THE EFFECT OF SUPPLEMENTING A RICE DIET WITH LYSINE, METHIONINE, AND THREONINE ON THE DIGESTIBILITY COEFFICIENT, BIOLOGICAL VALUE, AND NET PROTEIN UTILIZATION OF THE PROTEINS AND ON THE RETENTION OF NITROGEN IN CHILDREN

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Received September 10, 1963

Abstract

The effect of supplementing a rice diet providing about 1.3–1.4 g protein per kg body weight with lysine, methionine, and threonine individually or together on true digestibility coefficient (DC), biological value (BV), and net protein utilization (NPU) of the proteins has been studied in girls aged 8–9 years. The retention of nitrogen on the rice diet was very low (9.5% of intake in the first series and 8.5% in the second series). The BV and NPU of the proteins of rice diet were 64.1 and 52.9 in the first series and 66.6 and 54.9 in the second series. Supplementation of the rice diet with lysine or methionine or lysine + methionine brought about a significant improvement in N retention (12.6, 12.0, 13.5% of intake) and in the BV (68.3, 66.3, 69.3) and NPU (54.8, 55.7, 55.8). When the rice diet was supplemented with lysine and threonine, a highly significant improvement in the N retention (18.9% of intake) and in the BV (77.4) and NPU (63.4) was observed. Addition of methionine to rice diet containing lysine and threonine resulted in a further improvement in N retention, BV, and NPU of the diets. The net available protein (g/kg body weight) from the different diets were as follows: rice diet, 0.71; rice diet + lysine, 0.76; rice diet + methionine, 0.74; rice diet + lysine + methionine, 0.77; rice diet + lysine + threonine, 0.85; rice diet + lysine + threonine + methionine, 0.91; and skim milk powder diet, 0.96–0.98.

Introduction

The nutritional improvement of rice proteins by amino acid fortification has been the subject of investigation by a number of workers (1–4). Supplementation of rice proteins with lysine and threonine improved to a significant extent the biological value and protein efficiency ratio for the growing rat (1, 2). Neither lysine nor threonine alone was effective, while both together increased growth rates approximately threefold. In the above studies no beneficial effect was observed when methionine was added to rice diet fortified with lysine and threonine. Sure (3), however, found an increase in the protein efficiency ratio of rice proteins when methionine was added in addition to lysine and threonine. In the present investigation, the effect of supplementing a rice diet with lysine, threonine, and methionine individually or together on the digestibility coefficient (DC), biological value (BV), and net protein utilization (NPU) of the proteins has been studied in children.

Experimental

The metabolism studies were carried out in two series. The first series related to studies on the effect of addition of lysine and methionine individually or together to a rice diet on the N retention and NPU of the diets while the second series related to the effect of addition of lysine and threonine or lysine, threonine, and methionine.

Subjects

The subjects of the study were girls of age group 8–9 years who were residents of a local boarding home in Mysore city. The first series of studies was carried out with six girls and the second with eight girls. The ages, heights, and weights of the girls are given in Table I. All of them belonged to the low-income groups and were subsisting on a rice diet similar to the one used in this experiment. They were examined clinically and found free from diseases likely to interfere with the experiment.

TABLE I

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

Series I				Series II			
Girl No.	Age (years)	Height (cm)	Weight (kg)	Girl No.	Age (years)	Height (cm)	Weight (kg)
1	9	129.5	23.2	7	9	129.5	20.4
2	9	125.1	19.2	8	9	129.5	20.7
3	8	114.9	18.1	9	8	127.0	18.9
4	8	112.4	17.6	10	9	125.4	20.3
5	8	109.8	16.5	11	9	124.5	20.7
6	9	118.1	20.3	12	8	124.5	18.2
				13	9	121.9	19.2
				14	8	121.3	18.2

Experimental Diets and Feeding of the Children

The rice diet used in the present investigation was similar to that consumed habitually by the children in the boarding home. It consisted mainly of rice and contained small amounts of legumes, vegetables, oil, and skim milk powder and provided 1580 kcal/day. The composition of the experimental diets is given in Table II. The subjects were fed three times a day. The pattern of breakfast, lunch, and dinner was similar to that described by Reddy *et al.* (5). The amino acid supplements were incorporated in rice pudding and given in three equal doses along with breakfast, lunch, and dinner. The quantities of different amino acid supplements administered to each subject are given in Table II. Methionine, lysine, and threonine were added in amounts to bring the S-amino acid content of the fortified diet to a level present in FAO reference protein, and lysine and threonine to levels present in milk protein. The essential amino acid content of the different diets, determined according to Krishnamurthy *et al.* (6), is given in Table III. The intakes of essential amino acids (mg/kg body weight) from the different diets, as compared with their amino acid requirements, are given in Table IV.

The procedure adopted for the feeding of children and for the collection of

TABLE II

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

Foodstuffs	Rice diet‡	Skim milk powder
Rice, raw milled	250.0	—
Sugar	45.0	45.0
Peanut oil (fortified with vitamins A and D)	45.0	45.0
Salt mixture§	5.0	5.0
Vitaminized starch‖	5.0	5.0
Tur dhal (*cajanus cajan*)	12.4	—
Condiments	8.0	—
Tapioca flour (washed with dilute alkali)	—	125.0
Corn starch	—	80.0
Skim milk powder	5.0	70.0

*All the diets supplied in addition (g/day): common salt, 12.0; tamarind fruit pulp, 8.0; non-leafy vegetables (knol khol, brinjals, ladies finger, and radish white), 60.0; onions, 20.0.
†Each child received in addition 50 mg of ascorbic acid daily in the form of a sweetened drink.
‡In the I series, each child in addition received 316 mg *dl*-methionine during the 2nd period; 1128 mg *l*-lysine HCl during 3rd period; and 316 mg *dl*-methionine and 1128 mg *l*-lysine HCl during the 4th period. In the II series, each child in addition received 1128 mg *l*-lysine HCl and 452 mg *dl*-threonine during the second period; and 1128 mg *l*-lysine HCl, 452 mg *dl*-threonine, and 316 mg *dl*-methionine during the 3rd period.
§Osborne and Mendel salt mixture.
‖Provided the daily requirement of B vitamins as recommended by the (U.S.A.) National Research Council, Food and Nutrition Board (1958).

TABLE III

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

Amino acids	Diets A*	Diets G	FAO reference (1957) protein pattern	Ideal reference protein pattern
Arginine	5.75	3.86	—	6.6
Histidine	2.08	2.55	—	2.4
Lysine	4.49	7.45	4.2	7.5
Leucine	8.37	9.74	4.8	10.0
Isoleucine	4.81	6.25	4.2	6.6
Methionine	1.71	2.40	2.2	2.8
Cystine	1.26	0.94	—	2.0
Total sulphur amino acids	2.97	3.33	4.2	4.8
Phenylalanine	5.26	4.87	2.8	5.8
Threonine	3.82	4.53	2.8	5.0
Tryptophan	1.08	1.39	1.4	1.6
Valine	6.47	6.93	4.2	7.0
Protein score†				
FAO pattern	71	79	100	—
Ideal reference protein	60	69	—	100

*The essential amino acid contents (g/16 g N) of the diets B, C, D, E, and F were the same as those of diet A except in the following respects.
Diet B: methionine, 2.94; total sulphur amino acids, 4.20.
Diet C: lysine, 8.0.
Diet D: lysine, 8.0; methionine, 2.94; and total sulphur amino acids, 4.20.
Diet E: lysine, 8.0; and threonine, 4.70.
Diet F: lysine, 8.0; threonine, 4.70; methionine, 2.94; and total sulphur amino acids, 4.20.
†Protein scores of diets according to FAO and Ideal protein pattern were as follows: B, 77 and 60; C, 71 and 62; D, 71 and 62; E, 77 and 67; F, 77 and 67.

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

	Diets			
Amino acids	Basal low-protein diet	A (rice diet)	G (low-protein diet + skim milk powder)	Amino acid requirements† (mg/kg)
Arginine	7.3	77.1	53.6	—
Histidine	2.1	27.6	35.4	—
Lysine	4.7	59.9	103.1	60.0
Leucine	10.9	111.9	134.9	45.0
Isoleucine	5.7	64.6	87.0	30.0
Methionine	2.1	22.9	33.3	27.0
Cystine	1.6	16.7	13.0	—
Total sulphur amino acids	3.7	39.6	46.3	27.0
Phenylalanine	6.3	70.3	67.7	27.0
Threonine	4.7	51.0	63.0	35.0
Tryptophan	1.6	14.5	19.3	9.0
Valine	8.9	86.5	95.8	33.0

*The mean daily intakes of essential amino acids by the children from diets B, C, D, E, and F were nearly the same as diet A, except in the following respects.
Diet B: methionine, 39.1; total sulphur amino acids, 55.8.
Diet C: lysine, 109.9.
Diet D: methionine, 40.1; total sulphur amino acids, 57.3; and lysine, 109.3.
Diet E: lysine, 107.6; and threonine, 63.3.
Diet F: lysine, 108.6; methionine, 39.8; total sulphur amino acids, 57.2; and threonine, 63.8.
†Data of Nakagawa *et al.* (13–16).

excreta was similar to that of Tasker *et al.* (7). The composition of the low-protein diet was similar to the one used by Parthasarathy *et al.* (8). The first series of experiments consisted of six periods: period 1, rice diet; period 2, rice diet + methionine; period 3, rice diet + lysine; period 4, rice diet + lysine + methionine; period 5, low-protein diet + skim milk powder; and period 6, low-protein diet. The second series consisted of five periods: period 1, rice diet; period 2, rice diet + lysine + threonine; period 3, rice diet + lysine + threonine + methionine; period 4, low-protein diet + skim milk powder; and period 5, low-protein diet. Each period was of 10 days duration. The first 5 days were considered as a preliminary period, the faeces and urine being collected during the last 5 days. Since the experimental periods were of short duration, the design adopted was not likely to affect the interpretation of results. A similar procedure was followed by Scrimshaw *et al.* (9) in their studies on the effect of amino acid supplementation of a maize diet on nitrogen retention in children. The nitrogen in the diet, urine, and faeces was determined by the micro-Kjeldahl method. The digestibility coefficient and biological value of the proteins of the diets were calculated according to Tasker *et al.* (7). The net protein utilization (operative) ($NPU_{(op)}$) was calculated according to Platt, Miller, and Payne (10) by using the following formula:

$$NPU_{(op)} = (\text{true digestibility coefficient} \times \text{biological value}) \div 100.$$

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 were tested for significance by using a one-sided or two-sided *t* test, whichever is appropriate.

Results

Data regarding the endogenous urinary and faecal N are given in Table V and those of digestibility coefficient, biological value, and net protein utilization in Table VI. The net available protein in children on the different diets is given in Table VII.

TABLE V

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

Series I				Series II			
Girl No.	Urinary	Faecal	Total	Girl No.	Urinary	Faecal	Total
1	1.28	0.84	2.12	7	1.16	0.89	2.05
2	1.01	0.73	1.74	8	1.22	0.73	1.95
3	0.95	0.75	1.70	9	1.10	0.83	1.93
4	0.90	0.79	1.69	10	1.03	0.89	1.92
5	0.85	0.72	1.57	11	1.11	0.75	1.86
6	1.11	0.77	1.88	12	1.10	0.72	1.82
				13	1.05	0.72	1.77
				14	1.03	0.69	1.72
Mean value	1.01* ±0.103	0.77* ±0.076	1.78* ±0.079		1.10† ±0.023	0.78† ±0.028	1.88† ±0.038

*Mean value with its standard error (5 d.f.).
†Mean value with its standard error (7 d.f.).

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

The rice diet provided daily 25.7 g protein (N × 6.25); of this rice, red gram dhal, and skim milk powder contributed 17.5, 3.1, and 1.75 g protein respectively. The proteins of the rice diet contained (g/16 g N) lysine, 4.49; total sulphur amino acids, 2.97; and threonine, 3.82; while rice protein contained (g/16 g N) lysine, 3.77; total sulphur amino acids, 3.05; and threonine, 3.75. The data show that the lysine content of the rice diet proteins was slightly higher while threonine and total S-amino acid contents were nearly the same as those of rice proteins.

The protein scores of the different diets as compared with FAO reference protein pattern (11) and Ideal reference protein pattern (12) suggested by one of us, calculated according to the method of FAO Committee, are as follows: rice diet, 71 and 60; rice diet supplemented with lysine, threonine, and methionine, 77 and 67; and milk diet, 79 and 69.

Data regarding the essential amino acid intake and requirements of the children are given in Table IV. It is evident that the rice diet at a level of 1.3 g

TABLE VI

Mean daily balance of nitrogen and digestibility coefficient, biological value, and net protein utilization of the proteins of rice diet supplemented with amino acids, *l*-lysine, *dl*-methionine, and *dl*-threonine

Diets*	Intake		Excretion (g)			Balance			Apparent digestibility (%)	True digestibility (%)	Biological value	$NPU_{(op)}$
	g	mg/kg	Urinary	Faecal	Total	g	mg/kg	% intake				
Series I												
Rice diet	4.11	215	2.27	1.45	3.72	0.39	20.6	9.5	64.8	82.6	64.1	52.9
Rice diet + methionine	4.08	213	2.21	1.38	3.59	0.49	25.9	12.0	66.1	84.0	66.3	55.7
Rice diet + lysine	4.22	220	2.13	1.56	3.69	0.53	27.9	12.6	62.9	80.4	68.3	54.8
Rice diet + lysine + methionine	4.21	220	2.09	1.55	3.64	0.57	30.1	13.5	63.2	80.6	69.3	55.8
Skim milk powder	4.26	223	1.73	1.35	3.08	1.18	62.3	27.8	68.4	85.7	81.3	69.7
Standard error of the mean (20 d.f.)						±0.03	±1.73	±0.81	±0.98	±1.00	±0.67	±0.82
Series II												
Rice diet	4.05	207	2.22	1.49	3.71	0.34	17.6	8.5	63.3	82.5	66.6	54.9
Rice diet + lysine + threonine	4.22	215	1.88	1.54	3.42	0.80	40.8	18.9	63.5	82.0	77.4	63.4
Rice diet + lysine + threonine + methionine	4.26	217	1.72	1.56	3.28	0.98	50.1	23.0	63.5	81.7	82.1	67.1
Skim milk powder	4.28	218	1.70	1.38	3.08	1.20	61.3	28.0	67.7	85.9	83.7	71.8
Standard error of the mean (20 d.f.)						±0.03	±1.58	±0.70	±0.73	±0.73	±0.70	±0.70

*Calorie intake 1580 kcal.

TABLE VII

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

Diets	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
Series I								
Rice diet	25.7	1.3	13.6	0.71				
Rice diet + methionine	25.5	1.3	14.2	0.74				
Rice diet + lysine	26.4	1.4	14.5	0.76				
Rice diet + methionine + lysine	26.3	1.4	14.7	0.77				
Skim milk powder	26.6	1.4	18.5	0.96	0.6	0.90	0.64	0.96
Series II								
Rice diet	25.3	1.3	13.9	0.71				
Rice diet + lysine + threonine	26.4	1.3	16.7	0.85				
Rice diet + lysine + threonine + methionine	26.6	1.4	17.8	0.91				
Skim milk powder	26.8	1.4	19.2	0.98				

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

protein/kg body weight met all the essential amino acid requirements of children as assessed by Nakagawa *et al.* (13–16).

Nitrogen Retention

Nitrogen retention on the rice diet was only 9% of the intake. Supplementation of the rice diet with lysine and methionine individually or together resulted in a significant increase in the retention of nitrogen ($P < 0.01$, $P < 0.05$, and $P < 0.01$ respectively). However, supplementation of the diet with lysine and threonine significantly ($P < 0.001$) increased the nitrogen retention (18.9% of intake). Addition of methionine to the above diet resulted in a further significant improvement ($P < 0.001$) in nitrogen retention (23.0% of the intake), comparing well with that (28.0%) obtained on a milk protein diet.

True Digestibility Coefficient

The true digestibility coefficient of the proteins of the rice diet and the amino acid supplemented rice diets ranged from 80.4 to 84.0, there being no significant difference between the values.

Biological Value

The biological value (BV) of the proteins in the rice diet was 64.1 in the first series and 66.6 in the second series. Supplementation of the diet with lysine and methionine, individually or together, resulted only in a slight increase in the

BV of the proteins, the increases being statistically significant ($P < 0.001$, $P < 0.05$, and $P < 0.001$ respectively). However, supplementation of the rice diet with lysine and threonine resulted in a highly significant increase ($P < 0.001$) in the BV of the proteins to 77.4. Addition of methionine to the above diet caused a further significant increase ($P < 0.001$) in the BV of the proteins to 82.1, compared with a value of 83.7 obtained for milk protein.

Net Protein Utilization

The net protein utilization of the proteins of the rice diet was 52.9 in the first series and 54.9 in the second series. There is a slight increase in the NPU of the rice diet supplemented with lysine and (or) methionine, the increases with methionine or methionine and lysine supplementation being significant at $P < 0.05$. Supplementation of the rice diet with lysine and threonine brought about a highly significant increase ($P < 0.001$) in the NPU of the rice diet to 63.4. Addition of methionine to the above diet resulted in a further significant increase ($P < 0.001$) in the NPU of the diet to 67.1, as compared to NPU of 71 on milk protein diet.

Net Available Protein

The protein intake ranged from 1.29 to 1.39 g/kg body weight on the different diets. The net available protein (g/kg body weight) was as follows: rice diet, 0.71; rice diet supplemented with lysine and (or) methionine, 0.74–0.77g; rice diet supplemented with lysine and threonine, 0.85; rice diet supplemented with lysine, threonine, and methionine, 0.91; and milk diet, 0.98.

Discussion

The rice diet, at a level of 1.3 g protein/kg body weight, met all the essential amino acid requirements of children as assessed by Nakagawa *et al.* (13–16). It is, however, to be pointed out here, that Nakagawa *et al.* (13–16) in their studies on the assessment of amino acid requirements in children have used pure amino acids which are absorbed completely. The extent of digestibility of the proteins and availability of amino acids in the diet is a factor which has to be taken into consideration in determining the adequacy of the diet to meet the amino acid requirements. When the digestibility coefficient is taken into consideration, the rice diet becomes limiting in lysine.

The results obtained in the present investigation have shown that supplementation of a rice diet (providing about 1.3–1.4 g protein per kg body weight) with a mixture of lysine and threonine or lysine, threonine, and methionine brought about a highly significant increase in N retention, biological value, and NPU of the proteins. The net available protein (g/kg body weight) from the rice diet (0.71) was equal to the minimal protein requirements of FAO reference protein (0.6) or Ideal reference protein (0.64), while that from the rice diet supplemented with lysine, threonine, and methionine (0.91) and milk diet (0.98) was nearly equal to that of the optimal requirements (0.90–0.96) of these proteins.

The present study has shown that the primary limiting amino acids in the rice diet are lysine and threonine, and the secondary deficiency is methionine. These results are in conformity with those reported by Sure (3) in experiments with albino rats. From the amino acid composition, one would expect improvement in the quality of rice protein by the addition of lysine alone, as has been reported in the case of wheat proteins (17, 18). Since the nutritive value of rice proteins for albino rats can be improved only by the addition of both lysine and threonine, Flodin (19) suggested that about 40% of the threonine present in rice protein is not available for growth and both lysine and threonine are equally limiting. Further work on the availability of threonine present in rice proteins is therefore indicated.

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