

# Comparison of Tropical Forages of Known Composition with Samples of These Forages Collected by Esophageal Fistulated Animals<sup>1</sup>

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## Highlight

The effect of saliva on esophageal fistula samples was minimal. Regression equations are presented which compare crude protein and crude fiber contents of esophageal fistula samples with those of forage of known composition. Squeezing esophageal fistula samples, air drying the solid portion, and treating the liquid portion with phenol will preserve samples where laboratory facilities are not available.

Most previous studies with esophageal fistulas (Van Dyne and Torell, 1964) have been conducted in the temperate zone with sheep and British-type cattle. The principal breed of cattle in Uganda is the Zebu which has been indigenous for several centuries. Since saliva is the major contaminant in all esophageal fistula collections, its effect on tropical forage consumed by Zebu cattle was studied. As most field sampling was carried out at sites remote from laboratory facilities it was necessary to develop a method of sample preservation suitable for field use.

## Methods and Procedures

Esophageal fistulas were installed in six mature Zebu steers using the method described by Van Dyne and Torell (1964) and plugs as shown in their Fig. 3-C. Collection bags similar to their Fig. 2 but without drainage were used. The animals were accustomed to stall feeding but not always to the particular grasses used. To insure collection of a sufficiently large sample without contamination with ruminal contents the steers were not allowed feed for 18 hr prior to the trial. Collections were limited to less than one hr to avoid possible changes in the sample and excessive contamination with saliva.

Comparisons of chemical composition of samples obtained from fistulas with that of herbage offered were carried out on various freshly-cut grasses and sun-cured hays fed to steers kept in stalls. Dry matter, crude protein, crude fiber, and ash were determined on what was offered and the fistula sample.

Dry matter of sample was determined at 105 C. Crude protein was determined by the semi-macro Kjeldahl method. Crude fiber was determined by the method prescribed in the Fertilizers and Feeding Stuffs Act (1926). A correction factor as suggested by Todd (1961) and confirmed by Dougall (1955) and Bredon and Juko (1961) was used to compensate for lower boiling point due to altitude. Ash was determined by dry ashing.

Several sampling methods

were used on the fistula collections. The first method was to remove from a 4,800 g sample eight subsamples of equal weight while the sample was being stirred. A second method involved separating the liquid (plant and saliva) from the plant portion by squeezing through a coarsely woven cotton cloth. The plant portion was then divided into seven 300 g subsamples. Dry matter and crude protein were determined on each subsample to determine consistency.

Samples of the liquid portion were divided into seven parts and crude protein and dry matter were determined on one portion immediately. Five samples were stored at 4 C. for seven days with no preservative, with 20% ethyl alcohol, with 0.5% chloroform, with 0.5% toluene, or with 5% formalin. The seventh sample from each animal (3) was left at room temperature for seven days. A second set of samples was divided in half, the first portion treated with 1% phenol and kept at room temperature for seven days, the second half analyzed immediately for crude protein and dry matter.

Comparisons were made between herbages of a known chemical composition and esophageal fistula samples of four *Chloris gayana* hays, freshly cut *Chloris gayana*, three freshly cut and chopped *Pennisetum purpureum*, *Themeda triandra* hay, *Hyparrhenia dissoluta* hay, and freshly cut and chopped *Paspalum notatum*. Twenty-nine observations were made on these 11 forages.

Saliva samples were collected both before and after feeding in plastic bags that lined the collection bag. Dry matter, nitrogen, and ash were determined immediately.

The esophageal fistula samples were squeezed. The solid portion was oven dried and the liquid portion was analyzed immediately.

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## Results

**Techniques and Sampling.**—When fistula subsamples were taken from a whole sample by extracting portions, the dry matter content decreased from 12.0% on the first to 10.4% on the eighth while crude protein increased from 7.7% to 8.7% on a dry matter basis.

The second method of subsampling, after expressing the liquid portion, was more consistent with dry matter at 27.6% S.D. = 0.49, crude protein at 7.3% S.D. = 0.039, and crude fiber at 37.6% S.D. = 0.836. Considering the degree of accuracy in chemical determinations this method of sampling proved to be satisfactory.

The liquid sample left at room temperature became mouldy and was discarded. There were no differences between samples processed immediately and those stored at 4 C. for seven days irrespective of preservative used or with no preservative. A subsequent processing also proved objectionable in the presence of chloroform, toluene, and formalin. Ethyl alcohol in the concentration used precipitates the protein in the liquid. One % phenol in unrefrigerated liquid preserved the samples with no significant difference in dry matter and crude protein from those processed immediately.

**Herbage Offered vs. Fistula Sample.**—Percentages of crude protein and crude fiber were used for comparison of chemical composition of feed offered with samples obtained from the fistula. To calculate the composition of the whole sample, the total amount of crude protein and dry matter in the liquid portion was added to that in the solid portion. The percent crude protein content was calculated from the resulting figures on a dry-matter basis.

Crude fiber in the liquid is neg-

Table 1. Saliva composition (percent) quoted by various workers.

Reference	Description	Dry Matter	Nitrogen	Crude protein	Total Ash
Present work	Cattle	1.0362	0.0145	8.74	0.8233
McDougall (1948)	Sheep	1.28	0.020	9.77	0.97
Somers (1958)	Sheep	1.1064	0.03183	17.98	0.6677
Bailey, et al. (1961)	Steer	1.05	0.00757	4.51	0.91
	Cow	1.02	0.010	6.13	0.89

ligible, and the crude fiber comparisons are on the solid portion only.

Since saliva is approximately 80% ash on dry-matter basis (Table 1) and the amount of saliva excreted varied greatly between animals, the crude protein and fiber results are on an ash-free basis.

**Crude Protein.**—The crude protein content of the herbage used ranged from 3.0% to 7.8% for the hays and 8.5% to 11.1% for the freshly-cut green grasses. Since the crude protein percentages do not overlap, the regression equations are calculated for both hay and green grasses; however, they are also calculated together since under most grazing situations there will be both dried and green grass available and eaten by the animal.

Fig. 1 presents linear regression equations when the esophageal fistula samples have been squeezed and only the solid or fiber portion used.

Linear regression equations in Fig. 2 represent calculations combining liquid and solid portions. Where the solid sample is used, both saliva and some of the plant juices are removed in the squeezing process. This is especially true with green grass; however, the saliva-saturated hay will also lose some of the nutrients. Since crude protein content of the fistula sample is less than is fed, comparatively more nitrogen compounds have been removed than non-nitrogen compounds.

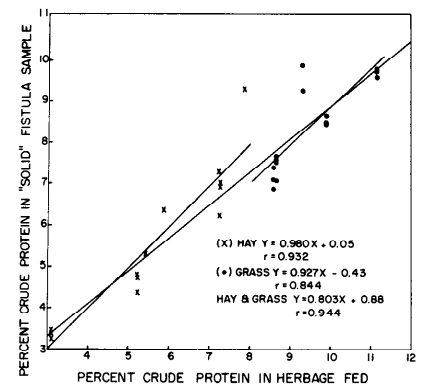


FIG. 1. Regression of crude protein of herbage fed on crude protein of "solid" portion of forage collected through esophageal fistula (Ash-free basis).

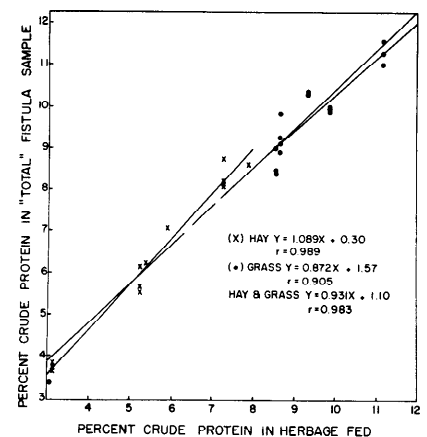


FIG. 2. Regression of crude protein of herbage fed on crude protein of "total" forage collected through esophageal fistula (Ash-free basis).

In contrast, when the squeezed liquid portion is added to the solid portion, the esophageal fistula sample is higher in crude protein than the herbage fed. This is true for both hay and green grass, and the differences are highly significant (t-test for paired comparisons).

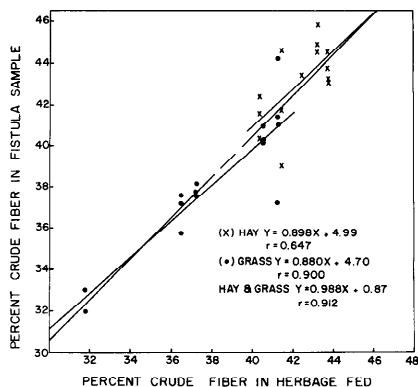


FIG. 3. Regression of crude fiber of herbage fed on crude fiber of forage collected through esophageal fistula (Ash-free basis).

**Crude Fiber.**—Fig. 3 presents crude fiber regression equations for hay and green grass and a combination of the two. The coefficient of linear correlation ( $r$ ) for hay was low ( $r=0.65$ ); however, crude fiber values for hay range from 40.4% to 43.8% as compared with green grass values of 31.8% to 41.3%, a much wider range.

Esophageal fistula samples contained more crude fiber than the animals were offered. This difference is explained under "Saliva Yield."

In some cases under limited laboratory facilities it might be advantageous to use a regression based only on dry matter and crude protein or crude fiber.

Regressions based on the whole sample are as follows:

% C.P. in herbage = 1.008  
 (% C.P. solid + liquid portion) - 0.677;  $r = 0.984$

% C.P. in herbage = 1.107  
 (% C.P. solid only) - 0.200;  
 $r = 0.972$

% C.F. in herbage = 0.873  
 (% C.F.) + 3.920;  $r = 0.907$

**Saliva Composition.**—There is considerable literature referring to yield and composition of saliva; however, most references are for work with sheep and suggest that the small quantity of nitrogen in saliva would have little influence on samples collected from esophageal fistulas.

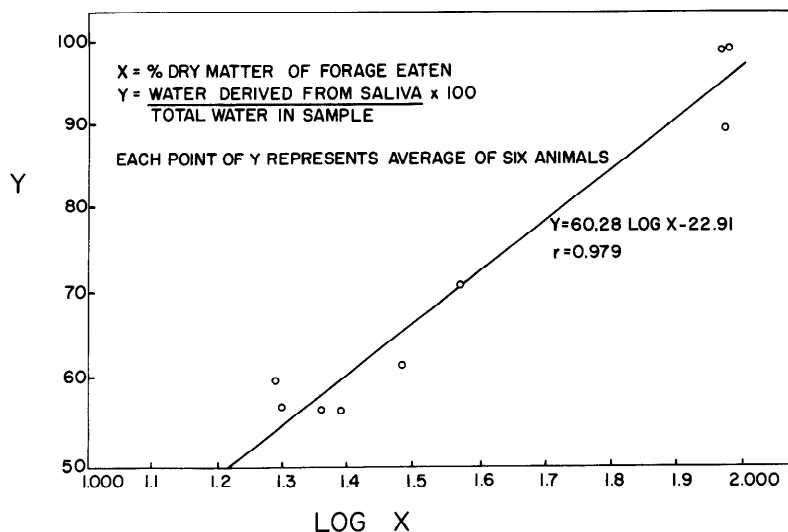


FIG. 4. Regression of yield of saliva on dry matter of forage consumed.

Results of this study are presented with results from other workers in Table 1. Considerable variations in values are obtained by various authors. Dry matter values tend to fall in the lower range in this study. Influence of saliva on chemical composition of samples from the fistula would depend on the ratio of saliva to forage as well as percent nitrogen on a dry-matter basis. If the nitrogen shown in Table 1 were divided by the dry matter and then multiplied by 6.25 to put it on a crude protein basis, the crude protein values would range from 4.5% to 18.0%. Saliva in this experiment contained 8.7% protein.

If the herbage in the fistula sample had a crude protein content less than 8.7%, saliva would be adding nitrogen to the fistula sample. In herbage with higher crude protein content, saliva would be adding more dry matter than nitrogen. The result then is less crude protein in the fistula sample. The further the crude protein value of the herbage sample gets from the saliva value the greater will be the influence to the fistula sample. This is substantiated by Shumway et al. (1963) who showed that alfalfa hay before consumption was 18.0% crude protein and

after 17.1%, whereas cottonseed hulls were 4.7% crude protein before and 5.2% after consumption.

**Saliva Yield.**—The influence of saliva is also correlated with the amount of saliva produced per weight of herbage consumed. Since the water content of the herbage of this experiment was known and the total sample was collected, the saliva yield was determined.

It was found that the yield of saliva is related to the dry matter of the forage fed and the relationship may be expressed by the equation in Fig. 4 ( $Y = 60.28 \text{ LOG } X - 22.91$ ).

The dry matter of forage eaten varied between 94.5% and 19.2% and, as expected, hay samples contained more saliva than did green grasses.

Using this equation, the amount of saliva and the amount of added nitrogen to the esophageal fistula sample can be determined. For example, the means for the hays contained 5.6% crude protein and 90% dry matter. For every 90 g of hay in the fistula sample there were 10 g free water and 190 g saliva ( $100X \div [10 + X] = 95$ ; then  $X = 190$ ). The crude protein content of the saliva was approximately 0.001% so the crude pro-

tein in the fistula sample would be  $90 (.0563) + 190 (0.001) = 5.26$  g and the dry matter would be  $90 (100.0) + 190 (.01) = 91.9$  g. The g crude protein  $\div$  g dry matter  $\times 100 =$  percent crude protein fistula sample ( $5.26 \times 100 \div 91.9 = 5.72$ ). The forage fed contained 5.63% crude protein. Addition of saliva increased the percentage to 5.72, then the error created by contamination with saliva was 1.6% ( $[5.72 - 5.63] \div 5.63 = .016$ ).

A similar calculation for green grass with an average of 9.47% crude protein and 20% dry matter will show an error of 0.3% due to added saliva. Because the grass was green less saliva was produced and percent crude protein in the grass and in the saliva was nearly the same.

McManus (1961) and Nelson (1962) suggested that the chemical composition of esophageal fistula samples be expressed on an ash-free basis. This suggestion is desirable for all constituents except crude protein since saliva contains mainly nitrogen and minerals. Using the previous calculations for saliva error in crude protein, if results are on an ash-free basis, the 5.26 g protein would be divided by 90 g of dry matter or a loss of 1.9 grams of saliva ash. This calculation shows a crude protein content of 5.85%. The error due to the saliva is 3.9% ( $[5.85 \div 5.63] \div 5.63 = .039$ ) compared with an error of 1.6%

on a non-ash-free basis. This error is still small so it would be more desirable to use ash-free basis rather than the more complicated costly silica-free method.

Most of the difference between the crude protein in forage fed and fistula sample must be due to factors other than saliva contamination.

#### Summary

Six mature Zebu steers were fitted with esophageal fistulas and were stall fed herbage of varying quality to enable the sample collected to be compared chemically with forage fed.

Various techniques were tried and those adopted for use under local conditions are described in detail.

Regression equations were calculated which compare crude protein and fiber content of samples collected from fistulas with those of forage eaten.

Figures present the composition of saliva before and after feeding and an equation correlates yield of saliva to the dry matter of forage eaten.

Possible reasons for the differences between the herbage fed and the forage collected are discussed.

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