DIGESTIBILITY OF DRY WEATHERED RANGE GRASS BY BEEF CATTLE AS DETERMINED BY INDICATOR METHODS

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INTRODUCTION

Native range grass is the basic source of roughage for beef cattle in the Southwest, both summer and winter. The most profitable method of wintering beef cattle in Oklahoma is on dry, weathered grass, properly supplemented with feeds that supply the nutrients lacking in the weathered forage. Cottonseed meal for protein and phosphorus, and milo for energy are two supplements widely used for that purpose.

More information is needed on the availability of nutrients in dry, weathered range grass as affected by protein or energy supplements. An experiment was conducted with beef cows to determine the effect of two different kinds of supplement, one high in protein and the other rich in both protein and N.F.E., on the digestibility of a ration based on dry grass. In these studies, it was possible to compare the chromic oxide and lignin ratio techniques as indicator methods.

REVIEW OF LITERATURE

Digestibility of range grass

Digestibility of dry weathered range grass by beef cattle as affected by supplemental feed has been studied by different workers. It is well known that when grass becomes mature, its digestibility and nutritive value decline. Experiments show that the addition of protein and energy may increase the digestibility of some nutrients, but decrease others.

Crampton and Jackson (1944) found a seasonal change in herbage and a decline in the digestibility and nutritive value of pasture grass as the season advanced.

Briggs *et al.* (1948) studied the digestibility of prairie hay harvested in June, July, August and September and fed to steers, plus 2.0 pounds of cottonseed meal per head daily. The digestibility of the nutrients in the hay was improved by the addition of cottonseed meal, and an increase in palatability was noted.

Studying the apparent digestibility of prairie hay of variable protein cotent, Gallup and Briggs (1948) found that the total digestible nutrients in the hay increased in a fairly uniform manner from 41% to 56% as the protein content of the hay increased from approximately 3% to 6%. Likewise, crude fiber digestibility increased from negative values to 41%. When cottonseed meal, in amounts varying from 0.5 pounds to 3 pounds per head daily,

was fed as a supplement to 10 pounds of hay, differences in digestibility as related to the protein content of the hay were again apparent. The digestibility of dry matter in the high protein hay was equal to that in rations made up of average to low-protein hay supplemented with 2 pounds of cottonseed meal. A marked increase in hay consumption was found when a protein supplement, even in such small amounts as 0.5 pound per hay daily, were added to the ration.

Swift et al. (1947) found that when starch was fed as an addition to alfalfa and timothy hay of excellent, uniform quality the digestion of crude fiber was definitely depressed. Hamilton (1942) suggested that this decrease in digestibility of fiber is due to preference for sugar by the microorganisms of the rumen. Hungate et al. (1952) found that the number of cellulolytic, bacteria in the rumen of sheep decreased markedly after consumption of a large amount of grain.

Despite numerous studies that have demonstrated the beneficial effect by additions of protein to mature forages, further experiments are necessary with mature bluestem and associated grasses because of their economic importance for wintering beef cattle in the Southwest. Limited data are available on the T.D.N. content of dry, weathered range grass, which is the chief source of energy for wintering beef cattle. Further studies are needed to show the effects of different kinds and levels of supplemented feed on digestibility of nutrients in mature and weathered grass.

The Use of Lignin as an Indicator

Lignin is a polymeric, noncarbohydrate material whose structure is unknown. It is believed that p-hydroxyphenylpropanes, derived from coniferol alcohol or some closely related compound, are the fundamental repeating units; methoxy groups usually are present ortho to the phenolic hydroxyl group (Fruton et al., 1960).

In higher plants, shikimic acid has been found to be a precursor of the phenol residues of lignins. Brown et al. (1955) working with wheat and ash-leaved maple found that shikimic acid is readily convertible by the plant to some compound closely allied to phenylalanine, which is a lignin precursor. Eberhardt et al. (1956) incorporating 2, 6-C^{14} shikimic acid into a living sugar cane plant found that the lignin isolated contained radioactivity. They concluded that shikimic acid is an intermediate in the pathway from carbohydrates formed by photosynthesis, to the aromatic rings of the lignin.

Diverse results have been observed when lignin was used as an indicator in the determination of digestibility coefficients. Crampton and Maynard (1938) fed clipped grass to rabbits and alfalfa hay-grain ration to a steer and found that 97.8 and 99.3% of the dietary lignin respectively were recovered in the feces.

Hale, Duncan and Huffman (1939) claimed that the lignin ratio technique was not a reliable measure of total digestion. Using Holstein cows fed alfalfa hay, they found that lignin was digested in variable amounts up to 23.7 percent and calculations of total digestion with lignin ratios

resulted in low coefficients. The method used in determining lignin was essentially that outlined by Crampton and Maynard (1938). Maynard (1940) found that digestion of lignin by rabbits and guinea pigs fed alfalfa hay was practically nil, but that a lamb digested 28% of the lignin from the same hay.

Using the method of Crampton and Maynard (1938), Forbes and Swift (1943) found that lignin varied in digestibility from negative values to plus 28%. In a metabolism experiment in which several different forage plants were fed to sheep, Bondi and Mayer (1943) obtained digestibility coefficients that ranged from 35.1 to 64% indicating that the sheep digested lignin comparatively well.

Crampton and Jackson (1944) stated that lignin, as estimated by either the method of Crampton and Maynard (1938) or the method of 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) considered the apparent lignin digestibility to be due largely to an inefficient method of determination and proposed a standard "72 percent sulfuric acid" method of determination. Using timothy hay and Sudan grass in a limited number of digestion trials, they found that lignin was not digested by the cow, sheep or rabbit; the recovery of lignin varied from 94 to 106 percent. Since lignin was apparently not digested, the lignin values in feeds and feces might prove useful in determining the coefficient of digestibility of other feed constituents. They called the new method the "Lignin Ratio Method". They suggested also

that the feed consumption could be measured provided the feces were quantitatively collected.

Forbes et al. (1946) using Merino wethers fed clover-timothy hay found that the average digestibility of lignin determined by the method of Ellis et al. (1946) was -1.0% and determined by the method of Crampton and Whiting (1942) was 24.4 percent.

Davis et al. (1947) found that the digestion coefficients of the lignin in pea vines was 16.2 percent and that in the lima-bean vines was 10.6 percent when they were fed in the dehydrated state to yearling and 2-year-old Hampshire ewes.

Swift et al. (1947), in a study made of the effect of supplements on the digestibility of a mixed ration for sheep, found that the digestibility coefficients of lignin apparently were not affected by the various supplements and were close to zero in all cases; the extreme values being -6.2 and +5.2 percent. Twelve values out of 17 did not exceed $\pm$ 3%. From these data they stated that lignin might be considered indigestible and that variations in the digestion coefficients above and below zero were due to experimental error.

Forbes and Garrigus (1948) used the lignin ratio technique to study the digestibility of pasture forages by steers and wethers. In a series of seven digestion trials with steers, the average recovery of lignin was found to be 102 $\pm$ 7 percent. Dry matter digestibility and total digestible nutrient content of the various forages were found to vary inversely with the lignin content of the forage.

Pazur and De Long (1948) reported that lignin in the earlier stages of growth in trefoil was more readily metabolized by ruminants than the lignin in more mature plants.

Kane et al. (1950) working with cows fed alfalfa silage or hay found no significant difference between digestibility coefficients obtained by the use of lignin and Cr_2O_3 ratios and those calculated by the standard total collection procedure. The recovery of lignin was 98.8 percent and the recovery of Cr_2O_3, 99.9 percent. Since the indicators could be fully recovered by the chemical procedures used in that study, they considered both substances indigestible, and suitable for the ratio technique. However, they stated that "the use of lignin should be adopted with some reserve, since it is not a chemical entity of a definite composition. The make-up of lignin in plants may vary depending on the species and maturity of the plant, which might in turn affect the chemical determination of lignin. Similarly, these factors might also affect the digestibility of lignin."

Cook and Harris (1951) compared the lignin ratio technique and the chromogen method of determining digestibility and forage consumption of desert range plants by sheep. It was found that chemical analysis of the lignin and chromogen content of range forage could be determined with equal accuracy. The difference between duplicate samples and duplicate determinations of the same samples were insignificant (at the 1% level in all cases). However, when differences among animals were compared by the two methods for various species, the lignin technique gave consider-

ably less variability than the chromogen method. The coefficient of variation for lignin content of feces among animals grazing big sagebrush was 11.9%, as compared to 21.3% for chromogen content. A similar relationship was found when comparing the lignin and chromogen content of feces of animals grazing shadscale. The coefficients of variation in this comparison were 8.3% for lignin and 20% for chromogen.

Kane et al. (1953) comparing various digestion trial techniques with dairy cattle found that the fecal recoveries of lignin were somewhat less than 100%, which resulted in lower digestibility coefficients calculated by the lignin ratio technique than those calculated by the standard consumption-excretion method.

Ely et al. (1953) reported that the apparent digestibility coefficient of lignin in rations containing orchard grass hay cut at four stages of maturity ranged from 3.8 to 16.0%.

Sullivan (1955) studying the cellulose and lignin in forage grasses and their digestion coefficients found that lignin itself had a considerable degree of digestibility with coefficients exceeding 10 in many cases. He suggested that this partial and variable digestibility of lignin renders it unsuitable as a reference substance for determining the digestibility of other constituents. He found also that the lignin of the feces was attacked more readily by strong acids and weak alkalis than the lignin isolated from the grass; this would be an indication that the lignin was modified in its passage through the sheep. Concerning this, Csonka et al. (1929) reported that lignin prepared from corn

cobs by the alkali method was fed to cows and dogs. The methoxyl group determination made on the lignin fed and that eliminated in the feces indicate that lignin suffers a loss of the methoxyl group in passing through the animal body and, hence, is broken down by the animal.

In studies at Utah, in which winter browse plants were fed to deer, Smith et al. (1956) found that the digestion values determined by the lignin ratio differed markedly from those obtained by conventional analysis.

Comparing the chromium oxide ratio and the lignin ratio techniques for determination of digestibility of hays, Archibald et al. (1958) reported that the digestibility coefficients for dry matter, protein, energy, crude fiber and nitrogen-free-extract from 16 trials showed that Cr_2O_3 method for determining digestibility of forages gave somewhat more uniform results than did the lignin method, as indicated by generally lower standard errors of the mean values.

Elam and Davis (1961) found that steers fed a mixed ration digested an average of 12.9 percent of the lignin fed.

Hill et al. (1961) reported that the percentage of lignin recovery in the feces for collections of 8-hour intervals during 7 days varied from 88.87 to 134.32, indicating considerable overstimulation in fecal output which was reflected in lowered feed digestibility, especially in the crude fiber fraction.

Using wethers fed a complete pelleted ration composed of 60% alfalfa hay and 40% ground barley, and mixed with 0.5% of chromic oxide,

Elam et al. (1962) reported that the chromic oxide recovery averaged 100.7% whereas dietary lignin recovery averaged 90.2%.

The study of the literature available fail to definitely answer whether or not lignin is an accurate indicator which is useful in digestibility trials. Further studies concerning its use, recovery and analysis are needed.

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

Chromic oxide has been used as an indicator in calculating digestibility of rations. Its use in the ratio technique obviates the need for data on the amounts of feed consumed and feces voided. The use of chromic oxide as such an index substance was first proposed by Edin (1918).

Hamilton, Mitchell, Kick and Carman (1927-28), from experiments with sheep, found a good agreement between the conventional (quantitative) fecal collection and the chromic oxide method, provided collection periods were of 3 days or longer in duration.

Kane et al. (1950), using cows fed alfalfa silage or hay, found no significant difference between digestibility coefficients obtained by the use of chromium oxide ratio and those calculated by the standard total collection procedure. The average recovery of chromic oxide was 99.9%.

Using rats, Schurch et al. (1950) found that the apparent digestion coefficients of the dry matter of the diet calculated by the conventional method and by the index method were similar.

Crampton and Lloyd (1951) studied the use of chromic oxide as an

index of digestibility of ruminant rations with sheep. Their studies indicated that the chromic oxide method might be successfully employed, provided the ration includes ground feed with which the chromic oxide may be premixed. On an unground, all-roughage ration, the chromic oxide administered as such tended to be partially retained inthe digestive tract, thus leading to unreliable and low estimates of digestibility. They suggested also that not less than 5 days following first ingestion of the index material must elapse before feces collection for analysis should be saved.

Kane et al. (1952) found that significant diurnal variations in the rate of chromic oxide and lignin excretion occur in dairy cattle. They confirmed, also, that one fecal sample was inadequate for the determination of digestibility. By confining the selection of sampling to hours when the diurnal variations of the indicators were at a minimum, indicator amounts recovered in the excreta should correspond more accurately to the 24-hour average.

Hardison and Reid (1953) reported that working with grazing steers, fecal samples procured at 6:00 A.M. and 4:00 P.M. and compounded on an equal net weight basis during 7 or more days, resulted in what were considered to be reliable estimates of dry matter intake. The mean rates of recovery of oxide from the feces taken from grazing steers at 6:00 A.M. and 4:00 P.M. were 71.8 and 129.3%, respectively. Wet bulking of the feces samples taken at these two times of the day resulted in an average recovery of 99.95% of the chromic oxide ingested by the grazing steers. The results ofthe

experiment indicated that the sampling of feces at two convenient times of the day (one time when the recovery of chromic oxide is less than 100% and another when the recovery is greater than 100%) would tend to effect a predictable recovery.

Kane et al. (1953) reported that in the study of the digestibility of orchard grass hay by milking dairy cows, the digestion coefficients calculated by the chromic oxide ratio compared very closely with the standard total collection technique for all the constituents of the proximate analysis.

Brannon et al. (1954) found a very close agreement between the estimated values of the chromic oxide-chromogen technique for estimating total intakes and those derived from the total collection. Five grams of chromium oxide in a gelatin capsule were given orally to each steer at 7:00 A.M. daily and "grab" samples of feces were taken at 6:00 A.M. and 4:00 P.M. daily.

Working with pigs, Clawson et al. (1955) found that digestion coefficients computed from the mean concentrations of chromic oxide in fecal samples taken from pigs individually during one-day periods agreed closely with those determined in a total collection trial of 7-day duration.

Smith and Reid (1955) conducted a series of five trials to determine the adequacy of chromic oxide as an indicator of fecal output by grazing cows. The Cr_2O_3 concentration of feces taken rectally at 6:00 A.M. and 4:00 P.M. on seven consecutive days and bulked on an equal-weight basis provided an accurate estimate of total fecal output. The mean rate of re-

covery of the ingested Cr_2O_3 from the combined feces sampled at 6:00 A.M. and 4:00 P.M. was 100.58 $\pm$ 0.87%. A study of the Cr_2O_3 concentration-time excretion pattern of grazing cows revealed that from 65 to 141% as much chromic oxide was voided in the feces at various hours of the day as was found in well-mixed feces, representing the total amount of feces voided during a 7-day period. This precluded the indiscriminate adoption of times for the sampling of feces for the purpose of measuring the total output of feces by grazing cows. No difference in the accuracy of the estimated output of feces was found between the administration of Cr_2O_3 in capsules and that in concentrate feeds.

Hardison et al. (1956), working with dairy cows, reported that when the chromic oxide was given twice daily the variability in the excretion rate was reduced substantially. In these studies the recovery of Cr_2O_3 ranged from 97 to 103%. When the indicator was administered twice daily and partial fecal samples taken at 6:00 A.M. and 4:00 P.M. the random error was about 1/16 of the error for the same sampling times, but with once-daily administration. They considered also that it would be more accurate to use more animals during less days, instead of less animals over more days, in digestibility trials.

Studying the variation in the excretion of chromic oxide by ruminants, Kameoka et al. (1956) reported that when hay and concentrate containing Cr_2O_3 were administered to goats at 9:00 A.M. and 4:00 P.M. or at 7 and 17-hour intervals, one peak of Cr_2O_3 occurred during the daytime. When the concentrate containing the Cr_2O_3 was fed alone, the chromic

oxide excretion curves were roughly similar to those when concentrate and hay were fed.

In an experiment to determine the effect of frequency of administration of chromic oxide on its fecal excretion pattern by grazing wethers, Pigden and Brisson (1956) reported that the Cr_2O_3 content of grab samples varied from 45 to 180% with once-a-day dosing and 65 to 135% with twice-a-day dosing. There was no diurnal trend in the excretion pattern when animals were dosed 6 times a day. They reported also that the length of preliminary period required for average daily Cr_2O_3 recovery to equal intake was found to be 10 days although average daily recovery reached 90% within 4 days after dosing began.

Balch et al. (1957) reported that the posture in which the animal received the capsules of Cr_2O_3 exerted no marked effect on the concentration of Cr_2O_3 in the feces. Administration of Cr_2O_3 before a single daily feed caused a more even excretion of Cr_2O_3 in the feces than administration immediately after feeding. It was recommended that in grazing experiments, Cr_2O_3 should be given at the beginning of the main daily periods of grazing. It was also suggested that the suitability of any given scheme of Cr_2O_3 administration and of feces sampling should be checked against a complete collection of feces under the conditions of each experiment.

Studying the effects of various hay-concentrate ratios on nutrient utilization and production responses of dairy cows, using Cr_2O_3 as an indicator mixed with the concentrate, Bloom et al. (1957) reported that the

maximum excretion of Cr_2O_3 was observed in samples taken at 5:00 A.M. or 9:00 A.M. and the minimum excretion was observed in samples at 5:00 P.M. or 9:00 P.M. They used for analysis the fecal collection made at 9:00 A.M. and 5:00 P.M.

Lambourne (1957a) studied the rate of passage of Cr_2O_3 through the sheep digestive tract and found that Cr_2O_3 first appeared in the feces 5-8 hours after dosing and the concentrations rose rapidly to peak values about 10 to 18 hours after dosing. Concentrations fell thereafter by about 50% each 6 to 10 hours until 60 to 70 hours after dosing when marker content could no longer reliably be determined by the method used. He reported also that the overall quality of the feed affected the basic pattern in that peak concentrations occurred progressively later on poorer pastures and on hay.

Working with sheep and estimating feces output with Cr_2O_3, Lambourne (1957b) found that if marker doses were administered and feces samples were taken twice daily at alternate intervals of 9 and 15 hours approximately, the mean marker concentration of these samples would yield an essentially unbiased estimate of the general level of marker in the feces.

Murdock et al. (1957) reported that yearling Holstein heifers grazing orchard grass without supplement and receiving Cr_2O_3 by capsule at a uniform level of 16 gm. daily in two equal doses at 6:00 A.M. and 4:00 P.M., the average percentages of Cr_2O_3 in fecal samples collected at 6:00 A.M. and 4:00 P.M. were in very good agreement with the average of all collections. The total recoveries of Cr_2O_3 from the individual animals

for the 4-day period were 94.0, 90.3 and 98.4%.

Studying the diurnal excretion of Cr_2O_3 given in gelatin capsules by cows fed rations containing from 35 to 100% roughage, Putnam et al. (1957) found that the Cr_2O_3 of the samples of feces varied in a diurnal fashion with the highest concentrations occurring at about 2:00 P.M. and the lowest concentration at about 2:00 A.M.. The percentage recovery of Cr_2O_3 for the 16 cow days was 99.6%.

Comparing the chromic oxide ratio and the lignin ratio techniques for determination of digestibility of hays, Archibald et al. (1958) reported that digestibility coefficients for dry matter, protein, energy, crude fiber and nitrogen-free-extract from 16 trials showed that Cr_2O_3 method for determining digestibility of forages gave somewhat more uniform results than did the lignin method, as indicated by generally lower standard errors of the mean values.

Bradley et al. (1958) reported that the Cr_2O_3 fed in a complete pelleted ration for steers was less variable in the excretion than the Cr_2O_3 given in capsules. The digestibility coefficients were similar to those obtained by total collection.

Studying the use of Cr_2O_3 in a sustained release pellet to reduce variation in its diurnal excretion pattern in cattle fed fresh grass, Brisson and Figden (1958) found no significant differences between the concentrations of Cr_2O_3 and that so-called physiological diurnal effects were of little importance. They suggested that a 12-hour sampling procedure would be more accurate than unequal fecal sampling times.

Elam et al. (1959) studied the fecal excretion pattern of chromic oxide administered to Hereford heifers in a completely pelleted ration. Chemical analysis of feces samples collected at 3 hour intervals for 48 hours indicated a significant time-concentration variation in the fecal pattern of Cr_2O_3 excretion, even though the indicator was mixed with the entire ration.

Studying the use of Cr_2O_3 for estimating the fecal output of dairy animals, Hardison et al. (1959) found that it would be necessary to allow from 3 to 7 days to reach a stable level of the Cr_2O_3 in the feces. The mean recovery rates of Cr_2O_3 from totally collected feces from the combined 6:00 A.M. and 6:00 P.M. samples showed a close agreement in three trials.

In 1960, Corbett et al. compared Cr_2O_3 given to sheep in three different forms: In capsules, in a sheet of Cr_2O_3 paper, and in Cr_2O_3 shredded paper. During the last 12 days of the experiment, feces were obtained directly from the rectum of each sheep once daily, at a different time determined at random each day. The 12 samples from each sheep provided estimates of the concentration of Cr_2O_3 in the feces at the 2 hour intervals from 2:00 to 24:00, which are independent of one another. Variability in concentration was much less for the treatment with Cr_2O_3 shredded paper than for the treatment with capsules, and the treatment with a sheet of Cr_2O_3 paper was intermediate. Also Corbett et al. (1958) considered the administration of Cr_2O_3 in paper to be superior to the administration in powder form. They believed that the

general pattern of the passage of chromium sequioxide derived from the digestion of the paper might more nearly approach that of the products of digestion of the food. Studying the factors affecting the excretion pattern of chromic oxide in feces of dairy cows on pasture, Linnereud and Donker (1961) found that cows on high concentrate had an excretion pattern that was more variable and about 2 hours ahead of the cows on low concentrate. Also they found that cows fed Cr_2O_3 once daily displayed a curve with one high and one low per day whereas cows fed twice daily had curves with two high and two low periods and with about half as wide a range in values.

Bertrand et al. (1962) reported that chromic oxide as an external indicator was relatively accurate in predicting fecal output. Working with dairy cows, the total fecal collections on five cows showed an average chromic oxide recovery of 99.5% with a range of 86.8 to 109.1%.

Elam et al. (1962), using sheep, found that coefficients of digestibility determined from single-day feces samples by the chromic oxide ratio method agreed well with those obtained by the total collection method. Chromic oxide recovery during the experiment averaged 100.7%.

Despite considerable variation in results obtained by various workers the review of literature indicates in that when chromic oxide is given twice daily, mixed with a concentrate, and partial fecal samples are taken in the morning and in the evening, a reasonable good recovery of the indicator can be expected.

EXPERIMENTAL PROCEDURE

TRIAL I

Four mature Hereford cows, weighing approximately 1000 pounds each were used as experimental animals. They were placed in a barn in individual pens where they remained during the 12-day pre-collection period and the 7-day collection period. A two-week period of adjustment was allowed in order that the cows could become accustomed to the pens before the pre-collection period.

Each cow received 25 pounds of chopped prairie hay, once daily in the morning after the collection of feces. The concentrates were fed twice daily i.e. in the morning and evening after the collection of feces. At approximately 4-day intervals, the refused hay was collected, weighed and recorded. The rations fed and the chemical composition of the feeds used in this experiment are given in Tables I and III.

Prairie hay was primarily little bluestem (Andropogon scoparius), big bluestem (Andropogon gerardi) and Indiangrass (Sorghastrum nutans). The high protein concentrate fed (A) was cottonseed meal containing molasses and a small amount of Cr_2O_3, fed at the rate of one pound daily ($\frac{1}{2}$ pound in the morning and $\frac{1}{2}$ pound in the evening). The high protein and energy supplement (B) contained 1 pound of cottonseed meal mixed with the chromic oxide plus $1\frac{1}{2}$ pounds of C.S.M. and 3 pounds of ground

milo. This was also fed in equal parts in the morning and the evening. Chromic oxide was mixed with the cottonseed meal in a premix in proportions of 80% C.S.M., 10% chromic oxide and 10% molasses. Cows 1 and 2 were fed with supplement A ration and cows 3 and 4 with supplement B ration.

Fecal samples were obtained twice daily at 8:00 A.M. and 4:00 P.M. from each cow, usually by stimulation of defecation or directly from the rectum. About 200 gm. of each sample were immediately placed in parafined containers, powdered with thymol crystals to prevent putrefaction and stored under refrigeration until prepared for analysis. During the trial, frequent samples of the concentrates and hay offered the animals were taken for chemical analysis.

All samples of feces were placed in a forced air oven at 50 degrees Centigrade until completely dry. Following this, they were ground in a Wiley mill and stored. Proximate analysis of the feeds and feces were conducted by the methods described by the A.O.A.C. (1955). Chromic oxide in feeds and feces was determined by the colorimetric method described by Kimura *et al*. (1957). Lignin in the feeds and feces were determined by the method described by Van Soest (1962) which is considered to be a more rapid, but as reliable, a method as those commonly used.

TRIAL II

At the completion of the first trial the cows were switched to the opposite feed level and a second trial was initiated in which cows 3

and 4 received the concentrate A and cows 1 and 2 received the concentrate B. After 15 days of pre-collection period, the feces were collected as described previously for 7 days. Rations fed, time of feeding and feces collections were the same as described for the first trial. Chemical composition of feeds and feces of the second trial are shown in Tables II and III. The chemical analysis followed the same procedures used in the first trial. The statistical methods as described by Steel and Torrie (1960) were used to analyze the data.

RESULTS AND DISCUSSION

The average coefficient of apparent digestibility for each nutrient obtained with four cows on each treatment are shown in Table IV. In this study the coefficients of apparent digestibility for the ration supplemented with concentrate B were higher than for concentrate A for dry matter, crude protein, ether extract and nitrogen-free extract, but lower for crude fiber.

In the case of dry matter, the ration supplemented with concentrate A had a coefficient of apparent digestibility of 49.85 percent as determined by the chromic oxide ratio method, and 43.76 percent according to the results of the lignin method. The ration supplemented with concentrate B had a coefficient of apparent digestibility of dry matter of 52.68 and 46.23 percent for the chromic oxide and lignin ratio methods, respectively. These differences between the rations supplemented with concentrates A and B were not significant at the 10 percent probability level.

For protein, the coefficients of apparent digestibility, as determined by the chromic oxide and lignin methods for concentrate A were 37.37 and 29.10 percent, whereas for the concentrate B supplemented ration they were 49.06 and 41.55 percent, respectively. These differences between treatments were significant ($P < 0.05$).

The coefficients of apparent digestibility for ether extract of the ration supplemented with concentrate A were 51.64 percent using the chromic oxide method, and 45.58 percent using the lignin ratio method. For rations containing concentrate B the values were 62.43 and 56.85 percent, respectively. These differences between treatments were significant ($P < 0.05$).

For nitrogen-free extract, the coefficients of apparent digestibility of the ration supplemented with concentrate A were 47.94 percent using the chromic oxide ratio method, and 41.63 percent using the lignin ratio method. For rations supplemented with concentrate B the coefficients were 54.96 and 48.84 percent, respectively. The differences between treatments were not significant ($P > 0.1$).

Apparently, some of the lignin was digested, as indicated by the results of the chromic oxide ratio method. This amounted to 10.91 percent for the concentrate A ration and 12.82 percent for the concentrate B ration. This difference between treatments was not significant ($P > 0.1$).

The only nutrient that showed a lower coefficient of apparent digestibility when the dried grass hay was supplemented with concentrate B versus concentrate A was crude fiber. Digestibility of this nutrient in the concentrate A ration was 61.11 percent using the chromic oxide ratio method, and 56.51 percent by the lignin ratio method; with concentrate B these values were 57.30 and 51.63 percent, respectively. These differences between treatments were not significant at the 10 percent level of probability.

These results are in agreement with those obtained by other workers. Bloom et al. (1957), studying the effects of various hay-concentrate ratios on nutrient utilization in dairy cows, reported that increasing the level of concentrates significantly depressed crude fiber digestibility ($P < 0.05$). Although the effect of amount of concentrate was not significant, there was a trend for greater crude fiber digestibility by the cows fed lower levels of concentrate.

Gallup and Briggs (1948) found succeeding increases in the digestibility of nutrients in prairie hay as the protein content of the ration increased from 3 percent to approximately 6 percent. However, they reported a close relationship between protein content and digestibility of nutrients in hay. In the study reported herein, crude fiber digestibility decreased when the total ration increased in protein.

Briggs et al. (1948) have shown that when prairie hay was supplemented with 2.0 pounds of cottonseed meal daily, the digestibility of the nutrients in the hay by steers was improved.

In the present study, the ration supplemented with concentrate B contained more N.F.E. than the ration supplemented with concentrate A. There are many studies reported in the literature in which the addition of readily digestible carbohydrates adversely affected the digestibility of crude fiber. Mitchell et al. (1940) reported that when glucose was added to a basal ration of timothy hay, cottonseed meal and corn fed to beef calves, the digestibility of the insoluble carbohydrate was decreased. Hamilton (1942) suggested that this decrease in digestibility

of fiber is due to preference for sugar by the microorganisms of the rumen. Swift et al. (1947) found that when starch was fed as an addition to alfalfa and timothy hay of excellent uniform quality, the digestion of crude fiber was definitely depressed. Hungate et al. (1952) found that the number of cellulolytic bacteria in the rumen of sheep decreased markedly after administration of a large amount of grain.

Relative to the influence of the hay consumed on the digestibility of the nutrients, Briggs et al. (1948) fed prairie hay of somewhat similar species and composition to that used in this experiment, but harvested in September. The hay was fed to steers at the rate of 12 pounds and supplemented with 2 pounds of C.S.M. Coefficients of digestibility for all nutrients were higher for the supplemented hay as compared to the weathered grass hay used here.

Such differences in digestibility might be due to several factors. The physical nature of the hay, which was chopped in these trials, might increase the total amount consumed. It has been shown that grinding, for example, may increase the consumption of coarse roughage. In the experiment reported here, the cows ate an average of 18.13 pounds of chopped hay per head daily which is higher than the amount normally expected (approximately 1.5 pounds per 100 pounds body weight daily). Early studies of Eckles (1913) showed that digestibility in the dairy cow was slightly lower at full feed than that at maintenance.

The rate of passage through the digestive tract also may influence the results obtained. Blaxter et al. (1956) found a decrease in digesti-

bility when grass was ground and pelleted, which caused it to pass through the digestive tract more quickly. Maynard and Loosli (1956) suggested that digestibility of the nutrients in a roughage may be limited by the lack of time to complete digestive action on the less easily digestible substances.

The coefficients of digestibility of different nutrients determined by the Cr_2O_3 ratio method and the lignin ratio method were considerably different (Table IV). The determination of digestibility of lignin by the chromic oxide ratio method showed that it had a variable but considerable digestibility ranging from -0.16 to 28.01 percent. This may explain the lower coefficients of digestibility obtained with the lignin ratio technique.

In the review of literature, it has been pointed out that both methods have some defects and that the recovery of indicators in the feces is variable. Thus, the coefficients of digestibility obtained with either method will not give an accurate result if some precautions and adjustments are not made. A comparison of these results with those obtained with a complete collection method would have shown the difference between the coefficients of digestibility obtained using the ratio technique and the actual coefficients of apparent digestibility. Due to the nature of this experiment, a total collection of feces was not possible. On the other hand, when cattle are maintained in metabolism stalls, they eat less feed than under natural conditions. Hence some difference in coefficients of digestibility might be obtained than are actually occurring under field conditions and on higher roughage intake.

From the published digestion coefficients of Morrison (1959) for cottonseed meal and milo, an estimate can be made of the T.D.N. value of the dry, weathered range grass hay fed in these trials. Using the chromic oxide ratio technique, the values for such hay fed with A and B supplements were 49.12 and 47.5 percent T.D.N., respectively; using the lignin ratio technique these values were 43.4 and 39.8 percent T.D.N., respectively. The difference in values due to method or technique of measurement is apparently the result of some digestibility of lignin or other errors. The above values, using the lignin ratio method agree closely with the 40.1 percent T.D.N. cited by Morrison (1959), but the values obtained by the chromic oxide ratio method are higher. Based on the above values, it can be concluded that a dry beef cow consuming 1.5 pounds of such dry grass per 100 pounds body weight daily would obtain approximately 90 percent of her T.D.N. or energy requirement, based on Morrison's minimum allowance for a 1000 pound cow.

SUMMARY AND CONCLUSIONS

Two trials were conducted to study the digestibility of dry, weathered, range grass hay as affected by supplements high in protein, or both protein and grain. Four, mature Hereford cows were fed individually in each trial, with two cows per treatment. The same cows were used and the treatments were reversed in the second trial. Native range grass hay, predominantly little bluestem (Andropogon scoparius), big bluestem (Andropogon gerardi) and Indiangrass (Sorghastrum nutans), was harvested in early December and chopped by passing through a ½-inch screen hammer mill. Both chromic oxide and lignin were used as indicators to determine the apparent digestibility of the ration components by the ratio method. Supplement A, fed at a level of 1.0 pound per head daily, contained 80 percent cottonseed meal, 10 percent chromic oxide and 10 percent molasses. Supplement B, fed at the rate of 5.5 pounds per head daily contained 1.0 pound of the cottonseed meal-chromic oxide-molasses mix, plus 1½ pounds C.S.M. and 3 pounds ground milo. Both supplements were fed twice daily, with equal parts in the morning and evening. Hay consumption was recorded and any hay not consumed was weighed back. Fecal samples were taken morning and evening for 7 consecutive days following a 12-15 day preliminary period.

The results show that the dry grass ration supplemented with concen-

trate B, had a significantly higher coefficient of digestibility for crude protein ($P < 0.05$) and ether extract ($P < 0.05$) versus the dry grass supplemented with concentrate A. Digestibility of dry matter and N.F.E. were also improved by the higher level of supplementation, but not significantly ($P > 0.1$). Crude fiber digestibility was depressed by feeding supplement B versus A, but the difference was not significant ($P > 0.1$).

The coefficients of digestibility, as determined by the chromic oxide ratio method were higher than those obtained by the lignin ratio method. However, both indicator methods revealed the same trends from supplementation. An apparent digestibility of lignin, ranging from -0.16 to 28.01 percent was obtained. An estimation of the T.D.N. content of dry, weathered, range grass was calculated by the difference between the results obtained and the published values for cottonseed meal and milo. It appears that such forage contains 49.1 to 47.5 percent T.D.N., when supplemented with A and B concentrate mixtures, respectively, as determined by the chromic oxide method and 43.4 to 39.8 percent as determined by the lignin method.

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APPENDIX

TRIAL I - CHEMICAL COMPOSITION OF FEED AND FECES, PERCENT.

FEEDS	Dry Matter	Dry Matter Basis						
		Ash	Crude Protein	Ether Extract.	Crude Fiber	N.F.E.	Chromic Oxide	Lignin
Concentrate A[1]	94.45	18.31	35.00	2.60	7.44	36.65	11.31	5.30
Concentrate B[2]	92.64	5.87	24.56	3.29	5.03	61.25	1.90	3.94
Prairie Hay[3]	94.04	8.36	4.05	2.13	35.13	50.33	-----	10.30
FECES								
Cow 1	97.16	13.08	6.72	2.17	26.05	51.98	1.04	17.095
Cow 2	96.99	13.26	6.57	1.83	27.71	50.63	1.09	17.06
Cow 3	97.42	13.15	9.59	1.77	24.97	50.52	0.84	14.47
Cow 4	97.04	12.43	9.24	1.71	25.91	50.71	0.89	14.59

[1]Concentrate A composed of: C.S.M. 80%, Chromic Oxide 10% and Molasses 10%.

[2]Concentrate B composed of: C.S.M. 41.82%, Ground Milo 54.55%, Chromic Oxide 1.82% and Molasses 1.82%.

[3]Prairie Hay, weathered, composed primarily of little bluestem (Andropogon scoparius), big bluestem (Andropogon gerardi) and Indiangrass (Sorghastrum nutans).

TRIAL II - CHEMICAL COMPOSITION OF FEED AND FECES, PERCENT

	Dry Matter	Dry Matter Basis						
		Ash	Crude Protein	Ether Extract	Crude Fiber	N.F.E.	Chromic Oxide	Lignin
FEEDS								
Concentrate A[1]	93.96	17.26	39.35	2.66	7.76	32.97	10.88	6.37
Concentrate B[2]	92.57	5.84	24.33	3.00	5.05	61.78	2.07	3.72
Prairie Hay[3]	92.31	6.67	2.74	2.00	38.32	50.27	-----	9.59
FECES								
Cow 3	97.80	12.43	6.52	1.98	27.55	51.52	1.27	18.30
Cow 4	97.61	12.96	6.79	2.14	26.87	51.24	1.38	16.78
Cow 1	97.47	12.23	8.88	2.01	26.00	50.88	1.08	17.195
Cow 2	97.29	12.05	9.34	2.03	26.87	49.71	1.14	17.05

[1]Concentrate A composed of: C.S.M. 80%, Chromic Oxide 10% and Molasses 10%.

[2]Concentrate B composed of: C.S.M. 41.82%, Ground Milo 54.55%, Chromic Oxide 1.82% and Molasses 1.82%.

[3]Prairie Hay, weathered, composed primarily of little bluestem (Andropogon scoparius), big bluestem (Andropogon gerardi) and Indiangrass (Sorghastrum nutans).

TRIAL I AND II - DAILY FEED INTAKE OF EACH COW AND CHEMICAL COMPOSITION ON A DRY MATTER BASIS, OF THE TOTAL RATION

Supplement	Cow No.	Dry Matter in Total Ration	Daily Intake lb.			Constituents in Ration Percent						
			Hay	Concentrate	Total	Cr_2O_3	Lignin	C. Protein	.E. Extract	C. Fiber	Ash	N.F.E.
						TRIAL I						
A	1	94.07	17.48	1.06	18.54	0.61	10.1	5.82	2.16	33.55	8.90	49.56
	2	94.04	18.08	1.06	19.14	0.59	10.03	5.75	2.16	33.59	8.88	49.62
B	3	93.66	16.60	5.94	22.54	0.50	8.61	9.45	2.42	27.20	7.72	53.21
	4	93.68	17.48	5.94	23.42	0.48	8.67	9.26	2.41	27.50	7.73	53.09
						TRIAL II						
A	3	92.42	17.14	1.06	18.20	0.60	9.39	4.89	2.02	36.54	7.25	49.30
	4	92.41	18.32	1.06	19.38	0.56	9.44	4.75	2.05	36.64	7.22	49.34
B	1	92.38	18.61	5.94	24.55	0.44	8.15	7.96	2.24	30.26	6.48	53.06
	2	92.38	21.37	5.94	27.31	0.40	8.31	7.43	2.23	31.09	6.50	52.75

TABLE IV

COEFFICIENTS OF APPARENT DIGESTIBILITY, PERCENT. AVERAGE OF THE FOUR COWS IN EACH TREATMENT.

Treatment	Constituent	Chromic Oxide Ratio	Lignin Ratio
Supplement A	Dry Matter	49.85 ± 3.96[1]	43.76 ± 1.72
	C. Protein	37.37 ± 1.98	29.10 ± 3.18
	Ether Extract	51.64 ± 3.62	45.58 ± 2.51
	Crude Fiber	61.11 ± 3.78	56.51 ± 2.17
	N.F.E.	47.94 ± 4.18	41.63 ± 5.33
	Lignin	10.91 ± 5.91	
Supplement B	Dry Matter	52.68 ± 5.66	46.23 ± 3.3
	C. Protein	49.06 ± 3.81	41.55 ± 1.9
	Ether Extract	62.43 ± 7.56	56.85 ± 0.5
	Crude Fiber	57.30 ± 5.91	51.63 ± 4.0
	N.F.E.	54.96 ± 5.41	48.84 ± 3.15
	Lignin	12.82 ± 5.83	

[1]Standard error of mean.

TABLE V

TRIAL I AND II - TOTAL DIGESTIBLE NUTRIENTS IN THE RATION OF EACH COW CALCULATED BY THE CHROMIC OXIDE RATIO AND LIGNIN RATIO METHOD, PERCENT

Supplement	Trial No.	Cow No.	Total Digestible Nutrients	
			Chromic Oxide Ratio	Lignin Ratio
	I	1	41.32	41.23
		2	45.62	41.47
A	II	3	52.72	49.03
		4	58.75	44.87
	I	3	42.30	42.30
		4	46.91	41.96
B	II	1	59.53	53.52
		2	64.54	52.18
Average of both Trials				
A	I and II		49.58	44.17
B	I and II		53.32	47.49

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