THE DIGESTIBILITY OF ALFALFA HAY WITH SPECIAL REFERENCE TO RUMEN DIGESTION

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The Digestibility of Alfalfa Hay with Special Reference to Rumen Digestion

I Rumen Digestion in the Bovine.

II The Digestibility of Alfalfa Hay with Reference to the Crude Fiber and Ether Extract Fractions and the Plane of Nutrition.

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The Digestibility of Alfalfa Hay with Special Reference to Rumen Digestion

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I Rumen Digestion in the Bovine.

INTRODUCTION

The rumen together with its associative organs and activities has served to set the ruminant apart from other domestic animals. Despite the importance of the functions of this organ, less than two decades ago there was an utter lack of dependable experimental evidence pertaining to the physiology of rumen digestion.

Recent investigators, however, have made rapid progress in illuminating the mechanical factors concerned in digestion within the rumen. Determinations of the products of the fermentation of fiber, the chief constituent of the feeds consumed by ruminants, have been made for many years. These studies have primarily involved in vitro experiments, and the products formed may not necessarily be identical in either quality or quantity to those produced in the rumen itself. Attempts to explain the fattening value of digestible fiber to the animal in terms of the in vitro products of fiber fermentation have failed.

Actual chemical studies of rumen digestion in vivo have progressed more slowly, and it has been only within the past several years that attempts have been made to follow the digestion of various feed constituents in the rumen. Even these findings have been limited, and the data obtained only indicated the comparative rate at which the various constituents disappeared from the rumen.

This study was undertaken to further the knowledge of the chemical factors involved in rumen digestion and to attempt to ascertain the quantitative digestion occurring in the rumen, together with some observations of the effect of the plane of nutrition and type of feed on rumen fill and barrel size.

REVIEW OF LITERATURE

The ruminant stands alone among the domestic animals as an efficient converter of coarse roughages into products that are highly palatable and nutritious human foods. Ruminants hurriedly consume relatively large amounts of roughage, subject it to a softening and fermentation process within their large rumen, and in ruminating return it to the mouth to be thoroughly remasticated. As a result of this activity a fraction of the roughage that is of very little value to many species of domestic animals serves to supply a large portion of the energy requirements of the ruminant. Enumerable microorganisms decompose enormous amounts of roughages to nutritive products within an astonishingly short period of time. When one considers the high place among domestic animals that is occupied by the ruminant and the numerous rumen activities that make this place possible, one readily realizes that the study of the various phases of rumen digestion is exceedingly important.

Passage of Food Material Through the Rumen

Course Followed by the Food

The physiology of the rumen has received considerable attention from investigators. With the development of the rumen fistula technique of making observations, the physiological studies were greatly facilitated. In the latter part of the last century Colin (15) made rumen fistulas into the ox and performed extensive studies. Schalk and Amadon (103), Magee (74), Bergman and Dukes (17) and Mangold (75)

have more recently contributed to this field.

The course followed by various food materials varies considerably and is influenced mainly by the nature of the food material itself. Passage of the food is primarily the result of the never ceasing and rhythmical movements of the rumen and reticulum. Schalk and Amadon (103) observed that "The general motility of the reticulum and rumen is quite characteristic and definitely established for each phase of activity." These investigators discussed the movements in detail.

It is agreed by a number of observers (103) (74) that food material swallowed in the normal manner goes to the anterior dorsal sac of the rumen. The course followed from there depends upon the character of the ingesta. The light bouyant forage is carried posteriorly into the rumen and the heavier concentrated food quickly finds its way to the reticulum. As the rumen is an adaptation by nature for the more complete utilization of roughage, the course of this type of food material will be considered first. The forage after being carried posteriorly moves through the entire reticulo-rumen cavity at a rapid rate. In this process it becomes thoroughly mixed with the ingesta of previous meals, is saturated with water, and is subjected to softening, maceration, and fermentation. This processed roughage is now ready for remastication during which the feed is more thoroughly crushed and macerated than can be accomplished by a horse, for example, in which there is no preliminary preparation. Schalk and Amadon (103) pointed out that the space made available during the processing of the roughage is filled on eating with a fresh supply of material. Following this, rumination usually occurs. It must be

remembered, however, that the boli formed at this period consist of ingesta which was consumed twelve to twenty-four hours earlier and which has been subjected to the activities of the rumen for that time. The mechanism of rumination has been discussed in detail by several investigators (103) (74) (17), and all agree to the importance of reticular activity in this function. Remasticated food is deposited in the anterior dorsal sac and, with some little exception, readily passes into the reticulum and on into the third and fourth stomach compartments. All hay particles seem to follow this course with little variation.

Schalk and Amadon (103) observed that green forages rapidly passed through the rumen and arrived at the reticulum to be regurgitated. Fermentation of such material is very rapid, and the residue may reach the reticulum in such a finely divided state as to escape rumination. The findings of Columbus (23), that green feeds accelerate passage through the digestive tract, bear out the above observation.

In contrast to roughages, concentrated feed soon finds its way into the reticulum and some portion of it may pass there directly. A small amount of the concentrates may pass to the other stomachs during the course of the meal (103) (89). Nevens (89) found corn was evenly dispersed throughout the rumen of animals fed shortly before slaughter and also observed that the preparation of such material, whether finely ground or shelled, seemed to bear no relation to the path followed in the stomach. He found that regardless of whether the corn was fed mixed with hay or separately, the course it took was essentially the same. Kick and associates (56) reported that whole corn kernels might remain in the rumen for six days. The age of the animal, the amount of corn fed, or the corn-hay ratio had no effect on the time of retention. Moore and associates (84) studied bulk as a factor in formulating dairy rations and found bulk was unnecessary in the make up of the grain mixture. The rumen had the ability to break up and dissolve boli even of the most cohesive feeds. Grains are not remasticated except as they are accidentally caught in the meshes of the roughage (103). Therefore, nearly 50 per cent of ingested corn kernels pass through the animal intact, for Schalk and Amadon (103) have shown that only about 50 per cent is crushed during eating. That concentrates take a short cut through the first two stomach compartments was further illustrated by the work of Moore and Winter (85) in which they found iron oxide, which might represent a concentrate, appeared more rapidly in the feces and more speedily reached its high point of excretion than did rubber rings, which might represent roughage. Mitchell and co-workers (80) observed that heavy feeds, which supposedly do not enter the rumen, passed through cattle at a relatively rapid rate.

The function of rumination seems to be dependent upon roughage. When concentrates are fed alone, rumination is not even a physiological necessity to the animal. The feeding experiments of Schalk and Amadon (103) quite conclusively demonstrated that a ration composed exclusively of concentrates abolished rumination, though the animals remained in good health. Ritzman and Benedict (100) reported that animals were unable to form boli when deprived of coarse roughages. Kick and co-workers (56) observed that steers without roughage ruminated listlessly. Ritzman and Benedict (100) found that the grinding of hay to a meal stopped rumination as the animal was apparently unable to form a bolus sufficiently cohesive for regurgitation. This was also ob-

served by Powell (96) and to some extent by Kick and associates (56).

The general conclusion of Beach (9) that "digestible nutrients in concentrated feeds are more valuable pound for pound than digestible nutrients in roughages, because less energy is required by the animal in the former case to render them available" is of importance. Ritzman and Benedict (100) pointed out that Zunts showed that 11.3 per cent of the energy of hay and only 2.8 per cent of the energy of oats was used in mastication, and that 48 per cent of the digested energy from hay and only 19.7 per cent of the digested energy from oats was used in the work of mastication and digestion. Ritzman and Benedict observed that fermentation losses in the form of methane remained about the same when animals received a concentrate meal as the sole feed as when the cows were fed hay only. The above difference in utilization was, therefore, due to the extra energy required for preparation of the roughage and not to a greater loss through fermentation.

Liquids, and thus highly soluble feeds as molasses, may remain in the rumen-reticular cavity for but a brief space of time or for several hours depending upon conditions (103). When the ingesta has been sufficiently divided into small particles it passes to the omasum. This passage is probably due to an aspiratory act on the part of the omasum (103) (74) and occurs at approximately 60 second intervals (103).

The possible function of the esophageal groove in the passage of ingesta has attracted the attention of many investigators. Early workers supposed that this structure conveyed liquid and semi-liquid ingesta directly from the cardia to the reticulo-omasal orfice without its having to traverse the rumen and reticulum. Recently Schalk and Amadon (103) demonstrated that it performs an important function in the calf during the nursing period and guides milk directly to the omasum. In the mature animal, however, the groove was not functional except upon special occasions as in the administration of saline solutions. Lenkeit and Columbus (72) studied the esophageal groove reflex and found the more effective the reflex (the coming together of the edges of the groove) the quicker the rate of passage of food. In young ruminants liquids and gruels caused the reflex, but dry food had no effect. The reflex was rarely obtained in adult animals. These workers (73) observed regurgitation of the fluid from the abomasum of young animals into the rumen occurred less than an hour following its ingestion. Wester (127) found certain chemical substances in solution caused a closing of the groove and he recommends the use of such substances in the administration of drugs intended for the abomasum.

Time Required for Passage

The significance of the rate of passage through the rumen and the whole of the alimentary tract under normal conditions is its effect on the degree of digestion and the absorption of the digested nutrients. Ewing and Wright (35) found the time that feed material remained in a certain organ depended primarily upon the functional activity of the organ. In organs least active functionally, the feed residue remained for a considerable period. This explains the stagnation of roughage in the rumen. These investigators found that feed residue remained in the rumen and reticulum for an average of 61 hours, in the abomasum for 2.8 hours. Columbus (23) studied the process of rumen evacuation in detail in sheep and goats. He found the test meal to be uniformly distributed throughout the rumen after one to two hours, and passage

into the abomasum began in two to five hours. Most of the meal had left the rumen in 24 hours and completely in eight to twelve days. Withdrawing of roughage delayed emptying of the rumen to 17 to 20 days. As Schalk and Amadon (103) observed the ingesta to remain in the rumen in a process of preparation for 12 to 24 hours, the total time required for evacuation was possibly between 24 to 30 hours.

Ewing and Smith (34) found the rate of passage through the digestive tract varied from 2.9 to 5.2 days depending upon the nature of the ration and quantity fed. Moore and Winter (85) found rubber rings appeared in 11 to 20 hours, reached a maximum excretion in 1 to 3.5 days, and were completely excreted in six to nine days. As was observed by Schald and Amadon (103) and Columbus (23) green forage increases the rate of passage. Concentrates pass more rapidly than roughages because of the short cut they take through the rumen, and thereby, they gain 12 to 24 hours on the roughage (103) (85). Mitchell (13) observed a more rapid passage of concentrated feeds as compared to roughages

Mitchell and associates (80) are of the opinion that the level of feeding is very important in determining the rate of passage. This is in harmony with the conclusion of Ewing and Smith (34). These observers also noticed that finer particles of feed and finer ground feed passed more rapidly than coarse ones. Ewing and Smith reported that in general a more complete digestion was associated with a more rapid rate of passage; however, crude fiber digestion seemed to decrease with a more rapid passage of the residue. It was suggested by Mitchell and co-workers that a lower level of feeding, and hence a slower rate of

passage, resulted in greater digestion and absorption.

Comminution of Food Material that Occurs in Passage

The comminution of the feed residue of average rations containing coarse feeds was thoroughly investigated by Ewing and Wright (35). They found that more than half of the comminution that took place in the average ration occurred as a result of mastication. When rations were incompletely masticated, a higher percentage of the comminution was found in the rumen and reticulum. The extent of comminution which occurred before the feed passed from the rumen and reticulum amounted to 58.5 per cent to 70.9 per cent of the total comminution. The highest percentage of break-down was observed on the smaller rations. The amount of comminution was not alone dependent upon the time feed residue remained in the several organs but it also depended on the functional activity of the several organs. The comminution of rations of silage alone during the process of digestion was over 90 per cent efficient, with 2 mm. as the dividing line.

Relation of Rumen Fill and Total Fill to Live Weight

Knowledge regarding the weight and character of fill in the ruminant at various planes of nutrition and during fast is of particular experimental importance not only to an estimation of live weight but, in the case of fasting, also to show at what stage absorption ceases. Ewing and Wright (35) found the rumen contents in animals receiving fibrous rations varied from 46.3 Kgm. to 51.15 Kgm. and constituted 12.8 per cent of the total live weight. Total fill constituted 17.7 per cent of the live weight. In 1862 Grouven (39)

studied the weight and character of fill and observed that the relation of total fill to live weight was 15.6 per cent in non-fasting cows and 12.7 per cent in oxen fasted for five to eight days. The animals were fed rye straw. Nevens (89) found that 13, 14, and 10 per cent of the live weight of non-fasting animals was fill. Trowbridge and co-workers (118) determined the percentage of live weight represented by the fill in five steers fed hay and grain and found the fill to be 12.0 per cent and 12.8 per cent in animals on submaintenance, 9.5 per cent and 8.3 per cent on maintenance, and 8.0 per cent on supermaintenance. These percentages are lower than most of the others reported. Ritzman and Benedict (100) concluded from their results that true fill in lactating dairy cows consuming large quantities of hay alone or a mixed ration would be in the neighborhood of 20 per cent of the live weight. Voit was quoted by Ritzman and Benedict as stating that the fill of herbivora may amount to 20 per cent or more of the total live weight. Ritzman and Benedict also referred to Seuffert and his co-workers who reported that the intestinal fill of the sixteen cows and bulls they studied represented from 10.96 per cent to as much as 31.0 per cent of the live weight. Grouven (39) concluded that there are about 60 Kgms. of fill in a 500 Kgm. ox, which is about the average for all cattle reported by different investigators.

Nevens (89) found the amount of dry matter in the digestive tract of fasting animals to be roughly 20 per cent of the daily dry matter in the feed when the animals were not fasting. He observed that about 70 per cent of this dry matter of the fill was found in the rumen. The results of Ewing and Wright (35) indicated that in full fed animals slightly over 72 per cent of the fill was in the rumen. In Grouven's

animals 77.5 per cent of the fill was in the stomach compartments of non-fasting animals and 85.6 per cent in the fasting animals.

Ritzman and Benedict (100) drew attention to the fact that the two main factors affecting the weight of fill are the character of the ration and its volume. The character of the ration concerns the ratio between hay and concentrates. In general the percentage of live weight that the fill constituted was relatively high in those animals whose feed was bulky. At maintenance feeding, when the ration comprised more hay than grain, 11 to 15 per cent of the live weight was fill; when the ration contained more grain than hay, from 9 to 12 per cent; and on grain alone, an even smaller percentage. Nevens (89) found that the form in which alfalfa hay was fed to the animal seemed to have little or no effect upon the dry matter content of the rumen.

The results of Ewing and Wright (35) indicated a lack of correlation between dry matter intake and rumen fill as is observed in the following table.

Steer	Wt. in kgms.	Ave. daily dry matter intake in kgms.	Wt. of rumen contents	% of dry matter in rumen
52	385	1.6565	49.124	9.2
49	380	2.6340	51.150	9.7
46	362	3.4730	46.255	11.9
45	385	3.8400	47.000	11.2

These figures readily demonstrate that although the dry matter intake varied considerably, the weight of both the rumen contents and dry

matter of the rumen varied but little. The same results followed out for total fill. This is not in agreement with the conclusion of Ritzman and Benedict (100) to the effect that the volume of feed consumed is important in determining the weight and character of fill.

Grouven (39) pointed out that the fill of his fasting oxen was about 95 per cent water. This is in agreement with the observation of Nevens (89) who noticed a large amount of free liquid in the rumen of fasted animals and practically none in full fed animals. The dry matter content of his fasted animals was one fourth to two thirds as much as that of full-fed animals.

Ritzman and Benedict (100) drew attention to the imperative need of further study on the amount and character of fill in large domestic ruminants, particularly after feeding at different nutritional levels.

Enzymatic Digestion in the Rumen

The rumen is not a secreting organ and the only digestive juice normally present is saliva. That the saliva of the ox does not contain ptyalin was shown by Trautman and Albrechet (120). Antoniani (3) did not find any evidence of cellobiase in the mucosa of the rumen; yet the presence of this enzyme was readily demonstrated in the expressed fluid. He concluded that cellobiase was not exclusively of bacterial origin but that a part was of vegetative origin. All cellobiase found in the rumen was of alimentary origin and the optimum pH for the hydrolysis of cellulose by it was pH 5.0 which is somewhat below the normal pH of the rumen. Brown (19) advanced the idea that celluloses in the grains were of importance in fiber digestion. That such cellulases do not take part in the digestion of fiber was demonstrated by Scheunert and

Grimmer (105). Woodman (128) found that even if conditions in the animal body were favorable for the action of plant cytases they did not take part in fiber digestion.

As the ruminant does not secrete any enzymes which might be active in the rumen and as enzymes of plants do not find conditions favorable for their activity, it is logical to conclude that the only enzymes active in the rumen are those of bacterial origin.

Hydrogen-ion Concentration of the Rumen Contents

The earlier pH values that were reported represented the rumen as always being alkaline in reaction. More recent data have not confirmed this, however, and the indication is that the reaction of the rumen is slightly acid or neutral. Some of the pH values reported in the literature are summarized in the following table.

pH Values for rumen contents of cattle

Investigators	pH Value	Reference
Schwarz and Gabriel	8.89	109
Schwarz and Stremnitzer	8.28	110
Stalfors	always alkaline	117
Kreipe	7.5 - 8.0	66
Knoth	6.95	59
Mangold and Usuelli	7.5 - 7.8	78
Monroe and Perkins	6.3 - 7.0	82
Koffman	6.0 - 8.1	63
Monroe and Perkins	6.2 - 7.4	83
Kick and co-workers	5.5 - 7.7	56

Schwarz and co-workers (109) (110), Stalfors (117), and Kreipe (66) reported that the reaction of the rumen was always alkaline. As is readily seen from the above table, however, most investigators give

a value tending to be neutral or weakly acid. Knoth (59) was the first investigator to make this observation. He also observed that when the rumen contents were exposed to the air, carbonic acid escaped and the reaction became alkaline (pH 7.82).

Monroe and Perkins (82) studied pH on different rations and found the following values:

Grain and hay	7.00
Grain, hay and corn silage	6.92
Grain, hay and A. I. V. silage	6.98
Grain, A. I. V. silage, and corn silage	6.86
Grain, A. I. V. silage, corn silage and limestone	6.97
Grain and blue grass pasture	6.30

Attention is called to the fact that the feeding of acid silage had little effect on the pH. Kick and co-workers (56) reported variations from pH 5.5 to 7.7, depending upon the ration. When alfalfa hay alone was fed the ingesta was most alkaline. As the amount of corn was increased, the reaction lowered. Stalfors (117) found the reaction was alkaline on rations of hay, straw and oat bran and also on hay alone. Koffman (63) observed an increase in pH when hay was fed. Soya meal, linseed, maise and crushed oats lowered the pH,and silage acted as a buffer between the other foods. Mangold and Usuelli (78), <u>in vitro</u> experiments, added milk and its separate components, as well as olive oil, butyric acid and lactic acid to rumen contents and observed a drop in pH from 7.5 - 7.8 to 5.9 - 6.1 in twenty-four to twenty-eight hours. This indicates acid formation in the rumen when milk forms part of the ration, and that the pH of the

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rumen content of calves is probably distinctly acid.

Diurnal variations were recorded by Kick and co-workers (56) and the results indicated that such variations were dependent upon the time of feeding. After the animals were fed the pH fell for a period of eight hours. Following the second feeding the pH again fell at a slower rate for about eight hours. Then it began to rise and reached a peak just before feeding in the morning. Monroe and Perkins (83) studied variations during the day and found there was an acid trend after feeding in the morning which reached a maximum in three to four hours. There was then a turn toward alkalinity and the peak was reached just before feeding in the evening. This trend held on all the rations studied except when pasture was included in the ration.

Monroe and Perkins (83) also found the pH to be fairly uniform throughout the rumen. The variability between samples was less than pH 0.1. In general the posterior region was slightly more alkaline. In experiments with sheep Ferber (36) observed the pH varied from only 7.6 to 7.7 in different parts of the rumen. The pH of the reticulum was 7.9.

Joshland (50) observed that bloat may be associated with a decrease in the normal acidity of the rumen contents. Koffman (63) found a low pH value in animals affected with ketosis and suggested a relationship between pH and ketosis. Ferber (36) followed the rumen pH of wethers during six days of starvation and a subsequent feeding period of fifteen days. The way that the pH of the rumen of the two wethers stayed together was remarkable. There was a slight increase in pH during starvation, but on the sixth day of fasting the lowest pH values were obtained. Following feeding the pH values gradually

increased and at the end of the fifteenth day were 8.5 and 8.4. The normal pH of the rumen of the wethers was 7.6 and 7.5 respectively.

The secretion of saliva is considered an important factor in maintaining the pH of the rumen and counteracting the acidity of fermentation. The saliva of the ox is decidedly alkaline. Schwarz and Henmann (108) gave a pH value of 7.99 to 8.27, average 8.10 for the saliva of the cow. Schwarte was quoted by Dukes (28) as finding the saliva of oxen to have a pH value of 8.23. Workers at Ohio (56) also found a high value, that is pH 8.4. Smith (115) estimated that the ox secreted something like 56,000 gm. (about 13 gallons) of saliva in twenty-four hours. One can readily see that with such a large amount of highly alkaline solution entering the rumen in a twenty-four period, the pH would tend to be fairly well maintained near a neutral reaction, particularly when the additional buffer action of some feeds is considered. Kick and co-workers (56) also observed that grinding hay causes more saliva to be mixed with it.

Rumen Microflora

As ruminants do not secrete any enzymes which might be active in the rumen and as plant enzymes are not active in this organ, one must conclude that microorganisms are responsible for the chemical changes which occur in this large compartment. The rumen with its many gallons of water and saliva, a favorable temperature for bacterial growth, a reaction suitable for the action of numerous organisms, and a large supply of hay and grain to serve as a medium for such organisms, should be teeming with enumerable bacteria and infusoria. That this is true is illustrated by the findings of Mangold (74) who pointed out that of

the latter type of organism alone, some thirty species were found in the rumen to the extent of one million per cubic centimeter.

Koffman (62) stated that the temperature of the rumen was 39°C. By continuous thermo-electric measurements of the rumen contents of sheep, Krzywanek (68) showed that the rumen temperature was normally about 40.5°C. The consumption of fibrous feeds as hay led to a rise in temperature while the feeding of watery feeds as roots caused a decided lowering. This established the rumen temperature as near 40°C.

Cellulose Decomposition by Bacteria

That fiber may be digested by animals was first observed by Sprengel (116) in 1832. Somewhat later Haubner (44) showed that cellulose may be digested and utilized by the animal, particularly by ruminants which digested 50 per cent of it. His results were quickly confirmed by Henneberg and Stohmann (46) who made exact chemical studies. Armsby (4) pointed out that Widt's investigations upon the digestive process in sheep showed that the solutions of cellulose occurred chiefly in those portions of the alimentary canal where the feed stagnates --- that is in the rumen of ruminants and in the cecum and colon of other animals. Popoff (95) demonstrated the formation of methane in the rumen which was an indication of bacterial fermentation. However, Armsby refers to Tappeiner as having been the first to demonstrate experimentally that the disappearance of cellulose in the digestive tract is effected by a fermentation brought about by microorganisms inhabiting the digestive tract. These findings were confirmed by Kellner (52) who found that the feeding of straw pulp to cattle resulted in a marked increase in the amount of methane eliminated. These findings definitely

established that the disappearance of cellulose was due to fermentation by microorganisms.

The nature of the bacterial flora concerned was carefully investigated by Ankersmit (2) and Hopffe (47). Omeliansky (91) obtained two cellulose splitting anaerobes from horse feces. Carbon dioxide and various organic acids as acetic, butyric, valeric, and higher fatty acids were formed, and in addition B. fossicularum produced hydrogen, and B. methanicus produced methane. Langwell and Lymn (70) found the products of cellulose fermentation by thermophibic organisms were, in addition to acetic and butyric acids, lactic acid, ethyl alcohol, carbon dioxide, methane and hydrogen. Their organisms also attacked hemicelluloses. Cowles and Rettger (24) observed an anaerobe from horse feces digested cellulose and produced carbon dioxide and organic acids. Veldhuis and associates (123) recently studied an anaerobic organism with an optimum pH of fermentation of 7.2 for acid production and found the products to be ethyl alcohol (26 per cent), acetic acid (24 per cent), butyric acid, higher volatile acids, and some non-volatile acids. Carbon dioxide, methane, and hydrogen were also present.

Kellerman and McBeth (51) demonstrated that cellulose may be decomposed under aerobic conditions as well as anaerobic. They isolated 36 active species from various sources which rapidly decomposed cellulose and other carbohydrates with the production of organic acids but no gas. Scott and associates (111) found the end products of cellulose fermentation to be acetic acid (45 to 65 per cent), carbon dioxide and smaller amounts of alcohol, lactic acid, and residual acids as succinic. Glucose was demonstrated as an end product and in one case was equivalent to 20 per cent of the fermented cellulose. Purification of the culture increased the production of acetic acid and non-volatile acids, decreased the production of alcohol and carbon dioxide, and caused the production of glucose as an end product.

The thermophibic bacteria studied by Viljoen, Fred and Peterson (124) produced acetic acid, representing up to 50 per cent of the cellulose destroyed, and small amounts of butyric acid, ethyl alcohol, carbon dioxide, hydrogen, and a fatty pigment. Fermentation was at pH 7.6 which approximates the pH of the rumen. Khouvine (54) studied the digestion of cellulose by the bacteria flora of man. Among the products of digestion were carbon dioxide, hydrogen, ethyl alcohol, acetic and butyric acids, and a yellow pigment corresponding to about 60 per cent of the cellulose fermented. Lactic acid was formed in traces. The digestion occurred at 35 to 51°C. This investigator reported that cellulose fermenting organisms were more efficient when in association with other bacteria. Cellulose digestion was five times greater in a mixed than in a purified culture. Sanborn (102) demonstrated that several species of bacteria, themselves not cellulose destroyers, exerted a stimulating effect upon C. folia both in growth and in cellulose-decomposing ability.

Larwynowicz (71) found that aerobic fermentation was very rapid whereas anerobic fermentation proceeded more slowly. At 37°, the temperature of the rumen, aerobic fermentation occurred in less than two to three days, and anaerobic fermentation required more than two days. In cattle he found cytophages to be the most important cellulose destroyers. Simola (113) isolated from feces an anerobe that destroyed cellulose optimally between 30 and 42° and between pH 6.5 and 7.5 with

the production of acid but not of gas. It should be noted that the conditions of its optimal activity were the same as those found in the rumen.

Pringsheim (97) recovered 45 per cent of fermented cellulose as acetic acid, small amounts of formic acid and large amounts of carbon dioxide and hydrogen. By arresting growth of the organism with a volatile antiseptic he proved that glucose and cellobiose were intermediate products of cellulose fermentation. Kellner (52) found that one pound of pure, finely divided cellulose would form as much body fat as would one pound of starch or other forms of digestible polysaccharide. Woodman (128) attempted to explain this value of cellulose in forming body fat by suggesting that glucose, instead of organic acids and gaseous products, was the main product of cellulose fermentation and based his theory upon Pringsheim's work (97). Later Woodman and Stewart (131) demonstrated the production of glucose from cellulose by stopping bacterial action at the head stage of fermentation, and proposed the following theory of cellulose breakdown. Cellulose $\longrightarrow$ cellobiose $\longrightarrow$ glucose. This theory was prominant for a number of years. In the light of subsequent investigations, however, Woodman and Evans (130) abandoned this theory. Although support was found that glucose was a primary breakdown product of cellulose fermentation, they considered it unlikely that this product would escape further breakdown before absorption could occur. These investigators showed that glucose, pyruvic acid, lactic acid, and volatile fatty acids (formic, acetic, and butyric) arise during the breakdown of cellulose by fermentation <u>in vitro</u> by rumen bacteria. They also found that fermentation occurred more quickly by organisms from sheep feces

acting at 37°, which is near the rumen temperature, than by thermophibic organisms. They did not attempt to purify the culture as such is not the condition in the rumen.

Pochon (93) isolated a cellulose splitting organism, Plectridium cellulolyticium, from the rumen of the ox and sheep. He very convincingly demonstrated by using this facultative anaerobic organism in in vitro experiments that the major products of cellulose fermentation were the lower fatty acids (94). Of the products formed 73 per cent was formic acid and 27 per cent acetic acid. At the end of the process 6 per cent propionic acid and 5.4 per cent ethyl alcohol, but no reducing sugars, were formed. There was not any gas formation, and fatty acids represented 80 per cent of the cellulose utilized. In order to follow digestion as it would occur in passage from the rumen (neutral reaction) to the abomasum (acid reaction) the medium was brought to a pH of 4 to 5 by either spontaneous acidification or by the addition of hydrochloric acid. Under these conditions glucose was produced in an optimum amount of 10 per cent. This was explained on the basis that when the ingesta entered the abomasum, cellulase of bacterial origin transformed cellulose to glucose.

The fatty acids seem to be the most important products formed from cellulose fermentation, with acetic acid being most predominant and with butyric, formic, propionic, lactic, pyruvic, valeric, and succinic acids being formed in small amounts. Glucose, ethyl alcohol, carbon dioxide, methane, and hydrogen are also formed. Various in vitro findings also tend to indicate the production of fatty acids in the rumen as a result of cellulose breakdown. Nicholson and Shearer (90) observed on post mortem examination of cows affected with lactation tetany that the rumen contents contained much volatile fatty acids, the nature of which was not

determined. Bruggemann and Buss (20) found that after allowance was made for the lactic acid present in the abomasum of the sheep, indication of the presence of ether soluble organic acids was found. Benedict and Ritzman (15) stated that their results suggested again the early view of Grouven that not only cellulose but other carbohydrates are absorbed through the fermentative processes, and this results in flooding the body with a large number of the lower fatty acids, which stimulate metabolism.

Other Food Constituents attacked by Rumen Microflora

Although cellulose is the major feed constituent that undergoes fermentation in the rumen, it is not the only one affected by fermentative processes. The hemicelluloses which include primarily pentosans, and various hexosans are also attacked. Armsby (4) reported Stone as the first to show that pentosans were digestible. About 60 per cent was digested in ordinary feeds by the rabbit. Since that time pentosans have been shown in some instances to be not only digested but highly digestible. Brahm (18) was the first to observe that pentosans were fermented by rumen bacteria. Baker and Martin (7) found hemicelluloses as well as cellulose to be disentegrated by microorganism in the rabbit ceacum. Iwata (49) isolated several species of xylan decomposing bacteria from the ceacum and rumen. The products were xylose and a small amount of lactic, acetic, formic and carbonic acids. Growth and activity were optimum at 35 to 40°C, and at pH 6.8 to 7.0. As there is no evidence of animal enzymes which attack hemicelluloses and as these substances not only are digestible in the animal but are fermented by bacteria from the rumen, ceacum and manure, the conclusion that hemicelluloses undergo bacterial fermentation in the rumen to yield substantually the same products as cellulose fermentation is warranted.

Schieblich (106) observed that pectins were also acted upon by bacteria in the digestive tract. Such bacteria were shown by Ankersmith (2) to occur in the digestive tract of the horse, and by means of pectinase they separated the plant cells and rendered cellulose available for digestion. Baker and Martin (7) proved the disentegration of pectin by microorganism in the rabbit ceacum. These results indicate rumen fermentation of pectins.

Lignin is the most resistant part of the cell membrane and by several investigators, including Rogozinski and Starzewska (101), it has been found to be indigestible. That it may be attacked, however, was indicated by the early work of Konig (61) who found the digestibility of lignin by sheep was 16 to 23 per cent in hay, 4 to 13 per cent in clover hay, and 28 per cent in pea straw. Haberlandt (42) found lignified cell walls were more highly digestible on passage through the digestive tract than usually is supposed. Baker and Martin (7) observed that lignin was sometimes digested by the activity of organisms in the rabbit ceacum. Lignin, though it normally is highly inert, may be and often is attacked to some extent by the rumen and intestinal flora.

Haberlandt (42) found cutinized cell walls to be wholly indigestible. On the other hand, Konig (6) reported the digestibility of cutin in roughage as varying from 7.27 to 20.93. per cent. As an animal enzyme capable of digesting cutin has not been demonstrated, this plant material probably is acted upon by rumen microflora.

At one time organisms producing methane fermentation were believed to attack cellulose specifically and not to act upon other carbohydrates. Armsby and Fries (6), however, found that the inclusion of starch in a

ration resulted in increased methane fermentation. Kellner (52) observed that 3.07 parts of methane were formed per 100 grams of digestible starch which indicates destructive fermentation of starch and similar compounds. Woodman (128) pointed out that about 10 per cent of the digested carbohydrate is fermented to waste products. This parallels the destructive fermentation of cellulose. In 1864 Grouven (39) suggested that carbohydrates for the large part pass through the fatty acid stage of fermentation in the rumen. The results of Benedict and Ritzman (15) tend to confirm this theory. They observed a striking increase in the metabolism of steers on a protein-poor and carbohydrate-rich maintenance ration. This increases in metabolism could be accounted for readily on the basis that the path of absorption of carbohydrates in the ruminant is through the fermentative processes, which would result in flooding the body with lower fatty acids which stimulate metabolism. As it is well known that carbohydrates are attacked by rumen bacteria, it is unlikely, since a considerable portion may remain in the rumen for several hours, that they would escape fermentation and more likely that they would be fermented to the fatty acid stage just as is cellulose.

Gaseous By-products of Fermentation

In herbivorous animals, whose food contains large portions of carbohydrates, the production of gas from the fermentation of the carbohydrate material reaches comparatively large proportions. The characteristic gaseous by-products of such fermentation are methane and carbon dioxide, of which the former plays far the greater role --- so much so that other waste products are generally disregarded. The value of fermentation from the viewpoint of the animal lies in the fact that wholly indigestible foods, primarily cellulose, are converted into substances which can be absorbed and utilized. This gain, however, is obtained only at the cost of a certain portion of the energy available in the food material.

Popoff (95) was the first to demonstrate methane formation in the rumen. Kellner (52) showed that the consumption of crude fiber (straw pulp) by cattle caused a marked increase in the production of methane. The composition of the gases contained in the rumen was studied by Markoff (79). He found variations over the following ranges:

CO_2	14.95% to 54.32%
CH_4	19.88% to 42.55%
H_2	.05% to 4.07%

Ritzman and Benedict (100) stated that Armsby and Fries in 1902 showed convincingly that there was no hydrogen in the gases of fermentation. Krogh and Schmit-Jensen (67) pointed out that Mollgaard and Anderson did not find any hydrogen to result from rumen fermentation and discredited Markoff's results to the effect the hydrogen was produced. More recently Washburn and Brody (125) reported the following composition of the rumen gases of cows fed alfalfa hay: CO_2 - 28.48% to 67.81%, CH_4 - 22.25% to 40.67%, and H_2 - 0.00% to 2.60%. Similar results were obtained on other feeds and with goats. Hydrogen, though not always, is often a product of rumen fermentation.

Markoff (79) observed ratios between carbon dioxide and methane in the rumen of goats to vary from .75 to 3.68. Krogh and Schmit-Jensen (67) found a ratio of 2.6 and used this value as a correction factor in studying the respiratory exchange. Klein (57) found the ratio to be 3.68 for the gases excreted by the ox.

Kellner (53) reported the formation of 3.17 grams of methane per 100 grams of starch digested, 4.5 grams per 100 grams of digested straw pulp and 4.29 grams per 100 grams of a mixed ration. Armsby and Fries (5) found 4.8 grams of methane to be produced from 100 grams of digested roughage and 4.7 grams for 100 grams of digested concentrates. Forbes and associates (38) observed the following production of methane per 100 grams of digestible carbohydrates in an alfalfa and corn ration; 5.37 grams on half maintenance; 4.68 on maintenance; 4.32 on one and one-half maintenance; and above this level there was little change in methane production.

Armsby and Fries (5) observed that carbohydrates were attacked to a greater extent on light than on heavy or medium rations. Markoff (79) found that carbon dioxide was usually in excess of methane, but on feeding hay alone and during starvation, methane was predominant. Feeding oats increased the carbon dioxide, and adding sugar or protein had no influence on the ratio. Washburn and Brody (125) concluded the kind of feed and the time after feeding caused a considerable but characteristic variation in the losses of gas and in the carbon dioxide methane ratio. Ritzman and Benedict (100) showed that, in general, methane losses were somewhat less on highly nitrogenous feeds. Armsby and Fries (6) reported an increase in methane production and a decrease in cellulose digestion on adding starch to the ration.

According to Armsby (4) one gram of methane contains 13.34 calories or one liter contains 9.56 Calories. In addition Krogh and Schmit-Jensen (67) showed that for every liter of methane produced in the rumen

3.97 Calories of heat were evolved, and this heat is usually regarded as waste. Ritzman and Benedict (100) pointed out that the ruminant excretes a large portion of methane directly by way of the gullet. Klein (57) showed that about one-third of the methane formed was resorbed and then expired by the lungs. Armsby (4) gave the methane losses for roughages as representing 7.29 per cent of the gross energy content. The loss from concentrates varied greatly depending upon their carbohydrate content. Ritzman and Benedict (100) found methane losses in cattle to average 9.93 per cent of the digestible energy of roughages and 8.00 per cent of that in the concentrates. Washburn and Brody (125) reported total energy losses as gases as amounting to 12 to 16.5 per cent of the feed energy intake or 25 to 40% of maintenance requirements.

Benedict and Ritzman (15) concluded on the basis of numerous long fasts in the large ruminant that digestive activity had essentially ceased before the production of methane ceased. On the basis of decrease in methane production in the fasting cow on various feeds, they later concluded that the cow usually reached a post-absorptive state by the end of the third day of fasting, irrespective of the type of feed, and that in the case of concentrates this condition may be reached in two days (100).

Function of Rumen Infusoria

In 1843 Gruby and Delafond (40) discovered the protozoan fauna of the rumen and reticulum of domestic ruminants, and since then numerous investigations of the activities of these organisms have been made. Becker and co-workers (14) found up to 2,000,000 infusoria per cc. of rumen contents, and Mangold (76) pointed out that upwards of thirty species have been found in the rumen. Gruby and Delafond (40) es-

timated the infusoria in the rumen were equal to approximately one-fifth of the total weight of the rumen contents. Mowry and Becker (87) reported that in some instances infusoria accounted for well over 30 per cent of the volume of the rumen content.

Infusoria are transmitted from animal to animal by salivary infection of the common food and water supply (76). Mowry and Becker (87) found that in fasting goats the number of infusoria fell rapidly. On a hay ration the numbers were comparatively low, and as grain was added to the ration, there was a proportionate increase in infusoria. Adding materials rich in animal protein also increased the numbers. Recently Koffman (64) demonstrated that NaCl had a toxic effect on the protozoa of the abomasum of cows. The administration of 250 g. of salt daily for thirty-three days reduced the number of fauna in half and the number of species by 25 per cent.

The question of the importance of infusoria in the digestion of cellulose has long been debated. Cleveland (21) referred to Everlein who observed the ingestion of plant fiber by certain of the rumen infusoria. He concluded, as did others who followed him, that infusoria could digest cellulose. Cleveland's classic work (21) proved the importance of the intestinal protozoa of termites in cellulose digestion within their host. He then concluded that the infusoria harbored in the ruminant stomach may aid their host in the digestion of cellulose. Koffman (62) was of the opinion that ciliates helped considerably in the digestion of cellulose by ruminants. Weineck (126) recently studied cellulose digestion by ciliates of the rumen and reported that they digested cellulose.

On the other hand, however, Becker and associates (14) quoted investigators who suggested that although infusoria may ingest cellulose they did not necessarily disintegrate it. Becker and associates in studies with animals with normal rumen flora and with infusoria-free animals found that cellulose digestion in the host was neither due to, nor assisted materially by infusoria. Usuelli (121) confirmed his own previous work in finding that no appreciable amount of cellulose was digested by infusoria. In feeding test with sheep Dyakov and co-workers (29) failed to find sufficient differences in digestibility in animals with and without infusoria to indicate any favorable influence on digestion. The results of Becker and associates (14) preclude the suggestion of several workers that there may be a symbiosis between cellulose digesting bacteria and infusoria as cellulose was digested normally in animals which had been free from infusoria for weeks. Although there is a possibility that infusoria are capable of digesting cellulose, the evidence that they actually do digest it in the rumen is lacking, and several recent investigations of such an activity indicate it is absent.

Koffman (62) suggested that there is some definite relationship between the numbers of protozoa present in the rumen and the general health of the cow, and that protozoan bodies are readily assimilated as food. He noted a quick improvement in the condition of sick cows following the introduction of protozoa from healthy cows. The optimum temperature and pH for their activity is within the range of rumen conditions. Usuelli (122) incubated the contents of sheep's rumen and added starch and starch containing foods. The starch was assimilated in four hours and glycogen formation reached a maximum in six hours.

Becker and Everett (13) found that lambs grew as rapidly (in fact somewhat more rapidly) than did infusoria-harboring lambs. Mowry and Becker (87) did not find a correlation between the infusoria numbers and

the weights of adult goats. They concluded that the reactions of infusoria to the conditions in the rumen connote commensalism rather than symbiosis.

In studying the major controversial questions as to the value of infusoria to their host, Becker and associates (14) found no reason for regarding these organisms either as constituting a useful endofauna or a true parasite. They concluded "we must consign them definitely, for the present, to the status of mere commensals." As the usefulness of infusoria seldom has been demonstrated in *in vivo* experiments and as infusoria-free animals appear to be unaffected by their absence, this conclusion of Becker and associates is justified.

Utilization of Non-protein Nitrogen

The possible synthesis of protein from non-protein compounds by rumen bacteria has been considered for many years. Armsby (4) concluded the early work in this field indicated that ruminants converted non-protein netrogenous compounds into protein by the action of the microorganism of the digestive tract. Morris (86) reported that the work of Muller gave considerable weight to this theory as he grew rumen bacteria in a medium with ammonium tartrate as the sole source of nitrogen. From the results of feeding experiments with dogs he concluded that the bacteria protein that had been formed was of value. Morris (86) recently reviewed the subject and also presented the possibility of the function of rumen flora in the utilization of such material. That bacteria and infusoria may constitute a large portion of the total rumen nitrogen was indicated by the findings of Schwarz (107) who found that infusoria contained 20 per cent and bacteria 11.7 per cent of the rumen nitrogen.

Mangold and Schmitt-Krahmer (77) demonstrated that 10 per cent of the rumen nitrogen was constituted by bacterial nitrogen. No evidence was found, however, that rumen infusoria contribute any appreciable amount to the total nitrogen content. Ferber and Winogradowa-Fedorowa (37) found the total infusorial mass amounted to from 10 to 20 per cent of the nitrogen present in the rumen. Morris (86) pointed out that since microorganisms multiply at a rapid rate with the formation of protein, it is possible that non-protein nitrogen may be synthesized into protein. He also drew attention to the fact that a majority of the investigations in feeding non-protein nitrogen have dealt only with the addition of non-protein nitrogen to a ration or the partial substitution of the protein in the ration with it. In such instances the protein nitrogen might not have been reduced below the actual requirements of the animal. In such experiments more care should also be exercised in assuring that the non-protein nitrogen does not contain any of the essential amino acids.

Recently Klein and associates (58) fed sheep a ration of straw, molasses and starch (a ration low in protein but high in non-protein nitrogen). As the animals were maintained in good health and showed normal fattening and wool growth, these investigators concluded the amides of the feed must have been utilized as a result of bacterial action in the rumen. Richter and Herbst (99) found the substitution of 50 per cent of the crude protein in a basic feed ration by urea did not entirely supply the necessary nitrogen compounds. Although glycocoll feeding resulted in a decrease in milk production, the decrease was much less than with urea feeding. Ehrenberg and co-workers (33) supplied only half of the maintenance requirements in the form of protein and the rest as

ammonium bicarbonate. Milk continued to be secreted in normal amounts with the normal percentage of protein, and there was little loss in weight. Ehrenberg and Prittwitz (22) later observed a 15 per cent decrease in milk production when 100 grams of ammonium bicarbonate replaced oil cake meal. Bartlett and Cotton (8) observed that urea was a good substitute for protein in meeting the nitrogen requirements of dairy heifers. Hart and associates (43) obtained similar results with ammonium bicarbonate and urea. They explained the results on the basis of protein synthesis by rumen bacteria. Nehring (88) found ammonium acetate, urea, and glycine could be substituted for part of the nitrogen or proteins in the feeding of ruminants.

Woodman and Evans (129) were unable to show that rumen bacteria form cystine. Sjollema and Van der Zande (114) studied the ability of bacteria from the ox rumen to synthesize tryptophane and tyrosine from urea, and from ammonia plus asparagine. In vitro results were positive. They stated "It is uncertain whether this synthesis is equally intense in the paunch." Kotake (65) observed that certain bacteria are able to synthesize tryptophane while others are not. Although there is considerable evidence indicating protein synthesis in the rumen, the proof that microorganisms make essential amino acids is lacking.

Synthesis of the Vitamin B Complex by Rumen Microflora

Although the vitamin B complex has been shown to be an important factor in the growth and general well being of many species of animals, investigations into its value for dairy animals have yielded negative results. Eckles and associates (30) found that the use of yeast to supplement rations fed dairy calves did not increase the rate of growth

or have any beneficial effect on health. Eckles and Williams (31) also found the results with lactating animals to be negative. Bechdel and associates (10) demonstrated that calves would grow normally to maturity and produce normal offspring on a ration that contained an insufficient amount of the vitamin B complex to support growth and health in rats.

In 1918 Pacini and Russel (92) showed certain bacteria as being capable of synthesizing vitamin B. Later Heller and associates (45) found evidence of bacteriological synthesis of vitamin B in the intestinal tract of the rat. In view of this evidence of synthesis of B vitamins by bacteria and the negative results obtained as to their value in dairy animals, Bechdel and associates (10) proposed the theory of vitamin B complex synthesis by microorganism in the rumen to account for their results obtained with calves. That the vitamin B complex content of milk is not dependent upon the presence of B vitamins in the ration of the cow was demonstrated by Bechdel and Honeywell (11). Bechdel and associates (12) very convincingly demonstrated that the vitamin B complex was produced in the rumen of cattle by bacterial fermentation. They fed alcoholic extracts of fermented rumen contents to rats and proved that the bacterial cells were highly potent in the vitamin B complex.

Guerrant and associates (41) and Abdel-Salaam and Leong (1) were more recent investigators to further demonstrate synthesis of the B vitamins by bacteria in the digestive tract of the rat.

It is a fairly definitely established fact that ruminants are able to synthesize their needed vitamin B factors through bacterial synthesis in the rumen. This offers a satisfactory explanation as to why cattle, unlike many species, have the ability to grow, reproduce normally and produce milk on rations deficient in the vitamin B complex.

Studies on the Comparative Digestibility of Various Feed Constituents in the Rumen

Methods of Study

Through the use of rumen fistula animals investigations have been made concerning the comparative digestibility of the various feed constituents within the rumen.

This method of study was used by Silver (112) to determine the effect of the preparation of alfalfa hay upon its digestibility and absorption in cattle. In order to follow the digestion of the ingested feed, a sample was removed from the rumen just prior to feeding and it represented the residue from previous feeding. The second sample was taken after feeding and represented a mixture of the hay eaten and the residue in the rumen. Subsequent samples were taken at two hour intervals. Results were compared on the percentage composition of the rumen contents as the period of digestion progressed.

Quittek (98), in studies with sheep, employed the rumen fistula technique to follow the digestion of hay. Rumen contents were removed and sampled at 3, 6 and 9 hours after feeding, and the results were interpreted in terms of the percentage composition of the contents as compared with the composition of the hay fed. Krzywanek and Quittek (69) used the same method of study and in addition compared the crude fiber to fat ratio in following hay digestion in sheep.

Kick and Gerlaugh (55) recorded the chemical and physical composition of the residual rumen ingesta in rumen fistula steers receiving alfalfa hay. Rumen contents were removed in the morning at the beginning of the test and again at the end of a twenty-four period. The contents were weighed, mixed, sampled, and returned as quickly as possible. Results were compared on the basis of the percentage of daily intake that the nutrients in the rumen represented.

Results of Studies on Rumen Digestion

Silver (112) observed that as the ingesta remained in the rumen for a longer time there was a rise in crude fiber content and a decrease in protein content, and just prior to feeding the percent of crude fiber was relatively high. The per cent of protein was relatively low compared with the analysis of the alfalfa hay fed. Quittek (98) found that despite a decrease in dry matter content in the rumen, both fat and nitrogen content of the dry matter increased proportionately to the duration of digestion. He concluded that the increase in fat and nitrogen was related to the breakdown of the cellulose fraction of the hay by rumen infusoria. In subsequent experiments, however, Krzywanek and Quittek (69) revised this conclusion and ascribed the increase of fat and nitrogen percentages as being due to the disappearance of carbohydrates by rumen fermentation rather than to a new formation of fat. They observed the percentage of nitrogen and crude fiber in the dry matter to increase rapidly as the digestion period progressed. Also as digestion progressed the ratio of crude fiber to fat decreased slightly as compared with the ratio in the feed. An increase in percentage moisture was attributed to the constant secretion of saliva.

Kick and Gerlaugh (55) reported data that indicated proteins disappeared from the rumen more quickly than crude fiber. The amount of

fiber retained in the rumen at the end of a twenty-four hour period decreased in passing from long hay to 1/4 inch hay but rose again when ground hay was fed. The protein retained also tended to diminish with fineness of the hay but in the oldest of three animals there was not any marked alteration. Dry matter passed from the rumen more rapidly as the particle size of the hay decreased. They suggested that fineness of the feed is probably associated with speed of the chemical reactions which occur in the rumen and,thereby, may affect the rate of flow of nutrients from this organ.

Scheunert (104) reported that 1,000 grams of rumen contents contained only one gram of protein in a digested form which indicated protein materials were digested to only a very slight extent in the rumen.

Absorption from the Rumen

Trautman (119), on the basis of experiments involving the introduction of solutions of water and of drugs into fistulas of the various stomach compartments of ruminants, reached the conclusion that the rumen, reticulum and omasum are capable of rapid absorption of water and substances in solution. The abomasum was found to absorb more slowly. He pointed out that this conclusion was contraty to current conception and stated that the widely held view that protective mucosa are only permeable with difficulty to water and aqueous solutions must be revised. More recently Davey (27) compared the osmotic pressure of the blood of sheep with that of the rumen, reticulum and abomasum by means of the freezing point method. In one animal the values were identical in all four instances and in another only the abomasal contents gave a slightly lower value. He concluded that the approximate identity of these two sets of values (blood and stomach contents) was best explained on the basis that

absorption takes place from the rumen and other stomach compartments.

Summary of Review of Literature

Studies of the physiology of rumen digestion have demonstrated the importance of the rumen and the functions associated with it in establishing the ruminant as the most efficient converter of roughages into human food.

Investigations of the mechanical processes of rumen digestion have revealed the continuous and rhythmical movements which aid in the mixing, softening, maceration and fermentation of roughages prior to their being subjected to remastication. Concentrates escape rumination and pass more rapidly from the rumen. The function of rumination has been demonstrated as being dependent upon roughage. The esophageal groove is not normally functional in the adult ruminant. More than half of the comminution of feed occurs as a result of mastication, and about 58 to 71 per cent of the total maceration occurs before the ingesta leaves the rumen. The total fill of cattle constitutes about 12 per cent of the live weight and even 20 per cent in lactating cows. Rumen fill accounts for approximately 70 per cent of the total fill.

A review of the data concerning the chemical factors of rumen digestion indicates that bacterial enzymes are the only enzymes active in the rumen. The reaction of rumen contents has been observed to be neutral or slightly acid and is influenced slightly by the type of feed and time after feeding. The saliva of cattle which is distinctly alkaline is secreted to the extent of about 13 gallons a day. This large amount of saliva is important in neutralizing the acid of fermentation. The rumen temperature is about 39 to 40°C. which is somewhat

higher than the rectal temperature of approximately 38.2°C.

Numerous microorganisms of a bacterial and protozoan nature inhabit the rumen. Cellulose-splitting organisms produce primarily acetic acid with smaller amounts of glucose and butyric, propionic, formic, lactic, pyruvic, valeric, and succinic acids and ethyl alcohol, carbon dioxide, methane and hydrogen. In addition, bacteria attack hemicelluloses, pectins, even lignin and cutin, and all carbohydrates to at least some extent. Of the gaseous products methane is the most important, and losses from this source represent about 10 per cent of the energy in the feeds.

Although numerous activities have been ascribed to rumen infusoria, they must be classed, for the present, as mere commensals. The literature reveals that ruminants are capable of utilizing non-protein nitrogen as protein substitutes, and some data indicate protein synthesis in the rumen. However, the proof that microorganisms form essential amino acids is lacking. Data have been presented which prove the synthesis of the vitamin B complex in the rumen.

Methods of studying the chemistry of rumen digestion have involved the use of the rumen fistula technique. The results have been interpreted in terms of the percentage composition of rumen contents as compared with either the percentage composition of previous samples of ingesta or the composition of the hay consumed. Also the percentage of total daily intake that is represented by the rumen contents has been used. Proteins disappear rapidly while fiber and fat leave the rumen slowly. Recent investigations furnish evidence of the possibility of absorption from the rumen.

OBJECT

The purpose of this study was (1) to investigate possible methods of evaluating the quantitative digestion which occurs in the rumen and to observe the effect of the plane of nutrition upon such digestion, (2) to observe the hydrogen-ion concentration and temperature of the rumen contents in relation to the variations occurring during the day at various levels of feeding and in different regions of the rumen, and (3) to study the effect of the level of feeding and the character of feed on rumen fill and barrel circumference.

EXPERIMENTAL PROCEDURE

Animals Used

Holstein cows in the Michigan State College experimental herd were used in this investigation. One heifer (Fig. 5 in the Appendix) in which a rumen fistula had been made was used for the direct observations of rumen digestion. In addition, five animals were used in metabolism trials to obtain data to supplement the studies with the rumen fistula animal. Several animals which were being used on various other experiments were utilized for obtaining information concerning barrel circumference and fill as affected by the character and level of feed.

Feeding of Animals

The feed used in this study was alfalfa hay, the chemical composition of which is given in Tables I, III, V, VII, IX, XI and XIII.

The rumen fistula animal and the other five cows were fed alfalfa hay alone at levels of 10, 20 and 30 pounds a day. In one trial alfalfa-brome hay was used instead of alfalfa. Some of the animals which were used for barrel measurements and body weight studies received feeds other than alfalfa and were not necessarily fed at the levels mentioned above.

Collection of Rumen and Feces Samples

During the investigations of rumen digestion the rumen fistula animal was fed for a preliminary period of at least 12 days on the level of feeding to be studied. Samples were taken before feeding in the morning and represented an elapse of 14 hours since the preceeding feeding. The animal was weighed and the barrel circumference was measured. The contents were removed, weighed, mixed, sampled and returned as rapidly as possible. In order to obtain a more accurate weight of the rumen contents, the animal was weighed before and after removal of the ingesta and the difference in weight recorded.

Following removal of the contents a sample of feces was collected but not on a quantitative basis except when the animal was on metabolism trials. Both rumen and feces samples were collected on two series of the 10, 20 and 30 pound levels, and in one instance a sample was removed 24 hours after feeding during a period when the animal was receiving 10 pounds of hay a day.

Metabolism Trials

Three metabolism trials were conducted to supplement data collected during the rumen studies. In the first trial one animal received 30 pounds and two received 20 pounds of hay a day; in the second, two re-

ceived 30 pounds and two others 20 pounds of hay; and in the third two received 20 pounds and two 10 pounds of hay a day. The rumen fistula animal was used in all three trials.

Chemical Analyses

The chemical analyses which were made on the hay, rumen contents and feces were moisture, protein, ash, ether extract, crude fiber, nitrogen free extract (by difference), cellulose, lignin, other carbohydrate (by difference), an enzymatic crude fiber determination, nitrogen free extract (by difference), total fatty acids and iron. Of these determinations moisture, protein, ash, ether extract, crude fiber, nitrogen free extract and iron were determined by standard A.O.A.C. methods (132).

Cellulose and lignin were determined by the methods of Crampton and Maynard (26). In the cellulose determination, 1 gm. of an air-dried sample was placed in a 200 ml. pyrex ignition test tube. The original method called for a 150 ml. round-bottom flask, but the above modification eliminated a subsequent transfer of the material. The test tube was fitted to a reflux condenser, and 15 ml. of 80 per cent acetic acid and 1.5 ml. of concentrated HNO_3 were added. The mixture was boiled gently for 20 minutes. Twenty ml. of alcohol was then added and the test tube was centrifuged for 10 minutes. The liquid was decanted and the residue washed (in test tube) with alcohol. Then the residue was transferred (with the aid of a stream of alcohol from a wash bottle) into an alundum crucible and washed successively with hot benzene, hot alcohol, and ether --- using suction. The sample was dried at 110°C., cooled in a dessicator and then weighed. Cellulose was calculated by

determining the loss on ignition.

The procedure used for lignin follows: One gram of oven-dried, alcohol-ether extracted material was placed in a 50 ml. glass stoppered Erlenmeyer flask, and 40 ml. of a 2.0 per cent solution of pepsin in N/10 HCl was added. This was digested for 12 hours at 39°C. with frequent shaking during the first four or five hours. The original procedure called for 40°C. but the rumen temperature of the animal used in this investigation was found to be approximately 39°C., therefore this temperature was selected. The non-digested residue was recovered by filtration through bolting silk and was washed successively with hot water, hot alcohol, hot benzene, hot alcohol and ether. The washed residue was transferred to a 100 ml. beaker and the last traces of ether removed with mild heat. The residue was moistened with four ml. of 40 per cent formaldehyde followed by the addition of 4 ml. of 72 per cent H_2SO_4 which was allowed to penetrate the sample (two minutes). Six ml. of concentrated H_2SO_4 were added and stirred vigorously with a glass rod to aid in solution of the sample which was complete in 10 to 15 minutes. On adding the 72 per cent and concentrated H_2SO_4, the beaker was immersed in a cold water bath to prevent the temperature from rising above 70°C. When the residue was dissolved, 35 ml. of a granulating reagent consisting of a 1:6 mixture (by volume) of chloroform and acetic acid was stirred in and the whole was poured into 500 ml. of distilled water in an 800 ml. beaker. The mixture was boiled gently until the chloroform had been driven off (15 minutes), after which the solution became clear and the lignin settled in granular form. The material was filtered through an alundum crucible with suction and washed in not less than 200 ml. of 5 per cent HCl. The residue was dried at

110°C., cooled in a dessicator and weighed. The lignin was determined by loss on ignition.

The crude fiber was determined by the enzymatic method of Horwitt and co-workers (48). Three grams of a dried sample were incubated with 0.5 gm. of pepsin in 500 ml. of N/10 hydrochloric acid at 39°C. (selection of temperature was discussed under lignin) for 48 hours. This mixture was then neutralized to pH 7.0 with sodium hydroxide, brought to pH 4.5 with hydrochloric acid, and treated with 0.1 gm. of taka-diastase for 48 hours. In neutralizing the solution and in reducing the pH to 4.5, it was found that the amount of alkali or acid required to bring about the desired pH could be readily determined by use of a pH meter or paper indicators. Once the volume necessary was established this part of the determination was greatly facilitated by adding the desired amount without the need of checking the pH of the solution afterwards. At the end of the 48 hours the digest was filtered on bolting silk, the residue returned to the digestion flask and treated with 500 cc. of faintly alkaline solution (0.2 gm. of sodium carbonate and 4.25 gm. NaCl) containing 0.5 gm. of trypsin. Toluene was added and the material was incubated for four days. The residue from this digest was washed with distilled water until the filtrate no longer gave a test for chloride, then with alcohol and ether. Bolting silk, which had been previously weighed with a 300 ml. beaker, was used in filtering. The residue was dried to constant weight at 110°C., cooled in a dessicator and weighed.

Total fatty acids were determined by a method developed by Horwitt and associates (48). Ten grams of the dried material were placed in a 500 ml. round-bottom flask, fitted to a reflux condenser, and boiled with 100 ml. of an alcohol-ether (4 + 1) mixture for 10 to 20 minutes. The blue-black supernatant fluid was decanted into a filter paper and the filtrate was collected in a 250 ml. beaker. The residue was extracted for 5 to 10 minutes with 75 ml. of boiling alcohol-ether mixture and twice again with 25 ml. portions.

The total filtrate was evaporated to a volume of about 100 ml. and to this solution 20 ml. of approximately 10 N potassium hydroxide were added. During saponification the mixture was heated on the open steam bath until the volume was reduced to about 30 ml. At this point 30 ml. of water was added and the saponification continued until the total time elapsed on the bath was one to two hours.

The total saponified mixture, which should not contain more than 20 per cent alcohol, was acidified with HCl. A large excess of HCl should be avoided as it would cause formation of chlorophyll products which are more soluble in petroleum ether. The acidified mixture was transferred to a 125 ml. separatory funnel and extracted with petroleum ether, twice with 50 ml. and twice with 30 ml. This extract was filtered and evaporated to a volume of 50 ml.

The petroleum ether extract which was evaporated to 50 ml. was extracted with 50 ml. of N/10 potassium hydroxide in 50 per cent alcohol. This in turn was twice extracted with 25 ml. of petroleum ether. The petroleum ether fractions were combined and extracted with 25 ml. of the alcoholic potassium hydroxide solution.

The combined potassium hydroxide fractions were acidified with HCl, using a trace of phenolphthalein, and extracted with 50 ml., 25 ml. and 20 ml. portions of warm petroleum ether. The petroleum ether extract of the fatty acids was evaporated in a tared dish, dried at 100°C. for 30 minutes and weighed.

Estimation of Quantitative Digestion

Two methods were used to evaluate the quantitative digestion occurring in the rumen. These were lignin ratios and iron ratios. Iron ratios as a method of evaluating digestion has been previously used with humans and small animals with apparently satisfactory results (16). Iron balances were determined to give some idea of its stability in the hay material. Lignin was selected because of the comparatively large amounts that occur in roughages and its relative inert character (25) (26). Several lignin balances were also determined.

Calculations were made by comparing the ratio of the percentage of lignin, or iron, to the percentage of the various constituents in the hay, with the ratio of the percentage of lignin, or iron, to the percentage of the constituents in the rumen. For example, if there were 15 per cent lignin and 15 per cent protein in the hay while the percentages in the rumen were 30 per cent lignin and 15 per cent protein, the digestibility would be calculated as follows:

15 per cent ÷ 15 per cent = 1

30 per cent ÷ 15 per cent = 2

1 ÷ 2 = 50 per cent of the protein was not used, and thus 50 per cent was digested.

Rumen pH and Temperature Values

In this experiment pH values were followed from just before feeding in the morning until two hours after feeding in the afternoon. Samples for pH values were taken at two-hour intervals throughout this period and were usually, though not always, accompanied by temperature readings. Variations in different regions of the rumen and reticulum

were recorded. Values were obtained for alfalfa hay alone on 10, 20 and 30 pound levels of hay feeding. In addition the pH was followed during the day on a ration of alfalfa hay, silage and soy bean oil meal. All pH values were obtained by use of a Beckman pH meter.

Barrel Circumference and Body Weight

Barrel measurements and the weights of the animal were recorded in order to obtain some idea of the effect on the level of feeding and character of the ration on fill and barrel size. These data were taken in the afternoon just prior to feeding. Although the body weight was not included from the beginning, it was recorded after the study had progressed. At first measurements were taken daily, but it was observed that measurements taken every third day were just as representative. In some instances more extended periods passed from one measurement to another.

RESULTS

Estimation of Quantitative Digestion

Analysis of the alfalfa hay, rumen contents and feces for the first trial, during which the animal received 30 pounds of hay a day are given in Table I.

Table I Chemical analyses on dry matter basis at the 30-pound level

	Alfalfa hay	Rumen contents	Feces
	%	%	%
Protein	17.8	13.9	12.8
Ash	6.7	10.0	11.6
Ether extract	2.3	2.8	4.3
Crude fiber	34.1	48.0	41.4
N-free extract	38.9	25.1	29.6
Cellulose	33.1	36.4	32.7
Lignin	14.9	31.1	28.9
Other carbohydrate	24.9	5.6	9.3
New crude fiber	50.7	73.6	72.3
N-free extract	22.2	-0.4	-1.1
True fat	.518	1.089	.628
Iron	.025	.033	.0638

The coefficients of rumen digestion and of total digestion on the basis of both lignin and iron ratios are presented in Table II. In addition, the percentage of the total digestion that occurred in the rumen is shown.

Calculations on the basis of the lignin ratios indicate that other carbohydrate and new nitrogen free extract disappeared from the rumen at a very rapid rate and, in the latter instance, to the extent

Table II Coefficients of digestion at the 30-pound level

	In rumen, lignin ratio	Total, lignin ratio	Per cent of the digest. occurring in rumen	In rumen, iron ratio	Total iron ratio	Per cent of the digest. occurring in rumen
Protein	62.4	62.7	99.4	40.0	71.7	55.7
Ash	27.7	10.0	275.3	-15.3	31.8	-32.5
Ether extract	43.1	6.2	695.3	9.1	28.8	31.7
Crude fiber	32.1	37.2	86.4	-8.3	52.3	13.6
N-free extract	68.8	60.6	113.5	50.2	70.1	71.6
Cellulose	46.9	48.7	96.2	15.3	61.1	25.0
Lignin	0.0	0.0		-59.6	24.1	-71.1
Other carbohydrate	89.0	80.6	110.4	82.5	77.2	100.0
New crude fiber	30.1	26.4	114.1	-11.5	44.2	-20.7
New N-free extract	100.0	100.0	100.0	100.0	100.0	100.0
True fat	-1.2	37.3	-3.2	-61.6	52.5	-42.0

of 100 per cent. Nitrogen free extract and protein were digested to a lesser extent, while crude fiber and new crude fiber were only slightly over 30 per cent digestible. Almost half of the cellulose was digested in the rumen. It should be noted that there was no disappearance of true fat from the rumen, although a considerable portion of the ether extract was digested.

The lignin ratios gave values for total digestion that were only slightly above or considerably below those for rumen digestion. This resulted in the rumen digestion accounting for up to 695 per cent of the digestion of ether extract and slightly over 100 per cent of several other constituents.

Calculations on the basis of iron ratios gave lower values for rumen digestion and higher values for total digestion than did the lignin ratios. However, the amount of digestion of the various nutrients followed the same sequence. Iron ratios resulted in several negative coefficients of digestion in the rumen.

Table III Chemical analyses on dry matter basis at the 20-pound level

	Alfalfa hay	Rumen contents	Feces
	%	%	%
Protein	17.8	14.4	12.2
Ash	7.5	9.3	9.1
Ether extract	1.7	2.8	3.0
Crude fiber	30.1	45.1	40.3
N-free extract	42.7	28.2	35.1
Cellulose	33.4	36.8	31.5
Lignin	15.0	28.0	29.2
Other carbohydrate	24.3	8.4	14.8
New crude fiber	53.2	79.2	67.8
New N-free extract	19.7	-5.8	7.6
True fat	.475	.730	.330
Iron	.0155	.0375	.0503

The analyses of the alfalfa hay used in the second trial which was conducted at a 20-pound level of feeding is given in Table III, and it is readily seen that the composition of this hay very closely approximates that of the hay used on the 30-pound level. The lignin content was practically identical.

Table IV Coefficients of digestion at the 20-pound level

	In rumen, lignin ratio	Total, lignin ratio	Per cent of the digest. occurring in rumen	In rumen, iron ratio	Total iron ratio	Per cent of the digest. occuring in rumen
Protein	56.3	64.5	87.3	66.4	78.8	84.3
Ash	33.4	37.4	89.4	48.9	62.5	78.1
Ether extract	10.3	8.8	117.9	31.1	45.4	68.5
Crude fiber	19.5	31.0	62.8	38.2	58.8	64.9
N-free extract	64.4	57.7	111.6	72.7	74.6	97.4
Cellulose	40.6	51.4	79.0	54.4	71.0	76.6
Lignin	0.0	0.0		23.2	40.2	57.6
Other carbohydrate	81.2	68.7	118.2	85.6	81.9	104.4
New crude fiber	19.8	34.2	57.8	38.4	60.6	63.3
New N-free extract	100.0	80.0	125.0	100.0	88.0	113.5
True fat	17.2	64.1	26.9	36.4	88.5	41.1

Lignin ratios gave coefficients of rumen digestion at the 20-pound level, Table IV, lower than those observed at the 30-pound level except in the case of the new nitrogen free extract and true fat. It is of interest to note that while only 64.5 per cent of the old nitrogen free extract disappeared from the rumen, all of the new nitrogen free extract did. Although total digestion coefficients indicated crude fiber, other carbohydrates, and new nitrogen free extract as being slightly more digestible and the enzymatic crude fiber less digestible than at the higher level, the digestibility of the other constituents was practically the same. True fat was considerably more digestible on the lower level.

Results obtained by using iron ratios followed about the same trend as did those with lignin, but the values were higher for both rumen and total digestion.

Table V Chemical analyses on dry matter basis at the 10-pound level

	Alfalfa hay	Rumen contents	Feces
	%	%	%
Protein	15.5	10.2	8.8
Ash	7.4	9.3	9.0
Ether extract	1.9	2.6	1.3
Crude fiber	34.4	51.0	50.5
N-free extract	40.4	26.6	30.1
Cellulose	36.9	41.1	39.5
Lignin	17.7	31.5	32.0
Other carbohydrate	20.2	8.0	6.2
New crude fiber	57.7	83.7	86.0
New N-free extract	17.1	-3.0	-8.2
True fat	.590	.836	.370
Iron	.013	.0256	.038

The chemical composition of the hay, rumen contents, and feces for that period during which the rumen fistula animal received 10 pounds

of hay a day is given in Table V. The hay was slightly higher in fibrous material than the previous samples. In general, lignin ratios resulted in data that indicate a lesser degree of digestion than on either of the higher levels --- the exceptions being true fat, ether extract and protein, while new nitrogen free extract was 100 per cent digested on all three levels. There was less difference between the 10 and 20-pound levels than between the 20 and 30-pound levels. Several total digestion coefficients were lower than those for rumen digestion. The smallest percentage of total digestion that occurred in the rumen was obtained with true fat. Rumen digestion on the basis of iron ratios very closely approximated that found with the lignin ratios, but total digestion values were considerably higher.

Table VI Coefficients of digestion at the 10-pound level

	In rumen, lignin ratio	Total, lignin ratio	Per cent of the digest. occurring in rumen	In rumen, iron ratio	Total iron ratio	Per cent of the digest. occurring in rumen
Protein	62.7	68.5	91.5	66.5	80.6	82.4
Ash	39.1	32.3	121.1	36.3	58.3	62.2
Ether extract	25.0	61.7	40.5	32.6	76.4	42.6
Crude fiber	16.3	18.5	88.1	24.7	49.8	49.6
N-free extract	62.8	58.5	107.2	66.5	74.5	89.3
Cellulose	37.1	40.5	91.4	43.4	63.4	68.4
Lignin	0.0	0.0		10.0	38.4	26.1
Other carbohydrate	77.5	82.9	93.4	79.7	89.5	89.1
New crude fiber	18.1	17.2	105.2	26.3	49.0	53.7
New N-free extract	100.0	100.0	100.0	100.0	100.0	100.0
True fat	19.9	65.1	30.6	28.0	78.5	35.6

In Table VII the composition of the hay, rumen contents and feces for the fourth trial are shown. The animal again received 30 pounds of the hay. This hay contained about 40 per cent cellulose.

Table VII Chemical analyses on dry matter basis at the 30-pound level

	Alfalfa hay	Rumen contents	Feces
	%	%	%
Protein	14.5	13.0	10.5
Ash	6.8	8.9	10.1
Ether extract	2.7	3.0	4.3
Crude fiber	36.4	48.0	44.7
N-free extract	39.4	26.9	30.1
Cellulose	39.5	35.8	33.7
Lignin	16.4	28.6	33.1
Other carbohydrate	19.9	11.4	8.0
New crude fiber	55.6	73.6	74.8
New N-free extract	20.3	1.4	0.0
True fat	.498	1.097	.307
Iron	.017	.024	.0426

Calculations on the basis of lignin ratios are presented in Table VIII and these show a consistently lower digestibility than on the first trial with 30 pounds of hay except in the case of cellulose which remained about the same. The most important figure was that obtained for true fat. True fat failed to show any digestibility and remarkably increased 26.39 per cent over the amount originally con-

Table VIII Coefficients of digestion at the 30-pound level

	In rumen, lignin ratio	Total, lignin ratio	Per cent of the digest. occurring in rumen	In rumen, iron ratio	Total iron ratio	Per cent of the digest. occurring in rumen
Protein	48.6	63.8	76.2	36.6	71.9	50.9
Ash	24.3	26.2	92.6	6.5	40.7	16.1
Ether extract	36.9	20.2	182.9	22.1	35.8	61.8
Crude fiber	24.2	39.0	62.2	6.5	50.9	12.7
N-free extract	60.9	62.1	98.0	51.7	69.5	74.4
Cellulose	47.9	57.6	83.0	35.6	65.9	54.1
Lignin	0.0	0.0		-23.4	19.5	-54.5
Other carbohydrate	67.0	79.9	83.8	59.2	83.8	70.6
New crude fiber	24.1	33.2	72.4	6.3	46.3	13.6
New N-free extract	96.0	97.8	98.1	95.1	98.2	96.8
True fat	-26.3	69.4	-27.5	-56.0	75.4	-42.7

Table VII Chemical analyses on dry matter basis at the 30-pound level

	Alfalfa hay	Rumen contents	Feces
	%	%	%
Protein	14.5	13.0	10.5
Ash	6.8	8.9	10.1
Ether extract	2.7	3.0	4.3
Crude fiber	36.4	48.0	44.7
N-free extract	39.4	26.9	30.1
Cellulose	39.5	35.8	33.7
Lignin	16.4	28.6	33.1
Other carbohydrate	19.9	11.4	8.0
New crude fiber	55.6	73.6	74.8
New N-free extract	20.3	1.4	0.0
True fat	.498	1.097	.307
Iron	.017	.024	.0426

Calculations on the basis of lignin ratios are presented in Table VIII and these show a consistently lower digestibility than on the first trial with 30 pounds of hay except in the case of cellulose which remained about the same. The most important figure was that obtained for true fat. True fat failed to show any digestibility and remarkably increased 26.39 per cent over the amount originally con-

Table VIII Coefficients of digestion at the 30-pound level

	In rumen, lignin ratio	Total, lignin ratio	Per cent of the digest. occurring in rumen	In rumen, iron ratio	Total iron ratio	Per cent of the digest. occurring in rumen
Protein	48.6	63.8	76.2	36.6	71.9	50.9
Ash	24.3	26.2	92.6	6.5	40.7	16.1
Ether extract	36.9	20.2	182.9	22.1	35.8	61.8
Crude fiber	24.2	39.0	62.2	6.5	50.9	12.7
N-free extract	60.9	62.1	98.0	51.7	69.5	74.4
Cellulose	47.9	57.6	83.0	35.6	65.9	54.1
Lignin	0.0	0.0	--	-23.4	19.5	-54.5
Other carbohydrate	67.0	79.9	83.8	59.2	83.8	70.6
New crude fiber	24.1	33.2	72.4	6.3	46.3	13.6
New N-free extract	96.0	97.8	98.1	95.1	98.2	96.8
True fat	-26.3	69.4	-27.5	-56.0	75.4	-42.7

tained in the feed. Figures for total digestion followed more as would be expected than was true in any previous trials. With the exception of ether extract and true fat, from 72.5 per cent to 98 per cent of the total digestion occurred in the rumen. The iron ratios gave somewhat lower values for rumen digestion and higher values for total digestion.

In the fifth trial alfalfa-brome hay was used instead of alfalfa hay. As is seen in Table IX, this hay was considerably lower in cellulose than the alfalfa and was higher in true fat, protein, other carbohydrate and new nitrogen free extract.

Table IX Chemical analyses on dry matter basis at the 20-pound level

	Alfalfa-brome hay	Rumen contents	Feces
	%	%	%
Protein	18.4	15.1	12.7
Ash	7.3	10.5	10.2
Ether extract	2.5	2.1	5.1
Crude fiber	32.3	45.3	34.3
N-free extract	39.4	26.7	37.4
Cellulose	24.9	32.3	26.1
Lignin	16.5	33.5	29.5
Other carbohydrate	30.2	6.3	16.0
New crude fiber	42.8	64.3	67.9
New N-free extract	28.7	7.8	3.8
True fat	.908	.934	.351
Iron	.0125	.0293	.053

The values for rumen digestion coefficients as determined by lignin ratios, Table X, were higher than on the 30-pound level of alfalfa hay except in the case of cellulose and new nitrogen free extract. This does not follow as did the results for the previous 30 and 20-pound levels when both hays were alfalfa. Also the disappearance of true fat amounted to 49.1 per cent which is considerably higher than any previous values. Although total digestion coefficients indicate a negative digestibility for ether extract, true fat was very highly di-

tained in the feed. Figures for total digestion followed more as would be expected than was true in any previous trials. With the exception of ether extract and true fat, from 72.5 per cent to 98 per cent of the total digestion occurred in the rumen. The iron ratios gave somewhat lower values for rumen digestion and higher values for total digestion.

In the fifth trial alfalfa-brome hay was used instead of alfalfa hay. As is seen in Table IX, this hay was considerably lower in cellulose than the alfalfa and was higher in true fat, protein, other carbohydrate and new nitrogen free extract.

Table IX Chemical analyses on dry matter basis at the 20-pound level

	Alfalfa-brome hay	Rumen contents	Feces
	%	%	%
Protein	18.4	15.1	12.7
Ash	7.3	10.5	10.2
Ether extract	2.5	2.1	5.1
Crude fiber	32.3	45.3	34.3
N-free extract	39.4	26.7	37.4
Cellulose	24.9	32.3	26.1
Lignin	16.5	33.5	29.5
Other carbohydrate	30.2	6.3	16.0
New crude fiber	42.8	64.3	67.9
New N-free extract	28.7	7.8	3.8
True fat	.908	.934	.351
Iron	.0125	.0293	.053

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gested. Coefficients for both rumen and total digestion were higher on the basis of iron ratios than those with lignin.

Table X Coefficients of digestion at the 20-pound level

	In rumen, lignin ratio	Total, lignin ratio	Per cent of the digest. occurring in rumen	In rumen, iron ratio	Total iron ratio	Per cent of the digest. occurring in rumen
Protein	59.2	61.1	96.9	64.8	83.5	77.6
Ash	28.7	21.5	133.3	38.5	67.0	57.4
Ether extract	59.7	-12.3		65.2	52.7	123.7
Crude fiber	30.5	40.3	75.6	40.1	74.9	53.5
N-free extract	66.4	46.7	142.1	71.0	77.5	91.5
Cellulose	35.8	41.0	87.3	44.6	75.2	59.3
Lignin	0.0	0.0		13.7	57.9	23.7
Other carbohydrate	89.6	70.1	127.8	91.0	87.4	104.1
New crude fiber	25.8	11.1	232.6	36.0	62.6	57.5
New N-free extract	86.6	92.5	93.5	88.4	96.8	91.3
True fat	49.1	78.3	62.7	56.1	90.8	61.7

Alfalfa hay was fed at a 10-pound level in the sixth trial, and its composition, along with that of the rumen contents and feces is given in Table XI. Calculations on the basis of the lignin ratios,

Table XI Chemical analyses on dry matter basis at the 10-pound level

	Alfalfa hay	Rumen contents	Feces
	%	%	%
Protein	17.0	11.8	11.8
Ash	6.5	9.4	9.5
Ether extract	1.3	1.9	2.9
Crude fiber	38.1	52.0	45.0
N-free extract	36.9	24.7	30.7
Cellulose	31.3	37.8	33.2
Lignin	17.5	34.8	32.5
Other carbohydrate	26.1	4.0	10.0
New crude fiber	50.0	80.0	61.7
New N-free extract	25.0	-3.3	13.9
True fat	.755	.753	.690
Iron	.011	.0205	.035

Table XII, gave values that very closely approximated those on the 20-pound level of alfalfa-brome, with the exceptions of a lower digestibility of ether extract and crude fiber, and a greater digestibility of new nitrogen free extract. Several of the total coefficients of digestion were lower than the values obtained for rumen digestion. Again, as in the fifth trial, a negative digestion coefficient was obtained for ether extract, while the true fat was digested to a considerable extent.

Table XII Coefficients of digestion at the 10-pound level

	In rumen, lignin ratio	Total, lignin ratio	Per cent of the digest. occurring in rumen	In rumen, iron ratio	Total iron ratio	Per cent of the digest. occurring in rumen
Protein	64.8	62.4	103.7	62.6	78.1	80.0
Ash	27.4	21.2	128.9	22.9	54.2	42.2
Ether extract	23.8	-20.1		19.1	30.2	63.1
Crude fiber	31.1	36.1	86.2	26.8	62.9	42.7
N-free extract	66.1	55.0	120.2	64.0	73.8	86.7
Cellulose	38.9	42.6	91.4	35.1	66.6	52.7
Lignin	0.0	0.0		-6.2	41.9	-12.9
Other carbohydrate	92.1	79.2	116.2	91.6	87.9	104.2
New crude fiber	19.2	33.2	57.8	14.2	61.2	23.2
New N-free extract	100.0	69.7	143.3	100.0	82.4	121.3
True fat	49.6	50.5	98.2	46.5	71.2	65.2

Rumen digestion coefficients calculated on the basis of iron ratios were very similar to those with lignin, but total digestion coefficients were considerably higher.

The data presented in Tables XIII and XIV represent the same level of feeding and the same feed as those in Table XI and XII, the difference being that the rumen contents were removed 24 hours after feeding on the seventh trial rather than the usual 14 hours. There was only a relatively small difference between the digestion coefficients in this trial and those in the previous one. Ether extract was more highly

Table XIII Chemical analyses on dry matter basis at the 10-pound level 24 hours after feeding

	Alfalfa hay	Rumen content	Feces
	%	%	%
Protein	17.0	11.0	11.8
Ash	6.5	11.2	9.5
Ether extract	1.3	1.5	2.9
Crude fiber	38.1	51.5	45.0
N-free extract	36.9	24.6	30.7
Cellulose	31.3	34.6	33.2
Lignin	17.5	33.0	32.5
Other carbohydrate	26.1	8.5	10.0
New crude fiber	50.0	79.2	61.7
New N-free extract	25.0	-2.9	13.9
True fat	.755	1.167	.690
Iron	.011	.0148	.035

Table XIV Coefficients of digestion at the 10-pound level 24 hours after feeding

	In rumen, lignin ratio	Total, lignin ratio	Per cent of the digest. occurring in rumen	In rumen, iron ratio	Total iron ratio	Per cent of the digest. occurring in rumen
Protein	°65.4	62.4	104.8	51.8	78.1	66.2
Ash	8.6	21.2	40.6	-27.4	54.2	-33.5
Ether extract	38.5	-20.1		14.3	30.2	47.4
Crude fiber	°27.9	36.1	77.2	-0.5	62.9	-.8
N-free extract	°64.4	55.0	117.1	50.4	73.8	68.2
Cellulose	°40.9	42.6	96.1	17.7	66.6	26.5
Lignin	0.0	0.0		-39.4	41.9	-48.4
Other carbohydrate	-82.6	79.2	104.2	75.7	87.9	86.1
New crude fiber	-15.6	33.2	47.0	-17.6	61.2	-22.3
New N-free extract	°100.0	69.7	143.3	100.0	82.4	121.3
True fat	17.6	50.5	34.8	-14.9	71.2	-17.3

digestible, but other carbohydrate, new crude fiber, and true fat showed lower digestion coefficients. The most significant difference was the drop of the digestibility of true fat from 49.7 per cent at 14 hours to 17.6 per cent at 24 hours. Total digestion coefficients indicate a negative digestibility of ether extract, while true fat is over 50 per cent digestible. The iron ratios gave considerably lower results

for rumen digestion than the lignin ratios. Several negative coefficients of rumen digestion were obtained. Total digestion coefficients were considerably higher on the basis of iron than on the basis of lignin.

The average coefficients of rumen digestion, on the basis of lignin ratios, and of total digestion, as obtained by metabolism trials, are given for each level of feeding in Table XV. In addition the percentage of the digestion which occurred in the rumen is presented.

These data indicate that the plane of nutrition did not affect the rumen digestion of all the constituents in the same manner. As the level of feeding increased the digestibility of protein and true fat decreased. On the other hand, the digestibility of ether extract, crude fiber and new crude fiber increased as the plane of nutrition increased. Other carbohydrate was least digestible on the highest level of feeding but was equally digested on the other two levels. Cellulose was digested to about the same extent on the 20 and 30-pound levels but less digestible at the 10-pound level. Nitrogen free extract was digested to the same extent on all three levels and new nitrogen free extract was digested independently of the level of feeding.

Total digestion coefficients varied but little from the 20 to 30-pound levels of feeding. The differences were a slightly lower digestibility of nitrogen free extract, new nitrogen free extract and true fat on the higher level. On the other hand, cellulose was more digestible at the 30-pound level. At the 10-pound level total digestion coefficients were consistently lower for all constituents than those observed at either of the two higher levels. Although the ether extract gave a negative coefficient of digestion, the true fat was digested to the extent of 48 per cent.

Table XV Summary of rumen digestion and total digestion at different levels of feeding

	Coefficient of rumen digestion on basis of lignin	Coefficient of total digestion as obtained from metabolism trials	Percentage of the digestion occurring in the rumen
		30-pound level	
Protein	55.5	68.9	80.6
Ether extract	40.0	17.2	232.3
Crude fiber	28.2	45.6	62.0
N-free extract	64.9	60.0	108.0
Cellulose	47.4	50.9	93.3
Lignin	0.0	17.0	
Other carbohydrate	78.0	85.0	91.8
New crude fiber	27.1	39.5	68.8
New N-free extract	98.0	86.6	113.2
True fat	-13.8	65.3	-17.5
Iron	27.5	-21.6	
		20-pound level	
Protein	57.8	68.4	84.5
Ether extract	35.0	13.4	262.1
Crude fiber	25.1	44.9	55.8
N-free extract	65.5	66.6	98.4
Cellulose	38.3	51.4	74.4
Lignin	0.0	17.4	
Other carbohydrate	85.4	84.7	100.9
New crude fiber	22.8	37.1	61.6
New N-free extract	93.9	90.0	103.7
True fat	33.2	72.5	45.8
Iron	-22.8	-68.6	
		10-pound level	
Protein	63.8	64.7	98.6
Ether extract	24.5	-18.6	179.9
Crude fiber	23.7	36.2	65.7
N-free extract	64.5	56.9	113.4
Cellulose	38.1	44.1	86.2
Lignin	0.0	-0.6	
Other carbohydrate	84.9	80.2	105.8
New crude fiber	18.7	32.9	56.9
New N-free extract	100.0	72.3	138.3
True fat	34.8	48.0	72.5
Iron	-2.6	-72.5	

On all levels of feeding the more highly digestible materials as nitrogen free extract, other carbohydrate and new nitrogen free extract

showed over 100 per cent of the digestion as occurring in the rumen in about two-thirds of the cases, and well over 90 per cent in the other instances. At the lowest level of feeding over 98 per cent of the digestible protein was digested in the rumen while at the higher levels approximately 80 to 85 per cent was digested. About 93 per cent of the total cellulose digestion occurred in the rumen at the 30-pound level while lesser percentages of the total were digested on the lower levels. Between 60 to 70 per cent of the digestible crude fiber and new crude fiber were digested in the rumen. This contrast between cellulose and lignin digestion is of interest. It should be observed that the percentage of the total digestion of cellulose that occurred in the rumen was considerably higher than that of the new crude fiber, which indicates that the additional digestion occurring after the fiber passed from the rumen was the result of the digestion of the lignin content of the new crude fiber. The data indicate that the ether extract was much more highly digested from the rumen contents than from the feces. The rumen digestion of true fat accounted for a considerable amount of the total digestion of this material at the lower levels, but at the 30-pound level a negative digestion coefficient was observed in the rumen.

The average coefficients of digestion for lignin varied from -0.6 to 17.4 per cent. Individual variations were from -5.1 to 23.7 per cent. Both of these extreme values were obtained with A19, the rumen fistula cow. The lowest digestibility of lignin occurred at the 10-pound level. Iron balances indicated a highly variable retention of from -8.9 per cent to -99.8 per cent. On the basis of the lignin ratios the iron retention in the rumen was highly variable, just as was true with the feces.

Hydrogen-ion Concentration and Temperature of the Rumen

The results obtained from studies of the variation of pH during the day at different levels of feeding are given in Fig. 1. These data indicate that the level of feeding alfalfa hay had no definite effect upon rumen pH. After feeding in the morning, the pH fell for a period of five to six hours. An alkaline trend then began which reached a maximum either two hours prior to feeding or just before feeding in the afternoon. Feeding in the afternoon was followed by a sharp drop in pH. Variations in pH during the day did not exceed pH 0.5 at any of the levels of nutrition.

The pH values on the ration of alfalfa hay, silage and soy bean oil meal were distinctly lower than the values obtained with alfalfa hay alone. There was less variation during the day on the mixed ration but the acid trend and subsequent alkaline trend followed the same order.

The variations of pH in the different regions of the rumen are shown in Table XVI. The variation between samples was less than pH 0.3 for any one level, and for the average figures the maximum difference was pH 1.7. Although any one region was not consistently more alkaline than the others, generally the posterior dorsal region was most alkaline. The reticulum was slightly more alkaline than the rumen.

Table XVI Variations in pH values in different regions of the rumen

Region	Alfalfa hay			Mixed	
	30 pounds	20 pounds	10 pounds	ration	Average
Anterior dorsal	6.83	6.91	7.07	6.34	6.79
Anterior ventral	6.77	6.85	7.03	6.53	6.79
Center	6.81	6.88	6.94	6.37	6.75
Posterior dorsal	6.93	6.96	7.13	6.28	6.83
Posterior ventral	6.66	6.95	6.96	6.38	6.74
Reticulum	6.71	6.97	7.05		6.91

Fig. 1. pH values of the rumen contents during the day on different levels of feeding.

Variations in rumen temperature during the day and at different levels of feeding are given in Table XVII. The temperature was little affected by the time of day, although the highest readings were usually observed in the morning just following feeding. On the 20-pound level

Table XVII Variations in rumen temperature during the day at different levels of feeding and on a mixed ration

Hour	Alfalfa hay 30 pounds	Alfalfa hay 20 pounds	Alfalfa hay 10 pounds	Algalfa hay silage and S.B.O.M.
5 AM	39.2	38.7	38.7	
7 AM	39.6	36.8*	39.4	39.5
9 AM	38.8	38.2	38.4	38.8
11 AM	39.2	38.7	38.4	39.0
1 PM	39.0	39.0	38.7	39.1
3 PM	39.0	38.8	38.6	39.1
5 PM	38.8	36.0*	38.3	39.4
Average	39.1	38.0**	38.7	39.1
Rectal temp.	38.4	38.2	38.1	38.6

*Cow drank water just before temperature was taken
**Excluding two low values, the average is 38.7

two low values were obtained as a result of the animal's having drunk considerable water just prior to the taking of the temperature. The average rumen temperatures for the 10 and 20-pound levels, excluding the two low values, were identical, while at the 30-pound level and on the mixed ration higher temperatures were observed. Rumen temperature values varied from 36.0 to 39.6°C. with an average of 38.8°C. The rectal temperature averaged about 0.5°C. below the rumen temperature with an average value of 38.3°C.

Effect of the Level of Feeding on Rumen and Total Fill

The data presented in Table XVIII illustrates that the dry matter intaken did not affect either the weight of the rumen contents or the

dry matter contained therein. Despite large variations in the level of feeding, the weight and dry matter of the rumen contents varied only slightly with one exception. This indicates the constancy of rumen fill. When the contents were removed 24 hours after feeding, the weight and dry matter content were about half the amount usually found. The per cent of dry matter was not altered.

Although the weight of the animal and the barrel circumference were not affected by the changes in level of feeding for the first series of the three levels, on the second series both the weight and barrel circumference decreased as the level of feeding decreased.

Table XVIII Effect of the level of feeding on rumen fill, live weight and barrel circumference

Weight of Animal	Dry matter intake	Weight of rumen content	Per cent dry matter	Total dry matter	Rumen fill as per cent of live weight	Barrel circumference
Kgm.	Kgm.	Kgm.		Kgm.		cm.
367	11.9	61.7	14.3	8.8	16.8	197.6
377	8.2	62.1	15.6	9.7	16.5	198.3
376	4.1	63.1	16.0	10.1	16.8	196.7
406	12.2	62.1	13.7	8.5	15.3	208.4
404	7.7	51.3	12.9	6.6	12.7	204.0
383	4.0	59.9	14.2	8.5	15.6	193.3
368	4.0	29.3*	14.5	4.2	8.0	191.4

*Rumen contents removed 24 hours after feeding

With one exception, rumen fill accounted for about 16 per cent of the live weight. The rumen contents removed 24 hours after feeding, however, represented only 8.0 per cent of the live weight.

The effect of the level of feeding alfalfa hay on the barrel circumference is shown in Fig. 2. From the data presented, it is noted that the barrel circumference increased and decreased as the level of feed-

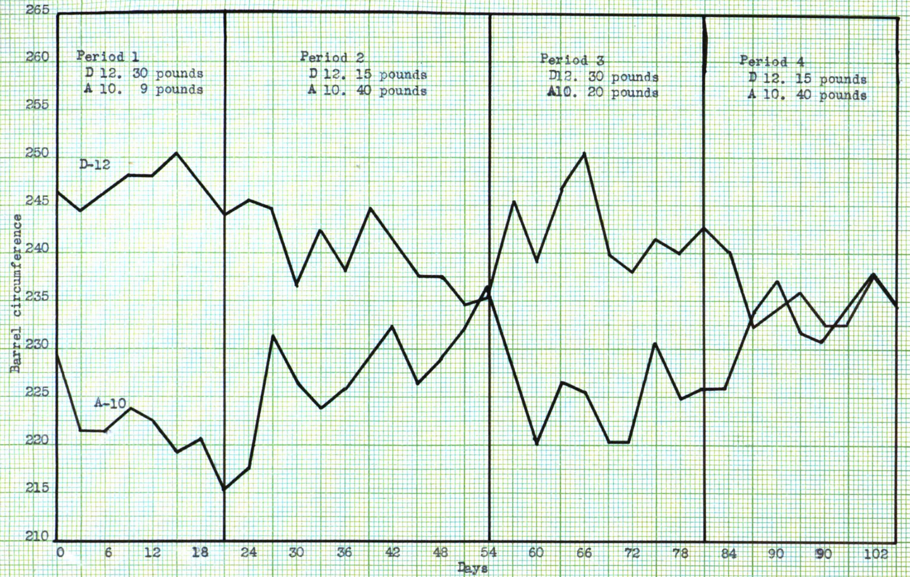

Fig. 2 Effect of the level of alfalfa hay feeding on the barrel circumference.

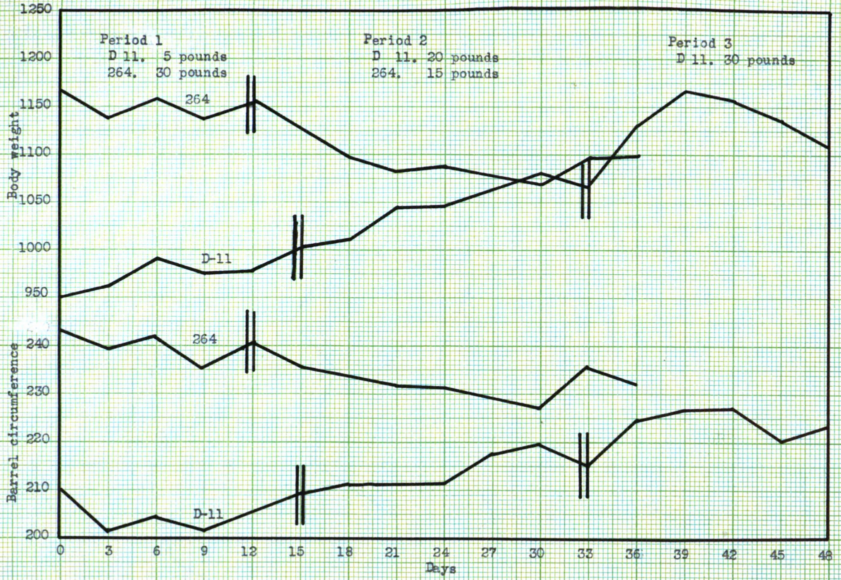

Fig. 3. Effect of the level of alfalfa hay feeding on barrel circumference and body weight.

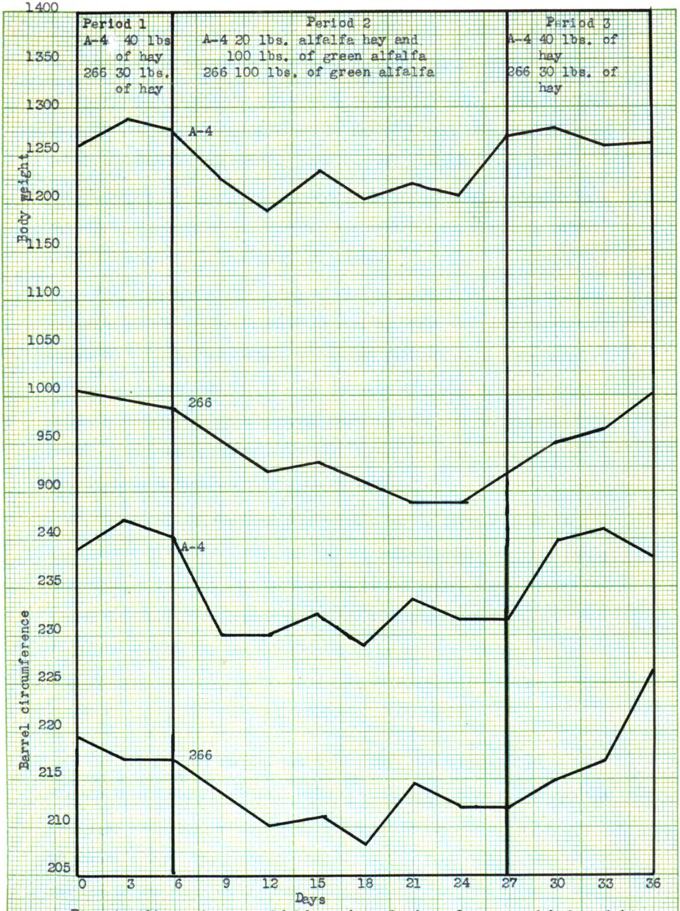

Fig. 4. Effect of green alfalfa on barrel circumference and body weight.

ing increased or decreased. An increase from nine pounds to 40 pounds of hay a day with A10 was followed by an approximately 15 cm. increase in barrel circumference. A 10 cm. change in barrel circumference following a change in the feeding level was not uncommon. Measurements for A4 during the third and fourth periods are not representative as she was approaching parturition and was normally increasing in barrel size independently of the level of feeding.

The influence of the level of feeding on body weight as well as on barrel circumference is shown in Fig. 3. With each successive increase in the level of feeding D11 showed an increase in both barrel circumference and body weight. An increase of almost 100 pounds in weight occurred in five days after changing from a 20 to 30-pound level. A decrease in body weight and barrel size was observed with 264 following a decrease in the amount of hay.

The effect of changing from alfalfa hay to green alfalfa is illustrated in Fig. 4. Although the dry matter intake was practically unchanged, a distinct fall in both weight and barrel circumference occurred immediately following the change to the green alfalfa. A return to alfalfa hay was accompanied by an increase in both values.

DISCUSSION

In the studies carried out during this investigation one rumen fistula animal and five normal animals were used. Alfalfa hay was the only feed that they received and it was fed in amounts of 10, 20 and 30 pounds a day. The rumen fistula animal was used to determine digestion in the rumen at the various levels and she was also used with

the other animals on metabolism trials to determine the coefficients of total digestion at different levels of feeding.

The rumen contents were removed in the morning before feeding, weighed, and samples taken for analysis. In one instance the rumen contents were removed 24 hours after feeding rather than at the usual 14 hour period. The digestibility of the feed in the rumen and total digestion was calculated by using lignin and iron ratios. These data were supplemented and checked by comparing them with the coefficients of total digestion as determined by the metabolism trials. Iron and lignin balances were made to further substantiate the accuracy of the calculated iron and lignin ratios.

Three different digestion coefficients are referred to in this discussion: (1) the coefficient of rumen digestion, calculated with either lignin or iron ratios; (2) coefficient of total digestion, calculated by lignin or iron ratios and referred to as such when mentioned, and (3) coefficients of total digestion, as determined by the metabolism trials.

The chemical analyses used in this study included the determination of crude fiber by an enzymatic method and the resulting fraction is referred to as new crude fiber and the nitrogen free extract remaining as new nitrogen free extract. Hydrogen-ion concentrations and temperature readings were made and rumen fill as influenced by various factors was studied.

Value of Iron Ratios in Studying Rumen Digestion

Although Bergeim (16) concluded from his studies that the iron index was a satisfactory method of studying digestion in certain species,

Knott and associates (60) recently reported that the iron ratio was not suitable for digestion studies in the ruminant. The results of this investigation confirm the findings of the latter investigators. As shown in Table XV, iron balances were highly variable and in most cases more iron was secreted than consumed. Because of the extremely variable excretion of iron in the feces, the application of the iron index to the determination of total digestion coefficients gave unusually high values which indicates that the iron index is not a reliable method for determining the coefficient of total digestion in the ruminant.

Although the iron index was shown to be unsuited for the estimation of total digestion, it was still possible that it might be useful in estimating rumen digestion. In this instance, however, the results indicated otherwise. The numerous negative coefficients of rumen digestion (Tables II and XIV) which were obtained by the application of the iron index to rumen digestion studies suggest the passage of iron from rumen ahead of the ingested material. In several instances, however, iron ratios gave slightly higher rumen digestion coefficients than the lignin ratios, which may indicate a slight tendency for iron to accumulate in the rumen. This variable and often considerable loss or concentration of iron in the rumen renders the iron index an unsuitable measure of rumen digestion and food utilization.

Value of Lignin Ratios in Studying Rumen Digestion

A review of the literature failed to reveal any previous attempts to ascertain either rumen or total digestion by means of lignin ratios. The lignin balances conducted in this study show that lignin is of

variable digestibility ranging from slightly negative coefficients of digestibility to over 20 per cent digestibility which is in agreement with the findings of other investigators (25) (26). The lignin index as a measure of total digestion may not be dependable because of the variable and often appreciable digestion of lignin. A comparison of Tables XII, XIV, and XV shows that the coefficients of digestion obtained by using the lignin index at the 10-pound level are almost identical to those found by actual metabolism trials. On the other levels, however, calculations with lignin ratios resulted in slightly lower total digestion coefficients than were actually found in the digestion trials.

The digestibility of lignin and the resulting error which is sometimes obtained in estimating total digestion by the use of lignin ratios suggest a possible similar error in rumen digestion calculations. The data obtained in these studies indicate a much smaller error in the results of determining rumen digestion than in total digestion evaluations. A comparison of the lignin and cellulose content of the alfalfa hay with the new crude fiber content reveals that the sum of the first two closely approximates the amount of the new crude fiber in the hay. Further observations (Table XV) show that a much larger portion of the digestible cellulose is digested in the rumen than of the new crude fiber. As the new crude fiber apparently consists of the cellulose and lignin fractions of the hay, the above observation indicates the additional digestion of new crude fiber after passing from the rumen is only slightly due to cellulose digestion and largely due to the digestion of lignin. It would seem that most of the lignin disappearing from the digestive tract was digested after the sample of rumen

contents had been removed. Because of this, any error which might occur in estimating rumen digestion would be much less than that obtained in the calculation of total digestion.

Any error resulting from the use of the lignin index would always be in the same direction, that is, rumen digestion would be underestimated and not overestimated. On the basis of the difference in the digestion of new crude fiber and of cellulose after leaving the rumen, the possible error resulting from the application of the lignin index was calculated for the 20-pound level. Similar calculations at other levels of feeding were not satisfactory. The figures obtained, however, showed that even with 10 per cent digestibility of lignin in the rumen and over 23 per cent in the feces, the coefficients of rumen digestion of the highly digestible materials as nitrogen free extract, other carbohydrate and new nitrogen free extract would not be in error. The percentage error in rumen digestion coefficients of ether extract, protein, and true fat was not appreciable. The largest error was in calculating the digestibility of fibrous materials and of these cellulose coefficients were more nearly correct. Differences would probably be even less for the other levels as more lignin was digested on the 20-pound level used in calculating the possible error than at any other.

These facts illustrate that the lignin index is a useful tool in ascertaining the utilization of food materials in the rumen. Any errors that might occur would be slight and negligible with the possible exception of fibrous materials, and in this instance, the error would always be in the same direction and could be allowed for.

Digestion of Various Nutrients in the Rumen

The coefficients of rumen digestion as calculated by using the lignin index reveal a disparity in the speed at which the various nutrients disappear from the rumen. The carbohydrate materials included in the nitrogen free extract, other carbohydrate, and new nitrogen free extract fractions rapidly enter solution and pass from the rumen. In most instances, the new nitrogen free extract disappeared to the extent of 100 per cent. The difference in the character of the new nitrogen free extract and the old nitrogen free extract is shown by the fact that the former almost completely disappeared from the rumen while less than 70 per cent of the latter disappeared. As shown in Table XV, more than 100 per cent of the digestible nitrogen free extract and related materials disappeared from the rumen in most cases. This indicates the rapid solution of these materials and their passage to the other stomach compartments. However, the rapidity with which such readily available material could be fermented by rumen bacteria should also be considered of possible importance in causing the speedy disappearance of these materials.

From 80 to 100 per cent of the digestible protein went into solution before leaving the rumen. The findings of Scheunert (104) indicated that this material was not digested in the rumen and, in view of the absence of protein splitting enzymes in the rumen, one would not suspect protein digestion there. The protein material which is digestible, however, is readily separated from the other food material and rapidly passed to the true stomach and intestine to be acted upon by proteolytic enzymes. Apparently that protein material which is not separated from the ingested hay while in the rumen is not digested to

any particular extent in subsequent passage through the remainder of the digestive tract.

A negative digestion coefficient of the ether extract (Table XV) has been recorded from time to time in the literature (4), and has frequently been referred to as being the result of the excretion of metabolic fat in the feces. The determinations of true fat which were made along with those of the ether extract, indicate that the so-called metabolic fat is of a non-fat character. Despite the increase in ether extract in the feces, the fat content was relatively low, and true fat was often digested to a high degree even though there was a negative digestibility of ether extract. The considerable excretion of ether soluble materials was thus not due to true fat materials but may have been due in part, at least, to the presence of unresorbed bile constituents.

Because of the inaccuracy of the methods that have been used in studying rumen digestion in the past, varying conclusions have been drawn from the same data. This is particularly true in regard to possible fat synthesis in the rumen (98) (69). By the use of lignin ratios a distinct increase was observed in the fat content of the rumen in one instance and a slight increase in another (Tables II and VIII). Of the seven samples taken in these studies, only two showed more than 20 per cent digestibility of true fat in the rumen and this is as would be expected, since fat is only removed from food material with difficulty. Krzywanek and Quittek (69) observed that fat disappeared from the rumen more slowly than did crude fiber. When the rumen contents were removed 24 hours after feeding, an increase in the fat in the rumen over that observed 14 hours after feeding was noted. These facts indicate that

the conclusion of Quittek (98) to the effect that fat is synthesized in rumen by microorganisms may be correct. It was also observed that the largest increase in fat in the rumen was noted on the highest level of feeding when the digestive processes would be progressing at the most rapid rate. The increase in fat brings up the consideration that all of the true fat in the feed may have been digested and that the fat which occurred in the feces was other fat material which possibly was synthesized by rumen bacteria.

Of the digestible fibrous materials, digestible cellulose was digested to a greater extent in the rumen than other materials. In some instances (Table XV) practically all of the digestible cellulose was digested in the rumen. This was to be expected in view of the special adaptation of the rumen for digesting such material and of the large number of cellulose splitting bacteria present. As any error in the calculation of the rumen digestion coefficient of cellulose would underestimate its digestibility, it is probable that an even larger amount was digested in the rumen than was indicated by the lignin ratios. In contrast to cellulose, only about 60 per cent of the digestible crude fiber and new crude fiber was usually digested in the rumen. Here again, as the digestibility of these two fractions would be underestimated more than those of any other constituents, they were probably somewhat more digestible in the rumen than indicated. Also it must be remembered that these rumen contents samples would have been subjected to further digestion in the rumen before passage to the other stomach compartments. Even considering these possibilities, a portion of the crude fiber fractions (probably lignin) is probably digested after passing from the rumen. This would lend weight to the conclusion of Csonka

anc co-workers (133) to the effect that the degradation of lignin occurs in the stomach of the cow and is not brought about by bacteria but possibly some enzyme of the gastric juice.

In Tables XII and XIV data are presented which show that the delay in the removal of rumen contents resulted in a slightly increased digestibility of the nutrients in the hay. This fact together with the relatively high percentages of total digestion which occurs in the rumen indicate that only a small portion of the digestible nutrients pass from the rumen without having been either completely digested or at least having entered into solution.

An increase in the plane of nutrition (Table XV) resulted in a decrease in the rumen digestion of protein and true fat. On the other hand, however, crude fiber, new crude fiber and ether extract were digested to a greater extent as the level of feeding increased. Cellulose digestion in the rumen was not affected except on the 10-pound level at which less was digested. Other carbohydrate was least digestible at the 30-pound level but was not variable on the other levels. New nitrogen free extract was most digestible in the rumen at the 20-pound level while the rumen digestion of nitrogen free extract was not affected by the plane of nutrition.

Total digestion coefficients at the 20 and 30-pound level were affected to a lesser degree than the rumen coefficients, but at the 10-pound level there was a decreased digestibility of all nutrients. The effect of the plane of nutrition on total digestion is discussed in detail in Part II of this thesis.

Rumen pH and Temperature Readings

The pH values obtained during this investigation tend to be neutral or slightly acid which is in agreement with most recent findings (56) (64) (82). The level of feeding did not seem to have any definite effect upon rumen pH.

Considerably higher pH values were observed on alfalfa hay alone than on the mixed ration. This difference has been previously observed by several investigators (56) (31). Since Koffman (62) observed that soy meal and several other concentrates lowered rumen pH, and as Monroe and Perkins (82) found acid silage only slightly affected pH, it is probable that the soy bean oil meal was of more influence in lowering the pH than the silage. The greater ease with which concentrate materials are fermented may be a factor in the lowered pH.

The maximum acidity during the day was reached about five to six hours after feeding as compared with eight hours as observed by Kick and associates (56) and three or four hours as observed by Monroe and Perkins (83). It would seem that on the average the lowest pH reading is found about six hours after feeding which suggests a decrease in fermentation activity near this time.

The variations between samples from different regions of the rumen was greater than those observed by other investigators and, as was also noted by Monroe and Perkins (83), the posterior region was generally a little more alkaline than the other regions. Data were obtained which indicate that the reticulum is slightly more alkaline than the rumen, which is in agreement with the reports of Ferber (36).

Rumen temperature readings varied independently of the time of

feeding and averaged 38.8°C. The consumption of water caused a considerable drop in temperature but the readings rapidly returned to normal. The temperature was higher at the 30-pound level and on the mixed ration than at the 10 and 20-pound levels. This was also true in the case of rectal temperature which indicates that the variations might not have been entirely due to the feeding. The average temperature is about the same as the value of 39°C. reported by Koffman (62). Rumen temperature was 0.5°C. higher than rectal temperature, a difference which probably is due to the more active fermentation process of the rumen.

Effect of the Level of Feeding and Character of the Feed on Rumen Fill

The data found in connection with rumen fill indicate that neither the amount of total content nor the dry matter content of the rumen is affected by the level of feeding (Table XVIII). As great a variation as from 10 to 30 pounds of hay a day did not alter rumen fill. These findings are supported by data reported by Ewing and Wright (35). When the rumen contents were removed 24 hours after feeding rather than 14 hours the weight was reduced one-half. The percentage, however, remained the same.

Rumen fill accounted for about 16 per cent of the live weight. The results of Ewing and Wright (35) and Nevens (89) indicate that about 70 per cent of the total fill is rumen fill. On the basis of this value the total fill in this animal would be almost 23 per cent of the live weight. This value is much higher than the average of 12 per cent usually found in the literature. Ritzman and Benedict (100) suggested that the fill in lactating dairy cows would probably account for 20 per cent of the body weight, and quoted some instances where it accounted for about 30 per cent. The above results indicate that even for non-lactating dairy animals the fill may be well over 20 per cent of the live weight.

This fairly constant weight of the rumen contents was not indicated, however, by the changes in body weight and barrel measurements. In experiments with the normal cows an increase in the level of feeding alfalfa hay resulted in a progressive increase in body weight and barrel size, and as the feed was decreased both of these values declined (Figs. 2 and 3). Similar variations were usually observed with the rumen fistula cow.

When the dry matter in alfalfa hay was replaced with the dry matter in green alfalfa a distinct drop in both body weight and barrel size occurred which is as would be expected with green feeds.

These data show that changing to rations where less water is consumed results in decreased barrel size and body weight. The moisture content of the rumen, however, remained the same on different levels of feed.

SUMMARY AND CONCLUSIONS

1. One rumen fistula cow and five normal cows were used to study rumen digestion. They received alfalfa hay as the only feed, and the hay was fed at 10, 20 and 30-pound levels.
2. Rumen contents were removed 14 hours after feeding, weighed, and samples taken for analyses on six occasions. The chemical analyses which were made were moisture, ash, protein, ether extract,

crude fiber, nitrogen free extract (by difference), cellulose, lignin, other carbohydrate (by difference), new crude fiber (enzymatic) and new nitrogen-free extract (by difference).

3. Rumen digestion coefficients were determined by means of iron and lignin ratios. These calculated coefficients were supplemented and checked by the total digestion coefficients and iron and lignin balances which were determined by metabolism trials.

4. The iron index is not a reliable method for determining either the coefficient of rumen digestion or of total digestion.

5. The lignin index is a useful and relatively accurate tool in ascertaining the coefficients of rumen digestion.

6. Digestible nitrogen-free extract, other carbohydrate and new nitrogen-free extract disappear very rapidly and in most cases completely from the rumen.

7. From 80 to 100 per cent of the digestible protein entered solution while in the rumen.

8. True fat ordinarily left the rumen slowly and in one instance a 26.4 per cent increase in true fat was observed. This seems to indicate the possible synthesis of fat in the rumen.

9. Cellulose was approximately 50 per cent digestible and as would be expected practically all of the digestion occurred in the rumen. The digestible portions of the crude fiber and new crude fiber were only slightly over 60 per cent removed by rumen digestion. This indicates that the digestible lignin may be only partially digested in the rumen and largely digested after passing on to the other parts of the digestive tract.

10. As the plane of nutrition increased, the rumen digestion of protein and true fat decreased, while that of crude fiber, new crude fiber

and ether extract increased. Cellulose was least digested at the lowest level of feeding and other carbohydrate at the highest level. The rumen digestion of nitrogen-free extract did not vary with the plane of nutrition and new nitrogen-free extract was most digestible on the medium level.

11. The pH of the rumen contents is neutral or slightly acid and does not seem to be affected by the level of hay feeding. Alfalfa hay causes a more alkaline reaction than a ration of hay, silage and concentrate. During the day pH values decrease for about six hours after feeding and then rise until a maximum is reached just before feeding in the afternoon.

12. Rumen temperature readings averaged 38.8°C. which was 0.5°C. higher than the rectal temperature.

13. Rumen fill, which accounted for 16 per cent of the live weight, was unaffected by the amount of hay consumed. However, an increase in hay consumption resulted in an increased body weight and barrel circumference. Green feeds cause a decrease in body weight and barrel size when replacing the dry matter of hay.

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APPENDIX

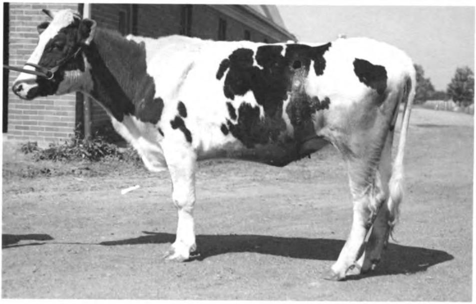

Fig. 5

A 19, the rumen fistula cow

used for studies on rumen digestion

The Digestibility of Alfalfa Hay with Special Reference to Rumen Digestion

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II The Digestibility of Alfalfa Hay with Reference to the Crude Fiber and Ether Extract Fractions and the Plane of Nutrition

INTRODUCTION

Alfalfa hay and other roughages consist predominately of fibrous materials and contain a very small amount of ether extract. The crude fiber may contain non-lignified cellulose which is highly digestible or it may consist of lignified cellulose which is of low digestibility. Also by the present method, some of the crude fiber fraction is found in the nitrogen-free extract. A further division of the carbohydrates into lignin, a practically inert material, and cellulose and nitrogen-free extract, more highly digestible fractions, has been suggested. The data supporting such a separation, however, is limited.

The ether extract of roughages is made up largely of chlorophyll and waxes with a small amount of true fat. Since chlorophyll and waxes are of little biological value, it is apparent that the determination of true fat would be more significant from the standpoint of nutrition.

It has been generally assumed that the various nutrients are less digestible at the higher planes of nutrition. However, the data supporting this are conflicting and indicate some nutritents may be affected while others are not. Also recent reports indicate that the level of feeding may affect rations of roughage alone differently than mixed rations. More studies along this line are needed.

REVIEW OF LITERATURE

The Composition and Digestibility of Alfalfa Hay

In 1912 Fraps (18) summarized the data on the composition and digestibility of alfalfa as reported by various experiment stations. The average data follows:

	Protein	Fat	Crude fiber	N-free extract	Water	Ash
			Composition of alfalfa hay			
Alfalfa hay, average	14.42	1.97	29.98	35.81	9.61	8.41
Alfalfa hay, 1/10 bloom	16.88	1.42	29.38	34.01	8.77	9.54
Alfalfa hay, 1/2 bloom	15.88	1.25	31.41	34.23	7.71	9.49
Alfalfa hay, full bloom	13.23	1.30	33.11	36.34	8.29	7.75
			Digestibility of alfalfa hay			
Alfalfa hay, average	75.27	40.57	46.37	68.43		
Alfalfa hay, 1/10 bloom	78.52	60.00	46.10	75.31		
Alfalfa hay, 1/2 bloom	75.14	30.30	50.44	71.99		
Alfalfa hay, full bloom	76.78	51.65	50.63	75.24		

Morrison (37) gave the following composition and digestibility of alfalfa hay:

	Protein	Fat	Crude fiber	N-free extract	Water	Ash
Alfalfa hay, average	14.7	2.0	29.0	36.4	9.6	8.3
Alfalfa hay, before bloom	19.0	2.7	22.3	36.6	9.8	9.7
Alfalfa hay, 1/10-½ bloom	14.9	1.7	30.1	35.0	8.9	8.9
Alfalfa hay, ¾-full bloom	14.0	2.0	30.3	35.8	8.3	8.3
Alfalfa hay digestion coefficients	72.0	32.0	43.0	71.0		

These data not only show the average composition and digestibility of alfalfa but also show that as it approaches full bloom, there is a decrease in protein content and an increase in crude fiber. The other constituents followed no definite trend.

Sotola (59) compared the composition and digestibility of alfalfa hay of the first, second, and third cuttings. The following data were obtained:

Cutting	Dry matter	Crude protein	Crude fiber	N-free extract	Fat
			Composition		
1st	87.81	11.96	34.54	35.64	1.46
2nd	86.08	14.05	36.37	29.60	2.02
3rd	81.46	15.81	23.53	40.02	2.83
			Coefficients of Digestion		
1st	52.8	62.5	43.3	65.2	14.4
2nd	55.5	69.8	34.3	70.5	39.5
3rd	60.5	74.0	42.3	77.2	28.2
Ave.	56.3	69.7	40.0	71.0	24.0

Headden (24) determined the chemical composition of alfalfa hay in the early bloom stage. A rather complete chemical study was made, and the composition was as follows:

	Per cent
Invert sugar	None
Sugar	Trace
Dextrin	Trace
Starch	11.1
Xylan, inverted by dilute alkali	3.76
Xylan, soluble in alkaline solution	0.15
Lignine, rendered soluble by chlorine	6.66
Cellulose	25.59
Moisture	7.21
Ash	8.81
Ether extract	1.15
Proteids	15.16
Soluble in alcohol	13.87
Soluble in water (starch, etc. deducted)	11.88
Not determined	3.65

Fraps and Rather (21) studied the components of the ether extract of alfalfa hay and other roughages. He found the following percentage of the ether extract of alfalfa.

Total ether extract	1.26
Unsaponifiable	67.00
Saponifiable	33.00
Free fatty acids	33.00

The unsaponifiable material was composed mainly of wax alcohols, and the saponifiable contained fatty acids, chlorophyll products and perhaps other substances. The average digestibility of the constituents of the ether extract were:

Total ether extract	4.9 per cent
Saponifiable	59.1 per cent
Unsaponifiable	Indigestible

These investigators attributed the low digestibility of the ether extract of hays and fodders to the presence of wax alcohols, waxes, chlorophyll, and other substances not as easily digested as the free fatty acids.

Wilson and Webb (67) observed that the water soluble carbohydrate content of alfalfa at the silage stage was 4.32 per cent and when beginning to bloom, 4.26 per cent. Fraps (19) made detailed studies of the composition and digestibility of the constituents of the nitrogen-free extract of alfalfa hay.

Alfalfa hay	Reducing sugars	Polysaccharoses	Starch	Pentosans in N-free extract	Resid. N-free extract	Total pentosans	Pentosans in crude fiber
Percentage composition	1.92	1.65	1.90	9.21	22.44	14.00	4.91
Coefficients of digestion	97.	98.	86.	56.	69.	53.	41.

He pointed out that the nitrogen-free extract contained some chlorophyll, organic acids, and ligno-cellulose as well as sugars, starch, pentosans and other carbohydrates. Roughages in general contain about 20 per cent pentosans and, as a rule, those pentosans in the nitrogen-free extract are digested to a greater extent than those in the crude fiber. The lowered digestibility of the nitrogen free extract was accounted for in part by its pentosan content.

Guanzon and Sandstrom (22) recently determined the composition of the nitrogen-free extract of alfalfa and presented the following results:

				Sugars and Starch			
Crude fiber	Pentosans in crude fiber	Nitrogen -free extract	Uronic acid anhydrides	Reducing sugar	Sucrose	Starch	Total
30.31	5.03	40.37	10.40	1.76	1.65	1.44	4.85

Composition of nitrogen-free extract

Uronic acid anhydrides	Sugars and starches	Pentosans total	Residual nitrogen-free extract
25.76	12.01	25.51	62.50

These investigators suggested that the lowered digestibility of the nitrogen-free extract in roughages may be due to their high content of uronic acids.

Headden (24) found the cellulose content of alfalfa hay to be 25.59 per cent and the lignin (rendered soluble by chlorine) content 6.66 per cent. Williams and Olmstead (66) studied the composition of alfalfa leaf meal and found it to be as follows:

	Per cent
Cellulose	32.5
Lignin	15.0
Hemicelluloses (S)	15.5
Hemicelluloses (L)	3.7
Starch	6.8
Protein	6.6
Ash	4.4
Soluble in alcohol benzene	3.5
Moisture	8.9

(L) Soluble in solutions of pH 8
(S) Insoluble in solutions of pH 8

The composition of alfalfa varies considerably with the stage of maturity. Recently Hunt and co-workers (27) reported that with an advance in the stage of maturity the protein decreased from 22.31 to 12.94 per cent; the ether extract fell intermittently from 3.01 to 1.63 per cent; the crude fiber increased steadily from 18.58 to 34.76 per cent; and the nitrogen-free extract varied only slightly. Other investigators have observed similar changes (60).

Although the growth of the alfalfa plant has not been followed with separate determinations for cellulose and lignin, the above increases in crude fiber indicate a considerable increase of these constituents as Headden (24) found the crude fiber of alfalfa contained 79 per cent cellulose and 21 per cent lignin. Norman (40) observed 97 per cent of the crude fiber of hay was constituted by cellulose and lignin to the extent of 85.7 and 10.9 per cent, respectively.

The cellulose, lignin, and hemicellulose contents of other plants have been followed as the plant matured, however, and such results probably indicate a somewhat similar process of development in alfalfa. Norman (39) followed the cell wall constituents of barley from one to fifteen weeks of growth. Cellulose increased from 26.0 to 39.8 per cent and lignin had increased from 14.4 to 19.7 per cent by the end of thirteen weeks following which it fell to 17.6 per cent. Xylan, the chief hemi-

cellulose of many feeds, increased from 3.3 to 11.3 per cent. More recently Norman (41) made similar studies with rye grass and found the same general trends in composition. Phillips and Goss (44) observed the pentosans of barley increased from 9.04 to 23.87 per cent from the seventh to the eighty-sixth day of growth. During the same period cellulose rose from 19.0 to 31.6 per cent and lignin from 1.48 to 7.74 per cent. The methoxyl content of the lignin increased with age. Lignin in the young plant differed from that in the old plant in that the latter contained a much higher percentage of methoxyl. In mature plants 75 to 80 per cent of the firmly bound methoxyl groups are found in lignin.

Nutritive Value of Crude Fiber

Limitations of the Present Crude Fiber Determination

That the inadequacy of the Weende method has long been recognized was pointed out by Williams and Olmstead (65). These investigators drew attention to the fact that the crude fiber obtained by this method has an inconstant chemical composition; that is, it determines a variable amount of cellulose, hemicellulose, and lignin. Remy (49) demonstrated that a considerable portion of the fiber in the Weende and Koenig methods was decomposed and hence escaped reckoning in the biological evaluation of the material, and, thereby, was likely to render the calculation of the energy value foodstuffs erroneous. Norman (40) pointed out that the crude fiber fraction does not bear a definite relationship to any particular plant constituent or group of constituents, or to the crude fiber of any other plant material. He found that 97 per cent of the crude fiber fraction was accounted for by lignin and cellulose in the amounts of 85.7 per cent cellulose and 10.9 per cent lignin. Headden (24) reported that crude fiber contains 79 per cent cellulose and 21 per cent lignin. Norman (40) observed that there was a highly variable lignin content in crude fiber, and a crude fiber high in lignin was not necessarily from a highly lignified material. He studied the recovery of cellulose and lignin in the crude fiber of several plant materials as compared with the amount in the original material. Cellulose, with one exception, represented a recovery of 60 to 80 per cent, and the lignin showed a much greater variability of from 4 to 67 per cent recovery of that present in the original material. This illustrated that the cellulose was partially attacked and the lignin was extensively removed by the present crude fiber method. In hay only 72 per cent of the cellulose and 35 per cent of the lignin was recovered in the crude fiber. Norman also pointed out that in view of the inert character of lignin and its influence on the digestibility of other plant constituents, the essential requisite of any method for evaluating the more resistant cell wall material is that a fraction should be given which includes all lignin and not a small and variable portion as does the present crude-fiber determination. The Weende method also results in the extensive but variable removal of hemicelluloses (9) (49) (65).

Although crude fiber is supposedly the poorly digested portion of rations and feeds and the nitrogen-free extract the highly digested fraction, Fraps (19) observed that the crude fiber of roughages often was more digestible than the nitrogen-free extract. Fraps (20) presented data to show that there was no correlation between the amount of crude fiber in a feed and the extent of its digestibility. He also observed a species difference in the ability of animals to utilize crude fiber.

Feed	Fiber	N-free extract	Species	Digestibility fiber	Digestibility N-free extract
	%	%		%	%
			pigs	33	93
Corn	3	72	ruminants	31	92
			poultry	13	90
			pigs	11	79
Oats	11	60	ruminants	42	82
			poultry	7	69
			pigs	21	66
Alfalfa	33	35	ruminants	46	69
			poultry	1	34

Crampton and Maynard (9) summarized the digestion coefficients of crude fiber and nitrogen-free extract found in Morrison's standards.

Relative digestibility of Weende crude fiber and nitrogen-free extract

Kind of feed	Number Averaged	Average Coefficient of digestibility Crude fiber	N-free extract	Per cent of cases with crude fiber showing as complete digestion as N-free extract
Dry roughages	110	52.4	59.5	39
Green roughages	61	63.5	76.3	20
Pasture herbage*	12	75.5	71.4	67
Silages	25	58.2	64.6	28
Concentrates	88	53.3	78.5	10
All feed	284	55.6	69.5	25

*Crampton (35)

It should be noted that dry roughages, the major feed of ruminants, showed as complete a digestion of crude fiber as nitrogen-free extract in over one-third of the cases and that of pasture in two-thirds of the cases. The attempt to divide roughages into highly digestible and poorly digestible portions by use of the present crude fiber method is of questionable value.

In order to overcome the faults and limitations of the present method, Remy (49) developed an enzymatic method. Williams and Olmstead (65) discussed the methods intended as improvements on the old Weende technique and pointed out that all of these methods, except Remy's, depended upon the incomplete solubility in certain reagent of one or more constituents of indigestible residue. Remy's determinations gave values about 100 per cent higher than the Weende or Koenig method, although the materials obtained by the three methods were practically identical in composition. Williams and Olmstead (65) modified Remy's method and separated the components of the indigestible residue into three fractions. More recently Horwitt and co-workers (26) slightly modified Remy's determination, and, with determinations of the crude fiber in the spinach leaf, found that the enzymatic method gave results which were more than three times as large as those obtained by the present official method. The following table from Williams and Olmstead (65) illustrates the greatly increased effectiveness of the enzymatic method in recovering the components of crude fiber.

Material Analysed	Enzyme method (1)	Weende method (2)	Difference 2/1
	mg.	mg.	per cent
25 ml. stool suspension 1	212.5	118.0	55.5
25 " " " 2	249.0	121.0	48.5
25 " " " 6	181.0	117.7	64.7
25 " " " 10	226.3	88.0	39.0
0.5 gm. (80 mesh) wheat bran	273.7	94.6	34.6
0.5 " " " cellu flour	490.0	276.2	56.4
0.5 " " " filter paper	490.0	499.5	100.0

Crampton and Maynard (9) proposed the partition of the carbohydrate fraction of feed into cellulose, lignin, and other carbohydrates. The greater biological significance of this method over the Weende method

is well illustrated in the following table from Crampton (8).

Comparison of schemes of carbohydrate partition
in feed analysis

Animals	Digestion of Carbohydrates				
	Proposed Scheme			Standard Analysis	
	Lignin	Cellulose	Other carbohydrate	Crude fiber	Nitrogen-free extract
4 steers	34	68	92	66	67
6 rabbits	18	33	62	24	45
60 rabbits	3	25	67	22	44

This method separated (1) a highly digested fraction, other carbohydrate, (2) a poorly digested fraction, lignin, and (3) cellulose, the digestibility of which may be expected to vary inversely with the lignin. The crude fiber determination, on the other hand, did not indicate a sharp biological division of the carbohydrate, particularly in the case of steers.

Mangold (34) presented the results of Koenig which showed the variable digestibility of the various materials present in the crude fiber which further illustrates the advantage of separating at least the most predominant of such materials.

Comparative digestibility of various fractions
of crude fiber

Animal	Feed	Crude fiber	Cellulose	Pentosan	Lignin	Cutin
	Meadow hay	70.27	83.40	82.06	16.69	7.27
Sheep	Clover hay	56.96	56.72	76.41	23.73	----
	Pea straw	42.45	51.09	44.02	28.36	20.93

Digestibility of Cellulose

The chemical and physical properties of cellulose were recently reviewed by Norman (42). Normal cellulose belongs to that class of carbohydrates known as polysaccharides and has a chemical formula of $(C_6H_{10}O_5)n$. Miller (35) discussed the various types of cellulose found in plants. Cellulose makes up from 40 to 60 per cent of mature plants and woods. In young green plants the cellulose content is rather low, but even in these cellulose is the chief structural constituent.

In 1855 Haubner (23) found that cellulose was digested and utilized by several species, particularly by ruminants which digested 50 per cent of it. The importance of the rumen microflora in cellulose digestion has been discussed in detail in Part I of the thesis. The extent to which cellulose is digested may vary from almost zero to over 90 per cent, depending upon the character of the plant material, which in the same plant is materially affected by the stage of maturity. Miller (35) pointed out that as the plant grows the tissue consists of cells with cell walls of protein and cellulose, filled largely with protein; adjacent cells are cemented with pectins and hemicellulose material. As the plant matures, protein of the walls disappears and is replaced by a deposit of hemicellulose and later by lignin, and all cell contents tend to disappear. In the ultimate condition, as reached in wood, the cell wall consists almost entirely of lignin and encrusting hemicellulose.

This process of lignification as the plant matures was demonstrated by Norman (39) who observed an increase in lignin from 14.4 to 19.7 per cent followed by a drop to 17.6 per cent. Cellulose rose from 26.0 to

29.8 per cent, and xylan, the chief hemicellulose of many plants, from 3.3 to 11.3 per cent. Phillips and Goss (44) not only found an increase in lignin of barley from 1.48 to 7.74 per cent but also observed that the nature of the lignin changed as the plant matured. That is, the methoxyl content of lignin increased with age. That the lignin and content of plants increases rapidly with approaching maturity has been demonstrated by many other investigators (41) (60) (27).

Because of the low digestibility of lignin, the process of lignification results in the lowering of the digestibility of the crude fiber of plant materials. Woodman (68) pointed out that lignification has more far reaching consequences than this. As the cell wall will be less readily broken down in the lignified plant, the cell contents may not be readily accessible for digestion and, for this reason, the protein and carbohydrate in lignified fodders as hay and straw are poorly utilized. His results which are shown in the following table, demonstrate the effect of lignin on the digestibility of all plant constituents.

Digestion coefficients of constituents of grass in its young, leafy stage of growth and at the hay stage of maturity

Constituent	Young, leafy grass per cent	Grass at hay stage of maturity per cent
Fiber	84.2	52.4
Carbohydrate	87.4	53.0
Protein	85.4	50.0
Oil	60.0	30.0

Crampton (8) presented data illustrating the effect of lignin on cellulose digestion in rabbits. These data are shown in the following table.

Effect of lignin on the digestibility of cellulose

Date of clipping		Ave. 28-day gain of rabbits	Per cent lignin in diet	Coefficients of Digestibility: Dry matter	Protein	Ether ex-tract	Cellulose	Lig-nin	Other carbo-hydrate
May	12	214	11.1	44	64	35	27	2	66
June	3	230	10.6	44	70	39	30	5	53
June	20	173	11.9	44	60	10	25	7	69
July	9	104	13.0	38	60	16	14	0	66
July	24	205	11.2	45	62	18	28	3	70
Aug.	20	239	11.5	44	53	18	27	3	76

Prjanischnikov and Tomme (46) found that the digestion coefficient of the fiber of rye straw after treatment with Cl O_2 was increased from 15 to 79 per cent. As there was a marked fall in lignin content following treatment, the increase in digestibility of the fiber was attributed to partial removal of the lignin. Kellner (30) removed the incrusta of straw (silica and lignocellulose) by treating it with alkaline reagents and observed the digestibility of cellulose in the residual product was extremely high --- that is, 95.8 per cent by cattle. Ustyantzev (61) reported that by freeing cellulose of wheat straw, low in nutritional value, from its incrusting substances the value of the straw became equal to the isodynamic qualities of starch and sugar as a protector of protein and fat. Fingerling and associates (14) showed that pigs digested crude fiber of treated straw better than sheep, but the fiber of untreated grass and wheat chaff was digested much better by the sheep. He ascribed the difference to the inability of the pigs to disintegrate the lignified coating of cellulose.

These results indicate that the digestibility of cellulose and crude fiber is inversely proportional to the lignin content of the plant

material consumed. This is further borne out by the data of Woodman (68) which shows the digestibility of cellulose in certain lignified and non-lignified feeding stuffs.

Digestion coefficients of cellulose in non-lignified and lignified feeding stuffs (trials with sheep).

	Digestion coefficient of cellulose
Non-lignified feeding stuffs	
Wet sugar beet pulp	89.8
Dried sugar beet pulp	89.7
Mangolds	78.0
Cabbage	74.0
Sugar beet tops	71.0
Lignified feeding stuffs	
Oat straw	54.0
Wheat straw	50.0
Undecort. cotton cake	37.0
Linseed cake	32.0
Wheat bran	26.0
Palm kernel cake	21.0

Woodman and Stewart (70) observed that the digestibility of fiber did not vary directly with the lignin content of the plant and concluded that it is not necessarily the amount of lignin but the manner of its deposition that determines the digestibility of fiber. Norman (40), however, pointed out that the results of Woodman and Stewart were more than likely due to the faults of the crude fiber determination rather than to the deposition of lignin. Williams and Olmstead (65) stated native cellulose and hemicellulose in the absence of lignin vary greatly in their resistance to chemical treatment, and variable results might be due to this rather than to the protective effect of lignin.

Woodman and Stewart (70) observed that the process whereby green crops become lignified and of reduced digestibility is associated with the production of relatively small amounts of lignin. This fact together with the observation of Phillips and Goss (44) that the lignin of mature plants contains a much higher percentage of methoxyl groups than does that of young plants suggested the possibility that the nature of the lignin as well as the amount present may be a factor in its lowering the digestibility of other constituents. More recently Crampton and Maynard (9) have suggested the antiseptic action of lignin, resulting from its phenolic nucleus, as being a factor in preventing lignified plant material from being attacked by alimentary bacteria.

Nutritive Value of Cellulose

The value of cellulose to the animal was demonstrated by Kellner (29) in 1900 by means of a respiration chamber. He found that one pound of pure, finely divided cellulose added to the maintenance ration of a bullock formed as much fat in the body of the animal as was produced by feeding one pound of starch or other forms of digestible polysaccharide. The results of Kellner's work are summarized in the following table.

Fat-producing capacities of digestible food nutrients

Digestible nutrient	Lbs. fat produced per lb. digestible nutrient	Starch equivalent of 1 lb. of digestible nutrient
Starch	0.240	1
Fiber	0.253	1
Cane sugar	0.188	0.76
Glucose	0.188	0.76
Protein	0.235	0.94
Oil from seeds and oil cake	0.598	2.41
Oil from cereal grains and by-products	0.526	2.12
Oil from coarse fodders and roots	0.474	1.91

Ustyantzev (61) reported that freeing the cellulose of straw from the incrusting substances caused its value to become equal to the iso-dynamic qualities of starch as a protector of fat and protein. Iwata (28) reported xylan, the predominant hemicellulose of many plants, caused as much fat formation in rabbits as did starch. Kellner (29) also reported the fattening effect of pentosans.

Although one pound of cellulose freed from incrusting substances is equal to starch for fattening, Kellner (29) found that the value of digested cellulose as it occurs in the natural plant was of widely varying magnitude in different feeding stuffs. In the case of linseed meal, for example, the starch equivalent was 0.97, for barley meal 0.98, for bran 0.77, good meadow hay 0.67, and for oat straw 0.43. The difference between these values is roughly proportionate to the fiber content of the feeds. This indicated that under ordinary feeding conditions, when cellulose is incrusted in lignin, the net starch equiv- is less than one, as a deduction must be made for the energy used by the animal during mastication to free the cellulose from the incrusta.

In a previous section of this thesis the predominant products of

cellulose fermentation from in vitro experiments were shown to be predominantly acetic acid with small amounts of glucose, butyric acid, propionic acid, formic acid, lactic acid, pyruvic acid, valeric acid, succinic acid, ethyl alcohol, and the gases carbon dioxide, methane, and hydrogen. The gaseous by-products are excreted as waste products and are to be regarded as valueless (54) (68). The significance of the remaining products of cellulose fermentation as fat precursors does not appear great enough to account for the high value of digestible cellulose.

Glucose, if a major product, would account for the value of cellulose. This sugar has been found to be produced in small quantities by several investigators, and Woodman and Evans (69) found it to be an important intermediate product. Woodman (68) proposed the theory that glucose was the main end product in the animal body. Baker and Martin (3) recently suggested that in nature the bacterial cleavage products of cellulose fermentation did not stop at the carbohydrate stage, but that in the intestinal tract the further resolution of carbohydrates was held in check --- thus making them available for nutrition. Woodman and Evans (69) concluded that, although glucose was an important intermediated product of cellulose fermentation, it was unlikely that it would escape further breakdown before being absorbed by the animal. The experiments of Pochon (45) demonstrated that the cellulose entering the abomasum was there converted to glucose by enzymes. However, only relatively small amounts would be washed thru the rumen to be acted upon in this way.

Acetic acid, the predominant product of cellulose fermentation is of doubtful value from the standpoint of fat production. Deuel and

Milhorat (12) reported acetic acid as being ineffective as a glucose former and suggested that it was practically completely oxidized in the animal body. Deuel and Milhorat discussed the results of Geelmuyden's experiments on phlorhizinized dogs which he believed proof of the formation of glucose from acetic acid. His results were very convincing, however, as the animals received a carbohydrate containing diet, and the increased sugar resulting from sodium acetate was not greater than on some other days. The question of glycogen formation from the liver from acetic acid was recently re-examined by Deuel and associates (11). They reached the conclusion that the even carbon-chained fatty acids, including acetic acid, did not produce glycogen.

Rittenberg and co-workers (53) found evidence that butyric acid was not deposited in fat tissues either as such or as higher fatty acids, but was probably rapidly burned. Other investigations have indicated butyric acid as valueless as a fat or glucose former (52) (11). Ringer and Jonas (52) observed formic acid was not a glucose precursor.

Ringer (50) proved that propionic acid was completely converted into glucose. This work is of additional interest as it was the first proof that fatty acids may be converted into glucose. The value of propionic acid as a glucose precursor has been repeatedly confirmed (52) (11). Ringer and Jonas (52) concluded that the fatty acids with an uneven number of carbon atoms gave rise to glucose while those with an even number did not. Valeric acid was demonstrated as a glucose former, and this has been confirmed. Lactic and pyruvic acids have long been recognized and accepted as fat and glucose precursors (11) (51).

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Woodman (68) contended that, although the major organic acids arising from bacterial fermentation of cellulose may, by virtue of their

heat value, be useful in the maintenance of body warmth, and indeed, in the case of the under-fed animal, may spare the oxidation of body fat for the production of heat, yet they will have no value for fat production in the body of the productively fed animal. Thus, of the lower fatty acids, only the uneven number carbon atom acids (propionic, caleric, lactic, and pyruvic) are able to function as fat or glucose precursors. These glucose forming fatty acids, however, constitute only a small percentage of the total end products of in vitro cellulose fermentation as compared to the amount of the non-glucose precursors, and, therefore, would not be sufficient to account for the fat forming value of digestible cellulose in the animal body.

Woodman and Evans (69) called attention to the fact that too much importance has been attached to the end-products of fermentation and that since absorption from the digestive tract is a continuous process it is of primary importance that the nature and behavior of all intermediate products that arise be investigated. These investigators observed that glucose was fermented by rumen bacteria at 37.0°C. to pyruvic acid which gradually disappeared from the medium by reduction to lactic acid and / or by breakdown to volatile fatty acids and gaseous products. Cellulose fermentation followed a similar course, the main difference being a much smaller accumulation of lactic acid. This was explained by the difference in the rate of its production from its precursor and its further breakdown into gases and volatile fatty acids. They pointed out that a conclusion as to whether the pyruvic and lactic acids produced from cellulose in the rumen are absorbed from the digestive tract promptly enough to preclude their further breakdown into volatile fatty acids was a question that must await further investigations. This

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idea might offer a possible explanation of the fattening value of cellulose. As yet, however, there is no plausible explanation that is compatible with Kellner's finding that digestible cellulose and digestible starch possess equal values for fattening.

A review of the literature suggests a possible relationship between the products of rumen digestion and the common occurrence of ketosis in cattle. Reilly and associates (48) observed that in carbohydrate fermentation acetic and butyric acids were intermediate products in the formation of acetone and in butyl alcohol. Hoffman (33) concluded from his experiments that acetone may be formed in the rumen as a result of carbohydrate metabolism. Brouwer and Dijkstra (5) produced alimentary ketosis in cows by feeding butyric acid silage. It is generally accepted that fatty acids with an even number of carbon atoms are ketogenic in nature while those with an odd number are antiketogenic. On this basis Brentano and Markees (4) recently demonstrated in experimental rabbits and dogs that immediately after the feeding of fatty acids having an even number of carbon atoms (C_2 to C_{18}), a considerable increase in blood ketone followed; whereas with acids of odd number carbon atoms (C_7, B_9) and with unsaturated acids (oleic and linoleic) such ketogenesis did not occur. *In vitro* studies indicate that the fatty acids of cellulose fermentation are primarily even number carbon atom acids (45) (68) (69) which are ketogenic in nature. These results indicate the possibility of an alimentary ketosis in cattle resulting from the normal products of rumen digestion and the additional possible formation of acetone from carbohydrate fermentation in the rumen.

Digestibility of Lignin

Lignin is one of the most inert plant tissues and plays no role other than that of adding stiffness to the plant. The combination in which it exists in the plant is a subject of controversy (42) (38). The structure of lignin is complex and difficult to determine. Hibbert (25), Phillips (43) and Norman (42) discussed the chemical nature of lignin.

Studies concerning the behavior of lignin in animal nutrition have yeilded conflicting results. However, Koenig and Becker (32) observed that although free lignin was digested by rabbits to the extent of 12.8 per cent, the lignin of wheat bran was not digested by these animals. In digestion experiments with sheep Koenig (31) found the digestibility of lignin to be 16 to 23 per cent in hay, 4 to 13 per cent in clover hay, and 28 per cent in pea straw. Rogozinski and Starzewska (55) found in experiments with sheep that the lignin of oat straw was excreted quantitatively in the feces. Williams and Olmstead (65) observed lignin was indigestible by humans, and Crampton and Maynard (9) reported lignin was found indigestible by steers and rabbits. Crampton (8), however, presented values of 20 to 34 per cent digestibility in steers and 0 to 17 per cent in rabbits. Csonka and associates (10) reported a measurable digestion of lignin by cattle and concluded it caused an increased elimination of hippuric acid in the urine. Rogozinski and Starzewska (55), on the other hand, contended that lignin plays no part in the formation of hippuric acid.

Phillips (43) concluded that lignin was, at least in part, broken down by the digestive process of the animal body --- a conclusion that is in agreement with a large portion of the literature. Csonka and

associates (10) suggest that some enzyme of the gastric juice digested lignin. Crampton and Maynard (9) pointed out, however, that proof for or against its utilization by the animal is difficult to establish, for until its molecular structure is known, no criterion of the accuracy of a quantitative test for lignin is possible. The influence of lignin on the digestibility of the other constituents of the plant was discussed in relation to the digestibility of cellulose.

Nutritive Value of the Ether Extract

That the ether extract of roughages may contain substances other than fat has long been recognized. Rather (47) referred to Stellwaag who determined the unsaponifiable constituents of the ether extract of several feeding stuffs and found the ether extract of hay to contain 30.8 per cent unsaponifiable material. Schulze (56) reported the ether extract of hays to be rich in wax like substances, free fatty acids, cholesterin and related compounds. He concluded the nutritive value of the ether extract of roughages and roots must be considered as low. Fraps and Rather (21) further attempted to separate fat and the non-fatty substances of hays and fodders. They found the saponifiable material contained fatty acids, chlorophyll products and perhaps other substances. The unsaponifiable matter of roughages was composed mainly of wax alcohols, waxes and other substances. This material accounted for 36 to 72 per cent of the ether extract, with an average of 58 per cent.

Rather (47) developed improved methods for the determination of the total fatty acids and other constituents of the ether extract. He also reviewed the earlier attempts to separate the various fractions in the ether extract. The ether extracts of concentrates contained

saponifiable material which did not appear to be fatty acid and which averaged about 8 per cent of the extract, and with 6 per cent unsaponifiable matter, the total non-fat of the ether extract was 14 per cent. The total non-fat in the roughage extract was approximately 68 per cent which meant only 32 per cent of the ether extract was fat.

Horwitt and associates (26) pointed out that the ether extract contains not only true fat, but also lecithins, waxes, alkaloids, sterols, chlorophyll, xanthophyll and carotene. They observed the fatty acids accounted for only 41 per cent of the ether extract of the spinach leaf. Chibnall and Channon (7) reported only 27 per cent of the ether extract of leaf protoplasm as fatty acids. The fatty acid content of several roughages analysed by Fraps and Rather (21) varied from 13 to 44 per cent and averaged 25.5 per cent. Stellwaag was quoted by Rather (47) as finding fatty acids of hay to make up 37.3 per cent of the ether extract.

Fraps and Rather (21) found the digestibility of the unsaponifiable material varied from 0 to 86.6 per cent with an average of 29.1 per cent. The digestibility of the saponifiable matter, however, varied from 8.6 to 92.3 per cent with an average of 66.4 per cent. These investigators attributed the low digestibility of ether extract to the presence of wax alcohols, waxes, chlorophylls and other substances. Rather (47) reported that the fatty acids of the ether extract were digested to the extent of 60.5 per cent by sheep. Fatty acids extracted by alcoholic soda, but not by ether, had an average digestibility of 11.2 per cent --- the digestibility in four cases being zero. Horwitt and co-workers (26) found less than 45 per cent of the ether extract materials to be

digested. Seshan (57) reported the fatty acids to be highly digestible (78 to 84 per cent), but the unsaponifiable matter was poorly digested. Unsaturated acids were digested to a greater extent than the saturated ones.

Horwitt and associates (26) reviewed the value of the components of ether extract as possible sources of energy. They concluded that the only parts of the ether soluble fraction which were available as food were the fatty acids or compounds of the fatty acids. The usefulness of separating the fatty compounds from the other acid components of ether extract in evaluating the biological value of feeding stuffs is indicated by the low digestibility of the non-fats and by the fact that they are not sources of energy to the animal body.

Smith and Chibnall (58) made fairly complete studies of the composition of the fatty acid content of cocksfoot and ryegrass. They found the mixed acids were highly unsaturated and contained a relatively low portion of saturated acids. The saturated acids of ryegrass consisted of 70 per cent palmitic, 20 per cent stearic, and 10 per cent "cerotic acid." The composition of the fatty acids of cocksfoot is presented in the following table.

Composition of the glyceride fatty acids
of cocksfoot as suggested by bromation experiments

	Unsaturated fraction	Original mixed fatty acids
	per cent	per cent
Saturated acids (Bertram)	12	15
Oleic acid	--	--
Alpha linoleic acid	38.5	29.5
Beta linoleic acid	21.0	16.
Alpha linolenic acid	21.0	16.
Beta linolenic acid	7.5	6.
	100.0	82.5*

*There was a loss of 17 per cent in the initial fractionation into saturated and unsaturated acids.

By means of the thiocyanometric analysis, the presence of oleic acid up to 16.5 per cent was suggested. These data illustrate that the fatty acids of roughages are high in the unsaturated fatty acids linoleic, linolenic and oleic. This may be important in the nutrition of cattle as these fatty acids have been shown to be essential to the animal body (6).

Digestibility as Influenced by the Plane of Nutrition

The influence of the plane of nutrition on the digestibility of both productive rations and rations of roughage alone has received the attention of investigators working with cattle. Eckles (13) fed two animals mixed rations on full-feed and on maintenance levels. With one animal the digestibility was 7.52 per cent higher on maintenance than on full-feed, and the other showed a 5.24 per cent higher digestibility on the maintenance level.

Mumford and associates (38) studied the effect of the level of feeding on the digestibility of productive rations with varying ratios of roughage to concentrates. When clover hay and ground corn were fed in the ratio of 1:1, the digestion coefficients of the dry substance and carbohydrates varied inversely to the amount of feed consumed. With the ratio of hay to corn 1:3 or 1:5 the digestion coefficients of the dry substance and carbohydrates showed no difference on 1/3, 2/3 or full-feed but was slightly greater on maintenance. The digestibility of the protein and the fat of the ration did not vary with the level of feeding in any of the above instances. The digestibility of the nutrients was not affected by the level of feeding a ration of hay, corn and oil meal in the ratio of 1:4:1. Armsby and Fries (1) reported

mixed rations of hay and hominy feed decreased only slightly in digestibility as the level of feeding increased, while a considerable decrease was observed with a mixture of hay and maize meal. With rations of alfalfa hay and starch Armsby and Fries (2) found the digestibility of the rations, the losses in the urine, and the extent of methane fermentation, showed an increase as the amount of the ration was reduced.

Forbes and co-workers (16) reported that as the plane of nutrition increased there was a slight decrease in the digestibility of dry matter, protein, nitrogen-free extract, and energy. The crude fiber increased in digestibility as the level of feeding increased but dropped somewhat at the higher levels. The ether extract varied independently of the plane of nutrition.

Mitchell and Hamilton (36) fed a mixed ration of alfalfa, corn, linseed meal, and molasses and observed the lowest level of feeding was associated with the most complete digestibility of all nutrients. However, there was a progressive decrease in digestibility from the lowest to the highest only in the case of the nitrogen-free extract, ether extract, and dry substance. Very recently Watson and associates (64) fed productive rations of equal parts of hay and ground barley at five levels of feeding varying from 1.0 to 5.0 kgms. of each feed a day. The digestibility of the nitrogen decreased as the plane of nutrition was increased. The digestibility of nitrogen-free extract showed a slight but insignificant lowering as the level of feeding increased and the digestibility of the dry matter, organic matter, ether extract and crude fiber were not significantly affected by the plane of nutrition.

Forbes and associates (17) reported that with steers the digestibility of protein, total energy-producing units, dry matter, organic

matter, and carbon was highest at the plane of maintenance, was lower at half maintenance, and also diminished at each point of observation above maintenance when six different planes were used. The digestibility of the same ration (one-half corn meal and one-half alfalfa hay) by cows diminished with a rise in the plane of nutrition above maintenance.

These data are not in agreement. As the plane of nutrition increased there was, in some cases, a marked and progressive decrease in digestibility. In many instances only a slight drop occurred at the higher levels, and, on some occasions, the decrease was intermittent rather than progressive. All of the nutrients were decreased in some instances and in others only a few were affected by the level of feeding. Watson and associates (64) concluded that generally in the case of productive rations at the higher levels of feeding, the digestibility of some, at least, of the nutrients is decreased. The question remains, however, as to the amount of the reduction, at what level it develops, and what nutrients are most affected.

Watson and associates (62) pointed out that results with roughages alone should not be confused with experimental results on mixed rations consisting of both roughage and concentrates. Armsby and Fries (1) observed that on smaller rations of hay, the loss in the feces was less, and the losses in the urine and methane were decidedly greater than with larger rations. Watson and associates (63) refer to the work of Honcamp and his collaborators who in 1920 found roughages fed to sheep showed a slight decrease at the higher levels of feeding. It was not possible, however, to estimate the significance of this. The authors concluded that, except in the case of crude fiber, the changes were not signigicant.

Forbes and associates (15) studied the digestibility of alfalfa hay and observed a higher apparent digestibility of the hay on the low levels of feeding in the case of dry matter, organic matter, crude fiber, ether extract, and energy. The protein and nitrogen-free extract varied independently of the level of feeding. Recently Watson and his collaborators (62) reported that for roughage alone (mixed clover and grass hay) the plane of nutrition did not influence markedly the coefficients of digestibility. Four levels of feeding varying from 10 pounds to 19 pounds were used. Watson and co-workers (63) further studied the digestibility of mixed clover and grass hay at five levels of feeding varying from 2.5 kgm. to 9.0 kgm. a day. For the range of 4.5 to 9.0 kgms. a day the plane of nutrition did not significantly affect the coefficients of digestibility. At the 2.5 kgm. level the average coefficients of digestibility were slightly lower than at other levels. This was suggested as being due to the low state of nutrition rather than to the plane of nutrition, per se. Watson (63) concluded on the basis of his work and the data in the literature that generally the plane of nutrition had no very marked effect upon the digestibility of dried roughages.

Summary of Review of Literature

Numerous data are available in the literature regarding the composition and digestibility of the constituents of alfalfa hay. These data, however, are primarily limited to the standard feed analyses usually determined. The nitrogen-free extract has been separated into various components and its low digestibility in hays has been attributed to its pentosan and uronic acid content.

Present methods of determining crude fiber are chemically inaccurate and the attempt to divide the carbohydrates of roughages into highly digestible and poorly digestible portions by use of this method is questionable as crude fiber is often more digestible than nitrogen-free extract. The partition of the carbohydrate fraction into cellulose, lignin and other carbohydrate gives a sharper distinction of the digestibility of the various portions of this fraction. Enzymatic methods of determining crude fiber more completely remove the fibrous materials and are thereby of greater biological value than present methods.

The digestibility of cellulose seems to be inversely related to the lignin content of the plant. Lignification occurs as the plant matures and causes the decreased digestibility of practically all plant constituents. Because of this, any satisfactory crude fiber determination should include all of the lignin.

Kellner (29) found one pound of cellulose was equal to one pound of starch for fat formation in the animal body. The <u>in vitro</u> products of cellulose fermentation would fail to account for this value as the gases are waste products and the even number carbon atom fatty acids which are not glucose or fat formers are the major products. The possible absorption of pyruvic and lactic acids before their further breakdown to volalite fatty acids might offer an explanation. A relationship between the products of cellulose digestion and the common occurrence of ketosis in cattle has been suggested.

Although studies concerning the behavior of lignin in animal nutrition have yielded negative results, it seems the lignin is, at least in part, broken down by the digestive processes of the animal body.

The usefulness of separating the fatty acid compounds from the other constituents of ether extract in evaluating the biological value of feeding stuffs is indicated by the low digestibility of non-fats and by the fact that they are not sources of energy to the animal body. The fatty acids of roughages are high in the unsaturated and essential fatty acids.

At the higher levels of feeding, the digestibility of some, at least, of the nutritents of productive rations is decreased. The question remains, however as to the amount of reduction, at what level it develops, and what nutrients are most affected. A review of the literature indicates that generally the plane of nutrition had no marked effect upon the digestibility of dried roughages.

OBJECT

In view of the unsatisfactory methods being used for the determination of crude fiber and ether extract, it is planned to apply methods which offer possibilities of having more biological value to studies with alfalfa hay. An enzymatic method of determining crude fiber, the further partition of crude fiber to lignin and cellulose; and a determination for true fat will be investigated. The effect of the plane of nutrition upon the digestibility of a ration of alfalfa hay alone will also be studied.

EXPERIMENTAL PROCEDURE

Animals Used and Feeding of the Animals

Six Holstein cows of the Michigan State College herd were used

in this investigation. One of these animals was a rumen fistula cow.

Alfalfa or alfalfa-brome hay was the only feed that the cow received and it was fed in amounts of 10, 20 and 30 pounds a day. The hay was fed twice daily and its chemical composition is given in Table 1.

Digestion Trials

The experiment was divided into three trials, each of which consisted of a preliminary period of 10 days followed by a 10-day collection period. Samples of hay for chemical analyses were taken at the beginning of the experiment. The feces was collected by means of metabolism stalls. During the first trial one cow received 30 pounds of hay and two received 20 pounds. A fourth cow was started on this trial but as she refused a considerable portion of the 30 pounds of hay fed a day she was removed. The cow which remained on the 30-pound level refused about one pound of hay a day. Two cows on the second trial received 30 pounds of hay and two 20 pounds. During the third trial two animals received 20 pounds of hay and two 10 pounds. With the exceptions mentioned, all animals exhibited a good appetite for the full amount of hay given them.

Chemical Analyses

The chemical analyses which were made on the hay and feces were moisture, ash, protein, crude fiber, nitrogen-free extract (by difference) cellulose, lignin, other carbohydrate (by difference) new crude fiber, new nitrogen-free extract (by difference) and true fat. Moisture, ash, protein, crude fiber and nitrogen-free extract were de-

termined by standard A.O.A.C. methods (71). Cellulose and lignin were determined by the method of Crampton and Maynard (9). The enzymatic method of Horwitt and associates (26) was used to determine crude fiber and this fraction is referred to as new crude fiber and that material determined by difference as new nitrogen-free extract. True fat was determined by a method developed by Horwitt and co-workers (26). All of these determinations except the standard A.O.A.C. methods are given in detail in Part I of this thesis.

RESULTS

Composition of the Alfalfa Hay

The chemical analyses of the hay used during this investigation is given in Table I. It is seen that the alfalfa hay contained a

Table I Composition of the alfalfa hay on dry matter basis

	Trial 1	Trial 2*	Trial 3
Protein	16.99	18.42	17.02
Ash	7.80	7.32	6.55
Ether extract	3.14	2.58	1.32
Crude fiber	32.50	32.33	38.16
N-free extract	39.57	39.55	26.94
Lignin	16.18	16.57	17.59
Cellulose	28.38	24.91	31.33
Other Carbohydrate	27.51	30.20	26.19
New crude fiber	46.03	42.89	50.08
New N-free extract	26.04	28.79	25.03
True fat	0.768	0.908	0.755

*This hay contained about one-third brome grass

considerable amount (16.18 to 17.59 per cent) of lignin. The cellulose content was variable and ranged from 24.91 to 31.33 per cent. In

Table 11 a lot of hay, which was not used in the digestion trials, contained almost 40 per cent cellulose. Both the new crude fiber and the lignin plus cellulose content were considerably higher than the value obtained by the standard crude fiber method. About 10 to 14 per cent more fiber was obtained by the enzymatic determination of crude fiber than by the standard method, and the sum of cellulose and lignin was about two per cent lower than the new crude fiber content. The highest value obtained for the true fat in alfalfa hay was 0.908 per cent and the lowest was 0.475 per cent. The high value was found in hay with a low crude fiber and cellulose content and a high protein content. There did not appear to be any direct correlation between the true fat and ether extract content of the hay.

The cellulose and lignin content of alfalfa hay and feces is compared with the new crude fiber in Table 11 and the per cent difference is calculated. As a rule in both hay and feces the enzymatic method for crude fiber removes a little higher percentage of fibrous material. The percentage difference was considerably larger in the feces than in the hay. From data on the analyses of the rumen contents which were presented in Part I of this thesis it was noted that the difference was greater for the rumen contents than for the hay.

Table 11 Comparison of the cellulose and lignin content of hay and feces with the crude fiber determined by enzymatic methods

	Feed				Feces			
Sample	Cellulose (1)	Lignin (2)	Crude fiber (3)	Per cent Difference (3)/(1)+(2)	Cellulose (1)	Lignin (2)	Crude fiber (3)	Per cent Difference (3)/(1)+(2)
1	33.10	14.98	50.78	5.62	32.79	28.99	72.32	17.06
2	33.47	15.07	53.20	9.51	31.51	29.23	67.89	11.77
3	36.97	17.79	57.77	5.50	39.51	32.01	86.01	4.60
4	39.52	16.44	55.62	-0.61	33.71	33.13	74.81	11.92
5	24.91	16.57	42.89	3.40	26.19	29.55	67.99	21.97
6	31.33	17.59	50.08	2.37	33.20	32.50	61.74	-6.03

Comparison of the various methods of analyses

The digestion coefficients of the various crude fiber fractions as found by different methods of analysis are presented in Table III. The digestibility of the materials obtained by difference is also given. The standard method divides the carbohydrate fraction of the hay into crude fiber and nitrogen-free extract. The nitrogen-free extract averaged about 20 per cent more digestible than the crude fiber. The digestibility of crude fiber varied from 32.83 per cent to 51.16 per cent while that of nitrogen-free extract varied from 47.56 per cent to 70.94 per cent.

Table III Digestibility of alfalfa hay at various levels of feeding and by different methods of analyses

Procedure and nutrients	Level of feeding: 10 pounds	20 pounds	30 pounds	Average (11 samples)
Standard				
Protein	64.71	68.38	68.92	67.86
Ether extract	-18.59	13.36	17.23	8.61
Crude fiber	36.15	44.92	45.56	43.50
N-free extract	56.94	66.55	60.06	63.03
True fat*	48.01	72.52	65.25	66.08
Modified I**				
Protein	64.71	68.38	68.92	67.86
Ether extract	-18.59	13.36	17.23	8.61
Cellulose	44.14	51.44	50.87	49.96
Lignin	-0.16	17.35	16.98	12.39
Other carbohydrate	80.22	84.66	85.02	83.95
Modified II***				
Protein	64.71	68.38	68.92	67.86
Ether extract	-18.59	13.36	17.23	8.61
New crude fiber	32.87	37.09	39.47	36.97
New N-free extract	72.79	89.97	86.60	85.84

*True fat is included under the standard procedure for convenience.
**Partition of carbohydrates by the method of Crampton and Maynard (9).
***Enzymatic determination of crude fiber by the method of Horwitt and associates (26).

The separation of the carbohydrate of the hay into lignin, cellulose and other carbohydrate made a sharper distinction in the digestibility of the various fractions. Lignin was digested to a slight extent, cellulose averaged practically 50 per cent digestibility and other carbohydrate 84 per cent. The digestibility of lignin varied from -5.12 per cent to 23.71 per cent while cellulose varied from 39.68 to 57.23 per cent and other carbohydrate from 78.19 to 87.27 per cent. It should be noted that the digestibility of the three fractions did not overlap as was true for the two fractions determined by the standard method. The other carbohydrate averaged almost 21 per cent more digestibility than the nitrogen-free extract.

The determination of crude fiber by an enzymatic method resulted in the separation of the carbohydrates into two substances of more distinct biological difference than the standard method. While the average digestibility of crude fiber and nitrogen-free extract only differed about 20 per cent, the new nitrogen-free extract was almost 49 per cent more digestible than new crude fiber. New crude fiber varied from 29.83 to 43.3 per cent digestible and the new nitrogen-free extract from 68.21 to 93.12 per cent with an average of 85.84 per cent.

True fat averaged 66.08 per cent digestible as compared with a value of 8.61 for ether extract. The digestion of true fat varied from 47.99 to 82.93 per cent while ether extract varied from -26.00 to 34.50 per cent.

Effect of the Plane of Nutrition on Digestibility

The summary of 11 coefficients of digestibility obtained at levels of 10, 20 and 30 pounds of hay a day are given for each

nutrient in Table III. Digestion coefficients varied but little at levels of 20 to 30 pounds a day but the digestibility at the 10-pound level was lower for each nutrient than on either of the higher levels. The only differences which occurred in changing from 20 to 30 pounds of hay a day were a decrease in the digestibility of nitrogen-free extract, new nitrogen-free extract and true fat and an increased digestibility for ether extract. New crude-fiber was slightly more digestible at the highest level. A negative digestion coefficient of ether extract was obtained at the 10-pound level.

DISCUSSION

Analyses of the alfalfa hay used in this investigation and also that used in studies with rumen digestion show that alfalfa contains an average of about 32 per cent cellulose and about 16 per cent lignin on the dry basis. The cellulose content was highly variable while lignin only varied slightly over two per cent. Other carbohydrate and new nitrogen-free extract usually accounted for 20 to 25 per cent of the hay.

The partition of the carbohydrates of alfalfa hay into lignin, cellulose and other carbohydrate by the method of Crampton and Maynard (9) confirms previous results with this method. Although the crude fiber of the hay studied was usually less digestible than the nitrogen-free extract when the standard method was, the distinction between the various components was much greater by the modified procedure. A poorly digestible fraction, lignin; a highly digestible portion, other carbohydrate; and a fraction of medium digestibility, cellulose, were obtained. Other carbohydrate averaged 21 per cent more digestible than nitrogen-free extract. The cellulose of the hay averaged about 50 per cent digestible and lignin was from -5.1 to 23.7 per cent digested. These data together with that in the literature indicate that lignin is usually digested to some extent in the animal body. The digestibility is highly variable and the conflicting values reported in the literature (8) (43) are not surprising.

The enzymatic method of determining crude fiber also gives a sharper distinction between the carbohydrate fractions than the standard method. An average difference of only 20 per cent was observed in the digestibility of crude fiber and nitrogen-free extract while the new nitrogen-free extract and new crude fiber, determined by the enzymatic method, showed a difference of 49 per cent. New crude fiber averaged 6 per cent less digestible than the old crude fiber and new nitrogen-free extract was about 23 per cent more digestible than old nitrogen-free extract. Results with rumen digestion studies which were presented in Part I of this thesis also indicate the difference in the character of the two nitrogen-free extractive fractions as 100 per cent of the new nitrogen-free extract was usually removed and only 60 to 70 per cent of the old nitrogen-free extract. There was a difference of about 25 per cent between the highest digestion coefficient of new crude fiber and the lowest coefficient for new nitrogen-free extract while with the standard method the two fractions which were determined overlapped. The greater biological value of this method is indicated.

That this method meets the requirements suggested by Norman (40) that any crude fiber determination should include all of the lignin is indicated by the data presented in Table II. The new crude fiber con-

tent of the hay was usually slightly more than the combined cellulose and lignin content. Remy (49) found crude fiber determined by the method he developed, and from which Horwitt and associates (26) adapted their determination, was of about the same chemical composition as that of the standard methods which usually contains 97 to 100 per cent lignin and cellulose (24) (40). These facts would indicate that the new crude fiber consists largely of cellulose and lignin and that practically all of both constituents are included. In the feces the variation between the cellulose plus lignin and the new crude fiber is greater which indicates the latter material may contain some poorly digestible material or materials other than cellulose andlignin. Likewise, the new nitrogen-free extract obtained differs somewhat from the other carbohydrate fraction. Although new nitrogen-free extract varied more in digestibility, it averaged five per cent more digestible in over half of the cases. The difference was even better illustrated by the results in Part I of this thesis as an average of 14.5 per cent more of the new nitrogen-free extract disappeared from the rumen than other carbohydrate. The new nitrogen-free extract was often 100 per cent removed.

Of these two methods, the enzymatic determination of new crude fiber requires less labor for the determination, but the time required to obtain the results is much longer as a total of eight days of incubation are necessary as compared to 12 hours incubation for lignin. Both of these methods are of greater value in predicting the feeding values of roughage than the present division of carbohydrates into an imperical crude fiber and nitrogen-free extract.

The various samples of analyzed material contained from 0.475 to 0.908 per cent true fat by the method of Horwitt and associates (26).

There was no correlation between the amount of ether extract present and the amount of true fat. The greater biological significance of separating the fats and non-fats was illustrated by the metabolism trials which closely agreed with the results of other investigators (47) (57). True fat averaged 66.08 per cent digestibility as compared with 8.61 per cent for the ether extract. At the 10-pound level even though ether extract was -18.59 per cent digested, true fat was digested to the extent of 48.01 per cent which indicated that the so-called metabolic fat was of a non-fatty nature. This method seems to give a fraction of more biological value than the ether extract determination.

Although this method shows the biological value of true fat attention should be brought to the fact that the present methods of fat extraction used for feed materials do not completely remove all of the fat and related substances. In view of the low values for true fat obtained in these studies it is suggested that methods of extraction which are more complete in extracting fatty substances be studied in connection with feed analyses.

As the level of feeding increased from 20 to 30 pounds of hay a day the digestibility of protein, crude fiber, cellulose, lignin, and other carbohydrate was not affected and new crude fiber showed a slight increased digestion at the higher level. Only the more digestible carbohydrates, represented by nitrogen-free extract and new nitrogen-free extract, and the true fat decreased in digestibility with an increase in the level of feeding. These results indicate that the general reduction of digestibility often observed at the higher levels of feeding productive rations (17) (36) may not follow for

rations of roughage alone. This is in agreement with the findings of Watson and co-workers (62) (63) but conflicts with the reports of Forbes and associates (15).

At the 10-pound level the digestibility of each nutrient was lower than at either of the higher levels. It should be pointed out, however, that this was a sub-maintenance and the influence of the low state of nutrition may have been a factor. Forbes and associates (17) and Watson and co-workers (63) also observed a lowered digestibility on sub-maintenance rations.

SUMMARY AND CONCLUSIONS

1. Digestion trials were conducted in which the cows received 10, 20 and 30 pounds of alfalfa hay a day.
2. Alfalfa hay contained an average of about 32 per cent cellulose and 16 per cent lignin. The cellulose averaged 50 per cent digestible and the lignin was of highly variable digestibility up to 23.7 per cent.
3. The separation of the carbohydrate fraction into cellulose, lignin and other carbohydrate is a better index to the biological value of feeding stuffs than the present division into crude fiber and nitrogen-free extract.
4. The enzymatic determination of crude fiber gives a much sharper distinction between the two carbohydrate fractions than the standard method and its usefulness in estimating the biological value of feeding stuffs is thereby indicated.
5. The coefficient of digestibility of the true fat averaged 66.1 per

cent, while the ether extract averaged only 8.6 per cent. It is apparent, therefore, that a true fat determination is biologically significant.

6. Increasing the level of feeding from 20 to 30 pounds of hay a day caused a decreased digestibility of the nitrogen-free extract, new nitrogen-free extract and true fat, while ether extract increased in digestibility. The other fractions were not affected.

7. Digestibility at the 10-pound level was lower than at either of the higher levels. This lowered digestibility was attributed to the sub-maintenance level of nutrition.

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