STUDIES ON THE DIGESTIBILITY OF ORCHARD GRASS WITH DAIRY CATTLE

I. A comparison of several techniques for determining digestibility coefficients with dairy cattle.

II. Studies on the composition of lignin isolated from orchard grass hay cut at four stages of maturity and from the corresponding feces.

III. A study of the crude fiber and nitrogen-free extract fractions of orchard grass hay and the digestibility of some of the constituents by milking cows.

by

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A THESIS

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FORWARD

The results reported in this thesis were secured from investigations conducted over a period of the past three years.

In order to simplify presentation, the results have been divided into three parts. Part I was an investigation of several different techniques for determining the digestibility coefficients of forage for dairy cattle studied with orchard grass hay cut at four stages of maturity. Part II was a study of the digestibility of lignin in orchard grass hays cut at various stages of maturity and an investigation of the composition of the lignin isolated from these orchard grass hays and the corresponding feces. In Part III a more detailed analysis was made of the crude fiber and nitrogen-free extract fractions and the digestibility of the various constituents reported.

STUDIES ON THE DIGESTIBILITY OF ORCHARD

GRASS WITH DAIRY CATTLE

By

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AN ABSTRACT

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STUDIES ON THE DIGESTIBILITY OF ORCHARD GRASS WITH DAIRY CATTLE

A comparison of several techniques for determining digestibility coefficients with dairy cattle was made with four stages of orchard grass hay fed to three milking cows. Digestibility coefficients calculated by the conventional ten day consumption-excretion ratios were compared with the coefficients calculated by the chromium, lignin and plant pigment ratio techniques with the total collection samples and also with partial collections or "grab" samples.

Comparable digestion coefficients were secured with the standard ten day consumption-excretion method and the chromium and plant pigment ratio techniques. Digestion coefficients calculated by the crude lignin and the corrected lignin ratio techniques using the total fecal collection samples were both significantly lower than the digestion coefficients calculated by the standard consumption-excretion method.

Digestibilities of the various constituents in the four stages of orchard grass hay of increasing maturity were; dry matter, 69.0, 70.4, 63.9 and 59.4 percent; protein, 65.7, 60.7, 55.4 and 54.3 percent; crude fiber, 82.5, 78.6, 72.0 and 69.1 percent; and nitrogen-free extract, 69.3, 74.7, 68.0 and 57.3 percent, respectively.

The apparent digestion coefficients of lignin in the orchard grass hays fed ranged from 3.8 to 16.0 percent. The lignin isolated from the four stages of orchard grass of increasing maturity had nitrogen contents of 4.25, 4.15, 3.53 and 2.41 percent. The average nitrogen contents of the lignin isolated from the corresponding feces were 5.19, 4.13, 3.62 and 2.91 percent.

The benzoic acid excretion in the urine per 100 grams of lignin fed was lower when feeding the most mature hay than when feeding the other three less mature hays. There was no relation between the digestion coefficients of lignin and the urinary excretion of benzoic acid.

The total methoxyl content of the hay fed was lower than the total methoxyl content of the corresponding feces on a dry matter basis. The methoxyl contents of the lignin isolated from the various hays was higher than the methoxyl contents of the lignin isolated from the corresponding feces.

Digestibility of the total methoxyl content and the non-lignin methoxyl content of the ration decreased with increasing maturity of the orchard grass hay. Digestion of the lignin methoxyl groups ranged from 20.3 to 31.6 percent and showed no consistent trend in relation to the stage of maturity of the hay fed.

A more detailed system of analysis that included determinations of the starch, sugars, organic acids, pentosans, alpha cellulose, and lignin on the orchard grass hays and the corresponding feces was used to study the constituents of the crude fiber and nitrogen-free extract fractions.

This system of analysis accounted for a greater percentage of the dry matter of the immature orchard grass hay than with the more mature orchard grass hay. Higher percentages of the dry matter were also determined in the feces of animals fed the immature orchard grass hay than in the feces of animals fed the mature orchard grass hay. Considerably higher percentages of the total dry matter of feces were determined by this system of analysis than were determined with the same analytical techniques on the forage fed these animals.

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PART I

A COMPARISON OF SEVERAL TECHNIQUES FOR DETERMINING DIGESTIBILITY COEFFICIENTS WITH DAIRY CATTLE

INTRODUCTION

One of the most common methods used to evaluate the nutritive value of feeds is to determine their digestible nutrient content. Compilation of the average proximate composition, average digestion coefficients, and average total digestible nutrient content values for a large number of feeds have been made by Morrison (126) and Schneider (161). While these average values are in many instances derived from a large number of trials, many are of necessity taken from a limited number of trials and perhaps for a single species. Many research workers need to know the digestible nutrient content of a given feed for specific animals under certain controlled conditions or under widely varying conditions. For example, it is desirable to know the nutritive value of various pasture mixtures under widely varying conditions of rainfall, temperature, soil types, soil treatments, and grazing management with different animals which vary quite widely in their nutritive requirement. Schneider *et al.* (165) analyzed the published data for five classes of feeds fed to four species of animals to determine the value of average digestibility data in determining the nutritive value of particular feed samples. They concluded that the practice of applying average digestion coefficients for a given feed to particular samples of that feed is warranted despite the fact that a high within-feed variability in digestibility occurs. Schneider and Lucas (163) also studied published digestibility data to determine the relative magnitude of certain sources of variation. They found that 25 to 45 percent of the within-feed variance in digestibility is associated with variations in proximate composition, 20 to 40 percent associated with authors, 20 to 35 percent associated with samples of feed and 10 to 25 percent associated with the trial. They pointed out that average digestion coefficients should be adjusted for the proximate composition of the particular sample being used. They also suggested that a greater amount of the variance could be related to composition of the feed if other nutrients, in addition to those determined in the usual proximate analysis, were ascertained.

Interest in the so-called inert material "ratio techniques" of determining the digestibility of the various constituents of feed has been stimulated largely by the fact that it will permit digestion trial studies to be made under the same conditions and with the same animals that would normally be used for the feed investigated. Furthermore, addition of an inert material to the daily ration of a grazing animal would permit calculation of the daily dry matter excretion from "grab" samples collected at convenient intervals. The determination of the concentration of an inert naturally-occurring constituent of the feed on both the feed and feces samples would permit a calculation of the dry matter intake of the animal studied and calculation of the dry matter digestibility.

The use of the inert material ratio technique for determining the digestibility of a feed or ration would facilitate studies of various feeds with different species and under widely varying conditions that are not readily feasible with the conventional total fecal collection technique, if direct comparisons could be established between these methods.

REVIEW OF LITERATURE

DIGESTION TRIAL TECHNIQUES

Total Fecal Collection Technique

Digestion trial techniques involving the total collection of feces over a specified period of time while the animals are on a constant feed intake and calculation of the digestibility of the various nutrients from the nutrient intake-excretion ratio's have changed but little since their adoption by Henneberg and Stohmann (91).

The length of the collection period in digestion trials using total fecal collections for securing satisfactory digestibility coefficients is still a controversial subject. Relatively few comparisons of the accuracy of different lengths of trials have been made. Hamilton, Mitchell and Kammlade (86) used collection periods of five or six days and stated that they were too short for accurate results with steers. Schneider and Ellenberger (162) made a study of the length of a digestion trial on apparent digestibility. They used at least a week preliminary period and rations that were near maintenance level compared with higher levels of nutrition. They found the greatest error in calculating the digestion coefficients was the irregularity in the quantity of feces excreted which was more pronounced at low nutritional (maintenance) levels. They found the digestion coefficients from a nine-day lactation trial (high plane of nutrition) gave approximately the same accuracy as results from a twenty-four-day maintenance trial. Hamilton, Mitchell, Kick and Garmon (87) used a ten-day collection period for sheep and a fourteen-day collection period for steers preceded by several weeks of preliminary feeding. Sotola (17[illegible]) used ten-day collection periods preceded by ten-day preliminary periods with sheep on alfalfa hay. Fourteen-day preliminary periods and fourteen-day collection periods were used by Mumford et al. (127) in digestion trials with steers. Knight, Hepner and Morton (105) used seven- and eight-day preliminary periods and five-day collection periods with wethers. These trials do not give much information on the relative accuracy of different lengths of collection periods since the trials are not directly comparable.

Staples and Dinusson (174) have recently reported on the relative accuracy of seven- and ten-day collection periods in digestibility trials with steers. They calculated coefficients of apparent digestibility with the data secured from seven- and ten-day collection periods with 27 individual trials. The degree of accuracy secured with a seven-day collection period may be sufficient for many studies of apparent digestibility since they reported that "With a possible exception in the case of nitrogen-free extract the seven-day collection period gave coefficients comparable in accuracy to the ten-day collection period".

Chromic Oxide Ratio Technique

Several studies have been made with different species of animals on the addition of known amounts of chromic oxide to the ration and determining the concentration of chromic oxide in the resulting feces to allow this material to serve as an index of digestibility. The use of chromic oxide as an inert tracer substance was first proposed by Edin (59) who fed chromium oxide impregnated in blotter paper and reported recoveries of 99.1 percent with slight variation in 26 experiments. A brief description of Edin's chromic oxide indicator technique was published in English by Edin, Kihlen and Nordfeldt (61). This report gives rather complete discussions of the preparation of the chromic oxide macaroni, mixing of feed, collection of feces and the determination of chromium oxide in feeds and feces.

Hamilton, Mitchell, Kick and Garman (87) found good agreement between the chromic oxide ratio method and the total fecal collection method with sheep, provided the collection periods were of three days or longer in duration.

Satisfactory results with the chromic oxide ratio technique have been reported more recently by Andersen (1), Andersen and Frederiksen (2), and Skulmowski, Szymanski and Wyszynski (166) using sheep and horses; by Barnicoat (19) and Lloyd (112) with swine; by Olsson, Kihlen and Cagell (142) with horses; by Kane, Jacobson and Moore (102,103) with cows; by Schurch, Lloyd and Crampton (167) with rats; by Irwin and Crampton (96), Kreula (108), and Virtanen (181) with humans; by Chanda et al. (37) with goats; and by Axelsson and Eriksson with ruminants (15).

Barnicoat (19) obtained consistently low digestion coefficients with sheep and calves when the chromic oxide was given in a gelatin capsule. They checked the possibility of retention of chromic oxide in the digestive tract and reported increased concentrations in the stomach folds of a lamb.

Kane, Jacobson and Moore (103) made a direct comparison of the digestibility coefficients obtained by using chromic oxide as an indicator with the total fecal collection technique on samples representing the total collection of feces from cows. This was done to evaluate the indicator method and to be able to eliminate the errors due to sampling while studying the errors due to the methods being compared. The results of their investigation showed that there were no significant differences between the digestion coefficients obtained by the total collection method and those secured by the chromic oxide ratio procedure. Recoveries of chromic oxide were remarkably close to 100 percent.

They fed the chromium oxide as a 15 percent mixture with wheat flour baked into a hard bread which was dried, ground in a Wiley Mill and then mixed with the grain in such amounts that the cows received approximately 15 grams of chromic oxide daily or 100 grams of the chromium bread mixed with the daily grain ration.

Comparisons of various methods of feeding may serve to emphasize the importance of this phase of the "Ratio" procedures particularly with the use of chromic oxide.

Variable recoveries of chromic oxide from sheep were secured by Crampton and Lloyd (45) with different methods of administration of the chromic oxide, but their results indicated that the chromic oxide may be accurately used in determining the digestibility of a sheep ration, provided the chromic oxide can be premixed with ground grain in the ration, but not when it is given separately as was necessary on an entirely roughage ration. This is shown in the following table from Crampton and Lloyd (45) where the chromic oxide on the roughage alone ration was fed as a concentrated pellet (sheep land 2) and on the roughage plus grain ration the chromic oxide was mixed with the grain (sheep 3 and 4).

Table 1

Recovery of Chromic Oxide[1]

Sheep	Ration	Recovery (%)
1	Roughage + chromium pellets	85
2	Roughage + chromium pellets	87
3	Roughage + grain + chromium	98
4	Roughage + grain + chromium	99

[1]From Crampton and Lloyd (45).

This may partially explain the low digestibility coefficients obtained by Barnicoat (19) with sheep and calves when the chromic oxide was given in a gelatin capsule which would be comparable to the pelleted chromic oxide concentrate feeding reported by Crampton and Lloyd (45). The roughage plus grain plus chromium reported by Crampton and Lloyd (45) would be comparable to the method of feeding employed by Kane _et al_. (103) with cows which also gave satisfactory results.

Jarl (99) felt that the chromic oxide indicator method was superior to the total fecal collection method since it is not influenced as greatly by variations in the daily amount of feces voided. He stated that "About the same methodological accuracy is achieved in six repeated experiments (72 collection days) with the 'quantitative' method as in one experiment conducted according to the indicator method". He felt that there was a constant relation between the chromic oxide and the various nutrients in the ration and feces, so that a variation in feces quantity on two successive days would not affect the calculated digestibilities. Recent data of Kane, Jacobson and Moore (104) showed a variation in the concentration of chromic oxide in individual passages of feces throughout a 24-hour period which does not agree with Jarl's (99) assumption that there was a constant relation between the chromic oxide and the various constituents in the feces.

Andersen and Winther (3) found that a preliminary period of eight or nine days was sufficient for representative samples when using the chromic oxide ratio method. They made a quantitative feces collection from four individual cows during 20 days while they received a constant amount of feed containing 14.82 grams of chromic oxide. The average excretion per day was calculated to be 14.61 grams of chromic oxide which is 98.6 percent of the amount consumed. Since they found the recovery of chromium to be always low they proposed a correction by raising the amount of chromium found by two percent of the value. They presented tables which showed that the chromic oxide indicator method gave the same coefficients of digestibility as the total fecal collection method. They also conducted three experiments with nine cows in which they analyzed the feces from each cow that was excreted during the day and then the feces that were excreted during the following night. They found the ratios of chromic oxide to the various nutrients in the feces varied between day and night feces, but were not always higher for one than the other and suggested that the partial collection of feces samples be done throughout the entire 24-hour day.

Chanda, Clapham, McNaught and Owen (37) used the chromic oxide ratio technique to study the digestibility of carotene by cows and goats fed dried grass. With goats the total fecal collection technique was compared with the chromium ratio technique. When mixed with the concentrate portion of the ration of lactating goats, 100.0, 99.1, 99.1 and 96.0 percent of the chromic oxide fed was recovered in the feces in four experiments with four different goats. The apparent digestibility of carotene by the goat was determined by total collection in metabolism crates and also by the chromium ratio technique. By the total collection technique the digestibilities of carotene in four goats were 68.7, 62.7, 61.8 and 59.6 percent. The corresponding digestibilities obtained from the same feces samples by the chromic oxide ratio method were 67.4, 62.9, 61.8 and 58.9 percent.

Eriksson (63) compared the digestion coefficients obtained by the total fecal collection method in difference trials with wethers with those calculated for cows by the chromium ratio technique and concluded that the total collection method gave the most reliable results.

Edin (60) reported that the chromium ratio method of calculating the digestion coefficients of feeds when using a two day collection period gave as accurate coefficients as the total collection method in a seven to eight day period. Axelsson and Kivimae (17) calculated digestion coefficients by the chromium ratio procedure and from the nutrient consumption-excretion ratios on the same samples collected at two day intervals from three wethers over a period of 44 days. The average digestion coefficients of the 22 periods were nearly the same for the two methods of calculation. The digestion coefficients calculated by the total collection and the chromium ratio techniques respectively were; organic matter 71.7 and 71.2; protein, 74.5 and 74.2; ether extract, 70.1 and 69.3; nitrogen-free extract, 77.2 and 76.8; and crude fiber, 53.7 and 52.9. They further showed that the average digestion coefficient was independent of the length of the collection period (two to ten days) but that the standard deviation decreased rapidly with increased length of the period.

Lignin Ratio Technique

Lignin has been quite widely studied during the past few years since, if it could be established that it is an indigestible substance, it would also meet the requirements of being a natural constituent of the plant material.

Lignin has been reported to be indigestible by steers (46); by cows on a mixed feed and by sheep on dried Sudan grass (62); by cows on alfalfa hay (84); by sheep (83); by sheep on mixed feeds (178); by sheep on Korean lespedeza hay cut at four stages of maturity (41); by steers on pasture forage (69); by cows on a ration of alfalfa silage, corn silage and grain, and also on a ration of alfalfa hay, corn silage and grain (103); and by cows fed wheat straw (66).

Swift *et al.* (178) had 16 trials using sheep on mixed rations and compared the digestibility coefficients calculated by the lignin ratio method with the conventional total collection method and found the two to check very closely and standard errors of the digestion coefficients determined by the two methods were of the same general magnitude.

Forbes and Garrigus (70) used the lignin ratio technique to study the digestibility of pasture forages by steers and wethers. The average recovery of lignin in seven digestion trials with steers was 102 ± 7 percent. Comparisons of the dry matter intakes and the digestibility coefficients of the dry matter as calculated by the lignin ratio method and the plant pigment ratio showed extreme variations in the study reported by Cook and Harris (40). Forage consumption as determined by the lignin ratio technique, however, agreed closely with the calculated dry matter intakes commonly accepted for the various sizes of sheep. In addition, the calculated digestibility of the dry matter by the use of the lignin ratio technique compared favorably with the results obtained by other investigators on similar forages. This was not true with all species studied for calculations made by the plant pigment ratio technique.

Ellis, Matrone and Maynard (62) determined digestibility coefficients by the conventional total fecal collection technique and also by the lignin ratio technique using various rations with cows, rabbits and sheep. They secured very close checks between the two methods for digestibility of the dry matter and the other feed constituents. They further showed very small daily variations in the lignin content of feces from three sheep fed timothy hay, which would make the shortened collection period of the partial fecal collection technique satisfactory.

Hale, Duncan and Huffman (84) proposed that lignin be used as an indicator for the study of digestibility in the rumen since lignin did not appear to be appreciably digested in the rumen even through total digestion ranged up to 23.7 percent. Digestibility of lignin ranging from negative values to 29 percent were reported by Forbes and Swift (72).

Forbes and Garrigus (70) reported a total of 70 grazing trials with steers and wethers using the lignin ratio technique and conducted periodic trials in digestion stalls using cut forage and total fecal collections to confirm the reliability of the lignin ratio technique. The average recovery of lignin from steers fed in the digestion stalls was 106 $\pm$ 5.8 percent, and from lambs the average recovery was 111 $\pm$ 8.7 percent. They did not present data, but stated that they investigated the methoxyl content of the forage lignin and the feces lignin but could not account for the slightly high recoveries. Actual comparisons of the digestibility coefficients obtained by the conventional method and the lignin ratio method are made in a later paper by Forbes and Garrigus (71) and very good agreement between the two methods are evident. Digestion coefficients obtained for lignin by the total collection technique were low except for high negative coefficients in four trials with wethers bedded with wood shavings.

Marshall (116) used the lignin ratio method to study the digestibility of pentosans of hay in various areas of the digestive tract of two sheep.

Various degrees of digestion of lignin have been reported by various workers, but comparisons between different experiments are difficult to evaluate since different analytical methods and different animal species were used.

Crampton and Maynard (46) reported lignin to be as high as 30 percent or more digestible in feed barley, wheat screenings and feed oats when determined by their method, but was almost completely indigestible when determined "by difference" as proposed by Crampton and Whiting (47).

Evidence that some change may occur in lignin as it passed through the digestive tract of ruminants has been presented by several investigators. Csonka, Phillips and Jones (48) reported a decrease in methoxyl groups of lignin that had been through the ruminant digestive tract. Pazur and DeLong (145) found an increased excretion of benzoic and hippuric acids in the urine of sheep fed increased amounts of lignin. The increased urinary concentration of aromatic acids were believed to be excretory products of the aromatic rings from the degradation of lignin. Lignin isolated from forages yielded up to 20 percent of its weight as aldehydes when treated with nitrobenzene in alkali, but lignin isolated from feces yielded no aldehydes when comparably treated in experiments of Bondi and Meyer (28). They also found a slight increase in nitrogen content, but no change in the methoxyl content of lignin isolated from feces as compared to that isolated from forages. Forbes and Garrigus (71) found no change in the methoxyl content of forage lignin in the digestive tract of sheep.

Paloheimo (143, 144) and Phillips (146) have determined the nitrogen content of crude lignin and deducted from the crude lignin percentage on the assumption that the nitrogenous residue is a protein. The principal difficulty in obtaining reliable figures for lignin in protein-rich materials is the removal of the protein without removing a part of the lignin. Norman and Jenkins (139) felt that a greater error might be made in calculating this nitrogen as protein than by not correcting at all. The fact that no one had ever succeeded in isolating a nitrogen-free lignin preparation from succulent plant tissue led MacDougall and DeLong (114) to give strong consideration to the possibility that nitrogen is a fundamental component of the lignin of succulent plant materials. Bondi and Meyer (28) prepared lignins from a number of annual grasses and legumes by extraction with dilute alkali. The preparations contained nitrogen which they felt was not due to contamination with proteins, but seemed to be a characteristic component of the lignin molecule itself. They believed that the constancy of the nitrogen content of their lignin preparations from the same plant material in consecutive extractions supported their opinion. They also removed most of the protein from the plant material by treatment with very dilute sodium hydroxide or by the action of pepsin in dilute hydrochloric acid before extracting the lignin and found that the lignin from plants given these preliminary treatments showed approximately the same nitrogen content as that from plant material which did not receive it. De Man and De Heus (52) hydrolyzed lignin preparations from a sample of Lolium perenne with strong sodium hydroxide and determined the tyrosine and tryptophane contents and also calculated the tyrosine-nitrogen and tryptophane-nitrogen as percentage of the total nitrogen in these preparations and compared these figures with the composition of the true protein of the grass. The tyrosine-nitrogen calculated as percentage of the total nitrogen of the preparations corresponded closely with the figure for the true protein of Lolium perenne. The corresponding figures for tryptophane checked only in the case of lignin prepared according to the method of Bondi and Meyer (28) which uses no strong acid to destroy tryptophane. These results lend support to the calculation of protein equivalent from the nitrogen residues in the lignin. Thomas and Armstrong (179) compared the Norman and Jemkins (139), the Crampton and Maynard (46), and the Ellis, Matrone and Maynard (62) methods of determining lignin all of which involve the use of 72 percent sulfuric acid. The lignin yields obtained with the method of Ellis, Matrone and Maynard were appreciably higher than those of the method of Norman and Jenkins. When a protein correction factor was used the results of the two methods were comparable. Thomas and Armstrong (179) further showed that acid hydrolyzates from crude lignin isolated from plant material and from feces contained amino-acids.

Indigestible Protein Ratio Technique

Several approaches have been made to the use of forage or feces protein, or a combination of these, for the calculation of forage digestibility. The principal advantage of this technique for pasture digestibility studies is that it eliminates a large part of the pasture sampling error. Since grazed forage is extremely difficult to sample that which is representative of the forage grazed by the animal, some error is permissable in the calculation of feed intake or digestibility of pasture by the nitrogen content and still secure greater accuracy than may be attained by attempts to sample the grazed forage.

Gallup and Briggs (78) believed that a constant relationship existed between the amount of fecal nitrogen and the dry matter intake, regardless of the amount of protein in the forage. Both Forbes (67) and Blaxter and Mitchell (26) have presented data to show that a constant relationship does not hold. Forbes (67) found that the data for fresh grass indicated that the total fecal nitrogen varied too widely to be used as suggested by Gallup and Briggs (78). He also found that with dried roughages there was a steady rise in fecal nitrogen excretion per 100 grams of dry matter intake as the percentage of protein in the roughage increased. Lancaster (111) proposed a method for the calculation of organic matter digestibility

Heller, Breedlove and Likely (90) used the normal iron content of a ration as the indicator substance for determining the digestibility of rations for rats.

Silica Ratio Technique

The silica ratio technique for the determination of digestibility of feeds has utilized both the naturally-contained silica and also added amounts of silica.

Gallup (77) used the silica ratio technique with rats on a diet containing one percent of added silica and secured results which checked closely with those obtained by standard methods. Gallup and Kuhlman (80) used cows on a ration which contained approximately three percent of naturally-occurring silica and 0.5 percent of added iron oxide and secured results which indicated that the silica naturally contained in the feed was superior to iron oxide as an indicator substance in digestion trials. A later trial with mung bean silage was conducted by Gallup and Kuhlman (81). This mung bean silage contained approximately 20 percent of silica which was more than six times the concentration previously used. They found the silica ratio technique unsatisfactory because the ratio of food constituent to silica ratio of the feed varied from day to day. The ratio of feces constituents to silica content showed such wide variations during a 24-hour period that random sampling of the feces was not justifiable. While they did not determine the cause of these variations, they postulated that they could be due to an uneven distribution of the silica in the feed and also to stratification and loss of silica in its passage through the intestinal tract. Gallup, Hobbs and Briggs (79) made a study of a series of rations using steers and sheep for comparison of the silica ratio technique with the standard method and for calculation of silica recovery. The recovery of ingested silica was practically quantitative when the steers were in stanchions and the feces collected semimanually. When the steers were in dry lot and the feces collected in canvas sacks, the average excretion of fecal silica exceeded the calculated intake by about 10 percent. When the steers were on pasture, fecal silica excretion was 36 percent higher than the calculated intake, probably due to the ingestion of dirt by the grazing animals. In the sheep trials in metabolism crates, fecal silica content averaged 107 percent of the calculated intake. They felt that contamination occurred when the feces were exposed to barn dust during the day of collections. Their results indicate that the silica ratio technique is adaptable to digestion trials with steers and sheep if the recovery of silica is reasonably close to 100 percent. Extra precautions are apparently necessary in using the silica ratio technique to keep contamination of the sample at a minimum. Druce and Willcox (56) secured good recovery of natural feed silica from the fecal material of rabbits and thus the silica ratio technique gave results comparable with the conventional total collection method. They were aware of certain objections to the silica ratio method, such as the variation in the recovery values between individual animals and the possibility of silica contamination from the soil in pasture studies.

Plant Pigment Ratio Technique

The use of a naturally-occurring plant pigment or group of pigments which are fully recoverable in the feces has the same advantage as other naturally-occurring feed constituents in that, in addition to determining the digestibility of the feed studied, they will also permit an indirect measurement of the quantity of pasture herbage consumed by grazing animals.

The first report of an indigestible plant pigment or group of pigments was made by Reid *et al.* (155, 156) using 85 percent acetone as the solvent for extracting the pigment or pigments from the feed and feces. This method will be referred to as the plant pigment ratio technique. Estimations of the concentration of plant pigment present in the 85 percent acetone extracts of the feed and feces were made from the amount of light absorbed by the diluted extracts at 406 millimicrons wave length using a Beckmann spectrophotometer.

The recovery of the extracted plant pigments in the feces were very close to 100 percent in all of the materials studied and the calculated digestion coefficients from the plant pigment ratios showed excellent agreement with the conventional total fecal collection technique. Comparisons were made between field-cured hay, barn-cured hay, oven-dried hay, hay-crop silage, ladino clover hay, and three stages of pasture grass.

While Reid et al. (156) did not report on the identity of the pigment or pigments that were responsible for the light absorption at 406 millimicrons, they felt that one substance was largely responsible for the absorption observed.

McCullough, Elliot and Bastin (122) have made application of the plant pigment ratio technique described by Reid et al. (156) for studying the seasonal changes in the digestibility of the dry matter and protein of pasture herbage treated with various amount of barnyard manure, but did not make a comparison with any other method of determining digestibility coefficients.

The committee of American Societies of Agronomy, Dairy Science, Animal Production and Range Management (39) reported on pasture and range research technique and proposed that the plant pigment ratio method should replace the lignin ratio technique. Cook and Harris (40) made a direct comparison of the lignin ratio method and the plant pigment ratio method using seven wether sheep grazed on five forage species in northwestern

Utah that were typical of the northern desert shrub vegetation. All species were in a dormant stage and did not represent typical lush growing vegetation. Feces were collected from fecal bags that were emptied twice daily and the samples composited over a six-day grazing period following an eight-day preliminary period. Forage samples were taken by manually plucking the portions of the plant that were observed to be grazed by the animals. The amount of plant pigment recovered in the feces of sheep grazing big sagebrush and black sage was considerably less than that consumed. This gave negative digestion coefficients and extremely low quantities of estimated dry matter intake. The plant pigment recovery in the feces of sheep grazing squirrel tail grass seemed very high and gave a calculated coefficient of digestibility for dry matter of 68.7 percent which was markedly higher than any other of the mature dormant desert species studied and seems erroneously high since it is approximately 50 percent higher than the digestibility coefficient obtained by the lignin ratio technique. This is the first indication that the plant pigment ratio technique will not work with certain species of forage. It is significant that they secured close checks between the lignin ratio method and the plant pigment ratio method when using alfalfa hay.

Reid _et al._ (154, 155, 156) have recently shown that the plant pigment content of the grass consumed and the feces voided are related to the degree of digestibility, to the stage of growth, and to the leaf to stem ratio. The relationship between the plant pigment content of dry feces and that of the consumed forage on a dry weight basis was established with conventional digestion trials and is expressed by the equation: $Y=(0.0925\ X + 137.3383 \log X) - 242.1181$, where

Y = plant pigment content per unit of dry matter of consumed forage and X = plant pigment content per unit of fecal dry matter.
Considering the difficulties encountered in manually sampling pasture forage that is comparable to that actually grazed by the animal, this method should offer an excellent means of obtaining a representative concentration of plant pigment in the forage consumed simply by analyzing the feces and applying the equation. Reid et al. (157, 158) have also found that a different relationship holds between the plant pigment concentration in dry hays and their corresponding feces than that reported with fresh green grasses.

The plant pigment ratio technique has been used by Noller, Hill and Lundquist (130) to determine the apparent digestibility of forage grazed by milking cows, but the method was not compared with any other technique.

Other Digestion Trial Techniques

Increased interest in the application of the ratio techniques utilizing either naturally-occurring plant substances or added materials as indicators for the determination of digestibility in ruminants will undoubtedly reveal a number of useful substances in both of these classes.

Corbin and Forbes (42) fed eleven lambs ground alfalfa twice daily and gave nine test lambs each 0.5 gram of anthraquinone violet before each feeding and the two control lambs received placebos. The dye was extracted from the feces with benzene in a Waring micro-blender and density determinations were made with a Beckman spectrophotometer on the benzene solution of the dye at 578 millimicrons. Recoveries of anthraquinone violet from the feces of nine lambs ranged from 96.4 to 106.0 percent with an average of 100.5 percent. This assured that digestibility coefficients calculated by the dye ratio and by the total fecal collection technique would be practically identical. In a study of the variation in dye concentration of feces excreted at different times of the day, it was evident that the noon feces had a higher dye concentration than the morning or night feces which indicates that adequate samples should be collected at different times during the day when sampling by a partial collection technique.

Schneider, Lucas, Pavlech and Cipolloni (166) published equations for predicting the digestion coefficients of nutrients in feeds fed to cattle and sheep and in concentrates fed to swine. They took into account the percentages of several nutrients and the type of feed involved. Equations were presented for estimating the digestibility coefficients for organic matter, crude protein, crude fiber, nitrogen-free extract, ether extract, and the content of total digestible nutrients in feeds, using their percentages of crude protein, crude fiber, nitrogen-free extract, and ether extract as predicting factors. Their calculations indicated that the use of equations will result in prediction error variances which are 25 to 45 percent below those secured if average digestion coefficients from prepared tables are employed.

Francois (74) also presented formulas and tables for obtaining the coefficient of digestibility of organic matter and protein as a function of the amount of cellulose in the dry matter.

Schneider _et al._ (164) simplified the equation formerly presented (166) to estimate the digestion coefficients of crude protein, crude fiber, nitrogen-free extract, and ether extract and the content of total digestible nutrients in feeds for which few or no digestibility data are available. The equation uses the percentage composition of crude protein, crude fiber, nitrogen-free extract and ether extract as predicting factors and the appropriate constant for the digestion coefficient being calculated.

The authors point out that this equation does not give as precise an estimate of the digestion coefficient as the equation previously reported which was designed for use with feeds on which sufficient digestibility data are available with the animal species desired.

FACTORS AFFECTING THE DIGESTIBILITY OF ROUGHAGES

Effect of Crude Fiber on Digestibility

The definition of crude fiber set up by Henneberg and Stohmann (91), based on a method devised by them and known as the Weende method for estimating crude fiber, consists of the residue after treatment with 1.25 percent sulfuric acid, 1.25 percent potassium hydroxide, water, alcohol and ether and is essentially the method most widely used today. This includes a mixture of chemical substances from the plant cell membrane which is primarly cellulose, hemicellulose and other encrusting substances, such as lignin, cutin, suberin and pectins.

It was originally intended that the crude fiber fraction should represent the relatively indigestible fraction of the carbohydrates while the nitrogen-free extract should include the more readily digestible portion. It is now well-known that the crude fiber fraction is digested to a considerable extent and, in some cases, to a greater extent than the nitrogen-free extract. The nitrogen-free extract also contains varying amounts of relatively indigestible constituents, including lignin. Crampton (43) reported that in roughages, particularly pastures, the crude fiber fraction is frequently more digestible than the nitrogen-free extract. Crampton and Maynard (46) reported the digestibility coefficients of crude fiber and nitrogen-free extract for three classes of roughages and for concentrates and the percent of cases in which the crude fiber was as highly digested as the nitrogen-free extract. These data are summarized in Table 2.

Table 2

Average Digestion Coefficients of Feeds by Ruminants[1]

Kind of Feed	No.of Cases	Coeff. of Digestibility Crude Fiber	N.F.E.	% with Crude Fiber as high as N.F.E.
Dry Roughages	110	52.4	59.5	39
Pasture Herbage	12	75.5	71.4	67
Silage	25	58.2	64.6	28
Concentrates	88	53.3	78.5	10

[1]From Crampton and Maynard (46).

Armsby (5) stated that crude fiber contained most of the cellulose, lignin, cutin, and some of the more difficultly soluble hemicelluloses. Headden (89) found that the crude fiber of alfalfa cut in early bloom stage contained 79.0 percent cellulose and 21.0 percent lignin.

Norman (133) concluded that the crude fiber determination is misleading because the fraction obtained does not bear any definite relationship to the structural constituents of the material from which it is obtained. He reported the cellulose and lignin contents of the crude fiber from several feeds and found that the more highly lignified materials do not necessarily yield a crude fiber high in lignin.

Louw (113) made a study of the digestibility of the various cell-wall constituents of grasses as affected by the stage of maturity, using material from earlier digestibility trials. The grass samples were a mixture of veld grasses grown in South Africa and were cut after periods of 1, 2, 3 and 4 months of undisturbed growth. In these grass mixtures and in the feces from sheep consuming them as their entire ration, estimations were made of cellulose, lignin, total furfural yield, xylan from cellulose, uronic acid anhydrides, pentosans, hemicelluloses, cold water soluble fraction, crude protein, ether extract and total ash; as well as the amount and composition of the crude fiber in the grass and feces. With increasing maturity of the grass, the percentage of natural cellulose and the ratio of xylan to true cellulose increased slightly. The increase in the lignin and hemicellulose contents were relatively small. The digestibility trials showed the true cellulose to be the most digestible and the lignin the least digestible of the cell-wall constituents, but all the constituents showed decreasing digestibility with advancing maturity. The digestibility of the hemicelluloses lay between the values for true cellulose and lignin, being considerably lower than that of true cellulose. Examination of the crude fiber isolated from the grass or from the feces showed that the material from grass contained an average of 96.5 percent natural cellulose, the remainder consisting mostly of lignin. The material isolated from feces contained 92.8 percent natural cellulose and about 10 percent lignin. It is evident from their data that the various constituents of the cell-wall showed a varying resistance to the reagents used in the Weende method for estimating crude fiber and therefore the amounts of these constituents recovered in the crude fiber showed considerable variation. From 86 to 93 percent of the true cellulose, 33 to 40 percent of the xylan in cellulose, 12 to 15 percent of the lignin and only about 1 to 4 percent of the pentosan in hemicellulose were recovered.

Comparisons by various investigators on the effect of the content of crude fiber on the digestibility of roughages must take into consideration that different analytical methods for crude fiber give widely different results. This has been pointed out by Nordfeldt _et al._ (132) who showed that three different methods for the determination of crude fiber gave

effect of crude fiber content on digestibility may be expressed by the equation, $Y = 90.1 - 0.897X$, where

Y = digestibility of the dry matter and

X = crude fiber content of the feed.

Duckworth (57) found a close negative correlation between the crude fiber content and the digestibility coefficients of roughages. Dijkstra (53) has calculated formulas for estimating the starch equivalent of artificially dried grass from the percentages of crude protein and crude fiber or from the crude fiber alone. Homb (92) found a close correlation between the crude fiber content and digestibility. With hays and silage, the relation between lignin content and digestibility seemed more marked than between crude fiber and digestibility.

Hallsworth (85) used the published results of digestibility trials by other workers to show a linear relation between the percentage of crude fiber and the digestibility of the organic matter of feeding stuffs. A linear relation was also established between the starch equivalent of pasture herbage and the crude fiber content. The relation between starch equivalent and crude fiber for a wide range of pasture species and fiber contents could be expressed by the equation, Starch Equivalent = $73.56 - 0.62X$, where

X = the percentage of crude fiber.

Similar results were reported by Jarl (98, 100) using the results of previously conducted digestion trials to compare the fiber content of hays with the digestibilities. He found a negative correlation between the fiber content and the digestibilities of the hays. Axelsson (11, 12, 13, 14) and Axelsson and Eriksson (15) reported that the crude fiber content of roughages shows a high negative correlation with the dry matter digestibility. Axelsson (13, 14) compiled the data from 757 digestion trials made in different parts of the world and compared the digestibility coefficients of crude protein, ether extract, nitrogen-free extract, crude fiber and organic matter to the crude fiber content of the dry matter of alfalfa, clover, clover-timothy mixtures and timothy fed green, as silage or as hay. The crude fiber content influenced the digestibility of all the nutrients, that of the organic matter decreasing by 0.71 for each increase of one percent in crude fiber content.

Nordfeldt et al. (132) investigated the effect of lignin, cellulose, pentosans and crude fiber on the digestibility of the dry matter of the ration and found that crude fiber was as closely correlated with digestibility of the dry matter as any of the constituents studied. If lignin, cellulose and pentosans were determined separately and their total influence upon the digestibility calculated by partial regression, the digestibility of the dry matter was found to have less deviation around the line of regression than when calculated from crude fiber alone.

There is considerable evidence that the optimum fiber content of the diet varies with the species of animal, the type of ration fed and other environmental factors. Axelsson (8, 9, 10) has proposed that a certain optimum level of fiber is needed in a ration if the energy is to be utilized most efficiently. He believes the optimum level of crude fiber to be between 18 to 23 percent and points out that in digestibility trials where values are obtained by difference, it is desirable that both the basal and the supplementary rations contain the optimum amounts of protein and fiber. Axelsson (13) has also compared the relative abilities of the pig, horse, cattle and sheep to digest the organic matter of the ration and showed that with sheep and cattle the digestibility fell as the crude fiber increased in the diet. The relation between the digestibility of the organic matter and the content of crude fiber in the ration of cattle and sheep is shown by the equation, $Y = 87.8 - 0.83X$, where Y is the digestibility of the organic matter and X is the percentage of crude fiber in the diet.

Nordfeldt et al. (131) conducted a feeding experiment with different levels of crude fiber in the ration of dairy cows. Other components of the ration (digestible protein and total digestible nutrients) were kept constant. Four different rations were fed, as follows: A with 60 pounds of Napier grass, B with 40 pounds of Napier grass, C with 20 pounds of Napier grass daily per cow and D with no Napier grass. The necessary amounts of protein and total digestible nutrients were made up with pineapple bran, soybean oil meal, meat meal, fish meal and molasses. The crude fiber content of these four rations in percentage of dry matter was as follows: A, 22.7; B, 19.6; C, 16.1; and D, 12.0 percent. The effect of the level of crude fiber in the feed upon milk production was highly significant. When the crude fiber content of the feed was increased above 16 percent, a drop in milk production occurred. The average daily 4 percent fat corrected milk production per cow for 12 weeks was 20.6 pounds on ration A, 21.7 pounds on ration B, 23.4 pounds on ration C, and 23.8 pounds on ration D. Whether other combinations of feeds might have a different optimum level of crude fiber in the ration has not been shown.

Lancaster (11) gave analytical and digestibility data for 11 succulent feeds and six roughages. Lignin, cellulose and crude fiber all had a depressing effect upon the digestibility of organic matter as shown by the correlation coefficients of -0.978, -0.808 and -0.944 for the digestibilities of the organic matter and the percentage content of lignin, cellulose and crude fiber, respectively.

McMeekan (123) also calculated a correlation coefficient of -0.944 between the crude fiber content and the digestibility of the organic matter of feeds reported by Woodman (187) which is identical with the correlation coefficient reported by Lancaster (110).

Phillips and Loughlin (151) analyzed 25 samples of essentially pure stands of forage from various laboratories in the United States that had served as the sole ration in feeding experiments with cattle. The lignin, protein, cellulose and crude fiber contents of the samples were all more or less closely related to their yield of energy, but at different levels of these constituents for timothy and alfalfa. Within a species, crude fiber was less closely correlated with digestibility of energy and dry matter than was the lignin content.

Steensberg and Winther (175) conducted digestibility trials with lucern taken at early, normal and late first cuttings. The later cuttings increased in crude fiber content and the digestibility of the organic matter decreased.

Sotola (171) did not get a consistent relation between the crude fiber content of wheat, oats and barley hays and digestibility of the various nutrients with increasing maturity. This study included ripe grains which produced an increased crude fiber content of the stems concomitant with increases in the readily digested carbohydrates of the maturing heads. The relationship between the digestibility of the dry matter and the crude fiber content of the stems and leaves only would likely give consistent results. This assumption is partially substantiated by later work by Sotola (173) with digestibility studies of three stages of smooth bromegrass. Crude fiber increased from 19.77 to 35.92 percent while the plant grew from 4.5 to 36 inches in height. The dry matter was 81 percent digestible at the 4.5-inch stage and only 55.8 percent digestible at the 36-inch stage.

Swift _et al._ (178) conducted digestibility trials with lambs and sheep fed a basal diet of good quality of alfalfa and timothy hay, corn meal and linseed cake meal. When crude fiber supplements in the form of straw were added, the digestibility coefficients of the protein, ether extract, crude fiber and energy of the whole ration decreased and the total amount of protein and ether extract digested was reduced.

Forbes and Garrigus (70) found correlation coefficients of -0.74 for steers and -0.64 for wethers between the digestibility of the organic matter and the crude fiber content of grazed forages. The best correlation between chemical composition and organic matter digestibility was obtained with lignin.

Reid (153) recently reported a correlation coefficient of +0.885 between the crude fiber content and the total digestible nutrients content of the grass.

Crampton and Jackson (44) did not find the crude fiber content of pasture herbage to be closely correlated to seasonal changes in the digestibility of these forages.

A compilation of the regression equations relating the crude fiber content of a feed to the digestibility of the dry matter is given in Table 3.

Table 3.

Regression equations relating digestibility of dry matter to crude fiber content of ration

Regression Equation	Correl. Coeff.	Material	Reference
Y = 83.0 - 0.70X	-0.784	Alfalfa	Phillips & Loughlin (151).
Y = 130.3 - 2.14X	-0.842	Timothy	" " "
Y = 87.8 - 0.83X	---	---	Axelsson (13).
Y = 86.4 - 0.84X	-0.97	---	Nordfeldt (132).
Y = 90.1 - 0.897X	--	---	Olofsson (140).
Y = 96 - 1.00X	-0.74	Pasture (Steers)	Forbes and Garrigus (70).
Y = 94 - 1.01X	-0.64	Pasture (Wethers)	Forbes and Garrigus (70).

Effect of Cellulose Content on Digestibility

Cellulose comprises such a large amount of the total dry matter of most forages that its availability to the animal and its effect on the digestibility of the other feed constituents is of major importance, especially in ruminant nutrition. Although there is a paucity of information on the relation of cellulose in forages to their nutritive value, there are several reports which indicate that the estimation of cellulose, lignin, hemicelluloses and other carbohydrates would give a better indication of forage value than a determination of the crude fiber content and calculation of the amount of nitrogen-free extract. (46, 62, 101, 117, 133, 187, 191).

True cellulose in a chemical sense is a glucose polysaccharide yielding only glucose on hydrolysis. Cellulose is formed in most plants in very close association with other polysaccarides and has hexosans and pentosans intermixed in the cellulose fibers which form a normal part of the cellulosic structure. These substances that are closely associated with the true cellulose, and under some conditions are extracted and retained with the true cellulose, have been called the cellulosans by Hawley and Norman (88). Norman (135) believes that the cellulosans are laid down with the cellulose as it is formed and the two form the micellae of the fibers. The molecular size of the cellulosans is considerably less than that of true cellulose. The sugar units are joined by secondary valencies weaker than those joining the true cellulose units and since the chain length is shorter, they may be largely extracted under conditions which do not extract the true cellulose. Norman and Fuller (138) believe it unlikely that cellulose and the associated polyuronide hemicelluloses and lignin are chemically linked or that so-called "compound" celluloses occur, but that the normal structure is such that inter penetrating systems are found.

The nutritive value of cellulose is indicated by Woodman and Stewart (190) who reported that over 90 percent of the digestible cellulose becomes available in the form of glucose and only about 8 percent undergoes fermentation to gases and organic acids. Cellulose is attacked by various bacteria that form extracellular enzymes that attack the cellulose molecule. Both aerobes and anaerobes are known to be capable of decomposing or are even dependent on cellulose (94, 121, 138). Woodman and Stewart (191) studied the bacterial decomposition of the crude fiber isolated from various feeds and found the fermentation coefficients in all cases higher than the digestion coefficients determined in animal trials. Also, the crude fiber fermentation coefficients for the untreated feeds were much lower than the corresponding value obtained from the separated fiber. This would indicate that association of the cellulose with other encrusting cell-wall constituents in plant materials would be a limiting factor in the cellulose utilization by the animal. Woodman (187) also reported on the difference in the digestibility of lignified and non-lignified feeding stuffs. Nonlignified feeds such as sugar beet pulp, mangolds, cabbage and sugar beet tops showed coefficients of digestibility of the cellulose as 89.7, 78.0, 74.0 and 71.6 percent, respectively. The coefficients of digestibility of the cellulose of such lignified feeds as oat straw, wheat straw, cottonseed meal, linseed meal and wheat bran were only 54.0, 50.0, 37.0 and 26.0 percent, respectively.

Naumann (128) made a study of the crude fiber fractions from rations used in several digestibility trials with sheep and of the corresponding feces. The digestibility of the crude fiber and its pentosan and cellulose components showed wide variation with different feeds and was inversely proportional to the lignin content, which was indigestible in nearly all cases. Crampton and Maynard (46) reported feed cellulose to be 62.5 percent digested by a steer on equal parts of alfalfa and grain. Other carbohydrates calculated by difference were 94.8 percent digested. Reid (153) reported the digestion coefficients of cellulose from early, medium and late-cut grass as 77.8, 68.5 and 61.0, respectively. Similar decreases occurred in the digestibility of the dry matter.

The cellulose content of grasses, expressed as a percentage of the dry matter or of the carbohydrate fraction, is much higher than that of legumes; thus giving higher crude fiber contents in grasses than in legumes (6).

Much of the reported work on the digestibility of cellulose may have been influenced by other factors in the ration affecting digestibility. Recent work has shown that the digestion of roughage by cattle was increased by addition of protein, (31, 34, 36) and also by a water extract of alfalfa hay and the ash from good quality alfalfa hay (33).

Burroughs _et al._ (30) have recently reported an artificial rumen technique for studying cellulose digestion in roughages. Using this technique, Burroughs _et al._ (35) studied the effect of supplements of available nitrogen, a complex mineral mixture and an autoclaved extract of cow manure on the digestion of four poor quality roughages (corn stover, wheat straw, corncobs, and mature timothy-blue grass hay) and four good quality dehydrated forages (clover hay, rye hay and two lots of alfalfa hay). The cellulose in the good quality roughages was digested efficiently without supplementation, with no increased digestion occurring when the complex minerals and autoclaved manure extract were included. The cellulose in the poor quality roughages was not digested efficiently without supplementation. The addition of complex minerals, manure extract or available nitrogen greatly increased the digestion of cellulose in these poor quality roughages.

Burroughs _et al._ (36) also studied the effect of several cereal grains and protein-rich foods with respect to their ability to increase the digestion of cellulose by rumen microorganisms. Their results showed that dried distillers solubles, soybean oil meal and linseed oil meal gave the greatest increase in roughage digestion. Cane molasses, corn, wheat bran and cottonseed meal had less effect and meat scraps, fish meal, liver meal and oats had little or no influence on roughage digestion by microorganisms in the artificial rumen.

Effect of Lignin Content on Digestibility

Lignin has been quite widely studied in forage plants and is quite logically associated with the digestibility of the other feed constituents since it is generally considered as a biologically inert constituent which increases with advancing maturity of the plant, (6, 46, 134, 148, 159, 191).

An increase in the lignin content of plant material is usually associated with a decrease in its digestibility (18, 43, 46, 69, 110, 119, 120, 141, 176, 187).

Lignin has been reported to be indigestible by steers (46); by cows on alfalfa hay (84); by sheep (83); by cows on a mixed feed and by sheep on dried Sudan grass (62); by sheep on mixed feeds (178); by sheep on clover-timothy hay (68); by sheep on Korean lespedeza hay cut at four stages of maturity (41); by cows on a ration of alfalfa silage, corn silage and grain and also on a ration of alfalfa hay, corn silage and grain (103).

Csonka, Phillips and Jones (48) felt that lignin was demethoxylated or that degradation of lignin occurred in the ruminant digestive tract since determinations of the methoxyl group on the lignin fed and that eliminated in the feces indicated that lignin lost a portion of the methoxyl groups in passing through the animal body. When lignin was fed to cows an increase in benzoic acid elimination in the urine was observed which was believed to be derived from lignin degradation products. Pazur and DeLong (145) determined the hippuric and benzoic acid excretion of sheep fed clover hay cut at five stages of maturity and found that about three times as much hippuric acid was excreted by the sheep fed the early-cut clover as by the sheep fed the mature clover.

Since they felt that these were excretory products of lignin breakdown, it indicated a greater degradation of the lignin in the younger clover than in the more mature clover fed to sheep.

McAnally (119) reported a loss of lignin during digestion of straw in the rumen and also with rumen contents <u>in vitro</u>. Bondi and Meyer (27) reported that the digestibility coefficients of lignin with sheep ranged between 35 and 64, depending upon the plant material fed. They also reported that feces lignin gave lower methoxyl values than plant lignin which they believed indicated an attack on the lignin molecule during passage through the digestive tract. Louw (113) reported that sheep were able to digest 24.5 percent of the lignin in grass one-month-old and only 11.6 percent when the same grass was four months old. Davis <u>et al</u>. (50) found the digestibility of lignin in pea vines and lima bean vines as 16.0 and 10.6 percent, respectively.

Ellis, Matrone and Maynard (62) and Gray (83) have used an enzymatic digestion followed by a dilute acid treatment before allowing the material to react with the strong acid in the determination of lignin. They have shown with this method of determination that the lignin of certain forages is completely indigestible. Crampton and Maynard (46) had previously reported lignin to be digestible to the extent of more than 30 percent in feed barley, wheat screenings and feed oats, but was almost completely indigestible by the modification of Crampton and Whiting (47) in which the lignin is determined by difference. The principal source of discrepancy may be the varying proportions of non-lignin materials accounted for by the different methods of determination. The negative digestion coefficients reported occasionally are probably explained on the basis of smaller nitrogen contents of the lignin isolated from the feeds than are obtained from the lignin isolated from the feces. Armitage, Ashworth and

Ferguson (4) have suggested a preliminary treatment with trypsin and dilute acid to minimize this error.

Lancaster (110) reported the correlation coefficients of the amount of lignin with the content and digestibility of other plant constituents. The results are shown in Table 4.

Table 4.

Correlation Coefficients of Lignin With Other Feed Constituents[1]

Percent digestion of organic matter	-0.978
Percent digestion of crude fiber	-0.954
Percent digestion of cellulose	-0.919
Percent digestion of pentosans	-0.905
Crude fiber content of feed	+0.935
Cellulose content of feed	+0.770

[1]From Lancaster (110).

Swanson and Herman (176) found the digestibility of practically all of the determined nutrients of Korean lespedeza hay decreased as the maturity advanced and the lignin content increased.

A compilation of published regression equations relating the lignin content and the digestibility of the dry matter is given in Table 5.

Table 5.

Regression Equations Relating Digestibility of Dry Matter to the Lignin Content of Ration

Regression Equation	Correl Coeff.	Material	Reference
Y=93.9 - 3.04X	-0.939	Alfalfa	Phillips and Loughlin (151).
Y=120.3 - 5.96X	-0.954	Timothy	Phillips and Loughlin (151).
Y=84.9 - 1.15X	--	Sulphite Cellulose	Hvidsten (95).
Y=95.9 - 4.06X	-0.97		Nordfeldt et al. (132).
Y=96.4 - 4.44X	---		Forbes and Garrigus (70).
Y=100 - 4.71X	-0.95	Steers, Pasture	Forbes and Garrigus (71).
Y=100 - 5.24X	-0.93	Wethers, Pasture	" " "
Y=97 - 4.33X	---	Bromegrass Pasture	" " "
Y=100 - 5.32X	---	Orch. Grass Pasture	" " "
Y=96 - 4.81X	---	Ky. 31 Fescue Pasture	" " "
Y=100 - 4.92X	---	Alfalfa Pasture	" " "
Y=94 - 2.95X	---	--	Lancaster (110).

Effect of Methoxyl Content of Plants on Digestibility

There is some indication that changes in the lignin molecule or in its distribution through the cell wall may have more effect on digestibility of other constituents than the quantity of lignin present. Mangold (115) and Woodman and Stewart (190, 191) have shown that the decrease in digestibility of grasses with increasing maturity may be accompanied by rather small increases in the lignin content. As plants mature, the lignin content usually increases and there is also an increase in the methoxyl content of the lignin. (136, 147, 148, 149).

Francois <u>et al</u>. (75) found that approximately 50 percent of the total methoxyl content, 50 percent of the methoxyl content of the lignin, and 90 percent of the methoxyl content of the pectin in rations fed to two calves disappeared, as indicated from analysis of the urine and feces. They used 10-day collection periods on rations containing alfalfa hay alone, alfalfa plus dried beet pulp, alfalfa plus wheat, and alfalfa plus dried beet pulp and wheat. Phillips <u>et al</u>. (150) and Csonka, Phillips and Jones (48) had previously reported a 15 percent loss of methoxyl in the digestive tract of a dog and a 37 percent loss by a cow.

The methoxyl content of grass and feces has been reported by Reid (153) to show a high negative correlation to the total digestible nutrients content of the grass. The correlation coefficients relating the methoxyl content of grass with the apparent digestion coefficients of several constituents of the grass are shown in Table 6.

Table 6.

Correlation of Methoxyl Content of Grass with the Apparent Digestion Coefficients of Several Grass Constituents[1]

Constituent	Correlation Coefficient[2]
Cellulose	-0.900
Hemicelluloses	-0.896
Crude Fiber	-0.887
Pectins	-0.865
Starch	-0.921
Crude Protein	-0.933
Total digestible nutrients	-0.96

[1]From Reid (153).

[2]All coefficients highly significant.

Reid (153) also pointed out that the methoxyl content of grass cut at a late stage had increased 32.9 percent in methoxyl content over that which it contained when cut at an early stage.

Phillips and Goss (148) have shown that the methoxyl content of plants is not entirely associated with the lignin molecule and the proportion of the total amount which is associated with the lignin molecule increases with more mature plants.

Effect of Stage of Maturity on Digestibility

Scientific workers and livestock feeders have undoubtedly been aware of the difference in nutritive value of roughages harvested at different stages of maturity long before experimental data were collected on this subject. The primary purpose of this section is to review those experiments which investigated changes in the digestibility of the dry matter of forages or of the various constituents of the dry matter and will not attempt to review all of the studies which have investigated changes in chemical composition with various stages of maturity of plants.

Mills (124) and Foster and Merrill (73) at the Utah Agricultural Experiment Station cut alfalfa when the first blossoms appeared, when in full bloom, and when half of the blossoms had fallen. They produced the greatest amounts of beef per unit of hay from the earliest stages and the smallest amounts from the most mature stage. The earliest stage also contained the highest amount of protein. Salmon, Swanson and McCampbell (160) cut alfalfa at the bud, one-tenth bloom, full bloom and seed stages. The ash and protein content decreased as the maturity advanced and the crude fiber content increased. Sixty-to ninety-day feeding tests conducted for the various cuttings on three different years with steers on the alfalfa hay without any other feed showed that 1628 pounds of bud stage hay produced

100 pounds of gain in live-weight as compared to 2,086 pounds of one-tenth bloom hay, 2.163 pounds of full-bloom hay, and 3,910 pounds of seed-stage hay. Digestion trials showed an average digestion coefficient of 78 percent for the protein in hay cut at the bud stage, 73 percent for that in the one-tenth bloom and full-bloom hays, and 67 percent for that in the hay cut at the seed stage. The crude fiber in the bud stage hays had an average digestion coefficient of 35 percent, as compared to an average of 48 percent for the one-tenth bloom, 43 percent for the full bloom, and 41 percent for the seed-stage hay. Woodman, Evans and Norman (188, 189) conducted digestion trials with sheep on alfalfa cut at the prebud, bud, and flowering stages. Alfalfa cut at a prebud stage was superior to later cuttings and compared favorably with young pasture grass in nutritive value. They also pointed out that the alfalfa plant produced fiber at an early stage of growth and that the digestibility of the fiber diminished rapidly as a result of lignification. The average crude protein content was 28.1 percent for five cuttings of prebud hay as compared to 21.5 and 19.3 percent for the bud stage and bloom stage cuttings, respectively. The crude fiber content averaged 19.3 percent for the prebud stage, increased to 26.5 for the bud stage, and 27 for the bloom stage hay. The digestion coefficients were considerably higher for hay cut in the prebud stage than for the hays cut at the later stages. The digestion coefficients for crude protein averaged 80 percent for the prebud stage, 78 percent for the bud stage, and 76 percent for the bloom stage hay. Digestion coefficients for crude fiber averaged 64, 48 and 43 for the three stages, respectively. Sotola (170) cut irrigated alfalfa hay at one-fourth, one-half, and three-fourth bloom stages. The protein content decreased and the fiber content increased as the plants matured. The hay cut at the one-half bloom stage was highest in digestible crude protein

and in total digestible nutrients. The digestion trials of Newlander et al. (129) with cows on sun-cured alfalfa that was cut at the one-half bloom stage gave an average digestion coefficient for crude protein of 78.3 for the no-bloom and 65.8 for the one-half bloom hay. The digestion coefficients of the nitrogen-free extract compared closely for both hays, but crude fiber averaged 55.3 for the no-bloom stage and 53.2 for the one-half bloom hay. The total digestible nutrient content of the dry matter averaged 63.3 percent for the no-bloom hay and 58.3 percent for the one-half bloom hay. Dawson, Kopland and Graves (51) reported the digestibility of alfalfa hay cut in three stages, as follows; (1) initial bloom stage, when not more than 10 percent of the plants were in bloom; (2) one-half bloom stage; and (3) full bloom stage, when 90 to 100 percent of the plants were in bloom. Digestion trials of fifteen-days collections were conducted with sheep. The average digestion coefficient for the crude protein was 77.7 for the initial bloom hay as compared to 77.1 for the one-half bloom and 75.4 for the full bloom hay. The digestion coefficients for the crude fiber averaged 47.7 for the initial bloom, 41.4 for the one-half bloom, and 38.3 for full bloom hay. The total digestible nutrients of the dry matter was 59.0 percent for the initial bloom, 56.7 for the one-half bloom, and 53.9 for the full bloom hay.

Sotola (171) has shown that the digestibility of wheat and barley plants does not decrease progressively with advancing maturity since the milk and dough stages of the grain formation yield highly digestible nutrients that later decrease in digestibility due to maturation of the vegetative part of the plant. Sotola (172) also compared the digestion coefficients for crested wheat grass clipped at four-inch and ten-inch heights. The coefficients of apparent digestibility for the four-inch and ten-inch cuttings were; dry matter, 66.7 and 60.7; crude protein, 74.3 and 59.7; crude fiber, 63.5 and

67.8; and nitrogen-free extract, 74.5 and 74.8, respectively. This comparison illustrates the value of a comparison of the digestibility of the various constituents in contrast to a comparison of only the dry matter digestibilities in a pasture evaluation study. Sotola (173) studied changes in chemical composition and apparent digestibility of nutrients in smooth brome grass cut at regular intervals during the growing season. The crude protein decreased from 13.6 to 4.0 percent while the crude fiber increased from 19.8 to 35.9 percent during growth of the plants from 4.5 to 36 inches in height. The dry matter was 81 percent digestible at the four- and ten-inch stages and only 55.8 percent digestible at the 36-inch stage. Digestibility of the crude protein, crude fiber and nitrogen-free extract followed similar trends.

The digestibility of a mixture of veld grasses cut after periods of 1, 2, 3 and 4 months growth was studied by Louw (113). With increasing maturity of the grass the percentage of natural cellulose and the ratio of xylan to true cellulose increased slightly. The increase in the lignin and hemicellulose contents were relatively small. The digestibility of cellulose, pentosans and hemicellulose all decreased with advancing maturity. Dijkstra and Brouwer (54) reported on changes in chemical composition and feeding value of grass mowed at different stages of maturity. With increasing age of the grass the percentage of protein decreased and the percentage of cellulose increased. Digestibility and feeding value decreased with increasing maturity. They also reported the digestibility of the crude cellulose as not appreciably different from the starch in digestion trials with sheep. Burkitt (27) conducted digestibility trials with sheep on beardless wheat grass cut at an early (3 to 5 inches), medium (7 to 10 inches) and late stage of growth. The coefficient of apparent digestibility of all the various nutrients dropped as the grass matured. Swenson and Herman (176) found the digestibility of practically all the nutrients of Korean lespedeza hay decreased with advancing maturity and increasing lignin content. The digestibility trials were conducted with dairy heifers. Underwood and Moir (180) conducted digestion trials with sheep on wheat and oat hay cut at various periods of growth ranging from before flowering to when the grain was practically mature. The first cutting of wheat hay had the highest digestion coefficient for all constituents, except the ether extract. The crude protein and fiber had decidedly lower digestion coefficients in the later cuttings of both the wheat and oat hay. A decline in the digestibility of pasture herbage dry matter from about 75 percent for early spring grass to 60 percent about six weeks later was reported by Crampton and Jackson (44). Drapala, Raymond and Crampton (55) made histological examinations of the plant residues isolated from the feces of sheep on clover cut at various stages of maturity and found the increase in the number of undigested bits of plant tissues in the feces and their increase in size was correlated with the increase in maturity of the forage eaten and its increasing lignification. Decreases in digestibility have been shown to be associated with advancing maturity in flat pea forage by Daniel *et al.* (49), timothy hay by Colovos *et al.* (38), and Pampas grass by Dunlop and Coup (58). Reid (153) has also recently reported that the digestion coefficients of the dry matter of early, medium and late cut grass was 72.2, 66.6 and 56.0, respectively.

Effect of Plant Ash on Digestibility.

Burroughs *et al.* (33) first presented evidence which indicated the presence of nutrients in alfalfa hay that had a favorable influence on the digestibility of a poor quality roughage when fed to cattle. Their previous experience (32) in finding that corn starch exerted little or no effect upon the digestibility of the dry matter of good quality alfalfa hay; but when added to a poor quality roughage (corn cobs), the corn starch caused a marked decrease in the digestibility of the roughage dry matter. These results indicated that alfalfa hay contained certain non-protein material essential for the growth of rumen microorganisms necessary for roughage digestion since approximately the same amounts of protein were fed with each of the roughages studied. They felt that if a roughage is fed with starch the digestibility will not be lowered if there is a sufficient amount of the unknown nutrients to supply the requirements of microorganisms subsisting on both the starch and the roughage, however, if sufficient quantities of these nutrients are not present in the roughage, the starch will decrease the digestibility of the roughage since the microorganisms attacking starch will deplete the roughage of nutrients needed for the apparently slower acting microorganisms concerned in roughage digestion. Burroughs et al. (35) reported a series of three digestion trials using grade Hereford steers as follows; (1) digestion of corncobs in rations containing variable amounts of hay, (2) digestion of corncobs in rations with and without additions of a water extract of alfalfa hay, and (3) the influence of alfalfa ash upon corncob digestion. In the first series each increment of alfalfa hay (0.5 to 5.0 pound) in the respective rations resulted in increased digestion of corncobs. Similar effects were secured with the addition of a water extract of alfalfa to the ration although the response was quite slow and the increased digestibility continued for a short time after removal of the water extract of alfalfa. The addition of alfalfa ash approximately equivalent to that found in four pounds of alfalfa meal was fed to each of four steers in two digestion trials. The addition of the alfalfa ash increased the digestibility of the dry matter from 31.6 percent in the control period to 43.5 percent in the supplemented period.

Swift *et al.* (177) extended the work reported by Burroughs (33) to a comparison of the metabolizable energy and methane production of sheep fed a basal ration containing 40 percent ground corn cobs and the basal ration plus alfalfa ash. They reasoned that an increase in the apparent digestibility of dry matter and crude fiber would be of no value to the animal if there was an increase in methane production corresponding to the decrease in fecal output. The digestibility of all of the ration constituents was increased by alfalfa ash supplementation, but the increase in the digestibility of crude fiber was the only one found to be highly significant. The increase in the crude fiber digestibility was accompanied by a small increase in methane production which was small enough that the net effect of alfalfa ash supplementation was to increase the amount of feed energy available to the animal.

Associative Effect of Feeds and Digestibility

Associative digestibility is the term applied to a change in the digestibility of a feed when incorporated in a ration with one or more other feeds as compared to its digestibility when fed alone. Ewing and Wells (65) were apparently the first to use the term, "associative digestibility", when they studied mixed rations composed of corn silage, cottonseed meal, and starch. The coefficients of digestibility for the cottonseed meal calculated from the mixed ration with silage were higher than the values for cottonseed meal fed alone, but the differences were reported to be not significant. They later (64) investigated rations composed of corn silage, alfalfa, and velvet bean meal in various combinations and proportions. In the silage and alfalfa ration, silage and velvet bean meal ration, velvet bean meal and alfalfa ration, and the silage, alfalfa and velvet bean meal ration, the coefficients of digestibility of the dry matter, nitrogen, crude fiber, and nitrogen-free extract of the mixed rations were less than the values calculated from the coefficients of the individual components. The differences were only two to four absolute percent.

Watson et al. (184) studied the associative digestibility of dry and succulent roughage such as mixed hay, oat straw, oat hulls, corn silage, and mangels when fed to steers. The coefficients of digestibility were determined when these feeds were fed as the sole ration and when combinations of various pairs of these feeds made up the ration. The digestibility of one of the individual components in each mixed ration was then compared with its digestibility when fed alone. They concluded that among mixed hay, oat straw, corn silage and mangels there was no associative effect on total digestibility or total digestible nutrients. They also concluded that the digestibility of oat hulls, calculated from a mixed ration with either hay, corn silage, or mangels, was lower than the digestibility observed when oat hulls constituted the sole ration. Watson et al. (185) determined the digestion coefficients of barley, oats, oil cake, barley plus oats, and barley plus oats and oil cake in a basal ration of hay by using steers. They concluded that there was no associative effect between barley and oats and only slightly higher digestibilities of the dry matter of the barley plus oats and oil cake ration were found experimentally than were calculated. Using rations of barley, oats and oil cake alone and also when they were fed in a mixed ration with hay, Watson et al. (182) reported on the associative digestibility of hay and these grains. With oil cake there were no statistically significant differences between the values obtained by calculation from the mixed ration and those obtained when the oil cake constituted the entire ration. There were also no statistically significant differences in the case of barley, except for the ether extract. The digestibility of the ether extract, calculated from the mixed ration with hay, was higher than that determined by feeding the barley alone. With oats, except for nitrogen and ether extract, the values calculated from the mixed ration with hay were significantly less than the values obtained when the oats were fed alone. For all three feeds, with the exception of ether extract, the values calculated from mixed rations with hay tended to be lower than the values obtained by feeding the grains alone. It was only in the case of oats that such differences were significant. Watson _et al._ (183) also determined the coefficients of digestibility for wheat bran, gluten feed, soybean oil meal, and a mixture consisting of equal parts of these grains. From the values of the individual grains a calculation was made of the coefficients of digestibility of the grain mixture which was practically identical with the values determined by digestibility trials. They concluded that there was no associative digestibility between these three grains. Watson _et al._ (186) reported on the relative associative effects of timothy and alfalfa hays with barley when using steers and sheep and rations of barley, timothy hay, alfalfa hay and mixed rations of barley plus timothy and barley plus alfalfa. The digestibility of barley when it was fed alone was compared with the digestibility when calculated from the barley plus timothy ration and the barley plus alfalfa ration. The digestibility of barley was the same when fed with timothy, when fed with alfalfa or when fed alone to sheep and steers.

Swift _et al._ (178) studied the effect of various supplements on the digestibility of a mixed ration for sheep, however, oat straw was the only natural feed supplement used. The addition of crude fiber to the ration in the form of oat straw reduced the total amount of protein and ether extract digested.

Graves et al. (82) did not conduct digestion trials in connection with their experiments, but reported what appeared to be very little difference in efficiency for milk production of the total digestible nutrients derived from the alfalfa and that derived from the grain, hay, and silage ration. Fifteen Holstein cows were kept on alfalfa hay alone for two or three lactations and although the cows were unable to consume as much total digestible nutrients as in a hay and grain ration, they were able to utilize the nutrients consumed with about the same efficiency.

Effect of Antibiotics on Digestibility

Many recent reports have shown increased gains and accelerated growth of several species of animals receiving various antibiotic supplements. Some of these experiments tend to indicate that a more efficient utilization of nutrients by the animal are responsible for the increased growth or weight gains, but since digestibility comparisons with and without these supplements have not been made these experiments will not be reviewed. Several experiments with different species of animals have shown that certain antibiotics have a pronounced effect on the microorganisms in the intestinal tract. Since the ruminant is primarily dependent upon an active microbial flora for efficient digestion of certain feed constituents, it seems reasonable that antibiotics might affect the digestibility of these nutrients depending upon the groups of organisms that were most greatly affected by the antibiotic material used.

Bell, Whitehair and Gallup (20,21) studied the digestibility of a basal ration of prairie hay, corn, soybean oil meal, bonemeal and salt with three steers. The same basal ration plus 0.2 gram of crystalline aureomycin hydrochloride was fed to three additional steers, one receiving only aureomycin and two 45 grams urea plus aureomycin. The steers were kept in metabolism stalls and fed a constant amount of ration during a ten-day preliminary and a fifteen-day collection period. The third day after aureomycin feeding was started two steers developed mild anorexia and diarrhea. About the fifth day all three steers became slightly constipated with symptoms of bloat and their feces became dry and fibrous. The effect of aureomycin on the digestibility of various feed constituents by steers is illustrated by material in Table 7 taken from the data of Bell _et al._ (21).

Table 7.

Effect of Aureomycin on Digestion Coefficients with Steers[1]

Steer No.	3-Day Period	Dry Matter	Crude Protein	Ether Ext.	Crude Fiber	N-Free Ext.
		Percent Apparent Digestibility				
4 and 5		(Basal Ration)				
	1	69.07	51.93	63.31	66.35	74.91
	2	68.38	52.68	58.38	62.77	74.93
	3	70.69	55.42	65.94	67.01	76.20
	4	69.37	55.15	66.93	65.45	75.40
	5	72.67	60.83	71.52	70.60	77.96
	Av.	70.04	53.20	65.26	66.44	75.88
3--		(Basal Ration + Aureomycin)				
	1	76.36	65.64	74.63	66.29	81.86
	2	63.71	56.27	62.76	49.71	70.48
	3	58.00	47.60	66.31	35.88	67.25
	4	54.06	45.31	60.50	30.72	63.49
1 and 6		(Basal Ration + Urea + Aureomycin)				
	1	74.58	72.10	71.58	69.98	79.65
	2	66.23	67.46	62.77	52.70	73.52
	3	63.84	65.05	65.44	51.37	70.20
	4	59.53	68.42	67.57	39.78	66.79
	5	59.58	67.86	65.13	42.86	66.89

[1]From Bell *et al.* (21).

It appears that the most pronounced effect of aureomycin was on the digestibility of crude fiber which might suggest that it had a detrimental effect on the cellulose digesting microorganisms of the intestinal tract. The digestibility of the dry matter and crude fiber of the rations was markedly reduced in the latter part of the experiment.

SUMMARY OF THE REVIEW OF LITERATURE

Digestion trials involving the total collection of feces during a ten day period of constant feed intake have become a standard method for determining the digestibility of various feeds.

Several inert materials have been proposed and studied as a means of conducting digestion trials by the so-called inert materials "ratio technique". A satisfactory inert material should be fully recoverable in the feces and should have analytical methods available that are easily conducted and accurate. In some instances it would be highly desirable to have the inert material a naturally occurring constituent of the plant being studied.

Chromium, iron, silica, lignin, indigestible nitrogen, certain plant pigments and various synthetic dyes have been proposed and studied as inert materials for use in the ratio technique for digestibility studies.

Controversial results have been reported with each of these materials that have been studied by independent workers. Chromic oxide has given satisfactory results for several investigators. The method of preparing and feeding the chromium may be an important factor in its successful use as an inert material with ruminants. Wide ranges in the recoveries of lignin have been reported for various feeds when fed to different animals and when the lignin analyses were made by different methods. The successful use of lignin as an inert material depends upon a previous knowledge of its recovery from the feed in question and with the animal species involved. Digestion coefficients calculated from iron ratios have been highly variable and in some cases iron recoveries have been very low. Unsatisfactory results with the silica ratio technique have been reported when using the naturally contained feed silica and also when using added amounts of silica. A naturally occurring plant pigment or groups of pigments have been reported which are fully recoverable in the feces and have also given satisfactory results when used as an inert material in digestibility trials with several forages. Only limited comparisons have been reported with the plant pigment ratio technique of studying digestibility. Further studies of synthetic dyes are necessary to evaluate their usefulness in digestibility studies. No studies have been reported in which more than two inert materials have been studied in the same digestion trial using the same feed and the same animals.

Lignin, crude fiber and cellulose all show high negative correlations with the digestibility of the dry matter of feeds. Analysis of feeds for their content of crude fiber has been criticized because the crude fiber is not of uniform composition when isolated from different feeds and the distribution of the various constituents between the crude fiber and nitrogen-free extract fractions varies widely with different feeds.

PLAN OF EXPERIMENT AND METHODS USED

This study was planned to make a comparison of the digestion coefficients determined with milking dairy cows fed orchard grass hay cut at four stages of maturity by the following digestion trial techniques:

(1) Total fecal collection technique.

(2) Chromium ratio technique (total collection).

(3) Plant pigment ratio technique (total collection).

(4) Lignin ratio technique (total collection).

(5) Chromium ratio technique (Grab samples).

(6) Plant pigment ratio technique (Grab samples).

The orchard grass hay used in this study was cut from a second growth that had been previously sprayed with 2,4-D for the control of weeds and clover. Ammonium sulphate was applied at the rate of 100 and 150 pounds per acre approximately one month and one week respectively, before the first experimental cutting was made. The different stages of maturity were harvested at approximately two week intervals as follows:

Stage 1	6 to 7 inches high.	(Pasture Stage)	Cut May 19, 1949.
Stage 2	8 to 10 inches high.	(Late Pasture)	Cut May 31, 1949.
Stage 3	10 to 12 inches high.	(Early hay)	Cut June 14, 1949.
Stage 4	12 to 14 inches high.	(Mature Hay)	Cut June 27, 1949.

The first cutting was cut in the field with a forage field chopper and the chopped material blown directly into wagons and hauled to the barn for artificial drying. The last three cuttings were left in the field for approximately one day and then windrowed with a side delivery rake and chopped from the windrow with a forage field-chopper. All lots were dried on a slatted floor barn drier with supplemental heat. After drying, each lot of hay was baled and stored until fed.

Digestion trials were conducted on each lot of hay using three milking cows in digestion stalls that permitted mechanical separation of the feces and urine. Ten day preliminary periods and ten day experimental periods were used for each lot of orchard grass studied. The animals were kept in the digestion stalls during the entire preliminary and experimental periods except for a daily period of walking for exercise. The feeding schedule for the various stages of orchard grass hay fed is given in Table 8 and the composition of the grain mixture fed is given in Table 9.

Table 8.

Animals used and quantities of feed fed during the digestion trials

Animal	Orchard grass hay	No. 86 grain
X54 *	20 lbs.	7 lbs.
X65 **	25 lbs.	5 lbs.
2408	26 lbs.	9 lbs.
N635	16 lbs.	7 lbs.

* Used with stage 4 hay only.

** Not used with stage 4 hay.

Table 9.

Composition of No. 86 Grain Mixture

Ground Corn	97 parts.
Bone meal	2 parts.
Salt	1 part.

Chromium oxide was added to the grain mixture by mixing 15 percent chromium oxide with wheat flour and baking into a hard bread which was dried and ground through a medium mesh screen in a wiley mill and then mixed with each individual cows ration in amounts that would give daily intakes of approximately 15 grams of chromium oxide per cow.

The feeding schedule for the different stages of orchard grass hay is given in Table 10.

Table 10.

Feeding Schedule of the Orchard Grass Hay Cut at Various Stages of Maturity

Dates (inclusive)	Period	Material Fed
1-24-50 to 2-2-50	Preliminary Period	Stage 4 Orchard Grass
2-3-50 to 2-13-50	Experimental Period	" " " "
2-14-50 to 2-22-50	Preliminary Period	Stage 3 Orchard Grass
2-23-50 to 3-4-50	Experimental Period	" " " "
3-5-50 to 3-14-50	Preliminary Period	Stage 2 Orchard Grass
3-16-50 to 3-25-50	Experimental Period	" " " "
3-26-50 to 4-4-50	Preliminary Period	Stage 1 " "
4-5-50 to 4-14-50	Experimental Period	" " " "

During each 10 day experimental period daily aliquots of feces samples were collected in duplicate as follows; (1) dried to constant weight in a circulating air sample drier for daily determinations of dry matter content and composited for the 10 day period for chemical analysis. (2) frozen daily and composited for the 10 day period for determination of the plant pigment concentration, and (3) composited in concentrated sulfuric acid for nitrogen determinations. Two samples of the hay fed daily were collected in duplicate as (1) a 200 gram sample dried to constant weight for determination of the dry matter content and composited for the 10 day period for chemical analysis, and (2) a daily sample composited as fed for determination of the plant pigment concentration.

Two samples of a daily aliquote of the urine were collected in duplicate and stored while composited as follows; (1) frozen daily for benzoic acid determinations and (2) acidified daily with dilute (20 percent) sulfuric acid for nitrogen determinations. Daily determinations of the specific gravity of the urine and total volume of the urine excreted were made. Aliquote amounts of milk from each milking were preserved with formaldehyde and were composited for each 10 day period for butterfat and nitrogen determinations.

In addition to the daily aliquotes of the feces, "grab" samples were taken from two passages daily of each cow on three successive days during each ten day experimental period. The "grab" samples were taken from two forenoon and two afternoon passages of feces at approximately the time of day that had been shown to give representative concentrations of chromium and lignin in the feces of previous trials (104). Duplicate samples were dried for moisture determinations and chemical analysis and duplicate samples were frozen as voided for determination of the plant pigment concentration with the exception of the stage 4 orchard grass hay. Each duplicate daily grab sample was handled and analyzed as an individual sample designated as "first day partial, second day partial and third day partial" for each stage of orchard grass hay fed. Corrections were made for the dry matter content of the "grab" sample to the total fecal dry matter excreted during the ten day collection period.

The dates of collection of the "grab" samples are given in Table 11.

Table 11

Collection Dates of the "Grab" Samples taken of each Stage of Orchard Grass Hay

Orchard Grass	Dates of Fecal "Grab" Samples		
	1st day	2nd day	3rd day
Stage 1	Apr. 10	Apr. 11	Apr. 12
Stage 2	Mar. 21	Mar. 22	Mar. 23
Stage 3	Feb. 28	Mar. 1	Mar. 2
Stage 4	Feb. 8	Feb. 9	Feb. 10

ANALYTICAL METHODS

Proximate Analyses

Proximate analyses of the feeds and feces were made according to the procedures described by the Association of Official Agricultural Chemists (7) for crude protein, ether extract, crude fiber, ash and nitrogen-free extract.

Lignin

Lignin was determined by the method of Ellis, Matrone and Maynard (62).

Plant Pigments

The extraction of plant pigments in the feed and feces were made by the method of Reid *et al.* (158) but a unit was arbitrarily defined as that amount of plant pigment per milliliter of 85 percent acetone extract that gave a density of 1.0 with a Beckmann model DU spectrophotometer when read in a 1 centimeter cell. Absorption curves of the extracts from the feces and orchard grass hays were very similar to those reported by Reid *et al* (158) for mixed hay and corresponding feces.

Chromium

The method used for the determination of chromium oxide was a modification of the method of Edin et al. (61). Sufficient sample to contain 20 to 40 mgs. of chromium oxide was weighed into nickel crucibles and ashed until free of carbon. The crucibles had a volume of 125 milliliters, a height of 6 centimeters, and a diameter of 6 centimeters. The ashed material was fused with a flux consisting of 4 grams of potassium hydroxide, 2.7 grams of sodium hydroxide, and 5.8 grams of a mixture of 138 grams (1 mol) of potassium carbonate, 106 grams (1 mol) of anhydrous sodium carbonate, 202 grams (2 mol) of potassium nitrate, and 170 grams (2 mol) of sodium nitrate. The total 12.5 grams of the fusion mixture was weighed out and added to each crucible with precautions to limit the water uptake of the fusion mixture. The crucibles were then heated on a hot plate until the mixture became liquid and swirled at 5 minute intervals during an additional heating period of 20 minutes. While the material was still hot a nickel wire, 1 millimeter thick and 15 centimeters long and formed into a spiral at one end, was placed in the fused material. After the mass had solidified, the crucible was quickly heated with a glass flame to melt the material around the walls so it could be lifted out with the nickel wire.

The melted material was placed in a 600 milliliter glass beaker, the crucible was thoroughly washed with warm distilled water, and the washings transferred to the beaker. The material was then dissolved in 150 to 200 milliliters of water. One milliliter of 30 percent hydrogen peroxide was added to the solution for reduction of any 6 valent manganese to the 4 valent state. When the solution had boiled for one minute, it was filtered through a sintered glass filter funnel into a 500 milliliter flat-bottom flask in order to remove the precipitated manganese dioxide. Then 8.3

milliliter of concentrated sulfuric acid and 2.5 milliliter of hydrogen peroxide (30 percent) were added, and the solution heated to and kept at boiling temperature for 5 minutes in order to destroy nitrites present. When the solution had cooled, 2 milliliters of hydrogen peroxide (30 percent) and 34.5 milliliters of 6.75 normal sodium hydroxide were added and the mixture heated again and boiled for 5 minutes to oxidize the reduced chromium salts to the chromate. Without interrupting the boiling, 5 milliliters of 5 percent nickel nitrate were added, and the boiling was continued for 5 minutes longer to destroy the excess hydrogen peroxide. After cooling, the solution was acidified slowly with 18.3 milliliters of concentrated sulfuric acid, which had been boiled previously for 4 hours. When the solution had cooled, 10 milliliters of the 10 percent potassium iodide solution were added and the solution titrated with 0.05 normal sodium thiosulfate using a 1 percent starch solution as the indicator.

EXPERIMENTAL RESULTS AND DISCUSSION

The average daily dry matter consumption of each animal receiving the four stages of orchard grass hay and the fecal dry matter excreted are given in Table 12. The dry matter consumed in the grain fed each individual cow is the same for each digestion trial while the dry matter consumption of the various stages of orchard grass hay varies slightly for the same animal due to slight variations in the dry matter content of the four stages of orchard grass hay fed. The chemical composition of the grain and the four stages of orchard grass hay fed and the corresponding feces for each animal used in the digestion trials is presented in Table 13. Each analysis is the average of at least four determinations secured by making replicate analyses on A and B samples of each material. The range in the stage of maturity represented by the four stages of orchard grass hay is indicated by the decrease in protein content from 24.78 percent to 12.38 percent and the increase in the crude fiber content from 26.88 percent to 35.05 percent with increasing maturity.

The apparent digestion coefficients of the constituents of the total ration calculated from the consumption-excretion ratios on samples from total fecal collection are given in Table 14. It is evident that there was less change in the digestibility of the various constituents between the stage 1 and 2 orchard grass hays than occurred between the stages 2, 3 and 4 orchard grass hays. The apparent digestion coefficients of the various stages of the orchard grass hay alone were calculated by correcting the figures for the total ration using the digestion coefficients for corn reported for cattle by Schneider (161) and are given in Table 15. All of the constituents of the proximate analysis have a considerably higher digestion coefficient in the orchard grass hay cut in early maturity than in the hay cut in late maturity.

Digestion coefficients were also calculated for the total collection samples using the ratio technique with crude lignin, corrected lignin (crude lignin - Nitrogen x 6.25), chromium oxide and plant pigments. Digestion coefficients for the inert materials ratio techniques were calculated from the following formula:

$$\text{Digestion Coefficient} = 100 - 100 \text{ X } \frac{\%\text{ Inert material in Feed}}{\%\text{ Inert Material in Feces}} \text{ X } \frac{\%\text{ Nutrient in Feces.}}{\%\text{ Nutrient in Feed.}}$$

The concentrations of these constituents in the feeds and feces used for calculating the digestion coefficients on the total collection samples are given in Table 16. The composition of the feces collected as "partial collection samples" on three days of the ten day total collection period and used for calculation of the digestion coefficients by the ratio techniques on the partial collection samples are given in Table 17. Lignin analysis of the partial collection samples were not made since incomplete recoveries of lignin were secured with the total collection samples, thus giving somewhat lower digestion coefficients when calculated by the lignin ratio technique than when calculated by the conventional total consumption-excretion ratios of the various constituents.

The digestion coefficients of the various constituents for each individual cow when calculated by the ratio technique on the total collection samples are given in Table 18 and the digestion coefficients for each cow on each of the three partial collection days calculated by the ratio technique with chromium oxide are compiled in Table 19 and when calculated by the plant pigment ratio technique are given in Table 20. A summary of these various techniques of calculating the digestion coefficients is given in Table 21, using the average digestion coefficients of the three cows used with each stage of orchard grass hay. The digestion coefficients calculated by the chromium ratio and pigment ratio techniques on both the total collection samples and the three day partial collection samples compare very closely with the standard total collection technique for all of the constituents of the proximate analysis. Digestion coefficients calculated by the lignin ratio technique using the crude lignin contents of the ration and feces gave slightly lower digestion coefficients than were obtained by the total fecal collection technique. The digestion coefficients calculated by the true lignin ratio technique are slightly lower than those calculated by the crude lignin ratio technique.

A review of the literature on various digestion trial techniques indicates that previous comparisons of digestion trial techniques have not included a comparison of several methods of determining digestion coefficients in the same digestion trial. The present study has made a comparison of seven different methods of calculating digestion coefficients with each of four different stages of orchard grass hay fed with a uniform amount of grain.

Analysis of variance by the method of Snedecor (169) shows a significant difference between these seven methods of calculating digestion coefficients. There is a close check, however, between the digestion coefficients calculated by the standard total fecal collection, the chromium ratio, and the plant pigment ratio techniques. The results showed that the fecal recoveries of lignin are somewhat less than 100 percent which gave lower digestion coefficients calculated by the lignin ratio technique than those obtained by the total fecal collection technique. Further studies would be necessary to ascertain if the digestibility of lignin in orchard grass hay is due to differences in the nature of the lignin isolated from the feed and feces studied; whether the metabolism of lignin in orchard grass hay is different than lignin metabolism in some other plants; or if lignin metabolism of second growth orchard grass hay might be different than that of a first growth of the plant. Satisfactory results with the lignin ratio method of calculating digestion coefficients have been reported for other rations (62, 69, 71, 103, 178).

The dry matter digestibilities of the rations containing Stage 1 and Stage 2 hays were similar (Table 21). The slightly higher dry matter digestibility of the Stage 2 hay compared with the Stage 1 hay was accompanied by a decrease in the lignin content. The Stage 2 hay was presumably cut during the period of the most rapid rate of growth of the grass. Huffman (93) has pointed out that the decreased digestibility of the dry matter of plants is believed to be associated with lignification of the cell wall although the total amount of lignin does not appear to be the determining factor.

The apparent digestibility of the crude protein and crude fiber was higher in the ration containing Stage 1 orchard grass than in the ration containing Stage 2 orchard grass but the digestibilities of the nitrogen-free extracts were similar. Digestibility of the dry matter, protein, crude fiber, and nitrogen-free extract fractions decreased progressively in the Stage 2, Stage 3 and Stage 4 orchard grass hays.

SUMMARY

A comparison of seven different methods of determining digestibility coefficients was made with each of four digestion trials using three milking cows fed orchard grass hay cut at four stages of maturity and a simple grain mixture. The conventional ten day total fecal collection method was used as a standard for comparison with the results of the other techniques. Chromium oxide was added to the grain ration as an exogenous inert substance. Lignin and certain plant pigments were determined as inert materials normally present in the ration. The concentrations of these three inert materials in the ration fed and in the fecal samples taken from an aliquote of the total collection were used in calculating the digestion coefficients by the inert material ratio technique. Additional "grab" samples taken on three days of the ten day collection period were analyzed and the digestion coefficients calculated on each of these days by the chromium ratio and plant pigment ratio techniques. Incomplete recoveries of lignin with each of the total collection trials made it impractical to calculate the digestion coefficients by the lignin ratio technique with the "grab" samples.

Comparable digestion coefficients were secured with the standard ten day consumption-excretion method and the chromium and plant pigment ratio techniques when calculated with both the total collection samples and the averages of three days partial collection or "grab" samples.

Digestion coefficients calculated by the crude lignin and the corrected lignin ratio techniques using the total fecal collection samples were both significantly lower than the digestion coefficients calculated by the standard consumption-excretion method in these trials.

The change in chemical composition of the orchard grass hay with increasing maturity resulted in a decrease in protein content of from 24.78 to 12.38 percent and an increase in crude fiber content of from 26.88 to 35.05 percent.

Digestibility of the various constituents in the four stages of orchard grass hay of increasing maturity was; dry matter 69.0, 70.4, 63.9 and 59.4 percent; protein, 65.7, 60.7, 55.4 and 54.3 percent; crude fiber, 82.5, 78.6, 72.0 and 69.1 percent; and nitrogen-free extract, 69.3, 74.7, 68.0, and 57.3 percent respectively.

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T A B L E S

Table 12.

Average Daily Dry Matter Consumed and Excreted by Cows Fed Four Stages of Orchard Grass Hay.

Hay Fed	Animal	Dry Matter Consumed: Hay	Dry Matter Consumed: Grain	Dry Matter Consumed: Total	Dry Matter Excreted
		gms.	gms.	gms.	gms.
Stage 1	X65	10,052	1,981	12,033	3,433
	2408	10,454	3,557	14,011	3,782
	N635	6,433	2,786	9,219	2,326
Stage 2	X65	10,315	1,981	12,296	3,471
	2408	10,727	3,557	14,284	3,668
	N635	6,601	2,786	9,387	2,318
Stage 3	X65	10,241	1,981	12,222	4,117
	2408	10,651	3,557	14,208	4,348
	N635	6,554	2,786	9,340	2,649
Stage 4	X54	7,290	2,496	9,786	3,316
	2408	10,530	3,557	14,087	4,955
	N635	6,480	2,786	9,266	3,102

Table 13.

Composition of the Dry Matter of Feeds and Feces used in Digestion Trials of Orchard Grass Hay.

Material	Protein	Fiber	N.F.E.	Ether Extract	Ash
	%	%	%	%	%
Grain 86	9.18	2.17	81.14	3.63	3.88
Orchard Grass					
Stage 1	24.78	26.88	35.02	3.99	9.33
Stage 2	15.80	28.15	45.82	3.46	6.77
Stage 3	13.00	31.77	44.24	3.91	7.08
Stage 4	12.38	35.05	41.16	4.24	7.17
Feces					
Stage 1					
X65	24.86	15.60	35.72	8.09	15.73
2408	26.45	14.26	37.45	7.27	14.80
N635	26.97	14.69	35.79	7.67	14.88
Stage 2					
X65	20.19	20.25	38.18	7.65	13.73
2408	20.73	18.92	41.42	6.82	12.47
N635	19.38	18.44	41.74	7.19	13.25
Stage 3					
X65	15.96	24.47	39.27	7.84	12.46
2408	16.84	22.69	41.11	7.18	12.18
N635	15.46	22.93	41.74	7.53	12.34
Stage 4					
X54	12.64	24.76	41.88	7.43	11.14
2408	14.34	23.76	43.88	7.06	10.96
N635	14.50	24.22	43.84	7.09	11.35

Table 14.

Apparent Digestion Coefficients of the Ration Constituents by Cows Fed Four Stages of Orchard Grass Hay

	Dry Matter	Protein	Fiber	N.F.E.	Ether Extract
Stage 1					
X65	71.5	68.1	80.5	76.1	41.2
2408	73.0	66.0	81.3	78.4	49.8
N635	74.8	66.1	80.9	81.6	50.0
Average	73.1	66.7	80.9	78.7	47.0
Stage 2					
X65	71.8	61.3	76.2	79.1	38.0
2408	74.3	63.1	77.7	80.4	51.1
N635	75.3	65.4	77.7	81.8	48.4
Average	73.8	63.3	77.2	80.4	45.8
Stage 3					
X65	66.3	56.5	69.5	73.7	31.6
2408	69.4	57.0	71.5	76.5	43.0
N635	71.6	63.0	71.7	78.6	44.1
Average	69.1	58.8	70.9	76.3	39.6
Stage 4					
X54	66.1	59.8	67.5	72.1	35.7
2408	64.8	56.2	68.8	69.9	39.4
N635	66.5	60.6	67.6	72.4	41.4
Average	65.8	58.9	68.0	71.5	38.8

Table 15

Apparent Digestion Coefficients of the Constituents of Orchard Grass Hay* cut at Four Stages of Maturity

	Dry Matter	Protein	Fiber	N.F.E.	Ether Extract
Stage 1					
X65	68.6	67.6	81.5	69.3	33.1
2408	68.6	64.8	83.0	68.4	38.2
N635	69.9	64.6	83.0	70.3	35.6
Average	69.0	65.7	82.5	69.3	35.6
Stage 2					
X65	69.1	59.8	77.0	75.0	28.2
2408	71.4	59.1	79.1	74.4	37.2
N635	70.8	63.1	79.6	74.7	32.7
Average	70.4	60.7	78.6	74.7	32.7
Stage 3					
X65	62.5	54.0	70.1	67.5	21.7
2408	63.8	52.8	72.7	67.6	29.1
N635	65.5	59.3	73.2	68.9	27.2
Average	63.9	55.4	72.0	68.0	26.0
Stage 4					
X54	59.3	55.6	68.5	59.3	20.1
2408	57.6	51.5	69.8	55.9	25.5
N635	58.2	55.9	69.0	56.6	24.7
Average	58.4	54.3	69.1	57.3	23.4

*Digestion coefficients of Orchard Grass Hay calculated "by difference" using the digestion coefficients of corn reported by Schneider (1947).

Table 16

Composition of the Dry Matter of Feeds and Feces used for Calculating Digestion Coefficients by the Ratio Technique on Total Collection Samples

Material	Lignin Content: Crude Lignin	Lignin Content: Corrected Lignin	Chromium Oxide	Plant Pigment
	%	%	%	units/gm.
Grain 86				
X65	.518	.505	.7341	5.41
2408	.518	.505	.4318	5.41
N.635	.518	.505	.5620	5.41
X54	.623	.607	.5200	7.63
Orchard Grass				
Stage 1	7.77	5.71	-	901.3
Stage 2	6.74	5.12	-	595.3
Stage 3	7.97	6.32	-	562.1
Stage 4	9.56	8.12	-	484.0
Feces				
Stage 1				
X65	21.61	14.77	.4367	2634.0
2408	20.20	13.69	.4246	2203.3
N635	20.63	13.74	.6667	2414.2
Stage 2				
X65	19.04	13.23	.4320	1779.0
2408	16.99	12.11	.4362	1742.5
N635	17.63	12.71	.6315	1897.1
Stage 3				
X65	19.32	14.52	.3352	1268.9
2408	17.36	13.18	.3356	1274.7
N635	18.12	13.36	.5262	1395.0
Stage 4				
X54	18.83	15.09	.3974	963.5
2408	17.88	14.72	.3157	994.7
N635	18.64	15.25	.4852	997.0

Table 17.

Composition of the Dry Matter of Feces used for Calculation of Digestion Coefficients by the Ratio Technique on Partial Collection Samples

Stage and Animal	Day	Protein	Fiber	N.F.E.	Ether Extract	Chromium Oxide	Plant Pigment
		%	%	%	%	%	units/gm.
Stage 1							
X65	1	22.65	15.31	40.24	7.73	.4634	2370.6
	2	23.78	14.41	39.05	8.02	.4353	2494.6
	3	24.31	15.60	37.64	8.06	.4459	2433.1
2408	1	22.91	14.48	42.30	6.74	.4541	2049.9
	2	22.60	14.23	44.29	6.54	.4231	1983.3
	3	22.84	13.77	43.31	7.28	.4299	2192.1
N635	1	24.31	13.64	41.66	6.71	.6672	2167.6
	2	25.54	12.52	41.52	6.86	.6340	2147.6
	3	23.84	13.02	39.85	8.29	.6692	2239.0
Stage 2							
X65	1	17.24	20.66	41.78	7.63	.4555	2080.5
	2	17.65	20.22	41.77	7.55	.4386	2005.8
	3	17.87	20.35	41.71	7.69	.4289	2058.6
2408	1	20.34	18.92	41.52	6.75	.4527	1881.2
	2	17.56	19.02	45.34	7.08	.4716	1816.4
	3	17.57	18.74	45.40	7.00	.4713	1788.6
N635	1	19.46	17.35	42.43	7.75	.6979	2127.1
	2	18.35	18.12	44.27	7.16	.6820	1939.5
	3	17.45	18.15	44.43	7.55	.6875	1884.5
Stage 3							
X65	1	14.80	25.70	39.91	7.90	.3560	1460.0
	2	14.98	24.36	40.81	8.11	.3735	1486.2
	3	14.91	23.32	41.54	8.31	.3768	1532.2
2408	1	16.08	22.70	40.43	8.54	.3803	1405.7
	2	15.13	23.91	41.15	8.08	.3470	1363.3
	3	14.56	23.18	42.79	7.77	.3511	1379.5
N635	1	16.02	21.49	43.13	7.65	.5745	1292.2
	2	13.71	24.00	42.94	7.92	.5749	1388.4
	3	15.70	21.25	43.71	7.92	.5878	1450.7
Stage 4							
X54	1	12.46	24.21	44.26	7.00	.4193	--
	2	12.31	24.40	44.72	7.16	.4400	--
	3	12.74	23.59	44.40	7.33	.4264	--
2408	1	12.85	24.29	46.37	6.87	.3271	--
	2	13.00	23.99	46.10	6.91	.3320	--
	3	13.66	24.24	44.54	7.41	.3259	--
N635	1	13.23	23.70	45.44	7.14	.5000	--
	2	12.42	24.21	45.64	7.30	.4714	--
	3	13.46	23.64	44.65	7.23	.4883	--

Table 18.

Digestion Coefficients Calculated by the Ratio Technique on the Total Collection Samples

Method	Hay	Animal	Dry Matter	Protein	Fiber	N.F.E.	Ether Extract
Chromium	Stage 1	X65	72.3	73.6	81.1	74.4	43.0
		2408	74.2	67.4	82.1	79.3	51.3
		N635	74.6	65.8	80.7	81.4	49.6
	Stage 2	X65	72.6	62.5	76.9	79.7	39.8
		2408	76.6	66.6	80.0	82.0	55.7
		N635	74.8	64.9	77.6	81.2	47.2
	Stage 3	X65	64.5	54.3	67.8	72.2	27.9
		2408	67.9	54.9	70.1	75.4	40.2
		N635	68.2	58.5	68.2	76.0	37.3
	Stage 4	X54	66.6	64.3	68.9	71.1	36.6
		2408	65.7	57.3	69.6	70.7	41.0
		N635	65.3	59.2	66.6	71.4	39.3
Pigments	Stage 1	X65	71.4	72.7	80.4	73.5	41.1
		2408	69.7	61.8	79.1	75.8	43.0
		N635	73.9	64.9	80.2	80.9	48.2
	Stage 2	X65	71.9	61.5	76.2	79.1	38.2
		2408	74.3	63.2	78.0	80.2	51.2
		N635	77.8	69.2	80.4	83.5	53.6
	Stage 3	X65	62.8	52.1	66.3	71.9	24.5
		2408	66.8	53.4	69.1	74.6	38.2
		N635	71.6	63.0	71.6	78.6	44.0
	Stage 4	X54	62.5	59.9	65.0	67.5	28.7
		2408	63.5	54.5	67.6	68.8	37.1
		N635	65.2	59.0	66.5	71.3	39.1
Crude Lignin	Stage 1	X65	69.6	72.0	79.2	71.9	37.4
		2408	70.6	63.0	79.7	76.5	44.7
		N635	73.0	63.6	79.5	80.2	46.4
	Stage 2	X65	69.9	58.7	74.5	77.6	33.7
		2408	70.1	57.0	74.0	77.2	43.3
		N635	72.8	61.5	75.5	80.1	43.1
	Stage 3	X65	65.0	54.9	68.3	72.6	28.9
		2408	65.3	51.2	67.7	73.4	35.2
		N635	68.7	59.2	68.8	76.4	38.4
	Stage 4	X54	60.7	58.0	63.3	65.9	25.2
		2408	58.8	48.6	63.4	64.8	29.0
		N635	63.4	57.0	64.8	69.9	36.0

Table 18. Continued.

Method	Hay	Animal	Dry Matter	Protein	Fiber	N.F.E.	Ether Extract
Corrected Lignin	Stage 1	X65	67.2	68.7	77.6	69.6	32.4
		2408	68.0	59.6	77.8	74.3	39.7
		N635	69.9	59.6	77.2	78.0	40.0
	Stage 2	X65	67.7	55.8	72.7	76.1	29.0
		2408	68.0	54.0	72.2	75.6	39.3
		N635	71.2	59.2	74.0	78.8	39.7
	Stage 3	X65	63.6	53.2	67.0	71.5	26.0
		2408	63.7	49.0	66.2	72.2	32.3
		N635	66.2	55.9	66.3	74.5	33.5
	Stage 4	X54	58.9	56.1	61.7	64.4	21.9
		2408	58.6	48.4	63.2	64.6	28.3
		N635	63.1	56.5	64.4	69.5	35.3

Table 19.

Digestion Coefficients Calculated by the Chromium Ratio Technique on the Partial Collection Samples

Hay	Animal	Collection Day	Dry Matter	Protein	Fiber	N.F.E.	Ether Extract
Stage 1	X65	1	73.9	73.4	82.5	75.3	48.7
		2	72.2	70.3	82.5	74.5	43.3
		3	72.9	70.3	81.5	76.0	44.4
	2408	1	75.8	73.4	73.0	78.1	57.8
		2	74.1	71.8	82.1	75.4	56.1
		3	74.5	72.0	83.0	76.4	51.9
	N635	1	74.6	69.2	80.8	81.4	49.6
		2	73.3	66.0	82.8	77.3	53.6
		3	74.7	60.9	83.0	79.6	47.0
Stage 2	X65	1	74.0	69.6	77.6	78.9	43.1
		2	73.0	67.7	77.2	78.1	41.5
		3	72.4	66.6	76.6	77.6	39.1
	2408	1	76.3	72.3	79.4	79.9	51.8
		2	77.2	71.6	80.0	81.1	54.1
		3	77.2	71.7	80.3	81.1	54.7
	N635	1	76.1	66.4	79.7	82.0	47.0
		2	75.5	67.6	78.3	80.8	49.9
		3	75.7	69.4	78.4	80.8	47.7
Stage 3	X65	1	66.6	60.1	68.2	73.4	31.6
		2	68.1	61.5	71.2	74.1	33.1
		3	68.4	62.0	72.7	73.9	32.0
	2408	1	71.6	62.0	73.5	78.5	37.0
		2	68.9	60.8	69.5	76.0	34.6
		3	69.2	62.7	70.7	75.7	37.9
	N635	1	70.8	60.7	72.7	77.2	41.5
		2	70.9	61.5	69.5	77.4	39.6
		3	71.5	62.4	73.6	77.5	40.9
Stage 4	X54	1	68.4	66.7	71.2	72.5	43.4
		2	69.9	68.6	72.3	73.5	44.8
		3	68.9	66.5	72.4	72.8	41.7
	2408	1	66.7	62.9	69.8	69.9	44.2
		2	67.2	63.0	70.6	70.5	44.7
		3	66.6	60.4	69.9	71.0	39.6
	N635	1	66.1	60.9	68.1	71.1	40.3
		2	64.1	61.0	65.4	69.2	35.2
		3	65.3	59.2	67.4	70.9	38.1

Table 20

Digestion Coefficients Calculated by the Pigment Ratio Technique on the Partial Collection Samples

Hay	Animal	Collection Day	Dry Matter	Protein	Fiber	N.F.E.	Ether Extract
Stage 1	X65	1	68.2	67.6	78.7	69.9	37.5
		2	69.8	67.7	80.9	72.3	38.3
		3	69.0	66.1	78.8	72.6	36.5
	2408	1	67.1	63.8	76.9	70.3	42.6
		2	66.0	63.1	76.6	67.8	42.4
		3	69.3	66.2	77.5	71.5	42.0
	N635	1	70.9	64.8	78.0	78.8	42.3
		2	70.6	62.7	81.1	75.1	49.1
		3	71.8	66.6	81.1	77.4	41.2
Stage 2	X65	1	76.0	71.9	79.3	80.5	47.3
		2	75.1	70.1	79.0	79.8	45.9
		3	75.7	70.5	79.4	80.3	46.4
	2408	1	76.2	72.2	79.3	79.8	51.6
		2	75.3	69.3	78.4	79.5	50.3
		3	74.9	68.9	78.3	79.2	50.2
	N635	1	80.2	72.3	83.2	85.1	56.3
		2	78.3	71.3	80.8	83.0	55.7
		3	77.7	71.9	80.2	82.4	52.0
Stage 3	X65	1	67.7	61.4	69.2	74.3	33.9
		2	68.3	61.6	71.3	74.2	33.3
		3	69.2	63.0	73.4	74.5	33.7
	2408	1	69.9	59.8	72.0	77.3	33.7
		2	69.0	61.0	69.6	76.2	34.9
		3	69.4	62.9	70.9	75.5	38.2
	N635	1	69.4	58.7	71.3	76.1	38.6
		2	71.5	67.1	70.2	78.0	40.9
		3	72.7	63.6	74.7	78.4	43.5
Stage 4(1)							

(1) Suitable samples were not available for determination of the plant pigment concentration of the feces for all animals used in the trial with Stage 4 Orchard Grass hay.

Table 21

Comparison of Digestion Coefficients Calculated by several methods on Four Stages of Orchard Grass Hay plus Grain

Constituent	Total Collection Samples: Standard	Chromium Ratio	Lignin Ratio: Crude	Lignin Ratio: Corrected	Pigment Ratio	Partial Collection: Chromium Ratio	Partial Collection: Pigment Ratio
Stage 1							
Dry Matter	73	74	71	68	72	74	69
Protein	67	69	66	63	67	70	65
Fiber	81	81	80	78	80	81	79
N.F.E.	79	78	76	74	77	77	73
Ether Ext.	47	48	43	38	44	50	41
Stage 2							
Dry Matter	74	75	71	69	75	75	77
Protein	63	65	59	56	65	69	71
Fiber	77	78	75	73	78	79	80
N.F.E.	80	81	78	77	81	80	81
Ether Ext.	46	48	40	36	48	48	51
Stage 3							
Dry Matter	69	67	66	65	67	70	70
Protein	59	56	55	53	56	62	62
Fiber	71	69	68	67	69	71	71
N.F.E.	76	75	74	73	75	76	76
Ether Ext.	40	35	34	31	36	36	37
Stage 4							
Dry Matter	66	66	61	60	64	67	--
Protein	59	60	55	54	58	63	--
Fiber	68	68	64	63	66	70	--
N.F.E.	72	71	67	66	69	71	--
Ether Ext.	39	39	30	29	35	41	--

PART II

STUDIES ON THE COMPOSITION OF LIGNIN ISOLATED FROM ORCHARD GRASS HAY CUT AT FOUR STAGES OF MATURITY AND FROM THE CORRESPONDING FECES

INTRODUCTION

Lignin has been reported to be indigestible by a number of workers (7, 9, 13, 14, 16, 17, 19, 38). Other reports indicate that varying amounts of lignin may be digested or broken down in the ruminant digestive tract (4, 8, 10, 11, 18, 21, 22, 27).

Since the chemical structure of lignin has not been established it is not feasible to isolate or determine a product of uniform composition from different materials. Certain characteristics of crude lignin preparations vary considerably depending upon the material from which it is isolated and the method used for its isolation. Extensive studies have been made of the characteristics of lignin isolated from various woods but only limited studies have been reported on lignin prepared from forage plants important in animal feeding.

Phillips *et al.* (33) found 4.34 percent nitrogen in the lignin obtained from oat plants 7 days old which decreased to 1.26 percent nitrogen in the lignin from plants 84 days old. Bondi and Meyer (4) reported from 1.18 to 1.63 percent nitrogen in the lignin from four grass species and from 2.92 to 3.36 percent nitrogen in the lignin from four legumes. The lignin isolated from the corresponding feces all had slightly higher nitrogen contents. They also showed that the lignin nitrogen was resistant to hydrolysis by strong acid (5 N sulfuric acid) and weak acid (0.5 N hydrochloric acid) and by pepsin added to the weak acid. These results led them to conclude that the nitrogen in crude lignin preparations was not present as protein nitrogen. Furthermore, the digestive enzymes of the ruminant did not reduce the nitrogen content of the lignin.

Norman and Jenkins (25) believed that protein degradation products following the treatment of plant material with strong acid resulted in high lignin yields rather than protein per se. Paloheimo (26), Waksman and Stevens (40), Phillips (28), and DeMan and DeHeus (12) have corrected crude lignin values by deducting the nitrogen content multiplied by the factor 6.25. Phillips (29) later felt that because there was not a constant relation between the amount of protein added to samples for the determination of lignin and the size of the error involved that the apparent lignin values should not be corrected but the percent of nitrogen in the lignin should be reported.

Thomas and Armstrong (39) found amino acids in the hydrolysates from crude lignin and proposed that the factor 6.25 for converting lignin nitrogen to protein was valid. DeMan and DeHeus (12) found the tyrosine and the tryptophane nitrogen in the lignin isolated from Lolium perenne was in agreement with that for the true protein of the grass. The tryptophane nitrogen was determined on lignin isolated without the use of concentrated sulfuric acid.

Bondi and Meyer (4) found about 5 percent methoxyl in the lignin obtained from legumes and about 10 percent methoxyl in the lignin isolated from various grasses. They also found very little difference in the methoxyl content of the lignin isolated from the corresponding feces which would indicate that there is very little breakdown of the ether linkages in the lignin molecule during passage through the digestive tract. Oxidation of the lignin from various plants gave yields of from 4.2 to 21.6 percent aldehydes while oxidation of the fecal lignins gave negligible quantities of aldehydes, indicating a change in the side chains attached to the lignin molecule during passage through the intestinal tract. The lignin from the grasses gave a much higher percentage of aldehydes following oxidation than the legumes but the ratio of vanillin to p-hydroxy-benzaldehyde was approximately 2 to 1 in all cases.

Stone, Blundell and Tanner (37) found that the ratios of vanillin to syringealdehyde did not remain constant in the wheat plant at different stages of growth since the percentage of syringealdehyde was lower than vanillin in the young plants and higher in the more mature plants. Concurrent increases in the methoxyl content of the lignin were observed.

Csonka, Phillips and Jones (10) and Phillips et al. (34) reported a loss of 36.7 percent of the methoxyl groups associated with the lignin in a ration of hay, grain and silage fed to a cow. Francois, Leroy and Levy (15) reported that 50 percent of the methoxyl content of the lignin disappeared in the digestive tract of ruminants fed alfalfa hay alone and in combination with wheat, and dried beet pulp. The apparent digestibility of the lignin of alfalfa was approximately 8 percent.

Pazur and DeLong (27) Phillips et al. (34) and Csonka, Phillips and Jones (10) have reported increased urinary excretion of hippuric acid when increased amounts of lignin are fed and felt that this indicated a metabolism of lignin in the animal body. Rogozinski and Starzewska (36) felt that lignin was indigestible by sheep and played no part in the formation of hippuric acid.

This study was conducted to determine certain characteristics of the lignin isolated from orchard grass hay cut at four stages of maturity and from the corresponding feces of dairy cows fed these forages.

EXPERIMENTAL PROCEDURE

The method of conducting the digestion trials and the collection and handling of the samples have been previously presented (Part I).

The nitrogen content of the lignin was determined by the microkjeldahl method of the Association of Official Agricultural Chemists (3) on samples of the lignin isolated from the various materials by the method of Ellis, Matrone and Maynard (13).

The total methoxyl groups in the feeds fed and the feces voided and also the methoxyl content of the lignin isolated from these materials were determined by the method of Clark (5, 6).

The benzoic acid concentration in the urine was determined by the method of Kingsbury and Swanson (20).

RESULTS AND DISCUSSION

The amounts of lignin consumed and excreted and the percent recovery of lignin in the feces and the percent of lignin digested are given in Table 22. Since lignin recoveries were all somewhat lower than is desirable for a material to be suitable for use as an inert tracer material it explains the tendency for the lower digestion coefficients previously presented (Part I) that were calculated by the lignin ratio technique. When planning this experiment it was postulated that if the lignin of orchard grass was partially digested that there might be a range in the digestibility of lignin that would show higher digestibilities of the lignin in immature orchard grass than in the more mature stages. Since lower digestibility of the lignin in the immature orchard grass hay ration was secured it seemed desirable to investigate some of the characteristics of the lignin isolated from the feeds and feces from these digestion trials. The nitrogen content of the lignin isolated from the four stages of orchard grass hay and the corresponding feces are presented in Table 23. The lignin isolated from the immature orchard grass had a higher nitrogen content (4.25 percent) than the lignin isolated from the most mature orchard grass (2.41 percent). Lignin isolated from the corresponding feces had average nitrogen contents of 5.19 and 2.91 percent respectively. The protein content of the dry matter of the immature grass was 24.78 percent while the protein content of the most mature orchard grass was 12.38 percent. The protein content of the corresponding feces averaged 26.09 and 13.83 percent respectively. Small differences were obtained between the nitrogen content of the lignin from each stage of orchard grass hay and the nitrogen content of the lignin isolated from the corresponding feces. The "true lignin" contents of each stage of hay and the corresponding feces were calculated by correcting the crude lignin content for the nitrogen content of the lignin multiplied by the factor 6.25. While there is considerable controversy concerning the nature of the nitrogen present in the lignin preparations, the work of DeMan and DeHeus (12) and Thomas and Armstrong (39) indicates that the use of a protein factor may be justified in converting the nitrogen present in the lignin to protein.

The crude lignin contents of the four stages of orchard grass hay of increasing maturity were 7.77, 6.74, 7.97, and 9.56 percent respectively. Lignin is generally considered as a constituent that increases with advancing maturity of the plant (2, 9, 23, 30, 31, 32, 33, 35, 41). Other work (2, 24) with orchard grass has shown that the lignin content initially decreases and then progressively increases with advancing maturity and these changes are concommitant with progressive increases in the crude fiber content and progressive decreases in the crude protein content. These results are in agreement with those found in this investigation. There is evidence that similar changes occur in timothy (31), oats (33), perennial ryegrass (2), tall fescue (2) and burnet (2). This pattern of change has not been observed in alfalfa, trefoil and yarrow (2). Whether these changes are a physiological characteristic of the growth of certain grasses or whether there is an inherent error in the determination of lignin in immature grasses has not been determined.

The true lignin intakes and excretion for each stage of hay fed was calculated and these data with the recoveries and digestibilities of the true lignin are presented in Table 24. The apparent digestion coefficients for true lignin are higher than those calculated for crude lignin. If the recoveries of the true lignin were higher than those found for the crude lignin one could assume that a higher digestibility of the nitrogen containing fraction of the crude lignin had resulted in greater losses of this portion in passing through the digestive tract. Comparison of the nitrogen contents of the lignin isolated from the various orchard grass hays and the corresponding feces (Table 23) does not indicate that this occurred.

Since other studies (10,27,34) have indicated that degradation products of lignin may cause increased urinary excretion of benzoic acid, determinations were made of the benzoic acid excretion by each animal fed the four stages of orchard grass hay. Table 25 gives the amounts of crude lignin and true lignin fed and digested by each animal and the urinary excretion of benzoic acid per 100 grams of lignin fed and digested. The benzoic acid excretion per unit of lignin fed is somewhat lower in the mature hay than in the other three stages of less mature hays. If the amount of urinary benzoic acid excretion is influenced largely by the degree of lignin degradation these figures would indicate a lower digestibility of lignin in the more mature orchard grass hay. This is not supported by the consumption-excretion figures for crude lignin given in Table 22. It is possible that other constituents of immature orchard grass contribute to the formation and excretion of benzoic acid.

The methoxyl contents of the feed and feces on a dry weight basis are given in Table 26. A progressive increase in the total methoxyl content of the hay occurs with increasing maturity from 1.41 percent to 1.85 percent and similar increases occur in the methoxyl content of the feces from animals fed these hays. The methoxyl content of the lignin from the immature hay is lower (7.84 percent) than that from the other three stages which are similar in methoxyl content (10.60, 10.73 and 10.41 percent). Comparable changes occur in the methoxyl content of the lignin isolated from the corresponding feces. Bondi and Meyer (4) found approximately 10 percent methoxyl groups in the lignin isolated from several species of grass. Their work also indicates that each structural unit of the lignin molecule from grasses contains two methoxyl groups which would give a theoretical methoxyl content of 9.9 percent. Phillips et al. (33) found the methoxyl content of the lignin of oats increased from around 4 to approximately 15 percent during growth to 84 days of age. Similar changes occurred in the lignin of barley plants (32) and timothy plants (31) during development. The percentages of the total methoxyl groups that were associated with the lignin in the four stages of orchard grass hay increasing in maturity were 31.9, 35.1, 40.7 and 45.4 percent, respectively. Adams and Castagne (1) found 82.4 percent and 81.7 percent of the total methoxyl groups of wheat and oat straw, respectively, in the lignin fraction. Phillips and Goss (28) found from 52.0 to 81.1 percent of the total methoxyl groups associated with the lignin fraction of barley plants at various stages of development. From 39.3 to 85.7 percent of the total methoxyl content of the oat plant was found in the isolated lignin by Phillips et al. (29).

There is not much change, however, in the percentage of the total methoxyl groups which are associated with the lignin isolated from the feces of cows fed orchard grass hay cut at four stages of maturity. This would suggest that there is a higher digestibility of the non-lignin methoxyl in the immature forage and this is evident in a comparison of the digestion coefficients of the various fractions of the methoxyl groups that are also given in Table 26. Digestibility of the total methoxyl content of the ration and the methoxyl groups not associated with the lignin both decrease with increasing maturity of the plant while there is practically no change in the digestibility of the methoxyl groups associated with the lignin.

SUMMARY

The apparent digestion coefficients of lignin in rations containing orchard grass hay cut at four stages of maturity ranged from 3.8 to 16.0 percent.

The lignins isolated from four stages of orchard grass of increasing maturity had nitrogen contents of 4.25, 4.15, 3.53 and 2.41 percent. The average nitrogen contents of the lignins isolated from the corresponding feces were 5.19, 4.13, 3.62 and 2.91 percent. Apparently no degradation of the nitrogen associated with the lignin in orchard grass hay occurred during digestion by dairy cows. There may be errors in the determination of lignin that are unaccounted for in the analysis of materials differing widely in other constituents.

The apparent digestion coefficients for the lignin corrected for nitrogen content was somewhat higher (7.5 to 19.8 percent) than the digestion coefficients for crude lignin.

The benzoic acid excretion in the urine per 100 grams of lignin fed was lower when feeding the most mature hay than when feeding the other three less mature hays. There was no relation between the digestion coefficient of lignin and the urinary excretion of benzoic acid.

The total methoxyl content of the hay fed was lower than the total methoxyl content of corresponding feces on a dry matter basis. The methoxyl contents of the lignin isolated from the various hays was higher than the methoxyl contents of the lignin isolated from the corresponding feces.

The percent of the total methoxyl content in the hay that was associated with the lignin increased with increasing maturity of the orchard grass. Higher proportions of the total methoxyl content of the feces was associated with the lignin in the feces and only slight changes in this percentage occurred when hays of a wide range of maturity were fed.

Digestibility of the total methoxyl content and the non-lignin methoxyl content of the ration decreased with increasing maturity of the orchard grass hay. Digestion of the lignin methoxyl groups ranged from 20.3 to 31.6 percent and showed no consistent trend in relation to the stage of maturity of the hay fed.

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T A B L E S

Table 22.

Crude Lignin Fed and Excreted by Each Animal Receiving Various Stages of Orchard Grass Hay plus Grain.

Hay Fed	Animal	Average Daily Crude Lignin Consumed			Average Daily Lignin Excreted	Lignin Recovery	Lignin Digested
		Hay	Grain	Total			
		gms.	gms.	gms.	gms.	%	%
Stage 1	X65	781.0	10.3	791.3	741.8	93.7	6.3
	2408	812.3	18.4	830.7	763.9	92.0	8.0
	N635	499.8	14.4	514.2	479.7	93.3	6.7
Stage 2	X65	695.6	10.3	705.9	660.8	93.6	6.4
	2408	723.4	18.4	741.8	623.2	84.0	16.0
	N635	445.2	14.4	459.6	408.7	88.9	11.1
Stage 3	X65	816.3	10.3	826.6	795.4	96.2	3.8
	2408	848.9	18.4	867.3	754.8	87.0	13.0
	N635	532.4	14.4	536.8	480.0	89.4	10.6
Stage 4	X54	696.9	15.5	712.4	624.5	87.7	12.3
	2408	1006.6	18.4	1025.0	886.0	86.4	13.6
	N635	619.4	14.4	633.8	578.2	91.2	8.8

Table 23.

Nitrogen Content of Crude Lignin Isolated from Various Stages of Orchard Grass Hay and the Corresponding Feces

Stage of Maturity	Material	Animal	Crude Lignin	Nitrogen	True[1/] Lignin
			%	%	%
Stage 1	Hay	----	7.77	4.25	5.71
	Feces	X65	21.61	5.07	14.77
		2408	20.20	5.16	13.69
		N635	20.63	5.34	13.74
Stage 2	Hay	----	6.74	4.15	5.00
	Feces	X65	19.04	4.35	13.23
		2408	16.99	4.07	12.11
		N635	17.63	3.98	12.71
Stage 3	Hay	----	7.97	3.53	6.21
	Feces	X65	19.32	3.49	14.92
		2408	17.36	3.69	13.18
		N635	18.12	3.67	13.36
Stage 4	Hay	----	9.66	2.41	8.12
	Feces	X54	18.83	3.18	15.09
		2408	17.88	2.69	14.72
		N635	18.64	2.85	15.25

1/ Crude Lignin minus (N x 6.25)

Table 24.

True Lignin Fed and Excreted by Each Animal
Receiving Various Stages of Orchard Grass Hay
plus Grain

Hay Fed	Animal	Average Daily True Lignin Consumed Hay	 Grain	 Total	Average Daily Lignin Excreted	Lignin Recovery	Lignin Digested
		gms.	gms.	gms.	gms.	%	%
Stage 1	X65	573.4	10.0	583.4	507.0	86.9	13.1
	2408	596.4	18.0	614.4	517.7	84.3	15.7
	N635	367.0	14.1	381.1	319.5	83.8	16.2
Stage 2	X65	515.3	10.0	525.3	459.2	87.4	12.6
	2408	535.9	18.0	553.9	444.2	80.2	19.8
	N635	329.8	14.1	343.9	294.7	85.7	14.3
Stage 3	X65	636.3	10.0	646.3	597.8	92.5	7.5
	2408	661.7	18.0	679.7	573.1	84.3	15.7
	N635	407.2	14.1	421.3	353.9	84.0	16.0
Stage 4	X54	592.0	15.2	607.2	500.4	82.4	17.6
	2408	855.1	18.0	873.1	729.4	83.5	16.5
	N635	526.2	14.1	540.3	474.1	87.8	12.2

Table 25.

Urinary Excretion of Benzoic Acid by Animals Fed Orchard Grass Hay Cut at Four Stages of Maturity

Stage and Animal	Average Daily Crude Lignin		Average Daily True Lignin			Benzoic Acid Excretion			
						Gms/100 gms. Crude Lignin		Gms./100 gms. True Lignin	
	Fed	Digested	Fed	Digested	Total Daily	Fed	Digested	Fed	Digested
	gms.	gms.	gms.	gms.	gms.				
Stage 1									
X65	791	49.5	583	76.4	139	17.6	280.8	23.8	181.9
2408	831	66.8	614	96.7	115	13.8	172.2	18.7	118.9
N635	514	34.5	381	61.6	80	15.6	231.9	21.0	129.9
Stage 2									
X65	706	45.1	525	66.1	136	19.3	301.6	25.9	205.7
2408	742	118.6	554	109.7	156	21.0	131.5	28.2	142.2
N635	460	50.9	344	49.2	115	25.0	225.9	33.4	233.7
Stage 3									
X65	827	31.2	646	48.5	122	14.8	391.0	18.9	251.5
2408	867	112.5	680	106.6	118	13.6	104.9	17.4	110.7
N635	537	56.8	421	67.4	97	18.1	170.8	23.0	143.9
Stage 4									
X54	712	87.9	607	106.8	104	14.6	118.3	17.1	97.4
2408	1025	139.0	873	143.7	92	9.0	66.2	10.5	64.0
N635	634	55.6	540	66.2	61	9.6	109.7	11.3	92.1

Table 26.

Methoxyl Content of the Feed and Feces Dry Matter and the Lignin Isolated from these Materials, and Digestion Coefficients for Various Fractions of Methoxyl Groups

Stage and Material	Animal	Total Methoxyl	Lignin Methoxyl	Lignin Methoxyl in Total	Digestion Coefficients		
					Total Methoxyl	Lignin Methoxyl	Non-Lignin Methoxyl
		%	%	%			
Stage 1							
Hay		1.41	7.84	31.9			
Feces	X65	1.84	7.75	63.6	58.4	16.0	77.3
	2408	1.77	7.39	59.3	59.7	23.5	75.4
	N635	1.72	7.70	63.4	61.0	21.5	79.2
	Av.	1.78	7.61	62.1	59.7	20.3	77.3
Stage 2							
Hay		1.51	10.60	35.1			
Feces	X65	2.09	8.80	58.4	56.4	28.4	70.8
	2408	2.08	8.78	53.4	57.8	35.1	69.1
	N635	2.08	8.88	56.7	57.9	31.3	71.3
	Av.	2.08	8.82	56.2	57.4	31.6	70.4
Stage 3							
Hay		1.62	10.73	40.7			
Feces	X65	2.25	9.30	62.2	47.5	20.7	65.2
	2408	2.08	8.68	57.2	52.7	33.0	65.3
	N635	2.40	9.52	55.4	47.4	27.2	59.9
	Av.	2.24	9.17	58.3	49.2	27.0	63.5
Stage 4							
Hay		1.85	10.41	45.4			
Feces	2408	2.35	9.77	60.9	45.5	22.8	62.8
	N635	2.50	9.96	59.6	42.4	17.7	60.6
	Av.	2.43	9.87	60.3	44.0	20.3	61.7

PART III

A STUDY OF THE CRUDE FIBER AND NITROGEN-FREE EXTRACT FRACTIONS OF ORCHARD GRASS HAY AND THE DIGESTIBILITY OF SOME OF THE CONSTITUENTS BY MILKING COWS

INTRODUCTION

Certain limitations of the widely used system of feedstuffs analysis inaugurated by Henneberg and Stohmann (22) have undoubtedly been recognized since the method was first proposed. The value and usefulness of this system of analysis has also been recognized and demonstrated through many years accumulation of results on a wide variety of feed materials. The extensive compilation of feed composition and digestibility data by Morrison (36) and Schneider (44) are a tribute to the past and present usefulness of the Henneberg system of feed analysis. The studies of Axelsson (3, 4, 5, 6, 7), Dijkstra (12), Duckworth (13), Hallsworth (20), Homb (24, 25), Jarl (26, 27), Lancaster (29), McMeekan (34), Nordfeldt et al. (37) and Olofsson (40) all indicate definite value in determining the crude fiber content of feeds since the high negative correlation between the crude fiber content and the digestibility and feeding value of a feed certainly justify the continued use of the crude fiber determination in the absence of a more desirable scheme of analysis.

There are numerous reports in the literature discussing the merits and limitations of partitioning the carbohydrates of feeds into crude fiber and nitrogen-free extract fractions. Several authors (8, 9, 10, 29, 31, 33, 35, 37, 38, 39, 49, 51) have criticized the Weende system of determining the content of crude fiber in a feed and calculating the amount of nitrogen-free extract as being unsatisfactory principally because the constituents that make up the crude fiber and nitrogen-free extract fractions vary greatly with different plant species and different materials and also with different conditions of determining the crude fiber fraction.

Crampton and Maynard (10) have compared the average digestion coefficients of the nitrogen-free extract and crude fiber fractions in various classes of feeds. Dry roughages, pasture herbage and silage had 39, 67, and 28 percent, respectively, of the cases in which the crude fiber was as high or higher in digestibility than the nitrogen-free extract. This demonstrated the variability in the composition, digestibility, and feeding value of crude fiber from various sources.

Norman (38) pointed out that crude fiber determined by the conventional method was not a single compound but consisted of cellulose and lignin with some hemicellulose and pectin. Several modifications of the Weende system of analysis for crude fiber have been proposed but they all have the same criticism even though the reproductibility is satisfactory. Nordfeldt _et al._ (37) compared several methods for the determination of crude fiber and found them to give good reproductibility but different methods gave different values for crude fiber. They also showed that the composition of the crude fiber was different in different feeds and the crude fiber from a particular feed was different from that of the corresponding feces. They also demonstrated a selective digestibility of the lignin, cellulose and pentosan fractions which are the main components of the crude fiber. This work emphasized the value of lignin, cellulose and pentosan determinations to supplement the crude fiber determination and thus would give more useful information of the nature of the carbohydrate fraction in feeds and feces.

Knowledge of these limitations associated with the determination of crude fiber has stimulated interest in other systems of feedstuffs analysis that would not leave such a large portion of the feed analytically undetermined. A few investigators have attempted to account for nearly all the dry matter of various feeds and feces by analytical determinations which leave comparatively small amounts of undetermined substances.

Probably the earliest studies of the summative analysis of common feeding stuffs in the United States were done by Headden (21) and Widtsoe and Stewart (50). Headden (21) partitioned clover hay into sugars, dextrins, starch, xylans, lignones, cellulose, moisture, ash, proteins, materials extracted by ether and materials extracted by alcohol and water corrected for sugar and dextrins. He had 4.8 percent of the total plant material undetermined by this system of analysis. Widtsoe and Stewart (50) partitioned the dry matter of alfalfa into the ether extract, water extract, acid extract, alkali extract, fiber, and ash in the fiber fractions. They made progressive extractions with each solvent on the residue remaining after previous extractions thus accounting for all of the dry matter. This sytem would not be too useful for digestibility studies since the material extracted by each solvent from the feed and feces would probably vary widely.

Crampton and Whiting (11) proposed a scheme of analysis which determined the percentage of lignin by difference after making analytical determinations of cellulose and soluble carbohydrates. Ferguson (15) analyzed wheat straw for its content of ether extract, crude protein, ash, lignin, true cellulose, pentosans in cellulose and furfuraldehyde. With this system of analysis he had 13.7 percent of the dry matter undetermined which he called "other carbohydrates". Ferguson (16) also analyzed several forage samples for the individual structural constituents of the cell wall and also the non-structural constituents and secured variable quantities of undetermined constituents. Analysis of very young high protein grass gave undetermined fractions amounting to only 4.5 percent of the dry matter while the more mature forages had over 16 percent of the dry matter undetermined. Reid (42) reported that larger amounts of the dry matter of mature grass than of younger grasses was undetermined by the sum of the lignin, cellulose, hemicellulose, pectin, starch, total sugar, ash, crude protein and ether extract percentages.

Flanders (17) recently reported a summative analysis of timothy hay by determining ash, benzene-alcohol extractives, water extractives, pectic substances, hemicelluloses, alpha cellulose, lignin and proteins. A study of the four hemicellulose fractions extracted indicated that they were mixtures and their composition varied from one plant source to another (18). The hemicellulose fractions showed comparable variations in the uronic acid anhydride and pentose contents within plant families but not between plants of different families.

The present status of our knowledge of the carbohydrate constituents of forage has been well described by Percival (41) in a recent discussion of the subject. He stated that, "Although an immense amount of routine analytical work has been carried out on forage crops in the past, we are still ignorant, in the main, of what is measured under such headings as crude fiber, total carbohydrates and so on."

It seemed desirable to study certain of the chemical constituents of the crude fiber and nitrogen-free extract fractions that could be determined as either definite chemical substances or that would be representative of similar substances when determinations are made on widely different materials such as feeds and feces. While such a method of analysis is not readily applicable to routine determinations it would give useful information on the relative digestibilities of various fractions of the carbohydrate complex of forage plants and also might permit logical grouping of certain of these constituents that would be biologically similar and also might be easily grouped for convenient chemical determinations.

The object of this investigation was to determine the digestion coefficients of certain constituents that are ordinarily grouped in the crude fiber and nitrogen-free extract fractions and also to ascertain what percentage of the dry matter of the feed and feces would be accounted for by a more detailed system of analysis.

EXPERIMENTAL PROCEDURE.

The method of conducting the digestion trials and the collection and handling of the samples has been previously described (Part I).

Cellulose was determined by the method of Matrone, Ellis and Maynard (32).

Pentosans were determined as the furfural yielding constituents according to the official method of the Association of Official Agricultural Chemists (1) and the weight of furfural phloroglucide calculated as the pentosan equivalent. The pentosan content of the crude Matrone cellulose was determined on a sample of crude cellulose which was divided into separate samples for determinations of the pentosan and cellulose contents.

Starch was determined on the grain samples by the official diastase method with subsequent acid hydrolysis of the Association of Official Agricultural Chemists (1). Starch was determined on the hay and feces samples by the method of Hoffpauir (23).

Determinations of the reducing sugars were made by extracting the material with 80 percent alcohol according to the method of Thomas, Melin and Moore (48), inversion with hydrochloric acid, and determining the reduced copper after boiling the extract with copper sulfate-alkaline tartrate solution by the dichromate-ferric sulfate-diphenylamine procedure described by Leonard *et al.* (30).

Organic acids were determined by the method of Somers (45). Aqueous extractions of the material were made in a Waring blender after addition of sufficient 3 normal hydrochloric acid to bring the pH to approximately 1.0. The acidified extracts were heated in a steam bath to remove carbon dioxide, made to a convenient volume at 25 degrees centigrade and then a 50 milliliter aliquote was adjusted to pH 7.8 with sodium hydroxide (3 normal followed by 0.05 normal sodium hydroxide) and titrated to pH 2.6 with 0.05 normal nitric acid. The organic acids were calculated as the equivalent amount of malic acid.

RESULTS AND DISCUSSION.

The composition of the crude fiber and nitrogen-free extract fractions of the feeds fed during the digestion trials conducted on the orchard grass hay cut at four stages of maturity are given in Table 27. The conventional proximate system of analysis for feeds includes determinations of the protein, ash, ether extract, and crude fiber percentages and calculation of the nitrogen-free extract fraction of the feed by difference. With this system, the percentages that were determined by difference (nitrogen-free extract) ranged from 35.0 to 45.8 percent of the dry matter of the hays. When analyses were made of the sugar, starch, organic acid, pentosan, alpha cellulose and lignin contents of the hay the undetermined portion of the total hay ranged from 8.6 to 18.5 percent of the dry matter. These percentages of the undetermined portion of the dry matter of feeds are within the same range as reported by Ferguson (15, 16). Reid (42) accounted for somewhat higher percentages of the total dry matter of forages.

A greater percentage of the dry matter of the immature orchard grass hay was accounted for by the more detailed system of analysis than in the more mature orchard grass hay used in this study. This trend has also been reported by Reid (42). Differences in the percent of dry matter accounted for in the immature and mature forages by this system of analysis may be partially due to the increase in the proportion of the dry matter of the hay which the crude fiber and nitrogen-free extract fractions represent with increasing maturity. The total of these two fractions increased from 61.9 percent in the immature hay to 76.2 percent in the mature hay. Errors due to conversion of the nitrogen content of young plants to protein equivalent may also be greater in young plants than in older plants. It is also recognized that the undetermined fraction includes any analytical errors involved in each of the individual analyses. With the proximate system of analysis these errors are included in the nitrogen-free extract fraction.

Several authors (2, 19, 28, 43, 47, 52) have given summative analyses of woods that are remarkably close to 100 percent of the original material. Most of these experiments have included the total carbohydrate fraction of woods in preparations of holocellulose. There is evidence that properly isolated holocellulose retains nearly all of the pentosans, acetyl groups and the carbon dioxide forming material originally present in the wood (43). Another approach to the summative analysis of forages would be to include several of the carbohydrate fractions present in forages in a single determination of related materials such as those included in holocellulose preparations. The feasibility of such a system would depend upon suitable techniques for isolating the holocellulose fraction from forages and would also necessitate a knowledge of the digestibility of the various constituents of the holocellulose fraction.

The lignin, total cellulose, and pentosan content of the immature orchard grass hay was lower than that of the mature orchard grass hay. The organic acid content of the orchard grass hay decreased with advancing maturity. The sugar content of the most immature orchard grass hay was lower than in the hays cut at later stages of maturity. The sugar content of the hays cut at the three later stages of maturity progressively decreased with advancing maturity. The low sugar content of the immature hay is probably due to its being placed on the barn dryer at a higher moisture content than the other stages thus permitting plant cell respiration to continue for a longer period than in the other three stages which were partially dried in the field before being placed on the barn dryer.

The detailed composition of the crude fiber and nitrogen-free extract fractions of the feces of each animal used in the digestion trials is shown in Table 28. The nitrogen-free extract in these feces ranged from 35.7 to 44.6 percent. When a more detailed system of analysis was used including the determinations of sugars, starch, organic acids, pentosans, alpha cellulose, beta and gamma cellulose, and lignin, the undetermined portion of the feces dry matter ranged from 0.1 to 9.6 percent. The more detailed system of analysis accounted for a higher percentage of the dry matter of fecal material than of the hays.

When orchard grass hays of increasing maturity were fed the alpha cellulose, pentosan and lignin contents of the feces increased. The increase in the lignin content of the feces is small compared to the change in the lignin content of the hays since decreases in the digestibility of the total dry matter reduce this change.

The content of reducing sugars was very low in all of the feces. The starch content of the feces ranged from 1.2 to 3.8 percent and showed about the same range of values for the three cows when fed each of the four orchard grass hays.

The cellulose aggregate isolated from plant material by the Matrone, Ellis and Maynard (32) method includes a large part of the hemicellulose and related materials which are furfural yielding constituents and are included in the pentosan fraction in this study. This does not permit a summative analysis of the forage to be made using the crude cellulose since it would duplicate a portion of the pentosan fraction in the crude cellulose fraction. Determinations of the pentosan content of crude cellulose isolated by the method of Matrone, Ellis, and Maynard (32) were made in order to calculate a "corrected Matrone cellulose" which was free of pentosans and would not cause duplication in a system of summative analysis which also included the pentosan fraction. Alpha cellulose was determined on separate samples of crude cellulose and the balance of the crude cellulose which was not determined as alpha cellulose and pentosans was calculated as beta and gamma cellulose. These data are shown in Table 29. Apparently the proportions of alpha cellulose and pentosans in the cellulose material isolated by the Matrone, Ellis and Maynard (32) method does not change appreciably with increasing maturity. Furthermore, the ratio of alpha cellulose to pentosans in the crude Matrone Cellulose isolated from the feces does not differ greatly from that isolated from orchard grass hay. The fraction designated as the "beta and gamma cellulose" was calculated as the residue from the crude Matrone Cellulose that was not determined as alpha cellulose or pentosans. This fraction is quite variable in the amount present in various materials.

The content of "corrected Matrone cellulose" follows the same trends in the hay and the corresponding feces as the crude Matrone cellulose.

The apparent digestion coefficients of the various constituents of the carbohydrate fraction and related constituents are given in Table 30. It is evident that the apparent digestion coefficients of the crude Matrone cellulose, corrected Matrone cellulose, alpha cellulose, holocellulose, pentosans, total carbohydrate, organic acids and undetermined fractions all decreased with advancing maturity of the orchard grass hay. The digestibility coefficients of the starch and sugar fractions were high with all of the four stages of maturity. A comparison of the apparent digestion coefficients of the crude Matrone cellulose and the corrected Matrone cellulose would indicate that there is very little difference in the digestibility of the alpha cellulose and pentosan fractions of the crude Matrone cellulose. A comparison of the apparent digestion coefficients of the total pentosan fraction of the feed and the crude Matrone cellulose indicates that the pentosans not associated with the crude Matrone cellulose had a somewhat lower digestion coefficient than the pentosans included with the crude Matrone cellulose.

The apparent digestion coefficients of the undetermined fractions were calculated by assuming that these fractions were of the same composition in the feed and feces. The digestibility of the undetermined fraction of the immature forage indicated that this fraction is as highly digested as the starch and sugar. The digestibility of this fraction decreased with increasing maturity and was digested to about the same extent as the alpha cellulose in the most mature stage of orchard grass.

A comparison of the digestion coefficients of the corrected Matrone cellulose, alpha cellulose, holocellulose, and pentosan fractions of any one stage of maturity of orchard grass hay would indicate that the digestibility of these groups are similar enough to justify grouping them into one fraction of similar substances such as might be accomplished in a holocellulose determination. The object of a more detailed analysis of the carbohydrate fraction is not to increase the analytical determinations necessary but to allow intelligent grouping of various constituents into groups of substances that would be similar from an analytical, biological and chemical standpoint.

SUMMARY

A more detailed system of analysis that included determinations of the starch, sugars, organic acids, pentosans, alpha cellulose, and lignin was used with orchard grass hay cut at four stages of maturity and the corresponding feces to study the constituents ordinarily grouped in the crude fiber and nitrogen-free extract fractions.

A greater percentage of the dry matter of the immature orchard grass hay was accounted for by this system of analysis than with the more mature orchard grass hay used in this study.

Higher percentages of the dry matter were accounted for by the detailed system of analysis in the feces of animals fed the immature orchard grass hay than in the feces from animals fed the mature orchard grass hay.

Considerably higher percentages of the total dry matter of feces were determined by this system of analysis than were determined with the same analytical techniques on the forage fed to these animals.

Cellulose isolated from the orchard grass hays cut at four stages of maturity and from the corresponding feces by the method of Matrone, Ellis and Maynard contained approximately 25 percent of furfural yielding constituents calculated as pentosans. The apparent digestion coefficients of the cellulose corrected for pentosans was not appreciably different from the digestion coefficients of the crude cellulose fraction.

The apparent digestion coefficients of the crude Matrone cellulose, corrected Matrone cellulose, alpha cellulose, pentosans, total carbohydrates, organic acids and undetermined fractions all decreased with advancing maturity of the orchard grass hay. The digestion coefficients of the starch and sugar fractions of the orchard grass hay were high with all of the four stages of maturity.

The similarity in the digestion coefficients of the alpha cellulose, holocellulose, pentosan, and crude Matrone cellulose fractions indicate that a large part of the forage that is ordinarily included in the crude fiber and nitrogen-free extract fractions could justifiably be grouped into a holocellulose fraction that could be analytically determined.

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T A B L E S

Table 27.

Composition of the crude Fiber and Nitrogen-free Extract Fractions of the Feeds used for Digestion Trials on Various Orchard Grass Hays

	Orchard Grass Hay				
	Stage 1	Stage 2	Stage 3	Stage 4	No. 86 Grain
Protein	24.8	15.8	13.0	12.4	9.2
Ash	9.3	6.8	7.1	7.2	3.9
Ether Ext.	4.0	3.5	3.9	4.2	3.6
Sugars	2.1	9.5	5.4	2.4	1.4
Starch	1.2	1.4	0.8	0.9	65.0
Organic Acids	6.3	6.0	5.4	5.0	--
Pentosans	15.1	15.8	16.8	18.1	6.4
Alpha Cellulose	19.5	19.8	19.1	27.7	2.3
Beta & Gamma Cellulose	3.4	5.4	3.8	2.5	--
Lignin	5.7	5.0	6.2	8.1	0.5
Undetermined	8.6	11.0	18.5	11.5	7.7
Crude Fiber	26.9	28.2	31.8	35.0	2.2
N.F.E.	35.0	45.7	44.2	41.2	81.1
Total Carbohydrates(1)	49.9	63.0	64.4	63.1	82.8
Holocellulose(2)	46.5	52.0	58.2	59.8	16.4

(1) Total Carbohydrates = (Crude Fiber + N.F.E.) - (Lignin + Organic Acids)

(2) Holocellulose = Total Carbohydrates - (Starch + Sugar)

Table 28.

Composition of the Crude Fiber and Nitrogen-free Extract Fractions of the Feces of Cows Fed Orchard Grass Hay Cut at Four Stages of Maturity

	Stage 1			Stage 2			Stage 3			Stage 4		
Constituent	X65	2408	N635	X65	2408	N635	X65	2408	N635	X54	2408	N635
Protein	24.9	26.5	27.0	20.2	20.3	19.6	15.7	16.8	15.5	12.6	14.3	13.5
Ash	15.7	14.8	14.9	13.7	12.5	13.2	11.4	12.2	12.3	11.1	11.0	10.9
Ether Ext.	8.1	7.3	7.7	7.6	6.8	7.4	7.9	7.2	7.5	7.4	7.1	6.8
Sugars	0.5	0.3	0.2	0.2	0.5	0.5	0.3	0.2	0.3	0.4	0.4	0.3
Starch	1.2	3.8	2.0	2.1	3.7	2.7	1.2	3.6	2.3	1.6	3.0	2.0
Organic Acids	8.3	8.4	8.2	7.1	7.4	8.3	6.5	6.8	6.9	5.9	5.8	5.9
Pentosans	10.5	10.5	10.5	14.2	13.3	13.7	16.1	15.7	15.8	16.9	16.1	16.6
Alpha Cellulose	11.5	11.5	10.2	13.4	13.9	12.8	15.3	15.4	15.2	17.2	16.5	17.4
Beta & Gamma Cellulose	2.5	2.5	3.0	3.9	1.8	5.0	3.2	3.1	3.3	1.6	4.0	3.2
Lignin	15.4	14.3	14.4	14.2	12.7	13.6	15.4	13.6	14.0	15.7	15.0	15.6
Undetermined	1.4	0.1	1.9	3.4	7.1	3.2	7.0	5.4	6.9	9.6	6.8	7.8
Crude Fiber	15.6	14.3	14.7	20.3	18.9	18.4	24.5	22.7	22.9	24.8	23.8	24.2
N.F.E.	35.7	37.1	35.7	38.2	41.5	41.4	40.5	41.1	41.8	44.1	43.8	44.6
Total Carbohydrates[1]	27.6	28.7	27.8	37.2	40.3	37.9	43.1	43.4	43.8	47.3	46.8	47.3
Holocellulose[2]	25.9	24.6	25.6	34.9	36.1	34.7	41.6	39.6	41.2	45.3	43.4	45.0

[1] Total Carbohydrates = (Crude Fiber + N.F.E.) - (Lignin + Organic Acids).

[2] Holocellulose = Total Carbohydrates - (Starch + Sugar)

Table 29.

The Crude Matrone Cellulose Content of Orchard Grass Hays and the Corresponding Feces and the Distribution of its Various Constituents

		Constituents of Matrone Cellulose			
	Crude Matrone Cellulose	Alpha Cellulose	Pentosans	Beta & Gamma Cellulose	Corrected Matrone Cellulose (1)
O.G. Hay		%	%	%	
Stg. 1	34.0	62.6	26.3	11.1	25.1
Stg. 2	34.7	61.0	28.6	10.4	24.8
Stg. 3	37.7	61.7	25.8	12.5	28.0
Stg. 4	40.3	67.5	26.4	6.1	29.7
Feces					
Stage 1					
X65	17.8	61.4	25.1	13.5	13.3
2408	17.8	61.4	25.1	13.5	13.3
N635	16.8	58.4	24.4	17.2	12.7
Stage 2					
X65	23.9	57.9	25.4	16.7	17.8
2408	22.2	64.2	27.4	8.4	16.1
N635	21.8	55.5	22.6	21.9	16.9
Stage 3					
X65	26.6	61.6	25.4	13.0	18.8
2408	26.2	61.7	25.7	12.6	19.5
N635	27.1	61.6	25.2	13.2	20.3
Stage 4					
X54	27.7	67.1	26.8	6.1	20.3
2408	27.1	63.5	24.2	12.3	20.5
N635	27.2	60.8	24.7	14.5	20.5

Table 30.

Apparent Digestion Coefficients of Carbohydrates and Related Constituents in Orchard Grass Hay Cut at Four Stages of Maturity

Stage and Cow	Crude Cellulose	Corrected Cellulose	Alpha Cellulose	Holo-cellulose	Pentosans	Starch	Sugar	Total Carbohydrates	Organic Acids	Undetermined Fraction
Stage 1										
X65	82	82	80	82	78	97	96	86	55	95
2408	82	81	80	83	79	94	97	87	52	99
N635	83	82	82	83	79	98	94	88	53	94
Av.	82	82	81	83	79	96	96	87	53	96
Stage 2										
X65	77	76	78	79	72	95	99	84	60	91
2408	79	78	77	79	75	95	98	85	58	82
N635	79	77	78	79	74	97	98	86	52	92
Av.	78	77	78	79	74	96	98	85	57	88
Stage 3										
X65	72	73	69	73	64	97	98	79	52	85
2408	72	72	68	75	66	93	99	81	49	90
N635	72	71	69	74	67	97	98	82	48	87
Av.	72	72	69	74	66	96	98	81	50	87
Stage 4										
X54	70	70	73	69	64	97	95	77	46	69
2408	69	68	73	69	63	94	93	76	45	77
N635	69	68	71	68	62	97	95	77	44	75
Av.	69	69	72	69	63	96	94	77	45	74