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**NITROGEN CONTENT
AND AMINOACIDS PROTEIN COMPOSITION
OF GRAIN OF WINTER WHEAT FOLIAR FERTILIZED
WITH UREA AND MICROELEMENTS FERTILIZERS**

**ZAWARTOŚĆ AZOTU I SKŁAD AMINOKWASOWY
BIAŁKA ZIARNA PSZENICY OZIMEJ
DOKARMIANEJ DOLISTNIE MOCZNIKIEM
I NAWOZAMI MIKROELEMENTOWYMI**

Abstract: The field experiment was carried out over 2003–2005 on the lessive soil (Haplic Luvisols), defective wheat complex, with a randomised block method in four random replications. The soil was characterised with an acid reaction ($\text{pH}_{\text{KCl}} - 5.3$) and a natural content of trace elements (characteristic for the geochemical background). A Rywalka (class A) high quality variety of winter wheat was used as a test crop. The aim of this study was to determine the influence of foliar feeding with urea and nickel chelate EDTA-Ni(II) and Plonvit Z for the total nitrogen content and endogenous amino acid composition of winter wheat grains. The differentiation of the total nitrogen content and amino acid composition of protein under the influence of experimental factors proved to be statistically insignificant. Under the influence of EDTA-Ni, the amount of endogenous amino acids and content of leucine and lysine in the protein increased, while reducing the overall protein content.

Keywords: nitrogen, amino acids, winter wheat, foliar feeding, urea, micronutrients

Protein plays a key role in almost all biological processes. The basic structural units of proteins are amino acids [1]. The human diet must contain not only a sufficient amount of protein – about 0.8 g of protein per kilogram of body mass – but the protein must be of the appropriate type. The human organism is not able to synthesize the nine basic amino acids (exogenous); therefore, it must be supplied additionally to the diet [2, 3]. The organism may produce certain amino acids (endogenous amino acids), such as in the case of cystine, which are the main building blocks of hair. However, the amounts that the human organism can produce are sometimes too low. Plants can synthesize all amino acids; however, the majority of plant proteins show a deficit in one or more of

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the basic amino acids, in the case of wheat it is the lysine amino acid that is limited. Protein content and total amino acid composition may change depending on the species of plants, variety, habitat, and fertilization [4].

In the preparation of feed mix, which is the bases for animal foodstuffs, cereals are the most frequently used. The nutritional value of cereal proteins, which is largely determined in the composition of amino acid, is a measure of the degree of utilisation of proteins required for the synthesis of specific systemic proteins. Therefore, it is important to strive for better use of not only protein, but for all nutrients so that it will be possible to obtain physiological, economic, and environmental benefits [5].

Nickel was found as an essential elements for plants in 1987 [6], quite recently and in 1991 was rated in the group of micronutrients [7]. Its most important function is the physiological activation of urease, which is so far the only known enzyme that contains nickel [8]. Nitrogen contained in urea is not available in plants until the hydrolysis of urea catalysed by urease to ammonia and carbon dioxide [9]. There are reports that the addition of nickel can increase several times the activity of urease in the leaves [10, 11].

The aim of this study was to determine the influence of foliar feeding with urea and nickel chelate EDTA-Ni(II) and Plonvit Z for the total nitrogen content and endogenous amino acid composition of winter wheat grains.

Materials and methods

The field experiment was carried out over 2003–2005 on the lessive soil (Haplic Luvisols), defective wheat complex, with a randomised block method in four random replications. Size of plots: $6 \times 5\text{m} = 30\text{m}^2$ gross, including $5,2 \times 3\text{m} = 15,6\text{m}^2$ to harvest.

The soil was characterised with an acid reaction ($\text{pH}_{\text{KCl}} = 5.3$) and a natural content of trace elements (characteristic for the geochemical background). A Rywalka (class A) high quality variety of winter wheat was used as a test crop. The following fertilization per one hectare was used: soil application per 1 ha: pre-sowing, 35 kg P in the form of triple superphosphate and 120 kg K in the form of potassium salt, per capita before start of vegetation 60 kg N in ammonium nitrate (solid form), and after vegetation started in spring 60 kg N in the form of $\text{CO}(\text{NH}_2)_2$ (3×20 kg) by the experiment scheme (foliar application). Deadlines of foliar feeding: I. height spring tillering (KD 25); II. stem formation (KD 32) (2. node); III. beginning of ear formation (KD 51).

Experimental objects:

1. urea (3×20 kg N \cdot ha⁻¹) soil application in solid + $\text{MgSO}_4 \cdot \text{H}_2\text{O}$ + water (spray 3 \times);
2. urea (3×20 kg N \cdot ha⁻¹) in a solution + $\text{MgSO}_4 \cdot \text{H}_2\text{O}$;
3. urea (3×20 kg N \cdot ha⁻¹) in a solution + chelate nickel + $\text{MgSO}_4 \cdot \text{H}_2\text{O}$;
4. urea (3×19.9 kg N \cdot ha⁻¹) in a solution + Plonvit Z + $\text{MgSO}_4 \cdot \text{H}_2\text{O}$;
5. urea (3×19.9 kg N \cdot ha⁻¹) in a solution + Plonvit Z + chelate nickel + $\text{MgSO}_4 \cdot \text{H}_2\text{O}$.

The concentration of urea solution used in the different development stages of plants was: I – 15 %, II – 7.5 % and III – 5 %. Magnesium sulphate was used only in the 1st

spring period (3 % solution of magnesium sulphate monohydrate). Plonvit Z was used in quantities of $1 \times \text{dm}^3 \text{ ha}^{-1}$, and Ni chelate $1 \times \text{dm}^3 \text{ ha}^{-1}$ ($5 \text{ g Ni} \cdot 1 \text{ dm}^{-3}$). The content of elements [$\text{g} \cdot \text{kg}^{-1}$] in Plonvit Z was as follows:

N	Mg	S	Mn	Fe	Zn	Cu	B	Ti	Mo	Na
100	24	20	11	10	10	9	0.7	0.1	0.05	13

Plant protection products Juwel TT 483 SE (epoksykonazol $83 \text{ g} \cdot \text{dm}^{-3}$, krezoksym metylowy $83 \text{ g} \cdot \text{dm}^{-3}$, fenpropimorf $317 \text{ g} \cdot \text{dm}^{-3}$), Tango Star 334 SE (fenpropimorf $250 \text{ g} \cdot \text{dm}^{-3}$, epoksykonazol $84 \text{ g} \cdot \text{dm}^{-3}$), Maraton 375 SC (pendimetalina $250 \text{ g} \cdot \text{dm}^{-3}$, izoproturon $125 \text{ g} \cdot \text{dm}^{-3}$), Alert 374 SC (flusilazol $125 \text{ g} \cdot \text{dm}^{-3}$, karbendazym $250 \text{ g} \cdot \text{dm}^{-3}$) were used.

The weather conditions in the years of experiments were different. The rainfalls were quite diverse, and the air temperature in both years was similar to average from perennial. In year 2003 during vegetation period draught occurred, but rainfalls which occurred in September and October greatly increase the humidity of soil as precipitation was greater than perennial average. In those weather conditions the emergence of winter wheat was regular. Before entering the winter break period, plants made the spreading stage well. The spring (except from May) and the first half of the 2004 summer was characterized by higher precipitation than perennial average. In those conditions the development of winter wheat was proper. High rainfalls which occurred in July lengthen the vegetative period of about 2 weeks. In September and partly in October high rainfalls deficiency has taken place. In those conditions the emergence of winter wheat was delayed and very irregular. In the third decade of September and in the October rainfalls which occurred made the emergence more regular and made the development of plants further. April in year 2005 was characterized by high deficiency of rainfalls and the second decade of this month was cold with ground froze up to $-8 \text{ }^\circ\text{C}$ occurred. Higher rainfalls took place in May but with the periodical cold the vegetation was not getting better as expected. However, June was quite hot with heavy rains which made the harvest delayed.

The winter wheat was harvested 13.08.2004 and 18.08.2005. In the wheat grain we determined the total protein content with Kjeld-Tec Auto Plus 1030 apparatus, using the 6.25 conversion. Moreover, the composition and content of amino acids was measured by using an ion exchange chromatography Microtech 339 M apparatus in the Central Apparatus Laboratory of University of Life Sciences in Lublin. The results were statistically analysed with variance analysis method with a level of relevance equalling to 0.05. To compare the statistical significant differences the Tukey test was used.

Results and discussion

The differentiation for the total nitrogen content and amino acid protein composition under the influence of experimental factors proved to be statistically insignificant. Upon application of EDTA-Ni increased the amount and content of endogenous amino acids

as well as leucine and lysine in protein, while reducing the total protein content. Endogenous amino acids are very important in wheat, not only forms the protein but is also related to wheat protein quality. Li [12], has shown that gliadin contains proline, glutamic acid, and cysteine; albumin contains aspartic acid, arginine, and histidine; and globulin contains alanine, aspartic acid, glycine, and cysteine. Non-essential amino acids are also related to the gluten, which affect the wheat baking quality [13, 14].

The total protein content in both years of study was highest at sites 1, 2 and 4, where chelate nickel was not used. The variability of weather conditions in years had a significant impact for the total protein content in grain, as illustrated by the results of research across the country (Table 1).

Table 1

The content of total protein in winter wheat grain [$\text{g} \cdot \text{kg}^{-1}$]

Years	Average results of own study	Average results for country (winter varieties)*
2004	102.1	116.0
2005	121.0	123.0

* [15, 16].

The course of the weather in 2005 was more conducive for protein storage in wheat grain than in 2004, because the protein accumulation in grain, which gives it more desirable baking properties, is higher in small amounts of rainfall and high temperature during the period from heading until the wax maturity of wheat [17, 18]. In 2004, there was double the amount of precipitation in June than in 2005, and a lower temperature in July of up to a few degrees.

Table 2

The share of amino acids protein composition of grain of winter wheat [%]

Amino acids		Experimental objects				
		1	2	3	4	5
		Amino acids percentage in protein				
		Endogenous				
Asparagine	<i>Asp</i>	5.05	4.60	4.55	3.92	4.66
Tyrosine	<i>Tyr</i>	3.32	3.23	3.48	3.23	2.63
Arginine	<i>Arg</i>	4.40	4.57	4.82	4.53	4.60
Serine	<i>Ser</i>	4.53	4.18	4.04	3.72	4.23
Glutamine	<i>Glu</i>	30.06	29.23	30.41	26.68	30.33
Proline	<i>Pro</i>	4.93	5.36	5.38	4.78	5.81
Glycine	<i>Gly</i>	3.70	3.46	3.73	3.64	4.03
Alanine	<i>Ala</i>	3.06	2.91	3.07	2.89	3.31

Amino acids		Experimental objects				
		1	2	3	4	5
Amino acids percentage in protein						
Exogeneous						
Threonine	<i>Thr</i>	3.47	3.44	3.29	3.00	3.38
Valine	<i>Val</i>	4.21	3.75	4.08	3.90	4.09
Isoleucine	<i>Ile</i>	3.32	3.30	3.31	3.20	3.09
Leucine	<i>Leu</i>	7.93	7.54	7.98	7.21	8.17
Phenylalanine	<i>Phe</i>	6.31	6.28	6.04	5.79	5.94
Histidine	<i>His</i>	2.51	2.87	2.86	2.63	2.47
Lysine	<i>Lys</i>	2.49	2.57	2.75	2.55	2.72
Sulphur (exogenous)						
Cysteine sulfonic acid	$CySO_3H$	3.35	3.46	3.18	3.95	3.28
Methionine sulphone	$MeSO_3$	6.71	3.35	3.45	3.87	3.56
The sum of amino acids		95.94	94.04	96.37	89.41	96.23
Total protein [$g \cdot kg^{-1}$ d.m.]		107.55	116.5	108.3	112.4	103.95
LSD _(0.05) between objects – n.s.						

The different fertilization factors had an influence on the amount of amino acids in wheat protein (Fig. 1). Places where chelate Ni was used, were distinguished with the largest sum of amino acid; however, the use of Plonvit Z trace element fertilizer (object 4) was characterised with less than over 50 [$g \cdot kg^{-1}$] of the sum of amino acids in

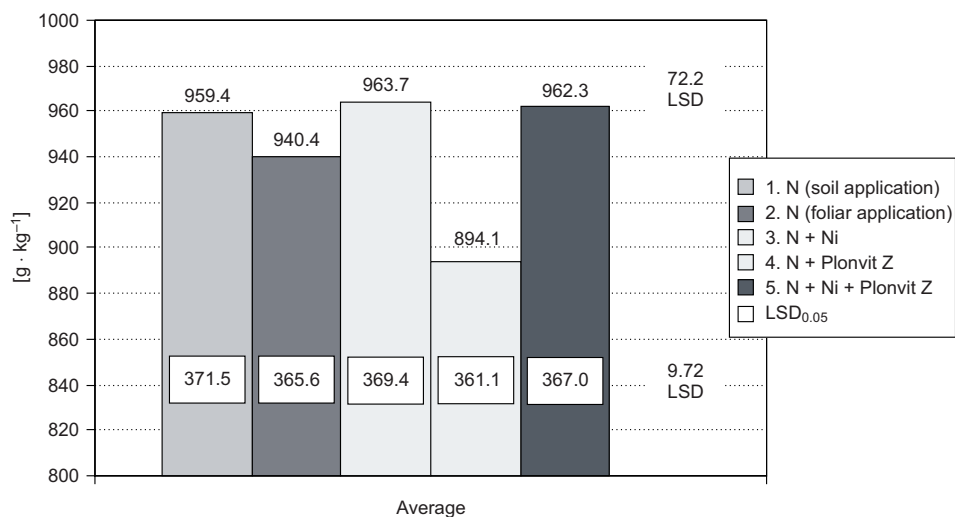


Fig. 1. The total sums of amino acids in protein of winter wheat (in white squares are exogenous sums)

relation to other objects. Among the essential exogenous amino acids, threonine, leucine and phenylalanine were reduced.

The lysine was limiting amino acid, which is usually the first limiting amino acid for cereals [19]. The largest of its contents were in chelate nickel addition. This element also affects the increase in leucine – amino acid involved in the process of muscle growth.

The largest sum of exogenous amino acid (among objects with foliar feeding) were characterised with the protein of wheat foliar fertilized with urea including EDTA-Ni(II) – 3 and 5 objects. The fertilizer trace element Plonvit Z influenced the reduction in the amount of these amino acids mainly due to reduction of threonine, leucine and phenylalanine content as well as endogenous amino acids as a result of low asparagine, serine, glutamine and proline content. The composition of grain protein in the last experimental object was characterized by highest content of proline, glycine, alanine and leucine amino acids but lowest isoleucine and histidine. Soil application of urea comparing with rest of objects had highest asparagine, serine, threonine, valine and CySO_3H but lowest arginine and lysine contents.

Conclusions

1. The fertilizer factors did not have a statistically significant effect on the total protein content and amino acid composition of winter wheat grain, but some trends did appear.
2. Both chelate Ni and Plonvit Z influenced the reduction in the amount of the total protein content in winter wheat grain in comparison with foliar feed with urea.
3. Under the influence of urea foliar application with chelate Ni, a gradual increase was witnessed in the amount of amino acids, mainly through the endogenous non-essential amino acids.
4. An addition of the fertilizer trace element Plonvit Z to urea resulted in a decrease in the amount of amino acids both endo- and exogenous (threonine, leucine and phenylalanine).

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ZAWARTOŚĆ AZOTU I SKŁAD AMINOKWASOWY BIAŁKA ZIARNA PSZENICY OZIMEJ DOKARMIANEJ DOLISTNIE MOCZNIKIEM I NAWOZAMI MIKROELEMENTOWYMI

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Abstrakt: Doświadczenie polowe przeprowadzono w latach 2003–2005 na glebie płowej (Haplic Luvisols), kompleks pszenney wadliwy metodą bloków losowych w 4 powtórzeniach. Gleba miała kwaśny odczyn ($\text{pH}_{\text{KCl}} - 5,3$) i naturalną zawartość pierwiastków śladowych. Rośliną testową była pszenica ozima odmiana jakościowa (klasa A) – Rywałka. Celem badań było określenie wpływu dokarmiania dolistnego mocznikiem oraz chelatem niklu EDTA-Ni(II) i Plonvitem Z na zawartość azotu ogółem i skład aminokwasowy ziarna pszenicy ozimej. Zróżnicowanie zawartości azotu ogółem i składu aminokwasowego białka pod wpływem czynników doświadczalnych okazało się statystycznie nieistotne. Pod wpływem EDTA-Ni zwiększeniu ulegała suma aminokwasów endogennych oraz zawartość leucyny i lizyny w białku, zaś zmniejszeniu zawartość białka ogólnego.

Słowa kluczowe: azot, aminokwasy, pszenica ozima, dolistne dokarmianie, mocznik, mikroelementy