Chemical Composition and Nutritional Evaluation of the Lupine Seeds (*Lupinus albus* L.) from Low-Alkaloid Varieties

DANIEL MIERLITA[1], DANIEL SIMEANU[2], IOAN MIRCEA POP[2], FLORIN CRISTE[3], CECILIA POP[2], CRISTINA SIMEANU[2], FLORIN LUP[1]*

[1]University of Oradea, Department of Animal Science, 1 Universitatii Str., 410087, Oradea , Romania
[2]University of Agricultural Sciences and Veterinary Medicine, 3 Mihail Sadoveanu Alley, 700490, Iasi, Romania
[3]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Manastur, 400372, Cluj, Romania

The aim of this paper is to analyse the chemical compositional and nutritional profiles of a two number of white lupine cultivars (Lupinus albus, cultivars Amiga and Energy) suited to the pedoclimatic conditions in Romania, collected in the year 2015. No significant differences were observed among lupine cultivars in their dry matter (DM), crude ash or alkaloid contents. The highest protein content (36.4±1.1% of DM) and crude fat (10.1±1.2% of DM) was found in seeds from lupines belonging to cv. Amiga, while the highest crude fibre content (15.2±1.7% of DM) was found in cv. Energy. Both varieties examined were characterised by a shortage of methionine and lysine, but lysine deficiency was higher in cv. Energy. Amiga cultivar was found to be a nutritionally more valuable crop than cv. Energy by the standards of nutrition for mature human and animals (chicken broilers and growing pigs). Amiga lupine was characterised by a higher essential amino acid index (EAAI) as well as chemical score (CS) of lysine, and the high nutritional index (NI) and biological value (BV) of protein as compared to cv. Energy. The white lupine seeds examined can serve as a source of good quality food protein for adult humans, meet the requirement for exogenous amino acids (EAA) and Lys in chicken broilers and to a lesser degree in the case of growing pigs, according to the standards of nutrition used. Fatty acid (FA) composition showed that oleic acid (C18:1 n-9) was the major fatty acid, followed by linoleic (C18:2 n-6) and linolenic (C18:3 n-3) acids. Apart from the highest level of polyunsaturated fatty acids (PUFA) the seed oil of Amiga cultivar showed and the largest content of linolenic acid (n-3) and the most favourable report n-3/n-6 FA.

Keywords: white lupine seeds, nutrients, amino acids profile, protein quality, fatty acids profile

In Europe, the deficit of feed protein sources is considered as important problem. The current global situation encourages EU countries to find ways to reduce the dependence on imported protein feeds. In this regard, seeds of lupine can be alternative protein sources replacing genetically modified soybeans in animal feed production and human diet [1].

The use of lupine seeds has been limited due to the presence of alkaloids. Plant breeders have worked on improving lupine by selecting varieties high in protein and low in alkaloids [2]. Among the species of the genus *Lupinus*, white lupine (*Lupinus albus* L.) is most often the subject of interest for experts in human and animal nutrition, mainly because of its seed yield potential, protein and oil content [3-5].

The seeds of white lupine cultivars contain 28 to 42% crude protein (CP) in dry matter (DM), which depends on the lupine cultivar and climatic conditions [6]. Lupine seed proteins are high in lysine and arginine [7]. The profile of amino acids is characterised by a lower level of sulphur containing amino acids and threonine in comparison with soy; in contrast, arginine content is markedly higher [8].

The oil content in seeds which ranges from 5.7 to 12.1% [4, 5]. The most frequent fatty acids in white lupine seeds included C18:1 and C18:2. Oils contained in lupine seeds, although less discussed, might perform an important nutritional role regarding their fatty acid profile. An important criterion for oil assessment for dietary purposes is represented by the contents and proportions of polyunsaturated fatty acids (PUFA), which are important for both human and animal nutrition [9-13]. Special emphasis is laid on sufficient intake of n-3 FA and proportion of n-3/n-6 FA in the diet. The recommended ratio is 1:4 [14].

In Europe cultivation of lupine species remains far behind that of other leguminous plants, although is the only highly productive plant, on exhausted or heavy soils which can be used for food and fodder production [2]. Apart from the high protein content, lupine has a strong capability for nitrogen fixation and organic phosphorus release from soil and improves the soil value for further cropping [1].

Although over recent decades, a growing body of research on sweet lupine has begun, mainly to produce species characterised by a low alkaloid content and enrich their nutritional values, in Romania the level of cultivation of this plant is still considered to be low [15]. The aim of this paper is to analyse the chemical compositional and nutritional profiles of a two number of white lupine cultivars (*Lupinus albus*, cultivars Amiga and Energy) suited to the pedoclimatic conditions in Romania, collected in the year 2015.

Experimental part

Raw material

Lupine cultivars (Amiga and Energy) were chosen from the European species most suited to the pedoclimatic conditions in Romania. Lupine seeds for analyses were obtained from Agricultural and Development Research Station (47°32' N; 21°56' E) of the University of Oradea. The seeds were collected in the year 2015. The weather temperature mean was 19.3°C and total precipitation was 315 mm during the plant growth season (April - August). The seeds were cleaned and rendered free of dust, then stored in tightly closed glass jars at room temperature until used [2].

* email: lup_florin@yahoo.com

Proximate chemical composition

Nutrient composition of the lupine seed sample was determined using the standard procedures of Association of Official Analytical Chemists [16]. Dry matters (DM) were determined by drying in an oven at 105°C until a constant weight was obtained [17, 18]. Crude fat (EE - ether extract) was determined by Soxhlet extraction with petroleum ether (boiling point, 40 to 60°C) using Soxhlet apparatus (Gerhardt) (Method No 930.09) [19]. The nitrogen content was estimated by the Kjeldahl method (Method No 978.04) and the crude protein (CP) content was calculated (N × 6.25) [20, 21]. The ash content was determined by heating 2 g of the dried sample in a silica dish at 580°C for 8 h (Method No 930.05). Crude fibber was determined after digesting a known weight of fat-free sample in refluxing sulfuric acid and sodium hydroxide (Method No 930.10). The nitrogen-free extract (N-FE) was calculated as 100 - crude protein - crude fat - crude fibre - crude ash.

Neutral detergent Fibber (NDF), acid detergent fibber (ADF) and acid detergent lignin (ADL) were determined according to Van Soest [22], using an Ankom 220 Fibber Analyser (ANKOM Technology Corp., Fairport, New York, USA). Hemicellulose content was estimated by subtracting ADF from NDF and cellulose content by substracting lignin from ADF [21].

For extraction of alkaloids from lupine seeds (freeze-dried) was used trichloroacetic acid, dichloromethane and internal standard (*n*-eicosane) according to the method described by Maknickiene [23]. Cromatographic analysis was performed em-ploying the modiûed method of Lee [24].

All determinations were expressed on a dry matter basis.

Amino acid analysis

Amino acids analysis of lupine seeds was determined using a Mikrotechna AAA 881 automatic amino acid analyser (Model 118/119 CL, CR). Prior to analysis, samples were hydrolysed in 6 M HCl at 110°C for 24 h under a nitrogen atmosphere. Cysteine and methionine were determined as cysteic acid and methionine sulfone, respectively, following sample oxidation with performic acid and hydrolysis in 6 M HCl at 110°C for 24 h. Tryptophan was determined after NaOH hydrolysis at 110°C for 22 h according to the method described in the Official Methods of Analysis of the Association of Analytical Chemists [25]. Amino acid determinations were expressed on a g/16 g N basis, equivalent to g/100 g of protein [26].

Protein quality evaluation

The quality of proteins was estimated by determination of total amino acids (AA), the fraction of the exogenous amino acids (EAA), the protein chemical score (CS) as well as the essential amino acid index (EAAI) [2, 27, 28]. The nutritional values were referred to the whole egg protein amino acid standard (Lys - 7, Met+Cys - 5.7, Thr - 4.7, Ile - 5.4, Trp - 1.7, Val - 6.6, Leu - 8.6, His - 2.2, Phe+Tyr - 9.3; EAA=51.2 g/16 g N [28], the standard for mature human (Lys - 5.5, Met+Cys - 3.5, Thr - 4, Ile - 4, Trp - 1, Val - 5, Leu - 7, Phe+Tyr - 6; EAA=36 g/16 g N - [26]) and to two different standards for animal feeding.

The protein usability for animal feeding was estimated on the basis of standard for 6-8 weeks chicken broilers (Lys - 4.7, Met+Cys - 3.3, Thr - 3.8, Ile - 3.4, Trp - 0.9, Val - 3.9, Leu - 5.2, His - 1.5, Phe+Tyr - 5.8; EAA=32.5 g/16 g N [30] as well as the standard for 20-50 kg growing pigs (Lys - 7, Met+Cys - 3.6, Thr - 4.5, Ile - 4, Trp - 1.2, Val - 5.2, Leu - 8, His - 2.5, Phe+Tyr - 8; EAA=44 g/16 g N [31].

The EAAI (Essential Amino Acid Index) was calculated as the geometric mean of all the concentrations of participating exogenous amino acids (EAA) compared to the concentration of a corresponding standard (in g/16 g N)

$$EAAI = \sqrt[n]{\left(\frac{a_1}{a_{1s}}\right) \times 100 \times \cdots \ldots \ldots \times \left(\frac{a_n}{a_{ns}}\right) \times 100},$$

where a_n is the AA content in the protein tested and a_{ns} the AA content in the reference protein.

The CS values were calculated for all amino acids according to the following formula [32]:

$$CS = {}^{a_n}/_{a_{ns}} \times 100$$

To emphasise the value of the lupine seed protein in terms of the content of limiting amino acids for animal feeding, only the CS_{Lys} and $CS_{Met+Cys}$ values are presented in the paper.

Protein efficiency ratio (PER) of lupine seeds were calculated according to the equations developed by Alsmeyer [33]:

$$PER = 0.06320\ [X_{10}] - 0.1539$$

where X_{10} = Thr + Val + Met + Ile + Leu + Phe + Lys + His + Arg + Tyr

Biological Value (BV) were computed according to the methods of Oser [32], respectively. The following equation was used for BV determination:

BV = 1.09 (EAAI) – 11.7.

The nutritional index (NI) of the lupine samples was calculated using the formula below as described by Crisan and San

$$Nutritional\ index\ (\%) = \frac{\mathbf{EAAI} \times \mathbf{\%\ protein}}{\mathbf{100}}$$

Lipid extraction and fatty acid analysis

Seed sample were ground and subjected to oil extraction using a Soxhlet apparatus (Gerhardt) according to AOAC [16]. Oil extracted from the seeds was subjected to esterification according to the method described by Andrzejewska [4]. At the first stage fat was saponified using a 0.5N KOH methanol solution at 70°C. Esterification with methanol was conducted in the presence of sulphuric acid as a catalyst.

Chromatographic analysis was performed using a Shimadzu GC-2010 gas chromatograph (Shimadzu Corporation, Tokyo, Japan) equipped with a DB-23 column (60m x 0.25mm i.d. and 0.25µm film thickness). Column and detector temperatures were 190 and 240°C, respectively. Carrier gas was helium at 1.0 ml/min ratio. The temperature of injection port was 230°C with the split ratio of 1:80, temperature program was 80°C/5 min, 200°C/30 min, and 230°C/15 min. Fatty acid methyl esters FAME peaks on the GC were identified by comparison against standard FAME mixture (Supelco 37 Component FAME mix; Supelco Bellefonte, PA, USA). The peak areas were used to calculate the percentage of FA expressed as the percentage of total fatty acid (FA).

The percentage values of the considered groups of FA were obtained from summation of the percentage of appropriate FA: SFA, sum of percentage values of total saturated FA, i.e., palmitic acid + stearic acid + arachidic acid + behenic acid; MUFA, sum of the percentage values of monounsaturated FA, i.e., palmitoleic acid + oleic acid + gadoleic acid + erucic acid; PUFA, sum of the percentage values of polyunsaturated FA, i.e., linoleic acid + linolenic acid. Ratio n-3/n-6 was expressed as the ratio linolenic acid/linoleic acid.

The polyunsaturated index (PI) of the lupine samples was calculated using the formula below as described by Timmons [35]:

$$PI = C18{:}2n\text{-}6 + (C18{:}3n\text{-}3 \times 2)$$

Statistical analysis

All chemical analysis are reported as an average of eight analyses (n=8). The data obtained was statistically analysed by an ANOVA using SAS (Statistical Analysis

Software, version 9.1.) [36] for significant F-statistics. If the overall F-test was significant ($p < 0.05$), a Fisher's t-test was performed to discern differences between the cultivars.

Results and discussions

Proximate chemical composition

The proximate composition of white lupine seed (cv. Amiga and cv. Energy) samples is presented in table 1. The results obtained in this study show that the variety had significant influence on the levels of crude protein, crude fat, crude fibre and on N-free extractives but didn't influence crude ash and alkaloides. Strakova et al. [8] found the influence of the cultivar on crude protein, crude oil and on crude fibre content for the cultivars grown in Europe.

The analysed white lupine seeds were characterised by high protein content. The highest protein content was found in the seeds lupine belonging to Amiga cultivar (36.4% DM), being lower than that reported for Butan cultivar (37.6 to 38.4% DM - [2, 37]) but higher than the protein levels in Amiga cultivar (34.1% DM - [6]) and other varieties of white lupine seeds (25.7 to 35.5% DM - [6, 38, 39]).

In cv. Amiga seeds the amount of oil was found to be significantly higher than those in cv. Energy lupines ($p < 0.05$). Oil content found for cv. Amiga (10.1±1.2% DM) was lower in comparison to Andean lupine (14.9%) [39] and lower than that reported by Sujak et al., [2] for cv. Boros, but similar to that reported by Rybinski et al., [5], Brenes at al., [6] and Grela et al., [39] for other cultivars white lupine. In the case of cv. Energy, the value of 8.4 ± 0.9% was similar to that reported previously for lupine varieties cultivated in Ramania [14]. The content of crude fat can be affected by a genotype within the same species [41] as well as by environmental factors during plant growth and development (temperature, air moisture, rainfalls level) [5, 42]. Boschin et al. [43] in the field trial with six white lupine cultivars in two locations indicated, that variance of genotypic effects was much larger than genotype-environment interaction for oil content and fatty acid composition.

The crude fibre content of white lupine seeds was relatively high and were 12.9 and 15.2% for cv. Amiga and cv. Energy respectively. The highest NDF (22.7%), ADF (16.2%) and CEL (14.4%) contents were found in the cultivar Energy. Similar content of NDF and ADF in white lupine seeds was found by Brenes et al., [6], Pisarikova et al. [37] and Grela et al. [39] Wasilewko and Buraczewska [44] they shown that the fat level in white lupine seeds was negatively correlated with ADF content ($r = - 0.687$), this conclusion being confirmed in the present study. The level of fibre fractions did not differ significantly for ADL and HCEL.

Nitrogen-free extract differed significantly between cultivars ($p < 0.05$) and was as follows: cv. Amiga 36.5% and cv. Energy 38.1% of DM. Apart from starch, sugars and pectin, this fraction contains water-soluble non-starch polysaccharides (NSP) as well as oligosaccharides [2]. Experiments on young piglets show that, they can negatively affect digestibility and nutrient absorption and act as anti-nutritional factors [44].

Alkaloids in fodder should not exceed 0.02–0.04% as a high alkaloid content can cause a significant decrease in protein digestibility and may also result in neurological disorders [2, 3]. The concentrations of alkaloids in analysed white lupine seeds did not exceed the recommended level and varied between 0.018 and 0.021% of dry matter. The level of alkaloids did not differ significantly for analysed cultivars.

Amino acid profile and protein quality of lupine seeds

The amino acid composition of the protein (in g/16 g N, equivalent to g/100 g protein) from the examined white lupine seeds and nutritional values of the seed protein from seeds determined on the basis of the egg protein standard (CS, EAAI) are shown in table 2 and table 3, respectively. Generally, proteins from the examined white lupine are a good source of Lys (6.1 - 5.7 g/16 g N) but they are deficient in other essential amino acids, especially in sulphur amino acids containing (2.3 - 2.1 g/100 g protein). Values were statistically significant ($p <$v0.05) for Lys, Trp and Arg. Amiga cultivar had the biggest content of Lys and Trp, while the Arg content was higher at cv Energy. The available literature shows a high proportion of lysine and a relatively low level of sulphur amino acids and tryptophan in white lupine seeds [2, 6, 34, 36, 43]. This was largely confirmed by the present results, as the CS_{Lys} value for all the analysed lupine seeds was high (82.0 - 87.2%), and sulphur amino acids were the limiting amino acids ($CS_{Met+Cys}$: 38.4 - 40.7%).

Differences in CS values for white lupine seeds depended on the standards of nutrition used: egg protein and also based on ideal protein for mature human, chicken broiler and growing pigs (table 3). In any of the above-mentioned cases Met+Cys were the limiting amino acids. Therefore, lupine should be combined with foods or fodders rich in methionine or supplemented with Met.

The protein of white lupine seeds is characterised by a lower value than that of animal protein. This was confirmed in the present study by content of exogenous amino acids

Table 1

CHEMICAL COMPOSITION OF THE TWO WHITE LUPIN CULTIVARS (% OF DRY MATTER)

Specification	cv. Amiga			cv. Energy			p -value
	Mean	(min. - max.)	SD	Mean	(min. - max.)	SD	
Dry matter (DM)	93.21	(89.1 - 94.3)	1.74	92.02	(89.3 - 94.6)	1.65	ns
Crude protein (CP)	36.41	(32.6 - 38.8)	1.14	34.06	(32.6 - 37.3)	1.09	**
Ether extract (EE)	10.17	(7.6 - 13.7)	1.27	8.46	(7.2 - 12.3)	0.94	*
Crude fibre (CF)	12.96	(9.4 - 15.7)	1.06	15.27	(12.8 - 18.3)	1.73	**
NDF	20.41	(16.7 - 23.2)	2.23	22.71	(18.1 - 26.4)	3.58	*
ADF	13.63	(10.8 - 15.1)	1.42	16.23	(13.6 - 18.2)	1.84	*
ADL	1.88	(1.0 - 2.7)	0.23	1.76	(1.1 - 2.6)	0.16	ns
HCEL	6.78	(4.4 - 8.0)	2.12	6.48	(4.5 - 8.1)	1.73	ns
CEL	11.75	(9.3 - 13.1)	1.73	14.47	(12.0 - 17.2)	2.41	**
Crude ash (CA)	3.92	(3.1 - 4.4)	0.14	4.11	(3.0 - 4.6)	0.27	ns
N-FE	36.54	(31.6 - 39.0)	2.9	38.10	(32.7 - 41.2)	3.6	*
Organic matter (OM)	96.08	(93.4 - 97.6)	1.12	95.89	(93.6 - 96.4)	1.27	ns
Alkaloides	0.018	(0.01 - 0.03)	0.01	0.021	(0.02 - 0.03)	0.01	ns

NDF neutral-detergent fibber, ADF acid-detergent fiber, ADL lignin, HCEL hemicellulose (calculated values: NDF-ADF), CEL cellulose (calculated values: ADF-ADL), N-FE nitrogen-free extract (calculated values: 100 - CP - EE - CF - CA)

Table 2
AMINO ACID COMPOSITION OF THE WHITE LUPIN SEEDS

Specification	cv. Amiga			cv. Energy			p -value
	Mean	(min. - max.)	SD	Mean	(min. - max.)	SD	
Essential amino acids (g/16 g N)							
Lys	6.11	(5.7 - 6.7)	0.43	5.74	(5.6 - 6.0)	0.45	*
Met + Cys	2.19	(1.9 - 2.5)	0.22	2.32	(2.1 - 2.7)	0.20	ns
Cys	1.49	(1.3 - 1.7)	0.12	1.67	(1.5 - 2.1)	0.17	ns
Thr	3.68	(3.1 - 3.8)	0.25	3.52	(3.2 - 3.9)	0.22	ns
Ile	4.39	(3.8 - 4.5)	0.31	4.27	(3.7 - 4.8)	0.40	ns
Trp	0.95	(0.8 - 1.1)	0.16	0.78	(0.6 - 0.9)	0.21	*
Val	4.23	(4.0 - 4.6)	0.23	4.45	(4.1 - 4.9)	0.27	ns
Leu	6.18	(5.9 - 6.6)	0.47	6.23	(6.0 - 6.6)	0.42	ns
His	2.31	(2.1 - 2.7)	0.22	2.53	(2.2 - 2.8)	0.27	ns
Phe + Tyr	8.09	(7.3 - 8.6)	1.03	7.94	(7.4 - 8.3)	0.93	ns
Tyr	3.98	(3.5 - 4.3)	0.20	3.91	(3.6 - 4.5)	0.24	ns
Non-essential amino acids (g/16 g N)							
Arg	9.89	(9.6 - 10.3)	0.75	10.32	(9.7 - 10.8)	0.91	*
Asp	10.17	(9.7 - 11.5)	0.67	10.30	(9.8 - 11.0)	0.69	ns
Ser	5.12	(4.6 - 5.6)	0.31	5.03	(4.7 - 5.4)	0.30	ns
Glu	23.37	(23.0 - 24.1)	0.84	23.61	(23.2 - 24.3)	0.89	ns
Pro	4.19	(3.7 - 4.5)	0.30	4.02	(3.6 - 4.5)	0.37	ns
Gly	4.17	(3.6 - 4.5)	0.24	4.05	(3.7 - 4.4)	0.31	ns
Ala	3.50	(3.1 - 3.8)	0.21	3.32	(3.1 - 3.9)	0.19	ns

Table 3
THE NUTRITIONAL VALUES OF PROTEINS OF THE WHITE LUPIN SEEDS

Specification		cv. Amiga		cv. Energy		p -value
		Mean	SD	Mean	SD	
AA (g/16 g N)		98.54	6.02	98.43	5.83	ns
Standard[1]						
	EAA (g/16 g N)	38.13	2.48	37.78	3.91	ns
	CS_{Lys}	87.28	5.14	82.0	4.03	**
	$CS_{Met+Cys}$	38.42	3.21	40.70	3.75	ns
	EAAI (%)	71.71	5.93	70.41	6.18	ns
	P-BV	66.46	4.61	65.04	3.52	ns
	Nutritional index %	26.11	1.89	23.98	2.18	*
Standard[2]						
	EAA (g/16 g N)	35.82	2.56	35.25	2.93	ns
	CS_{Lys}	111.09	8.31	104.36	7.54	**
	$CS_{Met+Cys}$	62.57	4.11	66.28	5.16	*
	EAAI (%)	94.98	6.03	92.16	5.83	*
	P-BV	91.82	5.68	88.75	5.71	*
	Nutritional index %	34.58	4.07	31.39	3.90	**
Standard[3]						
	EAA (g/16 g N)	38.13	2.48	37.78	3.91	ns
	CS_{Lys}	130.0	9.12	122.13	7.28	**
	$CS_{Met+Cys}$	66.36	4.71	70.30	6.07	*
	EAAI (%)	113.44	7.54	111.41	8.12	*
	P-BV	111.95	8.31	109.73	7.83	*
	Nutritional index %	41.30	2.84	37.95	2.70	**
Standard[4]						
	EAA (g/16 g N)	38.13	2.48	37.78	3.91	ns
	CS_{Lys}	87.28	5.14	82.0	4.03	**
	$CS_{Met+Cys}$	60.83	4.36	64.44	5.72	*
	EAAI (%)	84.45	6.57	83.05	7.15	*
	P-BV	80.35	7.18	78.82	6.83	*
	Nutritional index %	30.75	3.54	28.29	4.21	*
P-PER		2.73	0.21	2.73	0.27	ns

[1]Based on egg standard [29];
[2]Standard based on nutrient requirement for mature human [26];
[3]Standard based on nutrient requirement of 6-8 weeks chicken broilers [30];
[4]Standard based on nutrient requirement for growing pigs 20-50 kg [30];
CS_{Lys} - lysine chemical score; $CS_{Met+Cys}$ - methionine + cysteine chemical score;
P-BV - Predicted-Biological Value; P-PER - Predicted-Protein Efficiency Ratio.

Table 4
FATTY ACID COMPOSITION OF WHITE LUPINE SEEDS (% OF TOTAL FATTY ACIDS)

Fatty acid (FA)	cv. Amiga			cv. Energy			p -value
	Mean	(min. - max.)	SD	Mean	(min. - max.)	SD	
Palmitic C16:0	5.86	(4.6 - 8.8)	0.58	5.57	(4.1 - 6.7)	0.62	ns
Palmitoleic C16:1	0.32	(0.2 - 0.5)	0.03	0.38	(0.2 - 0.5)	0.03	ns
Stearic C18:0	2.98	(2.1 - 3.8)	0.48	3.41	(2.6 - 5.2)	0.62	ns
Oleic C18:1 n-9	47.65	(42.8 - 53.2)	2.13	51.11	(40.9 - 58.2)	2.67	*
Linoleic C18:2 n-6	19.97	(15.3 - 23.7)	1.91	20.43	(14.6 - 25.1)	2.36	ns
α-linolenic C18:3 n-3	10.93	(7.8 - 12.7)	1.07	8.07	(6.6 - 11.2)	1.26	**
Arachidic C20:0	0.90	(0.6 - 1.7)	0.21	1.23	(0.8 - 2.1)	0.33	ns
Gadoleic C20:1 n-9	6.82	(5.6 - 7.9)	0.51	4.63	(3.1 - 5.9)	0.65	**
Behenic C22:0	3.15	(2.3 - 5.2)	0.40	3.42	(2.1 - 5.3)	0.44	ns
Erucic C22:1 n-9	1.42	(0.8 - 2.4)	0.43	1.76	(0.9 - 2.6)	0.51	*
∑ SFA	12.89	(9.1 - 14.7)	1.26	13.62	(11.2 - 17.3)	1.81	ns
∑ MUFA	56.21	(48.7 - 62.4)	4.14	57.88	(46.3 - 61.7)	3.67	ns
∑ PUFA	30.90	(28.2 - 35.3)	2.98	28.50	(24.1 - 32.9)	2.07	*
n-3/n-6 FA	0.55	(0.47 - 0.58)	0.03	0.40	(0.36 - 0.43)	0.03	**
PI	41.83	(30.9 - 49.1)	4.02	36.57	(27.8 - 47.5)	4.35	**

SFA - saturated fatty acids, MUFA - monounsaturated fatty acids, PUFA - polyunsaturated fatty acids, PI (polyunsaturated index) = C18:2n-6 + (C18:3n-3 x 2)

(EAA) which was 37.7 - 38.1 g/16 g N, compared with the value of 51.2 g/16 g N in hen's egg white used in the assessment as an amino acid standard [28]. Similar results were obtained in the investigations conducted by Sujak et al. [2], where the EAA content in different white lupine seeds ranged from 36.0 to 37.7 g/16 g N.

The predicted nutritional values of the white lupine seed protein (EAA, CS_{Lys}, $CS_{Met+Cys}$, EAAI, BV and NI) were calculated based on standards of nutrition for 6-8 week broilers [30] and the nutrition standard for growing pigs [31]. Edible cultivars were compared against standard based on nutrient requirement for mature human [26]. Among analysed white lupine cultivars the highest EAAI, CS_{Lys}, BV and NI value was found for cv Amiga, and the highest $CS_{Met+Cys}$ for cv Energy both for mature human as well as for young chicken and pigs. According with these standards white lupine is rather a good source of the protein for chicken broilers nutrition (EAAI amounting 113.4%, CS_{Lys} 130.0 and BV 111.9). To a lesser degree white lupine protein covers the nutritional requirements for exogenous amino acids for mature human nutrition (EAAI amounting 94.9 and BV 91.8) and for young animals in particular of the pigs (20-50 kg), because it is deficient in sulphur containing amino acids (Met+Cys). This fact is expressed by the lowest EAAI, CS, BV and NI indices.

The mean values of CS_{Lys}, $CS_{Met+Cys}$ and EAAI were higer than those reported by Grela et al. [39] (78, 38.5 and 65.5) and similar with results reported by Sujak et al. [2], for white lupine cultivars grown in Poland.

The statistical analysis did not confirm the difference between the white lupine cultivars for value protein efficiency ratio (PER).

Nutritionally, a protein-based fodder is said to be of good nutritional quality when its essential amino acid index (EAAI) is >0.70, protein efficiency ratio (PER) is Ã2.7, biological values (BV) is >70% [43]. The nutritional outcome of examined white lupine seeds in terms of essential amino acid index, protein efficiency ratio and biological value showed that the white lupine contain proteins of an appreciable quality, however, the seeds cannot be used for animal feeding alone without complementing with other protein-based forages.

Fatty acid composition of lupine seeds

Generally, the quality of fat depends on fatty acid (FA) profile and content, and the ratios between individual acids [5, 47, 48]. For polyunsaturate FA (PUFA) the n-3/n-6 ratio (linolenic/linoleic fatty acids) is very important with respect to human and animal feeding. According to results presented in table 4, fatty acid composition showed that oleic acid (C18:1 n-9) was the major fatty acid, followed by linoleic (C18:2n-6) and α-linolenic (C18:3 n-3) acids. The individual fatty acid concentrations were influenced by the variety, but on average the prevailing FA included oleic, linoleic and linolenic acids, which is also confirmed by Rusnikova et al. [9].

The highest contents of C18:3 n-3 were detected in cv Amiga seed ($p < 0.01$) compared to cv Energy which contained the highest concentrations of C18:1n-9 ($p < 0.05$). A broad range of variation (6.6–12.7%) indicates on possibility of selection for high content of desired linolenic acid (n-3). Similar range of variability (5.6–12.8%) was obtained by Rybinski et al. [5].

Content of C22:1 (erucic acid) in seeds is considered undesirable for human and animal nutrition [4]. This work as well as data given by Rybinski et al. [5] indicates that a advantage of white lupine is the presence of small amounts of erucic acid. Minimum and maximum values for erucic acid ranged from 0.8 to 2.6% (table 4). The highest erucic acid content was found in the seeds lupine belonging to Energy cultivar (1.76 %) ($p < 0.05$). Similar results as in our study concerning FA concentrations in lupine were published [2, 3, 5, 9].

The quantities of MUFA, as assumed, prevailed over SFA and PUFA in examined white lupine cultivars. The SFA and MUFA no were significantly different in examined white lupine cultivars (p Ã 0.05); whereas in the cv Amiga showed higher proportions of PUFA (30.9%) compared to cv Energy (28.5%) ($p < 0.05$). Presented results are generally in agreement with values obtained by Erbas et al. [3], Rybinski et al. [5], Uzun et al. [41] and Zraly et al. [45], respectively. High content of polyunsaturated fatty acids indicates that lupine can be a potential source of considerable amount of useful fats. Moreover, the high content of linoleic and linolenic fatty acids makes lupine seed a good source of essential fatty acids.

The n-3/n-6 fatty acid ratio is important for human health and should be 1:1–1:4 [13]. White lupine seeds for examined meet this desirable ratio criterion: 1:1.83-2.53 (table 4). The fat from cv. Amiga was characterized by a highest content of á-linolenic acid, and thus a more favourable n-3/n-6 value as compared with cv. Energy ($p < 0.01$).

In the present study, it was observed that the polyunsaturated index (PI) of the lupine seeds samples were high, which indicated that seeds contain large amount

of PUFA. The highest values of PI were detected in cv Amiga seed ($p < 0.01$). Inclusions of polyunsaturated in human diets are recommended for preventing cardio-vascular diseases [14]. The polyunsaturated fatty acids are precursors of long chain n-3 PUFA in the eicosanoids biosynthesis, which are important in bio regulators of many cellular processes and immune system [43].

Conclusions

The results obtained in this study show that the variety had significant influence on the levels of crude protein, crude fat, crude fibre and on N-free extractives but didn't influence crude ash and alkaloides. Based on the experiments presented in this study, the cultivar affected amino acid content (Lys, Trp and Arg) and on nutritional values of the protein measured by means of CS, EAAI, BV and NI indices. Both varieties examined were characterised by a shortage of methionine and lysine, but lysine deficiency was higher in cv. Energy. The important point to be made here is that white lupine seeds examined can serve as a source of good quality food protein for adult humans, according to the standard of nutrition used. The examined white lupine meet the requirement for exogenous amino acids and Lys in 6-8 weeks' chicken broilers and to a lesser degree in the case of growing pigs (20-50 kg). The dominating fatty acid in examined white lupine seeds was monounsaturated oleic acid (47.6 - 51.1%). Among polyunsaturated fatty acids dominated linoleic FA (19.9 - 20.4%) followed by linolenic FA (8.0 - 10,9%). The fat from cv. Amiga was characterized by a highest content of á-linolenic acid, and thus a more favourable n-3/n-6 value for health as compared with cv. Energy (1:1.87 vs. 1:2.53) ($p < 0.01$). The two cultivars of white lupine studied are good protein sources, as having good quality protein and as profile of fatty acids and n-3/n-6 fatty acids ratio are suitable both for human and animal nutrition.

References

1.KASPROWICZ-POTOCKA, M., ZAWORSKA, A., KACZMAREK, S.A., RUTKOWSKI, A., Arch. Anim. Nutr., **70,** no**.** 3, 2016, p. 209.
2.SUJAK, A., KOTLARZ, A., STROBEL, W., Food Chem., **98**, 2006, p. 711.
3.ERBAS, M., CERTEL, M., USLU, M.K., Food Chem., **89**, 2005, p. 341.
4.ANDRZEJEWSKA, J., IGNACZAK, S., BARZYK, P., Acta Scientiarum Polonorum, Agricultura, **15**, 2016, p. 3.
5.RYBINSKI, W., SWIECICKI, W., BOCIANOWSKI, J., BORNER, A., STARZYCKA-KORBAS, E., STARZYCKI, M., Genet. Resour. Crop. Evol., **64**, 2017, p. 1.
6.BRENES, A., MARQUARDT, R.R., MUZQUIZ, M., GUENTER, W., VIVEROS, A., ARIJA, I., Span. J. Agric. Res., **3,** no**.** 2, 2005, p. 203.
7.SOBOTKA, W., STANEK, M., BOGUSZ, J., Ann. Anim. Sci., **16,** no. 1, 2016, p. 197.
8.STRAKOVA, E., SUCHY, P., VECEREK, V., SERMAN, V., MAS, N., JUZL, M., Acta Vet. Brno **75**, 2006, p. 489.
9.RUSNIOVA, L., STRAKOVA, E., SUCHÝ, P., Acta Vet. Brno, **82**, 2013, p. 283.
10.BRAHMA, J., BRAHMA, B.K., International Journal of Conservation Science, 7, no. 1, 2016, p. 137.
11.DRAGAN, F., LUPU, V.V., PALLAG, A., BARZ, C., FODOR, K., Edited by: Lemle, L.D., INNOVATIVE IDEAS IN SCIENCE 2016, Book Series: IOP Conference Series-Materials Science and Engineering, Volume: 200, 2017, Article Number: UNSP 012063, DOI: 10.1088/1757-899X/200/1/012063.
12.NEGI, P.S., SUBRAMANI, S.P., International Journal of Conservation Science, **6**, no. 4, 2015, p. 657.
13. KONUSKAN, O., KONUSKAN, D.B., LEVAI, C.M., Rev. Chim. (Bucharest), **68**, no. 9, 2017, p. 2073
14.SIMOPOULOS, A.P., World Rev. Nutr. Diet, **92**, 2003, p. 1.
15.MIERLITA, D., Bulletin UASVM Anim. Sci. Biotech, **72**(2), 2015, p. 188.
16.*** AOAC (Association of Official Analytical Chemists), Official Methods of Analysis In: W. Horowitz, Editor, Official methods of analysis, 18th ed., Gaithersburg, MD USA, 2005.
17.MANOLACHE, F.A., HANGANU, A., DUTA, D.E., BELC, N., MARIN, D.I., Rev. Chim. (Bucharest), **64**, no. 1, 2013, p. 45.
18.DOLIS, M.G., SIMEANU, C., USTUROI, AL., SIMEANU, D., Rev. Chim. (Bucharest), **68**, no. 1, 2017, p. 151.
19.RATU, R.N., USTUROI, M.G., SIMEANU, D., SIMEANU, C., USTUROI, AL., DOLIS, M.G., Mat. Plast., **54**, no. 2, 2017, p. 368.
20.IOVAN, M., RADU, F., ROTARU, L., Rev. Chim. (Bucharest), **67**, no. 5, 2016, p. 902.
21.DOLIS, M.G., BOISTEANU, P.C., SIMEANU, D., Rev. Chim. (Bucharest), **68**, no. 6, 2017, p. 1459.
22.VAN SOEST, P.J., ROBERTSON, J.B., LEWIS, B.A., J. Dairy Sci., **74**, 1991, p. 3583.
23.MAKNICKIENE, Z., ASAKAVICIUTE, R., BAKSIENE, E., RAZUKAS, A., Arch. Biol. Sci., **65**, no. 1, 2013, p. 107.
24.LEE, M.J., PATE, J.S., HARRIS, D.J., ATKINS, C.A., J. Exp. Bot., **58**, 2007, p. 935.
25.*** AOAC, Official Methods of Analysis of the AOAC. 15th ed., Association of Official Analytical Chemists, Arlington, VA, USA 1990.
26.*** FAO/WHO, Protein quality evaluation. Report of Joint FAO/WHO Export Consultant, FAO food Nutrition paper, **51**, 1991, p. 19.
267.KOTLARZ, A., SUJAK, A., STROBEL, W., GRZESIAK, W., Vegetable Crops Research Bulletin, **75**, 2011, p. 57.
28.SIMEANU, C., SIMEANU, D., POPA, A., USTUROI, A., BODESCU, D., DOLIS, M.G., Research regarding content in amino-acids and biological value of proteins from Polyodon spathula sturgeon meat, Rev. Chim. (Bucharest), **68,** no. 5, 2017, p. 1063.
29.***NRC (National Research Council), Recommended Dietary Allowances, Protein and Amino Acids, 10th ed. ISBN-10: 0-309-04633-5, DC: National Academy Press, Washington, 1989.
30.*** NRC (National Research Council), Nutrient Requirements of Poultry, 9th rev. ed., ISBN-10: 0-309-04892-3, Ed. National Academy Press. Washington, DC, 1994, pp. 19-34.
31.BOISEN, S., HVELPLUND, T., WEISBJERG, M.R., Livest. Prod. Sci., **64**, 2000, p. 239
32.OSER, B.L., An integrated essential amino acid index for predicting the biological value of proteins, in A.A. Albanese (Ed.), Protein and amino acid nutrition, New York: Academic Press, 1959, pp. 295-311.
33.ALSMEYER, R.H., CUNNINGHAM, A.E., HAPPICH, M.L., Food Technol., **28**, 1974, p. 34.
34.CRISAN, E.V., SANDS, A., Biology and cultivation of edible mushrooms, Hangeri Academic Press, New York, 1978, pp. 137-142.
35.TIMMONS, J.S., WEISS, W.P., PALMQUIST, D.L., HARPER, W.J., J. Dairy Sci., **84**, 2001, p. 2440.
36.SAS, SAS Users Guide, version 9.1. Cary. NC, SAS Institute Inc, 2000.
37.PISARIKOVA, B., ZRALY, Z., BUNKA, F., TRCKOVA, M., Vet. Med., **53**, 2008, p. 124.
38.YILMAZ, S., ATAK, M., ERAYMAN, M., Comparison of chemical composition in lupine (Lupinus spp.) species, Rev. Chim. (Bucharest), **66**, no. 3, 2015, p. 324.
39.GRELA, E.R., KICZOROWSKA, B., SAMOLINSKA, W., MATRAS, J., KICZOROWSKI, P., RYBINSKI, W., HANCZAKOWSKA, E., Eur. Food Res. Technol., **243**, 2017, p. 1385.
40.RYBINSKI, W., RUSINEK, R., SZOT, B., BOCIANOWSKI, J., STARZYCKI, M., Int. Agrophys., **28**, 2014, p. 491.
41.UZUN, B., ARSLAN, C., KARHAN, M., TOKER, C., Food Chem, **102**, no. 1, 2007, p. 45.
42.CHIRA, N.A., BRATU, A., MIHALACHE, M., TODASCA, M., DORNEANU, A., ROSCA, S.I., Rev. Chim. (Bucharest), **65**, no. 7, 2014, p. 774.
43.BOSCHIN, G., D'AGOSTINA, A., ANNICCHIARICO, P., ARNOLDI, A., Food Chem., **108**, 2008, p. 600.
44.WASILEWKO, J., BURACZEWSKA, L., J. Anim. Feed Sci., **8**, 1999, p. 1.
45.ZRALY, Z., PISARIKOVA, B., TRCKOVA, M., HERZIG, I., JUZI, M., Vet. Med., **52**, no. 1, 2007, p. 29.
46.IJAROTIMI, O.S., NATHANIEL, F.T., FARAMADE, O.O., J. Hum. Nutr. Food Sci., **3**, no. 2, 2015, p. 1060.
47.RAICIU, A.D., POPESCU, M., IVOPOL, G.C., BORDEI, N., ALEXANDRU, G., CRISAN, I., MANEA, S., DIMA, S.O., Rev. Chim. (Bucharest), **67**, no. 12, 2016, p. 2449.
48.COPOLOVICI, D., BUNGAU, S., BOSCENCU, R., TIT, D.M., COPOLOVICI, L., Rev. Chim. (Bucharest), **68**, no. 3, 2017, p. 507.

Manuscript received: 5.09.2017