THE INFLUENCE OF FERTILIZATION ON THE AMINO ACID COMPOSITION OF ALFALFA AND OF SUDAN GRASS

By

ANN E. WYNNGATE

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Thesis Approved:

Robert J. Sirny
Thesis Adviser

Robert MacVicar

Robert MacVicar
Dean of the Graduate School

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INTRODUCTION

The present thesis reports the results of continued work on a project initiated in 1951 to determine the effect of fertilization on the amino acid composition of proteins of plants.

Inasmuch as the protein composition of the tissues of a particular plant species remains remarkably constant, it was thought that careful re-evaluation of the effects of fertility on the amino acid content of plants should be made, particularly in view of the claims of Sheldon, Blue and Albrecht (11) that quality, as well as yield, of plant proteins can be improved by the use of inorganic fertilizers.

Hollis, a previous worker on this project, reported his findings (5) on the effects of fertilization on the proteins of rye, oats, wheat, and alfalfa, as reflected in their methionine, lysine, threonine, glutamic acid, and isoleucine content. In essence, his findings indicated that there was no appreciable alteration in the amino acid pattern of the proteins when certain inorganic fertilizers were added to the soil on which these crops were grown.

The results of further studies employing Sudan grass and alfalfa for methionine, lysine and threonine analyses are reported in this thesis.

REVIEW OF LITERATURE

The amino acid composition of a protein indicates its quality and thereby, to some degree, its nutritive value. The effects of fertilization on the protein of a plant may be comparable to the effects of different rations upon an animal protein. The amino acid composition of a given type of animal tissue was found by Lyman and Kuiken (8) to be very nearly the same whether it came from beef, pork or lamb, with the exception of histidine which exhibited wide variations. The findings of Chibnall (3), Tristram (17), and Lugg and Weller (6, 7) suggest that, in general, there was a marked uniformity in the amino acid content of the proteins contained in the leaves of herbage species. Reber (11) found that the general pattern of distribution of various amino acids in the total leaf protein was similar for most cereals.

The influence of mineral nutrients upon the amino acid composition of cereal grasses and alfalfa has been studied by several workers. Sheldon et al. (12) reported that substantial differences in the amino acid content of lespedeza occurred when the plants were grown on five different types of soil in Missouri. Alfalfa grown on a single soil exhibited, in general, an increase in amino acid content when treated with manganese, or boron, or a mixture of these with cobalt, copper, and zinc. Blue and co-workers (2) reported variations in the concentration of amino acids present in alfalfa due to fertilization with manganese, boron, phosphorus and potassium. The minor elements studied, manganese and boron, gave the greatest increase without altering the concentration of nitrogen. They interpreted their data as indicating that the quality as well as the yield of the protein could be affected by relatively small applications of inorganic nutrients. Tisdale _et al_. (16), studied two clonal lines of alfalfa grown in a greenhouse on flint-shot quartz sand in nutrient solutions in which the concentration of sulfate ion varied from 0 to 81 ppm. Under these conditions, significant differences in the relative ability of the plants to synthesize methionine were observed. Total nitrogen was higher at the lower levels of sulfur, decreasing as the concentration of sulfur was increased. The percentage of methionine and cystine in the alfalfa increased with increasing concentrations of sulfur. Sheldon _et al_. (14) also found that the methionine content of alfalfa increased progressively as sulfur was applied; this increase seemed to occur at the expense of other amino acids. Sheldon and co-workers further reported (13) that the percentage of tryptophan varied widely with the inorganic composition of the substrate upon which alfalfa was grown and that the concentration of tryptophan decreased when magnesium, boron, manganese and iron were withheld from the culture solutions. The synthesis of tryptophan appeared to be increased when the calcium content of the nutrient solutions was increased. Smith and Agiza (15) reported that samples of first growth of the grasses and clovers contained more leucine, isoleucine and arginine and less glutamic acid and aspartic acid than the second growth. Nitrogenous fertilizers decreased the aspartic and glutamic acid yields and increased the leucine, isoleucine, phenylalanine, arginine, lysine and tryptophan in young rye grass and late clover.

SOURCE OF MATERIALS

Sudan grass (Andropogon sorghum sudanensis) was grown on the agronomy farm west of Stillwater, Oklahoma on soil of low fertility. There were four separate plots; fertilization treatment was randomly distributed within each plot. The treatments were as follows: (1) none, symbol Ck; (2) 40 lbs. of nitrogen per acre, as ammonium nitrate, applied as a top dressing in the spring, symbol N; (3) 40 lbs. of phosphorus pentoxide per acre, as superphosphate applied at seeding, symbol P; (4) 40 lbs. of nitrogen and 40 lbs. of phosphorus pentoxide, as in (2) and (3), symbol NP; (5) 80 lbs. of nitrogen and 40 lbs. of phosphorus, as in (2) and (3), symbol 2NP.

The plots were twelve feet long, and in each plot there were four rows of plants twelve inches apart treated with the respective fertilizers. At the ends of the rows were alleys three feet wide.

The initial planting was made October 12 and 13, 1951.

Alfalfa (Medicago sativa, var. Oklahoma Common) was grown at the Heavener branch station of the Oklahoma Agricultural Experiment Station in triplicate blocks, each block containing twelve subdivisions. Of these twelve subdivisions, two were control plots, and the remainder were subjected to the following fertilizer treatments: no fertilizer added (Ck); phosphorus at three levels consisting of 160 pounds of superphosphate per acre (symbol P_1), 320 pounds (P_2) and 480 pounds (P_3); potassium at 120 pounds of potassium chloride per acre (K); magnesium at 480 pounds of magnesium sulfate per acre (Mg); boron at 40 pounds of borax per acre (B); manure at 5 tons per acre (M). The following combinations of treatments were studied by random distribution within each of 3 blocks: Ck (duplicated within each block), P_1, P_2, P_3, P_1K, P_2K, P_3K, P_2M, P_2KB, P_2KB Mg and M.

COLLECTION AND TREATMENT OF MATERIALS

Four clippings were made of Sudan grass at times to correspond to the periods such fields would be grazed. The dates of the four Sudan grass collections were June 11, July 18, August 9, and September 4, 1952. Two alfalfa clippings were April 3 and May 21, 1953 just prior to first and second "cutting." Plants were clipped to about 1½ inches above the ground.

The cost of analysis precluded the sampling of all treatments. Therefore, the samples were culled from the following plots so selected as to be representative of all the treatments: both Ck plots from each block, P_2, P_3, P_2K, P_2KB and M plots. Plant materials from the various treatments within each block were collected separately and brought to the laboratory. The leaves and stems of the alfalfa were separated from each other by hand. All samples were dried to constant weight at 180° F in a forced-draft oven, weighed and the tissue ground in a Wiley mill.

SAMPLING PROCEDURE FOR ANALYSIS

Cost of analysis also dictated the pooling of tissue from identical treatments from the replicate blocks. It was felt that if differences between treatments were revealed by analysis of the pooled sample, the individual samples could then be analyzed to permit an evaluation of the variation between replications. Therefore, five grams of the dried and ground tissue from each replicate of a particular treatment and a given clipping were pooled and thoroughly mixed. This sample (referred to hereafter as the composite) was analyzed for nitrogen and certain amino acids. In several cases, the replicates were analyzed individually for nitrogen and amino acids, to obtain an index of variation between replicates.

ANALYTICAL METHOD

Total nitrogen was determined by the macro-Kjeldahl method of the A. O. A. C. (1). The amount of protein present was calculated from the total nitrogen by multiplying by the conventional factor of 6.25. It is recognized that in studies of this kind there are undesirable aspects of this method of evaluating the crude protein, but no more suitable method was found.

Hydrolysates of the samples were prepared by autoclaving 2.5 grams of dry sample with 50 ml. of 3 N HCl for 10 hours at 15 pounds pressure. After adjusting the pH of the cooled hydrolysate to 7 with 6 N NaOH, using a Beckman Model H pH meter, the volume was brought to 100 ml. and the insoluble material removed by gravity filtering through E.&D. paper No. 615. The residue was not washed. The hydrolysate was then ready to be assayed, except that it was usually necessary to make dilutions in order to bring the concentration of the amino acid being assayed within range of the method. Following a short period of autoclaving to sterilize the hydrolysates, they were stored at about 4^{o} C. in cotton-plugged Erlenmeyer flasks.

Aliquots of the hydrolysate (diluted, if necessary) of 1, 2, 3, 4, and 5 ml. volume were put into test tubes and water added to bring the volume in each tube to 5 ml. Then 5 ml. of double strength medium was added to each tube, so that the total volume in all tubes was 10 ml.

Standards and blanks were similarly prepared; the L-form of the amino acid was used to prepare the standards, except in the case of threonine, in which case DL-threonine was used.

All tubes were then covered, autoclaved, inoculated when cool with a suspension of the washed organism, and incubated about 72 hours at 37° C.

The medium for lysine and threonine assay was basically that of Henderson and Snell (4), which consists of a citrate-phosphate-buffered system of glucose, salts, vitamins and commercially available amino acids; it was modified by substituting potassium acetate and potassium citrate for sodium acetate and sodium citrate in order to reduce the concentration of the sodium ion. The medium used for methionine was further modified by replacing the amino acids with peroxide-treated peptone (cf. Lyman et al. (9).

The following table supplies information regarding the organisms used, range of concentration of the standard, etc. The amount of growth attained was measured by titrating with 0.1 N NaOH to a sea green endpoint with bromthymol blue as indicator.

TABLE I

SUMMARY OF ANALYTICAL CONDITIONS EMPLOYED

Amino Acid Assayed	Assay Organism	Medium	Standard range (L-form of amino acid, micrograms)	Number of Assays
Methionine	Streptococcus faecalis R	Peroxide-treated peptone	0-50	24
Methionine	"	"	0-10	3
Methionine	"	"	0-30	5
Lysine	Leuconostoc mesenteroides P-60	Henderson & Snell	0-200	21
Lysine	"	"	0-160	9
Lysine	"	"	0-60	2
Threonine	Streptococcus faecalis R	Henderson & Snell	0-50	1
Threonine	"	"	0-30	11
Threonine	Lactobacillus brevis	"	0-30	7

RESULTS AND DISCUSSION

The results of analysis for methionine, lysine and threonine of Sudan grass and alfalfa grown with different fertilizer treatments are presented in Tables II to V. All values are expressed as grams per 100 grams of crude protein, (N x 6.25).

Sudan Grass

Nitrogen. The nitrogen content of the clippings decreased sharply from the first clipping to the second and remained fairly constant thereafter. There seemed to be little effect of nitrogenous fertilizer on the nitrogen content of the first clipping. In the second, third and fourth clippings, the addition of nitrogen did result in a higher nitrogen content of the plant tissue.

Methionine. The methionine content of Sudan grass ranged from 0.79 to 1.36 percent. Mean values for each of the four clippings were 0.96, 1.08, 1.01, and 1.13 percent, in order of their collection (Table II). When values found for the composite samples were plotted (Figure II) by treatment and collection, a trend toward an increase in methionine content in Sudan grass grown on phosphorus-treated soil was noted. The average increase in methionine content on phosphorus-treated soil over the average value of methionine content on untreated soil is 11.6 percent; only in the case of clipping number 4 was the former value lower than the control value.

It should also be noted that the two fertilizer mixtures containing both nitrogen and phosphorus caused a decrease in methionine content, though not so marked or consistent as the increase in methionine content on phosphorus-treated soil.

<u>Lysine</u>. The range of values for lysine in Sudan grass was from 2.76 to 5.67 percent (Table II and Figure III). Mean values for each of the four clippings were 3.31, 4.61, 4.37, and 5.26 percent, respectively. Here, again, there is a consistent trend toward increased amino acid content on phosphorus-treated soil; lysine values for plants grown on such soil are higher than the control values for each clipping made. The difference in average lysine values for phosphorus-treated and for untreated soils is 13.4 percent.

In contrast to methionine, moreover, the NP and 2NP treatments show, on the average, a slight increase in lysine content.

Lysine shows a more marked inversion of the nitrogen phenomenon than methionine; that is, the lysine value for the first clipping is markedly lower than that for subsequent clippings, while the reverse is true of nitrogen.

<u>Threonine</u>. The range of threonine content was 3.62 to 5.45 percent (Table II and Figure IV). The mean values for each of the four clippings were 4.89, 4.11, 4.48, and 5.42 percent respectively. There were no consistent trends from one clipping to the next, but the mean values for the sums of the four clippings from the N, P, and NP treated plots show a distinct and equal increase of about 10 percent over the mean of the sum of the four clippings from the untreated plots (see Figure IV).

TABLE II

EFFECT OF FERTILIZATION ON THE AMINO ACID COMPOSITION OF SUDAN GRASS

Treatment	% N	Grams of amino acid per 100 grams protein			Ratios M:L:T (N = 1)		
		Methionine	Lysine	Threonine			
		First Clipping (June 11)					
None	4.11	0.99	3.07	4.59	0.24	0.76	1.12
N	4.08	1.05	2.76	5.42	0.26	0.68	1.33
P	3.71	1.09	3.76	4.95	0.29	1.02	1.34
NP	3.84	0.79	3.40	5.17	0.21	0.89	1.35
2NP	3.96	0.90	3.57	4.32	0.23	0.90	1.09
Average	3.94	0.96	3.31	4.89	0.24	0.84	1.24
Av. Devn.	0.13	0.08	0.30	0.35			
% Av. Dev.	3.3	8.3	9.1	7.2			
		Second Clipping (July 18)					
None	2.21	1.01	4.63	3.97	0.46	2.09	1.80
N	2.44	1.03	4.35	4.15	0.42	1.78	1.70
P	2.23	1.20	4.65	4.45	0.54	2.09	2.00
NP	2.53	1.14	4.53	3.94	0.45	1.79	1.56
2 NP	2.65	1.02	4.91	4.02	0.39	1.85	1.52
Average	2.41	1.08	4.61	4.11	0.45	1.91	1.71
Av. Devn.	0.15	0.07	0.14	0.16			
% Av. Dev.	6.2	6.5	3.0	3.9			

Table II (Continued)

Treatment	%N	Grams of amino acid per 100 grams protein			Ratios M:L:T (N = 1)		
		Methionine	Lysine	Threonine			
		Third Clipping (August 9)					
None	2.36	0.96	4.05	4.79	0.41	1.72	2.03
N	2.51	1.05	4.08	3.62	0.42	1.63	1.44
P	2.34	1.20	5.34	4.15	0.51	2.28	1.77
NP	2.42	0.92	4.43	5.05	0.38	1.83	2.09
2NP	2.54	0.90	3.97	1.80	0.35	1.56	1.89
Average	2.43	1.01	4.37	4.48	0.42	1.80	1.85
Av. Devn.	0.07	0.10	0.41	0.48			
% Av. Dev.	3.9	10.0	9.4	17.1			
		Fourth Clipping (September 4)					
None	2.09	1.14	4.92	4.39	0.54	2.36	2.10
N	2.16	1.36	5.67	6.45	0.63	2.63	2.99
P	2.06	1.11	5.15	5.92	0.54	2.50	2.87
NP	2.24	0.94	5.61	5.43	0.42	2.50	2.42
2NP	2.20	1.10	4.95	4.90	0.50	2.25	2.23
Average	2.15	1.13	5.26	5.42	0.53	2.44	2.52
Av. Devn.	0.06	0.10	0.30	0.62			
% Av. Dev.	2.8	8.9	5.7	11.4			

Alfalfa

It is more difficult to interpret the data on the effect of fertilization on alfalfa because of an influence of treatments on the leaf-stem ratios of the plant. In every case fertilization decreased the leaf to stem ratio (dry weight basis), for both clippings, as compared to the ratio for untreated soil (Table VI). The proteins of the leaves differ in their amino acid composition from those of the stems. (Table VII).

Therefore, increased yield per acre, attained by means of fertilization, may or may not bring an increase in the total protein content of the crop, since more of each plant then consists of stems, which have a much lower protein content than leaves.

It is obvious, moreover, that this may cause a variation in the amino acid composition of the total plant tissue without affecting the composition of the proteins of either part individually.

Nitrogen. The average percent of nitrogen present in both stems and leaves remained remarkably constant from one clipping to the next, as data presented in Table III and the curves in Figure V show. There was little variation between the various fertilizer treatments. The nitrogen content of alfalfa leaves was more than double that of alfalfa stems, exhibiting a range of values from 3.79 to 4.19 percent as compared to a range of 1.53 to 1.93 percent for stems.

Methionine. Extreme values found for methionine were: leaves, 1.15 and 1.39; stems, 0.78 and 1.18 percent. Averages were: leaves, 1.23 and 1.33 (first and second clippings, respectively), and stems, 0.87 and 1.10 percent (first and second clippings). There was neither a consistent

TABLE III

COMPARISON OF AMINO ACID COMPOSITION OF SUDAN GRASS AS AFFECTED BY FERTILIZATION

Clipping Number	Treatment None	N	P	NP	2NP	Average for Clipping
			Percent Nitrogen			
1	4.11	4.08	3.71	3.84	3.96	3.94
2	2.21	2.44	2.23	2.53	2.65	2.41
3	2.36	2.51	2.34	2.42	2.54	2.43
4	2.09	2.17	2.06	2.24	2.20	2.15
Average for treatment	2.69	2.80	2.59	2.76	2.84	
			Methionine (g/100 g protein*)			
1	0.99	1.05	1.09	0.79	0.90	0.96
2	1.01	1.03	1.20	1.14	1.02	1.08
3	0.96	1.05	1.20	0.92	0.90	1.01
4	1.14	1.36	1.11	0.94	1.10	1.13
Average for treatment	1.03	1.12	1.15	0.95	0.98	
			Lysine (g/100 g protein*)			
1	3.07	2.76	3.76	3.40	3.57	3.31
2	4.63	4.35	4.65	4.53	4.91	4.61
3	4.05	4.08	5.34	4.43	3.97	4.37
4	4.92	5.67	5.15	5.61	4.95	5.26
Average for treatment	4.17	4.22	4.73	4.49	4.35	
			Threonine (g/100 g protein*)			
1	4.59	5.42	4.95	5.17	4.32	4.89
2	3.97	4.15	4.45	3.94	4.02	4.11
3	4.79	3.62	4.15	5.05	4.80	4.48
4	4.39	6.45	5.92	5.43	4.90	5.42
Average for treatment	4.44	4.91	5.92	5.43	4.90	5.42

* N = 16% of protein

increase nor decrease of methionine content as a result of any one fertilizer treatment.

It should be noted that there was an increase in methionine content between the first and second clippings for both stems and leaves. This increase amounted to about 25 percent in the case of stems (Figure VI). Indeed, the amount of methionine present in the stems of the second clipping approaches that of the leaves of the first clipping.

Lysine. Values range from 3.93 to 5.52 percent for leaves, and from 2.24 to 4.19 percent for stems. Correlation of Figure VII and Table VII reveals that the boron-containing fertilizer increased the lysine content of the leaves of alfalfa quite markedly, and also maintained the leaf-stem ratio about the same as the control.

All treatments reduced the lysine content of the stem, as compared to the control.

No other consistent effect of fertilizer treatment appeared.

An increase in the lysine content of the stem portion of the second clipping with respect to the first was found. This was an even larger increase than was found with respect to methionine.

Threonine. Values for threonine range from 4.56 to 5.65 percent for alfalfa leaves, and from 3.35 to 4.43 percent for stems. Average values for first and second clippings, respectively, are 4.83 and 5.37 percent for leaves, and 3.83 and 4.18 percent for stems.

Fertilization seemed to have little effect on threonine content, according to Figure VIII, but Table VIII shows that the potassium-containing fertilizers decreased the total amount of threonine in the total plant. This emphasizes the need for very careful examination and interpretation

TABLE IV

EFFECT OF FERTILIZATION ON THE AMINO ACID COMPOSITION OF ALFALFA STEMS AND LEAVES

Treatment	%N	Grams of amino acid per 100 grams protein			Ratios M:L:T (N = 1)		
		Methionine	Lysine	Threonine			
			Alfalfa Leaves				
			First Clipping (April 3)				
None	3.83	1.23	4.43	4.98	0.32	1.16	1.30
P_2	4.03	1.22	4.68	4.84	0.30	1.16	1.20
P_3	4.19	1.15	4.54	4.88	0.27	1.08	1.17
P_2K	3.97	1.38	4.27	4.76	0.35	1.08	1.20
P_2KB	3.97	1.18	4.83	4.56	0.30	1.22	1.15
M	4.14	1.22	4.29	4.95	0.30	1.04	1.20
Average	4.02	1.23	4.51	4.83	0.31	1.12	1.20
Av. Devn.	0.10	0.05	0.18	0.11			
% Av. Devn.	3	4	4	2			
			Second Clipping (May 21)				
None	3.79	1.39	4.52	5.65	0.38	1.19	1.49
P_2	3.92	1.29	3.93	5.22	0.33	1.00	1.33
P_3	3.97	1.23	4.03	5.24	0.31	1.01	1.32
P_2K	4.03	1.33	3.97	5.12	0.33	0.98	1.27
P_2KB	3.96	1.38	5.52	5.60	0.35	1.39	1.42
M	3.88	1.37	4.79	5.41	0.35	1.23	1.39
Average	3.93	1.33	4.49	5.37	0.34	1.14	1.37
Av. Devn.	0.06	0.05	0.49	0.18			
% Av. Devn.	2	4	11	3			

Table IV (Continued)

Treatment	%N	Grams of amino acid per 100 grams protein			Ratio		
		Methionine	Lysine	Threonine	M:L:T (N = 1)		
Alfalfa Stems							
First Clipping (April 3)							
None	1.71	0.78	3.20	3.53	0.46	1.87	2.06
P_2	1.78	0.87	3.01	4.14	0.49	1.69	2.33
P_3	1.63	0.85	2.97	3.35	0.52	1.82	2.06
P_2K	1.86	0.81	2.50	3.92	0.44	1.35	2.11
P_2KB	1.75	0.92	2.57	3.86	0.53	1.47	2.21
M	1.81	0.99	2.24	4.20	0.55	1.24	2.32
Average	1.76	0.87	2.75	3.83	0.50	1.56	2.18
Av. Devn.	0.06	0.06	0.31	0.26			
% Av. Devn.	3	7	11	7			
Second Clipping (May 21)							
None	1.58	1.14	4.19	4.30	0.72	2.66	2.72
P_2	1.93	1.07	3.94	4.22	0.55	2.04	2.19
P_3	1.90	1.03	4.15	3.96	0.54	2.18	2.08
P_2K	1.77	1.13	4.08	3.74	0.64	2.31	2.12
P_2KB	1.53	1.18	4.02	4.41	0.77	2.63	2.88
M	1.73	1.02	3.89	4.43	0.59	2.25	2.56
Average	1.74	1.10	4.05	5.18	0.63	2.33	2.40
Av. Devn	0.13	0.06	0.11	0.22			
% Av. Devn.	7	5	3	5			

TABLE V

COMPARISON OF AMINO ACID COMPOSITION OF ALFALFA LEAVES AND STEMS AS AFFECTED BY FERTILIZATION

Clipping Number		Treatment: None	P_2	P_3	P_2K	P_2KB	M	Average for Clipping
				Percent Nitrogen				
1	Leaves	3.83	4.03	4.19	3.97	3.97	4.14	4.02
2	Leaves	3.79	3.92	3.97	4.03	3.96	3.88	3.93
1	Stems	1.71	1.78	1.63	1.86	1.75	1.81	1.76
2	Stems	1.58	1.93	1.90	1.77	1.53	1.73	1.74
				Methionine*				
1	Leaves	1.23	1.22	1.15	1.38	1.18	1.22	1.23
2	Leaves	1.39	1.29	1.23	1.33	1.38	1.37	1.33
1	Stems	0.78	0.87	0.85	0.81	0.92	0.99	0.87
2	Stems	1.14	1.07	1.03	1.13	1.18	1.02	1.10
				Lysine*				
1	Leaves	4.43	4.68	4.54	4.27	4.83	4.29	3.51
2	Leaves	4.52	3.93	4.03	4.97	5.52	4.79	4.49
1	Stems	3.20	3.01	2.97	2.50	2.57	2.24	2.75
2	Stems	4.19	3.94	4.15	4.08	4.02	3.89	4.05
				Threonine*				
1	Leaves	4.98	4.84	4.88	4.76	4.56	4.95	4.83
2	Leaves	5.65	5.22	5.24	5.12	5.60	5.41	5.37
1	Stems	3.53	4.14	3.35	3.92	3.86	4.20	3.83
2	Stems	4.30	4.22	3.96	3.74	4.41	4.43	4.18

* g/100 g of protein; N = 16%

TABLE VI

ALFALFA LEAF-STEM RATIOS

Clipping and Treatment	Dry Weight of Clipping in Grams			Ratio
	Leaves	Stems	Total	Leaves:Stems
First Clipping				
None	356.0	172.8	528.8	2.06
P_2	159.2	103.0	262.2	1.54
P_3	233.8	153.1	386.9	1.53
P_2K	53.8	42.2	96.0	1.27
P_2KB	229.8	178.4	408.2	1.29
M	227.2	123.7	350.9	1.84
Second Clipping				
None	199.1	246.0	445.1	0.81
P_2	119.5	185.0	304.5	0.65
P_3	117.1	217.8	334.9	0.54
P_2K	90.1	154.0	244.1	0.58
P_2KB	105.3	168.5	273.8	0.63
M	115.1	182.5	297.6	0.63

TABLE VII

RATIOS OF AMINO ACIDS IN ALFALFA LEAVES AND STEMS

Clipping and Treatment	Grams of Amino Acid in Total Grams Dry Weight of Sample								
	Methionine			Lysine			Threonine		
	Leaves	Stems	L:S	Leaves	Stems	L:S	Leaves	Stems	L:S
First Clipping									
None	1.04	0.14	7.4	3.78	0.59	6.4	4.24	0.65	6.5
P_2	0.49	0.10	4.9	1.88	0.35	5.4	1.94	0.47	4.1
P_3	0.70	0.13	5.4	2.78	0.46	6.0	2.98	0.52	5.7
P_2K	0.18	0.04	4.5	0.57	0.12	4.8	0.64	0.19	3.4
P_2KB	0.67	0.18	3.7	2.75	0.50	5.5	2.60	0.75	3.5
M	0.72	0.14	5.1	2.52	0.31	8.1	2.91	0.59	4.9
Second Clipping									
None	0.66	0.28	2.4	2.13	1.02	2.1	2.66	1.04	2.6
P_2	0.38	0.24	1.6	1.15	0.88	1.3	1.53	0.94	1.6
P_3	0.36	0.27	1.3	1.07	1.07	1.0	1.51	1.02	1.5
P_2K	0.30	0.19	1.6	0.90	0.70	1.3	1.16	0.64	1.8
P_2KB	0.36	0.19	1.9	1.44	0.65	2.2	1.46	0.71	2.1
M	0.38	0.20	1.9	1.34	0.77	1.7	1.51	0.87	1.7

TABLE VIII

COMPUTED AMINO ACID COMPOSITION OF WHOLE ALFALFA PLANT

Treatment	Methionine	Lysine	Threonine
	(Mg/g Dry Weight)		
	First Clipping		
None	2.2	8.3	9.3
P_2	2.2	8.5	9.2
P_3	2.1	8.3	9.0
P_2K	2.3	7.2	8.6
P_2KB	2.0	8.0	8.2
M	2.4	8.1	10.0
	Second Clipping		
None	2.1	7.1	8.3
P_2	2.0	6.6	8.1
P_3	1.9	6.4	7.6
P_2K	2.0	6.6	7.4
P_2KB	2.0	7.6	7.9
M	1.9	6.9	8.0

of data in this kind of study and may account for some of the conflicting observations of other workers.

Whole Plant. The total amount of amino acid present, in leaf and stem combined, is shown as milligrams per gram of dry material in Table VIII. This table reveals the following facts:

1) There was no significant change in methionine content with fertilization.
2) Fertilization with phosphorus and potassium caused an appreciable decrease from control values in lysine and threonine contents; this was noted in both clippings.
3) Further addition of boron (P_2KB) resulted in a further diminution of threonine.
4) Manure treatment showed the only increased value for threonine and this only in the first clipping.
5) In no case was there more of a particular amino acid present in the second clipping than in the first.

General

There seems to be no ideal way in which to express the amino acid composition of plant tissue. It seems inadequate to express the amino acid content as percent of dry weight, and is perhaps equally misleading to express it as percent of protein.

The crux of the difficulty is the lack of constant proportion of the various nitrogen fractions in plants. The Kjeldahl method measures total nitrogen and hence provides no information about variation in the ratios of various forms of nitrogen in plants. MacVicar (10) and Reber (11) found, however, that most of the nitrogen is present as proteins or amino acids, and hence this may be less serious in interpreting data than might be generally thought.

For our purpose, which was to unmask any change in the pattern of the amino acids in the proteins, the expression of the amounts of the amino acids present as milligrams per gram of protein was much to be preferred.

Sheldon et al. (14) found values of 2.44 and 2.20 respectively for percent of nitrogen in Sudan grown on soil which was adequate in phosphate and had been treated with flowers of sulfur, and on the same soil untreated. These figures compare very well with those reported herein, for all clippings but the first. The first clipping contained an average of 3.94 percent nitrogen, and all treatments resulted in a slight lowering of nitrogen content from the control value. For the other clippings the effect of fertilizer treatment was of no greater magnitude than for Sheldon's sulfur-treated soil. In all later clippings, addition of nitrogen produced a plant material of higher nitrogen content.

The effect on alfalfa was the reverse. All treatments showed a slight increase in percentage of nitrogen in the leaves of the first clipping. There was almost no change between the first clipping and the second in the nitrogen content of the leaves. The effect of treatment was greater on the nitrogen content of stems.

Comparison of values for the nitrogen content of the whole alfalfa plants was made more difficult by the separate analysis of leaves and stems. However, by weighting the values found separately for leaves and for stems according to the leaf-stem ratio, the percentage of nitrogen for the whole plant was calculated. The results indicate a somewhat surprising uniformity as shown below:

Treatment	First Clipping	Second Clipping
None	3.14	2.57
P_2	3.14	2.72
P_3	3.18	2.62
P_2K	3.05	2.60
P_2KB	3.00	2.47
M	3.07	2.56

Smith and Agiza (15) found 3.1-3.7 percent nitrogen in dry alfalfa. Tisdale et al. (16) reported a range of 2.84-3.98 percent nitrogen in alfalfa grown in the presence of the sulfate ion, the percentage decreasing with increasing amounts of sulfate ion. The percentage of nitrogen in alfalfa reported by Blue, Sheldon and Albrecht (2) is much higher: 4.65-5.95 percent.

The methionine content of the first clipping of Sudan grass was much like that found by Sheldon et al. (14) on untreated soil; it averages 2.46 mg/gram of sample, as compared to his 2.04 mg/gram of sample. But in no case was a value found which approached his for the sulfur-treated soil: 4.06 mg/g sample.

Blue et al. (2) state that the concentration of the amino acids, when expressed as percent of the total dry weight, was increased by soil treatments in most cases. These observations were not confirmed by our studies. As shown in Table VIII, increased fertility resulted in a decrease somewhat more frequently than it produced any increase.

Blue reports in the same paper (2) that B increases the amino acid content of alfalfa, and this increase appears in the case of lysine in the leaves of alfalfa grown on the P_2KB plot, as mentioned above. Here again, however, care in interpreting the data must be taken. By "reconstituting" the plant mathematically, it was found that the lysine content of the whole plant, expressed as mg of lysine per gram of protein, was

3.84 for the first clipping and 4.60 for the second clipping, as compared to 4.03 and 4.33 for the respective clippings on untreated soil. More extensive studies will be required to validate the significance of their finding.

The conclusion to be drawn from the data and a study of the literature is that the effects of such factors as stage of growth and method of collecting and treating on the nitrogen distribution and protein content of a plant must be better understood before the effect of elemental nutrients on the amino acid composition of protein can be adequately assessed.

A study of the leaf-stem ratio reveals the importance of knowing more of the feeding habits of animals given alfalfa hay, or allowed to graze on it; that is, whether or not they tend to reject the stems.

The lowering of the nitrogen content and, presumably, of the protein content of the plant, which generally accompanies fertilizer treatments, suggests that a careful assessment of the benefits of increased yields brought about by such treatments should be made.

In reviewing the entire study, emphasis seems more appropriately placed on the lack of effect of fertilizer treatment on amino acid composition than the reverse. To be sure, in a few cases changes which probably represent real and significant differences were revealed. This would indicate a change of some kind with respect to the proteins of the plant, since variation of the amino acid composition of a single protein is unlikely. This change may be a change in amounts of proteins or may be the result of the initiation of or cessation of elaboration of one or more specific proteins.

SUMMARY

This thesis reports the results of an evaluation of the effect of the addition of major plant nutrients on the amino acid composition of two important Oklahoma forages, Sudan grass and alfalfa. The major findings are summarized as follows:

Sudan Grass. Phosphorus treatment resulted in increased values of about 10 percent for methionine and lysine.

A similar increase was observed in the threonine content where either nitrogen, phosphorus or both in combination were applied.

These increases are of doubtful significance from a statistical viewpoint. However, the trend shown in all four clippings toward an increased methionine and lysine content of the proteins of plants grown on phosphorus-treated soil is so consistent that it seems highly probable that a real change in content is being observed.

Alfalfa. Different plant parts (leaves vs. stems) when analyzed separately did show changes which are believed to be sufficiently great to represent real differences. This emphasizes the need for careful study of plants of this type prior to drawing conclusions as to the effect or lack of effect of plant nutrients on protein composition.

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A P P E N D I X

Figure I. Effect of various fertilizer treatments on the nitrogen content of Sudan grass (composite samples).

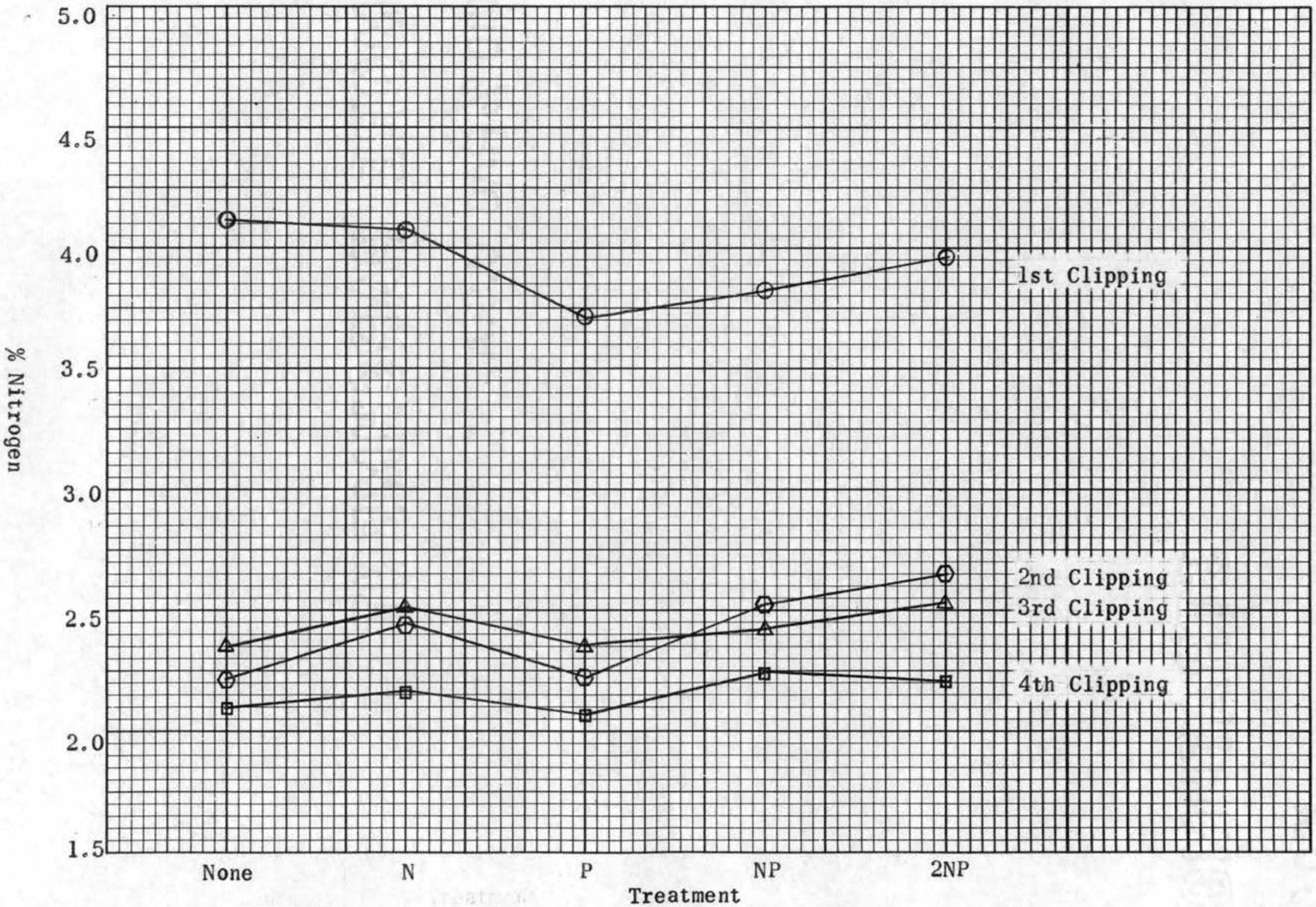


Figure II. Effect of various fertilizer treatments on the methionine content of Sudan grass (composite samples).

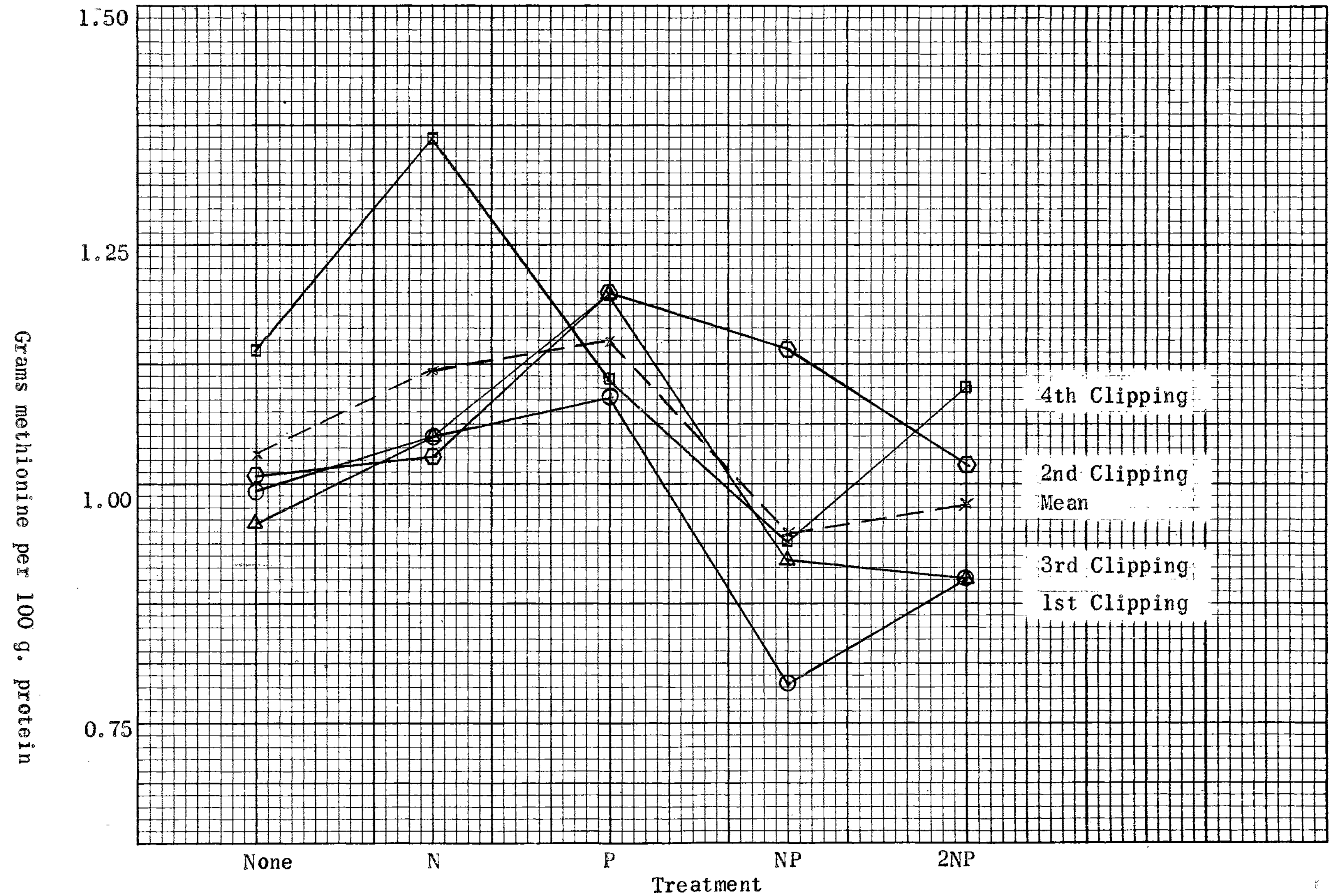


Figure III. Effect of various fertilizer treatments on the lysine content of Sudan grass.

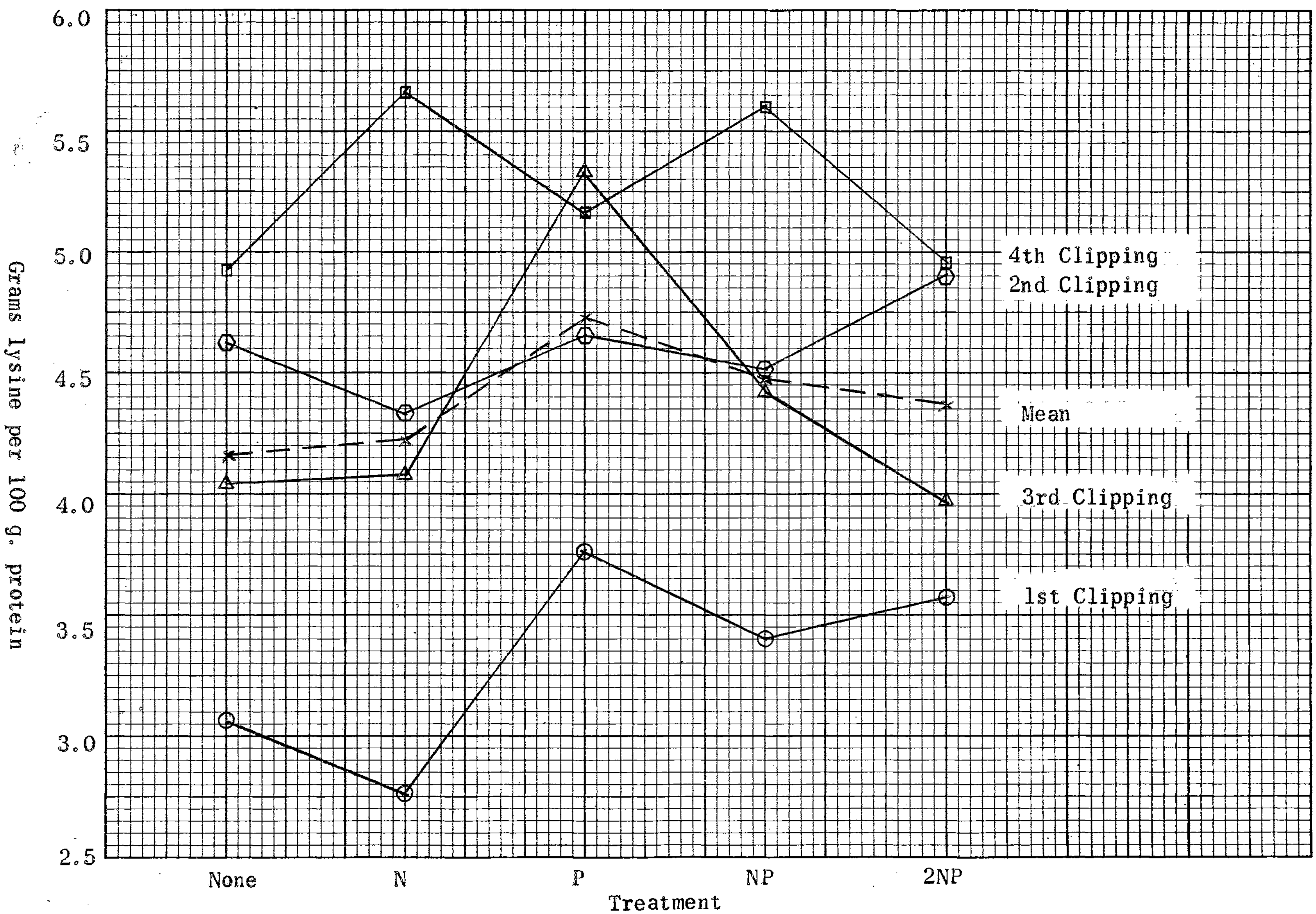


Figure IV. Effect of various fertilizer treatments on the threonine content of Sudan grass.

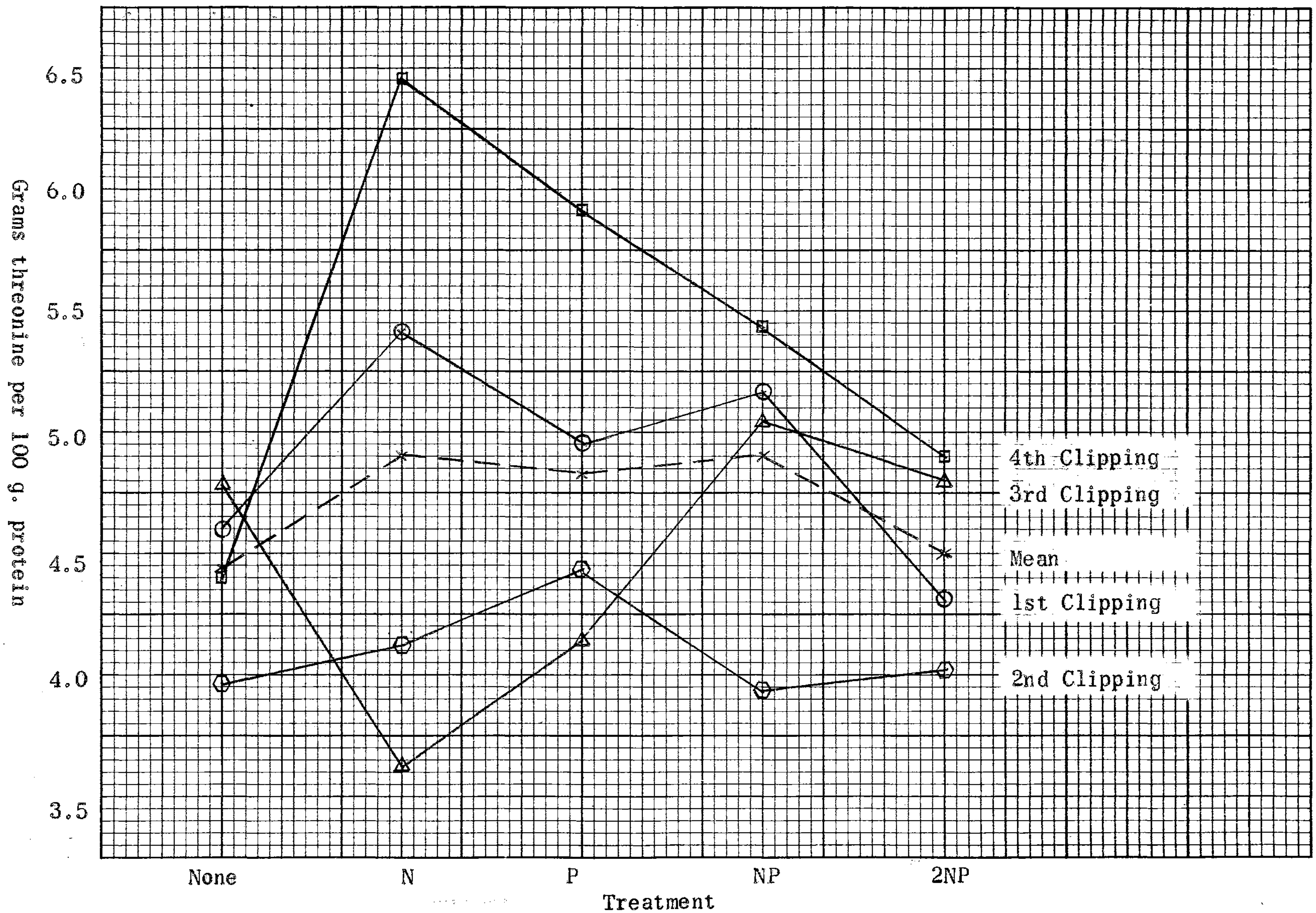


Figure V. Effect of various fertilizer treatments on the nitrogen content of alfalfa (composite samples).

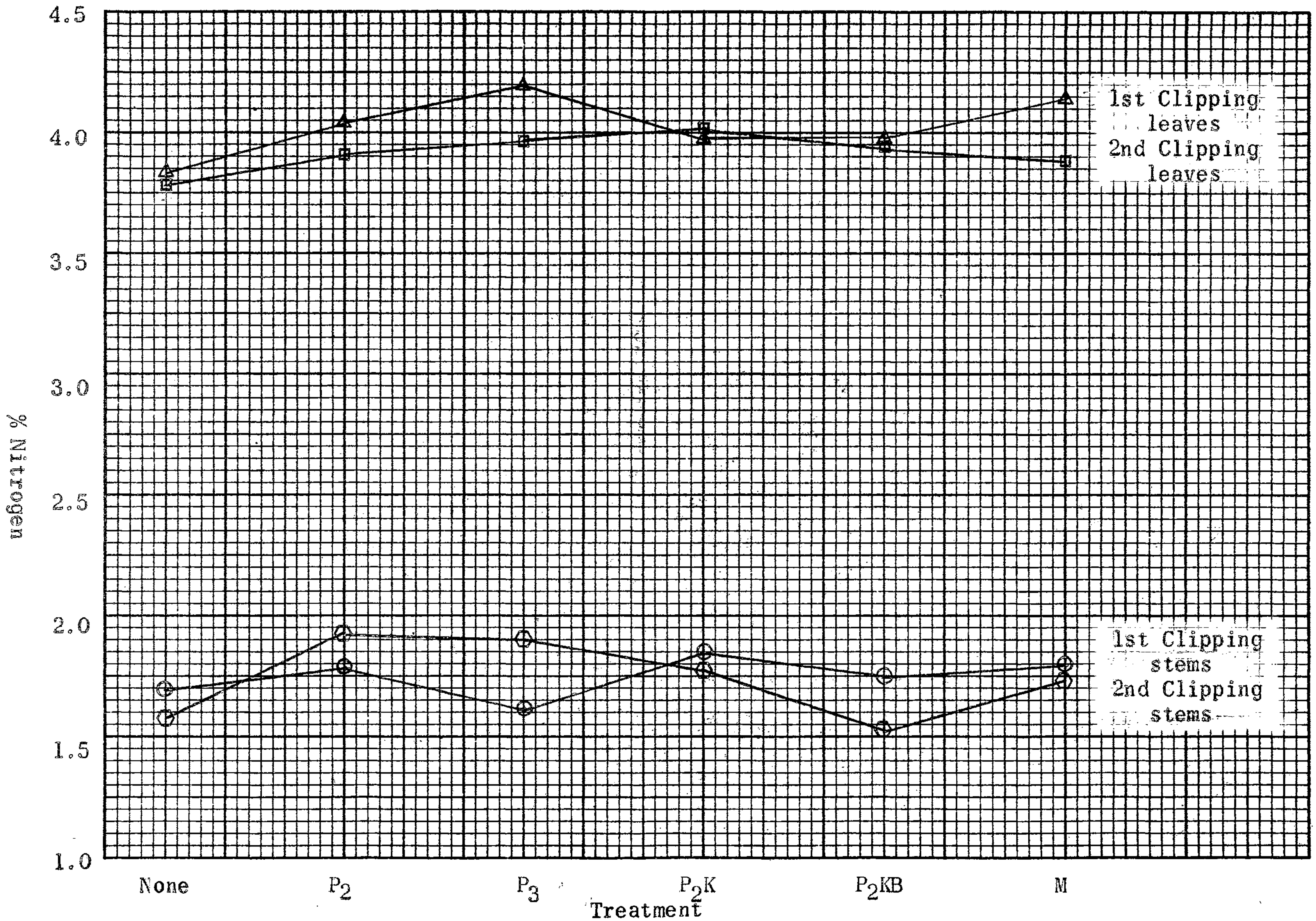


Figure VI. Effect of various fertilizer treatments of the methionine content of alfalfa.

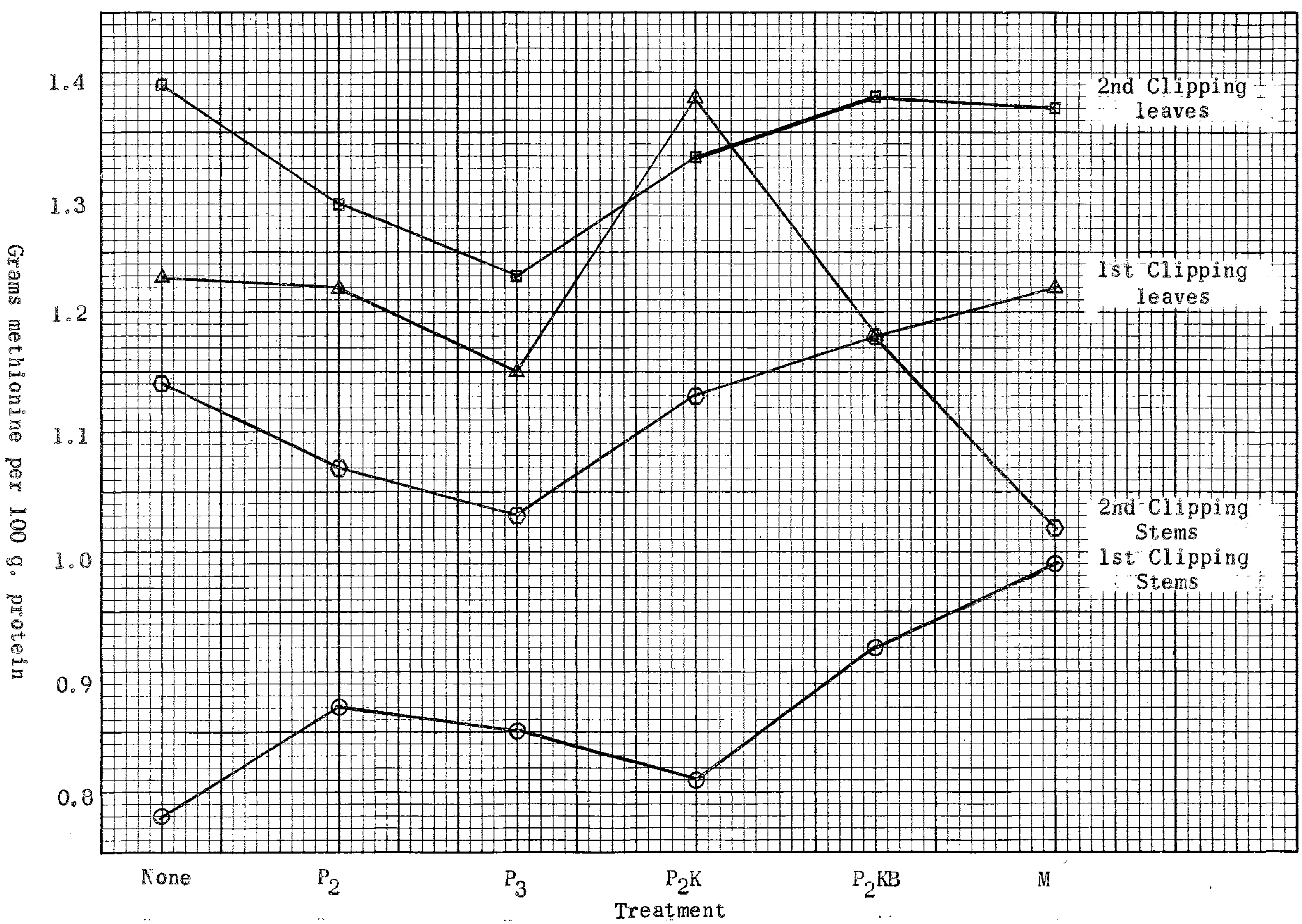


Figure VII. Effect of various fertilizer treatments on the lysine content of alfalfa.

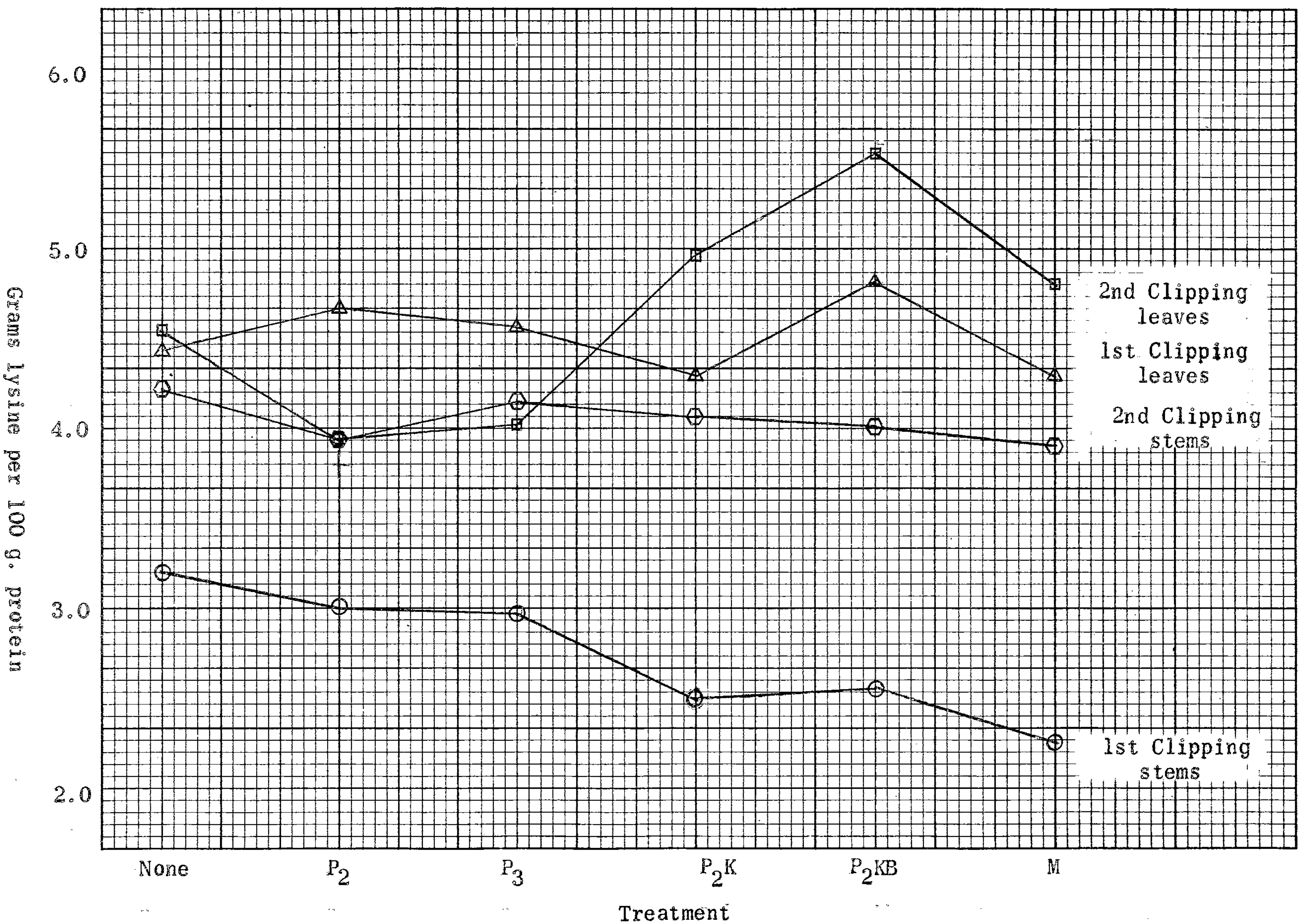
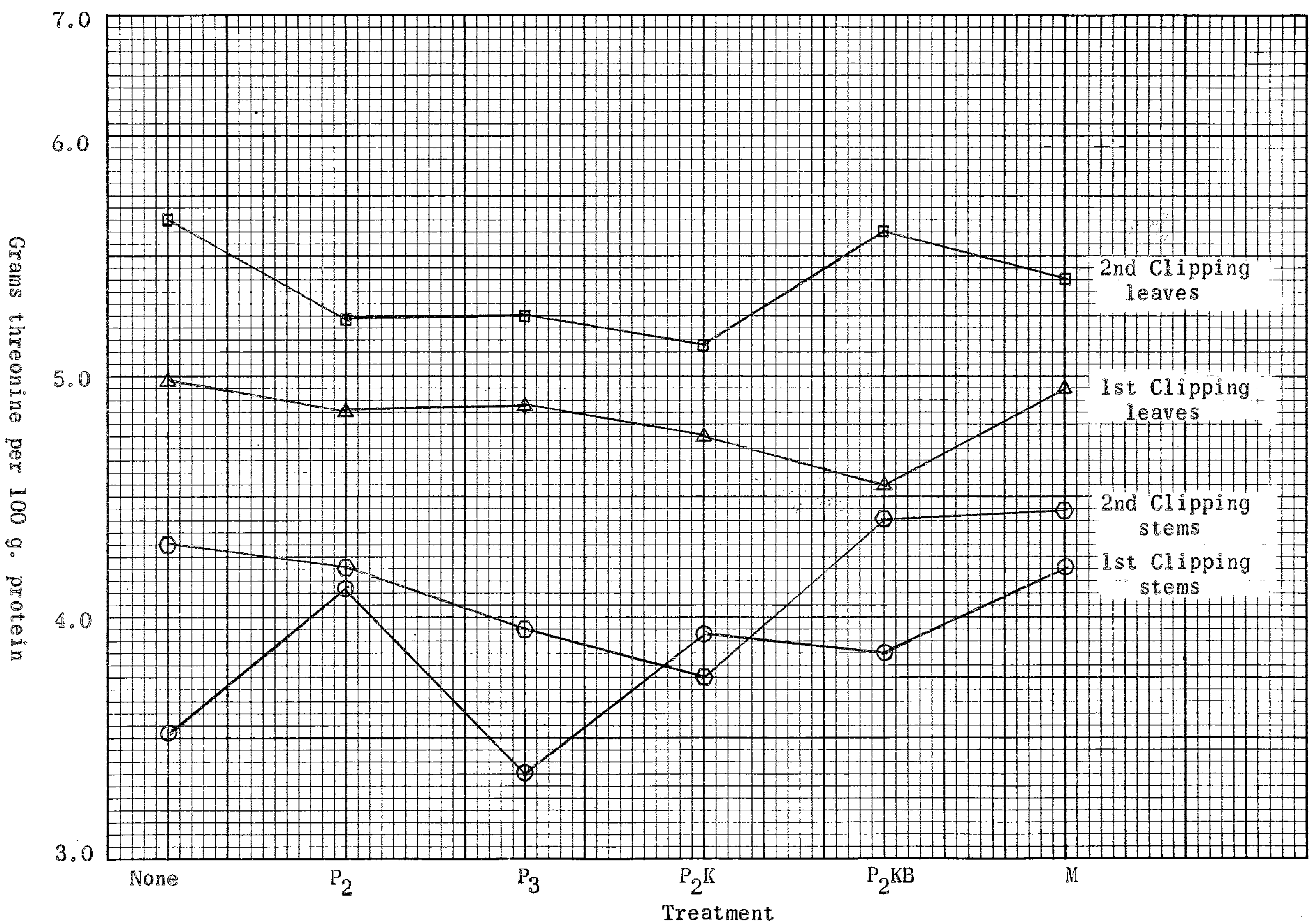


Figure VIII. Effect of various fertilizer treatments on the threonine content of alfalfa.



VITA

Ann Elizabeth Wynngate

Candidate for the Degree of

Master of Science

Thesis: THE INFLUENCE OF FERTILIZATION ON THE AMINO ACID COMPOSITION OF ALFALFA AND OF SUDAN GRASS

Major Field: Chemistry

Biographical:

Born: October 13, 1919 at Fort Worth, Texas.

Education:

Undergraduate Study: University of Oklahoma, 1939-1941; Columbia University, 1946-1947; University of Oklahoma, 1947-1950.

Graduate Study: Oklahoma Agricultural and Mechanical College, 1952-1954.

Date of Final Examination: June 14, 1957.