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The variance of the amino acids in some lucerne (*Medicago sativa* L.) populations

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SUMMARY – It is probably theoretically possible to select lucerne materials with the better amino acid composition for the different uses. The highest coefficients of variance were observed for isoleucin (31.52), phenylalanin (30.43), arginin (30.35) and methionin (25.88). It is necessary to check the heredity of these traits. The differences in the total content of the amino acids among populations were from 85.89 to 110.11% (100% = 39233 g/kg). The highest total content of amino acids and the content of indispensable amino acids was observed in populations *ZE 24.5*, *Palava*, *N $_2$ III*, *SAP II* and *HOLÝ 1*.

Key words: Lucerne, quality, amino acid.

RESUME – *"Variation de la teneur en acides aminés de populations de luzerne (*Medicago sativa *L.)". La sélection de luzerne, possédant une meilleure composition en aminoacides, et destinée à différentes applications, est en principe théoriquement possible. Les coefficients de variabilité les plus élevés ont été observés dans l'isoleucine (31,52), dans la phénylalanine (30,43), dans l´arginine (30,35) et dans la méthionine (25,88). La confirmation de l´hérédité de ces caractères est indispensable. Les différences dans la teneur totale en aminoacides entre les populations analysées variaient de 85,89 à 110,11%. La teneur totale la plus élevée en aminoacides et la teneur en aminoacides indispensables ont été trouvées dans les populations* de ZE 24.5, Palava, N $_2$ III, SAP II *et* HOLÝ 1.

Mots-clés : *Luzerne, qualité, acide aminé.*

Introduction

The protein constitutes 80-90% of all organic substances in the animal body. Protein cannot be compensated for by another nutrient. The protein of the animal organism is created mostly from the plant protein (Koudela *et al*., 1964).

The increase in fodder yield and quality are the most important targets for the lucerne breeding. It mainly means improving the digestibility, increasing the protein content, improving the amino acid profile, increasing beta-carotene content, decreasing the hemolytic saponins content (Petrík, 1987).

The lucerne breeding for a higher feed quality may target either a higher protein content or a higher digestibility. A better way is by means of earlier and more frequent cuts. The protein yield and the preceding crop worth are possible to increase by the higher symbiotic nitrogen fixation of lucerne (Chloupek, 1995).

An understanding of amino acid requirements is important to minimize wastage of dietary protein and to optimize animal productivity (Chalupa and Sniffen, 2000). A 650 kg cow producing 45 l milk with 3.2% crude protein requires 2918 g metabolizable protein, 59 g methionine, 181 g lysine and 144 g isoleucine, every day.

The main objective of this paper was to evaluate the amino acid composition in the different newly bred materials of lucerne with the improved yield and quality.

Material and methods

The standard varieties and newly bred materials of lucerne (*Medicago sativa* L.) were analyzed for their quality characteristics. The amino acids composition in lucerne hay (first harvest year, first cut in bud stage) was compared with those of the soya (*Glycine max* L.) seeds, white lupine (*Lupinus albus* L.) and andaen lupine (*Lupinus mutabilis* Sweet) seeds.

The analyzed plant materials were:

(i) Lucerne: standard varieties *Palava* and *Zuzana*, newly bred materials *ZE 24.5*, *KMOP 30, PAST* (a grazing type lucerne), *N $_2$ III* (the population with the highest symbiotic nitrogen fixation), *SAP II* (the population with the lower content of the hemolytic saponins), *HOLÝ 1*, *HOLÝ 2.*

(ii) Soya (*Glycine max* L.): Varieties *Gieso*, *Maple Arrow*, *Portage 94*, *Wilkin*, *Polanka*.

(iii) White lupine (*Lupinus albus* L.): Variety SU-*L 94002*.

(iv) Andean lupine (*Lupinus mutabillis* Sweet): New Czech variety *Anda*.

The automatic aminoanalyzer was used for the determination of the 16 amino acids (Table 1). The amino acid analysis was performed at the Veterinary and Pharmaceutical University of Brno, Institute of Nutrition, Dietetic and Hygiene. The automatic analyzer AAA T 339 (Mikrotechna Praha, CZ) was used with hydrolysat system, which is determined for fixed amino acids analysis (after acid and oxidative hydrolysis).

Results and discussion

The biologic value of the protein is important for each fodder. It may be expressed as a degree of protein exploitation by animal organism. It depends on the content and proportion of the individual amino acids (AA) (Table 1). The animal protein is universally full-valued (milk, egg). The plant protein is biologically non full-valued; there is a deficit of some essential amino acids. Both animal and plant protein considerably differ in amino acids composition between and within species and populations.

The highest total content of the amino acids (% AA) was observed in the lucerne populations ZE *24.5* (110.11%), *N $_2$ III* (106.29%), *Palava* (106.29%), *HOLÝ 1* (104.50%) and *SAP II* (103.74%) in comparison with the mean of the observed lucerne materials (Table 1).

The evaluation of the amino acids was performed in the three different ways (Table 2):

(i) The amino acid evaluation according to Koudela and Labuda (1964): essential amino acids (vital importance) (phe, ileu, leu, lys, met, thr, try, val), amino acids compulsory for growth of organism (the first group plus arg and his), "dispensable" amino acids (glyc, asp, ala, ser, pro). Our lucerne populations had the highest content of the vital importance and for the growth indispensable amino acids as follows: *ZE 24.5* (111.28%), *Palava* (111.28%), *N $_2$ III* (110.68%), *SAP II* (105.20%) and *HOLÝ 1* (104.00%).

(ii) For the human being essential amino acids are valine, leucine, isoleucine, lysine, methionine, threonine, phenylalanine and tryptophan according to Karlson (1971). From this point of view the ranking of the observed lucerne populations was Palava (111.00%), *ZE 24.5* (110.25%), *N $_2$ III* (110.25%), *SAP II* (105.75%) and *HOLÝ 1* (103.50%).

(iii) Evaluation of the amino acids according to Chalupa and Sniffen (2000), standardized methionine, lysine and isoleucine for the cow feeding. It led practically to the same ranking of the best lucerne populations: *Palava* (114.27%), *ZE 24.5* (111.94%), *N $_2$ III* (111.94%), *SAP II* (107.27%) and *HOLÝ 1* (104.95%).

The highest total content of the amino acids and content of the most important amino acids was observed in the home variety *Palava* and related population *ZE 24.5* (*Palava Europe*). Then following material with the higher symbiotic nitrogen fixation *N $_2$ III* and with the lower content of the hemolytic saponins *SAP II.* On average, the last population was *HOLÝ 1*.

Table 1. Amino acids (AA) in the lucerne, soya and lupine materials from the Plant Breeding Station Zelešice (g/kg)

Amino acids	Lucerne												Soya							Lupine			
	Palava	Zuzana	ZE 24.5	KMOP 30	PAST	N_2 III	SAP II	HOLÝ 1	HOLÝ 2	Mean	Var. coef.	% of AA	Gieso	Maple Arrow	Portage 94	Wilkin	Polanka	Mean	% of AA	White lupine	Andean lupine	Mean	% of AA
Asparagic acids	7.40	7.50	8.30	7.20	7.50	7.30	7.50	8.50	6.60	7.533	7.539	19.20	44.34	44.84	46.44	42.05	41.17	43.77	13.38	35.27	33.67	34.47	13.60
Threonine	2.10	1.90	2.10	1.80	1.90	2.10	2.10	2.00	1.90	1.989	5.867	5.07	14.33	14.28	14.88	13.69	12.93	14.02	4.29	12.19	11.21	11.70	4.62
Serine	2.20	2.00	2.20	1.90	1.90	2.20	2.10	2.00	1.90	2.044	6.523	5.21	18.22	18.15	19.30	17.69	16.55	17.98	5.50	17.20	14.72	15.96	6.30
Glutamic acid	3.90	3.50	3.90	3.30	3.40	4.00	3.80	3.80	3.50	3.678	6.902	9.37	62.62	62.81	68.47	60.41	59.43	62.75	19.19	72.91	57.99	65.45	25.83
Proline	3.50	3.40	4.10	3.20	3.30	3.60	3.60	3.70	3.40	3.533	7.489	9.01	16.59	20.49	19.33	17.06	12.79	17.25	5.27	7.01	9.05	8.03	3.17
Glycine	1.90	1.80	1.90	1.70	1.70	2.00	1.90	1.80	1.70	1.822	5.998	4.64	16.08	16.28	16.23	14.91	14.40	15.58	4.76	14.39	12.08	13.23	5.22
Alanine	2.50	2.30	2.60	2.20	2.20	2.50	2.50	2.30	2.20	2.367	6.680	6.03	18.64	19.10	19.56	18.56	17.26	18.62	5.69	6.63	4.92	5.78	2.28
Valine	2.50	2.30	2.60	2.20	2.20	2.50	2.40	2.40	2.20	2.367	6.337	6.03	17.93	18.41	20.31	18.63	19.16	18.89	5.77	6.15	6.12	6.14	2.42
Methionine	0.40	0.20	0.40	0.20	0.30	0.40	0.40	0.30	0.30	0.322	25.880	0.82	3.17	2.82	4.52	0.87	2.73	2.82	0.86	0.61	0.90	0.75	0.30
Isoleucine	2.00	1.70	1.90	0.30	1.70	1.90	1.80	1.80	1.60	1.633	31.524	4.16	16.43	16.42	17.68	16.56	17.11	16.84	5.15	14.71	12.80	13.76	5.43
Leucine	3.20	2.70	3.10	2.70	2.70	3.20	3.00	3.00	2.80	2.933	7.233	7.48	27.69	28.15	29.50	27.13	27.07	27.91	8.53	23.73	22.61	23.17	9.14
Tyrosine	2.00	1.70	1.90	1.70	1.60	1.90	2.00	1.80	1.70	1.811	8.023	4.62	11.59	11.07	11.88	10.46	10.20	11.04	3.38	12.27	10.71	11.49	4.53
Fenylalanine	2.10	0.40	2.10	1.70	1.70	2.10	2.00	1.90	1.80	1.755	30.434	4.47	15.48	16.39	16.83	15.68	15.46	15.97	4.88	4.25	4.01	4.13	1.63
Histidine	1.40	1.20	1.30	1.10	1.20	1.30	1.20	1.20	1.10	1.222	7.953	3.11	9.21	9.32	8.83	8.83	7.97	8.83	2.70	9.14	6.61	7.88	3.11
Lysine	2.50	2.20	2.50	2.10	2.20	2.50	2.40	2.40	2.20	2.333	6.777	5.95	22.30	23.52	24.00	22.27	20.60	22.34	6.89	18.99	14.58	16.79	6.63
Arginine	2.10	2.10	2.30	0.40	1.90	2.20	2.00	2.10	1.90	1.889	30.346	4.81	13.77	13.51	12.71	10.79	10.40	12.24	3.74	16.77	12.51	14.64	5.78
Total AA	41.70	36.90	43.20	33.70	37.40	41.70	40.70	41.00	36.80	39.233		100.00	328.40	335.59	341.64	306.77	305.24	327.06	100.00	272.22	234.50	253.36	100.00
% AA	106.29	94.05	110.11	85.89	95.33	106.29	103.74	104.50	93.80	100.00	7.987		100.41	102.61	104.46	93.80	93.33	100.00		107.44	92.56	100.00	
Crude protein in DM	217.1	219.3	217.9	209.9	213.2	215.3	216.0	211.5	214.8	215.5			417.30	417.60	434.40	400.30	410.60			441.40	354.90		
% AA from crude prot.	19.21	16.83	19.83	16.05	17.54	19.37	18.84	19.38	17.13	18.25			78.70	80.36	78.65	76.63	74.34			61.67	66.08		

Table 2. Evaluation of the amino acid content in the lucerne, soya and lupine materials

Evaluation	Percentage of the mean value of the lucerne populations									Mean value (g/kg)		
	Palava	Zuzana	ZE 24.5	KMOP	PAST	N_2 III	SAP II	HOLÝ 1	HOLÝ 2	Lucerne hay	Soya seeds	Lupine seeds
Total amino acid content	106.29	94.05	110.11	85.89	95.33	106.29	103.74	104.50	93.80	39.23	327.06	253.00
Koudela and Labuda (1964)[†]	111.28	89.39	111.28	76.01	96.01	110.68	105.20	104.00	96.08	16.44	136.53	98.95
Karlson (1971)[††]	111.00	85.50	110,25	82.50	95.25	110.25	105.75	103.50	96.00	13.33	118.99	76.43
Chalupa and Sniffen (2000)[†††]	114.27	95.61	111.94	55.97	97.95	111.94	107.27	104.95	95.61	4.29	42.00	31.29

[†]Koudela and Labuda (1964): amino acids of vital importance and indispensable for the growth of the organism (phe, ileu, leu, lys, met, thr, try, val + arg, his).
[††]Karlson (1971): amino acids indispensable for the human being (val, leu, ileu, lys, met, thr, phe, try).
[†††]Chalupa and Sniffen (2000): amino acids standardized for dairy cattle (met, lys, ileu).

Depending on the inheritability of the amino acid content breeding for the better amino acids composition could be successful. Especially for the amino acids with the largest variability. The highest coefficients of the variance were observed for isoleucine (31.52), phenylalanine (30.43), arginine (30.35) and methionine (25.88) (Table 1). Heritability for protein concentration was the highest of all the quality traits investigated by Hill and Barnes (1977).

The percentage contents of the individual amino acids in the lucerne, soya and lupine can be compared in our results. Alfalfa with 20% crude protein and 40% NDF (neutral detergent fiber) is ideal for formulating rations for high producing dairy cows. The acreage of the soya and lupine are very small in the Czech Republic, therefore the lucerne is one of the most important protein feeds.

The conclusions of Rotili *et al*. (1994) was that it is almost impossible to obtain positive results in the breeding for protein and fiber content because of very low genetic variability. This opinion was corrected by Rotili *et al.* (1996). It is possible to improve this trait by indirect selection, i.e. for physiological traits such as leaf persistency, onset of senescence and resistance to an early cutting regime (in our trial the pasture lucerne).

Heinriches (1970) found that protein concentration was higher in the leaves than in the stems. Selection for protein concentration without considering forage yield might lead to a decrease in total forage yield due to an increase in the leaf-to-stem ratio.

In this paper we studied differences among populations (varieties and newly bred materials) and not among genotypes within populations. We will eventually be able to choose the best populations to recommend farmers and do not intend to improve our knowledge of the genetic control of these traits.

Conclusions

(i) Based on the variability of the results obtained (Tables 1 and 2) it is theoretically possible to select lucerne materials with the better amino acid composition for the different utilization.

(ii) The way of lucerne selection may probably influence the amino acid composition.

(iii) The highest total content of amino acids as well as the content of indispensable amino acids was observed in population *ZE 24.5*, *Palava, N_2 III*, *SAP II* and *HOLÝ 1*.

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