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1963. Supplementation of Cereal Proteins with Amino Acids. V. Effect of Supplementing Lime-treated Corn with Different Levels of Lysine, Tryptophan and Isoleucine on the Nitrogen Retention of Young Children.

J. Nutrition, v. 80, no. 1, May.
Pages 80–84.

PRESS OF
THE WISTAR INSTITUTE
OF ANATOMY AND BIOLOGY
PHILADELPHIA, PA., U.S.A.

Reprinted from THE JOURNAL OF NUTRITION
Vol. 80, No. 1, May 1963

Supplementation of Cereal Proteins with Amino Acids

V. EFFECT OF SUPPLEMENTING LIME-TREATED CORN WITH DIFFERENT LEVELS OF LYSINE, TRYPTOPHAN AND ISOLEUCINE ON THE NITROGEN RETENTION OF YOUNG CHILDREN[1,2]

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ABSTRACT A lime-treated corn diet was supplemented with two levels each of lysine and of tryptophan, as well as two levels of isoleucine with a constant lysine and tryptophan supplement. Comparison of corn protein with the FAO reference protein indicates tryptophan to be the first limiting amino acid and lysine the second. In 18 3-day balance periods in two children lysine supplementation alone somewhat improved nitrogen balance, but tryptophan addition had no effect. Analysis of 12 3-day balance periods in 4 children indicated that the addition of lysine to give 180 mg/g N in the diet (0.30% L-lysine·HCl) and of tryptophan to a total of 75 mg/ g N (0.28% DL-tryptophan) approximately doubled the percentage of nitrogen retention compared with the results when the basal diet was fed. Increasing the lysine to 270 mg/g N (0.56% L-lysine·HCl) or the tryptophan to 90 mg/g N (0.35% DL-tryptophan) either singly or together, did not improve significantly the nitrogen balance compared with that obtained with these amino acids at the lower levels of supplementation. The addition of isoleucine to 270 mg/g N (0.45% DL-isoleucine) to the 270 mg lysine/g N plus 25 mg tryptophan/g N resulted in a slightly higher nitrogen balance. The addition of isoleucine to a level of 225 mg/g N gave an intermediate value for nitrogen retention. None of the amino acid combinations used to supplement corn-masa resulted in as high nitrogen retention as those obtained from feeding isonitrogenous levels of milk. The variable results with supplementation of different cereals with essential amino acids to the level in the FAO reference protein provide further evidence that reference amino acid patterns should include both upper and lower limits for the amount of each amino acid per gram of nitrogen and specify the range of protein intake over which they apply.

The effect of amino acid supplementation of a food cannot always be predicted from comparison with a reference amino acid pattern. In many instances supplementation with the first limiting amino acid to the reference level will accentuate the deficiency of the second limiting amino acid in the protein. This was clearly shown in the studies of wheat flour supplementation of Bressani et al. (1) and Rosenberg (2). The latter concluded that the amount of the most limiting amino acid to add to a deficient protein for maximal response in nutritive value should just balance the second most limiting amino acid. Since at low levels, at least, protein intake affects amino acid requirement for maximal response (3), the optimal amount of the most limiting amino acid in a given experimental situation may also depend on the protein level.

Previous studies in children (4, 5) indicated that the nutritive value of lime-treated corn could be best improved by the simultaneous addition of lysine, tryptophan and isoleucine. Similar results have been reported for adults (6) and experimental animals (7–10). In our previous studies the levels for lysine, tryptophan and isoleucine used for supplementation of lime-treated corn were those of the amino acid pattern of the FAO reference protein (11). It was the purpose of this study to find

Received for publication October 17, 1962.
[1] This investigation was supported by grant A-3811 from the National Institutes of Health.
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TABLE 1

Age and weight of subjects at start of experiment and protein and caloric intakes during the experimental periods

Case no.	Age	Weight	Protein intake	Calorie intake/ kg/day
		kg	*g/kg/day*	*Cal.*
77	2 years, 10 months	8.06	3.0	100
82	1 year, 5 months	8.70	2.0	80
102	5 years, 2 months	15.76	2.0	100
112	5 years, 1 month	13.52	2.0	100
113	3 years	10.91	2.0	100

out whether lower levels of the 3 amino acids in combination would result in nitrogen retention values as high or higher than those obtained from the use of the levels of the FAO reference amino acid pattern.

MATERIALS AND METHODS

Two children were fed lime-treated corn diets supplemented with 2 different levels of lysine alone and of tryptophan alone. One of these and three additional children were used to study the effect of feeding lime-treated corn diets supplemented with different combination of lysine and tryptophan and of lysine, tryptophan and isoleucine. The age and weight of the subjects at the start of each experiment and their protein and caloric intakes during the experimental periods are shown in table 1.

In all cases nitrogen balance studies using previously discussed techniques (1, 4–5) were carried out with a 2-day adaptation period followed by two 3-day balance periods for subjects PC-77 and PC-82 and by three 3-day balance periods for subjects PC-82, 102, 112 and 113. The basal diet in grams per 100 g consisted of lime-treated corn, 85; corn gluten, 5; L-glutamic acid, 2; glycine, 3; and cornstarch, 5. The various amino acids were substituted for an equal amount of cornstarch, the nitrogen from these replacing glycine nitrogen so that all the diets remained isocaloric and isonitrogenous.

The basal diet contained 14.4% protein and 405 Cal./100 g. Its essential amino acid composition has been described previously (4). The test diets supplied the specific level of protein and part of the calories which were adjusted to the desired level of caloric intake by the addition of sugar and vegetable fat. The children were fed 3 times a day and a vitamin and mineral capsule given daily.[4] Two levels of lysine, tryptophan and isoleucine were added to the basal diet. One level brought the amount to that in the FAO reference protein (11). The other gave a 1:4 ratio of tryptophan to lysine and a 1:3 ratio of tryptophan and isoleucine using a lower level of tryptophan as the reference base rather than the amount in the FAO reference protein (11). Corrections for the D-form of the amino acids[5] were made by doubling the amount added. The amount of lysine was corrected for the hydrochloride molecule present in the form used.

At the end of each 3-day balance period, the pooled urine and feces collections and aliquots of the diet were each analyzed for nitrogen by the Kjeldahl method. Only averages for each treatment are given since the variations among 3-day periods with the same diet combination were similar to those previously reported (1, 4–5).

RESULTS

Table 2 presents the nitrogen balance results of the individual addition of 2 levels of lysine and 2 of tryptophan to the basal diet. With subject PC-77 tryptophan was tested first and with subject PC-82 lysine was the first supplement. Lysine alone at either level improved nitrogen balance, but tryptophan addition alone did not. Table 3 presents nitrogen balance results of the children fed combinations of lysine and tryptophan or of lysine, tryptophan and isoleucine.

The addition of lysine to give 180 mg/g N in the diet (0.30% L-lysine·HCl) and

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

[5] Donated by DuPont de Nemours, Wilmington, Delaware.

TABLE 2

Nitrogen balance of subjects PC-77 and 82 fed lime-treated corn supplemented with two levels each of lysine and tryptophan

Diet	No. of balances	Weight	Nitrogen				
			Intake	Fecal	Urinary	Absorption	Retention
		kg	*mg/kg/day*	*% of intake*		*% of intake*	*% of intake*
			Subject PC-77				
Milk	1	8.06	486	93	320	80.9	15.0
Basal	1	8.00	474	185	349	61.0	−12.7
Basal + DL-tryptophan[1]	3	8.00	466	101	368	78.3	0.6
Basal + DL-tryptophan[2]	3	8.20	482	116	337	75.9	6.0
Basal	2	8.50	479	111	346	76.8	4.6
Basal + L-lysine·HCl[3]	2	8.50	485	131	343	73.0	2.3
Basal + L-lysine·HCl[4]	2	8.80	479	110	305	77.0	13.4
			Subject PC-82				
Milk	2	8.70	392	45	295	88.5	13.3
Basal	2	8.50	320	56	273	82.5	−2.8
Basal + L-lysine·HCl[3]	2	8.50	320	45	238	85.9	11.6
Basal + L-lysine·HCl[4]	2	8.93	350	63	276	82.0	3.1
Basal	1	8.84	346	63	287	81.8	−1.2
Basal + DL-tryptophan[1]	2	8.79	342	49	305	85.7	−3.5
Basal + DL-tryptophan[2]	2	8.80	333	56	311	83.2	−10.2

[1] To give 75 mg tryptophan/g N.
[2] To give 90 mg tryptophan/g N.
[3] To give 180 mg lysine/g N.
[4] To give 270 mg lysine/g N.

TABLE 3

Average nitrogen balances of subjects PC-77, 102, 112 and 113 fed lime-treated corn supplemented with several combinations of lysine, tryptophan and isoleucine

Diet	No. of balances[1]	Nitrogen				
		Intake	Fecal	Urinary	Absorption	Retention
		mg/kg/day	*% of intake*		*% of intake*	*% of intake*
Milk	9	338	55.4	255.5	83.6 ± 0.6[2]	24.4 ± 2.9
Basal	12	337	77.8	317.8	76.9 ± 1.1	5.7 ± 3.1
Basal + DL-tryptophan + L-lysine·HCl[3]	12	334	66.8	279.2	80.0 ± 1.0	16.4 ± 1.3
Basal + DL-tryptophan + L-lysine·HCl[4]	10	342	72.2	281.5	78.9 ± 1.4	17.7 ± 1.9
Basal + DL-tryptophan + L-lysine·HCl[5]	6	330	59.7	271.6	81.9 ± 0.4	17.7 ± 2.9
Basal + DL-tryptophan + L-lysine·HCl[6]	10	333	66.9	278.1	79.9 ± 0.9	16.5 ± 1.2
Basal	14	367	82.9	353.4	77.4 ± 2.0	3.7 ± 4.2
Basal + DL-tryptophan + L-lysine·HCl[7] + DL-isoleucine	14	363	68.6	291.5	81.1 ± 1.0	19.7 ± 2.5
Basal + DL-tryptophan + L-lysine·HCl[8] + DL-isoleucine	14	364	74.6	289.4	79.5 ± 1.0	20.5 ± 2.5
Basal	6	308	57.9	256.9	81.2 ± 1.3	16.6 ± 3.4
Milk	9	363	55.2	277.7	84.8 ± 0.9	23.5 ± 1.3

[1] Each balance was of a 3-day duration.
[2] Mean ± SE.
[3] Total tryptophan, 75 mg/g N and total lysine, 180 mg/g N in diet.
[4] Total tryptophan, 75 mg/g N and total lysine, 270 mg/g N in diet.
[5] Total tryptophan, 90 mg/g N and total lysine, 180 mg/g N in diet.
[6] Total tryptophan, 90 mg/g N and total lysine, 270 mg/g N in diet.
[7] Total tryptophan, 75 mg/g N, total lysine, 270 mg/g N and total isoleucine, 225 mg/g N in diet.
[8] Total tryptophan, 75 mg/g N, total lysine, 270 mg/g N and total isoleucine, 270 mg/g N in diet.

of tryptophan to 75 mg/g N (0.28% DL-tryptophan) approximately doubled the percentage of nitrogen retention over the results with the basal diet. Neither increasing lysine to 270 mg/g N of diet (0.56% L-lysine·HCl) nor tryptophan to 90 mg/g N (0.35% DL-tryptophan) singly or together significantly improved nitrogen balance over that obtained at the lower levels of supplementation with these amino acids. The addition of isoleucine to 270 mg/g N (0.45% DL-isoleucine) to the 270 mg lysine/g N plus 75 mg tryptophan/g N resulted in slightly higher nitrogen balance than lysine and tryptophan supplementation alone. The addition of isoleucine to the lower level of 225 mg isoleucine/g N in the diet gave an intermediate value for nitrogen retention which was not significantly different from either. None of the amino acid combinations used to supplement corn-masa resulted in as high nitrogen retention values as those obtained from feeding isonitrogenous levels of milk.

DISCUSSION

Previous work by Scrimshaw et al. (4) and by Bressani et al. (5) indicated that the single addition of either tryptophan or lysine to the basal lime-treated corn protein used in these studies did relatively little to restore or improve the nitrogen balance observed with the basal diet, and it was difficult to conclude from the data which of the two amino acids was first limiting. The present results indicate that lysine is definitely more limiting than tryptophan in lime-treated corn protein since in both children lysine at either level of supplementation gave better nitrogen retention values than tryptophan. This is contrary to the conclusion to be drawn from comparison of the amino acid pattern of lime-treated corn with that of the FAO reference protein and suggests once again that the amount of tryptophan in the FAO reference protein is too high relative to the other amino acids present.

When the protein intake was higher as in subject PC-77, the response to the addition of tryptophan resulted in positive nitrogen balance, whereas at the lower level of protein intake, as in subject PC-82, the single addition of tryptophan resulted in a negative balance which worsened with increased tryptophan. Similar results have been obtained in experiments with rats (12–14). These results are interpreted to mean that at the lower level of intake the relative deficiency of lysine in the diet became greater when tryptophan was added, resulting in greater amino acid imbalance. The fact that the extent to which an amino acid is limiting is affected by protein intake is now well recognized (3, 15, 16)[6] and is one of the important qualifications to the use of reference amino acid patterns for amino acid supplementation studies of foods. As previously pointed out (1), a reference amino acid pattern would be more useful if an upper as well as lower limit were specified for the amount of each amino acid per gram of nitrogen and if the range of protein intake to which the pattern applies were specified.

In the present study the addition of lysine and tryptophan together markedly improved the nutritive value of corn protein as reported previously by Scrimshaw et al. (4), Bressani et al. (5) in children and by Truswell et al. (6) in adults. The effect of isoleucine was small in all of these studies. The present data give no indication that levels of lysine, tryptophan and isoleucine as high as those in the FAO reference protein have any advantage over somewhat lower levels. However, the optimal levels for maximal nitrogen balance in children cannot yet be stated with confidence because the study of a sufficient number of children for statistical validity is so time-consuming and costly that it has not been made.

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