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Supplementation of Cereal Proteins with Amino Acids

VI. EFFECT OF AMINO ACID SUPPLEMENTATION OF ROLLED OATS AS MEASURED BY NITROGEN RETENTION IN YOUNG CHILDREN[1,2]

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ABSTRACT As a further test of amino acid supplementation of a protein, using the amino acid levels of the FAO reference protein as a guide, children were fed diets in which rolled oats, supplemented with potentially limiting amino acids, were the sole protein source. The series began and ended with isonitrogenous, isocaloric periods in which milk served as the protein source and basal periods of the unsupplemented oat diet were interspersed between each test period. Each diet was fed for 3 successive 3-day periods. At intakes of 2 g of protein/kg, no significant effect on nitrogen retention was observed with additions to 308 mg/g N of lysine, to 296 of methionine or both together. At a level of 222 mg/g N of threonine alone or in combination, the increase was significant and the nitrogen retention only slightly below that with milk. At 1.5 g of protein/kg, some increase in retention was observed with either lysine alone or methionine alone to these levels. Threonine alone was not tested. The combination of either lysine and methionine or all 3 amino acids gave a further increase in retention. A tendency for a higher retention in a basal period following an experimental one in which methionine had been one of the supplementary amino acids was noted. This was interpreted as due to an adverse effect of methionine supplementation at the level of this amino acid in the FAO reference protein, as previously observed with the methionine supplementation of corn diets.

Since the nutritive value of a protein depends primarily upon its amino acid composition, comparison with a good amino acid pattern is a helpful way of obtaining an estimate of the deficient amino acids of most proteins. The value of such comparisons is often limited, however, because the biological availability of the amino acids must be taken into account. It is necessary, therefore, to confirm by biological trial the amino acid deficiencies of proteins for animal and human feeding. Such determinations also help to evaluate and improve amino acid reference patterns.

Using the nitrogen balance technique with young children, the effects of amino acid supplementation of lime-treated corn (1–3), and of wheat flour have been studied (4, 5). The results indicated discrepancies between the order of deficient amino acids as determined experimentally and that predicted from comparison of the amino acid pattern of the cereal protein with that of the FAO reference protein (6). For example, by comparison with the FAO reference protein, both corn and wheat are deficient in methionine. Nevertheless, children fed diets based on wheat protein showed no change in nitrogen retention when the diet was supplemented with methionine and those receiving corn consistently showed a decrease.

Because of the less marked amino acid deficiencies of rolled oats, amino acid supplementation of this protein source was chosen as a further and more sensitive test of the efficiency of the FAO amino acid reference pattern (6). Studies carried out in rats by Tang et al. (7) indicated that rolled oats are deficient in lysine, methionine and threonine, in this order, but the results of other investigators suggest that after lysine, threonine, tryp-

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tophan and methionine are next limiting to an equal extent.[4] Tang et al. (7) reported that threonine addition to rolled oats supplemented with lysine and methionine resulted in an improvement in weight gain and protein efficiency ratio (PER) because this amino acid is not completely available to the rat.

Using 6 women as subjects, Leverton and Steel (8) recently obtained nitrogen balance results using adequate protein intakes and semipurified diets whose essential amino acid patterns simulated those of the FAO reference protein and rolled oats. Their results are not comparable, however, because they used ratios of essential amino acid nitrogen to total nitrogen varying from 1:9 to 1:19 instead of the 1:4.2 specified in the FAO reference protein.

MATERIAL AND METHODS

Techniques described previously (1–5) were used to measure the effect of amino acid addition to rolled oats on nitrogen retention in children. The first of 2 series of studies involved 4 children fed 2.0 g protein/kg/day throughout ten 9-day experiments. The 3 children in the second series were fed 1.5 g protein/kg/day. The initial age, weight, protein and calorie intake of each are shown in table 1. They were fed a basal diet containing 92% of rolled oats,[5,6] 3% of glycine and 5% of cornstarch. The basal diet contained: (in per cent) 17.0 protein, 5.5 fat; 65.7 carbohydrate and 430 kcal/100 g. The added amino acids were substituted for the starch, and the nitrogen from these replaced glycine nitrogen so that all diets remained isocaloric and isonitrogenous. All of the protein was furnished by rolled oats, whereas the additional calories were provided by dextrose and margarine added to the experimental diets before cooking.

[4] Bressani, R., unpublished data.
[5] Supplied by the Quaker Oats Company, Chicago.
[6] Chemical composition of the rolled oats: (in per cent) moisture, 10.6; ether extract, 6.0; crude fiber, 2.0; nitrogen, 2.590; ash, 1.8.

TABLE 1
Age, weight, protein and caloric intake of experimental subjects

Case	Age	Weight	Protein intake	Calorie intake
		kg	*g/kg/day*	*cal/kg/day*
PC-105	4 yr 6 mo	15.14	2.0	89
PC-108	1 yr 11 mo	9.75	2.0	90
PC-110	3 yr 7 mo	12.47	2.0	89
PC-110	3 yr 3 mo	15.06	2.0	90
AT-7	1 yr 7 mo	8.05	2.0	100
AT-7	2 yr 1 mo	9.25	2.0	100
AT-7	2 yr 4 mo	10.91	2.0	100
PC-116	2 yr 1 mo	11.54	1.5	90
PC-117	2 yr 5 mo	11.17	1.5	89
PC-118	2 yr 6 mo	13.12	1.5	90

TABLE 2
Essential amino acid content of rolled oats, FAO reference pattern and rolled oats basal diet

Amino acid	Rolled oats	FAO pattern	Difference	Basal rolled oats diet
	g/g N	*g/g N*	*g/g N*	*g/g N*
Isoleucine	0.343	0.270	—	0.315
Leucine	0.515	0.306	—	0.474
Lysine	0.233	0.270	0.037	0.214
Cystine	0.135	0.270	0.049	0.203
Methionine	0.086			
Phenylalanine	0.301	0.360	—	0.410
Tyrosine	0.145			
Tryptophan	0.068	0.090	0.022	0.062
Threonine	0.203	0.180	—	0.187
Valine	0.294	0.270	—	0.270

A multivitamin and mineral capsule [7] was also given daily. The handling of the food and the collection of feces, urine and food for analysis have been described (2, 5).

The essential amino acid content in milligrams of amino acid per gram of nitrogen of rolled oats as determined microbiologically (10) and of the FAO reference protein (6) are shown in table 2. Oats contain only 82% of the methionine plus cystine, 86% of the lysine and 75% of the tryptophan contained in the FAO reference protein. Because of reports that threonine added to oat protein increased the growth of rats (7),[8] children were also fed rolled oats supplemented with threonine alone or in combination with lysine and methionine. The quantities of L-lysine·HCl, DL-methionine and DL-threonine added alone or in combination were 0.34, 0.27 and 0.20%, respectively, values which are slightly higher than needed to bring the pattern of oats to that of the FAO reference protein. In the case of threonine, 0.20% was added to meet the presumed deficiency of this amino acid due to its reduced availability (7). Amino acid proportions were the same with the 2 levels of protein intake. Tryptophan was not studied because previous results with lime-treated corn (1–3) and wheat flour (4, 5) indicated that a lower level of this amino acid than observed in oats was sufficient for maximal response.

RESULTS

Table 3 shows the effect of different amino acid additions to rolled oats as measured by the nitrogen balance method at a protein intake of approximately 2.0 g/kg of body weight/day. All nitrogen values pertaining to the same dietary treatment from all the children studied were pooled. Of the additions to the rolled oat basal diet only threonine improved nitrogen balance significantly ($P < 0.05$). When the 3 possible combinations of 2 amino acids were added to the basal diet, only those containing threonine gave higher nitrogen retention values than the basal diet. The response is not greater, however, than

[7] Gevral, donated by Lederle Laboratories, American Cyanamid Co., Pearl River, New York.
[8] See footnote 4.

TABLE 3

Effect of supplementing rolled oats with lysine, methionine and threonine alone and in combination on nitrogen balance in children fed 2.0 g of protein/kg/day

Diet	No. children tested	No. balance periods[1]	Nitrogen Intake	Nitrogen Fecal	Nitrogen Urine	N retained[2]	Significance of comparison to basal diet
			mg/kg/day	*mg/kg/day*	*mg/kg/day*	*% of intake*	
Milk	4	25	327±4	51±3	203±7	22.3±1.9	$P < 0.01$
Basal	4	82	330±1	68±1	208±3.2	16.4±0.9	—
Basal+lysine	4	12	344±3	72±4	223±8	14.1±1.6	NS[3]
Basal+methionine	4	15	331±3	70±4	208±4	15.9±1.7	NS
Basal+threonine	3	10	328±7	65±4	193±4	20.9±2.0	$P < 0.05$
Basal+lysine +methionine	4	12	326±4	65±4	210±9	15.7±2.5	NS
Basal+methionine +threonine	2	6	324±4	65±4	199±5	18.6±2.4	NS
Basal+lysine +threonine	2	7	315±7	61±4	190±12	20.7±3.9	NS
Basal+lysine +methionine +threonine	2	6	328±3	66±2	197±6	20.0±2.0	NS
Milk	2	5	315	35	211	21.9	$P < 0.01$

[1] Each of 3-day duration.
[2] Average nitrogen absorbed as percentage of nitrogen intake: milk, 86.6; all basal rolled oats diet, 79.5; all basal rolled oats diet plus amino acid supplements, 79.5.
[3] NS = not significant.

TABLE 4

Effect of supplementing rolled oats with lysine, methionine and threonine on the nitrogen balance of children fed 1.5 g protein/kg/day[1]

Diet	Nitrogen			N retained[2]	Significance of comparison to basal diet
	Intake	Fecal	Urine		
	mg/kg/day	*mg/kg/day*	*mg/kg/day*	*% of intake*	
Milk	253 ± 5	27 ± 2	158 ± 5	26.8 ± 2.2	$P < 0.01$
Basal[3]	251 ± 1	49 ± 2	164 ± 3	15.1 ± 1.7	—
Basal + lysine	251 ± 2	49 ± 2	157 ± 4	17.9 ± 2.3	NS[4]
Basal + methionine	250 ± 3	53 ± 2	153 ± 4	18.8 ± 1.9	NS
Basal + lysine + methionine	244 ± 2	51 ± 4	129 ± 5	26.2 ± 2.9	$P < 0.01$
Basal + lysine + methionine + threonine	249 ± 4	53 ± 4	134 ± 5	24.9 ± 3.0	$P < 0.01$
Milk	242 ± 2	31 ± 3	125 ± 8	35.5 ± 3.6	$P < 0.01$

[1] Three children, 3 balance periods of 3-days each.
[2] Average nitrogen absorption expressed as percentage of nitrogen intake: milk, 88.1; rolled oat basal diet, 80.6; rolled oat basal diet plus amino acid supplements, 79.9.
[3] Nine balance periods of 3-days duration each.
[4] NS = not significant.

that observed from the addition of threonine alone.

No further improvement in nitrogen balance over the values observed from the threonine addition alone or from the combination of threonine with either lysine or methionine was obtained by supplementing with all 3 of these amino acids. The nitrogen balance values from threonine addition alone or in combinations were only slightly lower than those obtained from the isocaloric, isonitrogenous feeding of milk. The difference is entirely accounted for by the slightly greater nitrogen absorption with milk.

Table 4 presents the results of supplementing the rolled oat basal diet fed at a level of approximately 1.5 g/kg of body weight, a level which is considered low for the children studied. At this level there is an apparent tendency for both lysine and methionine to increase nitrogen retention when added to the basal diets although this effect fell short of statistical significance. With the combination of lysine and methionine the increase was highly significant and did not differ from that when threonine was added to the combination. The level again fell short of the average retention with milk by about the difference in nitrogen absorption between the 2 types of diets.

DISCUSSION

That the amino acid pattern of oats is better, even without supplementation, than that of corn is indicated by the higher nitrogen balances relative to milk when the oat protein basal diet was fed. The results of the present study in children indicate that instead of lysine, methionine and tryptophan as predicted from comparison with the FAO reference protein, only threonine is deficient in oats fed at a protein intake of 2.0 g/kg/day. At a level of 1.5 g protein/kg/day, both lysine and methionine also appear to be slightly deficient although only with the two added together is the difference significant.

Tang et al. (7) suggested that threonine is limiting in oats because its biological availability to the rat is only 70%. It is probable that even though acid hydrolyzates of oats appear to contain adequate amounts when judged by the 180 mg threonine/g of nitrogen in the FAO pattern, its availability to the human is sufficiently reduced that this amino acid becomes limiting in this cereal. Apparently at lower levels of nitrogen intake both methionine and lysine become limiting to some degree.

A 9-day basal period was introduced between each experimental period. This gave an opportunity to note the effect of amino acid supplementation on the nitro-

TABLE 5

Change in nitrogen retention from an experimental to a subsequent basal diet period

Basal period following:	Protein intake, g/kg/day	
	2.0	1.5
	% N intake retained	
Milk	−10.3	−16.1
Basal+lysine	− 3.5	− 1.4
Basal+methionine	+ 3.1	+ 2.0
Basal+threonine	− 3.4	—
Basal+lysine+methionine	− 0.9	—
Basal+threonine+lysine	− 5.0	—
Basal+threonine+methionine	+ 7.7	—
Basal+threonine+methionine+lysine	− 5.8	—

gen retention during the following basal period. In previous INCAP studies, the basal period tended to be lower in nitrogen retention at the same level of intake when the preceding experimental period, although isonitrogenous, furnished protein of high quality and resulted in relatively high retention. Depletion due to infection, or trauma, as well as low protein intakes, also resulted in high retentions in immediately subsequent periods even with diets of relatively poor protein quality. The poorer nitrogen retention observed by Leverton and Steel (8) in young women fed an oat diet can be explained by the overly diluted essential amino acid nitrogen to total nitrogen in the diets used by them.

In the present studies the decrease in nitrogen retention with the basal diet was greatest following either milk or the basal supplemented with threonine in any combination and least when the basal period followed methionine addition (table 5). This suggests that the added methionine may have been producing a latent imbalance which, although not sufficient to lower nitrogen retention during the experimental period of supplementation with methionine, nonetheless had an effect. This was observed in all basal periods which followed methionine addition regardless of simultaneous addition of threonine and lysine and is similar to the effect previously encountered during periods of methionine supplementation of corn. In the future attention should be given to the possible significance and value of the differences in retention of nitrogen observed on the same basal diet depending upon the quality of protein in preceding isonitrogenous periods.

Since an increased nitrogen retention during supplementation with threonine alone was evident only at low levels of protein intake, it would be pertinent to learn whether this effect is related to caloric consumption. Methionine addition to low nitrogen diets of low caloric density has been reported to decrease urinary nitrogen excretion in rats (10, 11) and dogs (12), but not in adult man (13).

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