COMPARISONS OF HERBAGE SAMPLING METHODS AND DIGESTION TRIAL TECHNIQUES WITH BEEF CATTLE

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INTRODUCTION

Grasses and forage of the grazing lands are the main source of nutrients in beef cattle production. Crampton *et al.* (1960) stated that herbage contributes approximately 65 percent of the nutrient requirements of beef cattle, 55 percent of that of dairy cattle and 90 percent of that of sheep. Yet, despite the importance of the need for an understanding of the nutritional problems of grazing animals, there is only limited knowledge of the actual composition of the diet of grazing animals. An important factor in the lack of knowledge is the lack of a suitable method of sampling and measuring the nutrient content of grazed forage.

The selective grazing habits of animals make accurate sampling of the herbage consumed extremely difficult. Herbage samples collected by a foraging animal should be more representative of the grazing animal's diet than herbage samples obtained by other methods.

The purpose of this study was to evaluate several techniques which may prove helpful in determining the composition and nutritive value of the forage consumed by cattle. The study includes: (1) a comparison of hand-plucked samples with esophageal-fistula samples of bermuda grass (*Cynadon dactylon*); (2) collection of various feedstuffs through an esophageal-fistula in cattle and a determination of the percent recovery of that eaten; and (3) a test for differences between hand-plucked samples obtained by two technicians.

REVIEW OF LITERATURE

Methods of Sampling Pasture Herbage

Herbage clipping has been the most widely used technique in attempting to ascertain the herbage consumed by the grazing animal. Clipping techniques measure only the herbage available to the grazing animal and may not give an accurate estimate of that consumed since the grazing animal may preferentially select various plants and plant parts. The problem of selective grazing was recognized by Kennedy and Dinsmore (1909) who reported that sheep fed clipped herbage in digestion crates did not show the selectivity exhibited by sheep grazing on the range.

Hooper and Nesbitt (1930) and Cook and Harris (1950) noted that forages normally consumed by grazing animals were much higher in protein and ash than the whole plant. Cook et al. (1948) found that sheep grazing on desert range selected mainly leaves and tender stems and rejected the fibrous portions of the plant.

In further investigations of selective grazing of steers, Hardison et al. (1954) concluded that clipped herbage was an unreliable sample of herbage selectively grazed. Because of differences in chemical composition between clipped herbage and the herbage selected by the grazing animal, they believed that the digestibility of the two samples would differ with an advantage in favor of the grazed herbage.

The hand-plucking method has been adopted by several workers for field use in an attempt to compensate for the selective grazing habits of the animal. The hand-plucking method of determining the diet of a grazing animal is accomplished by a technician who attempts, as a result of close observation of the grazing animal, to collect portions of the plants similar to those grazed by the animal. This method has given satisfactory results under some conditions. Cook et al. (1948) observed that hand-plucked samples were similar to the herbage grazed by sheep on sparse desert range. The hand-plucking method has also given satisfactory results with pure stands, but according to Cook et al. (1951), it is wholly inadequate for complex mixtures of grasses.

Halls (1954) reported that samples obtained by two technicians working separately in similar pastures, but with two different cow herds, differed widely in both chemical and botanical composition. Schneider et al. (1955) questioned the value of the hand-plucking method in studies with sheep.

It has been suggested by many workers that one or more foraging animals will select a diet typical of that grazed by all animals on a pasture. The use of the esophageal-fistula and the rumen-fistula as means of sampling the diet has evolved from this concept.

Saltonstall (1948) and Lesperance et al. (1960 a, b) estimated the chemical composition of a grazing steer's diet using a rumen fistula. The need to evacuate the rumen contents prior to collection requires a considerable amount of time and labor, and may also affect the normal grazing habits of the animal.

Torell (1954) developed the esophageal-fistula technique, which involved the fistulating of the esophagus of a sheep in such a way that feed ingested by the animal could be collected. Collection of forage samples through the fistula was successful, although it was difficult to close the fistula following collection periods. Cook et al. (1958) developed a plastic cannula to aid in opening and closing the fistula in sheep. These workers were able to control leakage to a large degree by closing the cannula when it was not in use.

Heady and Torell (1959), using esophageal-fistulated sheep, were able to determine the percentage botanical composition of ingested forage. They reported a seasonal effect on preference for plant species by foraging animals. Torell and Weir (1959) noted a change in the chemical composition of grazed forage when esophageal-fistulated sheep were rotated between pastures, and also a change during the grazing period within the same pasture.

Weir and Torell (1959) reported that, under a variety of pasture conditions, sheep consistently selected a forage higher in protein and lower in crude fiber than that obtained by hand-clipping. Cook et al. (1961) observed the nutrient content of the diet of foraging ewes changed from paddock to paddock under summer range conditions. However, the data were confounded with time as the paddocks were grazed at different periods during the grazing season.

Lesperance et al. (1960b) observed that esophageal-fistulated steers grazing a mixed clover and grass pasture consumed a smaller percentage of clover and a larger percentage of grass as the grazing period progressed. Chemical analyses of the samples indicated that protein content decreased and crude fiber content increased as the grazing season progressed.

Edlefsen et al. (1960) concluded that only a small number of animals would be required to provide a reliable estimate of herbage intake, due to the uniformity of samples collected when using esophageal-fistulated sheep on saltbrush and sagebrush vegetation. These workers concluded that corrections should be made for the phosphorus and calcium added to the fistula samples from the saliva.

Lesperance et al. (1960a) reported a significant difference in the chemical composition between esophageal-fistula samples and the feed as offered. There were significant changes in the amount of crude fiber, nitrogen-free-extract, energy, and ash. McManus (1961), on the other hand, noted no significant change in physical composition of the diet after extrusion of samples from the esophageal-fistula. The chemical composition of the esophageal samples differed from the feed only in ash content when the samples were collected by the esophageal-fistula method. He reported an average recovery of hand-fed forage from esophageal-fistula to be between 36 and 82 percent, depending on forage type and cannula used. No change in nitrogen content of the collected samples was noted.

Lesperance et al. (1960a) noted several difficulties when esophageal-fistulated cattle were used to sample herbage. Among those listed were: difficulty in swallowing, protrusion of the cannula ends through the skin, the inside diameter of the cannula too small and enlargement of the fistula openings. Eng (1962) reported protrusion of the ends of the cannula through the skin, low recovery, and frequent plugging of the cannula opening while collecting dry forage through esophageal-fistulae in steers.

McManus (1962) stated that these difficulties were related to the structure and location of the cannula and the lack of an esophageal serosa at the fistula area. Control of leakage is a major factor in preventing loss of saliva which leads to digestive dysfunction and dehydration. According to McManus (1962) the esophageal-fistula must remain functional for at least 3 months to be satisfactory. This requires prudent daily care and maintenance. He also found 52 percent of esophageal-fistulated sheep to be unsuitable for collection of samples.

Rusoff and Foote (1961) found the unbreakable stainless steel esophageal-fistula cannula more satisfactory than the plastic type. They also reported increased amounts of ash in samples collected through the esophageal-fistula.

Although there are still many problems to be solved, the esophageal-fistula method appears to offer great promise for estimating the actual intake of the grazing animal.

MATERIALS AND METHODS

Installing the Esophageal Fistula and Cannula

Two steers fitted with esophageal fistulae, into which modified plugs (Eng, 1962) had been installed, were available for collection when the study was initiated. However, due to a decrease in the size of the fistula, the two steers were refitted with lucite cannulae similar to that described by Cook et al. (1958).

The animals were tranquilized with protiopromazine hydrochloride (Tranvet-Abbott) by intravenous injection, then anesthetized with a solution of thiopental sodium pentobarbital sodium (Combuthal-Abbott) by intravenous injection. Surgical anesthesia was maintained with methoxyflurane (Penthrane-Abbott) administered with an auto-circuit auto-inhalation system via endotracheal catheter. The endotracheal catheter was used to prevent aspiration of rumen contents.

A 6-inch incision was made on the ventral mid-line of the throat region. The esophagus was exposed by blunt dissection of the sternothyro-hyoideus muscles, the esophagus was elevated and a 3-inch longitudinal incision was made. A sterile lucite cannula was inserted into the lumen, followed by the vertical portion which was screwed into the horizontal portion to form a "T", which completed the cannula. The mucosa was sutured to the skin with simple interrupted sutures. At each end of the incision the esophageal mucosa was brought into apposition by horizontal mattress sutures to insure a snug fit around the cannula.

In the later modification of the cannula into the wedge type (McManus, 1961) a similar procedure was followed, with the exception that the cannula was installed as a complete unit of three portions. The wedge type cannula is composed of a forward inverted "L," a rear inverted "L," and a center wedge. The wedge may be placed between the inverted "L" portions to form a "T" by two horizontal screws.

The animals were treated with an antibiotic for 5 days after the operation to prevent infection. During this time they received a pelleted ration to which they had become accustomed prior to the operation. The wounds were sufficiently healed in about 3 weeks to allow the use of the animals for experimental purposes.

The cannulae were of two types, as shown in Figures 1 and 2. Type I, of tubular lucite was made into the form of a "T" while Type II, a wedge-like cannula, was composed of three parts, a forward inverted "L," a rear inverted "L," and a center wedge.

Type I cannula was built from 2-inch lucite tubing by cutting an 8-inch piece of 2-inch lucite tubing into longitudinal halves, affixing a threaded collar approximately 2 1/4-inches from one end into which a second piece of lucite tubing was threaded, to serve as the neck. A threaded cap was screwed onto the neck to seal it off. This type was modified by using a 2 1/4-inch lucite tubing with 1/8-inch walls and by using left hand threads on the collar and upper end of the "T" and right hand threads on the lower end of the "T" for the cap.

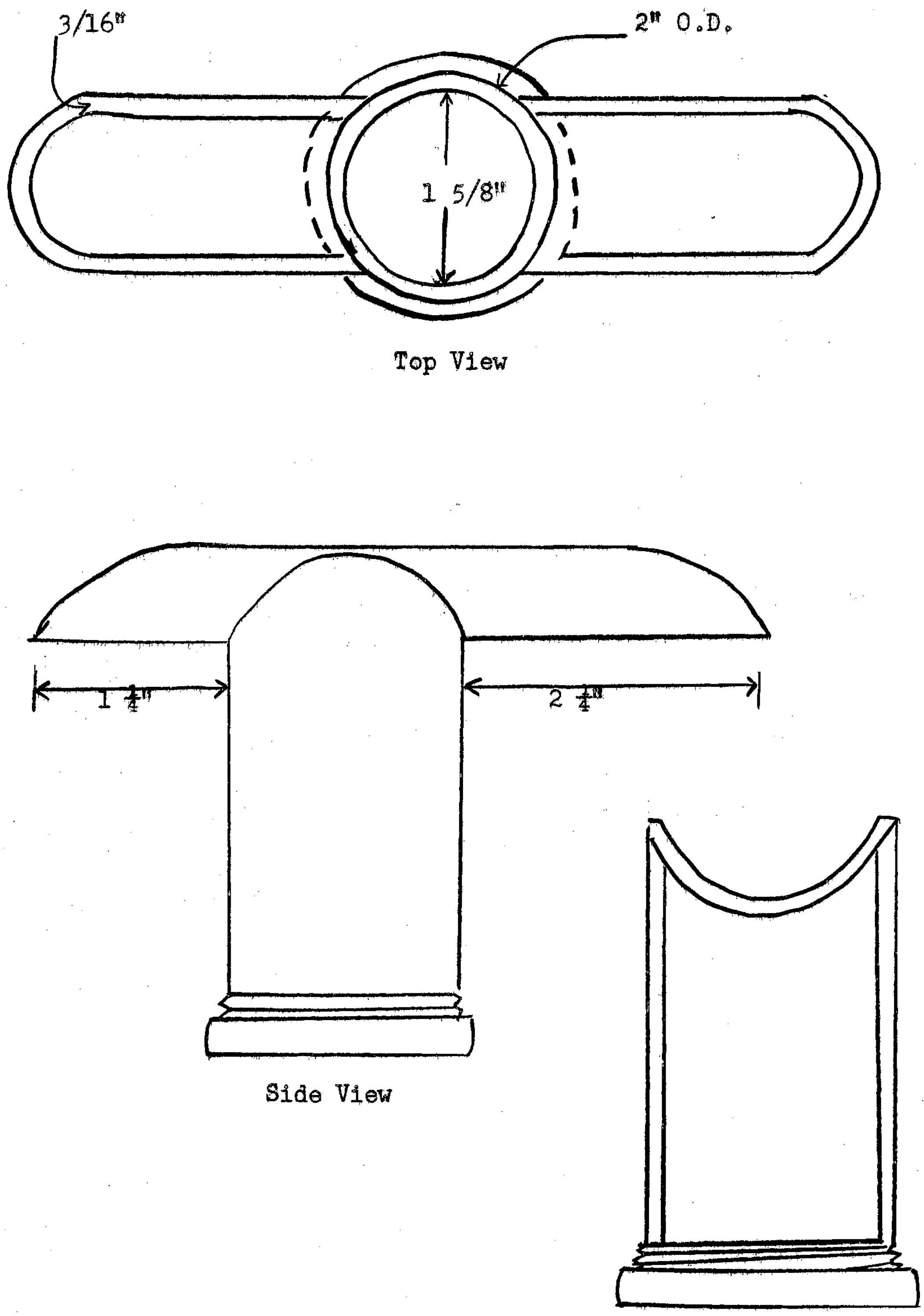

Figure 1. Type I Tubular Cannula

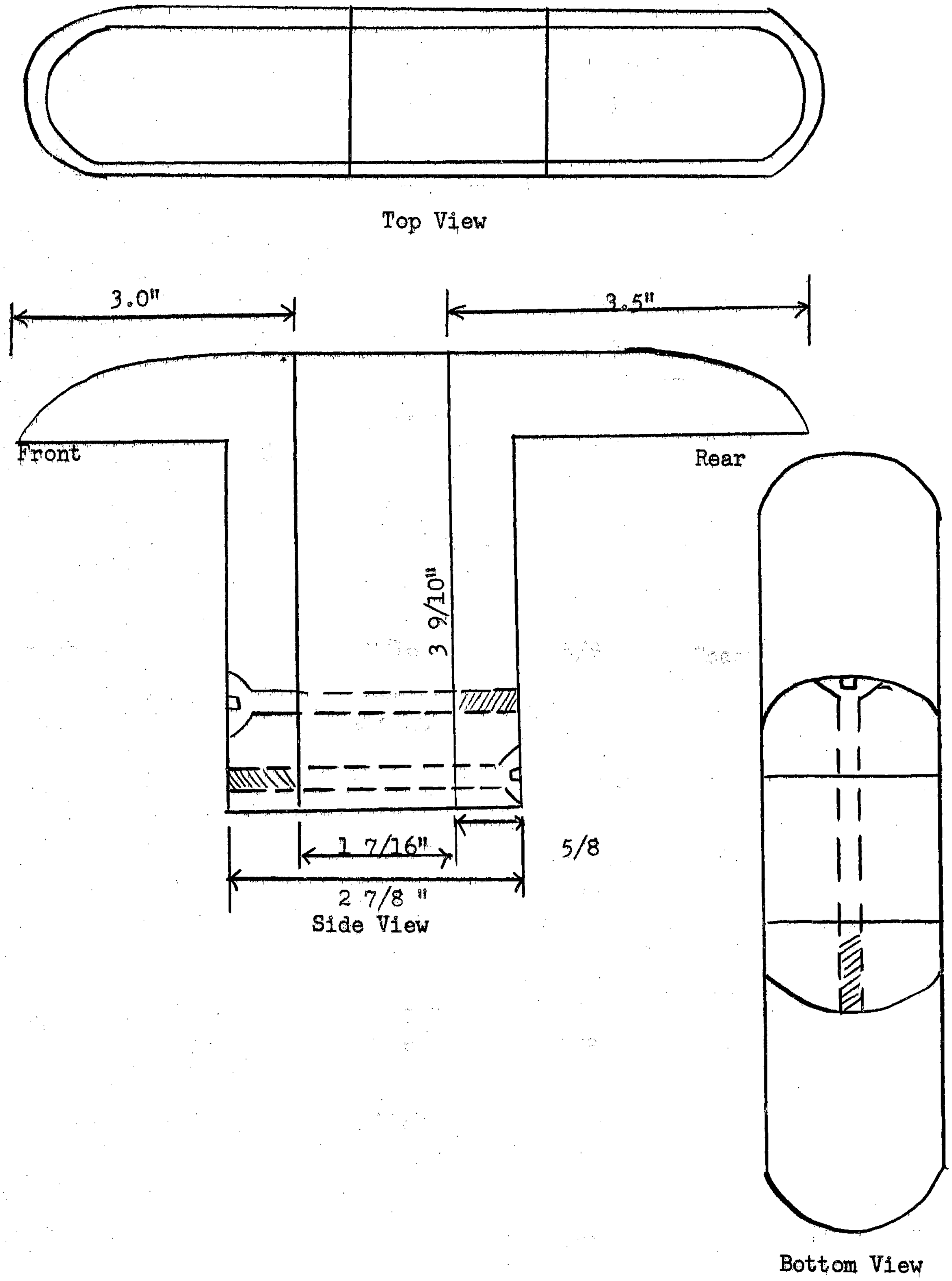

Figure 2. Type II Wedge Cannula

Thin wall tubing was found to be too flexible since the threads would spring apart under the tension exerted by the animal's movements. The left hand threads in the upper part of the "T" were useful in preventing the unscrewing of the neck piece from the collar when removing the cap.

Type II cannula was built from 1-inch lucite tubing and solid sheets of 3/16-inch lucite material, from which the wedges were constructed. While the general contour of the "T" was retained, an oblong plug was substituted for the round one used in Type I. By using different size wedges, the size of the opening could be varied to fit the fistula size.

Of the seven animals fistulated, (four steers and three cows) only four were suitable for collection of herbage samples. Because of difficulties with the cannula, three of the original animals were not used in the herbage sampling trial. In one animal the thin wall of the two parts of the cannula lacked sufficent rigidity to remain attached once installed in the animals' esophagus. The normal movements of the animal seemed to be sufficient to cause the threads holding the two portions together to become disengaged. A steer retained from a previous study could not be used because of the excessive scar tissue remaining from other operations. When this animal was fitted with a new cannula the excessive scar tissue and necrosis of the adjacent tissue caused failure of the fistula. The third animal, a cow, was not fistulated in time to be available for the collection period.

No loss of appetite was encountered in the animals fitted with esophageal-fistulae. However, difficulty in swallowing did develop in several of the animals after a time.

Satisfactory esophageal-fistulae were established in five animals, although the functional time varied considerably between animals. The main cause of failure of the esophageal-fistula apparently was due to an inability of the animal to swallow or some other dysfunction of the esophagus. The functional time of the satisfactory esophageal-fistulae ranged from 12 weeks to over one year. One animal, in which an esophageal-fistula was established during the spring of 1961, was still operative at the conclusion of this experiment. This steer had been subjected to at least three operations in establishing various types of esophageal cannulae.

One cow in which an esophageal-fistula seemed to be satisfactorily established was sacrificed after about 12 weeks because of extreme difficulty in swallowing. A second cow with an esophageal-fistula which operated for 27 weeks was sacrificed after she lost her cannula and the esophagus became impacted. The other cow with an operative esophageal-fistula was sacrificed after 15 weeks because she developed difficulty in swallowing. One steer remained functional as a sampling unit for approximately 24 weeks, after which he became unable to swallow.

McManus (1962) has proposed that if an esophageal-fistula is to be satisfactory, it should remain functional for at least 3 months. On this basis, there were five satisfactory esophageal-fistulae established in seven animals during the course of this experiment.

Construction of Collection Apparatus

The container described by Eng (1962) was used to collect esophageal samples. The container was constructed of 15-ounce waterproof canvas and was 6 inches wide and 12 inches deep. In place, the anterior end was higher than the posterior end. Leather stiffenings were placed in each side of the bag to aid in holding it forward. Three canvas straps extended over the animal's neck and fastened to the opposite side of the bag to secure it. A surcingle around the heart girth, with canvas straps running from the surcingle to the container, was used to secure the posterior part of the container to the animal and to prevent it from sliding forward when the animal's head was lowered.

Trial I

To test the operation of the esophageal-fistula cannula, feeds of different physical makeup (alfalfa hay, cottonseed hulls, and a pelleted ration) were hand-fed to two animals equipped with esophageal-fistulae. The samples collected were then compared to the feed as consumed.

Experimental Procedure: A representative sample of feed that could be consumed in a relatively short period of time was fed. Six hundred grams of cottonseed hulls, 300 grams of alfalfa hay, or 300 grams of completely pelleted ration were offered to each animal each collection period. The collection period was for no longer than 20 mintues. If the animal failed to clean up the amount of feed offered during this time the remaining feed was weighed and this amount subtracted from the original amount fed. McManus (1962), using sheep, suggested a collection period of 15 minutes to minimize the loss of saliva during the collection period. Feed for collection was offered once daily at 5:00 p.m. After collections were made, the animals were fed their total daily ration. The percent recovery of dry matter or organic matter was calculated by dividing the amount recovered by the amount consumed.

All samples were weighed immediately after collection and dried in a forced air oven at 60^{o} C. The samples were removed from the oven and allowed to reach equilibrium with the air, weighed, and stored in sealed glass jars. The samples were ground in a Wiley mill and dry matter, ash, and organic matter were determined on each sample.

Duplicate samples of each feed were subjected to similar analyses. The "T" test was utilized in the analysis of the differences between feedstuff and fistula samples.

Results and Discussion: The animals consumed the alfalfa hay and pelleted rations readily, but the cottonseed hulls were consumed with difficulty. This difficulty was overcome by mixing 250 ml. molasses with each 15 pounds of cottonseed hulls. The average recovery of dry matter consumed was 81.1, 85.2 and 83.3 percent, respectively, for cottonseed hulls, the pelleted ration, and alfalfa hay when these feeds were collected through the esophageal-fistula (Table 1). In the same order, the average amount of saliva added to each feed was 253, 189, and 394 grams per 100 grams of feed dry matter, respectively. The percent recovery of organic matter consumed was 79.8 percent for cottonseed hulls, 84.4 percent for the pelleted ration, and 81.5 percent for alfalfa hay.

TABLE I

DRY MATTER, ORGANIC MATTER, AND ASH AS FED AND COLLECTED BY MEANS OF ESOPHAGEAL FISTULA[1,4]

Constituent	Feed: Cottonseed Hulls	Feed: Pelleted Ration	Feed: Alfalfa Hay
Feed Dry Matter, %	90.3	98.7	91.5
Dry Matter Offered, gm.	542.0	236.2	274.5
Dry Matter Consumed, gm.	393	236	246
Dry Matter Recovered, %	81.1±5.6[2]	85.2±8.2	83.3±3.8
Dry Matter Recovered, gm.	319	224	205
Organic Matter Consumed, gm.	380	250	221
Organic Matter Recovered, %	79.8±5.6	84.4±6.8	81.5±3.9
Organic Matter Recovered, gm.	304	211	180
Ash Consumed, %	2.9	2.9	10.1
Ash Consumed, gm.	11.2	13.0	25.0
Ash Recovered, %	4.5	6.7	12.6
Ash Recovered, gm.	14.5	15.0	25.9
Saliva per 100 gm. Dry Matter Collected, gm.	253[3]	189	394

[1]Two animals per feed and three collections per animal for cottonseed hulls and alfalfa hay. Two animals per pelleted ration and two collections for one and three collections for the other animal. Loss of one sample was due to regurgitation.

[2]Standard error of the mean.

[3]Saliva calculated in the following manner:

$$\frac{\text{Wet sample gm.} - \text{Sample as consumed gm.}}{\text{Dry sample weight gm.}} \times 100 = \text{Saliva/100 gm. dry matter}$$

[4]Organic matter and ash determined on dry matter basis.

McDougall (1948), working with sheep, reported that total nitrogen added by the saliva was minor when compared to total daily nitrogen intake. Since little change in protein, ether extract, and crude fiber has been reported in esophageal-fistula samples when feeds of known composition were fed (Eng, 1962; McManus, 1961; Weir and Torell, 1959), the samples were analyzed only for dry matter, ash, and organic matter. A comparison of these three fractions of the extruded samples with those of the original feed indicated a large increase ($P < .01$) in ash content of the extruded samples. This is in agreement with Eng (1962), who reported an increase in ash content of the esophageal-fistula sample over the sample of feed as fed.

Slight difficulty was encountered from regurgitating and contaminating the sample, unless the animal began to consume the feed as soon as offered. An attempt was made to obtain three samples of each feed from each animal; however, one animal regurgitated and only two samples of the pelleted ration were obtained with that animal. No difficulty was encountered by plugging of the cannula when the inside diameter was at least 1 5/8 inches. The overall operation of the cannula seemed adequate for field trials.

Trial II

Lesperance (1960a) and Eng (1962) compared hand-plucked samples to esophageal-fistula samples and obtained results which substantiate the view that the latter are more representative of what the foraging animal consumes; however, the hand-plucking method of estimating intake is often used experimentally. This experiment was designed to compare esophageal-fistula and hand-plucked samples and to test the feasibility of the esophageal-fistula cannula under pasture conditions. Six Hereford cattle were grazed on a pure stand of Midland bermuda (Cynadon dactylon) in this experiment.

Experimental Procedure: Initially, an experiment was designed to compare esophageal-fistula samples with hand-plucked samples using two Hereford steers fitted with esophageal-fistulae. The pasture lots were approximately 150 by 105 ft. and consisted of a pure stand of Midland bermuda in full flower and about 10 inches in height at the time of sampling. The pasture had previously been cut for hay but had not been grazed. A small pen in one corner of the pasture was used to confine the cattle between sampling periods. Salt and water were available during confinement.

The steers were given a short period to become adjusted to the new area prior to sampling. Samples were obtained for three consecutive days, and the animals were then removed from the area until the next collection phase. The chemical composition of forage available was estimated by clipping three 1-sq. ft. areas at ground level. This forage contained 91.4 percent organic matter and 7.2 percent protein (Table II).

The steers were observed by two technicians, each making independent observations of the portions of the plants consumed while the steers were grazing. Attempts were then made to obtain a hand-plucked sample similar to that consumed by the grazing animal. Sampling periods were two times each day for three days, at 8:00 a.m. and 5:30 p.m. The animals were penned overnight and allowed to graze after the 8:00 a.m. collection. At 1:00 p.m.

TABLE II

CHEMICAL COMPOSITION OF HERBAGE AVAILABLE DURING COLLECTION TRIALS
AS ESTIMATED BY TOTAL CLIPPING METHOD
(DRY MATTER BASIS)

Collection Phase	Portion of Plant	Protein (%)	Ether Extract (%)	Crude Fiber (%)	N.F.E. (%)	Ash (%)	Organic Matter (%)
I	Top 6 inches	8.6	2.0	35.9	46.1	7.4	92.6
	Bottom	6.7	2.1	31.4	50.7	9.1	90.9
	Total	7.2	2.0	32.7	49.4	8.7	91.4
II	Total	7.1	1.0	29.8	53.7	8.4	91.6

the animals were again penned until 5:30 p.m. when the second daily collection was made. The animals were allowed to graze until about 8:00 p.m., then penned until the next morning collection period. The animals were confined without feed in an effort to prevent regurgitation and possible refusal to eat while collecting samples. The sampling period did not exceed 20 minutes duration for any one grazing period. Each steer was individually observed during the collection period.

After collection, the samples were transferred to tared plastic bags, sealed, weighed, and immediately frozen. The samples were later dried in a forced air oven at 60° C. and proximate analyses were determined by A.O.A.C. (1960) procedures.

The same area was used during the second phase of the sampling study with four animals, two Hereford steers and two Hereford cows. The collection procedure, time of sampling, and method of handling the samples were similar to those used in the first phase. The forage available during this phase contained 91.6 percent organic matter and 7.1 percent crude protein on a dry basis (Table II). The data from fistula and hand-plucked samples were paired for each animal within each phase, and the differences analyzed statistically by Student's "T" test.

Results and Discussion: The animals equipped with the esophageal-fistula appeared to perform in a satisfactory manner as no stoppage was observed and the samples were apparently not contaminated by regurgitation. The animals appeared to graze normally and showed little concern toward the technician who was hand-plucking a sample.

The differences in percentage organic matter and crude protein between the samples hand-plucked by the two technicians observing the same animal were not significant (Table III).

The composition of hand-plucked and esophageal-fistula samples are shown in Table IV. In the first trial, the animals selected a diet consistently higher in crude protein ($P < .05$) and ash ($P < .01$), lower in organic matter ($P < .05$) and N.F.E. ($P < .05$) than the samples obtained by hand-plucking. During the second phase, significant differences were noted for several components. The animals selected a diet significantly higher in crude protein ($P < .05$) and ash ($P < .01$), but significantly lower in N.F.E. ($P < .01$), crude fiber ($P < .01$), and organic matter ($P < .01$) than the hand-plucked material. Differences in ether extract of the esophageal-fistula and hand-plucked samples were small and nonsignificant.

These results agree with those of Eng (1962) and Edlefsen et al. (1960) who found that fistula samples have a higher crude protein and ash content, and less crude fiber and N.F.E. than hand-plucked samples. The differences between esophageal-fistula and hand-plucked samples with regard to N.F.E. and organic matter may be influenced by the large increase of minerals in the fistula collected material. The added minerals from saliva would lower the percentage of N.F.E. and organic matter.

TABLE III

CRUDE PROTEIN AND ORGANIC MATTER OF HAND-PLUCKED SAMPLES OBTAINED BY TWO TECHNICIANS OBSERVING THE SAME ANIMAL[1,2] (Dry Matter Basis)

Constituent	Organic Matter		Crude Protein	
Technician	1	2	1	2
	%	%	%	%
$\bar{x}$	92.1	91.2	11.7	10.6
$s_{\bar{x}}$	1.1	0.4	0.7	0.6

[1]Two technicians observing same animal for 12 collections on bermuda grass. The "T" test was used to test the differences between technicians.

[2]The differences in organic matter and crude protein between technicians were different at the $P < .2$ and $P < .1$ levels, respectively

TABLE IV

CHEMICAL COMPOSITION OF SAMPLES OBTAINED BY THE ESOPHAGEAL-FISTULA AND HAND-PLUCKING TECHNIQUE WITH CATTLE GRAZING BERMUDA GRASS (DRY MATTER BASIS)

Trial Number	Method of Sampling	Protein	Ether Extract	Crude Fiber	N.F.E.	Ash	Organic Matter
I[1]	Fistula						
	Steer #7	13.88 ± 1.07[3]	2.49 ± .60	30.31 ± .69	43.27 ± 1.35	10.05 ± .14	89.95 ± .14
	Steer #94	12.45 ± 1.06	3.06 ± .93	29.06 ± .98	43.60 ± 1.65	11.83 ± 1.70	88.17 ± 1.70
	Steer x̄	13.16* ± .75	2.77 ± .45	29.68 ± .60	43.45 ± 1.02	10.94** ± .86	89.06 ± .86
	Plucked	11.12 ± .69	2.27 ± .18	30.87 ± .40	47.47* ± 1.01	8.27 ± .60	91.73* ± .35
II[2]	Fistula						
	Steer #7	12.97 ± 1.19	2.63 ± .30	29.34 ± .27	42.74 ± 1.18	12.32 ± .67	87.68 ± .67
	Steer #94	8.87 ± .67	1.80 ± .30	27.88 ± 1.08	47.97 ± 2.37	13.48 ± 1.65	86.52 ± 1.62
	Cow #68	9.87 ± .26	1.90 ± .34	29.97 ± .71	43.11 ± 2.25	15.15 ± 2.70	84.85 ± 2.70
	Cow #46	11.10 ± .30	2.64 ± .41	27.44 ± .26	44.61 ± .69	14.21 ± .39	85.79 ± 1.79
	Animal x̄	10.64* ± .98	2.74 ± .17	28.66 ± .38	44.39 ± .87	14.04** ± .78	85.96 ± .89
	Plucked	8.03 ± .90	1.79 ± .16	30.57** ± .38	51.15 ± .59	8.47 ± .20	91.53** ± .20

[1]Two animals and 6 collections per animal.

[2]Four animals and 6 collections per animal. Comparisons are between hand-plucked and fistula samples in each trial. Differences between fistula and hand-plucked samples significantly different at levels shown.

[3]Standard error of the mean

*(P < .05)

**(P < .01)

SUMMARY

Two trials were conducted for the purpose of studying sampling techniques of grazed herbage. In the first trial, a wedge type cannula was tested by feeding a known amount of feed and determining the amount recovered by use of the esophageal-fistula. The percent of organic matter recovered was 79.8, 84.4, and 81.5 percent of that consumed, respectively, for cottonseed hulls, pelleted ration, and alfalfa hay. The esophageal-fistula appeared to function satisfactorily for field trials as no stoppage of the fistula occurred in the cannula of the wedge type when known amounts of roughages were fed.

The second trial was conducted for testing the esophageal-fistula under field conditions and to compare esophageal-fistula samples with hand-plucked samples. Two Hereford steers, fitted with esophageal-fistulae, were used for sampling the herbage available in the first phase of this study. Two technicians independently observed each animal during the collection periods and attempted to take a sample similar to that of the grazing animal. The animals selected forage which was higher ($P < .05$) in crude protein and ash but lower ($P < .05$) in organic matter and N.F.E. than the hand-plucked samples. Differences between the two technicians observing the same animal were not significantly different.

In the second phase of this trial, two Hereford steers and

two Hereford cows were fitted with esophageal-fistulae and allowed to graze Midland bermuda. Esophageal-fistula and hand-plucked samples were obtained twice daily over a 3-day period. The animals consistently selected a diet higher in crude protein and ash ($P < .05$), but lower in crude fiber and N.F.E. ($P < .01$) than those obtained by hand-plucking.

The esophageal-fistula in beef cattle appeared to function in a satisfactory manner. About 70 percent of the tests in establishing a functional esophageal-fistula were successful. The functional time of the esophageal-fistula varied from 16 weeks to more than 52 weeks.

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DIGESTION TRIAL TECHNIQUES WITH BEEF CATTLE

INTRODUCTION

The conventional method of determining digestion coefficients of nutrients cannot be easily adapted to forage research under range or pasture conditions; however, the introduction of indicator methods for determining herbage intake and digestibility have contributed much to our ability to evaluate herbage.

Kane et al. (1952) sets two primary conditions as requisites of an external indicator if it is to be useful in obtaining accurate digestibility values. The inert indicator must pass through the digestive tract in a manner similar to that of the ingested nutrients, and the inert indicator must be evenly distributed with the ingesta. Other characteristics of a satisfactory indicator are that it has no physiological action on the digestive tract, that it be indigestible, and that it be easily determined chemically.

The purpose of this study was to compare chromic oxide in two forms, a powder form administered in gelatin capsules and a special chromic oxide impregnated paper administered in shreds, as indicators of digestibility. Lignin, as found in the ration, was also used as an indicator for determining ration digestibility.

REVIEW OF LITERATURE

Use of Chromic Oxide and Lignin as Indicators

Chromic oxide is the most widely accepted of the many indicators thus far tested. Corbett et al. (1950) stated that chromic oxide is the best external reference substance now available. Chromic oxide may be used to obtain an estimate of total fecal output and to determine ration digestibility under specific conditions.

Edin (1918) proposed the use of chromic oxide as an indicator in the calculation of the digestibility of rations. Later workers have reported satisfactory agreement between digestion coefficients determined by the conventional quantitative method and the chromic oxide method (Kane et al., 1950; Crampton and Lloyd, 1951; Chanda et al., 1951; Hamilton et al., 1955; Elam et al., 1962).

Kane et al. (1952) listed the following advantages of the ratio techniques for determining digestibility: (1) elimination of total collections; (2) conduction of digestion trials in the field; (3) substantial savings of time and expense; and (4) the animals are under natural conditions.

Archibald et al. (1958) recommended the chromic oxide method over several other digestion trial procedures on the basis of ease of chemical determination and uniformity of results.

The variation in excretion rate of chromic oxide is a recognized shortcoming. Kane et al. (1952) attempted to measure this variation and to determine the possible cause(s). These investigators reported that fecal chromic oxide concentrations were highest at 9:00 a.m. and lowest at 9:00 p.m. The so-called diurnal variation in chromic oxide excretion has been studied by several workers (Hardison and Reid, 1953; Linkous et al., 1954; Smith and Reid, 1955; Kameola et al., 1956; Lambourne, 1957a, 1957b), and high and low peak concentrations have been observed by most workers. These observations lead to the suggestion by Smith (1955) and Lambourne (1957b) that by sampling at low and high points of excretion each day, one would be able to correct for the diurnal variation.

Balch et al. (1957) studied factors influencing the chromic oxide excretion pattern in steers and noted that gelatin capsules containing chromic oxide entered the anterior rumen or reticulum and dissolved within 15 minutes. Administering chromic oxide immediately before a single daily feeding caused a more even chromic oxide excretion than administering immediately after the feeding due to a greater mixing with the ingesta. The rapid removal of a portion of the administered chromic oxide is believed to contribute to the diurnal variation in chromic oxide excretion.

Corbett et al. (1959) studied the distribution of chromic oxide in the reticulo-rumen and found the concentration of chromic oxide to be higher in the solid portion of the ingesta than in the liquid.

Bloom et al. (1957) observed a larger range in fecal chromic oxide concentration as the ratio of roughage to concentrate in the ration of dairy cows increased, although the general excretion pattern did not change. Lambourne (1957a) reported that the quality of the feed affected the general excretion pattern; the peak concentration occurred progressively later on poor quality pasture and hay. Davis <u>et</u> <u>al</u>. (1958) also compared once and twice daily administration of chromic oxide. Although twice daily administration decreased the extremes in variation in excretion pattern, a large diurnal variation occurred with both methods.

Putman <u>et</u> <u>al</u>. (1958) concluded that the time of administering chromic oxide was the important factor in determining its exretion pattern. They reported that time of feeding and proportion of roughage had no effect; however, this is not in agreement with other workers.

Hardison <u>et</u> <u>al</u>. (1959) found a significant variation in excretion of chromic oxide with time of sampling. Mean recovery of chromic oxide varied from 84.7 percent to 87.4 percent in the combined 6:00 a.m. and 6:00 p.m. samples. Average percent recovery of chromic oxide from total collections was 99.9 percent. In other studies with chromic oxide in a pelleted ration, Elam (1959) and Elam and Davis (1961) observed an effect of feeding time on the excretion pattern.

Putman <u>et</u> <u>al</u>. (1958) reported highly variable patterns of chromic oxide excretion when it was administered to grazing cows. Linnereud and Donker (1961) observed a difference in excretion pattern in cows on pasture related to concentrate. Cows on a higher concentrate level exhibited a more variable excretion pattern. Linnereud and Donker (1961) also noted a difference in excretion patterns between cows fed twice daily and cows fed once daily.

The site of deposition of the gelatin capsule containing the chromic oxide, and the resulting rapid removal of a large percent of the chromic oxide from the reticulo-rumen within a short period of time, seem to be the primary factors contributing to the diurnal variation in excretion of chromic oxide. Hence, a means of reducing the rate of release of the chromic oxide may alter the diurnal variation. In an effort to control the diurnal variation, Pigden and Brisson (1957) prepared a sustained release pellet (SRP) by mixing chromic oxide with plaster of paris and water. This SRP method allowed a slow but sustained release of chromic oxide. The diurnal variation in chromic oxide excretion was non-existent, and recovery of chromic oxide was 100.4 percent.

Eng (1962) reported 95 percent recovery of chromic oxide administered as SRP and the diurnal variation was reduced in comparison to chromic oxide administered in gelatin capsules. However, the calculated digestion coefficients, using SRP as the means of administering chromic oxide, were low.

Troelson (1961) observed a consistently low (67-94 percent) recovery of chromic oxide when using chromic oxide SRP. He believed the incomplete recovery was due to regurgitation of the pellets.

Pigden *et al*. (1959) reported a significant reduction in the variation of chromic oxide excretion under stall-feeding conditions. However, when the ruminants were placed on pasture, many of the pellets were regurgitated.

Corbett *et al*. (1958) stated that the general pattern of the passage of chromic oxide from chromic oxide impregnated paper may more nearly approach that of the undigested portion of the ingested food. A large proportion of the chromic oxide in the feces appeared to be attached to the partially digested cellulose fibers. The chromic oxide was closely associated with the organic debris in the duodenum. In further studies of the distribution of chromic oxide in the reticulo-rumen, Corbett et al. (1959) found the distribution of chromic oxide approximated the dry matter of the rumen contents closer than did polyethyleneglycol. When chromic oxide is mixed with some other material, it may be carried into the rumen and become more evenly mixed with the rumen contents than when it is administered alone (Corbett et al., 1959).

Corbett et al. (1959) described a method for preparation of the chromic oxide paper. To 100 parts, by air-dry weight, of Kraft woodpulp were added 75 parts chromic oxide and 2 parts aluminum sulphate. The pulp was moderately beaten to 300-500 degrees Canadian Freeness, to assist in retention of chromic oxide and to give added strength to the sheets of paper. The pulp was processed into paper of substance weight of approximately 170 gm. per square meter. In the process, the water was not recirculated in order not to increase the chromic oxide retention of the paper above the standard amount. The paper contained a mean of 39.5 $\mp$ .14 percent chromic oxide in a group of random strips. By using this special chromic oxide paper, variability in fecal concentration of chromic oxide was much less than for chromic oxide administered as a powder in capsules. Corbett (1960) was able to reduce errors in the fecal index technique by using this paper. He administered chromic oxide in gelatin capsules, chromic oxide in SRP, whole chromic oxide paper, and shredded chromic oxide paper.

Shredded paper resulted in the least variation of chromic oxide excretion. By the use of shredded chromic oxide paper he was able to take random samples of feces and estimate fecal output over a 7-day period with a coefficient of variation not greater than 5 percent. Corbett and Greenhalgh (1960) reported that chromic oxide, administered in chromic oxide impregnated paper sheets, was excreted at a more constant rate than when it was administered in a powder form in gelatin capsules. A further improvement in uniformity was observed when paper shreds were administered.

Chromic oxide has generally been reported to be an acceptable indicator if total collection of feces is made or if appropriate feces sampling times are chosen. The control of factors affecting the excretion pattern is of utmost importance so that a uniform excretion pattern can be obtained. Presently, the chromic oxide impregnated paper seems to offer a means of reducing the diurnal variation.

Lignin appears to be an encrusting material of the plant which is built up mainly of phenylpropane units derived from coniferol alcohol or closely related compounds. The structure of lignin is not yet known. It is nonhydrolyzable by acid, readily oxidizable in hot alkali and bisulfite and it condenses readily with phenols and thio compounds (Brauns, 1952).

Brown et al. (1955) found shidimic acid was readily convertible in the plant to compounds closely allied with phenylalanine, which is a lignin precursor.

The analysis for lignin in plants has presented widely differing results and varying digestion coefficients are reported. The most widely used method for lignin analysis in nutritional studies is that described by Ellis et al. (1946) as modified by Thacker (1954). Van Soest (1961) proposed a new analysis for lignin based on the work of Moon (1952). This method consists of an acid detergent analysis for lignin. Crampton and Maynard (1938) reported a recovery of dietary lignin of 97.8 to 99.3 percent in the feces while feeding clipped grass to rabbits and an alfalfa hay-grain ration to a steer.

Hale et al. (1940) found that up to 23.7 percent lignin was digested by Holstein cows. These workers reported low digestion coefficients using the lignin ratio technique of Crampton and Maynard (1938), and observed a marked difference in lignin digestion between rabbits and guinea pigs fed alfalfa hay and a lamb fed a similar hay. The digestion of lignin by rabbits and guinea pigs was negligible, but 28 percent was digested by the lamb. Forbes et al. (1946) observed lignin digestion varied from -2.5 to 2.4 percent. Forbes and Garrigus (1948) studied digestibility of pasture herbage by steers and wethers using the lignin ratio technique. The average recovery of lignin was found to be 102± 7 percent.

Ellis et al. (1946) proposed a standard "72 percent sulfuric acid" method for lignin determination. Recoveries of 94 percent to 106 percent were reported for sheep, rabbits and cows; and these workers concluded that lignin may prove useful in determining digestion coefficients of feedstuffs. Kane et al. (1950) used dairy cows to compare the lignin and chromic oxide methods to the conventional method of determining digestion coefficients. As both indicators were fully recovered by chemical procedures, those workers considered both substances indigestible and satisfactory. The recovery of lignin was 98.8 percent and the recovery of chromic oxide was 99.9 percent.

A comparison of the lignin ratio technique and the chromogen method of determining digestibility and forage consumption was conducted by Cook and Harris (1951). They found the calculated values, using the lignin ratio technique, to be well within the limits of accepted values and concluded that lignin was satisfactory as an indicator. The lignin ratio technique has been widely used by these workers in determining digestibility and intake of grazed herbage by sheep (Cook et al., 1951, 1952, 1961, 1962; Cook and Stoddard, 1961).

Not all researchers have reported satisfactory recoveries of lignin. Pigden and Stone (1952) observed that the species of the plant affected lignin recovery. A larger percent of dietary lignin was recovered with dicotyledons studied than with monocotyledons. They postulated that this difference could be due to a higher percentage of easily oxidizable aldehydes in the monocotyledonous plants.

In a comparison of several indicators by Kane et al. (1953), incomplete recoveries of lignin were reported in the feces. These workers postulated that lignin of orchard grass may be metabolized in a different manner than the lignin of other plants. Van Soest (1961) has indicated that small differences in conditions of analysis may influence the recovery of lignin. The low recoveries of lignin caused the digestibility coefficients calculated by the lignin method to be lower than those calculated by the conventional method. Incomplete recovery of lignin has been reported by several workers (Archibald et al., 1958; Elam and Davis, 1961; Hill et al., 1961; Elam et al., 1962). Archibald et al. (1958) preferred chromic oxide to lignin as an indicator in digestion trials due to incomplete recovery of lignin.

Ely et al. (1953) observed lignin in orchard grass cut at various stages of growth to range from 3.8 to 16.0 percent. Rusoff and Foote (1961) also found lignin progressively increased with plant maturity in sudan and millet forages.

Sullivan (1955) observed that lignin digestion coefficients varied 10 percentage points, and reported that lignin of feces was attacked more readily by strong acids and weak alkali than the lignin isolated from grass, indicating that passage through the digestive tract of sheep altered lignin in some manner.

Cairnie (1963) using Hereford cows and the chromic oxide method found lignin of cottonseed meal and mature prairie hay to have a digestibility of from -.2 to 28.0 percent.

Due to the reported effect of age and plant species on lignin digestibility, along with the differing recoveries reported by researchers, more information is needed on the use of lignin as an indicator in digestibility studies. A lignin analysis giving consistent results would be very beneficial in the use of lignin as an indicator; therefore, the acid detergent method of Van Soest (1961) was utilized in the study to determine lignin content of the feed and feces.

MATERIALS AND METHODS

Although chromic oxide has been shown to be a reliable indicator when total collections are made, the diurnal variation in excretion limits its use in herbage evaluation when total feces are not collected. The diurnal excretion pattern of chromic oxide may be overcome to some extent by sampling at "appropriate times"; however, this procedure could introduce errors because of the difficulty in predicting these sampling times in advance.

The following experiment was designed to compare the chromic oxide and lignin methods to the conventional total collection method of determining digestibility. Chromic oxide was administered as a powder in gelatin capsules and as shredded chromic oxide impregnated paper.

Experimental Procedure

Eight grade Hereford steers averaging 685 pounds were placed in metabolism stalls (Nelson et al., 1954) and fed the experimental ration for 21 days prior to the 5-day collection period. This ration consisted of 3500 gm. prairie hay, 500 gm. cottonseed meal, 25 gm. dicalcium phosphate, and 25 gm. salt. Two methods of administering the chromic oxide were studied. Four of the steers received 15.0 gm. chromic oxide by gelatin capsule at 5:00 p.m. each day while the remaining steers each received 45 gm. of chromic oxide impregnated paper which contained 17.5 gm. of chromic oxide.

Total feces were collected in metal trays and rectal "grab" samples were taken every 2 hours. The amount of feces collected by each method was recorded for further use in determining digestibility coefficients. A small composite sample of the 5-day total collection period was placed in a plastic bag and frozen for future protein analysis. The remainder of the composite sample was placed in flat containers and dried at 60^{o} C. until a constant weight was obtained. The samples were allowed to reach equilibrium with the air then ground through a Wiley mill and mixed thoroughly. A representative sample was then stored in a sealed glass jar for further chemical analyses.

The "grab" fecal samples were weighed and frozen in moisture proof plastic bags until dried and ground as described above.

Proximate, lignin, and chromic oxide analyses of feed and feces were determined by the methods of A.O.A.C. (1960), Van Soest (1961), and Kimura and Miller (1957), respectively. Digestion coefficients determined by the chromic oxide and lignin methods were calculated using the equation reported by Kane _et_ _al_. (1953):

$$\text{Digestibility} = 100 - 100 \quad \frac{\%\ \text{indicator in feed}}{\%\ \text{indicator in feces}} \times \frac{\%\ \text{nutrient in feces}}{\%\ \text{nutrient in feed}}$$

The data were analyzed statistically by analysis of variance.

Results and Discussion

Recoveries of chromic oxide and lignin are shown in Table V. The recovery of chromic oxide administered in the special impregnated paper was 99.5 percent, whereas the recovery of chromic oxide administered by gelatin capsule was 98.6 percent. A larger variation

TABLE V

FECAL RECOVERY OF LIGNIN AND CHROMIC OXIDE, ADMINISTERED IN GELATIN CAPSULES OR IN SPECIAL CHROMIC OXIDE IMPREGNATED PAPER (PERCENT)

Animal Number	Chromic Oxide: Total Collection	Chromic Oxide: Grab Sample Collection	Lignin
	CHROMIC OXIDE ADMINISTERED IN GELATIN CAPSULES		
1	99.7	94.1	88.8
2	100.6	100.9	96.2
3	97.0	93.6	86.9
4	96.9	102.5	94.2
$\bar{x}$	98.6	97.8	91.5
	SPECIAL PAPER		
5	94.5	93.3	83.7
6	101.5	97.4	89.6
7	96.1	100.7	100.0
8	105.8	108.9	107.3
$\bar{x}$	99.5	100.0	95.1
$\bar{x}$[1]	99.0	98.9	93.3

[1]Mean of eight steers.

between animals was found in the steers receiving the special chromic oxide paper than in those receiving chromic oxide in a gelatin capsule.

Table VI shows the digestion coefficients for the various components. When less than 100 percent of the indicator fed was recovered from an animal, a corresponding decrease in the calculated digestion coefficient was observed. This is in agreement with Kane et al. (1953), Eng (1962), and Elam (1962). Therefore, the lower the recovery of the administered indicator the larger the difference between the digestion coefficients calculated by indicator methods and by total fecal collections.

The average percent lignin recovery, as shown in Table V, was 93.3 percent. These results agree with those of Csonka et al. (1929), Crampton and Maynard (1938), Hale et al. (1940), Bondi and Meyer (1943), Eng (1962), and Cairnie (1963). As a result of the low average percent recovery of lignin, the digestion coefficients calculated by the lignin ratio method are lower than those calculated by the chromic oxide or conventional methods. The lower lignin recovery is probably due to some change in the lignin as it passes through the digestive tract (Sullivan, 1955). As the structure of lignin is not known, some researchers have questioned the indiscriminate use of lignin as an indicator for digestion studies (Archibald et al., 1958; Kane et al., 1953; Hill et al., 1961; Elam, 1962; Eng, 1962). The technique employed in lignin analysis is very empirical and varying results may be obtained by only slight changes in the procedure used (Van Soest, 1961). However, some researchers have been able to obtain very good recoveries

TABLE VI

COEFFICIENTS OF APPARENT DIGESTIBILITY CALCULATED BY CONVENTIONAL, CHROMIC OXIDE, AND LIGNIN METHODS

Animal	Method	Dry Matter	Organic Matter	Crude Protein	Ether Extract	Crude Fiber	N.F.E.
1	Conventional	58.8	62.6	58.7	48.8	67.8	64.6
	Chromic Oxide	58.7	62.4	58.4	48.8	67.6	64.5
	Lignin Ratio	53.6	58.0	53.4	42.6	63.8	60.2
2	Conventional	56.6	60.5	58.5	62.8	69.8	58.3
	Chromic Oxide	57.1	60.7	58.8	62.9	69.9	58.5
	Lignin Ratio	55.1	58.9	56.9	61.2	68.6	56.6
3	Conventional	61.0	65.4	62.5	61.2	73.9	63.9
	Chromic Oxide	59.8	64.3	61.4	60.1	73.9	62.7
	Lignin Ratio	55.1	60.2	57.0	55.5	69.9	58.4
4	Conventional	53.4	56.7	55.6	79.8	64.5	55.1
	Chromic Oxide	51.9	55.3	54.3	79.4	63.4	52.6
	Lignin Ratio	50.5	54.1	52.9	78.5	62.4	51.3
5	Conventional	58.8	62.5	58.7	76.7	70.7	55.7
	Chromic Oxide	56.5	60.3	56.6	75.6	69.5	53.1
	Lignin Ratio	50.8	55.2	50.6	72.5	65.6	47.0
6	Conventional	57.1	60.8	57.5	79.1	70.5	52.4
	Chromic Oxide	57.1	60.8	57.6	79.3	79.3	52.4
	Lignin Ratio	52.1	56.3	52.6	76.9	67.6	46.9
7	Conventional	56.9	61.4	57.0	72.9	72.1	52.9
	Chromic Oxide	56.0	59.8	55.3	71.7	71.7	51.0
	Lignin Ratio	57.7	61.4	57.0	72.8	72.6	52.9
8	Conventional	50.5	55.2	51.1	70.5	67.3	46.6
	Chromic Oxide	53.2	57.7	53.7	72.0	69.7	49.5
	Lignin Ratio	53.8	58.2	54.3	72.4	58.6	50.2
$\bar{x}$[1]	Conventional	56.7	60.6	57.4	69.1	69.6	56.2
	Chromic Oxide	54.5	58.5	55.2	67.4	68.1	54.0
	Lignin Ratio	53.6	57.8	54.3	66.6	68.4	52.9

[1]No significant differences were observed between methods of calculating digestion coefficient.

of lignin and believe it to be an acceptable indicator (Ellis _et al._, 1946; Forbes _et al._, 1946; Kane _et al._, 1950; Cook and Harris, 1951).

The curves of percent recovery of ingested Cr_2O_3 in the feces at 2-hour intervals for the capsule and paper methods are shown in Figure 3. The percent recovery of Cr_2O_3, when administered in special paper, had a smaller range than did that administered in gelatin capsules. This could be due to a more even distribution and mixing of the chromic oxide in the ingesta. Corbett _et al._ (1960) suggested this reduction in diurnal variation may be due to the chromic oxide being released from many places in the rumen rather than from one position.

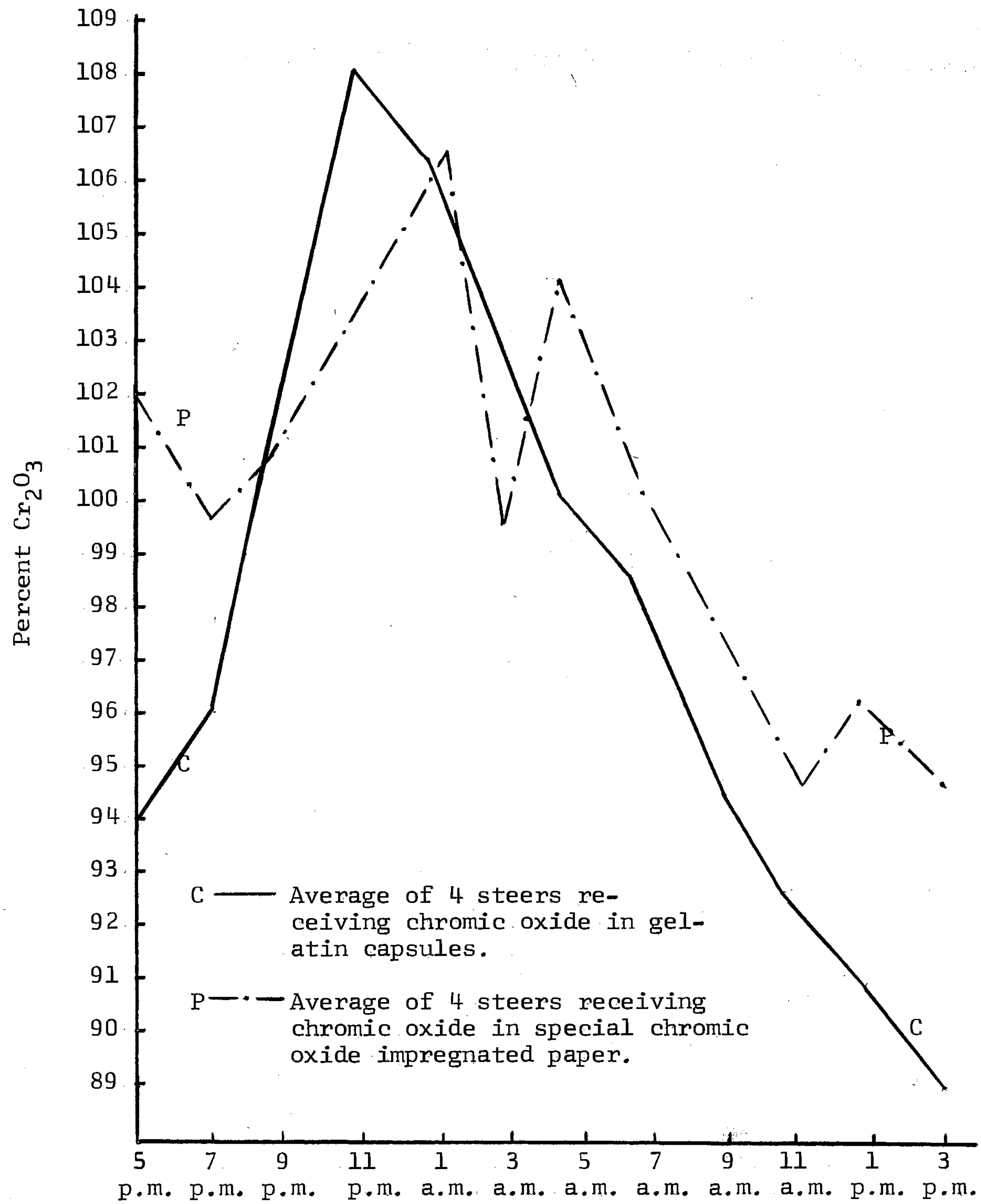

Figure 3. Percent Recovery of Ingested Cr_2O_3 in Feces at 2-hour Intervals (5-day Average for each method).

SUMMARY

An experiment was conducted to compare various indicator techniques with the total collection method for determining digestibility of rations by steers. Chromic oxide, administered in gelatin capsules or by feeding impregnated paper, and lignin were used as indicators. The indicator methods were compared to the total collection method for determining the digestibility of a ration composed of prairie hay and cottonseed meal. Four steers received 15.0 gm. of chromic oxide daily in gelatin capsules while four other steers were fed 45 gm. of chromic oxide impregnated paper containing 17.5 gm. chromic oxide daily.

The range of chromic oxide concentration in the feces of steers receiving chromic oxide in capsules, when expressed as a percent of the daily average, was 88.9 to 108.1 percent, while that of steers receiving special chromic oxide impregnated paper shreds varied from 94.6 to 106.6 percent.

Average recovery of chromic oxide administered in a powder form in gelatin capsules was 98.6 percent when total feces were collected. The percent of chromic oxide recovered in the feces when special chromic oxide impregnated paper was administered was 99.5 percent. The average recovery of dietary lignin in the feces was 93.3 percent.

The digestion coefficients calculated by the various methods were not significantly different ($P > .05$). However, a decrease

in the calculated digestion coefficients was noted, as compared to the total collection method of determining digestion coefficients, when the recovery of the indicator in the feces fell below 100 percent.

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APPENDIX

APPENDIX TABLE I

RECOVERY OF CHROMIC OXIDE IN FECES OF STEERS FED COTTONSEED MEAL AND PRAIRIE HAY (PERCENT)

	Steer Number											
Time	1	2	3	4	$\bar{x}$	s	5	6	7	8	$\bar{x}$	s
Gelatin Capsule												
5 p.m.	84.4	97.0	87.7	106.9	94.0	10.3	93.9	89.8	96.9	124.1	101.6	15.6
7 p.m.	86.7	95.1	90.8	112.0	96.2	11.1	95.1	90.3	97.8	116.0	99.8	11.2
9 p.m.	94.2	97.0	94.8	115.6	100.5	17.0	90.8	97.8	99.8	115.0	100.7	10.2
11 p.m.	102.1	113.3	104.9	112.1	108.1	5.4	92.3	104.2	101.0	118.5	104.0	11.2
1 a.m.	96.5	110.3	99.7	118.9	106.3	23.1	101.1	105.5	105.7	114.1	107.0	5.4
3 a.m.	98.2	105.5	100.8	108.9	103.3	4.8	90.9	101.0	105.1	107.0	99.7	4.1
5 a.m.	96.6	105.9	93.5	104.2	100.1	5.9	100.8	105.5	108.8	100.8	104.0	26.1
7 a.m.	95.5	102.5	94.3	96.9	98.3	3.6	97.2	102.3	109.5	96.8	100.4	5.9
9 a.m.	98.5	95.9	92.3	92.9	94.7	30.3	92.5	100.3	100.8	96.8	97.6	12.8
11 a.m.	92.1	101.6	92.2	85.0	92.7	6.8	93.9	91.0	96.9	98.2	98.2	3.2
1 p.m.	94.8	95.7	87.2	86.8	91.1	4.8	86.1	92.8	96.0	107.0	95.6	8.2
3 p.m.	87.6	91.3	85.3	91.1	88.8	29.3	84.1	90.4	90.6	113.2	94.6	12.8
$\bar{x}$[1]	94.1	100.9	93.6	102.6			93.3	97.4	100.7	109.0		
s	5.1	7.3	5.8	11.7			5.0	7.1	5.6	9.2		

[1]Mean and standard deviation of each animal over twelve 2-hour collection periods.

APPENDIX TABLE II

CONCENTRATION OF CHROMIC OXIDE IN FECES OF STEERS RECEIVING PRAIRIE HAY AND COTTONSEED MEAL EXPRESSED AS MG. CHROMIC OXIDE PER GM. OF DRY MATTER

Method	Steer Number 1		2		3		4		5		6		7		8	
Gelatin Capsule																
Day[1]																
1	8.97	.9	8.92	1.1	9.52	.1	7.95	1.5	10.22	1.5	10.29	1.2	10.70	1.7	11.21	.8
2	9.51	1.2	9.40	.7	9.31	1.3	8.59	1.4	11.09	1.0	10.29	1.3	11.86	1.1	10.22	1.3
3	9.55	1.2	9.48	1.0	10.03	.7	8.82	.7	10.27	1.9	10.52	1.2	11.37	1.3	9.37	1.0
4	8.47	.9	9.51	1.1	9.70	1.1	9.29	1.2	10.62	1.0	10.71	1.1	10.73	1.3	10.18	1.2
5	9.41	1.8	9.58	1.0	9.63	.7	9.55	2.1	10.41	.9	10.90	1.1	10.71	1.3	10.18	1.4
$\bar{x}$[2]	9.18	.5	9.40	.3	9.64	.3	8.84	.4	10.52	.3	10.54	.3	11.08	.5	10.22	.7

[1] Time variable fixed.

[2] Mean concentration of Cr_2O_3/ gram of dry feces in mg. for collection period.

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