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To the Graduate Council:

I am submitting herewith a thesis written by Atalla Said Mohammed entitled "Effect of Nitrogen Fertilization and Time of Harvest on In Vitro Rumen Microbial Utilization of Some Bermuda Grasses." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirement for the degree of Master of Science, with a major in Animal Husbandry.

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EFFECT OF NITROGEN FERTILIZATION AND TIME OF HARVEST
ON IN VITRO RUMEN MICROBIAL UTILIZATION
ON SOME BERMUDA GRASSES

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CHAPTER I

INTRODUCTION

The ruminant's diet consists mainly of roughages and forages. While there are a large number of grasses in the world, in any given area, only a relatively small number of them are used. Investigators are constantly striving to increase the number of valuable forage species in their area by the introduction of new (to that region) species and/or the hybridization and improvement of old species.

Before a forage under investigation is recommended for an area it must undergo severe testing for a number of things. Besides general adaptability to soil and climatic conditions, the nutritive value of the forage should be thoroughly studied.

Utilization of the forage by the animals it is intended for, is of course, necessary for the final evaluation of a new or improved forage. However, animal feeding trials involve long periods of time and are expensive. It is not possible to evaluate very many forages at a given time with this technique. Thus the artificial rumen technique has been proposed as a technique to get preliminary information as to the possible value of forages for animals.

The major carbohydrate constituents of roughages are cellulose, hemicellulose and related compounds. Animals, so far as it is known, do not possess enzymes capable of breaking down these complex substances into simple compounds which can be absorbed. The degradation

of complex carbohydrates is one aspect of the vital role performed by rumen bacteria in the digestion of forages. It is known that the extent to which rumen bacteria will break down cellulose and related compounds is dependent to a large degree upon the presence and availability of various nutrients needed by the bacteria. As the bacteria and the host animals may have different nutritive requirements, the "in vitro" results are not necessarily the same as from "in vivo" experiments. However, a number of experiments which will be discussed later have shown a close positive correlation between "in vivo" and "in vitro" forage digestion experiments.

Attention has been focused on the use of nitrogen fertilization to increase forage yields, and forage yields primarily have been the basis for the evaluation of nitrogen applications. It is well known that nitrogen fertilization up to certain levels will increase the yield of forage crops. However, very little is known about the effects of nitrogen fertilization of specific forages on microbial utilization of the forages and thus on the nutritional value of the forages to the animal.

The purpose of the experiments reported in this thesis was to compare rumen microbial utilization of three varieties of Bermuda grasses when fertilized with different levels of nitrogen and harvested at different stages of maturity. The percentage of the cellulose digested in the forages by rumen bacteria was used as the criterion of evaluation.

CHAPTER II

REVIEW OF LITERATURE

Artificial Rumen Technique

Studies on the role of rumen microorganisms in the nutrition of ruminants have led to the development of the artificial rumen techniques. Marston (1948) was probably the first to study the metabolism of rumen microorganisms by culturing the population of the rumen "in vitro" and by determining the end products formed.

Many studies have been conducted regarding the use of the artificial rumen. These investigations include the study of different techniques and various modifications of these techniques concerning the actions of various organic and inorganic substances on cellulose digestion.

Early artificial rumen techniques involved the use of whole rumen liquid as an inoculum. Since rumen liquid contains nutrients needed by bacteria, it is difficult to determine what nutrients have been supplied by the forage or other material being studied. Another limitation of this method is that a long fermentation time is required. As a result of these limitations, Cheng et al. (1955) developed the washed suspension technique.

One advantage of this method is that any unknown factors present in the rumen fluid are removed. The suspension is also relatively free of metabolic end products produced by the microorganisms while they

were in the rumen.

Burroughs et al. (1950) compared cellulose digestion in good and poor quality roughages in vitro. Supplements, including available nitrogen, a complex mineral mixture, and an autoclaved water extract of cow manure were added singly and in combination to each of the good and poor quality roughages. The cellulose in the good quality roughages was digested efficiently without supplementation. The cellulose in the poor quality roughages failed to be digested efficiently without supplementation. The addition of a complex mineral solution and manure extract along with available nitrogen greatly increased cellulose digestion in the poor quality roughages.

Burroughs stressed the limitations of the in vitro technique with respect to the evaluation of forages and suggested that it could serve only as a screening device from which the most promising results should be checked in animal experimentation.

Hershberger et al. (1959) compared the cellulose digestibility of 35 forages in vitro and in vivo. The in vitro system utilized bovine rumen microorganisms, all-glass fermentation vessels, and a 24-hour fermentation period, whereas the in vivo system utilized conventional 11-day digestion trials with two to four sheep per trial. The forages tested were orchard grass, Kentucky bluegrass, timothy, alfalfa, and ladino clover. He found a correlation coefficient of 0.97 indicating a close linear relationship between cellulose digestion in vitro and in vivo. He also found a correlation coefficient of 0.92 between cellulose digestion and the digestible energy of these forages.

Kamstra et al. (1955) showed that 48 hours in vitro and in vivo cellulose digestion were of similar magnitude. Likewise, Pigden and Bell (1955), in evaluating forage quality, using 11 forages, obtained a close agreement of the per cent organic matter digested by sheep and per cent anthrone-determined carbohydrate fermented in vitro.

Barnett (1957) reported a similiar study in which a reasonable degree of correlation was obtained between cellulose digestion in vitro using 27 samples of silage, and the digestibility of the crude fiber of the silages as measured in feeding trials.

Quicke et al. (1959) determined the digestibility of the cellulose in seven grass hays and six legume hays by an in vitro technique and in digestion trials with sheep. He found no significant difference between results obtained in vitro and in vivo with grass hays, but in some of the legume hays, cellulose digestibilities, as measured by the two methods, were significantly different. He suggested the possible use of in vitro techniques as tools in the evaluation of the nutritive value of forages.

Nitrogen Fertilization and Forage Quality

Enlow et al. (1929) reported that the protein content of pasture grass in a grazed condition can be increased and maintained at a somewhat higher level by frequent light application of a nitrogen fertilizer. They stated that rainfall is a limiting factor in forage production, as the ratio between the total nitrogen content of the grass and the pounds of forage produced is flexible.

Barth (1958) studied in vitro rumen microbial utilization of

several nitrated (120 pound nitrogen per acre) and non-nitrated forages at different stages of maturity. He reported that when cut at the pasture stage the non-nitrated Sudans were utilized to a highly significantly greater extent than non-nitrated millets. At this stage of maturity, nitration had no significant effect on the digestion of the millets while it had an inhibiting effect on the digestion of the Sudans. The decrease in digestion of Sweet Sudan was highly significant while the decrease in the case of Piper Sudan was not significant statistically.

When cut at the hay stage there was no significant difference between any of the species or varieties tested, and nitration had no significant effect. At this stage of maturity there was no decrease in the digestion of the Sudans due to nitration.

Comparisons made between non-nitrated forages cut at the pasture stage and the hay stage showed that maturity had a highly significantly negative effect on the digestibility of all of the forages tested except German millet. In the case of German millet the decrease in digestion due to maturity was not quite significant.

Comparisons made between the nitrated forages cut at the pasture and the hay stage showed that maturity had a significantly negative effect upon the digestion of the millets and Piper Sudan. The Sweet Sudan was digested to a significantly greater extent when mature than when cut at the pasture stage, because there was no inhibition due to nitration in the case of the mature plants.

Hall et al. (1960) examined the effects of nitrates and nitrites

upon forage utilization by rumen microorganisms and upon ration digestibility by lambs. They found that addition of 2.8 milligrams of sodium nitrate to fermentation flasks containing solka-floc markedly decreased in vitro cellulose digestion and that 1.4 milligrams of sodium nitrite and above, completely inhibited in vitro microbial cellulose digestion. They also indicated that there was poorer utilization of heavily nitrated young Sudan forage (120 pounds of nitrogen per acre) by microbes in vitro due to the presence of nitrates and/or nitrites in the forage.

At the same time they fed 2.5 grams of sodium nitrate per head per day to lambs on orchard grass hay and reported significantly lower hay dry matter and cellulose utilization than with the control lambs. However, lambs fed 5.0 grams of sodium nitrate per head daily digested as much cellulose and dry matter as did the control lambs.

Hopkins et al. (1960) evaluated nitrogen fertilized mountain meadow hay in a 117-day wintering experiment with sixteen Hereford heifers. They found that heifers fed nitrated (90 pounds per acre) hay gained 0.73 pound per day while a similiar group fed non-fertilized hay gained 1.00 pound per day. The following winter in a 117-day feeding period nine Hereford heifers consuming nitrogen fertilized hay gained 1.04 pounds per day while the control group gained 1.22 pounds per day.

The digestibility of these hays, determined in digestion trials with sheep was as follows: nitrogen fertilized hay-dry matter 65.6 per cent, protein 67.1 per cent, crude fiber 75.5 per cent, nitrogen free

extract 59.81 per cent, ether extract 48.9 per cent, and ash 53.3 per cent. Non-fertilized hay-dry matter 66.3 per cent, protein 62.1 per cent, crude fiber 78.3 per cent, nitrogen free extract 61.2 per cent, ether extract 19.2 per cent and ash 49.9 per cent.

The above workers compared the two hays in an artificial rumen, using gas production as a measure of total microbial activity. These results showed significantly greater gas production from the non-fertilized hay than from the fertilized hay. They stated that the difference in animal gain may be due to a physiological disturbance caused by nitrogen fertilization of mountain meadow hay, but not necessarily due to the nitrogen in the hay.

Alexander et al. (1961) conducted digestibility and feeding trials with cattle and sheep for a period of 3 years to study the effect of 50 and 100 pounds of nitrogen per acre and two dates of harvesting on the nutrient composition, digestibility and feeding value of Coastal Bermuda grass hay. They found that the crude protein content and digestibility of crude protein in the hay were increased up to 30 per cent by increasing the rate of nitrogen fertilization from 50 to 100 pounds per acre, but it was lowered by the late cutting. Hay consumption decreased and weight losses occurred with late-cut, low-nitrogen fertilization. The apparent digestibility of crude protein and the total digestible nutrients decreased with increasing stages of maturity. Dry matter and energy digestibility followed trends similar to the protein digestibility. The TDN values increased as the nitrogen level was increased from 50 to 100 pounds per acre.

Markley et al. (1957) used four 18-month old dairy heifers in a total collection trial. The four rations, fed twice daily, were second cutting alfalfa hays and 3 bromegrasses--the latter grown with 25, 125 and 225 pounds of nitrogen per acre. A nitrogen balance was determined for each hay. The crude protein and digestion coefficient of the second cutting bromegrass increased with increasing levels of nitrogen. Ether extract followed a trend similar to protein while the per cent fiber and nitrogen free extract decreased with increasing nitrogen fertilization. The digestibility of energy, protein, ether extract, and fiber increased with increasing nitrogen fertilization.

In 1948 experiments were carried out by Moon (1954) in Scotland to study the effect of a late application of nitrogen fertilizer on nutritive value of hays grown in the east of Scotland. Digestibility measurements of forty-one hays showed that increased protein content resulting from late treatment with nitrogenous fertilizer was associated with a higher digestibility of the protein. Nitrogen fertilization had little effect on the digestibility of non-protein fractions. The fertilizer nitrogen absorbed by the plants appeared to be converted into completely digestible protein.

Poulton et al. (1957) carried out a digestion trial using four yearling wether sheep. Orchard grass hays that had been fertilized with 100, 200, and 400 pounds of nitrogen per acre were compared with standard alfalfa hay.

Poulton stated that the apparent digestibility of crude protein in the orchard grass hays was significantly lower than that of alfalfa

hay and digestibility of crude fiber for orchard grass hays was much higher than for the alfalfa. The differences observed in the digestibility of the energy, ether extract, and nitrogen free extract components of these hays were not significant. The three orchard grass hays were equal in their TDN and digestible energy contents and they were superior to the alfalfa hay.

Burton et al. (1956) studied the effect of nitrogen and age of growth upon the palatability of Coastal Bermuda grass, using the per cent of grass consumed as a measure of palatability. A Hereford herd and several Jersey cows were used as test animals. They found that the amount of forage consumed increased as the nitrogen rate increased. There was no evidence to indicate that the heaviest rate of nitrogen reduced the palatability of the grass.

Burton et al. (1952) stated that Coastal and several other Bermuda grass hybrids had produced more than twice as much forage as Common Bermuda grass at moderate fertility levels. This suggested that they might efficiently use greater quantities of nitrogen than Common Bermuda grass.

The application of 50, 100, 200, and 400 pounds of nitrogen per acre for a period of 3 years, increased the yield from one ton with no nitrogen to 8 tons with 400 pounds of nitrogen. The results indicated that nitrate of soda and ammonium nitrate were equally effective as nitrogen sources and were superior to uramon and cyanamid. Increases in nitrogen application increased the crude protein percentage in the hay from 7 per cent crude protein in the nonfertilized

hay to 13 per cent in the hay produced with a single application of 400 pounds of nitrogen per acre from nitrate of soda. This significantly increased the fiber content and significantly reduced the N.F.E. and ash content. It had no significant effect upon the percentage of fat, calcium, phosphorus and potassium in the hay. The 100 pound rate of nitrogen gave the greatest yield per pound of nitrogen whereas the 200 and 400 pound rates produced the most crude protein per pound of nitrogen. The cheapest hay was produced by applying 200 pounds of nitrogen per acre and the cheapest crude protein by applying 400 pounds of nitrogen per acre.

In experiments by Burton et al. (1962) Coastal Bermuda grass fertilized annually with 600 pounds of nitrogen and 1500 pounds of 0-10-20 was clipped at frequencies of 3, 4, 5, 8, 12, and 24 weeks for 24 week periods in the summers of 1959, 1960, and 1961. Nitrogen applications split the same number of times as the clipping frequencies were made in March and after each cutting. Three-year average dry matter yields of 6.8, 7.2, 7.9, 8.9, 9.0, and 6.5 tons per acre were obtained from plots cut every 3, 4, 5, 6, 8, 12, and 24 weeks, respectively. The respective 3-year average analyses on a dry basis were: for crude protein 18.5, 16.4, 15.4, 13.3, 10.7, 9.0, and 8.4 per cent; and for vitamin A equivalent 199,900; 164,900, 162,200; 131,900; 86,500; 61,600; and 32,800 I.U. per pound. In vitro analysis of the 1960 forage gave dry matter digestibility values (based on cellulose for 36 hours) of 62.6, 60.3, 56.1, 54.7, and 52.9 per cent for forage cut every 3, 4, 6, 8, and 12 weeks, respectively. Respective crude

protein digestibilities of 72.6, 70.9, 65.6, 58.9, and 51.2 per cent were obtained for this material.

Cullison et al. (1962) reported that Coastal Bermuda grass hays produced at two different levels of nitrogen fertilization (125 pounds and 675 pounds of nitrogen per acre) were used in a wintering trial with weanling calves. Two groups of 10 steer calves each were fed on either the 125 pound nitrogen or 675 pound nitrogen-fertilized hays on an ad libitum basis, along with 2 pounds of cottonseed meal per head daily over a period of 140 days. The two groups consumed 11.49 and 11.86 pounds of their respective hays daily and had average daily gains of 0.96 pounds and 1.11 pounds, respectively. No outward manifestations of any specific deleterious effects from the feeding of either hay were noted. However, both groups showed significant increases in carotene and decreases in vitamin A levels in the blood after 84 days on test. The condition was most apparent with the group fed the heavily nitrated hay, but it tended to correct itself toward the end of the wintering period. The two hays were similar in composition except that the heavily nitrated hay contained considerably more crude protein, carotene, and nitrate. The calves which received the heavily nitrated hay gained significantly slower than did the other group during a subsequent summer grazing period.

Stage of Growth and Nutritive Value of Forages

Kamstra et al. (1955) studied the effect of stage of maturity upon plant cellulose digestion by rumen microorganisms in vitro. Alfalfa, timothy and orchard grass were harvested at three stages of

maturity, air dried and ground to 40 mesh. Cellulose fractions were isolated from these samples. The rate of cellulose digestion was determined by the artificial rumen technique for both the cellulose fractions and the whole plant materials. As much as a 50 per cent decrease in the rate of cellulose digestion was observed between the first to the third samplings of orchard grass and a 30 per cent decrease with comparable samples of timothy and alfalfa. Isolated cellulose from these same samples showed less variation in the rate of cellulose digestion. The digestion of the isolated cellulose proceeded at a more rapid rate than the whole plant cellulose, especially the cellulose in the most mature samples. The results obtained indicate that lignification is an important factor in depressing the rate of digestion of cellulose in mature plants.

Sullivan (1955) stated that lignin has been considered important from two points of view: it is very poorly digested if at all, and it influences the digestibility of other substances.

Kamstra et al. (1958) conducted in vitro studies with orchard grass, alfalfa, and timothy hays that were cut at three stages of maturity. The result indicated that the stage of maturity of the plant at harvest had a marked effect on the in vitro digestibility of the cellulose in orchard grass, alfalfa and timothy hays. However, when cellulose was isolated from the same plant materials and used as the cellulose substrate in the fermentation flasks, the digestibility was found to be improved over the corresponding sample of whole plant material. The isolated cellulose was more digestible than the

cellulose in the whole plant. These results indicate that lignin in the plant is an inhibiting factor. They also suggested that the extent of fermentation over 24 hours did not appreciably alter the amount of cellulose digested in a given forage sample. Maturity of the plant material has an effect upon cellulose digestion, the cellulose in the young plant being the more digestible.

Knox and Burton (1958) studied the effect of nitrogen rate and clipping frequency upon lignin content and digestibility of Coastal Bermuda grass when fed to lambs. They found when the grass was fertilized with 100 pounds of nitrogen per acre, clipping frequently did not affect the lignin content; at higher nitrogen rates, lignification increased with age of the grass. This is due to the fact that nitrogen decreases the per cent of leaf and increases the per cent of stem which is high in lignin.

Prime and Burton (1956) studied the effect of nitrogen rate and clipping frequency upon the yield, protein content, and certain morphological characteristics of Coastal Bermuda grass. They found that an increase in nitrogen increased the hay yield, crude protein percentage, crude protein yield, and nitrate content, but reduced the per cent of nitrogen fertilizer recovered in the hay. Increasing the nitrogen rate resulted in a decrease in per cent of leaf. Increasing the length of the clipping interval increased the hay yield and lowered the crude protein percentage, but had no effect on nitrate content, crude protein yield, and per cent of nitrogen recovered.

Bird (1943) stated that the stage of maturity is one of the

most important considerations in connection with the harvesting of forage crops. Both the increased production of stem and resulting changes in proportion of stem to leaf as a plant matures have a great effect upon the nutritive value of the crop. Stage of maturity has a great effect on chemical composition, palatability, and digestibility of a grass plant.

CHAPTER III

EXPERIMENTAL PROCEDURE

Source of Forages

The forages used in this study were grown at the West Tennessee Experimental Branch Station during 1961 in agronomic experiments with Dr. Henry A. Fribourg as project leader. The varieties of grasses grown were Coastal Bermuda, Common Bermuda, and Midland Bermuda.

The levels of nitrogen fertilization used were: 0, 60, 120, 240, 360, 480 and 720 pounds of nitrogen per acre. The nitrogen was applied in three applications. Half of the total amount was applied at seeding time on April 15; one fourth of the amount was applied on May 30, while the other fourth was applied on July 8.

Four cuttings were obtained. The first cutting was obtained on May 27; the second cutting was obtained on July 7; the third cutting was obtained on August 11; and the fourth cutting was obtained on October 14.

Preparation of the Digestion Flasks

Four tenths gram of the ground and dried forages was weighed, the exact weight recorded, and added to numbered 125 milliliter Erlenmeyer digestion flasks. The digestion flasks were fitted with a rubber stopper, through which passed two separate pieces of glass tubing which served as carbon dioxide lines. One length of glass tubing reached almost to the bottom of the flask. This served as the carbon

dioxide inlet tube and carried carbon dioxide down into the bacterial suspension. The second piece of glass tubing was simply a short piece extending just below the rubber stopper and served as an escape tube.

In advance of going to the barn to collect the rumen juice, 15 milliliters of a one per cent solution of sodium bicarbonate solution in distilled water was pipetted into each flask. Each flask was placed on a magnetic stirrer and the contents agitated rapidly for exactly one minute.

The flasks were stoppered and placed in a shallow water bath set at 39.5° Centigrade. This water bath was located beneath a series of gas outlet fittings attached to a tank of carbon dioxide. A stream of carbon dioxide was started through the flasks as soon as possible in advance of processing the bacteria.

Before going to the barn, one flask containing 900 milliliters of one per cent sodium bicarbonate buffer solution and one flask containing 450 milliliters of the one per cent sodium bicarbonate solution were placed in the same water bath set at 39.5° Centigrade and carbon dioxide was bubbled through the solutions. These buffer solutions were used to wash the rumen microorganisms.

Preparation of Rumen Bacteria

Rumen contents obtained from a fistulated steer maintained on orchard grass hay and concentrates were strained through four layers of cheese cloth into previously warmed thermos flasks. About 2000 milliliters of rumen juice were collected.

The whole rumen juice was centrifuged in an Ivan Servall angle

centrifuge at a speed of about 1000 r.p.m. for one minute. By this process partially digested feed particles in the liquid as well as most of the protozoa were sedimented and were discarded.

The supernatant, which contained the cellulolytic bacteria, was then poured off and strained through eight layers of cheese cloth into a warm flask situated in a water bath set at 39.5° Centigrade. The supernatant was then centrifuged again at a speed of 25,000 r.p.m. in a Sharples super-centrifuge. The resulting sediment consisting principally of rumen bacteria was then suspended in 900 milliliters of a one per cent sodium bicarbonate solution which had been saturated with carbon dioxide gas. This fluid was passed through the super-centrifuge for a second time to sediment the bacteria. The final sediment was suspended in 450 milliliters of a one per cent sodium bicarbonate solution and saturated with carbon dioxide gas.

Fifteen milliliters of the bacterial suspension were pipetted into each digestion flask. The flasks were then returned to the water bath and reconnected to the source of carbon dioxide. After a 24-hour fermentation period, the amount of cellulose remaining in each flask was chemically determined.

Cellulose Determination

After the fermentation period, the entire contents of the digestion flasks were transferred to 75 milliliter pyrex centrifuge tubes. The flasks were washed several times with small amounts of distilled water to remove all of the forage. The tubes were centrifuged for four minutes at a speed of about 1000 r.p.m. in an Ivan

Servall angle centrifuge and the supernatant discarded.

Twenty five milliliters of 1.25 per cent sodium hydroxide solution in distilled water were added to each tube and each tube was placed in a boiling water bath for exactly 20 minutes to digest the lignin. As soon as it was possible, the tubes were centrifuged at 1000 r.p.m. for five minutes and the sodium hydroxide poured off. Then, 12 milliliters of glacial acetic acid and 2.5 milliliters of 70 per cent nitric acid were added and the tubes were placed in a boiling water bath and left for exactly twenty minutes.

The entire contents of each tube was filtered through a Gooche crucible with each tube being washed with a stream of ethanol. The crucibles were then left in a 100° Centigrade oven overnight. After being cooled in a dessicater and weighed, they were ignited in a muffle furnace at 650° Centigrade, cooled again in a desicater and reweighed. The undigested cellulose was determined by the loss of weight upon ignition.

One gram of each original sample was weighed in duplicate. The amount of cellulose in the various forages was determined in a similar manner to that described above. The per cent of cellulose digested by the bacteria was obtained by the difference between the amount of cellulose in the original forages and the amount of cellulose left after the fermentation period.

Statistical Analysis of Data

Duplicate flasks for each treatment within an experiment were set up as previously described, and the entire experiment was repeated

three times. Analysis of various techniques were employed and treatment means were partitioned by "D" tests according to Snedecor (1956).

CHAPTER IV

RESULTS AND DISCUSSION

Cellulose Content of the Forages

The cellulose content of the various forages used in these studies is shown in Table I. The amount of cellulose in the forages varied from about 29 per cent to 34 per cent. The time of cutting had considerable effect on the cellulose content of the forage. Generally, the third cutting of each of the forages contained significantly more cellulose than did samples of the forages cut at other times during the growing season. Level of nitrogen fertilization or variety of the forage apparently had very little effect on the per cent of cellulose in the forages.

Nitrate Content of the Forages

As previously stated, the presence of nitrates and/or nitrites in a forage sample may possibly interfere with rumen microbial utilization of that forage in vitro. Therefore all of the forages used in these studies were tested qualitatively for nitrates using diphenylamine. If nitrates are present, a blue color develops when a drop of the reagent is added to the forage. The intensity of the color gives some indication of the relative amounts of nitrates present. When the tests were conducted on the experimental forages used in these studies, the observed color intensity was subjectively assigned a numerical value of zero to four. Zero was used to indicate a negative test and a value

TABLE I

CELLULOSE CONTENT OF THE BERMUDA GRASSES (PER CENT)

Pounds nitrogen added/acre	Cutting	Variety of Bermuda grass		
		Coastal	Common	Midland
0	I	28.75	--	29.38
	II	30.33	29.70	29.66
	III	32.50	31.07	31.92
	IV	30.10	30.83	31.04
60	I	29.70	--	--
	II	30.72	--	--
	III	32.00	--	--
	IV	30.02	--	--
120	I	29.70	29.70	29.63
	II	30.40	29.59	30.09
	III	33.00	31.37	31.96
	IV	30.05	31.27	31.12
240	I	30.22	29.81	29.75
	II	30.88	30.55	31.26
	III	33.20	31.54	32.20
	IV	30.26	31.42	31.58
360	I	30.32	29.92	29.95
	II	31.29	31.00	31.49
	III	32.52	32.02	32.41
	IV	31.24	31.48	31.82
480	I	30.28	--	--
	II	31.59	--	--
	III	--	--	--
	IV	--	--	--
720	I	31.01	--	--
	II	32.24	--	--
	III	33.90	--	--
	IV	31.65	--	--

of four was used to indicate the presence of large amounts of nitrates in the forage.

The results of the above tests are shown in Table II. As shown by the data, the first three cuttings of most of the forages fertilized with 120 pounds of nitrogen per acre contained some nitrates. The first cutting of all three Bermuda grasses which had been fertilized with 360 pounds of nitrogen per acre contained large amounts of nitrates. It will be observed that the fourth cuttings of most of the forages fertilized with either 240 pounds or 360 pounds of nitrogen per acre had only small amounts (relatively speaking) of nitrates.

Nitrogen Content of the Forages

Dr. Henry A. Fribourg of the Agronomy Department of the University of Tennessee analysed most of the forage samples for total nitrogen content using the standard A.O.A.C. method (1950). Since these data help to further characterize the forage samples, they are shown in Table III. As shown by the data, time of cutting had a very marked effect on the amount of nitrogen in the forages. First cuttings of the control forages (no added nitrogen) contained about 2.22 to 2.94 per cent nitrogen as compared to less than 0.97 to 1.11 per cent for the fourth cutting forage samples. The effects of level of nitrogen fertilization on total nitrogen content of the forages were variable.

Rumen Microbial Utilization of the Forages

The extent to which rumen bacteria utilized the various forages

TABLE II
RESULTS OF THE QUALITATIVE TEST FOR NITRATES FOR
THE BERMUDA GRASSES^a

Pounds nitrogen added/acre	Cutting	Variety of Bermuda grass		
		Coastal	Common	Midland
0	I	0	0	0
	II	0	0	0
	III	0	0	0
	IV	0	0	0
60	I	1	--	--
	II	0	--	--
	III	0	--	--
	IV	0	--	--
120	I	2	2	1
	II	2	0	2
	III	1	1	0
	IV	0	0	0
240	I	3	4	3
	II	3	0	2
	III	3	0	2
	IV	1	0	0
360	I	4	4	4
	II	3	2	3
	III	4	1	3
	IV	1	0	1
480	I	4	--	--
	II	3	--	--
	III	--	--	--
	IV	--	--	--
720	I	4	--	--
	II	3	--	--
	III	4	--	--
	IV	2	--	--

^aZero denotes a negative test; a numerical value of four denotes the maximum amount of color intensity observed in the test and thus high levels of nitrate.

TABLE III
NITROGEN CONTENT OF THE BERMUDA GRASSES (PER CENT)

Pounds nitrogen added/acre	Cutting ^a	Variety of Bermuda grass		
		Coastal	Common	Midland
0	I	2.94	2.91	2.52
	II	1.38	1.31	1.52
	III	1.74	1.41	1.32
	IV	0.97	1.01	1.11
60	I	2.80	—	—
	II	1.44	—	—
	III	1.53	—	—
	IV	0.98	—	—
120	I	3.33	3.22	3.11
	II	2.92	2.05	2.64
	III	1.57	2.00	1.49
	IV	0.84	1.00	1.05
240	I	3.43	3.78	3.44
	II	2.31	1.84	2.79
	III	1.71	2.06	2.19
	IV	1.39	1.27	1.15
360	I	2.81	3.92	3.59
	II	2.48	2.56	2.70
	III	2.09	1.94	2.45
	IV	1.23	1.39	1.76
480	I	3.28	—	—
	II	1.96	—	—
	III	—	—	—
	IV	—	—	—
720	I	3.78	—	—
	II	2.33	—	—
	III	2.43	—	—
	IV	1.93	—	—

^aThe cutting dates were: I, May 27; II, July 7; III, August 11; and IV, October 14.

are summarized in Tables IV through VII. As indicated previously, cellulose digestion was used as the criterion to evaluate microbial activity. After the analyses of variances of the data from a specific series of experiments was completed, "D" tests (Snedecor, 1956) were performed to determine differences among treatment means. Two "D" values were determined and these are listed at the bottom of the tables. D1 is based on the error term and D2 is based on the interaction of nitrogen fertilization and time of harvest or cutting.

Coastal Bermuda grass--first and second cuttings. As shown by the data in Table IV and Figure 1, rumen microbes digested significantly more of the cellulose of Coastal Bermuda grass harvested early in the growing season, as compared to samples harvested later in the growing season. This trend was consistent for all levels of nitrogen fertilization.

Nitrogen fertilization up to 240 pounds of nitrogen per acre of the above grass appeared to improve the quality of this forage for rumen bacteria in vitro. Thus for the control forage, 59 per cent of the cellulose was digested by the bacteria for cutting I, as compared to a value of 68 per cent for cutting I of the forage fertilized with 240 pounds of nitrogen per acre. Higher levels of nitrogen did not result in further increase in microbial activity. A slight depression in cellulose digestion occurred in the sample fertilized with 720 pounds of nitrogen per acre when compared to the non-nitrated sample (59 vs 48 per cent). It is possible that the nitrate content of the heavily fertilized forage reached a point which depressed rumen microbial

TABLE IV

IN VITRO RUMEN MICROBIAL UTILIZATION OF COASTAL BERMUDA
GRASS FERTILIZED WITH DIFFERENT LEVELS OF NITROGEN
AND CUT AT DIFFERENT TIMES DURING THE GROWING
SEASON (CUTTINGS I AND II)

Pounds nitrogen added/acre	Cutting ^a	Cellulose digested, %			
		Exp. I	Exp. II	Exp. III	Mean ^b
0	I	60	58	58	59
	II	30	36	32	33
60	I	64	62	62	63
	II	45	46	40	44
120	I	65	66	64	65
	II	50	53	50	51
240	I	67	68	68	68
	II	46	45	42	44
360	I	55	61	60	59
	II	44	42	37	41
480	I	52	57	50	53
	II	41	39	35	38
720	I	48	55	41	48
	II	37	38	31	35

^aThe cutting dates were: I, May 27; and II, July 7.

^bFor nitrogen fertilization effects, D1=6.00 and D2=25.91; for cutting effects, D1=2.25 and D2=14.31.

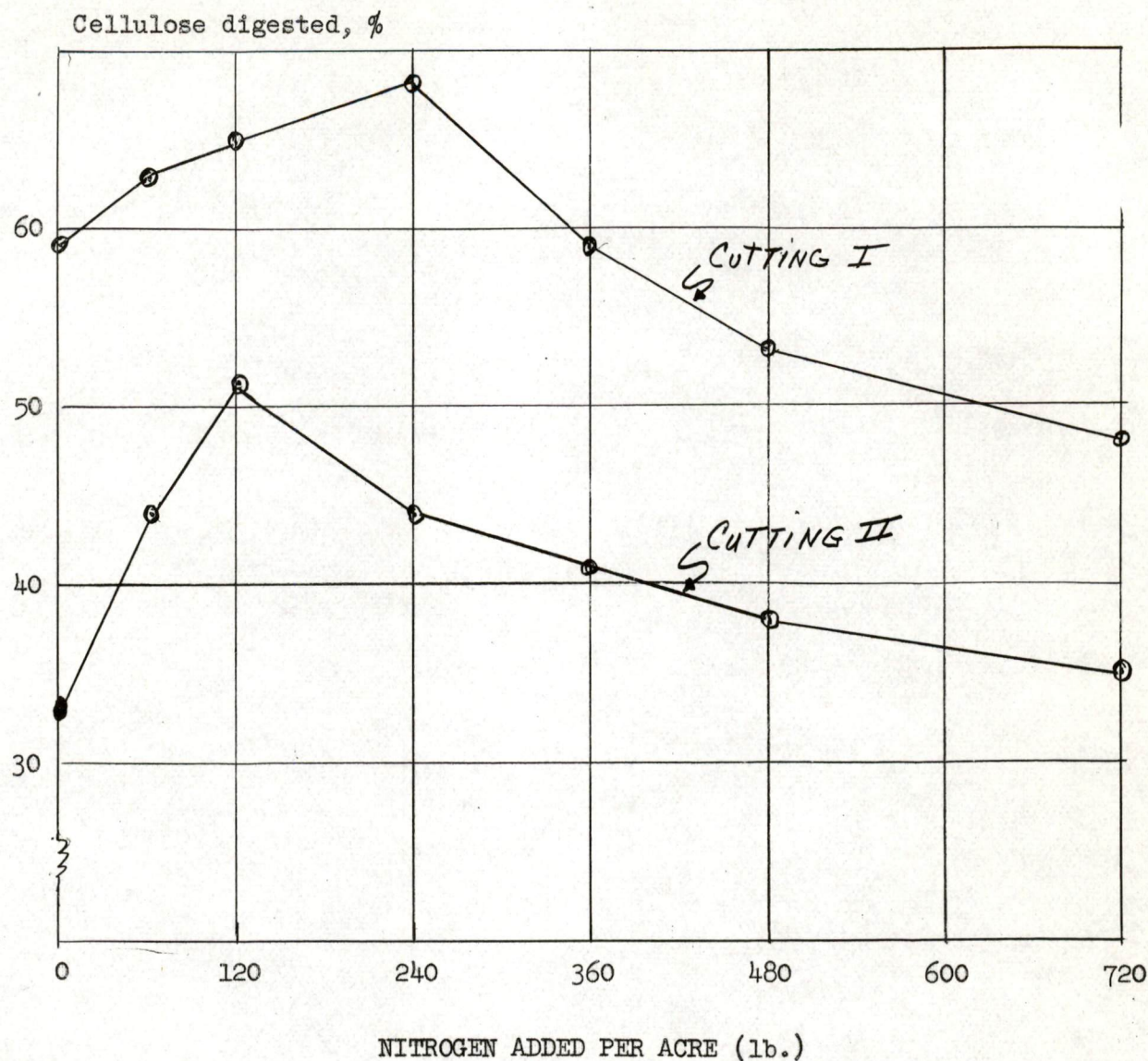


FIGURE 1

RUMEN MICROBIAL UTILIZATION (BASED ON CELLULOSE DIGESTION)
OF FIRST AND SECOND CUTTINGS OF COASTAL BERMUDA GRASS
FERTILIZED WITH DIFFERENT LEVELS OF NITROGEN

breakdown of the cellulose.

Coastal Bermuda grass--third and fourth cuttings. Rumen microbial utilization of two additional cuttings of Coastal Bermuda grass fertilized with different levels of nitrogen is shown in Table V and Figure 2. Forage samples represented by cutting III were obtained on August 11 and those represented by cutting IV were obtained on October 14. Except for the control forage (no added nitrogen), the fourth cutting forage samples appeared to be utilized equally as well by rumen microbes as were the samples obtained on August 11 (cutting III). The mean square for cutting x level of nitrogen fertilization for this series of experiments was highly significant indicating that time of harvesting had a pronounced effect on the cellulolytic response due to nitrogen fertilization. Thus when the results for cutting III are considered, it will be observed that cellulose digestion remained about the same or decreased slightly with increasing amounts of nitrogen fertilizer whereas with cutting IV, cellulose digestion increased with increasing levels of nitrogen fertilizer up to 120 pounds of nitrogen per acre. Beyond this level, rumen microbial cellulolytic response decreased as the amount of nitrogen applied was increased.

Common Bermuda grass. The effects of four levels of nitrogen fertilization and four dates of harvesting on in vitro rumen microbial utilization of Common Bermuda grass are shown in Table VI and Figure 3. Forage samples fertilized with 480 and 720 pounds of nitrogen per acre were omitted from this and the subsequent series of experiments so that

TABLE V

IN VITRO RUMEN MICROBIAL UTILIZATION OF COASTAL BERMUDA
GRASS FERTILIZED WITH DIFFERENT LEVELS OF NITROGEN
AND CUT AT DIFFERENT TIMES DURING THE GROWING
SEASON (CUTTINGS III AND IV)

Pounds nitrogen added/acre	Cutting ^a	Cellulose digested, %			
		Exp. I	Exp. II	Exp. III	Mean ^b
0	III	39	42	40	40
	IV	32	32	29	31
60	III	38	39	38	38
	IV	39	37	41	39
120	III	39	38	37	38
	IV	48	45	48	47
240	III	38	37	33	36
	IV	40	40	40	40
360	III	34	35	32	34
	IV	35	39	37	37
720	III	34	35	32	34
	IV	32	38	35	35

^aThe cutting dates were; III, August 11; and IV, October 14.

^bFor nitrogen fertilization effects, D1=3.13 and D2=31.44; for cutting effects, D1=1.80 and D2=18.20.

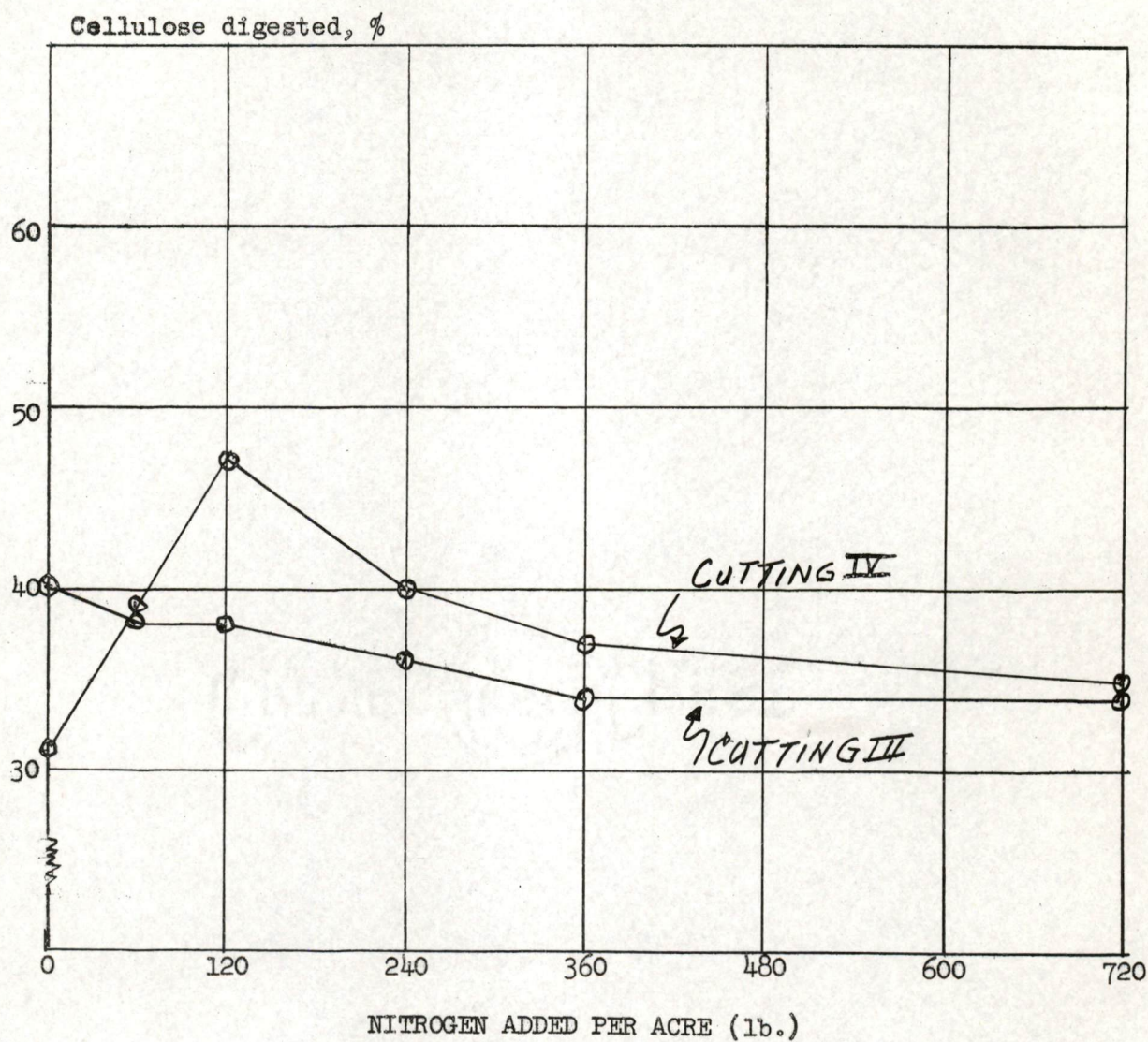


FIGURE 2

RUMEN MICROBIAL UTILIZATION (BASED ON CELLULOSE DIGESTION)
 OF THIRD AND FOURTH CUTTINGS OF COASTAL BERMUDA GRASS
 FERTILIZED WITH DIFFERENT LEVELS OF NITROGEN

TABLE VI

IN VITRO RUMEN MICROBIAL UTILIZATION OF COMMON BERMUDA
GRASS FERTILIZED WITH DIFFERENT LEVELS OF
NITROGEN AND CUT AT DIFFERENT TIMES
DURING THE GROWING SEASON

Pounds nitrogen added/acre	Cutting ^a	Cellulose digested, %			Mean ^b
		Exp. I	Exp. II	Exp. III	
0	I	--	--	--	--
	II	32	34	36	34
	III	34	34	37	35
	IV	30	32	32	31
120	I	56	57	56	56
	II	40	41	39	40
	III	39	40	38	39
	IV	34	41	36	37
240	I	60	58	60	59
	II	44	43	45	44
	III	38	36	37	37
	IV	32	33	34	33
360	I	53	55	51	53
	II	34	41	37	37
	III	33	34	32	33
	IV	30	31	32	31

^aThe cutting dates were: I, May 27; II, July 7; III, August 11; and IV, October 14.

^bFor nitrogen fertilization and cutting effects D1=1.88 and D2=7.97.

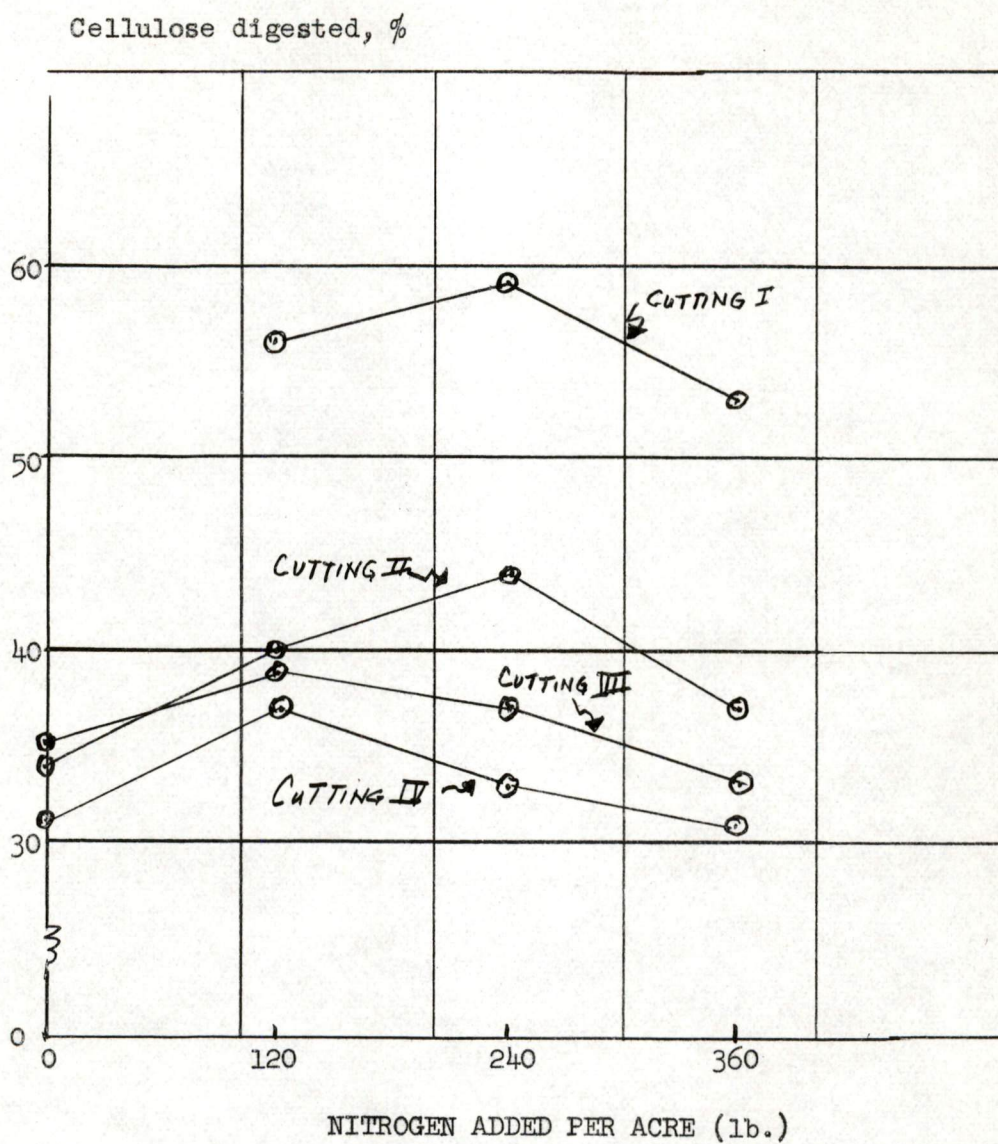


FIGURE 3

RUMEN MICROBIAL UTILIZATION (BASED ON CELLULOSE DIGESTION)
OF FOUR CUTTINGS OF COMMON BERMUDA GRASS FERTILIZED
WITH DIFFERENT LEVELS OF NITROGEN

all four cuttings or times of harvesting could be studied simultaneously.

At levels of nitrogen fertilization varying from 120 to 360 pounds per acre, the first cutting of forages supported significantly more rumen microbial activity than did those forages harvested later in the growing season. Third and fourth cuttings of forages fertilized with 240 pounds of nitrogen per acre supported significantly less microbial activity than did cutting II of forages but this trend was not evidenced for the other levels of nitrogen fertilization.

Considering the effects of nitrogen fertilization on cellulose digestion it will be observed that 44 per cent of the cellulose was digested for cutting II of forages as compared to 34 per cent for the corresponding control forage. The effect of nitrogen fertilization on microbial utilization of the other forages was not significant statistically.

Midland Bermuda grass. Results of rumen microbial utilization studies of Midland Bermuda grass followed a somewhat similar pattern to the results obtained from Common Bermuda grass (Table VII and Figure 4). Thus the first cutting of forages supported significantly more microbial activity than did the second, third or fourth cuttings over all levels of nitrogen fertilization. Although differences among cellulose digestion coefficients for the second, third and fourth cuttings were not as marked as those listed above, there was a consistent trend for the amount of cellulose that was digested by rumen microbes to decrease as the stage of maturity or time of cutting increased.

TABLE VII

IN VITRO RUMEN MICROBIAL UTILIZATION OF MIDLAND BERMUDA
GRASS FERTILIZED WITH DIFFERENT LEVELS OF
NITROGEN AND CUT AT DIFFERENT TIMES
DURING THE GROWING SEASON

Pounds nitrogen added/acre	Cutting ^a	Cellulose digested, %			Mean ^b
		Exp. I	Exp. II	Exp. III	
0	I	55	58	57	57
	II	41	45	40	42
	III	37	41	37	38
	IV	32	35	35	34
120	I	59	63	61	61
	II	46	52	46	48
	III	42	49	40	44
	IV	39	41	40	40
240	I	65	67	64	65
	II	52	61	52	55
	III	39	46	39	41
	IV	36	40	35	37
360	I	57	57	56	57
	II	46	49	43	46
	III	35	41	36	37
	IV	33	33	31	32

^aThe cutting dates were: I, May 27; II, July 7; III, August 11; and IV, October 14.

^bFor nitrogen fertilization and cutting effects, D1=1.84 and D2=9.09.

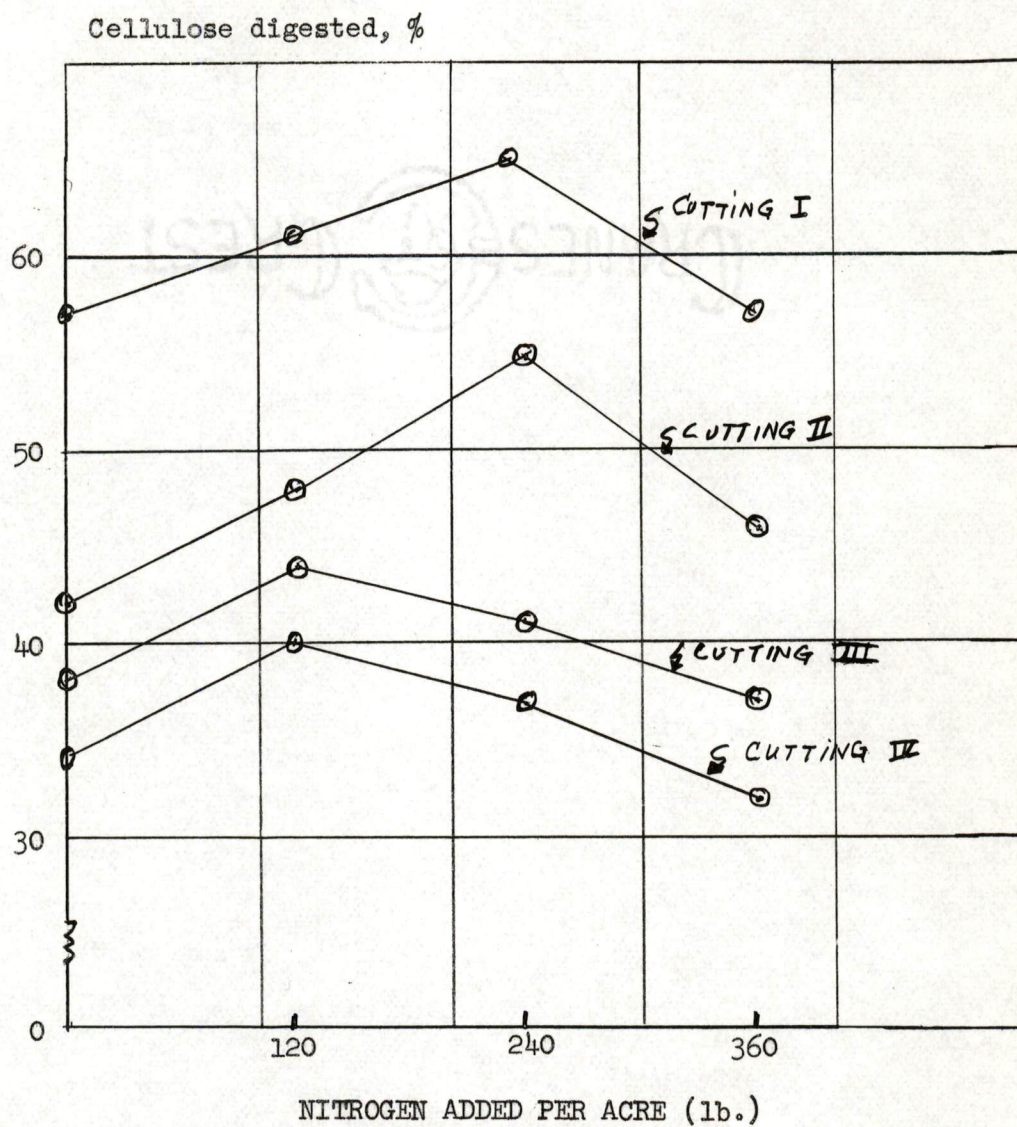


FIGURE 4

RUMEN MICROBIAL UTILIZATION (BASED ON CELLULOSE DIGESTION)
OF FOUR CUTTINGS OF MIDLAND BERMUDA GRASS FERTILIZED
WITH DIFFERENT LEVELS OF NITROGEN

The first two cuttings of Midland Bermuda grass fertilized with 240 pounds of nitrogen per acre supported significantly more microbial activity as evidence by cellulose breakdown than did the forages which had not received nitrogen fertilizer. Differences apparently due to level of nitrogen fertilization for third and fourth cuttings were not significant statistically. It can also be noted from the data in Table VII that rumen microbial activity supported by all cuttings of the forage fertilized with 360 pounds of nitrogen per acre was very similar to that supported by the non-nitrated forage.

CHAPTER V

SUMMARY

Rumen microbial utilization of Coastal, Common and Midland Bermuda grasses fertilized with 0, 60, 120, 240, and 360 pounds of nitrogen per acre and cut at four different times during the summer period (May 27, July 7, August 11 and October 14) was studied in four series of in vitro experiments. Additional levels of nitrogen fertilization of Coastal Bermuda grass (up to 720 pounds per acre) were also studied. The forage samples were supplied by the Agronomy Department of the University of Tennessee and all forages had been grown under carefully planned experimental conditions. Each experimental forage used in the studies represented a pooled sample prepared by combining forage obtained from four replicated agronomic plots.

Rumen liquid was obtained from a fistulated steer maintained on a good quality grass-legume hay and about 3.0 pounds of concentrates per head daily. The bacteria were separated from the mother liquor by a process of differential centrifugation. The bacteria were washed in a sodium bicarbonate-carbonic acid buffer and then equal volumes of the washed bacterial suspension were used to inoculate fermentation flasks containing the finely ground forages suspended in sodium bicarbonate-carbonic acid buffer. Cellulose disappearance during a 24-hour fermentation period due to cellulolytic bacteria was determined and used as the criterion of microbial activity.

All forage samples used were chemically analyzed for nitrogen and cellulose. A qualitative test for nitrates using diphenylamine was also conducted on each forage.

Results of the chemical analyses of the forage samples showed that time of harvest had a more pronounced effect on the cellulose content of the forages than did the level of nitrogen fertilization. The cellulose content of the forages harvested May 27 contained about 29 per cent cellulose as compared to 32-33 per cent cellulose for the forages harvested August 11. Cellulose content of the forages harvested October 14 was approximately 30 to 31 per cent. There was a trend for the cellulose to increase slightly with increasing levels of nitrogen fertilization. According to the results of the qualitative nitrate test, small quantities of nitrates had apparently accumulated in the first two cuttings of the forages fertilized with 120 pounds of nitrogen per acre. The first cuttings of all forages fertilized with 360 pounds of nitrogen per acre apparently contained relatively large amounts of nitrates. The content of nitrates in subsequent cuttings of forages fertilized with the above level of nitrogen and higher levels was variable.

Based on cellulolytic response, rumen bacteria utilized all forages harvested early in the growing season (May 27) to a significantly greater extent than they did forages harvested later in the growing season (July 7, October 11, and August 14). This was true even though some of the first cuttings of the forages apparently contained high levels of nitrates (based on a qualitative test).

Generally, nitrogen fertilization up to 240 pounds per acre of first and second cuttings of all three varieties of Bermuda grasses resulted in an improvement in the microbial utilization of the forages based on cellulose breakdown by the bacteria.

Third and fourth cuttings of the forages (with exception of the third cutting of Coastal Bermuda grass) fertilized with 120 pounds of nitrogen per acre supported slightly more microbial activity in vitro than did non-nitrated forages harvested at the same time. First and second cuttings of Coastal Bermuda grass fertilized with 720 pounds of nitrogen supported less microbial activity than did comparable non-nitrated forage. However, this difference was not evidenced in subsequent cuttings of the forage. Based on the results of these experiments it would appear that nitrogen fertilization of 120-240 pounds per acre may improve the quality of Bermuda grass for rumen bacteria in vitro and that even higher levels might be used without adversely affecting rumen microbial activity significantly.

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LITERATURE CITED

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