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Blue lupine seeds protein content and amino acids composition

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ABSTRACT

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Lupine seeds are promising soy replacement in food products and feeds. Eighteen cultivars of blue lupine seeds were examined to indicate the one most favourable in protein content and quality. Two parameters were studied, i.e. total protein content (with the Kjeldahl method) and amino acids composition (with the ultra performance liquid chromatography technique). Both parameters studied were variable and depended both on the cultivar and on the place of cultivation. Protein content was in the range of 28–41% and the worst cultivar, regardless of cultivation place, was cv. Kalif (average total protein content 29.37 ± 1.14%), while the best cultivar was Boruta (average total protein content 37.43 ± 0.98%). The blue lupine seeds were rich especially in leucine (5.3 ± 0.5–9.7 ± 0.5 g/16 g N), threonine (2.4 ± 0.7–4.9 ± 0.1 g/16 g N) and lysine (2.7 ± 0.4–5.6 ± 0.1 g/16 g N). The richest among all amino acids studied were the cv. Bojar seeds from Wiatrowo and cv. Oskar from Przebędowo. It was not possible to choose one cultivar preferable from the nutritional point of view, because a strong influence of cultivation place on protein content and quality was observed, especially as to the precipitation sum.

Keywords: legume seeds; protein nutritional value; chemical score; protein composition; weather conditions

A positive impact of legumes on the environment and using them as an additional source of protein in feeds are strongly emphasized in a discussion on the legume crop recovery in Europe. At the same time, cereals in Europe constitute ~70% of the crop rotation (compared to 46% in the USA) (Sońta and Rekiel 2016), and European feed management strongly depends on the import of soybean meal obtained from seeds after oil extraction. Because the harvesting of soy in Europe is small, an increasing market interest in native legumes is observed. It is supposed to make the economy of individual countries at least partially independent of imported soybean meal. The value of soy meal import in Poland reaches the level of 2–3 million tons per year, and the expected goal is to reduce it by ~30% (www.farmer.pl).

One of the most interesting legumes, rich in protein, is lupine, perfectly developing in the Central European climate, which is thus convenient for soy replacement. Lupine seeds contain a huge amount of protein – up to 42% for some species and cultivars. The interest in lupine cultivation is related not only to its use for feeding purposes (Sobotka et al. 2016). Due to the scientifically proven health-promoting properties of lupine seeds, an increasing interest in food production with lupine seeds content is observed in Europe. Currently, the production of food containing lupine ingredients in European Union is ~500.000 t. Its addition in this food is relatively small, even less than 5% (Department of Agriculture and Food 2016). The food is mainly produced for people with special dietary needs: in athlete nourishment and various foods dedicated to celiac individuals, vegans and vegetarians. The hypocholesterolemic and antidiabetic activity of the lupine proteins

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was also demonstrated, which suggests their application in food for special medical purposes (Fontanari et el. 2012, Millán-Linares et al. 2014, Bouchoucha et al. 2016).

Due to the composition of lupine protein in some South American countries, addition of lupine seeds into the basic food products is regulated by governmental programs. Of course, these proteins, like other plant proteins, are deficient in some exogenous amino acids, but they may supplement very well other vegetable proteins (e.g. cereal proteins) in lysine, arginine, leucine, glutamic and aspartic acid (Pisarikova et al. 2008, Stanek et al. 2012, Khalid et al. 2016).

Finding the lupine cultivars with higher nutritional values could increase the use of lupine as a food and feed component and this will certainly result in the growing interest of farmers (Pisarikova et al. 2008, Sujak et al. 2016). Thus, the aim of the study was to evaluate the variability of amino acid composition in 18 cultivars of blue lupine registered in Poland grown in controlled conditions in two cultivation areas, to indicate the potentially most nutritionally valuable cultivar by comparing total protein and selected amino acids content in the studied seeds.

MATERIAL AND METHODS

Blue lupin seeds of 18 cultivars (Table 1) registered in the National Varieties Register (in Poland) in the years 1999–2015 were obtained from two places of cultivation (Przebędowo and Wiatrowo, Wielkopolska Province), in the year of harvest 2015 and 2016. These cultivars differ in morphological characteristics (e.g. type of growth) and utility features (e.g. alkaloids content) and maturation form (Table 1). The vegetation period of examined cultivars lasted from 104 to 121 days.

The cultivation conditions of the lupine plants were controlled and were identical for these two experimental farms. Lupine was planted on Luvisols developed from glacial tills (the class of the soil according to the Polish classification was 3B in Wiatrowo and 4B in Przebędowo). Winter triticale was used as a forecrop in both years in Wiatrowo and in 2016 in Przebędowo, where maize was used in 2015. Standard pre-sowing fertilization was applied i.e. in Wiatrowo: N – 18 kg/ha, P – 70 kg/ha, K – 100 kg/ha, in Przebędowo: P – 45 kg/ha, K – 72 kg/ha. Weather conditions (daily precipitation sum and average daily temperature) were controlled by the local meteorological points.

Table 1. Characteristics of the studied cultivars of lupine (COBOR 2015 and experimental data)

	Cultivar	Type of growth	Alkaloids content (%)	Group of maturity	Flowering		Maturation time
					onset	end	
1	Zeus	traditional	0.016	early	June, 5th–6th	July, 5th	July, 29th–31th
2	Neptun	traditional	0.017	early	June, 3th–4th	July, 4th – 5th	July, 29th–30th
3	Heros	traditional	0.020	early	June, 5th–6th	July, 5th	July, 29th–August 4th
4	Regent	self–completing	0.013	early	June, 3th–4th	July, 4th	July, 28th–August 2th
5	Emir	traditional	0.013	early	June, 5th–6th	July, 6th	August, 5th
6	Kalif	traditional	0.017	late	June, 5th–6th	July, 8th	August, 3th–6th
7	Dalbor	traditional	0.019	early	June, 5th–6th	July, 6th	July, 28th–August 3th
8	Karo	traditional	1.167	medium	June, 6th–7th	July, 5th	August, 4th–6th
9	Kurant	traditional	0.024	medium	June, 4th–5th	July, 6th	July, 31th–August 5th
10	Bojar	traditional	0.016	medium	June, 5th	July, 6th	July, 30th–August 6th
11	Graf	traditional	0.020	late	June, 5th–6th	July, 6th	August, 3th–4th
12	Oskar	traditional	1.001	early	June, 6th–8th	July, 8th	August, 5th–6th
13	Boruta	self–completing	0.019	medium	June, 5th–8th	July, 6th	July, 26th–30th
14	Kadryl	traditional	0.021	late	June, 5th–8th	July, 5th	July, 30th–August, 5th
15	Tango	traditional	0.028	early	June, 6th	July, 8th	August, 6th–10th
16	Lazur	traditional	0.014	early	June, 5th–6th	July, 5th	July, 29th–30th
17	Salsa	traditional	0.018	early	June, 5th–6th	July, 6th	July, 30th–August 2th
18	Rumba	traditional	0.031	early	June, 6th	July, 7th	August, 6th–7th

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Total protein content was determined with the Kjeldahl method (FAO 2003).

Amino acid composition was determined after acidic hydrolysis (110°C, 23 h) (AOAC 2014). After the sample was evaporated at 80°C, dilution and derivatization of amino acids was conducted (with AccQ•Tag reagents, No. 186003836, Waters) according to the protocol obtained from the manufacturers.

Samples prepared in the presented manner were analyzed with ultra performance liquid chromatography (UPLC, Shimadzu Nexera 2.0, Kyoto, Japan) equipped with a binary solvent manager, an autosampler, a column heater, and a PDA detector (Kyoto, Japan). As a separation column AccQ-Tag Ultra C18 1.7 μm was used (2.1 mm i.d. × 100 mm, 1.7 μm particles, Waters). The column temperature was set at 55°C, the mobile phase flow rate was maintained at 0.6 mL/min. The non-linear separation gradient was used, formed by mixing 5% and 100% AccQ•Tag Ultra solvent (Waters). One microliter of sample was injected for the analysis. The PDA detector was set at 260 nm, with a sampling rate of 20 points/s.

A quantitative analysis of amino acids was performed with amino acid standards, which contained 2.5 μmol/mL for each amino acid in 0.1 mol/L HCl (088122, Waters). The standards were diluted 25 times with ultra pure water. Next, 10, 20 or 60 μL of the diluted standard were mixed with 70 μL of borate buffer and 20 μL of AccQ•Tag reagents, to conduct the standard amino acids derivatization. One microliter of the obtained sample was injected 5 times for the UPLC analysis, to prepare a calibration curve with the LabSolution program (Shimadzu Corp., Kyoto, Japan). Amino acids content was expressed in g/16 g N (which is equivalent to g/100 g of protein).

Data were processed using the Statistica 13.0 for Windows (Statsoft Inc., Tulsa, OK) package.

All analyses were repeated three times and data obtained were subjected to the analysis of variance (ANOVA). The significance of ANOVA was checked with the *F*-test. In case of significant differences, a post-hoc analysis (Tukey's test) was performed to determine homogeneous groups. The data were expressed as an average ± standard deviation.

RESULTS

The year 2015 was unfavourable for lupine yields, even worse than 2016. Weather conditions allowed for early sowing of legumes, but the temperatures in early April extended the lupine emergence. Weather in the later periods (Figures 1 and 2) decreased yielding and weight of thousand seeds. Poor quality of seeds was confirmed all over Poland and it resulted mainly from the water deficiency and high temperatures in July (COBOR 2015). Moreover, significant differences were noted in the precipitation sum between these two cultiva-

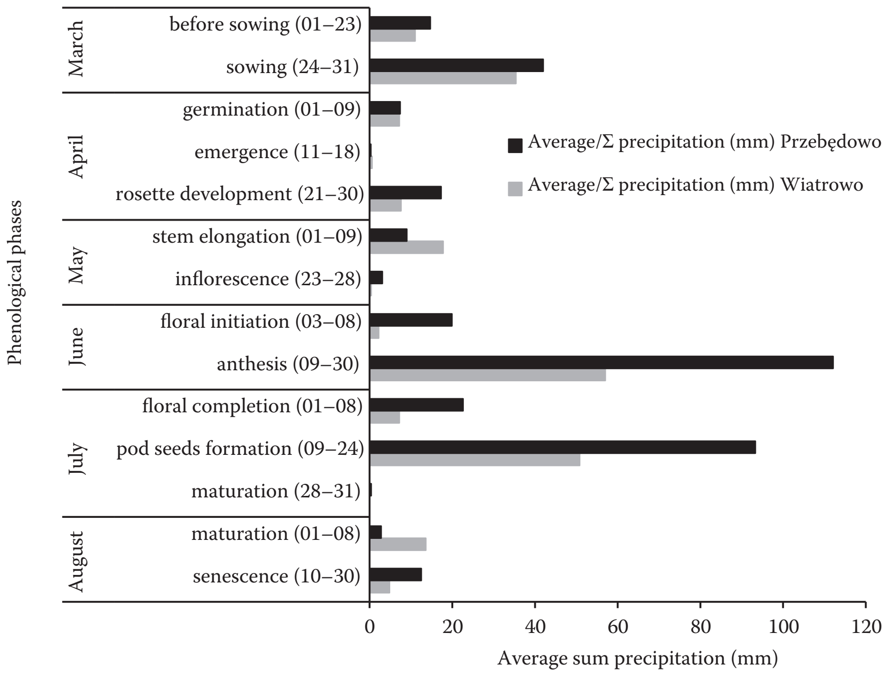

Figure 1. Precipitation sum from March to August 2015 noted in Przebędowo and Wiatrowo. Data recorded according to the vegetation period

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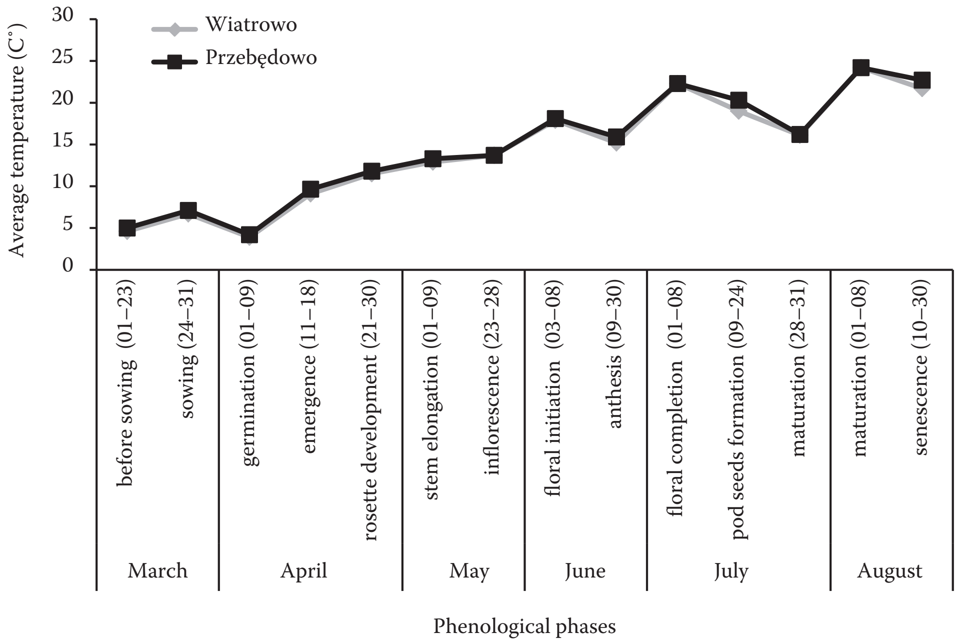

Figure 2. Average daily air temperature noted in Przebędowo and Wiatrowo from March to August 2015. Data recorded according to the lupine vegetation period

tion places (Figure 1). The air temperature in the period from March to August ranged from 5.0°C to 24.2°C in Przebędowo and 4.6°C to 24.2°C in Wiatrowo (Figure 2). The observed high temperatures in June and July could adversely affect lupine disease, but that was not investigated in the presented experiments.

The nutritional value of lupine seeds, both to humans and animals, results mainly from the quantity and quality of the seed proteins. An analysis of the total protein content showed that it varied in the examined materials and was dependent both on the cultivar and place of cultivation. Experiments confirmed high protein content in the examined blue lupine seeds (between 28% and 41% for the cultivars grown in Przebędowo, and between 29% and 39% in Wiatrowo) (Table 2). The worst cultivar in terms of the protein content, regardless of the field on which the cultivar had grown, was Kalif (average total protein content 29.37 ± 1.14%), while the best cultivar was Boruta (average total protein content 37.43 ± 0.98%). An unexpected effect was observed: cultivars with the lowest and the highest protein content differed depending on the place of cultivation. Even when the applied cultivation conditions were the same, the place of cultivation influenced the protein content in the derived seeds. The lowest content of protein in Wiatrowo was found in cvs. Salsa and Bojar seeds, the highest – in cv. Karo. In Przebędowo, the highest and lowest content of protein was

Table 2. Protein content (%) in seeds of the analysed blue lupine cultivars, depending on the place of cultivation

Cultivar	Wiatrowo	Przebędowo
Zeus^{A,B,C,D}	31.7 ± 0.2^{d,e,f}	30.7 ± 0.3^{c}
Neptun^{A,B,C,D,E,F}	35.2 ± 0.1^{n}	32.3 ± 0.5^{g,h,i}
Lazur^{A,B,C}	33.2 ± 0.1^{j,k}	28.2 ± 0.2^{a}
Heros^{A,B}	31.2 ± 0.1^{c,d}	29.7 ± 0.1^{b}
Rumba^{A,B,C,D}	31.3 ± 0.1^{d}	31.6 ± 0.5^{d,e}
Salsa^{A,B,C,D}	29.7 ± 0.1^{b}	32.7 ± 0.2^{g,h,i,j}
Regent^{E,F}	33.4 ± 0.1^{l,m}	40.7 ± 0.2^{t}
Emir^{A,B,C,D,E,F}	32.1 ± 0.1^{e,f,g}	34.2 ± 0.2^{m}
Kalif^{A}	30.7 ± 0.1^{c}	28.1 ± 0.1^{a}
Dalbor^{A,B,C,D,E}	32.9 ± 0.0^{i,j,k}	32.8 ± 0.2^{i,j,k}
Karo^{C,D,E,F}	38.7 ± 0.0^{s}	31.7 ± 0.1^{d,e,f}
Kurant^{D,E,F}	37.2 ± 0.2^{r}	34.1 ± 0.1^{m}
Bojar^{A,B,C,D}	29.4 ± 0.1^{b}	33.3 ± 0.1^{k,l}
Graf^{B,C,D,E,F}	30.6 ± 0.1^{c}	39.2 ± 0.2^{s}
Oskar^{A,B,C,D,E,F}	33.9 ± 0.0^{m}	32.2 ± 0.2^{f,g,h}
Boruta^{F}	35.9 ± 0.0^{o}	39.0 ± 0.3^{s}
Kadryl^{B,C,D,E,F}	32.2 ± 0.1^{f,g,h}	36.3 ± 0.2^{o,p}
Tango^{E,F}	36.2 ± 0.1^{o,p}	36.6 ± 0.2^{p}

Capital letters in the column show statistically significant differences at $P < 0.05$ between the studied cultivars (after one-factor analysis of variance); lowercase letters in the columns show statistically significant differences at $P < 0.05$ after a multivariate analysis of variance taking into account cultivars and place of cultivation

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found in cvs. Regent and Kalif, respectively. Many studies confirmed the influence of precipitation on maturation and protein content in the seeds of many crops, not only lupine (e.g. Podleśny and Podleśna 2011, Zielińska-Dawidziak et al. 2012). The highest water demand was observed during bud formation, through flowering to the formation of pods. Access to water is particularly important in the last days of vegetation (maturation seeds in pods), when it influences the final protein content in formed seeds. During that period, the precipitation sum was 0.4 mm in Przebędowo, and 0 mm in Wiatrowo (Figure 1, July 28–31), while the average air temperature was 16.2°C and 16.1°C, respectively (Figure 2). Precipitation differences were huge and could explain such variability of the determined protein content (COBOR 2015). The lack of influence of nitrogen fertilization on protein content in lupine seeds, which was suggested in cultivation directions, was confirmed.

However, for human nutrition not only the protein content, but also the amino acid composition is important (Sujak et al. 2016). Thus, it was decided to analyse the amino acids content variability in the examined cultivars. The content of 14 amino acids was studied (L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-phenylalanine, L-proline, L-serine, L-threonine, L-valine).

The most important for the nutritional aim is the content of essential amino acids (EAA) (listed here according to the U.S. National Library of Medicine (2015)). An analysis of the seeds obtained from Wiatrowo showed that the most valuable cultivars in terms of the nutritional value of the protein were: cvs. Kurant, Bojar and Tango (marked grey in Table 3). However, the results obtained for seeds from Przebędowo varied significantly. The most valuable cultivars here were: Dalbor, Oskar and Kadryl (marked grey in Table 3). This suggests, unfortunately, that it is impossible to identify cultivars with significantly higher content of EAA in seeds. There was also no correlation observed between the content of EAA and the total protein content. Among the cultivars that were considered best in terms of quality protein (i.e. cvs. Kurant, Bojar and Tango from Wiatrowo and cvs. Dalbor, Oskar and Kadryl from Przebędowo), cultivars with a high protein content can be found (such as cv. Kurant from Wiatrowo) and those of low content compared to the other studied cultivars (such as cv. Dalbor from Przebędowo). It cannot be also clearly indicated which cultivar was characterized by significantly lower contents of essential amino acids and the total protein in seeds obtained from both places of the experiment. An influence of climate conditions both on the protein content and quality in crops was proved many times (Adomas et al. 2005, Lizarazo et al. 2015); however, the inability to identify the desired cultivars in the studies presented is discouraging. The only one of amino acids which represents a stable content in the material examined is L-histidine.

Comparing the content of the analysed amino acids in the studied seeds and protein standard proposed by the FAO/WHO (2007), it can be concluded that blue lupine seeds studied were rich especially in leucine, threonine and lysine (Table 4), and may be proposed for supplementation of food raw materials poor in lysine (i.e. cereals), as it was frequently suggested (Mahmoud et al. 2012).

The lowest calculated chemical score among the studied amino acids was 42% for valine in the cv. Lazur seeds delivered from Wiatrowo (Table 4).

Table 4. Comparison between the content of essential amino acids in seeds of the studied blue lupine cultivars with their content in the FAO/WHO standard

Amino acid	Content in FAO/WHO standard (g/16 g N)	Wiatrowo		Przebędowo	
		content (g/16 g N)	calculated CS (%)	content (g/16 g N)	calculated CS (%)
Ile	3.69	2.1–6.0	57–162	2.7–5.4	73–146
Leu	5.26	5.3–9.7	101–184	5.6–9.9	106–188
Lys	3.72	2.7–5.6	73–151	3.3–5.4	89–145
Thr	2.69	2.4–4.9	89–182	3.1–4.8	115–178
Val	4.53	1.9–4.5	42–99	2.5–4.6	55–101

Ile – isoleucine; Leu – leucine; Lys – lysine; Thr – threonine; Val – valine; CS – chemical score

Vol. 64, 2018, No. 4: 147–155

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Table 3. Determined essential amino acids composition (g/16 g N) in seeds of the studied blue lupine cultivars grown in Wiatrowo and Przebędowo

Amino acid	Zeus	Neptun	Lazur	Heros	Rumba	Salsa	Regent	Emir	Kalif	Dalbor	Karo	Kurant	Bojar	Graf	Oskar	Boruta	Kadryl	Tango
Wiatrowo																		
Ile	2.7 ± 0.5^{a,b}	3.9 ± 0.3^{b,c,d}	3.7 ± 0.1^{a,b,c,d}	2.1 ± 0.0^{a}	6.0 ± 0.1^{e}	3.6 ± 1.4^{a,b,c,d}	4.1 ± 0.1^{b,c,d}	4.4 ± 0.5^{b,c,d}	3.5 ± 0.3^{a,b,c,d}	2.7 ± 0.2^{a,b}	3.6 ± 0.1^{a,b,c,d}	4.6 ± 0.6^{c,d,e}	4.7 ± 0.2^{c,d,e}	4.6 ± 0.1^{c,d,e}	4.0 ± 0.1^{b,c,d}	3.8 ± 0.8^{a,b,c,d}	3.7 ± 0.2^{a,b,c,d}	4.6 ± 0.2^{c,d,e}
Leu	5.8 ± 0.2^{a,b,c,d}	7.9 ± 0.1^{b,c,d,e,f,g,h,i,j}	5.3 ± 0.5^{a}	6.5 ± 0.3^{a,b,c,d,e,f}	7.0 ± 0.5^{a,b,c,d,e,f,h}	8.0 ± 0.9^{c,d,e,f,g,h,i}	8.1 ± 0.4^{c,d,e,f,g,h,i}	8.1 ± 0.4^{d,e,f,g,h,i}	7.5 ± 0.4^{a,b,c,d,e,f,g,h}	5.8 ± 0.0^{a,b,c}	5.8 ± 0.8^{a,b,c,d,e}	8.6 ± 0.4^{g,h,i}	9.7 ± 0.5^{h,i,}	8.1 ± 0.1^{d,e,f,g,h,i}	7.4 ± 0.2^{a,b,c,d,e,f,g,h}	7.3 ± 1.4^{a,b,c,d,e,f,g}	7.6 ± 0.7^{b,c,d,e,f,g,h}	8.2 ± 0.3^{e,f,g,h,i}
Lys	3.5 ± 0.3^{a,b,c}	4.5 ± 0.4^{a,b,c}	2.7 ± 0.4^{A}	3.4 ± 0.1^{a,b}	3.3 ± 0.3^{a,b}	4.8 ± 0.5^{a,b,c}	3.5 ± 0.1^{a,b,c}	4.0 ± 0.7^{a,b,c}	4.4 ± 0.^{2a,b,c}	3.4 ± 0.0^{a,b}	4.0 ± 0.7^{a,b,c}	4.9 ± 0.2^{b,c}	5.6 ± 0.1^{c}	5.1 ± 1.5^{b,c}	4.3 ± 0.1^{a,b,c}	4.4 ± 1.1^{a,b,c}	4.5 ± 0.5^{a,b,c}	4.9 ± 0.3^{a,b,c}
Thr	3.4 ± 0.2^{a,b,c,d,e}	4.0 ± 0.1^{b,c,d,e,f}	2.6 ± 0.2^{a,b}	3.1 ± 0.1^{a,b,c}	3.2 ± 0.5^{a,b,c,d}	4.5 ± 0.4^{c,d,e,f}	3.9 ± 0.3^{b,c,d,e,f}	4.3 ± 0.2^{c,d,e,f}	4.0 ± 0.0^{b,c,d,e,f}	2.4 ± 0.7^{a}	3.5 ± 0.3^{a,b,c,d,e,f}	4.5 ± 0.0^{c,d,e,f}	4.9 ± 0.1^{f}	4.3 ± 0.1^{c,d,e,f}	4.4 ± 0.6^{c,d,e,f}	4.0 ± 0.6^{b,c,d,e,f}	3.8 ± 0.2^{a,b,c,d,e,f}	4.4 ± 0.3^{c,d,e,f}
Val	2.5 ± 0.1^{a,b,c,d}	4.0 ± 0.1^{e,f}	1.9 ± 0.3^{A}	2.1 ± 0.1^{a,b}	2.2 ± 0.5^{a,b,c}	3.5 ± 1.0^{b,c,d,e,f}	4.0 ± 0.0^{d,e,f}	3.5 ± 0.4^{b,c,d,e,f}	3.8 ± 0.1^{d,e,f}	3.0 ± 0.3^{a,b,c,d,e}	3.1 ± 0.2^{a,b,c,d,e,f}	4.3 ± 0.6^{e,f}	4.4 ± 0.3^{e,f}	4.5 ± 0.5^{f}	3.7 ± 0.1^{c,d,e,f}	3.7 ± 0.7^{c,d,e,f}	3.6 ± 0.1^{b,c,d,e,f}	4.3 ± 0.4^{e,f}
His	2.8 ± 0.1^{a}	2.3 ± 0.2^{a}	2.9 ± 0.4^{a}	2.6 ± 0.1^{a}	2.4 ± 0.3^{a}	3.4 ± 0.0^{a}	2.7 ± 0.5^{a}	2.7 ± 0.3^{a}	2.4 ± 0.2^{a}	2.6 ± 0.7^{a}	2.4 ± 0.3^{a}	3.4 ± 0.1^{a}	3.0 ± 0.4^{a}	2.3 ± 1.3^{a}	2.8 ± 0.1^{a}	2.8 ± 0.5^{a}	2.5 ± 0.6^{a}	3.3 ± 0.0^{a}
Phe	3.6 ± 0.5^{a,b,c,d,e,f}	3.9 ± 0.2^{a,b,c,d,e,f}	2.6 ± 0.1^{a,b}	2.7 ± 0.2^{a,b,c}	2.5 ± 0.2^{a}	4.3 ± 0.3^{a,b,c,d,e,f}	3.3 ± 0.5^{a,b,c,d,e}	3.8 ± 0.2^{b,c,d,e,f}	3.7 ± 0.1^{a,b,c,d,e,f}	3.6 ± 1.0^{a,b,c,d,e,f}	2.5 ± 0.8^{a,b}	4.3 ± 0.0^{b,c,d,e,f}	4.9 ± 0.0^{e,f}	4.7 ± 0.4^{d,e,f}	3.9 ± 0.1^{a,b,c,d,e,f}	3.9 ± 0.6^{a,b,c,d,e,f}	3.8 ± 0.1^{a,b,c,d,e,f}	4.3 ± 0.2^{b,c,d,e,f}
Przebędowo																		
Ile	2.7 ± 0.5^{a,b}	4.7 ± 0.1^{c,d,e}	3.7 ± 0.1^{a,b,c,d}	4.7 ± 0.3^{c,d,e}	3.9 ± 0.2^{b,c,d}	4.5 ± 0.3^{b,c,d,e}	3.0 ± 0.1^{a,b,c}	4.1 ± 0.4^{b,c,d}	4.7 ± 0.6^{c,d,e}	5.5 ± 0.1^{d,e}	4.3 ± 0.4^{b,c,d,e}	4.6 ± 0.6^{c,d,e}	4.2 ± 0.1^{b,c,d,e}	4.4 ± 0.4^{b,c,d,e}	4.9 ± 0.2^{d,e}	4.1 ± 0.3^{b,c,d}	4.8 ± 0.3^{d,e}	4.4 ± 0.6^{a,b,c,d,e}
Leu	5.8 ± 0.2^{a,b,c,d}	8.3 ± 0.2^{f,g,h,i,j}	7.6 ± 0.1^{a,b,c,d,e,f,g,h,i}	8.6 ± 0.7^{f,g,h,i,j}	7.3 ± 0.5^{a,b,c,d,e,f,g,h}	8.5 ± 0.1^{f,g,h,i,j}	5.6 ± 0.1^{a,b}	7.4 ± 0.9^{a,b,c,d,e,f,g,h,i}	8.8 ± 0.7^{g,h,i,j}	9.3 ± 0.1^{f,g,h,i,j}	7.9 ± 0.9^{b,c,d,e,f,g,h,i,j}	8.8 ± 1.3^{f,g,h,i,j}	8.6 ± 0.2^{f,g,h,i,j}	7.9 ± 0.4^{b,c,d,e,f,g,h,i,j}	9.1 ± 0.1^{h,i,j}	7.9 ± 0.5^{b,c,d,e,f,g,h,i,j}	10.0 ± 0.5^{j}	8.2 ± 1.1^{a,b,c,d,e,f,g,h,i,j}
Lys	3.5 ± 0.2^{a,b,c}	5.0 ± 0.1^{b,c}	3.5 ± 1.2^{a,b,c}	4.8 ± 0.5^{a,b,c}	4.2 ± 0.8^{a,b,c}	4.2 ± 1.2^{a,b,c}	3.3 ± 0.1^{a,b}	4.2 ± 0.5^{a,b,c}	4.9 ± 0.5^{b,c}	5.0 ± 0.1^{a,b,c}	4.8 ± 0.8^{a,b,c}	4.7 ± 0.1^{a,b,c}	4.2 ± 0.1^{a,b,c}	4.4 ± 0.1^{a,b,c}	5.4 ± 0.5^{b,c}	4.5 ± 0.3^{a,b,c}	4.9 ± 0.2^{a,b,c}	4.8 ± 0.7^{a,b,c}
Thr	3.4 ± 0.2^{a,b,c,d,e}	4.5 ± 0.3^{c,d,e,f}	4.3 ± 0.8^{c,d,e,f}	4.8 ± 0.5^{e,f}	4.1 ± 0.2^{c,d,e,f}	4.4 ± 0.4^{c,d,e,f}	3.1 ± 0.1^{a,b,c}	3.9 ± 0.6^{b,c,d,e,f}	4.6 ± 0.4^{d,e,f}	4.6 ± 0.0^{c,d,e,f}	4.4 ± 0.3^{c,d,e,f}	4.3 ± 0.4^{c,d,e,f}	4.0 ± 0.0^{b,c,d,e,f}	3.9 ± 0.1^{a,b,c,d,e,f}	4.7 ± 0.2^{e,f}	4.1 ± 0.4^{c,d,e,f}	4.7 ± 0.2^{e,f}	4.1 ± 0.52^{a,b,c,d,e,f}
Val	2.49 ± 0.1^{a,b,c,d}	4.4 ± 0.1^{e,f}	3.5 ± 0.1^{b,c,d,e,f}	4.3 ± 0.3^{e,f}	3.7 ± 0.3^{c,d,e,f}	4.6 ± 0.1^{f}	3.1 ± 0.1^{a,b,c,d,e,f}	3.9 ± 0.5^{d,e,f}	4.2 ± 0.5^{e,f}	4.3 ± 0.1^{d,e,f}	4.1 ± 0.6^{e,f}	4.0 ± 0.1^{d,e,f}	3.5 ± 0.1^{b,c,d,e,f}	3.7 ± 0.1^{c,d,e,f}	4.3 ± 0.3^{e,f}	3.8 ± 0.3^{d,e,f}	3.9 ± 0.3^{d,e,f}	4.2 ± 0.5^{b,c,d,e,f}
His	2.2 ± 0.1^{a}	3.1 ± 0.3^{a}	2.8 ± 0.2^{a}	3.4 ± 0.1^{a}	2.8 ± 0.1^{a}	3.3 ± 0.2^{a}	2.2 ± 0.1^{a}	3.1 ± 0.5^{a}	3.3 ± 0.7^{a}	3.2 ± 0.1^{a}	3.0 ± 0.4^{a}	2.8 ± 0.2^{a}	2.5 ± 0.1^{a}	2.7 ± 0.1^{a}	3.3 ± 0.2^{a}	2.8 ± 0.2^{a}	3.1 ± 0.1^{a}	3.1 ± 0.4^{a}
Phe	3.6 ± 0.5^{a,b,c,d,e,f}	4.4 ± 0.4^{c,d,e,f}	3.9 ± 0.1^{a,b,c,d,e,f}	4.6 ± 0.1^{d,e,f}	3.7 ± 0.1^{a,b,c,d,e,f}	4.5 ± 0.2^{c,d,e,f}	2.9 ± 0.1^{a,b,c,d}	4.0 ± 0.3^{e,f}	4.8 ± 0.3^{e,f}	4.9 ± 0.1^{e,f}	4.9 ± 1.5^{e,f}	4.9 ± 0.5^{e,f}	5.3 ± 0.1^{f}	4.5 ± 0.4^{c,d,e,f}	4.8 ± 0.3^{e,f}	4.9 ± 0.1^{e,f}	5.3 ± 0.2^{f}	4.5 ± 0.6^{d,e,f}

*Letters in rows show statistically significant differences at $P < 0.05$. Ile – isoleucine; Leu – leucine; Lys – lysine; Thr – threonine; Val – valine; His – histidine; Phe – phenylalanine

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Table 5. Determined conditionally essential amino acids composition (g/16 g N) in seeds of the studied cultivars of blue lupine grown in Wiatrowo and Przebędowo

Amino acid	Zeus	Neptun	Lazur	Heros	Rumba	Salsa	Regent	Emir	Kalif	Dalbor	Karo	Kurant	Bojar	Graf	Oskar	Boruta	Kadryl	Tango
Wiatrowo																		
Ser	4.6 ± 0.2^{a,b*}	6.1 ± 0.1^{a,b,c,d}	4.9 ± 0.3^{a,b,c}	5.3 ± 0.3^{a,b,c,d}	5.6 ± 1.1^{a,b,c,d}	7.2 ± 0.3^{c,d}	7.3 ± 1.2^{c,d}	5.9 ± 0.5^{a,b,c,d}	4.7 ± 0.0^{a,b}	6.1 ± 0.3^{a,b,c,d}	6.8 ± 0.1^{a,b,c,d}	7.7 ± 0.2^{d}	7.0 ± 0.2^{b,c,d}	6.2 ± 0.2^{a,b,c,d}	6.2 ± 0.2^{a,b,c,d}	6.0 ± 0.8^{a,b,c,d}	6.3 ± 0.6^{a,b,c,d}	6.4 ± 0.2^{a,b,c,d}
Gly	4.7 ± 0.1^{a,b,c,d,e}	6.4 ± 0.1^{d,e,f,g}	4.1 ± 0.2^{a,b,c}	4.7 ± 0.1^{a,b,c,d,e}	4.9 ± 0.1^{a,b,c,d,e,f}	6.2 ± 0.9^{c,d,e,f,g}	6.0 ± 0.1^{c,d,e,f,g}	6.1 ± 0.2^{c,d,e,f,g}	7.1 ± 1.5^{e,f,g}	3.5 ± 1.1^{a,b}	4.8 ± 0.1^{a,b,c,d,e}	5.7 ± 0.1^{c,d,e,f}	6.8 ± 0.4^{e,f,g}	6.2 ± 0.0^{c,d,e,f,g}	5.5 ± 0.3^{b,c,d,e,f}	5.0 ± 0.6^{a,b,c,d,e,f}	5.9 ± 0.9^{c,d,e,f,g}	5.7 ± 0.2^{b,c,d,e,f}
Gln	25.2 ± 0.4^{b,c,d,e,f,g,h}	27.8 ± 0.8^{c,d,e,f,g,h,i,j}	20.6 ± 0.2^{a,b}	22.5 ± 0.6^{a,b,c,d}	22.6 ± 1.9^{a,b,c,d,e}	29.3 ± 0.9^{f,g,h,i,j}	27.3 ± 0.1^{c,d,e,f,g,h,i,j}	28.0 ± 1.8^{d,e,f,g,h,i,j}	27.3 ± 0.1^{c,d,e,f,g,h,i,j}	21.4 ± 0.9^{a,b,c}	24.0 ± 1.0^{a,b,c,d,e,f}	30.2 ± 1.2^{f,g,h,i,j}	33.2 ± 0.8^{j}	31.5 ± 2.4^{h,i,j}	26.8 ± 0.7^{b,c,d,e,f,g,h,i,j}	22.8 ± 1.0^{a,b,c,d,e}	27.7 ± 2.4^{c,d,e,f,g,h,i,j}	29.0 ± 1^{e,f,g,h,i,j}
Arg	9.1 ± 0.5^{a,b,c,d}	13.1 ± 0.2^{c,d,e,f,g,h,i}	9.0 ± 1.9^{a,b}	11.8 ± 0.1^{a,b,c}	11.5 ± 1.3^{a,b,c,d,e,f}	15.5 ± 0.5^{e,f,g,h,i}	12.8 ± 0.1^{g,h,i}	12.6 ± 0.4^{f,g,h,i}	12.1 ± 0.2^{c,d,e,f,g,h,i}	9.8 ± 0.2^{a,b,c,d,e}	10.6 ± 0.8^{a,b,c,d,e,f,g,h,i}	14.5 ± 0.5^{g,h,i}	14.5 ± 2.8^{i}	14.3 ± 0.5^{h,i}	13.1 ± 0.8^{c,d,e,f,g,h,i}	12.3 ± 1.1^{e,f,g,h,i}	12.1 ± 0.5^{f,g,h,i}	14.2 ± 1.1^{g,h,i}
Pro	3.6 ± 0.2^{b,c,d,e}	4.4 ± 0.3^{b,c,d,e}	3.3 ± 0.4^{b,c}	3.7 ± 0.1^{b,c,d,e}	3.6 ± 0.2^{b,c,d,e}	5.0 ± 0.1^{c,d,e}	5.4 ± 1.1^{E}	1.0 ± 0.7^{a}	5.1 ± 0.6^{d,e}	2.8 ± 0.8^{b}	3.3 ± 0.4^{b,c,d}	4.9 ± 0.3^{c,d,e}	5.3 ± 0.1^{e}	4.5 ± 0.6^{b,c,d,e}	4.4 ± 0.1^{b,c,d,e}	4.4 ± 0.9^{b,c,d,e}	3.7 ± 0.9^{b,c,d,e}	4.7 ± 0.1^{c,d,e}
Przebędowo																		
Ser	4.6 ± 0.22^{a,b}	7.0 ± 0.1^{b,c,d}	6.7 ± 1.1^{a,b,c,d}	7.0 ± 0.4^{b,c,d}	6.3 ± 0.4^{a,b,c,d}	6.9 ± 0.5^{a,b,c,d}	4.5 ± 0.8^{a}	6.5 ± 1.4^{a,b,c,d}	7.1 ± 0.9^{b,c,d}	6.5 ± 0.1^{a,b,c,d}	6.8 ± 1.3^{a,b,c,d}	6.8 ± 0.2^{a,b,c,d}	6.0 ± 0.8^{a,b,c,d}	6.0 ± 0.2^{a,b,c,d}	7.0 ± 0.5^{b,c,d}	6.0 ± 0.5^{a,b,c,d}	7.0 ± 0.2^{a,b,c,d}	6.5 ± 0.7^{a,b,c,d}
Gly	4.7 ± 0.1^{a,b,c,d,e}	6.1 ± 0.3^{c,d,e,f,g}	5.5 ± 0.9^{b,c,d,e,f}	6.1 ± 0.43^{c,d,e,f,g}	5.5 ± 0.19^{b,c,d,e,f}	5.7 ± 0.49^{b,c,d,e,f}	3.3 ± 0.5^{a}	4.9 ± 0.41^{a,b,c,d,e}	6.3 ± 0.3^{d,e,f,g}	5.4 ± 0.1^{a,b,c,d,e,f,g}	5.2 ± 0.76^{a,b,c,d,e,f}	5.2 ± 0.16^{a,b,c,d,e,f}	4.6 ± 0.21^{a,b,c,d}	4.7 ± 0.16^{a,b,c,d}	5.7 ± 0.3^{b,c,d,e,f}	5.4 ± 1.26^{a,b,c,d,e,f}	5.3 ± 0.261^{a,b,c,d,e,f}	5.0 ± 0.61^{a,b,c,d,e,f}
Gln	25.1 ± 0.4^{b,c,d,e,f,g,h}	29.3 ± 0.4^{f,g,h,i,j}	26.2 ± 2.9^{b,c,d,e,f,g,h,i}	28.3 ± 3.4^{d,e,f,g,h,i,j}	26.0 ± 1.7^{b,c,d,e,f,g,h,i}	28.4 ± 1.2^{d,e,f,g,h,i,j}	18.6 ± 0.3^{a}	25.0 ± 1.4^{b,c,d,e,f,g}	30.8 ± 2.0^{g,h,i,j}	29.8 ± 0.2^{d,e,f,g,h,i,j}	27.1 ± 2.8^{c,d,e,f,g,h,i,j}	27.7 ± 1.4^{c,d,e,f,g,h,i,j}	25.4 ± 0.7^{b,c,d,e,f,g,h,i}	26.9 ± 0.1^{b,c,d,e,f,g,h,i,j}	31.7 ± 0.2^{i,j}	27.0 ± 2.5^{b,c,d,e,f,g,h,i,j}	30.6 ± 0.7^{g,h,i,j}	29.0 ± 3.7^{b,c,d,e,f,g,h,i,j}
Arg	9.1 ± 0.5^{a,b,c,d}	14.6 ± 0.2^{f,g,h,i}	12.1 ± 0.7^{b,c,d,e,f,g,h}	13.9 ± 2.3^{f,g,h,i}	12.6 ± 0.7^{b,c,d,e,f,g,h,i}	13.9 ± 0.9^{e,f,g,h,i}	8.7 ± 0.3^{a}	11.4 ± 0.5^{a,b,c,d,e,f,g,h}	14.8 ± 0.7^{g,h,i}	14.6 ± 0.1^{b,c,d,e,f,g,h,i}	13.4 ± 0.6^{b,c,d,e,f,g,h}	13.4 ± 0.7^{b,c,d,e,f,g,h,i}	12.2 ± 0.2^{a,b,c,d,e,f,g}	12.9 ± 0.1^{b,c,d,e,f,g,h,i}	15.1 ± 1.3^{f,g,h,i}	13.0 ± 1.0^{b,c,d,e,f,g,h,i}	14.4 ± 0.6^{f,g,h,i}	13.5 ± 1.8^{a,b,c,d,e,f,g,h,i}
Pro	3.6 ± 0.2^{b,c,d,e}	5.1 ± 0.1^{E}	4.4 ± 0.2^{b,c,d,e}	5.1 ± 0.5^{d,e}	4.4 ± 0.3^{b,c,d,e}	5.0 ± 0.1^{c,d,e}	3.3 ± 0.1^{b,c}	4.3 ± 0.5^{b,c,d,e}	5.2 ± 0.4^{e}	4.8 ± 0.1^{b,c,d,e}	4.6 ± 0.5^{b,c,d,e}	4.4 ± 0.1^{b,c,d,e}	4.0 ± 0.1^{b,c,d,e}	4.3 ± 0.1^{b,c,d,e}	5.2 ± 0.3^{e}	4.3 ± 0.5^{b,c,d,e}	4.8 ± 0.2^{c,d,e}	4.6 ± 0.5^{b,c,d,e}

Letters in rows show statistically significant differences at $P < 0.05$. Ser – serine; Gly – glycine; Gln – glutamine; Arg – arginine; Pro – proline

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Simultaneously, the cultivars indicated as most valuable (marked grey in Table 3) had a higher content of the studied EAA than the FAO/WHO standard, which was not always listed for lupine proteins (Monteiro et al. 2014). However, it should be remembered that a lowered content of sulfur-containing amino acids characterizes lupine seeds, an thus the protein value of the studied seeds cannot be determined only on the basis of the presented analysis (Sujak et al. 2016). The applied method of amino acids determination was not sufficient for the determination of methionine, tryptophan and cysteine.

Since lupine protein is often proposed as an ingredient of food dedicated to consumers with special dietary requirements, controlling of the conditionally essential (CEAA) amino acids in humans, i.e. essential in stress conditions and in some illnesses (U.S. National Library of Medicine 2015) is also worth considering. Among them arginine, glutamic acid, glycine, proline and serine contents were determined (Table 5). The observed trends were the same as for the studies of essential amino acids – the impact of both the field and the cultivar on the content of these amino acids. Moreover, the cultivars pointed as rich in EAA were not always rich in CEAA. Cultivars richest in CEAA were cvs. Bojar (from Wiatrowo), Kalif and Oskar (from Przebędowo).

Finally, after analysing the contents of other, i.e. endogenous amino acids (alanine and aspartic acid) (Table 6), two cultivars can be indicated as the richest in all amino acids studied, i.e., cv. Bojar (from Wiatrowo) and cv. Oskar (from Przebędowo). There is also one cultivar which showed significantly lower content of all amino acids tested, i.e. cv. Zeus from both cultivation places. It can be expected that this cultivar could be rich in non-protein nitrogenous substances, which, however, must be confirmed after an analysis of the remaining amino acids and urea content. Thus, this cultivar has the lowest nutritional value among the tested cultivars. Moreover, Oskar and Karo, as cultivars with high alkaloid content (Table 1), should be also excluded from the human and animal nutrition if more interesting cultivars could be indicated. The results were presented and discussed for the year of harvest that was worse for lupine cultivation, which is more interesting for good agricultural practice. Data for 2016 are not presented, because they also did not allow indicating the most favourable cultivar, confirming the conclusions presented for 2015.

Table 6. Determined endogenous amino acids composition (g/16 g N) in seeds of the studied cultivars of blue lupine grown in Wiatrowo and Przebędowo

Amino acid	Zeus	Neptun	Lazur	Heros	Rumba	Salsa	Regent	Emir	Kalif	Dalbor	Karo	Kurant	Bojar	Graf	Oskar	Boruta	Kadryl	Tango
Wiatrowo																		
Asp	9.1 ± $0.4^{a,b,c}$	13.1 ± $0.2^{c,d,e,f,g}$	9.0 ± $1.9^{a,b}$	11.8 ± $0.1^{a,b,c,d,e,f,g}$	11.505 ± $1.3^{a,b,c,d,e,f,g}$	15.5 ± $0.5^{g,h}$	12.8 ± $0.1^{b,c,d,e,f,g}$	12.6 ± $0.4^{a,b,c,d,e,f,g}$	12.1 ± $0.2^{a,b,c,d,e,f,g}$	9.8 ± $0.1^{a,b,c,d}$	10.6 ± $0.8^{a,b,c,d,e}$	14.5 ± $0.5^{e,f,g,h}$	14.4 ± $2.8^{e,f,g,h}$	14.2 ± $0.5^{e,f,g,h}$	13.1 ± $0.8^{c,d,e,f,g}$	12.3 ± $1.1^{a,b,c,d,e,f,g}$	12.1 ± $0.5^{a,b,c,d,e,f,g}$	14.2 ± $1.1^{e,f,g,h}$
Ala	3.2 ± $0.1^{a,b,c,d,e}$	3.8 ± $0.3^{a,b,c,d,e,f}$	2.8 ± 0.5^{a}	3.6 ± $0.0^{a,b,c,d,e,f}$	3.4 ± $0.4^{a,b,c,d,e}$	4.8 ± $0.4^{e,f,g}$	4.2 ± $0.1^{a,b,c,d,e,fg}$	4.3 ± $0.1^{b,c,d,e,f,g}$	3.9 ± $0.2^{a,b,c,d,e,f}$	3.1 ± $0.2^{a,b,c,d}$	3.0 ± $0.4^{a,b,c}$	4.4 ± $0.1^{d,e,f,g}$	4.9 ± $0.1^{e,f,g}$	4.0 ± $0.4^{a,b,c,d,e,f,g}$	3.9 ± $0.1^{a,b,c,d,e,f}$	4.0 ± $0.9^{a,b,c,d,e,f}$	3.6 ± $0.3^{a,b,c,d,e,f}$	4.1 ± $0.3^{a,b,c,d,e,g}$
Przebędowo																		
Asp	9.1 ± $0.4^{a,b,c}$	14.6 ± $0.2^{e,f,g,h}$	12.1 ± $0.7^{a,b,c,d,e,f,g}$	13.9 ± $2.3^{e,f,g}$	12.6 ± $0.6^{a,b,c,d,e,f,g}$	13.9 ± $0.9^{e,f,g}$	8.7 ± 0.3^{a}	11.4 ± $0.5^{a,b,c,d,e,f}$	14.8 ± $0.7^{f,g,h}$	14.6 ± $0.0^{d,e,f,g,h}$	13.5 ± $1.6^{d,e,f,g}$	13.4 ± $0.7^{d,e,f,g}$	12.2 ± $0.1^{a,b,c,d,e,f,g}$	12.8 ± $0.0^{b,c,d,e,f,g}$	15.1 ± $1.3^{f,g,h}$	13.0 ± $1.0^{b,c,d,e,f,g}$	14.4 ± $0.6^{e,f,g,h}$	13.5 ± $1.8^{a,b,c,d,e,f,g}$
Ala	3.2 ± $0.1^{a,b,c,d,e}$	4.4 ± $0.1^{d,e,f,g}$	4.0 ± $0.5^{a,b,c,d,e,f,g}$	4.4 ± $0.6^{c,d,e,f,g}$	3.9 ± $0.5^{a,b,c,d,e,f}$	4.5 ± $0.1^{d,e,f,g}$	3.0 ± $0.0^{a,b}$	3.7 ± $0.4^{a,b,c,d,e,f}$	4.4 ± $0.3^{d,e,f,g}$	4.3 ± $0.1^{a,b,c,d,e,f,g}$	4.3 ± $0.4^{b,c,d,e,f,g}$	4.1 ± $0.2^{a,b,c,d,e,f,g}$	3.8 ± $0.0^{a,b,c,d,e,f}$	3.7 ± $0.0^{a,b,c,d,e,f}$	4.5 ± $0.3^{e,f,g}$	3.9 ± $0.4^{a,b,c,d,e,f}$	4.4 ± $0.2^{d,e,f,g}$	4.0 ± $0.5^{a,b,c,d,e,f}$

Letters in rows show statistically significant differences at $P < 0.05$. Asp – aspartic acid; Ala – alanine

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In conclusion, all the studied parameters were strongly dependent on both the cultivar and weather conditions, especially the precipitation sum. These factors influence the content of the total protein as well as amino acids composition. Some of the examined materials could be identified as significantly richer in the analysed amino acids – cv. Bojar seeds from Wiatrowo and cvs. Dalbor and Oskar from Przebędowo, and one that was significantly poorer – cv. Zeus in both cultivation places. Unfortunately, the experiment carried out does not allow for the identification of one best cultivar common to both cultivation places and nutritionally desired, due to the strong influence of weather conditions on the protein content and quality of the studied blue lupine seeds.

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