INFLUENCE OF TEMPERATURE AND FERTILIZERS ON YIELD, CARBOHYDRATE, PROTEIN CONTENT, AND *IN VITRO* DIGESTIBILITY OF MIDLAND BERMUDAGRASS

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CHAPTER I

INTRODUCTION

There is considerable concern by farmers, ranchers, and research workers in regard to the low animal gains on bermudagrass (Cynodon dactylon L.), Pers., in late summer and fall (25). There are five million acres of bermudagrass being utilized in Oklahoma by animals. If it were possible to improve the quality of forage obtained during this time of year and thus to increase animal gains, it would represent a tremendous economic gain in livestock production.

The term "quality" is a widely-used term and one which is not very well understood. Quality will be used in this discussion to describe the individual animals in terms of the amount of body weight maintained or gained (41). Plant factors which influence or relate to quality in terms of individual animal gains are the nutritive value of the forage and the rate of intake (40). The nutritive value is influenced by the chemical composition and digestibility, while the rate of intake by the animal is influenced by the acceptability of the forage and the rate of passage of the forage through the animal. The rate of intake is also influenced by grazing pressures and the environmental effects on the animal.

The objectives of this experiment were to determine the influence of nutrients on protein and carbohydrate contents, especially sugars and starch. The total production and quality as measured by in vitro

digestion were also observed in this experiment. The temperature effects on the above measurements were also studied. The bermudagrass variety, Midland, was the forage plant chosen for study because of its widespread use in the southern part of the United States.

CHAPTER II

LITERATURE REVIEW

A number of chemical tests have been used to ascertain quality of forages. Among the tests which have been used are crude protein, crude fiber, cellulose, lignin, silica, and carbohydrate contents. In general, plants which are young and vigorously growing are high quality, possess high protein and carbohydrate contents along with low lignin, silica, cellulose, and crude fiber. Studies of the relationship of one or more of these chemical tests show varying degrees of success.

Crude fiber has long been used as an estimate of forage quality in the belief that it represented the indigestible part of the forage; however, this is not entirely true since a part of the crude fiber is digested by herbivores (51). Increases in lignin content are associated with reduced digestibility, and lignin is believed to form a lignin-cellulose compound of some type in the plant cell wall (50).

Crude protein as estimated by the Kjeldahl nitrogen method provides an acceptable method of quality estimation, particularly in hays where protein may be a limiting factor; however, in very high quality forage, increasing protein beyond a certain level may not produce any response by the animal on feed (47). The Kjeldahl nitrogen method also measures some non-protein nitrogen in addition to true protein nitrogen. This is not a problem in forage for ruminants since this non-protein nitrogen can be used by the microflora in the rumen and, thus, made

available to the animal.

Lignin is a substance in plants which has little, if any, digestibility and also has the property of lowering digestibility of other cell wall constituents (47). The correlation coefficient between the dry matter digestibility and percent acid-insoluble lignin in 60 samples of grass was much higher than between dry matter digestibility and percent crude fiber. Most procedures for lignin determination are based on insolubility of lignin and solubility of cellulose in 72 percent sulfuric acid; these procedures are tedious and have a very low precision level. Problems also exist in trying to make comparisons between grasses and alfalfa. Alfalfa is more highly lignified than grass with a lower digestion coefficient for the cellulosic constituents; however, the digestion coefficient would not be as low as would be expected from the lignin content. When a sample of alfalfa has a higher digestibility of dry matter than a grass sample, the higher digestibility is usually associated with higher digestion of protein in the alfalfa rather than greater digestion of cell wall substances (47). Even though the determination of lignin has some weaknesses, it offers merit as a measurement of the digestibility of forages.

Increased silica content has been implicated in reduced quality of forages. In a study of ryegrass--tall fescue hybrids and tall fescue varieties, the hybrid possessed lower percent silica and higher percent digestibility than the tall fescue varieties, with the backcross to the tall fescue varieties being intermediate between the varieties and hybrids (8). In forages with low digestibility which could not be explained by such factors as lignin or crude fiber, silica content was found to be high (50). There has been only limited work relating

silica to quality.

Water soluble carbohydrates such as glucose, fructosans, and starch may be regarded as highly digestible (47). The relationship of the soluble carbohydrates with quality does not appear to have been widely explored and will be examined in some detail in this paper.

A number of external factors affect the quality of forages. Environmental factors producing the greatest effects are moisture and temperature, along with age of the plant. Many experiments show that the percentage of cellulose and lignin increased as the amount of available moisture decreased. Plants grown at low temperatures have a lower cellulose and lignin content than plants grown at higher temperatures.

The effects of plant nutrients on quality of grass have also been widely studied. The effect of certain nutrients on forage quality will be reviewed later.

Plant nutrients produce certain physiologic effects as well as affecting forage quality. Nitrogen produces very obvious effects in that it promotes vigorous vegetative growth and increased fruit and seed yields of the plants. Nitrogen is necessary for protein and enzyme production in the plant. Since most of the physiologic processes are enzyme-mediated, many plant processes are affected by nitrogen level. Phosphorus tends to promote early growth, counterbalances nitrogen fertilization, and hastens plant maturity. Potassium is involved in enzyme reactions in the plant, and a high concentration is present in the cells, suggesting some sort of ionic balance property in the cell. Low potassium levels depress the translocation of nitrogenous and carbohydrate constituents in the plant. Calcium is present as a part of the cell wall and with calcium deficiency in most plants, root growth is

reduced; frequently root rotting occurs, and the terminal bud may die back. Magnesium is a constituent of chlorophyll, and it affects the rate of photosynthesis and is also involved in certain enzyme reactions in carbohydrate metabolism. Deficiency of sulfur reduces growth of plants, and many plants respond to sulfur only at high nitrogen levels. Sulfur is also a constituent of protein in plants (35).

Plant nutrients also affect the quality of the plants, and especially carbohydrate and protein metabolism. Phosphorus and nitrogen promote growth and plant maturity, suggesting involvement in carbohydrate metabolism.

Higher temperatures generally result in lower carbohydrate content in grasses. Total soluble carbohydrates in the leaves of many species of grass have been shown to decrease under rising temperatures (5, 15, 16, 23, 45). Schmidt and Blaser (42) reported the carbohydrate reserves in bermudagrass, a tropical species, to be higher in high temperatures. He attributed this to higher leaf-root ratio found in bermudagrass grown at higher temperatures. Smith (45) found carbohydrate (total sugars plus fructosans) percentages to be as high as 30 percent in some leaf blades under cool temperatures.

In a separate experiment (23), Bahiagrass (Paspalum notatum Flugge), bermudagrass, Italian ryegrass (Lolium multiflorum Lam.), and timothy (Phleum pratense L.) were grown in pots in a greenhouse and then transferred to growth chambers at 15, 20, and 25°C. In like manner, fructosan, total sugar, reducing sugar, and non-reducing sugar contents were lower at the higher temperatures. Higher temperature was also shown to depress carbohydrates in two other species, Lolium perenne L., a temperate grass, and Brachiaria ruzizienses, a tropical species (15).

Timothy grown under 18.5-10°C and 29.5-21°C day-night temperatures, respectively, had greater dry matter production and a higher percentage of total non-structural carbohydrates in all plant parts under the cooler temperature treatment. Temperature influenced the level of fructosans accumulated, but had little effect on the level of total sugars and starch. (44)

Davidson and Milthorpe (16) studied the growth of orchardgrass (Dactylis glomerata L.) under 14, 22, and 26°C, respectively. It was found that water-soluble carbohydrates accumulated in the plants more rapidly under the lower temperatures. Temperature also appeared to influence accumulation of fructosans and non-structural polysaccharides in grasses of temperate origin. Blaser et al. (5) reported from their research that carbohydrates were higher in grasses grown under cool temperatures.

Temperature also affects photosynthesis and growth rate of many plants. Schmidt and Blaser (42) reported that the highest net photosynthesis of Tifgreen bermudagrass occurred at 24-18°C day-night temperature, and the lowest occurred at 12-10°C day-night temperature. Miller (38) found that the relative rate of apparent photosynthesis was greatest at 25°C in Seaside bentgrass (Agrostis palustris Huds.) and at 35°C in bermudagrass. Deinum (15) reported that high temperature resulted in increased dry matter of a tropical grass, Brachiaria ruziziensis. Ehara and Tanaka (23) reported that dry matter production of Italian ryegrass and timothy was greatest at 15°C, while bermudagrass and Bahiagrass dry matter production was greatest at 35°C.

Protein content is also affected by temperature. Ehara and Tanaka (23) found crude protein content of a temperate grass, Italian ryegrass,

was higher at 25°C than at 15 or 20°C, while crude protein of timothy and the tropical grasses, Bahiagrass and bermudagrass, decreased with increasing temperature. Deinum (15) found the same trends for Lolium perenne L. and Brachiaria ruziziensis. McCroskey et al. (36) found crude protein in Midland bermudagrass to be highest in April. Crude protein content may also be influenced by moisture and soil fertility.

Digestibility of the plant is also affected by temperature. Smith and Jewiss (44) grew timothy at 18.5-10°C and 29.5-21°C with and without nitrogen fertilization. It was found that the dry matter digestibility decreased as the temperatures increased. In general, dry matter digestibility is influenced by the lignin content of the grass. Ehara and Tanaka (23) found that crude lignin content increased with increasing temperatures in timothy, Italian ryegrass, Bahiagrass, and bermudagrass. Walster (53) grew barley plants in sand culture at two temperature levels, 15 and 20°C. There were no significant differences in lignin content due to these temperatures. Brown (6) studied four species, Kentucky bluegrass (Poa pratensis L.), Canada bluegrass (Poa compressa L.), orchardgrass, and bermudagrass grown in growth chambers. Crude fiber content increased for all species as the temperature rose from 40 to 60°F and changed little with further rises in temperature.

In field experiments, the seasonal termperature also affects the crude fiber percentage of the plants. Brown (7) found the increasing temperature from April to May increased crude fiber content from 15.9 to 23.4 percent on a dry weight basis in Kentucky bluegrass. McCroskey et al. (36) reported that field-grown Midland bermudagrass had a lignin percentage of 2.8 in April and increased each month to a high of 6.1 percent in January and February. The in vitro digestibility of Midland

bermudagrass dry matter reached a high of 70.2 percent in April and reached a low of 37.5 percent in January.

Fertilizers influence yield of dry matter, carbohydrates, protein, and digestibility of most plants. This is especially true for some major nutrients in fertilizers such as nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur. Nitrogen, phosphorus, and potassium have been shown to influence herbage yield in grasses more so than other elements. Evans et al. (27) found applications of nitrogenous fertilizers greatly increased yields of Coastal bermudagrass in Alabama. Smith and Kapp (43) reported that application of phosphorus and potassium fertilizers in combination with nitrogen gave increases in yield of Coastal bermudagrass over that fertilized with nitrogen alone. Jackson et al. (30) found the rate of depletion of soil phosphorus and potassium when omitted from the fertilizer increased with increasing rates of nitrogen application. Potassium became critical sooner than phosphorus on Tifton loamy sand.

It has been shown in many experiments that calcium and magnesium are necessary for normal plant growth. Colwell et al. (13) reported that soils supplied with available calcium equivalent to 1,200 pounds lime per acre produced a normal yield of peanut kernels, but when the calcium supply was below this value, kernel yields were increased by adding gypsum. Jacob (31) proposed that magnesium acts as a carrier of phosphorus in plants. It would then be expected that the response to phosphorus would be limited if the enzyme systems mediating its metabolism were limited by magnesium supply.

Sulfur often produces an effect on plants. Jordan and Reisenauer (33) found that small grains often show sulfur deficiency if high rates

of nitrogen fertilizer are applied and the nitrogen carrier is free of sulfur. They reported that in some instances negative responses were obtained from nitrogen application when sulfur was deficient, and when sulfur was supplied there was an excellent response to the nitrogen fertilizer. Jordan and Ensminger (34) cited a number of experiments in which grasses and cereals responded to sulfur at high nitrogen levels but showed little or no response at low nitrogen levels.

Carbohydrate contents of plants are also influenced by fertilizer applications. Blaser et al. (5) found increasing nitrogen nutrition decreased the total water soluble carbohydrates of the grass. Burris et al. (9) grew Midland bermudagrass under 30 and 300 pounds of nitrogen fertilizer per acre, respectively. With 300 pounds of nitrogen per acre, total carbohydrates declined the first 14 days, then increased for the next 7 days. With 30 pounds nitrogen per acre, total carbohydrates declined the first 7 days, then increased the next 14 days. Plants were then placed in darkness for two weeks, and total carbohydrates declined under both regimes. Smith and Jewiss (44) found that increasing nitrogen nutrition decreased the total water soluble carbohydrates of timothy grass. Adegbola and McKell (2) fertilized Coastal bermudagrass with nitrogen rates of 0, 100, and 250 pounds per acre. Plant tissues were analyzed for reducing sugar (glucose and fructose) and non-reducing sugar (sucrose) every 2 weeks during the 1963 growing season. They stated that leaves contained reducing sugar at 5.7, 7.5, and 11.5 percent on a dry mater basis at nitrogen fertilizer applications of 0, 100, and 250 pounds per acre, respectively. The highest content of sucrose occurred in the leaves 2 weeks after fertilizer application.

Eaton (19) found phosphorus deficiency in sunflower (Helianthus

annuus L.) caused increased concentration of total sugars, reducing sugar, sucrose, and starch in young plants, but found in old plants the reverse of the above was true or there were no effects at all. Eaton (20) also found phosphorus deficiency in soybean (Glycine max.) increased the concentration of all carbohydrate fractions. It was found that phosphorus deficiency in black mustard (Brassica nigra) increased total sugars, reducing sugar, and sucrose, but not starch (21).

Eaton (22) found that sunflowers often accumulated carbohydrates in the early stages of growth when potash was deficient. Cooil and Statlery (14) found guayule (Parthenium argentatum) plants accumulated starch in the phloem, cortex, and medullary rays, but in later stages of potassium deficiency, starch disappeared from the plants. Hartt (28) found potassium deficiency in sugarcane (Saccharum officinarum) led to increased proportions of reducing sugar, while sucrose levels were decreased. Wall (52) found in tomato (Lycopersicon esculentum) that potassium deficiency led to higher carbohydrates in the early stages, followed by a sharp decline in carbohydrate content of the plant.

Joham (32) concluded from his studies with cotton (Gossypium hirsutum) that calcium has an effect on translocation of carbohydrates analogous to that postulated for boron. Kermit and Parker (35) found that magnesium is involved in movement of carbohydrates from leaves to the stems of many plants.

Eaton (18) found that sulfur deficiency led to increased starch content and a decrease in total sugar in sunflower. In general, there were decreases in reducing sugars and increases in total carbohydrates. It was found that sulfur deficiency in soybean decreased the content of total and reducing sugars but increased starch and crude hemicellulose

(17).

Protein content of plants may be influenced by addition of fertilizers, especially those containing nitrogen. Adams et al. (1) reported an increase from 9.44 percent to 16.37 percent protein in Coastal bermudagrass from 0 to 400 pounds nitrogen per acre, respectively. Worker and Peterson (55) found nitrogen fertilizer increased protein, reduced ash and nitrogen-free extracts, but had no effects on fat, calcium, potassium, and phosphorus content in Coastal bermudagrass hay.

Eaton (22) found potassium deficiency depressed protein synthesis in sunflower. Cooil and Statlery (14) found potassium deficiency in guayule decreased protein assimilation. Hartt (28) reported similar results associated with potassium deficiency in other plant species.

Burstrom (10) found calcium functioned by increasing uptake of nitrate for protein synthesis. Anderson and Spencer (3) found sulfur deficiency limited the nodulation of subterranean clover (Trifolium subterraneum). Hewitt (2θ) concluded that insoluble nitrogen was generally decreased in all species by sulfur deficiency. Ergle (26) observed that sulfur deficiency in cotton decreased protein content and soluble sulfur compounds.

Forage quality is greatly affected by fertilizers and age of the plants. Lignin, cellulose, and hemicellulose are the most important plant fractions determining the digestibility of a forage. There is an abundance of evidence indicating that the main structural constituents of grasses increase progressively with age. Burton et al. (11) found digestibility of Coastal bermudagrass decreased with age and dropped rapidly in plants over six weeks old. In vitro digestibility of the forage gave values ranging from 62.2 down to 43.2 percent cut at 3 to

24-week intervals. Burton et al. (12) found that 23 clones of bermudagrass cut at 2, 3, 4, and 6-week intervals differed significantly in dry matter digestibility using the nylon bag technique. Miller et al. (39) reported similar results with Coastal bermudagrass harvested at 3, 5, and 7 weeks through the season. He found digestibility decreased as the age of the grass increased at harvesting time. Digestibility of organic matter was 66.0, 60.7, and 56.8 percent, and dry matter digestibility was 65.9, 60.7, and 56.6 percent at 3, 5, and 7-week harvest intervals, respectively.

The effect of fertilizer application on the percentage of structural components in the plant is variable. Interpretation of the experimental evidence is complicated because the herbage is cut at several different times through the season. Also, in some experiments fertilizer was applied only once, while in others it was applied several times during the season. In a 9-year study in Virginia, Eheart and Ellett (24) found no significant difference in the percentage of crude fiber in fertilized and non-fertilized forage when either nitrate of soda, ammonium sulfate, or urea was used or when 50, 100, or 150 pounds of nitrogen per acre were applied. However, at the first cutting in all years there was a significantly higher percentage of crude fiber in grass from the fertilized plots than in grass from the unfertilized check plots. The mean crude fiber content of samples from all fertilized plots was 17.8 percent on a dry matter basis at the first cutting, 20.8 percent at mid-season, and 19.1 percent at the last of the season compared to 16.8, 20.4, and 17.9 percent, respectively, for the unfertilized plots.

Archibald and Bennett (4) conducted extensive experiments using

complete fertilizers (N, P, K) on grasses and found the percentage of crude fiber decreased with fertilizer application. They found 6 percent more crude fiber in the dry matter of grass from check plots than from fertilizer plots. The per acre production of fiber, however, was about 24 percent greater on the fertilized plots.

Swift et al. (48) applied ammonium nitriate to plots of orchardgrass after three cuttings had been taken on May 15, May 26, and June 23. The crude fiber content on these dates was 20.8, 27.2, and 29.6 percent. Subsequent to the fertilizer application, the percentage of crude fiber in samples cut on July 20, August 24, and October 10 was 28.2, 28.2, and 26.8 percent, respectively. The fact that crude fiber content failed to increase further after June 23 was attributed to the fertilizer application.

Season and soil moisture also affect digestibility of forages. McCroskey et al. (36) found the lignin content of Midland bermudagrass rose from a low in April to a high in February. There was an inverse relationship with percentage of <u>in vitro</u> digestibility of the grass. Brown (7) observed that a period of drought caused a marked increase in the lignin content of Kentucky bluegrass. Grass harvested July 1, July 31, and August 16 contained 6.3, 12.1, and 10.8 percent lignin, respectively. Rainfall following drought resulted in resumption of growth, and lignin content was down to 6.2 percent on August 30.

CHAPTER III

MATERIALS AND METHODS

Short rhizomes of Midland bermudagrass (Cynodon dactylon (L).), Pers., were obtained from the Agronomy Research Station at Perkins, Oklahoma. Five rhizomes were planted in each one-gallon can six inches in diameter and containing 3,100 grams of sand and vermiculite at 3:1 ratio by volume. The sand, of the Eufaula series, was analyzed for soil nutrients and was found to contain 35 pounds potassium per acre, 145 pounds calcium per acre, 23 pounds magnesium per acre, 15 pounds sodium per acre, 4.71 pounds phosphorus per acre, and had a pH of 7.5. The test was set up to contain 8 treatments with 4 replications, and the experiment was duplicated at two temperature regimes. Before the rhizomes were planted in the cans, nutrients were added as shown in Tables I and II.

Sufficient quantities of each nutrient element were added to insure that the minus nutrient would be the only limiting factor to normal growth of the plants. After each harvest, all treatments except check (treatment No. 1) and minus nitrogen received 11.1 ml. of 1 molar ammonium nitrate. Because of an error in timing, the fertilizer was not added until 5 days after the first harvest. This probably resulted in a change in the composition of the plants in the second harvest.

The rhizomes were planted in the cans on June 5, 1968. The material was placed in growth chambers and subjected to two temperature

TABLE I

AMOUNTS OF NUTRIENT ELEMENTS ADDED
TO EACH TREATMENT*

Nutrients	Treatments							
	Check	-N	-P	-K	-Ca	-Mg	-S	Complete
$Ca(NO_3)_2 4H_2O$			3.7	3.7		3.7		3.7
KNO_3							8.9	
KH_2PO_4		5.8			5.8	5.8	5.8	5.8
MgO							66.5	
CaO		207.4					207.4	
K_2SO_4		9.0	9.0		9.0	9.0		9.0
$MgSO_4$		1.65	1.65	1.65	1.65			1.65
KOH			5.4					
H_3PO_4				5.8				
H_2SO_4				4.5		1.5		
NH_4NO_3					11.1			

*ml. of 1 molar solution for all nutrients except MgO and CaO which were applied as mg. per can. Treatments designated as "minus" are missing the element so designated.

TABLE II

AMOUNTS OF NUTRIENT ELEMENT IN POUNDS PER ACRE ADDED TO EACH CAN*

		Lbs./A.
1.	Nitrogen	67
2.	Phosphorus	116
3.	Potassium	600
4.	Calcium	96
5.	Magnesium	26
6.	Sulphur	220

*Nitrogen (as NH_4NO_3) added to all treatments at rate of 200 lbs./A. after each harvest except for check and minus nitrogen treatments.

regimes, 85-70°F and 100-85°F day-night temperatures. The day length was regulated to 14 hours with a light intensity of 3,000 foot candles. Plants were watered every other day to insure adequate moisture for growth.

Three weeks after planting, all grasses were uniformly clipped at a height of 2 inches above the top of the cans. Two weeks after the initial clipping, July 9, 1968, the first samples were taken for yield and analysis. Six harvests were obtained at 3-week intervals. Weights both on a green and dry weight basis were obtained for each treatment. Harvest dates are given in Table III below.

TABLE III

HARVEST NUMBER AND HARVEST DATES
INVOLVED IN THE EXPERIMENT

Harvest	Harvest Date
1	July 9, 1968
2	July 30, 1968
3	Aug. 20, 1968
4	Sept. 10, 1968
5	Oct. 1, 1968
6	Oct. 22, 1968

After the fourth harvest the plants in check and -N were in very poor condition, and chances for appreciable regrowth appeared remote. To assure continued growth of plants in these treatments, 3 ml. of nitrogen fertilizer as 1 molar ammonium nitrate were added. Phosphorus fertilizer, 1.5 ml. of 1 molar phosphoric acid, was added to the check

treatment. Increased herbage yield and compositional change were noted in these treatments for the 5th and 6th harvests.

Chemical analyses were determined for alcohol soluble sugar, starch, protein, and *in vitro* digestibility was determined.

Alcohol Soluble Sugar

Alcohol soluble sugar content was determined by the anthrone method of Yemm and Willis (56). A representative sample of 2 grams of grass material (leaves and stems) was selected from each treatment, put in 15 ml. of 80-percent ETOH, and stored in a cool place. Each sample was ground, boiled gently for 20 minutes, and filtered through No. 40 Whatman paper. All alcohol extract samples were made up to a standard volume with 80-percent ETOH, and the alcohol soluble sugar content determined.

Starch

The insoluble residue from the alcohol soluble sugar sample was used for starch determination by the method of Weiman (54) as modified. Nineteen ml. of distilled water were added to the samples, and the samples were boiled to remove alcohol and to gelatinize the starch. Two ml. of amylase solution[1] and 9 ml. of 0.4 M acetic acid buffer were added to each sample. The final solution had a pH of 4.45. All samples were incubated at 38°C for 44-48 hours. Starch hydrolized from

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[1]One gm. of the following obtained from Sigma Chemical Company was mixed in 200 ml., 0.13 M acetic acid buffer: 1) alpha-amylase activity--1 mg. will liberate approximately 4.2 mg. of maltose from starch in 3 minutes at pH 6.9 at 20°C. 2) beta-amylase--1 mg. will liberate approximately 1.1 mg. of maltose from starch in 3 minutes at pH 4.8 at 20°C.

each sample was measured as glucose, using the anthrone color method reported by Yemm and Willis (56).

Protein

Each dry sample was ground with a Wiley mill to a fineness to pass a 60 mesh screen. Nitrogen determinations were made, using the Micro-Kjeldahl method. Crude protein percent was obtained by multiplying Kjeldahl nitrogen by the factor 6.25.

In Vitro Digestibility

The method of Tilley and Terry (49) was used to determine in vitro digestibility. For in vitro rumen digestion, 1 gm. each of ground oven dry material was placed in a 250 ml. centrifuge bottle. Eighty ml. of buffer solution of McDougall's (37) artificial sheep saliva and 20 ml. strained rumen liquor were added to each bottle. All were made anaerobic with CO_2, sealed with a cork gas release valve, maintained at a pH of 6.7 to 6.9 with 1N Na_2CO_3, and incubated 48 hours in darkness at a temperature of 38°C. The samples were agitated gently at approximately 4-hour intervals to mix the contents.

For pepsin digestion, bacterial activity in all tubes was stopped by placing the bottles under refrigeration. The tubes were centrifuged 15 minutes at 1,800 g., the supernatant discarded, 100 ml. pepsin (2 gm. 1:10,000 pepsin in 1,000 ml. of 0.1N HCl) added, and incubated at 38°C for 48 hours with occasional shaking. Most of the supernatant was discarded, and the residue along with the remaining super, was transferred to a tared weighing container, and dried at 67°C. The weight of the

blanks was then subtracted from the sample. The percentage of digestibility was calculated for each 100 grams of sample dry matter.

CHAPTER IV

RESULTS AND DISCUSSION

Temperature had very little effect on green weight yields under either temperature regime. The moisture content was, however, higher in the herbage produced under the cool temperature regime (70-85°F). The high temperature regime (85-100°F) resulted in a slightly greater yield of dry herbage (Fig. 1), but yield between the two temperature regimes were not statistically significant (Table IV). After the fourth harvest the growth chamber set at the high temperature regime was infested with insects, which resulted in lower yields of herbage for this treatment.

Previous work of Miller (38) has shown that the photosynthesis rate in bermudagrass is highest at 35°C. Since temperatures in the second regime reached 38°C (100°F) yields may have been depressed because of exceeding the optimum temperature for photosynthesis.

Fertilizers had a marked effect on herbage yield, but there were no differences in yields due to temperature (Table X and Figure 1). Data for yield are presented in Table IV, and Duncan's multiple range tests (46) for all observations are presented in Table XVI. Dry matter yields were very low in check and -N treatments for each harvest except the fifth and sixth harvest, where they increased slightly (Fig. 6). These treatments received an application of nitrogen and phosphorus fertilizer after the fourth harvest. Thus, nitrogen was the first limiting

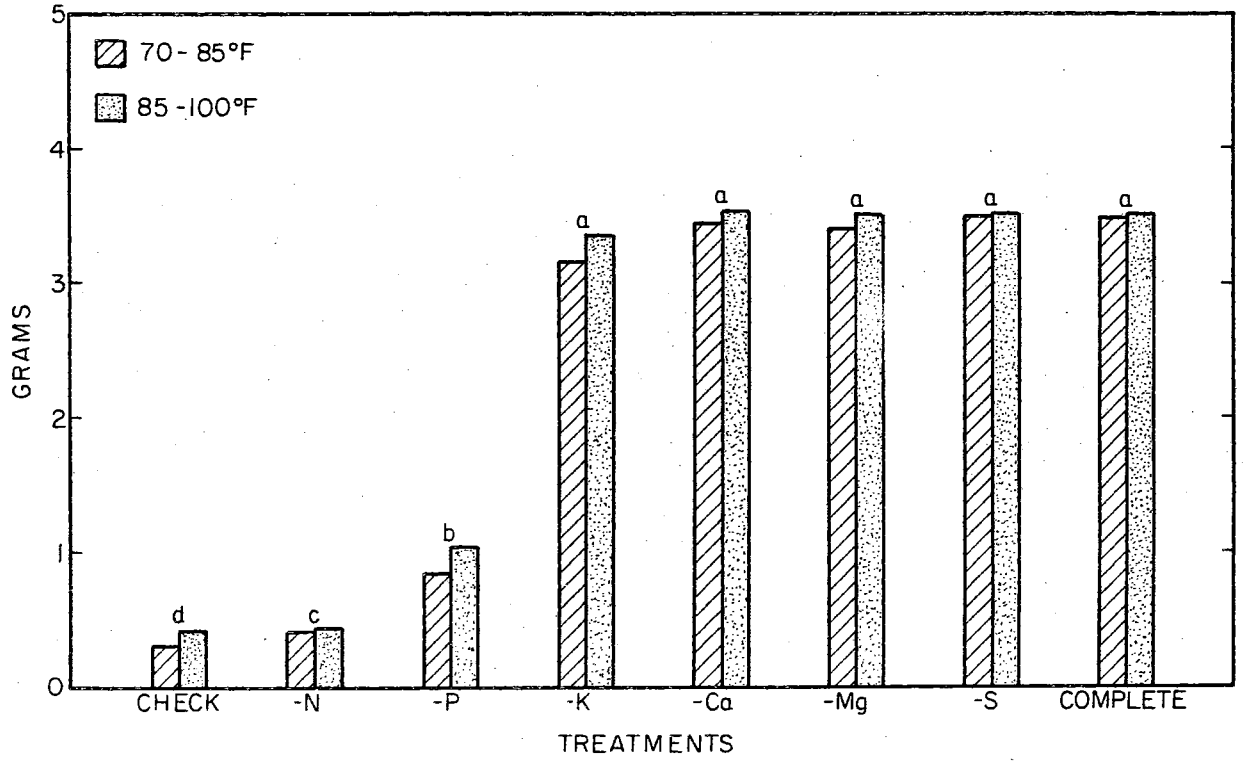

Figure 1. Dry Matter Yields in Response to Nutrient and Temperature Treatments (Average of Six Cuttings). Treatment averages with the same letter not significantly different due to nutrients at 5% level using Duncan's Multiple Range Test. No significant difference at 5% level due to temperature regimes.

element for growth. The treatment of -P was only slightly higher than the check and -N treatments. Phosphorus apparently was sufficient for limited growth in the first three harvests, but not sufficient to support a continued growth rate (Fig. 6). The yields of -K, -Ca, -Mg, -S, and complete fertilizer treatments were high and very close together (Fig. 1).

Yields for all fertilizer treatments decreased at the fifth and sixth harvests. A number of factors could have depressed yields: 1) the quality of water used, 2) the pH of solution and soil in the cans could have changed to the point that some nutrient element became unavailable to the plants, 3) the nutrient ions could have been washed out of the cans since the watering covered 4½ months of experiment, and it was difficult to prevent excessive watering of the sandy soil, 4) root volume in the cans could have become excessive during the period of the experiment, and 5) the plants were maturing in spite of the favorable growing conditions.

Statistically, the average yield of all treatments occurred as two groups. The check, -N, and -P treatments yielded the least, while the highest yields were obtained from treatments -S, -Mg, and -Ca (Table XVI). The growth patterns of the plants under the various temperatures and fertility treatments may be observed in Figures 2 and 3.

High temperature produced plants which were higher in dry matter content, and there was a significant difference in response to temperature treatments (Table XI). There are general upward trends in percent dry matter although the data are erratic (Table V). The plants apparently were maturing even though under controlled conditions (Fig. 4). The moisture, nutrients, temperature, and light levels were maintained

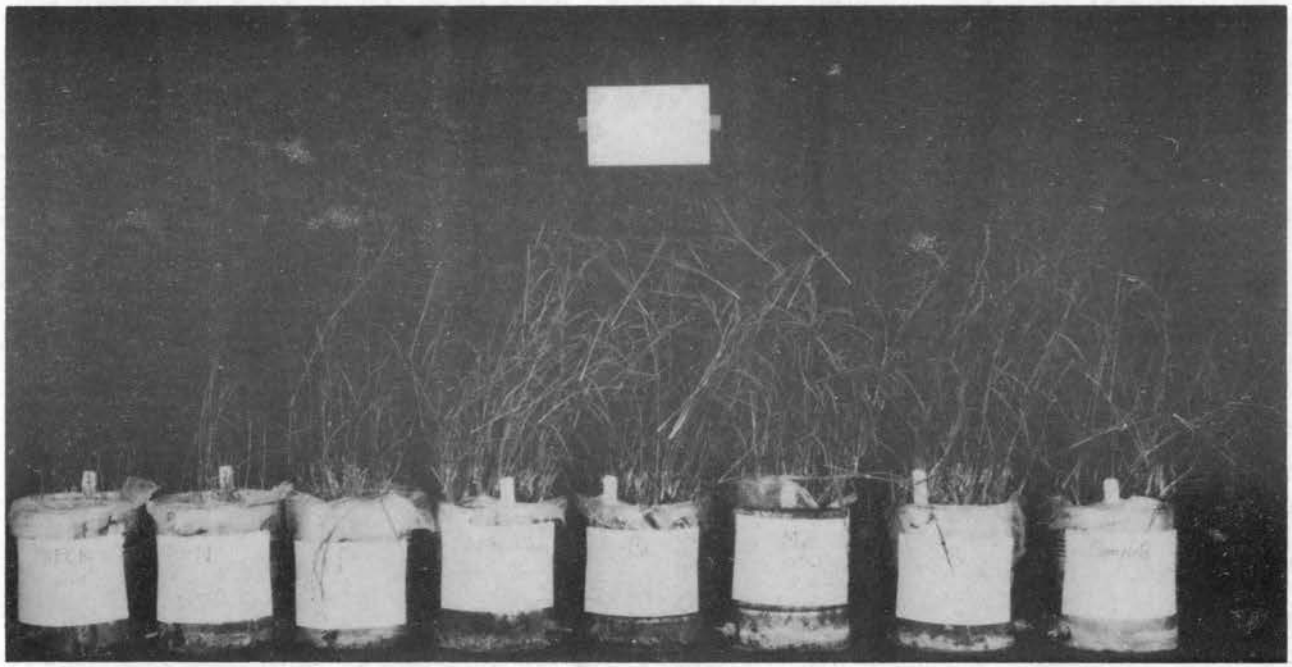

Figure 2. Relative Growth Patterns at 70-85°F (night-day) for Bermudagrass Due to Fertility Treatments. Left to right: Check, -N, -P, -K, -Ca, -Mg, -S, and Complete.

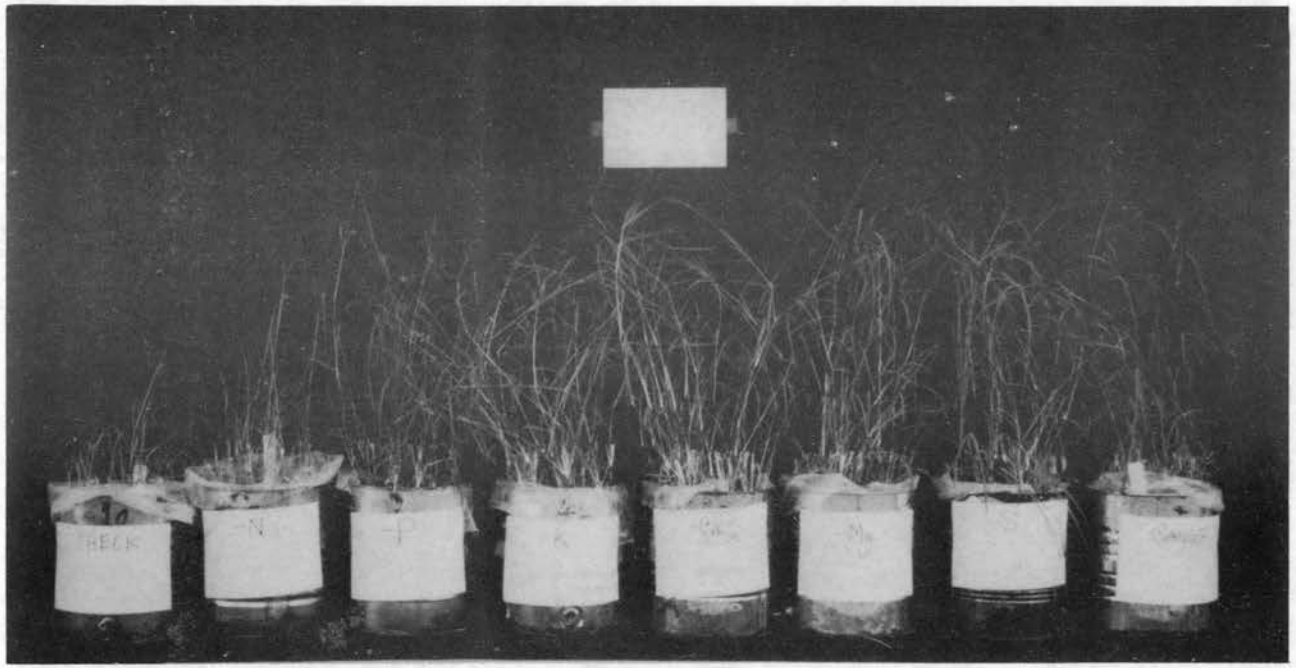

Figure 3. Relative Growth Patterns at 85-100^{O}F (night-day) for Bermudagrass Due to Fertility Treatments. Left to right: Check, -N, -P, -K, -Ca, -Mg, -S, and Complete.

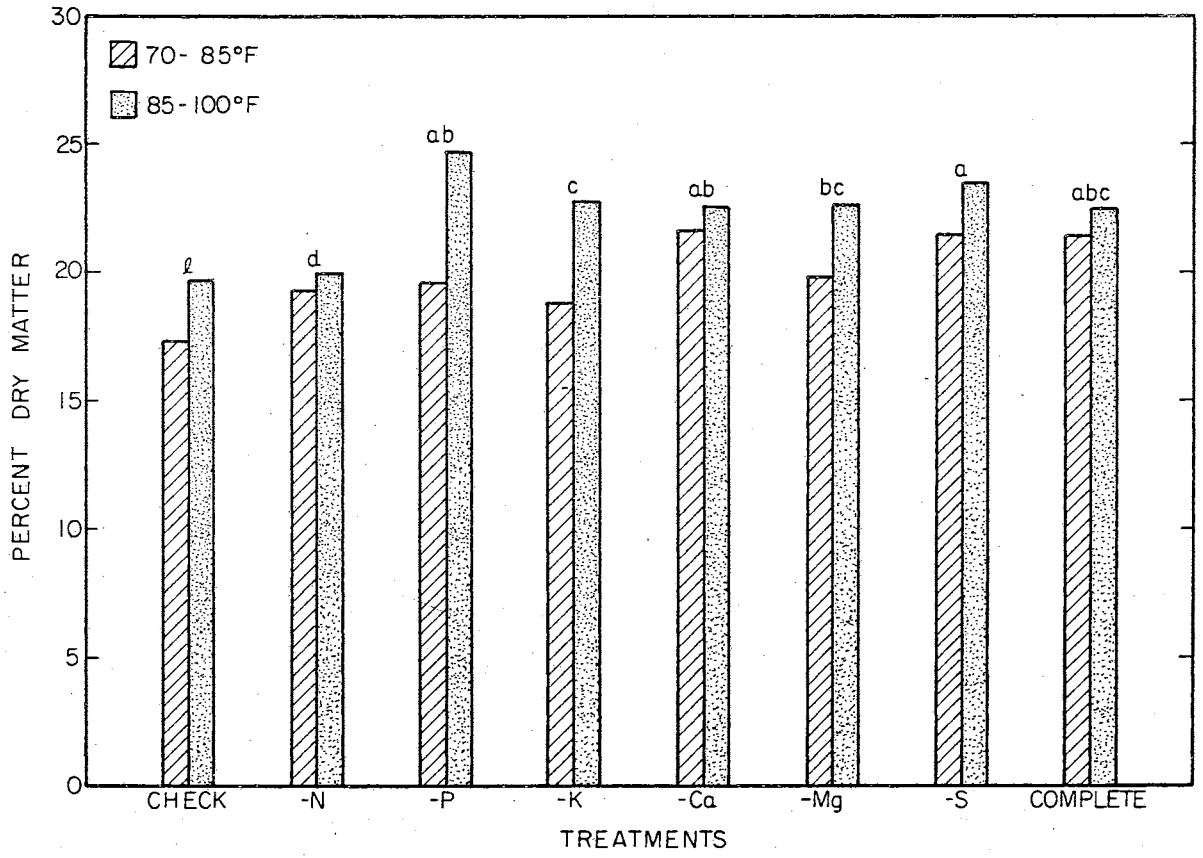

Figure 4. Percent Dry Matter in Response to Nutrient and Temperature Treatments (Average of Six Cuttings). Treatment averages with the same letter not significantly different due to nutrients at 5% level using Duncan's Multiple Range Test. Temperature effect on percent dry matter is also significant at 5% level.

at levels which should have been optimum for a continued high rate of growth of a high quality product. It is conceivable that over the period of time of the experiment, 4 months 17 days, the root population reached too high a level and was exerting a pressure toward maturity. It is also conceivable that the plants would seek a rest period or maturity stage even though they were growing under very favorable conditions. Fertilizer treatments showed some effects on percent dry matter. There were no differences between check and -N, and these treatments were significantly less than the other treatments.

The two temperature regimes did not establish a pattern for protein (Fig. 5), and there was no significant difference due to temperature (Table XII). The various fertilizers, however, greatly influenced protein content in the forage (Table VI). The treatments of -K, complete, -Mg, and -Ca possessed the highest protein content, while check, -N, and -P were lowest in protein content. When nitrogen (3 ml. of 1 molar ammonium nitrate) and phosphorus (1.5 ml. of 1 molar phosphoric acid) were applied to check and -N treatments after the fourth harvest, which was about 1/3 of the levels in other treatments, it was interesting to note that the increase in protein was approximately 1/3 of the difference between these treatments and treatments receiving high nitrogen and phosphorus levels (Fig. 6).

The protein contents for the fertilizer treatments were separated into four groups, using Duncan's Multiple Range Test (46): 1) check, 2), -N, 3) -P, and 4) five treatments with high protein content -S, -Ca, -Mg, complete, and -K (Table XVI and Fig. 5). Protein content apparently was declining with age (Fig. 6) even though the growth was new growth and was harvested at each 3-week interval. This trend was

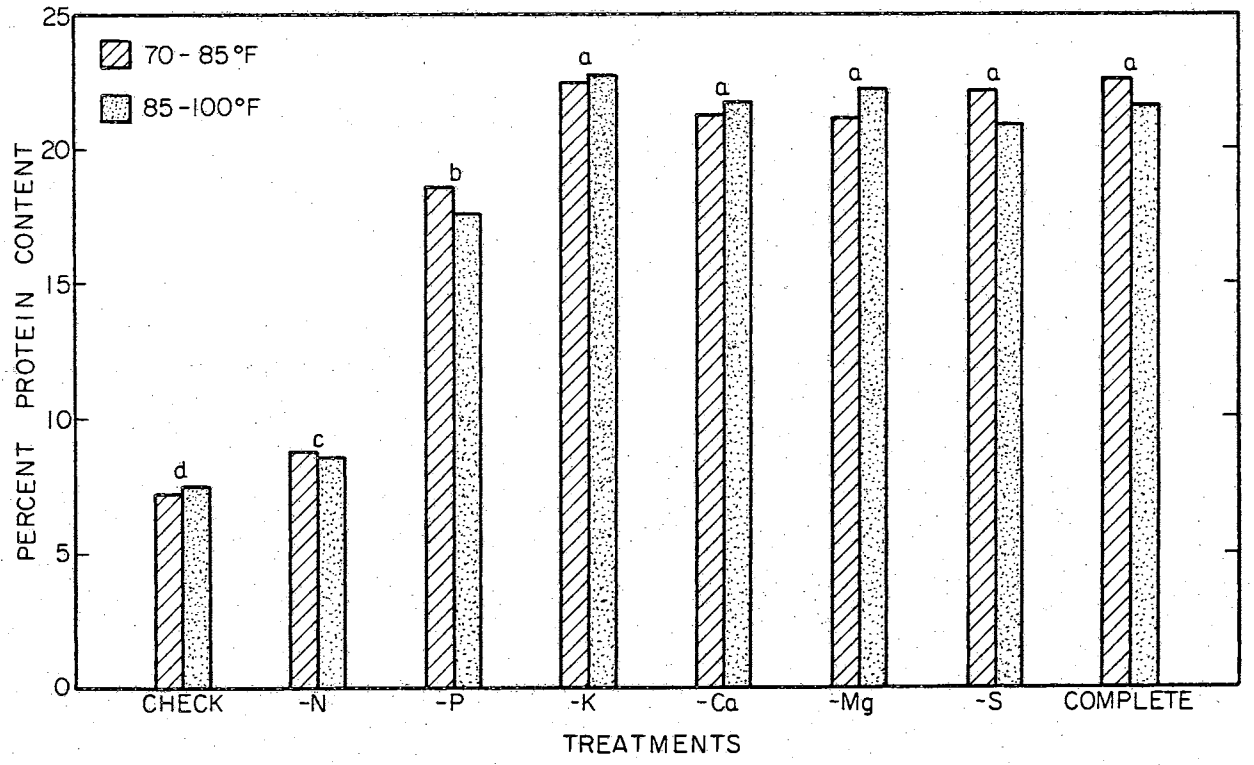

Figure 5. Percent Protein Content (D.M. Basis) in Response to Nutrient and Temperature Treatments (Average of Five Cuttings). Treatment averages with the same letter not significantly different due to nutrients at 5% level using Duncan's Multiple Range Test. No significant difference at 5% level due to temperature regimes.

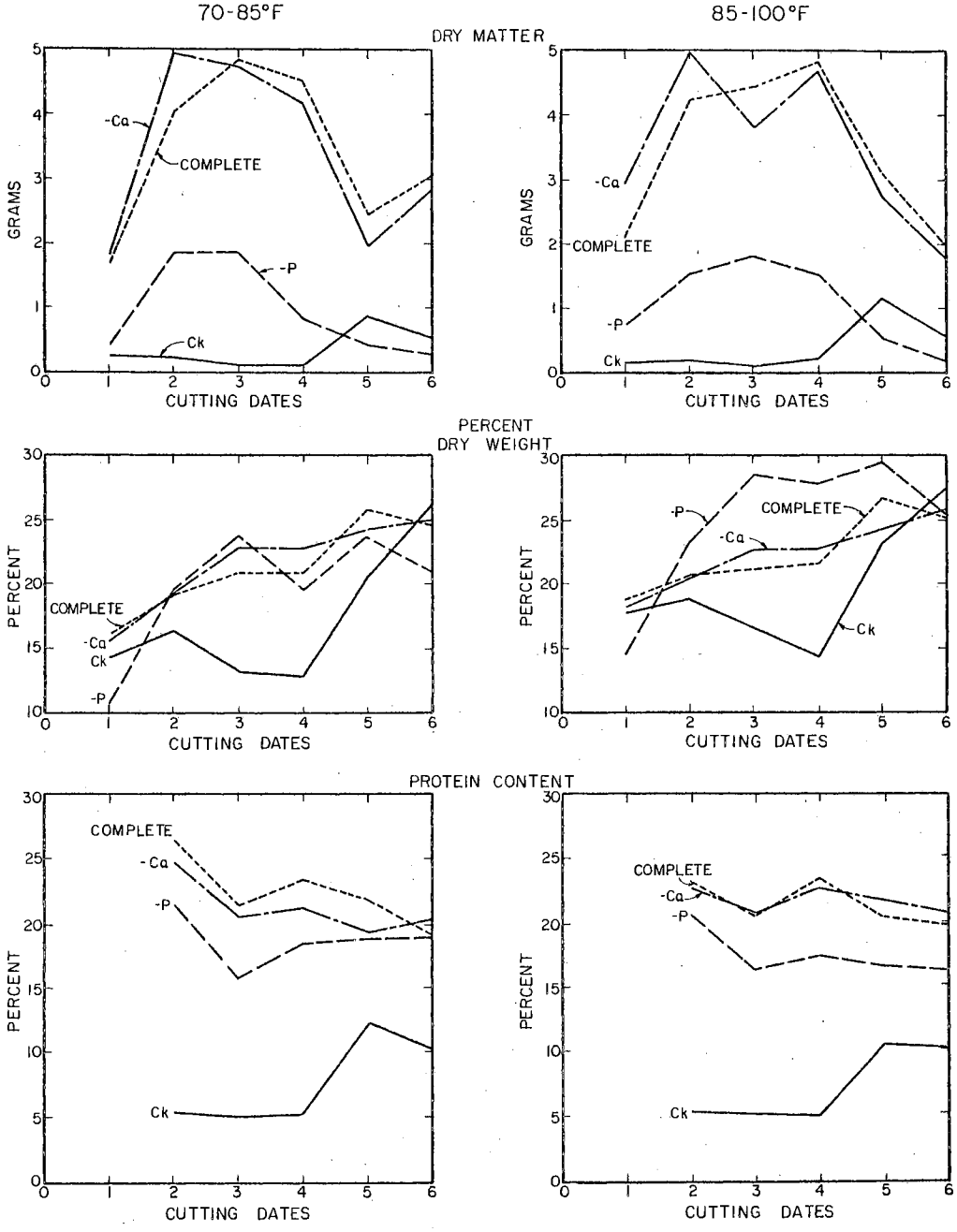

Figure 6. Dry Matter Yields, Percent Dry Weight and Protein Content for Bermuda Grass at 70-85°F and 85-100°F (night-day) Temperature Treatments at Six Sampling Dates

interrupted for check and -N since these treatments received some nitrogen and phosphorus fertilizer after the fourth harvest. The addition of these nutrients produced a marked increase in protein content.

High temperature greatly reduced the percent alcohol soluble sugar in the plants at all fertilizer levels (Fig. 7). Research by previous workers has shown similar results (5, 15, 16, 23, 45). The fertilizer treatments produced significant differences in percentage of alcohol soluble sugar between each fertilizer treatment and under both temperature regimes (Table VII). The treatments of check, -N, and -P had lower alcohol soluble sugar, while the complete treatment was intermediate with other treatments -K, -S, -Ca, and Mg being much higher in every harvest (Fig. 10).

The patterns of starch accumulation were very similar to those of alcohol suluble sugar. Plants grown at higher temperatures contained significantly less starch than those grown at lower temperatures (Table XIV). However, the range of amounts of starch on respective samplings was not so great under the 85-100^{o}F temperature as under the cooler temperature treatment. Fertilizer treatments greatly influenced the percentage of starch in the forage in both temperature regimes (Table VIII). The starch content of each harvest showed the same trend as alcohol soluble sugar in that the check, -N, and -P were lower in starch content than the other treatments (Fig. 10). All fertilizer treatments were significantly different from each other at .05 level (Table XVI). The determinations of sugars and starches were much lower at harvest 2 than harvest 1 (Fig. 10). At least four explanations are possible:

1. Dry matter production was much greater on harvest 2 with the starches and sugars converted to total growth.

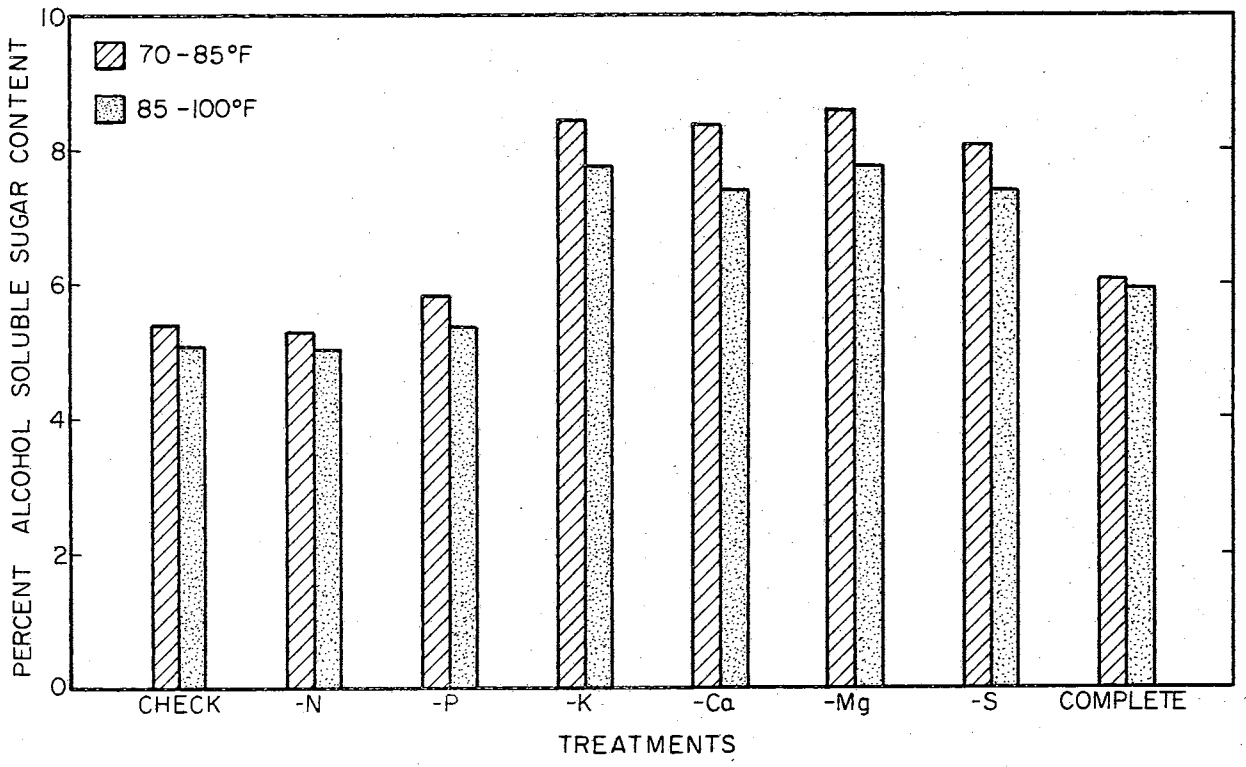

Figure 7. Percent Alcohol Soluble Sugar (D.M. Basis) in Response to Nutrient and Temperature Treatments (Average of Six Cuttings). Each treatment significantly different from each other due to nutrients at 5% level using Duncan's Multiple Range Test. Temperature effects are also significantly different at 5% level.

2. Some management practice such as too much or too little watering may have adversely affected the plants.

3. An error may have been made in preparing the plant samples.

4. Nitrogen fertilizer was applied one week after cutting instead of one day after first harvest.

The first explanation appears more logical and is probably responsible for the decline in sugars and starch.

Inverse relationships between sugar and starch and dry matter yields exist. On harvests 2 and 4 sugar and starch contents were low, and dry matter yields were high, while the reverse was generally true for harvest 5. This may be explained in that the starch and sugars were used for increased growth, and the level of these constituents were more erratic than the growth level, the trend for this relationship appears real.

Temperature caused no significant differences in digestion (Table XV), however, there was a trend for digestion to be lower at the higher temperature (Fig. 9). If harvest 5 and 6 are excluded from the data (Table IX) for check and -N treatments, again the four general response levels are noted with check being low, -N slightly higher, and -P higher than these but lower than the remaining treatments. The harvesting date did not show much difference in digestibility except harvests 5 and 6 of check and -N that received some fertilizer after the fourth harvesting (Fig. 10).

Digestibility was quite uniform across harvests for the respective treatments except for the check and -N on harvests 5 and 6. In like manner, protein content was uniform across harvest dates for the

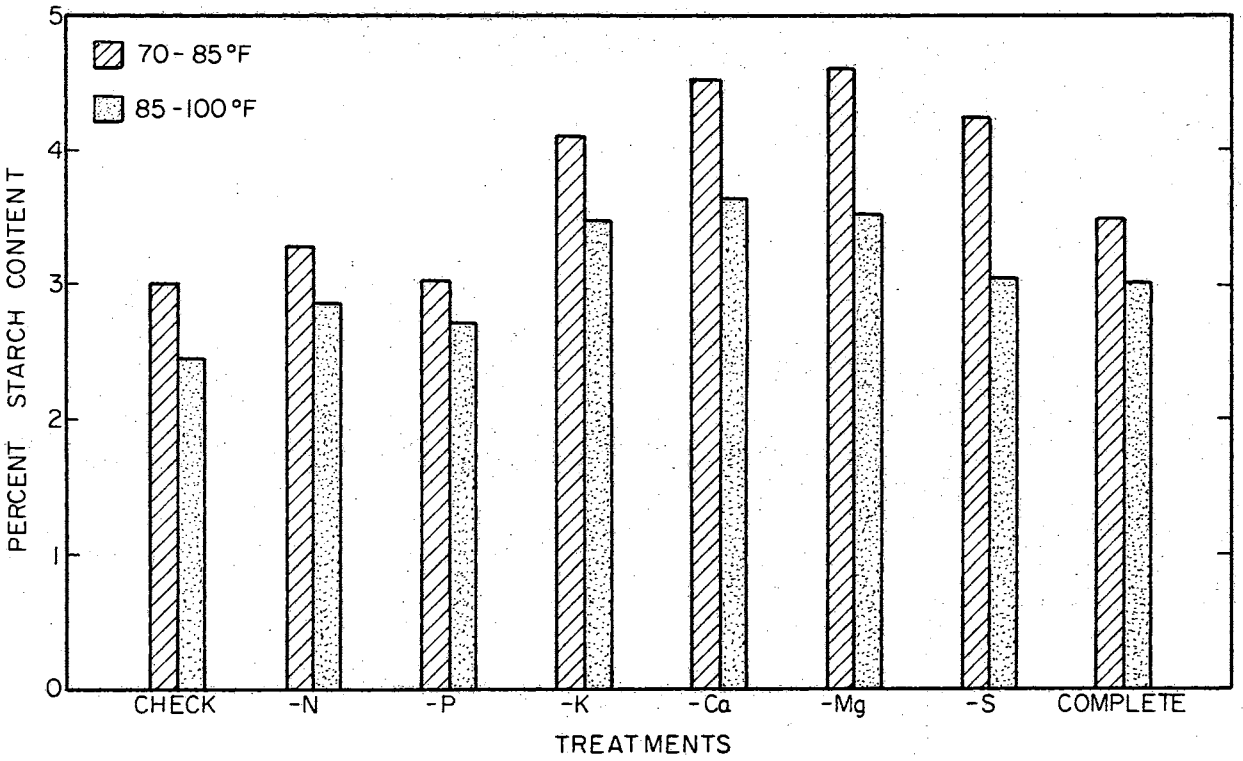

Figure 8. Percent Starch Content (D.M. Basis) in Response to Nutrient and Temperature Treatments. Each treatment significantly different from each other due to nutrients at 5% level using Duncan's Multiple Range Test. Temperature effects are also significantly different at 5% level.

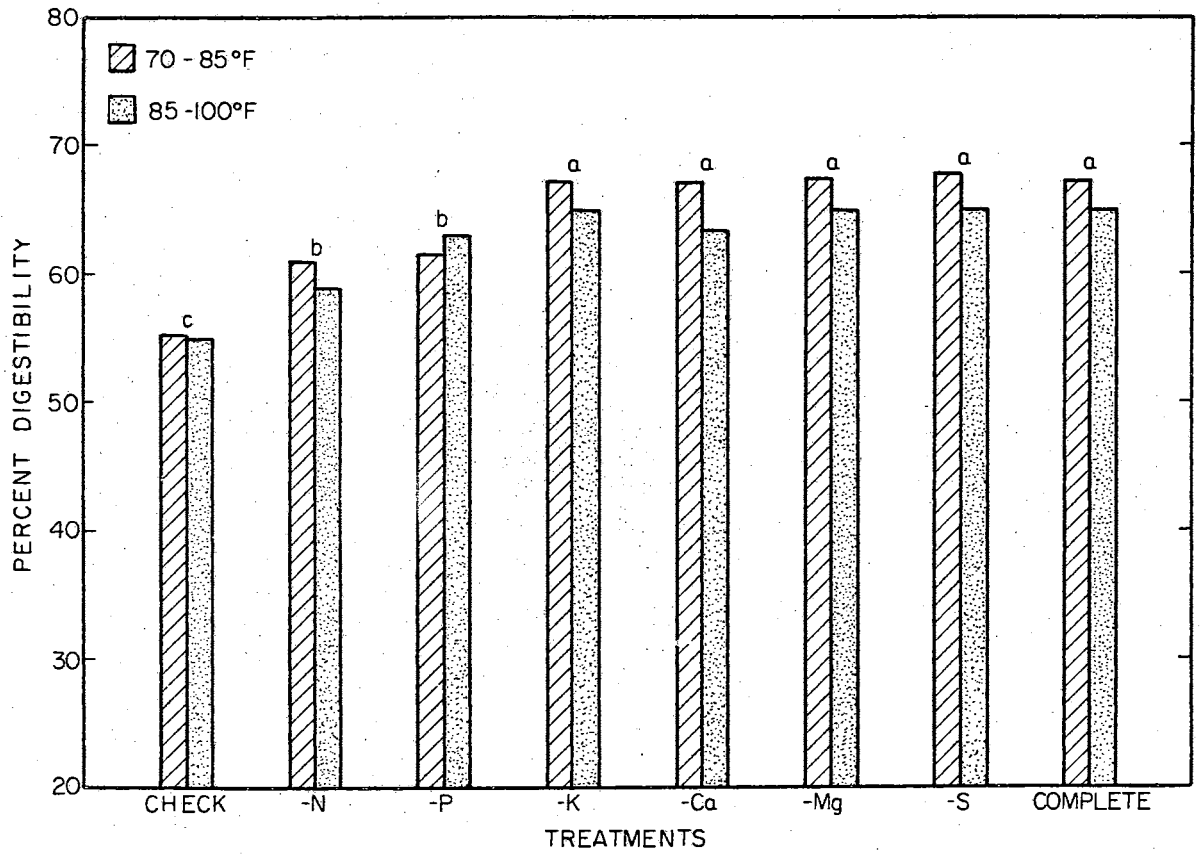

Figure 9. Percent Digestibility (D.M. Basis) in Response to Nutrient and Temperature Treatments. Treatment averages with the same letter not significantly different due to nutrients at 5% level using Duncan's Multiple Range Test. No significant difference at 5% level due to temperature regimes.

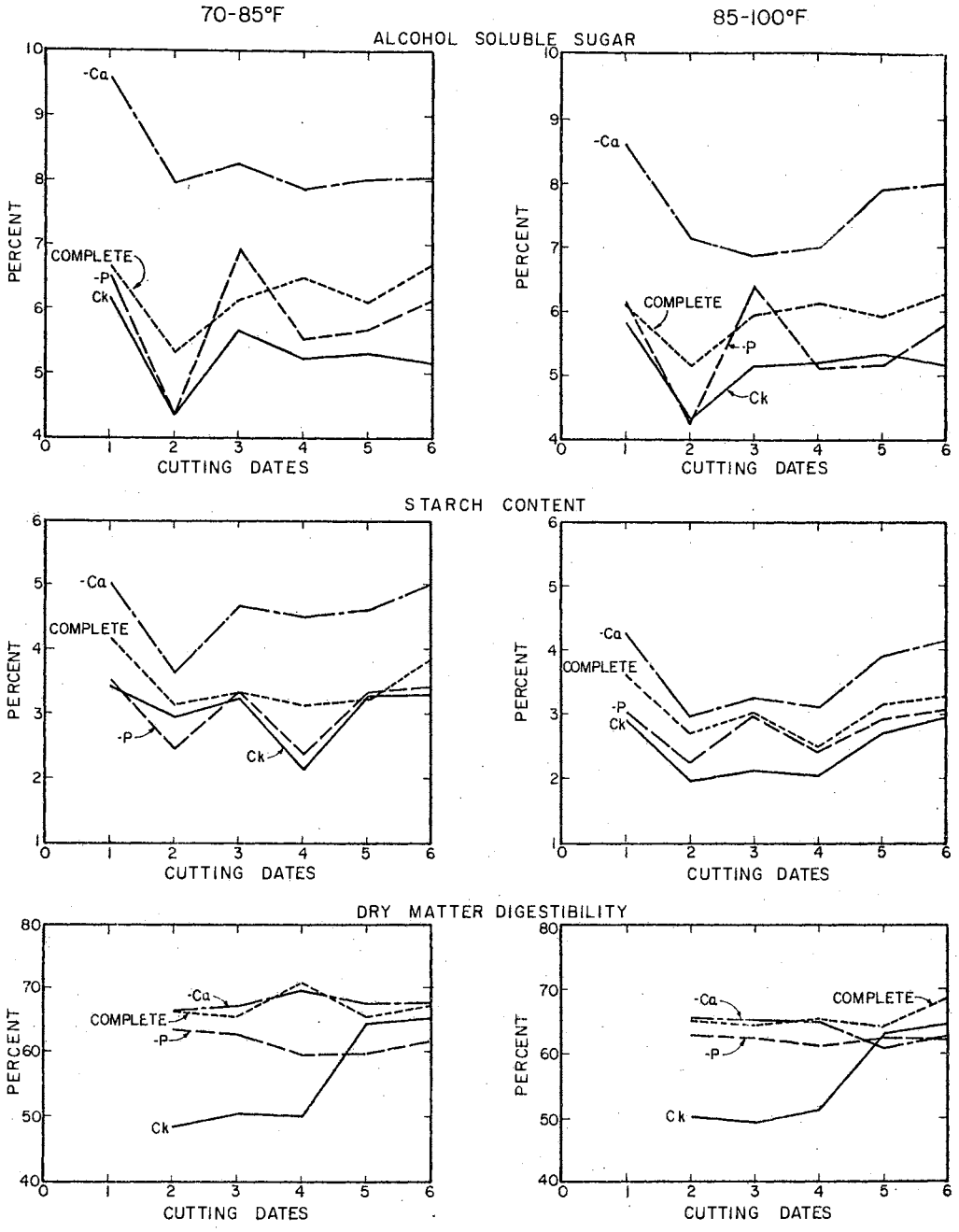

Figure 10. Alcohol Soluble Sugar, Starch Content, and Dry Matter Digestibility for Bermuda Grass at 70-85°F, and 85-100°F (night-day) Temperature Treatments at Six Sampling Dates

treatments, but the starch and sugar contents were quite erratic. Thus, protein content of the forage would appear to be closely related with digestibility and be the first limiting factor for quality. However, in the case of -P treatment, the protein content in the forage of the -P treatment was lower than the protein in the complete and -Ca treatments even though these treatments all received the same levels of nitrogen. The -P treatment had lower starch and sugar contents than the complete or -Ca as well as lower digestion. Thus, the sugars and starch contents do appear to influence the quality of the forage as evidenced by the dry matter digestion even though their levels were erratic. Since the sugars and starch are utilized for total growth and dry matter production, it is conceivable that their levels will not be uniform if growth is not uniform. This physiologic response may make it difficult to obtain consistent relationships between starch and/or sugars and digestibility.

These data would indicate that protein is the first limiting factor in quality of forages. Sugars and starch appear to influence quality, but this effect is secondary and not to the extent of the protein effect.

CHAPTER V

SUMMARY AND CONCLUSIONS

The objective of this study was to determine the influence of nutrients on total production and quality of bermuda forage as evidenced by protein content, carbohydrates (especially sugar and starch) and dry matter digestibility under the two temperature regimes, 70-85°F and 85-100°F (night-day). The eight nutrient treatments consisted of: check (no added nutrients), -N, -P, -K, -Ca, -Mg, -S with all the other elements being at a high level and complete. Midland bermudagrass was chosen for the study.

Green and dry weight yields of the grass were affected very little by the temperature. Under high temperature, dry matter percentages of the plants were higher than under low temperature. Fertilizer treatments had large effects on yield, and nitrogen appeared to be the first limiting element for growth. Low yields were observed for the check, -N, and -P treatments, and high yields were observed for -K, -Ca, -Mg, -S, and complete treatments.

Temperature greatly influenced the alcohol soluble sugar and starch content. Both constituents were higher under cool temperature, and all nutrient treatments were significantly different from each other at .05 level. It is noted that alcohol soluble sugar and starch are food reserves of the plants and that they are greatly influenced by both temperature and plant nutrients.

Temperature did not produce any effect on protein content of the grass, but the plant nutrients, especially nitrogen, influenced the amount of the substance. Nitrogen was the first limiting element for protein synthesis. Forage from the check, -N, and -P treatments was very low in protein content, while all other treatments were rather high and close together.

Temperature had very little effect on percent dry matter digestibility, but it was slightly higher under the cool temperature. The percent digestibility of the check, -N, and -P treatments was low, while digestibility for all other treatments was high and quite close together. Thus, low nitrogen and phosphorus levels depressed the quality of the forage, with the quality being high for the -S, -Mg, -Ca, and -K treatments in this experiment. These elements either have limited effects on forage quality, or the soil supplied enough to meet the quality needs of the plants.

In general, on the basis of data obtained, forage quality as evidenced by the percent dry matter digestibility is more closely related to protein content, with sugar and starch having secondary effects on quality.

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APPENDIX

TABLE IV

DRY MATTER YIELD (GM. PER CAN)

Temperature 70-85°F (21-29.6°C)
1968

Cutting Date	Treatment									
	Check	-N	-P	-K	-Ca	-Mg	-S	Complete	Total	Mean
July 9	0.26	0.21	0.40	1.84	1.81	1.23	1.64	1.71	9.10	1.14
July 30	0.21	0.27	1.82	3.13	4.95	4.14	4.03	4.04	22.59	2.82
Aug. 20	0.11	0.14	1.84	4.38	4.76	5.11	4.67	4.82	25.83	3.23
Sept 10	0.11	0.20	0.83	4.22	4.19	4.40	5.05	4.54	23.54	2.94
Oct. 1	0.86	0.85	0.41	2.79	1.96	2.59	2.61	2.45	14.52	1.82
Oct. 22	0.56	0.70	0.28	2.59	2.85	2.87	2.88	3.04	15.77	1.97
Total	2.11	2.37	5.58	18.95	20.52	20.34	20.88	20.60	111.35	
Mean	0.35	0.40	0.93	3.16	3.42	3.39	3.48	3.43		

Temperature 85-100°F (29.6-38°C
1968

Cutting Date	Treatment									
	Check	-N	-P	-K	-Ca	-Mg	-S	Complete	Total	Mean
July 9	0.18	0.26	0.77	1.88	2.97	2.32	2.44	2.10	12.92	1.62
July 30	0.20	0.27	1.52	4.26	4.98	4.46	4.42	4.22	24.33	3.04
Aug. 20	0.11	0.19	1.80	4.25	3.80	4.06	4.30	4.42	22.93	2.87
Sept 10	0.20	0.22	1.46	4.65	4.65	4.71	4.94	4.81	25.64	3.21
Oct. 1	1.15	0.98	0.55	2.70	2.74	2.73	3.15	3.12	17.12	2.14
Oct. 22	0.58	0.61	0.18	2.29	1.78	2.89	1.83	1.96	12.12	1.52
Total	2.42	2.53	6.28	20.03	20.92	21.17	21.08	20.63	115.06	
Mean	0.40	0.42	1.05	3.34	3.49	3.53	3.51	3.44		

Each figure is the mean of four replications.

TABLE V

PERCENT DRY MATTER

Temperature 70-85°F
1968

Cutting Date	Treatment Check	-N	-P	-K	-Ca	-Mg	-S	Complete	Total	Mean
July 9	14.27	11.72	10.93	15.37	15.58	15.52	15.16	16.09	114.64	14.33
July 30	16.67	18.67	19.64	17.88	19.40	19.73	20.01	19.33	151.33	18.92
Aug. 20	13.18	14.23	23.73	18.90	22.83	20.23	20.39	20.73	154.22	19.28
Sept 10	12.85	18.18	19.55	20.35	22.83	20.95	23.95	20.74	159.40	19.93
Oct. 1	20.45	21.56	23.65	20.54	24.07	22.44	25.82	25.77	184.30	23.04
Oct. 22	26.15	30.56	20.59	20.30	24.98	20.81	23.25	24.75	191.39	23.92
Total	103.57	114.92	118.09	113.34	129.69	119.68	128.58	127.41	955.28	
Mean	17.26	19.15	19.68	18.89	21.62	19.95	21.43	21.24		

Temperature 85-100°F
1968

Cutting Date	Treatment Check	-N	-P	-K	-Ca	-Mg	-S	Complete	Total	Mean
July 9	17.84	13.61	14.62	18.77	18.26	20.49	20.54	18.87	143.00	17.88
July 30	18.89	18.56	23.10	20.70	20.39	20.51	23.17	20.59	165.91	20.73
Aug. 20	16.69	15.95	28.68	22.10	22.80	20.87	20.36	21.08	168.53	21.07
Sept 10	14.44	18.53	27.92	22.96	22.86	20.93	24.62	21.66	173.92	21.74
Oct. 1	23.14	22.23	29.63	26.16	24.13	26.39	26.69	26.78	205.18	25.65
Oct. 22	27.74	31.03	25.38	26.63	25.76	26.61	25.22	25.08	213.45	25.68
Total	118.74	119.94	149.33	137.32	134.20	135.80	140.60	134.06	1069.99	
Mean	19.79	19.99	24.89	22.89	22.37	22.63	23.43	22.34		

Each figure is the mean of 4 replications.

TABLE VI

PROTEIN CONTENT (% DRY MATTER)

Temperature 70-85°F (21-29.6°C)
1968

Cutting Date	Treatment									
	Check	-N	-P	-K	-Ca	-Mg	-S	Complete	Total	Mean
July 30	5.13	6.36	21.37	25.72	24.81	23.04	25.89	26.44	158.76	19.85
Aug. 20	5.00	6.23	15.84	20.78	20.41	20.66	21.16	21.47	131.55	16.44
Sept 10	5.02	6.10	18.36	22.62	21.17	21.18	21.89	23.45	139.79	17.47
Oct. 1	10.24	12.83	18.75	22.34	19.47	20.50	21.17	21.97	147.27	18.41
Oct. 22	10.06	12.89	18.88	20.53	20.22	20.11	20.13	19.22	142.04	17.76
Total	35.45	44.41	93.20	111.99	106.08	105.49	110.24	112.55	719.41	
Mean	7.09	8.88	18.64	22.40	21.22	21.10	22.05	22.51		

Temperature 85-100°F (29.6-38°C)
1968

Cutting Date	Treatment									
	Check	-N	-P	-K	-Ca	-Mg	-S	Complete	Total	Mean
July 30	5.27	6.51	20.59	25.42	22.78	24.87	21.95	23.03	150.42	18.80
Aug. 20	5.20	6.28	16.38	20.26	20.73	21.33	20.05	20.61	130.84	16.36
Sept 10	5.04	6.33	17.46	24.15	22.86	23.23	21.98	23.50	144.55	18.07
Oct. 1	10.78	12.45	16.71	22.67	21.89	21.98	20.95	20.48	147.91	18.49
Oct. 22	10.44	11.75	16.44	21.24	20.91	19.69	19.32	19.91	139.70	17.46
Total	36.73	43.32	87.58	113.74	109.17	111.10	104.25	107.53	713.42	
Mean	7.35	8.66	17.52	22.75	21.83	22.22	20.85	21.51		

Each figure is the mean of 4 replications.

TABLE VII

ALCOHOL SOLUBLE SUGAR CONTENT (% DRY MATTER)

Temperature 70-85°F (21-29.6°C)
1968

Cutting Date	Treatment									
	Check	-N	-P	-K	-Ca	-Mg	-S	Complete	Total	Mean
July 9	6.19	5.26	6.56	9.24	9.60	9.90	7.80	6.63	61.18	7.77
July 30	4.31	5.02	4.35	7.46	7.99	7.73	7.59	5.36	49.81	6.23
Aug. 20	5.68	5.64	6.95	8.62	8.23	8.63	8.44	6.17	58.36	7.30
Sept 10	5.21	4.65	5.54	7.77	7.86	7.82	7.90	6.47	53.22	6.65
Oct. 1	5.30	5.51	5.68	8.61	8.06	8.27	7.93	6.12	55.48	6.94
Oct. 22	5.19	6.11	6.19	8.68	8.08	8.57	8.43	6.70	57.95	7.24
Total	31.88	32.19	35.27	50.38	49.82	50.92	48.09	37.45	336.00	
Mean	5.31	5.36	5.88	8.40	8.30	8.49	8.02	6.24		

Temperature 85-100°F (29.6-38°C)
1968

Cutting Date	Treatment									
	Check	-N	-P	-K	-Ca	-Mg	-S	Complete	Total	Mean
July 9	5.81	5.67	6.16	8.92	8.60	9.10	7.02	6.13	57.41	7.18
July 30	4.30	4.77	4.24	6.57	7.19	6.12	7.19	5.16	45.54	5.69
Aug. 20	5.16	5.24	6.40	8.01	6.94	8.31	8.04	5.94	54.04	6.75
Sept 10	5.20	4.12	5.13	7.13	7.00	7.28	7.03	6.17	49.06	6.13
Oct. 1	5.34	5.15	5.18	8.16	7.96	7.98	7.16	5.93	52.86	6.61
Oct. 22	5.19	5.92	5.80	8.07	8.00	8.17	8.44	6.26	55.85	6.98
Total	31.00	30.87	32.91	46.86	45.69	46.96	44.88	35.59	314.76	
Mean	5.17	5.15	5.49	7.81	7.33	7.83	7.48	5.93		

Each figure is the mean of 4 replications.

TABLE VIII

STARCH CONTENT (% DRY MATTER)

Temperature 70-85°F (21-29.6°C)
1968

Cutting Date	Treatment									
	Check	-N	-P	-K	-Ca	-Mg	-S	Complete	Total	Mean
July 9	3.43	3.88	3.53	4.51	5.00	5.28	4.68	4.15	34.46	4.31
July 30	2.97	2.87	2.41	3.48	3.64	3.70	3.55	3.13	25.75	3.22
Aug. 20	3.23	3.18	3.35	4.46	4.65	4.67	4.49	3.37	31.40	3.93
Sept 10	2.12	2.96	2.39	3.66	4.48	4.32	3.96	3.14	27.03	3.38
Oct. 1	3.26	3.41	3.36	4.17	4.62	4.57	4.36	3.22	30.97	3.87
Oct. 22	3.28	3.53	3.42	4.34	5.01	4.98	4.62	3.85	33.03	4.13
Total	18.29	19.83	18.46	24.62	27.40	27.52	25.66	20.86	182.64	
Mean	3.05	3.31	3.08	4.10	4.57	4.60	4.28	3.48		

Temperature 85-100°F (29.6-38°C)
1968

Cutting Date	Treatment									
	Check	-N	-P	-K	-Ca	-Mg	-S	Complete	Total	Mean
July 9	2.91	3.29	3.02	4.12	4.26	4.09	3.62	3.60	28.91	3.61
July 30	1.99	2.15	2.23	2.65	2.98	2.17	2.02	2.70	18.89	2.36
Aug. 20	2.13	3.01	2.98	3.60	3.24	3.93	3.16	3.03	25.08	3.14
Sept 10	2.05	2.64	2.40	3.13	3.12	3.18	2.99	2.49	22.00	2.75
Oct. 1	2.68	3.11	2.91	3.63	3.94	3.84	3.26	3.18	26.55	3.32
Oct. 22	2.94	2.87	3.08	3.50	4.15	3.86	3.39	3.27	27.06	3.38
Total	14.70	17.07	16.62	20.63	21.69	21.07	18.44	18.27	148.49	
Mean	2.45	2.85	2.77	3.44	3.62	3.51	3.07	3.05		

Each figures is the mean of four replications.

TABLE IX

PERCENT DRY MATTER DIGESTIBILITY

Temperature 70-85°F
1968

Cutting Date	Treatments									
	Check	-N	-P	-K	-Ca	-Mg	-S	Complete	Total	Mean
July 30	48.10	55.26	63.63	67.37	66.22	66.92	68.28	66.19	501.97	62.75
Aug. 20	50.12	58.49	62.44	66.05	66.68	67.35	67.65	65.89	504.67	63.08
Sept 10	50.06	56.24	59.85	67.46	69.78	69.31	68.35	70.84	511.89	63.99
Oct. 1	64.28	65.45	59.86	67.50	66.81	66.91	67.16	65.53	523.50	65.44
Oct. 22	65.85	69.59	61.98	68.62	67.93	66.86	68.22	67.79	536.84	67.11
Total	278.41	305.03	307.76	337.00	337.42	337.35	339.66	336.24	2578.87	
Mean	55.68	61.01	61.55	67.40	67.48	67.47	67.93	67.25		

Temperature 85-100°F
1968

Cutting Date	Treatments									
	Check	-N	-P	-K	-Ca	-Mg	-S	Complete	Total	Mean
July 30	50.14	55.78	62.78	66.64	65.83	65.30	64.45	65.22	496.14	62.02
Aug. 20	49.45	52.64	62.58	66.31	65.69	65.81	64.47	64.27	491.22	61.40
Sept 10	51.12	56.42	61.34	64.83	65.03	66.83	66.17	65.52	497.26	62.16
Oct. 1	63.03	66.04	62.25	64.02	60.72	63.51	64.97	64.06	508.60	63.58
Oct. 22	64.32	64.94	62.10	63.92	62.38	65.17	65.72	68.30	516.85	64.61
Total	278.06	295.82	311.05	325.72	319.65	326.62	325.78	327.37	2510.07	
Mean	55.61	59.16	62.21	65.14	63.93	65.32	65.16	65.47		

Each figure is the mean of 4 replications.

TABLE X

AN ANALYSIS OF VARIANCE OF DRY WEIGHT
IN GM. PER CAN.

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F. Value
Total	383	3156.668		
Temperature (Temp.)	1	0.799	0.799	7.198
Replication (Rep.)	3	22.777	7.592	68.396**
Temp. x Rep.	3	0.334	0.111	
Fertilizer (Fer.)	7	727.917	103.988	97.641**
Fer. x Rep.	21	22.381	1.065	
Cutting (Cut.)	5	180.516	36.103	10.383**
Cut. x Rep.	15	52.160	3.477	
Fer. x Cut.	35	123.552	3.530	7.354**
Fer. x Cut. x Rep.	105	50.433	0.480	
Fer. x Temp.	7	0.516	0.073	0.078
Fer. x Temp. x Rep.	21	19.646	0.935	
Cut. x Temp.	5	11.426	2.285	3.335*
Cut. x Temp. x Rep.	15	10.275	0.685	
Fer. x Cut. x Temp.	35	14.356	0.410	0.022
Fer. x Cut. x Temp. x Rep.	105	1919.580	18.281	

* Significant at .05 level.
** Significant at .01 level.

TABLE XI

AN ANALYSIS OF VARIANCE OF PERCENT DRY MATTER

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F. Value
Total	319	178,676.279		
Temperature (Temp.)	1	538.580	538.580	23.577*
Replication (Rep.)	3	33.930	11.310	0.495
Temp. x Rep.	3	68.529	22.843	
Fertilizer (Fer.)	7	645.363	92.194	20.311**
Fer. x Rep.	21	95.323	4.539	
Cutting (Cut.)	4	3,583.200	716.640	100.061**
Cut. x Rep.	12	107.440	7,162	
Fer. x Cut.	28	1,744.931	49.855	17.773**
Fer. x Cut. x Rep.	84	294.618	2.805	
Fer. x Temp.	7	208.477	29.782	7.494**
Fer. x Temp. x Rep.	21	83.468	3.974	
Cut. x Temp.	4	40.390	8.078	2.025
Cut. x Temp. x Rep.	12	59.822	3.988	
Fer. x Cut. x Temp.	28	156.215	4.463	0.002
Fer. x Cut. x Temp. x Rep.	84	171,015.993	1628.723	

* Significant at .05 level.
** Significant at .01 level.

TABLE XII

AN ANALYSIS OF VARIANCE OF PROTEIN CONTENT
IN PERCENT OF DRY MATTER

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F. Value
Total	319	122,080.282		
Temperature (Temp.)	1	1.780	1.780	0.157
Replication (Rep.)	3	39.375	13.125	1.158
Temp. x Rep.	3	33.990	11.330	
Fertilizer (Fer.)	7	11,042.179	1577.454	579.946**
Fer. x Rep.	21	57.127	2.720	
Cutting (Cut.)	4	299.604	74.901	8.115**
Cut. x Rep.	12	110.755	9.229	
Fer. x Cut.	28	924.916	33.032	10.426**
Fer. x Cut. x Rep.	84	266.195	3.168	
Fer. x Temp.	7	54.045	7.720	2.912*
Fer. x Temp. x Rep.	21	55.682	2.651	
Cut. x Temp.	4	22.832	5.708	1.952
Cut. x Temp. x Rep.	12	35.086	2.923	
Fer. x Cut. x Temp.	28	68.204	2.435	0.001
Fer. x Cut. x Temp. x Rep.	84	109,068.512	1298.434	

* Significant at .05 level.
** Significant at .01 level.

TABLE XIII

AN ANALYSIS OF VARIANCE OF ALCOHOL SOLUBLE SUGAR IN PERCENT OF DRY MATTER

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F. Value
Total	383	18,466.534		
Temperature (Temp.)	1	16.813	16.813	560.433**
Replication (Rep.)	3	0.035	0.011	0.366
Temp. x Rep.	3	0.092	0.030	
Fertilizer (Fer.)	7	551.858	78.836	4637.411**
Fer. x Rep.	21	0.362	0.017	
Cutting (Cut.)	5	92.300	18.460	710.000**
Cut. x Rep.	15	0.394	0.026	
Fer. x Cut.	35	61.621	1.760	135.384**
Fer. x Cut. x Rep.	105	1.414	0.013	
Fer. x Temp.	7	9.775	1.396	60.695**
Fer. x Temp. x Rep.	21	0.492	0.023	
Cut. x Temp.	5	5.294	1.058	24.045**
Cut. x Temp. x Rep.	15	0.663	0.044	
Fer. x Cut. x Temp.	35	21.281	0.608	0.003
Fer. x Cut. x Temp. x Rep	105	17,704,140	168.610	

* Significant at .05 level.
** Significant at .01 level.

TABLE XIV

AN ANALYSIS OF VARIANCE OF STARCH CONTENT
IN PERCENT OF DRY MATTER

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F. Value
Total	383	4820.228		
Temperature (Temp.)	1	48.763	48.763	903.018**
Replication (Rep.)	3	0.039	0.013	0.240
Temp. x Rep.	3	0.162	0.054	
Fertilizer (Fer.)	7	89.817	12.831	442.448**
Fer. x Rep.	21	0.610	0.029	
Cutting (Cut.)	5	0.347	12.069	524.739**
Cut. x Rep.	15	0.352	0.023	
Fer. x Cut.	35	8.841	0.252	10.956**
Fer. x Cut. x Rep.	105	2.441	0.023	
Fer. x Temp.	7	10.440	1.491	8.875**
Fer. x Temp. x Rep.	21	3.536	0.168	
Cut. x Temp.	5	1.002	0.204	7.846**
Cut. x Temp. x Rep.	15	0.395	0.026	
Fer. x Cut. x Temp.	35	4.733	0.135	0.003
Fer. x Cut. x Temp. x Rep.	105	4588.730	43.702	

* Significant at .05 level.
** Significant at .01 level.

TABLE XV

AN ANALYSIS OF VARIANCE OF DIGESTIBILITY
IN PERCENT OF DRY MATTER

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F. Value
Total	319	1,305,825.650		
Temperature (Temp.)	1	236.534	236.534	6.118
Replication (Rep.)	3	143.850	47.950	1.240
Temp. x Rep.	3	115.981	38.660	
Fertilizer (Fer.)	7	4,569.926	652.847	52.934**
Fer. x Rep.	21	258.997	12.333	
Cutting (Cut.)	4	608.833	152.208	14.758**
Cut. x Rep.	12	123.756	10.313	
Fer. x Cut.	28	2,780.654	99.309	11.068**
Fer. x Cut. x Rep.	84	753.678	8.972	
Fer. x Temp.	7	133.334	19.048	1.492
Fer. x Temp. x Rep.	21	268.070	12.765	
Cut. x Temp.	4	25.932	6.483	0.583
Cut. x Temp. x Rep.	12	133.226	11.102	
Fer. x Cut. x Temp.	28	221.236	7.901	0.0005
Fer. x Cut. x Temp. x Rep.	84	1,295,451.643	15422.043	

* Significant at .05 level.
** Significant at .01 level.

TABLE XVI

MEAN DIFFERENCES DUE TO FERTILIZER EFFECTS*
(AVERAGE OF TWO TEMPERATURE REGIMES)

Dry Weight (Gm. Per Can.)

Check	-N	-P	-K	Complete	-Ca	-Mg	-S
0.375	0.410	0.990	3.250	3.435	3.455	3.460	3.495

Percent Dry Matter

Check	-N	-K	-Mg	Complete	-Ca	-P	-S
18.52	19.57	20.89	21.29	21.79	21.99	22.28	22.43

Alcohol Soluble Sugar (% Dry Matter)

Check	-N	-P	Complete	-S	-Ca	-K	-Mg
5.24	5.26	5.68	6.09	7.75	7.95	8.11	8.16

Starch Contents (% Dry Matter)

Check	-P	-N	Complete	-S	-K	-Mg	-Ca
2.75	2.93	3.08	3.26	3.68	3.77	4.06	4.10

Protein Contents (% Dry Matter)

Check	-N	-P	-S	-Ca	-Mg	Complete	-K
7.22	8.77	10.08	21.45	21.53	21.66	22.01	22.57

Percent Digestibility

Check	-N	-P	-Ca	-K	Complete	-Mg	-S
55.64	60.08	61.88	65.70	66.27	66.36	66.39	66.54

*Means underlined by the same line are not significantly different from each other at .05 level according to Duncan's Multiple Range Test.

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