Effect of amino acid supplementation of white rice fed to children[1]

RICARDO BRESSANI,[2] DOROTHY M. WILSON,[3] FERNANDO VITERI,[4]
LUIS MOSOVICH[3] AND JORGE ALVARADO[5]
Institute of Nutrition of Central America and Panama (INCAP),
Guatemala, C. A.

SUMMARY

A total of six studies were carried out with 14 children to determine the amino acid deficiencies of rice protein. In all feeding trials, rice was fed as the sole source of protein, at the level of 2 g/kg of body weight in 5 studies, and at 1.5 g/kg of body weight in one of them, maintaining a calorie intake level of 100 Kcal/kg/day. The effect of amino acid supplementation was measured by the nitrogen balance method. The results were most variable and did not allow clear conclusions to be reached. However, findings suggest that rice protein is deficient to an equal degree in lysine, methionine and threonine. Even with the simultaneous addition of the three amino acids, nitrogen retention did not reach the values observed when feeding equal amounts of milk protein. This effect was attributed to the lower digestibility of rice protein as compared to that of milk. It was also concluded that the protein quality of rice protein is relatively good, which rendered difficult the determination of its limiting amino acids. Rice is, for all practical purposes, more deficient in total protein than in specific amino acids.

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2 Chief, Division of Agricultural and Food Sciences, Institute of Nutrition of Central America and Panama.

3 When this research was carried out, Dr. Wilson and Dr. Mosovich served as Medical Officers in the Division of Clinical Research of INCAP.

4,5 Chief and Associate Chief of the Biomedical Division, INCAP, respectively.

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INTRODUCTION

Previous amino acid supplementation studies carried out in children fed single cereal grains as the only source of protein, showed that their protein quality can be improved by the addition of the respective limiting amino acids (1-6). The present paper describes the results of studies in which the grain fed was rice.

It is well known that rice is the main staple food for large sections of the world's population, providing significant amounts of calories and protein. It is therefore very important to determine its amino acid deficiencies as tested in children, and to be able to correct them, either by selection of varieties with better protein quality, or through supplementation with protein concentrates or individual amino acids.

Several investigators (7-13) have indicated, from studies with experimental animals, that rice protein is deficient in lysine. Others, however, have shown that lysine addition alone is not as effective as when added in combination with threonine. Harper *et al.* (8) and Rosenberg, and Rosenberg and Culik (10, 11) have found that it is essential to maintain a strict balance between lysine and, threonine when added to rice protein, if a response in its protein quality is to be obtained. A relative excess of one will result in a deficiency of the other and vice versa.

Studies with human subjects on the amino acid supplementation of rice are relatively few. Hundley *et al.* (14) in their work with adults, were not able to determine which were the deficient amino acids in rice protein, because of the variability of responses observed to the addition of lysine and threonine. They concluded that rice is more deficient in total protein than in specific amino acids. On the other hand, Parthasarathy and associates (15), in their studies with children, reported an increase in the biological value of rice when this was supplemented with lysine, and a higher utilization when lysine, threonine and methionine were added together.

The present paper renders the results obtained in a series of studies carried out with children fed rice protein supplemented with various amino acids. The effects of the supplement, as measured by the nitrogen balance method, are also discussed.

MATERIAL AND METHODS

Subjects

A total of 14 male children were included throughout the study, which consisted of six feeding trials. Their age, weight and height, as well as their serum protein and albumin levels when the study began, are given in Table 1. All of the children were admitted with protein-calorie malnutrition at the Metabolic Unit of INCAP, but at the time of study they were considered to be fully recovered by the usual biochemical tests and medical examinations. Three children were used for each of the first five trials, and four, in the sixth. The same children (PC-120, 125 and 126) were included in the first and second trials, and only the last two were used for the third. The weight for height increments observed during the various phases of the study have little meaning in terms of biological value of the experimental diets tested, since very often higher weight incre-

TABLE No. 1

AGE AND WEIGHT OF CHILDREN USED IN STUDIES

Case PC No.	Age	Weight kg	Height cm	Total serum protein	Serum albumin %	Experiment No.
120	3 yr 8 mo	15.25	100.0	6.70	3.92	1, 2
125	3 yr	10.82	87.0	7.16	2.97	1, 2, 3
126	3 yr 7 mo	9.48	82.0	6.97	-	1, 2, 3
AT12	2 yr 6 mo	10.55	75.0	6.74	4.20	3
148	2 yr 1 mo	8.71	74.0	6.32	-	5
154	2 yr	9.84	74.0	6.85	-	5
121A	3 yr 7 mo	13.85	91.1	6.20	-	5
149	2 yr 3 mo	11.32	83.0	7.03	3.96	4
150	2 yr 6 mo	12.66	85.2	6.46	3.79	4
151	2 yr	12.04	85.0	6.54	4.09	4
162	2 yr 11 mo	13.90	88.8	7.00	3.86	6
169	2 yr 6 mo	10.92	77.9	7.20	3.43	6
170	2 yr 1 mo	10.63	79.3	7.00	3.37	6
171	3 yr 8 mo	14.25	87.2	7.10	3.56	6

ments were observed with lower nitrogen retention. A more complete analysis of these variables will be discussed in another communication.

Diet

White polished rice, as produced and milled in Guatemala, was used as the only source of protein in the diet. Its proximate composition and essential amino acid content are shown in Table 2. The chemical composition was determined by the AOAC Official Methods of Analysis (16), and the amino acids by the microbiological techniques described previously (17). The rice was used as a fine flour by grinding it in a stone mill so as to pass 80 mesh. The composition of the basal diet, in terms of percentage, was: rice flour, 96.0, cornstarch, 1.0, and glycine, 3.0. The amino acids were added to the basal diet so as to replace equal weight of cornstarch and equal amounts of nitrogen from glycine; thus, all diets were isonitrogenous. The amino acids used were DL-methionine, DL-tryptophan, L-lysine HCl and DL-threonine, and the amounts added were obtained by comparing the amino acid pattern of rice protein with the 1957 FAO pattern (18), on the basis of the protein derived exclusively from rice.

The basal diets, as well as those supplemented with amino acids, were then used in specific amounts, according to the level of protein intake to be fed and to the child's weight. They were then mixed with sugar, margarine, salt and water. An example of a diet fed for a 24-hour period consisted of 200 g of a protein source (either milk, the rice basal diet or the supplemented rice) ; 111 g sugar; 37 g margarine; 1 g salt and 1.151 g of water.

These preparations were then cooked for approximately 20 minutes. After cooking, the diets were distributed into three equal weight portions to meet the required calorie and protein intake for each child. One portion was fed at 8:00 a.m., the second at noon and the last at 5:00 p.m. Because of the thickness of the gruel, additional known and constant amounts of water were added when feeding the diets. Their acceptability was good; however, when refused, the amounts were taken into consideration for the calculation of the nitrogen balances.

In all of the studies, except in the last one, the children were fed 2.0 g of protein and 90-100 Kcal/kg of body weight/day. In the last feeding trial, protein intake was reduced to 1.5 g/kg/

TABLE No. 2

CHEMICAL COMPOSITION AND AMINO ACID CONTENT OF RICE FLOUR

Main components			Amino acids mg/g N	Amino acids g/16 g N
Moisture, %	13.10	Arginine	509	8.14
Protein (Nx6.25)%	7.14	Histidine	196	3.14
Crude fat, %	0.40	Isoleucine	324	5.18
Ash, %	0.60	Leucine	475	7.60
Crude fiber, %	0.60	Lysine	225	3.60
Carbohydrate, %	78.16	Methionine	193	3.09
Calories/100 g	345	Phenylalanine + tyrosine	468	7.49
		Threonine	221	3.54
		Tryptophan	82	1.31
		Valine	360	5.76

Cystine = 1.81 g/16 g N.

day with the same calorie intake. Furthermore, a complete vitamin supplement and 0.16 g of ferrous sulfate were administered daily to all of the children.

Nitrogen Balance

Children were first placed on a milk-protein diet, fed at intakes equal to those indicated above for rice in all of the studies. The rice basal diet and amino acid supplemented diets were tested only after the milk-diet balances were performed, and in most studies according to the calculated degree of amino acid limitation. Each dietary treatment was applied for 13 days, save for the last study, during which the treatments covered 10 days. The first four days were used as an adaptation period to the diet, and the last 6 or 9 for the quantitative collection of feces and urine, pooled every 3 days, in order to obtain 2 or 3 three-day balance periods. The urine was collected in dark bottles containing 1 ml of concentrated acetic acid and placed in an ice bath. The 3-day pools of urine and feces were separately homogenized, and aliquots were taken for nitrogen analysis. Similarly, samples of the diet fed every 3 days were analyzed for nitrogen concentration.

RESULTS

As shown in Table 3, the results of the first study revealed a slight effect on nitrogen retention when rice was supplemented with 0.10% methionine. The response was greater when 0.03% tryptophan was added in the presence of methionine, and significantly higher when the supplements used were 0.05% lysine, 0.10% methionine, and 0.03% tryptophan; however, they did not reach the level of retention observed in the last milk-feeding period.

Table 4 summarizes the results of the second study. In this case no effect was observed on nitrogen retention when lysine was the only supplement used, and a decrease occurred with the simultaneous addition of lysine and threonine. However, supplementation with lysine, methionine, and threonine, gave a significant response, above the nitrogen retention obtained with the basal diet. The addition of tryptophan together with the other three amino acids did not alter nitrogen retention. As in the first study, the best response obtained did not reach the values resulting from milk protein fed at similar levels of protein intake.

The results of the third study are summarized in Table 5. As observed, the sequence of diet feeding differed from the previous studies in that a basal diet feeding preceded each amino acid treatment. As the data reveal, lysine addition alone improved nitrogen retention slightly, whether compared to the nitrogen balance values resulting from the basal diet alone, or to those obtained when both lysine and threonine were added together. The addition of threonine alone decreased nitrogen retention significantly. A large retention of nitrogen was observed with the basal diet which followed the threonine addition.

As shown in Table 6, where results of the fourth study are presented, the addition of threonine caused a response in nitrogen retention when compared to the value obtained from the basal diet. Such response was found similar in all children. The addition of lysine and threonine resulted in a small response in nitrogen retention, while that of lysine and methionine gave

TABLE No. 3

AMINO ACID SUPPLEMENTATION OF RICE PROTEIN

(Experiment 1)

Diet	Nitrogen Intake	Nitrogen Fecal	Nitrogen Urine	Nitrogen Absorbed	Nitrogen Retained	Weight
	mg/kg/day			% of intake		kg
Milk	294	38	209	87.1	16.0	11.91
Basal	330	61	230	81.5	11.8	12.16
Basal + methionine	336	71	217	78.9	14.3	12.30
Basal + methionine + tryptophan	312	63	189	79.8	19.2	12.47
Basal + methionine + lysine + tryptophan	317	54	175	83.0	27.8	12.55
Milk	325	41	160	87.4	38.1	12.66

Levels of amino acids added:

L-lysine HCl	0.05%.
DL-methionine	0.10%.
DL-tryptophan	0.03%.

TABLE No. 4

AMINO ACID SUPPLEMENTATION OF RICE PROTEIN

(Experiment 2)

Diet	Nitrogen Intake	Nitrogen Fecal	Nitrogen Urine	Nitrogen Absorbed	Nitrogen Retained	Weight
	mg/kg/day			% of intake		kg
Basal	300	56	175	81.3	23.0	12.80
Basal + lysine	329	71	183	78.4	22.8	13.04
Basal + lysine + threonine	297	64	180	78.5	17.8	13.07
Basal + lysine + threonine + methionine	329	70	165	78.7	28.6	13.20
Basal + lysine + threonine + methionine + tryptophan	309	67	160	78.3	26.5	13.21
Milk	321	44	133	86.3	35.5	13.51

Levels of amino acids added:

L-lysine HCl	0.05%.
DL-methionine	0.10%.
DL-tryptophan	0.03%.
DL-threonine	0.08%.

TABLE No. 5

EFFECTS OF LYSINE AND THREONINE SUPPLEMENTATION OF RICE PROTEIN
(Experiment 3)

Diet	Nitrogen Intake	Nitrogen Fecal	Nitrogen Urine	Nitrogen Absorbed	Nitrogen Retained	Weight
	mg/kg/day			% of intake		kg
Basal	326	74	197	77.3	16.9	12.53
Milk	306	45	180	85.3	26.5	12.70
Basal	327	66	187	79.8	22.6	12.79
Basal + lysine	311	59	168	81.0	27.0	12.28
Basal	326	70	201	78.5	16.9	12.82
Basal + threonine	315	78	210	75.2	8.6	13.07
Basal	318	67	178	78.9	22.9	13.26
Basal + lysine + threonine	322	60	186	81.4	23.6	13.38
Basal	316	63	175	80.1	24.7	13.54
Milk	318	42	158	86.8	37.1	13.85

Levels of amino acids added:
L-lysine HCl 0.05%.
DL-threonine 0.08%.

a response similar to that observed from threonine addition alone. The results obtained in the fifth study are summarized in Table 7. In this experiment, only lysine and threonine additions to rice gave a slight response. Lysine, methionine and threonine induced no change, while other dietary treatments decreased nitrogen retention. Table 8 presents the findings of the last trial carried out at a protein intake of 1.5 g/kg of body weight/day. Some response was observed by supplementing rice protein with lysine and methionine, but not from the addition of lysine alone. The decreasing effect caused by tryptophan — when added to the lysine and methionine supplemented diet — was possible to reverse by the addition of threonine. As in the previous experiments, the best response induced by the amino acid supplemented rice, did not reach the levels obtained with milk protein.

TABLE No. 6

AMINO ACID SUPPLEMENTATION OF RICE PROTEIN
(Experiment 4)

Diet	Nitrogen Intake	Nitrogen Fecal	Nitrogen Urine	Nitrogen Absorbed	Nitrogen Retained	Weight
	mg/kg/day			% of intake		kg
Milk	324	52	186	83.9	26.5	12.06
Basal	316	85	192	73.1	12.3	12.34
Basal + threonine	323	73	182	77.4	21.0	12.38
Basal + lysine + threonine	347	79	211	77.2	16.4	12.57
Basal + lysine + methionine	344	78	192	77.3	21.5	12.80

Levels of amino acids added:
- L-lysine HCl 0.05%.
- DL-methionine 0.10%.
- DL-threonine 0.08%.

TABLE No. 7

AMINO ACID SUPPLEMENTATION OF RICE PROTEIN
(Experiment 5)

Diet	Nitrogen Intake	Nitrogen Fecal	Nitrogen Urine	Nitrogen Absorbed	Nitrogen Retained	Weight
	mg/kg/day			% of intake		kg
Milk	317	47	203	85.2	21.1	10.80
Basal	322	62	201	80.7	18.3	10.92
Basal + lysine + threonine	342	77	198	77.5	19.6	11.07
Basal + lysine + threonine + methionine	369	78	224	78.9	18.1	11.25
Basal + lysine + threonine + tryptophan	343	71	226	79.3	13.4	11.51
Basal + threonine + tryptophan + methionine	305	62	210	79.7	10.8	11.78
Milk	328	46	208	86.0	22.6	12.01

Levels of amino acids added:
- L-lysine HCl 0.05%.
- DL-methionine 0.10%.
- DL-tryptophan 0.03%.
- DL-threonine 0.08%.

TABLE No. 8

AMINO ACID SUPPLEMENTATION OF RICE PROTEIN
(Experiment 6)

Diet	Nitrogen Intake	Nitrogen Fecal	Nitrogen Urine	Nitrogen Absorbed	Nitrogen Retained	Weight
	mg/kg/day			% of intake		kg
Milk	265	32	162	87.9	26.8	12.37
Basal	235	38	157	83.8	17.4	12.33
Basal + lysine	249	50	157	79.9	17.7	12.40
Basal + lysine + methionine	260	46	146	82.3	22.7	12.47
Basal + lysine + methionine + tryptophan	245	50	159	79.6	15.1	12.67
Basal + lysine + methionine + tryptophan + threonine	254	40	159	84.2	21.6	12.83
Milk	267	31	133	88.4	38.6	12.98

Levels of amino acids added:

L-lysine HCl	0.05%.
DL-methionine	0.10%.
DL-tryptophan	0.03%.
DL-threonine	0.08%.

DISCUSSION

The findings of the series of studies described above are not consistent enough to indicate which are the deficient amino acids in rice protein, as tested in children. One of the probable reasons for the variable responses observed is that the level of protein intake of 2 g/kg/day is relatively high for the ages and weights of the children included in our trials, thus making it quite possible that their requirements for the amino acids which are limiting in rice, were already being met. As indicated by several workers (19,20), the effects of amino acid supplementation are maximum when the amino acids added improve and maintain the appropriate balance with the other essential amino acids. Of all amino acid supplementation studies carried out with cereal grains, those with corn and wheat flour gave definite responses to specific amino acids (1-5), while the results with oat protein showed a large variability of response (6).

Thus, it would appear that the better the quality of the protein, the more difficult it is to show a definite and conclusive effect of the supplement. This is not a surprising finding, since the better the quality of the protein, the better the balance of essential amino acids, and the easier to cause an imbalance by amino acid addition. One way to discover the deficient amino acids in the better-quality proteins is to feed them at low levels of intake. This approach was tried in the study reported herein. However, the results obtained do not allow to conclude which are the deficient amino acids in rice protein. It would seem that even at the lower level, rice protein was able to supply the essential amino acid needs of these children, and more consistent results would probably have been obtained if the tests had been carried out at the level of 1 g of protein/kg/day. It is also possible that the variable responses observed might have been due to the relatively low levels of the amino acid suplements added. It would be of interest, therefore, to learn if better and more consistent responses are obtained when increasing the levels. The nitrogen retention values which resulted from the basal diet feeding, although lower than those induced by milk, indicate that the quality of rice protein is relatively good. It would thus appear that the main limitation of rice is total protein rather than specific amino acids, a conclusion that has also been reached by Hundley and coworkers (14).

Examination of results derived from the series of trials in our study, indicate that the sequence of feeding the different diets might have some effect on the responses obtained. Although a four-day adaptation period always preceded any diet change, it will be noticed that in those experiments where milk was the first and the last diet fed, the last milk-feeding period usually resulted in a higher N retention than the first. This may suggest that a slight protein depletion occurred during the rice-diet feeding treatments, with or without amino acid supplementation. It is possible that such depletion may have occurred, particularly if the amino acids added to improve the quality of rice protein, actually induce other deficiencies or caused amino acid imbalances. It is also interesting to observe the results of the third experiment (Table 5). In this case the basal diet preceded each diet supplemented with amino acids. Apparently, when the supplement was appropiate, a positive response was obtained, but when it caused an imbalance the response was lower than that obtained from the basal diet. Similar observations have been reported before in studies with other cereal grains, particularly with oats (6). It seems, therefore, that the sequence of diet feeding may influence in some way the results. These aspects undoubtedly merit further investigation.

Although the evidence presented in this paper is not altogether conclusive, examination of the data suggests that in some cases slightly higher retentions of nitrogen resulted when rice was supplemented with lysine, threonine and methionine.

Of some interest was the observation that fecal nitrogen from rice amounted to 18-24% of the nitrogen intake, while fecal nitrogen from milk amounted to 13-16% of the nitrogen ingested. These figures indicate that rice proteins have a lower digestibility than milk protein, a finding also evident from the figures of nitrogen absorbed as percentage of intake. This observation was also appreciated in similar studies with other cereal grains (1-6).

RESUMEN

Efectos de la suplementación con aminoácidos del arroz blanco administrado a niños

Con el propósito de determinar las deficiencias de aminoácidos de la proteína del arroz, se llevó a cabo una serie de seis estudios en 14 niños. En todos los ensayos alimenticios se administró arroz como fuente única de proteína, al nivel de 2 g/kg de peso corporal, en cinco estudios, y al de 1.5 g/kg de peso corporal, en uno de ellos, manteniendo siempre la ingesta calórica a un nivel constante de 100 Kcal/kg/día. El efecto de la suplementación de aminoácidos se midió por medio del método de balance de nitrógeno. Los resultados fueron muy variables por lo que no permitieron llegar a conclusiones claras. Sin embargo, los datos sugieren que la proteína del arroz es deficiente en igual grado en lisina, metionina y treonina. Según pudo establecerse, la retención de nitrógeno no alcanzó los valores observados al administrar cantidades similares de proteína de leche, ni aún con la adición simultánea de los tres aminoácidos citados. Este efecto se atribuyó a la menor digestibilidad que, en comparación con la proteína de la leche, tiene la proteína del arroz. Se concluyó también que la calidad proteínica de la proteína del arroz es relativamente buena, lo que dificultó la determinación de sus aminoácidos limitantes. Desde el punto de vista práctico, el arroz es más deficiente en su contenido de proteína total que en el de aminoácidos específicos.

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