Reprinted from THE JOURNAL OF NUTRITION
Vol. 70, No. 2, February 1960

Supplementation of Cereal Proteins with Amino Acids

III. EFFECT OF AMINO ACID SUPPLEMENTATION OF WHEAT FLOUR AS MEASURED BY NITROGEN RETENTION OF YOUNG CHILDREN[1,2]

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Previous work published by the Institute of Nutrition of Central America and Panama (INCAP) on the effect of amino acid supplementation of corn masa (Bressani et al., '58c; Scrimshaw et al., '58) demonstrated that the biological value of corn protein as measured by nitrogen balance in children could be increased by the simultaneous addition of lysine and tryptophan and by the further addition of isoleucine. Recently, Truswell and Brock ('59) confirmed these results using adult subjects. The studies of amino acid supplementation of corn protein were undertaken in part to test the usefulness of the amino acid proportions of the FAO "reference protein" ('57). This "reference protein" represents the initial attempt of an Expert Committee on Protein Requirements of the Food and Agriculture Organization of the United Nations (FAO) to devise an optimal amino acid pattern for human growth and maintenance based on the composition of proteins of known high biological value, and on the various studies of amino acid requirements in man and experimental animals.

To obtain further knowledge of the applicability of this proposed amino acid pattern, studies of the effect of amino acid supplementation of other proteins are required. Because of the importance of wheat in human nutrition, this was selected as the next staple food to be studied. The many reports based on studies in experimental animals, which indicate that the biological value of wheat flour can be significantly improved by the addition of lysine, have been summarized recently by Rosenberg and Rohdenburg ('52), Flodin ('53, '56), Rosenberg ('59) and Harris and Burress ('59). The present paper describes the results of the supplementation of wheat flour with essential amino acids in the proportions of the FAO "reference protein" ('57). The observations were made on hospitalized children who had recovered from severe protein malnutrition.

MATERIALS AND METHODS

Techniques described previously (Bressani et al., '58c; Scrimshaw et al., '58) were used to measure the nitrogen balance in 6 boys in 8 experiments involving, in most cases, two-day adaptation periods followed by three three-day balance periods with each diet combination. The age and weight of the children at the start of the experiments were as follows:

Case no.	Experiment no.	Age	kg
PC-83	1	2 years, 2 months	9.2
	2	2 years, 4 months	10.5
PC-88	1	3 years, 1 month	10.6
	2	3 years, 5 months	12.7
PC-89		1 year, 5 months	7.7
PC-91		5 years, 9 months	11.9
PC-92		2 years, 11 months	10.3
PC-97		3 years, 3 months	12.6

In preliminary feeding trials, a basal diet containing 5% of wheat gluten and

Received for publication August 8, 1959.
[1] Assisted by grants-in-aid from the National Millers' Federation, Washington, D. C., and from E. I. du Pont de Nemours and Company, Wilmington, Delaware.
[2] INCAP Publication I-142.

2% of L-glutamic acid, with the same amounts of the other ingredients, was used. This basal diet was, however, refused by the children because of the unpleasant flavor of L-glutamic acid. This was, therefore, omitted from the basal diet and the level of wheat gluten increased to 7%. Accordingly, the basal diet used in all experiments contained, in grams per 100 gm: wheat flour,[3] 85; wheat gluten,[4] 7; glycine, 3; and corn starch, 5. Also a vitamin and mineral capsule[5] was given daily. Nitrogen content of the basal diet was 3%. The amino acids added were substituted for corn starch, and the nitrogen from these replaced glycine nitrogen so that all diets remained isocaloric and isonitrogenous. They were fed at the rate of 2 gm of protein/kg/day with caloric intakes of 80 to 100/kg/day, according to the estimated requirement of the child.

The essential amino acid content of the wheat flour, the wheat gluten, the basal diet, and the FAO "reference protein" is given in table 1. According to the pattern of the "reference protein," the order of deficiency in the basal diet from the greatest to the least limiting amino acids was as follows: lysine, tryptophan, methionine, isoleucine, valine and threonine, the three latter appearing limiting to an equal degree. Also shown in the table is the amount of each amino acid in milligrams per gram of nitrogen of the basal diet, needed to make up the difference between the reference amino acid level and the amount present in the basal wheat diet. The amino acids were added progressively to the basal diet in approximately these amounts. Because initial calculations of the amino acid content of the wheat flour–wheat gluten basal diet were based on values from the literature, the quantities added were very slightly different from the amounts shown in table 1, based on subsequent analyses by microbiological methods previously referred to (Bressani and Scrimshaw, '58). Corrections were made for the D-form of the amino acids used, by doubling the amount added, except for DL-methionine which was

[3] Wheat flour milled in Guatemala, having the following chemical composition in gm/100 gm: moisture, 14.0; nitrogen, 1.88; ether extract, 1.30; crude fiber, 0.40; ash, 0.58. This flour is prepared from a mixture of locally grown and imported wheat.

[4] Chemical composition in gm/100 gm: moisture, 6.0; nitrogen, 12.88; ether extract, 1.60; crude fiber, 0.60; ash, 0.84.

[5] VI-Syneral, courtesy of the U. S. Vitamin Corporation.

TABLE 1

Essential amino acid composition of the wheat flour, wheat gluten, the basal wheat diet and of the FAO "reference protein"

Amino acid	Wheat flour	Wheat gluten[1]	Basal diet	Basal diet	FAO "reference protein"	Adequacy of basal diet	Amount of amino acids to be added
	gm %	*gm %*	*gm %*	*mg/gm nitrogen*	*mg/gm nitrogen*	*%*	*mg/gm nitrogen of basal diet*
Arginine	0.430	3.481	0.610	203	—	—	—
Histidine	0.292	1.825	0.374	125	—	—	—
Isoleucine	0.450	3.677	0.640	213	270	79	57
Leucine	0.705	5.993	1.018	339	306	—	—
Lysine	0.299	1.530	0.361	120	270	44	150
Methionine	0.190	1.389	0.259				
				149	270	55	121
Cystine	0.210[1]	1.726	0.189				
Phenylalanine	0.610	4.351	0.827		180		
				377		—	—
Tyrosine	0.359[1]	2.596	0.305		180		
Threonine	0.345	2.119	0.441	147	180	82	33
Tryptophan	0.098	0.856	0.143	48	90	53	42
Valine	0.460	3.789	0.656	219	270	81	51

[1] Orr, M. L., and B. K. Watt. Amino Acid Content of Foods. Home Economics Research Report no. 4, U.S.D.A., Washington, D. C., December, 1957.

TABLE 2
Nitrogen balance results of case PC-83 fed wheat flour supplemented with amino acids

Diet fed	Weight change	Nitrogen						
		Intake	Fecal	Absorbed	Absorption	Urinary	Retained	Retention
	kg	*mg/kg/day*			*%*	*mg/kg/day*		*%*
Experiment 1								
Milk	9.16	320	77	243	76.0	186	57	18.0
Basal	9.47	345	47	298	86.4	257	41	11.7
Basal	9.47	310	39	271	87.5	248	23	7.6
Basal	9.55	295	31	264	89.4	261	3	0.8
Basal + 0.64% L-lysine·HCl	9.55	323	49	274	84.9	243	31	9.8
Basal + 0.64% L-lysine·HCl	9.55	324	27	297	91.7	249	48	14.8
Basal + 0.64% L-lysine + 0.97% DL-valine	9.72	331	34	297	89.6	202	95	28.6
Basal + 0.64% L-lysine + 0.97% DL-valine	9.72	309	38	271	87.7	189	82	26.5
Basal	10.09	279	36	243	87.1	216	27	9.7
Basal	10.34	311	38	273	88.0	211	62	20.1
Experiment 2								
Basal	10.49	323	35	288	89.0	220	68	21.1
Basal	10.49	327	40	287	87.9	263	24	7.6
Basal	10.49	327	38	289	88.5	279	10	3.4
Basal + 0.64% L-lysine·HCl	10.74	272	42	230	84.6	178	52	19.1
Basal + 0.64% L-lysine·HCl	10.74	340	38	302	88.8	202	100	29.3
Basal + 0.64% L-lysine·HCl	10.74	325	33	292	90.0	232	60	18.5
Basal + 0.64% L-lysine + 0.97% DL-valine	11.03	356	38	318	89.4	259	59	16.8
Basal + 0.64% L-lysine + 0.97% DL-valine	11.03	345	39	306	88.7	215	91	26.6

assumed to be fully utilized. The amount of lysine added was corrected for the hydrochloride molecule present in the form used.

RESULTS

Case PC-83. The results of two experiments are given in table 2 and summarized in figure 1. The child retained 18% of nitrogen when milk protein was fed, while with the basal diet, nitrogen retention decreased. Lysine added to the basal diet increased nitrogen retention. When the studies were initiated, the original FAO "reference protein" value of 360 mg/gm of total nitrogen was used as the reference level for valine. This was later changed to 270 mg on the basis of additional data available prior to publication of the report. The 360-mg value made valine the second limiting amino acid in wheat, and the effect of adding valine to the basal plus lysine diet was studied with PC-83. With the lysine plus valine diet the retention appeared to increase. When the child was again fed the basal diet, retention decreased to about 15%. In the second experiment, the lysine effect was more marked and valine had no further effect.

Cases PC-88 and PC-91. The values of the nitrogen balances, obtained with cases PC-88, experiment 1, and PC-91, are presented jointly in table 3 and summarized in figure 1. Nitrogen retention when fed the milk diet was 26% of the intake. Upon feeding the basal diet, nitrogen retentions decreased. The next diet consisted of the supplementation of the basal diet with lysine; addition of this amino acid caused an increase only in the second period.

The results of case PC-91 indicate that an average retention of 23% of the nitrogen in the form of milk was observed. The basal diet resulted in a decreased nitrogen retention. The addition of lysine to the basal diet increased retentions to an average of 22% of the intake. According to the FAO "reference protein," the next limit-

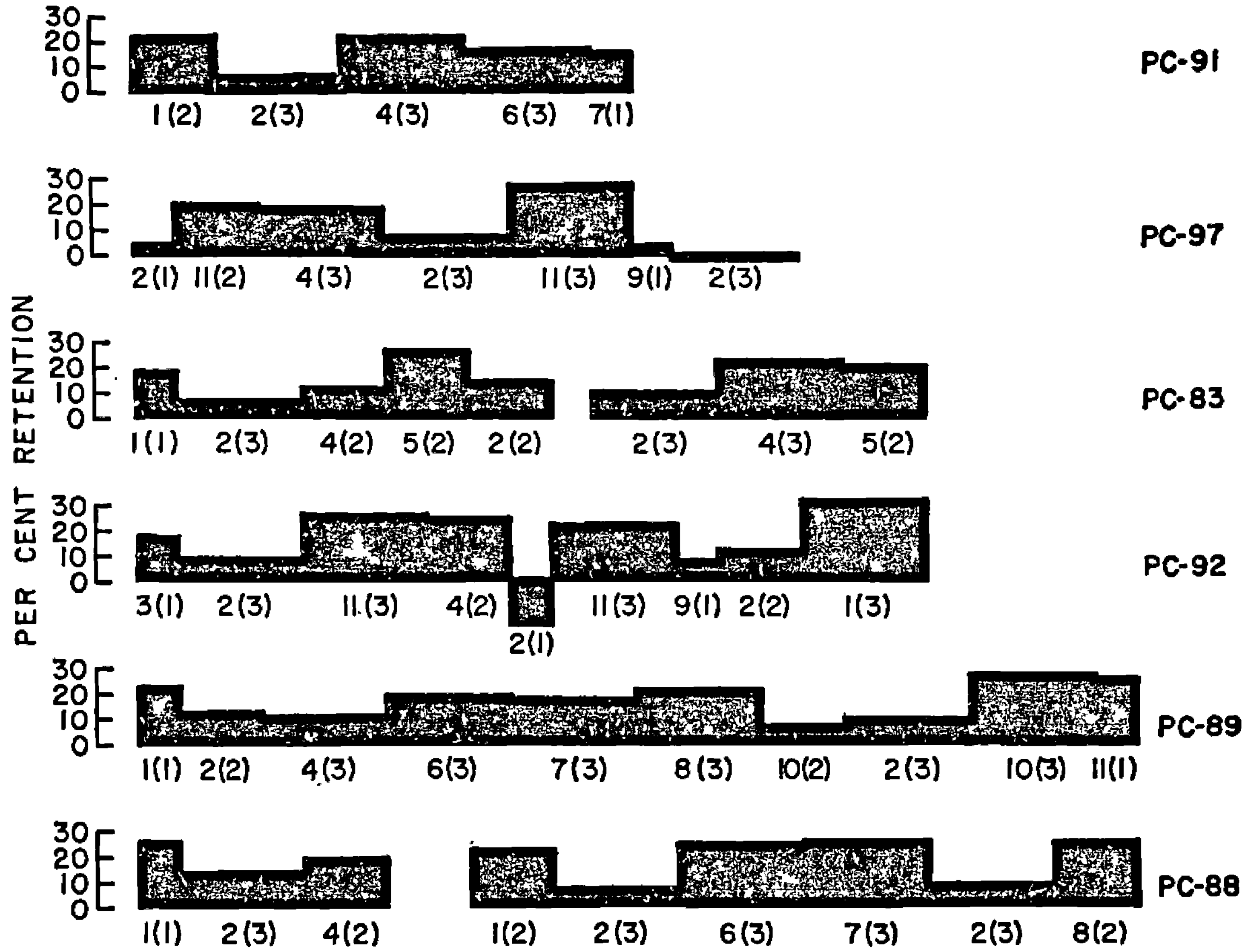

Fig. 1 Percentage of nitrogen retention of intake of children fed wheat diets supplemented with various essential amino acids.

Key to diets: The figures beneath the retention bars represent the diet fed, while those in parentheses represent the number of three-day balance periods. Amounts of amino acids added are listed in table 1.

1 Milk
2 Basal
3 INCAP vegetable mixture 9
4 Basal + lysine
5 Basal + lysine + valine
6 Basal + lysine + tryptophan
7 Basal + lysine + tryptophan + methionine
8 Basal + lysine + tryptophan + methionine + threonine
9 Basal + tryptophan + methionine + threonine + isoleucine + valine
10 Basal + lysine + tryptophan + methionine + threonine +isoleucine
11 Basal + lysine + tryptophan + methionine + threonine + isoleucine + valine

ing amino acid after lysine was tryptophan, and the third, methionine. Addition of these two amino acids to the basal plus lysine diet did not alter retentions from those previously obtained with the basal wheat diet supplemented with lysine alone.

Case PC-88. The results of a second experiment are presented in table 4 and summarized in figure 1. The nitrogen retentions observed with milk protein were similar to those of the previous cases. The child was then fed the basal wheat diet, and retention decreased to an average of 8% of the intake. The next diet consisted of supplementing the basal diet with lysine and tryptophan. The results show that retentions increased to an average value of 25%. As in the previous cases, methionine addition to the basal diet plus lysine plus tryptophan did not alter nitrogen retentions. When the child was again fed the basal diet, retentions dropped to an average value of 12%. Upon feeding the basal diet supplemented with lysine, tryptophan, methionine and threonine, the child retained an average of 25% of the nitrogen intake,

TABLE 3

Nitrogen balance results of cases PC-88 and PC-91 fed wheat flour supplemented with amino acids

Diet fed	Weight change	Nitrogen						
		Intake	Fecal	Absorbed	Absorption	Urinary	Retained	Retention
	kg	mg/kg/day			%	mg/kg/day		%
Case PC-88								
Milk	10 6	321	69	252	78 5	169	83	26 0
Basal	11 1	329	50	279	84 8	248	31	9 4
Basal	11 1	324	61	263	81 2	214	49	15 1
Basal	10 9	326	39	287	87 9	228	59	17 7
Basal + 0 67% L-lysine HCl	10 9	326	60	266	81,6	240	26	8 0
Basal + 0 67% L-lysine HCl	10 9	350	51	299	85 4	198	101	28 9
Case PC-91								
Milk	11 9	292	64	228	78 2	148	80	27 6
Milk	11 9	288	61	227	78 9	174	53	18 5
Basal	12 3	349	38	311	89 2	279	32	9 2
Basal	12 3	339	69	270	79 7	276	–6	–1 8
Basal	12 3	316	23	293	92 9	249	44	14 1
Basal + 0 67% L-lysine HCl	12 6	340	51	289	84 9	225	64	18 6
Basal + 0 67% L-lysine HCl	12 6	327	35	292	89 2	187	105	32 0
Basal + 0 67% L-lysine HCl	12 6	329	50	279	84 7	229	50	15 1
Basal + 0 67% L-lysine + 0 20% DL-tryptophan	12 9	321	36	285	88 8	227	58	18 0
Basal + 0 67% L-lysine + 0 20% DL-tryptophan	13 0	331	61	270	81 7	219	51	15 4
Basal + 0 67% L-lysine + 0 20% DL-tryptophan	13 0	344	60	284	82 6	225	59	17 2
Basal + 0 67% L-lysine + 0 20% DL-tryptophan + 0 35% DL-methionine	13 2	305	50	255	83 6	204	51	16 7

a retention which was similar to that obtained with lysine and tryptophan supplementation

Case PC-89 As shown in table 5 and summarized in figure 1, feeding milk protein resulted in a retention of 23% The basal diet produced a decrease in nitrogen retention to an average of 13% The addition of lysine to the basal diet resulted in an average retention of 11%, and when this diet was further supplemented with tryptophan, retentions increased to an average of 20% The basal diet was then supplemented with lysine, tryptophan and methionine, resulting in an average retention of 20% which is equal to that obtained when the basal diet was supplemented with lysine and tryptophan The addition of threonine to the lysine, tryptophan and methionine–supplemented diet resulted in an average retention of 22%, and when this diet was supplemented with isoleucine, retention decreased to an average of 7% The basal diet fed for the next period maintained retentions at an average of 10%, whereas feeding the basal diet supplemented with 5 and 6 of the limiting amino acids gave a retention of approximately 28%

Case PC-97 As shown in table 6 and summarized in figure 1, feeding of the basal diet resulted in a low retention as in the previous cases The basal wheat diet supplemented with all 6 of the limiting amino acids gave an average retention of 20% When the child was fed the basal diet supplemented with lysine alone, retentions were as high as with the basal diet sup-

TABLE 4

Nitrogen balance results of case PC-88 fed wheat flour supplemented with amino acids in a second experiment

Diet fed	Weight change	Nitrogen						
		Intake	Fecal	Absorbed	Absorption	Urinary	Retained	Retention
	kg	*mg/kg/day*			*%*	*mg/kg/day*		*%*
Milk	12.7	314	60	254	80.8	166	88	27.8
Milk	12.7	312	59	253	81.1	194	59	18.9
Basal	12.8	333	41	292	87.7	263	29	8.8
Basal	12.8	346	47	299	86.4	267	32	9.1
Basal	12.8	323	40	283	87.7	268	15	4.7
Basal + 0.67% L-lysine·HCl + 0.20% DL-tryptophan	13.0	329	47	282	85.7	207	75	22.8
Basal + 0.67% L-lysine·HCl + 0.20% DL-tryptophan	13.0	328	29	299	91.2	212	87	26.4
Basal + 0.67% L-lysine·HCl + 0.20% DL-tryptophan	13.0	334	36	298	89.2	214	84	25.0
Basal + 0.67% L-lysine·HCl + 0.20% DL-tryptophan + 0.35% DL-methionine	13.4	327	38	289	88.4	217	72	22.0
Basal + 0.67% L-lysine·HCl + 0.20% DL-tryptophan + 0.35% DL-methionine	13.4	350	40	310	88.5	211	99	28.2
Basal + 0.67% L-lysine·HCl + 0.20% DL-tryptophan + 0.35% DL-methionine	13.4	344	41	303	88.2	211	92	26.7
Basal	13.8	305	46	259	84.9	233	26	8.3
Basal	13.8	338	42	296	87.6	253	43	12.7
Basal	13.8	294	38	256	87.1	243	13	4.4
Basal + 0.67% L-lysine·HCl + 0.20% DL-tryptophan + 0.35% DL-methionine + 0.32% DL-threonine	13.9	320	35	285	89.1	202	83	25.9
Basal + 0.67% L-lysine·HCl + 0.20% DL-tryptophan + 0.35% DL-methionine + 0.32% DL-threonine	13.9	324	36	288	88.9	210	78	24.1

plemented with all 6 of the limiting amino acids. Again the basal diet gave a low nitrogen retention of around 9% of the intake. Upon supplementation with all 6 amino acids, an average retention of 30% was observed. When lysine was omitted, nitrogen retention dropped to 4%. Continuation was not possible because the child persistently vomited in the next 6 days and balance data could not be obtained. He continued to do so even when fed the basal diet, with the result that nitrogen balances were negative.

Case PC-92. As shown in table 7 and summarized in figure 1, feeding INCAP Mixture 9, a combination of vegetable ingredients, containing 27.5% of protein of high biological value (Institute of Nutrition of Central America and Panama, '58), resulted in a retention of 18%. The basal diet gave an average retention of 9%, and when this was supplemented with all of

TABLE 5

Nitrogen balance results of case PC-89 fed wheat flour supplemented with amino acids

Diet fed	Weight change	Nitrogen						
		Intake	Fecal	Absorbed	Absorption	Urinary	Retained	Retention
	kg	*mg/kg/day*			*%*	*mg/kg/day*		*%*
Milk	7.7	318	61	257	80.9	186	71	22.2
Basal	8.1	269	28	241	89.7	207	34	12.8
Basal	8.1	337	39	248	88.4	250	48	14.4
Basal + 0.67% L-lysine·HCl	8.1	321	49	272	84.6	247	25	7.5
Basal + 0.67% L-lysine·HCl	8.1	343	34	309	90.1	248	61	17.6
Basal + 0.67% L-lysine·HCl	8.1	321	39	282	87.7	257	25	7.8
Basal + 0.67% L-lysine·HCl + 0.20% DL-tryptophan	8.6	358	39	319	89.2	242	77	21.5
Basal + 0.67% L-lysine·HCl + 0.20% DL-tryptophan	8.6	354	27	327	92.3	244	83	23.2
Basal + 0.67% L-lysine·HCl + 0.20% DL-tryptophan	8.6	347	34	313	90.2	267	46	13.5
Basal + lysine + tryptophan + 0.35% DL-methionine	8.8	343	39	304	88.7	266	38	11.1
Basal + lysine + tryptophan + 0.35% DL-methionine[1]	8.8	353	22	331	93.7	243	88	24.8
Basal + lysine + tryptophan + methionine + 0.32% DL-threonine	9.1	342	22	320	93.6	224	96	28.1
Basal + lysine + tryptophan + methionine + 0.32% DL-threonine	9.1	339	36	303	89.4	228	75	22.1
Basal + lysine + tryptophan + methionine + 0.32% DL-threonine	9.1	363	55	308	85.0	253	55	15.2
Basal + lysine + tryptophan + methionine + threonine + 0.43% DL-isoleucine[2]	9.4	320	53	267	83.4	241	26	8.1
Basal + lysine + tryptophan + methionine + threonine + 0.43% DL-isoleucine[2]	9.4	315	38	277	87.9	258	19	6.0
Basal	9.5	330	42	288	87.4	245	43	13.3
Basal	9.5	345	44	301	87.2	256	45	13.0
Basal	9.5	354	50	304	85.8	289	15	4.4
Basal + lysine + tryptophan + methionine + threonine + 0.43% isoleucine	9.9	373	43	330	88.6	225	105	28.3
Basal + lysine + tryptophan + methionine + threonine + 0.43% isoleucine	9.8	320	43	277	86.6	195	82	25.6
Basal + lysine + tryptophan + methionine + threonine + 0.43% isoleucine	9.8	349	64	285	81.8	187	98	28.2
Basal + lysine + tryptophan + methionine + threonine + isoleucine + 0.43% DL-valine	10.2	323	36	287	88.9	202	85	26.3

[1] Six-day balance period. [2] Vomiting.

TABLE 6

Nitrogen balance results of case PC-97 fed wheat flour supplemented with amino acids

Diet fed	Weight change	Nitrogen						
		Intake	Fecal	Absorbed	Absorption	Urinary	Retained	Retention
	kg	*mg/kg/day*			%	*mg/kg/day*		%
Basal	12.6	348	49	299	86.0	281	18	5.3
Basal + lysine + tryptophan + methionine + threonine + isoleucine + valine	12.7	362	39	323	89.2	243	80	22.1
Basal + lysine + tryptophan + methionine + threonine + isoleucine + valine	12.7	349	54	295	84.5	231	64	18.3
Basal + L-lysine·HCl	13.1	374	61	313	83.8	241	72	19.2
Basal + L-lysine·HCl	13.1	393	57	336	85.6	249	87	22.1
Basal + L-lysine·HCl	13.1	376	56	320	85.1	254	66	17.6
Basal	13.6	361	92	269	74.6	277	−8	−2.1
Basal	13.6	338	48	290	85.8	234	56	16.7
Basal	13.6	348	54	294	84.4	254	40	11.3
Basal + lysine + tryptophan + methionine + threonine + isoleucine + valine	13.8	368	56	312	84.9	180	132	35.4
Basal + lysine + tryptophan + methionine + threonine + isoleucine + valine	13.8	369	59	310	84.0	200	110	29.8
Basal + lysine + tryptophan + methionine + threonine + isoleucine + valine	13.8	370	56	314	84.8	226	88	23.6
Basal + tryptophan + methionine + threonine + isoleucine + valine[1]	14.2	321	64	257	80.1	243	14	4.3
Basal	14.5	318	64	254	79.9	256	−2	−0.6
Basal	14.5	270	61	209	77.4	240	−31	−11.5
Basal	14.5	332	67	265	79.9	264	0	0

[1] This diet was fed for two additional 3-day periods, but balances were not carried out due to excessive vomiting.

the amino acids deficient by comparison with the FAO "reference protein," the average retention was 27%. The next diet consisted of the basal wheat diet supplemented with lysine, which gave an average retention of 25%. Return to the basal diet resulted in a negative nitrogen retention, as a consequence of vomiting. When the basal diet was supplemented with all of the amino acids found limiting according to the FAO pattern, an average retention of 23% was observed. Retentions dropped to 9% when the basal wheat diet was supplemented with the 5 amino acids next limiting after lysine. The last diet consisted of feeding milk protein, and in this instance retentions were similar to those observed with the basal wheat diet supplemented with lysine alone or with all of the amino acids deficient by comparison with the FAO "reference protein."

TABLE 7

Nitrogen balance results of case PC-92 fed wheat flour supplemented with amino acids

Diet fed	Weight change	Nitrogen						
		Intake	Fecal	Absorbed	Absorption	Urinary	Retained	Retention
	kg	*mg/kg/day*			*%*	*mg/kg/day*		*%*
Vegetable mixture	10.3	330	96	234	70.9	174	60	18.2
Basal	10.5	341	48	293	85.9	263	30	8.8
Basal	10.5	364	55	309	85.0	271	38	10.6
Basal	10.5	347	51	296	85.3	270	26	7.5
Basal + lysine + tryptophan + methionine + threonine + isoleucine + valine	10.9	365	50	315	86.4	204	111	30.5
Basal + lysine + tryptophan + methionine + threonine + isoleucine + valine	11.0	333	57	276	82.8	211	65	19.4
Basal + lysine + tryptophan + methionine + threonine + isoleucine + valine	11.0	346	61	285	82.3	175	110	31.9
Basal + lysine	11.6	346	55	291	84.0	205	86	24.9
Basal + lysine	11.6	345	51	294	85.1	207	87	25.0
Basal[1]	11.9	295	102	193	65.6	245	–52	–17.6
Basal + lysine + tryptophan + methionine + threonine +isoleucine + valine	11.9	310	52	258	83.2	196	62	20.0
Basal + lysine + tryptophan + methionine + threonine +isoleucine + valine	11.9	345	45	300	87.0	203	97	28.1
Basal + lysine + tryptophan + methionine + threonine +isoleucine + valine	11.9	343	52	291	84.8	217	74	21.6
Basal + tryptophan + methionine + threonine + isoleucine + valine[1]	12.6	253	42	211	83.5	189	22	8.6
Basal	12.3	332	43	289	87.0	230	59	17.8
Basal[1]	12.3	268	38	230	85.8	210	20	7.4
Milk	12.6	307	47	260	84.6	129	131	42.7
Milk	12.6	269	58	211	78.4	150	61	22.5
Milk	12.6	311	61	250	80.6	151	99	32.0

[1] Vomiting.

DISCUSSION

The nitrogen balance data presented show that the nutritive value of wheat proteins can be improved markedly by the addition of lysine alone to the wheat diet; in some cases the retention approximated that obtained with milk protein. Apparently, however, more consistent results are obtained if tryptophan is added together with lysine, and also with the addition of the other limiting amino acids according to the FAO "reference protein" levels. This result was to be expected, since the amount of added lysine exceeded that required to bring its relative proportion to that of tryptophan, the second limiting amino acid in wheat, according to the amino acid pattern of the reference protein. Therefore, lysine deficiency could have caused an imbalance which resulted in a decrease in nitrogen retention. Yang, Clark and Vail[6] have shown, in rats, that the mean growth rate, food and ntirogen intake, nitrogen efficiency ratio, nitrogen retention and bio-

[6] Yang, S. P., H. E. Clark and G. E. Vail 1959 Effect of level and method of lysine supplementation on the nutritive value of wheat proteins for young rats. Federation Proc., *18:* 553 (abstract).

logical value of a 10% protein wheat diet were at a maximum when supplemented with 0.20% of lysine and decreased when less or more lysine was added.

Amino acid supplementation is often less effective because the amount of amino acids added frequently is larger than actually needed and causes imbalances. As has been pointed out repeatedly by Rosenberg ('59), the biological value of cereal grains is improved by adding the amount necessary to bring the level of the first limiting amino acid to that of the second. He showed also that 90% wheat diets could be improved significantly by adding 0.3 to 0.4% lysine, respectively, to rations fed to female and male rats. The level of protein intake is probably also a very important factor in determining the optimal level of lysine or any other amino acid. This has been clearly shown in rats (Bressani and Mertz, '58; Harris and Burress, '59), chicks (Grau, '48) and swine (Becker et al., '57), using wheat, corn and other diets.

The importance of supplementing wheat protein with lysine, and the significance of its relation to the proportions of other essential amino acids, are particularly apparent from the results obtained in the last two cases, PC-97 and PC-92, since the feeding of the wheat basal diet supplemented with all the limiting amino acids according to the FAO "reference protein" gave nitrogen retentions similar to those obtained with the lysine supplementation alone. Omitting lysine from this diet, however, immediately resulted in vomiting and refusal of the food offered. This effect was probably due to the fact that the addition of other amino acids increased the imbalance of the diet with respect to lysine. Kumta et al. ('58) have shown that rats immediately refuse to consume imbalanced diets.

The amino acid pattern of the FAO "reference protein" or any other theoretical pattern of amino acids for the estimation of the order of amino acid deficiency in a test protein is very useful in interpreting the nitrogen balance results of the amino acid supplementation of food proteins. The results of supplementing wheat protein to meet the content of the FAO "reference protein" with respect to a given amino acid are quite different from those observed with the supplementation of corn protein in exactly the same manner. For example, comparison with the FAO pattern indicated that both corn masa and wheat proteins were deficient in methionine. Not only did the addition of methionine fail to bring about a positive or improved nitrogen balance in either study, but also its addition to corn masa protein resulted in a decreased nitrogen balance even when the basal diet was supplemented with lysine and tryptophan (Bressani et al., '58; Scrimshaw et al., '58). No such effect was observed in the studies with wheat.

In corn masa proteins, other amino acids besides lysine, tryptophan and isoleucine are apparently needed to counteract the effect of even a small excess of methionine, but in wheat protein these or other amino acids seem to be present in sufficient amounts so that a small excess of methionine has no effect. These results suggest that the 270 mg of methionine plus cystine in the FAO "reference protein" are too high.

The use of three three-day nitrogen balance periods to test the effect of the addition of the limiting amino acids to wheat has an advantage over the one and two three-day periods used in the previous studies. The results suggest that a good amino acid supplementation tends to produce a sustained response, while a poor or less satisfactory supplementation causes a negative balance or a transient increase in nitrogen retention. This is owing presumably to a temporary benefit from filling nitrogen needs with incomplete proteins which do not require the same amino acid proportions as overall growth and maintenance. A decrease in nitrogen retention following an initial rise due to an amino acid change suggests that the amino acid supplementation is inadequate. More extensive experiments are required to obtain the necessary data for quantitative evaluation of these phenomena.

SUMMARY

Six children recently recovered from severe protein malnutrition, ranging in age from one year, 5 months to 5 years, 9 months and in weight from 7.7 to 12.7 kg, were fed a simplified wheat diet in which the protein was contributed by both the

flour and gluten from wheat, in 8 experiments. All of the children were fed 2 gm of protein and 80 to 100 Cal./kg of body weight/day; a vitamin and mineral capsule was also given. The effect of the addition of the limiting amino acids according to the FAO "reference protein" was measured in most cases using three three-day balance periods. Comparison of the essential amino acid pattern of the wheat basal diet to the FAO "reference protein" showed that the order of the limiting amino acids was: lysine, tryptophan, methionine, isoleucine, valine and threonine. The results supply further evidence that in the utilization of wheat protein, lysine is the most limiting amino acid, since in most of the cases described in this paper, its addition to the basal wheat diet produced a sustained nitrogen retention sometimes similar to that obtained with milk feeding or observed when the basal diet was supplemented with all of the limiting amino acids according to the pattern of the FAO "reference protein." In one of two trials an improvement in nitrogen retention was obtained upon the addition of tryptophan to the lysine–supplemented diet. The refusal and vomiting of the diet fed and a reduction in nitrogen retention when the basal diet was supplemented with all the limiting amino acids except lysine, was further evidence of the importance of the lysine deficiency of wheat diets. For wheat protein, at least, the level of methionine in the FAO "reference protein" appeared to be too high since its addition did not improve nitrogen retention.

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