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EQUATIONS AS A BASIS FOR FEED PROTEIN QUALITY EVALUATION FROM AMINO ACID ANALYSIS

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Abstract

The aim of the research was to establish the amino acid profile of proteins in white lupine beans from low alkaloid varieties and their nutritional evaluation, as an alternative to proteins from soy products and by-products. The amino acid composition of the proteins in the analyzed lupine samples showed that lupine is a good source of lysine (5.2 - 6.3 g / 16 g N), but is deficient in other essential amino acids, especially sulfur amino acids (methionine + cystine: 2.0 - 2.4 g / 100 g protein) and tryptophan. This is confirmed by the high value of CS_{Lys} *(85.28%), and sulfur amino acids were limiting amino acids (*$CS_{Met + Cys}$*: 38.07%), when egg protein was used as a standard. Similar aspects were found when used as standard, the nutritional requirements of the adult human, the nutritional requirements of broilers or the nutritional requirements of fattening pigs. Regardless of the standard used, in all cases mentioned Met + Cys were limiting amino acids. According to the data obtained in this study, it can be concluded that white lupine grains are rather a good source of protein for feeding broilers (EAAI is 107.49%,* CS_{Lys} *is 127.00 and P-BV is 105.46). The protein in lupine grains covers to a lesser extent the nutritional requirements of essential amino acids for the diet of mature man (EAAI in proportion of 89.01; and P-BV 85.32) but also for young animals especially for fattening pigs. (20-50 kg), because it is deficient in essential amino acids, this fact being expressed by the low values of the analyzed nutritional indices.*

Key words: essential amino acids, protein standard, limiting amino acids, EAAI, CS_{Lys}

INTRODUCTION

It is well known that the feeding of birds in an intensive system is dependent on conventional protein sources, respectively soybean meal which has a high protein content (42-46%) with a balance of essential amino acids close to the nutritional requirements of birds. However, in the context of the ban on animal meal in poultry feed, the ban on the cultivation of genetically modified plants (soy) and the tendency to limit the use of genetically modified soy products and by-products, it is necessary to evaluate unconventional sources of protein with good biological value. which can be available locally and at the same time be economical. Thus, alkaloid-free white lupine berries are a promising alternative.

Many authors consider that white lupine is a valuable source of cheap protein for animal feed (Podleśna et al., 2014) and is a suitable crop not only in Romania but also in other European countries (Mierliță, 2012; Voisin et al., 2014; Faligowska and Szukała 2015, Reckling et al., 2016), because they have low requirements for soil and climate and leave the land relatively quickly, making it possible to prepare the land for the establishment of autumn crops. The data from the literature mention that the improved varieties of white lupine produce 3500-4800 kg of grains / ha, contain 31-43% protein and 6-11% fat (depending on climatic conditions), but the lower biological value of the protein is underlined. from lupine compared to soy (Nalle et al., 2010).

The aim of the research was to establish the amino acid profile of proteins in white lupine beans from low alkaloid varieties and their nutritional evaluation, as an alternative to proteins from soy products and by-products.

MATERIALS AND METHODS

The amino acid content of lupine grain proteins was determined using an automatic amino acid analyzer type Biochrom 20 Plus and complying with the current standard PN-EN ISO 13903: 2006. Prior to analysis, the samples were hydrolyzed in 6 M HCl at 110 ^{0}C for 24 hours under a nitrogen atmosphere, according to the method described by Sobotka et al. (2016). Cystine and methionine were determined as cysteic acid and methionine sulfonate, respectively, after oxidation of the sample with performic acid for 16 hours at 0 ^{0}C and neutralization with hydrobromic acid before hydrolysis with 6 M HCl at 110 ^{0}C for 24 h was determined after hydrolysis of the sample with NaOH at 110 ^{0}C for 16 h, according to the current standard PN-EN ISO 13904: 2005. All analyzes were performed in four repetitions. The amount of amino acids was expressed in g / 16 g N, equivalent to g / 100 g of protein (FAO / WHO, 1991).

The nutritional quality of lupine grain proteins was estimated based on (Mierliță et al., 2018):

- total amino acid (AA) content;
- protein content in essential amino acids (EAA);
- EAAI (essential amino acid index), using as a standard egg protein (NRC, 1989), the nutritional requirements of 6-8 week old broilers (NRC, 1994), the nutritional requirements of meat pigs weighing 20-50 kg (Boisen et al., 2000) and the nutritional requirements of the adult human (FAO / WHO 1991);
- chemical score for limiting amino acids (CS_{Lys} - lysine chemical score; $CS_{Met + Cys}$ - methionine + cysteine chemical score);
- expected protein efficiency ratio (P-PER: Predicted-Protein Efficiency Ratio);
- predicted biological value (P-BV: Predicted-Biological Value);

• nutritional index.

EAAI (Essential Amino Acids Index) was calculated as the geometric mean of all participating essential amino acid concentrations (EAA) compared to the concentration of an appropriate standard (in g/16 g N) according to the following formula (Oser, 1959):

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

where a1… an, is the AA content of the tested protein and a1s… .ans is the AA content of the reference protein; n is the number of essential amino acids considered.

CS (chemical score) values were calculated for lysine and methionine + cystine, respectively, according to the following formula (Block and Mitchell, 1946):

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

where an is the AA content of the tested protein and ans is the AA content of the reference protein.

The Predicted-Protein Efficiency Ratio (P-PER) was calculated according to the equations developed by Alsmeyer et al. (1974):

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

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

The Predicted Biological Value (PMV) was calculated according to the methods of Oser (1959). The following equation was used to determine P-BV:

$$\text{P-BV} = 1{,}09\,(\text{EAAI}) - 11{,}7.$$

The nutritional index (NI) of lupine grain proteins was calculated using the mathematical relationship proposed by Crisan and Sands (1978):

$$NI\ (\%) = \frac{\text{EAAI} \times \%\ \text{proteină brută}}{100}$$

RESULTS AND DISCUSSION

The amino acid composition of the proteins in the analyzed lupine samples, expressed in g/16 g N, equivalent to g/100 g protein, is shown in Table 1. Table 2 shows the nutritional value of the proteins in lupine grains assessed on the basis of standards. presented in Table 3.

In general, lupine grains are a good source of lysine (5.2 - 6.3 g / 16 g N), but are deficient in other essential amino acids, especially sulfur amino acids (methionine + cystine: 2, 0 - 2.4 g / 100 g protein) and tryptophan (Fig. 1).

Table 1

Amino acid content of white lupine protein (g / 16 gN)

	Own results			Bibliographical references*		
	Media	(Min - Max)	SD	1	2	3
Essential AA						
Lysine	**5,97**	(5,21 - 6,37)	0,181	5,80	5,10	6,11
Methionine + Cystine	**2,17**	(2,02 - 2,46)	0,094	2,80	2,50	2,19
Arginine	**8,28**	(7,31 - 9,11)	0,209	8,96	11,1	9,89
Treonine	**3,72**	(3,41 - 3,92)	0,174	3,83	3,10	3,68
Izoleucine	**3,67**	(2,87 - 4,51)	0,161	3,06	4,10	4,39
Tryptophan	**0,84**	(0,75 - 0,89)	0,037	0,65	0,70	0,95
Valine	**4,03**	(3,47 - 5,09)	0,195	3,57	3,80	4,23
Leucine	**5,74**	(5,38 - 6,41)	0,254	7,20	8,20	6,18
Histidine	**2,40**	(2,18 - 2,69)	0,112	2,71	3,10	2,31
Phenylalanine+Tyrosine	**7,49**	(6,76 - 8,24)	0,275	6,12	5,50	8,09
Non-essential AA						
Aspartic acid	**10,56**	(9,40 - 11,67)	0,491	12,20	9,90	10,17
Serine	**5,19**	(4,72 - 5,84)	0,207	5,60	4,10	5,12
Glutamic acid	**21,31**	(19,15 - 23,4)	0,518	18,54	24,20	23,37
Proline	**3,87**	(3,21 - 4,15)	0,142	3,74	3,80	4,19
Glicine	**3,96**	(3,46 - 4,81)	0,196	3,83	4,30	4,17
Alanine	**3,49**	(2,78 - 4,11)	0,132	3,71	3,10	3,50
Tyrosine	**3,61**	(3,02 - 4,26)	0,097	2,79	1,50	3,98
Cystine	**1,45**	(1,15 - 1,92)	0,088	1,12	1,80	1,49

* 1- Grela et al., 2017; 2 - Sujak et al., 2006; 3 - Mierliță et al., 2018.

The available literature shows a high proportion of lysine and a relatively low level of amino acids with sulfur and tryptophan in white lupine grains (Brenes et al., 2005; Sujak et al., 2006; Zraly et al., 2007; Pisarikova et al. , 2008; Grela et al., 2017; Mierliță et al., 2018). This was largely confirmed by the present results, as the CS_{Lys} value for the proteins in the lupine grains analyzed was high (85.28%) and the sulfur amino acids were limiting amino acids ($CS_{Met + Cys}$: 38.07%), when egg protein was used as a standard (NRC, 1989). Similar aspects were found when used as a standard, the nutritional requirements of adult humans (FAO / WHO 1991), the nutritional requirements of broilers (NRC 1994) or the nutritional requirements of fattening pigs (Boisen et al. 2000) (Table 2). Regardless of the standard used, in all cases mentioned Met + Cys were limiting amino acids. Therefore, alkaloid-free white lupine grains should be combined with methionine-rich foods or feeds or supplemented with synthetic methionine (Kotlarz et al., 2011). Similar aspects were recorded in studies conducted by other authors, in the white lupine of the Amiga variety (Mierliță et al., 2018) and in the white lupine of other varieties cultivated in Europe (Sujak et al., 2006 - in the cultivated Boros variety in Poland; Grela et al., 2017 - in the Bhutan variety, cultivated in the Czech Republic).

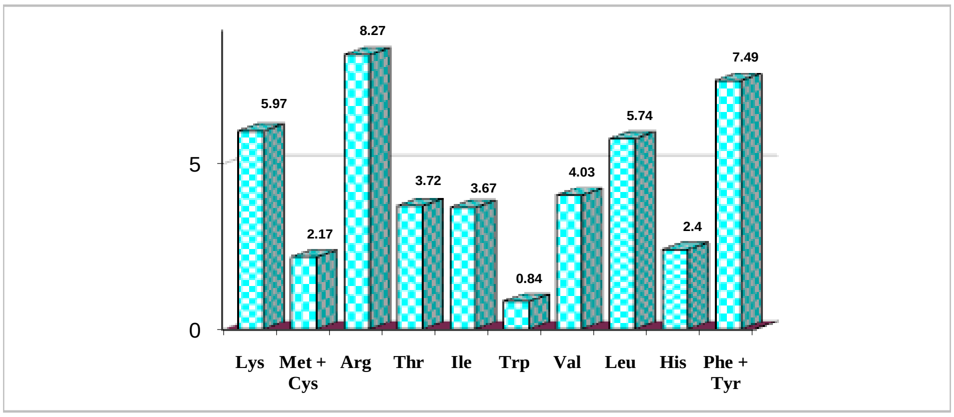

Fig. 1. Essential amino acid content of lupine grain proteins (g/16 g N).

Comparing the amino acid content of lupine grains proteins obtained in the agro-climatic conditions in Transylvania, with the data provided by other researchers, we find some differences that may be due on the one hand, the dosing methods used and on the other hand due to the conditions meteorological (humidity, temperature), soil type, agrotechnics used and last but not least, the variety used. Larger differences were found in the content of arginine, leucine and phenylalanine + tyrosine.

Protein from white lupine grains has a lower biological value than animal protein. This was confirmed in the present study by the lower content of essential amino acids (EAA), which was 36.03 g / 16 g N (Table 2), compared to the value of 51.2 g / 16 g N in chicken egg white used in evaluating the quality of lupine proteins as a standard (Hidvegi and Bekes, 1984). The results obtained in this study confirm the data previously obtained in other studies (Sujak et al., 2006; Grela et al., 2017; Mierliță et al., 2018), in which the EAA content in white lupine grains of different varieties, a ranging from 35.74 to 38.13 g / 16 g N.

The expected biological value (P-VB) of lupine grain proteins was 62.19 in our study, while in the literature are cited values between 61.00 and 66.46 (Table 2), which confirms and strengthens the results obtained in this study.

Table 2

Nutritional characterization of proteins from white lupine grains

	Own results	Bibliographical references*		
		1	2	3
Total AA (g/16 g N)	**92,68**	91,32	98,54	96,60
Standard[1]				
EAA (g/16 g N)	**36,03**	35,74	38,13	36,10
CS_{Lys}	**85,28**	82,85	87,28	72,86
$CS_{Met+CYS}$	**38,07**	39,12	38,42	43,86
EAAI (%)	**67,79**	66,70	71,71	67,55
P-BV	**62,19**	61,00	66,46	61,92
IN (%)	**24,67**	24,27	26,11	24,58
Standard[2]				
EAA (g/16 g N)	**33,63**	33,03	35,82	33,00
CS_{Lys}	**108,54**	105,45	111,09	92,72
$CS_{Met+CYS}$	**62,00**	80,00	62,57	71,43
EAAI (%)	**89,01**	85,91	94,98	85,86
P-BV	**85,32**	81,94	91,82	81,88
IN (%)	**32,40**	31,27	34,58	31,25
Standard[3]				
EAA (g/16 g N)	**36,03**	35,74	38,13	36,10
CS_{Lys}	**127,02**	123,40	130,0	108,51
$CS_{Met+CYS}$	**65,76**	84,84	66,36	75,75
EAAI (%)	**107,49**	105,43	113,44	107,14
P-BV	**105,46**	103,21	111,95	105,08
IN (%)	**39,12**	38,37	41,30	39,00
Standard[4]				
EAA (g/16 g N)	**36,03**	35,74	38,13	36,10
CS_{Lys}	**85,28**	82,85	87,28	72,86
$CS_{Met+CYS}$	**60,28**	77,78	60,83	69,44
EAAI (%)	**79,89**	78,57	84,45	79,69
P-BV	**75,38**	73,94	80,35	75,16
IN (%)	**29,07**	28,60	30,75	29,00
P-PER	**2,55**	2,60	2,73	2,71

EAA - total essential AA; CS - chemical score; EAAI - index of essential AA; P-BV - predicted biological value; P-PER - predicted protein efficiency ratio.
Standard[1] - egg protein (NRC, 1989),
Standard[2] - standard based on adult nutritional requirements (FAO / WHO 1991)
Standard[3] - standard based on the nutritional requirements of broilers, 6-8 weeks (NRC, 1994)

Standard[4] - standard based on the nutritional requirements of fattening pigs (20-50 kg) (Boisen et al., 2000)

* 1- data processed after Grela et al., 2017; 2 - Mierliță et al., 2018; 3 - data processed according to Sujak et al., 2006.

The nutritional index (IN) of lupine grain proteins, calculated as a percentage of the product between the crude protein content and the protein content of essential amino acids, was 24.67, according to data obtained in other studies (Sujak et al., 2006; Grela et al., 2017).

The expected nutritional values for white lupine protein (EAA, CS_{Lys}, $CS_{Met + Cys}$, EAAI, P-BV and IN) were calculated using egg protein as a standard but also based on standards for nutritional requirements for broilers (NRC, 1994); those related to the nutrient requirement for mature man (FAO / WHO 1991) and nutritional requirements for fattening pigs weighing 20-50 kg (Boisen et al. 2000) (Table 3). The values recorded in this study regarding the nutritional value of lupine protein, respectively the value for EAAI, CS_{Lys}, $CS_{Met + Cys}$, P-BV and IN, were comparable to those cited in the literature, which mentions numerous studies conducted in different pedoclimatic areas in Europe and in which different varieties of white lupine free of alkaloids have been studied (Sujak et al., 2006; Grela et al., 2017; Mierliță et al., 2018) (fig. 2). It should be noted that in most situations, especially the value of $CS_{Met + Cys}$ was at the lower limit of its range of variation, mentioned in other studies.

Table 3

Standards used to evaluate lupine seeds proteins

Standards used	Amount of essential AA (g/16 gN)									EAA (g/16 gN)
	Lys	Met + Cys	Thr	Ile	Trp	Val	Leu	His	Phe + Tyr	
Egg proteins (NRC, 1989)	7,0	5,7	4,7	5,4	1,7	6,6	8,6	2,2	9,3	51,2
Nutritional requirements for adults (FAO/WHO, 1991)	5,5	3,5	4,0	4,0	1,0	5,0	7,0	-	6,0	36,0
Nutritional requirements for broilers (NRC, 1994)	4,7	3,3	3,8	3,4	0,9	3,9	5,2	1,5	5,8	32,5
Nutritional requirements for pigs 20-50 kg (Boisen et al., 2000)	7,0	3,6	4,5	4,0	1,2	5,2	8,0	2,5	8,0	44,0

According to the data obtained in this study, using the standards mentioned above (nutritional requirements for humans, broilers and fattening pigs), it can be concluded that white lupine grains are rather a good source of protein for feeding chickens (EAAI is 107.49%, CS_{Lys} is 127.00 and P-BV is 105.46) (Fig. 3).

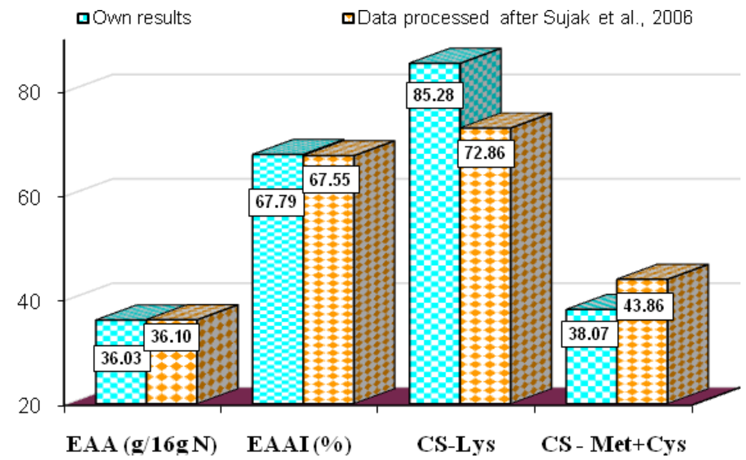

Fig. 2. The value of quality nutritional indices of lupine proteins, using egg proteins as standard (NRC, 1989).

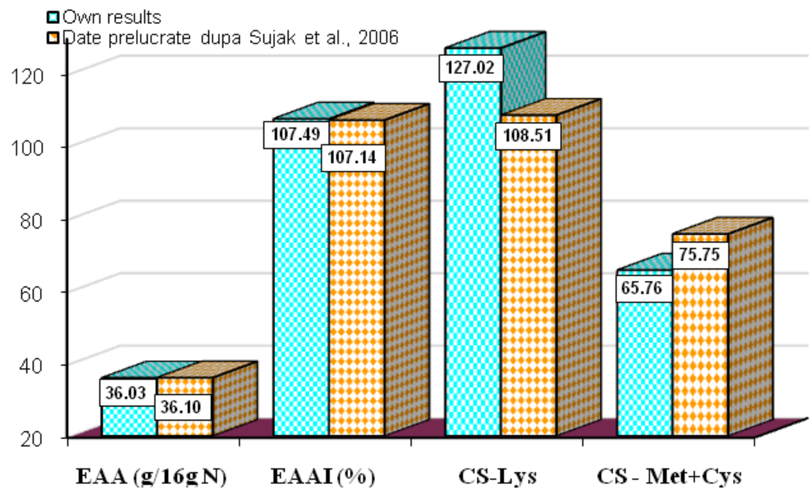

Fig. 3. The value of quality nutritional indices of lupine proteins, using as standard the nutritional requirements of broilers (NRC, 1994).

The protein in lupine grains covers to a lesser extent the nutritional requirements of essential amino acids for the diet of mature man (EAAI in proportion of 89.01; and P-BV 85.32) but also for young animals especially for fattening pigs. (20-50 kg), because it is deficient in the content of essential amino acids, this fact being expressed by the lower indices of EAAI (79.89%); CSLys (85.28); CSMet + Cys (60.28); P-BV (75.38) and IN (29.07).

From a nutritional point of view, food proteins have a good nutritional value when the index of essential amino acids (EAAI) is> 70.0%; the protein efficiency ratio (PER) is >2.7; expected biological value (P-BV) is> 70% (Ijarotimi et al., 2015).

CONCLUSIONS

The results obtained in this study, respectively the values recorded for EAAI, PER and P-BV, showed that white lupine from low alkaloid varieties contains proteins of appreciable quality, but still they cannot be used as the only source of protein for animal nutrition must be combined with feeds whose proteins have a higher biological value, but also with feeds that have a higher content of sulfur amino acids.

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