A STUDY OF SOME NITROGEN FRACTIONS OF WHEAT GRASS

by

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TABLE OF CONTENTS

INTRODUCTION

Schrenk (14) obtained data which show that there are great differences in crude protein content of wheat grass grown in various parts of Kansas. Presumably this is due to a greater supply of available nitrogen in some Kansas soils. His data were obtained by determining nitrogen by the Kjeldahl method and multiplying by 6.25. Hence, the greater apparent protein content of wheat grass in western Kansas might have been due partially to accumulation of greater amounts of non-protein nitrogen in the plants. Such accumulation of non-protein nitrogen was found to occur in bromegrass which was heavily fertilized with nitrogen (4).

When grown under the proper conditions, some plants accumulate sufficient quantities of nitrates that the crop will be toxic to livestock. Mayo (9) reported death of cattle due to ingestion of corn fodder containing 18.3 per cent potassium nitrate. Bradley, Eppson, and Beath (3) reported that plants containing over 1.5 per cent potassium nitrate (nitrate nitrogen calculated as KNO_3) were toxic to animals. They found that many weeds and cultivated plants in Wyoming contained sufficient nitrates to be toxic.

The purpose of this investigation was to determine the effect of location of growth on some of the types of nitrogen which are found in wheat grass. Of particular interest was the possibility that accumulation of nitrates may be responsible for wheat grass poisoning which occurs periodically in western Kansas.

EXPERIMENTAL

Experimental Plots

Pawnee wheat was planted in September, 1950 on unfertilized plots at the experiment stations located at Colby, Garden City, Hays, Manhattan, and Mound Valley. Figure 1 indicates the location of these stations in the state. At the Manhattan station, Pawnee wheat also was planted on a plot which received the following fertilization: at the time of planting, 75 pounds per acre of superphosphate containing 48 per cent P_2O_5, and in March, 100 pounds per acre of ammonium nitrate containing 33 per cent nitrogen.

The temperature during the season was at times relatively low, but no prolonged periods of extreme temperatures occurred during the growing season. The precipitation from September 1, 1950 to January 1, 1951 was considerably below normal, but from January 1, 1951 to June 1, 1951 it was about normal, Table 1.

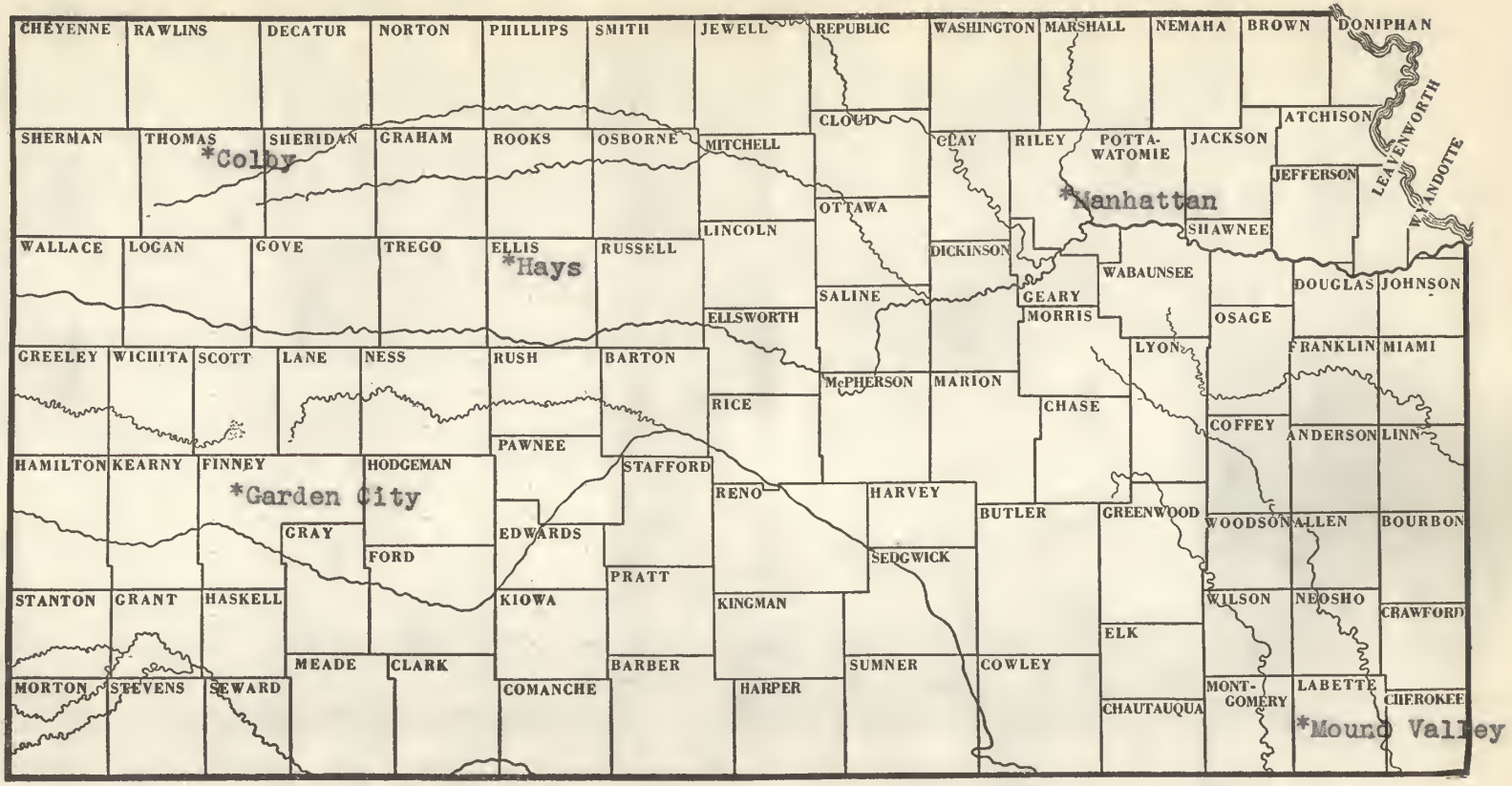

Fig. 1. Location of the experiment stations from which samples were collected.

Table 1. Rainfall at the five experiment stations from which samples were collected.

	Colby	Garden City	Hays	Manhattan	Mound Valley
	inches				
Sept.-1950	0.55	1.05	0.22	0.30	2.33
Oct.	0.23	1.14	2.46	1.63	0.97
Nov.	0.04	0.06	0.23	0.53	0.05
Dec.	0.13	0.02	0.03	0.03	0.00
Total	0.95	2.27	2.94	2.49	3.35
20 year av.	3.32	4.42	5.30	8.81	11.79
Jan.-1951	0.33	0.99	0.62	0.47	1.39
Feb.	0.33	1.22	1.48	1.36	2.37
March	0.24	0.57	1.75	2.62	1.00
April	1.13	2.71	3.47	3.45	2.85
May	4.96	4.96	7.29	8.62	3.10
Total	6.99	10.45	14.61	16.52	10.71
20 year av.	6.38	6.86	7.71	10.64	15.68

Collection and Preparation of Samples

The first samples were collected in November when plants were 3 to 5 inches high. The aerial portion of the plant, including the crown, was taken. The second samples were collected in April at the start of the spring growing season when plants were 3 to 5 inches high. The third samples were taken at the beginning of the jointing stage, and the fourth samples were collected at the early blooming stage. The samples were packed in dry ice at each of the locations and were shipped to the laboratory. Upon arrival at the laboratory, the samples were autoclaved at five pounds pressure for five minutes to inactivate

enzymes, and were 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 in the dark at -20° C. until analyzed.

Analytical Procedure

Total nitrogen was determined by the Gunning modification of the Kjeldahl method (8). Non-protein nitrogen was determined by placing the dry plant tissue in a 250 ml Erlenmeyer flask, adding 100 ml of water, and refluxing for ten minutes. One ml of 6 N acetic acid was added and the solution again was brought to a boil to insure precipitation of soluble protein. The mixture was filtered, and an aliquot of the filtrate was subjected to a Kjeldahl determination. This method was compared to that used by Miller (10), who exhaustively extracted the dried tissue with boiling water. Both methods gave essentially the same results. Since Miller's method was more time consuming, the method described above was used. Protein nitrogen was calculated by subtracting the non-protein nitrogen from the total nitrogen.

Ammonium nitrogen and amide nitrogen were determined by the method of Pucher, Vickery, and Leavenworth (12). The color intensity was measured with a Beckman spectrophotometer at 430 mu. Nitrate nitrogen was determined by the colorimetric method of Gilbert, Eppson, Bradley, and Beath (6). The intensity of the color produced was measured at 408 mu by means of a Beckman spectrophotometer.

Nitrite nitrogen was determined by the method recommended by the Association of Official Agricultural Chemists (1). This method was modified by using the clarified extract obtained from the nitrate determination for development of the color with the alpha-naphthylamine hydrochloride reagent.

RESULTS

Because of the small amount of rainfall, winter kill, and injury due to green bug and wheat mosaic, no samples were obtained from the Colby station for either the fall or spring growing seasons. The second spring sample from the Hays station was not received due to a misunderstanding concerning the number of samples to be collected.

Table 2 shows the relationship which was found between the total nitrogen of the plants and their location of growth. The results show a steady decline in total nitrogen during the period of this investigation. The greatest differences between the four stations were found in sample 3. Table 2 also shows that there was a greater amount of total nitrogen in western Kansas wheat than in eastern Kansas wheat when analyses were made at an equivalent stage of growth. These results are in agreement with those of Schrenk (14). Application of a nitrogen fertilizer at Manhattan increased the total nitrogen of the wheat grass, a response which is similar to that observed in bromegrass (4).

Table 2. The total nitrogen content of wheat grass from four locations in the state. (expressed as gm/100 gm. dry wt.)

Station	Sample no. 1	2	3	4
Garden City	5.120	5.120	3.930	2.173
Hays	4.620	4.720	-----	1.953
Manhattan, fertilized	5.720	4.700	3.510	1.890
Manhattan, unfertilized	5.420	4.570	3.450	1.480
Mound Valley	4.750	4.487	2.540	1.680

Tables 3 and 4 show the manner in which protein and non-protein nitrogen varied during growth. It will be seen that in general they followed the same trend that was found for total nitrogen.

Table 3. The non-protein nitrogen of wheat grass from four locations in the state. (expressed as gm/100 gm dry wt.)

Station	Sample no. 1	2	3	4
Garden City	.955	1.130	.510	.493
Hays	.840	.740	-	.493
Manhattan, fertilized	1.280	.860	.584	.350
Manhattan, unfertilized	.950	.732	.689	.318
Mound Valley	.810	.608	.420	.329

Table 4. The protein nitrogen of wheat grass from four locations in the state. (expressed as gm/100 gm dry wt.)

Station	Sample no. 1	2	3	4
Garden City	4.165	3.990	3.300	1.680
Hays	3.780	3.880	-	1.500
Manhattan, fertilized	3.460	3.840	2.827	1.540
Manhattan, unfertilized	4.550	3.840	2.752	1.162
Mound Valley	3.940	3.872	2.120	1.351

Tables 5 and 6 show the changes in ammonium and amide nitrogen for the four experiment stations. The ammonium content was relatively low and followed no consistent trend with regard to growth or location. Amide nitrogen was appreciably higher and appeared to be at a maximum at the time of the first spring sampling. This is consistent with the theory that plants store excess ammonium nitrogen in the form of amides until needed (5).

Table 5. The ammonium nitrogen of wheat grass from four locations in the state. (expressed as mgm/100gm dry wt.)

Station	Sample no. 1	2	3	4
Garden City	27.15	23.20	25.80	24.80
Hays	16.55	33.90	-	13.08
Manhattan, fertilized	19.65	30.10	6.70	3.90
Manhattan, unfertilized	22.90	19.50	9.50	3.30
Mound Valley	24.10	15.68	12.10	12.40

Table 6. The amide nitrogen of wheat grass from four locations in the state. (expressed as mgm/100 gm dry wt.)

Station	Sample no. 1	2	3	4
Garden City	47.30	81.40	36.90	53.10
Hays	45.55	66.10	-	50.52
Manhattan, fertilized	49.85	66.20	49.80	44.40
Manhattan, unfertilized	47.30	53.40	51.10	39.60
Mound Valley	31.60	63.62	53.30	21.10

Although nitrite determinations were made on each sample throughout the growth period, no nitrite was detected in the growing plant. This is in agreement with the many reports that nitrites will accumulate in plants only after severe carbohydrate starvation followed by luxury consumption of nitrates (2).

Table 7 shows the comparison of nitrate nitrogen for the four experiment stations. Nitrate nitrogen increased up to approximately the jointing stage (sample 3), and then decreased markedly. Nitrate nitrogen at no time during the period of study accounted for more than 4 per cent of the total nitrogen present in the plants. Never was there enough nitrate present to cause the plants to be toxic to livestock. Table 7 also shows the effect of fertilization on growing wheat. This effect is in agreement with the results of Carey (4), who found that fertilization of bromegrass increased the nitrate content considerably.

Table 7. The nitrate nitrogen of wheat grass from four locations in the state. (expressed as mgm/100 gm dry wt.)

Station	Sample no. 1	2	3	4
Garden City	23.70	19.90	30.40	20.50
Hays	18.35	64.50	-	26.50
Manhattan, fertilized	12.50	47.30	116.40	9.46
Manhattan, unfertilized	18.00	26.20	78.60	6.60
Mound Valley	10.60	14.63	9.67	7.43

Some interesting observations can be made by comparing total and non-protein nitrogen with the sum of ammonium, amide and nitrate nitrogen of samples from a given location. Since the three latter types of nitrogen are intermediate materials of nitrogen metabolism in plants, their concentration would be expected to be conditioned by the general metabolic rate and the rate of absorption of nitrogen from the soil. The data for all the samples were recombined as shown in Tables 8 and 9 to indicate the proportion of the non-protein and total nitrogen that was in the form of ammonium, amide and nitrate. It is apparent that the relative amount of the sum of these forms of non-protein nitrogen changed during the growth of the plants. It reached a maximum at about the early jointing stage (sample 3) and then decreased. This, in general, was true regardless of location of growth.

These data suggest either that the wheat plants were absorbing nitrogen at the greatest rate at the early jointing stage, or the rate of conversion to amino acids and protein had started to decrease. Haigh (7) reported that the greatest absorption of

nitrogen by wheat and timothy occurred during the early stages of growth, but did not specify what these stages were.

Table 8. Sum of ammonium, amide, and nitrate nitrogen expressed as per cent of non-protein nitrogen.

Location	Sample no. 1	2	3	4
Garden City	10.0	12.7	18.3	19.8
Hays	9.6	22.1	-	18.3
Manhattan, fertilized	6.4	16.7	25.3	16.5
Manhattan, unfertilized	9.3	13.5	22.1	15.5
Mound Valley	8.2	15.4	17.9	12.4

Table 9. Sum of ammonium, amide and nitrate nitrogen expressed as per cent of total nitrogen.

Location	Sample no. 1	2	3	4
Garden City	1.9	2.8	2.4	4.5
Hays	1.7	3.5	-	4.6
Manhattan, fertilized	1.4	3.1	4.9	3.1
Manhattan, unfertilized	1.6	2.2	4.0	3.4
Mound Valley	1.4	2.5	3.0	2.4

DISCUSSION

It is customary in determining the protein content of feedstuffs to subject the sample to a Kjeldahl determination, and to multiply the resulting nitrogen content by 6.25 to convert to protein. However, such a determination will include ammonium, amide, and most of the nitrate nitrogen. Ranker (13) reported that approximately 90 per cent of the nitrates are reduced to

ammonium by the ordinary Kjeldahl method, and thus are reported as protein. From Table 9 it will be seen that ammonium, amide, and nitrate in the wheat grass varied from 1.4 to 4.9 per cent of the total nitrogen. This range would be only the minimum error in expressing the results as protein. Undoubtedly other non-protein nitrogenous compounds are present to make the error much greater. Non-protein nitrogen actually determined in this study amounted to as much as 25.2 per cent of the total nitrogen. Although the major portion of the non-protein nitrogen probably was amino acids, which would behave similarly to proteins in nutrition, it still would be incorrect to designate it as crude protein. Thus, the error in the determination of protein by the ordinary Kjeldahl method at times may be quite significant.

Although this investigation showed that nitrate nitrogen did not accumulate in the 1950-'51 crop in sufficient quantities to be toxic to livestock, it is believed that the conditions under which the wheat was grown could not be considered normal, and that the results of this study are inconclusive. An investigation of this type should be carried out for several years to obtain conclusive results concerning the possibility of livestock poisoning from wheat pasturing. There were a few scattered cases of livestock poisoning from wheat pasturing during the 1950-'51 growing season. However, the investigator was unable to obtain samples from these isolated cases for analysis. The small number of cases of livestock poisoning reported may be due to the fact that there was very little pasturing of wheat because of the drought

conditions in most areas. It is believed that if proper conditions for growth prevailed, nitrates might be shown to be the cause of livestock poisoning due to pasturing livestock on young wheat.

SUMMARY

Samples of Pawnee wheat were collected from four experiment stations in the state, and were analyzed for various types of nitrogen.

Total, protein, and non-protein nitrogen declined steadily as plants matured. Wheat grass grown in western Kansas contained more total nitrogen than did that grown in eastern Kansas. Application of a nitrogen fertilizer at Manhattan increased the total nitrogen of the wheat grass.

The ammonium content was relatively low and followed no consistent trend with regard to growth or location. Amide nitrogen was appreciably higher than ammonium nitrogen and appeared to be at a maximum at the time of the first spring sampling.

No nitrites were detected in any of the samples during the investigation. At most stations nitrate nitrogen increased up to approximately the jointing stage, and then decreased markedly. Nitrates did not accumulate in sufficient quantities to be toxic to livestock.

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Pawnee wheat was planted in September, 1950 on unfertilized plots at experiment stations located at Colby, Garden City, Hays, Manhattan, and Mound Valley. At the Manhattan station, a plot also was planted which received the following fertilization: at the time of planting, 75 pounds per acre of superphosphate containing 48 per cent P_2O_5, and in March, 100 pounds per acre of ammonium nitrate containing 33 per cent nitrogen. Samples were taken at different stages of growth, and total, protein, non-protein, ammonium, amide, nitrite, and nitrate nitrogen determined.

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