THE EFFECT OF FORTIFICATION OF PROCESSED SOYA FLOUR WITH *dl*-METHIONINE HYDROXY ANALOGUE OR *dl*-METHIONINE ON THE DIGESTIBILITY, BIOLOGICAL VALUE, AND NET PROTEIN UTILIZATION OF THE PROTEINS AS STUDIED IN CHILDREN

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Abstract

The true digestibility coefficient, biological value, and net available protein of diets based on processed soya flour supplemented with *dl*-methionine hydroxy analogue (MHA) or *dl*-methionine (at a level of 1.2 g/16 g N) have been determined in children aged 8–9 years. The mean daily intake of protein by the children on the different diets was maintained at a level of about 1.2 g/kg body weight. Supplementation of soya flour with *dl*-methionine brought about a marked increase in the biological value and net protein utilization of the proteins. MHA was, however, slightly less effective than *dl*-methionine in this respect. The biological value and net protein utilization of the different proteins were as follows: soya flour, 63.5 and 53.3; soya flour + MHA, 71.5 and 61.4; soya flour + methionine, 74.9 and 64.7; and skim milk powder, 82.6 and 72.0.

Introduction

During recent years, studies have been carried out by several workers on the use of oilseed meals and legumes as supplements to human diets and also for the treatment of protein malnutrition in children (1–3). Legume proteins, in general, are deficient in methionine (4). It has been shown by certain workers in experiments with animals that fortification of legume proteins with *dl*-methionine brings about a marked improvement in their nutritive value (5, 6). In an earlier publication from this laboratory, it was reported that fortification of soya-bean proteins with *dl*-methionine hydroxy analogue (MHA) increased the protein efficiency ratio and net protein utilization in albino rats almost to the same extent as that obtained with *dl*-methionine (7). The present paper describes the results of studies with children.

Experimental

Materials

Spray-dried skim milk powder of good quality was used. Processed full-fat soya flour was prepared according to Narayana Rao *et al.* (8). The essential amino acid composition of the proteins of the soya flour, skim milk powder, and the low-protein diet was determined according to the methods used by Krishnamurthy *et al.* (9). The mean intakes of the essential amino acids from the different diets were calculated by using the above values.

The sample of calcium salt of *dl*-methionine hydroxy analogue (90% purity) used in this study was kindly supplied by Monsanto Chemical Company, U.S.A. The product was reported by the manufacturers to contain 78.8% pure acid. *dl*-Methionine (E. Merck, U.S.A.) was used as the source of methionine. The required quantities of soya-bean flour were fortified with *dl*-methionine or MHA at a level of 1.2 g/16 g N by dry mixing in a mechanical mixer.

Subjects

The subjects were eight girls aged 8–9 years and were residents of a boarding home in Mysore city. The ages, heights, and weights of the girls are given in Table I. All of them belonged to the low-income groups of the population and were accustomed to consuming diets based on cereals, millets, and legumes.

TABLE I
Ages, heights, and weights of the children at the beginning of the test

Girl No.	Age (years)	Height (cm)	Weight (kg)
1	9	127.7	23.8
2	9	124.8	21.8
3	9	122.6	21.5
4	9	122.8	20.9
5	9	120.6	19.6
6	8	118.8	18.7
7	8	117.1	20.5
8	8	114.3	17.7

Diets

The composition of the low-protein diet used in this study is given in Table II. This diet provided 1459 calories and contained about 2.8 g protein (N × 6.25). The children received the low-protein diet throughout the experiment. In addition, the children received either soya flour (with or without added *dl*-methionine or MHA) or skim milk powder as a source of protein during the first four periods of the experiment. They were fed three times a day, i.e. in the morning, noon, and night. The mineral salts and vitaminized starch were mixed with the tapioca flour and corn starch. Tapioca flour was given in the form of unleavened bread while the corn starch was made into sweet and savory vermicelli-like preparations. In addition, the children received a vegetable soup and a sweetened drink containing ascorbic acid. Vitamins A and D were added to the vegetable oil. Full-fat soya flour (41.0 g) was given in three equal doses along with breakfast, lunch, and dinner in the form of sweet pudding. Skim milk powder (56.8 g) was also given in three equal doses (after reconstitution in 6 times the weight of water and addition of cane sugar) along with the three meals. The mean daily intake of protein on the soya flour or skim milk powder diets was maintained at a level of about 1.2 g/kg body weight.

Feeding of Children and Collection of Urine and Faeces

The metabolism period consisted of five periods of 10 days each; period 1,

TABLE II

Mean daily intake (g) of foodstuffs by the children on different diets*†

Foodstuffs	Low-protein diet	Diets based on Soya flour‡	Diets based on Skim milk powder
Basal low-protein diet			
Tapioca flour (washed with dilute alkali)	134.0	115.0	105.0
Corn starch	120.0	91.0	91.0
Sugar	58.0	58.0	58.0
Peanut oil (fortified with vitamins A and D)	37.0	37.0	37.0
Salt mixture§	5.0	5.0	5.0
Vitaminized starch‖	5.0	5.0	5.0
Supplements			
Processed full-fat soya flour	—	41.0	—
Skim milk powder	—	—	56.8

*All the diets supplied in addition (g/day): common salt, 8.0; onion, 14.0; tamarind fruit pulp, 5.0; non-leafy vegetables (knolkhol, brinjals, ladies finger, and radish white), 60.0; condiments (red chillies and mustard) 3.0. The protein content (N × 6.25) of the different diets were as follows: low-protein diet, 2.8 g; soya flour diet, 24.4 g; and skim milk powder diet, 25.8 g.

†Each child received 50 mg of ascorbic acid daily in the form of a sweetened drink.

‡In the 2nd and 3rd period, each child in addition was given 260 mg of *dl*-methionine or methionine hydroxy analogue respectively.

§Osborne and Mendel salt mixture.

‖Provided the daily requirements of B vitamins as recommended by the (U.S.A.) National Research Council, Food and Nutrition Board (1958).

soya flour diet; period 2, soya flour + *dl*-methionine diet; period 3, soya flour + MHA diet; period 4, skim milk powder diet; and period 5, low-protein diet. The first 5 days on each diet were treated as a preliminary period for the children to get accustomed to the diet and the collection of urine and faeces was confined to the last 5 days in each period. Carmine was used as a marker for the collection of faeces. The daily excretion of creatinine in urine was determined as a check for the quantitative collection of urine. The daily excretion of creatinine in the subjects ranged from 457 mg to 511 mg per day and of creatine 48 to 75 mg per day. In the same subject the daily excretion of creatinine and creatine on different days during the metabolism period did not differ by more than 4%, indicating thereby that the collection of urine was almost quantitative. Duplicate samples of the different diets consumed daily by each child were collected and dried at 60–65° C in a cabinet drier. They were powdered and kept in glass-stoppered bottles for analysis. The urine and faeces were preserved according to Murthy *et al.* (10). Total nitrogen in diet, urine, and faeces were determined by the micro-Kjeldhal method. The pattern of diets consumed by the children during the different periods is given in Table II. The essential amino acid composition of the diets is given in Table III. Data regarding the amino acid intake of children on the different diets, as compared with children's amino acid requirements as reported by Nakagawa *et al.* (11–14), are given in Table IV.

The digestibility coefficient, biological value, net protein utilization (NPU)

TABLE III
Essential amino acid content (g/16 g N) of the mixed proteins of different diets

Amino acid	Diets based on: Soya flour	Soya flour + methionine or MHA	Skim milk powder	FAO reference protein pattern (15)	Ideal reference protein pattern (16)
Arginine	7.3	7.3	4.1	—	6.6
Histidine	2.6	2.6	2.3	—	2.4
Lysine	6.6	6.6	7.8	4.2	7.5
Leucine	7.7	7.7	9.9	4.8	10.0
Isoleucine	5.5	5.5	6.7	4.2	6.6
Methionine	1.5	1.5 + 1.2*	2.4	2.2	2.8
Cystine	1.7	1.7	0.9	—	2.0
Total sulphur amino acids	3.2†‡	3.2 + 1.2*	3.3†‡	4.2	4.8
Phenylalanine	5.0	5.0	5.5	2.8	5.8
Threonine	3.9	3.9‡	4.5	2.8	5.0
Tryptophan	1.3	1.3†	1.4	1.4	1.6
Valine	5.3	5.3	5.0	4.2	7.0
Protein score	{67	78	68	—	100
	{76	93	79	100	—

*Methionine or MHA.
†Amino acids limiting as compared to FAO pattern.
‡Amino acids limiting as compared to Ideal reference protein pattern.

TABLE IV
Mean daily intake (mg/kg) of essential amino acids by the children from the different diets as compared with the amino acid requirements

Amino acid	Basal low-protein diet	Diets based on: Soya flour	Soya flour + methionine or MHA	Skim milk powder	Amino acid* require-ments
Arginine	6.8	86.5	91.3	51.5	—
Histidine	1.9	31.3	33.0	28.7	—
Lysine	4.4	77.8	82.2	97.1	60
Leucine	10.2	92.0	96.9	124.1	45
Isoleucine	5.3	65.1	68.7	84.6	30
Methionine	1.9	17.6	18.6 + 13.3†	29.8	27
Cystine	1.5	20.4	21.5	11.6	—
Total sulphur amino acids	3.4	38.0	40.1 + 13.3†	41.4	—
Phenylalanine	5.8	59.3	62.5	68.3	27
Threonine	4.4	46.3	48.9	55.8	35
Tryptophan	1.5	15.1	16.0	17.1	9
Valine	8.2	62.7	66.0	62.9	33

*Data of Nakagawa *et al.* (11–14).
†Methionine or MHA.

and net available protein were calculated according to the following formulae:

$$\text{Apparent digestibility coefficient} = 100 \times \frac{\text{N intake} - \text{faecal N}}{\text{N intake}}$$

$$\text{True digestibility coefficient} = 100 \times \frac{\text{N intake} - (\text{faecal N} - \text{endogenous faecal N})}{\text{N intake}}$$

$$\text{Biological value} = 100 \times \frac{\text{N intake} - (\text{faecal N} - \text{endogenous faecal N}) - (\text{urinary N} - \text{endogenous urinary N})}{\text{N intake} - (\text{faecal N} - \text{endogenous faecal N})}$$

$$\text{NPU}_{(\text{op})} = \frac{\text{true digestibility coefficient} \times \text{biological value}}{100}$$

$$\text{Net available protein} = \frac{\text{protein intake} \times \text{NPU}_{(\text{op})}}{100}$$

Statistical Treatment of Data

The data were analyzed by the analysis of variance method appropriate for randomized block design, considering each subject as a block and differences tested for significance by using a one-sided or two-sided t test, whichever is appropriate.

Results

Data regarding the daily urinary and faecal endogenous nitrogen on the low-protein diet are given in Table V. The mean daily balance of nitrogen, digestibility coefficient, biological value, and net protein utilization of the protein in children fed on diets based on soya (with or without added MHA or *dl*-methionine) or on skim milk powder is given in Table VI. The net available protein on the different diets is given in Table VII.

TABLE V

Daily urinary and faecal excretion (g) of nitrogen by the children on the low-protein diet

Girl No.	Urinary	Faecal	Total
1	1.14	0.78	1.92
2	1.14	0.72	1.86
3	1.05	0.75	1.80
4	1.05	0.74	1.79
5	0.98	0.76	1.74
6	0.98	0.76	1.74
7	0.99	0.71	1.70
8	1.06	0.63	1.69
Mean value with its standard error (7 d.f.)	1.05±0.023	0.73±0.016	1.78±0.028

Essential Amino Acid Intake and Requirements (Tables III and IV)

The protein scores of the different diets as compared with FAO reference protein pattern and Ideal reference protein pattern (16) calculated according to the method of FAO Committee (15) are as follows: soya flour diet, 76 and 67; soya flour + methionine or MHA diet, 93 and 78; and milk diet, 79 and 68 respectively.

Data regarding the essential amino acid intakes and requirements of the children are given in Table IV. It is evident that soya-bean protein at a level of 1.2 g/kg body weight provided the essential amino acid requirements of children as assessed by Nakagawa *et al.* (11–14) even after allowance is made for the loss of 16% of the protein in digestion.

TABLE VI

Mean daily balance of nitrogen and digestibility coefficient, biological value, and net protein utilization of the proteins of diets based on soya flour supplemented with MHA or *dl*-methionine

Diets*	Intake g	Intake mg/kg	Excretion (g) Urinary	Excretion (g) Faecal	Excretion (g) Total	Balance g	Balance mg/kg	Balance % intake	Apparent digestibility	True digestibility	Biological value	$NPU_{(op)}$
Soya flour	3.91	190	2.25	1.36	3.61	0.30	15.1	7.7	65.3	84.0	63.5	53.3
Soya flour + methionine	4.11	200	1.95	1.28	3.23	0.88	43.5	21.4	68.7	86.4	74.9	64.7
Soya flour + MHA	4.11	200	2.05	1.32	3.37	0.74	36.3	18.1	68.0	85.8	71.5	61.4
Skim milk powder	4.13	200	1.68	1.26	2.94	1.19	58.6	28.8	69.4	87.1	82.6	72.0
Standard error of the mean (21 d.f.)						±0.03	±1.67	±0.75	±0.86	±0.76	±0.90	±0.75

*Calorie value: 1460 kcal.

TABLE VII

Mean protein intake and net available protein in children on different diets

Diet	Protein intake g	Protein intake g/kg	Net available protein* g	Net available protein* g/kg	FAO reference protein requirements† (g/kg) Minimum	FAO reference protein requirements† (g/kg) Optimum	Ideal protein requirements‡ (g/kg) Minimum	Ideal protein requirements‡ (g/kg) Optimum
Soya flour	24.4	1.19	13.0	0.63				
Soya flour + methionine	25.7	1.25	16.6	0.81	0.6	0.90	0.64	0.96
Soya flour + MHA	25.7	1.25	15.8	0.77				
Skim milk powder	25.8	1.26	18.6	0.90				

*(Protein intake × NPU) ÷ 100.
†FAO rept. No. 16. FAO, Rome, 1957.
‡M. Swaminathan. Indian J. Pediat. **30**, 189 (1963).

Nitrogen Balance in Children and Digestibility Coefficient and Biological Value of the Proteins (Table VI)

The mean daily N intake from the different diets ranged from 3.91 to 4.13 g (about 200 mg/kg body weight). The mean daily N retention ranged from 0.30 g on soya flour diet to 1.19 g on milk diet (15.1 mg to 58.6 mg/kg body weight). The mean true digestibility coefficient of the proteins ranged from 84.0 to 86.4 on the soya flour diet and the same fortified with *dl*-methionine and MHA as compared with 87.1 for milk diet. The biological value of soya proteins was 63.5, which significantly increased ($P < 0.001$) to 74.9, when fortified with *dl*-methionine and to 71.5 ($P < 0.001$) when fortified with MHA. *dl*-Methionine hydroxy analogue was, however, significantly less effective ($P < 0.01$) than *dl*-methionine in increasing the biological value of soya proteins.

Net Protein Utilization and Net Available Protein (Tables VI and VII)

The $NPU_{(op)}$ of diet based on soya flour + MHA (61.4) was significantly less ($P < 0.01$) than that of a diet based on soya flour + *dl*-methionine (64.7), which in turn was significantly less ($P < 0.001$) than that (72.0) of milk proteins. The net available protein (g/kg body weight) from the different diets were as follows: soya flour, 0.63; soya flour + *dl*-methionine, 0.81; soya flour + MHA, 0.77; and skim milk powder, 0.90 as compared with FAO reference protein requirements (15) of 0.6 g (minimal) and 0.9 g (safe practical allowance) and Ideal reference protein requirements of 0.64 g (minimal) and 0.96 (optimal) suggested by one of us (16).

Discussion

The results obtained in the present study with children have shown that fortification of soya flour with *dl*-methionine or *dl*-methionine hydroxy analogue (MHA) (at a level of 1.2 g/16 g N) brings about a significant increase in the biological value and net protein utilization of the proteins. *dl*-Methionine hydroxy analogue, however, was significantly less effective than *dl*-methionine in this respect. The biological value and net protein utilization of soya protein fortified with *dl*-methionine or MHA were significantly less than those of milk proteins. Studies reported earlier with albino rats, however, showed that supplementation of soya flour with *dl*-methionine or MHA increased the protein efficiency ratio and net protein utilization, almost to the same extent as those of milk proteins (7). The results obtained with children in the present study, therefore, differ to some extent from those obtained with albino rats.

The mean daily intake of protein was maintained at a level of about 1.2 g/kg. The net available protein from soya flour diet (0.63 g) was nearly equal to the 'minimal' protein requirements as FAO reference protein (0.6 g) or Ideal reference protein (0.64 g) but less than the 'optimal' requirements (0.9 and 0.96 g respectively). Supplementation of soya flour with MHA or *dl*-methionine increased the net available protein to 0.77 g and 0.81 g/kg respectively, as compared with a value of 0.90 obtained for milk proteins. Even though the results obtained with children in the present short term study have shown that

MHA is an effective supplement to soya proteins deficient in methionine, there is, nevertheless, need for conducting long-term studies with albino rats and other animals and also with human subjects to ascertain whether MHA will be as effective as *dl*-methionine as a supplement to proteins deficient in sulphur amino acids over long periods of feeding.

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