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Methionine supplementation of soya products: effects on nitrogen balance parameters

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SUMMARY. Soybean protein is one of the best quality foods available. Contribution of soy to human nutrition increases because of its overall positive nutritional profile, low cost, high protein and excellent functional properties. Addition of methionine to rat soybean diets improve biological value of soy protein. Few studies on methionine fortification of soya protein were carried in infants, but fortification of baby formulas with this amino acid is usually found. This study was carried out to demonstrate in malnourished children that the effect of methionine supplementation of soya milk and soy isolated protein, as well as to compare with their results to cows' milk. A total of 30 malnourished children, 1 to 3 years old, admitted to our metabolic unit and distributed in groups of 6 children were studied. They were fed experimental formulas with cows' milk, soya milk, soya milk plus methionine, soya isolated and soya isolated plus methionine. Nutrient compositions of formulas were calculated to be similar to mothers' milk. DL-methionine, 1.5 g per 100 g protein content was added to soya milk and soya isolated formulas. Two nitrogen balances, 3 days each, were carried out. Fecal and urinary nitrogen, serum proteins, creatinine and urea in serum and urine were followed during the study. Results showed differences of intake and retention of nitrogen between some of the groups, but there were no statistically significant differences on protein absorption in the groups. No differences were demonstrated in serum proteins, total nitrogen and other serum and urine parameters analyzed. Cows' milk fed children presented the highest nitrogen retention in both balance studies. The addition of methionine to the soya milk formula increased the nitrogen retention, not reaching the cows' milk levels and did not have the same effect when added to the isolate soy protein.
Key words: Soybean protein, fortification, methionine, soya milk formula

SUMÁRIO. Suplementação de productos de soja com metionina: Efeito sobre os parâmetros do balanço nitrogenado. A soja é considerada um dos melhores alimentos disponíveis. A contribuição da soja na nutrição humana aumenta devido as suas várias propriedades nutricionais, como seu alto teor protéico, alido ao baixo custo. A adição de metionina aumenta, em ratos, o valor biológico da proteína de soja, entretanto, poucos estudos sobre a fortificação de metionina tem sido realizados em humanos. Este estudo buscou demonstrar em crianças desnutridas o efeito da suplementação de metionina em formulados de soja, comparando-os com o leite de vaca. Um total de 30 crianças desnutridas, entre 1 e 3 anos de idade, foram admitidas na unidade metabólica, sendo distribuidas em cinco grupos de seis crianças. Elas receberam formulas contendo leite de vaca, leite de soja, leite de soja mais metionina, isolado proteíco de soja e isolado proteíco de soja mais metionina. A composição nutricional das formulas foram calculadas para serem similares ao leite materno, tendo 1.5% de proteína, 3.5% de gordura e 7.5% de carboidratos, a DL-metionina foi adicionada em 1,5 grama por 100 gm de proteína nas formulas do leite de soja e do isolado proteíco de soja. Dois balanços nitrogenados de 3 dias cada, foram realizados, sendo que o primeiro balanço iniciou no décimo dia e o segundo balanço iniciou no vigésimo dia depois da admissão das crianças. Os parâmetros determinados foram o nitrogênio total nas fezes e urina, proteína total sérica, albumina e glubulina séricas e a creatinina e uréia no soro e na urina. A ingestão, absorção e retenção de nitrogênio foram determinadas nos dois períodos de balanço. A absorção proteíca foi similar em todos os grupos mas a retenção aumentou no grupo que recebeu o leite de soja mais metionina no primeiro balanço, no segundo balanço a ingestão foi diferente nos grupos que receberam leite de vaca e no grupo do leite de soja mais metionina. As crianças que receberam leite de vaca apresentaram a maior retenção nos dois períodos. Nenhuma diferença estaística foi demonstrada nas proteínas séricas. Os dados urinários mostraram uma grande variação quanto ao nitrogênio total, creatinina e uréia, entretanto não houve diferença estaística entre os grupos. O balanço nitrogenado mostrou variação quanto a ingestão proteíca, mas a absorção de nitrogênio foi similar para todos os grupos. A retenção nitrogenada foi maior no grupo que recebeu leite de vaca do que naqueles que receberam leite de soja. A adição de metionina nas formulas de soja aumentaram a retenção proteíca, entretanto o efeito da suplementação não ficou muito claro nestas crianças. A suplementação da soja com metionina não foi capaz de levar os níveis da retenção nitrogenada aos mesmos níveis achados para o leite de soja.
Palavras chave: Soja, suplementação, metionina, leite de soja.

INTRODUCTION

Soya beans and soy products are widely utilized as human foods. It is shown in animals, mainly in rats, the nutritive value of soya bean protein to be lower than cow's milk protein, ranging 65 to 85% of casein (1,2). Addition of methionine improves the biological value of soy protein. Soy proteins are known to be sulfur aminoacid deficient (2). Our experience with soya milk, produced and industrialized in Brazil, is similar to other workers. Addition of 0.15 or 0.30% of DL Methionine to a rat soya milk diet containing 10% protein will raise the protein efficiency (PER) to that of cows' milk (3).

Normal infants, children and adults are able to utilize the protein and other nutrients present in soya, when fed products in amounts sufficient to cover their needs. Milk or lactose intolerance children and adults could benefit from the high nutritive value and low cost of cow milk (4-6).

Soybean protein has also hypocholesterolemic effects and improves human diabetes control (7,8). A long term metabolic study (11 wks) to assess nutritional value and immunological tolerance of 2 soy protein concentrates in adults, revealed good nutritive results and absence of allergic reactions (9).

Need of methionine supplementation of soya protein formulas when fed to infants and children has been questioned. Few studies on the nutritional effects of methionine fortification of soy proteins have been carried out on human infants (10,11). The question relates to the fact that methionine requirements of rats are different from those of humans. Experimental rat data seems to be the main support for the addition of methionine to soya infant foods available on the market of developed countries.

Dutra-de-Oliveira and co-workers did not show differences on nitrogen retention, serum albumin level values and clinical recovery in 1-3 years old malnourished children fed soya milk, without methionine, as compared with cows' milk (4,5).. Casein and soya bean protein have different, but acceptable effects on whole body protein turnover (12).

The present study was carried out to demonstrate, in malnourished children and through different parameters, the effect of methionine supplementation of soya milk and soy isolated protein formuals, compared with cows' milk.

EXPERIMENTAL

Malnourished children, 1 to 3 years of age, in the acute phase of malnutrition were admitted to our metabolic unit for these studies. The experiment was statistically planned to include a total of 30 pre-diagnosed malnourished children, distributed in five groups of 6 children each. They received experimental formulas of cows' milk, soya milk, soya milk plus methionine, soya isolate and soya isolate plus methionine.

Formulas were calculated to have the protein, fat and calorie values of mothers' milk. Soy milk was a water extraction product from whole soybeans and soy isolated processed in Brazil. Both products were thoroughly tested in rat assays and their FER values improved significantly by the addition of 1.5g of DL-methionine per 100g of protein (6).

The nutrient composition of all experimental formulas had 1.5% protein, 3.5%/fat and 7.5% carbohydrate. Soya oil and sucrose were used to match the necessary nutrient composition. No mineral or vitamin supplements were added. DL-methionine 1.5g/100g protein was added to the formulas containing soya milk and soya isolate. Soya milk was flavored with vanilla and soy isolate with vanilla, strawberry and coconut. All children were kept on the same feeding regime during 25 days.

The amount of feeding was calculated to supply 2.0g protein and 90 calories per kg of body weight. No other foods were given to the children during the experimental period.

Two nitrogen balances, 3 days each, were carried out in the children. The first balance started on the 19th day and the second on the 20th day after admission of the child to the metabolic unit. During the 24h after admission and during the balance studies children were kept in metabolic beds.

During the first day after admission a hydroelectrolyte solution was offered to all children. Blood samples and 24-hours urine collection were obtained during the first day. Carmine and charcoal were used as stool markers. Blood samples were obtained by venipuncture during the balances. Nitrogen content in formulas, stools and urine was determined by a semimicro Kjeldahl method (13). Total serum protein, albumin and globulin were estimated by biuret reaction (14). Creatinine in serum and urine by Jaffé reaction (15). Serum and urine urea by diacetyl monoxine reaction (16). Results of the 5 groups were statistically compared by variance analysis (17).

RESULTS

Age and weight of children are shown in Table 1. There were no acceptance problems with soya milk (with and without methionine). The soya isolate formulas were not so well accepted, in spite of different flavors, and some children did not consume the total amount offered.

TABLE 1
Age and weight of the children

Formula	n	Age (Months)	Weight (kg)
Cow's Milk	6	22.2±5.9 [a]	7.8±0.86 [a]
Soya Milk	6	21.3±5.8 [a]	7.0±1.00 [a]
Soya Milk + Meth	6	16.0±2.4 [a]	6.9±1.10 [a]
Soya Isolate	6	22.5±7.4 [a]	8.2±1.70 [a]
Soya Isolate + Meth	6	20.2±6.0 [a]	7.9±1.20 [a]

Means ± SD of six children. Means in the same column with different scripts differ significantly ($p<0.05$)

Results of 3-days nitrogen balance trials in malnourished children are presented (Table 2). The protein intake varied a little in the balances, soya milk and soya milk plus methionine group had higher mean nitrogen intake/kg/body weight. Percent absorption was similar in all groups. Highest retention was found with cows' milk formula. Retention of nitrogen increases in the first and second balance when methionine was added to soya milk. The same effect was not found with soy isolate, there was even a decreased retention when methionine was added.

TABLE 2
Three-day metabolic nitrogen balance studies with malnourished children fed different formulas

	1st Nitrogen balance		
Formula	Intake (mg/kg/d)	Absorption %	Retention %
Cow's Milk	322.3±18.0 a	80.3±8.30 a	34.7±12.4 b
Soya Milk	362.3±14.0 b	71.8±9.80 a	15.3±6.55 a
Soya Milk +M	353.3±22.7 ab	69.0±6.00 a	24.8±9.63 ab
Soy Isolate	324.2±27.2 a	76.3±7.10 a	14.7±10.7 a
Soy Isolate +M	325.5±19.1 a	68.3±11.4 a	9.7±5.04 a

	2nd Nitrogen balance		
Formula	Intake (mg/kg/d)	Absorption %	Retention %
Cow's Milk	320.3±13.8 ab	77.8±5.40 a	31.8±12.0 b
Soya Milk	359.5±26.7 b	73.0±12.3 a	10.5±8.25 a
Soya Milk +M	349.5±34.5 ab	70.0±10.1 a	17.2±4.63 ab
Soy Isolate	315.5±20.6 a	75.3±13.7 a	16.5±10.9 ab
Soy Isolate +M	309.2±20.4 a	72.8±11.7 a	11.0±3.70 a

Means ± SD of six children. Means in the same column with different scripts differ significantly ($p<0.05$)

Values of total protein, albumin and globulin are shown in Table 3. No differences were demonstrated in protein among all groups, although the group of cows' milk started the experiment with the lowest level.

Concentration of creatinine and urea in serum and nitrogen, creatinine and urea nitrogen in urine are presented in Table 4 and 5. Values of creatinine in serum showed larger variations within the soya groups, with higher values compared with the group receiving cows' milk. Serum urea did not demonstrated variation in the 10th day but in the 20th day urea values were higher in a soya isolate group. Cows' milk had the lowest mean values. Values of total nitrogen, creatinine and urea nitrogen in urine showed variations but did not demonstrated well-defined differences in the study. Mean values of cows' milk and soya milk plus methionine groups were among the lowest.

TABLE 3
Total protein, albumin and globulin levels in serum of malnourished children fed cow's milk and soya products

		Total protein (g/100 ml)	
Formula	0 day	10 day	20 day
Cow's Milk	5.72±1.7 a	6.34±1.1 a	6.85±0.50 a
Soya Milk	6.78±0.7 a	7.25±0.7 a	7.10±0.66 a
Soya Milk +M	6.38±1.4 a	6.59±0.8 a	7.18±0.72 a
Soy Isolate	6.61±0.8 a	6.39±0.8 a	6.15±13.0 a
Soy Isolate +M	6.37±2.0 a	6.25±2.1 a	6.21±15.1 a

		Albumin (g/100 ml)	
Formula	0 day	10 day	20 day
Cow's Milk	2.40±1.0 a	2.87±0.6 a	3.26±0.4 a
Soya Milk	3.50±0.6 a	3.38±0.6 a	3.71±0.8 a
Soya Milk +M	3.23±0.7 a	3.40±0.3 a	3.82±0.4 a
Soy Isolate	3.26±0.3 a	3.32±0.7 a	3.16±0.7 a
Soy Isolate +M	3.20±1.3 a	3.12±1.5 a	3.21±1.2 a

		Globulin (g/100 ml)	
Formula	0 day	10 day	20 day
Cow's Milk	3.32±0.8 a	3.47±0.5 a	3.69±0.5 a
Soya Milk	3.27±0.7 a	3.87±0.4 a	3.39±0.7 a
Soya Milk +M	3.15±0.9 a	3.19±0.6 a	3.36±0.5 a
Soy Isolate	3.35±0.6 a	3.07±0.4 a	2.99±0.6 a
Soy Isolate +M	3.17±0.8 a	3.13±0.7 a	3.00±0.8 a

Means ± SD of six children. Means in the same column with different scripts differ significantly ($p<0.05$)

TABLE 4
Creatinine and urea concentration in serum of malnourished children

	Creatinine (mg/100 ml)	
Formula	10 day	20 day
Cow's Milk	0.25±0.05 a	0.22±0.04 a
Soya Milk	0.31±0.12 ab	0.32±0.14 ab
Soya Milk +M	0.25±0.04 a	0.24±0.03 a
Soy Isolate	0.44±0.14 b	0.47±0.21 ab
Soy Isolate +M	0.37±0.10 ab	0.53±0.24b

	Urea (mg/100 ml)	
Formula	10 day	20 day
Cow's Milk	13.7±0.3 a	14.6±0.3 a
Soya Milk	19.4±0.3 a	20.9±0.2 ab
Soya Milk +M	14.7±0.3 a	16.9±0.4 ab
Soy Isolate	17.9±0.4 a	21.5±0.5b
Soy Isolate +M	18.2±0.7 a	16.5±0.3ab

Means ± SD of six children. Means in the same column with different scripts differ significantly ($p<0.05$)

TABLE 5
Total nitrogen, creatinine nitrogen and urea nitrogen excretion in urine of malnourished children

Formula	Total nitrogen (mg/day) 10 day	20 day
Cow's Milk	1155.0±326.5 [a]	1285.2±442.8 [a]
Soya Milk	1465.5±310.5 [a]	1573.5±397.8 [a]
Soya Milk +M	1098.5±389.1 [a]	1286.8±315.5 [a]
Soy Isolate	1767.3±798.6 [a]	1618.3±398.4 [a]
Soy Isolate +M	1509.8±320.1 [a]	1552.0±265.4 [a]

Formula	Creatinine (mg/day) 10 day	20 day
Cow's Milk	28.8±6.25 [a]	26.7±7.20 [a]
Soya Milk	25.3±7.64 [a]	27.2±7.52 [a]
Soya Milk +M	21.5±8.33 [a]	24.2±7.13 [a]
Soy Isolate	24.5±10.1 [a]	22.5±10.2 [a]
Soy Isolate +M	26.2±2.90 [a]	25.2±4.66[a]

Formula	Urea (mg/day) 10 day	20 day
Cow's Milk	868.0±317.7 [a]	872.5±263.3 [a]
Soya Milk	163.0±319.3 [a]	1052.2±433.8 [a]
Soya Milk +M	787.7±379.6 [a]	991,0±213.1 [a]
Soy Isolate	1127.8±494.5 [a]	1189.8±393.0 [a]
Soy Isolate +M	1097.5±339.7 [a]	1158.3±214.0 [a]

Means ± SD of six children. Means in the same column with different scripts differ significantly ($p<0.05$)

DISCUSSION

There is no boubt that soybean products should be and will be more used as food for humans in many forms. Hydrolysate of isolated soy protein is a well balance source of aminoacid used in human enteral nutrition (18). Rat bioassays, such as the protein efficiency ratio (PER), generally underestimates the protein quality of soy protein for humans because the rat have a higher relative amino acid requirements for sulfur amino acids, methionine and cystine, limiting amino acids of soy proteins. Soya formulated products are already used in several countries and their nutritive values compare well with those of more conventional foods. Soy foods should be considered not only good sources of protein, but also energy, some vitamins and minerals. Parameters used in our soya experiments are clinical findings, nitrogen balance trials and data on nitrogen metabolites in blood and urine (19).

The appropriateness of soy protein for children and infants was also evaluated by Torun (20,21), who conclude that well processed soya products have comparable protein quality to milk, for preschool children and adolescents. For full term infants it is generally accepted that soy protein formulas promote growth to the same extent as do cows' milk formulas (20,21).

Nitrogen balances have been employed in most of these metabolic studies following standard techniques (19). However there has been some question regarding the validity of a 3-day balance period as against a 5 to 7 or more days trial. This reasoning, although logical, seems not practical. To keep a malnourished child in a metabolic bed for a period longer than 3 to 4 days seems unreasonable both from experimental and human point of view. On the other hand it seems of importance in nitrogen balance studies to carry along with any study of food protein value, a control group receiving a well know protein source, as it is cow's milk in the same experimental conditions. The level of protein in the diet given to the children seems to be another point of concern. Lower levels seem to be more sensitive than higher in bioassays for protein quality. In the present study we used a formula having a protein composition similar to mother's milk, because previous experience with soya milk containing 3.5% of protein did not show differences to cow's milk on the same protein level.

The importance and usefulness of different parameters in metabolic balance studies have been discussed and questioned. It has been accepted that changes in different nitrogen and protein values should bring better results. Urea nitrogen in serum of normal infants receiving adequate calories intake reflects recent intakes of dietary nitrogen and protein quality. Creatinine reflects muscle metabolism. Relationship among different nitrogen compounds have been used as indicators of protein nutriture.

Results obtained in the present study with malnourished children fed soya milk and soy isolate formulas having a composition similar to that of mother's milk showed that the acceptability of soya milk is independent of the addition of methionine. The soy isolate formula is not so well accepted, but the addition of different fllavors increases its acceptability.

Analyzing the data of nitrogen balance shows variation of nitrogen intake in all groups of children, but nitrogen absorption were similar and the nitrogen retention of the children fed cow's milk formula was greater than that of the soya groups. The addition of methionine in the soya milk formula increased the protein retention. The soy isolate formula showed higher nitrogen retention, compared to soy isolate plus methionine. Feeding of soya milk formula to these malnourished children showed a lower nitrogen retention than cow's milk group. The effect of soya methionine supplementation is not clear. Methionine fortication of soya was not sufficient to bring the nitrogen retention to the same level as that of the cow's milk formula group.

Analyses of total protein, albumin and globulin values in serum did not change drastically during the study. However, in one of these children the albumin value increased three times during the period of cow's milk feeding. On the other hand, in a child receiving the soy isolate formula plus methionine the albumin value decreased. Concentrations of urea and creatinine in serum were lower in the groups which received cow's milk and soya milk plus methionine formulas. Average urinary excretion of nitrogen and urea nitrogen were also lower in the same two groups. These were also the groups with better nitrogen balances. It is possible these results bear relationship to protein utilization. Higher values in urinary urea and creatinine were found in groups receiving soy isolate formula, with no relation to methionine supplementation. Serum concentrations of urea nitrogen are less when proteins of higher quality are fed than when equal intakes of nitrogen are provided from proteins of poor quality (1).

Larger variation in nitrogen retention was not reflected in excretion of nitrogen products in urine, probably because of the short study period.

Fomon and coworkers (10,22) studied in infants the effect of supplementing soybean formulas with 5 mg of L-methionine per gram of protein. Intakes were of the order of 2.4 g protein/kg/day. Methionine supplementation did not influence retention of nitrogen, growth rate or serum concentration of albumin. However, serum concentration of urea nitrogen was also lower, which may suggest an improvement in protein quality through supplementation, although it is difficult to judge the health significance of that finding (10,22).

Overall consideration of our children study showed a clear difference between the cow's milk formula as compared to the four soya formulas. A few points could be further discussed, such as the amount of methionine added, the period of the study and the biochemical parameters used. They could influence the results. Another aspect would be the level of protein in our formula. In our earlier studies, soya milk was formulated with a protein level similar to that of cow's milk (4). Lower level of protein as found in mother's milk could be more sensitive to demonstrate the nutritive value of food proteins. At the present lower level of intake the cow's milk protein showed higher nutritive value than our tested soya products, with or without added methionine. Addition of methionine to the soya milk formula tested increased its protein value. This was not found with the isolated soy protein.

In a recent review Young (11,23) conclude that well-processed soy protein products, such as isolated soy proteins have high nutritional value. Soy flour and soy isolates when they are the sole or major source of protein in diet containing adequate energy and others essential nutrients are fully capable of promoting adequate growth in young infants. He also concludes that methionine supplementation of soy protein is clearly unnecessary to adults. Soy proteins consumed as isolates or concentrates are excellent sources for meeting nitrogen and aminoacid needs when consumed at physiologically intakes of total protein. Methionine supplementation of soy-based infants formulas may, however, be desirable, considering the small amount of methionine required to achieve higher utilization of soy protein (11,23).

This benefits of methionine supplementation could not always be true. This is not shown in our findings, considering different soya products and several parameters. The use of two soy proteins, as the only source of protein at low level of intake to malnourished children, showed different results. Cow's milk protein produced better nitrogen utilization than the soya products. Methionine supplementation seems to improve the nutritive value of soya milk protein but not of soya isolated protein.

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