CHEMICAL COMPOSITION OF NATIVE GRASS AS INFLUENCED BY NITROGEN FERTILIZATION

By

Jorge Carlos Dickinson

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Thesis Approved:

Wilfred E McMurphy
Thesis Adviser

Robert M. Reed

Dean of the Graduate School

593408

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CHAPTER I

INTRODUCTION

There is more land in the United States used for pasture than for all other farm crops grown. Throughout history it has always been recognized that well managed pastures are an economical source of feed. Any means of improving or increasing the efficiency of this great natural resource will obviously be of enormous value.

It is generally accepted that the application of nitrogen fertilizer to forages usually increases yield. However, there have been variable results when the nutritive value of the feed is considered. This is especially true when several plant species are considered.

The chemical composition of a feed has long been known to be closely related to its nutritive value. Recently, many investigators have been recommending the use of a lignin analysis as a good indicator of feed quality. A protein determination has long been used for this purpose as well.

The object of this study was to observe the effect of three rates of nitrogen fertilizer, applied on three different dates, on the nutritive value of big bluestem (Andropogon gerardi Vitman), little bluestem (Andropogon scoparius Michx.) and indiangrass (Sorghastrum nutans (L.) Nash) when they were harvested on three separate dates.

CHAPTER II

REVIEW OF LITERATURE

Ward (1959) made an extensive summary of literature pertaining to fertilization of forages. He stated that the effects of fertilization vary with soil types, relative levels of fertility within soil types, ratios of available nutrients, forages, and climatic conditions. The chemical composition of forages may be altered in any or all of several ways. The botanical composition of mixed herbage may be changed. One nutrient may be so plentiful that luxury consumption occurs, resulting in the forage containing an abnormally large proportion of the nutrient. The various species react independently to differences in soil fertility; thus the results of variations in soil fertility vary with the species and proportion of species in the stand.

Several authors (Colovos and Keener, 1961; Meyer, et al. 1957; Morrison, 1959) reported that the nutritive value of forage depended primarily on the morphological and physiological development of grasses and legumes. Blaser (1964) stated in his review that published data generally showed that there was a progressive decline in digestibility as plants changed from a leafy vegetative to a morphologically stemmy growth as plants grew to maturity. Nitrogen compounds, with advanced stages of growth, made up progressively less of the dry matter and there was a net loss in protein in the mature stages of growth. This occurred because of leaf losses and large decreases in leaf/stem ratios, as well as the increase in structural materials.

Reid (1959) reported that concurrent with maturation changes in plant composition there was a rapid decline in digestibility of protein and the utilizable energy of forages. He worked on several perennial grasses and legumes.

With the addition of nitrogen fertilizer, results have been inconsistent when related to chemical composition. Not all species have yielded the same results. Several authors (Poulton et al., 1957; Vinall and Wilkens, 1936; Leukel et al., 1934) generally found that nitrogen fertilizer increased the nitrate components and their apparent digestibility in grasses. However, Poulton et al. (1957) and Chalupa et al. (1961) found that high applications had little or no beneficial effect on the average value of the herbage. This was due to the fact that the content of crude fiber and its digestibility was not altered. There was also a tendency for the nitrogen free extract to decrease as the nitrogen content increased. Results obtained by Eheart and Pratt (1942) on bluegrass pasture made them conclude that "fertilizer should be applied to get high yields of desired grasses and legumes but fertilizers '<u>per se</u>' generally do not improve the energy value of forages."

Brown et al. (1963) reported that the amount of soluble carbohydrates in harbage influenced the energy value of grasses. Some workers (Alberda, 1960; Waite, 1958) determined that high nitrogen fertilization had little or no beneficial effect on the energy value of grasses. An increase in crude protein content, concurrent with added nitrogen, was associated with a decrease in highly digestible fructosan in grasses. Blaser (1964) in his review on the subject, concluded that the drastic reduction in soluble carbohydrates with added nitrogen could be attributed to increased respiration and utilization of soluble carbohydrates for synthesis of protein and structural materials in increased yield.

Colovos and Keener (1961) showed that stage of growth, regardless of fertilizer, had a decided effect on the digestible energy of bromegrass hay. The addition of the nitrogen fertilizer did not alter the high digestibility of early nor the low digestibility of late cut grass.

Blaser (1964) similarly reported that structural carbohydrates and lignification were apparently not reduced by nitrogen application; thus the energy value to animals of these components in plants was not improved by liberal nitrogen fertilization.

Patton and Giesecker (1942) reported that lignin affected the digestibility of feeds in three main ways:

(1) The lignin may incrust the other digestible constituents, "putting them in a nutshell" which the animal can't crack.

(2) Lignin may combine chemically with other constituents, thus forming unavailable compounds.

(3) Digestion may be retarded through local inhibition of digestive enzymes due to toxic action of phenolic groups resulting from partial decomposition of lignin.

Another possible adverse effect of lignin is its action on total intake. Forbes and Carrigus (1948) reported a decrease of the maximum intake of 8.2 percent with steers and 9 percent with wethers for each percentage unit increase in lignin content of the forage.

Brown (1961) reported that there have been many complicating factors in the study of this substance. One of these was the heterogeneity of lignin. Even if simple polymars were considered, he stated that there were three different lignins. Patton and Giesecker (1942) said that the lignin which they isolated from the woody portion of plants was in reality a group of similar organic substances. These contained methoxy groups, aromatic nuclei, and a carbon to oxygen ratio higher than that of the carbohydrates. Brown (1961) pointed out that another factor was the multiplicity of products that arise from degradative reactions. Most of these have successfully resisted purification.

Because its chemical composition is not known, the quantitative determination is still somewhat empirical. However, this is a small deterrent to its practicality in feed analysis as is evidenced by results in feeding trials. Crampton and Maynard (1938) recovered 99.3% of the ingested lignin in feeding trials with steers and they suggested that it replace the analysis of crude fiber. Sullivan (1955) found that although lignin was of low digestibility it did have some. He discovered that its coefficient of digestibility was a little over 10 in some cases. However, this was extremely low when compared to his value for true cellulose that had a coefficient of about 67. Moon and Abou Raya (1952) have reported that lignin is a mixture with various degrees of condensation and that upon acid treatment some is lost by hydrolysis. But at the same time apparent lignin-like substances such as condensation products of carbohydrates, nitrogenous compounds, and other substances are included. Therefore, the end product is more or less the same.

This was confirmed by Bondi and Meyer (1943) who observed that while lignin was almost completely recovered after going through the animal's body its chemical composition had been slightly changed. The feces lignin yielded a lower methoxy value than the plant lignin. Armstrong et al. (1950) concluded that lignin was not merely the least digestible of organic plant nutrients, but it probably afforded the best available criterion of digestibility when a feeding trial was not possible. Several authors (Crampton and Maynard, 1938; Heller and Wall, 1940; Forbes and Garrigus, 1950) have approved lignin analysis for predicting feeding values of forage plants.

Sullivan (1964) after working on several grasses such as ryegrass, sudangrass and orchardgrass, stated that protein not only was an essential nutrient but its quantity was positively correlated with the dry matter digestibility.

Kivimae (1960) pointed out that protein content was relatively easy to determine and provided a satisfactory estimate of the digestibility of protein itself, both in legumes and grasses. He also noted that it indicated the digestibility of organic matter in grasses more accurately than did crude fiber.

According to Forbes and Garrigus (1948) the next best measure of the nutritive value of forages after lignin was the protein content.

Sullivan (1962) stated that the crude protein of forages seemed to be an acceptable measure of quality. Usually the higher the protein content the higher the nutritive value of the forages. He recommended an analysis for crude protein, lignin and moisture.

It must be noted that the many advantages attributed to a protein analysis are probably more related to the high degree of negative correlation between lignin and protein contents than to any specific effect of the protein itself upon total digestibility. However, this fact does not detract from its value. For these reasons a lignin analysis and a nitrogen determination were chosen as measures of the nutritive value of the forages in this research.

CHAPTER III

MATERIALS AND METHODS

Fertilizer treatments on a native grass hay meadow were started in 1962. They consisted of 40 and 80 pounds of actual nitrogen per acre applied annually at three dates: May 15, June 1 and June 15, plus an unfertilized check. In the following discussion these treatments will be referred to as the check, 40N - May 15, 80N - May 15, etc.

The plots were located in Payne County, Oklahoma, near Lake Blackwell on the N 1/2, SW 1/4, Sect. 2, R 1 W, T 19 N. The soil was a Norge loam with a 1 - 3 percent slope. It was in an open hay meadow with typical vegetation for the area, the dominant species being big bluestem, little bluestem and indiangrass.

In 1964 forage samples of big bluestem, little bluestem and indiangrass were taken from these plots on July 1, July 21 and August 11.

Additional samples of native grass were obtained for nitrogen analysis from a fertilizer study at Poteau, Oklahoma. Cuttings were made on July 1 in 1963 and 1964. Treatments analyzed were a check, 50 and 100 pounds of actual nitrogen per acre. Detailed information on yield and vegetation are reported by Ball (1965).

Ammonium nitrate was used as the source of nitrogen fertilizer at Poteau and Lake Blackwell.

Plants were clipped at a height of two inches from the ground and then dried in a forced draft oven at 65 C. Lignin samples were ground in a Wiley mill to pass through a 60-mesh screen. Nitrogen samples were ground to pass through a 20-mesh screen.

Only the check, 40N - May 15 and 80N - May 15 treatments of big and little bluestem which were cut on July 1 and August 11 were analyzed for lignin. All samples were analyzed for nitrogen and reported as crude protein. The method of lignin analysis chosen was basically the same as that developed by Ellis et al. (1946). It was modified by Moon and Abou Raya (1952) and further changed by Sullivan (1959). The main advantage of this method over other similar ones is that it is relatively shorter. This largely overcomes the main drawback of the lignin analysis which is that it takes too much time. It is estimated that in a given period of time twice as many samples can be analyzed for acid insoluble lignin as for total lignin. Sullivan (1964) noted that acid insoluble lignin does not include that part which dissolves in 72 percent sulphuric acid and which is precipitated when the acid is diluted with water, but total lignin does contain this fraction. A one-half gram sample was weighed into a 200 ml beaker, and 30 to 40 ml of benzene alcohol in a ratio of 2.5 to 1 were added. The samples were allowed to stand overnight. After boiling for about five minutes they were filtered through a Gooch crucible (fitted with an asbestos mat) under suction. The residue was rinsed three times with benzene alcohol, three times with acetone, and three times with water. The material was then transferred back to the beaker and about 40 ml of 0.1 N pepsin solution were added.

The samples were then placed in a water bath for 24 hours at a temperature of 45 C. Again the samples were filtered through the Gooch crucibles and rinsed twice with water. Concentrated hydrochloric acid was then filtered through, under suction, for 2 1/2 minutes. About 10 to 15 ml of acid passed through in this time. The samples were washed three times with water, alcohol and ether. The samples were then placed in the suction flasks and treated with 72 percent sulphuric acid. The acid had previously been cooled to approximately 0 C. When it was noted that the acid had passed through the sample the crucible was removed from the suction flask. Then the acid was allowed to percolate slowly for three hours. At the end of this period the crucible was returned to the suction flask and the undiluted acid was removed by filtration. The residue was washed with 60 ml of hot water to remove all traces of acid. The samples were dried overnight at 105 C. After weighing them they were ashed in an oven for 5 hours at 500 C., and the acid insoluble lignin was determined by the loss in weight of the sample. All samples were run in duplicate.

Nitrogen was determined by the Kjeldahl method as reported in the Official Methods of Analysis (1955).

Statistical procedure was according to Steel and Torrie (1960).

CHAPTER IV

RESULTS AND DISCUSSION

Preliminary results from the acid insoluble lignin analysis appeared variable; therefore, a methods check was made using only one sample of forage which had 10.09 percent acid insoluble lignin content.

Also, 1/2 gram samples were compared to 1 gram samples as it was thought that this would enable the acid to more readily come in contact with all of the particles. However, the maximum variation of results ranged from 9.65 percent to 10.33 percent lignin, and, therefore, was considered negligible. It was noted that the water filtered through the smaller sample in less than half the time required for the larger one. Therefore, a 1/2 gram sample was adopted.

It was thought that most of the variation arose from one or both of two factors. The first possibility was that the sulphuric acid may not have been washed out completely. Therefore, washings were made with 60 and 120 ml of water. However, no significant differences were detected between washing treatments, (Appendix Table III).

After the last ether washing was applied the samples had been allowed to dry out before the sulphuric acid was added. However, it was noted that when the acid was added many particles formed into clumps. Therefore, the acid did not come in complete contact with the sample. For this reason samples were stirred gently with a rounded glass rod in order to break up any conglomerations. This also appeared to have no effect (Appendix Table III).

During this check on methods all samples were treated with sulphuric acid immediately after the last drop of ether had been filtered out. This caused the sample to still be relatively moist and not hard and compact as it would get if allowed to dry out. This may have had an influence on the high degree of uniformity that was achieved in this methods check. This practice was continued throughout the analysis.

The samples of the trial were divided into two groups and run at different times. This allowed the variation between dates to be estimated. It was found that there was a difference at the .10 probability level. This indicated that the method used may have varied slightly from day to day even though every effort was made to keep conditions identical (Appendix Table III).

Some crucibles with thick asbestos mats were treated with sulphuric acid. They were then washed with 60, 120 and 240 ml of water. This was done in order to determine if any acid was being held by the mats themselves. No acid was being retained by the asbestos mats.

Nitrogen fertilization did not significantly influence the lignin content of big bluestem or little bluestem at either of the sampling dates (Appendix Table IV). The lignin content of little bluestem was significantly greater than that of big bluestem at both sampling dates. Lignin content of both species increased with maturity. The average lignin content of big bluestem was 8.85 percent on July 1 and increased to 6.90 percent by August 11. Little bluestem had 13.01 percent on July 1 which increased to 14.76 percent lignin on August 11 (Table I).

TABLE I

LIGNIN CONTENT OF BIG AND LITTLE BLUESTEM FROM LAKE BLACKWELL ON JULY 1 AND AUGUST 11

	BIG BLUESTEM			LITTLE BLUESTEM		
	Check	40N-May 15	80N-May 15	Check	40N-May 15	80N-May 15
July 1	8.53	8.57	9.45	12.52	12.77	13.74
	Mean	8.85		Mean	13.01	
August 11	8.90	9.63	10.27	14.26	14.37	15.66
	Mean	9.60		Mean	14.76	

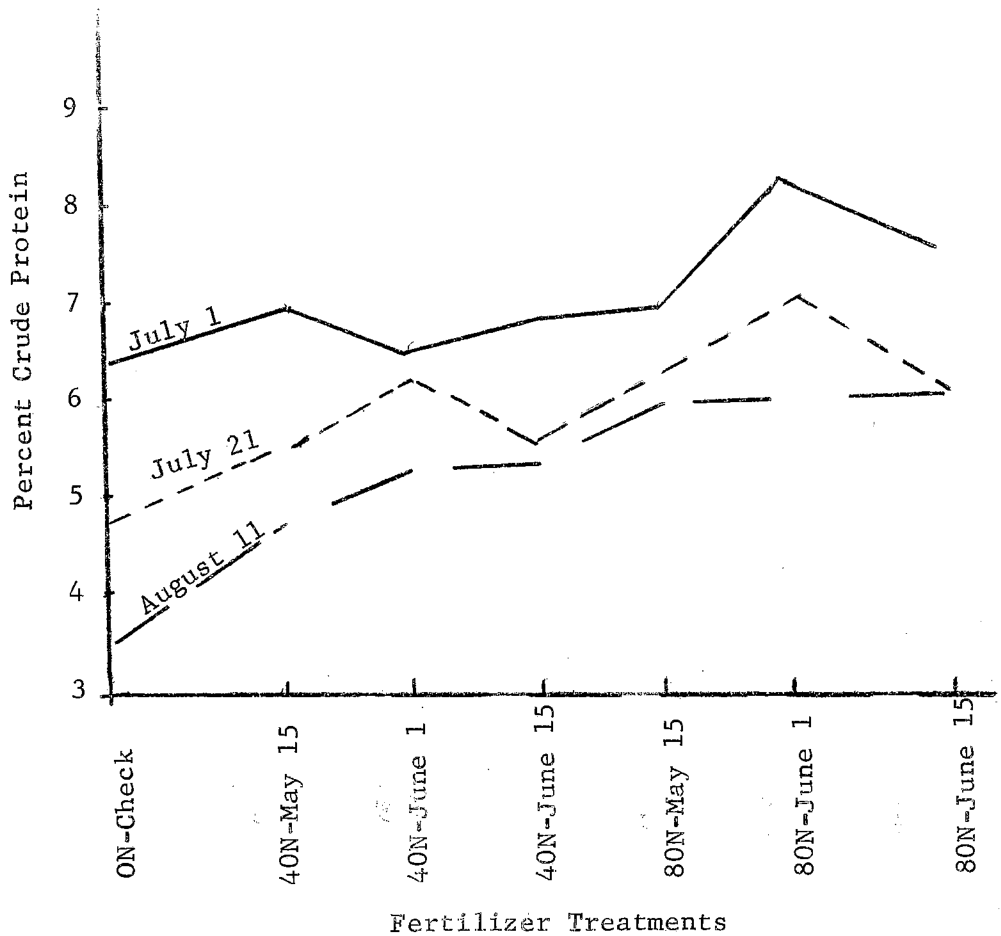

Figure 1. Crude protein of big bluestem from Lake Blackwell as affected by fertilizer treatments and harvest date.

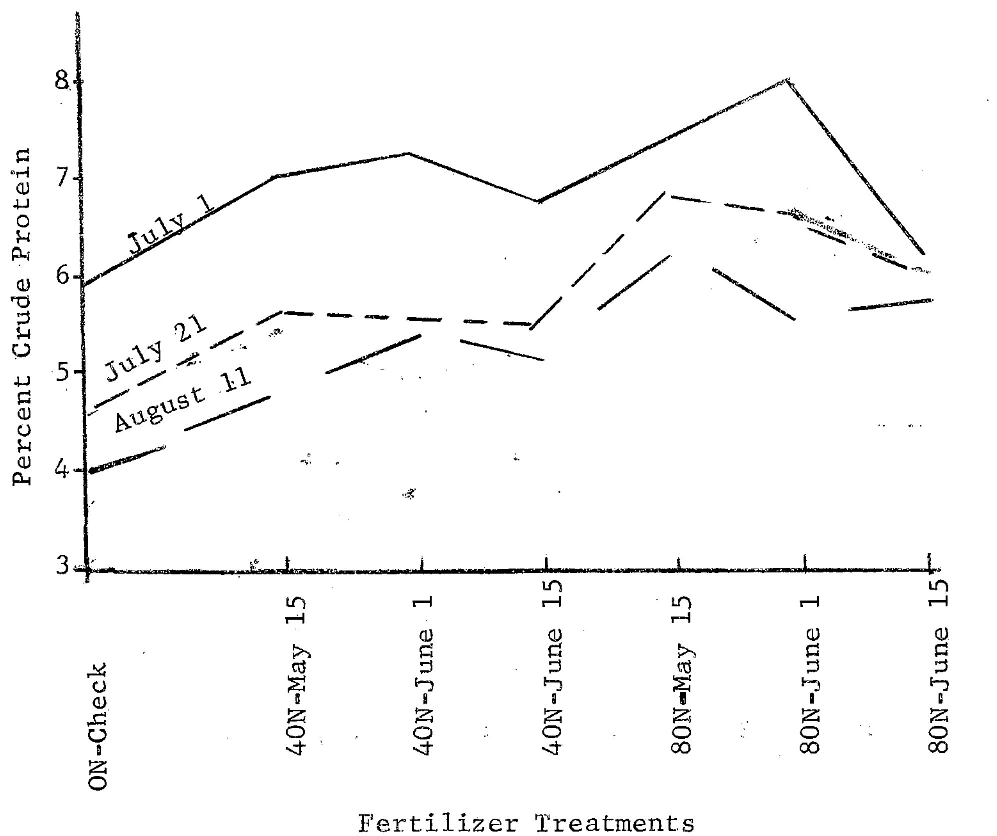

Figure 2. Crude protein of indiangrass from Lake Blackwell as affected by fertilizer treatments and harvest date.

Crude protein content of big bluestem was significantly influenced by nitrogen fertilization (Table II) and date of cutting (Appendix Table V). The 80N-June 1 treatment was the most effective early in the season, but by August 11 all fertilizer treatments had significantly increased the crude protein content. Crude protein of unfertilized big bluestem was 6.31 percent on July 1 and dropped to 3.50 percent by August 11 (Figure 1). By August 11 big bluestem from all fertilized plots except the 40N-May 15 had at least 5.00 percent crude protein (Table II).

Crude protein of little bluestem was not significantly influenced by nitrogen fertilization at any of the cutting dates (Table II). Crude protein of unfertilized little bluestem was 6.37 percent on July 1 and dropped to 4.89 percent by August 11. The highest crude protein content of any fertilized plot on July 1 was 7.12 percent and 5.25 percent on August 11. This apparent inability of little bluestem to increase its protein content under fertilization is unusual. It is usually found, as was the case with the other two species in this project, that nitrogen fertilizer increases the protein content of forage. The average crude protein content of little bluestem for all treatments on July 1 was 6.37 percent and dropped to 4.89 percent by August 11 (Table II).

Crude protein of indiangrass was not significantly increased by nitrogen fertilization on July 1. However, by July 21 the 80N treatments had significantly increased the crude protein. On August 11 the unfertilized indiangrass had 3.50 percent crude protein while that from all 80N treatments had over 5.00 percent crude protein (Figure 2).

TABLE II

CRUDE PROTEIN[1/] OF FORAGES FROM LAKE BLACKWELL AS INFLUENCED BY FERTILIZER TREATMENTS

Cliping Date	Check	40N-May 15	40N-June 1	40N-June 15	80N-May 15	80N-June 1	80N-June 15	Mean
<u>Big bluestem</u>								
July 1	6.31a	6.81a	6.12a	6.50a	6.56a	8.12b	6.87a	6.77
July 21	4.87a	5.37a	5.81ab	5.26a	5.93ab	6.56b	5.50a	5.61
August 11	3.50a	4.56b	5.00b	5.00b	5.81b	5.81b	5.68b	5.05
<u>Little bluestem</u>								
July 1	5.75a	6.00a	6.25a	5.50a	6.94a	7.12a	6.06a	6.37
July 21	4.75a	5.44a	5.94a	5.06a	5.25a	6.06a	5.44a	5.42
August 11	4.18a	4.93a	5.06a	4.56a	5.25a	5.12a	5.19a	4.89
<u>Indiangrass</u>								
July 1	5.44a	6.31a	6.56a	6.12a	6.62a	7.25a	5.93a	6.32
July 21	4.06a	5.00ab	5.00ab	4.93ab	6.18c	5.93bc	5.62bc	5.24
August 11	3.50a	4.06ab	4.93bc	4.50abc	5.56c	5.25bc	5.37c	4.74

1/ Means for a species within a date followed by the same letter are not significantly different at the .05 level of probability.

The average crude protein content for all treatments on July 1 was 6.32 percent and decreased rapidly to 4.74 percent by August 11 (Table II).

No significant difference in crude protein content existed between species in each of the dates (Appendix Table VI). Apparently these three major native grasses were quite similar in crude protein content during normal hay harvest time. From the standpoint of trying to determine the quality of native grass hay it appears that a composite sample of the hay would give a satisfactory estimate of crude protein content.

The 80N fertilizer treatment usually stimulated a higher protein content in all the forages, although this increase was not always significant (Table II).

The best dates of application for both the 40 and 80 pounds per acre of nitrogen from the standpoint of crude protein content of the forage were May and June 1. Applications of nitrogen on June 1 resulted in the best increase of crude protein early in the season but by August 11 there were no significant differences due to date of application.

From May 15 to August 11, when the last cutting was made, only 2.88 inches or rain fell. The normal precipitation for the period is 11.15 inches (Appendix Table VII). Because of this lack of moisture the forage was quite dry on July 1 when the first cutting was made and became even more so as the season progressed. Between the first and last sampling dates little growth was noted. However, in spite of this fact there was a drop in protein content as the season progressed. This abnormally low precipitation may well have influenced the effect of the fertilizer on the chemical composition of the forage.

The crude protein content of the unfertilized forage in 1963 was 6.18 percent at Poteau, Oklahoma (Figure 3). With 50 pounds of nitrogen per acre crude protein increased to 8.69 percent and with 100 pounds of nitrogen 9.52 percent crude protein was recorded. In 1964 the crude protein content was 7.23 percent for the unfertilized check, 9.45 percent with 50 pounds of nitrogen and rose to 11.36 percent with 100 pounds of nitrogen fertilizer per acre. Although there were no significant differences between years, the difference between treatments was considerable (Appendix Table VIII). Figure 3 shows the almost straight line relationship between crude protein and nitrogen fertilizer. At this location the precipitation was below normal in both years.

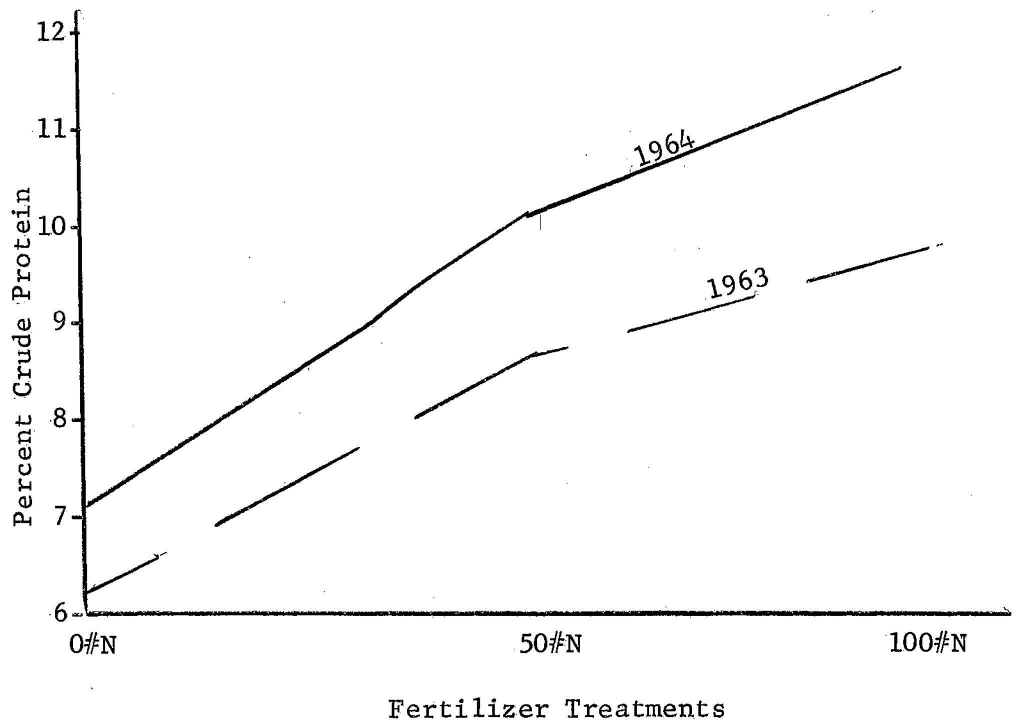

Figure 3. Crude protein of native range on July 4 as influenced by fertilizer treatments at Poteau, Oklahoma.

CHAPTER V

SUMMARY AND CONCLUSIONS

At the Blackwell area samples of big bluestem, little bluestem and indiangrass were collected on July 1, July 21 and August 11. These were analyzed to determine the effect of fertilizer application on crude protein and lignin content. Fertilizer treatments included the unfertilized check and ammonium nitrate applied at rates of 40 and 80 pounds of actual nitrogen per acre on May 15, June 1 and June 15. All forage samples were analyzed for crude protein and some were also analyzed for lignin.

A methods check was run on the method used for lignin analysis to determine the source of variation. Differences from one day to the next were detected at the .10 probability level.

Native grass samples were collected from a fertility experiment at Poteau, Oklahoma, and analyzed for crude protein. The fertility treatments were 0, 50, and 100 pounds of actual nitrogen per acre and samples were collected in 1963 and 1964.

Lignin content of the forage from Lake Blackwell was not affected by nitrogen fertilization. Little bluestem had a greater percent lignin than big bluestem.

Crude protein content of big bluestem and indiangrass was increased by nitrogen fertilization. In big bluestem the 80N-May 15 treatment was consistently higher in protein than all other treatments at all three dates of sampling. Crude protein content of indiangrass was not significantly increased by nitrogen fertilization on July 1. However, by July 21 all three 80N treatments were significantly higher in crude protein than the check. The high level of fertilizer application consistently had a greater effect on the protein level of the forage than the low level. The greatest increase of crude protein over the check in big bluestem and indiangrass was observed on the August 11 sampling date. From the standpoint of crude protein content best results with nitrogen fertilizer application at both 40 and 80 pounds per acre were from May 15 and June 1 dates of application. June 15 applications produced the least crude protein increase.

Crude protein content of little bluestem was not significantly influenced by nitrogen fertilization at any of the cutting dates.

There were no differences in protein content between grass species and apparently these three species were quite similar in crude protein content during normal hay harvest time. From the standpoint of trying to determine the quality of native grass hay it appears that a composite sample of the hay would give a satisfactory estimate of the crude protein content.

Lignin content increased and crude protein decreased with increasing maturity.

Crude protein increase with fertilization was more pronounced at Poteau, Oklahoma.

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CHAPTER VI

APPENDIX

TABLE III

ANALYSIS OF VARIANCE FOR METHODS CHECK ON LIGNIN ANALYSIS

Source	df	MS	F calc.
Total	14		
Trials	1	5439.06	7.80*
Treatments	3		
Stirring	(1)	68.06	.01
Washing	(1)	855.56	3.04
Stir. x Wash.	(1)	27.56	
Error	3	697.23	
Duplicates	7	281.64	

* Significant at .10 level.

TABLE IV

ANALYSIS OF VARIANCE FOR ACID INSOLUBLE LIGNIN

Source	df	MS	F calc.
Total	47	0.00080917	
Replication	3	0.00105046	
Fertilization	2	0.00065102	2.43
Error (a)	6	0.00026785	
Grass species	1	0.02588658	430.55**
Error (b)	9	0.00006012	
Cutting	1	0.00190890	10.51*
Error (c)	18	0.00018163	

* Significant at the .05 level.

** Significant at the .01 level.

TABLE V

ANALYSIS OF VARIANCE OF CRUDE PROTEIN SAMPLES

FROM PAYNE COUNTY, OKLAHOMA

Source	df	MS	F calc.
Total	251	0.00031398	
Replications	3	0.00003625	
Fertilization	6	0.00268945	8.114**
Error (a)	18	0.00033145	
Grass species	2	0.00079092	5.592**
Error (b)	42	0.00014144	
Cutting	2	0.01372524	114.082**
Error (c)	126	0.00012031	

** Significant at the .01 level.

TABLE VI

ANALYSIS OF VARIANCE OF THE CRUDE PROTEIN CONTENT OF

THE SPECIES FROM LAKE BLACKWELL ON

JULY 1, JULY 21 AND AUGUST 11

Source	df	MS	F calc.
July 1			
Grass species	2	0.00046394	3.017
Error	42	0.00015377	
July 21			
Grass species	2	0.00025804	3.039
Error	42	0.00008490	
August 11			
Grass species	2	0.00014934	1.053
Error	42	0.00014180	

TABLE VII

CLIMATOLOGICAL DATA FROM STILLWATER, OKLAHOMA

Month	Precipitation	Departure from Normal
January	0.54	-0.62
February	1.61	0.26
March	1.02	-0.87
April	1.71	-1.15
May	4.04	-0.58
June	1.17	-3.07
July	0.28	-3.25
August	7.30	4.09

TABLE VIII

ANALYSIS OF VARIANCE OF CRUDE PROTEIN OF NATIVE GRASS FROM POTEAU, OKLAHOMA

Source	df	MS	F calc.
Total	23		
Replications	3		
Fertilization	2	0.00287009	17.258**
Error (a)	6	0.00016629	
Years	1	0.00089916	3.665
Treat. x Years	3	0.00031329	1.277
Error (b)	6	0.00024534	

** Significant at the .01 level.

VITA

Jorge Carlos Dickinson

Candidate for the Degree of

Master of Science

Thesis: CHEMICAL COMPOSITION OF NATIVE GRASS AS INFLUENCED BY NITROGEN FERTILIZATION.

Major Field: Agronomy (Range Management)

Biographical:

Personal Data: Born in Buenos Aires, Argentina on January 11, 1943, the son of George and Muriel Dickinson.

Education: Attended grade school in Buenos Aires, Argentina; graduated from St. Georges College in 1959; received the Bachelor of Science degree from Oklahoma State University, with a major in Animal Science, in May, 1964; completed requirements for the Master of Science degree in August, 1965.

Experience: While an undergraduate the writer worked at the Beef Barn and Meat Laboratory of Oklahoma State University in 1961 and 1962.

Professional Organizations: The writer is a member of the American Society of Range Management.