N analyses of steer fecal samples were made to enable indirect calculation of forage digestibility using the data obtained in conventional trials with sheep. The number of observations for each calculation was 12, i.e., grazed forage from three sampling periods fed to four sheep.

Details regarding these experimental conditions and analytical procedures are reported by Wallace and Denham (1970). Ether extract determinations were made but because of their well known variability they are not included in this paper.

**Table 2.** Number of sheep required in conventional digestion trials for estimating various digestion coefficients of cattle-grazed forages within 5% of the mean with 95% confidence, for 3 dates of collection.

Component digested by sheep	Dates		
	June	July	Sept.
Dry matter	1	1	8
Organic matter	1	3	9
Acid-detergent fiber	1	2	7
Gross energy	1	2	14
Crude protein	1	9	111

## Results and Discussion

### Sampling Reliability: Fecal N Method

Accuracy and precision of results obtained using the fecal N method depend upon animal-to-animal variability (1) in digestive ability in the conventional trials, (2) in fecal N concentrations in field trials, and (3) the predictability of digestion coefficients from fecal N in the conventional trials. For (1) and (2) calculations were made of the number of animals required for providing reasonable assurance ( $P \leq .05$ ) that sample means were within 5 or 10% of the true means (Steel and Torrie, 1960). In the conventional digestion trial the calculated number exceeded the actual number of sheep (four) for protein in the July diet and for all components in the September diet (Table 2). There was more variation among sheep in their ability to digest the September diet compared to June and July diets (Wallace and Denham, 1970). Van Dyne and Lofgreen (1964) found that either four wethers or two steers were sufficient in drylots to estimate most digestion coefficients within 10% of the mean with 95% confidence.

Although the fecal N method is generally used only for estimating the digestibility of dry matter or organic matter, we developed regression equations for each component studied (Table 3). More than 80% of the variability in digestion coefficients could be predicted from fecal N.

Fecal N decreased significantly ( $P < .01$ ) between

**Table 3.** Relation of fecal nitrogen to digestibility of various forage components established by conventional digestion trials with sheep fed three cattle-grazed forages.

Component	Regression equation <sup>1</sup>	Correlation Coef.
Dry matter	$Y = 19X + 31$	0.95**
Organic matter	$Y = 19X + 35$	0.94**
Fiber	$Y = 15X + 36$	0.90**
Gross energy	$Y = 21X + 27$	0.94**
Protein	$Y = 36X - 4$	0.91**

<sup>1</sup> Where X is % fecal N, Y is the digestion coefficient, n for each equation was 12, and \*\* means  $P < 0.01$ .

**Table 4.** Number of grazing steers required for sampling fecal nitrogen and lignin within 5% and 10% of the mean with 95% confidence during each summer sampling period.

Component	Percent of mean	Period		
		June	July	Sept.
Nitrogen	5	6	3	11
	10	2	1	3
Lignin	5	11	18	2
	10	3	4	1

sampling dates through the summer. Digestibility of all components in the cattle diets decreased markedly from June to September (Wallace and Denham, 1970). Thus, strong positive relationships between fecal N and various digestibility values were natural in this case.

Means (and standard deviations in parentheses) for the steer fecal N percentages were 1.98 (.09), 1.56 (.06), and 1.19 (.07) for the June, July, and September periods, respectively. These values were essentially the same as those found in feces from sheep fed cattle diets representing these same periods (Wallace, 1969). The number of steers needed to determine fecal N values within 5% or 10% of the true mean with 95% confidence was calculated (Table 4). Since 8 steers were used to sample fecal N at pasture, the required number for estimating within 5% of the mean were met in June and July but not in September. More steers were used than was necessary to estimate sample means of these fecal components within 10% of the true mean.

### Sampling Reliability: Lignin Ratio Method

The animal-to-animal and day-to-day variability in concentrations of nutrients and lignin in (1) the diet and (2) the feces influence the precision of the lignin ratio method. Generally more steers were needed on the range to sample fecal lignin than fecal N (Table 4). Fecal N and lignin means were estimated within 10% for all three summer periods. Estimates of the number of days required to sample various forage components within 5% of the mean with 95% confidence indicate that the number of days used for sampling were reasonable (Table 5).

**Table 5.** Number of days required using eight esophageal-fistulated steers to sample forage components within 5% of the mean with 95% confidence during 3 periods.

Forage component	Period		
	June	July	Sept.
Protein	6	7	3
Fiber	2	1	1
Lignin	2	1	5

**Table 6.** Apparent digestion coefficients (%) for lignin in range forage determined in conventional digestion trials with sheep.

Dates cattle diets collected	Forage crude protein (%)	Forage lignin (%)	Apparent digestion coefficient <sup>1</sup>
June	15.3	5.0	46 (1.0)
July	10.4	6.2	42 (1.6)
Sept.	6.3	8.2	29 (2.6)
Dec.	4.1	9.0	4 (3.0)

<sup>1</sup> Apparent digestion coefficients are the mean of 4 sheep shown with standard deviations in parentheses. Forage lignin and crude protein are expressed on an organic matter basis.

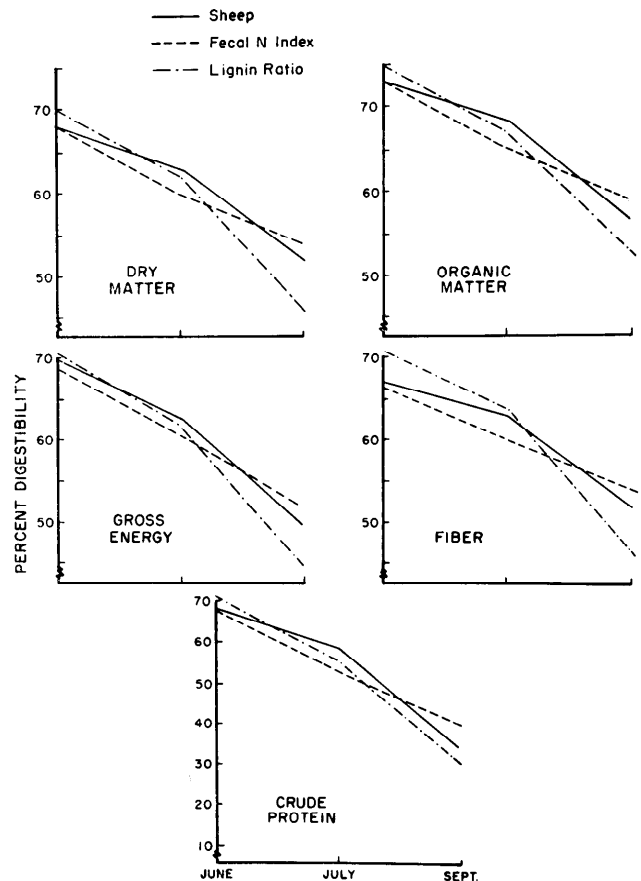
More grazing steers are required to sample fecal N or lignin than are required of sheep in digestion rates to measure digestibility at the same level of precision. These results agree with those of Van Dyne and Lofgreen (1964) who found variability among animals on dry, annual range was 50% greater than those fed alfalfa pellets in drylot.

#### Apparent Digestibility of Lignin

The lignin ratio method was proposed on the assumption that lignin is indigestible. An investigator using this method under field conditions ordinarily has no way of knowing whether or not the lignin is being recovered completely. Since the same forage grazed by steers was fed in conventional trials to sheep, an excellent opportunity was provided to determine apparent digestibility of lignin for each sampling period.

Apparent lignin digestion coefficients varied from 46% down to 4% for June, July, September, and December cattle diets as measured with sheep in digestion crates (Table 6). The decreasing lignin digestibility with advancing plant maturity in the cattle diets agrees with that found for plants harvested at varying growth stages (see Table 1: Ely et al., 1953; Sullivan, 1955; Waite et al., 1964). Waite et al. (1964) reported apparent lignin digestion coefficients of 42 to 0% for ryegrass ranging from a young, leafy stage to the seed-setting stage.

The digestion coefficients in Table 6 were used to correct lignin values in steer fecal samples for each of the summer periods. The usual assumption was made that digestibility of lignin is the same by steers and sheep. Corrected and uncorrected fecal lignin values from the grazing steers were used to estimate dry matter digestibility of summer diets by the ratio procedure. Dry matter digestion coefficients, calculated on the usual assumption that lignin was indigestible (using uncorrected fecal lignin values), were about 30% below those found in sheep trials for all summer diets. Alternately, digestion coefficients calculated with steer fecal



**Fig. 1.** Digestibility of various components in summer cattle diets by a direct procedure with sheep, by indirect fecal nitrogen index method, and by indirect adjusted lignin ratio procedure.

values adjusted for lignin digestion agreed well with those from the sheep trials.

Lignin digestibility may have been influenced by the procedure used for lignin analyses (potassium (K) permanganate). We found no published reports of lignin digestibility where this procedure was used for lignin analyses. It is possible that lignin was not digested but that the method was incapable of accounting for all fecal lignin. However, this is probably true for all lignin procedures as lignin digestibility has been found with all other methods for lignin analysis advanced to date (Table 1). Bement (1968) used K permanganate lignin values to estimate digestion by steers on blue grama range. His data show very low digestibility of immature herbage suggesting that lignin was digested to about the same extent as in the present study, but lignin recovery was not measured in Bement's work. Conversely, Stenquist (1968) used K permanganate lignin values in a ratio procedure to estimate digestibility of a high concentrate ration by cattle and obtained results that were very close to those obtained on similar rations in conventional trials.

### Comparison of Direct and Indirect Estimates of Cattle Diet Digestibility

Two factors contribute to the similarity of digestion coefficients of cattle diets estimated by direct and indirect methods (Fig. 1): (1) regression equations used in the fecal N index method were developed in conventional trials with sheep fed the forage selected by steers at pasture; and (2) lignin values for steer feces were adjusted for digestibility of lignin as determined in sheep trials. Note, too, that the data in Fig. 1 are not to display the time trend in digestibility, but simply to compare among estimation techniques. Digestion coefficients estimated by the lignin ratio with steers were about equal to those estimated with sheep or with the nitrogen fecal index for June and July diets. But lignin ratio method digestion coefficient estimates for September were appreciably lower than those obtained by the other two techniques. However, no statistical test is available for a period-by-method interaction because only one estimate of digestion coefficients is available with the lignin or nitrogen methods.

Digestibility values for most components estimated by the fecal N method were closer to conventional results for June and September diets than those derived by the lignin ratio procedure. The reverse was true on the July diet for all components. The greatest differences between conventional results and those attained by lignin ratio were noted on the September diet. This may reflect a small difference in apparent lignin digestibility between cattle and sheep on more mature forage. Other differences among methods in Fig. 1 may indicate that forage samples fed to sheep were not exactly the same as total forage grazed by steers.

Either indirect method can provide fairly reliable estimates of digestibility if adequate precautions are taken. First, adequate numbers of animals must be used to sample forage and fecal components. With the fecal N method, diets of animals used to establish regression equations should be as similar as possible to diets of pasture animals (Langlands, 1967b). Where the lignin ratio procedure is used, some measure of apparent lignin digestibility is necessary, particularly with immature forages. In our study the fecal N method provided greater overall accuracy than the adjusted lignin ratio procedure when compared to results of conventional trials.

#### Precision of Indirect Estimates of Digestion Coefficients

No direct measure of variability of digestion coefficients is available for the steers, whereas such measures are available for the sheep. By the lignin ratio and fecal nitrogen index procedures, only one estimate of the digestibility of the forage is available for each period. An indirect measure of precision

could be calculated using probabilistic methods, similar to Van Dyne's (1965) calculation of forage intake variances. Alternately, approximate Taylor-expansion methods could be used to estimate variances for the items computed by equations [1] and [2]. In either procedure of estimating variances of the left-hand sides of equations [1] and [2], one must have estimates of the variance-covariance relationships for all the parameters in the equations. Examinations of the applications of these analytical procedures to range nutrition problems are much needed but are beyond the scope of this paper.

#### Summary

Grazed forage was collected from eight esophageal-fistulated steers on sandhill range over 5-day periods in June, July, September, and December. The grazed forage later was fed to sheep in conventional digestion trials. The degree of lignin digestibility and the regression equations for the fecal N method were established in the sheep digestion trials. Fecal grab samples were taken daily from the steers during each sampling period and used to estimate the digestibility of forage components by the fecal N method and by the lignin ratio procedure.

More steers were required to sample fecal lignin than fecal N. With 8 steers and twice daily sampling, from 1 to 7 days would be required to sample dietary protein, fiber, and lignin within 5% of the mean with 95% confidence. Fewer sheep were required to measure digestibility of cattle-grazed forage conventionally as compared to the number of steers at pasture to indirectly estimate digestibility by either the fecal N or lignin ratio method.

Digestibility values estimated by the fecal N method were in close agreement with those found in the conventional trials. The usual lignin ratio method gave invalid digestibility results; but good results were obtained by correcting steer fecal lignin values for apparent digestibility of lignin as found in the sheep trials. Apparent lignin digestion was 46, 42, 29, and 4% on the June, July, September, and December diets, respectively. Digestibility estimated by the fecal N method was generally closer to conventional results than were values derived by the adjusted lignin ratio.

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