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The influence of liming and mineral fertilization on the content of various nitrogen forms in spring barley

Wpływ wapnowania i nawożenia mineralnego na zawartość różnych form azotu w jęczmieniu jarym

Abstract. The aim of the experiment was to find out the influence of liming and fertilization with ammonium sulphate and calcium nitrate on the content of total protein, true protein, $N\text{-}NO_3$ and $N\text{-}NH_4$ in spring barley. The study was based on the chemical analysis of plant material obtained from a two-year pot experiment. The factors of the study were: liming fertilization with ammonium and nitrate nitrogen at two levels and fertilization with phosphorus at two doses. The obtained results indicated that the content of total protein and true protein was higher in plants fertilized with ammonium sulphate than in plants fertilized with calcium nitrate. Liming caused a decrease of protein content in the tested plant. The content of ammonium and nitrate nitrogen in the plant was lower in limed than non-limed combinations. Ammonium sulphate influenced a significant increase of the content of $N\text{-}NH_4$ whereas calcium nitrate caused an increase of the concentration of $N\text{-}NO_3$ in spring barley.

Key words: nitrogen forms, protein, liming, spring barley

INTRODUCTION

From among all the mineral components applied in fertilizers, nitrogen, due to the nature of compounds it is a component of, has the greatest effect upon the quality of cultivated plants. This element occurs primarily in the form of organic compounds. Only a small amount of it remains in the mineral form of (NH_4^+, NO_3^-). The products of mineral nitrogen transformations to organic nitrogen compounds are in. a. amino acids, proteins, nucleotides, nucleic acids and porphyrin compounds. In using plants as fodder it is significant to obtain yields with high participation of protein, with simultaneous low content of nitrates (V), because their excessive amount may lead to the decreased animal healthiness and productivity. Among many factors determining the effectiveness of metabolic transformations of nitrogen a significant role is played by mineral fertilization, especially the dose and form of a nitrogen fertilizer [Borowski *et al*. 1991, Uziak *et al*. 1991, Gąsior and Kaniuczak 1996, Krzywy *et al*. 1996, Noworolnik and Sułek 1999, Bednarek 2005].

The condition of the correct course of nitrogen metabolic processes in a plant is also the optimum supply of the remaining macro- and microelements. Their availability for the plants depends on soil reaction. Liming, through its influence upon the pH change and, consequently, change of nutrient availability may significantly modify the proportions between protein and non-protein nitrogen in the plant [Mazur 1983, Kopeć and Mazur 2006].

The objective of the studies was to determine the effect of liming and fertilization with ammonium sulphate or calcium nitrate upon the content of total protein, true protein, as well as $N-NO_3$ and $N-NH_4$ in spring barley.

MATERIAL AND METHODS

The studies were conducted on the basis of two-year pot experiment. The experiment was established on the soil material with granulometric composition of light loamy sand. The soil was characterized with very acid reaction (pH_{KCl} 4.00), low abundance with available phosphorus and potassium and very low content of available magnesium. The total nitrogen content was 0.73 g kg^{-1}, the content of mineral nitrogen: 15.02 mg kg^{-1}, the content of $N-NH_4$: 11.82 mg kg^{-1} and $N-NO_3$: 3.20 mg kg^{-1}.

For our tests we used pots that held 5 kg of soil material. The scheme of experiment comprised 9 combinations in 4 repetitions on two series of soil: acid and limed:

1. K Mg 0 – control
2. K Mg P_1 N_1 – NH_4
3. K Mg P_1 N_1 – NO_3
4. K Mg P_1 N_2 – NH_4
5. K Mg P_1 N_2 – NO_3
6. K Mg P_2 N_1 – NH_4
7. K Mg P_2 N_1 – NO_3
8. K Mg P_2 N_2 – NH_4
9. K Mg P_2 N_2 – NO_3

Phosphorus was applied in the form of granulated triple superphosphate (20.1% P) in two rates (P_1 – 0,06 g P kg^{-1}, P_2 – 0,12 g P kg^{-1} d.m. of soil), nitrogen as ammonium sulphate (20% N) and calcium nitrate (15.5% N) at two levels (N_1 – 0,1 g N kg^{-1}, N_2 – 0,2 g N kg^{-1} d.m. of soil), potassium (0.1 g K kg^{-1} d.m. of soil) in the form of potassium high-percentage potash salt (49.8% K) and magnesium (0.025 g Mg kg^{-1} d.m. of soil) as magnesium sulphate (9.6% Mg). The experimental factors were applied against the background of control object. Liming with $CaCO_3$ was applied once, before establishing the experiment in the amount calculated according to 1 Hh. Fertilization with nitrogen, phosphorus, potassium and magnesium was applied every year of the studies, before sowing the plants. During vegetation constant soil moisture was maintained on the level of 60% field water capacity. The test plant was spring barley Bryl variety, which was harvested at its full ripeness.

The dry mass content was determined by means of the drier method, by drying the plant material in the temperature of 105°C. The plant material was mineralized in concentrated sulphuric acid (VI) with an addition of perhydrol. In the mineralizates the content of total nitrogen was determined with the use of Kjedahl's method, the total protein content was calculated with the use of 6.25 conversion factor. We determined the N protein true content with the use of Kjedahl's method with trichloroacetic acid, $N-NH_4$ with the use of Nessler reactant and $N-NO_3$ with the use of the method with sodium salicylate.

The influence of experimental factors on the examined plant properties was determined by means of variance analysis with the application of Tukey's confidence half-intervals ($p = 0.05$). The results stated in tables constitute mean values from the two-year experiment.

RESULTS AND DISCUSSION

In the conducted experiment, the total protein content in spring barley grain, straw and roots dependent significantly upon liming and nitrogen fertilization. However, the rate of phosphorus was the factor that determined the concentration of total protein only in the dry mass of straw and roots. The effect of cooperation between the kind of soil and mineral fertilization was the greatest in the combinations, which were not limed and fertilized with ammonium sulphate (tab. 1). The application of calcium carbonate contributed to the statistically proven decrease in the total protein content. Undoubtedly, this was caused by the so-called „nitrogen dilution effect". Similarly, in the studies conducted by Łabuda [1988] the decreased soil pH enhanced the increased concentration of the analyzed compound, whereas Mazur [1983] fund that the total protein content increases in plants after the application of soil liming. Irrespectively of the analyzed part of the index plant, the use of ammonium sulphate enhanced the increase of total protein content in spring barley. A more advantageous effect of the ammonium than nitrate form of nitrogen was most probably related to the fact that the process of amino-acid formation from NH_4^+ ions is faster and less energy consuming than the process of creating these compounds out of NO_3^- ions. In this experiment the increased nitrogen doses contributed to the increase of total protein concentration by 16% in the grain, by 41% in the straw, and by 20% in the roots of test plant. The obtained results confirm the observations of many authors [Czerniawski 1983, Dziamba *et al.* 1983, Szurpicka-Połtarzewska and Koc 1997, Noworolnik and Sułek 1999]. In our own studies the increased rates of triple superphosphate with simultaneous increase of nitrogen doses positively influenced the total protein concentration in the vegetative parts of spring barley. Similar dependencies are indicated by the results of Czerniawski's [1983] and Dziamba *et al.* [1983] studies.

Among the applied experimental factors, what had the statistically proven effect upon the content of true protein in spring barley was liming and nitrogen fertilization (tab. 2). Irrespectively of the used form of nitrogen fertilizer, the application of $CaCO_3$ contributed to a significant decrease of true protein concentration in the dry mass of the test plant. The increased content of the compound under discussion, together with the decreased pH are also indicated by the results of studies conducted by Łabuda [1988]. In the analyzed experiment the greatest effect upon the true protein content was that of the nitrogen form. The ammonium sulphate contributed to the increased content of the discussed compound by 24%, 62% and 55% respectively in the grain, straw and roots of spring barley, as compared to its concentration in plants fertilized with calcium nitrate.

Table 1. Effect of the experimental factors on the content of total protein in spring barley (g kg^{-1} d.m.)
Tabela 1. Wpływ czynników doświadczalnych na zawartość białka ogólnego w jęczmieniu jarym (g · kg^{-1} s.m.)

Object Obiekt	Grain Ziarno				Straw Słoma				Roots Korzenie			
	G_1		G_2		G_1		G_2		G_1		G_2	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
P_1N_1	153.13	122.05	101.91	103.45	103.15	18.21	35.33	33.81	80.48	48.45	50.00	42.68
P_1N_2	176.11	148.75	120.23	122.98	124.11	68.15	47.81	38.15	90.47	53.75	68.45	50.83
P_2N_1	164.55	112.85	102.21	103.45	80.65	47.22	35.63	31.25	74.05	43.60	46.61	41.63
P_2N_2	175.18	137.85	113.93	123.15	118.12	52.05	51.25	41.92	82.05	49.52	67.82	49.42
$\bar{x}$ G	148.79		111.41		76.47		39.39		65.30		49.68	
$\bar{x}$ F			138.40	121.81			74.51	41.35			69.99	47.49
LSD (p = 0.05) NIR (p = 0,05)	N, F, G – 3.02 GF, GN, PF, NF, GP –5.64 GNF, GNP – 9.36				N, F, P, G – 4.17 GF, NF, GN, PF, PN – 7.81				N, F, P, G – 2.41 GF, NF, GP, GN, PN – 4.51 GNF – 8.43			
K	103.45		85.95		81.11		24.68		53.61		39.05	

G_1 – acid soil, gleba kwaśna; G_2 – limed soil, gleba wapnowana; F_1 – ammonium sulphate, siarczan amonu; F_2 – calcium nitrate, saletra wapniowa; N_1, N_2 – nitrogen rates, dawki azotu; P_1, P_2 – phosphorus rates, dawki fosforu; K – control, kontrola

Table 2. Effect of the experimental factors on the content of true protein in spring barley (g N kg^{-1} d.m.)
Tabela 2. Wpływ czynników doświadczalnych na zawartość białka właściwego w jęczmieniu jarym (g N · kg^{-1} s.m.)

Object Obiekt	Grain Ziarno				Straw Słoma				Roots Korzenie			
	G_1		G_2		G_1		G_2		G_1		G_2	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
P_1N_1	23.33	16.71	15.15	14.33	6.58	4.25	3.50	3.08	7.05	4.25	5.13	4.15
P_1N_2	26.41	20.31	16.95	15.98	8.25	5.75	4.85	3.08	8.42	4.54	5.55	4.71
P_2N_1	24.91	15.82	15.50	14.51	8.08	4.28	3.50	2.85	7.89	4.13	4.43	3.63
P_2N_2	25.61	18.95	17.00	16.33	11.45	4.85	4.80	3.40	8.93	4.53	5.28	4.08
$\bar{x}$ G	21.61		15.72		6.68		3.63		6.22		4.62	
$\bar{x}$ F			20.61	16.63			6.38	3.94			6.59	4.25
LSD (p = 0.05) NIR (p = 0,05)	N, F, G – 0.31 GF, GN, NF, GP – 0.62 GNF, GPN – 1.18				N, F, G – 0.24 GF, NF, GN, GP – 0.46 GNF – 0.91				N, F, P, G – 0.23 GF, NF, GP – 0.38			
K	19.53		11.75		5.00		2.23		4.35		3.55	

Explanations – see table 1
Objaśnienia – patrz tabela 1

Undoubtedly, this phenomenon was connected with less complicated involvement of NH_4^+ ions than NO_3^- ions to the process of protein biosynthesis. We should not also disregard the fact that with ammonium sulphate the plants were supplied with sulphur, indispensable for the formation of the III-part protein structure. Similarly, in the experiment conducted by Uziak *et al*. [1991], fertilization plants with the ammonium form of nitrogen caused the increased content of the discussed compound in the above-ground parts of the plants and in their roots in comparison to the concentration in plants fertilized with the nitrate form of nitrogen. In this experiment the increased nitrogen rates contributed to the statistically confirmed increase of true protein content in the dry mass of spring barley. Similar dependencies are indicated by study results obtained by Krzywy *et al*. [1996], Szurpicka-Poltarzewska and Koc [1997] as well as Bednarek [2005], whereas Domska and Rogalski [1993] related the influence of nitrogen doses upon the content of true protein to the species of cultivated crops. In our own studies the increased phosphorus rates significantly affected the content of the discussed compound only in the test plant roots. Positive cooperation of increased N and P doses in the formation of true protein concentration are indicated by the study results obtained by Czerniawski [1983], Dziamba *et al*. [1983] as well as Gąsior and Kaniuczak [1996]

The excessive accumulation of NH_4^+ ions in the plant reveals disorders in protein biosynthesis, lowers the content of the remaining cations, and the forming NH_3 toxically affects the plants. In the foregoing studies the significant and greatest effect upon the content of nitrogen in spring barley was exerted by liming and nitrogen fertilization (tab. 3). Calcium carbonate contributed to statistically proven decrease of the amount of $N\text{-}NH_4$ in the plant. Most probably this was caused by nitrification process, which was more intensive at higher soil pH values. Both in the grain and straw, as well as in the roots of the test plant the content of ammonium nitrogen significantly increased in the objects fertilized with ammonium sulphate, as compared to the combinations fertilized with calcium nitrate. Significant increase of $N\text{-}NH_4$ content in the above-ground parts and roots of the plants fertilized with the ammonium form of nitrogen is also indicated by the studies results obtained by Borowski *et al*. [1991] and Uziak *et al*. [1991]. In our own studies the phosphorus rate significantly influenced only the content of ammonium nitrogen in spring barley straw and roots, whereas, irrespectively of the applied form of nitrogen, the increase of its doses enhanced the increase of nitrogen content in the test plant. The increase was much greater in the combinations fertilized with $(NH_4)_2SO_4$ than with $Ca(NO_3)_2$. The positive effect of increased rates of nitrogen fertilizers upon the concentration of $N\text{-}NH_4$ was also reported by Łabuda [1988] and Bednarek [2005].

One of the basic plant quality assessment criteria is the content of nitrates in them. The nitrate ions (V) may be reduced to nitrates (III), which contribute to the formation of methemglobin. Besides, nitrates (III) participate in generating carcinogenic nitrozoamines. In our own studies the influence of the experimental factors upon the content of $N\text{-}NO_3$ in spring barley was various and depended on the index part of the test plant (tab. 4). The application of calcium carbonate contributed to the statistically proven decrease of the content of the analyzed nitrogen form in the dry mass of grain and straw, but also to the significant increase of $N\text{-}NO_3$ in the roots. The decreased content of nitrate nitrogen in above-ground parts of the plants grown on limed soil can be justified by increased assimilability of molybdenum – a component of nitrate reductase in these soil conditions. Different study results were obtained by Kopeć and Mazur [2006]. For these authors observed the increased $N\text{-}NO_3$ concentration in plants cultivated on limed soil.

Table 3. Effect of the experimental factors on the content of $N-NH_4$ in spring barley (mg N kg^{-1} d.m.)
Tabela 3. Wpływ czynników doświadczalnych na zawartość $N-NH_4$ w jęczmieniu jarym (mg N · kg^{-1} s.m.)

Object Obiekt	Grain Ziarno				Straw Słoma				Roots Korzenie			
	G_1		G_2		G_1		G_2		G_1		G_2	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
P_1N_1	290.36	232.64	287.35	261.33	419.04	277.31	274.93	229.80	198.43	170.36	147.36	136.24
P_1N_2	408.21	293.92	323.71	213.47	418.74	267.63	314.98	203.92	220.32	216.35	187.02	161.07
P_2N_1	373.48	234.42	279.04	209.44	448.53	275.93	285.51	216.69	201.32	182.38	171.28	137.30
P_2N_2	240.00	288.98	295.96	245.23	579.79	324.37	330.38	233.23	236.76	177.58	233.31	161.04
$\overline{x}$ G	302.09		264.44		393.79		261.18		200.44		166.83	
$\overline{x}$ F			319.08	247.43			401.36	253.61			199.48	175.26
LSD (p = 0.05) NIR (p = 0,05)	G, N, F – 21.58 GF, GN, PF – 43.25 GNF – 83.24				G, N, F, P –16.16 GF, NF, GN, GP – 30.31 GNF, GPN – 56.85				G, N, P – 13.06 GF – 22.68			
K	296.45		278.39		343.06		234.09		187.65		131.00	

Explanations – see table 1
Objaśnienia – patrz tabela 1

Table 4. Effect of the experimental factors on the content of N-NO_3 in spring barley (mg N kg^{-1} d.m.)
Tabela 4. Wpływ czynników doświadczalnych na zawartość N-NO_3 w jęczmieniu jarym (mg N · kg^{-1} s.m.)

Object Obiekt	Grain Ziarno				Straw Słoma				Roots Korzenie			
	G_1		G_2		G_1		G_2		G_1		G_2	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
P_1N_1	16.12	18.41	16.11	17.91	153.08	141.24	51.38	82.86	45.67	26.84	40.98	57.20
P_1N_2	17.21	20.43	17.24	18.52	171.78	118.23	48.77	103.39	54.07	41.54	52.24	89.04
P_2N_1	21.81	18.21	16.90	19.05	124.78	90.39	58.85	74.35	58.92	34.77	44.62	57.51
P_2N_2	16.21	21.26	18.42	20.90	134.40	105.67	62.22	83.73	53.09	42.25	51.98	77.96
$\bar{x}$ G	19.04		18.13		129.91		70.69		44.64		58.94	
$\bar{x}$ F			17.83	19.34			100.63	99.98			50.20	53.38
LSD ($p = 0.05$) NIR ($p = 0,05$)	N, F, P, G – 0.90 GF –1.81				P, G – 10.91 GF, GN, GP – 20.09				N, G – 13.06 GF – 22.68			
K	20.67		15.75		126.94		66.58		54.32		30.24	

Explanations – see table 1
Objaśnienia – patrz tabela 1

From among the applied fertilizers, more stimulating effect upon the content of nitrate nitrogen in spring barley was achieved by the use of calcium nitrate than ammonium sulphate. Positive influence of the nitrate form of nitrogen upon the $N\text{-}NO_3$ concentration is confirmed by studies results obtained by Warchołowa and Mroczkowski [1982] as well as Uziak *et al*. [1991]. In this experiment the increased nitrogen rates contributed to the statistically proven increase of the nitrate nitrogen content in the grain and roots of the tested plant, whereas no significant effect of nitrogen doses upon $N\text{-}NO_3$ concentration in the straw was observed. Łabuda [1988] and Bednarek [2005] found that the concentration of nitrate nitrogen in plants increased with the increase of N rates, whereas the results of Kopeć and Mazur [2006] studies indicate lack of significant effect of nitrogen fertilization upon the content of $N\text{-}NO_3$ in plants. In the analyzed studies applying double dose of phosphorus led to a significant increase of nitrate nitrogen amounts in the above-ground parts of spring barley. Similar dependencies are indicated by study results obtained by Bednarek [2005].

CONCLUSIONS

1. The total protein content in the dry mass of spring barley decreased as a result of liming. The ammonium form of nitrogen enhanced the increased concentration of the analyzed component, irrespectively of the soil pH.

2. The greatest influence upon the increase of true protein content in the test plant was that of the application of ammonium sulphate. Calcium carbonate contributed to the decrease in the concentration of the component under discussion. The plants fertilized with increased rate of nitrogen had higher content of true protein.

3. Significant factors modifying the content of $N\text{-}NH_4$ were liming and nitrogen fertilization. The application of the ammonium form of nitrogen caused a distinct increase of $N\text{-}NH_4$ in the dry mass of spring barley. The increased rates of this component had a similar effect, whereas liming contributed to statistically proven decrease of ammonium nitrogen concentration in the plant.

4. The influence of experimental factors upon the content of $N\text{-}NO_3$ was differentiated and depended on the index part of spring barley. Generally, it can be stated that the application of calcium nitrate, as well as greater N and P rates enhanced the increase of nitrate nitrogen in the plant. The use of calcium carbonate had a different effect.

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Streszczenie. Celem badań było określenie wpływu wapnowania oraz nawożenia siarczanem amonu i saletrą wapniową na zawartość białka ogólnego, białka właściwego oraz N-NO_3 i N-NH_4 w jęczmieniu jarym. Badania oparto na analizie chemicznej materiału roślinnego otrzymanego z dwuletniego doświadczenia wazonowego. Czynnikami doświadczalnymi były wapnowanie, nawożenie formą amonową i azotanową azotu stosowane na dwóch poziomach oraz nawożenie fosforem w dwóch dawkach. Rezultaty badań wskazały, że zawartość białka ogólnego i właściwego była większa w roślinach nawożonych siarczanem amonu niż saletrą wapniową. Wapnowanie przyczyniło się do zmniejszenia zawartości białka w roślinie testowej. Zawartość azotu amonowego i azotanowego w roślinie była mniejsza w kombinacjach wapnowanych niż niewapnowanych. Siarczan amonu wpływał istotnie na wzrost zawartości N-NH_4, a saletra wapniowa na zwiększenie koncentracji N-NO_3 w jęczmieniu jarym.

Słowa kluczowe: formy azotu, białko, wapnowanie, jęczmień jary