EFFECT OF NITROGEN FERTILIZATION ON THE CHEMICAL COMPOSITION OF BROMEGRASS

by

VIRGINIA SWARTZ CAREY

B. S., Illinois Wesleyan University, 1948

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1950

TABLE OF CONTENTS

INTRODUCTION

Unpublished data of the department of Agronomy of Kansas State College show that the nitrogen and carotene contents of bromegrass were increased markedly by fertilization with ammonium nitrate. Eggleton (6) has shown that the addition of nitrogenous salts to the soil increased the content of the various nitrogen fraction of grassland crops. It was thus of interest to know if heavy applications of ammonium nitrate on bromegrass affect the type of nitrogen compounds that accumulate in the plant.

A potential use of bromegrass is for dehydration and incorporation into poultry feeds. If used in this manner, the riboflavin content of bromegrass meal would be of considerable importance, since this nutrient frequently is deficient in poultry rations. Inasmuch as nitrogen fertilization resulted in an increased carotene content, it seemed desirable to determine if the riboflavin content would be increased also.

EXPERIMENTAL

Early in the 1948 growing season, duplicate plots of bromegrass were fertilized with 300 pounds of nitrogen per acre in the form of Uramon; 300 pounds as cyanamide; and 100, 200 and 300 pounds of nitrogen as ammonium nitrate. One set of plots was left untreated as control. Samples were cut semi-monthly from the plots which received the different rates and kinds of fertilizers. Equal samples from duplicate plots were combined, autoclaved at

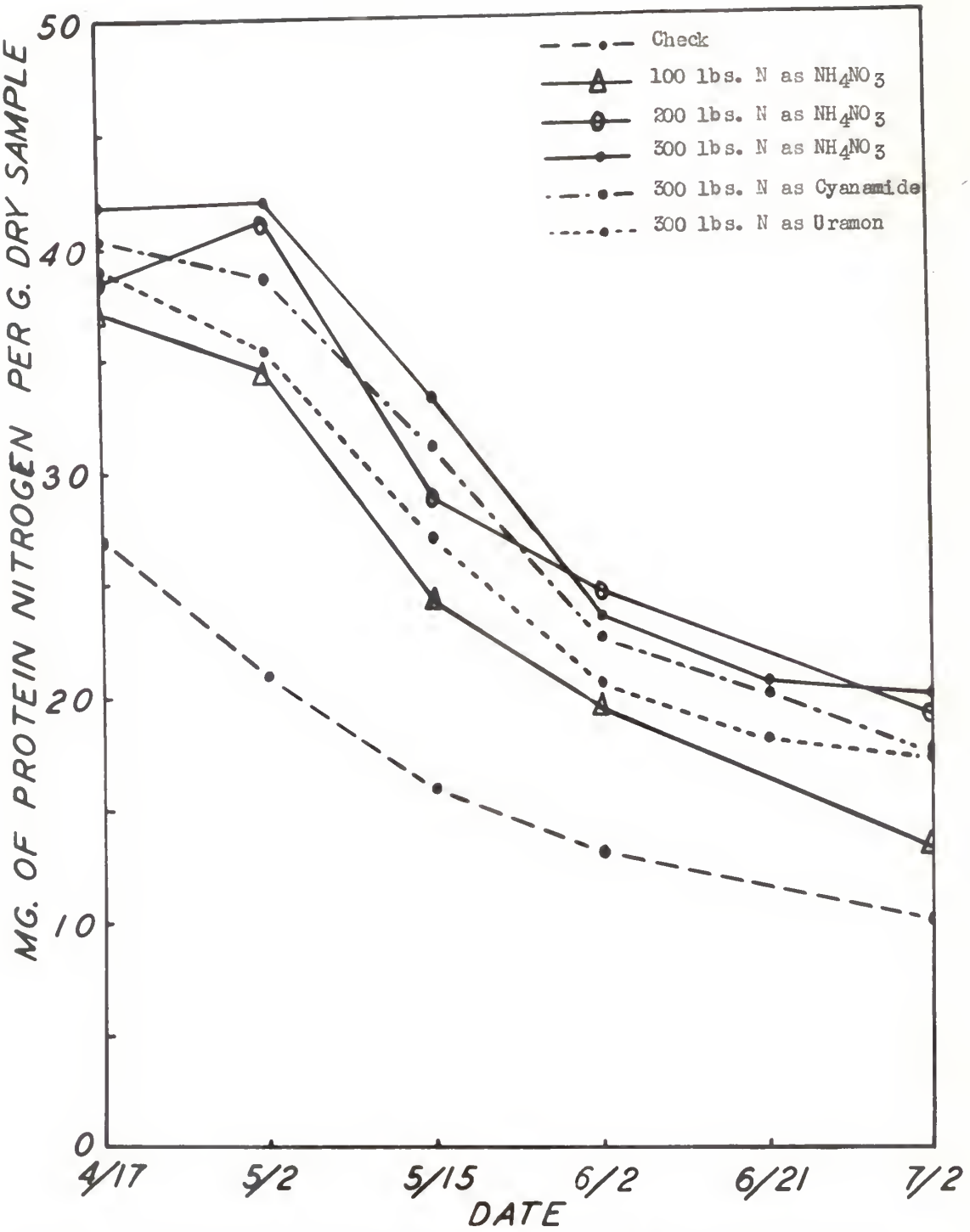

FIG. I. EFFECT OF NITROGEN FERTILIZATION ON THE PROTEIN CONTENT OF BROMEGRASS.

five pounds pressure for five minutes, and dried for four hours at 65° C. in a circulating air oven. The dried samples were ground to pass through a 20-mesh screen and were stored at -20° C. in the dark until needed.

Changes in the Nitrogen Fractions

Protein Nitrogen. Protein nitrogen was determined by the official method of the Association of Official Agricultural Chemists (1) for feedstuffs. The data presented in Fig. 1 show a steady decline of protein nitrogen as the plants matured. The amount present was directly related to the amount of nitrogen applied to the soil. The protein content of the plants receiving the 300 pounds of nitrogen as ammonium nitrate was slightly higher than those receiving Cyanamide and Uramon. This indicates that the nitrogen of ammonium nitrate is more readily available to the plant than when in the other two forms.

Ammonia Nitrogen. Ammonia nitrogen was determined by the method of Pucher, Vickery, and Leavenworth (13). This method consists of placing the dry plant tissue in a distilling flask, adding a phosphate-borate buffer, making the solution alkaline to liberate the ammonia, distilling the ammonia under reduced pressure into a standard acid solution, and determining it by Nesslerization. All determinations were corrected for a small apparatus blank.

From Fig. 2, it will be seen that increasing the ammonium nitrate applied to the soil resulted in increased amounts of ammo-

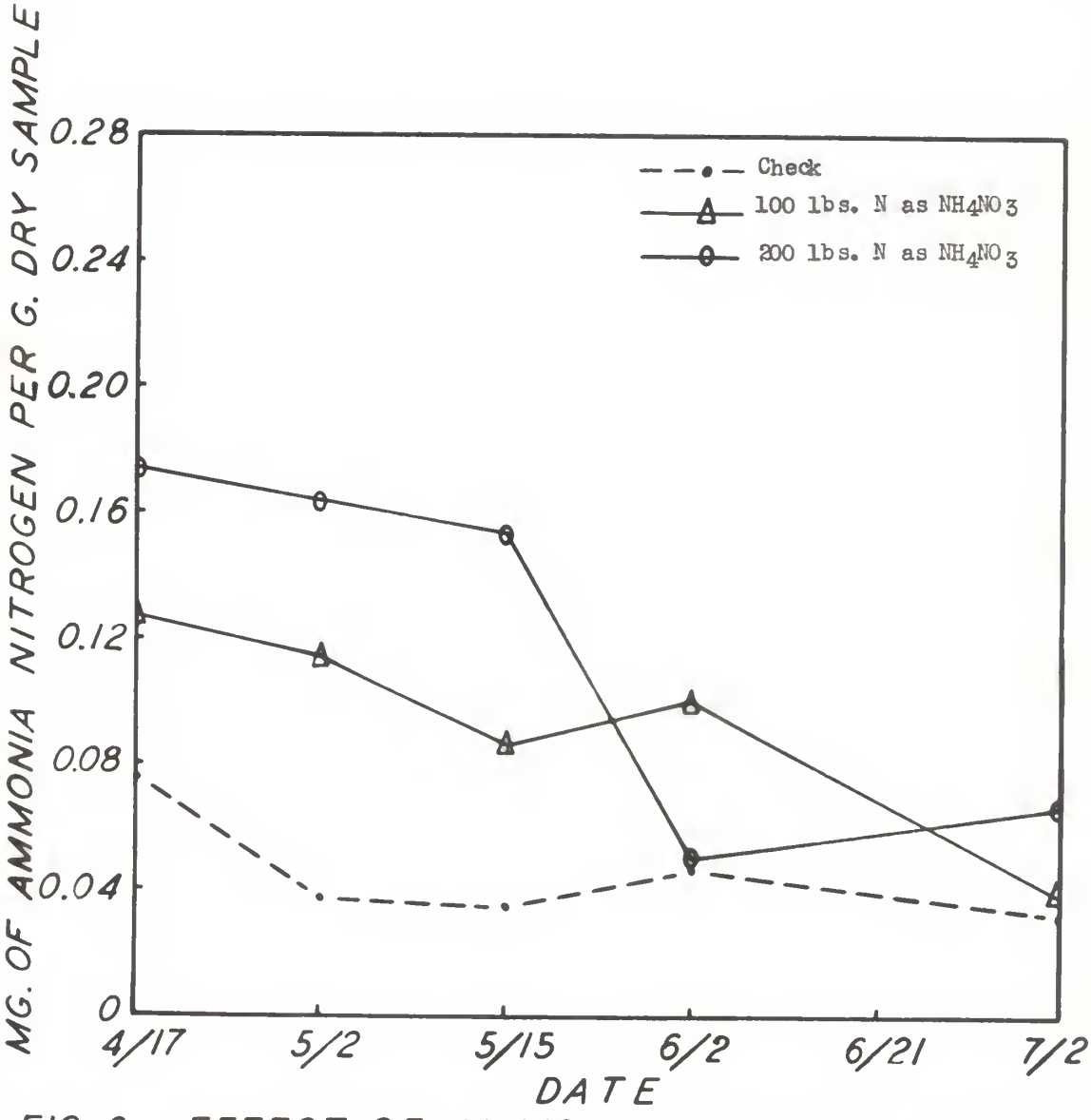

FIG. 2. EFFECT OE AMMONIUM NITRATE FERTILIZATION ON THE AMMONIA CONTENT OF BROMEGRASS.

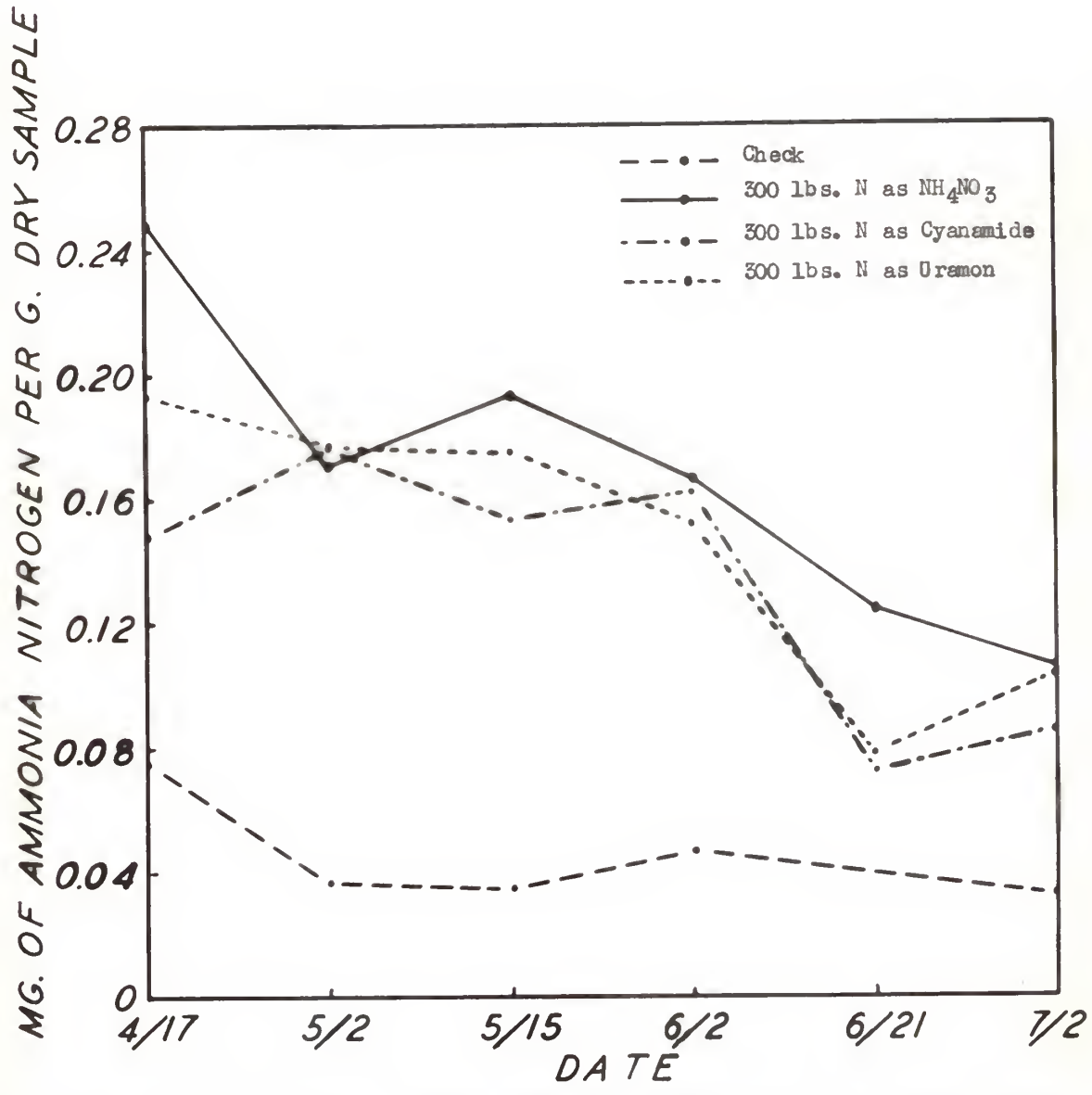

FIG. 3. EFFECT OF TYPE OF NITROGEN FERTILIZER ON THE AMMONIA CONTENT OF BROMEGRASS.

nium ion in the plants early in the season. At maturity, however, plants from the fertilized plots contained approximately the same quantity of ammonium nitrogen as the unfertilized plants. From Fig. 3, it will be seen that the kind of nitrogen fertilizer applied also influenced to some extent the amount of ammonium ion that accumulated in the plants.

Amide Nitrogen. Amide nitrogen determinations were made on a water extract of the dried plant tissue as recommended by Pucher, et al. (13). An aliquot of the extract was hydrolyzed for three hours with sulfuric acid, made alkaline, and the ammonia determined. Amide nitrogen is the difference between the ammonia present after hydrolysis and that originally present.

Figure 4 shows that the amide content of plants from fertilized plots increased markedly between the first and second cuttings, and then steadily decreased. Little change occurred in the unfertilized plants. The changes in amide content from one cutting to another were more pronounced than the changes in the ammonium content.

Nitrate Nitrogen. Nitrate nitrogen was determined by the method of Gilbert, Eppson, Bradley, and Beath (7), with certain modifications. This method consists of extracting the dried tissue with hot water; clarifying with silver sulfate solution, calcium hydroxide, and activated carbon; evaporating to dryness; adding phenoldisulfonic acid; and making alkaline with ammonium hydroxide to develop a yellow color. Color intensity was measured with a Beckman spectrophotometer at 408 mu.

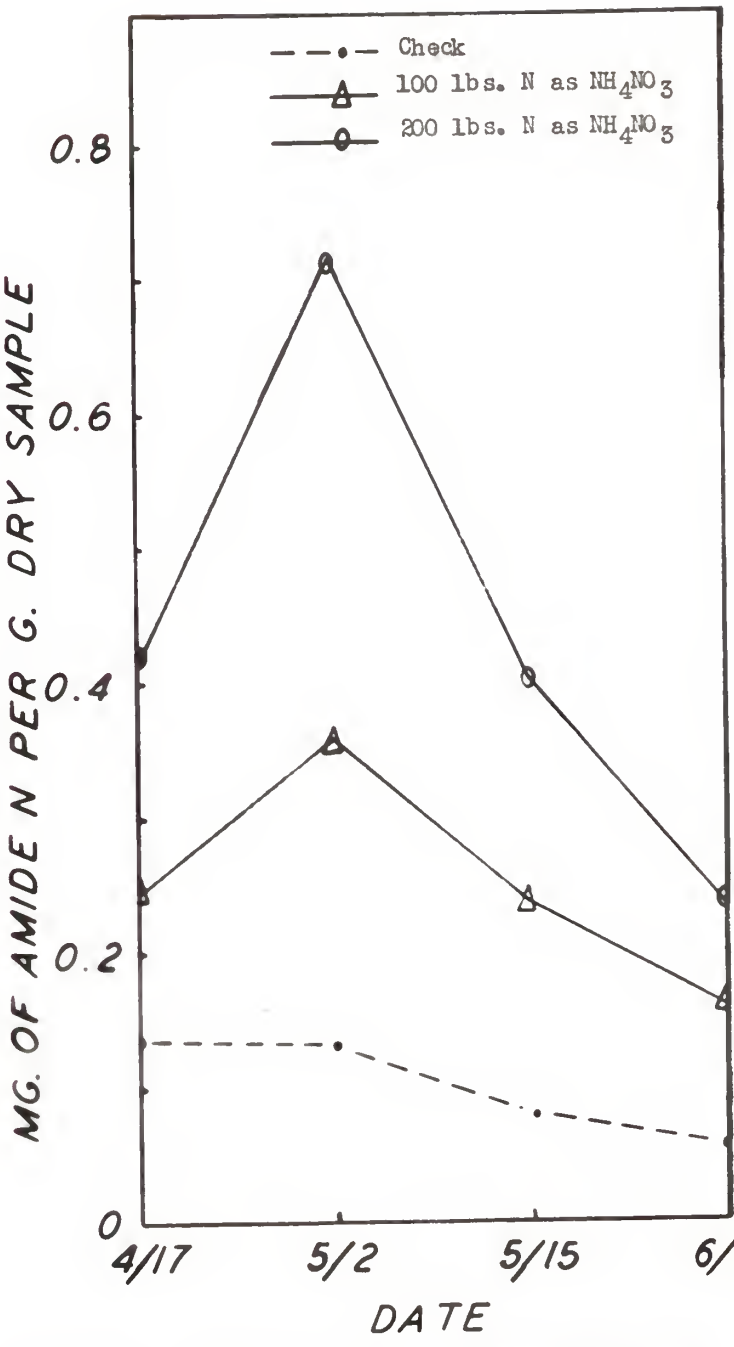

FIG. 4. EFFECT OF AMMONIUM NITRATE FERTILIZATION ON THE AMIDE CONTENT OF BROMEGRASS.

It was found that high values were obtained with a standard nitrate solution when activated vegetable charcoal was used for clarification. Carbon black gave adequate clarification, and did not contain soluble nitrates (16). Hence, it was used in the determinations.

A study was made by Chamot et al. (4) to determine errors due to variations in the amount of base used to develop the final color. Although he stated that ammonium hydroxide gave variable results, this difficulty was not encountered. It was found desirable to add the ammonium hydroxide after transferring the solution to a volumetric flask from the evaporating dish, rather than before, as recommended by Gilbert et al. This eliminated errors due to spattering.

The wavelength for determining the color intensity by means of a Beckman spectrophotometer was not known. Maximum light absorption was found to occur between 406 and 410 mu. This is in agreement with Berge (2) who used 400 to 410 mu with the Colman spectrophotometer.

Figures 5 and 6 represent the data obtained from the nitrate nitrogen determinations. The amount of nitrate in the bromegrass was related directly to the amount of ammonium nitrate applied to the soil. Cyanamide and Uramon applications resulted in little increase of nitrate nitrogen in the plant tissue.

Effect of High Nitrate Content on Protein Determination. Strowd (16) and Paul and Berry (12) have stated that the nitrate nitrogen in plant tissue is reduced partially to ammonia in the

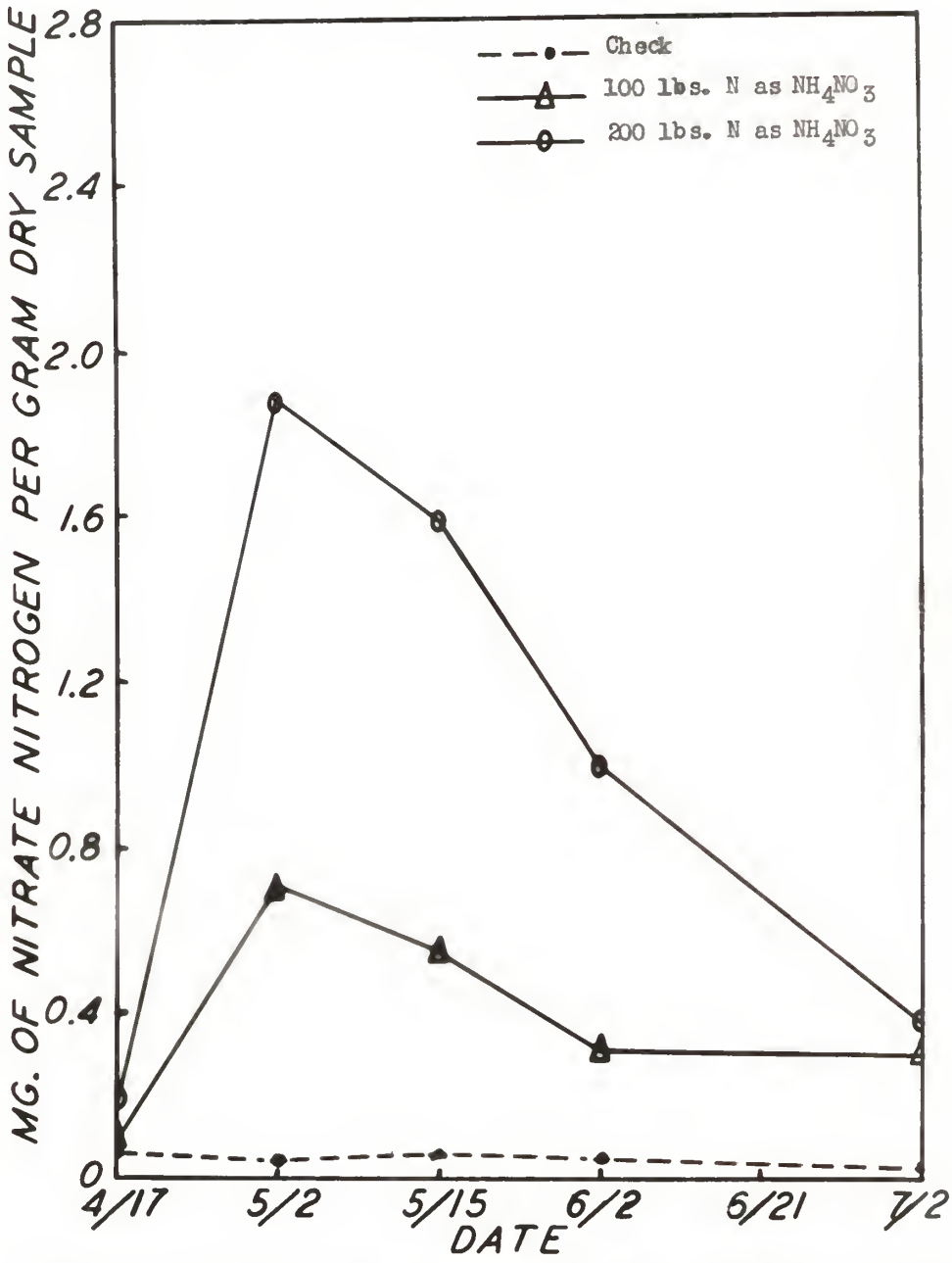

FIG. 5. EFFECT OF AMMONIUM NITRATE FERTILIZATION ON THE NITRATE CONTENT OF BROMEGRASS.

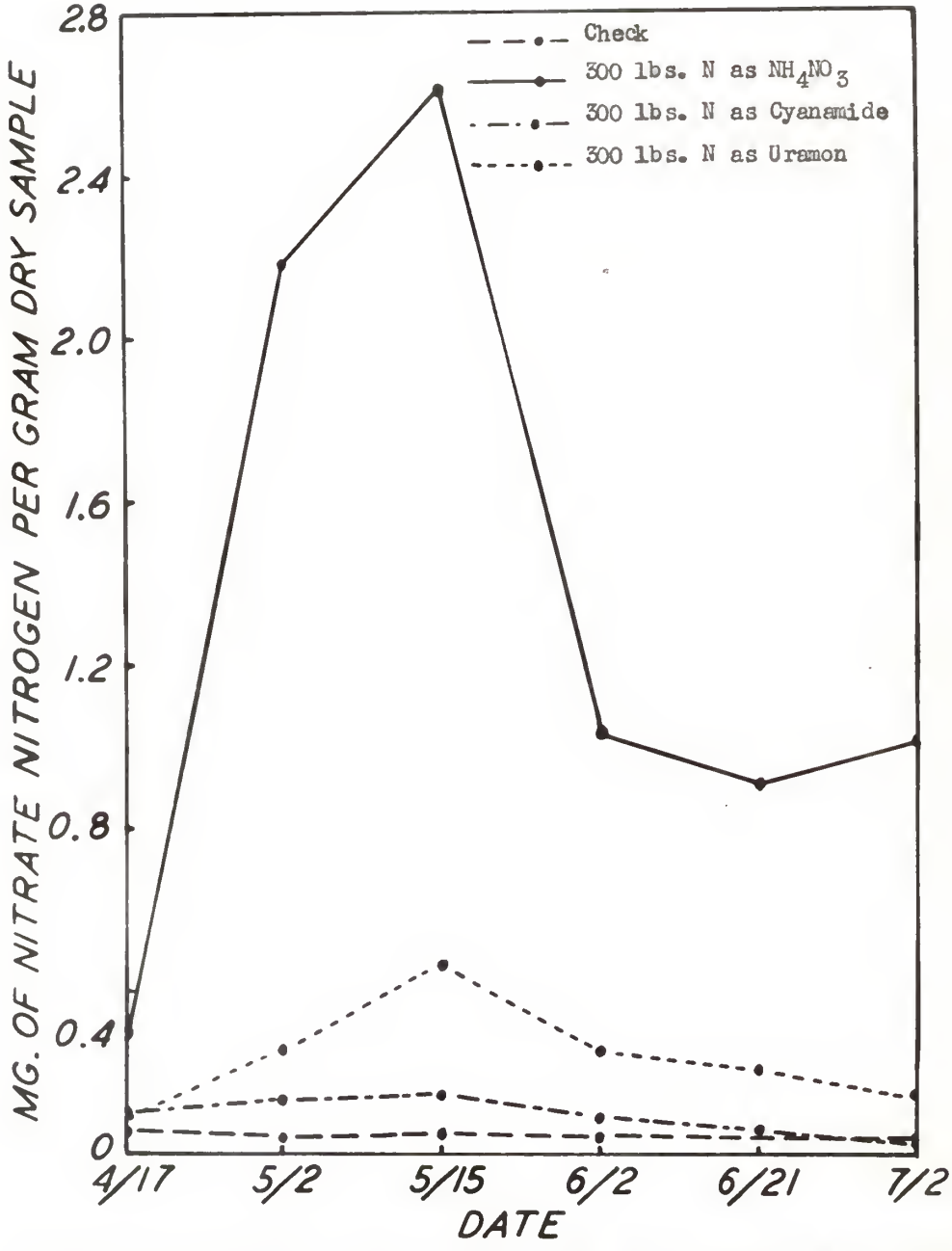

FIG. 6. EFFECT OF TYPE OF NITROGEN FERTILIZER ON THE NITRATE CONTENT OF BROMEGRASS.

Kjeldahl method of determining nitrogen. Since the nitrate nitrogen content was high in the plants from the plots fertilized with ammonium nitrate, it seemed advisable to determine the amount of nitrate that would appear as protein by the official method for feeds. This was done by determining nitrogen in a solution of potassium nitrate with salicylic acid added to include nitrates (1). Filter paper was added to each flask as a source of organic matter.

Table 1. Comparison of nitrate nitrogen of a potassium nitrate solution determined by the official method for feedstuffs and by the official method for inclusion of nitrate.

Official method for inclusion of nitrate	Official method for feedstuffs	Per cent determined by method for feedstuffs
Mg. N	Mg. N	% N
0.32	0.35	106.3
1.86	1.61	86.5
1.79	1.61	90.0
3.89	3.22	82.7
3.82	3.08	80.7

Table 1 indicates that at low levels almost all of the nitrate was reduced and appeared as protein. As the concentration increased, a smaller per cent of the nitrate was reduced. The nitrate nitrogen content of the bromegrass samples analyzed in this study ranged from 0.026 to 2.51 mg per g of dry sample. At the highest level, possibly 85 per cent, or 2.13 mg, of the nitrate nitrogen was determined as protein nitrogen. Consequently, this amount of nitrate nitrogen resulted in 6.4 per cent more protein than actually was present.

Changes in Riboflavin Content

Riboflavin was determined by the method of Loy (9). The samples were autoclaved in the presence of sulfuric acid, proteins were precipitated, the pH was adjusted to 6.8, and the fluorescence of the riboflavin was measured by means of a Coleman photofluorometer. Calculations were made according to the formula of Loy.

The effect of ammonium nitrate fertilization on the riboflavin content of bromegrass is shown in Fig. 7. Nitrogen fertilization up to 100 pounds per acre caused an appreciable increase in the riboflavin content of the plants. However, the application of 200 pounds of nitrogen caused only a small increase over that which was obtained with 100 pounds. The riboflavin content of the plants from all treatments decreased rapidly as the plants matured.

DISCUSSION

The mechanism for the synthesis of organic nitrogenous compounds from inorganic materials has been under investigation for a long time. Ammonium or nitrate nitrogen is the main source for nitrogenous compounds in higher plants. When nitrogen fertilizers in the form of ammonium ion are applied to the soil, nitrifying bacteria usually oxidize it to nitrate and little is absorbed as ammonium ion (10, 11). If the soil is sterile or has been fumigated, ammonium ions are absorbed without being changed to nitrate

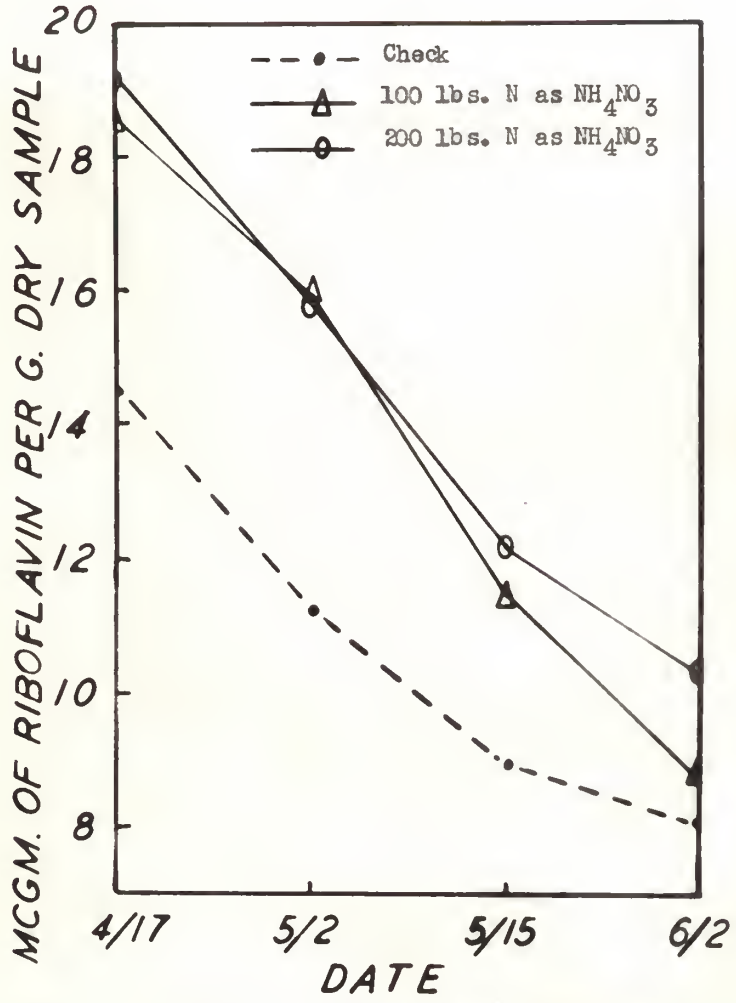

FIG. 7. EFFECT OF AMMONIUM NITRATE FERTILIZATION ON THE RIBOFLAVIN CONTENT OF BROME-GRASS.

(17). Ammonium ions are absorbed directly when present as such in solution culture (15). When absorbed as ammonia, it requires no reduction for use in protein synthesis, and therefore eliminates an energy-required reaction.

Ammonium ions are assimilated as rapidly as they enter the plant, but the nitrates are utilized more slowly and tend to accumulate (14). Nitrogen must be in the form of ammonium ions for biological synthesis. There is an enzyme, reducase, present which reduces the nitrate ion to the ammonium ion within the plant (5).

Ordinarily amino acids are the first detectable organic nitrogen compounds to be synthesized from ammonia. If luxury consumption of nitrogen compounds occurs, however, the excess ammonia may combine with α-keto acids to form asparagine and glutamine (18). The α-keto acids originate from the respiration of carbohydrates.

Nitrate determinations on samples from the plots fertilized with ammonium nitrate have shown that increasing the amount of fertilizer increased the nitrate content of the plants. Cyanamide (CaNCN) and Uramon (urea plus a conditioner) are hydrolyzed in the soil with the liberation of ammonia. This reaction presumably was not sufficiently rapid to supply the plants with all of the nitrogen they could absorb, since both the ammonia and protein nitrogen were somewhat lower than in plants fertilized with an equivalent amount of nitrogen as ammonium nitrate. However, the nitrate content of the plants from the Cyanamide and Uramon plots was but little greater than of plants from the unfertilized plots, which

suggests that absorption of the liberated ammonia was rapid enough that it was not oxidized to nitrate to any great extent in the soil before being absorbed. If, however, nitrate formation did occur in the soil, reduction to ammonia within the plant must have been more rapid than bacterial oxidation in the soil so that nitrates did not accumulate in the plant.

Nitrate absorption in the plants fertilized with ammonium nitrate was greatest in the first part of the growth period, as shown by an increasing nitrate content at each of the early cuttings. Later in the growth period, the rate of absorption was decreasing and the plants probably were utilizing the excess nitrate nitrogen, which had been absorbed earlier, for biological synthesis.

The ammonium ion concentration did not vary greatly from one cutting to another until after the third cutting. In general, ammonium concentration did not decrease appreciably until after the nitrate and amide nitrogen had decreased. This might indicate that the latter were being changed into ammonium ion for use by the plants.

According to Bradley, Eppson, and Beath (3), plant material containing over 1.5 per cent KNO_3 (nitrate nitrogen calculated to KNO_3) is potentially toxic to animals. Only in samples from plots fertilized with 300 pounds of nitrogen as ammonium nitrate was the concentration of nitrates ever that high, and it did not remain so as the plant matured. Thus, young bromegrass, which has been fertilized heavily with ammonium nitrate, may be toxic to livestock under some conditions.

SUMMARY

1. The protein content of bromegrass decreased as the plants matured. Increasing the amount of nitrogen applied to the soil caused a corresponding increase in protein nitrogen in the plants. Presumably, the nitrogen of ammonium nitrate was more readily available to the plant than the nitrogen of Cyanamide or Uramon when applied in equivalent amounts of nitrogen, since the protein content was slightly higher when ammonium nitrate was the fertilizer used.

2. The ammonium ion concentration of the plant tissue varied directly with the amount of nitrogen applied.

3. In the early stages of growth, amides increased rapidly, but as the plants approached maturity they decreased, indicating that the amides were formed from the excess ammonium absorbed and later were metabolized into protein.

4. Increasing the amount of ammonium nitrate applied to the soil resulted in increased amounts of nitrate in the plants. Uramon and Cyanamide fertilization had little influence on the nitrate content of the plants.

5. The official method of the Association of Official Agricultural Chemists for the determination of protein in feedstuffs was found to be inaccurate for plant material containing considerable quantities of nitrate.

6. Application of nitrogen fertilizer up to 100 pounds of nitrogen as ammonium nitrate per acre caused an appreciable increase in the riboflavin content of bromegrass.

ACKNOWLEDGMENT

The author wishes to express her gratitude to Dr. H. L. Mitchell, major instructor, for his helpful advice and constructive criticisms through the course of this investigation. The author is also particularly grateful to the Department of Agronomy for supplying the samples and to the Kansas Industrial Development Commission for sponsoring the research which made this study possible.

LITERATURE CITED

(1) Association of Official Agricultural Chemists, Methods of Analysis. 6th ed. Washington, D. C.: Assoc. Offic. Agr. Chemists. 1945.

(2) Berge, T. O.
Determination of nitrate nitrogen with a photoelectric colorimeter. Soil Sci. 52: 185-191. 1941.

(3) Bradley, W. B., H. F. Eppson, and O. A. Beath.
Livestock poisoning by oat hay and other plants containing nitrate. Wyoming Agr. Expt. Sta. Bul. 241. 1940.

(4) Chamot, E. M., D. S. Pratt, and H. W. Redfield.
A study on the phenoldisulfonic acid method for determination of nitrate in water. Amer. Chem. Soc. Jour. 33: 366-381. 1911.

(5) Eckerson, S.
Protein synthesis by plants. Botanical Gaz. 77: 377-390. 1924.

(6) Eggleton, W. G. E.
The assimilation of inorganic nitrogenous salts, including $NaNO_2$, by the grass plant. Biochem. Jour. 29: 1389-1397. 1935.

(7) Gilbert, C. S., H. F. Eppson, W. B. Bradley, and O. A. Beath.
Nitrate accumulation in cultivated plants and weeds. Wyoming Agr. Expt. Sta. Bul. 277. December, 1946.

(8) Harper, Horace J.
The accurate determination of nitrates in soils. Indus. and Engg. Chem. 16: 180-183. 1924.

(9) Loy, Henry W., Jr.
Report on chemical method for riboflavin. Assoc. Offic. Agr. Chemists Jour. 30: 392-397. 1947.

(10) Meyer, Bernard S., and D. B. Anderson.
Plant Physiol. New York: D. Van Nostrand, Inc., 1939.

(11) Nightingale, Gordon T.
The nitrogen nutrition of green plants. II. Botonical Review 14: 185-221. 1948.

(12) Paul, A. E., and E. H. Berry.
The Kjeldahl nitrogen method and its modifications. Assoc. Offic. Agr. Chemists Jour. 5: 108-132. 1921.

(13) Pucher, G. W., H. B. Vickery, and C. S. Leavenworth.
Determination of ammonia and amide nitrogen in plant tissue. Indus. and Engg. Chem., Analyt. Ed. 7: 152-154. 1935.

(14) Sideris, C. P., B. H. Krause, and H. Y. Young.
Assimilation of ammonium and nitrate by pineapple plants grown in nutrient solutions and its effects on nitrogenous and carbohydrate constituents. Plant Physiol. 13: 489-527. 1938.

(15) Sideris, C. P., H. Y. Young, and H. H. Q. Chun.
Effects of nitrogen on the nitrogenous fractions of Ananas comosus (L.) merr. Plant Physiol. 22: 127-148. 1947.

(16) Strowd, W. H.
The determination of nitrites and nitrates in plant tissue. Soil Sci. 10: 333-342. 1920.

(17) Tam, R. K., and H. E. Clark.
The action of calcium cyanide as a soil disinfectant. Soil Sci. 57: 359-365. 1944.

(18) Vickery, H. B., and G. W. Pucher.
The metabolism of amide in green plants. III. The mechanism of amide synthesis. Jour. Biol. Chem. 128: 703-713. 1939.