TECHNIQUES FOR SAMPLING AND MEASURING INTAKE AND DIGESTIBILITY OF GRAZED FORAGE

By

KENNETH S. ENG, JR.

Bachelor of Science
The University of Nebraska
Lincoln, Nebraska
June, 1958

Master of Science
The University of Nebraska
Lincoln, Nebraska
January, 1960

Submitted to the Faculty of the Graduate School of the Oklahoma State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY
August, 1962

OKLAHOMA
STATE UNIVERSITY
LIBRARY

NOV 7 1962

TECHNIQUES FOR SAMPLING AND MEASURING INTAKE

AND DIGESTIBILITY OF GRAZED FORAGE

Thesis Approved:

Arnold B. Nelson
Thesis Adviser

Allen D. Tillman

Dennis D. Gallup

Joe B. Wheeman

LeGrande C. Ellis

Dean of the Graduate School

504402

ACKNOWLEDGMENT

The author wishes to thank Dr. A. B. Nelson of the Animal Husbandry Department for his suggestions and guidance during the course of this study and in the preparation of this thesis.

The author is indebted to Dr. W. D. Gallup of the Biochemistry Department for his helpful suggestions and constructive criticisms of the thesis.

Appreciation is extended to Dr. E. W. Jones of the Veterinary Medicine and Surgery Department for the installation of the esophageal fistula cannulae.

Appreciation is also extended to Dr. D. L. Weeks of the Mathematics Department for his council concerning the statistical analysis of the data and to the author's wife, Pat, for assistance in the preparation and typing of this manuscript.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The total intake of forage by grazing animals is of primary importance in determining its feeding value. The usefulness of a forage for ruminants is, in most cases, measured by the extent to which it supplies the animal's energy needs. McCullough et al. (1959) indicated that total forage intake must be the beginning point in forage evaluation since it represents the initial available energy. Crampton (1957) concluded that the feeding value of forages can best be measured in terms of voluntary intake. However, a measure of the digestibility of a forage is also useful since the digestibility of certain components of a forage determines to a large extent the amount of the forage consumed.

Unfortunately, there is no way to apply the conventional methods of determining digestibility and intake to grazing animals for evaluation of pasture herbage. Thus, many questions concerning the evaluation of pasture herbage have gone unanswered. This lack of knowledge is not due to a reluctance on the part of researchers to study this important phase of livestock production, but is rather due to a lack of convenient and reliable methods which can be used to study the problem. The worker attempting to evaluate pasture herbage is faced with three basic problems: (1) What herbage does the animal consume? (2) How much herbage does the animal consume? and (3) What is the digestibility of the herbage consumed?

A number of workers have proposed procedures for estimating the nutritive value of grazed herbage. Generally, the measurement of digestibility

and intake is dependent on an indicator method. This method involves the use of a naturally occurring indicator to measure herbage digestibility and an external indicator to measure fecal output. By combining these two measures, the total intake can be estimated. An ideal indicator should be indigestible, have no physiological action on the digestive tract, pass through the tract at a uniform rate, and be easily determined chemically.

To ascertain the nutrient and indicator content of the herbage eaten, one must obtain a representative sample of the herbage grazed. This is extremely difficult because of the ability of the animal to graze selectively. Apparently, a representative sample of herbage can be obtained only by letting the animal forage it.

This thesis reports the results of studies of the development of satisfactory methods for sampling forage consumed and measuring forage intake by the grazing animal. Studies include (1) the preparation of and collection of samples through an esophageal fistula-cannula in steers, (2) a comparison of chemical composition of feeds offered and of samples of these feeds collected by means of the esophageal fistula-cannula, (3) the comparison of hand-plucked samples and samples collected by the esophageal fistula technique on various pasture types, (4) determining the suitability of chromic oxide, chromogens and lignin as indicators of forage digestibility and intake, and (5) measurement of diurnal variations in excretion pattern of chromic oxide and development of a method of lessening these variations.

REVIEW OF LITERATURE

Methods of Sampling Pasture Herbage

Researchers attempting to measure digestibility and intake of grazing animals must contend with the problem of how to determine accurately the nutrient and indicator content of the forage consumed by the grazing animal. Clipping has been the most extensively used method of obtaining a sample for determining the chemical composition of forage. However, because of animal preference for various plants and parts of plants, the clipping method does not give an accurate estimate of the nature of the forage consumed. The problem of selective grazing was first studied by Kennedy and Dinsmore (1909) who reported that feeding clipped forage to sheep in digestion crates did not adequately evaluate the diet under range conditions. They concluded that sheep fed clipped forage did not show selectivity which would be expected on the range.

Cook et al. (1948) observed that sheep grazing on desert range were highly selective in their diet and consumed mainly leaves and tender stems, and rejected the more fibrous portions of the plant. Therefore, the quality of the forage consumed was much higher than chemical analyses of the bulk samples indicated, the leaves being higher in protein and ash than the rest of the plant, as noted by Hooper and Nesbitt (1930) and by Cook and Harris (1950).

Hardison et al. (1954) investigated the degree of selective grazing by steers and concluded that clipped herbage was an unreliable index of chemical composition of herbage selected by grazing animals. They also believed that the digestibility of clipped herbage differed from the digestibility of grazed herbage.

The hand plucking method for determining diet composition has been adopted by some workers in an attempt to compensate for the selective grazing habits of the animals. In this method, a technician closely observes the grazing animal and attempts to collect portions of the plants similar to those grazed by the animal. Cook et al. (1948) reported that the method was accurate when used with sheep grazing on sparse desert range.

Cook et al. (1951) stated that hand plucking was satisfactory on pure stands but totally inadequate on pastures consisting of complex mixtures of grasses.

In an attempt to check the consistency of the hand plucking method, Halls (1954) employed two technicians working separately to collect forage samples from two cow herds grazing in separate but similar pastures. The samples of the two collectors differed widely in both chemical and botanical composition.

Schneider et al. (1955) reported that in spite of spending $1\frac{1}{2}$ to 2 hours daily with grazing sheep it was impossible to be sure that the sample plucked was representative of the forage eaten. They concluded that the hand plucking method was time consuming and of questionable value.

Many researchers suggest that a representative sample of herbage can be obtained only by letting the animal forage it. Procedures currently

being tested include the use of the rumen fistula, analysis of fecal material, and use of the esophageal fistula.

Saltonstall (1948) used a grazing steer equipped with a rumen fistula to estimate chemical composition of herbage. He noted that samples collected as swallowed by the fistulated animal were consistently higher in nitrogen content than were hand plucked samples.

Lesperance et al. (1960a, 1960b) reported that a rumen fistula method of determining the composition of a grazing steer's diet appeared to give satisfactory results. However, this method necessitated the complete evacuation of rumen contents, a procedure which is a laborious task and, in all probability, changes the normal grazing habits of the animal.

Crocker (1959) developed a direct method of measuring the botanical composition of the diet of grazing sheep. He compared the fragments of plant cuticle found in feces to a set of references prepared from leaves of known plants and thereby determined the botanical composition of the diet. The advantage of this method is that it in no way interferes with the normal grazing habits of the animal; however, it is obvious that it does not provide a direct measure of the chemical composition of the diet.

A relatively new direct method of sampling the diet of grazing animals is the use of the esophageal fistula. This technique was developed by Torell (1954). The esophagus of sheep was fistulated but no cannula was installed. The collection of forage samples through the fistula by means of a plastic collection bag was successful but trouble was encountered in closing the fistula following collection periods.

Using sheep, Cook et al. (1958) developed a plastic cannula to facilitate opening and closing of the fistula. Shrubs could be distinguished

from grasses in samples collected by this method, but individual species of grasses could not be identified.

Heady and Torell (1959) used sheep equipped with esophageal fistulae to determine the percentage botanical composition of grazed forage. By careful microscopic examination of the samples they were able to identify the plant species consumed. A seasonal effect on species preference was noted.

Torell and Weir (1959) used esophageal-fistulated sheep to study the changes in nutrient intake resulting from rotational grazing. The data of chemical analysis indicated a change in grazed forage when the animals were rotated between pastures and also a change during the grazing period within a pasture.

Weir and Torell (1959) compared the chemical composition of forage samples obtained by hand clipping and by esophageal-fistulated sheep on a variety of pastures and during various growing seasons. The sheep consistently selected forage which was higher in protein and lower in crude fiber than that obtained by hand clipping.

Lesperance et al. (1960b) using steers equipped with esophageal fistulae conducted grazing trials on a grass and clover pasture. Botanical analysis of samples indicated that as the grazing period progressed, the percentage of grass in the animals' diets increased and clover decreased. Chemical analysis of the samples indicated that protein content decreased and crude fiber content increased as the grazing season progressed.

Edlefsen et al. (1960) used sheep equipped with esophageal fistulae-cannulae in collecting samples of saltbrush and sagebrush vegetation. The uniformity of the samples collected indicated that only a small number of animals would be required to provide a reliable estimate of herbage intake.

It was concluded that correcting fistula samples for the phosphorus and calcium added by the saliva was desirable.

Using sheep, Bath et al. (1956) found only little change in chemical composition of the forage in the collection procedure. Lesperance et al. (1960a), however, reported a significant change in chemical composition of samples so collected. There were significant changes in the amount of crude fiber, nitrogen-free extract, energy and ash. They reported several difficulties in using the esophageal fistuale: (1) cannula too small, (2) swallowing difficulty, (3) enlargement of fistula openings, and (4) protrusion of the cannula ends through the skin.

McManus (1961) reported the average percent recovery of forage in esophageal-fistula samples to range from 36 to 82 percent, depending on forage type and type of cannula. There was no change in the nitrogen content of the collected samples, but a large amount of ash was added. He concluded that the esophageal fistulae technique could be used successfully under field conditions to indicate the quality of the forage selected by grazing animals.

Rusoff and Foote (1961) reported that an esophageal fistula cannula made of stainless steel was more satisfactory than the plastic type since it would not crack or break. Samples collected from grazing cows through an esophageal fistula contained a greater amount of ash than hand plucked samples.

Work thus far reported indicates that the esophageal fistula method of determining the make-up of a grazing animal's diet may be superior to older methods. However, since the technique is new, specific problems associated with the method need further study.

The Use of Indicators

The use of Chromium Sesquioxide (Cr_2O_3, chromic oxide) as an Indicator.

At the present time, chromic oxide is the indicator most widely used for the determination of total fecal output. Under certain conditions, it can also be used to calculate ration digestibility.

Edin (1918), a Swedish worker, was the first to use chromic oxide as an indicator of ration digestibility. He mixed chromic oxide with macaroni and administered it to dairy cows. An English translation of this publication is the report by Edin, Kihlen, and Nordfeldt (1944). Using various classes of livestock, Barnicoat (1945) calculated digestion coefficients by the chromic oxide method and noted that due to incomplete recovery of the indicator results were consistently lower than those obtained in a conventional trial. In contrast, Kane et al. (1950) using dairy cows found chromic oxide to be a satisfactory indicator. Digestion coefficients for dairy cows calculated by the chromic oxide ratio method and by the total collection method were almost identical and average recovery of the indicator was 99.9 percent. Using sheep, Crampton and Lloyd (1951) reported agreement between digestion coefficients determined by the conventional method and the chromic oxide method if the ration contained ground feed with which the chromic oxide could be premixed. However, if the ration was made up entirely of unground roughage, results were inconsistent because of poor recovery of the indicator. Chada et al. (1951) used chromic oxide to measure the apparent digestibility of carotenoids in dry grass. Using sheep and goats as experimental animals, the results obtained by the conventional and chromic oxide methods were in good agreement.

Kane et al. (1953a) using the conventional 10-day trial as the control compared various digestion trial techniques for dairy cows. There was no statistical difference between results when comparing the chromic oxide method with the conventional procedure. Using dairy cows, Archibald et al. (1958) compared various digestion trial techniques and recommended the chromic oxide method because of ease of chemical determination and uniformity of results.

One of the major objections to the chromic oxide technique is the variation in excretion rate of the indicator. Kane et al. (1951) were among the first investigators to attempt measurement of this variation and its possible cause(s). Using dairy cows, these workers observed that fecal concentration of chromic oxide was highest at 9:00 a.m. and lowest at 9:00 p.m. They concluded that this variation was not associated with time of feeding.

Hardison and Reid (1953) compared the diurnal variation of chromic oxide excretion of cattle grazing on pasture with cattle hand fed. Both were given chromic oxide once daily in a gelatin capsule. In the hand fed cattle, the time of highest chromic oxide concentration in the feces was 8:00 a.m. and of lowest concentration was from 4:00-6:00 p.m. This excretion curve differed considerably for cattle on pasture in which the highest concentration was at 6:00 p.m. and the lowest at 12:00 p.m. The excretion pattern of the pasture fed cattle was the more variable.

Kameoka et al. (1956) observed two daily peaks in the excretion of chromic oxide administered to goats at 12-hour intervals. If the chromic oxide was given at 7- and 17-hour intervals, only one daily peak was noted. Excretion patterns were similar when either concentrate alone or hay and concentrate were fed in combination.

Bloom et al. (1957) noted that the general excretion pattern of chromic oxide by dairy cows did not change with change in ratio of concentrate:hay of the ration. However, the variation in range of fecal chromic oxide concentration increased from high- to low-concentrate rations.

Putman et al. (1957) reported highly variable patterns of chromic oxide excretion when it was administered to grazing dairy cows. Their observations indicated that estrous periods may play a role in altering these excretion patterns.

Mahaffey et al. (1954) studied the effect of four feeding regimens on the chromic oxide excretion pattern. Cattle were hand fed roughage 1, 2, 3, and 6 times daily. The greatest diurnal variation in chromic oxide excretion resulted when the animals were fed 6 times daily and the least variation when they were fed once a day. Linkous et al. (1954) administered chromic oxide at various times relative to feeding and observed periods of peak concentration of fecal chromic oxide to occur at 12:00 p.m. - 2:00 p.m. and 2:00 a.m. - 4:00 a.m. Individual variations in excretion patterns were apparent. The results indicated an effect of feeding time on the chromic oxide excretion pattern.

Smith and Reid (1955) reported that chromic oxide concentration in grab samples of feces varied from 65 to 141 percent of the mean concentration and that the time and mode of chromic oxide administration had no effect on the accuracy of the chromic oxide method of determining digestibility.

Balch et al. (1957) in studying factors influencing the excretion rate of chromic oxide in steers noted that gelatin capsules containing chromic oxide entered the anterior rumen or reticulum and dissolved within 5 minutes after administration. Within 30 minutes, 67 percent of the

chromic oxide had moved out of the rumen and reticulum. However, the remaining 33 percent became mixed with the rumen contents and left at the same rate as the rest of the rumen dry matter. The administration of chromic oxide just before the meal caused more uniform excretion than administration immediately after a meal.

Bradley et al. (1959) noted that the chromic oxide excretion patterns were similar but total variation was less when chromic oxide was fed to steers as part of a pelleted ration than when administered by capsule. Using chromic oxide pelleted with the ration, Elam (1959) observed an effect of feeding time on the excretion pattern of chromic oxide in cattle. This observation was further substantiated by Elam and Davis (1961).

Pigden and Brisson (1956) used sheep to study the effect of frequency of administration of chromic oxide on excretion pattern. Chromic oxide was given one, two and six times daily in gelatin capsule form. Diurnal variation in chromic oxide excretion varied from 45 to 180 percent when chromic oxide was given once daily and from 65 to 135 percent when given twice daily. In the group receiving chromic oxide six times daily, diurnal variation in excretion was eliminated. Subsequent studies by Brisson et al. (1957) confirmed these results.

Putman, Loosli and Warner (1958) fed cattle rations containing varying amounts of roughage and administered chromic oxide at various times. They concluded that time of chromic oxide administration was the primary factor in determining the excretion pattern and that time of feeding or proportion of roughage had no effect on the pattern.

Using dairy cows, Davis, Beyers, and Luber (1958) compared once and twice daily administration of chromic oxide capsules. There was a large diurnal variation in chromic oxide excretion with both methods, but the

excretion patterns were not similar. Although giving chromic oxide twice daily lessened extremes in excretion variation, considerable fluctuation still occurred.

The variation in fecal chromic oxide excretion patterns which is so pronounced in ruminants appears to be of little consequence with simple-stomach animals. Using human subjects, Irwin and Crampton (1950) obtained dry matter digestibility coefficients of 88.0 percent by the chromic oxide method as compared to 88.3 percent by the conventional method. Schurch, Lloyd, and Crampton (1950) using rats compared chromic oxide and conventional methods. The coefficients calculated by the chromic oxide method appeared accurate regardless of the time of fecal sampling. Schurch et al. (1952) using the chromic oxide and conventional method found that results with swine were in close agreement. Clawson et al. (1955) using swine noted that results of the chromic oxide and the conventional methods agreed closely regardless of fecal sampling time.

The experimental results indicate that the complex stomach of the ruminant is responsible for a major portion of the variation in chromic oxide excretion. This is ironical when one considers that it is with ruminant animals that the use of chromic oxide has its greatest value.

In an attempt to lessen the variation in chromic oxide excretion, Canadian scientists prepared a sustained release pellet (SRP) by mixing chromic oxide with plaster of Paris and water. It was hoped that such a pellet would remain in the rumen or reticulum of cattle and sheep and dissolve at an even rate. Pigden and Brisson (1957) using the SRP observed that diurnal variation in chromic oxide excretion was non-existent. Recovery of chromic oxide was 100.4 percent. There was no evidence of regurgitation or passage of the pellets from the rumen before they dissolved. Pigden et al.

(1959) noted that using SRPs with stall-fed animals significantly reduced chromic oxide excretion variation, and observed no regurgitation of the pellets. However, when these animals were placed on pasture, many of the pellets were regurgitated. Troelson (1961) reported recovery of chromic oxide to be consistently low (67-94 percent) when the chromic oxide was administered in SRP form. He concluded that the incomplete recovery was at least partially due to regurgitation of the pellets.

Corbett et al. (1960) prepared a chromic oxide-impregnated paper and compared this to other methods of chromic oxide administration to sheep. Methods of administration were gelatin capsule, SRP, whole paper, and shredded paper. The use of the SRP was discontinued because of regurgitation of the pellets. Of the three remaining methods, administering chromic oxide in the shredded paper form resulted in the least variation in excretion, while the variation was greatest when chromic oxide was given by means of a capsule. The standard deviation of the concentration of chromic oxide in grab samples of feces (mg. chromic oxide/gm. organic matter) ranged from 0.5 to 0.9 in the group which received chromic oxide in shredded paper form. This was a notable improvement over the other methods of chromic oxide administration.

In general, results from the use of chromic oxide as an indicator have been acceptable if total collection of feces is made or if appropriate feces sampling times are chosen. However, if the technique is to be of consistent value in pasture studies either a successful method of predicting or of eliminating the diurnal variation in excretion of chromic oxide must be developed.

The Use of Chromogens as an Indicator.

Chromogens is a term used to designate a group of naturally occurring plant substances whose exact make-up is unknown. Reid et al. (1949) proposed use of the chromogen method. They observed that 85 percent acetone extracts of various forage contained compounds absorbing light at 406 mu which were completely recovered in the feces.

Using sheep fed a variety of forages, Woolfolk (1950) reported the average recovery of such chromogens to be 101.2 percent. However, he experienced increased difficulty extracting the chromogens from the forage as the plants reached maturity.

Woolfolk et al. (1950) concluded that the chromogen technique was a satisfactory method of evaluating forage digestibility in cattle and sheep. They observed that the level of chromogens in individual samples of feces taken at various hours of the day was comparable to that of the total sample. This indicated that grab samples of feces could be used to determine digestibility.

McCullough et al. (1951) used the chromogen-ratio technique to measure seasonal changes in digestibility of pasture herbage. They reported that digestibility of forage protein and dry matter was highest in May, declined during the summer months, increased during September, and then fell sharply during October.

Cook and Harris (1951) compared the chromogen- and lignin-ratio techniques of determining digestibility and forage consumption of desert range plants by sheep. They concluded that the chromogen method was not suitable because of incomplete recovery of the chromogen material. Analysis of the urine of the sheep indicated that a large portion of the

chromogens was absorbed from the digestive tract and eliminated in the urine.

As a result of a study of 18 pasture forages ranging in dry matter digestibility from 51.6 to 74.0 percent, Reid et al. (1952) established a mathematical relationship between the chromogen content of the feces and that of the forage consumed. This relationship can be expressed by the following formula:

$$Y = (0.925X + 137.3 \log X) - 242.12$$

Where Y = units of chromogen/gm. of dry forage, and X = units of chromogen/gm. of dry feces

The dry matter digestibility of the forage then can be determined by the ratio formula:

Percent digestibility of D.M. =

$$100 - 100\frac{(\text{units chromogen/gm. dry forage})}{(\text{units chromogen/gm. dry feces})}$$

The use of these formulas to predict the forage changes content is termed the fecal chromogen method.

Comparing various digestion trial techniques with the standard 10-day total collection method, Kane et al. (1953a) reported that the chromogen ratio method gave satisfactory results with dairy cows.

Soni et al. (1954) concluded that there was a diurnal variation in the concentration of chromogens in feces of sheep and steers. However, this variation did not cause variations in calculated dry matter digestibility of grazed forage when the fecal chromogen method was used since the chromogen content of the forage is predicted from the level of fecal chromogens.

Brisson et al. (1954) conducted a series of four digestion trials with sheep and steers to evaluate the chromogen ratio and fecal chromogen methods of determining dry matter digestibility of pasture herbage. If a slight wavelength adjustment was made (404 mu rather than 406), digestion coefficients obtained by both the chromogen ratio and the fecal chromogen methods were almost identical to those obtained by the conventional method.

Testing a total of 40 different herbages, Raymond et al. (1954) reported that the fecal chromogen method of determining digestibility with sheep gave satisfactory results and was particularly useful since it did not require forage sampling. However, they suggested reading the chromogen solutions at 415 mu wavelength rather than 406 mu.

Hamilton et al. (1955) compared the chromogen ratio and the conventional method for determining rumen digestion and total digestion. They reported that digestion coefficients determined by the chromogen method were low and inconsistent.

Squibb et al. (1958) compared the chromogen ratio method with the standard digestion trial for determination of nutrient digestibility of Kihagu grass and Ramie grass by sheep. The chromogen method appeared to be suitable with Kihagu grass, but in trials using Ramie grass, incomplete recovery of the chromogens led to erroneous results. This points out the necessity of proving the reliability of the chromogen method for various species of grass.

Richards et al. (1959) noted that with forages containing large amounts of ash, the fecal chromogen method was not reliable. However, if the chromogen concentrations were computed on an organic matter basis, results were in agreement with the conventional method.

Little is known about the structure and chemical make-up of the substances which are contained in the chromogen solutions. In his doctorial thesis, Woolfolk (1950) makes the following statement: "---for lack of better terms, 'chromogen(s)' and 'chromogenic substances' are employed throughout this report to refer to substances in solution absorbing light. Whether or not the substance(s) absorbing light at 406 mu is a natural plant pigment or is chromogenic in nature is not known."

Smart et al. (1953) reported that acetone extracts of feces and forage contain mainly chlorophylls and their degradation products. Seven different pigments were identified. They concluded that although the pigments are partially degraded in the digestive tract, this change has little effect on their light absorption at 406 mu.

Irvin et al. (1953) concluded that pheophytin, which is the first degradation product of chlorophyll, controls the character of the absorption curve of the fecal extracts. In the digestive tract, pheophytin results from the action of digestive juice on chlorophyll. Light absorption by pheophytin is maximum at a wavelength of 415 mu. These workers noted that individual pigments, although varying greatly between hay and feces, produce composite absorption curves comparable with one another.

By treating plant pigments with oxalic acid, Kane and Jacobson (1954) were able to convert the chlorophyll in the forage to pheophytin. They noted that oxalic acid treatment of the cattle feces was unnecessary since pheophytin occurs naturally in the feces. Extracts were read in a spectrophotometer at 415 mu, the wavelength at which pheophytin displays its maximum absorption. To determine the reliability of pheophytin as an indicator, four methods of determining digestibility were compared. The methods were: (1) total collection, (2) pheophytin ratio, (3) chromogen

ratio, and (4) fecal chromogen method. Three separate trials were conducted and in all cases the pheophytin method compared favorably with the total collection method. However, with certain forages, results obtained by the chromogen ratio or fecal chromogen methods were not in agreement with the total collection method.

Smart et al. (1954) reported that by allowing fecal and grass samples to stand for two hours in 0.1 M copper chloride solution, a very stable derivate of the chlorophylls and pheophytin was formed. This method introduces copper into the porphyrin ring of chlorophyll and pheophytin. It also eliminated other pigments, such as carotenoids. Using this technique on fecal and forage samples of winter grass, they obtained 101 percent recovery of the chromogens as compared to 55.2 percent recovery when samples were not treated with copper chloride.

The variability of experimental results obtained with the use of chromogens as an indicator indicate the need for further work before the technique can be recommended as an accurate method.

The use of Lignin as an Indicator.

Lignin, a naturally occurring plant substance, is a high molecular weight polymer containing carbon, hydrogen and oxygen. Although the exact structure is unknown, the nucleus in polyhydroxy aromatic and therefore lignin cannot be classified as a carbohydrate. According to Mertz (1959), recent studies suggest that the aromatic amino acids, especially phenylalanine, serve as precursors of lignin in the plant. As the demands for phenylalanine by the plant decrease, this amino acid is converted to compounds which serve as manomer units in the formation of lignin.

Many reports are in disagreement as to whether lignin is indigestible and therefore a suitable indicator. This disagreement could be partially due to differences in composition of products isolated by different methods from different sources and designated as lignin.

Using methods which they developed, Crampton and Maynard (1938) reported recoveries of ingested lignin to be 97.8 percent with rabbits and 99.3 percent with a steer. The rabbits were fed clipped grass and the steer fed an alfalfa hay-grain ration. Ellis et al. (1946) developed a modified procedure for the determination of lignin. Using sheep and rabbits and feeding a variety of grasses, they found lignin recovery ranged from 94 to 106 percent. Daily variations in lignin concentration of the feces were very small; therefore, it was suggested that three- to four-day fecal collections should be sufficient. On the basis of these results, they suggested that lignin be used as an indicator of both digestibility and consumption of pasture herbage. This is the first report in the literature of the possibility of using an indicator to determine consumption. Forbes et al. (1946) used the method of lignin determination suggested by Ellis et al. (1946) and found the average digestibility of lignin in clover-timothy hay by sheep to be -1.0 percent. In no cases did the digestion coeffecient calculated by the lignin ratio method differ significantly from those obtained by the conventional procedure. Swift et al. (1947) also used the procedure suggested by Ellis et al. (1946). In their investigation, the lignin digestibility of various rations fed to sheep varied from -2.5 to 2.4 percent. Digestion coefficients determined by the conventional and the lignin ratio method agreed closely. Using the lignin ratio method, Forbes and Garrigus (1948) calculated forage consumption values for sheep and steers on pasture. The average lignin recovery was

102 percent. Although their results were satisfactory, they emphasized the need to prove the method with each animal under investigation and on a variety of forages. Using dairy cows, Kane, Jacobson, and Moore (1950) compared the lignin and chromic oxide methods to the conventional method of determining digestibility and concluded that either method was satisfactory. In their work, recovery of lignin was 98.8 percent.

Cook and Harris (1951) compared lignin and chromogens as indicators of digestion and consumption values for sheep grazing on desert range. They found that values calculated via the lignin ratio method compared well with accepted values and concluded that lignin was an acceptable indicator. Since that time, Utah workers have used the lignin ratio method to determine digestibility and intake of grazed herbage by sheep in a number of investigations (Cook et al., 1951, 1952, 1961, 1962; Cook and Stoddart, 1961).

Although the aforementioned authors believe that the lignin ratio method is a satisfactory procedure for determination of forage digestibility and consumption, there are a number of workers who report that the lignin ratio method is too unpredictable to be of much practical value.

Various workers have reported a partial degradation of lignin in the animal body. Csonka, Phillips, and Jones (1929) observed that in dogs receiving lignin isolated from corn cobs, a loss of 13 to 20 percent of lignin methoxy groups occurred. In this same experiment, they reported that with a cow fed isolated lignin along with a mixed ration, a 36.7 percent loss of methoxy groups occurred. They felt this loss was due to the action of digestive juices in the stomach. Crampton (1939) fed dry pasture herbage to four steers and six rabbits and determined the digestibility of lignin to be 18 and 34 percent, respectively. Hale, Duncan, and Huffman

(1940) used lignin as an indicator of digestion in the rumen of cows fed alfalfa hay. They reported no degradation of lignin in the rumen; however, recovery of lignin in the feces was only 82.3 percent. Thus, considerable lignin digestion appeared to take place further down the digestive tract. This precluded the use of lignin as an indicator for determining total digestion, however, it may be useful in calculating rumen digestion.

Bondi and Meyer (1943) fed sheep four different forages in which lignin digestibility ranged from 35.1 to 64.0 percent. Since the fecal lignin was lower in methoxy values than the plant lignin, chemical change as it passed through the animal body was indicated. In later work, Bondi and Meyer (1949) reported no loss in methoxy groups of plant lignin as it passed through the digestive tract of sheep. However, they did find changes in the molecular weight, nitrogen percentage, and side chains of lignin during digestion.

Hale, Duncan, and Huffman (1947) fed two rumen-fistulated cows alfalfa hay. They used the Crampton and Maynard (1938) method to determine lignin and reported lignin digestibility to be 21.5 percent. Three and one-tenth percent of this digestion occurred in the rumen and the rest took place further down the digestive tract. On the basis of these results, they concluded that lignin was not a suitable indicator.

Several workers have shown that plant species can affect lignin recovery. Using sheep, Davis, Miller, and Lindahl (1947) used a lignin determination procedure similar to that of Ellis et al. (1946) and observed that digestibility of lignin in pea vines and lima bean vines was 16.2 and 10.6 percent, respectively. Forbes and Garrigus (1949, 1950) conducted a series of trials which demonstrated the effect of plant species or feed on the recovery of lignin. Using sheep fed a ration of corn and alfalfa hay

during the first phase of the experiment and cottonseed hulls, corn, cottonseed meal and alfalfa meal during the second phase, Forbes and Garrigus (1949) determined lignin recoveries to be 97.0 and 85.7 percent, respectively. Forbes and Garrigus (1950) fed wethers and steers various species of grass and found the lignin ratio method of determining intake led to variable results. In all cases, more lignin was recovered from the feces than was fed, the average recovery being 114 percent. Pigden and Stone (1952) also reported that plant species affected lignin recovery. Four cured hays were fed: alfalfa and Kochia (both dicotyledons), and crested wheat grass and smooth brome grass (both monocotyledons). They observed that with the dicotyledon hays, recovery of lignin was 100 percent. But, with monocotyledon plants, lignin was only partially recovered. They believed these differences in lignin recovery could be due to the higher percentage of easily oxidizable aldehydes present in the lignin of monocotyledon plants.

Kane et al. (1953a) using dairy cattle which were fed hay of four different stages of maturity compared a number of digestion trial techniques. They observed that both crude lignin and corrected lignin (crude lignin-N X 6.25) calculations resulted in low digestion coefficients due to incomplete recovery of lignin.

Archibald et al. (1958) compared chromic oxide and lignin methods and concluded that the chromic oxide method was preferable because it gave more uniform results. Also, chemical determinations were far simpler and less time consuming. Elam and Davis (1961) using cattle fed a mixed ration compared lignin and chromic oxide recoveries and reported lignin recovery to be 87.1 percent. Hill et al. (1961) reported that the average dairy recovery from heifers fed a mixed ration was from 114.0 to 134.3 percent.

It should be noted that in many of these trials in which recovery of lignin has been less than 100 percent, the rations have contained pasture herbage. Thus, in pasture experiments in which workers have merely assumed complete recovery of lignin, the consumption and digestibility values obtained may be erroneous. Further study of lignin recovery from specific plant species at various stages of maturity is needed.

The Use of a Combination of Indicators to Determine Herbage Intake.

Generally speaking, the measurement of forage intake of grazing animals is dependent upon a reliable indirect method which entails the combined use of two indicators. An external indicator such as chromic oxide is used to estimate fecal output and a naturally occurring indicator such as chromogens or lignin is used to estimate dry matter digestibility. By combining the two measures, dry matter intake can be calculated.

Numerous workers have calculated dry matter intake by equipping animals with fecal collection bags and estimating digestibility by the lignin or chromogen indicator method. In studies of the utilization of desert range, Cook and Harris (1951), and Cook et al. (1951, 1952, 1961, 1962) determined dry matter intake of sheep equipped with fecal collection bags by the lignin ratio method.

Total collection of feces by use of bags and harness eliminates errors due to estimating of fecal output by the indicator method. However, collection bags have the disadvantage of being difficult to handle; they are not readily adaptable to female animals, and they may influence grazing behavior.

The combined use of a naturally occurring indicator (chromogens or lignin) to measure digestibility and an external indicator (chromic oxide)

to measure fecal output appears to be the most feasible way of estimating the dry matter intake of grazing animals.

Nollor et al. (1951) combined the use of chromogens and chromic oxide to measure the forage consumption of milk cows. Data gathered indicated that the animals consumed more than enough forage to meet their requirements.

Hardison and Reid (1953) used the combined chromogen-chromic oxide method for measuring dry matter intake of grazing steers. Kane et al. (1953b) used the combination chromogen-chromic oxide method to determine intake of grazing cows and concluded that the simultaneous use of the two indicators gave excellent results in measuring dry matter consumption. In another phase of this same trial, they used chromic oxide combined with either chromogens or lignin to measure the intake of stall-fed cows. It was observed that dry matter consumption values calculated by the indicator method agreed closely with the actual intake.

Brannon et al. (1954) reported a positive correlation between dry matter intake of grazing steers as measured by the chromogen-chromic oxide method and the digestibility of the forage. The data indicated that the optimum times for fecal sampling were 6:00 a.m. and 4:00 p.m. Using grazing dairy cows as experimental animals, Smith (1955) concluded that the combined use of the indicators, chromogens-chromic oxide, was a satisfactory method of measuring dry matter consumption. These workers observed that grab samples of feces taken at 6:00 a.m. and 4:00 p.m. would give a close approximation of the true dry matter consumption.

Carter et al. (1960) conducted an experiment comparing the chromogen-chromic oxide indicator method of determining forage intake of grazing animals with the before and after clipping method. Results indicated that the

clipping method, as compared with the indicator method, overestimated forage intake by 26 to 50 percent.

Aforementioned data indicate that a combination of indicator methods have been used successfully to estimate dry matter consumption of grazing animals. However, it should be remembered that there are numerous factors that can affect the excretion rate of chromic oxide and the relative recovery of the various indicators, and thereby lead to erroneous conclusions.

EXPERIMENT I. SAMPLING OF FEED INTAKE

Materials and Methods

Establishment of Esophageal Fistula and Cannula.

Esophageal fistula-cannulae were established in four steers and a cow. The surgical operation was similar to that reported by Cook et al. (1958). The animal was placed under general anesthesia with Combuthal (Abbott) and was maintained in this state with ether. An endotracheal tube was placed in the trachea to prevent aspiration of rumen contents.

A 5-inch incision was made on the ventral mid-line in the mid-cervical region. The sterno-thyro-hyoideus muscles were separated by blunt dissection and the esophagus was exposed. The esophagus was elevated and a 2-inch longitudinal incision was made in it. A sterile stainless steel cannula was inserted into the esophageal lumen and was held in place with towel clamps. 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.

Following the operation, the animals were treated with antibiotics for 5 days to prevent infection. Prior to the operation, the animals had been fed a pelleted ration. These pellets were fed for approximately 3 weeks following the operation at which time it was thought the wounds were sufficiently healed so the animals could be used for experimental purposes.

The cannula installed (Figures 1 and 2) is similar to one described by Rusoff and Foote (1961). It is constructed of thin stainless steel. The design is a modified "T", the ends are flanged, and the neck is $3\frac{1}{4}$ inches long with an inside diameter of 1 3/8 inches. A number six rubber stopper is used to close the open end of the cannula neck.

No immediate postoperative complications were noted in any of the animals. However, less than two months following the operation, the anterior lip of the flanged cannula end protruded through the esophagus wall in one steer. The stainless steel cannula was replaced with a temporary plug. Eventually, three of the steers lost the original cannulae due to pressure erosion at the anterior lip of the cannula.

Recurrent inappetence was encountered in four of the five animals. This inappetence could be corrected initially by adjustment of rumen pH and administration of rumen transplants. The rumen pH of these animals was high. However, three of the animals, two of which still had the original cannulae, eventually failed to respond to therapy and died. An autopsy revealed ulceration of the rumen and reticulum and a fungoid invasion.

Studies of Kay and Phillipson (1957) have shown that pressure at various points in the esophagus can stimulate salivation. The abnormally high rumen pH evident in these cattle might have been caused by excessive salivation resulting from the presence of the cannula in the esophagus.

Because of the difficulties encountered in using the steel cannula, a modified plug was designed (Figure 3). Since coarse feeds often plugged the neck of the cannula, it was decided a plug which could be removed during collection periods would be desirable. Also, in an attempt to lessen pressure erosion, the modified plug was somewhat flexible. This type plug has the advantage of being comparatively easy to remove from the esophagus.

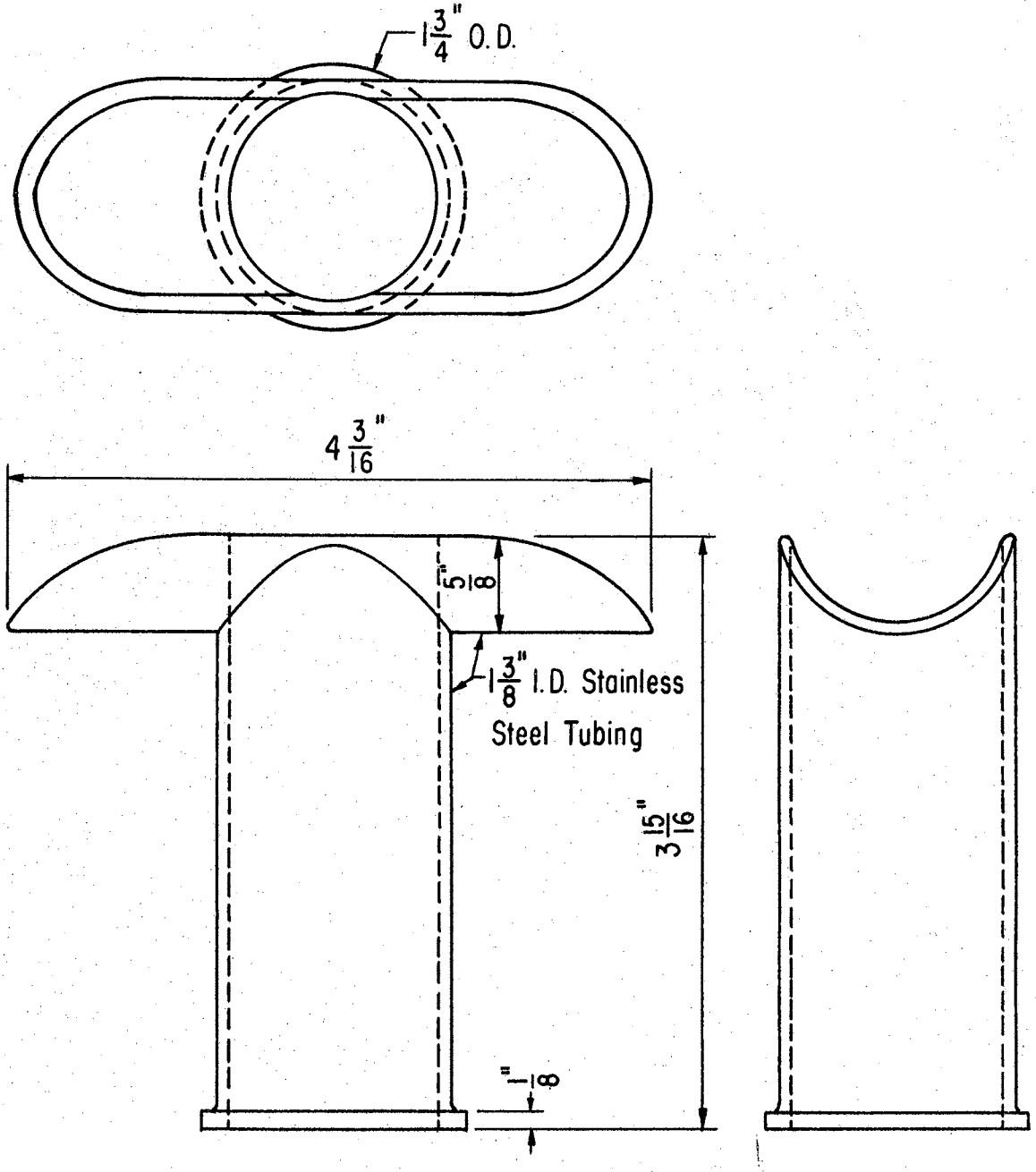

Figure 1. Stainless Steel Cannula for Esophageal Fistula

Figure 2. Steer Equipped with Cannula.

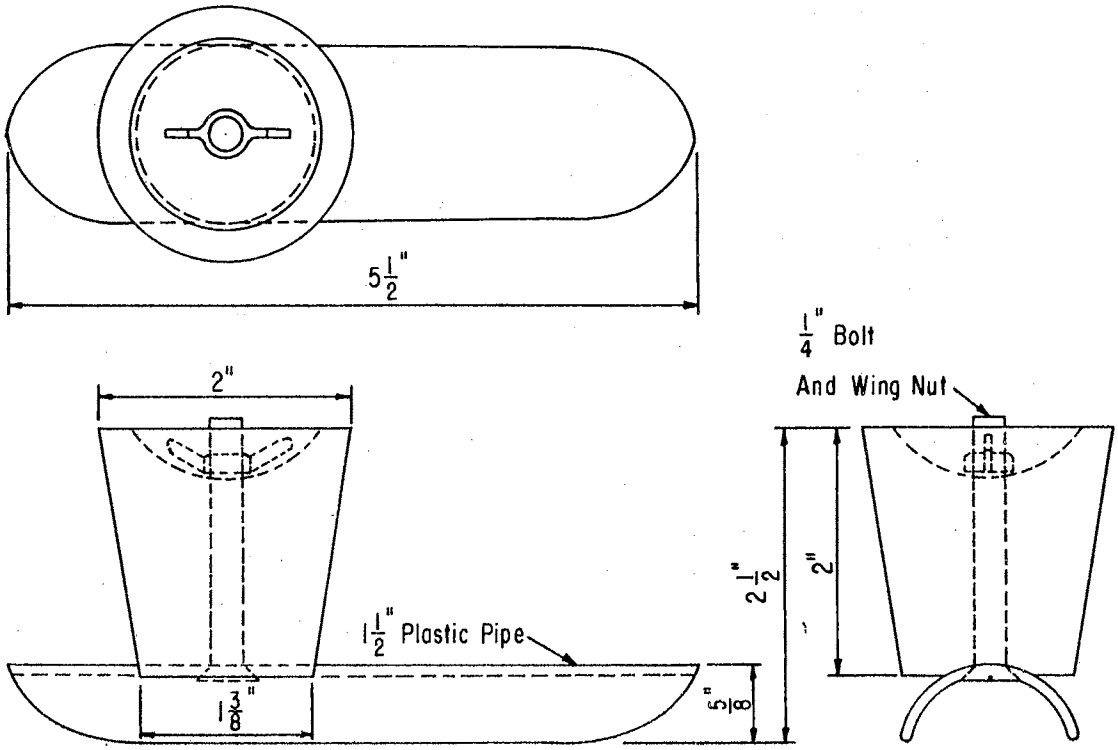

Figure 3. Plug for Closing Esophageal Fistula not Equipped with Cannula.

However, collections attempted approximately six months after the loss of the original cannula have not been completely successful because of the decrease in size of the fistula opening. Whether or not this will be a problem with all animals equipped with a plug of this type is a question yet unanswered.

Construction of Collection Apparatus.

A bag which would fit around the animal's neck was constructed so that a sample of ingesta which was extruded through the fistula-cannula was collected. Several modifications of the original bag were necessary because of the many positions and shapes which the animal's neck may assume.

The bag design ultimately used (Figure 4) was constructed of 15 ounce waterproof canvas. The bag is 6 inches wide and 12 inches deep. The posterior end is lower than the anterior end. A piece of leather stiffening was placed in each side of the bag to help hold it forward. The bag is secured to the animal's neck by three canvas straps running over the top of the neck and fastening on the opposite side of the bag. The posterior part of the bag is secured to the animal by a surcingle around the heart girth with canvas straps running from the surcingle to the bag. This prevents the bag from sliding forward when the animal's head is lowered (Figure 5).

Trial I

The use of the esophageal-fistula method of sampling grazed forage is a relatively new and little used technique. Therefore, there are questions concerning the method which must be answered before it can be used in pasture experiments.

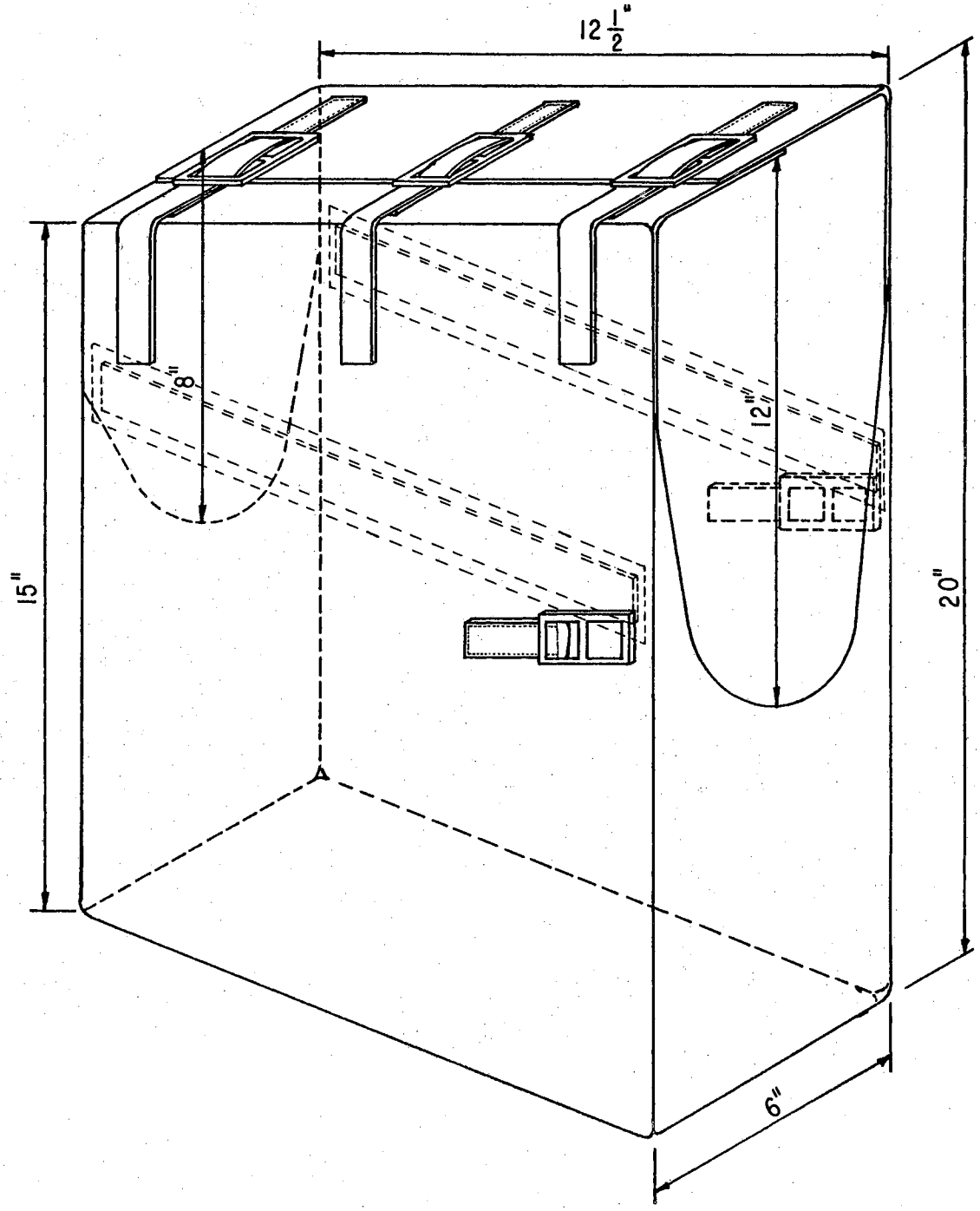

Figure 4. Pictorial View of Canvas Bag for Collection of Samples from Esophageal Fistula

Figure 5. Grazing Steer Equipped with Esophageal Fistula-Cannula and Canvas Collection Bag.

The chief requirements for the successful use of the esophageal-fistula technique are: (1) the fistula must not interfere with the animal's normal eating habits, (2) the sample extruded must be representative of the feed consumed, and (3) the sample collected must be large enough for analysis.

It appears that these factors can best be studied by the hand feeding of a number of different feeds of varying physical make-up and chemical composition and then comparing the properties of the feeds and extruded samples.

Experimental Procedure.

Using two yearling Hereford steers equipped with esophageal fistulae, collection of the following feeds was attempted: pelleted complete ration, ground complete ration, whole milo, pelleted prairie hay, and long alfalfa hay. The steers refused to eat the pelleted prairie hay, and, due to plugging of the cannula neck, no samples of the alfalfa hay were collected. However, satisfactory samples were obtained when the other feeds were offered.

Two pounds of feed were offered each steer at each collection period. It was observed that this amount of feed was consumed in a sufficiently short period of time to prevent contamination of the samples from regurgitation. Feed for collections was given twice daily at 8:00 a.m. and 4:00 p.m. At all other times the steers were fed the pelleted complete ration. They were not allowed water during collection periods.

Eight samples of freshly clipped bermuda grass were also fed and collected from these two steers. Shortly after the collection of these samples, one of the steers lost his cannula and was removed from the trial.

However, two more yearling Hereford steers equipped with esophageal fistulae were then available making a total of three steers which could be used for collection of samples. The next feed collected was freshly clipped native grass consisting mainly of a mixture of big bluestem (Andropogon gerardi), little bluestem (Andropogon scorparius), Indiangrass (Sorghastrum nutans), and switchgrass (Panicum virgatum). Four samples were collected from each of two steers but because of continual plugging of the cannula neck samples could not be obtained from the third steer.

All samples were weighed immediately after collection and dried in a forced air oven at 60° C. The samples were then reweighed, ground in a Wiley mill and subjected to proximate analysis. Representative samples of each feed were handled in the same manner. From these data, the percentage recovery of the various feeds was calculated and a comparison made of the composition of feeds and fistula samples. Differences between animals were tested for significance by analysis of variance, and differences between feeds and fistula samples were tested by the "t" test as described by Snedecor (1956).

Results and Discussion.

The collection of the concentrate type feeds, the pelleted complete ration, loose complete ration, and whole milo, by the use of the esophageal technique was satisfactory. The animals consumed the rations readily and no trouble was encountered with the cannulae plugging. The average recovery of the three rations was 95.48, 89.18, and 92.67 percent, respectively (Table I).

The grams of saliva obtained per 100 gm. of feed collected was 170.7 with the pelleted complete ration, 318.8 with the loose complete ration

TABLE I

DRY MATTER FED AND COLLECTED BY MEANS OF ESOPHAGEAL FISTULA[1]

Feed	Collect. time	Feed dry matter	Dry matter consumed	Fistula Sample			Dry matter recovery	Saliva per 100 gm D.M. collected
				Dry matter	Wet collect.	Dry matter		
	min.	%	gm.	%	gm.	gm.	%	gm.
Pelleted complete	15	90.20	819	35.51	2202	782	95.48 ± 1.88[2]	170.7[3]
Loose complete	20	90.15	657	23.27	2514	585	89.18 ± 1.78	318.8
Whole milo	10	85.68	778	53.17	1356	721	92.67 ± 2.10	71.4
Clipped bermuda grass	30	27.10	224	8.02	1023	82	36.61 ± 7.64	878.5
Clipped native grass	30	45.00	201	11.23	481	54	26.87 ± 7.65	668.5
Bermuda grass pasture	20	27.90	-	8.55	877	75	-	810.9
Native grass pasture	20	49.14	-	8.02	611	49	-	1,043.4

[1] Two steers per feed and four collections per steer for the first five feeds listed. Three steers and 12 collections per steer for bermuda grass pasture, and three steers and seven collections per steer for native grass pasture.

[2] Standard error of mean.

[3] $\frac{2202 - (782 \div .902)}{782} \times 100 = 170.7$.

and 71.4 with the milo.

A comparison of the proximate chemical composition of the extruded samples with that of corresponding feed samples indicated a large increase ($P < .01$) in the ash content of the extruded samples (Table II). The percentage increase was: pelleted complete, 42.18; loose complete, 32.52; and, milo, 27.86 percent. This increase in ash is probably the result of ash added by the saliva. Lesperance et al. (1960) reported that the ash content of bovine saliva was 0.85 percent.

There were only slight changes in the amount of protein, ether extract, fiber and nitrogen-free extract (N.F.E.) of the samples. An animal difference ($P < .05$) was noted in the ether extract content of the extruded milo samples.

In contrast with the relatively easy collection of the concentrate type rations, the collection of fistula samples of clipped bermuda grass and clipped native grass was quite difficult. Trouble was encountered with forage either partially or completely plugging the cannula neck. Also, two collections were discarded because of regurgitation.

The average recovery percentage was 36.71 for the bermuda grass and 26.87 for the native grass. With the bermuda grass, 878.5 gm. of saliva were added per 100 gm. of feed collected as compared to 668.5 for the native grass.

The fistula samples of bermuda grass and native grass differed from feed samples in ash ($P < .01$), protein ($P < .05$), ether extract ($P < .01$) and N.F.E ($P < .01$). A difference between animals ($P < .05$) was noted in the fiber content of fistula samples of native grass.

The large increase in the ash content of the fistula samples, 41.32 percent for bermuda grass and 52.04 percent for native grass, is most likely

CHEMICAL COMPOSITION OF FEED AND FISTULA SAMPLES[1, 2, 3]

Chemical constituent ...	Ash		Protein		Ether Extract		Fiber		N.F.E.	
Sample	Feed	Fistula	Feed	Fistula	Feed	Fistula	Feed	Fistula	Feed	Fistula
Feed	%	%	%	%	%	%	%	%	%	%
Pelleted complete	4.22	6.00**	11.69	11.93	3.00	2.71	22.06	22.29	59.03	58.09
Loose complete	4.92	6.47**	11.69	12.06	2.33	2.33	19.42	19.32	60.27	59.82
Whole milo	2.01	2.57**	11.50	11.62	3.33	3.02[4]	2.31	2.20	80.85	80.59
Clipped bermuda grass	10.77	15.52**	14.76	14.22*	3.27	1.49**	28.00	27.65	43.20	41.42**
Clipped native grass	5.85	8.77**	5.08	7.79**	2.29	1.89**	36.07	36.69[2]	50.70	45.86**

[1] Two steers per feed and four collections per steer.
[2] Differences in differences between chemical composition of feed offered and fistula samples were tested using the "t" test. *($P < .05$), **($P < .01$).
[3] Animal differences in differences in chemical composition between composition of feed offered and fistula sample were tested using analysis of variance.
[4] Animal difference ($P < .05$).

due to ash contamination from saliva. The change in protein and ether extract content may be the result of the incomplete recovery of the feed and not due to any changes taking place within the animal. Although saliva does contain a small amount of nitrogen, McDougall (1948) reported that with sheep, the total production of nitrogen in a 24-hour period is only about 20 milligrams. Therefore, saliva probably has little, if any, effect on the protein content of fistula samples.

Incomplete recovery of feed because of partial plugging of the cannula may explain why the N.F.E. content was less in the fistula samples than in the feed samples. However, since N.F.E. values are determined by difference, the large increase in ash content of the fistula samples would indirectly be responsible for at least part of the N.F.E. decrease.

Trial II

One of the major advantages of the esophageal-fistula technique of forage sampling is that the animal collects the sample and thus eliminates some of the problems of selective grazing. However, there is some question as to whether or not selective grazing on a relatively homogeneous pasture is a problem of sufficient magnitude to warrant the use of the esophageal-fistula technique. An experiment was designed to compare in chemical composition samples collected on a pasture containing only one grass species (Midland bermuda) by hand plucking with those obtained by the esophageal-fistula method.

Experimental Procedure.

Three yearling Hereford steers equipped with esophageal fistulae served as experimental animals. The pasture was a pure stand of Midland

bermuda (Cynadon dactylon) which had previously been cut for hay but had not been grazed. The grass at the beginning of the trial was approximately six inches high and very even. The area grazed was rectangular, 105 X 150 feet, and was enclosed by a woven wire fence which was approximately five feet high. This type of fence was used to eliminate the possibility of having the fistula-cannulae become entangled or caught in the fence. The steers grazed in the initial area for nine days. Because of the decreased availability of forage, the fence was moved back 20 feet three days before the completion of the trial. There was a small pen in one corner of the pasture which contained a water tank and loose salt.

Esophageal-fistula samples were collected once daily for 12 days. Initially, an attempt was made to collect grass samples from the steers at various times during the day. However, it was noted that unless the animals had been kept off feed for a considerable length of time, they refused to graze when the cannula plugs were removed. The following procedure was used thereafter. The steers were penned at 6:30 p.m. each evening. At 8:00 a.m. the next morning the cannula plugs were removed and collection bags were attached, and the animals were allowed to graze. After 20 minutes of grazing, they were put back in the pen, the collection bags removed, the cannula plugs replaced, and the animals were then allowed to graze normally during the remainder of the day.

During the 20-minute period in which esophageal-fistula samples were being collected, a technician followed the steers and attempted to collect plants and parts of plants similar to those grazed by the animals. This is referred to as the hand-plucking technique of grass sampling.

A total of 36 esophageal-fistula samples and 12 hand-plucked samples were collected. All samples were weighed immediately after collection,

placed in a forced air oven at 60° C. until completely dry and then re-weighed. Next, they were ground in a Wiley mill and stored until analyzed. The results of proximate analysis of both the esophageal-fistula and the hand-plucked samples were compared. Differences between methods of sampling, between collection periods, and between animals were tested by analysis of variance as described by Snedecor (1956).

Results and Discussion.

The esophageal fistulae appeared to function satisfactorily. The steers seemed to graze normally, no plugging of the cannulae was encountered and no samples were ruined by regurgitation.

The average size of the dried sample collected was 75 gm. (Table I). The grams of saliva added per 100 gm. of feed collected was 810.9.

A comparison of the proximate chemical composition of the fistula samples and the hand-plucked samples shows that the fistula samples were higher in ash and protein ($P<.01$) and lower in ether extract and N.F.E. ($P<.01$) than corresponding hand-plucked samples (Table III). The large percentage increase in ash (50.95 percent) is no doubt partially due to saliva contamination. However, in observing the grazing animals, it was noted that they would occasionally ingest bits of dirt from plant roots and this would also contribute to the increase in ash.

The higher protein content of the fistula samples is indication that the animal does graze selectively even though the pasture is of a homogeneous nature. There was also an animal difference ($P<.05$) in the protein content of the fistula samples. Samples from steer 2 contained more protein than samples from steers 3 and 4 ($P<.01$). When observing the grazing animals it was noted that steer 2 appeared to be more selective in

TABLE III

CHEMICAL COMPOSITION OF HAND-PLUCKED AND ESOPHAGEAL FISTULA SAMPLES WITH STEERS ON PASTURE

No. of Steers	Coll. per Steer	Ash		Protein		Ether Extract		Fiber		N.F.E.	
		Plucked	Fistula	Plucked	Fistula	Plucked	Fistula	Plucked	Fistula	Plucked	Fistula
		%	%	%	%	%	%	%	%	%	%
Bermuda Grass Pasture											
3	12	10.54	15.91**	13.69	15.98**[2,3]	3.03	1.84**	25.73	26.83	47.01	39.46**[3]
Native Grass Pasture											
3	7	5.69	14.83**[2]	4.94	8.80**[1]	2.10	2.15	35.87	29.45**	51.40	44.55**

** ($P < .01$).

1 Animal Differences ($P < .01$).

2 Animal Differences ($P < .05$).

3 Collection Period Differences ($P < .01$).

TABLE III (Continued)

Sample	No. Coll.	Ash %	Protein %	Ether Extract %	Fiber %	N.F.E. %
Bermuda Grass Pasture						
Plucked	12	10.54 ± .11[1]	13.69 ± 1.19	3.03 ± .27	25.73 ± .52	47.01 ± .89
Fistula						
Steer 2	12	15.77 ± .49	17.21 ± .90	1.78 ± .14	27.00 ± .60	38.30 ± .89
Steer 3	12	15.55 ± .52	15.72 ± 1.01	1.83 ± .11	27.13 ± .59	39.75 ± 1.32
Steer 4	12	16.41 ± .63	14.99 ± .87	1.91 ± .16	26.36 ± .52	40.33 ± .82
Steer $\bar{x}$	36	15.91 ± .98	15.98 ± 1.68	1.84 ± .25	26.83 ± 1.00	39.46 ± 1.84
Native Grass Pasture						
Plucked	7	5.69 ± .30	4.94 ± .26	2.10 ± .14	35.87 ± .57	51.40 ± .76
Fistula						
Steer 2	7	12.69 ± .36	11.13 ± .20	2.36 ± .17	28.95 ± .43	44.20 ± .79
Steer 3	7	15.69 ± .64	6.95 ± .20	1.95 ± .04	29.40 ± 1.14	46.01 ± .51
Steer 4	7	16.12 ± 1.22	8.31 ± .45	2.14 ± .04	30.04 ± 1.54	43.43 ± 1.29
Steer $\bar{x}$	21	14.83 ± 1.79	8.80 ± 1.93	2.15 ± .30	29.45 ± 1.98	44.55 ± 1.84

[1] Standard error of mean.

his grazing. Edlefsen et al. (1960) reported animal differences when using fistulated sheep grazing on a desert type range.

Although the ether extract content of the fistula-collected forage was lower than that in the hand-plucked sample, the variation in individual samples was large, making the interpretation of the data difficult and the value of any interpretation questionable.

It is surprising that the fiber difference between the fistula and hand-plucked samples was so small. Since it is apparent that the animals were selecting a sample higher in protein than was being plucked, one might also expect the fistula sample to be lower in fiber when compared to the plucked sample. However, no such difference was evident.

Again, the lower N.F.E values of the fistula samples ($P < .01$) is probably a partial reflection of the increased ash content of fistula samples.

Among the fistula samples, a significant difference between days was found for only protein and N.F.E. This was true even toward the end of the trial when the available forage became very sparse. The lack of more striking day differences is another indication of the animal's ability to select plants of similar and possibly most desirable composition.

Trial III

In Trial I, difficulty was encountered when hand-feeding clipped samples of native grass. The main trouble was caused by the plugging of the cannula neck. Because of this, it seemed advisable to test the use of the esophageal fistula technique on native pasture. Simultaneously with the collection of the esophageal-fistula samples, hand-plucked grass was also collected and the samples collected by the two methods compared in chemical composition.

Experimental Procedure.

The three steers used in Trial II were also used in this trial. The pasture was of native tallgrass which had been grazed lightly throughout the summer. The grass consisted chiefly of big bluestem (Andropogon gerardi), little bluestem (Andropogon scoparius), switchgrass (Panicum virgatum), and Indiangrass (Sorghastrum nutans). The height of grass ranged up to 3 feet and was quite variable. The area grazed was triangular and measured 204 X 162 X 126 feet. A small pen containing a water tank and loose salt was located in one corner of the pasture.

Twenty-one samples were collected by the esophageal-fistula method. The collections were made twice daily at 10:00 a.m. and 4:00 p.m. The steers were penned at 7:00 p.m. in the evening. At 10:00 a.m. the cannula plugs were removed, collection bags were placed on the steers, and they were allowed to graze. After a 20-minute grazing period, the animals were put back in the pen, the collection bags were removed and the cannula plugs replaced. The animals were then allowed to graze freely until 1:00 p.m. when they were again penned. At 4:00 p.m. the collection procedure was repeated after which the animals were allowed to graze freely until 7:00 p.m. when they were again penned until the morning collection.

The procedure for collecting hand-plucked samples was the same as that used in Trial II. Other procedures also were the same as those described in Trial II.

Results and Discussion.

Although the animals appeared to graze normally, the cannula neck was partially plugged when three of the fistula samples were collected. During one collection period, no samples were obtained from two steers because the

cannula necks were completely plugged. The average weight of the dried fistula samples was 40 grams. A larger sample would be desirable for purposes of analysis and might also be more representative of the animals' diets. However, to prevent excessive salivary contamination and contamination from regurgitation, the length of the collection periods (20 minutes) was not increased. The grams of saliva obtained per 100 grams of dried feed collected was 1043.4.

The fistula samples did not differ significantly from the corresponding hand-plucked samples in ether extract, but were higher in ash and protein ($P<.01$) and lower in fiber and N.F.E. ($P<.01$) than corresponding hand-plucked samples (Table III).

The fistula samples averaged 160 percent greater in ash than the plucked samples. This increase is probably a result of both salivary contamination and the ingestion of soil by the animal. There was a significant difference between animals in ash content of the fistula samples ($P<.05$), samples from steer 2 being lower than those from steers 3 and 4 ($P<.01$).

The average protein content of the fistula samples was almost double that of the plucked samples. Marked animal differences in the protein content of fistula samples were also evident ($P<.05$). Samples from steer 2 were higher in protein than samples from steers 3 and 4 ($P<.01$). And, samples from steer 4 contained more protein than samples from steer 3 ($P<.05$). Through careful observation of the grazing animals, it was evident that the grazing habits of the three steers differed. For example, steer 3 seemed to relish particularly the seed heads of little bluestem and Indiangrass while the other two steers rejected the seedheads.

The height of the pasture grass and the apparent differences in grazing habits between animals made it difficult to obtain representative hand-plucked samples. The accuracy of the method might be improved by assigning a technician to each animal.

The average fiber content of the fistula samples was much lower than that of the plucked samples, values being 29.45 and 35.87 percent, respectively. This difference and the higher protein content of the fistula samples, can be interpreted as evidence of intense selectivity on the part of grazing animals.

If N.F.E. content were expressed on a corrected-ash basis, the difference between fistula and plucked samples would decrease markedly. Thus, the lower N.F.E. content of extruded samples was probably a reflection of both ash contamination and animal selectivity.

The nutrient content of the fistula samples was not affected by collection period. The quantity of available forage cover was still quite high when the trial was terminated, and grazing pressure could be shifted from species to species.

Summary and Conclusions

In Trial I, five feeds, pelleted complete, loose complete, whole milo, clipped bermuda grass, and clipped native grass were hand-fed to two steers equipped with esophageal cannulae. Average recovery of the concentrate-type rations ranged from 89.18 to 95.48 percent. Recovery of bermuda grass was 36.61 percent as compared with 26.87 percent for the native grass. The grams of saliva added per 100 grams of feed collected were: pelleted complete, 170.7; loose complete, 318.8; whole milo, 71.4; bermuda grass, 878.5; and native grass, 668.5.

The addition of saliva greatly increased the ash content of fistula samples irrespective of the type of feed. However, with concentrate-type feeds, protein, ether extract, fiber and N.F.E. values of fistula samples closely approximated those of feed samples. When feeding clipped bermuda or native grass, the protein, ether extract, and N.F.E. values of the fistula samples differed from those of the feed samples. Although differences in protein content of the feed and fistula samples were statistically significant, they were not of sufficient magnitude to eliminate the possibility of using the esophageal fistula technique to sample herbage intake.

In Trial II the esophageal fistula technique and the hand-plucking technique of sampling herbage intake were compared with steers on a pure stand of bermuda grass, and in Trial III the techniques were compared with steers on native grass pasture.

Although cannula plugging occurred when bermuda grass was hand-fed (Trial I), plugging was not a problem when the fistulated steers were grazing bermuda pasture. This indicates that when the animals were on pasture, they consumed a more succulent type forage which was less apt to cause plugging of the cannula. Plugging of the cannula neck was a problem also when feeding clipped native grass, but was less frequent when the animals were grazing native pasture. Differences between feed and fistula samples in Trial I (hand-fed clipped grasses) probably resulted from plugging in the neck of the cannula and are not indicative of changes which may occur when the steers are grazing bermuda and native grass pastures (Trials II and III).

In Trial II, fistula samples contained more ash and protein and less ether extract and N.F.E. than hand-plucked samples. In Trial III, fistula

samples contained more ash and protein and less fiber and N.F.E. than the hand-plucked samples.

A significant difference between collection periods was evident only in Trial II, and this difference was limited to two of the proximate constituents, protein and N.F.E. This indicates that the steers were able, to a degree, to maintain the nutritive level of their diets in spite of nutritive changes in the forage. Animal differences in the protein content of fistula samples were evident in both Trials II and III. There was also an animal difference in ash content of fistula samples in Trial III. These data indicate that in studies with animals on pasture, grazing behavior of individual animals must be taken into account. This would hold true in collecting samples by either the esophageal fistula or hand-plucking technique with animals on either native grass or bermuda grass pastures.

In Trials II and III the animals grazed herbage of different composition than was hand-plucked. Whether or not the fistula samples represent the true nature of the forage which would be normally grazed is a matter of conjecture since it was necessary to use animals from which feed had been withheld for several hours before collecting fistula samples. This procedure probably affects an animal's normal grazing selectivity.

EXPERIMENT II. THE USE OF INDICATORS TO MEASURE DIGESTIBILITY AND INTAKE OF FEED

Method of Chemical Analysis for the Various Indicators

Chromic Oxide Analysis.

The method used for the analysis of chromic oxide was similar to that described by Kimura and Miller (1957). The procedure was as follows:

1. Weigh out 0.5 gram of a finely ground sample.
2. Add 1 to 5 mg. sodium molybdate ($NaMoO_4 \cdot 2H_2O$).
3. Add 10 ml. concentrated nitric acid and boil, using low heat, until sample is digested.
4. Allow to cool and add 5 ml. of 70 percent perchloric acid.
5. Boil, using high heat, to complete oxidation (solution turns orange).
6. Allow sample to cool, filter through glass fritted funnels into 100 ml. volumetric flasks and make up to volume with distilled water.
7. Read optical density (O.D.) with a Klett spectrophotometer at 440 mm wavelength.

The actual concentration of chromic oxide in the sample is determined by calculating K values from samples of known chromic oxide concentration and then using the formula; Concentration = K(O.D.) to calculate the concentration of chromic oxide in the unknown samples. The concentration of the known solutions from which the K values are calculated should be of the same approximate magnitude as the unknown samples.

Chromogen Analysis.

The extraction of the chromogens was carried out according to a procedure described by Reid et al. (1950, 1952). The analytical procedure was as follows:

1. Weigh out a 10-gram wet sample (feces).
2. Transfer sample to a Waring blender, add 250 ml. of 85 percent acetone and blend for 5 minutes.
3. Filter the blended material through a Buchner funnel fitted with Whatman no. 42 filter paper. Suction should be applied.
4. Return residue to Waring Blender.
5. Repeat steps 2 and 3. Three blendings are generally required to remove the chromogens from the feces.
6. Transfer filtrates to 1000 ml. volumetric flask and make up to volume with 85 percent acetone.
7. Read optical density with spectrophotometer at appropriate wavelengths.

The procedure for the chromogen extraction of forages is identical to the above procedure except that the number of blendings and the blending time should be increased. Care should be taken to keep light exposure of the extracts to a minimum since the pigments are light labile.

For the purpose of using the chromogen ratio method of calculating digestibility, the chromogen concentration of fecal and forage samples was expressed in units of absorbancy (optical density) per gram of dry matter. The formula described by Brisson et al. (1957) is as follows:

$$\text{Concentration (C)} = \frac{A}{W} \times \frac{F}{DM} \times 100$$

A = measured absorbance
W = weight of sample in grams
DM = dry matter content of sample in percent
F = dilution faction
C = units absorbancy per gram dry matter

Apparent digestibility can then be calculated using the formula:

$$100 - \left(\frac{a \cdot x \text{ in feces}}{b \cdot x \text{ in forage}} \, 100 \right)$$

a = units of chromogen/gm. forage
b = units of chromogen/gm. feces
x = percent of specific nutrient

Lignin Analysis.

The procedure followed for the analysis of lignin was a combination of the procedures reported by Phillips and Smith (1943) and Thacker (1954). The analysis entails the following steps:

1. Use 0.5 gram of a finely ground sample.
2. Extract with 50 ml. ethanol-benzene (1 part ethanol - 2 parts benzene) for 24 hours using soil-extracting flasks and air condensers.
3. Remove ethanol-benzene solution, add 40 ml. of 1 percent pepsin solution in 0.1 N HCl and incubate overnight at 37 to 40° C. (Note: for ease of operation, solutions can best be removed with the aid of filter sticks).
4. Remove pepsin solution and wash with hot water.
5. Add 100 ml. of 0.25 percent Na_2CO_3 solution and incubate overnight at 37 to 40° C.
6. Filter and wash with hot water.
7. Add 100 ml. of 5 percent (1N) H_2SO_4 and boil for one hour under reflux condenser. (Note: Use crude fiber digestors).
8. Filter and wash with ethanol and ether. Remove ether.
9. Return to dry Berselius beaker. Add 15 ml. of cold 72 percent (by weight) H_2SO_4. Stir frequently to assure moistening of sample. Continue treatment for two hours in water bath at approximately 18° C.
10. At the end of two hours, dilute the acid to approximately 1.0 normal by the addition of 350 ml. H_2O.
11. Boil gently under a reflux condenser for one hour.
12. Filter through gooch crucibles, wash with hot water, followed by ethanol, and then ether.

13. Remove ether by heat, dry in oven at 105° C., and weigh.

14. Determine lignin by loss of weight on ashing.

From the standpoint of equipment needed, the determination of lignin is relatively simple; however, the procedure is very time consuming.

Trial I

Research workers using chromic oxide as an indicator of nutrient digestibility and fecal output are confronted with the problem of diurnal variation in its excretion by experimental animals. They have attempted to resolve this problem by sampling the feces at "appropriate" times. However, this procedure has many sources of possible error.

Pigden and Brisson (1957) and Pigden et al. (1959) have sought to eliminate this variation in chromic oxide excretion rate by administering the chromic oxide in a sustained release pellet (SRP). This SRP is prepared by mixing chromic oxide with plaster of Paris and water. They report that such a pellet significantly lessens the diurnal variation.

The following experiment was designed to determine if a SRP could be prepared in our laboratory which would significantly lessen the variation in chromic oxide excretion of grazing steers.

Experimental Procedure.

Two yearling Hereford steers weighing approximately 550 pounds served as experimental animals. They were placed on a predominately bermuda grass pasture of about 3 acres. One steer received 13.5 gm. of loose chromic oxide in a gelatin capsule and another received 13.86 gm. of chromic oxide in the form of a SRP. The chromic oxide was administered at 8:00 a.m. each morning.

After a 10-day preliminary period, grab samples of feces were collected from each steer at 4-hour intervals for 3 days. The fecal samples (approximately 200 gm. in size) were placed in polyethylene bags, tagged, and frozen. An effort was made to prevent excessive exposure of the samples to light so that some of the samples could later be analyzed for chromogens.

Following the collection period, the treatments of the animals were reversed and the experiment was repeated.

The SRP used in this experiment was prepared in the following manner:

1. Measure 5 parts chromic oxide, 5 parts plaster of Paris and 6 parts water.
2. Place ingredients in large beaker and stir with a spatula until the mixture begins to harden.
3. Place the mixture into a No. 10 gelatin capsule and pack firmly with a hand plunger.
4. Allow the SRP to set a minimum of 24 hours before administration.

Results and Discussion.

The patterns of chromic oxide excretion are shown in Figure 6. When the steers received chromic oxide in the gelatin capsule, chromic oxide concentration in the feces was high at 8:00 a.m., then declined steadily until reaching a low point at 4:00 p.m. or 8:00 p.m. The concentration then increased and reached a maximum at 4:00 a.m. The excretion patterns of the two steers were almost identical.

When the steers were given chromic oxide in SRP form, no well-defined maximum or minimum points of chromic oxide concentration were evident. Table IV shows chromic oxide concentration (mg. chromic oxide/gm. of feces)

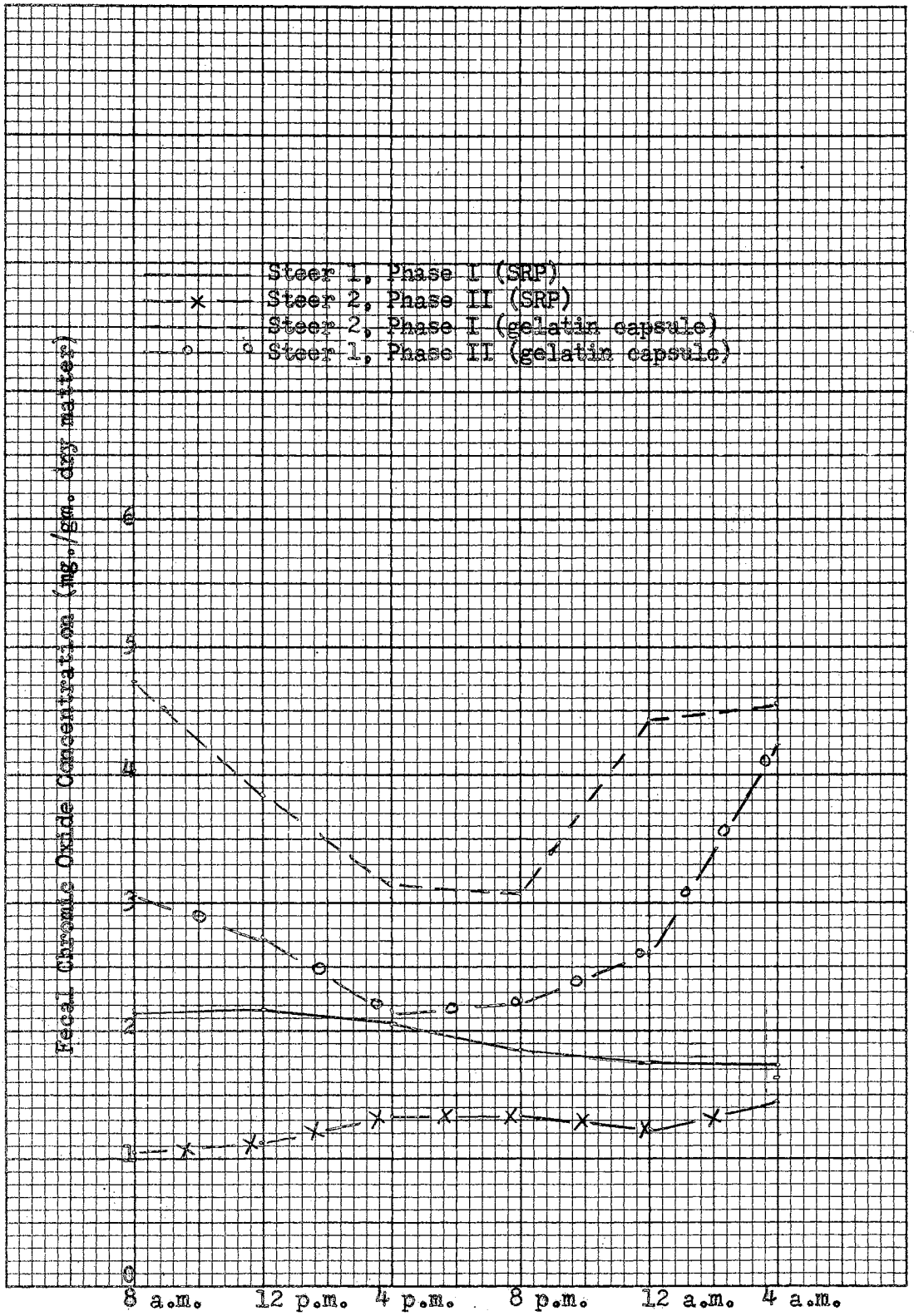

Figure 6. Diurnal Excretion of Chromic Oxide in Feces (3-day average)

TABLE IV

CHROMIC OXIDE IN FECES (mg. Cr_2O_3/gm. D.M.) OF STEERS GRAZING BERMUDA GRASS PASTURE

	Sustained Release Pellet				Gelatin Capsule			
	Steer 1		Steer 2		Steer 1		Steer 2	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
Day[1]								
1	1.493	.073	.691	.195	2.965	.849	4.228	1.010
2	2.473	.440	1.120	.238	3.212	.826	3.833	.989
3	1.899	.246	1.877	.224	3.033	1.035	3.944	.659
$\bar{x}$	1.966	.253	1.261	.219	3.062	.903	3.988	.886
Time of Coll.								
8 am	2.163	.640	1.063	.574	3.062	.354	4.742	.663
12 pm	2.178	.361	1.110	.585	2.746	.158	3.827	.406
4 pm	2.045	.636	1.375	.548	2.176	.693	3.154	.100
8 pm	1.830	.464	1.388	.784	2.232	.071	3.070	.510
12 am	1.772	.292	1.226	.632	3.643	1.070	4.460	.430
4 am	1.731	.265	1.445	.430	4.216	.346	5.036	.332

[1] Time variable fixed and not random.

and standard deviations. The standard deviations indicate that diurnal variation was less when the animals received chromic oxide in a SRP.

The chromic oxide concentration of grab samples taken at 4:00 a.m. and 4:00 p.m. from steers which received chromic oxide in the gelatin capsules closely approximated the average concentration in samples taken throughout the day. When the steers received the SRP, time of fecal sampling was of minor importance since the diurnal variation in chromic oxide excretion was so small.

Total collection of the feces was not made, therefore, the absolute recovery of chromic oxide could not be measured. However, differences in concentration of chromic oxide in feces indicate that recovery was less when steers received the SRP. The average chromic oxide concentration (mg. per gm. of feces) was 1.966 for steer 1 and 1.261 for steer 2 when chromic oxide was given in SRP form; when it was given in loose form, the concentration was 3.062 for steer 1 and 3.993 for steer 2. These differences in concentration occurred although each steer received approximately the same amount of chromic oxide. It is possible that the differences could be due to actual differences in the amount of feces excreted. It seems likely that the chromic oxide given in SRP was poorly recovered. Such low recovery may have been the result of regurgitation and loss of a portion of the pellets. Pigden et al. (1959) and Troelson (1961) reported that regurgitation of the SRP occurred when grazing cattle served as experimental animals.

In conjunction with this trial, grab samples of feces taken at various times of the day were analyzed for chromogen content. Irrespective of time of fecal sampling, the concentration of chromogens was almost constant. This indicates that diurnal variation of fecal chromogens was not a problem.

On the basis of the results of this trial, it was apparent that the use of the SRP lessened diurnal variation in excretion rate of chromic oxide. However, the recovery of chromic oxide administered in this manner was apparently low. Therefore, even though the SRP shows promise of overcoming the problem of diurnal variation, the method needs further study. Incomplete recovery of the indicator would presently preclude its use.

It appears that diurnal variation does not pose a problem in the use of fecal chromogens as an indicator of digestibility.

Trial II

The three indicators presently receiving the most attention for use in the measurement of forage digestibility and intake are chromic oxide, chromogens and lignin. The usefulness of lignin as an indicator is somewhat dependent on the plant species being studied (Forbes and Garrigus, 1949, 1950; Pigden and Stone, 1952). This may also be true of chromogens (Squibb et al., 1958). There is also some question as to which is the more reliable method of measuring digestibility, the chromogen ratio or the fecal chromogen method. The proper wavelength for reading O.D. of the chromogen solutions has also been questioned.

A digestion trial in which fresh clipped bermuda grass was hand-fed was conducted to compare lignin, chromogens, and chromic oxide as indicators. The chromic oxide was administered in both the gelatin capsule and SRP form so that the two methods of administration could be compared.

Experimental Procedure.

Four yearling Hereford steers were placed in metabolism stalls (Nelson

et al., 1954) and fed 20 pounds of fresh clipped Midland bermuda grass once daily at 4:00 p.m. Each morning at 8:00 a.m., two of the steers received chromic oxide in a gelatin capsule and two were given chromic oxide in the form of a SRP. The method of preparing the SRP was identical to that described in Trial I. Approximately 14 grams of chromic oxide was given each steer daily.

A 7-day collection period followed a 14-day preliminary period. Total feces were collected and grab samples were taken every 4 hours for the measurement of the variation in chromic oxide excretion. When taking grab samples, an attempt was made to completely empty the rectum. The amount of feces collected each time was measured to determine whether or not there was any relationship between the concentration of chromic oxide in the feces and the amount of dry matter excreted.

The recovery of lignin, chromogens, and chromic oxide was measured. The results using lignin, chromogens, and chromic oxide as indicators to determine digestibility and intake were compared to the results obtained by using the conventional method. In addition to the analysis of the grass and fecal samples for the indicators, they were also subjected to proximate analysis.

Special attention was given to the effect of light on the chromogen solutions. Precautions were taken to keep light exposure to a minimum. Duplicate acetone extractions were made on each fecal sample. Immediately after extraction, O.D. measurements at wavelengths of 406 and 414 mu were taken on each solution. Following the initial O.D. measurement, one of the duplicate solutions from each fecal sample was placed in the dark and the other left exposed to light. Additional O.D. readings were made at 1 hour, 24 hours, and 7 days after extraction.

Results and Discussion.

Chromic oxide as an indicator. The average diurnal variation in the chromic oxide excretion rate is shown in Figure 7. For steers 2 and 3 which received the chromic oxide in gelatin capsules, the peak concentration of chromic oxide in the feces occurred at 8:00 a.m. The concentration then declined until it reached a low point at approximately 8:00 p.m. or 12:00 midnight. Thereafter, the concentration increased. Steers 1 and 4, which received the SRP, showed no well-defined diurnal excretion pattern.

The range in the relative concentration of fecal chromic oxide at various hours for the steers receiving the SRP was much smaller (95.51 to 104.53 percent for steer 1 and 97.85 to 102.57 percent for steer 4) than the range for steers receiving gelatin capsules (87.16 to 112.75 percent for steer 2 and 84.35 to 115.85 percent for steer 3). The average standard deviation (mg. chromic oxide/gm. feces) of the daily mean (Table V) was .316 for both steers 1 and 4 as compared to .960 for steer 2 and .909 for steer 3. Statistical analysis indicated diurnal variation in the excretion of chromic oxide was less for steers receiving the SRPs than for the steers receiving the chromic oxide in gelatin capsules ($P < .01$).

The average chromic oxide concentration in grab samples taken at 8:00 a.m. and 8:00 p.m. from steers which received chromic oxide in gelatin capsules was approximately the same as that in samples from the total fecal collections. This was also true for steers 1 and 4 (given SRP); however, time of feces sampling with these steers is not critical because of the low diurnal variation.

In an effort to determine if the amount of feces excreted was a factor in the diurnal variation, correlations between the amount of dry matter

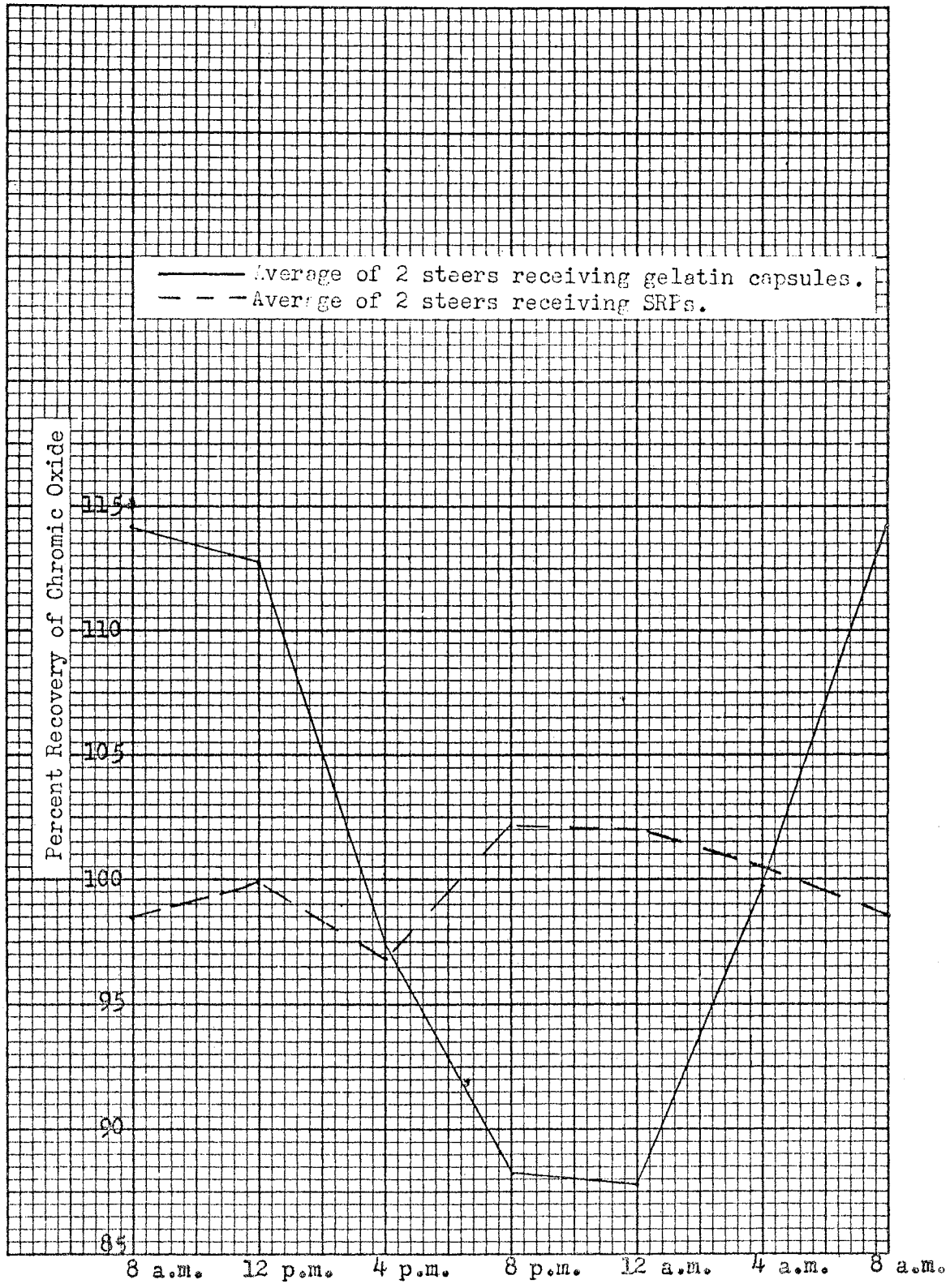

Figure 7. Percent Recovery of Ingested Chromic Oxide at Different Times of Fecal Sampling (average of 7 days)

TABLE V

CHROMIC OXIDE IN FECES (mg. Cr_2O_3/gm. D.M.) OF STEERS FED CLIPPED BERMUDA GRASS

	Sustained Release Pellet				Gelatin Capsule			
	Steer 1		Steer 4		Steer 2		Steer 3	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
Day[1]								
1	6.431	.184	6.245	.187	5.859	.986	5.509	.808
2	6.084	.228	5.446	.584	6.187	1.786	5.380	.710
3	5.913	.664	5.196	.347	5.479	.779	6.127	.403
4	6.682	.271	6.144	.182	5.148	.450	5.743	1.328
5	6.283	.314	6.548	.398	5.803	.648	5.101	1.127
6	4.884	.235	6.146	.198	5.354	1.112	4.664	1.078
$\bar{x}$	6.046	.316	5.954	.316	5.638	.960	5.621	.909
Time of Coll.								
8 am	5.948	.548	6.014	.539	6.214	.203	6.546	.455
12 pm	5.984	.860	6.024	.336	6.592	.921	6.014	.810
4 pm	5.893	.738	5.768	.657	5.512	.557	5.180	.401
8 pm	6.182	.709	6.103	.529	4.758	.871	4.800	.800
12 am	6.105	.742	6.029	.699	5.178	.811	4.441	.949
4 am	6.038	.728	5.787	.769	5.659	.897	5.418	.938

[1] Time variable fixed and not random.

excreted and the chromic oxide concentration of the feces grab samples were computed. The correlation values were .253, -.197, -.061, and .292 for steers 1, 2, 3 and 4, respectively. None of these correlations differed significantly from zero. Coefficient of variation values were: steer 1, 6.25; steer 2, 4.00; steer 3, 0.36; and steer 4, 8.41 percent. It is evident that the amount of variation in fecal chromic oxide concentration accounted for by the quantity of fecal excretion is of little consequence.

The recovery of chromic oxide in the feces is shown in Table VI. The recovery for animals receiving chromic oxide in the loose form was essentially 100 percent. However, the recovery of chromic oxide for animals receiving the SRPs was 95.23 percent for steer 1 and 95.22 percent for steer 4. This low recovery is probably a result of regurgitation and loss of a portion of the chromic oxide pellets. On two occasions, parts of the pellets were found in the feed boxes. Low recovery of chromic oxide administered in this form has also been reported by Pigden et al. (1959) and Troelson (1961).

A comparison of results using various methods of calculating ratio digestibility is shown in Table VII. With steers 2 and 3, digestion coefficients calculated by the chromic oxide method agree closely with those determined by the conventional method. This was true whether the chromic oxide concentration was determined by total collection or by appropriate grab samples of feces. With steers 1 and 4, recovery of chromic oxide in the feces was low, and therefore, digestibility coefficients calculated by the chromic oxide method were slightly lower than those determined by the conventional method.

Table VIII shows the results obtained in using the chromic oxide method closely approximated the measured fecal output. However, estimates of

TABLE VI

RECOVERY OF INDICATORS
(percent)

Animal	Chromic Oxide				Chromogen		Lignin
	Gelatin Capsule		S.R.P.				
	Total[1]	G. S.[2]	Total[1]	G. S.[2]	406 mu	414 mu	
Steer 1			95.23	95.92	146.16	138.22	73.04
Steer 4			95.22	95.97	144.41	147.15	74.93
Steer 2	99.97	99.91			154.52	151.51	73.01
Steer 3	100.20	102.53			147.89	150.50	78.85
$\bar{x}$ =	100.08	101.22	95.22	95.94	148.24	146.84	74.96

[1] Total collection of feces.
[2] Grab samples of feces taken at 8:00 a.m. and 8:00 p.m.

TABLE VII

COEFFICIENTS OF APPARENT DIGESTIBILITY CALCULATED BY SEVERAL METHODS

Animal	Constituent	Coefficients of Apparent Digestibility, percent							
			Chromic Oxide Ratio						
			Gelatin Capsule		SRP		Chromogen Ratio		
		Conventional	Total[1]	G. S.[2]	Total[1]	G. S.[2]	406 mu	414 mu	Lignin Ratio
Steer 1	D.M.	53.07			50.79	50.87	67.90	66.05	35.76
	Protein	55.05			52.86	52.94	69.25	67.48	38.46
	E.E.	9.40			4.98	5.14	38.01	34.45	-24.04
	C.F.	53.26			50.99	51.07	68.02	66.19	36.02
	N.F.E.	60.14			58.20	58.27	72.73	71.17	45.44
Steer 4	D.M.	53.39			51.10	51.40	67.72	68.32	37.79
	Protein	54.20			51.94	52.24	68.28	69.66	38.87
	E.E.	35.82			32.78	33.08	55.56	56.39	14.35
	C.F.	56.98			54.86	55.14	70.21	70.76	42.58
	N.F.E.	58.03			55.96	56.24	70.94	71.48	43.99
Steer 2	D.M.	52.63	52.64	52.64			69.35	68.74	35.12
	Protein	54.88	54.89	54.89			70.80	70.22	38.20
	E.E.	14.37	14.39	14.39			44.59	43.49	-17.28
	C.F.	54.40	54.40	54.40			70.49	69.90	37.53
	N.F.E.	57.89	57.90	57.90			72.75	72.21	42.33
Steer 3	D.M.	53.74	53.83	54.96			68.72	69.26	41.43
	Protein	50.69	50.80	52.00			66.66	67.24	37.47
	E.E.	18.51	18.67	20.66			44.90	45.85	- 3.35
	C.F.	56.31	56.40	57.47			70.46	70.97	44.59
	N.F.E.	59.89	59.98	61.06			72.89	73.36	49.14

TABLE VII (Continued)

$\bar{X}$	D.M.	53.21	53.22	53.80	50.94	51.14	68.42	68.09	37.52
	Protein	53.70	52.84	53.44	52.40	52.59	68.75	68.65	38.25
	E.E.	19.52	16.53	17.52	18.88	19.11	45.76	45.05	- 7.58
	C.F.	55.24	55.40	55.94	52.92	53.10	69.80	69.46	40.18
	N.F.E.	58.99	58.94	59.48	57.08	57.26	72.33	72.06	45.22

[1] Total collection of feces.
[2] Grab samples of feces taken at 8:00 a.m. and 8:00 p.m.

TABLE VIII

THE USE OF INDICATORS TO MEASURE FECAL OUTPUT AND DRY MATTER INTAKE

Steer Number	Fecal Output (gm.)			Dry Matter Intake (gm.)						
		Chromic Oxide			Chromogen-Cr_2O_3				Lignin-Cr_2O_3	
					406 mu		414 mu			
	Actual	Total[1]	G. S.[2]	Actual	Total[1]	G. S.[2]	Total[1]	G. S.[2]	Total[1]	G. S.[2]
				Chromic Oxide in Sustained Release Pellet						
1	9,266	9,729	9,708	19,746	30,308	30,244	28,654	28,595	15,145	15,112
4	9,181	9,641	9,587	19,698	29,867	29,700	30,432	30,262	15,478	15,391
$\bar{x}$	9,224	9,685	9,648	19,722	30,088	29,972	29,543	29,428	15,312	15,252
				Chromic Oxide in Gelatin Capsule						
2	9,390	9,394	9,394	19,824	30,649	30,649	30,051	13,051	14,479	14,479
3	9,207	9,188	9,138	19,902	29,373	29,214	29,889	29,727	15,687	15,602
$\bar{x}$	9,298	9,291	9,266	19,863	30,011	29,932	29,970	29,889	15,083	15,040

[1] Total collection of feces.
[2] Grab samples of feces taken at 8:00 a.m. and 8:00 p.m.

fecal output determined by the chromic oxide method with steers 1 and 4 were higher than the actual output because of the incomplete recovery of chromic oxide.

Chromogens as an indicator. To study the effect of light on chromogen solutions, extracts were prepared from fecal samples from four steers receiving a clipped bermuda grass ration. In Table IX the effect of light exposure on the O.D. readings of the chromogen solutions is shown. Exposure to light caused a decrease in the O.D. readings of the solutions. This decrease was evident 1 hour after extraction. The O.D. values of solutions exposed to light decreased with time. The solutions kept in darkness decreased in O.D. at 24 hours and at 7 days after extraction but not at 1 hour.

The results of this study are not in agreement with those reported by Kane and Jacobson (1955) who indicated that light exposure resulted in an increase in O.D. of fecal chromogen solutions.

For the calculation of digestibility and dry matter intake, chromogen solutions were prepared by acetone extraction of the fecal and grass samples. The absorption spectra of the fecal chromogen solutions indicated a well-defined maximum absorption at 414 mu wavelength (Figure 8). Similar results were reported by Kane and Jacobson (1954). These workers suggested calculation of digestion coefficients from O.D. readings at the point of maximum absorption rather than 406 mu as was suggested by Reid et al. (1950 and 1952). In light of this, digestion and intake values were calculated from O.D. readings at wavelengths of both 406 and 414 mu.

The absorption spectra of the grass extracts (Figure 8) differed markedly from that of the fecal extracts. The point of maximum absorption occurred at 430 mu and was not as well defined as the point of maximum

TABLE IX

EFFECT OF LIGHT ON FECAL CHROMOGEN SOLUTIONS

Wavelength mu	0 Hour Chromogen Units	1 Hour Chromogen Units	1 Hour Percent Decrease[1]	24 Hour Chromogen Units	24 Hour Percent Decrease[1]	7 Days Chromogen Units	7 Days Percent Decrease[1]
			Samples Kept in Dark				
406	806[2] ± 49.48[3]	806 ± 49.48	0.00	767 ± 49.26	4.84	733 ± 34.72	9.06
414	1,019 ± 58.60	1,019 ± 58.60	0.00	1,000 ± 43.01	1.86	978 ± 43.20	4.02
			Samples Exposed to Light				
406	806 ± 51.57	722 ± 55.16	4.21	700 ± 56.35	13.15	569 ± 46.90	29.40
414	1,017 ± 47.26	922 ± 48.22	2.46	919 ± 43.95	9.64	689 ± 44.34	32.25

[1] Percent decrease from 0 hour.
[2] Each value is the average of 4 observations.
[3] Standard error of mean.

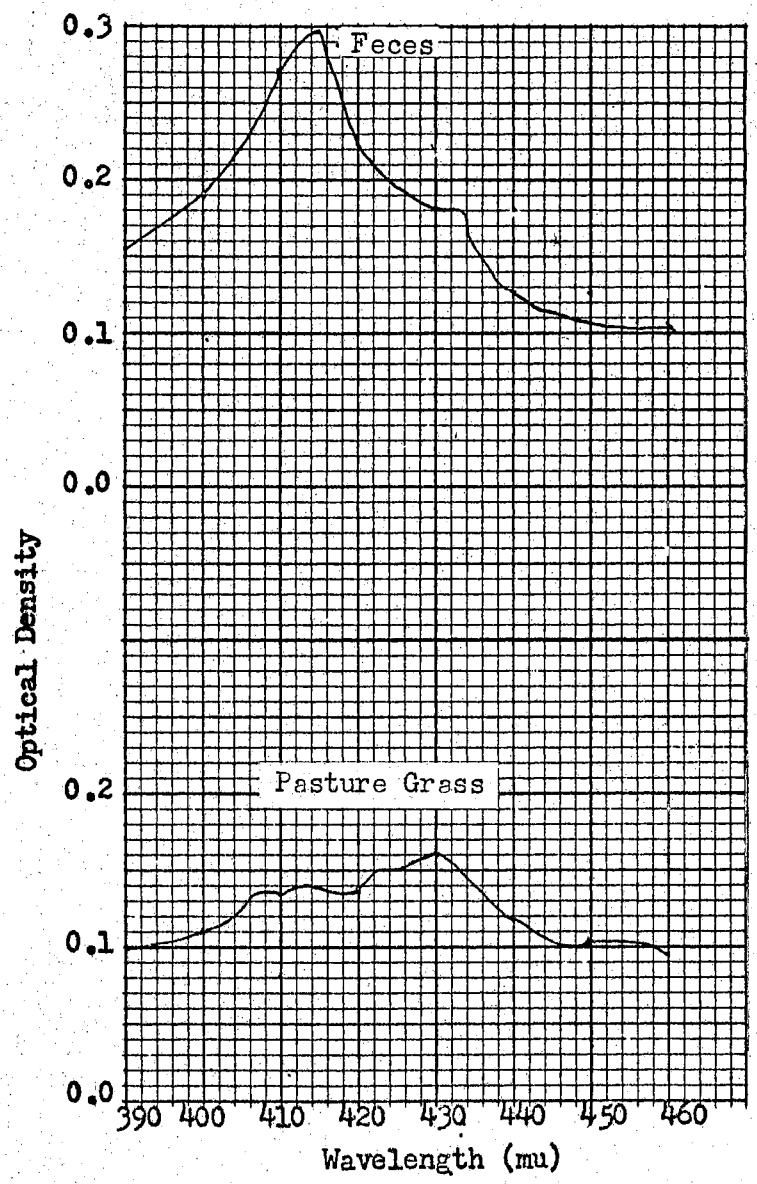

Figure 8. Absorption Spectra of Acetone Extracts

absorption of the fecal extracts. Reid et al. (1950) reported a similar absorption spectra for grass extracts.

Table VI gives the recovery of the various indicators studied in this trial. The recovery of chromogens at wavelengths of either 406 or 414 mu greatly exceeded 100 percent. This may indicate that low results were obtained in determining chromogens in the grass fed. It was noted that when chromogen extracts of the grass samples were being prepared that regardless of the number of extractions preformed on an individual sample, complete removal of the chromogens seemed impossible. This is in contrast to the fecal samples where three extractions per sample were apparently sufficient to completely remove the chromogens. Woolfolk (1950) reported similar problems in extracting chromogens from mature grasses.

Since calculated recovery of chromogens exceeded 100 percent, digestion coefficients calculated by the chromogen ratio method were higher than those determined by the conventional method (Table VII). Likewise, dry matter intake calculated by the chromogen ratio-chromic oxide method was higher than the actual intake (Table VIII). It is therefore apparent that the chromogen ratio technique of determining digestibility and intake was not successful in this trial.

The estimation of the chromogen content of the grass from the chromogen content of the feces by the equation given by Reid et al. (1952) requires calibration of the spectrophotometer used in our laboratory with that used by Reid. The calibration data are not presently available; therefore, calculations using the fecal chromogen method have not been made.

Lignin as an indicator. Lignin determinations were made on grass and fecal samples by a combination of methods described by Phillips and Smith

(1943) and Thacker (1954). The recovery of ingested lignin (Table VI) in the feces (total collection) was consistently low for individual steers. The average recovery of lignin for the four steers was 74.96 percent. Incomplete recovery of lignin has also been reported by Bondi and Meyer (1943), Crampton and Maynard (1938), Csonki et al. (1929), Davis et al. (1947), Hale et al. (1940) and Kane et al. (1953).

As a result of the low recovery of lignin, digestion coefficients calculated by the lignin ratio method were lower than those determined by the conventional method. Also, dry matter intake values calculated by the combination lignin ratio-chromic oxide method were also lower than the actual intake.

It is apparent that in this trial with bermuda grass, lignin was not an acceptable indicator for the calculation of digestion coefficients and dry matter intake. Whether or not this would hold true for bermuda grass at other stages of maturity is not known. Pazur and DeLong (1948) reported that the metabolism of lignin is affected by the stage of maturity of the grass.

Summary and Conclusions

Trial I was designed to study a method of lessening diurnal variation in fecal excretion of ingested chromic oxide by grazing steers. The chromic oxide was administered in loose form (gelatin capsule) and in the form of a sustained release pellet (SRP). The chromic oxide was given at 8:00 a.m. daily and grab samples of feces were taken six times daily at 4-hour intervals. Two 3-day collection periods were conducted.

When steers received the SRP, the maximum average deviations from the average chromic oxide concentration were: steer 1, 88.05-110.78 percent and steer 2, 84.03-114.59 percent. When the steers received chromic oxide in gelatin capsules, the values were: steer 1, 71.06-137.69 percent and steer 2, 76.88-126.12 percent. The average standard deviation from the daily average fecal chromic oxide concentration was .253 for steer 1 and .219 for steer 2, when they received the SRP. When the chromic oxide was given in the gelatin capsules, the standard deviations were .903 for steer 1 and .886 for steer 2.

When steers received the gelatin capsules, the chromic oxide concentration in grab samples taken at 4:00 a.m. and 4:00 p.m. closely approximated the average concentration of samples taken throughout the collection period. When the steers received the SRP, time of fecal sampling was of minor importance since diurnal variation was relatively small.

Collection of total feces was not made; therefore, recovery of chromic oxide could not be measured. However, the concentration of chromic oxide in the fecal dry matter when the steers received gelatin capsules was nearly twice the concentration of chromic oxide when the SRPs were administered. The amount of chromic oxide administered was the same in all cases. Therefore, the recovery of chromic oxide when given in SRP form was apparently about 50 percent. While it appears that the SRP does lessen diurnal variation, further studies must be undertaken to determine the reason for the low recovery of the chromic oxide before the use of the SRP can be recommended.

Examination of the chromogen content of a number of grab samples chosen at random showed little variation in chromogen concentration of samples taken at various times. Apparently, diurnal variation in chromogen

excretion does not pose a problem.

Trial II was designed to compare various indicator methods of calculating digestibility and dry matter intake with the conventional method. The chromic oxide recovery and excretion patterns for steers were determined and the relative merit of two methods of administering chromic oxide were compared. The possible relationship between amount of dry matter in a grab sample (total content of rectum) and its concentration of chromic oxide was investigated.

Four yearling Hereford steers received 20 pounds of freshly clipped Midland bermuda grass once daily. At 8:00 a.m. each day, steers 1 and 4 were given chromic oxide in SRP form and steers 2 and 3 were given chromic oxide in gelatin capsules. Grab samples of feces were taken six times daily at 4-hour intervals. Total feces were collected over each 24-hour period. The collection period was 7 days in duration.

With steers 1 and 4, the range in the relative concentration of chromic oxide in grab samples taken at various times was 95.51-104.53 and 97.85-102.57 percent, respectively. The average standard deviation from the daily mean chromic oxide concentrations was .316 for each steer. The range in relative chromic oxide concentration for steers 2 and 3 was 87.16-112.75 and 84.35-115.85 percent, respectively. The average standard deviation from the daily mean chromic oxide concentration was .960 for steer 2 and .909 for steer 3. Recovery of chromic oxide from total feces was 95.23, 99.97, 100.20 and 95.22 percent for steers 1,2,3 and 4, respectively. The low recovery for steers 1 and 4 is apparently due to regurgitation and loss of a portion of the pellets.

Grab samples taken at 8:00 a.m. and 8:00 p.m. contained approximately the same chromic oxide concentration as the total collections. However,

since diurnal variation was small in steers 1 and 4, fecal sampling time was relatively unimportant. The fact that the optimum grab sampling times are different in this trial from those in Trial I is evidence that the feeding regime may affect the excretion pattern.

The correlations between the amount of excreted dry matter and the chromic oxide concentration of grab samples were not significantly different from zero. Little if any of the diurnal variation in chromic oxide concentration could be accounted for by the amount of dry matter excreted.

The chromogen content of the grass and feces was measured at 406 and 414 mu wavelengths. Exposure of the fecal chromogen solutions to light resulted in a large and rapid decrease in the O.D. of the solutions. If the chromogen solutions were kept in darkness, O.D. readings taken at 24 hours and 7 days after extraction were only slightly lower than original readings. No trouble was encountered in the removal of the chromogens from the feces; however, complete extraction of the chromogens from the grass appeared to be impossible. The apparent recovery of chromogens in the total feces ranged from 138-154 percent. These high recovery figures, in all probability result from incomplete extraction of the chromogens from the grass.

Grass and fecal samples were also analyzed for lignin. The recovery of ingested lignin from feces of steers 1,2,3 and 4 was 73.04, 73.01, 78.85 and 74.93 percent, respectively.

Conclusions concerning the use of indicators to predict digestibility and dry matter intake from these experiments are:

1. Chromic oxide given in gelatin capsules was satisfactory as an indicator of digestibility and fecal output. However, sampling of feces irrespective of time of day was not practical because of the diurnal variation in excretion.

2. The administration of chromic oxide in SRP form eliminated the problem of diurnal variation. However, as the result of incomplete recovery of chromic oxide, digestion coefficients were lower and calculated fecal output figures higher than those determined by the conventional method.
3. The chromogen ratio technique resulted in higher digestion coefficients and higher dry matter intakes than those determined conventionally. These high results were due to the high apparent recovery of fecal chromogens.
4. Digestibility coefficients and dry matter intakes calculated by the lignin ratio method were lower than those calculated by the conventional method because of incomplete recovery of lignin in the feces.

GENERAL SUMMARY

Five trials with yearling Hereford steers were conducted to study techniques for sampling and measuring digestibility and intake of grazed forage. The first three trials were concerned with methods of sampling feed intake. Average recovery of concentrate-type feed offered to esophageal fistulated steers ranged from 89.18 to 95.48 percent. Average recovery of clipped bermuda grass and clipped native grass was 36.61 and 26.87 percent, respectively. The cannulae plugged frequently when grasses were fed. All fistula samples contained a large amount of saliva. Ash content of fistula samples was higher than that of the feed samples ($P<.01$). Other proximate constituents of fistula samples were similar to those of the feed when concentrate-type rations were fed, but with the exception of fiber were significantly different when grasses were fed.

Hand-plucked and esophageal fistula samples were compared with steers grazing a pure stand of bermuda grass. The fistula samples contained more ash and protein and less ether extract and NFE than the hand-plucked samples ($P<.01$). The former showed collection period differences in protein and NFE ($P<.01$) and animal differences in protein ($P<.05$).

Fistula samples from steers on a native grass pasture contained more ash and protein and less crude fiber and NFE than hand-plucked samples. There were animal differences in ash and protein of the fistula samples.

Indicators used to calculate forage digestibility and dry matter intake were studied in two trials. Chromic oxide given in a sustained release

pellet (SRP) to grazing steers showed less diurnal variation in fecal excretion than chromic oxide given by gelatin capsule; however, recovery was apparently less than 100 percent. Optimum feces sampling times were 4:00 a.m. and 4:00 p.m. when capsules were used. Fecal chromogen excretion did not show diurnal variation.

SRP gave less diurnal variation than the capsule when given to steers fed clipped bermuda grass in metabolism stalls; however, recovery of chromic oxide in SRPs was only 95 percent. Chromic oxide in gelatin capsules was a satisfactory indicator of digestibility and fecal output if total feces were collected or grab samples were taken at 8:00 a.m. and 8:00 p.m. SRP yielded low digestibility coefficients and high fecal output values.

Chromogens were not a satisfactory indicator of digestibility or dry matter intake, recoveries in feces being 138-154 percent. Recovery of lignin in feces was low, ranging from 73.01 to 78.85 percent, therefore, lignin was not a satisfactory indicator.

LITERATURE CITED

Archibald, J. G., D. F. Owen, H. Fenner, and H. D. Barnes. 1958. Comparison of chromium ration and lignin ratio techniques for determination of digestibility of hays. J. Dairy Sci. 41:1100.

Balch, C. C., J. T. Reid, and W. J. Stroud. 1957. Factors influencing rate of excretion of administered chromic oxide by steers. British J. Nutr. 11:184.

Barnicoat, C. R. 1945. Estimation of apparent digestion coefficients by means of an inert reference-substance. New Zealand J. Sci. Tech. 27:202.

Bath, D. L., W. C. Weir, and D. T. Torell. 1956. The use of the esophageal fistula for the determination of consumption and digestibility of pasture forage by sheep. J. Animal Sci. 15:1166.

Bloom, S., N. L. Jacobson, R. S. Allen, L. D. McGilliard, and P. G. Homeyer. 1957. Effects of various hay-concentrate ratios on nutrient utilization and production responses of dairy cows. II. Observations on ration digestibility and on the excretion pattern of chromic oxide. J. Dairy Sci. 40:240.

Bondi, A. H. and H. Meyer. 1943. On the chemical nature and digestibility of roughage carbohydrates. J. Agr. Sci. 33:123.

Bondi, A. and H. Meyer. 1947. Lignin in young plants. Biochem. J. 43:248.

Bradley, N. W., R. M. Forbes, W. W. Albert, G. E. Mitchell, Jr., and A. L. Neumann. 1958. Use of the chromic oxide method for determining digestible energy and protein in complete pelleted steer rations. J. Animal Sci. 17:1199. (Abstr.).

Brannon, W. F., J. T. Reid and J. I. Miller. 1954. The influence of certain factors upon the digestibility and intake of pasture herbage by beef steers. J. Animal Sci. 13:535.

Brisson, G. J., W. J. Angus, and P. E. Sylvestre. 1954. Plant pigments as internal indicators of digestibility of dry matter of pasture herbage. Can. J. Agr. Sci. 34:52.

Brisson, G. J. and G. Hatina. 1957. A method for the extraction of pigments (chromogens) from feces of cattle and sheep. Can. J. Agr. Sci. 37:136.

Brisson, G. J., W. J. Pigden, and P. E. Sylvestre. 1957. Effect of frequency of administration of chromic oxide on its fecal excretion pattern by grazing cattle. Can. J. Agr. Sci. 37:90.

Carter, T. F., D. W. Bolin, and D. Erikson. 1960. The evaluation of forages by the agronomic "difference" method and the chromogen-chromic oxide "indicator" technique. N. Dak. Agr. Exp. Sta. Bul. 426.

Chanda, R., H. M. Claphan, M. L. McNaught, and E. C. Owen. 1951. The use of chromium oxide to measure the apparent digestibility of carotene by goats and cows. British J. Nutr. 5:4.

Clawson, A. J., J. T. Reid, B. E. Sheffy, and J. P. Willman. 1955. Use of chromium oxide in digestion studies with swine. J. Animal Sci. 14:700.

Cook, C. W. and J. L. Harris. 1950. The nutritive content of the grazing sheep diet on summer and winter ranges in Utah. Utah. Agr. Exp. Sta. Bul. 342.

Cook, C. W. and L. E. Harris. 1951. A comparison of the lignin ratio technique and the chromogen method of determining digestibility and forage consumption of desert range plants by sheep. J. Animal Sci. 10:565.

Cook, C. W., J. L. Harris and L. A. Stoddart. 1948. Measuring the nutritive content of a foraging sheep's diet under range conditions. J. Animal Sci. 7:170.

Cook, C. W., J. Mattox, and L. Harris. 1961. Comparative daily consumption of summer range forage by wet and dry ewes. J. Animal Sci. 20:866.

Cook, C. W. and L. A. Stoddart. 1961. Nutrient intake and livestock responses on seeded foothill ranges. J. Animal Sci. 20:36.

Cook, C. W., L. A. Stoddart, and L. Harris. 1951. Measuring the consumption and digestibility of winter range plants in sheep. J. Range Management 4:335.

Cook, C. W., L. A. Stoddart, and L. E. Harris. 1952. Determining the digestibility and metabolizable energy of winter range plants by sheep. J. Animal Sci. 11:578.

Cook, C. W., K. Taylor, and L. E. Harris. 1962. The effect of range condition and intensity of grazing upon daily intake and nutritive value of the diet on desert ranges. J. Range Management. 15:1.

Cook, C. W., J. L. Thorne, J. T. Blake, and J. Edlefsen. 1958. Use of an esophageal-fistula cannula for collecting forage samples by grazing sheep. J. Animal Sci. 17:189.

Corbett, J. L., J. F. Greenhalgh, I. McDonald, and E. Florence. 1960. Further studies on the administration of chromic oxide as a component of paper. British J. Nutr. 14:289.

Crampton, E. W. 1939. The nutritive value of pasture herbage. Sci. Agr. 19:345.

Crampton, E. W. 1957. Interrelationships between digestible nutrient and energy content, voluntary dry matter intake, and overall feeding value of forages. J. Animal Sci. 16:546.

Crampton, E. W. and L. E. Lloyd. 1951. Studies with sheep on the use of chromic oxide as an index of digestibility of ruminant rations. J. Nutr. 45:319.

Crampton, E. W. and L. A. Maynard. 1938. The relation of cellulose and lignin content to the nutritive value of animal feeds. J. Nutr. 15:383.

Croker, B. H. 1959. A method of estimating the botonical composition of the diet of sheep. New Zealand J. Agr. Res. 2:72.

Csonka, F. A., M. Phillips, and D. B. Jones. 1929. Studies in lignin metabolism. J. Biol. Chem. 85:65.

Davis, C. L., J. H. Byers, and L. E. Luber. 1958. An evaluation of the chromic oxide method for determining digestibility. J. Dairy Sci. 41:152.

Davis, R. E. and C. O. Miller, and I. L. Lindahl. 1947. Apparent digestion by sheep of lignin in Pea and Lima bean vines. J. Agr. Res. 74:285.

Edin, H. 1918. "Orienterande foroak over en pa 'ledkroppsprinciper' grumdad method att bestamma en foderblandnings smalbarhet. Centralansten for forsoksvasendet pa jordbruksomradet." Stockholm Medd. Nr. 165:1 (Cited by Edin et al., 1944).

Edin, H., G. Kihlen, and S. Nordfeldt. 1944. A summarized description of 'Edin's indicator method' for the determination of the digestion of feeds and feed mixtures. Lantbrukshogskolans Husdjursforsoksanstalt. 12:166.

Edlefsen, J., C. W. Cook, and J. T. Blake. 1960. Nutrient content of diet as determined by hand plucked and esophageal fistula samples. J. Animal Sci. 19:560.

Elam, C. J. and R. E. Davis. 1959. Chromic oxide excretion on a pelleted ration. J. Animal Sci. 18:718.

Elam, C. J. and R. E. Davis. 1961. Lignin excretion by cattle fed a mixed ration. J. Animal Sci. 20:484.

Ellis, G. H., G. Matrone, and L. A. Maynard. 1946. A 72% H_2SO_4 method for determination of lignin and its use in animal nutrition studies. J. Animal Sci. 5:285.

Forbes, E. B., R. Elliott, R. Swift, W. James, and V. Smith. 1946. Variation in determination of digestive capacity of sheep. J. Animal Sci. 5:298.

Forbes, R. M. and W. P. Garrigus. 1948. Application of a lignin ratio technique to the determination of the nutrient intake of grazing animals. J. Animal Sci. 7:373.

Forbes, R. M. and W. P. Garrigus. 1949. The digestibility and metabolizability by lambs of a standard ration of alfalfa and corn and one containing cottonseed hulls. J. Agr. Res. 78:483.

Forbes, R. M. and W. P. Garrigus. 1950. Some effects of forage composition on its nutritive value when cut and fed green to steers, as determined conventionally and by lignin ratios. J. Animal Sci. 9:531.

Hale, E. B., C. W. Duncan, and C. F. Huffman. 1940. Rumen digestion in the bovine with some observations on the digestibility of alfalfa hay. J. Dairy Sci. 23:953.

Hale, E. B., C. W. Duncan, and C. F. Huffman. 1947. Rumen digestion studies. J. Nutr. 34:747.

Halls, L. K. 1954. The approximation of the cattle diet through diet sampling. J. Range Management. 7:269.

Hamilton, P., B. Plumer, and H. Dickey. 1955. Measures of digestibility in the ruminant. Maine Agr. Exp. Sta. Bul 543.

Hardison, W. A., and J. T. Reid. 1953. Use of indicators in measurement of the dry matter intake of grazing animals. J. Nutr. 51:35.

Hardison, W. A., J. T. Reid, C. M. Martin, and P. G. Woolfolk. 1954. Degree of herbage selection by grazing cattle. J. Dairy Sci. 37:89.

Heady, H. F. and D. T. Torell. 1959. Forage preference exhibited by sheep with esophageal fistulas. J. Range Management. 12:28.

Hill, K. R., W. W. Repp, W. E. Watkins, and J. H. Knox. 1961. Estimation of feed intake and digestion by 4, 6 and 24-hour fecal collections using lignin as an indicator with heifers. J. Animal Sci. 20:667. (Abstr.).

Hooper, T. and L. Nesbitt. 1930. The chemical composition of some North Dakota pasture and hay grasses. N. Dak. Agr. Exp. Sta. Bul. 236.

Irvin, H. M., H. G. Wiseman, J. C. Shaw, and L. A. Moore. 1953. The role of plant pigments in digestion trial studies. J. Animal Sci. 12:541.

Irwin, M. I. and E. W. Crampton. 1950. The use of chromic oxide as an index material in digestion trials with human subjects. J. Nutr. 43:77.

Kameoka, K., S. Takahashi, and H. Morimoto. 1956. Variation in the excretion of chromic oxide by ruminants. J. Dairy Sci. 39:462.

Kane, E. A., R. E. Ely, W. C. Jacobson, and L. A. Moore. 1953a. A comparison of various digestion trial techniques with dairy cattle. J. Dairy Sci. 36:325.

Kane, E. A., and W. C. Jacobson. 1954. An improved method of using plant pigment as an indicator of digestibility. J. Dairy Sci. 37:672. (Abstr.).

Kane, E. A. and W. C. Jacobson. 1955. The effect of light on optical density of plant pigment extracts of feces in digestion studies. J. Dairy Sci. 38:625. (Abstr.).

Kane, E. A., W. C. Jacobson, R. E. Ely, and L. A. Moore. 1953b. The estimation of dry matter consumption of grazing animals by ratio techniques. J. Dairy Sci. 36:637.

Kane, E. A., W. C. Jacobson, and L. A. Moore. 1950. A comparison of techniques used in digestion studies with dairy cattle. J. Nutr. 41:583.

Kane, E. A., W. C. Jacobson, and L. A. Moore. 1951. Diurnal variation in the excretion of chromic oxide and lignin. J. Nutr. 47:263.

Kay, R. N. B. and A. T. Phillipson. 1957. Salivary secretion of anesthetized sheep during distention of the rumen and esophagus. J. Physiol. 139:7.

Kennedy, P. B. and S. C. Dinsmore. 1909. Digestion experiments on the range. Nev. Agr. Exp. Sta. Bul. 71.

Kimura, F. T. and V. L. Miller. 1957. Improved determination of chromic oxide in cow feed and feces. J. Agr. Food Chem. 5:216.

Lesperance, A. L., V. R. Bohman, and D. W. Marble. 1960a. Development of a technique for evaluating grazed forage. J. Dairy Sci. 43:682.

Lesperance, A. L., E. H. Jensen, V. R. Bohman, and R. A. Madsen. 1960b. Measuring selective grazing with fistulated steers. J. Dairy Sci. 43:1615.

Linkous, W. N., W. A. Hardison, G. C. Graf, and R. W. Engel. 1954. Fecal chromic oxide concentration in twelve dairy cows as related to time and frequency of administration and to feeding. J. Animal Sci. 13: 1009. (Abstr.).

Mahaffey, J. C., W. J. Miller, J. D. Donher, and M. L. Dalton. 1954. Effect of certain factors in diurnal excretion pattern of chromic oxide. J. Dairy Sci. 37:672. (Abstr.).

McCullough, M. E. 1959. Conditions influencing forage acceptability and rate of intake. J. Dairy Sci. 42:571.

McCullough, M., R. F. Elliott, and G. M. Bastin. 1951. Use of the chromogen(s) technique in the determination of seasonal changes in digestibility of dry matter and protein of pasture herbage. J. Dairy Sci. 34:494. (Abstr.).

McDugall, E. I. 1948. Studies of rumen saliva. I. Composition and output of sheep saliva. Biochem. J. 43:99.

McManus., W. R. 1961. Properities of roughage feedstuffs collected from oesophageal fistulas. Aus. J. Exp. Agr. Animal Husb. 1:159.

Mertz, E. T. 1959. Elementary Biochemistry. 1st. Ed. Burgen Pub. Co., Minneapolis, Minn.

Meyer, J. H., G. P. Lofgreen, and J. H. Hull. 1957. Selective grazing by sheep and cattle. J. Animal Sci. 16:766. [illegible]

Meyer, J. H., G. P. Lofgreen, and N. R. Ittner. 1956. Further studies on the utilization of alfalfa by beef steers. J. Dairy Sci. 15:64.

Nelson, A. B., A. D. Tillman, W. D. Gallup, and R. MacVicar. 1954. A modified metabolism stall for steers. J. Animal Sci. 13:504.

Noller, C. H., D. L. Hill, and N. S. Lundquist. 1951. Observations on the use of the chromogen technique in determining forage consumption of milking cows. J. Animal Sci. 10:1072. (Abstr.).

Pazur, J. H., and W. A. DeLong. 1948. Effect of lignin content and stage of maturity of dry clover forage on the urinary excretion of aromatic acid by sheep. Sci. Agr. 28:39.

Peterson, M. L., G. P. Lofgreen, and J. H. Meyer. 1956. A comparison of the chromogen and clipping methods for determining the consumption of dry matter and total digestible nutrients by beef steers on alfalfa pasture. Agron. J. 48:560.

Phillips, T. G. and T. O. Smith. 1943. The composition of timothy. Part I. Young grass and hay. N. H. Agr. Exp. Sta. Tech. Bul. 81.

Pigden, W. J. and G. Brisson. 1956. Effect of frequency of administration of chromic oxide on its fecal excretion pattern by grazing wethers. Can. J. Agr. Sci. 36:146.

Pigden, W. J. and G. J. Brisson. 1957. Chromic oxide in sustained-release pellet to reduce variation in its diurnal excretion pattern in cattle. Can. J. Animal Sci. 47:185.

Pigden, W. J., G. J. Brisson, K. A. Winter, and G. I. Pritchard. 1959. A sustained release pellet for oral administration of chromic oxide and physiologically active compounds to ruminants. Proc. Can. Soc. Animal Prod.

Pigden, W. J. and J. E. Stone. 1952. The effect of ruminant digestion on forage lignin. Sci. Agr. 32:502.

Putnam, P. A., J. K. Loosli and R. G. Warner. 1958. Excretion of chromic oxide by dairy cows. J. Dairy Sci. 41:1723.

Putnam, P. A., J. K. Loosli, R. G. Warner, and S. R. Sarle. 1957. Diurnal excretion of chromic oxide and ash by dairy cows. J. Dairy Sci. 40:618. (Abstr.).

Raymond, W. F., C. D. Kemp, A. W. Kemp, and C. E. Harris. 1954. Studies in the digestibility of herbage. IV. The use of fecal collections and chemical analysis in pasture studies. (b) Fecal index methods. British Grassland Soc. J. 9:69.

Reid, J. T., P. Woolfolk, W. Hardison, C. M. Martin, A. Brundage, and R. Kaufmann. 1952. A procedure for measuring the digestibility of pasture forage under grazing conditions. J. Nutr. 46:255.

Reid, T. J., P. G. Woolfolk, C. R. Richards, J. K. Loosli, K. L. Turk, J. I. Miller, and R. E. Blaser. 1949. A new indicator method for determination of digestibility and consumption of forages by ruminants. J. Animal Sci. 8:636. (Abstr.).

Reid, J. T., P. G. Woolfolk, C. R. Richards, R. W. Kaufmann, J. K. Loosli, K. L. Turk, J. I. Miller, and R. E. Blaser. 1950. A new indicator method for the determination of digestibility and consumption of forages by ruminants. J. Dairy Sci. 33:60.

Richards, C. R., G. F. Haenlein, and W. G. Crothers. 1959. The effect of forage ash and low chromogen level of feces upon the determination of digestion by the fecal chromogen method. J. Animal Sci. 18:683.

Rusoff, L. L. and L. E. Foote. 1961. An unbreakable esophageal-fistula cannula for collecting forage samples by grazing cows. Proc. Assn. So. Agr. Workers. P.118.

Saltonstall, L. 1948. The measurement of the quantity and quality of pasture herbage consumed by sheep. A technique study. Ph.D. Thesis, Cornell University, Ithaca, N. Y.

Schurch, A. F., E. Crampton, S. R. Haskell, and L. E. Lloyd. 1952. The use of chromic oxide in digestion studies with pigs fed ad. lib. in the barn. J. Animal Sci. 11:261.

Schurch, A. E., L. E. Lloyd, and E. W. Crampton. 1950. The use of chromic oxide as an index for determining the digestion of a diet. J. Nutr. 41:629.

Schnider, B. H., B. K. Soni, and W. E. Ham. 1955. Methods for determining consumption and digestion of pasture forages by sheep. Wash. Agr. Exp. Sta. Tech. Bul. 16.

Smart, W., G. Matrone, and V. Smart. 1954. Use of copper derivatives of chlorophylls in a ratio method to estimate digestibility of forages. J. Agr. Food Chem. 2:1331.

Smart, W. G., F. W. Sherwood, G. Matrone, and G. H. Wise. 1953. Pigments involved in the chromogen(s) ratio method. J. Agr. Food Chem. 1:318.

Smith, A. M. 1955. The use of chromic oxide and plant chromogens for determining intake of pasture herbage by grazing dairy cows. M.Sc. Thesis, Cornell University, Ithaca, N. Y.

Smith, A. M. and J. T. Reid. 1955. Use of chromic oxide as an indicator of fecal output for the purpose of determining the intake of pasture herbage by grazing cows. J. Dairy Sci. 38:515.

Snedecor, G. W. 1956. Statistical Methods. 5th Ed. The Iowa State College Press, Ames, Iowa.

Soni, B., F. R. Murdock, A. S. Hodgson, T. H. Blosser, and K. Mahanta. 1954. Dirunal variation in the estimates of digestibility of pasture forage using plant chromogens and fecal nitrogen as indicators. J. Animal Sci. 13:474.

Squibb, R. L., C. Rivera, and R. Jarquin. 1958. Comparison of chromogen method with standard digestion trial for determination of digestible nutrient content of Kikagu and Ramie forages with sheep. J. Animal Sci. 17:318.

Swift, R. W., E. J. Thacker, A. Black, J. W. Bratzler, and W. J. James. 1947. Digestibility of rations for ruminants as affected by proportion of nutrient. J. Animal Sci. 6:432.

Thacker, E. J. 1954. A modified lignin procedure. J. Animal Sci. 13: 501.

Torell, D. T. 1954. An esophageal fistula for animal nutrition studies. J. Animal Sci. 13:878.

Torell, D. T. and W. C. Weir. 1959. The effect of rotational grazing on animal nutrition. J. Animal Sci. 18:1177. (Abstr.).

Troelsen, J. E. 1961. Preliminary observations in the use of a sustained release pellet for administration of chromic oxide to sheep in digestion studies. Can. J. Animal Sci. 41:71.

Weir, W. C. and D. T. Torell. 1959. Selective grazing by sheep as shown by a comparison of the chemical composition of range and pasture forage obtained by hand clipping and that collected by esophageal fistulated sheep. J. Animal Sci. 18:641.

Woolfolk, P. G. 1950. Development and evaluation of the chromogen method for determining digestibility and consumption of feeds by ruminants. Ph.D. Thesis, Cornell University, Ithaca, N. Y.

Woolfolk, P. G., C. R. Richards, R. Kaufmann, C. Martin, and J. T. Reid. 1950. A comparison of fecal nitrogen excretion rate, chromic oxide, and chromogen(s) method for evaluating forages and roughages. J. Dairy Sci. 33:385. (Abstr.).

VITA

Kenneth S. Eng, Jr.

Candidate for the Degree of

Doctor of Philosophy

Thesis: TECHNIQUES FOR SAMPLING AND MEASURING INTAKE AND DIGESTIBILITY OF GRAZED FORAGE

Biographical:

Personal Data: Born at Newman Grove, Nebraska, March 1, 1937, the son of Kenneth and Janahn Eng. Married Patricia Bredthauer, September 7, 1958; the father of two children, Laura and Julie Eng.

Education: Received the Bachelor of Science degree from the University of Nebraska, with a major in Technical Science Agriculture in June, 1958; received the Master of Science degree from the University of Nebraska, with a major in Animal Nutrition, January, 1960.

Professional Organizations: The American Society of Animal Science, Alpha Zeta, Gamma Sigma Delta and Associate Member of the Society of Sigma Xi.

Experiences: Self employed as farmer in 1954. Graduate Assistant at the University of Nebraska, 1958-1960 and at Oklahoma State University of Agriculture and Applied Science, 1960-1962

Date of Final Examination: August, 1962.