DEVELOPMENT OF TECHNIQUES FOR EVALUATING GRAZED FORAGE

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SUMMARY

Techniques were developed to sample grazed forage with steers, using both esophageal and rumen fistulas. Comparisons between feeds of known composition and samples collected from these same feeds through fistulas indicated that some changes in composition occurred. These changes in composition of certain constituents were influenced by the type of feed and type of fistula. Regression equations for the various changes were calculated. The relative amounts of organic constituents were modified by fistula sampling. The amount of protein and ether extract was the same in the fistula sample as the feed, but significant changes were noted in the amount of crude fiber, NFE, and energy. Fistula samples were highly contaminated with mineral matter. The composition and amount of saliva secreted while consuming a sample of forage was investigated, to explain some of the chemical changes noted in fistula samples. Repeated feed sampling on the same day with the same animal had little effect on the composition of fistula samples. Due to the high variation between feeds, and the generally high correlations between feed and fistula samples, it was felt that the fistula sample represents the best estimate of the foraging animal's diet until adequate comparisons of grazed forage have been made. Fistulated animals were used as sampling agents on pasture. The fistula sample could be separated botanically, between grasses and broad-leaved plants.

The importance of grazing land in American agriculture is well established. It is desirable to obtain suitable methods by which the nutritive value of various range and pasture forages can be determined. A number of systems have been developed to determine the quality and quantity of the grazing animal's diet, but much of the work was based on unproved assumptions or other means of estimating the animal's grazing habits. The biases that occurred and consequent errors might not have been measurable if grazing animals were unable to select their diet. Several outstanding investigations have shown that animals selectively graze (3, 4, 5, 7, 8, 9). Many have felt that the only practical means of pasture and range forage evaluation is through the use of the animal as the sampling agent. The indicator method has gained wide acceptability in recent years as a method for determining both nutritive value and intake of grazed forage; however, a representative sample of the animal's diet can not be obtained with the use of the indicator method alone. Accurate forage sampling using the animal was accomplished by Torell (11), with the establishment of a successful esophageal fistula in a sheep. Recently (2) a method for establishing a cannula in esophageal-fistulated sheep for the enhancement of forage collection has been developed. Work by Bath (1) indicated that only ash appeared to be higher in fistula-collected samples when compared to feed samples. No changes were re-

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ported in other chemical constituents. Additional work (12) indicated that mineral contamination is very high in forage collected from esophageal-fistulated sheep.

The purpose of this study was to (1) develop suitable biological sampling techniques with steers, to further investigate the intake and nutritive value of grazed forages, and (2) make chemical comparisons between feeds of known composition and samples collected from these same feeds through fistulas.

EXPERIMENTAL PROCEDURE

Rumen fistulas were established in three yearling steers. The operation was similar to the two-phase method reported by Schalk and Amadon (10). Rumen fistulas were closed with a simple plug constructed with two pieces of masonite lined with foam rubber. One piece was placed inside the rumen and the other on the outside. Two or three smaller pieces of masonite, approximately the same size as the fistula, were used as fillers between the two larger plates and held in place with a 5/16-× 4-in. carriage bolt and wing nut. This system allowed the plug to be closed tightly enough to prevent most leakage, but not tightly enough to cause necrosis.

The following procedure was used to collect samples from rumen-fistulated animals: The animal was first restrained and the rumen plug removed; next, the entire contents from the rumen and reticulum were removed and placed in a suitable container. The sides of the rumen were cleaned by downward strokes with the hand. This step was essential to lessen contamination of the collected sample. After the rumen was evacuated, the animal was allowed to sample the forage for 30 to 45 min. At this time the animal was again restrained, the ingested sample removed, and the former rumen contents replaced in the rumen.

Esophageal-fistula cannulas were established in four steers. The operation was similar to that described by Cook et al. (2) for sheep. After the animal was anesthetized with a general anesthesia, an incision was made through the skin and subcutaneous fascia in the upper one-half of the cervical region, and then between the left margin of the sterno-hyoid muscle and the right margin of the sterno-cephalicus muscle. Passing a stomach tube into the rumen prior to the operation aided in locating the esophagus. An incision somewhat shorter than the length of the flanged end of the cannula was made in the esophagus and the cannula was then inserted into the esophageal lumen. The tissue was closed tightly around the cannula by suturing the anterior and posterior ends of the incision in the esophagus with No. 2 catgut. Next, the subcutaneous tissue was sutured tightly, which aided in securing the cannula. The incision through the skin was then closed with Vetafil\(^*\) and dusted with sulfanilamide. Following the operation the animals were maintained on pelleted feed to facilitate healing.

The first cannula used in an esophageal fistula consisted of a modified ‘‘T’’ made from plastic pipe. The inside diameter was 1½ in. and the flanged end was

\(^*\) Vetafil is a heavy-type suture (0.40-mm. diameter) manufactured by Fort Dodge Laboratories, Fort Dodge, Iowa.
approximately 5 in. long. The cannula was closed with a rubber stopper held in place with a water hose clamp. Cannulas used in a later operation were of a larger size. These were modified from a "T" made of a plastic material with an inside diameter of 2 in. and a flanged end 5 1/2 in. long. This material was ground to a thickness of approximately 1/8 in. to reduce weight and add flexibility. Closure of the cannula was accomplished with a screw cap. A two-piece esophageal fistula plug (11) was used to close fistulas upon removal of the cannula from certain animals.

Esophageal fistula collections were made simply by removing the screw cap, or plug, depending on the type, and placing a collection sack around the neck. The collection sack, with a capacity of approximately 3 gal., was constructed of rubberized canvas and held in place with a strap around the top of the neck. Time required for collection of samples from esophageal-fistulated animals was similar to rumen-fistulated animals.

Comparisons between feeds of a known composition and fistula samples of these feeds were made. Alfalfa-concentrate pellets, alfalfa hay, and grass hay were tested on both types of fistulas. A limited number of grass and alfalfa samples was fed in the succulent stages. The sample of feed to be tested was divided into two parts with one-half used as the control sample and the other half fed to the animal and collected through the fistula.

In a later study, alfalfa hay was used to determine both the effect of fistula sampling and the effect of consecutive sampling within a comparatively short time period on the chemical composition of the fistula-collected sample. A two by four factorial design with three replications was employed for this study. Three animals with each type of fistula were compared on four consecutive sampling periods with a time interval of 1.5 hr. between sampling periods. Rumen contents were withheld from the rumen-fistulated animals for the entire period. Animals were not allowed access to water during the entire sampling period.

In a related study, the amount of salivary contamination in rumen-fistula samples was determined with several levels of grass hay. Salivary secretion was determined by feeding a known quantity of hay which was recovered from the rumen along with the saliva secreted. The difference between the initial weight and the final weight was recorded as the amount of saliva secreted while consuming the sample. The experimental design for this study was a three by three factorial with three replications. Three different initial weights of hay were used with three animals. During this study, limited numbers of saliva samples were collected for chemical analysis. A 400-ml beaker was placed under the cardia in the anterior portion of the rumen. Several types of feed were presented to the animal, but he was not allowed to sample them in an attempt to stimulate the secretion of saliva comparable to that secreted when actually consuming forage.

4 Vuleathene "T's" (No. T34) were purchased from American Vuleathene, Division of the Nolge Co., Inc., Rochester, New York.
RESULTS AND DISCUSSION

Rumen-fistula techniques used in this study were satisfactory. However, esophageal fistulas were not entirely successful. The first cannula, 1.5 in. inside diameter, was successfully established but proved too small for a bolus to fall through. The cannula was removed and a plug was fitted to the fistula. The animal was maintained on pasture for 3 mo. under these conditions. On a grass hay diet, the animal developed trouble in swallowing. Several times the esophagus became compacted with food and the fistula opening gradually became larger. The animal could not be maintained under these conditions. The larger cannula was established in the next two operations. The cannula was accidentally removed from one animal shortly after the operation. Failure to devise an adequate plug resulted in death 2 mo. later. The operation appeared successful on the second animal in this series, the wound healed, and little scar tissue developed. The animal was used successfully for 75 days; however, the anterior lip of the flanged end finally protruded through the esophagus and skin, and the animal was sacrificed. A later attempt to establish a more modified 2-in. "T" also failed.

Sixty-two comparisons of fistula and feed samples indicate that some changes occur with fistula sampling by steers. The change in amounts of certain constituents through fistula sampling is influenced by the type of feed and type of fistula (Table 1). The regression equations for the various changes were calculated with \( X \), the independent variable, representing the fistula sample, and \( Y \), the dependent variable, representing the usual feed sample. Through the application of these regression equations to the appropriate feeds and types of fistulas, it will be possible to predict the chemical composition of the foraging animal's diet. When the regression equation is not significant, but significant changes occurred by fistula sampling, the composition can be determined simply by adding the recorded change.

The amounts of certain organic constituents were modified by fistula sampling. In all cases, the amounts of ether extract and protein were the same in the fistula sample as the feed, but significant changes were noted in the amount of crude fiber, nitrogen-free extract (NFE), and energy.

The amount of crude fiber in fistula samples of grass hay and alfalfa-concentrate pellets increased, but NFE decreased. It is possible that soluble portions of NFE might be leached from the feed by the saliva and lost in sampling. If this effect occurred, it would explain the results obtained, although these soluble carbohydrates should be collected with the esophageal fistula. Esophageal fistula samples usually contained more NFE than rumen fistula samples, but this effect was statistically significant only when alfalfa hay was fed. The reason for the increase in crude fiber in fistula samples, using alfalfa-concentrate pellets and grass hay, is not clear. The approximate analysis adds up to 100%; therefore, a change in the value of one constituent should cause relative changes in the values of the others. Thus, indirectly, the decrease in NFE may account for some of the increase of crude fiber in fistula samples. NFE and crude fiber accounted for more than 60% of the composition of roughages. Over-all NFE decreased significantly less with alfalfa hay than it did on other feeds involved in this
### TABLE 1
A comparison of feed and fistula samples of the same feed

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Type of feed</th>
<th>Type of fistula</th>
<th>Feed-fistula sample*</th>
<th>Regression equation</th>
<th>Correlation value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude fiber</td>
<td>Grass hay, alfalfa-concentrate pellets</td>
<td>Both</td>
<td>-2.27%±0.68**</td>
<td>( \hat{Y} = 2.02+0.867X ) **</td>
<td>.929**</td>
</tr>
<tr>
<td></td>
<td>Alfalfa hay</td>
<td>Esophageal</td>
<td>+4.06%±1.77**</td>
<td>( \hat{Y} = 7.94+0.906X ) **</td>
<td>.621**</td>
</tr>
<tr>
<td>NFE</td>
<td>Grass hay, alfalfa-concentrate pellets</td>
<td>Both</td>
<td>+4.19%±0.91**</td>
<td>( \hat{Y} = 21.04+0.590X ) **</td>
<td>.615**</td>
</tr>
<tr>
<td></td>
<td>Alfalfa hay</td>
<td>Rumen</td>
<td>+1.53%±1.36%</td>
<td>( \hat{Y} = 17.39+0.614X ) **</td>
<td>.622**</td>
</tr>
<tr>
<td>Energy (kcal/gram)</td>
<td>Alfalfa hay</td>
<td>Rumen</td>
<td>+0.059 ± 0.036**</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>All types of feed</td>
<td>Esophageal</td>
<td>+0.077 ± 0.028**</td>
<td>( \hat{Y} = 1.509X-2.132 ) **</td>
<td>.851**</td>
</tr>
<tr>
<td>Energy (ash-free)</td>
<td>All types of feed</td>
<td>Both</td>
<td>-0.086 ± 0.022**</td>
<td>( \hat{Y} = 2.298+0.515X ) **</td>
<td>.556**</td>
</tr>
<tr>
<td>Ash</td>
<td>Grass hay, alfalfa-concentrate pellets, succulent alfalfa</td>
<td>Both</td>
<td>-2.02%±0.38**</td>
<td>( \hat{Y} = 2.22 + 6.28X ) **</td>
<td>.630**</td>
</tr>
<tr>
<td></td>
<td>Fibrous alfalfa hay</td>
<td>Both</td>
<td>-3.57%±0.43**</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>All types of feed</td>
<td>Rumen</td>
<td>-0.18%±0.03**</td>
<td>( \hat{Y} = 0.853X-0.110 ) **</td>
<td>.851**</td>
</tr>
<tr>
<td></td>
<td>All types of feed</td>
<td>Esophageal</td>
<td>-0.16%±0.02**</td>
<td>( \hat{Y} = 0.867X-0.054 ) **</td>
<td>.970**</td>
</tr>
</tbody>
</table>

* = Significant at \( P < .05 \).
** = Significant at \( P < .01 \).
NS = Not significant.

* Difference is absolute and is determined by subtracting composition of fistula sample from feed sample, i.e., a change of -2.27% indicates fistula sample contained 2.27% more of the constituent than did the feed sample. Confidence limits are at the 5% level.

\( X = \) Fistula sample. \( Y = \) Feed sample.
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study; in fact, NFE did not decrease on esophageal-fistula samples of this feed. The majority of the alfalfa used in this study was low in NFE because soluble constituents were leached by rain. Less NFE was available at the time of collection which, in turn, resulted in less difference between the feed and the fistula sample. The decrease in crude fiber in esophageal-fistula samples may be caused by incomplete collection. The more fibrous parts of the bolus may have continued down the esophagus while the less fibrous and smaller portions were collected. When fistulated cattle were fed coarse alfalfa hay, the esophageal-fistula samples were 5% higher in protein than the rumen-fistula samples. Since the protein is primarily in the leaves, these data would support the above hypothesis. This difference did not approach significance because of the large variation associated with this coarse feed. More data are needed to test this hypothesis.

Energy decreased in all cases in fistula samples, although it was not significant with rumen-fistula samples of grass hay or alfalfa-concentrate pellets. This decrease was small and apparently due to ash contamination. When energy was calculated on an ash-free basis, the fistula samples were significantly higher in all cases than the feed. If the fistula samples were corrected for the caloric content of the saliva, there probably would be no change of energy when calculated on an ash-free basis, and on a dry weight basis the decrease due to the dilution of organic matter with ash would be even greater. The caloric content of the saliva was not determined.

The most striking changes in chemical composition of fistula samples occurred in the mineral matter. Ash increased highly significantly in all cases on fistula samples. Closer examination of the data indicated that ash increased significantly more on coarse, fibrous alfalfa than it did on collections made with succulent alfalfa, grass hay, and alfalfa-concentrate pellets. Phosphorus also increased significantly (P < .01) on all fistula samples; however, the increase was significantly greater on rumen-fistula than on esophageal-fistula collections. Calcium increased slightly in fistula samples, but not significantly.

The effect of hay consumption on salivary secretion (Table 2) indicated that for the first pound of hay consumed, there were 4.5 lb. of saliva secreted and

<table>
<thead>
<tr>
<th>Pounds of hay consumed</th>
<th>1.00</th>
<th>2.00</th>
<th>3.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pounds of sample collected</td>
<td>5.59</td>
<td>10.10</td>
<td>14.75</td>
</tr>
<tr>
<td>Pounds of saliva secreted</td>
<td>4.49</td>
<td>8.10</td>
<td>11.75</td>
</tr>
</tbody>
</table>

for every pound thereafter, up to 3 lb., an additional 3.6 lb. of saliva were secreted. Other roughages may behave differently in their effect on salivary secretion.

Analysis of saliva (Table 3) indicated that the dry matter is mainly ash. Calcium was recorded in only trace amounts. McDougall (6) obtained similar values for the same constituents in sheep’s saliva. With the assumption that all tests were involved with approximately 2 lb. of feed, an animal would secrete 8.1
lb. of saliva, which would account for the increase in ash, phosphorus, and calcium contamination of the fistula sample.

Those fistula samples of coarse type feeds containing more ash may have resulted from greater salivary secretion. Although quantitative data were not available for evaluating the effect of different roughages on salivary secretion, it appears that both the amount and kind of roughage influence the secretion rate. Greater phosphorus contamination of the rumen-fistula sample, when compared to the esophageal-fistula sample, may be due to contamination from the rumen wall.

Repeated fistula sampling had little effect on the composition of the fistula samples. The amount of calcium was significantly different from the fourth period when compared to Period One. No other changes were observed with any of the constituents.

The correlations between the composition of the feed and the fistula sample were high and statistically highly significant for most constituents. Since a variation exists between types of feed, with a high correlation between the composition of feed and fistula samples, the fistula sample probably represents the best estimate of the composition of grazed forage until adequate comparisons of grazed forage, or forage similar to grazed, have been made. It is doubtful if regression equations calculated from hay and fistula samples of hay could correctly apply to succulent pastures.

The collection techniques were successfully applied to pasture studies. Both rumen-fistulated and esophageal-fistulated animals were used as sampling agents. Botanical analyses of the collected samples on irrigated pasture indicated that grass could easily be distinguished from broad-leaved plants. No attempt was made to distinguish clovers from other broad-leaved plants.

**REFERENCES**


