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CHANGES IN DIGESTIBILITY AND BIOLOGICAL VALUE OF PUMPKIN SEED CAKE PROTEIN AFTER LIMITING AMINO ACIDS SUPPLEMENTATION

PROMJENE U PROBAVLJIVOSTI I BIOLOŠKOJ VRIJEDNOSTI BJELANČEVINA POGAČE SJEMENKI BUNDEVE NAKON DODAVANJA LIMITIRAJUĆIH AMINOKISELINA

Tatjana Pirman, Maja Marič, Andrej Orešnik

Original scientific paper - Izvorni znanstveni članak
Received - Primljeno: 3. may - svibanj 2007.

ABSTRACT

Pumpkin seed cake is a by-product in the production of a very tasty and special quality pumpkin oil. The amount of crude protein in the analysed pumpkin seed cake sample was 520.2 g in the dry matter. Lysine and methionine are the first and the second limiting amino acids in pumpkin seed cake proteins. The effect of one or two limiting amino acids addition into the test diet on the digestibility and biological value of pumpkin seed cake protein was determined in the experiment on laboratory rats. 16 young male Wistar laboratory rats (4 per group) were housed in individual balance cages and fed *ad libitum* either control (C) diet, or diet with added lysine (L), or methionine (M), or both amino acids (L+M). During the time of experiment the amount of consumed diet and the body weight of animals were registered. There was no significant difference in the consumed amounts of the diets between the control and experimental groups. The apparent and true digestibilities of protein were similar in all groups, in average 88.47 % and 93.69 %, respectively. Protein biological value (56.62, 59.71, 58.78 and 61.68) and net protein utilisation (53.10, 56.33, 55.01 and 57.38) were increased after the addition of amino acids into the diet. The most pronounced and statistically significant ($P < 0.05$) difference was obtained after the jointly addition of lysine and methionine. Protein efficiency ratio (PER) was increased from 1.53 g in the control group to 1.75 g, 1.70 g and 2.12 g in L, M and L+M groups respectively, but statistically significant ($P < 0.05$) only in L+M group. Also laboratory rats growth rate and efficiency of consumed diet were significantly ($P < 0.05$) increased in the diet supplemented with two amino acids together (L+M), but not in other two experimental diets. After additional limiting amino acids feeding, the amount of excreted N (mg/g of gain) was reduced from 38.97 mg in control group to 24.43 mg ($P < 0.05$) in L+M group.

Key words: nutrition / laboratory rats / pumpkin seed cake / digestibility / protein biological value / amino acids

Doc. dr. sc. Tatjana Pirman, prof. dr. sc. Andrej Orešnik, Zootechnical Department, Biotechnical Faculty, University of Ljubljana, Groblje 3, 1230 Domžale, Slovenia; Maja Marič, Kardeljev trg 9, 3320 Velenje, Slovenia.

INTRODUCTION

In north-eastern part of Slovenia (Prlekija and Prekmurje regions) and in regions across the Austrian, Hungarian and Croatian border, pumpkin (*Cucurbita pepo* L.) seeds are used for the production of edible oil of special taste and quality. In Slovenia about 500 to 700 tons of pumpkin seeds, about 70 % without hulls and 30 % with hulls, are processed yearly. More than one half of oil is produced by small local oil mills, which do not have a uniform technology of processing. Differences are in grinding, temperature, duration of roasting and in squeezing. The result of those differences is a different chemical composition of the by-product, pumpkin seed cake, fit for sale on the market.

Pumpkin seed cake can be used in different ways, for the dairy cow nutrition, fattening of pigs, in the poultry nutrition and also as bait for fish. It can also be used in human nutrition. Dry ground cake could be mixed with wheat flour and prepared as special bread (Kocjan Ačko, 1999, Mansour *et al*., 1999) or for improving the quality of bologna type sausages (Mansour *et al*., 1996).

Pumpkin seed cakes contain around 60 % of protein with a rather constant amino acid composition. Crude protein concentration is affected mainly by the proportion of hulls and crude fat content in the cake. The biological value of pumpkin seed protein is in the range 73-86 % (Mansour *et al*., 1993) and the first limiting amino acid in most cases is lysine (Zdunczyk *et al*., 1999, El-Adawy and Taha, 2001, Pirman *et al*., 2004b). The sulphur amino acids content is higher than in soybean meal. The pumpkin seed protein could be an important source of these amino acids in animal nutrition.

From many food or feed composition tables (Souci *et al*., 2000, Kunachowicz *et al*., 1998, Holland *et al*., 1998, DLG – Futterwerttabellen, 1997, Ewing, 1997) it is evident that data for pumpkin seed cake nutritional value are not yet published. In one publication (Harvey, 1970) we found the amino acid composition in pumpkin seed protein (*Cucurbita farinosa*) and in USDA (2007) 22 different items on pumpkin are presented, but not on pumpkin seed cake.

The aim of the present study was to determine the effect of adding one or two limiting amino acids into the test diet on the utilisation of pumpkin seed cake protein, on the growth rate of laboratory rats and on the amount of excreted nitrogen.

MATERIAL AND METHODS

Diets

The protein source of diets was pumpkin seed cake, purchased from one of the small local oil mills in southwestern part of Slovenia. Pumpkin seed cake sample contained 520.2 g of crude protein, 283.36 g of crude fat, 44.98 g of crude fibre and 69.48 g of ash in dry matter. The amount of phosphorus (15.16 g/kg dry matter) and potassium (12.89 g/kg of dry matter) was rather high. After the amino acid analysis, the first and second limiting amino acids were lysine and methionine, respectively (Pirman *et al*., 2004b). Four diets were prepared, a control diet (C) and three diets in which one or two limiting amino acids after NRC (1995) recommendations were added. In the lysine diet (L) the amino acid lysine was added up to the requirements, methionine diet (M) was prepared with the addition of methionine and the lysine and methionine diet (L+M) with the addition of both amino acids. Diets were designed to meet the nutritional requirements of growing rats.

Animals

All procedures were performed according to current legislation on animal experimentation in Slovenia. The permission for the experiment was given by the Veterinary administration of the Republic of Slovenia (VURS) with the number 3440-62/2006. Sixteen male Wistar rats (131.1 $\pm$ 4.15 g of body mass) from the Medical Faculty (Ljubljana, Slovenia) were housed in a ventilated room at a controlled temperature (21°C) with a 12 hour light-dark cycle (starting at 7.00 a.m.) and placed in individual balance cages with free access to drinking water and right away separated into four equal groups; the Control group (131.0 g $\pm$ 3.0 g of body mass, n = 4), Lysine group (131.2 g $\pm$ 4.3 g of body mass, n = 4), Methionine group (131.2 g ± 7.2 g of body mass, n = 4) and the Lysine and Methionine group (131.2 g ± 2.6 g of body mass, n = 4). In the adaptation period (8 days) and at the time of experimental measurements rats received control or experimental diets *ad libitum*. Food was changed every day at 9 a.m.

On the 8^{th} day of the experiment the balance study was performed, following the mode of operation introduced at the Chair of Nutrition (Orešnik *et al*., 1981, 1982; Stekar *et al*., 1984; Orešnik and Cvirn, 1984). Before taking an aliquot of the sample, faeces was homogenised in a ceramic holder. Urine was homogenised by shaking to prevent stratification. In faeces and urine nitrogen was determined by the Kjeldahl method (N * 6.25). From these data true protein digestibility, biological value of protein, net protein utilisation and protein efficiency ratio were calculated.

Data were analysed by the GLM procedure (SAS/STAT, 2000), taking into consideration the diet as the only main effect. Data are expressed as least square means (LSM) ± standard deviation. If not stated otherwise, a least significant difference of 0.05 was used to separate treatment impacts.

RESULTS AND DISCUSSION

In Table 1 composition and results of Weende analysis of test diets are presented. It can be seen that in the nutritional composition significant differences among the diets do not exist.

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

Composition - Sastav (g/kg)	Control	Lysine	Methionine	Lysine + Methionine
Pumpkin seed cake Pogače sjemenki bundeve	210.00	210.00	210.00	210.00
Lysine - Lizin		0.60		0.60
Methionine - Metionin			0.55	0.55
Wheat starch - Pšenična škrob	706.45	705.85	705.90	705.30
Sunflower oil - Suncokretovo ulje	15.00	15.00	15.00	15.00
$CaCO_3$	12.50	12.50	12.50	12.50
$FeSO_4 \cdot H_2O$	0.05	0.05	0.05	0.05
NaCl	1.00	1.00	1.00	1.00
Cellulose - Celuloza	50.00	50.00	50.00	50.00
Premix	5.00	5.00	5.00	5.00
Composition - Sastav (g/kg dry matter) (g/kg suhe tvari)				
Dry matter - Suha tvar (g/kg)	910.54	902.31	903.79	900.46
Crude protein - Sirova bjelančevina	119.45	120.77	121.25	121.05
Crude fat - Sirova mast	76.56	62.41	64.22	69.68
Ash - Pepeo	31.56	31.71	31.44	31.94
Dietary fibre - Vlaknina	51.71	53.82	53.42	52.82

The average initial body mass of laboratory rats was similar in all four groups. At the end of the experiment the average final body mass was not significantly different among the groups (Table 2). It can be seen Some increase in the L+M group (P = 0.0997) and L group (P = 0.1104) can be seen, but because of a higher variability in body weight among the animals in the groups, the differences were not significant. Similar situation was found in diet consumption. The average growth rate was significantly (P < 0.05) higher in the L+M group in comparison to the control group (4.69 g : 3.09 g).

The results demonstrate significantly better efficiency of consumed diet in L+M group in comparison to the control group (P = 0.007). The values for average diet consumed and average growth rate in the control group are in accordance with our previous experiment on pumpkin seed cake nutritional value (Svenšek, 2001).

Table 2. Body mass, amount of consumed diet and growth rate (average ± SD)

Tablica 2. Tjelesna masa, količina pojedene hrane i stopa rasta (prosjek ± SD)

	Control	Lysine	Methionine	Lysine + Methionine
Initial body mass (g) Početna tjelesna masa (g)	163.6 ± 5.8^{a}	167.9 ± 7.6^{a}	162.4 ± 10.4^{a}	168.9 ± 7.9^{a}
Final body mass (g) Završna tjelesna masa (g)	179.0 ± 8.7^{a}	186.4 ± 10.6^{a}	179.4 ± 14.7^{a}	192.3 ± 6.5^{a}
Average diet consumed (g/day) Prosječna konzumacija hrane (g/dan)	18.45 ± 1.25^{a}	19.16 ± 1.68^{a}	18.07 ± 2.86^{a}	20.26 ± 0.98^{a}
Average growth rate (g/day) Prosječan dnevni prirast (g/dan)	3.09 ± 0.63^{a}	3.69 ± 0.94^{ab}	3.41 ± 0.91^{a}	4.69 ± 0.61^{b}
Efficiency of diet consumation (%) Iskoristivost konzumirane hrane (%)	16.63 ± 2.32^{a}	19.09 ± 3.56^{ab}	18.71 ± 2.77^{a}	23.13 ± 2.50^{b}

a, b values with the same subscript are not significantly different (P < 0.05)

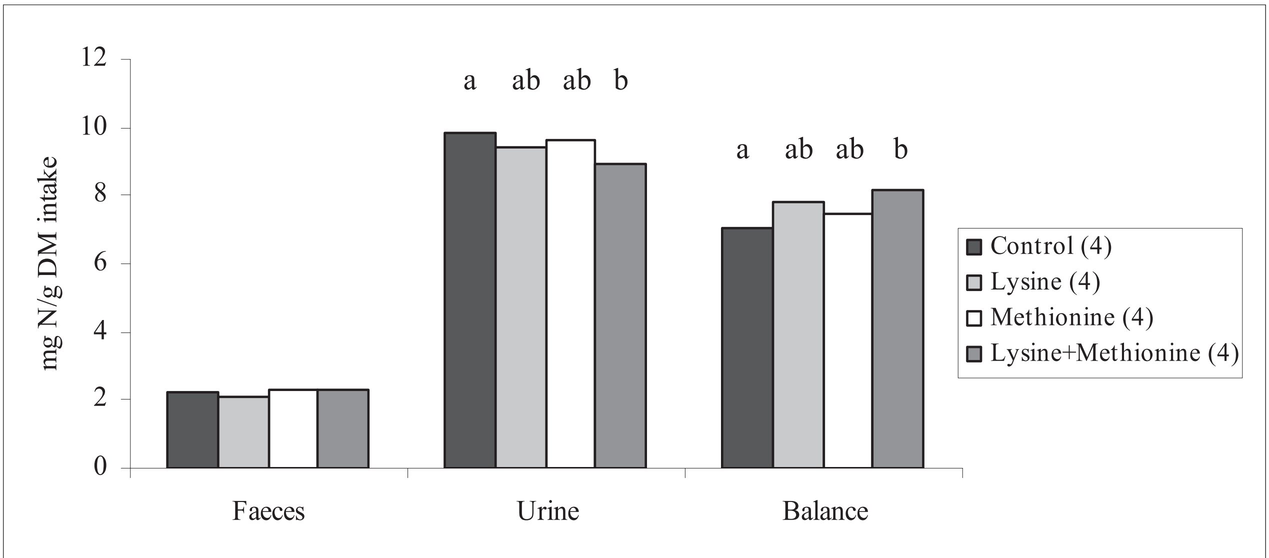

Figure 1. Nitrogen excretion and balance (mg N / g DM intake)

Slika 1. Izlučenje i ravnoteža dušika (mg N/suhe tvari)

Whereas nitrogen losses per g of dry matter intake were not different in faeces (from 2.10 mg to 2.32 mg in L and L+M group, respectively), they were lower in urine of all three experimental groups in comparison to the control group, with significant difference only in L+M group (Figure 1). Consequently the nitrogen balance was significantly ($P < 0.05$) favourable in L+M group. The protein digestibility, apparent and true, was similar in all groups, but biological value of protein and net protein utilisation was significantly ($P < 0.05$) higher in L+M group. In L and M group the trend of better protein utilisation can be seen, but the differences are statistically not significant (Table 3).

lower as compared to our previous results (Pirman *et al*., 2004a) and the results of Mansour *et al*. (1993). Calculated essential amino acids index (EAAI) of the analysed pumpkin seed cake was 68, which is again lower than in our previous analysis (Pirman *et al*., 2004b), where it was on average 78. Also those samples contained higher amount of crude protein (between 573.25 and 629.94 g per kg dry matter). The current results indicate that the crude protein content in the pumpkin seed cake samples inversely effect the protein digestibility. Contrary to this the higher crude protein content in the pumpkin seed cake sample improves the utilisation of digestible crude protein out of the consumed diet.

Table 3. Protein digestibility, biological value of protein and protein efficiency ratio (PER) (average ± SD)
Tablica 3. Probavljivost bjelančevina, biološka vrijednost bjelančevina i omjer djelotvornosti bjelančevina (PER) (prosjek ± SD)

	Control	Lysine	Methionine	Lysine + Methionine
Apparent protein digestibility (%)	88.47 ± 1.19^{a}	89.14 ± 0.74^{a}	88.25 ± 0.86^{a}	88.03 ± 0.44^{a}
True protein digestibility (%)	93.74 ± 1.26^{a}	94.35 ± 0.53^{a}	93.62 ± 1.08^{a}	93.03 ± 0.56^{a}
Biological value of protein (BV) (%)	56.62 ± 3.29^{a}	59.71 ± 1.12^{ab}	58.78 ± 4.06^{ab}	61.68 ± 1.32^{b}
Net protein utilisation (NPU) (%)	53.10 ± 3.69^{a}	56.33 ± 1.28^{ab}	55.01 ± 3.51^{ab}	57.38 ± 1.51^{b}
PER (g of growth rate /g CP)	1.53 ± 0.21^{a}	1.75 ± 0.33^{ab}	1.70 ± 0.25^{a}	2.12 ± 0.23^{b}
Excreted N (mg) per g of growth rate	38.97 ± 3.40^{a}	32.28 ± 6.84^{ab}	33.34 ± 6.95^{a}	24.43 ± 3.18^{b}
Difference mg (%)		- 6.69 (17.17)	- 5.63 (14.45)	- 14.55 (37.32)

a, b values with the same subscript are not significantly different ($P < 0.05$)

The obtained values of protein digestibility are higher in comparison to the results of our previous studies, where true protein digestibility in different (define) samples of pumpkin seed cake protein (no added amino acids) was between 87.52 to 90.10 % (Pirman *et al*., 2004a). The true digestibility of different products of pumpkin seeds reported by Mansour *et al*. (1993) was between 87.78 to 96.83 % and increased with the protein content in the sample. Those products contained between 720 and 960 g of crude protein in dry matter. In the present experiment we used pumpkin seed cake with a rather low amount of crude protein (520.20 g/kg dry matter) and high amount of crude fat (283.36 g/kg dry matter). Crude protein digestibility was higher and protein biological value (in control group) was

The PER value obtained in our experiment was the best in L+M group, up to 38 % higher than in the control group. Again, as in other parameters of protein utilisation, L group had some better values than M group.

Regarding nitrogen environmental pollution the addition of limiting amino acids into the diet (up to the animal requirements) hed favourable effects, since the excretion of nitrogen (per g of growth rate) was lower after the addition of lysine and methionine as compared to the control or methionine or lysine addition alone (Table 3). The addition of one amino acid reduced the nitrogen excretion by more than 10 %. Higher reduction of nitrogen excretion after the addition of two amino acids in comparison to addition of one amino acid was already proven by Orešnik and Blanchon (1999) in cereal protein diets. Addition of lysine and methionine to wheat and barley protein reduced nitrogen excretion per g of growth rate by 34.4 % and 35.4 %, respectively, which is in accordance with 37.32 % (Table 3) obtained in our experiment.

In the analysed pumpkin seed cake protein lysine and methionine were the first limiting amino acids. In regard to ideal protein (NRC, 1995) some other amino acids are missing. To find the best solution for improving pumpkin seed cake protein quality, more amino acids must be added or we have to find the balance with some other protein with the highest amount of amino acids deficient in pumpkin seed cake proteins. Zdunczyk *et al*. (1999) recommend the mixture of pumpkin seed cake protein with soybean protein in the ratio 1 : 1, which gives better PER value than with the soybean protein alone. Longe *et al*. (1983) suggest that pumpkin protein contains considerable quantities of tryptophan, but small amounts of lysine and isoleucine, the others (Mansour *et al*., 1993) find that isoleucine and valine are the first limiting amino acids, and the second is threonine. Sharma *et al*. (1986) find that the sulphur-containing amino acids are the first and lysine and threonine the second limiting amino acids. Those differences may be connected with protein quality variations in different samples.

CONCLUSION

Digestibility of pumpkin seed cake crude protein is rather high, on average 88 % (apparent) and 94 % (true), irrespective of the addition of limiting amino acids. After the addition of limiting amino acids the biological value of proteins NPU and PER increased. A better nitrogen balance was obtained (mg N/g DM intake) and consequently higher growth rate and efficiency of consumed diet after the addition of amino acids. The best effect was noticed with the addition of two amino acids, lysine and methionine, the addition of lysine alone increased those values a little more than the addition of methionine alone. Statistically significant ($P < 0.05$) differences to the control diet were obtained only with both amino acids in the test diet. Last, but not least, the nitrogen excretion (mg per g of growth rate) decreased after the addition of amino acids, by 17 %, 14 % and 37 % after the addition of lysine, methionine or both amino acids, respectively. This conclusion is important with regard to environmental pollution by nitrogen in animal excrements.

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SAŽETAK

Pogača od sjemenki bundeve je nusproizvod u proizvodnji vrlo ukusnog ulja bundeve, posebne kakvoće. Količina sirovih bjelančevina u analiziranom uzorku pogače od sjemenki bundeve iznosila je 520,2 g suhe tvari. Lizin i metionin su prva i druga ograničavajuća amino-kiselina u bjelančevinama pogače od sjemenki bundeve. Djelovanje dodatka jedne ili druge limitirajuće aminokiseline u pokusni obrok na probavljivost i biološku vrijednost bjelančevina u pogači određena je u pokusu na laboratorijskim štakorima. Šesnaest muških mladih laboratorijskih štakora Wistar (4 u skupini) smješteno je u pojedinačne jednake kaveze i hranjeno *ad libitum* kontrolnim obrocima (C) ili obrocima s dodatkom lizina (L) ili metionina (M) ili obje aminokiseline (L+M). Za vrijeme pokusa bilježeni su količina pojedenog obroka i tjelesna masa životinja. Nije bilo značajne razlike u količini pojedene hrane između pokusnih i kontrolne skupine. Prividna i prava probavljivost bjelančevina bile su slične u svim skupinama, u prosjeku 88,47 % odnosno 93,69 %. Biološka vrijednost bjelančevina (56,62; 5:,81; 58,78 i 61,68) i neto iskoristivost bjelančevina (53,10; 56,33; 55,01 i 57,38) povećale su se nakon dodatka aminokiselina u obroke. Najizraženija i statistički značajna razlika (P<0,05) dobivena je nakon istovremenog dodavanja lizina i metionina. Omjer djelotvornosti bjelančevina (PER) povećao se s 1,53 g u kontrolnoj skupini na 1,75 g, 1,70 g i 2,12 g u skupinama L, M odnosno L + M, ali statistički značajno (P<0,05) samo u L+M skupini. Isto tako stopa rasta laboratorijskih štakora i djelotvornost pojedene hrane značajno su porasli (P<0,05) u obrocima s dodatkom dvije aminokiseline zajedno (L+M), ali ne u druge dvije pokusne skupine. Nakon hranjenja s ograničavajućim aminokiselinama količina izlučenog N (mg/g prirasta) smanjena je od 38,97 mg u kontrolnoj skupini na 24,43 mg (P<0,05) u L+M skupini.

Ključne riječi: hranidba, laboratorijski štakori, pogača od sjemenki bundeve, probavljivost, biološka vrijednost bjelančevina, aminokiseline