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NUTRITIONAL VALUE OF BEANS AND LENTILS IN RATS

HRANIDBENA VRIJEDNOST GRAHA I LEĆE U ŠTAKORA

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SUMMARY

The nutritional value of Slovenian variety of common bean (Phaseolus vulgaris) Češnjevec and French variety of lentil (Lens esculenta Puyensis var. Anicia) was evaluated on young growing rats. Before the preparation of diets, both legumes were cooked and analysed on the crude nutrients and mineral elements. Diets were supplemented with lacking sulphur containing amino acids. Two experiments were performed with each legume and compared to a pair fed on casein. The growth rate and average dry matter intake were similar in all four groups of young growing rats in the experiments. The amount of faeces and faecal N excretion increased and nitrogen digestibility decreased significantly ($P \leq 0.001$) in legume fed rats as compared to their casein controls. In contrast, the biological value of protein was apparently unaffected in cooked bean diet, but biological value of lentil protein was significantly ($P \leq 0.001$) lower than in the casein group. Average net protein utilisation (NPU) of cooked lentil was 57.2% and of cooked bean 73.1% of their controls. Viscosity of small intestine content, and average amount and proportion of volatile fatty acids (VFA) in large intestine of cooked bean rats were higher than in other groups. Even if common bean and lentil had similar protein content, but different contents of fibre, starch and antinutritional factors, their metabolism in the body was different.

Key words: bean, lentil, protein, amino acids, rats, digestibility, biological value, viscosity, volatile fatty acids

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INTRODUCTION

Legumes are one of the world's most important sources of food supply, especially in developing countries, in terms proteins, some mineral elements and energy. On a global basis, plants supply over 65% of food protein and over 80% of food energy. Legumes have a special place in diets, because they contain nearly two to three times more protein than cereals.

Food legumes become notorious because of their antinutritional factors: enzyme inhibitors (protease inhibitors), lectins, tannins and others. Another limit in their utilisation includes a low protein apparent digestibility and deficiency in sulphur amino acids. However, leguminous seeds have now become interesting for human nutrition by the healthy effect of fibres and polysaccharides on the physiology of the digestive tract as well as on the glucose level and lipid metabolism. In the Western world consumers have recently shifted from animal fat to vegetable oil products because of their ability to lower the blood cholesterol level. Many food legumes have been shown to lower serum cholesterol level in the liver of rats (Evans and Cheung, 1993, Mongeau et al., 1993, Roberfroid, 1993, Glore et al., 1994). Although there is substantial evidence to support a protective role of dietary fibres against colorectal cancer, there is controversy and uncertainty as to which fibre types are protective and how they act.

In the mouth, high-fibre foods generally require more chewing. This slows down the process of eating and stimulates an increase flow of saliva. The saliva contributes to the volume of the swallowed food bolus. Once in the stomach, the fibre-rich food tends to absorb water and the soluble component starts to become viscous. In the small intestine the soluble fibre travels slowly because of increased viscosity; this prolongs the period of time available for the absorption of nutrients. Once in the large intestine, the soluble fibre becomes a food source for the growth and multiplication of the bacterial flora. The result of this process is an increase in faecal mass due to the multiplication of the bacteria, production of short-chain fatty acids and a decrease in colon pH. In addition CO_2, H_2 and some CH_4 are produced. These contribute to a sensation of bloating and flatulence. Insoluble fibre, which has reached the colon largely unchanged, swells by water holding and, adds further to the volume of the colonic contents. The faeces therefore are both bulkier and softer because of the increased water content (Barasi, 1997).

Fibres that increase the viscosity in the intestine decrease the blood glucose and insulin level. Foods which contain fibres have low glycemical index (Mongeau et al., 1993). Beans are the most suitable food to improve the glucose tolerance, because it causes the lowest and slowest increase of blood glucose and insulin level (Hughes, 1991). Diet rich in complex carbohydrates and containing non-starch polysaccharides is recommended in the management of diabetes (Barasi, 1997).

The aim of this study was to compare the nutrition utilisation of two legumes that are similar in their crude protein content and their energy content, but differ in their antinutritional factors, their fibres, tannins, starch etc; as described and commented by Pirman and Stibilj (2000). Beans and lentils are known to contain a relatively large amount of proteins, but the contents of some amino acids (arginine, tyrosine, valine, leucine and phenilalanine) in proteins are different (Deshpande and Damodaran, 1990; Pirman and Stibilj, 2000). Beans are very popular food in Slovenia. In France, especially in the province of Haute-Loire, from where green lentils originate, lentils are largely consumed. In the study cooked and freeze-dried legume seeds were used. Cooking is the way of utilisating legumes in human nutrition.

MATERIAL AND METHODS

In the experiments defined samples of legumes were used. The Slovenian common bean (Phaseolus vulgaris) variety Češnjevec was obtained from the 1997 harvest from Semenarna Ljubljana. A sample of green lentil (Lens esculenta Puyensis var. Anicia - Lentille verte du Puy), harvested in 1997, was obtained from the province of Haute-Loire (France), where this variety of lentil originates from. Beans were soaked in 2 volumes of water during the night and next day cooked in the same unsalted water for 50 minutes. Since this variety of lentil did not need soaking it was cooked according to the producer's instructions: 20 minutes in 3 volumes of unsalted boiling water. The cooked legumes were filtered and freeze dried.

Table 1. Composition of experimental diets (g/kg).
Tablica 1. Sastav pokusnih obroka

	Cooked bean Kuhani grah	Cooked lentil Kuhana leća	Casein Kazein
Cooked bean Kuhani grah	780		
Cooked lentil Kuhana leća		620	
Casein - Kazein			185
Wheat starch Pšenični škrob	108	258	665
Sunflower oil - Suncokretovo ulje	42	42	50
Cellulose - Celuloza	10	10	10
L-methionin - L-metionin	10	10	
Cystein - Cistein			10
Vitamin mixture* Vitaminska mješavina	10	10	10
Mineral mixture ** Mineralna mješavina	40	50	70

* Vitamins (IE/kg or mg/kg): 2,000,000IE A, 250,000IE D3, 2000mg BI, 1500mg B2, 7000mg B3, 1000mg B6, 15,000mg B7, 5mg B12, 80,000mg C, 17,000mg E, 4000mg K3, 10,000mg PP, 136,000mg cholin, 500mg folic acid, 5000mg acid APAB, 30mg biotin.

** Minerals (g/kg): 100g Ca, 60g K, 40g Na, 10g Mg, 3g Fe, 77.5g P, 0.8g Mn, 0.45g Zn, 125mg Cu, 0.9mg Co and 4.9mg J.

Samples of cooked lyophilised legumes were ground in a cyclone mill (Tecator, mesh 1 mm) into meal and mixed with other ingredients of the diets. Water was added for good homogeneity and semi-liquid consistency. Composition of the diets (Table 1) was designed to meet the nutritional requirements of the growing rats (NRC, 1995), and brought 18 MJ gross energy and 180 g crude protein per kg of diet in dry matter.

To the determine biological value of bean and lentil protein, two metabolic experiments were done. In each experiment there were only 12 rats, because of the limited number of cages. Male rats used were from the Wistar (Krka, Novo mesto) strain. The average body mass of animals for the first experiment was 161.2 g ± 21.9 g (42 days old), for second 118.6 g ± 7,4 g (35 days old), 7 animals were fed on a legume diet and 5 animals on a casein diet. Young growing male rats were individually housed in metabolic cages, which permit to collect the urine and the faeces during the experiment, normally without any contamination between the urine and faeces. In those cages water was always available. Animals on the legume diet were fed ad libitum and rats in the control casein diet were pair-fed. With two days of delay rats with the casein diet were offered the average amount (in dry matter) of diet eaten by rats in the legume diet.

During the experiment the room temperature was practically the same (about 21°C) and with special moisture condition (about 60%). The light was regulated by an automatic timeswitch during the experiment to obtain the same conditions every day (12 hours light/dark regime).

Nitrogen balance experiments were performed after the mode of work developed in the laboratory of the Institute for Nutrition (Orešnik et al., 1981, Orešnik et al, 1982, Stekar et al, 1984, Orešnik and Cvirn, 1984). Before the beginning of the balance experiment rats had pre-experimental period for 9 days in order to be adapted to all conditions in the experimental room, to the cage and diet. The experimental period that included also the collection of urine and faeces was 5 days long.

From the obtained data we calculated:

- apparent digestibility (AD, %) = (N intake - N faeces) / N intake

- true digestibility (TD, %) = (N intake - (N faeces - N endogenous)) / N intake

- endogenous N faeces (ENF, mg) = 15.2 x (average body mass / 0,075)

- endogenous N urine (ENU, mg) = 0,081 x (average body mass) + 3,01

- biological value (BV, %) = N intake - (N faeces - ENF) – (N urine – ENU)/ N intake (N faeces - ENF)

- net protein utilisation (NPU, %) = BV x TD

- protein efficiency ratio (PER) = gain in body mass (g) / protein consumed (g).

Table 2. Results for certified reference material.*
Tablica 2. Rezultati potvrđenih referentnih materijala

	Obtained value in lab Vrijednost dobivena u lab-u	Certified value Potvrđena vrijednost
Whole milk powder (CRM 380) - Neobrano mlijeko u prahu		
N-Kjeldahl (g/100g)	4.30 ± 0.22 (4)**	4.50 ± 0.04
Fat (g/100g) - Mast	26.8 ± 0.7 (6)	26.9 ± 0.3
Ash at 550°C (g/100g) Pepeo kod 550°C	6.06 ± 0.01 (4)	6.07 ± 0.05
Haricot Vertes (Beans) (CRM 383) Grah Haricot Vertes		
N-Kjeldahl (g/100g)	1.0 ± 0.02 (4)	1.1 ± 0.1
Ash at 550°C (g/100g) Pepeo kod 550°C	2.5 ± 0.0 (4)	2.4 ± 0.1
P (g/kg)	1.7 ± 0.05 (4)	(1.8)
Mg (g/kg)	1.1 ± 0.02 (4)	(0.9)
Ca (g/kg)	2.9 ± 0.1 (4)	2.9 ± 0.1
K (g/kg)	8.4 ± 0.4 (4)	7.8 ± 0.2

* Results are means ± SD - Rezultati se odnose na prosjeke + SD

** Number of determinations - Broj uzoraka

The crude nutrients content and mineral elements in legume samples and diets, and the N content in urine and faeces were determined by the standard procedures of the VDLUFA (Methodenbuch, III, 1993). The gross energy content of diets was determined by using an adiabatic bomb calorimeter (IKA Analysentechnik GmbH, Heitersheim, Germany). Tannin content was determined by the procedure for determination of tannin in sorghum (Methodenbuch, III, 1993). The extract viscosity of diets and viscosity of the contents of small intestine were done according to Dusel et al. (1997) with some modification explained elsewhere (Pirman and Stibilj, 2000). Volatile fatty acids (VFA) were determined by the modified method according to Anaerobe Laboratory Manual (1975).

The accuracy and reproducibility of the used methods were checked with BCR certified reference material CRM 380 - Whole Milk Powder, CRM 383 - Haricots Verts (Beans) and (BCR, 1992). The measured values of all methods are in good agreement with the certified values (Table 2).

Data from the rat experiments were analysed by the General Linear Models (GLM) procedures (SAS, 1990) of SAS® software (Release 6.12), taking into consideration the diet as the only main effect. The data are expressed as least square means (LSM) ± standard deviation. If not explained otherwise, the least significant difference 0.05 was used to separate treatment means.

RESULTS AND DISCUSSION

In Table 3 the composition of crude nutrients, gross energy and minerals in experimental diets are presented. The composition of the experimental diets is similar, they are isonitrogenous and isocaloric. However, they are different in the crude fibre, the legumes diets are 2 times higher than the casein diets. The tannin concentration is very weak, the lentil diet is 10 times higher than the bean diet. The viscosity of bean diet is 30 times mat of lentil diet and it is in accordance with the results of extract viscosity in cooked bean and cooked lentil sample. In sample of cooked bean Češnjevec high (189.6 $mPa.s^{-1}$) extract viscosity is found and cooked green lentil sample has 3.18 $mPa.s^{-1}$ (Pirman and Stibilj, 2000). Also the other characteristics have been shown to be specific of the grains which were used (Pirman and Stibilj, 2000). All those compounds (high fibre, light tannins and high viscosity) are of importance and can modify the process of digestion of the intake in the gut and their metabolic utilisation. Among the studied Slovenian bean cultivars, Češnjevec had the highest NDF and the tannin content (Stekar et al., 1997).

Table 3. Crude nutrients, gross energy, minerals, and extract viscosity of experimental diets (in dry matter).
Tablica 3. Sirove hranjive tvari, ukupna energija, minerali i ekstrahirani viskozitet pokusnih obroka (u suhoj tvari)

	Cooked bean Kuhani grah	Casein* Kazein	Cooked lentil kuhana leća	Casein** Kazein
Crude protein (g/kg) § - Sirova bjelančevina	175.59	187.08	173.47	185.99
Crude fat (g/kg) - Sirova mast	61.12	61.08	54.60	56.54
Crude fibre (g/kg) - Sirova vlaknina	61.82	24.92	60.21	26.50
Ash (g/kg) - Pepeo	53.99	50.94	46.40	44.18
NFE (g/kg) - NET	647.47	669.06	665.32	680.81
Gross energy (MJ/kg) - Ukupna energija	18.65	18.50	18.73	18.55
Tannins (g/100g) - Tanini	0.014		0.102	
Extract viscosity (mPa.s) - Ekstrahirani viskozitet	30.85	0.88	1.19	0.77

* Casein diet from the first experiment – Kazeinski obrok iz 1. pokusa

** Casein diet from the second experiment – Kazeinski obrok iz 2. pokusa

§ N * 6,25

f Nitrogen free extractives - NET

Table 4. Average body mass of rats, daily gain and consumed test diets. *
Tablica 4. Prosječna tjelesna masa štakora, dnevni prirast i konzumirani pokusni obroci

	1st experiment - 1. Pokus		2nd experiment - 2. Pokus	
	Cooked bean (7)§ Kuhani grah	Casein (5) Kazein	Cooked lentil (7) Kuhana leća	Casein (5) Kazein
Initial body mass (g) -D0 Početna tjelesna masa	154.0 ± 26.3^a	171.4 ± 7.5^a	117.0 ± 7.41^a	120.9 ± 7.47^a
Final body mass (g) - D5 Završna tjelesna masa	176.8 ± 25.5^a	195.0 ± 6.3^a	139.5 ± 7.10^a	145.5 ± 10.03^a
Average growth rate (g day^{-1}) Prosječna stopa rasta (g/dan^{-1})	4.56 ± 0.28^a	4.72 ± 0.38^a	4.49 ± 0.48^a	4.93 ± 0.64^a
Average dry matter intake (g day^{-1}) Prosječan unos suhe tvari (g/dan^{-1})	13. 18 ± 1.90^a	12.10 ± 0.77^a	14.16 ± 0.52^a	14.07 ± 0.88^a
Dry matter efficiency (%)** Djelotvornost suhe tvari	35.23 ± 5.71^a	39.18 ± 4.65^a	31.75 ± 3.53^a	34.97 ± 2.95^a

* Results are means ± SD - Rezultati se odnose na prosječne vrijednosti + SD

§ Number of animals in the group - Broj životinja u skupini

a, b Values with the same subscript are not significantly different ($P < 0.05$) far the same experiment - Vrijednosti s jednakom oznakom nisu značajne u tom pokusu ($P \leq 0.05$)

** Ratio of the growth rate to dry matter intake, expressed in percentage - odnos stupnja rasta naprama unosu suhe tvari prikazano je u postocima

As already mentioned there were two experiments comparing the legume contained diets with casein diet. The average initial body mass of young growing rats in both experiments was diverse because of different age of animals. At the beginning of the first experiment animals were 42 days old and in the second one 35 days old. Due to differences in the initial body mass there was different body mass of rats in each experiment in the end. On the average there were no differences in the dry matter intake, because pair feeding allowed for equal intake in the legumes fed animals and in their casein controls. Also the average growth rate in the groups was not significantly different ($P \leq 0.05$), but it was slightly better in both casein groups as compared to the belonging legume group.

In the conditions of those experiments, the dry matter efficiency was not significantly different between the legume diets and their casein controls. From the average values a trend of decreased dry matter efficiency in legume groups can be seen, but the variability in all groups increased, probably because of different body mass of animals. Legumes included in the diets were cooked and consequently some (heat labile) antinutritional factors were destroyed. Another reason for similar dry matter efficiency is evidently calculated well balanced diets which were supplemented with the lacking amino acids. If we compare both legume groups, consumption of cooked lentil diet was better, but efficiency of dry 'matter intake was slightly decreased, due to nearly the same average growth rate in both groups. We could compare it, because animals in those two groups were fed ad libitum.

The nitrogen intake was slightly higher in the second experiment, but similar in the groups of the same experiment because of pair feeding. Care was taken to prepare isoeneregetic diets to avoid possible interference with the energy supply. Intestinal absorption was diminished in rats fed on legume diets, which can be seen in the elevated values of faecal nitrogen. In spite of this more nitrogen was excreted through the urine than through the faeces, except in the bean group. Considering these results it can be said that faecal nitrogen excretion was enhanced in both legume fed groups, as compared to their casein controls. Moreover, in the conditions of our experiments the beans diet allowed a smaller excretion of urinary nitrogen than the casein diet, but this was not the case in the lentil diet, where both, faecal and urinary, nitrogen excretions were increased when compared to their casein controls.

Table 5. The average amounts of consumed nitrogen, nitrogen excretion by faeces and urine, and nitrogen balance. *
Tablica 5. Prosječne količine konzumiranog dušika, izlučivanje dušika fecesom i urinom i bilansa dušika

	1st experiment - 1. Pokus		2nd experiment - 2. Pokus	
	Cooked bean (7)§ Kuhani grah	Casein (5) Kazein	Cooked lentil (7) Kuhana leća	Casein (5) Kazein
Consumed nitrogen (mg/day) Konzumirani dušik (mg/dan)	370 ± 53^{a}	362 ± 23^{a}	394 ± 14^{a}	419 ± 26^{a}
Excreted faeces (g in 5 days) Izlučeni feces (g u 5 dana)	25.32 ± 10.01^{a}	4.93 ± 2.20^{B}	23.45 ± 5.42^{a}	4.23 ± 0.43^{B}
Excreted N by faeces (mg/day) N izlučeni fecesom (mg/dan)	119 ± 25^{A}	25 ± 8^{B}	99 ± 13^{a}	27 ± 3^{B}
Excreted urine (g in 5 days) Izlučeni urin (g u 5 dana)	120.35 ± 16.67^{a}	100.16 ± 20.38^{a}	90.72 ± 10.10^{a}	114.85 ± 21.74^{b}
Excreted N by urine (mg/day) N izlučeni urinom (mg/dan)	55 ± 4^{A}	68 ± 5^{B}	128 ± 6^{a}	80 ± 7^{B}
N - balance (mg/day) N bilansa (mg/dan)	193.8 ± 27.9^{a}	269.0 ± 19.9^{B}	166.7 ± 16.6^{a}	312.6 ± 18.7^{B}

* Results are means ± SD - Rezultati se odnose na prosječne vrijednosti + SD

§ Number of animals in the group - Broj životinja u skupini

a, b Values with the same subscript are not significantly different ($P \leq 0.05$) for the same experiment, A, B ($P \leq 0.001$) - Vrijednosti s istom oznakom ne razlikuju se značajno ($P \leq 0.05$) za isti pokus

A, B ($P \leq 0.001$)

Martinez and Larralde (1984) reported that rats fed on similar protein level, but different quality (casein or Vicia faba) had different faecal nitrogen content, enlarged in rats fed on Vicia faba, in two levels of protein content (approximately 18% or 12%). Grant et al. (1996) reported that rats fed on Phaseolus vulgaris lectins had significantly more faeces and N excreted in faeces per day, than control animals. The same was found with the Glycine max lectins, but not at the same level. In contrast, Vicia faba lectins had little or no effect upon metabolism. In our study the effect of legumes on the digestion of nitrogen was not much different, but there was much more nitrogen in the urine of rats fed on lentil. It seems that nitrogen from the bean is less digestible, but when it is digested it is utilised much better by the animals than the nitrogen from the lentil. This statement confirms that, firstly nitrogen balance is better in cooked bean diet than in cooked lentil diet fed rats and, secondly biological value of bean proteins is better (Table 6) as compared to the lentil.

The digestibility of bean proteins is a little lower than that of lentil. On the other hand the digestibility of casein proteins is higher than of legume proteins, which is known from the literature (Combe and Cvirn, 1995). Tomšič (1997) found better biological value in bean proteins (69,41%) as compared to the lentil (51,24%). She compared the same variety of bean and lentil as we did, but without S-containing amino acids. Nutritional values of dietary protein depends on their essential amino acid composition and availability. In this study the diets were supplemented with lacking sulphur containing amino acids (methionine or cysteine), therefore the biological value of protein in cooked bean (80.0) and casein (81.2) diets were similar. The amount of essential amino acids was not equally different in all diets except in the cooked lentil diet, where arginine was present in a slightly higher amount and phenylalanin and tyrosin in a slightly smaller amount (Table 7). Probably this is the reason why the biological value of protein in cooked lentil diet is rather small. Net protein utilisation (NPU) is significantly different among the groups of the same, experiment and higher in cooked bean group than in the cooked lentil group.

Table 6. Apparent digestibility (AD), true digestibility (TD), biological value (BV), net protein utilisation (NPU) and protein efficiency ratio (PER) of tested protein diets. *

Tablica 6. Prividna probavljivost (AD), prava probavljivost (TD), biološka vrijednost (BV), iskorištenje neto bjelančevina (NPU) i omjer djelotvornosti bjelančevina (PER) testiranih obroka bjelančevina

	1st. experiment - 1. Pokus		2nd experiment - 2. Pokus	
	Cooked bean (7)[§] Kuhani grah	Casein (5) Kazein	Cooked lentil (7) Kuhana leća	Casein (5) Kazein
Apparent digestibility (%) Prividna probavljivost	68.1 ± 2.5^{A}	93.2 ± 1.7^{B}	74.7 ± 3.6^{A}	93.7 ± 0.6^{B}
True digestibility (%) Prava probavljivost	70.01 ± 2.4^{A}	$95.3 \pm .8^{B}$	76.1 ± 3.6^{A}	95.0 ± 0.6^{B}
Biological value (%) Biološka vrijednost	80.0 ± 1.4^{a}	81.2 ± 2.3^{a}	58.2 ± 1.7^{A}	80.6 ± 1.2^{B}
Net protein utilisation (%) Iskorištenje neto bjelančevina	56.0 ± 1.9^{A}	77.4 ± 2.0^{B}	44.3 ± 3.2^{A}	76.6 ± 1.4^{B}
Protein efficiency ratio - Omjer djelotvornosti bjelančevina	2.01 ± 0.33^{a}	2.10 ± 0.25^{a}	1.83 ± 0.20^{a}	1.88 ± 0.1^{a}

* Results are means ± SD - Rezultati se odnose na prosječne vrijednosti + SD

§ Number of animals in the group - Broj životinja u skupini

a, b Values with the same subscript are not significantly different ($P \le 0.05$) for the same experiment, A, B ($P \le 0.001$) - Vrijednosti s istom oznakom ne razlikuju se značajno ($P \le 0.05$) za isti pokus,

A, B ($P \le 0.001$)

Table 7. Contents of amino acids in the diets (g/kg of diet) *

Tablica 7. Sadržaj aminokiselina u obrocima (g/kg obroka)

	Cooked bean Kuhani grah	Cooked lentil Kuhana leća	Casein Kazein
Lys	11.23	9.99	12.00
Met + Cys	14.21	14.15	12.68
Thr	5.98	4.64	7.08
Trp	2.11	1.55	1.88
Iso	7.27	6.44	8.01
Leu	12.92	11.20	14.45
Val	7.40	7.14	9.85
His	4.11	3.47	5.93
Arg	8.81	12.12	7.87
Phe + Tyr	15.13	10.48	16.26

* Contents of amino acids in the diets were calculated from the amino acid composition of the raw materials (Pirman and Stibilj, 2000) - Sadržaj aminokiselina u obrocima su izračunavani iz aminokiselinskog sastava sirovina (Pirman i Stibilj, 2000.)

In is well known from literature (Pusztai et al., 1991) that the efficiency of the utilisation of diets based on plant proteins is not easily predicted. Clearly, if the dietary proteins are not fully digested- in the small intestine, their nutritional value is predictably reduced. However, if the dietary proteins are not completely digested and if they react in either the gut or its bacterial content, a further deterioration in the efficiency of both digestion and absorption will occur. According to our present knowledge, some dietary components interact with the gut and modify the metabolism, digestion, absorption and probably some other functions of the small intestine and its bacterial content. One of those components are lectins, and legume seeds are a rich source of them and 30 - 40% of the naturally occurring lectins are difficult to inactivate by heating (Pusztai et al., 1991). Legume seeds are also well known for the protease (trypsin) inhibitors, which interfere with the proper digestion of dietary proteins in the small intestine. Already in the year 1950 Jaffe (cit. by Adsule et al., 1989) showed that lentil seed had the highest protein digestibility among all the legumes studied and the lowest trypsin inhibitor activity. Heating can reduce activity of trypsin inhibitor. In lentil seed trypsin inhibitor activity was reduced from 4.9 IU/mg in raw seeds to 0.51 IU/mg in cooked seeds, Češnjevec beans had reduction from 19.48 IU/mg in raw beans to 0.67 IU/mg in cooked beans (Combe, 1998).

Tannins may bind to the dietary proteins and/or inhibit the activity a digestive enzymes, and increase the secretion of endogenous proteins (Marquardt, 1989). They are also capable to bind on the cell wall of microorganisms and inactivate the microbe proteolysis. Alzueta et al. (1992) proved that adding the freeze-dried tannin extract in levels of 4 to 12% to the diet caused poor digestion of dietary protein, because of N in faeces. On the other hand the biological value of nitrogen was not influenced, since the urine N was decreased by the presence of tannins in the diet. In our study there were small differences in the tannin content, but levels their in the diets did not influence the digestibility of protein.

Viscosity is one of the parameters of digestion and metabolism in small intestine. The highest viscosity was found in the small intestine content bean diet fed rats similar to other three groups (Table 8). Those results were expected regarding the viscosity results of legumes and diets. Consumption of soluble polysaccharide can dramatically alter the reological properties of the gut contents and hence influence the absorption of nutrients, by slowing the dispersion of solid food. Simultaneous gastric emptying reduced the rate at which nutrients enter the small intestine (Blackburn and Johnson, 1981). Increased viscosity in the small intestine of bean fed rats mean more soluble dietary fibres in the diet, like guar gum and other galactomannans, which reduce the utilisation of nutrients from the feed. The mass of content of small intestine was almost 5 times higher in cooked bean group as compared to its casein groups and 2.2 times in cooked lentil as compared to its control group. Obviously, the viscosity influences not only the utilisation of nutrients, but also the mass of intestine content as well as the mass of intestine tissues.

It seems that in our study, because of the increased viscosity in the small intestine of bean fed rats, remains more nitrogen and probably other nutrients small intestine in the content and pass to the large intestine. Because of bigger nitrogen content in the large intestine, different microflora was developed. This observation can confirm the different volatile fatty acids composition of the large intestine content (Table 8). There were considerably more of all acids and especially of n-butyric acid in the large intestine content of cooked bean fed rats as compared to the control casein rats. The proportions among the acids in the content of large intestine were approximately the same in the casein or cooked lentil fed rats, but different in the cooked bean fed rats because of the increase especially of n-butyric acid in those rats. And even more, the mass of intestines was increased by feeding rats with legumes (Combe and Cvirn, 1995, Šimenc et al., 1996) as compared to the casein group and this increase was much wider by feeding bean than lentil (Pirman, 1999). The mass of the large intestine with caecum content was increased in cooked lentil group as compared to the casein group. Control rats had only 48% of mass than rats on cooked lentil diet. Regarding the results of mass of large intestine with caecum from both experiment it can be said that the mass of the large intestine with caecum content would be even more increased in the cooked bean group as compared to its casein group, but the measurement was not carried out. This can be confirmed by the results with the small intestine.

Table 8. Viscosity in small intestine content and volatile fatty acid content (mmol/kg) in content of large intestine with caecum. *

Tablica 8. Viskozitet u sadržaju tankog crijeva i sadržaj hlapljivih masnih kiselina (mmol/kg) u debelom crijevu sa slijepim crijevom

	1st experiment - 1. Pokus		2nd experiment - 2. Pokus	
	Cooked bean (7)§ Kuhani grah	Casein (5) Kazein	Cooked lentil (7) Kuhana leća	Casein (5) Kazein
Fresh mass of small intestine content (g) Svježa masa sadržaja tankog crijeva (g)	2.33 ± 0.43^{A}	0.47 ± 0.14^{B}	2.21 ± 0.24^{A}	0.99 ± 0.29^{B}
Viscosity of small intestine content ($mPa.s^{-1}$) Viskozitet sadržaja tankog crijeva	41.50 ± 23.38^{A}	0.97 ± 0.31^{B}	1.17 ± 0.33^{A}	1.05 ± 0.11^{A}
Fresh mass of content of large intestine with caecum (g) - Svježa masa sadržaja debelog crijeva sa slijepim crijevom			5.13 ± 0.80^{A}	2.44 ± 0.32^{B}
Acetic acid (AA) - Octena kiselina	33.57 ± 5.32^{a}	27.01 ± 5.26^{b}	30.68 ± 4.33^{a}	24.18 ± 5.89^{b}
Propionic acid (PA) - Propionska kiselina	10.95 ± 2.17^{A}	8.02 ± 1.59^{B}	7.85 ± 0.68^{a}	6.18 ± 2.07^{a}
n-butyric acid (BA) n-maslačna kiselina (BA)	18.12 ± 11.41^{A}	5.04 ± 2.18^{B}	5.03 ± 1.82^{a}	4.66 ± 1.38^{a}
Sum	63.75 ± 14.92^{A}	40.81 ± 20.96^{B}	43.56 ± 5.38^{a}	35.73 ± 9.53^{a}
AA : PA : BA	3:1:2	3 : 1 : 0.7	3 : 1 : 0.8	3:1: 0.9

* Results are means ± SD - Rezultati se odnose na prosječne vrijednosti + SD

§ Number of animals in the group - Broj životinja u skupini

a, b Values with the same subscript are not significantly different ($P \leq 0.05$) for the same experiment, A, B ($P \leq 0.001$) - Vrijednosti s istom oznakom ne razlikuju se značajno ($P \leq 0.05$) za isti pokus,

A, B ($P \leq 0.001$)

To summerise the amino acid composition of bean protein seems to be more suitable to the rats than lentil amino acid composition, when the meal is supplemented with the sulphur containing amino acids. It can be predicted that lectins, protease inhibitors, tannins or some other antinutritional factors are not major factors in mediating the changes in digestibility of proteins of cooked legumes. It is possible that changes in protein metabolism of different legume seeds are linked to the viscosity of the small intestine content and production of volatile fatty acids in the gut as the result of digestion dietary fibres, especially soluble ones, derived from legume or bean seeds diet.

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SAŽETAK

Hranidbena vrijednost slovenske vrste običnog graha (Phaseolus vulgaris) Češnjevec i francuske vrste leće (Lens esculenta Puyensis var. Anicia) ocjenjivana je na mladim štakorima u rastu. Prije pripreme obroka obje su mahunarke kuhane te analizirane sirove hranjive tvari i mineralni elementi. Obroci su nadopunjavani nedostatnim sumpornim amino kiselinama. Izvedena su dva pokusa sa svakom mahunarkom i uspoređena s parom hranjenim kazeinom. Stupanj rasta i prosječni unos suhe tvari bili su slični u sve četiri skupine mladih štakora u rastu u pokusima. Količina fecesa i izlučivanje fekalnog N se povećalo a probavljivost dušika znatno se smanjila ($P \leq 0.001$) u štakora hranjenih leguminozama u usporedbi s kontrolama hranjenih kazeinom. Nasuprot tome, biološka vrijednost bjelančevina prividno je bila nepromijenjena u obrocima kuhanog graha, ali biološka vrijednost bjelančevina leće bila je znatno niža $P \leq 0.001$) nego u skupini na kazeinu. Prosječno neto iskorištenje bjelančevina (NPU - NIB) kuhane leće bilo je 52,2% a kuhanog graha 73.1% od njihovih kontrola. Viskozitet sadržaja tankog crijeva i prosječna količina i omjer hlapljivih masnih kiselina (VFA - HMK) u debelom crijevu štakora na kuhanom grahu bili su viši nego u ostalim skupinama. Čak i ako su obični grah i leća imali sličan sadržaj bjelančevina ali različit sadržaj vlaknine, škroba i protuhranjivih čimbenika, njihov metabolizam u tijelu bio je drugačiji.

Ključne riječi: grah, leća, bjelančevina, aminokiseline, štakori, probavljivost, biološka vrijednost, viskozitet, hlapljive masne kiseline