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COMPARATIVE ANALYSIS OF THE BIOLOGICAL VALUE OF PROTEIN OF *Chenopodium quinoa* WILLD. AND *Chenopodium album* L. PART II. AMINO ACID COMPOSITION OF THE GREEN MATTER PROTEIN

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Abstract. Material for research was obtained from the field experiment conducted at the Experimental Station of Cultivar Testing in Chrząstowo (53°09' N; 17°35' E) in the years 2006-2008. Amino acid composition in the vegetation matter was determined after acid hydrolysis with high performance liquid chromatography (HPLC). High content of the total protein was found in the green matter. Profile analysis proved that protein of the analyzed species differs from each other in composition. However, it is characterized by a similar content of three exogenous amino acids (phenylalanine, isoleucine, valine) as well as two endogenous amino acids (alanine and serine). *Chenopodium quinoa* is distinguished from *Chenopodium album* by higher biological value of protein in the green matter, measured with the essential amino acid index (EAAI). The analyzed species are characterized by high yields of exogenous and endogenous amino acids in cultivation for green matter. *Chenopodium quinoa* as a crop plant is characterized by higher forage value than *Chenopodium album*. Small proportion of *Chenopodium album* in *Chenopodium quinoa* canopy will not significantly affect the change of quality of the produced green matter.

Key words: amino acid content, essential amino acid index, EAAI, limiting amino acid index, protein quality, pseudocereals

INTRODUCTION

An easy and low-cost protein production of high quality still poses a problem. It concerns not only seeds, but also green matter which after satisfying some requirements of nutritive value may be used for dried material production. The number of plant species that can be used for this purpose is limited. Most often these are plants from the family Fabaceae. *Chenopodium quinoa*, the new offered crop plant species in Poland, is

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equaled with regard to nutritive value by this group of plants, and even exceeds it, which gives greater possibilities of its application [Ahamed et al. 1998, Jacobsen 2003]. The use of plants from the family *Chenopodiaceae*, which *Chenopodium quinoa* belongs to, for green matter is common in Europe. However, in South America it is traditionally cultivated for seeds. *Chenopodiaceae* family includes pasture and industrial species of great economic significance like: fodder beet, sugar beet, red beet, garden beet and spinach [Grochowski 1996] and *Chenopodium album*, whose young leaves were used for salads, similarly to spinach, and for soups. Protein content in the green matter of *Chenopodium quinoa* is higher than in seeds. Also the green matter yield of this species is high. In Germany the yield of app. 70 $t \cdot ha^{-1}$ was obtained [Ritter 1986]. It shows that protein yield in cultivation for green matter is much higher than in the case of seed cultivation. Because of this and with regard to high protein quality of *Chenopodium quinoa* [Gorinstein et al. 2002, Drzewiecki et al. 2003, Balzotti et al. 2008], which is described as an alternative species, production of dried material from this species is subsidized in European Union countries. Unfortunately, because of the fact that this species used to be unknown in Poland, it has not been included in the list of subsidized plants. Deficiency of proteinaceous raw materials of plant origin is high, not only in Poland [Prusiński and Kotecki 2006]. One of the causes of a sudden increase of demand for protein are restrictions on application of meals of animal origin in animal nutrition, which resulted in a deficiency of this component on the market. This gap is currently filled by soybean. Protein from *Chenopodium* may be an alternative to the imported genetically modified soybean.

The reason for research concerning comparisons of *Chenopodium quinoa* and *Chenopodium album* is not only the nutritive value of *Chenopodium quinoa*, but also lack of literature concerning *Chenopodium album* despite its common occurrence. Under Polish conditions *Chenopodium album* is a resistant weed in the cultivation of *Chenopodium quinoa*. There are no such selective herbicides which would control *Chenopodium album*, not harmful at the same time to the crop plant. In cultivation for seeds, it has to be mechanically removed, as it competes for nutrients as well as for other environmental factors. In cultivation for green matter this treatment could be omitted. Assuming that *Chenopodium album* as a domesticated plant has a similar nutritive value as *Chenopodium quinoa*, its coexisting in a small number in a canopy would not significantly decrease the quality of green matter, and thus it would reduce costs of its control. On the basis of this research hypothesis was formulated. It assumed that green matter of *Chenopodium quinoa* and *Chenopodium album*, which would be used in nutrition, should be characterized by a similar amino acid composition of protein. It is indicated by the fact that it belongs to the same botanical genus, and also by many morphological and developmental similarities.

The aim of the research was determination of differences in the content of particular amino acids between *Chenopodium quinoa* and *Chenopodium album*, especially the proportion of exogenous amino acids. From the point of view of practical application, it seems to be important to know the amino acid yield of both species cultivated in Poland.

MATERIAL AND METHODS

Field experiment with cultivation for green matter was carried out at the Experimental Station of Cultivar Testing in Chrząstowo (53°09' N; 17°35' E) in the years 2006-2008. The experiment was set up as a single-factor experiment in a randomized subblock design, in four replications, on soil of the very good rye complex, according to methodology presented in the first part of the paper [Gęsiński and Nowak 2011]. Standard and dates of sowing were the same as in the cultivation for seeds. Two species were cultivated for green matter: *Chenopodium quinoa* cv. Sandowal and *Chenopodium album*. Characteristics of agricultural-functional properties of *Chenopodium quinoa* cv. Sandowal are presented in the paper of Gęsiński and Nowak [2011].

Harvesting of green matter was conducted at the milk stage. For both *Chenopodium* species it took place every year in the middle of August. Green matter yields were as follows: *Chenopodium quinoa* at the level of app. 43.5 $t \cdot ha^{-1}$, and *Chenopodium album* 38.2 $t \cdot ha^{-1}$. Forage samples were dried at a temperature of 50°C.

In dry matter samples from forage of both *Chenopodium* species, the total nitrogen content was determined with Kjeldahl method and next, using the conversion factor 6.25, the total protein content was calculated. Amino acid content in protein was determined with HPLC method after acid hydrolysis. On the basis of amino acid composition, the total of exogenous and endogenous amino acids was calculated. On the basis of chicken egg composition, for green matter protein of the analyzed *Chenopodium* species the essential amino acid index (EAAI) was calculated as well as the limiting amino acid index (CS). Detailed method of amino acid determination and of calculating indexes which were mentioned above is presented in the first part of the paper [Gęsiński and Nowak 2011].

Statistical evaluation of obtained results was done on the basis of comparison of the amino acid content, based on protein profiles of both species with multivariable method, profile analysis. For profile analysis, Cohen's similarity coefficient r_c was used, whose formula is presented in the first part of the paper [Gęsiński and Nowak 2011]. Next, formation of variations of particular amino acids in protein, in green matter and of the yield in both *Chenopodium* species, was compared with the use of t-student test. Results of this analysis are presented in tables.

RESULTS

It was found that the analyzed *Chenopodium* species were characterized by large proportion of the total protein in the dry matter of forage: *Chenopodium quinoa* – 216 $g \cdot kg^{-1}$ and *Chenopodium album* – 203 $g \cdot kg^{-1}$.

Comparative analysis of protein profiles of the green matter from *Chenopodium quinoa* and *Chenopodium album*, based on the amino acid proportion (Fig. 1), proved lack of similarity on the basis of the calculated Cohen's similarity coefficient r_c (coefficient value 0.334). Analysis of variations of particular amino acids in the green matter protein of *Chenopodium quinoa* and *Chenopodium album* proved lack of significant differences between these species with regard to the content of three exogenous amino acids: phenylalanine, isoleucine and valine. Proportion of other exogenous amino acids was significantly higher in the protein of *Chenopodium quinoa* than in the protein of *Chenopodium album* (Table 1). In consequence, the total of

exogenous and endogenous amino acids as well as the total of all amino acids was also significantly higher in the protein of *Chenopodium quinoa* than *Chenopodium album*. Both species did not differ significantly in the proportion of alanine and serine. Higher content of glycine, glutamic acid and tyrosine was found in the protein of *Chenopodium quinoa*, while of aspartic acid in the protein of *Chenopodium album*. Lack of significant differences between the analyzed species with regard to the content of isoleucine, valine and serine was proved. Proportion of other amino acids was also higher in *Chenopodium quinoa* than in *Chenopodium album*. The exception was aspartic acid whose content in the green matter protein of *Chenopodium quinoa* was lower than in *Chenopodium album*.

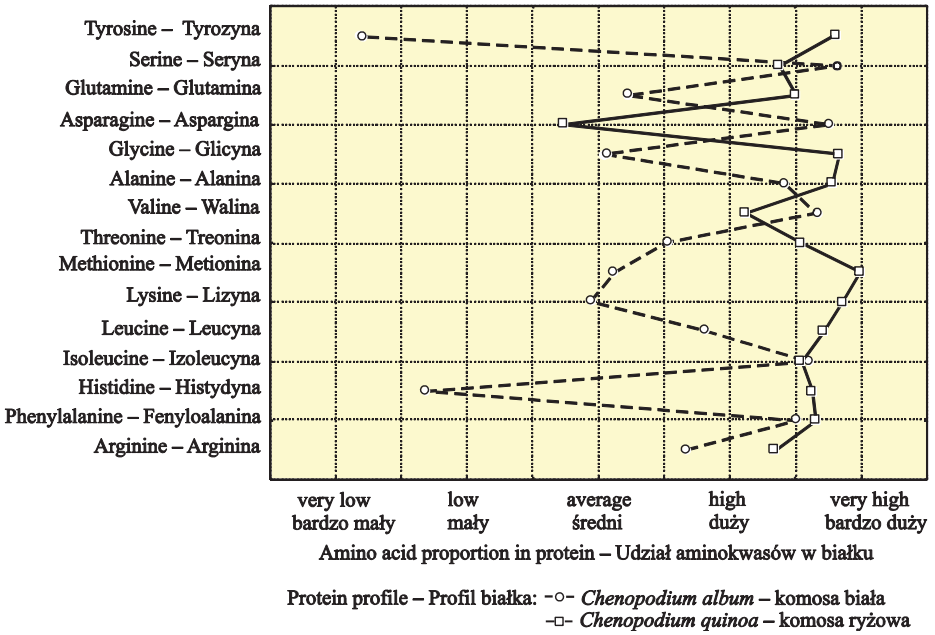

Fig. 1. Profiles of amino acid proportion in the green matter protein of *Chenopodium quinoa* and *Chenopodium album*

Rys. 1. Profile udziału aminokwasów w białku zielonej masy komosy ryżowej i komosy białej

Significant differences of the amino acid yields between the species were found (Table 1). The exogenous amino acid yield of *Chenopodium album* was lower than that of *Chenopodium quinoa* by 195 kg·ha^{-1}, and endogenous by 199 kg·ha^{-1}. Finally, the amino acid yield of *Chenopodium quinoa* was higher by 394 kg·ha^{-1} than that of *Chenopodium album*.

On the basis of the essential amino acid index, higher protein value of *Chenopodium quinoa* than *Chenopodium album* was found (Table 2). The value of the limiting amino acid index (CS) allows to state that methionine and isoleucine are amino acids which reduce the nutritive value of protein from the green matter of both *Chenopodium quinoa* and *Chenopodium album*.

Table 1. Content and yield of amino acids in the green matter of *Chenopodium quinoa* cv. Sandowal and *Chenopodium album*
Tabela 1. Zawartość i plon aminokwasów w zielonej masie komosy ryżowej odmiany Sandowal i komosy białej

Amino acid- Aminokwas	Content in protein – Zawartość w białku g·16 g^{-1} N			Content in green matter Zawartość w zielonej masie g·kg^{-1}			Yield – Plon kg·ha^{-1}		
	Chenopodium quinoa komosa ryżowa	*Chenopodium album* komosa biała	$LSD_{0.05}$ $NIR_{0,05}$	*Chenopodium quinoa* komosa ryżowa	*Chenopodium album* komosa biała	$LSD_{0.05}$ $NIR_{0,05}$	*Chenopodium quinoa* komosa ryżowa	*Chenopodium album* komosa biała	$LSD_{0.05}$ $NIR_{0,05}$
Arginine – Arginina	5.98	5.56	0.38	12.92	11.29	0.80	138.7	108.2	8.14
Phenylalanine – Fenyloalanina	4.62	4.56	ns – ni	9.98	9.26	0.56	107.2	88.8	5.71
Histidine – Histydyna	2.76	2.14	0.46	5.96	4.34	0.97	64.0	41.7	9.85
Isoleucine – Izoleucyna	2.62	2.64	ni	5.66	5.36	ns – ni	60.8	51.4	8.26
Leucine – Leucyna	7.17	6.62	0.34	15.49	13.44	0.71	166.3	128.9	7.28
Lysine – Lizyna	5.78	4.98	0.53	12.48	10.11	1.11	134.1	96.9	11.35
Methionine – Metionina	1.12	0.89	0.14	2.42	1.81	0.29	26.0	17.3	3.00
Threonine – Treonina	4.37	4.03	0.23	9.44	8.18	0.48	101.4	78.5	4.93
Valine – Walina	3.08	3.24	ns – ni	6.65	6.58	ns – ni	71.5	63.1	6.25
Σ exogenous amino acids Σ aminokwasów egzogennych	37.5	34.7	2.23	81.0	70.4	4.68	870	675	47.77
Alanine – Alanina	4.05	3.92	ns – ni	8.75	7.96	0.72	94.0	76.3	7.34
Glycine – Glicyna	6.35	5.38	0.62	13.72	10.92	1.30	147.3	104.7	13.28
Aspartic acid Kwas asparaginowy	4.46	5.71	0.38	9.63	11.59	0.80	103.5	111.2	8.14
Glutamic acid Kwas glutaminowy	9.16	8.29	0.26	19.79	16.83	0.55	212.5	161.4	5.57
Serine – Seryna	3.75	3.90	ns – ni	8.10	7.92	ns – ni	87.0	75.9	9.06
Tyrosine – Tyrozyna	7.57	4.70	0.65	16.35	9.54	1.37	175.6	91.5	13.92
Σ endogenous amino acids Σ aminokwasów endogennych	35.3	31.9	1.20	76.2	64.8	2.52	820	621	25.70
Σ studied – Σ badanych	72.8	66.6	2.16	157.2	135.2	4.54	1690	1296	46.27

ns – ni – non-significant differences – różnice nieistotne

Table 2. Values of the essentials amino acid index (EAAI) and limiting amino acid index (CS) of the green matter protein in *Chenopodium quinoa* and *Chenopodium album*.
Tabela 2. Wartości wskaźnika aminokwasów egzogennych (EAAI) i wskaźnika aminokwasów ograniczających (CS) białka zielonej masy komosy ryżowej i komosy białej

Index – Wskaźnik	*Chenopodium quinoa* Komosa ryżowa	*Chenopodium album* Komosa biała
EAAI ($LSD_{0.05}$ – $NIR_{0,05}$ 2,41)	67.0	61.1
CS I – methionine – metionina	31	25
CS II – isoleucine – izoleucyna	38	38

DISCUSSION

In European Union countries, similarly to Poland, there occurs high deficiency of proteinaceous raw materials of plant origin [Prusiński and Kotecki 2006]. Annually, the demand for high-protein components in Poland is in 80% covered by import of almost 2 million tons of soybean meal, which is obtained mainly from the genetically modified soybean cultivars [Święcicki et al. 2007]. Thus, it seems reasonable to introduce *Chenopodium quinoa* into cultivation in Poland as a valuable high-protein product. Possibilities of using *Chenopodium quinoa* in nutrition technology are great. This plant may be treated as a cereal and vegetable [Haber 1996]. Multidirectional application of this species results from its properties, but also from retaining its primary traits. Green matter of *Chenopodium quinoa* is a valuable high-protein product [Jacobsen 2003], which is another argument in favor of cultivation of this species in Poland. Total protein concentration in vegetation matter is app. 216 $g \cdot kg^{-1}$ and is higher than in seeds, in which it may range from 126 $g \cdot kg^{-1}$ to even in some cases 190 $g \cdot kg^{-1}$ [Ahamed et al. 1998, Aufhammer 2000, Balzotti et al. 2008]. High value of this product caused that production of dried matter from *Chenopodium quinoa* is subsidized in European Union countries. One of the most important criteria of the biological value of the yields [Klupczyński 1986, Domska and Rogalski 1993, Domska 1996], especially of plants cultivated for feed purposes and as raw materials for agricultural-food industry, is the content and quality of protein, determined on the basis of its amino acid composition. Similarly to seeds, green matter of both *Chenopodium quinoa* and *Chenopodium album* contains protein of well-balanced composition [Balzotti et al. 2008]. The greatest proportion is of lysine, though it is lower in the green matter than in seeds. High concentration of lysine is the result of a combined effect of its synthesis and accumulation in a soluble and protein form [Varisi et al. 2008]. Leaves of *Chenopodium quinoa* have been identified as the predominant site of nitrate reduction in this species. Profile analysis of amino acids in leaves and roots also proved the significant role of the soluble glutamine as a nitrogen transporting compound [Varisi et al. 2008]. When comparing amino acid content in the green matter protein of *Chenopodium quinoa* and *Chenopodium album* with its content in seeds [Gorinstein et al. 2002, Drzewiecki et al. 2003, Balzotti et al. 2008], some differences should be noted. The content of phenylalanine, isoleucine, leucine, threonine and valine in the green matter protein is even higher than in the protein of seeds. These results are in accordance with those obtained by Ahamed et al. [1998] as well as by Aufhammer [2000]. The total of exogenous amino acids in the green matter is lower but it results from the lower arginine content (app. 44%). The content of this amino acid in seeds is very high and,

except lysine and leucine, over twice exceeds other exogenous amino acids in respect of the amount. From endogenous amino acids in the green matter protein of both species there is also more alanine, glycine, aspartic acid and tyrosine than in seeds. In consequence, the total of endogenous amino acids is higher than in seeds, and the total of all studied amino acids for *Chenopodium quinoa* is the same both in the green matter and in seeds, and for *Chenopodium album* it is lower. Comparing values of the limiting amino acid index in green matter protein of the analyzed species with seeds, it can be stated that proportions between amino acids are more equaled in the green matter. Isoleucine and valine, which in seeds are the limiting amino acids, in the green matter protein have a higher value of the limiting amino acid index (CS). Nevertheless, isoleucine is the second limiting amino acid, while methionine is the first one. Comparing biological value of protein of the studied *Chenopodium* species with green matter of e.g. winter barley at the milk stage it should be stated that it is comparable but its concentration in the yield of barley is over twice as low (107 $g \cdot kg^{-1}$) [Barczak and Nowak 2006]. The above analysis results support the idea of introducing *Chenopodium quinoa* into cultivation not only because of the high biological value of the green matter protein, but also because of its high concentration. Own research results show that presence of a small proportion of *Chenopodium album* in the canopy of *Chenopodium quinoa* will not significantly affect the change of quality of the produced green matter. It is important as it allows cost reduction connected with weed control of the plantation.

High biological value of the green matter protein of *Chenopodium quinoa* and *Chenopodium album* indicates the alternative possibility of choice of production of this component compared with the seed production. It is confirmed not only by higher protein content in the green matter than in seeds, but first of all higher amino acid yields. In cultivation for green matter, *Chenopodium quinoa* gives over six times higher exogenous amino acid yields compared with the one cultivated for seeds. Similar relations concern endogenous amino acids and the yield of all amino acids. The amino acid yields of *Chenopodium album* in cultivation for green matter are even over eight times higher compared with the one cultivated for seeds. Regardless of this, the yield of all amino acids of *Chenopodium quinoa* in cultivation for green matter is over 30% higher than of *Chenopodium album*. *Chenopodium quinoa* is a species of higher biological value of the green matter protein than *Chenopodium album* and more yield-forming.

CONCLUSIONS

1. *Chenopodium quinoa* and *Chenopodium album* were characterized by high protein content in the green matter: 216 $g \cdot kg^{-1}$ and 203 $g \cdot kg^{-1}$ respectively, per dry matter.
2. On the basis of profile analysis it was stated that the green matter protein of the analyzed species differed in the amino acid composition. However, it was characterized by a similar content of three exogenous amino acids (phenylalanine, isoleucine, valine) as well as two endogenous amino acids (alanine and serine).
3. *Chenopodium quinoa* is distinguished by a higher biological value of the green matter protein measured with the essential amino acid index (EAAI).

4. Both species were characterized by high yield of exogenous and endogenous amino acids in cultivation for green matter. However, *Chenopodium quinoa* exceeded *Chenopodium album* with regard to this.

5. Presence of a small proportion of *Chenopodium album* in the canopy of *Chenopodium quinoa* will not significantly affect the change of quality of the produced green matter.

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ANALIZA PORÓWNAWCZA WARTOŚCI BIOLOGICZNEJ BIAŁKA KOMOSY RYŻOWEJ (*Chenopodium quinoa* WILLD.) I KOMOSY BIAŁEJ (*Chenopodium album* L.) CZ. II. SKŁAD AMINOKWASOWY BIAŁKA ZIELONEJ MASY

Streszczenie. Materiał do badań uzyskano z doświadczenia polowego przeprowadzonego w Stacji Badawczej Oceny Odmian w Chrząstowie (53°09' N; 17°35' E) w latach 2006--2008. Zawartość aminokwasów w masie wegetatywnej oznaczano po hydrolizie kwasowej metodą chromatografii wysokociśnieniowej HPLC. Stwierdzono wysoką zawartość białka ogólnego w zielonej masie. Analiza profilowa wykazała, że białko analizowanych gatunków różni się między sobą składem. Charakteryzuje się jednak podobną zawartością trzech aminokwasów egzogennych (fenyloalaniny, izoleucyny, waliny) oraz dwóch endogennych (alaniny i seryny). Komosa ryżowa różni się od komosy białej wyższą wartością biologiczną białka zielonej masy, mierzoną wskaźnikiem aminokwasów egzogennych (EAAI). Analizowane gatunki charakteryzują się wysokimi plonami aminokwasów egzogennych i endogennych w uprawie na zieloną masę. Komosa ryżowa jako roślina uprawna charakteryzuje się większą wartością zielonki niż komosa biała. Nieduży udziału komosy białej w łanie komosy ryżowej nie wpłynie znacząco na zmianę jakości produkowanej zielonej masy.

Słowa kluczowe: EAAI, jakość białka, pseudozboża, wskaźnik aminokwasu ograniczającego, wskaźnik aminokwasów egzogennych, zawartość aminokwasów

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