Amino Acid Supplementation of Cereal Grain Flours Tested in Children

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Introduction

Amino acid supplementation of foods is based on the results of the classical studies of Osborne and Mendel carried out over 50 years ago, on the nutritional quality of proteins and their constituent amino acids. They and many other investigators have given stimulus to numerous experiments with animals. These concern the mutual complementation of protein foods and the use of crystalline amino acids to improve the nutritive value of dietary protein as measured by protein utilization, as well as the improvement of growth and production and overall performance.

From these investigations, Rose[1,2] established, over 30 years ago, the concept that some of the dietary amino acids were essential and others dispensable. The essential amino acids are those which the animal body needs for its maximum growth or performance but cannot synthesize at the needed rate and, therefore, must be supplied by food. Dispensable or nonessential amino acids, on the other hand, are synthesized at a sufficient rate and thus do not limit the metabolic processes. These are important nutritionally as sources of nitrogen for the synthesis of tissue protein, and most protein foods provide sufficient amounts. The studies of Mitchell and Block[3] later led to the limiting amino acid concept. This is defined as the amino acid present in the smallest amount in comparison with a reference amino acid ratio or requirements. Harper[4,5] showed an adverse effect of an imbalance in amino acid pattern even when the absolute amounts of the individual amino acids are adequate.

This led to the general principle of amino acid supplementation[6,7] as a means for improving the quality of protein or the efficiency of its utilization. This principle states that the limiting essential amino acid should be added in such an amount that the total of this amino acid in the protein of the diet balances with the amount of the second limiting amino acid and the rest of the protein according to the needs of the organism.

It is well recognized that protein can be fully utilized only if there is sufficient energy in the diet from nonprotein sources to satisfy the requirements of the organism for calories. This can be of critical importance for the successful amino acid supplementation of foods, as it is the amount of protein in the diet that controls the amount of the first limiting amino acid that should be added.

Other important factors in the successful amino acid supplementation of protein in foods are the age and sex of the species and the biological availability of amino acids from the protein.

The majority of the principles or concepts mentioned have developed from numerous and extensive experimental studies in animals. This information has been considered as good evidence that amino acid supplementation is an effective way to improve the nutritive value of protein for man. However, the biological effectiveness of amino acid supplementation of foods for human feeding requires confirmation by appropriate studies in man. This has been hindered by the lack of certainty as to the optimum pattern of amino acids for human nutrition.

The approach of the 1955 FAO/WHO Expert Group on Protein Requirements was the establishment of a theoretical reference protein whose amino acid content would serve as a reference protein.[8] A subsequent FAO/WHO Committee recommended the use of the essential amino acid proportions of a protein known to be 100 percent absorbed and retained — that of egg.

The results presented in this paper summarize the work carried out to evaluate such a reference pattern by its use as a base for the supplementation of cereal grain proteins fed to children.[6,7,9–12] (See also, unpublished INCAP data.)

Materials

Children The studies to be described were carried out in hospitalized children who had recovered from protein-calorie malnutrition. They were studied in a metabolic ward with facilities for the quantitative collection of urine and feces. The children ranged from 8 kg to 16 kg in weight and from 1.5 to 5 years in age. Their total serum proteins ranged from 6.2 to 7.2 percent and their serum albumin from 3.0 to 4.5 percent.

Cereal Grain Flours The protein tested consisted of flours from lime-treated corn, wheat of 70 percent extraction, rolled oats and white polished rice. The samples were analyzed for their essential amino acid content, values for which are shown in Table 1, along with the nitrogen concentration. The lime-treated corn, white rice, and wheat flour were

Table 1 Essential amino acid composition of cereal grain flours*

Amino acid	Corn	Wheat flour	Oats (mg/g N)	Rice	FAO 1957†
Arginine	198	229	384	509*	–
Histidine	177	155	107	196*	–
Isoleucine	292	239	343	324	270
Leucine	607	375	515	475	306
Lysine	200	159	233	225	270
Methionine	150	101	86	193	270
Cystine	83	112*	135	81	–
Phenylalanine	271	324	301	298	180
Tyrosine	195	191*	145	132	180
Threonine	253	183	203	221	180
Tryptophan	16	52*	68	82	90
Valine	282	245	294	360	270

* Orr, M. L., and B. K. Watt. Amino acid content of foods. Washington, D.C.: U.S. Department of Agriculture, 1957. Home Economics Research Report No. 4.
† FAO 1957, Reference 8.

Table 2 Basal cereal grain diets (in percent)

Ingredients	Corn	Wheat	Oats	Rice
Cereal flour	85	85	92	96
Gluten	5	7	–	–
Glutamic acid	2	–	–	–
Glycine	3	3	3	3
Corn starch	5	5	5	1
Protein	14.4	18.7	17.0	10.4
Calories/100 g	375	385	430	337

all products from Guatemala, while the rolled oats were obtained from the United States. The table also shows the 1957 FAO reference pattern[8] which was used to predict the order of deficient amino acids as well as for the calculation of the amounts of amino acids to be added.

Method The addition of amino acids was evaluated by the nitrogen balance technique. In most studies the children were given each diet for a total of 9 days with a 4-day interval between treatments as an adjustment period to the new diet. Feces and urine were collected every 3 days, obtaining 3 balances per dietary treatment.

A basal diet from each cereal grain is described in Table 2. The weight of the amino acids added replaced an equal weight of cornstarch, and the nitrogen replaced an equivalent amount of nitrogen from glutamic acid or glycine or both.

In some studies, the amounts of amino acids added were calculated on the basis of the total nitrogen of the diet, while for rice, the calculations were based on the nitrogen for rice only. The level of protein intake varied between experiments but was held constant within each experiment. The calorie intake for all studies varied between 90 to 100 per kg body weight per day. The basal or amino acid supplemented diets were then included in the working diet described in Table 3 which provided 20 percent of the calories as derived from fat. The children received a multiple vitamin and mineral capsule daily.

Corn Supplementation

It is well accepted that corn proteins are deficient in both lysine and tryptophan as documented from the results of studies in experimental animals.[13,14] However, in tests with children to be described, comparisons were made of the essential amino acid content of lime-treated corn and the amino acid content of the 1957 FAO reference protein.[8] This comparison suggests the following order of deficiency: tryptophan, lysine, methionine, valine, isoleucine, and threonine, and the amounts of amino acids needed to reach the reference level. Therefore, the initial studies aimed to test the order of limitation, but results did not fully corroborate the FAO prediction.

Representative results from two children fed 3.0 g of protein per kilo body weight per day are shown in Table 4. There is an apparent response to the addition of 148 mg DL-tryptophan/g N which was very much improved by the simultaneous addition of tryptophan and lysine, the latter in the amount of 243 mg/g N. Addition of methionine

Table 3 Composition of diet fed to children*

Ingredients	g
Cereal diet†	213
Sugar	42
Margarine	29
Salt	1
Water	915
Total	1200

* Example: For 11.0 kg child fed 2 g protein/kg/day.
† With or without amino acids added.

Table 4 Addition of lysine, tryptophan and methionine to lime-treated corn*

Diet	Nitrogen balance				
	NI[1]	FN[2]	UN[3] (mg/kg/day)	NA[4]	NR[5]
Basal (B)	461	117	334	344	+10
B + tryptophan	457	115	289	342	+53
B + tryptophan + lysine	464	135	243	329	+86
B + tryptophan + lysine + methionine	459	135	272	324	+52

* Children No. 56–57. Amino acid used: DL-tryptophan: 0.34%; L-lysine HCl: 0.56%; DL-methionine: 0.34%.
[1] NI = nitrogen intake
[2] FN = fecal nitrogen
[3] UN = urinary nitrogen
[4] NA = nitrogen absorbed
[5] NR = nitrogen retained

Table 5 Response to lysine and tryptophan added alone*

Treatment	Nitrogen balance				
	NI	FN	UN (mg/kg/day)	NA	NR
Milk	586	93	320	393	73
Basal (B)	474	185	349	289	–60
B + tryptophan	474	108	352	366	14
B	479	111	346	368	22
B + lysine	482	120	324	362	38
Milk	392	45	295	347	52
Basal (B)	320	56	273	264	–9
B + lysine	335	54	257	285	24
B	346	63	287	283	–4
B + tryptophan	337	52	308	285	–23

* Levels added: to give 75–90 mg/tryptophan/g N and 180–270 mg/L-lysine HCl/g N.

decreased nitrogen retention. In other studies, nitrogen balances were carried out to learn about the response previously obtained by tryptophan addition alone. The results on two subjects are presented in Table 5 and clearly show that tryptophan had no effect on improving protein quality. The addition of lysine, on the contrary, appeared to give a small response, suggesting lysine to be more limiting than tryptophan.

Table 6 Addition of lysine, tryptophan, isoleucine and methionine to lime-treated corn*

Diet	Nitrogen balance NI	FN	UN (mg/kg/day)	NA	NR
Basal (B)	320	68	270	252	−18
B + tryptophan	320	91	241	229	−12
B + tryptophan + lysine	321	105	201	216	+15
B + tryptophan + lysine + isoleucine	321	90	207	231	+24
B + tryptophan + lysine + isoleucine + methionine	314	84	217	230	+13
B	319	98	242	221	−21

* Children No. 66–67: Amino acid levels added: DL-isoleucine 0.45 percent; other amino acids added in amounts shown in Table 4.

Similar studies were carried out by feeding children 2.0 g of protein per kilo per day. The results with respect to two children are summarized in Table 6. Tryptophan addition did not induce a positive nitrogen retention, but the addition of tryptophan and lysine with and without isoleucine improved nitrogen balance. Methionine addition decreased retention of nitrogen as previously demonstrated.

Nitrogen balance tests were performed at 1.5 g of protein intake/kg/day. The results on one child are shown in Table 7. Although lysine addition did not induce a positive balance, it did tend to cause a decrease in nitrogen losses. The improvement from lysine and tryptophan, with and without isoleucine, is also evident. The addition of methionine, even at this level of protein intake, decreased the nitrogen balance as previously indicated for higher intakes of protein.

Because of the consistency of the results, the data obtained per protein levels under the various dietary treatments were pooled; the results are shown in Table 8. There is a response to tryptophan addition only at the higher level of dietary protein, but the response to lysine is consistent at all levels of intake, suggesting that this amino acid is more deficient than tryptophan. The response from its single addition, however, is small and without nutritional significance.

A nitrogen level of 239 mg/kg/day intake is equivalent to 20 g of corn/kg/day, or about the 200 g of corn normally ingested by children. Supplementation with lysine alone would have little effect. When tryptophan is also added, however, the increase in nitrogen retention is significantly higher and even surpasses that of milk at the higher level of

Table 7 Amino acid supplementation of corn at 1.5 g protein/kg/day, level of intake

Treatment	Nitrogen balance				
	NI	FN	UN (mg/kg/day)	NA	NR
Basal (B)	241	71	187	170	−17
B + lysine* (0.56%)	239	59	184	180	−4
B + lysine† (0.30%)	239	48	193	191	−2
B + lysine + tryptophan	239	47	162	192	30
B + lysine + tryptophan + isoleucine	240	44	150	196	46
N + lysine + tryptophan + isoleucine + methionine	240	55	162	185	23
Basal	235	45	193	190	−3

* 0.56 percent L-lysine HCl. Other amino acids added in the amounts used before.
† 0.30% L-lysine HCl.

Table 8 Addition of amino acids to lime-treated corn at various levels of nitrogen intake*

Diet	Nitrogen balance					
	NI	NR	NI	NR (mg/kg/day)	NI	NR
Basal (B)	469	14	326	−5	238	−10
B + tryptophan	465	33	327	−17	–	–
B + lysine	482	38	335	24	239	−4
B + tryptophan + lysine	461	83	328	36	239	30
B + tryptophan + lysine + isoleucine	475	108	335	40	240	46
B + tryptophan + lysine + isoleucine + methionine	469	90	314	13	240	23
Milk	458	70	364	73	–	–

* Levels used: DL-tryptophan: 0.34 percent; L-lysine HCl: 0.56 percent; DL-isoleucine 0.45 percent; DL-methionine 0.34 percent.

dietary protein. The overall conclusion which can be reached from the results obtained by amino acid supplementation of corn is that both lysine and tryptophan must be added to obtain a significant response in protein quality as measured by nitrogen retention. It also appears that both amino acids are equally limiting in spite of the fact that the addition of lysine alone tended to improve protein quality slightly, while the results from the addition of tryptophan are inconsistent.

The effect of methionine is of interest and deserves further comment. It was interpreted as due to an amino acid imbalance, because corn already contains enough of this amino acid to meet requirements.

The results shown in Table 9 indicate that valine also decreased nitrogen retention and that its effect can be reversed by the addition of isoleucine and threonine. A more detailed study in dogs permits the conclusion that there is also a close interrelationship among all four of these amino acids.[15]

It is a point of major interest and importance that children are sensitive to such small changes in amino acid proportions that this is readily detectable in a short period by nitrogen balance. The data that have been presented emphasize the importance of establishing a proper

Table 9 Effect of multiple amino acid supplementation of corn

Diet	Nitrogen balance NI	FN	UN (mg/kg/day)	NA	NR
Basal (B)	471	117	315	354	39
B + lysine + tryptophan + methionine	451	223	244	228	−16
B + lysine + tryptophan + methionine* + valine	454	241	242	213	−29
B + lysine + tryptophan + methionine* + valine + isoleucine	460	128	265	332	67
B + lysine + tryptophan + methionine + valine + isoleucine + threonine	447	190	218	257	39
B + lysine + tryptophan + methionine† + valine + isoleucine + threonine	450	129	238	321	83
Basal	478	138	294	340	46

* 0.34 percent DL-methionine.

† 0.14 percent DL-methionine; other amino aicds added in amounts shown before. DL-valine: 0.90 percent; DL-threonine: 0.22 percent.

balance among the essential amino acids if a maximum retention of nitrogen is to be obtained. This is the principle of amino acid supplementation.

Wheat Flour Fortification

The approach to the studies on the amino acid supplementation of wheat flour was the same as that used in those with corn. Representative results for wheat are shown in Table 10 for one child.[10] Nitrogen balance improved upon addition of 0.67 percent L-lysine HCl, giving values similar to those obtained from milk. The addition of tryptophan or methionine in combination with lysine did not give nitrogen retention values above those previously obtained with lysine. Other studies have indicated that other amino acids in combination with lysine could further improve the quality of wheat flour protein above that observed with lysine. Representative results are shown in Table 11. In this example, improvement of nitrogen retention with lysine is observed as well as that resulting from the addition of this amino acid with tryptophan, methionine, threonine, isoleucine, and valine.

On account of the similarity of results among children, all data were pooled; the results are shown in Table 12. Results again show that the major effect of improving protein quality is due to the lysine addition, although the addition of tryptophan and methionine improves nitrogen retention further. The data are not enough to establish which amino acid or mixture of amino acids in addition to lysine will further increase protein quality and whether the effect is due to a correction of a deficiency of these amino acids in wheat protein or to other reasons. The

Table 10 Nitrogen balance of child fed wheat flour supplemented with amino acids*

	Nitrogen balance				
Diet	NI	FN	UN (mg/kg/day)	NA	NR
Milk	290	63	161	227	66
Basal (B)	335	43	268	291	23
B + 0.67% lysine	332	45	214	287	73
B + lysine + 20% tryptophan	332	52	224	280	56
B + lysine + tryptophan + 0.35% methionine	305	50	204	255	51

* Case PC-91: Average 33-day balance periods.

Table 11 Comparison of nitrogen balance results of milk and wheat flour supplemented with amino acids*

Diet	Nitrogen balance NI	FN	UN (mg/kg/day)	NA	NR
Milk	329	51	209	278	69
Basal (B)	338	49	260	289	29
B + lysine	328	42	245	286	41
B + amino acid mix	317	48	183	269	86
B + lysine + tryptophan	328	37	224	291	67
B	320	43	236	277	41
B + lysine + valine	304	41	210	263	53
Milk	321	71	165	250	85

* Child PC-98: Levels added to give: 270 mg lysine: 90 mg tryptophan; 270 mg isoleucine; 270 mg valine and 180 mg threonine/g N in diet.

Table 12 Amino acid supplementation of wheat flour; summary results*

Diet	Nitrogen balance NI	FN	UN (mg/kg/day)	NA	NR
Milk	307	61	172	246	74
Basal (B)	326	47	252	279	27
B + lysine	335	47	228	288	60
B + lysine + tryptophan	330	41	222	289	67
B + lysine + tryptophan + methionine	337	38	225	299	74
B + lysine + tryptophan + methionine + threonine	338	37	223	301	78
B + lysine + tryptophan + methionine + threonine + isoleucine + valine	336	49	204	281	83

* Amino acids added to give 270 mg lysine; 90 mg tryptophan; 270 mg methionine; 270 mg isoleucine; 270 mg valine and 180 mg threonine/g N in diet.

consistency of the results, however, suggests that valine might be important. It is very likely that some of the effects observed with the mixtures of amino acids were due either to their low biological availability from wheat or to an imbalance among them, as was the case for the multiple supplementation of corn proteins shown previously. It should be indicated that amino acid supplementation is often less effective because the amount of amino acids added is larger than that actually needed, hence causing an imbalance. A significant improvement is attained only by adding the amount needed to bring the level of the first limiting amino acid to that of the second. Since lysine addition was responsible for most of the effect, additional studies were carried out to determine the best amounts to be added to wheat flour. This was tested at two levels of dietary proteins and results are shown in Table 13.

The results at a protein intake of 2.0 g/kg/day suggest that 61 mg of lysine/g N will suffice to increase nitrogen retention. At a protein intake level of 3.0 g/kg/day, however, it appears that 107 mg of lysine/g N are needed. This may seem contradictory since it would be expected that at the higher level of protein intake less lysine is needed, as protein would be supplying more of the essential amino acid needs of the growing child. However, the response is dependent upon nitrogen intake and its relation to the intake of calories. In the particular case shown, calories remained constant, but nitrogen intake varied. This is responsible for the higher level of lysine required. However, the ratio of lysine need to nitrogen intake was similar, 0.19 for the 328 level of intake and 0.21 for the 505 level. This is in agreement with findings in experimental animals.[16]

Table 13 Effect of lysine addition to wheat flour*

	Nitrogen balance			
Lysine level (mg/g N)	NI	NR (mg/kg/day)	NI	NR
101	327	14	483	3
162	328	44	487	49
208	316	43	505	60
260	310	50	506	38
282	324	43	502	74

* Average three children/protein level.

Rolled Oat Supplementation

The techniques used in previous studies with corn and wheat flour were also used to measure the effect of amino acid addition to rolled oats on nitrogen retention in children. Table 14 shows the effect of adding lysine, methionine, and threonine alone and in combination at a protein intake of approximately 2.0 g/kg/day. Of the single additions, only threonine improved nitrogen balance significantly. When two 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 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 three 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. It has been suggested[17] that threonine is

Table 14 Supplementation of rolled oats with various amino acids*

	Protein intake			
	2.0 (g/kg/day)		1.5 (g/kg/day)	
Diet	NI (mg/kg/day)	NR (mg/kg/day)	NI (mg/kg/day)	NR (mg/kg/day)
Milk	327	73	253	68
Basal (B)	330	54	251	38
B + lysine	344	49	251	45
B + methionine	331	53	250	44
B + threonine	328	70	–	–
B + lysine + methionine	326	51	244	64
B + methionine + threonine	324	60	–	–
B + lysine + threonine	315	64	–	–
B + lysine + methionine + threonine	328	65	249	62
Milk	315	69	242	86

* Amino acids added: L-lysine HCl, 0.34 percent; DL-methionine, 0.27 percent; DL-threonine, 0.20 percent.

limiting in oat because its biological availability to the rat is only 70 percent. The question is yet to be answered as to whether its availability to the human is sufficiently reduced that this amino acid becomes limiting in oats.

Table 14 presents the results of supplementing the rolled oat basal diet fed at a level of approximately 1.5 g/protein/kg of body weight, a level which is considered low for the children studied. At this level there is a tendency for both lysine and methionine to increase nitrogen retention when added to the basal diets, although the difference is not statistically significant. 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 two types of diet. The data suggest that in oat protein, both lysine and threonine appeared equally limiting, with methionine taking the next position.

Rice Supplementation

The study of the effect of amino acid supplementation to rice was carried out at two levels of protein intake. Results from six experiments in ten children are summarized in Table 15. Of the amino acids added

Table 15 Amino acid supplementation of white rice*

Diet	Nitrogen balance				
	NI	FN	UN (mg/kg/day)	NA	NR
Milk	317	44	180	273	93
Basal (B)	320	67	193	253	60
B + methionine	336	71	217	265	48
B + threonine	319	76	196	243	47
B + lysine	320	65	176	255	79
B + methionine + tryptophan	312	63	189	249	60
B + lysine + threonine	327	70	194	257	63
B + lysine + methionine	344	78	192	266	74
B + lysine + methionine + threonine	349	74	194	275	81
B + lysine + threonine + tryptophan	343	71	226	272	46
B + threonine + tryptophan + methionine	305	62	210	243	33
B + lysine + methionine + tryptophan + threonine	309	67	160	242	82

* Amino acids added: 0.10 percent DL-methionine; 0.03 percent DL-tryptophan; 0.05 percent L-lysine HCl; 0.08 percent DL-threonine.

Table 16 Amino acid supplementation of rice protein* (Experiment 6)

Diet	NI	FN	UN (mg/kg/day)	NA	NR
Milk	265	32	162	233	71
Basal (B)	235	38	157	197	40
B + lysine	249	50	157	199	42
B + lysine + methionine	260	46	146	214	68
B + lysine + methionine + tryptophan	245	50	159	195	36
B + lysine + methionine + tryptophan + threonine	254	40	159	214	55
Milk	267	31	133	236	103

Nitrogen balance: NI, FN, UN, NA, NR

* Levels of amino acids added: see Table 15.

alone, only lysine induced and increased nitrogen retention over the level found for the basal diet. Threonine and methionine, on the other hand, decreased nitrogen balance. Of the combinations tested with two amino acids, none was capable of increasing nitrogen balance over the effect observed from lysine addition alone. Except for the addition of lysine and methionine together, others tended to decrease nitrogen retention values. The addition of three or four amino acids together did not significantly improve retention values above those obtained with lysine supplementation alone. These results suggest, therefore, that lysine is the limiting amino acid in rice protein for children.

The results at a lower level of protein intake are presented in Table 16. At this level, the addition of lysine alone did not influence nitrogen balance; however, the addition of lysine and methionine together improved nitrogen retention. Other amino acids did not have any effect. The results suggest that at the lower level of protein intake both lysine and methionine are limiting.

Some Interesting Aspects

Although the number of trials per cereal grain flour was limited and the periods short, the results were remarkably consistent and clear in their implications. Under experimental conditions children are sensitive to small changes in the amino acid content of their diets and reflect them in the nitrogen retention. The sensitivity to supplementation was observed when balance was improved by the addition of the limiting amino acids and also when the addition caused imbalances in the dietary protein and decreased nitrogen retention. Imbalances were observed with corn and wheat. An example for corn is shown in Table 17. The addition of methionine, before or after adding isoleucine to the lysine and tryptophan supplemented corn, always resulted in a decreased retention.

In other tests, it was found that valine addition also decreased retention and the effect of both amino acids and could be corrected by adding isoleucine and threonine. These results, also demonstrated in dogs[15] and pigs,[18,19] could probably help to explain the findings of various authors[20,21] that corn protein is also deficient in threonine. Although corn proteins are not necessarily deficient in isoleucine and threonine, some samples of corn may contain larger amounts of leucine, methionine, and valine, requiring the addition of isoleucine and threonine to improve their balance and thus their nutritive value.

A further interesting observation is related to the level of protein intake at which studies should be carried out, a point that can be shown by the results with lime-treated corn (Table 8). It appears that at the

Table 17 Methionine imbalance in corn masa*

Diet	Nitrogen balance		
	NI	NA (mg/kg/day)	NR
Basal (B) + tryptophan + lysine	470	336	112
B + tryptophan + lysine + methionine	462	316	83
Basal (B)	471	354	39
B + tryptophan + lysine + methionine	451	228	−16
Basal	453	330	−8
B + tryptophan + lysine	463	334	76
B + tryptophan + lysine + isoleucine	475	376	108
B + tryptophan + lysine + isoleucine + methionine	469	379	90
Basal	474	342	39

* Case PC–57: Amino acid levels added: see Tables 4 and 6.

higher level of protein intake, single additions give increases in nitrogen balance over the basal values, and at the lower levels of intake, multiple additions seem to be more effective. The results of the extensive animal experimentation indicate that to be able to show the order of deficiency in a protein it is necessary to decrease its level of intake. However, working with humans, which is more expensive and time consuming, it appears to be necessary to establish the level of intake that gives maximum retention to the addition of the supplement.

To be able to judge whether the addition of an amino acid or amino acid mixture is improving protein quality, it is necessary that its addition produce a stable response as measured by nitrogen balance.

Representative results with wheat flour are shown in Table 18; it can be seen that the combination of the four amino acids gave a high retention at first, which decreased as the feeding of the diet continued. The results also show the effect of feeding the same supplemented diet before and after a period on the basal. The diet before the basal gave a low retention, however, after the basal, retention was significantly higher. These results were interpreted to indicate that the basal diet caused a depletion of nitrogen, which was corrected by the supplemented diet. After repletion characterized by higher retentions, a decrease that will become stable after 9 or 12 days is expected. This interpretation is yet to be tested.

In studies on basal diet with rolled oats, nitrogen retention decreased most following either milk or supplementation with threonine or lysine alone, or combined, and least when the basal diet followed methionine alone or lysine and threonine combined. These results are shown in Table 19. The possible significance and value of the difference in retention of nitrogen observed on the same basal diet, depending upon the quality of protein in preceding isonitrogenous periods, are not fully understood. These observations can, however, be explained on the basis of the protein quality of milk. Since its protein quality is high, when fed it produced high store of nitrogen evidenced by high nitrogen retention. This nitrogen is lost when a poorer quality protein is fed, as in the case of protein depletion.

Therefore, the greater the decrease in nitrogen balance taking place upon feeding a higher followed by a lower quality protein, the better the quality of the diet fed preceding it. Thus, there is a large decrease after milk, an intermediate decrease when all three amino acids or when lysine and threonine are added, and a lower decrease when lysine and threonine are added alone. On the other hand, there is a rise when oats

Table 18 Nitrogen balance of children fed wheat flour supplemented with amino acids*

Nitrogen Balance

Diet	NI (mg/kg/day)	NR (mg/kg/day)	Diet	NI (mg/kg/day)	NR (mg/kg/day)
Child PC–89			Child PC–88		
Basal (B) + lysine + tryptophan + methionine + threonine	342	96	Basal (B)	323	15
B + lysine + tryptophan + methionine + threonine	339	75	B + lysine + tryptophan	329	75
B + lysine + tryptophan + methionine + threonine	363	55	B + lysine + tryptophan	328	87
B + lysine + tryptophan + methionine + threonine + isoleucine	320	26	B + lysine + tryptophan	334	84
B + lysine + tryptophan + methionine + threonine + isoleucine	315	19	B + lysine + tryptophan + methionine	327	72
Basal	330	43	B + lysine + tryptophan + methionine	350	99
Basal	345	45	B + lysine + tryptophan + methionine	344	92
Basal	354	15	Basal	305	26
B + lysine + methionine + threonine + isoleucine	373	105	Basal	338	43
B + lysine + methionine + threonine + isoleucine	320	82			
B + lysine + methionine + threonine + isoleucine	349	98			

* For amino acid levels added, see Table 12.

Table 19 Change in nitrogen retention from an experimental to a subsequent basal diet period*

	Nitrogen intake (mg/kg/day) 320	240
Basal period after	Nitrogen retention change % of intake	
Milk	–10.3	–16.1
Basal (B) + lysine	–3.5	–1.4
B + threonine	–3.4	–
B + methionine	+3.1	+2.0
B + lysine + methionine	–0.9	–
B + lysine + threonine	–5.0	–
B + methionine + threonine	+7.7	–
B + lysine + methionine + threonine	–5.8	–

* Levels of amino acid added: see Table 14.

were supplemented with methionine alone and with methionine and threonine without lysine, the latter amino acid considered to be limiting in oat protein. The change could therefore be very useful in learning about the effect of amino acid supplementation. The effect of methionine could be due to a latent imbalance which, although not sufficient to lower nitrogen retention during the period of supplementation with methionine, nonetheless had an effect. Actually, its removal improved the quality of protein because the basal diet gave higher nitrogen retention values.

Research Needs

For practical purposes, the results presented clearly show that deficient proteins can be improved by appropriate amino acid supplementation and that children under controlled conditions respond to changes in quality rather rapidly (Figure 1). However, there is still much to be done to understand some of the effects observed, and it is recommended that studies be carried out to measure amino acid availability in humans, to learn of the relationship between nitrogen intake and level to be added of the deficient amino acids, and of the relationship between protein and intake of calories in relation to amino acid supplementation.

Finally, amino acid supplementation studies of cereal grains should

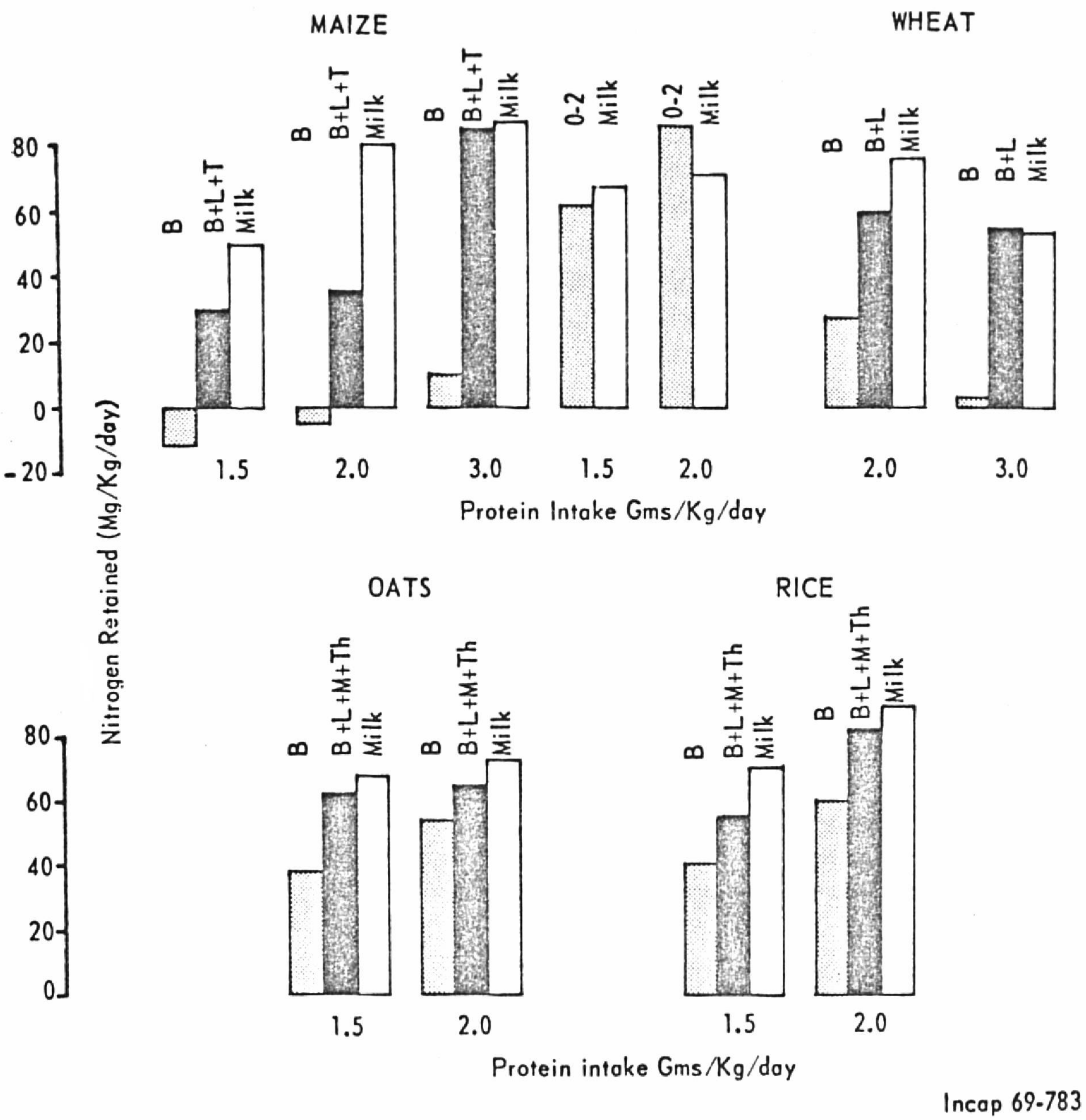

Figure 1. Nitrogen retention of children fed cereal proteins with and without their limiting amino acids.

be performed using diets rather than cereals alone. Preliminary results with the amino acid supplementation of animals fed a mixed, predominantly cereal diet have indicated that improvement in quality is obtained by supplementing the food component providing the largest amount of protein to the diet. Nevertheless, since small amounts of other protein foods are consumed also, they influence the level of amino acid to be added to the cereal grain to improve more efficiently the nutritive value of the protein of the whole diet. These small amounts of protein also alter the balance of essential amino acids of the diet. If this is not taken into consideration, the indiscriminate addition of amino acids could even decrease the protein quality of the diet.

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