THE NUTRITIVE VALUE OF BRAZILIAN SOY PRODUCTS TESTED IN MALNOURISHED CHILDREN[1]

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SUMMARY

The nutritive value of a soya milk and soya protein isolate available in Brazil was tested in formulas fed to 30 malnourished children during a period of 25 days. The chemical composition of all formulas was similar, including their energy and protein content. The protein (1.5 g/100 ml) was supplied by

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either cow's milk, unfortified soy milk, unfortified soy protein isolate, and methionine-enriched soy milk or methionine-enriched soy isolate. Methionine was added at the level of 1.5 g/100 g of protein. The trials included clinical, biochemical and nitrogen balance studies, and results indicated that these soy products formulas did not have the same nutritive value of cow's milk. However, the nutritional quality of soy milk was improved by methionine supplementation, but the soy protein isolate was not affected by the addition of the same amino acid. Average nitrogen retention as percent of intake ranged from 31.8 to 34.7 in the children fed cow's milk. Those that received soy milk retained 10.5 to 15.3%, and when methionine was added the average retention increased from 17.2 to 24.8%. When the isolated soy protein was fed, the average retention values were 14.7 to 16.5% without methionine, and 11.0% when the methionine-supplemented formula was used. Factors that could influence the results and the importance of the subject are discussed.

INTRODUCTION

Soybeans and soy products are widely utilized today for human consumption. Animal studies, mainly in rats, have shown that soybean protein has a lower nutritive value than cow's milk protein. The biological value of soy protein, however, is increased by the addition of methionine. In rat experiments, results obtained with soy milk produced and manufactured in Brazil, were similar to those of other investigators, i.e. when 0.15 or 0.30% DL-methionine was added to a soy milk diet containing 10% protein, the protein efficiency ratio (PER) rose to that of cow's milk (1).

In humans, Fomon (2) was unable to demonstrate any difference in weight gain and nitrogen retention between a group of infants (113 to 154 days of age) fed a full-fat soy flour extract without methionine, and another group of infants of similar age fed human milk.

In previous studies, we have also been unable to show differences in nitrogen retention, increased serum albumin levels, and clinical recovery among 1 to 3-year-old malnourished children fed soybean milk without methionine and children fed cow's milk, both diets containing 3.5% protein (3).

The results obtained by Parathasarathy *et al.* with 8 to 9-year-old children fed a local low-protein diet supplemented with soy flour or skim milk powder, have shown that supplementation of processed full-fat soy flour with DL-methionine markedly increases the biological value and net protein utilization of soy protein (4).

Earlier studies by Graham *et al.* (5) and more recently by Fomon *et al.* (6), using a diet containing protein derived almost exclusively from soy isolate, have shown that soy protein fortified with methionine is an adequate nutrient for both infants and children. These investigators, however, did not study their diets without methionine fortification.

Graham *et al.* (7) demonstrated, in 1971, that fortification of a commercial soy protein product with methionine increased nitrogen retention in infants aged 6-23 months.

In 1979 Fomon and Ziegler (8) reported the findings of their studies, the purpose of which was to evaluate the effect of methionine supplementation of a diet providing moderate intakes of soy protein isolates. Methionine suplementation did not show any influence on the nitrogen retention of infants in the age group of 67 to 307 days.

The objective of this work was to study the nutritive value and the effect of methionine supplementation of local protein soy milk and soy protein isolate formulas in malnourished children.

MATERIALS AND METHODS

The design of the experiment was statistically determined so as to include a total of 30 malnourished children. The testing was carried out in a selected group of 30 children, 12 to 36 months old, admitted to our metabolic unit during the acute phase of malnutrition. They were chosen from more than 50 children admitted to the hospital during a 2-year period. Each subjet was submitted to a complete physical examination after admission, and diagnosed as malnourished on the basis of predetermined clinical criteria. Any child, with fever or any health problem during the metabolic studies was taken out, and a substitute included. The children approved for the studies were randomly assigned to five groups of six subjects each, half of them with clinical edema, and half without it. Each group received one of five experimental formulas based on cow's milk, soy milk, soy milk plus methionine, soy isolate and soy isolate plus methionine, respectively. All children received a hydroelectrolyte solution 24 hours after admission. Blood and 24-hr urine samples were obtained at the moment of admission.

All formulas were designed to contain the same amount of protein, fat and calories and were based on powdered cow's milk, soy milk, and soy isolate. The soy milk used was an industrial product water-extracted from whole soybeans and later diluted and supplemented according to the design of the formula. The soy isolate was an industrial product manufactured following modern technological processes. Both products are commercially used in Brazil for human consumption. The protein content, protein efficiency ratio and amino acid composition of the soya products used are shown in Table 1.

TABLE 1

PROTEIN CONTENT, PER AND AMINO ACID COMPOSITION OF THE SOYA PRODUCTS USED IN OUR STUDIES

Amino acids (g/100 g prot.)	Soy milk powder[1]	Soy isolate[1]	Cow's milk powder[2]
Lysine	7.36	6.08	7.2
Threonine	6.72	3.39	4.9
Methionine	1.28	0.97	2.5
Cystine	2.56	0.88	0.9
Isoleucine	4.96	4.20	5.3
Leucine	7.36	7.33	9.9
Phenylalanine	5.44	5.66	4.8
Tryptophan	1.28	0.81	1.4
Valine	4.80	4.33	6.4
Histidine	2.56	2.35	2.9
Protein (g%)	39.7	90.1	35.9
PER	1.88	1.27	2.73

1 As analyzed.
2 Literature values.

The composition of all experimental formulas was calculated to contain 1.5% protein, 3.5% fat, and 7.5% carbohydrates. Soy oil, cornstarch and sucrose were added to obtain the necessary nutrient composition. Minerals and vitamins were not added to the formulas, but were administered separately through commercial medical products so as to satisfy the recommended daily intake of the children under study. DL-methionine (1.5 g/16 g nitrogen) was added to the soy milk and soy-isolate supplemented formulas.

The soy milk formula was flavored with vanilla, and the soy isolate with vanilla, strawberry or coconut.

All children were kept on the same diet throughout the experimental period of 25 days. They were either bottle-fed or cup-fed. The total daily amount was distributed over 4 or 5 feedings. No other food was offered to them during the experimental period.

Two nitrogen balance studies, each of three days duration, were carried out in each child. The first started on the 10th day, and the second on the 20th day after admission to the metabolic unit. During the first 24 hours of admission and in the course of the balance studies, the patients were kept in metabolic beds. Carmin and charcoal were used as stool markers. Blood samples were obtained by venipuncture upon admission and before each balance study. Nitrogen content of food, stools and urine was estimated by the semimicro Kjeldahl method, using selenium as a catalyst. Total serum protein (TSP) was estimated by a biuret reaction, and albumin was determined by electrophoresis.

Statistical Analysis of the Data

Analysis of the data obtained was carried out at the University Computer and Statistical Center, using standard techniques.

RESULTS

Acceptability and Clinical Findings

There was no acceptability problem with soy milk, with or without methionine supplementation. The soy isolate formula, with or without methionine, was not so well accepted, but acceptability improved when flavorings were added. General improvement and loss of clinical edema, when present, were about the same for all groups.

Nitrogen Balance Studies

The results of the nitrogen balance studies (2-3-day periods for each of the 30 children) are shown in Table 2. The overall nitrogen intake ranged from 2.42 to 2.63 g/day, showing thereby that food intake was similar for all children. Nitrogen absorption

TABLE 2

THREE-DAY NITROGEN BALANCE STUDIES WITH MALNOURISHED CHILDREN

Formula	Subjects	Average age, months	Average weight, kg	First nitrogen balance			Second nitrogen balance		
				Intake, g/day	Absorption, % intake	Retention, % intake	Intake g/day	Absorption, % intake	Retention, % intake
Cow's milk	6	22.2±5.9	7.8±0.9	2.49±0.23	80.3±5.5	34.7 ± 1.38	2.49±0.27	77.8± 3.8	31.8±11.5
Soya milk	6	21.3±5.8	7.0±1.0	2.55±0.34	71.8±8.4	15.3 ± 6.0	2.54±0.42	73.0±10.5	10.5± 8.7
Soya milk + *methionine*	6	16.0±2.4	6.9±1.1	2.45±0.42	69.0±5.0	24.8 ±11.1	2.42±0.44	70.0± 9.5	17.2± 4.6
Soy isolate	6	22.5±7.4	8.2±1.7	2.63±0.41	76.3±5.9	14.7 ±13.2	2.59±0.60	75.3±10.7	16.5±11.9
Soy isolate + methionine	6	20.2±6.0	7.9±1.2	2.58±0.40	68.3±14.6	9.7 ± 5.3	2.43±0.24	72.8±10.4	11.0± 3.9

was higher for those receiving the cow's milk formula during both balance studies as compared to the groups receiving soy milk and soy isolate, either with or without methionine. Individual nitrogen absorption from the soy milk and soy isolate formulas presented larger variations than those observed in the case of cow's milk.

Nitrogen retention was higher in children on cow's milk formula than on soy products. Again, a great variation in nitrogen retention was observed in children receiving soy products. The group which received soy milk plus methionine showed increased nitrogen retention. The same does not seem to be the case with the soy-isolate group. If anything, when methionine was added to the soy-isolate formula there was an apparent decrease in nitrogen retention.

Total Serum Protein and Albumin

Changes in serum protein and albumin values are shown in Table 3. As the data reveal, in seven patients (one receiving cow's milk, another soy milk, 3 soy isolate, and 2 soy isolate plus methionine) TSP values lower than 6 g/100 ml were found at the end of the study. The same patients had albumin values under 3 g/100 ml. One of the patients who received soy isolate plus methionine had a very low albumin level (1.08 g/100 ml) at the end of the study period.

Average TSP and albumin values were initially lower for the group that received cow's milk, but the increase was higher in this group than in all others. The final average values for these blood constituents were higher for the group receiving soy milk, with and without methionine, than for the group fed with the soy isolate.

Urea and Creatinine in Serum and Urine

Concentrations of serum urea and creatinine and total nitrogen, urea nitrogen, and creatinine nitrogen in urine are presented in Tables 4 and 5. Lower average values for serum urea and creatinine were found for the group which received cow's milk and soy milk plus methionine. Excretion of nitrogen and urea was also lower, both during the first and second balance studies in the groups of children fed cow's milk and soy milk plus methionine. Creatinine excretion exhibited small variations in all groups during both balance studies.

TABLE 3

TOTAL PROTEIN AND ALBUMIN LEVELS IN SERUM OF MALNOURISHED CHILDREN

Formula	Subjects	Total protein (g/100 ml)			Albumin (g/100 ml)		
		Admission	10th day	20th day	Admission	10th day	20th day
Cow's milk	6	5.72 ± 1.73	6.34 ± 1.09	6.85 ± 0.54	2.40 ± 1.02	2.87 ± 0.65	3.26 ± 0.40
Soya milk	6	7.25 ± 0.69	7.25 ± 0.69	7.10 ± 0.60	3.50 ± 0.66	3.38 ± 0.65	3.71 ± 0.84
Soya milk + methionine	6	6.38 ± 1.43	6.59 ± 0.84	7.18 ± 0.66	3.23 ± 0.70	3.40 ± 0.34	3.82 ± 0.36
Soy isolate	6	6.61 ± 0.80	6.39 ± 0.81	6.15 ± 1.30	3.26 ± 0.27	3.32 ± 0.68	3.16 ± 0.73
Soy isolate							
Soy isolate + methionine	6	6.37 ± 2.03	6.25 ± 2.06	6.21 ± 1.51	3.20 ± 1.29	3.12 ± 1.47	3.21 ± 1.22

TABLE 4

CREATININE AND UREA CONCENTRATION IN SERUM OF MALNOURISHED CHILDREN

Formula	Subjects	Urea (mg/100 ml)		Creatinine (mg/100 ml)	
		10th day	20th day	10th day	20th day
Cow's milk	6	13.7 ± 3.1	14.6 ± 2.7	0.25 ± 0.05	0.22 ± 0.04
Soya milk	6	19.4 ± 3.1	20.9 ± 2.1	0.31 ± 0.11	0.32 ± 0.14
Soya milk + methionine	6	14.9 ± 3.1	16.9 ± 4.1	0.25 ± 0.04	0.24 ± 0.03
Soy isolate	6	17.9 ± 4.2	21.5 ± 5.9	0.44 ± 0.14	0.47 ± 0.21
Soy isolate + methionine	6	18.2 ± 6.7	16.5 ± 3.3	0.37 ± 0.10	0.53 ± 0.24

TABLE 5

TOTAL NITROGEN, UREA NITROGEN AND CREATININE NITROGEN EXCRETION IN URINE OF MALNOURISHED CHILDREN

Formula	Subjects	Total nitrogen (g/day)		Urea nitrogen (g/day)		Creatinine nitrogen (mg/day)	
		1st balance	2nd balance	1st balance	2nd balance	1st balance	2nd balance
Cow's milk	6	1.2 ± 0.4	1.3 ± 0.4	0.9 ± 0.3	0.8 ± 0.3	28.8 ± 6.2	26.7 ± 7.2
Soya milk	6	1.5 ± 0.3	1.6 ± 0.4	1.2 ± 0.3	1.2 ± 0.4	25.3 ± 7.6	27.2 ± 7.5
Soya milk + methionine	6	1.1 ± 0.4	1.3 ± 0.3	0.8 ± 0.4	1.0 ± 0.2	21.5 ± 8.3	24.2 ± 7.1
Soy isolate	6	1.8 ± 0.8	1.6 ± 0.4	1.2 ± 0.5	1.2 ± 0.4	24.5 ± 10.1	22.5 ± 10.2
Soy isolate + methionine	6	1.5 ± 0.3	1.5 ± 0.3	1.1 ± 0.3	1.2 ± 0.2	26.2 ± 2.9	25.2 ± 4.6

DISCUSSION

The nutritive value of soy products may be tested by several methods. Chemical analysis, animal and human studies, are some of the methods used for such purpose. In each one different parameters may be used to compare the results obtained.

When malnourished children are the subjects, as in the present study, the assumption is that –due to their protein-depleted state– clearer differences are to be obtained when proteins of different nutritive values are compared.

In addition, it seems to be true that lower protein concentration of the tested proteins are more sensitive than higher levels in bioassays for protein quality. A lower protein level could also be a more useful tool to show the importance of methionine supplementation to protein foods.

The results of the present study revealed that the acceptability of the tested soy formulas by the malnourished children was fairly good and independent of the presence of methionine.

As shown in Table 2, the intakes of the various formulas were quite similar in all balances. It is true that at the beginning we had acceptance problems with the soy isolate products, both with and without methionine. Addition of different flavors to the formulas, however, solved these problems.

When all the nitrogen balance data were analyzed, the first observation was that none of the soya formulas had an average nitrogen retention equal to that obtained with the cow's milk formula. Statistical analysis of the five groups showed cow's milk retention to be significantly different in relation to that of children who received soy milk, isolated soy protein, and methionine-enriched soy protein. Our previous nitrogen balance studies with malnourished children fed unfortified soy milk and containing more protein (3.5%), showed nitrogen retention to be similar to that of a group fed cow's milk with the same protein content (3). This result points out differences in the protein value of these products at this low level of protein intake and concentration.

This finding –the difference among the cow's milk and the soy milk formulas– seems to be confirmed, because the addition of methionine to the soya milk formula increased the nitrogen retention and rendered it statistically similar to the group which received the cow's milk formula.

The soy-isolate product utilized in the present study produced a quite variable and low nitrogen retention. The addition of methionine to this product as opposed to what happened to soya milk, decreased the average nitrogen retention of the malnourished children who received it (Table 2).

No great differences were found in regard to the average serum protein and albumin values of the groups. But the analysis of individual values clearly showed increases in these parameters in the children with low initial concentrations when they received the cow's milk, soya milk and soya milk plus methionine formulas. Children with low initial values who received the soy isolate product, with or without methionine, kept low values throughout the study. Although the statistical analysis of all children did not indicate differences among the groups, our individual results again show that low initial levels of total protein, especially of albumin, are good parameters to test food proteins.

Concentrations of urea and creatinine in serum were found to be lower in the groups fed the cow's milk and soya milk plus methionine formulas. These were also the groups with better nitrogen retention. Average nitrogen and urea excretion were also lower in the same two groups. It is possible that these results may be related to a better utilization of the protein of the formulas.

All in all, our present studies showed a clear difference, as measured through different parameters, in the nutritive value of the soy milk protein and isolated soya protein available in Brazil, as compared to cow's milk.

Studies on the addition of methionine to the soya products indicated that although the nutritive value of the soy milk increased, it did not improve the value of the soy protein isolate.

Serum concentrations of urea and creatinine were lower in the groups receiving cow's milk and soy milk plus methionine. These were also the groups that exhibited better nitrogen balances. Average nitrogen and urea excretion were also lower in the same two groups of children. It is possible that these findings may be related to protein utilization. Considering that nitrogen intake and N absorption were about the same in all groups, and that while nitrogen retention was higher, urinary nitrogen was lower when fed the cow's milk formula, this would have reflected a better utilization of the protein in this formula.

In summary, our experimental studies showed a clear difference between the protein retention of the cow's milk formula and three of the four soy formulas. Among the latter, soy milk but not soy isolate improved with the addition of methionine.

The amount of methionine added to the soy formulas could be open to question: the levels used were based on our studies with rats, but we cannot be sure whether they were also adequate for children. On the basis of the amino acid analyses (Table 1) the amount added (1.5 g/100 g protein) can be considered adequate: it increases the level of methionine of the soy milk to 2.78 and that of the soy isolate to 2.47 g/100 g of protein. These values are similar to those determined for cow's milk. The amount we added was higher than that used by Fomon *et al.* (6). On the other hand, it is known that infant soya formulas commercially available in the United States provide from 0.015 to 0.025 g of methionine per 100 ml. These values are certainly similar to the ones in our formulas.

The protein level used in our formulas could also be subject to discussion. In previous studies we used soy milk with a protein level similar to that of cow's milk. It seems to us that when working with a lower protein level, it is useful to utilize a composition approaching that of mother's milk. Natural parameters such as cow's milk or mother's milk could facilitate comparison of results from different studies. With lower protein levels, the differences in nutritive value of several formulas fed to malnourished subjects could be detected in a more sensitive way. At this low level, cow's milk gave higher nitrogen retention than soy milk, a fact that had not been observed in our previous studies with formulas containing higher protein levels (3).

Finally, the quality of the soy products used could also be under discussion. Both the soy milk and soy isolate were prepared in Brazil by large companies claiming they were using the most modern technology. But our results with the isolate soy protein, for example, do not agree with those of Graham *et al.* (5). These investigators used an isolated soy-protein milk supplemented with DL-methionine in studies with malnourished infants and children, obtaining a nutritive value similar to that of a cow's milk formula. Later they showed the importance of methionine fortification of different soy products (7). The processing of soy products has a very important effect on their nutritional value, and the amount of methionine supplementation may also have an effect.

Our previous studies with these soya products demonstrated that they had a different amino acid composition and lower nutritive value than cow's milk when tested in rats (Table 1). In these experiments, we found that when methionine was added to the diet, the rats responded immediately. This did not happen with the present testing of the soya products in malnourished children, especially with the isolated soy protein. Since the amino acid composition of the soy milk and soy isolate are different, it is possible that the addition of methionine was sufficient to balance this amino acid in the first product, and not in the second. Considering that the addition of methionine to the soy isolate decreased the nitrogen retention, it could also be thought that the added amino acid resulted in a worse balance in that particular product.

Finally, we would like to emphasize herein that in order to demonstrate the nutritive value or the need for methionine supplementation of soy products, local tests in human subjects should be carried out in spite of modern technology or good results obtained elsewhere with similar products.

RESUMEN

VALOR NUTRITIVO DE PRODUCTOS BRASILEÑOS DE SOYA SOMETIDOS A PRUEBA EN NIÑOS DESNUTRIDOS

El valor nutritivo de la leche de soya y el del aislado proteínico de soya, industrializadas en Brasil, fue determinado en 30 niños malnutridos.

Los productos se incluyeron en fórmulas alimenticias que dichos niños recibieron durante un período de 25 días.

La composición química de todas las fórmulas fue similar, incluyendo el contenido de energía y proteína. Esta proteína se proporcionó (1.5 g/100 ml) en forma de leche de vaca, leche de soya no fortificada, aislado proteínico de soya no fortificado, leche de soya enriquecida con metionina o aislado proteínico de soya enriquecido también con metionina.

La investigación incluyó estudios químicos, bioquímicos y de balance nitrogenado.

Los resultados del estudio en cuestión indicaron que las fórmulas de productos de soya no tienen el mismo valor nutritivo que la leche de vaca. No obstante, la calidad nutritiva de la leche de soya mejoró con la suplementación de metionina. En cambio, el aislado de soya no se vio afectado por la adición del mismo aminoácido.

Varios son los factores capaces de influenciar los resultados, asunto cuya importancia se discute ampliamente.

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