INDICATOR TECHNIQUES FOR MEASURING VOLUNTARY INTAKE AND DIGESTIBILITY OF FORAGE

By

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TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

In recent years there has been increasing interest in the area of intensified forage production for ruminants because in the foreseeable future the human population may be in serious competition with animals for the grain which is produced. This has brought about the need for a more efficient and more accurate means of forage evaluation.

There are two main factors which determine the nutritive value of a forage: (1) Voluntary intake and (2) The extent to which the nutrients contained in the forage are digested. Unfortunately, conventional methods for measuring intake and nutrient digestibility of harvested forages are not applicable to pasture conditions. This has brought the use of indicators or reference materials into extensive use. In order to determine intake, one must be able to measure or estimate digestibility and fecal output. This can be accomplished by the use of a naturally occurring plant constituent (internal indicator) to determine forage digestibility and an external indicator to estimate fecal output. There are other methods of estimating forage digestibility such as the <u>in vivo</u> nylon bag technique (Van Keuren and Heinemann, 1962) and the <u>in vitro</u> digestion technique (Tilley and Terry, 1963).

An external indicator must be an inert material which

is not destroyed in or absorbed from the digestive tract of the animal, has no undesirable physiological effect on the digestive tract, passes through the digestive system at a uniform rate, and is relatively easily determined in feed or fecal samples.

Obtaining a sample of forage which is representative of that being consumed by the grazing animals has also been a problem. The common methods of forage sampling such as hand clipping or hand plucking give samples that are higher in crude fiber and lower in crude protein than those selected by the animals (Hardison et al., 1954 and Eng, 1962). Another technique, the esophageal fistula, has been unsatisfactory in some cases as a means of sampling grazed forage (Eng, 1962). One method which has been used successfully and shows promise is the rumen evacuation technique using rumen fistulated steers (Lesperance et al., 1960). Forage samples taken from a previously evacuated rumen differed only slightly in chemical composition from the forage before being eaten. The major difference was a higher ash content due to saliva contamination.

This thesis reports the findings of an experiment designed to study voluntary intake of cattle grazing under normal pasture conditions and to relate intake to forage properties and digestibility. In addition, two total collection digestion trials were conducted in metabolism stalls using rumen fistulated steers to compare various internal and external indicators for use in future grazing trials.

REVIEW OF LITERATURE

External Indicators

Chromic Oxide

Chromic oxide is the most widely used and accepted external indicator for nutrition studies. It was first used as an external indicator by Edin (1918). Since that time it has been used as an indicator of digestibility, as an indicator of feed intake and as a rumen marker.

Kane et al. (1952) stated that an external indicator such as chromic oxide had the following advantages for determining digestibility: (1) elimination of total collections; (2) conduction of digestion trials in the field; (3) substantial savings of time and expense; and (4) the animals are under natural conditions.

Chromic oxide has been used and reported to be successful with many different species of animals. Schurch et al. (1950) and Lloyd et al. (1955) in their work with rats, found that dry matter digestibility coefficients as calculated with chromic oxide and the conventional method did not differ significantly. Dansky and Hill (1952) using chromic oxide as an indicator of ration digestibility with chickens reported chromic oxide gave more accurate results than did the total collection method. Lloyd and McCay

(1954) reported very satisfactory results using chromic oxide to estimate digestion coefficients with horses, foxes, mink, and dogs. Huang et al. (1954) in a study with rabbits, reported close agreement between digestibility values obtained with chromic oxide and the total collection method. In work with human subjects, Irwin and Crampton (1951) reported dry matter digestibility of 88.0 percent using chromic oxide and 88.3 percent using the total collection technique. Clawson et al. (1955) found that with swine, chromic oxide gave digestion coefficients that were in close agreement with total collection values regardless of sampling time.

Most workers have reported relatively complete recovery of chromic oxide, however a few workers have reported that recovery depends to some extent upon the type of ration. Crampton and Lloyd (1951) in a study with sheep reported close agreement between digestion coefficients determined by chromic oxide and the conventional method when the ration was ground and chromic oxide uniformly mixed with the ration. However, when the ration consisted entirely of unground roughage and chromic oxide was administered once daily in a gelatin capsule, recoveries were rather poor. They also suggested that chromic oxide should be administered at least 5 days before starting to collect fecal samples.

Putnam et al. (1958) reported that the time of feeding or the proportion of roughage in the ration had no effect

on the recovery of chromic oxide; however, this does not agree with findings of other workers. Linnereud and Donker (1961) found a difference in the excretion pattern of chromic oxide in cows on pasture and cows on concentrate. They also found a difference in excretion patterns when cows were fed once or twice daily. Corbett et al. (1959) reported that a large proportion of the chromic oxide in the feces appeared to be attached to the partially digested cellulose fibers, this might explain the difference in chromic oxide excretion patterns between concentrate and all-roughage rations.

Using dairy cows fed alfalfa hay or silage, Kane et al. (1950) found chromic oxide to be a satisfactory indicator with recoveries of 99.9 percent. However, Barnicoat (1945) using various classes of livestock, found recoveries of chromic oxide to be consistently low.

The extremely variable excretion pattern of chromic oxide is recognized as a serious shortcoming of the indicator. Among the first workers to study the causes of this variation were Kane et al. (1951). These workers found that fecal concentration of chromic oxide was highest at 9:00 a.m. and lowest at 9:00 p.m. and concluded that this variation was not associated with time of feeding.

Davis et al. (1958) reported that the peak in the level of fecal chromic oxide concentration occurred from 12:00 a.m. to 6:00 p.m. and the lowest level was between 2:00 a.m. and 8:00 a.m. when administered to lactating

dairy cows. Smith and Reid (1955) in studies with grazing cattle reported a low fecal chromic oxide concentration of 65 percent at 2:00 p.m. and a high of 141 percent at 12:00 p.m. Putnam et al. (1957) found highly variable excretion patterns for chromic oxide when used with grazing dairy cows. They also indicated that the estrous periods of the cows seemed to influence these excretion patterns.

Balch et al. (1957) administered gelatin capsules containing chromic oxide to steers and noted that capsules entering the anterior rumen or reticulum were dissolved within five 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.

Hardison and Reid (1953) compared the chromic oxide excretion patterns of grazing cattle and cattle hand-fed in dry lot. Chromic oxide in a gelatin capsule was administered to both groups daily. Cattle on pasture had the highest concentration at 6:00 p.m. and the lowest at 12:00 p.m. This pattern was more variable and differed considerably from the hand-fed cattle which had a high concentration at 8:00 a.m. and a low concentration at 4:00-6:00 p.m. In work with dairy cows, Hardison et al. (1956) found that when chromic oxide was administered twice daily the variation in excretion rate was substantially reduced. They reported a recovery of 97 to 103 percent.

Many workers have recognized that the extreme variation in excretion pattern of chromic oxide is the primary disadvantage of the indicator, therefore attempts have been made to lessen the variation in excretion. These methods include the sustained-release pellet (SRP), chromic oxide impregnated paper and mixing chromic oxide uniformly with the ration and pelleting the complete ration.

The sustained-release pellet (SRP) was prepared by Canadian scientists by mixing chromic oxide with plaster of paris and water. It was hoped that the pellet would remain in the rumen of sheep or cattle and dissolve to release the chromic oxide at a slow even rate. Brissom and Pigden (1957, 1958) used the SRP and observed that diurnal variation was practically non-existant. Recovery of the chromic oxide was 100.4 percent. Regurgitation and passage of the pellet from the rumen before it was dissolved was reported for sheep but not for cattle. From the low diurnal variation of chromic oxide it was concluded that the SRP had performed the job for which it was created.

Eng (1962) reported 95 percent recovery of chromic oxide from the SRP and a much lower diurnal variation (84.03 to 114.5 percent) for the SRP than for the gelatin capsule (76.88 to 126.12 percent). However, the calculated digestion coefficients using SRP were low.

Troelsen (1961) reported serious disagreement between digestion coefficients calculated using the SRP and the total collection method. He also reported consistently

low recoveries of chromic oxide from the SRP (67-94 percent). He concluded that the incomplete recovery was due to regurgitation of the pellets.

Pigden et al. (1959) reported that feeding SRP to stall-fed animals significantly reduced the diurnal variation pattern of chromic oxide, and no regurgitation of the pellets was observed. However, when the animals were placed on pasture, many of the pellets were regurgitated. Pigden et al. (1964) reported using an extruded sustained-release pellet (ESRP) and a pressed sustained-release pellet (PSRP). They reported more diurnal variation for the PSRP than for the ESRP. However, more of the ESRP were regurgitated.

The method of chromic oxide-impregnated paper was described by Corbett et al. (1960). The paper was prepared by adding 75 parts of chromic oxide and 2 parts of aluminum sulphate to 100 parts, by weight, of Kraft woodpulp. The pulp was processed into paper of substance weight of 170 gm. per square meter. The paper contained a mean of 39.5$\pm$ 0.14 percent chromic oxide.

In a study by Corbett et al. (1960), the various methods of administration of chromic oxide; gelatin capsule, SRP, whole chromic oxide-impregnated paper, and shredded chromic oxide-impregnated paper; were compared using sheep. The use of the SRP was discontinued because of regurgitation of the pellets. Of the other three methods it was found that the variability in excretion pattern was much less for the shredded chromic oxide paper, followed by whole sheet

chromic oxide paper, and variation was the highest for gelatin capsules. Corbett and Greenhalgh (1960) and Campbell (1964) also reported that chromic oxide-impregnated paper gave much less variation in fecal chromic oxide concentration that did the gelatin capsule method.

Bradley et al. (1958) found that mixing chromic oxide in a ration and pelleting the ration, resulted in less variability of results than with capsule administration. Elam et al. (1959) using a pelleted complete ration containing chromic oxide, found a signifcant concentraion variation with time, even though the chromic oxide was pelleted with the complete ration. Crampton and Lloyd (1951) found that mixing chromic oxide with the ground concentrate portion of the ration provides a satisfactory method of administering chromic oxide to sheep.

Chromic oxide has had extensive use as an indicator for calculating fecal output. Most workers in the United States and Canada are satisfied with the chromic oxide technique (Putnam, 1962). However, it is recognized that chromic oxide may have serious limitations under certain conditions due to its variation among animals, extremely variable excretion patterns, and low recoveries. It has been shown that chromic oxide gives more repeatable results in the monogastric animal than in the complex digestive system of the ruminant. It has also been shown that the excretion of chromic oxide is less variable in animals on an all concentrate ration than for animals on pasture.

Polyethylene Glycol

The search continues for an external indicator which has a minimum diurnal variation in excretion pattern and in percent recovery. The use of a water soluble compound, such as polyethylene glycol (PEG), would seem to eliminate many of the problems encountered with water insoluble substances such as chromic oxide.

Sperber et al. (1953) studied the usefulness of polyethylene glycol as an indicator and found no significant absorption, precipitation, or uptake of PEG-4000 by rumen contents. Corbett et al. (1956) reported no PEG in the urine of cattle after 10 days of continuous dosing. Similarly Hyden (1956) reported no PEG in the urine or blood of animals receiving the indicator.

PEG is also reported by Sperber et al. (1953) to have no effect on the digestive tract. These workers found that neither bacteria nor protozoa were affected by PEG concentrations of 0.1 to 1.0 gm. per 100 ml. of media.

Unlike chromic oxide, PEG follows the fluid portion in the digestive tract; therefore, one would logically expect less variation in excretion rate. Unfortunately, this has not been found to be true. Corbett et al. (1958) reported more variability in excretion of PEG than for chromic oxide in their work with dairy cattle. Christie and Lassiter (1958) observed erratic excretion patterns for PEG and stated that no constant day-to-day excretion of PEG was ever reached.

Workers who have reported extremely variable excretion patterns and poor recoveries of PEG have used relatively small doses. Christie and Lassiter (1958) administered 15 gm. of PEG and concluded that much higher concentrations of the indicator would undoubtedly have given more accurate results. Corbett et al. (1956) stated that PEG may be a satisfactory indicator if it is fed to give not less than 250 mg. per 100 gm. of feces.

The excretion pattern for PEG has been reported to have extremes within a 24-hour period, however, this pattern is relatively predictable and constant from animal to animal. Christie and Lassiter (1958) found the lowest concentration at 4:00 p.m. and the highest concentration at 11:00 p.m. The high nocturnal readings were partially accounted for by the fact that dry matter content of the feces taken at the time was high.

Recoveries of PEG by all reporting workers have been consistently less than 100 percent. Christie and Lassiter (1958) concluded that the most likely mode of loss of PEG in the digestive tract was through some type of adsorption on the feces. Hyden (1956) concluded that PEG was destroyed to a limited extent in the digestive tract. However, he found no PEG in either urine or blood.

The chemical determination which is most commonly used for PEG is the turbidimetric procedure reported by Hyden (1956). The procedure consistently yields in vitro recoveries of PEG of near 100 percent, therefore the

procedure is assumed to be accurate. Corbett et al. (1956), Hyden (1956) and Christie and Lassiter (1958) all report excellent in vitro recoveries of PEG.

Much of the early work using PEG as an indicator used doses of 15 to 25 gm. per day. According to Sperber et al. (1953), Corbett et al. (1956), Christie and Lassiter (1958), and Downes and McDonald (1964), much more accurate results and more complete recoveries of the indicator are obtained when 100 to 500 gm. of PEG are administered daily, or at least sufficient administration to give at least 2.5 mg. of PEG per gm. of feces.

Internal Indicators

Lignin

Lignin is a naturally occurring plant constituent found to be almost completely undigestible. Its chemical structure is unknown, however, according to Friston and Simmons (1961) p-hydroxyphenylpropanes derived from coniferyl alcohol or some closely related compounds are fundamental repeating units of lignin. It is nonhydrolyzable by acid, readily oxidizable in hot alkali and bisulfite and it condenses readily with phenols and thio-compounds (Brauns, 1952).

Mertz (1959) states that recent studies suggest that 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 into compounds which serve as the units in the formation of lignin.

The value of lignin as an indicator is not well defined because of conflicting reports as to digestibility by ruminants. Satisfactory recoveries have been reported by Crampton and Maynard (1938); Ellis et al. (1946); Forbes et al. (1946); Swift et al. (1947); and Kane et al. (1950). Crampton and Maynard (1938), using the method which they developed reported lignin recoveries to be 97.8 percent with rabbits and 99.3 percent with a steer. The rabbits were fed clipped grass while the steers were fed an alfalfa hay-grain ration. Ellis et al. (1946) reported recoveries of 94 to 106 percent for sheep, rabbits and cows and concluded that lignin may prove useful in determining digestion coefficients. Forbes and Garrigus (1948) reported lignin recovery from pasture herbage to be 102 $\pm$ 7.0 percent. Maynard (1940) found that digestion of lignin by rabbits and guinea pigs fed alfalfa hay was practically nil.

However, many other workers have reported digestibility coefficients for lignin of from 10 to 65 percent. Hale et al. (1940) reported that up to 23.7 percent lignin was digested by Holstein cows. Maynard et al. (1940) found lignin digestion coefficients of 28 percent with sheep fed alfalfa hay. Ely et al. (1951) in studies with lactating dairy cows found lignin to be as high as 14 percent digestible. Hale et al. (1947) reported digestion coefficients as high as 32.7 percent, and concluded that this digestion

was taking place in the intestine since no ruminal lignin digestion occurred. Elam et al. (1962) reported lignin recoveries of 90.2 percent. Davis et al. (1947) using yearling ewes, found that the digestion coefficient of lignin was 16.2 percent in pea vines and 10.6 percent in lima bean vines.

There are probably several explanations for these conflicting reports, however, the most important ones are undoubtedly plant species, stage of plant maturity, and accuracy of lignin determination. Kane et al. (1951) and Pigden and Stone (1952) reported plant species differences in lignin recoveries. Lignin in alfalfa was found to be a reliable indicator while that in orchard grass was not. Ely et al. (1953) reported that the stage of maturity of the plant involved can influence lignin digestibility. Pazur and De Long (1948) showed that lignin found in the earlier stages of growth of clover were much more readily digested by ruminants than that found in mature clover. These workers also found lignin in the earlier stages of growth in trefoil was more readily metabolized by ruminants than the lignin in more mature plants. According to Kane et al. (1951) and Pigden and Stone (1952), lignin in alfalfa is a reliable indicator but the lignin in orchard grass was not found to be as reliable.

Methods for determination of lignin were outlined in early work by Crampton and Maynard (1938), however, Crampton and Jackson (1944) stated that lignin, as estimated by

either the method of Crampton and Maynard (1938) or by Crampton and Whiting (1942), cannot be relied upon to indicate the trends in digestibility of dry matter in a pasture herbage.

Ellis et al. (1946) developed a modified procedure for the determination of lignin. These workers proposed a standard "72 percent sulfuric acid" method of determination and reported 94 to 106 percent recovery from the cow, sheep, and rabbit fed timothy hay and sudan grass. The researchers called the new method the "Lignin Ratio Method" and suggested that lignin be used as an indicator of both digestibility and intake. This is the first suggestion in the literature of using an indicator to determine consumption. Van Soest (1961) proposed a new analysis for lignin based on the work of Moon (1952). This method involved an acid detergent analysis.

Ely et al. (1951) working with dairy cows fed orchard grass at different stages of maturity, showed lignin digestibilities up to 14 percent. Lignin prepared from orchard grass had higher methoxyl content than lignin isolated from corresponding feces. This indicated a degradation of the lignin molecule in the digestive tract. Only about 70 percent of the methoxyl content of lignin in the ration was recovered in fecal lignin.

Due to the inconsistency of the findings reported in the literature, no definite conclusions can be drawn as to the reliability of lignin as an internal indicator. It has

been found that many factors such as age, plant species, and type of lignin determination used can seriously affect lignin recoveries. With these factors in mind, a worker should not merely assume complete recovery of lignin but should study recoveries and type of analysis to be used for the type of forage being studied and seriously evaluate the reliability of lignin as an indicator in each individual situation.

Cellulose

In estimating forage dry matter digestibility by the use of an internal indicator it is desirable that the indicator be completely undigestible. The plant constituent which had been used most extensively as an internal indicator is lignin since it is assumed to be completely undigestible by the ruminant. However, as previously discussed, many workers have reported lignin digestibility of from 5 to 60 percent.

If a completely undigestible plant constituent does not exist, it seems logical that other plant constituents, present in relatively large quantities, may be used if a digestibility factor for that constituent is known.

Cellulose has had very little use as an internal indicator because of the lack of an accurate method for determining its digestibility. McCroskey and Brackett (1965 unpublished data) used cellulose and chromic oxide to determine the intake of Midland bermudagrass by range cows.

These workers used the nylon bag technique (Van Keuren and Heinemann, 1962) to determine the digestion coefficient for cellulose. Cellulose was then used to calculate forage digestibility and chromic oxide was used to estimate fecal output. McCroskey and Brackett reported that cellulose, with the digestibility factor determined by the nylon bag technique, was a satisfactory indicator. However, there was considerable variation in the nylon bag digestibility values.

Brackett (1966 unpublished data) examined the possibilities of using the in vitro digestion technique (Tilley and Terry, 1963) to determine the digestion coefficient for cellulose when using cellulose as an internal indicator. He found that this procedure was possible but not practical Furthermore, he concluded that a much simpler approach for determining voluntary forage intake was to use the in vitro digestion technique to directly determine dry matter digestibility for the grass being consumed and use chromic oxide (or other external indicators) to estimate fecal output.

EXPERIMENT I. METHODS FOR RELATING FORAGE PROPERTIES TO INTAKE AND DIGESTIBILITY

Experimental Procedure

This experiment was originally undertaken in an attempt to determine the voluntary intake of Midland bermudagrass by grazing cattle, and to correlate the seasonal intake with chemical composition and digestibility of the forage. Voluntary intake was determined by the use of chromic oxide to estimate fecal output and undigestible cellulose as an indicator of dry matter digestibility. Four rumen-fistulated Hereford steers were placed on a pasture of Midland bermudagrass. Two of the steers were designated as "forage collectors", and the remaining two were used for *in vivo* cellulose digestion by the nylon bag technique and for fecal grab sampling.

Forage Collection Technique

Two forage collecting steers were used to obtain a sample of forage which was representative of that being consumed at different times during the growing season. These forage samples were taken once during each of eight sampling periods throughout the growing season.

In preparing for the collection of forage samples, the

collector steers were placed in a work chute where the entire rumen contents were evacuated by way of the rumen fistula. The solid portion was removed by hand and the liquid portion was removed by vacuum pump. Rumen contents were placed in cans and held at near anaerobic conditions, to be returned to the rumen later. Following rumen evacuation the steers were turned back to the pasture and allowed to graze for two hours then returned to the chute and a sample of the grazed forage removed. The original rumen contents were then returned to the rumen and the steers turned back to pasture. A representative sample of the grazed forage was dried for determining cellulose digestion in the nylon bag. The remainder was placed in plastic bags and frozen for later chemical analysis.

Forage samples were analyzed for acid detergent fiber, acid detergent lignin (Van Soest, 1963), and neutral detergent fiber (Van Soest, 1967). Other analyses included crude protein (A.O.A.C., 1960), cellulose (Crampton and Maynard, 1938), and *in vitro* dry matter digestibility (Tilley and Terry, 1963).

Estimates of Forage Digestibility

The use of cellulose as an internal indicator necessitates some estimate of its digestibility. This was determined by the use of the nylon bag technique (Van Keuren and Heinemann, 1962). Five gram samples of forage that had been ground through a 1 mm. screen were placed in nylon

bags prepared from parachute material. Four bags containing the forage samples were suspended in the rumen of each of the two fecal collecting steers for a period of 48 hours at each sampling period. After the fermentation period, the bags were removed from the rumen, washed thoroughly to remove soluble material and placed in an oven at 55°C for 24 hours or until dry. The bags were then emptied and the contents weighed and analyzed for cellulose content.

Estimates of Fecal Output

Fifteen grams of chromic oxide was administered in a gelatin capsule to each of the fecal collecting steers, via the rumen fistula, at 8:00 a.m. each day. The indicator was administered at least five days before fecal collections began. An excretion curve for chromic oxide was established by taking fecal "grab" samples from each steer every four hours for a 24-hour period. Fecal samples, which were taken for estimation of forage intake, were taken from each steer at 8:00 a.m. and 4:00 p.m. for four consecutive days and this procedure was repeated eight times during the growing season.

All fecal samples were placed in properly identified plastic bags and frozen immediately. In preparation for analysis, the samples were dried in a forced air oven (55°C) and ground through a 1 mm. mesh screen and stored in plastic bags for subsequent analysis for chromic oxide and cellulose. Chromic oxide was determined using a

Perkin-Elmer Atomic Absorption Spectrophotometer.

The following formulae were used to calculate fecal output and forage intake:

$$\text{Fecal output (gm.D.M./day)} = \frac{Cr_2O_3 \text{consumed (gm./day)}}{Cr_2O_3 \text{in feces (gm./gm.D.M.)}}$$

$$\text{Forage intake (gm.D.M./day)} = \frac{\%\text{cellulose in feces}}{\%\text{cellulose not digested}} \times \text{fecal output}$$

After calculated forage intake was determined for the various periods during the growing season, intake was correlated with the percentages of lignin, acid detergent fiber, neutral detergent fiber, crude protein, and *in vitro* digestibility of the forage.

Forage Nutrient Changes After Ingestion

It has been demonstrated that the chemical composition of forage selected by cattle is different from that of hand clipped samples. The rumen evacuation technique of forage sampling is one method of collecting samples which closely approximates what the animal actually consumes. However, the question arises as to the possible loss of nutrients from the forage during mastication and ingestion. Therefore, a study was designed to measure the change in chemical composition of bermudagrass due to ingestion.

Four rumen-fistulated Hereford steers were placed in dry lot where the rumen evacuation technique was performed as described previously. Freshly clipped Midland

bermudagrass was then fed and allowed to remain in the rumen for two hours. The ingested forage was then removed from the rumen via the rumen fistula and samples taken for chemical analysis. This procedure was repeated three times. Samples were analyzed for crude protein, ether extract, cellulose and ash. These values were compared to the values for these components in samples of clipped forage before ingestion. These results are shown in Table I.

It was found that there was a slight decrease in percent crude protein and ether extract, and a slight increase in cellulose and ash. The increased ash content could be expected due to contamination with saliva. Decrease in percent crude protein was probably due to removal of water soluble compounds and to the relative increase in ash content. It is doubtful that any appreciable fermentation losses occurred in the short time the forage was in the rumen, especially since it was essentially a dry rumen. The apparent increase in percent cellulose was probably due to the relative decreases in some of the water soluble materials.

Results and Discussion

The seasonal variation in forage intake as determined by indicators is reported in Table II and is also shown graphically in Figure I. Chemical composition of forage samples is shown by season in Table III. It may be noted

TABLE I

CHANGE IN CHEMICAL COMPOSITION OF FRESHLY CLIPPED BERMUDAGRASS AFTER TWO HOURS IN AN EVACUATED RUMEN (PERCENT)

	Crude Protein	Ether Extract	Cellulose	Ash
Trial 1				
Freshly Clipped	12.54	7.65	30.43	0.089
After Ingestion[1]	10.87	5.93	32.63	0.142
Change	-1.67	-1.72	+2.20	+0.053
Trial 2				
Freshly Clipped	11.63	8.83	31.09	0.103
After Ingestion	11.33	8.38	32.63	0.103
Change	-0.30	-0.45	+1.54	0.000
Trial 3				
Freshly Clipped	10.20	8.02	29.91	0.093
After Ingestion	10.11	7.69	32.44	0.108
Change	+0.09	-0.33	+2.53	+0.015
Average Change	-0.69	-0.83	+2.09	+0.023

[1]Each value for "After Ingestion" is a mean of data from four steers.

TABLE II

CALCULATED VOLUNTARY INTAKE OF MIDLAND BERMUDAGRASS BY GRAZING STEERS AT VARIOUS TIMES DURING THE GROWING SEASON

Month	Calculated Intake (kg. D.M./day)		
	Steer 58	Steer 60	Mean
Mid June	7.74	7.35	7.54
Early July	9.22	6.67	7.94
Mid July	11.75	8.15	10.04
Late July	12.03	9.46	10.75
Early Aug.	10.50	8.66	9.64
Late Aug.	8.04	7.55	7.72
Mid Sept.	7.43	5.37	6.40
Mid Oct.	5.40	3.69	4.55

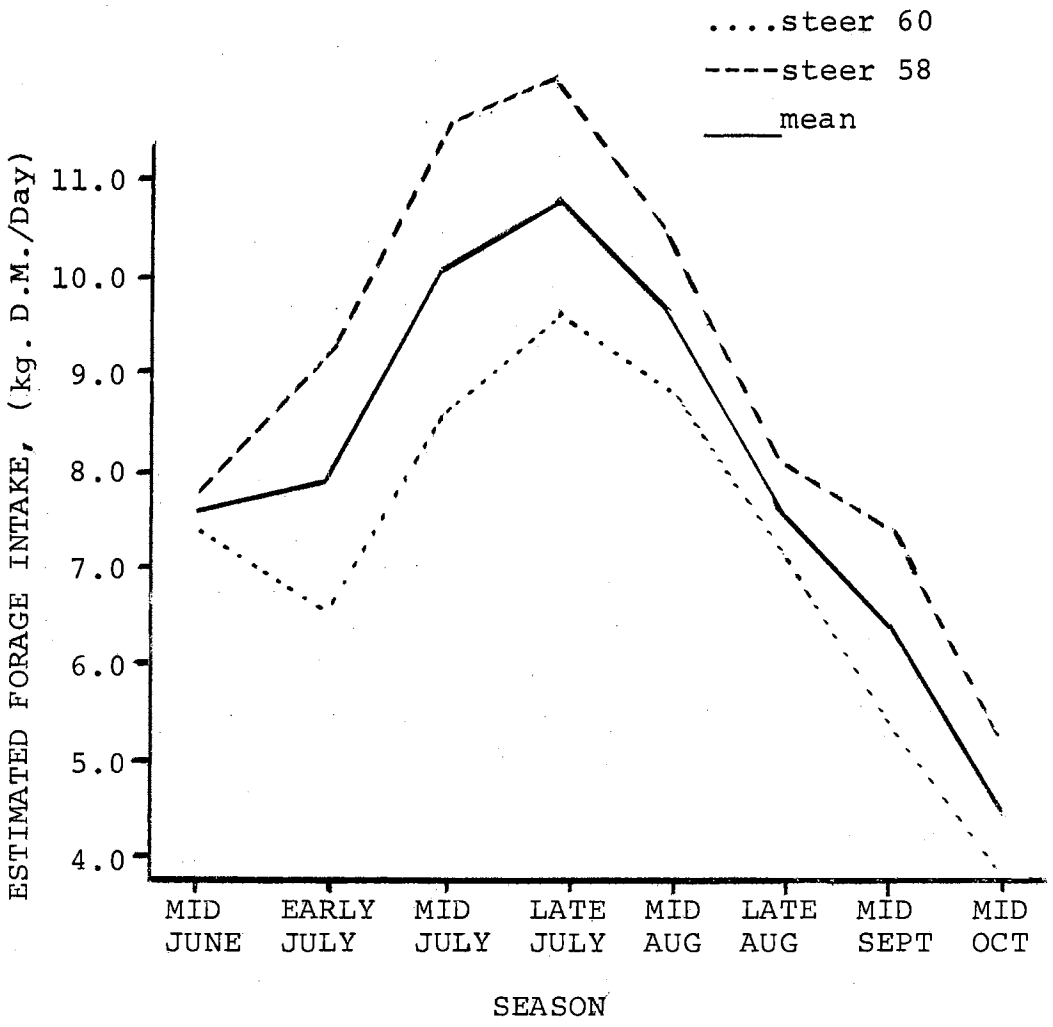

FIGURE 1. ESTIMATED SEASONAL INTAKE OF MIDLAND BERMUDAGRASS

TABLE III

CHEMICAL COMPOSITION OF MIDLAND BERMUDAGRASS BY SEASON[1] (PERCENT)

Season	Crude Protein	ADF	NDF	Lignin	Cellulose	In Vitro D.M. Digest.
Mid June	16.3	58.7	60.93	6.2	29.5	57.0
Mid July	14.3	61.6	62.65	7.4	27.0	52.2
Late July	9.9	56.0	61.85	6.1	31.2	60.4
Mid Aug.	7.2	54.7	57.78	6.0	32.0	52.4
Mid Sept.	7.6	59.7	59.70	6.2	31.8	54.9
Mid Oct.	5.9	55.7	59.10	7.1	30.5	47.5

[1]Composite samples from two steers obtained by rumen evacuation technique.

that the variation in acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin, cellulose, and in vitro dry matter digestibility of forage samples throughout the season is less than might be expected for bermudagrass. A possible explanation for this fact is that the forage samples were grazed samples taken by rumen evacuation technique. Since animals tend to select the highest quality forage available, it stands to reason that if any forage of relatively high quality were present in the pasture, it would be selected. Therefore, it is quite possible that these samples were not representative of the majority of the forage available.

The general pattern of seasonal forage intake, as shown in Figure I, is similar to growth patterns found by other workers (Elder et al., 1961), however, the peak in the curve is about one and one half months later than would normally be expected for bermudagrass. A possible explanation for this may be a combination of factors. The pasture on which this study was conducted was heavily fertilized and irrigated throughout the season. Also, the pasture was somewhat over-grazed. These factors combined would tend to produce a more succulent, higher quality forage in mid-summer than would normally be found. This may account for the fact that intake peaked in late July rather than mid-June as might normally be expected.

The correlation between forage intake and various forage constituents is shown in Table IV. The correlations were rather low and failed to produce significant

TABLE IV

SIMPLE CORRELATIONS BETWEEN FORAGE INTAKE AND VARIOUS FORAGE CONSTITUENTS

Constituent	Correlation[1] Coefficient
Crude Protein	+0.51
ADF	+0.24
NDF	+0.64
Lignin	-0.09
Cellulose	-0.35
In Vitro D.M. Digestibility	+0.67

[1]None of these values differ significantly from zero ($P < .05$)

relationships between seasonal intake and chemical composition. It is quite probable that the forage sampling technique used provided samples which were characteristic of forage being consumed, but were not representative of the bulk of forage available to the animals. Perhaps hand plucked or clipped samples would have given a more accurate estimate of actual change in chemical composition of the grass throughout the season.

Evaluation of Chromic Oxide and Undigestible Cellulose

Most of the literature cited indicates that chromic oxide is the most reliable external indicator in use currently. However, some workers have found that it is somewhat less accurate for pasture studies than in feedlot studies due to more diurnal variation in excretion patterns under pasture conditions. With highly variable excretion patterns, individual fecal samples may give erroneous concentrations of the indicator.

In work reported in this experiment, it was found that the excretion pattern for chromic oxide varied widely between sampling times, seasons and animals. An attempt was made to compensate for this variation by taking samples every four hours for 24 hours in order to establish an excretion curve for each animal. Once the curve was established, the deviation in concentration of the indicator at 8:00 a.m. and 4:00 p.m. (normal sampling times) from the mean was determined and this correction was applied to all

grab samples taken thereafter.

The use of undigestible cellulose as an internal indicator is a rather new approach. It was used in preference to lignin due to extremely variable recoveries reported in the literature. However, the accuracy of undigestible cellulose (determined in the nylon bag) as an indicator was questioned when compared with dry matter digestion coefficients determined by the *in vitro* technique (Table V). The nylon bag technique was not entirely satisfactory in this study due to frequent punctures of bags by grass stems which allowed two-way passage of large feed particles in and out of the bag. These observed weaknesses in this technique for determining intake of grazed forage causes considerable doubt as to the accuracy of individual values, but the differences between estimates during the study may well represent the relative variation in consumption throughout the growing season.

Summary and Conclusions

Four mature, rumen-fistulated Hereford steers grazing a pure stand of Midland bermudagrass for an entire growing season were used to estimate voluntary forage intake. Two of the steers were used to obtain forage samples by the rumen evacuation technique. The remaining two steers were used to determine forage digestibility and fecal output. These steers were administered chromic oxide in gelatin capsules, and fecal output was calculated by the indicator

TABLE V

ESTIMATES OF DIGESTIBILITY OF RUMEN FORAGE SAMPLES (PERCENT)

Season	D.M. Digestibility Calculated by Undigestible Cellulose Ratio[1]	In Vitro D.M. Digestibility
Mid June	45.4	57.0
Mid July	59.0	52.2
Late July	56.5	60.4
Mid Aug.	52.8	52.4
Mid Sept.	48.5	54.9
Mid Oct.	35.9	47.5

[1]Undigestible cellulose determined by nylon bag technique.

method. The nylon bag technique was used to determine cellulose digestibility. Undigestible cellulose was used as an internal indicator to estimate forage digestibility. Dry matter intake was calculated at eight times during the growing season using these indicators. Forage samples obtained by the rumen evacuation technique were analyzed for crude protein, acid detergent fiber, neutral detergent fiber, lignin, cellulose, and *in vitro* dry matter digestibility. Seasonal intake was correlated with each of the above mentioned constituents. None of these correlations was statistically significant from zero ($P < .05$).

The lack of significant correlations between chemical constituents and voluntary intake may be due to the fact that animals select only the tender parts of the plant and will eat the lower quality portion only when there is no other material available. Thus, if there is some tender, succulent plant material available the animal will limit his intake primarily to that portion. If this type of material is abundant, his intake is high; if it is limited, he may reduce intake accordingly. Hence, intake may be more highly correlated with components of hand-clipped samples than grazed samples.

Results of this study indicate that the indicators used in this experiment should be studied under controlled conditions before being used in further pasture studies.

EXPERIMENT II. COMPARISON AND EVALUATION OF INTERNAL AND EXTERNAL INDICATORS

After using chromic oxide and undigestible cellulose as indicators for calculating forage intake in the previous study (Experiment I), the accuracy of the indicators was seriously questioned. It was concluded that a study should be conducted to examine the accuracy of these and other indicators under controlled conditions.

Trial I

Experimental Procedure

Four mature, rumen-fistulated Hereford steers were placed in metal metabolism stalls and fed two kg. of bermudagrass hay, twice daily in long form. Lignin, undigestible cellulose, and *in vitro* dry matter digestibility were compared as internal indicators for estimating digestibility. Fifteen grams of chromic oxide was administered once daily in gelatin capsule, via the rumen fistula, at 8:00 a.m. Administration of chromic oxide began seven days prior to collection of fecal samples. The trial consisted of a 10 day preliminary period and a seven-day collection period. During the collection period, fecal "grab" samples were taken at 8:00 a.m. and 4:00 p.m. and were placed in properly

identified plastic bags and frozen. Total feces voided was determined daily for each steer and representative samples were taken at 4:00 p.m. each day during the seven-day collection period. During the last 24 hours of the collection period, "grab" samples were taken rectally every four hours to establish an excretion curve for the indicator. The samples were dryed in a forced air oven at 55°C then ground through a 1 mm. screen. Chemical analyses included lignin, cellulose, and determination of chromic oxide content using a Perkin-Elmer Atomic Absorption Spectrophotometer.

The digestion factor for cellulose was determined using the nylon bag technique. A representative sample of hay was ground through a 1 mm. screen and five grams placed in each of 16 nylon bags. Four bags were then suspended in the rumen of each of the four fistulated steers as described in Experiment I. Dry matter digestibility was also determined by the in vitro digestion technique (Tilley and Terry, 1963).

Fecal output and forage intake were calculated in the same manner as described in Experiment I and compared to actual intake and fecal output values determined during the conventional digestion trial.

Results and Discussion

Chromic oxide as an indicator. The average diurnal variation curve for the four steers is shown in Figure 2. The peak concentration occurred at 8:00 a.m. and the lowest

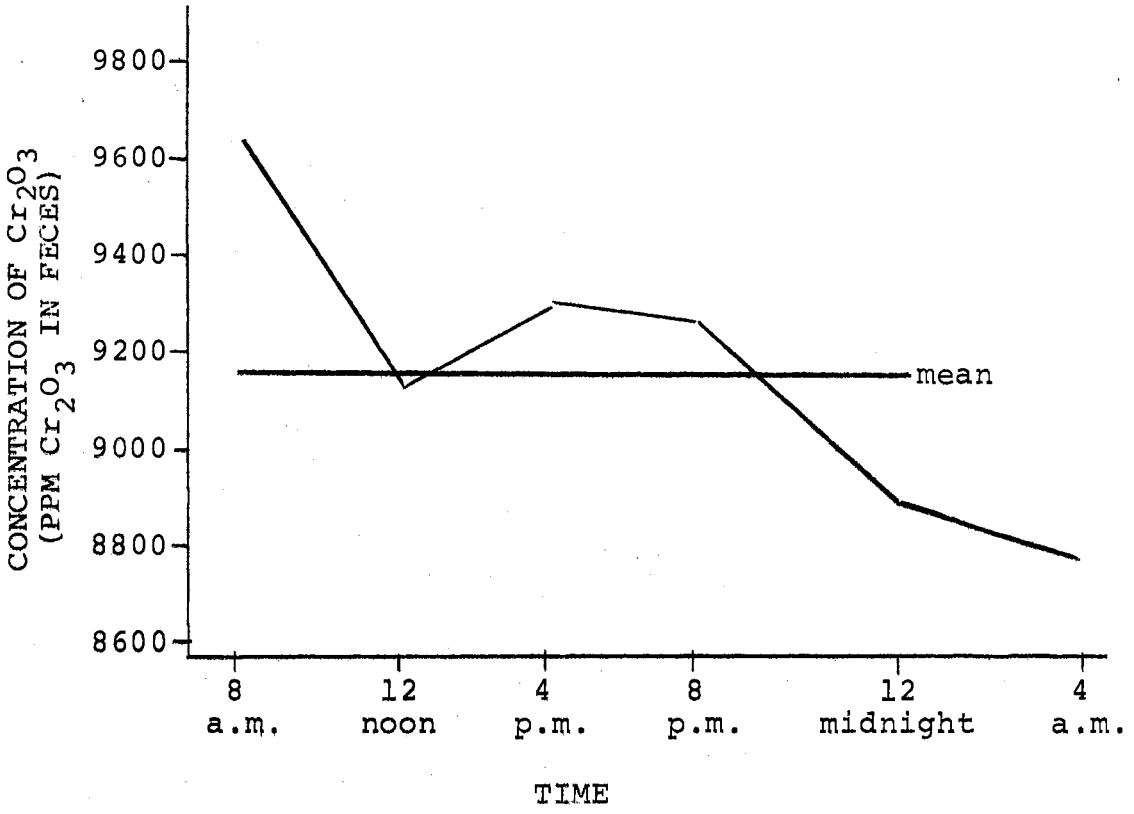

FIGURE 2. EXCRETION CURVE FOR CHROMIC OXIDE (AVERAGE OF 4 STEERS)

concentration at 4:00 a.m. It may be noted that both the 8:00 a.m. and 4:00 p.m. readings (normal sampling times) are both above the mean concentration. This is unlike excretion patterns established on pasture (Experiment I) and also unlike patterns reported for animals confined to metabolism stalls (Eng, 1962). The primary reason for selecting 8:00 a.m. and 4:00 p.m. as grab sampling times is the fact that in most studies, excretion of chromic oxide will be somewhat higher than the mean at 8:00 a.m. and will be nearly equidistantly lower than the mean at 4:00 p.m. Therefore, an average of these two readings should give a mean reading for the day which closely approximates the mean excretion of chromic oxide for that day.

The mean recovery of chromic oxide in this trial (85.47±9.31 percent) is shown in Table VI. Low recoveries of chromic oxide from animals fed hay rations have also been reported by Crampton and Lloyd (1951) and Corbett et al. (1959). There was no apparent problem encountered in technique or analysis which would tend to explain this poor recovery.

Fecal output as calculated using chromic oxide is shown in Table VII along with actual weighed fecal output. Due to poor recovery of chromic oxide, the calculated fecal output was 24.5 percent higher than actual weighed fecal output. This difference between calculated and actual output was shown to be statistically significant ($P < .005$).

Estimates of digestibility. The recovery of ingested

TABLE VI

RECOVERY OF CHROMIC OXIDE AND LIGNIN
(PERCENT)

Steer	Cr_2O_3	Lignin
16	74.80	68.48
18	82.13	69.00
58	88.46	80.56
105	96.53	74.59
mean	85.47	73.15
s	9.31	5.66

TABLE VII

MEASURES OF FECAL OUTPUT
(TRIAL I)

Steer	Fecal output kg.D.M./day	
	Actual	calculated using Cr_2O_3
16	1.14	1.57
18	1.37	1.78
58	1.46	1.75
105	1.59	1.84
mean*	1.39^a	1.73^b
s	0.63	0.12

*Values with different letters differ significantly ($P<.005$).

lignin is shown in Table VI. The mean recovery of lignin for the four steers was rather low (73.15 percent). Incomplete recovery of lignin has also been reported by Bondi and Meyer (1943), Crampton and Maynard (1938), Davis et al. (1947), Hale et al. (1940), Kane et al. (1953) and Eng (1962).

Dry matter digestion coefficients calculated by lignin ratio method were significantly lower ($P < .05$) than those determined by the conventional method (Table VIII) due to low recoveries. It has become apparent from this trial and a similar trial conducted at this station by Eng (1962), and from the literature, that lignin in bermudagrass is not an acceptable indicator of forage digestibility. According to Pazur and De Long (1948), the metabolism of lignin is affected by the stage of maturity of the grass. Therefore, one cannot say that the results of this trial could be expected for bermudagrass in other stages of maturity.

Undigestible cellulose, as determined by nylon bag technique, was also used as an estimate of forage digestibility. Dry matter digestion coefficients obtained using undigestible cellulose ration (Table VIII) were also significantly lower ($P < .05$) than those obtained by conventional method. Similar problems were encountered with the nylon bag in this trial as described in Experiment I, namely the puncturing of small holes in the bag by stems of hay in the rumen. This allowed some two-way passage of large particles through the bag. If more dry matter was deposited in the

TABLE VIII

MEASURES OF DRY MATTER DIGESTIBILITY (TRIAL I)
(PERCENT)

Steer	Conventional	Undigestible Cellulose	Lignin Ratio	*In Vitro*[1]
16	67.99	44.76	49.75	
18	64.72	46.81	50.25	
58	62.60	47.09	45.36	
105	59.01	47.65	46.24	
mean*	63.58[a]	46.57[b]	47.90[b]	55.26[c]

[1] *In Vitro* dry matter digestibility is the mean of two determinations.

*Values with different letters differ significantly ($P<.05$).

bag than escaped, this would account for the low apparent digestion coefficients obtained using undigestible cellulose ratio.

Dry matter digestion coefficients obtained by the *in vitro* dry matter digestion technique (Table VIII) were much closer to those obtained by the conventional method than those determined using undigestible cellulose or lignin. However, there was still a statistically significant ($P<.05$) difference between the two mean coefficients. Digestion coefficients determined by the conventional method appear rather high for the quality of forage fed. It is postulated that the digestion coefficients obtained by this method would have been much lower, and therefore close to those obtained by the *in vitro* technique had the steers been on a higher level of feed intake.

Estimates of dry matter intake. Table IX shows actual dry matter intake by the four steers and intake calculated using the following indicator combinations: chromic oxide-undigestible cellulose, chromic oxide-lignin, and chromic oxide-*in vitro* dry matter digestibility. Analysis of variances showed no statistically significant difference ($P<.05$) between actual intake and intake values calculated by the chromic oxide-*in vitro* digestibility indicator combination. There was, however, a significant difference ($P<.05$) between actual intake and values determined by the chromic oxide-undigestible cellulose and chromic oxide-lignin combinations.

TABLE IX

COMPARISON OF INDICATORS TO MEASURE DRY MATTER INTAKE (TRIAL I)

	Dry matter intake (kg.D.M./day)			
Steer	Actual	Cr_2O_3 Cellulose	Cr_2O_3 Lignin	Cr_2O_3 In Vitro
16	3.55	2.78	3.15	3.50
18	3.88	3.28	3.52	3.98
58	3.88	3.28	3.22	3.91
105	3.88	3.13	3.40	4.13
mean*	3.80^a	3.12^b	3.32^c	3.88^a
s	0.16	0.36	0.16	0.73

*Values with different letters differ significantly ($P<.05$).

The fact that there is no significant difference between actual dry matter intake and that calculated by the chromic oxide-*in vitro* digestibility indicator combination, does not necessarily denote accuracy of the indicators. This calculated intake is, in fact, a combination of two counter-balancing errors. Fecal output as calculated by the chromic oxide method was 24.5 percent higher than actual weighed fecal output. Also, *in vitro* dry matter digestibility was 12.9 percent lower than that obtained by conventional method. When the high calculated fecal output, was combined with a low estimate of digestibility the result was a relatively close estimate of dry matter intake. However, chance of repeating this circumstance would probably be rather low.

Trial II

The inconclusive findings in Trial I, indicated the need for more information on indicators for use in intake studies. This trial was designed to further study various indicator methods for calculating digestibility, fecal output, and dry matter intake. Undigestible cellulose and *in vitro* dry matter digestibility were compared as indicators of digestibility while chromic oxide and polyethylene glycol (PEG) were compared as external indicators for calculating fecal output.

Experimental Procedure

A conventional digestion trial was conducted as described in Trial I. Ten mature, rumen-fistulated Hereford steers were placed in metal metabolism stalls and fed 3.18 kg. of bermudagrass hay pellets twice daily. Chromic oxide (15 gm. in gelatin capsule) and PEG (225 gm., dry) were administered to each steer, via the rumen fistula, once daily at 8:00 a.m. Fecal "grab" samples were taken rectally at 8:00 a.m. and 4:00 p.m. during the seven-day collection period. During the last 24 hours of the collection period, "grab" samples were taken every four hours in order to establish an excretion curve for chromic oxide and PEG.

The procedure described by Hyden (1956) was used for PEG determination. All other determinations were conducted as described previously.

Results and Discussion

Estimate of fecal output. The average diurnal variation curve for chromic oxide excretion is shown in Figure 3. There was a rather extreme range of values in this trial, however, the mean excretion curve was nearly identical to that obtained in a similar trial by Eng (1962). Many workers have observed a larger diurnal range in chromic oxide concentration when animals were fed an increasing ratio of roughage to concentrate (Bloom et al, 1957). This was partially explained by Corbett et al. (1959) who stated that a large proportion of the chromic oxide in the feces

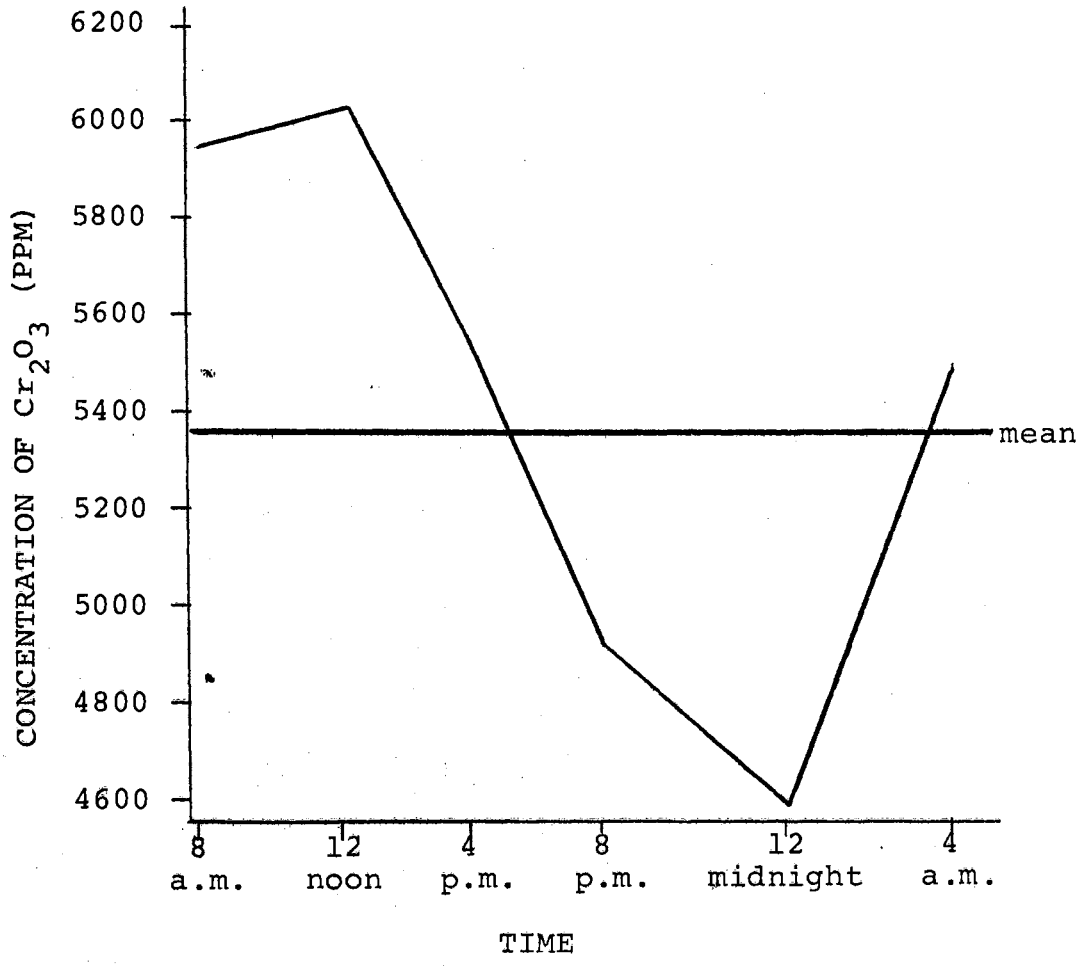

FIGURE 3. EXCRETION CURVE FOR CHROMIC OXIDE (AVERAGE OF 10 STEERS)

of animals fed high roughage rations, appeared to be attached to the partially digested cellulose fibers.

The mean recovery of chromic oxide in this trial was 112.34 percent (Table X). Procedures in this trial differed from Trial I only in that the bermudagrass hay was pelleted to reduce feed wastage, and the daily feed intake was greater. Except for these factors, the vast increase in chromic oxide recovery is unexplainable.

Calculated fecal output using chromic oxide is shown along with actual weighed fecal output in Table XI. There was no statistically significant difference ($P<.05$) between actual fecal output and that calculated using chromic oxide.

The average diurnal variation for polyethylene glycol (PEG) is shown in Figure 4. The lowest concentration occurred at 4:00 p.m. and the highest concentration at 12:00 midnight. This excretion pattern is very similar to that reported by Christie and Lassiter (1958). The excretion pattern for PEG had less variation than did chromic oxide in this study. PEG is reported to have less diurnal variation than is normally found for chromic oxide due to the fact that PEG is water soluble and follows the fluid portion of the ingesta (Sperber et al., 1953), whereas chromic oxide tends to follow the feed particles.

Recovery values for PEG are shown in Table X. The mean recovery was rather low (92.95 percent) which may be due partially to small losses of PEG during administration. Sudden rumen contractions occasionally occurred resulting

TABLE X

RECOVERY OF CHROMIC OXIDE AND POLYETHYLENE GLYCOL (PERCENT)

Steer	Cr_2O_3	PEG
1	119.47	95.59
2	115.93	112.00
3	127.73	111.89
4	138.20	83.80
5	98.87	68.19
6	97.40	93.83
7	103.93	95.46
8	108.40	77.66
9	111.33	102.20
10	102.13	82.91
mean	112.34	92.95
s	13.05	14.35

TABLE XI

MEASURES OF FECAL OUTPUT
(TRIAL II)

Steer	Fecal output kg.D.M./day		
	Actual	Cr_2O_3	PEG
1	2.82	3.05	3.49
2	3.01	3.92	4.28
3	3.33	3.33	3.66
4	3.03	3.02	3.54
5	3.27	3.07	4.12
6	3.38	3.23	3.55
7	2.96	3.10	3.64
8	2.86	3.01	3.51
9	3.25	3.04	3.05
10	3.15	2.91	3.24
mean*	3.11^a	3.17^a	3.60^b
s	0.23	0.29	0.37

*Values with different letters differ significantly ($P < .05$).

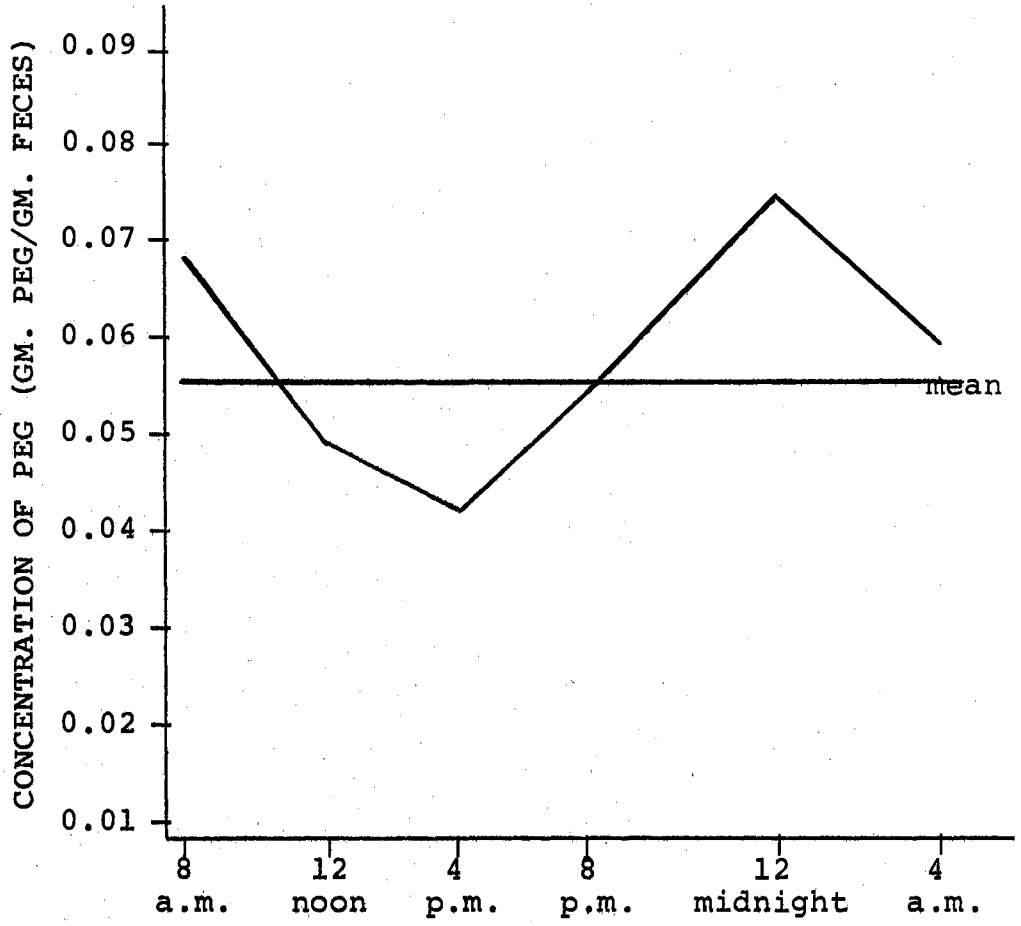

FIGURE 4. EXCRETION CURVE FOR PEG
(AVERAGE OF 10 STEERS)

in the loss of some PEG before the fistula could be recapped. According to Sperber *et al.* (1953) there is no significant absorption, precipitation, or uptake of PEG-4000 by rumen contents. Corbett *et al.* (1956) and Hyden (1956) reported no PEG in urine or blood of cattle receiving the indicator.

Fecal output as calculated using PEG is compared with actual weighed fecal output in Table XI. The incomplete recovery of PEG resulted in a calculated fecal output that was approximately 15.8 percent higher than actual output.

Estimates of digestibility. Digestion coefficients obtained by the conventional method are compared with those obtained by the undigestible cellulose ratio and *in vitro* techniques in Table XII. There were no statistically significant differences ($P<.05$) between any of the three methods of determining dry matter digestibility in this trial.

Estimates of dry matter intake. Dry matter intake values calculated using all combinations of indicators are compared with actual dry matter intake in Table XIII. Mean calculated intake values using chromic oxide-undigestible cellulose and chromic oxide-*in vitro* digestibility are the same and are not significantly different ($P<.1$) from actual intake. Mean intake values determined using PEG-undigestible cellulose or PEG-*in vitro* digestibility methods do not differ significantly ($P<.01$), however, both are significantly different ($P<.05$) from actual intake.

TABLE XII

MEASURES OF DRY MATTER OF DIGESTIBILITY (TRIAL II)
(PERCENT)

Steer	Conventional	Undigestible Cellulose Ratio	*In Vitro* D.M. Digestibility[1]
1	55.66	48.19	
2	52.68	48.98	
3	47.65	42.09	
4	52.36	48.72	
5	48.59	50.99	
6	46.23	51.22	
7	53.46	51.46	
8	55.04	49.24	
9	48.90	51.46	
10	50.48	47.65	
mean*	51.11[a]	49.50[a]	49.44[a]
s	3.29	1.66	

[1] *In Vitro* dry matter digestibility is the mean of two determinations.

*Values with different letters differ significantly ($P<.05$)

TABLE XIII

COMPARISON OF INDICATORS TO MEASURE DRY MATTER INTAKE
(TRIAL II)

	Dry matter intake kg.D.M./day				
Steer	Actual	CR_2O_3 Cellulose	CR_2O_3 In Vitro	PEG Cellulose	PEG In Vitro
1	6.36	5.87	6.04	6.74	6.91
2	6.36	5.74	5.78	8.43	8.47
3	6.36	6.31	6.59	6.92	7.24
4	6.36	5.91	5.98	6.90	7.00
5	6.36	6.26	6.08	8.40	8.16
6	6.36	6.58	6.40	7.27	7.02
7	6.36	6.41	6.14	7.49	7.20
8	6.36	5.91	5.96	6.92	6.94
9	6.36	6.27	6.02	8.34	6.03
10	6.36	5.57	5.76	6.19	6.41
mean*	6.36[a]	6.08[a]	6.08[a]	7.36[b]	7.13[b]
s		0.59	0.80	0.79	0.73

*Values with different letters differ significantly ($P<.1$).

Summary and Conclusions

Trial I was conducted to compare various indicator methods of calculating digestibility, fecal output and dry matter intake with the conventional method. Chromic oxide was used as an indicator for calculation of fecal output. Undigestible cellulose (determined by nylon bag), lignin ratio, and *in vitro* dry matter digestibility were compared for dry matter digestibility determination.

Four mature, rumen-fistulated Hereford steers were placed in metabolism stalls and fed 2 kg. of bermudagrass hay twice daily. Chromic oxide (15 gm. in gelatin capsule) was administered once daily at 8:00 a.m. The trial consisted of a 10-day preliminary period and a seven-day collection period. During the trial fecal "grab" samples were taken at 8:00 a.m. and 4:00 p.m. throughout the seven day collection period. During the last 24 hours of the collection period, grab samples were taken every four hours in order to establish an excretion curve for chromic oxide.

Mean recovery of chromic oxide was 85.47 percent. This poor recovery resulted in a calculated fecal output which was 24.5 percent higher than actual weighed fecal output values. Poor recovery of lignin (73.15 percent) resulted in dry matter digestion coefficients that were significantly lower ($P<.005$) than those obtained by the conventional method. Dry matter digestion coefficients calculated using undigestible cellulose were also significantly lower ($P<.005$) than those obtained by conventional

method. Dry matter digestion coefficients obtained by the *in vitro* dry matter digestion technique were much closer to those obtained by conventional method, but were still significantly lower ($P<.05$). The combination of chromic oxide and *in vitro* dry matter digestibility gave a mean calculated intake that was not significantly different ($P<.05$) from actual dry matter intake.

In Trial II, ten rumen-fistulated Hereford steers were used in a conventional digestion trial as described in Trial I. Pelleted bermudagrass hay was fed twice daily at the rate of 3.18 kg. per feeding. Chromic oxide (15 gm. in gelatin capsule) and PEG (225 gm., dry) were administered via the rumen fistula, once daily at 8:00 a.m. Fecal "grab" samples were taken at 8:00 a.m. and 4:00 p.m. during the seven-day collection period. During the last 24 hours of the collection period, grab samples were taken every four hours in order to establish an excretion curve for chromic oxide and PEG.

Recovery values for chromic oxide and PEG were 112.34 and 92.35 percent, respectively. Fecal output as calculated using chromic oxide was not significantly different ($P<.05$) from actual weighed fecal output. Due to incomplete recovery of PEG, fecal output calculated using PEG was significantly higher ($P<.05$) than actual weighed fecal output. There was no significant difference ($P<.05$) between dry matter digestibility coefficients calculated using undigestible cellulose, *in vitro* dry matter digestion technique,

and the conventional method.

Dry matter intake in Trial II as calculated using chromic oxide-undigestible cellulose and chromic oxide-*in vitro* dry matter digestibility were not significantly different ($P<.1$) from actual dry matter intake. Intake as calculated using PEG-undigestible cellulose and PEG-*in vitro* dry matter digestibility were not significantly different ($P<.05$) from each other, but they were significantly higher ($P<.05$) than actual intake.

Conclusions from Trial I and Trial II concerning the use of indicators to predict fecal output, digestibility, and dry matter intake are:

1. Chromic oxide administered in gelatin capsules was satisfactory as an indicator for calculating fecal output. However, diurnal variation in excretion is large, therefore an excretion curve should be established for each trial and appropriate correction factors applied.
2. The use of lignin in Midland bermudagrass as an internal indicator is not recommended. The mean recovery of lignin in Trial I was 73.15 percent. This is very similar to findings of other workers using lignin in bermudagrass.
3. Recovery of PEG was incomplete (92.35 percent), however this low recovery may be partially accounted for by small losses during administration.
4. The recommended indicator of dry matter digestion

is the *in vitro* dry matter digestion technique (Tilley and Terry, 1963). This recommendation is made due to the fact that values obtained by this method were consistently similar to those obtained by conventional method, and due to ease of determination.

GENERAL SUMMARY

In Experiment I, four mature rumen-fistulated Hereford steers grazing a pure stand of Midland bermudagrass for an entire growing season were used to estimate voluntary forage intake. Two of the steers were used to obtain forage samples by the rumen evacuation technique. The remaining two were used to determine forage digestibility and fecal output. Chromic oxide was used as an indicator for calculating fecal output, and undigestible cellulose (determined by nylon bag) was used as the internal indicator for calculation of dry matter digestibility.

Forage samples obtained by the rumen evacuation technique were analyzed for crude protein, acid detergent fiber, neutral detergent fiber, lignin, cellulose and *in vitro* dry matter digestibility. Seasonal intake was correlated with each of the above mentioned constituents. None of these correlations proved significantly different from zero ($P<.05$), possibly because the animals were able to select high quality forage at each sampling time. Hence, grazed samples may not have been representative of the total forage available in the pasture.

Experiment II was designed to compare various indicator methods of calculating fecal output, dry matter digestibility, and dry matter intake with the conventional method. Four

mature rumen-fistulated Hereford steers were used in Trial I while ten steers were used in Trial II. Long bermudagrass hay was fed in Trial I, whereas in Trial II the hay was pelleted. The indicator of fecal output in Trial I was chromic oxide (gelatin capsule), administered once daily at 8:00 a.m. In Trial II, chromic oxide and polyethylene glycol (PEG) were compared as indicators of fecal output. The indicators used for determining dry matter digestibility were undigestible cellulose (determined by nylon bag) lignin, and in vitro dry matter digestibility. The use of lignin as an indicator was discontinued in Trial II because of poor recoveries in Trial I.

The most accurate estimate of fecal output was obtained using chromic oxide. In Trial II there was no significant difference between actual weighed fecal output, and that obtained using chromic oxide. Calculated fecal output, using PEG, was significantly higher than actual fecal output, due to incomplete recovery of the indicator. There was however, less diurnal variation in excretion of PEG than for chromic oxide.

The most reliable estimate of forage digestibility was shown to be in vitro dry matter digestibility. Forage digestibility determined using the undigestible cellulose ratio was much lower than that determined by the conventional method in Trial I but there was no significant difference in Trial II. The use of lignin, in bermudagrass, as an internal indicator of forage digestibility, is not

recommended due to poor recoveries.

The only indicator combination in Trial I which produced a calculated dry matter intake that was not significantly different from actual intake was the chromic oxide-*in vitro* digestibility combination. However, this was a combination of a calculated digestibility that was 12.9 percent too low, and a calculated fecal output that was 24.5 percent too high. In Trial II there was no significant difference between actual intake and that calculated using chromic oxide-undigestible cellulose or chromic oxide-*in vitro* digestibility. All values calculated using PEG were significantly higher than the actual values because of incomplete recovery of the indicator.

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