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Supplementation of Cereal Proteins with Amino Acids

IV. LYSINE SUPPLEMENTATION OF WHEAT FLOUR FED TO YOUNG CHILDREN AT DIFFERENT LEVELS OF PROTEIN INTAKE IN THE PRESENCE AND ABSENCE OF OTHER AMINO ACIDS[1,2]

R. BRESSANI, D. WILSON, M. BÉHAR, M. CHUNG AND N. S. SCRIMSHAW
Institute of Nutrition of Central America and Panama (INCAP), Guatemala, Central America

In a previous report (Bressani et al., '60) the effect of supplementing wheat flour protein with its limiting amino acids according to the FAO reference protein was studied in children using the nitrogen balance technique. Compared with the essential amino acid pattern of the FAO reference protein (FAO, '57), the order of the limiting amino acids in the wheat basal diet was: lysine, tryptophan, methionine, isoleucine, valine, and threonine.

The results obtained with these children (Bressani et al., '60; Barness et al., '61) indicated clearly that lysine is the most limiting amino acid in wheat protein. The nitrogen retention values when lysine alone was added were considerably above those found when the basal diet was fed, although below those obtained with isoproteic amounts of milk (Bressani et al., '60; Rosenberg et al., '54). The addition of both tryptophan and lysine to the basal diet gave a slightly greater and more constant response.

Several reports (Bressani et al., '58; Almquist et al., '50; Becker et al., '57; Grau, '48; Brinegar et al., '50) from experiments with animals have shown that the relative amounts of the essential amino acids required for maximal response increase with the percentage of protein in the diet. Similar studies in humans have not been reported. It seemed pertinent, therefore, to determine whether a basal diet of wheat, supplemented with both lysine and tryptophan in appropriate amounts, would give consistent nitrogen retention values as high as those obtained with milk, and also whether the effect of a given amount of lysine per gram of nitrogen added to wheat flour is altered by the level of protein intake.

MATERIALS AND METHODS

The first experiment compared nitrogen retention observed in two children fed isoproteic levels of milk, of wheat flour supplemented with lysine, and of wheat flour supplemented with other limiting amino acids. The second experiment investigated the effect of adding different amounts of lysine to wheat flour at two levels of protein intake in 5 children. The age and weight of the children at the start of each experiment are shown in table 1.

The basal diet (in gm/100 gm) consisted of: wheat flour, 85; wheat gluten, 7; glycine, 3; and cornstarch, 5. The test diets supplied the specified level of protein and a part of the calories which were adjusted to the desired level by the addition of sugar and vegetable fat. The various amino acids were substituted for an equal amount of cornstarch, their nitrogen replacing glycine nitrogen so that all the diets remained isonitrogenous, as well as isocaloric. Corrections for the D- form of the amino acids were made by doubling the amount added, except for DL-methionine which was assumed to be fully utilized. The amount of lysine was corrected

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TABLE 1

Age and weight of children at start of each experiment

Case no.	Exp. no.	Age	Weight	Protein intake	Calorie intake
			kg	*gm/kg/day*	*kg/day*
97	1	3 years, 8 months	14.7	2	90
98	1	2 years, 9 months	10.6	2	90
92	2	3 years, 6 months	13.7	2	90
109	2	6 years, 4 months	18.4	2	90
103	2	3 years, 8 months	12.8	2	90
105	2	4 years, 6 months	15.3	3	90
115	2	2 years, 11 months	9.0	3	100

for the hydrochloride molecule present. Amino acid contents and supplements are expressed as milligrams of amino acids per gram of nitrogen to facilitate comparison with the amino acid pattern of the FAO reference protein.

In most instances, the diet was prepared for each three-day balance period and refrigerated until all was used. The two food preparations were a gruel and a pudding of a thicker consistency. The children were fed both preparations three times a day, and a multivitamin and mineral mixture[3] was given daily. The basal diet contained 101 mg of lysine/gm of nitrogen.

In experiment 1, the quantities of amino acids added to the basal diet were those needed to bring its essential amino acid pattern to that of the FAO reference protein. In experiment 2, different levels of L-lysine·HCl were added to wheat flour so that the ratios of lysine-to-trytophan in the diet were 2/1, 3/1, 4/1, 5/1 and 6/1. The 6/1 lysine supplement gave a total of 270 mg lysine/gm N, the amount contained in the essential amino acid pattern of the FAO reference protein.

In all trials weight either remained constant or increased slightly. Balance studies were usually carried out with a two-day adaptation period followed by three 3-day balance periods on each diet. At the end of each three-day balance period, the entire collections of urine and feces and samples of the diet were prepared for nitrogen analysis by the Kjedahl method.

Results for successive three-day periods on the same regimen are given as averages; variation among periods was similar to that described previously for identical procedures (Bressani et al., '60). Values for individual three-day periods were used in the analysis of variance.

RESULTS

Experiment 1

Table 2 presents the results with child PC-97, who was fed the basal wheat diet with additions of lysine, of lysine and tryptophan, and of a mixture of lysine, tryptophan, methionine, isoleucine, valine, and threonine. Nitrogen intake was relatively constant, but the absorbed nitrogen tended to be slightly lower during the milk feeding period than when the wheat protein was fed. Nitrogen retention with the wheat basal diet supplemented with lysine alone was only slightly lower than with any of the amino acid mixtures or with milk.

Table 2 also shows the nitrogen balance results obtained with child PC-98, who received the wheat basal diet with different amino acid supplements. Nitrogen intake remained relatively constant throughout the study, whereas absorbed nitrogen was slightly higher with the wheat flour diets than with milk. Nitrogen retention was similar with both milk and wheat flour supplemented simultaneously with lysine, tryptophan, methionine, valine, isoleucine, and threonine at the specified levels.

Nitrogen retention was greatest with wheat protein supplemented with lysine and tryptophan followed by lysine alone, by lysine and valine, and by the basal diet in order of decreasing retention.

[3] Gevral, donated by Lederle Laboratories, American Cyanamid Company.

TABLE 2

Comparison of nitrogen balance results with milk and a wheat flour basal diet supplemented with amino acids

Diet	No. of balance periods	Nitrogen Intake	Nitrogen Fecal	Nitrogen Urine	Absorption	Retention
		mg/kg/day			*% of intake*	*% of intake*
		Child PC — 97				
Milk	3	288	51	185	82.3	18.0
Basal + L-lysine·HCl[1]	4	302	58	221	87.4	14.2
Basal + amino acid mixture[2]	4	290	30	205	89.6	19.0
Basal + L-lysine·HCl + DL-tryptophan[3]	3	295	44	194	85.1	19.3
		Child PC — 98				
Milk	2	329	51	209	84.5	21.0
Basal	3	338	49	260	85.5	8.6
Basal + L-lysine·HCl[1]	8	328	42	245	87.2	12.5
Basal + amino acid mixture[2]	4	317	48	183	84.8	27.1
Basal + L-lysine·HCl + DL-tryptophan[3]	4	328	37	224	88.7	20.4
Basal	3	320	43	236	86.6	12.8
Basal + L-lysine·HCl + DL-valine[4]	3	304	41	210	86.5	17.4
Milk	2	321	71	165	77.9	26.5

[1] To give 270 mg lysine/gm N in diet.
[2] To give 270 mg lysine, 90 mg tryptophan, 270 mg methionine, 270 mg isoleucine, 270 mg valine and 180 mg threonine/gm N in diet.
[3] To give 90 mg tryptophan/gm N in diet.
[4] To give 270 mg valine/gm N in diet.

Experiment 2

Protein intake: 2 gm/kg/day. Table 3 shows the nitrogen balance results obtained with each of the three children fed the wheat basal diet at a protein intake of 2 gm/kg/day, supplemented with increasing levels of L-lysine·HCl. The supplementation to 162 mg lysine/gm N in the diet increased nitrogen retention above that observed with the basal diet alone. Higher levels of supplemental lysine failed to improve nitrogen balance significantly. Child PC-109 had a preliminary feeding of milk, which gave slightly higher balance results than those observed with the lysine supplemented wheat protein diet.

Protein intake: 3 gm/kg/day. Table 4 summarizes the nitrogen balance results obtained from feeding each of three children the wheat basal diet at a protein intake of 3 gm/kg/day, supplemented with increasing levels of lysine. The basal diet gave low nitrogen balance values, but the supplementation to 162 mg of lysine/gm N again improved nitrogen balance. It was further increased in one child by the addition to 208 mg lysine/gm N. In child PC-105 this level of lysine gave nitrogen values similar to those observed during the initial treatment with milk.

Statistical analysis of lysine supplementation results at the two levels. The analysis of variance of the nitrogen retention data, expressed as percentage of nitrogen intake, shows a significant difference in the percentage of retention among the various lysine levels of supplementation studied ($P = 0.01$). This difference can be ascribed to the increment in percentage of retention compared with that obtained with 101 mg lysine/gm N in the diet and was observed in all cases when lysine was added at the rate of 162 mg lysine/gm N in the total diet. Higher levels of lysine failed to produce significant additional increases in nitrogen retention. Since there is no statistical indication of either a lysine level × protein level interaction or a lysine level × child interaction, it can be concluded that within the range of protein intake studied a uniformly high level of percentage of nitrogen retention was attained starting with lysine additions to the level of 162 mg of lysine/gm N.

TABLE 3

Effect of increasing levels of supplemental lysine on nitrogen retention of children fed a wheat basal diet at a protein intake of 2 gm/kg/day (average of three 3-day balance periods)

Diet	Nitrogen			Absorption	Retention
	Intake	Fecal	Urine		
	mg/kg/day			*% of intake*	*% of intake*
	Child PC — 92				
Basal (101 mg lysine/gm N)[1]	325	47	274	85.5	1.2
Basal+lysine (162 mg lysine/gm N)	322	45	227	86.0	15.5
Basal+lysine (208 mg lysine/gm N)	313	49	211	84.3	16.9
Basal+lysine (260 mg lysine/gm N)	301	54	191	82.1	18.6
	Child PC — 103				
Basal (101 mg lysine/gm N)	330	44	269	86.7	5.2
Basal+lysine (162 mg lysine/gm N)	335	44	244	86.9	14.0
Basal+lysine (208 mg lysine/gm N)	322	52	239	83.8	9.6
Basal+lysine (260 mg lysine/gm N)	320	54	222	83.1	13.7
Basal+lysine (282 mg lysine/gm N)	317	36	224	88.6	17.9
	Child PC — 109				
Milk	312	68	201	78.2	13.8
Basal (101 mg lysine/gm N)	325	34	270	89.5	6.5
Basal+lysine (162 mg lysine/gm N)	328	27	264	91.8	11.3
Basal+lysine (194 mg lysine/gm N)	313	29	241	90.7	13.7
Basal+lysine (208 mg lysine/gm N)	318	27	258	91.5	10.4
Basal+lysine (282 mg lysine/gm N)	331	29	272	91.2	9.1

[1] Total lysine in diet.

TABLE 4

Effect of increasing levels of supplemental lysine on nitrogen retention in children fed a wheat basal diet at a protein intake of 3 gm/kg/day (average of three 3-day balance periods)

Diet	Nitrogen			Absorption	Retention
	Intake	Fecal	Urine		
	mg/kg/day			*% of intake*	*% of intake*
	Child PC — 103				
Milk	468	79	345	83.1	9.4
Basal (101 mg lysine/gm N)[1]	473	59	455	87.5	−8.7
Basal+lysine (162 mg lysine/gm N)	475	53	422	88.8	10.5
Basal+lysine (208 mg lysine/gm N)	495	65	379	86.9	10.3
Basal+lysine (260 mg lysine/gm N)	505	59	446	88.3	9.5
Basal+lysine (282 mg lysine/gm N)	493	52	384	89.5	11.6
	Child PC — 105				
Milk	482	77	341	84.0	13.3
Basal (101 mg lysine/gm N)	495	66	400	86.7	5.9
Basal+lysine (162 mg lysine/gm N)	479	48	382	90.0	10.2
Basal+lysine (208 mg lysine/gm N)	488	47	385	90.4	11.5
Basal+lysine (260 mg lysine/gm N)	508	40	390	92.1	16.3
Basal+lysine (282 mg lysine/gm N)	504	41	385	91.9	15.5
	Child PC — 115				
Basal (101 mg lysine/gm N)	481	61	398	87.3	4.6
Basal+lysine (162 mg lysine/gm N)	509	64	345	87.4	19.6
Basal+lysine (194 mg lysine/gm N)	530	70	379	86.8	15.3
Basal+lysine (208 mg lysine/gm N)	533	59	400	88.9	13.9
Basal+lysine (282 mg lysine/gm N)	509	60	363	88.2	16.9

[1] Total lysine in diet.

DISCUSSION

The nitrogen retention results of the first experiment confirm the previous report of Bressani et al. ('60) that in young children the addition of lysine to a basal wheat diet brings about a marked increase in nitrogen retention. Nevertheless, the increase is lower than that observed from feeding isonitrogenous amounts of milk. The present results indicate that the addition of lysine, tryptophan, methionine, isoleucine, valine and threonine to the wheat basal diet in amounts called for by the pattern of the FAO reference protein (FAO, '57) results in nitrogen retention values as high as those obtained with milk, or higher. They further show that, of the 6 amino acids added in these amounts, lysine and tryptophan together, added to wheat protein fed at intakes between 2 and 3 gm of protein/kg of body weight, produce about 90% of the nitrogen retentions obtained with the same amount of milk protein.

Contrary to the results with a previous child (Bressani et al., '60) valine did not improve nitrogen retention beyond that obtained with lysine alone in the child given the tryptophan and valine combination in the present study. The deficiencies of methionine, isoleucine, and threonine in wheat flour are also minimal. As in the previous study by Bressani et al. ('60), the addition of the 4 amino acids to wheat flour supplemented with lysine and tryptophan produced only a small response; nevertheless, the present experiments and those of investigators working with rats (Hutchinson et al., '58; Bender, '58; Rosenberg et al., '54; Ericson, '60) suggest the need of these amino acids to obtain maximal response with wheat flour protein.

One of the 6 children appeared to require 194 mg of lysine/gm N in the diet for maximal retention rather than 162 mg. The higher figure is equivalent to 102 mg of lysine/kg of body weight, very close to the 103 mg of lysine suggested for children by Snyderman et al. ('59) on the basis of nitrogen balance studies in children one to two months of age, and higher than the figures reported by Nakagawa et al. ('61) for children 11 years old.

The results suggest that when wheat is the only source and is fed at a level of 2.00 to 3.00 gm of protein/kg, the optimal quantity of lysine to be added to the diet is between 162 and 194 mg of lysine/gm N. Adding these quantities of lysine to those in the basal diet results in a lysine-to-tryptophan ratio between 3/1 and 4/1, as proposed in the FAO reference pattern. In a recent review on lysine in human nutrition, Jansen ('62) expressed in several ways the lysine requirements for man and rats observed by a number of investigators. Expressed as the relationship of lysine-to-tryptophan, most of the values were between 3/1 and 4/1.

That the maximal retention of nitrogen was obtained at a much lower level in the present studies helps to explain why the addition of L-lysine at a level of 270 mg lysine/gm N in the studies of Bressani et al. ('60) did not result in nitrogen retention values as high as those obtained with the lower level. It also suggests the reason that the addition of tryptophan to the higher levels of lysine supplementation improved balance; the higher additions of lysine probably caused tryptophan to become the first limiting amino acid for children. The addition of only 162 mg lysine/gm N to the diet was sufficient to balance the available tryptophan in the wheat flour. If larger amounts of lysine are supplied, other amino acids must be added to maintain favorable proportions among the essential amino acids. This appears to be a fundamental limitation in the use of the FAO or any other amino acid reference pattern.

In using any amino acid pattern for the evaluation of proteins, it is necessary to consider not only the adequacy of the amount of each amino acid per gram of nitrogen when others are present in optimal quantities, but also the effect of excesses. This agrees with Rosenberg's conclusion ('59) that the amino acid supplementation of a deficient protein gives an improved response only when it corrects the most limiting amino acid in such a way as to improve the balance or proportion among the other essential amino acids. Some of the amino acids may have little or no adverse effect on nitrogen utilization when they exceed the quantity per gram of nitrogen recommended in the FAO reference pattern; others, such as methionine, valine, and leucine, when present in excess, may under some conditions produce imbalances. An improvement in the present amino acid pattern concept would be to include upper, as well as lower, limits for each amino acid concentration per gram of nitrogen so that when used to evaluate foods or diets the pattern would warn of possible imbalances due to amino acid excesses, as well as adverse effects of deficiencies.

SUMMARY

In the first of two experiments using the nitrogen balance technique in children, it was shown that the addition of lysine to a basal wheat diet brings about a marked increase in nitrogen retention. Nitrogen retention values as high as or higher than those obtained with isonitrogenous feeding of milk were obtained by feeding a wheat basal diet supplemented with a mixture containing the FAO levels of lysine, tryptophan, methionine, isoleucine, valine, and threonine. About 90% of the response, however, was found to be due to the combined lysine and tryptophan supplement.

In the second experiment, the effect of protein level of intake on the minimal lysine supplement to be added to wheat flour was studied in 6 children. Maximal retention of nitrogen was obtained with the addition of 162 to 194 mg lysine/gm of nitrogen to a basal diet fed at a rate of 2 and 3 gm of protein/kg of body weight per day, respectively. Within the range of the protein fed, the amount of lysine per gram of nitrogen that must be added did not vary significantly with protein intake.

The results demonstrate once again that it is necessary in amino acid supplementation studies to consider not only the adequacy of the amount of each amino acid, when others are present in optimal quantities, but also the effect of excesses of some amino acids. Maximal response is obtained only when a correction is made in the amount of the most limiting amino acid in such a way as to improve the balance or proportion among the other essential amino acids. This is a fundamental characteristic of the FAO or any other amino acid reference pattern.

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