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Journal of Nutritional Biochemistry 15 (2004) 12–17

THE JOURNAL OF Nutritional Biochemistry

Differential effect of lentil feeding on proteosynthesis rates in the large intestine, liver and muscle of rats[1]

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Received 15 October 2002; received in revised form 7 July 2003; accepted 16 August 2003

Abstract

The aim of this work was to test the hypothesis that the trophic effect of lentil feeding on large intestine results from a stimulation of protein synthesis and to determine whether it interferes with protein metabolism in other splanchnic or peripheral organs. Two groups of growing Sprague Dawley male rats were pair-fed iso-caloric iso-nitrogenous balanced diets containing either cooked lentils (*Lens esculenta puyensis*) or casein as unique protein source. Protein synthesis rates were measured *in vivo*, in large intestine, liver and gastrocnemius at the postprandial state. In large intestine, protein and ribonucleic acid contents were higher in the lentil-fed group than in the control group, and the amount of proteins synthesized was also higher (+57%). By contrast, liver protein and ribonucleic acid contents as well as protein synthesis rates were significantly lower in the lentil-fed group than in the control group. In the gastrocnemius muscle protein and ribonucleic acid contents were significantly lower and the amount of protein synthesized was also lower (-18%) in the lentil fed group than in the control group. This study suggests that stimulation of protein synthesis in the large intestine is compensated for by a decrease in liver and muscle.

Keywords: Lentil; Protein synthesis; Muscle; Liver; Large intestine; Rat

1. Introduction

Lentils have been cultivated, harvested, stored and finally cooked for human nutrition purposes since thousands of years. They are now considered as one of the legumes most beneficial for health. The lentil seed is a valuable source of proteins, starch and non starchy carbohydrates, minerals and micronutrients, including some tannins [1,2]. The activity of its lectins and antitrypsic factors is low and non toxic [3] and they are destroyed by adequate cooking. The nutritional interest of whole lentil seed has been stressed for its beneficial effect in diabetes [4], and for its ability to develop intestinal microflora [5]. However, like other legumes, lentils are also known to have a lower protein efficiency for growth, as compared to animal protein sources. Moreover, they have a trophic effect on the tissues of large intestine [6], but their effect on small intestine is uncertain [6,7]. The aim of this work was to check if lentil feeding may affect protein metabolism in other splanchnic or peripheral organs and to test the hypothesis that this increase of tissues and particularly in the large intestine was caused by increased protein synthesis. Consequently, protein synthesis rates were measured in vivo in the tissues of rats fed either a cooked lentil diet, or a control diet.

2. Material and methods

2.1. Animals

All procedures were performed according to the guidelines formulated by the European Community for the use of experimental animals (L358-86/609/EEC). Sixteen Sprague Dawley male rats (76 g, 3 to 4 weeks old) purchased from IFFA CREDO (St Germain l'Arbresle, France) were housed in individual stainless steel wire mesh cages with free access to water, and maintained at 22 ± 1°C on a 12-hr

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[1] This work is part of a bilateral scientific collaboration between INRA France and University of Ljubljana, Slovenia.

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doi:10.1016/j.jnutbio.2003.08.006

Table 1
Composition of the balanced diets

Ingredients ($g.kg^{-1}$dry matter)	Diet group	
	Casein	Lentil
Cooked lentils	0	581
Casein	172.5	0
Wheat starch	653	227
Agar-agar	30	30
Mineral mixture[1]	47.9	29.2
Oligo-element mixture[2]	20	20
Vitamin mixture[3]	10	10
L-methionine	0	3.2
L-cystine	0.3	0
Groundnut oil	45	79
Corn oil	20	20

[1] Mineral mixture ($g.kg^{-1}$ of diet): 2.9 g $CaCO_3$, 29 g $CaHPO_4$, 5 g NaC1, 7.5 g $KHCO_3$, 3.5 g $MgCO_3$ for the casein diet [5] and 10 g $CaCO_3$, 18 g $CaHPO_4$ 1.2 g NaC1 for the lentil diet [12].

[2] Oligo-element mixture ($g.kg^{-1}$ of diet): 342.8 mg NH_4Fe citrate, 39.37 mg $CuSO_4$ $5H_2O$, 83.68 mg $MnCO_3$, 132.16 mg $ZnSO_4$ $7H_20$, 11.06 mg NaF, 0.2 mg $CoCO_3$, 0.14 mg SeO_2, 1.75 mg $A1_2(SO_4)3$ and 19.39 wheat starch [5].

[3] Vitamin mixture N°2, Usine Alimentation Rationnelle, (Villemoisson/Orge France): vitamins (A: 1,980,000 IU; D: 600,000 IU; B1: 2,000 mg; B2: 1,500 mg; Ca pathotenate: 7,000 mg; B6: 1,000 mg; Inositol: 15,000 mg; B12: 5 mg; C: 80,0000 mg; E: 17,000 mg; Menadione: 4,000 mg; Nicotinamide: 10,000 mg; Choline: 136,000 mg; Folic acid: 500 mg; *p*-aminobenzoic acid: 5,000 mg; Biotine: 30 mg) completed to 1 kg by Arbocel 800.

dark/light cycle starting at 09.00 AM. They were assigned to homogenous groups of 8 on the basis of body mass and received either the experimental diet (Lentil diet) or the control diet (Casein diet). Body weights were recorded daily during the experimental period.

2.2. Diets and feeding

The experimental diet and the control diet were isocaloric (18,800 kJ) and isonitrogenous (N x 6.25 = 160 g per kilo of dry matter); they were formulated in order to meet the essential amino acid and mineral requirements for the growing rat [8]. Composition of the diets is presented in Table 1. The sole source of protein was either casein (Union des Caséineries de Charente Maritime, Surgères, France) for the control diet or cooked lentils for the experimental diet.

Lentils (*Lens esculenta puyensis*, var. Anicia), provided by "Groupement Interprofessionnel de la Lentille verte du Puy" (Le Puy en Velay, France) were cooked according to their recommendations (rinsed, placed in two volumes of cool tap water, heated and kept boiling for 20 min) and drained. They were dried in a ventilated area at room temperature and ground to pass a 0.8 mm mesh.

Both diets were offered daily (at 09.00 AM) in a semi-liquid consistency. The lentil diet was offered *ad libitum* and individual measurements of food intake were performed for 16 days after a 2-day period of adaptation to the experimental diet. For the control group, the casein diet was offered according to the pair feeding procedure: the individual amount of casein diet offered to the paired rat was calculated after measurement of the actual individual dry matter intake of the *ad libitum* lentil fed paired rat; in the case of semi liquid diets, this requires a 2-day delay between the groups.

2.3. Administration of the tracer

Protein synthesis rates in the tissues of the rats were determined at the end of the experimental period, in a fed state, between 01.15 and 03.30 PM. They were measured *in vivo* by the flooding dose method [9] using ^{14}C valine. The radioisotope (L-[U^{14}C]valine), from Radiochemical Centre, Amersham, Buckingamshire, UK) was injected into the rat (150 μmoles, 543 kBq.100 g^{-1} body mass; radiochemical purity > 99.4%) via a tail vein. Incorporation times of 8 and 16 min were chosen, being short enough to avoid exportation of newly synthesized proteins from the liver. At the end of the incorporation period, the animals received a lethal intraperitoneal injection of pentobarbital sodium (Sanofi, Libourne, France) which produced rapid anesthesia. Blood was collected from carotid artery into heparinized tubes. The tubes were centrifuged and plasma removed. Liver was excised and rinsed with cold saline. Stomach, small intestine and large intestine were quickly placed on ice-cold dishes, separated from other organs, had their contents flushed out with cold saline, and were blotted. Gastrocnemius was dissected. All tissues samples were weighed prior to freezing in liquid nitrogen. Average time between freezing and the beginning of blood collection from the animal was 3.5 min for liver and 6.5 min for other tissues. The frozen samples were stored at –20°C until analysis for protein synthesis determination.

2.4. Analysis and tissue preparations

The determination of protein synthesis rates requires measurement of specific activities of free and protein-bound valine, and also determination of RNA and protein contents for connected criteria: ribosomal capacity and efficiency. Tissues were treated to separate the free valine from the protein-bound valine. Frozen tissues were homogenized in 8 volumes of ice cold trichloracetic acid (TCA) solution (0.6 M), either directly in a motor driven glass homogenizer (muscle and large intestine), or by sonication after being finely pulverized (liver). The acid soluble fraction containing the free amino acids was separated by 5,400 g centrifugation at 4°C (Sorvall superspeed RC2-B) and the TCA removed by ion exchange chromatography (AG 50 W, Bio Rad). Amino acids were eluted by NH_4OH (4 M), dried and resuspended in 0.2 M sodium citrate buffer (pH = 2.2). Specific activity of free valine was determined by simultaneous measurements of free valine concentration with an amino acid analyzer and measurement of the radioactivity in a flow counter fitted with a Y Si cell (Flo One Packard,

Paris, France). The precipitates were prepared for determination of the specific activity of the protein bound valine, the protein and ribonucleic acid (RNA) contents. Therefore, they were washed 3 more times with 8 volumes of perchloric acid (PCA) (0.2 M) and centrifuged, in order to remove free valine and TCA contaminants. The washed pellet was homogenized in NaOH (0.3 M) and incubated at 37°C for 1 hr with frequent shakings. The process was stopped by dipping into ice-cold water. Protein content was determined on an aliquot, by the colorimetric method with bicinchoninic acid [10]. The specific activity of the protein bound valine was measured after complete hydrolysis (HCl (5.5 M) for 48 hr at 110°C) of the protein pellet. The hydrolysate was dried, resuspended in sodium citrate buffer (0.2 M) pH 2.2 for chromatographic determination of valine concentration. Norleucine was added before hydrolysis to be used as internal standard for all quantitative valine determinations. Valine radioactivity was determined by counting hydrolysate radioactivity by liquid scintillation (beta counter, Kontron), given that it had been checked that more than 95% of the protein radioactivity was from the valine. RNA determination was performed on the alkaline homogenates, after treatment with PCA (2 M) to precipitate the proteins and centrifugation at 7,000 g. RNA concentration was measured in the supernatant by the UV absorption method [11]. Tissue ribosomal capacity (Cs) was calculated as the ratio of RNA (mg) to protein (g) amounts.

In order to check the postprandial state, and intestinal entry rates, individual stomach dry matter contents were determined and compared to corresponding dry matter intakes.

Glucose and urea concentrations were measured in plasma using respectively glucose dehydrogenase and urease (Merck, Darmstadt, Germany). Plasma insulin was measured by radioimmunoassay with the Bi-insulin RIAn kit (ERIA Diagnostics Pasteur, Marne la Coquette, France).

2.5. Calculation and data analysis

Fractional synthesis rate (FSR, in % /day) was calculated as follows: FSR = 100.SB/ (SA.t), where SA is the specific activity of the tissue free valine, SB is the specific activity of the protein-bound valine and t is the time of incorporation expressed in days. This parameter gives the turnover rate of the proteins in the tissue. The absolute synthesis rates (ASR, mg per day) of proteins were calculated as the product of FSR value by the total protein content of the tissue. It clearly takes into account the size of the tissue or organ considered. An estimate of the ribosomal efficiency was calculated from the ratio of the mass (mg) of protein synthesized per day to the mass (mg) of RNA.

As expected, the values of free valine specific activity were close (in a range of ±6% in the liver, muscle and large intestine), and not significantly different ($P > 0.53$). There were no differences between groups in the protein synthesis rates obtained at 8 and 16 min in each tissue and the results were pooled for presentation in the tables.

All data are given as means ± SD. Student's *t*-test was used to compared the two groups, except for insulin, where covariance analysis was used

Table 2
Growth rate, food efficiency and plasma parameters

	Diet groups		
	Casein	Lentil	p
Growth rate (g/d)	6.1 ± 1.0	5.6 ± 0.6	ns
Dry matter intake (g/d)	16.4 ± 1.6	16.7 ± 1.2	ns
Food efficiency (%)	37 ± 3	33 ± 2	<0.05
Plasma glucose (μmol.ml^{-1})	12.7 ± 2.6	11.5 ± 1.5	ns
Plasma urea (μmol.ml^{-1})	3.92 ± 0.57	4.53 ± 0.67	ns
Plasma insulin (μIU.ml^{-1})	42.7 ± 30.1	22.0 ± 17.5	<0.01*

Means ± SD; N = 8.

* Covariance analysis with intestinal entry rates as a covariable (see Methods).

3. Results

Growth rates were not significantly different ($P = 0.212$) in the lentil fed group as compared to its pair-fed casein control group (Table 2). However food efficiency ratio was more discriminating, because of lower within-group variability: it was lower in the lentil group than in the casein group by 12% and the difference was significant ($P = 0.015$).

In the plasma, glycemia values were slightly lower and uremia values were higher in the lentil fed group than in the controls, but the differences were not significant between the two groups. The insulin concentrations was lower in the lentil group than in the control group, and the difference was significant ($P < 0.01$), if a quadratic effect of intestinal entry rates (see methods) was taken into acount.

The rat body weights were slightly lower in the lentil group than in the control group (175 ± 11 vs. 199 ± 17 g), partly because of the 2 days of delay due to the pair feeding procedure. Consequently, comparisons of tissue weights and protein metabolism criteria in large intestine, liver and gastrocnemius muscle, were performed on data, expressed per 100 g of body mass (i.e., relative weight), in order to avoid emphasizing differences resulting mainly from whole body mass differences.

In the stomach, no significant differences were found for the relative fresh weights of the tissues. In the small intestine, relative fresh weight was significantly higher by 26% in the lentil group than in the casein group (4.39 ± 0.62 vs. 3.48 ± 0.37 g/100g of body weight, $P < 0.05$). In the large intestine (Table 3), relative weights of fresh tissues were significantly higher in the lentil fed group than in the control group by 35%. This difference would be only 28% if the sum of small intestine plus large intestine tissues were

Table 3
Effect of lentil feeding on protein metabolism in large intestine

Diets	Casein	Lentil	p
Wet weight (g/100g body weight)	0.99 ± 0.06	1.34 ± 0.07	<0.05
Protein content (mg/100g body weight)	100 ± 5	137 ± 13	<0.05
RNA (mg/100g body weight)	4.32 ± 0.71	6.7 ± 0.95	<0.05
Ribosomal capacity (mg RNA/g protein)	41.43 ± 5.53	47.8 ± 8.4	ns
FSR (%/d)	47.8 ± 6.7	55.5 ± 7.8	=0.05
ASR (mg protein synthesized/d)/100 g body weight	48.0 ± 8.6	75.6 ± 10.1	<0.05
Ribosomal efficiency (mg protein synthesized /d)/(mg RNA)	11.7 ± 2.2	11.8 ± 2.0	ns

Means ± SD; N = 8; FSR: Fractional Synthesis Rate; ASR: Absolute Synthesis Rate.

considered (5.73 ± 0.65 vs. 4.47 ± 0.37 g/100g of body weight, $P < 0.05$).

Protein and RNA relative weights in large intestine (Table 3) were significantly higher in the lentil fed group than in the control group by 37% and 55%, respectively. However, no significant ($P = 0.097$) difference appeared between the groups for ribosomal capacity. The fractional synthetic rate (FSR) in the large intestine tended to be higher in the case of the lentil fed group than in the control group ($P = 0.053$). When expressed in terms of absolute synthesis rate (ASR), the amount of proteins synthesized in the large intestine per 100 g of body weight per day, was larger by 57% in the lentil fed group than in the control group and the difference was highly significant ($P < 0.0001$). However, no significant ($P = 0.93$) difference appeared between the groups for ribosomal efficiency.

In the liver (Table 4), relative weights of fresh tissues and protein were significantly lower by 13% and 10% respectively in the lentil fed rats than in their controls. Also, the relative weight of RNA was lower by 13% in the liver of the lentil fed rats than in their controls and the difference was highly significant. However ribosomal capacity in the liver was not significantly different between the two groups ($P = 0.300$). The average value of FSR and of ASR of the liver were significantly lower in the case of the lentil fed group than in the control group by 24% and 32%, respectively. Ribosomal efficiency was not found to be significantly different between the 2 groups.

In the gastrocnemius muscle (Table 5), relative weight of fresh tissues and proteins were significantly lower in the lentil fed rats than in their controls, by 9% and 8% respectively. The relative weights of muscle RNA and ribosomal capacity, however, were not significantly different between the two groups. The amount of protein synthesized (ASR) in gastrocnemius tended to be lower in the lentil-fed rats than in the controls ($P = 0.06$). However, neither FSR nor ribosomal efficiency were different between the two groups.

4. Discussion

The nutritional value of the cooked lentil diet supplemented with sulfur amino acids according to the requirements of the growing rat, would appear almost as good as the control casein diet for growth of the rat, however food efficiency was lower (12%). This implies that metabolic utilization for growth of the proteins from the cooked lentils must be limited by some other factors which could be revealed by their specific effect on protein metabolism: a trophic effect on large intestine which resulted from an increase in protein synthesis rates and the opposite in liver and gastrocnemius.

We have reported that legumes included in balanced diets were able to sustain live weight gain for the growing rat [12], and that they had a trophic effect on the large intestine [6,13] and the small intestine [13]. Trophic effect of legumes on small intestine and large intestine is not always reported and seems to depend on the nature of the seed, the fraction which has been tested and the animal species [7,14,15]. Even if the trophic effect is not shown, a stimulation of protein metabolism could be expected. Cooked bean feeding has been reported to enhance intesti-

Table 4
Effect of lentil feeding on protein metabolism in liver

Diets	Casein	Lentil	p
Wet weight (g/100g body weight)	4.24 ± 0.18	3.76 ± 0.31	<0.05
Protein content (mg/100g body weight)	721 ± 71	648 ± 61	<0.05
RNA (mg/100g body weight)	39 ± 2	34 ± 2	<0.05
Ribosomal capacity (mg RNA/g protein)	54.5 ± 5.3	52.3 ± 2.3	ns
FSR (%/d)	95.3 ± 23.5	72.03 ± 17.86	<0.05
ASR (mg protein synthesized/d)/100g body weight	695 ± 209	472 ± 144	<0.05
Ribosomal efficiency (mg protein synthesized/d) (mg RNA)	17.8 ± 5.3	13.9 ± 3.7	ns

Means ± SD; N = 8; FSR: Fractional Synthesis Rate; ASR: Absolute Synthesis Rate.

Table 5
Effect of lentil feeding on protein metabolism in gastrocnemius

Diets	Casein	Lentil	p
Wet weight (g/100g body weight)	0.47 ± 0.02	0.43 ± 0.01	<0.05
Protein content (mg/100g body weight)	92 ± 4	85 ± 6	<0.05
RNA (mg/100g body weight)	0.92 ± 0.09	0.85 ± 0.08	ns
Ribosomal capacity (mg RNA/g protein)	10.0 ± 0.9	10.1 ± 0.5	ns
FSR (%/d)	17.3 ± 3.1	15.3 ± 2.5	ns
ASR (mg protein synthesized/d)/100g body weight	15.9 ± 3.2	13 ± 2.7	ns
Ribosomal efficiency (mg protein synthesized /d)/(mg RNA)	17.4 ± 3.5	15.2 ± 2.3	ns

Means ± SD; N = 8; FSR: Fractional Synthesis Rate; ASR: Absolute Synthesis Rate.

nal cell turnover in rats [16,17] and peas or beans have been proved to highly increase endogenous secretion in pigs [18]. Enteral infusion of soybean protein marginally increased amino acid uptake in the portal drained area of the pig [19]. In liver and skeletal muscles, different results were described. In liver, raw faba bean intake increased protein synthesis in rats [20] whereas enteral infusion of soybean protein decreased it in pigs [19]. Raw faba bean intake decreased protein synthesis rate in *gastrocnemius* [20], had no effect on *extensor digitorum longus* and increased it in *soleus* [21]. Enteral infusion of soybean protein resulted in higher aminoacid uptake by hind legs in pigs than casein infusion [19].

These discrepancies observed for the effect of the legumes on the same tissue may have several origins: the nature of the food, the animal model, the different aminoacids used for the labeling (tyrosine, phenylalanine, valine) and the methods involved to evaluate the effect on protein metabolism: constant intravenous infusion, flooding dose, arterio-venous differences.

The anabolic effect of lentil feeding on the large intestine has already been reported [6]. This effect of lentil feeding on large intestine is likely to be related to the microflora activity which is increased by legume feeding [5]. Microflora growth in the distal parts of the digestive tract seems to be promoted by the presence of undigested carbohydrates (α-galactosides, resistant starch, cellulose) or of proteins, either dietary proteins or endogenous proteins and glycoproteins which act as substrates for microflora. The activity of microflora is known to enhance enterocyte renewal in rats [22], and to stimulate protein synthesis in the small intestine and ceca of chicken [23]. This action of intestinal microflora has been proposed to be mediated through its metabolites such as short chain fatty acids [24] or ammonia [25], or by an indirect signal [26] which would regulate the increase of enterocyte proliferation (thymidine incorporation) and/or the increase of intestinal protein synthesis (amino acid incorporation). Also, the presence of nucleotides of microbial origin and dietary origin has been demonstrated to ensure normal ultrastructure in the intestinal mucosa, probably by serving as precursors for the nucleic acids [27].

Inhibition of protein synthesis in the liver is related to a concomitant decrease in RNA content. One possible explanation is that the portal flux of nutrients from the intestine was lower with lentil than with casein, since liver protein synthesis depends mostly on portal amino acid flux [28]. This type of difference in portal amino acid flux was also observed in pigs when comparing casein and soybean protein isolate [19]. In consequence, if the real digestibility does not differ between the legume and the control, a lower portal amino acid flux in the legume fed animals must result from an increase of intestinal capture. This is consistent with the trophic effect of the lentil diet on the small intestine found in the present experiment.

Protein mass and proteosynthesis in gastrocnemius were slightly reduced by lentil feeding at the postprandial state. This is also in agreement with the reduction of the food efficiency ratio. The consequence of lentil feeding observed on muscle protein synthesis in the postprandial state would be even more marked in the postabsorptive state, since the stimulation of protein synthesis in large intestine would persist during this period, enhancing the depletion of peripheral blood amino acids [29,30].

The trophic effect of the lentil diet on small and large intestinal tissues (+1.26 g) was much higher than its depressing effect on liver (-0.48 g). Enhanced anabolism in intestinal tissues could limit it in liver and/or muscle. In large intestine, liver and muscle, indeed, the effects of lentil feeding were similar on organ weights and on absolute protein synthesis rates (ASR). The differences seen in organ weights appear as a consequence of differences in protein synthesis rates. This suggests that the stimulation of protein synthesis in intestinal tissues would have limited protein synthesis in liver and muscle. However, the increase of ASR in the large intestine does not explain all the decrease found in the liver. Proper measurements of protein synthesis and degradation in the small intestine are necessary to improve our understanding of the relationships between these organs in the splanchnic area.

In conclusion, feeding cooked lentil seems to induce a partitioning of the flux for protein synthesis, preferentially to the benefit of the intestinal tissues, and mainly at the expense of liver and muscle in the postprandial state. This is in agreement with the important impact of the digestive tract on the availability of aminoacids for the support of other productive function, such as growth [31]. It would be of interest to investigate whether the low food efficiency of the lentil diet arises from stimulation of the degradation rates,

and/or from the decrease of synthesis in muscles at the postabsorptive state also, when nutrient flux is lower than in the postprandial state.

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