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Determination of Digestibility of Lignin by Mule Deer

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DETERMINATION OF DIGESTIBILITY OF LIGNIN BY MULE DEER

by

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INTRODUCTION

The conflicting problems involving the herds of mule deer (Odocoileus hemionus) are of major interest throughout Utah. Individuals who are affected the most are sportsmen, ranchers, fruitgrowers, sanitation engineers, home owners, federal administrators, and big game managers. Mainly, the problems arise as a result of insufficient forage, especially on the winter range. Here, factors such as increased deer numbers, decreased range productivity, severe winters, and expanded agricultural activity make more acute the problem of a naturally critical season.

Restoration of forage by artificial measures may be necessary as a management solution for certain areas. Range rehabilitation, rather than winter feeding during critical periods, has much in its favor, for it can lead to a proper balance between deer numbers and a sustained forage supply. By contrast, feeding leads to further unbalance.

If plants are to be established on the ranges as a source of deer food, more qualitative and quantitative information as to their nutritional value should be had by the game manager.

Studies of forage preference and chemical composition of plant parts have been the most popular means in the past by which to obtain knowledge of deer-forage relationships. Few investigators have resorted to digestion trials for measurements of nutritive quality and daily consumption of forages consumed by deer.

Certain advantages favor the digestion trial over other approaches used by animal nutritionists in determining forage nutrient values. Nevertheless, criticism still prevails owing to the methods of

partitioning forage into chemical fractions. All known digestion trials with deer to date have used the method of proximate chemical analysis called the Weende method, whereby the forage is partitioned into protein, ether extract, ash, crude fiber, and nitrogen-free extract, with the latter two constituents containing the carbohydrate fraction. A refinement in technique which may prove useful in deer nutrition is to partition the carbohydrate fraction of ingested forage and feces into lignin, cellulose, and "other carbohydrates". The technique is called the modified method of proximate analysis.

The lignin fraction of the modified analysis is regarded as indigestible. By the Weende method crude fiber is considered as the portion resistant to digestion; an assumption which is often unjustified, since crude fiber may be digested as much as or more than nitrogen-free extract. Digestion coefficients for lignin as determined from livestock studies indicate no digestibility of this complex organic substance which is present in all forage. However, owing to the anatomical differences in domestic ruminants and deer, it is possible that digestion of lignin may take place in the latter.

Lignin has recently been used as a "tracer" material for determining the digestibility and consumption of forage. The technique is based on the assumption that if lignin is completely indigestible a ratio may be made between lignin and any other constituent in the feed and feces from which the digestibility of the constituent under consideration may be calculated. The lignin-ratio technique is widely used in livestock nutrition research and has advantages over conventional digestion trials, the main advantage being that animals need not be confined to crates. Rather, they are free to graze in a more natural manner, and hence,

the results secured may more closely approximate actual digestion.

It was the purpose of this investigation to find out if lignin present in species providing winter forage for deer is digested to any degree by them.

REVIEW OF LITERATURE

Stoddart and Rasmussen (48) described areas in Utah where deer have increased beyond the capacity of their winter range, causing death to hundreds as a result of lack of forage. Smith (46) reported that rehabilitation of winter ranges will be necessary if they are to support a continuous high number of deer. Furthermore, he stated the need for more precise knowledge as to palatability and nutritional value of plants which are important in natural deer range improvement, and those which may be of use for artificial revegetation.

Smith (45) fed captive mule deer the common winter browse plants which are present on the ranges of northern Utah. The feeds were weighed and fed outdoors in deer pens. Forage preferences and average daily consumptions were determined. Curlleaf mahogany (Cercocarpus ledifolius) proved to be the most preferred plant, showing the highest daily consumption.

Nichol (39) fed captive deer in Arizona to determine daily feed consumption necessary to meet maintenance requirements. He reported 2.2 pounds per hundredweight to be sufficient to keep the animal in good condition. Metabolism crates were not employed, the animals being fed in pens.

Among investigators studying the chemical content of deer foods, Hellmers (27) working on whitetail deer range in Pennsylvania found the nutritive value of browse to decline from winter to spring. Einarsen (14) found the protein content of blacktail deer forage to decline from summer to winter. He also reported higher protein contents of forages

growing on burned areas and logged openings. Reynolds and Sampson (42) discovered that the water, mineral, and protein content of the vigorous sprout growth of chaparral was twice as great as in old growth. Aldous (1), working in Nevada, found that twig tips and leaves of bitterbrush were more nutritious than the stems. Atwood (3) tested whitetail deer food with rats, and suggested a possible shortcut for appraising the value of deer forages. Swift (50) observed that deer grazing on Pennsylvania grain and hay fields singled out areas where plants proved to be higher in fat, calcium, and phosphorus.

Nutritional studies of farm ruminants have been in progress for years. Nutritional studies of deer, on the other hand, are recent in origin with practically all research techniques obtained from knowledge accrued through livestock experimentation. Maynard (34) reported nineteenth century work including respiration studies, digestion trials, crude fiber studies, and chemical analysis of feeds. The method of proximate chemical analysis of feedstuffs has been a popular means of determining the latter and has been in use for over 75 years. Morrison (37), however, cites examples for certain feeds where crude fiber has a higher digestibility than nitrogen-free extract.

Other criticism of the partition of carbohydrates into crude fiber and nitrogen-free extract has been voiced (38), (36), (12), (15), and (34). Crampton and Maynard (10) proposed a method of partitioning of cell-wall carbohydrates into lignin, cellulose, and other carbohydrates. They felt this method to be biologically more sound than the Weende method, regardless of the difficulty in determining lignin.

Maynard et al. (35) working with whitetail deer in New York was the first to conduct digestion trials with deer. The animals were

confined in metabolism crates, and common livestock feed composed the bulk of the diets tested. Forbs et al. (17) later performed digestion trials in the same manner. It was found that goats were more efficient in the digestion of crude fiber than deer, whereas rabbits were the least efficient.

The first digestion trials using mule deer in metabolism crates were reported by Smith (44). Digestion coefficients were determined for diet consisting of leaves and twigs of big sagebrush (Artemisia tridentata subsp. typica). The diet was found to be of high nutritional value; however, all animals lost weight during the trials. Later, Smith (46) reported digestion trials for curlleaf mahogany, bitterbrush (Purshia tridentata), Utah juniper (Juniperus osteosperma), and alfalfa hay. Curlleaf mahogany proved to be superior to bitterbrush in both quality and quantity of digestible nutrients. Juniper appeared to be the poorest of the three.

Hagen (24) ran digestion trials in California using Columbian black-tailed deer. The plant fed was buck brush (Ceanothus cuneatus). The deer were not caged in metabolism crates but fed in pens 16 feet by 8 feet. With exception of protein, digestion of buck brush proved to be as efficient by deer as digestion of good hay by cattle. Hagen also compared the nutritive value of feeds based on chemical composition. He found plants which are considered as good forage to be consistantly high in protein, while those considered as emergency plants were low.

Digestion coefficients may be determined for grazing animals if a tracer material in the plant is indigestible and totally recoverable in the feces. Bergeim (4) recognized the possibilities of this procedure and used ferric oxide as the tracer material. Although the method appears

satisfactory for rats, it does not meet the requirements for grazing animals. The use of naturally occurring iron in the plant was proposed by other investigators (20), (25), and (29), but was found to have the same discrepancy as Bergeim's method; a failure of the iron in the feed to pass uniformly through the digestive system. Many investigators (20), (21), (22), (23), and (29) used silica as the tracer material. However, Knott (29) found the silica method impractical, owing to the contamination of the feed with dirt.

Reid et al. (41) proposed the chromogen method which employs the use of naturally occurring plant pigments that absorb light at 406 millimicrons as the tracer substance. Cook et al. (8) found the chromogen method to be unreliable when applied to winter range forage, since less chromogenic material was recovered in the feces than was consumed.

Many investigators (15), (16), and (18) have proposed that lignin apparently meets the requirement as a tracer substance. Their theory is supported from evidence that lignin is an indigestible plant constituent to livestock.

Hibbert (28) made an extensive review of the literature on the chemical and physical properties of lignin. Although much has been learned since, its organic structure still remains a mystery. Maynard (34) states that the woody parts of plants contain a complex, indigestible substance called lignin. The substance is composed of carbon, hydrogen, and oxygen; the proportion of carbon being much higher than in carbohydrates.

Brauns (6) defines lignin as that incrusting material of the plant which is built up mainly, if not entirely, of phenylpropane building stones, and carries the major part of the methoxyl content of the p

Dutcher et al. (13) describes lignin as a substance of unknown c

which is in combination with cellulose in woody tissue.

Quantitative determinations of lignin have resulted in much difficulty over the years. In 1935 Phillips (40) reviewed methods of lignin determinations for the Association of Official Agricultural Chemists and reported that until further studies could be made, no method could be recommended.

Much controversy has arisen over the digestibility of lignin by herbivorous animals. Among the many investigators who found an appreciable degree of digestibility, Csonka et al. (11) working with cows, noted an increase in benzoic acid in the urine when lignin was fed, and a loss of the methoxyl group from lignin in the feces. Their conclusion was that lignin must have been broken down by the digestive processes, by some enzyme or gastric juice rather than from bacterial action. Crampton (9) found digestibility coefficients for lignin as high as 34 percent in pasture grasses fed to steers. Maynard (33) reported digestibility of lignin near zero for alfalfa hay fed to rabbits and guinea pigs, whereas a lamb digested 28 percent of the lignin. Louw (31) reported sheep that were fed grass of one month's growth to yield digestion coefficients for lignin of 24.5 percent, whereas grass of four months' growth was 11.6 percent. However, Louw suggests that no reliable digestibility coefficient of lignin can be determined until the method of determination has been perfected. Heller and Wall (26) report the utilization of lignin by sheep as high as 49.3 percent, and Sullivan and Garber (49) found lignin digestibility to be 24.5 percent for sheep on pasture plants. Coefficients ranging from 35.1 to 64.0 percent were obtained from work done by Bondi and Meyer (5) from the lignin content of green roughages fed to herbivores.

Lancaster (30) working with sheep in New Zealand obtained many negative digestive coefficients for lignin, one as high as -40.5 percent. He also found positive coefficients as high as 32.4 percent. High variability between individual sheep on the same feed-stuffs was noted.

Crampton and Maynard (10) concluded, after studying previous work, that some form of the 72 percent sulphuric acid method was the most satisfactory quantitative estimation of the lignin content of animal forages. They found 97.8 and 99.3 percent of the lignin consumed in the forage to be recovered in the feces of rabbits and steers respectively.

Many investigators accepted Crampton and Maynard's (10) conclusions that lignin is not digested to any appreciable degree by herbivores. These investigators (15), (16), (18), and (7) show that digestion coefficients for lignin fluctuate slightly above and below zero, and the average approaches zero. The method of lignin determination for the above work was the 72 percent sulphuric acid method proposed by Ellis et al. (15) who attribute many of the early inconsistencies dealing with digestibility of lignin to the chemical procedures used in isolation of lignin from the feed and feces.

Woodman and Stewart (51) reported that lignified plant tissues are not attacked by alimentary bacteria.

METHOD AND PROCEDURE

Material and data used for this study were obtained from digestion trials conducted with mule deer (Odocoileus hemionus) during the winters of 1948-1949, 1952-1953, and 1953-1954. The scene of the feeding activity was at the Utah State Fish and Game Department deer pens, located four miles northeast of Logan, Utah.

The 1948-1949 trials were conducted by others, whereas the trials of 1952-1953 and 1953-1954 were by this investigator.

Plants fed during the trials were all native species except alfalfa hay. The species tested were curlleaf mahogany, birchleaf mahogany (Cercocarpus montanus), cliffrose (Cowania stansburiana), chokecherry (Prunus virginiana var. melanocarpa), gambel oak (Quercus gambelii), and Utah juniper.

Deer History

The deer used varied in age from under a year to mature animals. The relative age, sex, and identification numbers are given in Table 1. All of the trials included at least one fawn or yearling as a test animal, whereas the trials with cliffrose and gambel oak employed two fawns. Most of the animals tested were males, only four out of twelve being females.

Five deer were tested with curlleaf mahogany, cliffrose, gambel oak, and Utah juniper, whereas four were fed birchleaf mahogany, chokecherry, and alfalfa hay.

Each deer was weighed just prior to being placed in the metabolism cage and weighed again at the end of the collection period.

Table 1. Identification, relative age, and sex of deer used in digestion trials.

Year and deer number	Ear tag number	Relative age at time of test	Sex
'49 - 1	84	Adult	Male
'49 - 2	85	Adult	Male
'49 - 3	182	Adult	Female
'49 - 4	2384	Adult	Male
'49 - 5	2386	Yearling	Male
'53 - 9	2381	Fawn	Female
'53 - 10	2382	Fawn	Female
'53 - 11	2396	Adult	Female
'53 - 12	2397	Adult	Male
'53 - 13	2393	Adult	Male
'54 - 14	Untagged	Fawn	Male
'54 - 15	1133	Fawn	Male

Metabolism Cage

Four metabolism cages were used for the digestion trials, although on occasion only two or three were in use at one time, owing to the lack of animals.

The wooden cage used was five-feet square and of sufficient height within to allow plenty of room for a standing deer (Figure 1). An animal could move about freely within the cage. The floor was made of heavy steel mesh wire, under which was a slanted mesh screen for the interception of feces. A pan served to catch the feces. Below the screen was a tilted metal trough for the funneling of urine into a receptacle. A box for containing a water can was supported on one side of the cage, and a box for containing the feed was supported on the opposite side. Openings between boards of the cage were covered with cardboard, so as to darken the interior. The animals were more docile when in the dark.

Experimental Feeding

The native plants were collected from their natural sites in the mountains, foothills and canyons, and brought in to the feeding site. Here, the foliage and current year's growth of twigs were hand clipped into lengths varying from two to three inches (Figure 2). Owing to the growth form of the foliage, Utah juniper was cut into longer sections. Figures 3,4,5,6, and 7 show the forages used in the 1952-1953 and 1953-1954 trials as they appeared when presented to the animals.

All deer were given the feed to be investigated for a preliminary period of five to seven days prior to their being placed in the metabolism cages. The initial feeding in the pens enabled the investigator to judge how the animals might take to the feed after being placed in

Figure 1. A deer in a metabolism cage ready to start digestion trial. The feed box is removed.

Figure 2. Hand clipping of current year's growth of gambel oak

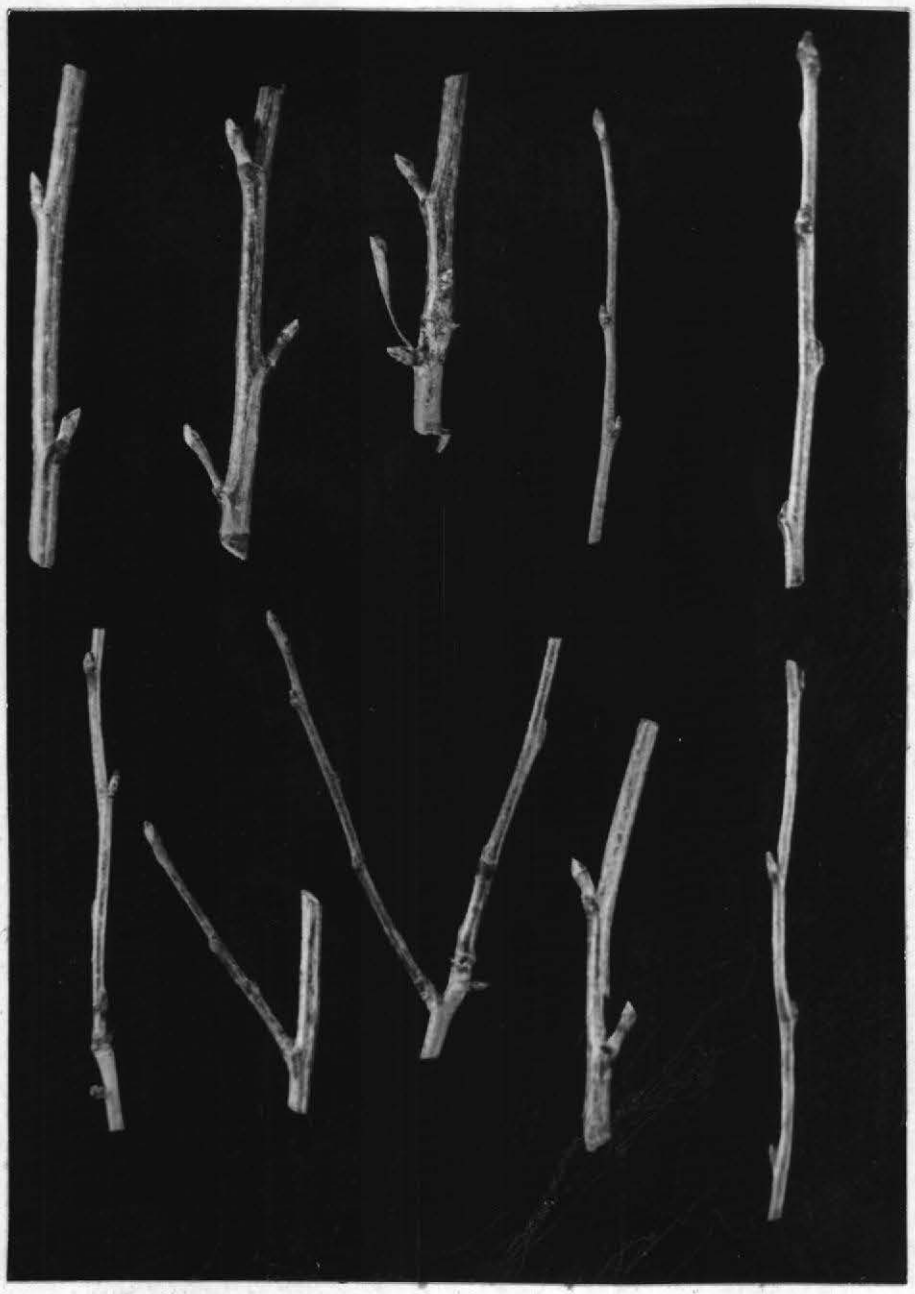

Figure 3. Current year's growth of birchleaf mahogany as it appeared clipped and ready to be fed

Figure 4. Current year's growth of cliffrose as it appeared clipped and ready to be fed

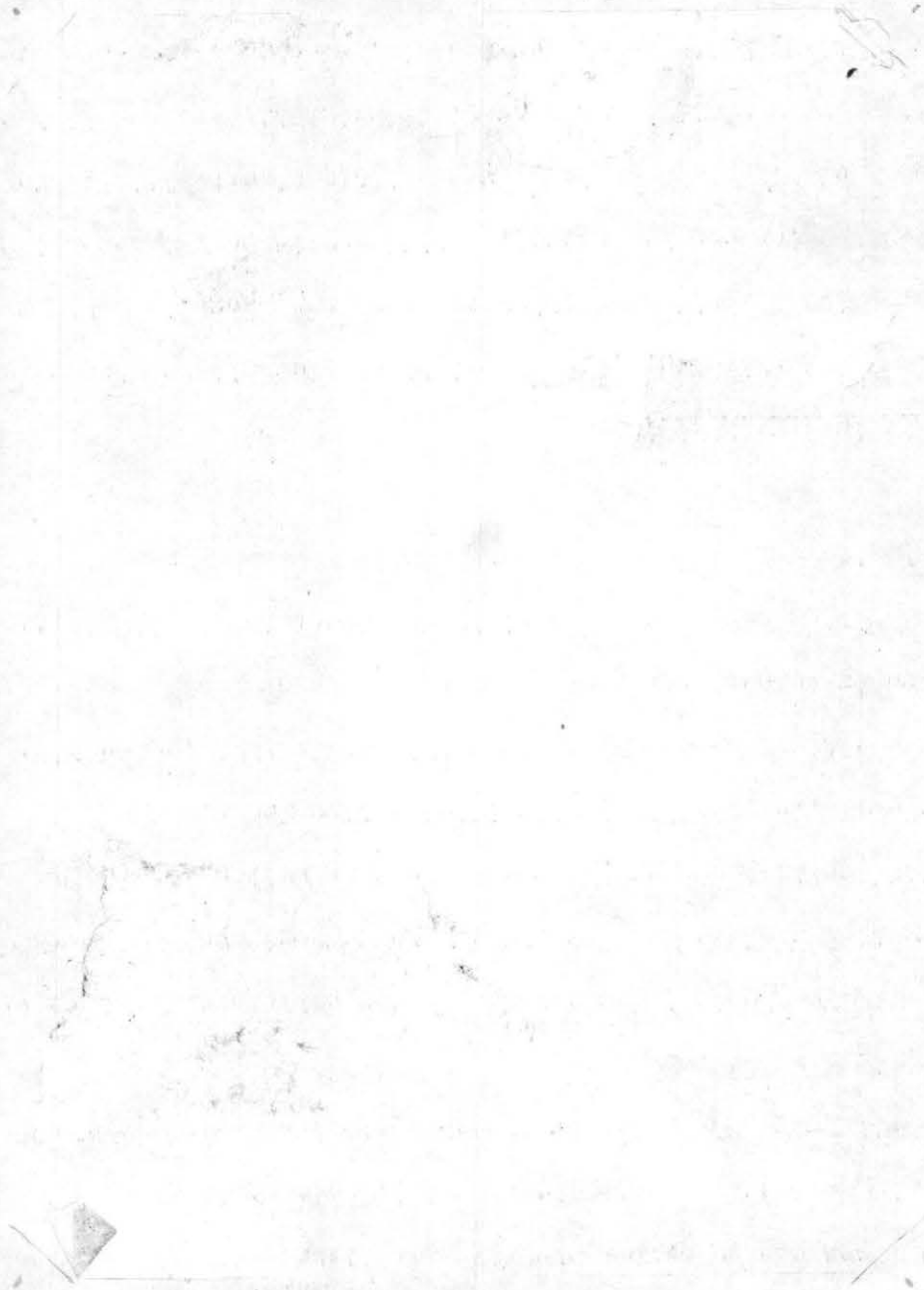

Figure 5. Current year's growth of chokecherry as it appeared clipped and ready to be fed

Figure 6. Current year's growth of gambel oak as it appeared clipped and ready to be fed

Figure 7. Foliage of Utah Juniper as it appeared clipped and ready to be fed

the cages. This preliminary feeding permitted the animals to become accustomed to the feed and free the digestive tract of previous feed eaten. After the deer were placed in the cages, the preliminary feeding was continued from two to five days before the collection period began, the duration depending on the length of feeding in the pens.

The collection period for the 1949-1950 trials was ten days, whereas the collection period for the 1952-1953 and 1953-1954 trials was seven days.

The animals were presented feed each morning and afternoon, feeding time being about the same for each day. In addition, the animals were fed at irregular times during the day if necessary in order to assure an available supply of feed at all times. The feed was weighed to the nearest gram at the time it was presented to the animals. Feed which remained uneaten at the afternoon feeding was removed from the feed box and weighed and saved for chemical analysis. Water was available at all times.

Collection of Samples

Samples of the feeds, feces, orts (weigh-back material), and urine were saved for chemical analysis from each deer.

Just prior to feeding, a representative sample of one tenth the feed weight was saved. At the end of the collection period the accumulated sample material was mixed together and a composite sample was taken. This composite sample was placed in an oven at 60 degrees centigrade for a period of 24 hours. It was then left exposed for a period of 24 hours in order to allow the moisture contained in the feed to come to equilibrium with the moisture in the air at room temperature.

The orts which were usually present in the feed box and removed each afternoon were weighed, composited, and dried in the same manner as was the feed sample.

The feces were collected from the pan at the end of each day and were weighed before being preserved against putrefaction and mold with 10 c.c. of a mixture of hydrochloric acid and alcohol. The feces collected in 1953-1954 were placed in a refrigerator each day with no addition of a preservative. At the end of the collection period the total amount of feces were weighed again for the determination of moisture loss. After being weighed and thoroughly mixed, a composite sample was taken and dried in the same manner as the feed sample.

After drying, the samples of feeds, orts, and feces were ground through a Wiley mill to a fineness which would permit the ground material to pass through a one-millimeter screen. The samples were then sent to the chemical laboratory for analysis.

Analytical Methods

For the method of proximate analysis (Weende method) plant and fecal samples were analyzed for nitrogen (from which crude protein was calculated), ether extract, crude fiber, and ash. Nitrogen-free extract was determined by difference. For the modified method of proximate analysis presented by Crampton et al. (10) and Louw (31) lignin and cellulose determinations were made and other carbohydrates computed by difference. Nitrogen, ether extract, and ash values were available from the Weende method.

Nitrogen was determined by the Gunning method as described by the Association of Official Agricultural Chemists (2) except that the titration process as outlined by Scales and Harrison (43) was used. Boric

acid was employed to hold the ammonia distilled in the process. Crude protein was obtained by multiplying the amount of nitrogen determined by the factor 6.25. Ether extract was determined by extracting the dry sample with anhydrous ether for 8 hours in a Goldfisch apparatus. Ash was determined by the Association of Official Agricultural Chemists (2) method, cellulose by the method of Matrone et al. (32), and lignin by the method suggested by Ellis et al. (15), with a modification by Forbs (19). A modification in the filtering process taken from Stamm and Harris (47) was employed for lignin determinations for the 1953-1954 samples. Crude fiber was determined by the method of the Association of Official Agricultural Chemists (2).

Nitrogen-free extract and other carbohydrates were determined from the following formulas:

Nitrogen-free extract = 100 percent oven dry basis - (Protein percent + Ether extract percent + Ash percent + Crude fiber percent).

Other Carbohydrates = 100 percent oven dry basis - (Protein percent + Ether extract percent + Ash percent + Cellulose percent + Lignin percent).

Calculation of Digestion Coefficients

The percentage of a nutrient in a feed which is utilized by an animal is known as a digestion coefficient. The following equations were used in determining the digestion coefficients.

(Percent nutrient in feed X Daily dry weight of feed) - (Percent nutrient in orts X Daily dry weight of orts) = Daily dry weight of nutrient consumed.

Daily dry weight of nutrient consumed - (Percent nutrient in feces X Daily dry weight of feces) = Daily dry weight of nutrient utilized

$$100 \times \frac{\text{Daily dry weight of nutrient utilized}}{\text{Daily dry weight of nutrient consumed}} = \text{Nutrient digestion coefficient}$$

Lignin-Ratio Technique

For comparisons of feed intake and nutrient digestibility between the actual measured feed and that calculated as a ratio to lignin, the following equations of the lignin-ratio technique as proposed by Forbs and Garrigus (18) and Ellis et al. (15) were used:

$$\text{Weight of forage consumed} = 100 \times \frac{\text{Weight of lignin in feces}}{\text{Percent lignin in forage}}$$

$$\text{Percent digestibility of a specific nutrient} = 100 - \left(\frac{\text{Percent lignin in forage}}{\text{Percent lignin in feces}} \times \frac{\text{Percent nutrient in feces}}{\text{Percent nutrient in forage}}\right) 100$$

Reanalyses of Samples

Owing to the apparent difficulty in obtaining consistent values for lignin in the chemical laboratory, reanalyses were conducted with one each of samples from two species (Table 2). Eight separate analyses were completed for lignin from the feed, orts, and feces collected from deer number 11 feeding on cliffrose and deer number 10 feeding on chokecherry. The first analysis was made in May, 1953 with the rest of the 1952-1953 samples; the second in August, 1953; the third, fourth, fifth, and sixth in January, 1954; and the seventh and eighth in February, 1954.

The third and fourth analyses were duplicate laboratory values reported on the same day. The fifth and sixth analyses were duplicate laboratory samples tested a day later. The seventh and eighth analyses were duplicate laboratory samples tested at a later day.

Table 2. Eight separate chemical analyses for the percentage of lignin in the feed, orts, and feces collected from two trials

Forage and deer number	Sample	Chemical analyses and Dates of analyses: 1st Analysis May 1953	2nd Analysis August 1953	3rd Analysis January 1954	4th Analysis January 1954	5th Analysis January 1954	6th Analysis January 1954	7th Analysis February 1954	8th Analysis February 1954	Mean	Range
53-11 Cliffrose	Feed	16.3	18.9	17.7	17.4	14.4	14.9	13.8	13.1	15.8	13.1 to 18.9
	Orts	14.5	22.9	25.0	24.8	22.1	22.3	22.1	21.4	21.9	14.5 to 25.0
	Feces	31.9	31.3	33.4	33.6	32.4	32.1	31.4	33.0	32.4	31.3 to 33.6
53-10 Chokecherry	Feed	27.7	27.5	28.4	28.1	25.5	26.5	24.8	24.5	26.6	24.5 to 28.4
	Orts	25.9	26.2	27.2	27.4	24.7	24.7	23.0	23.7	25.4	23.0 to 27.4
	Feces	35.6	33.7	33.9	35.2	32.5	33.9	32.9	33.3	33.9	32.5 to 35.6

RESULTS AND DISCUSSION

Response of Deer to Confinement

The anomaly of deer obtaining a diet in confined state, and having but one forage to subsist on resulted in a loss of weight by most of the animals tested. Changes in weights for the total collection period are shown in Table 3. The deer lost weight from all feeds except alfalfa hay, to which two animals responded with gains, the highest being 2.5 pounds by a yearling buck. The greatest loss for an individual trial was eleven pounds by a mature buck feeding on gambel oak.

A few of the deer did not adjust well to confinement in the crates, and consequently, consumed small amounts of forage daily (Table 4). A fawn feeding on birchleaf mahogany, a mature buck feeding on curlleaf mahogany, and a mature buck feeding on Utah juniper were especially low in consumption. In the cases of chokecherry and Utah juniper consumption was relatively low; however, for the purpose of this study sufficient amounts were eaten. All deer fed cliffrose and gambel oak equaled or exceeded a consumption of 2.2 pounds per hundredweight per day.

Chemical Analyses

Comparisons of the mean percent composition of the nutrient values as determined by the Weende method of chemical analysis and the modified method of chemical analysis are shown in Table 5. Protein, ether extract and ash are the same for both analyses.

The percentages for nitrogen-free extract and other carbohydrates would be identical if material isolated as crude fiber by the Weende method were the equivalent of material which appears as cellulose plus lignin by the modified method.

Table 3. Weights of deer during collection periods

Deer number	Weight	Birchleaf mahogany	Curlleaf mahogany	Cliffrose	Chokecherry	Gambel oak	Utah juniper	Alfalfa hay
49-1	Initial weight		120.0				114.0	114.0
	Final weight		115.0				112.0	117.0
49-2	Initial weight		119.0					
	Final weight		116.0					
49-3	Initial weight		87.5					84.0
	Final weight		83.5					85.0
49-4	Initial weight		100.0					106.0
	Final weight		92.5					103.5
49-5	Initial weight		62.0				57.0	59.0
	Final weight		54.0				53.0	61.5
53-9	Initial weight	50.0						
	Final weight	48.0						
53-10	Initial weight	64.5		64.0	62.0	68.0		
	Final weight	59.0		61.5	52.0	65.5		
53-11	Initial weight	121.5		114.0	111.5	120.0	105.0	
	Final weight	116.5		113.5	102.0	116.0	96.0	
53-12	Initial weight	114.5		110.0	101.5	108.5	94.0	
	Final weight	107.0		107.5	97.5	106.0	89.5	
53-13	Initial weight			143.0	138.0	135.0		
	Final weight			141.0	127.0	129.0		
53-14	Initial weight					78.0		
	Final weight					76.0		
54-15	Initial weight			62.0				
	Final weight			53.0				

Table 4. Average daily forage consumption, lbs./cwt.

Deer number	Forage						
	Birchleaf mahogany	Curlleaf mahogany	Cliffrose	Chokecherry	Gambel oak	Utah juniper	Alfalfa hay
49-1		1.97				.81	1.82
49-2		1.71					
49-3		1.45					1.88
49-4		.95					2.32
49-5		1.52				1.89	2.85
53-9	1.0						
53-10	1.94		2.90	1.26	2.26		
53-11	2.32		2.58	2.02	2.29	1.41	
53-12	2.40		2.51	1.43	2.28	1.52	
54-13			2.20	1.31	2.38		
54-14					2.68		
54-15			2.89				

Table 5. Mean chemical composition determined by the Weende method of analysis and the modified method of analysis

Forage	Plant constituent							
	Protein	Ether extract	Ash	Crude fiber	Nitrogen-free extract	Cellulose	Lignin	Other carbohydrates
Birchleaf mahogany	7.2	4.5	1.4	34.7	52.1	21.3	25.2	40.4
Curlleaf mahogany	11.0	9.4	3.3	19.1	57.2	16.7	18.2	41.4
Cliffrose	8.4	10.8	5.2	23.0	52.6	16.6	22.7	36.3
Chokecherry	9.9	2.4	5.0	29.1	53.6	18.9	26.5	37.3
Gambel oak	5.4	3.2	6.5	34.0	51.0	21.4	26.1	37.4
Utah juniper	6.2	14.1	4.4	24.9	50.3	18.2	16.2	40.9
Alfalfa hay	19.2	2.3	10.6	24.6	43.3	18.1	7.6	42.2

However, there is more lignin and cellulose together than there is of crude fiber, and a reduced amount of other carbohydrates as compared to nitrogen-free extract, since the last two are determined by difference.

Lignin or cellulose, or both, then, contain materials which appear as both crude fiber and nitrogen-free extract in the Weende method. The decrease in other carbohydrates does not indicate an estimation of lower quality of the feeds. Indeed, the opposite may result as shown in Table 6. Here, the mean digestion coefficients calculated from percent composition values are shown for the two methods of analysis. The coefficients of all feeds show that other carbohydrates are digested to a greater amount than nitrogen-free extract. Therefore, it appears that the refined method separates the carbohydrate fraction into more critical components from the standpoint of digestibility.

Since both cellulose and lignin proved to be digested for most of the feeds, the inherent digestibility appears to be as much disguised as with the divisions obtained by the Weende method. Table 7 shows a comparison of the mean digestible nutrients per hundred pounds of forage consumed calculated from data obtained by the two methods of analysis. The values were calculated by summing the products of specific nutrient composition times the corresponding digestion coefficients, followed by calculation of a mean. Ether extract percentages were multiplied by 2.25, owing to a higher energy content than other nutrients.

The data show that the refined (modified) method yields results practically the same as for the Weende method, when measured in terms of total digestible nutrients. The highest deviation noted between methods is only 1.3 pounds for cliffrose. Further, there is no evidence

Table 6. Mean digestion coefficients calculated from the chemical composition determined by the Weende method of analysis and the modified method of analysis

Forage	Plant constituents						
	Protein	Ether extract	Crude fiber	Nitrogen-free extract	Cellulose	Lignin	Other carbohydrates
Birchleaf mahogany	48.5	37.6	31.8	60.0	43.4	31.9	64.1
Curlleaf mahogany	54.3	42.9	35.9	76.3	41.2	18.9	96.3
Cliffrose	39.8	47.7	4.4	59.4	16.6	7.2	71.0
Chokecherry	48.4	23.3	8.8	56.1	21.2	26.5	56.3
Gambel oak	10.7	38.4	16.6	53.6	23.7	23.7	54.6
Utah juniper	16.8	58.9	33.7	70.4	39.3	19.7	84.5
Alfalfa hay	77.0	16.9	49.0	82.9	59.3	5.8	87.6

Table 7. Mean digestible nutrients in pounds per hundred pounds, calculated by the Weende method and the modified method of proximate analysis

Forage	Weende method	Modified method
Birchleaf mahogany	49.6	50.5
Curlleaf mahogany	67.8	66.5
Cliffrose	52.7	52.6
Chokecherry	38.6	39.8
Gambel oak	36.3	36.6
Utah juniper	63.5	64.7

of either of the two methods yielding a greater amount of total digestible nutrients. Hence, no increased precision is noted for the refined method based on the calculation of total digestible nutrients.

On the other hand, the low digestibility of lignin in alfalfa hay indicates that mainly cellulose and other carbohydrates contain the digestible portion of the carbohydrates. Thus, if the carbohydrate fraction of feed can be satisfactorily established into three classes distinguishable as poorly digestible (lignin), moderately digestible (cellulose), and easily digestible (other carbohydrates) material, a closer degree of precision may be possible.

The chemical percent values for cellulose and the corresponding digestion coefficients are likely to be more accurate for alfalfa, since difficulty in determining cellulose as well as lignin was noted by the chemist for the highly lignified native feeds.

It can be concluded from this study that the modified method of proximate analysis has little advantage over the Weende method for deer nutrition work except possibly for feeds low in lignin content, such as alfalfa hay.

Lignin Digestibility

Digestion coefficients of lignin for all trials are shown in Table 8. With the aid of statistical analyses shown in Table 9 a clearer understanding can be secured. Cliffrose is most interesting, where a low mean of 7.2 might indicate very little digestibility. However, the high standard deviation, widespread fiducial limits, and high coefficient of variation indicates the variability of the data and reduces the value of the mean. Further, the fact that results differed greatly in the two

Table 8. Digestion coefficients of lignin for all trials (percent)

Forage	49-1	49-2	49-3	49-4	49-5	53-9	53-10	53-11	53-12	54-13	54-14	54-15	Mean	Range
Birchleaf mahogany						31.0	23.8	30.8	42.1				31.9	23.8 to 42.1
Curlleaf mahogany	26.0	15.8	19.9	21.7	11.2								18.9	11.2 to 26.0
Cliffrose							-7.2	-2.7	-.1	32.8		13.0	7.2	-7.2 to 32.8
Chokecherry							16.8	36.2	35.8	17.0			26.5	16.8 to 36.2
Gambel oak							26.7	21.9	24.4	30.2	15.5		23.7	15.5 to 30.2
Utah juniper	16.9				9.3			27.6	25.1				19.7	9.3 to 27.6
Alfalfa hay	1.8		7.5	3.9	11.1								5.8	1.8 to 11.1

Table 9. Statistical analysis of digestion coefficients of lignin

Forage	Number of trials	Mean digestion coefficient	Standard deviation	Fiducial limits*	Coefficient of variation
Birchleaf mahogany	4	31.9	7.6	± 12.0	23.7
Curlleaf mahogany	5	18.9	5.7	± 7.05	30.2
Cliffrose	5	7.2	16.2	± 20.2	225.0
Chokecherry	4	26.5	11.0	± 17.5	41.5
Gambel oak	5	23.7	5.5	± 7.1	23.3
Utah juniper	4	19.7	8.4	± 11.5	42.6
Alfalfa hay	4	5.8	4.3	± 6.0	74.1

* 95 percent confidence level

winters in which tests were made suggests some procedural errors.

The trials with birchleaf mahogany, curlleaf mahogany, chokecherry, gambel oak and Utah juniper produced positive digestion coefficients which can be taken with a higher degree of confidence than those for cliffrose. Lignin in these feeds appears to be digested to an appreciable degree by deer.

The low mean of 5.8 percent, low standard deviation, and narrow fiducial interval for the digestion coefficients of alfalfa hay suggests that little digestion of lignin took place, a finding which is in line with studies made with domestic livestock.

Lignin-Ratio Technique

Daily feed consumptions calculated by the lignin-ratio technique are shown in comparison with measured consumptions in Table 10. The calculated consumptions are considerably lower than the measured amounts for the feeds which shows some degree of digestibility of lignin, since the ratio is based on an assumption of equal amounts of lignin in the forage and feces. Animals 10, 11, and 12 feeding on cliffrose are low in lignin digestibility and therefore, have calculated consumption values close to what was actually measured. Conversely, a high lignin digestibility such as 42.1 percent for deer number 12 feeding on birchleaf mahogany accounts for a little more than half of what was consumed daily.

The mean digestion coefficients of the feeds calculated by the lignin-ratio technique are also shown in Table 10. Here again the apparent digestibility of lignin affects the results, a reduction in digestibility of the nutrients resulting. Accordingly, the digestion coefficients for animals 10, 11, and 12 feeding on cliffrose are the only ones

Table 10. Daily feed consumption and digestion coefficients calculated by the lignin ratio technique and daily feed consumption measured from digestion trials

Forage	Deer number	Feed consumption (Grams) Measured intake	Feed consumption (Grams) Calculated by lignin ratio technique	Percent digestible Protein	Percent digestible Ether extract	Percent digestible Cellulose
Birchleaf mahogany	53-9	221.2	150.0	10.0	8.4	22.9
" "	53-10	546.9	417.5	28.2	7.7	18.8
" "	53-11	1252.9	865.9	18.2	7.0	7.5
" "	53-12	1208.9	700.0	28.4	1.9	-1.8
Curlleaf mahogany	49-1	1048.4	820.8	41.7	20.0	19.6
" "	49-2	910.3	747.6	52.3	36.1	29.6
" "	49-3	562.6	442.8	40.4	34.3	32.9
" "	49-4	414.3	323.1	36.4	24.1	26.6
" "	49-5	398.0	337.7	43.1	26.5	24.6
Cliffrose	53-10	823.9	833.5	38.9	49.1	6.1
"	53-11	1331.4	1346.0	39.3	57.0	12.1
"	53-12	1250.4	1247.0	38.8	44.0	11.8
"	54-13	1441.7	971.0	18.6	19.0	0
"	53-15	715.8	595.1	37.0	21.6	5.0
Chokecherry	53-10	328.4	298.3	24.0	0	1.1
"	53-11	981.2	648.2	27.5	3.8	-17.9
"	53-12	645.7	450.5	35.0	-25.3	1.2
"	54-13	784.4	630.7	41.0	24.1	23.3
Gambel oak	53-10	686.0	538.8	-25.0	12.1	19.0
" "	53-11	1226.9	963.6	-20.0	12.3	6.2
" "	53-12	1111.2	852.9	-12.0	7.6	20.3
" "	54-13	1423.6	1005.9	-9.3	15.6	10.0
" "	54-14	933.7	817.1	-10.9	22.9	15.5
Utah juniper	53-11	623.4	446.2	-14.8	46.1	28.7
" "	53-12	643.5	480.8	7.3	58.4	26.2
" "	49-1	416.7	348.8	.9	55.9	22.2
" "	49-5	469.7	491.8	-3.8	47.7	26.1
Alfalfa hay	49-1	952.8	775.0	68.3	-6.8	59.1
" "	49-3	724.0	517.3	63.8	-21.2	66.4
" "	49-4	1101.3	911.0	69.9	-13.5	57.0
" "	49-5	783.1	586.1	62.5	-34.2	53.4

approximating the nutrient digestibility determined from measured consumption.

The apparent digestibility of lignin causes forage consumption and nutrient digestibility calculations to be in error, where these are calculated by the lignin-ratio, since the ratio is based on no digestibility of the tracer material. Moreover, if lignin were not digested for any of the feeds tested, the difficulty of making lignin determinations in the laboratory make questionable the use of this approach.

Until laboratory determinations are more easily and accurately made, there may be question as to the reliability of the digestion coefficients secured for lignin. However, there is strong evidence from these data that lignin is not an indigestible material for deer.

Reanalyses of Samples

In order for lignin to be of use in estimating forage consumption and nutrient digestibility, and as a tracer material, the reliability of the chemical analysis is important. If the percentage of lignin present in feeds cannot be determined accurately, lignin is of little value in nutrition work, since misleading results would unavoidably occur. Discussion with the chemist who made all the analyses for this study disclosed that the separation of lignin from woody materials is done with difficulty. Table 2 illustrates the variability of percentages of lignin present in the feed, orts, and feces of the samples which were reanalyzed.

The most consistent results were obtained from laboratory duplicate values reported on the same day, such as the feed, orts, and feces of the third and fourth analysis of cliffrose (deer 11). A day later the same duplicate samples were reanalyzed, and again the percentages were consistent.

However, between days the percentages varied greatly. The minimum and maximum values obtained from the eight analyses show a marked difference; the high and low percentages of all homologous samples vary as much as 2.1 percent or greater.

When a chemical determination for lignin deviates only 2 or 3 percent from the actual percentage, no great error results. However, a range of percentages from 13.1 to 18.9 for deer 11 feeding on cliffrose is a cause for concern and casts doubt on the practicality of the chemical determination of lignin. Therefore, the use of lignin as a base for the fractionation of a feed sample is questionable. Time and expense prohibited a complete reanalysis of all samples, but the results observed tend to illustrate the need for a comprehensive chemical and statistical appraisal of the feasibility of determining the percentage of lignin present in highly lignified forages.

SUMMARY

The purpose of this study was to determine the digestibility of lignin by mule deer. Lignin is a complex organic constituent present in cell-wall material of plants and is believed to be indigestible by domestic livestock. When found to be indigestible, lignin serves well as a tracer material in estimating digestibility and forage consumption of animal forages.

Digestion trials using mule deer (Odocoileus hemionus) were conducted near Logan, Utah, for six of the common native winter browse species and alfalfa hay. Nutrient digestibility and forage consumption were determined. The species tested were birchleaf mahogany, curlleaf mahogany, cliffrose, chokecherry, gambel oak, and Utah juniper. The forages were collected in their native sites and brought to the feeding location, where current year's growth was removed and fed. Old leaves of Utah juniper were included with current year's growth as the portion fed. Metabolism cages were employed to measure the weight of ingested forage and egested fecal material.

The plant and fecal samples were chemically analyzed by the Weende method and the modified method of proximate analysis to include protein, ether extract, ash, crude fiber, cellulose, and lignin. Nitrogen-free extract and other carbohydrates were determined as remainders.

Lignin present in all of the browse species except cliffrose appeared to be digested to an appreciable degree by mule deer. The digestion coefficients for lignin in cliffrose indicated low digestibility; however, the results were highly variable for this species. Alfalfa hay was also

low in lignin digestibility with the digestion coefficients being low in variation.

The modified method of proximate analysis was found to be of no advantage over the Weende method for deer nutrition work except possibly for feeds low in lignin content, such as alfalfa hay. Total digestible nutrients per hundred pounds of forage consumed were calculated by both methods and found to be practically equal for each browse species, thus indicating no gain in precision.

Nutrient digestibility and consumption for each forage was compared by digestion trial measurements to values calculated by the lignin-ratio technique. The calculated results were much too low for the feeds which proved to be high in lignin digestibility. Three trials with cliffrose provided values which approximated actual measured amounts. In these instances the digestibility of lignin was negligible.

The lignin composition of the plant and fecal samples from a trial with cliffrose and a trial with chokecherry were reanalyzed seven times in the chemical laboratory. Difficulty was encountered in obtaining consistent results for the highly lignified homologous samples.

The results secured suggest that the lignin ratio technique is not applicable to digestion studies with mule deer on diets of winter browse plants. It is not entirely clear from these data whether this is due to inaccuracies of lignin determination or whether lignin is indeed digested. The weight of evidence suggests that the latter is true. In either case the lignin ratio offers no advantage as a line of investigation.

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APPENDIX

Scientific and common names of plants discussed

Scientific name	Common name
(*Cercocarpus ledifoluis*)	Curlleaf mahogany
(*Artemisia tridentata* subsp. *typica*)	Big sagebrush
(*Purshia tridentata*)	Bitterbush
(*Juniperus osteosperma*)	Utah juniper
(*Ceanothus cuneatus*)	Buck brush
(*Cercocarpus montanus*)	Birchleaf mahogany
(*Cowania stansburiana*)	Cliffrose
(*Prunus virginiana* var. *melanocarpa*)	Chokecherry
(*Quercus gambelii*)	Gambel oak