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# PROTEIN SYNTHESIS RATES IN SOME TISSUES OF GROWING RATS FED LEGUMES

# STUPANJ SINTEZE BJELANČEVINA U NEKIM TKIVIMA ŠTAKORA U RASTU HRANJENIH LEGUMINOZAMA

Tatjana Šimenc, E. Combe, Jasna.M.A. Stekar, P. Patureau Mirand

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## SUMMARY

The effects of diets containing casein or cooked lentils on the protein contents and synthesis rates in liver and large Intestine were compared in 2 groups of 10 pair-fed growing rats. Protein synthesis rates were determined by the $^{14}C$ valine flooding dose method. The protein and RNA contents were significantly higher ($P<0,05$) with lentils than with casein in the large intestine (means ± se were respectively: 137 ± 4 vs 98 ± 3 mg/100 g body weight and 6,5 ± 0,4 vs 4,0 ± 0,2 mg/100 g body weight) and lower in the liver (648 ± 22 vs 723 ± 22 and 33 ± 1 vs 39 ± 1 mg/100 g body weight). Compared to the casein group, the protein synthesis rates were higher in large intestine and lower in liver of the lentil fed rats.

## INTRODUCTION

Legumes are widely used as feed and scientific literature has shown their ability to develop intestinal microflora and to decrease glycemic index and cholesterolomy. However, it seems also of interest to study the metabolic utilisation of the essential amnoacids brought by these legume seeds. For this purpose, we compared the rates of protein synthesis in some tissues of growing rats fed either a lentil diet or a casein diet as control. Here are given first results dealing with tissues from the splanchnic area.

## MATERIAL AND METHODS

Sprague Dawley male rats, (N=20; body weight = 75,7 ± 3,9 g) from IffaCredo, St. Germain l'Arbresle, France, were individually housed in wired cages with free access to water. Temperature was regulated at 22°C and light kept off from 9 am to 9 pm in the animal room. The rats were assigned to one of the 2 homogenous (body weight based) groups and received the experimental (cooked lentil) diet ad libitum for 16 days or the control (casein) diet, in a pair fed procedure after the necessary 2 daxs of delay. Body weight was also registred daily for the whole period.

Composition of the diet (table 1), designed to meet the nutritional requirements of the growing rat (NAS NRC 1978) brought 4500 kcal/kg and 16% of crude protein.

Dipl. ing. kmet. Tatjana Šimenc and prof dr. Jasna M. A.Stekar: Univerza v Ljubljani. Biotehniška fakulteta, Oddelek za zootehniko, 61230 Domžale, Groblje 3, Slovenija, samostalni istraživač ing. Etlennette Combe and dr. Philippe Patureau Mirand: INRA, 63122 Theix, France

**Table 1. Composition of diets (g/kg dry matter)**
**Tablica 1. Sastav obroka (g/kg suhe tvari)**

| Diets<br>Obroci | Casein<br>Kazein | Lentil<br>Leća |
|---|---|---|
| Casein - Kazein | 172.5 | 0 |
| Lentil° - Leća | 0 | 581 |
| L-cystine | 0.3 | 0 |
| L-methionine | 0 | 3.2 |
| Wheat starch - Pšenična škrob | 637.2 | 246.6 |
| Agar agar | 30 | 30 |
| Mineral mix 1 - Mineralna smjesa 1 | 50 | 29.2 |
| Oligo mix 2 | 20 | 20 |
| Vit mix 3 | 10 | 10 |
| Peanut oil - Ulje od orašca | 60 | 60 |
| Corn oil - Ulje od kukuruza | 20 | 20 |

° couriesy of Comité Interprofessionnel de In Lontillo Vorte du Puy, Le Puy en Velay, France

1: composition of mineral mix for casein diet: (Combe et al 1965) and lentil diet (Combe et al 1991):

2: composition of oligo mix (Combe et al 1988);

3: vit mix N° 2.00: Usine Alimentation Rationelle, Villemoisson sur l'Orge, France.

The lentils (Lens esculenta, var. Anicia) were cooked 20 minutes in boiling water, dried in open air, and ground (0,8 mm mesh) before miscing with the other ingredients of the diet. Water was added for good homogeneity and semi- liquid consistency.

Protein synthesis rates were determined at the end of the feeding period, by the flooding dose method (Mac Nurlan 1979). In order to flood the free aminoacid pools so that their specific activities in the tissues remain close and fairly constant during the incorporation time, the injection of the radioactive aminoacid was combined with a large amount of the same unlabelled aminoacid. L-14C Valine (150 μ moles, 14,7 μ curies / 100 g body weight) was injected into a lateral vein of the rat.

The animals were anesthetised and exsanguinated 8 or 16 minutes after the injection. Tissues were quickly removed, rinsed with saline, blotted, weighed and frozen in liquid nitrogen.

The fractional synthesis rates of tissue protein (FSR, % per day) were calculated by: FSR = (Sb/Sa*t) * 100, where Sa and Sb respectively, are the specific activities of free and protein bound valine and t is the incorporation time expressed in days. Free and protein bound valines were separated by trichloracetic (10%) precipitation.

Trichloracetic extracts were purified by ion exchange chromatography (Dowex 50). The specific activity of free valine was determined by simultaneous measures of its concentration in the purified extract with the amino acid analyser and its radioactivity with a flow counter (Flo one Packard, equipped with a Y Si cell).

The trichloracetic precipitates were digested in NaOH (=,3 N). Protein content was determined on an aliquot by the bicinchoninic acid method (Smith and all. 1985). Proteins were precipitated with perchloric acid (0,6 M) and hydrolysed with chlorhydric acid (5,5 N) at 105°C for 48 hours. The specific activity of protein bound valine was calculated from the radioactivity of the HCl hydrolysate measured by liquid scintillation counting (Betamatic Kontron) and its valine concentration (580 and 486 μmoles/g of protein in liver and large Intestine, respectively).

In order to calculate the ribosomal capacity (mg RNA/g protein) and the ribosomal efficiency (mg of protein synthetised per day / mg of RNA), RNA concentrations were calculated (Manchester and Harris 1969) from the absorbance at 260 and at 275 nm of the perchloric acid supernatants.

## RESULTS AND DISCUSSION

### Animal response (see table 2)

The diets allowed high dry matter intake and growth rate for all the rats. No significant differences were obtained between the 2 groups for the 16 days of measuring or for the 10 last days. Only in the last 10 days period, the dry matter efficiency appears significantly smaller In the lentil group than in the casein group, due to the response of 8 out of the 10 pairs of rats.

**Table 2. Body weight, growth rates and dry matter intakes of the rats fed the casein or the lentil diets**
**Tablica 2. Tjelesna težina, stupanj rasta i uzimanje suhe tvari štakora hranjenih obrocima kazeina ili leće**

| Diets - Obroci | Casein - Kazein | Lentil - Leća |
|---|---|---|
| Initial body weight (g) - Početna tjelesna težina (g) | 75.5 ± 2.8 | 75.9 ± 5.0 |
| Final body weight (g) - Konačna tjelesna težina (g) | 197.5 ± 16.2 | 174.2 ± 13.1* |
| Average growth rate during the last 10 days (g/day)<br>Prosječna stopa rasta u zadnjih 10 dana (g/dan) | 6.1 ± 0.9 | 5.6 ± 0.7 |
| Average dry matter intake during the last 10 days g/day)<br>Prosječno uzimanje suhe tvari u zadnjih 10 dana | 16.7 ± 0.2 | 16.4 ± 0.2 |
| Dry matter efficiency during the last 10 days (%)<br>Djelotvornost suhe tvari u zadnjih 10 dana | 37 ± 3 | 33 ± 2* |

10 animals in each group; Means ± SD; *P<0.05
10 životinja u svakoj grupi; Prosjek ± standardna devijacija; * P < 0.05

**Table 3.Protein and RNA contents and protein synthesis rates in liver**
**Tablica 3.Sadržaj bjelančevina i RNA i stupanj sinteze bjelančevina u jetri**

| Diets - Obroci | Casein - Kazein | Lentil - Leća |
|---|---|---|
| Number of animals - Broj životinja | 9 | 8 |
| Fresh tissue weight g - Težina svježeg tkiva g | 8.44 ± 0.65 | 6.61 ± 0.85* |
| Relative weight g per 100 g body weight<br>Relativna težina g na 100 g tjelesne težine | 4.28 ± 0.18 | 3.76 ± 0.31* |
| Protein mg/g fresh tisue - Bjelančevina mg/g svježeg tkiva | 170.3 ± 15.0 | 172.2 ± 7.8 |
| Total protein content mg/100 g body weight<br>Ukupni sadržaj bjelančevina mg/100 g tjelesne težine | 723 ± 67 | 648 ± 61* |
| RNA mg/g fresh tissue - RNA mg/g svježeg tkiva | 9.11 ± 0.47 | 8.88 ± 0.31 |
| Total RNA in liver mg/100 g body weight<br>Ukupni RNA u jetrima mg/100 g tjelesne težine | 38.7 ± 1.9 | 33.4 ± 2.6* |
| Ribosomal capacity mg RNA/g protein<br>Kapacitet ribosoma mg RNA/g bjelančevina | 53.9 ± 5.1 | 51.7 ± 3.1 |
| Fractional rates of protein synthesis (% per day)<br>Frakcionirani stupanj sinteze bjelančevina (% dnevno) | 70.4 ± 14.5 | 54.6 ± 9.0* |
| Ribosomal efficiency mg prot. synt. per day/mg RNA<br>Efikasnost ribosoma na mg bjelan. sinteze dnevno/mg RNA | 13.0 ± 2.9 | 10.6 ± 1.7(*) |

Results are means ± SD; *P<0.05; (*)P<0.1
Rezultati su prosjek ± SD

**Protein metabolism in liver** (see table 3)

Lentil feeding resulted in significantly lower fresh and relative weight of liver, total protein and total RNA contents in liver (expressed for 100 g of body weight) when compared to the casein group. Although ribosomal capacity was not different between the 2 groups, fractional synthesis rates were significantly smaller in the lentil fed than in the casein group. Also ribosomal efficiency appeared to be smaller (at P = 0,1) in the lentil than in the casein group. This may partially explain the weaker protein synthetic rates in the liver of the lentil group.

**Table 4. Protein and RNA contents and protein synthesis rates in large intestine**

**Tablica 4.Sadržaj bjelančevina i RNA i stupanj sinteze bjelančevina u debelom crijevu**

| Obroci - Diets | Casein - Kazein | Lentil - Leća |
|---|---|---|
| Number of animals - Broj životinja | 9 | 8 |
| Fresh tissue weight g - Težina svježeg tkiva g | 1.92 ± 0.27 | 2.34 ± 0.19* |
| Relative weight g per 1oo g body weight | | |
| Relativna težina g na 100 g tjelesne težine | 0.97 ± 0.07 | 1.34 ± 0.07* |
| Protein mg/g fresh tissue | | |
| Bjelančevine mg/g svježeg tkiva | 100.4 ± 7.9 | 102.5 ± 8.1 |
| Total protein content mg/100 g body weight<br>Ukupni sadržaj bjelančevina mg/100 g tjelesne težine | 98 ± 9 | 137 ± 13* |
| RNA mg/g fresh tissue - RNA mg/g svježeg tkiva | 4.16 ± 0.44 | 4.85 ± 0.55* |
| Total RNA content mg/100 g body weight<br>Ukupni sadržaj RNA mg/100 g tjelesne težine | 4.1 ± 0.5 | 6.5 ± 1.0* |
| Ribosomal capacity mg RNA/g protein<br>Kapacitet ribosoma mg RNA /g bjelančevina | 41.6 ± 4.9 | 47.8 ± 7.9 (*) |
| Fractional rates of protein synthesis (% per day)<br>Djelomični stupanj bjelančevina (% dnevno) | 40.0 ± 10.1 | 54.4 ± 14.4* |
| Ribosomal efficiency mg prot. synt. per day / mg RNA<br>Djelotvornost ribosoma mg.sinteze bjelančevina na dan/mg RNA | 9.5 ± 2.1 | 11.3 ± 1.8 |

Results are means ± SD; *P<0.05; (*)P<0.1
Rezultati su prosjek ± SD; *P<0.05; (*)P<0.1

**Protein metabolism in large intestine** (see table 4)

Lentil feeding resulted in significantly larger fresh and relative weight of the large intestine as well as RNA concentration and total protein and total RNA contents in the large intestine, when compared to the casein group. Also ribosomal capacity of the large intestine was found higher (at P<0,1) in the lentil than in the casein group. Fractonal synthesis rates were found significantly higher in the lentil than in the casein group. This large increase of protein synthesis rates may involve partially the slight improvement of ribosomal capacity and efficiency.

## CONCLUSION

In this experiment, essential aminoacid supplementation and pair fed method were efficient enough to avoid differences in feed intake and growth rates beetween the groups. However, in the last period feed efficiency of the cooked lentil

showed to be weaker than that of the casein. At the time the protein synthetic rates were measured, liver and large intestine reacted in a different way to the lentil diet. Mechanisms of the large increase of protein synthesis rates in the large intestine must also be connected to some of the many metabolites of intestinal microflora (Muramatsu 1993), in connection with the other organs. The decrease of the protein synthesis rates in the liver of the lentil group could result from a lower nutrient flow, as compared to what the intestine would have caught in situ, even in the case of a low digestibility of the diet.

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## SAŽETAK


Utjecaj hrane koja je sadržavala kazein ili kuhanu leću na sadržaj bjelančevina i stupanj sinteze u jetri i debelom crijevu kompariran je u dvije skupine 10 parova hranjenih štakora u porastu. Stupanj sinteze bjelančevina utvrđivan je metodom $^{14}C$ valin dodanom dozom. Sadržaj bjelančevina i RNA (ribonukleinska kiselina) signifikantno je viši ($P<0,05$) s lećom nego s kazeinom u debelom crijevu (prosjek ± standardna pogreška (se) bila je za obadva dodatka (leća - kazein): 137 ± 4 odnosno 98 ± 3 mg/100 g tjelesne težine i 6,5 ± 0,4 odnosno 4,0 ± 0,2 mg/100 tjelesne težine). Uspoređujući kazeinsku grupu, stupanj sinteze bjelančevina je visok u debelom crijevu a malen u jetri u grupi štakora hranjenih lećom.