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Influence of Casein and Soy Flour Proteins on Aminoacid Content in the Liver of Experimental Animals

S. DUDÁŠOVÁ, E. GRANČIČOVÁ

Research Institute of Human Nutrition, Bratislava

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Summary

We have observed a significantly increased content of fats and decreased content of proteins in the liver of experimental rats fed a diet supplemented with 25 % casein proteins in comparison with the application of de-fatted soy flour. Casein proteins have a higher content of methionine in relation to cystine than baked soy flour. But the soy diet in contrast to the casein diet has a high content of free aminoacids which are not present in casein at all: aspartic acid, asparagine, α-aminoadipic acid, methionine, norleucine, lysine, phenylalanine, β-alanine, ethanolamine, histidine, proline, γ-aminobutyric acid, taurine. Differences in free valine, alanine, arginine, glycine, ornithine and cysteic acid are also significant. The content of free aminoacids in the liver of experimental animals fed a soy diet is high in the content of cystine, cystathionine, ornithine, β-aminoisobutyric acid, β-alanine, γ-aminobutyric acid, leucine. We have also found accumulation of methionine, glycine, α-aminobutyric acid, taurine and cytrulline in free aminoacids from the liver of animals fed a casein diet. Citrulline and glycine in free aminoacids from the liver of animals fed a soy protein supplement were not recorded. Our investigations have shown that the application of a soy diet enriched with cystine acts protectively on methionine and that methionine is preferentially utilized for protein synthesis. The catabolic pathway of methionine prevails in animals on a casein diet.

Key words

Liver – Dietary proteins – Amino acid metabolism

Introduction

Smith *et al.* (1983) demonstrated with isotope methods that the energy received from food is deposited in the body, in the ratio between sulphuric aminoacids of cysteine and methionine in the applied diet. They also observed that if cystine in the diet is present in higher amounts in relation to methionine than 3:7, the energy received from food is deposited not in the form of lipids, but of saccharides. This demand is fulfilled by the ratio of suphuric aminoacids in de-fatted baked soy flour, but not in casein (Tab. 2). The necessity of sulphuric aminoacids ratio introduced by cystine and methionine in the diet and not their total amount is evident from other reports which have confirmed that the high content of methionine itself in the applied diet can have anti-nutritional to toxic effects manifested by inhibition of growth (Friedman and Gulman 1988). But at the same time, the role of methionine in normal growth and development is well known and these results from its direct participation in a number of basic biological processes, e.g. protein synthesis, number of S-adenosyl-methionine dependent trasmethylation reactions, polyamine formation (spermidine, spermine), synthesis of cystathionine, cysteine and other metabolites of trans-sulphuration paths, formation of homocysteine which is necessary for folate metabolism and choline catabolism.

S-adenosyl-methionine is a primary donor of the methyl group in biochemical reactions. In spite of that, the formation of S-adenosyl-methionine from methionine is reversibly inhibited by its own product S-adenosyl-cysteine. It is evident that the ratio of S-adenosyl-methionine to S-adenosyl-homocysteine can regulate many physiologically significant reactions. Finkelstein (1970, 1978) demonstrated the possibility that enzyme levels influence methionine metabolism in the liver by some factors, e.g. dietary factors (protein content, methionine content), effect of hormones as well as the dependence of enzyme activity and their production on age. Methionine formation from homocysteine will increase by activation of betaine-homocysteine transferase if a diet with a low methionine content is used. The response of the organism is the same even when methionine is added to the diet. Mechanism of this unused two-phase response is not yet known. It seems that betaine-homocysteine transferase can play a significant role determined by nutritional conditions. With shortage of dietary methyl groups, betaine-homocysteine transferase can lead to methionine accumulation in the liver (Finkelstein 1988). Asi *et al.* (1987) have observed that the shortage of methionine in the diet can support the accumulation of RNA and increase RNA polymerase activity. These changes can participate in the initiation of nucleus replication of DNA. Diets supplemented by cystine block nucleus elargement as well as the activity of RNA polymerase.

Cysteine, as a non-esential aminoacid, originates from methionine by trans-sulphuration. But this catabolic pathway of methionine is not developed sufficiently or it is impaired. This is evident in prematurely born children and patients with liver diseases (cirrhotics). In these cases, cysteine is considered to be an essential aminoacid important for active sulphate and taurine formation (Stelile 1988).

Methods

Casein and de-fatted soy flour, treated at 180 °C temperature during 20 minutes to remove anti-nutritional compositions present in raw soy flour were used. Diets contained 25 % proteins, 10 % fats, 53.35 % saccharides, 4 % minerals, 0.4 % Fel-tauri, 1 % of vitamine mixture, 0.25 % cholesterol, 6 % cellulose (Tab. 1).

Table 1

Composition of applied diets

	Soy diet	Casein diet
Proteins	25.00	25.00
Fats	10.00	10.00
Saccharides	53.35	53.35
Minerals	4.00	4.00
Vitamin mixture	1.00	1.00
Cellulose	6.00	6.00
Cholesterol	0.25	0.25
Fel-tauri	0.40	0.40

Pork fat was used as a component and saccharose plus wheat starch as a saccharide component. Saccharose was used in the casein diet together with soy oil, calculated according to the content of these components in de-fatted and baked flour from soy, in amounts sufficient to eliminate the influence of other dietary components on metabolism of sulphuric aminoacids and lipids (Dudášová *et al.* 1991).

Diets were applied *ad libitum* to two groups of animals for 60 days (experimental rats), 19 animals in one group received de-fatted soy flour and 16 were fed a casein supplement in the diet. Animals were decapitated by a special guillotine without narcosis.

We determined free and bound aminoacids on an automatic aminoacids analyser T 339 (Mikrotechna, Prague) according to Spackman *et al.* (1958). Aminoacids from proteins were released by acid hydrolysis with 6N HCl at two different time intervals and extrapolated to zero time of hydrolysis. Free aminoacids were determined in tissues after removing proteins with 20 % sulphosalicylic acids. Phosphoenolpyruvatcarboxykinase was determined according to Flores and Alleyne (1971). The content of fats was determined by apparatus SOXTEK SYSTEM, from Tecator Co. Proteins in applied diets were determined from the nitrogen content received by automatic analyser RAPID N from Heraeus Co. The content of proteins in homogenates was determined by spectrophotometric method according to Lowry *et al.* (1951). Differences in the obtained values was statistically verified by Student t-test (Ekschlager 1971).

Results

In the liver of animals fed a casein diet a higher fat content 20.31 : 5.37 (in g/100 g of tissue), lower content of liquids, higher dry weight content 40.94 : 32.17 in g/100 g of tissue and a significantly higher increase in body weight 8.14 : 58.52 (in g) were found at almost normal food intake. This comprised 22.5 ± 1.06 g per animal and day on a casein diet and 23.6 ± 1.56 g per animal and day on a soy diet on the average during the 60 days' period.

Animals fed the casein diet had also a significantly lower content of proteins in the liver 16.81 g/100 g of tissue : 20.4 g/100 g of tissue, which is connected with preferential deposition of fat in the liver tissue - extensive steatosis (Tab. 2).

We have found that in the liver of animals fed a soy diet, phosphoenolpyruvatcarboxykinase activity is decreased, in relation to the activity in animals fed a casein diet (0.151:0.279) expressed in μkat.

After finding out the relation between sulphuric aminoacids and their possible influence on changes in lipid metabolism as well as changes in the metabolic turnover of methionine, we have made a comparison between glucogenous, ketogenous and sulphuric aminoacids bound in proteins (Tab. 3, 5). The difference of cistine and methionine content in the used diets results from the content of sulphuric aminoacids in the proteins of de-fatted and baked soy flour and casein (Tab. 3). We have recorded a high content of free aminoacids in de-fatted and baked soy flour, which are not present in casein in the free form at all (α-aspartic acid, asparagine, β-amino-adipic acid,

Table 2

Biological and biochemical indicators in relation to the applied diets, aminoacids as precursors of choline in the diets

	Soy diet	S.E.M.	Casein diet	S.E.M.	p<
Number of animals	19	-	16	-	-
Liver weight (g/100 g of animal)	3.07	0.06	3.37	0.05	not sign.
weight gain (g)	58.52	3.30	81.14	4.00	0.001
dry matter in liver (g/100 g of liver)	32.17	0.42	40.94	0.67	0.001
fat in liver (g/100 g liver)	5.37	0.44	20.31	0.49	0.001
phosphoenolpyruvat-carboxykinase (μkat)	0.151	0.013	0.279	0.018	0.001
proteins in liver (g/100 g of liver)	20.40	0.187	16.81	0.120	0.001
precurs. of choline/AA	0.148	0.124			
precurs. of choline/EAA	0.389	0.214			

(AA-sum of aminoacids in proteins, EAA-sum of essential aminoacids in proteins.)

Table 3
Content of suphuric acids in diet source

Aminoacid free [mg/100 g proteins]	Cystine	Methionine	Cysteic acid	Taurine
De-fatted and baked soy flour	-	7.32±0.05	14.69±0.019	5.77± +0.26
Casein	-	-	-	-
Bound aminoacid [mg/100 g proteins]				
De-fatted and baked soy flour	0.751+0.072	1.274+0.051	-	-
De-fatted and baked casein	0.909+0.215	2.199+0.327	-	-

methionine, norleucine, lysine, phenylalanine, γ-alanine, ethanolamine, histidine, proline, α-aminobutyric acid, taurine, cysteic acid - see Tab. 3).

Content of free aminoacids in the liver of animals fed the soy diet is interesting in its high content of cystine, cystathionine, ornithine, β-aminoisobutyric acid, γ-aminobutyric acid, β-alanine, leucine (Tab. 6). Accumulation of free methionine, glycine, α-aminobutyric acid, taurine and citrulline was observed in the liver of animals fed a casein diets. Citrulline and glycine in free aminoacids of animal livers were not followed after application of soy proteins.

Discussion

A high content of fat-extended steatosis was recorded in the liver of animals fed a casein diet in relation to animals with soy protein in the diet

Table 4

Free aminoacids and their representation in applied diets

	De-fatted and baked soy flour mg/100 g prot.	S.E.M.	Casein mg/100 g prot.	S.E.M.	p<
Cysteic acid	14.690	0.019			
Taurine	5.774	0.260			
Aspartic acid	39.400	0.011			
Asparagine	27.350	0.082			
Glutamic acid	4.990	0.015	5.131	0.200	0.020
α-aminoadipic acid	10.190	0.120	3.697	0.240	0.002
Alanine	24.630	0.023	2.182	0.082	0.001
Valine	79.580	0.019	6.641	0.038	0.001
Methionine	7.315	0.052	-	-	
Isoleucine	13.294	0.080	5.615	0.032	0.002
Leucine	11.000	0.076	10.385	0.040	0.020
Norleucine	11.850	0.132	-	-	
Tyrosine	30.710	0.090	-	-	
Phenylalanine	29.340	0.032	-	-	
β-alanine	30.940	0.032	-	-	
γ-aminobutyric acid	29.880	0.040	-	-	
β-aminoisobutyric acid	10.850	0.040	3.190	0.320	0.050
Ethanolamine	5.070	0.300	-	-	
Tryptophane	133.730	0.200	67.640	0.016	0.002
Ornithine	43.550	0.038	9.602	0.380	0.002
Lysine	17.460	0.120	-	-	
Histidine	19.140	0.008	-	-	
Argine	109.080	0.004	20.697	0.011	0.001
Proline	13.990	0.098	-	-	

Table 5

Content of aminoacids in used sources of proteins in relation to the possibility of metabolic influence (g/100 g proteins)

	Glucogenic[a]	Glucogenic[b]	Ketogenic	Glucogenic[a] EAA	Glucogenic[b] EAA	Ketogenic EAA
Casein	92.291	48.830	35.030	2.210	1.170	0.840
De-fatted and baked soy flour	81.257	32.940	27.040	2.385	0.967	0.794

Glucogenic[a] (sum of pure glucogenic aminoacids according to Harper 1977)
Glucogenic[b] (sum of essential and conditionally essential aminoacids which can be glucogenic and also ketogenic, Duchoň *et al.* 1985)

Table 6

Content of free aminoacids in the liver tissue of animals on a soy and casein diet

	Soy mmol/g	S.E.M. mmol/g	Casein	S.E.M.	p<
Cysteic acid	0.098	0.012	0.070	0.006	0.05
Taurine	0.558	0.051	1.128	0.147	0.002
Phosphoetanolamine	0.285	0.029	0.261	0.013	
Urea	0.439	0.035	0.337	0.023	0.020
OH-proline	19.969	2.462	15.343	1.015	
Threonine	0.913	0.096	0.620	0.046	0.020
Serine	1.284	0.136	0.900	0.063	
Glutamic acid	0.345	0.041	0.217	0.017	
α-aminoadipicacid	1.506	0.222	1.084	0.175	
Proline	20.041	2.381	17.817	1.761	
Glycine	0.124	0.040			
Alanine	0.174	0.048	0.056	0.009	0.020
Citrulline	1.277	0.090	0.001		
α-aminobutyric acid	0.080	0.010	2.065	0.168	0.001
Valine	1.412	0.219	0.051	0.011	0.001
Cystine	3.205	0.401	0.081	0.090	0.001
Methionine	0.113	0.019	0.676	0.054	0.001
Cystathionine	0.959	0.112			
Leucine	0.501	0.056	0.286	0.039	0.002
Tyrosine	0.720	0.078	0.463	0.044	
Phenylalanine	0.822	0.087	0.527		
β-alanine	1.117	0.118	0.602	0.064	0.002
β-aminoisobutyric acid	1.083	0.108	0.702	0.052	0.002
γ-aminobutyric acid	0.097	0.007	0.066	0.003	0.001
Ethanolamine	0.997	0.067	1.032	0.065	
Ammonia	5.434	0.279	5.595	0.271	
Ornithine	0.723	0.074	0.457	0.031	0.002
Lysine	1.055	0.124	0.667	0.053	
Histidine	0.541	0.067	0.342	0.025	

at which steatosis was followed. Iritani *et al.* (1988) observed with the help of isotope methods that the hypotriglyceridaemic effect of soy proteins in the liver of experimental animals was a consequence of decreased triacylglycerols biosynthesis under the influence of lowered fatty acid synthesis.

Many authors paid great attention to the influence of dietary proteins from casein and soya on lipid metabolism, these questions are discussed in the paper of Dudášová *et al.* (1991). Smith *et al.* (1983) observed that if the ratio between cystine and methionine in the diet is changed it causes a change not only in the metabolism of methionine itself, but also in the deposition of accessive energetical reserves. These authors stated that if the ratio of cystine and methionine in the diet is higher than 3:7, it leads to the deposition of excessive energetical reserves in the form of glycids but not fats. The ratio of cystine and methionine in soy flour is 4:6 which could correspond to the given affirmation on changes in deposition of excessive reserves of energy. Confirmation of these statements may be derived from the conclusions of Chiji *et al.* (1990), who found that fats accumulate in the animal liver after a soy diet if fortified by methionine. Stipanuk and Benevenga (1977) observed that adaptation of the organism to a higher cystine intake in the diet begins on the 11th day of diet intake. When animals are fed a diet containing cystine and a limiting content of methionine, a decrease of methionine-adenosyl-transferase and cystathionine-synthetase activity was found. A supplement of cystine into diets with low methionine content leads to an equal reduction of the oxidation of labelled carbons L/U-^{14}C) of methionine, L/1-^{14}C) valine and L/U-^{14}C) tyrosine and to a considerable increase in the velocity constant for muscle protein synthesis.

Finkelstein (1990) explains the cystein effects on methionine oxidation by decreased aminoacid catabolism as a result of preferential methionine incorporation into proteins. When comparing the metabolic pathways in the liver after adopting 3.5 % and 55 % diets of casein the increased intake of proteins decreased the half time of the desintegration of methyl groups occured between 9.3 to 4.8 minute. The percentage of transsulphuration was increased from 10 % to 70 %. The response of the organism to high protein and methionine intake was a high velocity of methionine cyclization with omitted cystathionine synthesis. The ability of rapid flow through the trassulphuration pathway could be of significance for the methabolism of large excesses of methionine with the production of non-toxic sarcosine which can be oxidized during glycine regeneration (Benevenga 1976). These conclusions confirm our observations showing a high content of glycine in the liver of animals after application of casein. It is evident from our observations that feeding soy diet containing a higher percentage of cystine, acts as a preventive agent towards methionine, and methionine is preferentially utilized for protein synthesis. On feeding a casein diet, a catabolic pathway of methionine prevails and this is accelerated, without cystathionine formation.

References

ASI S., THUNG WEN L., KUWAHABA T., TSUKADA K.: Alteration in the ribonucleic acids in rat liver induced by a methionine free total parenteral nutrition solution. *J. Nutr.* **117:** 673-677, 1987.

BENEVENGA N.J., HARPER A.E.: Alleviation of methionine and homocyteine toxicity in the rat. *J. Nutr.* **93:** 44-52, 1976.

COTTON R.H.: Soy products in bakery goods. *J. Am. Oil. Chem. Soc.* **51:** 116-119, 1974.

DUCHOŇ J., BARNA K., BERGENDI L.: Medical chemistry and biochemistry. Prague, Avicenum, 1985, 714 p.

DUDÁŠOVÁ S., GRANČIČOVÁ E., VOLKOVOVÁ K.: Influence of casein and soy proteins on lipids catabolism (in Slovak). *Potravinarske vedy* **9:** 269-277, 1991.

EKSCHLAGER K.: Failures of Chemical Analysis. Prague, SNTL, 1971, 140 p.

FINKELSTEIN J.D.: Control of sulphur metabolism. In: *Sulphur in Nutrition*, J.E. OLDFIELD, O.H. MUTH (eds). Westport, Avi Press, CT, 46- 60, 1970.

FINKELSTEIN J.D.: Regulation of methionine metabolism in mammals.In: *Transmethylation* E. USDIN, R.T BRONCHARDT, R.C. CREWELING (eds) New York, Elsewier, 49-58, 1978.

FINKELSTEIN J.D., MARTIN J.J., HARRIS B.J.: Methionine metabolism in mammals. The methionine sparing effect of cystine. *J. Biol. Chem.* **263:** 11750-11754, 1988.

FINKELSTEIN J.D.: Methionine metabolism in mammals. *J. Nutr. Biochem.* **1:** 228-237, 1990.

FLORES M., ALLEINE G.A.O.: Phosphoenolpyruvatcarboxykinase of kidney. *Bioch. J.* **123:** 35-39, 1971.

FRIEDMAN M., GUMLMANN M.: Nutritional value and safety of methionine derivates, isomeric dipeptides and hydroxyanalogs in mice. *J. Nutr.* **118:** 388-397, 1988.

GALIBOIS I., PARENT G., SAVOIE L.: Effect of dietary protein on time dependent changes in plasma aminoacids levels and on liver proteinsynthesis in rat. *J. Nutr.* **117:** 2027-2035, 1987.

HARPER H.A.: Survey of Physiological Chemie. Prague, Avicenum, 1977, 639 p.

CHIJI H., HARAJAMA K., KIRIJAMA S.: Effect of feeding rats low protein diets containing casein or soy protein isolate supplemented with methionine or oligo-L-methionine. *J. Nutr.* **120:** 166-171, 1990.

IRITANI N., SUGA A., FUKODA H., KATSURADA A., TAKARAKA K.: Effect of dietary casein and soy bean protein on triacylglycerol turnover in rats liver. *J. Nutr. Sci. Vitaminol.* **34:** 309-315, 1988.

LOWRY CH., ROSENBROUCH U.J., FARR A.L.: Protein measurement with the Folin reagent. *J. Biol. Chem.* **193:** 265-275, 1951.

SMITH J.T., ACCUFF R.V., BITTLE J.B., GILBERT M.L.: A metabolic comparison of cysteine and methionine supplements in the diet of rats. *J. Nutr.* **113:** 222-227, 1983.

SPACKMANN D.H., STEIN W.H., MOORE S.: Automatic recording apparatus for use in the chromatography of aminoacids. *Anal. Chem.* **30:** 1190-1206, 1958.

STELILE P., ALTERS S., POLLACK L., FURST P.: In vivo utilization of cysteine containing synthetic short-chain peptides after intravenous bolus injection in rat. *J. Nutr.* **118:** 1470-1474, 1988.

STIPANUK M.H., BENEVENGA N.J.: Effect of cystine on metabolism of methionine in rats. *J. Nutr.* **107:** 1455-1467,1977.

Reprint Requests

E. Dudášová, Institute of Human Nutrition, CS-833 37 Bratislava, Limbová 14.