EFFECT OF NITROGEN FERTILIZER ON NITROGEN FRACTIONS OF WHEAT AND FLOUR[1]

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ABSTRACT

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The effect of high nitrogen fertilization at seeding time on protein and nonprotein nitrogen fractions of hard red spring wheat and flour was investigated. With high nitrogen fertilization, there was a significant increase in content of protein, gluten, soluble protein, nonprotein nitrogen, and nitrate. Also the cultivar and year of growth had a significant influence on the fractions. Ammonia was not significantly increased. The proportion of the sample nitrogen found as protein or as nonprotein nitrogen was not significantly influenced by nitrogen fertilization, cultivar, location of growth, or crop year. Only a small error could have occurred in estimating the relative protein content by Kjeldahl nitrogen analysis because the proportion as protein nitrogen was quite constant among samples.

Protein content of wheat and flour is usually estimated by the well-established Kjeldahl procedure which measures nitrogen content of the sample. If nonprotein nitrogen varied with high nitrogen fertilization, the Kjeldahl method would not give an accurate estimate of relative protein content. Investigations on the effects of nitrogen fertilization on different nitrogenous fractions of wheat have been neglected. The objective of this study was to determine the effect of nitrogen fertilization at seeding time on protein and nonprotein nitrogen fractions of spring wheat. Wheat grown under high and low nitrogen fertilization and the flour from the wheat were analyzed for gluten N, soluble protein N, nonprotein N, ammonia N, and nitrate N.

MATERIALS AND METHODS

Wheat Samples

The cultivars used in this study are given in Table I. Samples used in this study were grown with low and high nitrogen fertilization at each location and year. Samples grown at low nitrogen fertilization had no NH_4NO_3 fertilizer added at seeding while those at high nitrogen fertilization had 120 lb of NH_4NO_3/acre added at Casselton, N. Dak., and 105 lb/acre added at Carrington, N. Dak. Also, 102 lb/acre of superphosphate and 100 lb/acre of 0-33-17 fertilizer were added to both low and high nitrogen fertilized plots. The experimental design was a randomized complete block with a split plot arrangement used with nitrogen treatments as whole plots, replicated three times.

Sample Preparation

Wheat samples were ground on a Udy cyclone mill (Udy Analyzer Co.) using a 0.024-in. sieve. For flour milling, 40 g of grain from each triplicate plot of a cultivar was blended into a composite, tempered to 16% moisture (18 hr), and milled on a Brabender Quadraplex mill to flour. Flour yield ranged from 51 to 65% with an average yield of 60.8%. Protein content of samples was determined

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by the macro-Kjeldahl method (1) using a factor of 5.7 to convert N to protein.

Extraction and Analysis for Protein and Nonprotein Nitrogen Fractions

For extraction, 12 g of ground grain or flour was homogenized 4 min with 36 ml of 0.1*M* NaCl in the 50-ml bowl of a Sorvall Omnimixer at 16,000 rpm while cooling the homogenizer bowl with an ice bath. Maximum extraction of N occurred in 4 min. The homogenate was centrifuged at 48,000 × *g* at 5° C for 20 min. Extract N was determined on 1 ml of supernatant by the micro-Kjeldahl procedures (1). Nitrogen not extracted was considered in this study to be gluten N. Gliadin solubility in water was suppressed by using 0.1*N* NaCl as extractant (2).

Nonprotein nitrogen was determined on the supernatant remaining after precipitating the protein from 2 ml of extract with tungstic acid (3). Soluble protein N was calculated as the difference between total extract N and nonprotein N.

For free ammonia analysis, protein was precipitated from 25 ml of extract by heating with boiling water under a reflux condenser for 5 min. After centrifuging at 31,000 × *g* for 10 min, the ammonia was determined on 10-ml aliquots of supernatant in a Labconco micro-Kjeldahl still using 10 ml of aqueous MgO suspension (16 g/600 ml H_2O) to make the supernatant weakly alkaline, a 3 min distillation time, and Antifoam A spray (Dow Corning Co.) to suppress foaming. A time study showed that distillation of ammonia was completed in 2 min and no additional ammonia from the hydrolysis of glutamine side-chain amide bonds was observed on extending the distillation time to 6 min.

Nitrate nitrogen in the extract was determined by an enzyme method employing nitrate reductase of soybean root nodules (4). Preliminary treatment of the extracts was necessary to prevent enzyme inhibition. For wheat, a 5-ml aliquot of extract was titrated with 1*N* HCl to pH 1, let stand for 1 min, and then titrated to pH 6.8 with 1*N* NaOH. After centrifugation at 12,000 × *g* for 5 min (5° C), the supernatant was heated in an 80° C water bath for 5 min, cooled, and recentrifuged. In the treatment of flour extracts, heating in the 80° C bath was not required.

The contents of all nitrogen fractions are expressed on a dry-weight basis.

Data Analysis

The wheat cultivars that were commonly grown at the two locations or for the

TABLE I
Location and Year Hard Red Spring Wheat Cultivars were Grown in Triplicate Plots

	Casselton 1967		Casselton 1968	Carrington 1967
Cultivar	Wis-255	TzPP[a]	Wis-255	TzPP[a]
	Justin	Chris	Justin	Chris
	Dakuru	Mexipak	Dakuru	Mexipak
	Crim	ND-442	Crim	ND-442
	ND-264	...	ND-264	ND-264

[a]Tezanos Pintos Precos.

2 years (Table I) were analyzed by the standard analysis of variance procedure for split-plot design. Also, for each analysis, the means of the low nitrogen and high nitrogen fertilized samples were analyzed for significance differences by the student's t-distribution test.

RESULTS AND DISCUSSION

Content of Nitrogen Fraction

As was expected, the protein content of wheat and flour from both locations grown during 1967 and 1968 was significantly increased by nitrogen fertilization

TABLE II
Effect of Nitrogen Fertilization on Protein and Gluten Content[a]

Location and Year	Mean Protein Content			Mean Gluten Content		
	Low N %	High N %	t[b]	Low N %	High N %	t[b]
Wheat						
Casselton, 1967	13.9	16.5	12.32**	11.0	13.4	9.18**
Casselton, 1968	13.2	15.8	6.20**	10.4	12.6	5.67**
Carrington, 1967	17.8	19.6	4.02**	14.6	16.0	2.98**
Overall	14.7	17.1	12.18**	11.8	13.9	9.81**
Flour						
Casselton, 1967	12.7	15.3	10.50**	10.7	13.3	7.95**
Casselton, 1968	12.5	14.3	3.72**	10.8	12.4	2.95**
Carrington, 1967	16.6	18.3	5.63**	14.8	15.7	1.93
Overall	13.7	15.8	10.58**	11.8	13.7	6.65**

[a]On dry-weight basis. For wheat, the means of the triplicate plots were used for the calculations.
[b]Test of significant differences between the mean of the low and the mean of the high nitrogen fertilized samples by the student's t-distribution test.

TABLE III
Analysis of Variance for Wheat Nitrogen Fractions as Proportion of Dry Matter—Casselton and Carrington 1967 Samples

Source of Variance	d.f.	Mean Squares					
		Protein	Gluten	Soluble protein	Non-protein	Free ammonia $\times 10^{-2}$	Nitrate
Location (L)	1	398.470**	947.813**	9.802**	1.318**	1.555*	9.700**
Error (a)	4	2.036	6.503	0.216	0.014	0.156	0.254
Nitrogen fertilizer (N)	1	114.406**	229.218*	6.246**	0.756**	0.294	7.310**
L × N	1	0.161	1.355	0.051	0.063	0.031	0.188
Error (b)	4	4.151	11.764	0.060	0.009	0.055	0.232
Cultivar (C)	4	54.446**	144.278**	3.402**	0.251**	3.228**	3.215**
L × C	4	7.517**	22.247**	0.101	0.006	0.172**	2.439**
N × C	4	1.888	7.713	0.542**	0.009	0.215**	0.643*
L × N × C	4	3.604	11.404	0.276**	0.012	0.046	0.916**
Error (c)	32	1.704	4.421	0.063	0.010	0.042	0.191

(Tables II, III, and IV). However, some samples showed a much larger increase in protein than others. Gluten content was significantly increased for all but the flour from Carrington in 1967 (Table II), whereas the content of soluble protein and nonprotein nitrogen increased significantly at both locations and for both years (Tables III, IV, and V). Since protein content as measured by Kjeldahl nitrogen analysis was significantly increased, one would expect a significant increase in the content of various nitrogen fractions. Also, cultivar and location of growth had a significant effect on the content of nitrogen fractions in wheat grain (Tables III and IV).

Ammonium nitrate as a fertilizer did not cause a dramatic increase in content of free ammonia or nitrate in wheat or flour, although some samples gave relatively large increases in nitrate (Table V). Since ammonia is known to be rapidly converted to nitrate in the soil, it would not be available to the plant. This fertilizer does appear to increase nitrate content of wheat and flour significantly (Tables III, IV, and V). The highest level of nitrate in wheat (2.6 μg/g, Mexipak wheat) and flour (2.3 μg/g TzPP flour) was far below levels used in studying nitrate poisoning where levels of 3,000 μg/g were used (5). The levels of nitrate found are near those reported in 1971 by McNamara *et al.* (6) for winter wheat.

Relative Distribution of Nitrogen Fractions

The proportion of sample N in wheat and flour found as nonprotein N, gluten N, and soluble protein N was, in most cases, not significantly changed by high nitrogen fertilization (Tables VI, VII, and VIII). For all samples, 95.9 to 97.5% of the nitrogen in wheat and 97.8 to 99.0% in flour were found as protein N and, with one exception (flour, Carrington, 1967), there was no significant change in the proportion of nitrogen in protein due to nitrogen fertilization (Tables VI, VII, and VIII). With one exception for wheat (Casselton, 1968, Table VI), there was also no significant change in the proportion of nonprotein N which occurred at 2.51 to 4.09% of the wheat N and 0.98 to 2.22% of the flour N.

Since protein is necessary for bread-baking and nutrition and is usually measured by nitrogen analysis, the ratio of protein N to nonprotein N should be

TABLE IV
Analysis of Variance for Wheat Nitrogen Fraction as Proportion of Dry Matter—Casselton 1967 and 1968 Samples

		Mean Squares					
Source of Variance	d.f.	Protein	Gluten	Soluble protein	Non-protein	Free ammonia $\times 10^{-3}$	Nitrate
Year (Y)	1	4.328	8.651	0.313	0.009	3.808*	1.998**
Error (a)	4	2.682	6.098	0.265	0.013	0.336	0.034
Nitrogen fertilizer (N)	1	225.201**	524.423**	5.800**	0.928**	3.413*	0.253*
Y × N	1	3.928	6.330	0.0923	0.004	0.043	0.055
Error (b)	4	1.674	6.258	0.031	0.005	0.282	0.028
Cultivar (C)	4	41.763**	92.091**	2.659**	0.480**	35.820**	0.461**
Y × C	4	41.117**	114.387**	0.641**	0.067**	0.673	0.422**
N × C	4	2.058*	5.578**	0.580**	0.003	2.006**	0.090
Y × N × C	4	0.793	2.706	0.060	0.010	0.031	0.101
Error (c)	32	0.586	1.353	0.119	0.014	0.353	0.075

TABLE V
Effect of Nitrogen Fertilization on Some Nitrogen Fraction of Wheat and Flour

Location and Year	Mean Content of Nitrogen in Fractions from Low and High N Fertilized Samples											
	Soluble protein			Nonprotein N			Ammonia N			Nitrate N		
	Low N	High N	t	Low N	High N	t	Low N	High N	t	Low N	High N	t
	mg N/g sample			mg N/g sample			mg N/g × 10^{-1}			μg N/g sample		
Wheat												
Casselton, 1967	4.00	4.43	3.14*	0.812	0.930	3.41**	0.384	0.348	−0.83	0.38	0.63	2.99*
Casselton, 1968	4.21	4.53	3.83*	0.742	0.925	5.45**	0.610	0.454	−1.23	0.50	0.76	2.62
Carrington, 1967	4.50	5.01	5.68**	0.955	1.359	15.30**	0.756	0.574	−1.31	0.88	1.47	2.06
Overall	4.19	4.61	9.81**	0.831	1.041	7.79**	0.542	0.487	−2.04	0.54	0.89	3.49**
Flour												
Casselton, 1967	2.60	2.95	3.96**	0.324	0.390	2.45*	0.270	0.343	2.03	0.52	0.68	2.58*
Casselton, 1968	2.57	2.90	3.01**	0.306	0.380	9.78**	0.280	0.300	0.95	0.51	0.60	0.93
Carrington, 1967	2.80	3.19	5.84**	0.367	0.523	5.32**	0.444	0.684	3.16*	0.80	1.63	3.19*
Overall	2.64	3.00	6.93**	0.330	0.422	5.14**	0.318	0.422	3.21**	0.59	0.91	2.97**

TABLE VI
Effect of Nitrogen Fertilization on Distribution of Nitrogen Fractions

Location and Year	Mean Distribution of Fractions from Low and High N Fertilized Samples											
	Nonprotein N			Gluten N			Soluble protein N			Total protein N		
	Low N	High N	t	Low N	High N	t	Low N	High N	t	Low N	High N	t
	% of sample N											
Wheat												
Casselton, 1967	3.34	3.22	−1.50	80.1	81.5	2.24	16.6	15.4	−2.33	96.7	96.8	1.37
Casselton, 1968	3.22	3.31	3.13*	78.3	80.3	3.00*	18.3	16.4	−2.76	96.8	96.7	−0.60
Carrington, 1967	3.09	3.38	2.92	82.3	82.0	−1.10	14.6	14.6	0	96.9	96.6	−2.86
Overall	3.24	3.28	1.15	80.2	81.3	2.04	16.5	15.4	−2.98**	96.8	96.7	−0.30
Flour												
Casselton, 1967	1.48	1.47	−0.12	86.7	87.5	1.68	11.8	11.1	−2.05	98.5	98.6	0.18
Casselton, 1968	1.41	1.53	1.71	86.7	86.7	0.02	11.9	11.7	−0.19	98.6	98.5	0.69
Carrington, 1967	1.25	1.67	1.63	89.1	88.1	−1.70	9.6	10.2	1.12	98.8	98.3	−3.93*
Overall	1.40	1.54	1.20	87.3	87.4	0.19	11.3	11.0	−0.77	98.6	98.5	−1.72

rather constant between samples to ensure comparable results. For all wheat samples, the standard deviation for protein N among samples was 0.38% of the sample N with a range of 1.6%. The standard deviation for protein N among flour samples was 0.29% of the sample N with a range of 1.2%. The variation between samples in the proportion of protein was rather small with a coefficient of variation of 0.39% for wheat and 0.29% for flour. The standard deviation and range for nonprotein N were just as small as for protein N. Thus, for the sample studied, the influence of variety, location of growth, year grown, and nitrogen fertilization would generally have caused only a small variation in relative Kjeldahl protein due to changes in the ratio of protein to nonprotein N present. The range of 1.6 and 1.2% of the wheat and flour N, respectively, as protein N

TABLE VII
Analysis of Variance for Wheat Nitrogen Fractions Calculated as Per Cent of Total Sample Nitrogen—Casselton and Carrington 1967 Samples

Source of Variance	d.f.	Mean Squares: Total protein	Gluten	Soluble protein	Non-protein	Free ammonia $\times 10^{-2}$	Nitrate $\times 10^{-10}$
Location (L)	1	0.003	56.856**	56.719**	0.031	3.739	56.581**
Error (a)	4	0.084	1.071	1.071	0.129	1.290	2.356
Nitrogen fertilizer (N)	1	0.320	0.936	2.976	0.383	11.644*	54.702*
L × N	1	0.936*	5.208	1.752	0.702*	0.014	0.049
Error (b)	4	0.075	3.827	2.675	0.091	0.794	2.937
Cultivar (C)	4	3.570**	69.336**	44.706**	3.501**	36.550*	60.659**
L × C	4	0.459*	8.276**	4.991**	0.363*	2.390**	25.324**
N × C	4	0.353	9.225**	7.527**	0.259	4.400**	6.188*
L × N × C	4	0.085	7.427**	4.984**	0.129	0.596	7.476**
Error (c)	32	0.139	1.509	1.150	0.123	0.526	1.807

TABLE VIII
Analysis of Variance for Wheat Nitrogen Fractions Calculated as Per Cent of Total Sample Nitrogen—Casselton 1967 and 1968 Samples

Source of Variance	d.f.	Mean Squares: Total protein	Gluten	Soluble protein	Nonprotein	Free ammonia $\times 10^{-2}$	Nitrate $\times 10^{-10}$
Year (Y)	1	0.001	1.027	3.960	0.001	2.269	25.181**
Error (a)	4	0.240	6.508	13.328	0.237	1.049	0.252
Nitrogen	1	0.096	55.081**	100.467*	0.126	17.250**	0.076
Y × N	1	0.085	0.331	0.832	0.093	0.256	0.992
Error (b)	4	0.189	2.513	7.347	0.182	0.408	0.370
Cultivar (C)	4	2.304**	21.017**	18.277	2.352**	32.871**	6.309**
Y × C	4	0.716*	24.437**	48.304**	0.659	0.489	5.214**
N × C	4	0.130	8.324	7.614	0.123	5.423**	2.090
Y × N × C	4	0.181	3.288	14.656	0.205	0.698	1.962
Error (c)	32	0.259	3.238	9.467	0.251	0.633	0.796

would have caused a maximum variation in relative protein content of about 0.2% for a 15% protein sample.

The proportion of some of the nitrogenous fraction, in grain samples of cultivars that vary in nitrogen content, has been found to follow the "regularity principle" reported by Bishop (7) for barley. Gluten, soluble protein, free ammonia, and nitrate nitrogen as per cent of the sample nitrogen were significantly correlated with sample nitrogen on most cultivars, and each cultivar had its own characteristic regression equation. Total protein and nonprotein nitrogen, however, remained relatively constant with increasing sample nitrogen.

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