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THE BIOLOGICAL AVAILABILITY OF ESSENTIAL AMINO ACIDS IN WHEAT, FLOUR, BREAD, AND GLUTEN[1]

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ABSTRACT

Rat-feeding experiments were conducted to determine the extent to which the essential amino acids of wheat and wheat products are utilized. This study reports the availabilities of methionine, threonine, tryptophan, valine, phenylalanine, leucine, and isoleucine in wheat, flour, bread, and gluten. Availability values were measured both by determining the proportion of sample amino acid excreted in the feces and by equating the increase in carcass nitrogen to the ingestion of the amino acid. The latter is believed to be the more reliable method. The increase in total carcass nitrogen content was found to vary directly with the amount of available amino acid consumed when that amino acid was limiting in the diet. Nitrogen gains on sample diets were referred to standard response curves obtained by feeding pure amino acids, and the percent availability was calculated by comparing the derived values to those obtained by microbiological analyses of the samples. Results by both methods show the biological availability of these amino acids to be generally high in the products studied. Availabilities tended to be highest in gluten and lowest in the whole wheat, perhaps because of differences in digestibility.

Within the past 15 years, methods of analysis have permitted reliable estimation of quantities of amino acids in cereal foods. However, it is widely known that the amount of a nutrient in a food as determined by analysis does not necessarily represent the amount of that nutrient which is utilizable when consumed as food. The extent of its utilization is termed in this paper the biological availability. A decrease in utilization may result from a number of factors, including failure or delay in complete digestion of the food, impaired absorption from the gastrointestinal tract, or inefficient utilization of the digested and absorbed amounts. The availability as found depends on the method of measurement. In this paper, the primary intention is to measure

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biological availability of essential amino acids in terms of their over-all effectiveness for protein formation as determined by the increase in carcass nitrogen.

Test procedures were reported previously (1) for determining the biological availability of lysine in wheat products to the growing rat. The general methods have been extended to the remaining essential amino acids (except arginine and histidine), and the present paper presents the availability results for isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, and valine.

Materials and Methods

Diets. Amino acid basal diets were prepared as shown in Table I.

TABLE I

COMPOSITION OF AMINO ACID DIETS

INGREDIENT	DIET LEVEL	EXPERIMENTAL AMINO ACIDS WHEN NOT SUBJECT OF TEST[a]	DIET LEVEL
	%		%
DL-Alanine	0.35	DL-Isoleucine (50% L-)	1.42
L-Arginine·HCl	0.81	L-Leucine	1.15
DL-Aspartic acid	0.56	DL-Methionine	0.47
L-Cystine	0.33	DL-Phenylalanine	0.84
L-Glutamic acid	5.65	DL-Threonine	1.02
Glycine	1.59	DL-Tryptophan	0.22
L-Histidine·HCl·H_2O	0.68	DL-Valine	1.46
L-Lysine·HCl	1.37		
L-Proline	0.39		
DL-Serine	0.73		
L-Tyrosine	0.50		
Salts, Hegsted[b]	4.00		
Corn oil[c]	5.00		
Urea	[d]		
Vitamins	[e]		
Wheat starch[f] to total	100		

[a] When under test, amounts shown replaced by: L-isoleucine, 0.20%; L-leucine, 0.25%; L-methionine, 0.05%; DL-phenylalanine, 0.12%; DL-threonine, 0.35%; L-tryptophan, 0.04%; and DL-valine, 0.28%, respectively.
[b] Nutritional Biochemicals Corporation, Cleveland, Ohio.
[c] Mazola, Corn Products Company, Argo, Ill.
[d] As required to maintain nitrogen level of basal diets at 2.70%.
[e] Vitamins were supplied in a portion of the starch in mg./100 g. of diet as follows: thiamine·HCl, 1; riboflavin, 1; pyridoxine·HCl, 1; nicotinic acid, 10; *i*-inositol, 20; *p*-aminobenzoic acid, 20; folic acid, 0.1; biotin, 0.1; menadione, 2; Ca pantothenate, 4; choline chloride, 150; vitamin B_{12}, 0.004. Each rat received weekly 2 mg. alpha-tocopheryl acetate dissolved in 2 drops of corn oil. Vitamins A and D were supplied by a drop of halibut liver oil given to each rat weekly.
[f] Aytex, General Mills, Minneapolis.

Amino acid concentrations were essentially those employed in the lysine study (1) but with 80% of the proline replaced by glycine (equivalent nitrogen basis) to reduce cost, and with the inclusion of lysine. In the present experiments each basal diet contained a small amount of the amino acid under study, in order to prevent weight loss in the groups receiving no supplement. With methionine only, a separate additional feeding test was also performed in which gluten, at 6% of the diet, was substituted for its complement of amino acids in the basal amino acid mixture and provided the only source of basal methionine. With each amino acid study a positive control diet was fed in which the amino acid mixture of Table I was replaced by 22.5% of gluten, supplemented with 1% of lysine; 0.2% each of L-histidine, DL-methionine, and DL-threonine; and 0.05% of DL-tryptophan.

Increments of the amino acid under test were added to the basal mixture to provide at least eight diets with which to establish a standard response curve. Test samples were added in amounts expected to produce rates of growth within the upper two-thirds of the fast-rising portion of the standard curves. In the methionine and tryptophan experiments, two sample concentrations were fed. In all others, three dietary levels were included to ensure that at least two of them would provide the desired response. All additions to the basal diet were made at the expense of wheat starch.

Sample Description. The wheat, flour, and gluten were from the same lots used in the lysine study. The wheat was a blend of hard red spring and hard red winter wheats. The flour was a 95% patent, commercially milled at an extraction rate of 72%, thus representing approximately 68% of the cleaned wheat. Bread was prepared from the flour as required, using 4 parts of nonfat dry milk and 2.5 parts of compressed yeast per 100 parts of flour. Loaves were sliced and dried in a slow stream of unheated air. Prior to incorporation into the diets, the wheat and bread were ground in a hammer mill using a 0.024-in. screen. Amino acids were determined microbiologically as previously described (2), and the results are shown in Table II along with the nitrogen values.

Experimental Procedure. Weanling, male, albino rats (Holtzman) of 40- to 50-g. initial weight were adjusted gradually to the amino acid diets. The readily accepted gluten diet was fed on the first day; an

TABLE II

NITROGEN AND AMINO ACID CONTENT OF WHEAT, FLOUR, BREAD, AND GLUTEN [a]
(As-fed moisture basis)

SAMPLE	NITROGEN	METHIONINE	TRYPTOPHAN	ISOLEUCINE	PHENYLALANINE	VALINE	LEUCINE	THREONINE
	%	%	%	%	%	%	%	%
Wheat	2.46	0.250	0.177	0.599	0.714	0.725	0.981	0.439
Flour	2.31	0.245	0.152	0.603	0.711	0.662	0.984	0.383
Bread	2.42	0.258	0.144	0.648	0.792	0.757	1.057	0.428
Gluten	12.00	1.251	0.677	3.202	3.841	3.284	5.197	1.863

[a] Nitrogen determined by Kjeldahl; amino acids by microbiological analysis.

amino acid diet was incorporated with it in increasing proportions over the next 3 days in the ratios (amino acid diet to gluten diet) of 1:3, 1:1, and 3:1, respectively, and 100% of amino acid diet was offered on the fifth day to end the adjustment period.

On the sixth day the experimental diets were assigned in random order to groups of five rats. Those in one group were killed to establish the average initial carcass weight and nitrogen content. Diets and distilled water were fed *ad libitum* for 3 weeks. Spilled food and feces were collected on absorbent towels placed beneath each cage. The papers were changed at the end of the second day of the experiment, and thereafter at the end of each week. After drying in air, the contents were sieved to separate the food from the feces. Feces for the first 2 days were discarded and those for the last 19 days were pooled by group, hydrolyzed, and assayed for their content of the amino acid under study. Food spillage was weighed and accounted for in the measurement of food consumption during the 19-day fecal collection period and over the entire 3 weeks.

At the completion of the feeding experiment, the animals were killed in random order using chloroform, the gastrointestinal contents were removed, and the empty carcass weights were taken. The carcasses were frozen and ground with dry ice. Ground samples were collected by group in polyethylene bags and were mixed thoroughly both before and after thawing. Nitrogen was determined in triplicate by the conventional Kjeldahl method. Carcass nitrogen gains were calculated by determining the increase in mean total carcass nitrogen of each experimental group over that found for the group sacrificed on the initial day of the experiment. Carcass fat was determined (except in the methionine studies) by extraction with a mixture of ether and petroleum ether after acid hydrolysis as described previously (3).

Results and Discussion

Maximum growth response was remarkedly consistent over the eight separate feeding trials. The gluten-based, positive control diet produced average weekly weight gains of 47–50 g. Maximum weight gains of 38–40 g./week were obtained with all amino acid diets when they were supplemented with adequate amounts of the amino acid under study. The uniformity of response indicates that experimental conditions were comparable between studies, and lends assurance as to the adequacy of the diets. The unusually high rate of gain supported by the amino acid diet was investigated during the course of these studies and was found to be associated with the relatively high content of glutamic acid and arginine (3).

Availability as Determined by Increase in Carcass Nitrogen. The results of the various methods of computing availabilities supported the conclusion made from the lysine study (1) that amino acid availability in the products tested is represented best by the relation between increase in carcass nitrogen and the amount of available amino acid consumed. This method again provided the best agreement between basal diets (in the methionine study) and also the best agreement between sample levels as compared to the other performance criteria and calculation methods previously described. Standard response curves were constructed by plotting gain in total carcass nitrogen against the total amount of the amino acid consumed, including that contributed by the basal level (Figs. 1–3). The methionine in the gluten basal was taken to be 96% available in determining the total

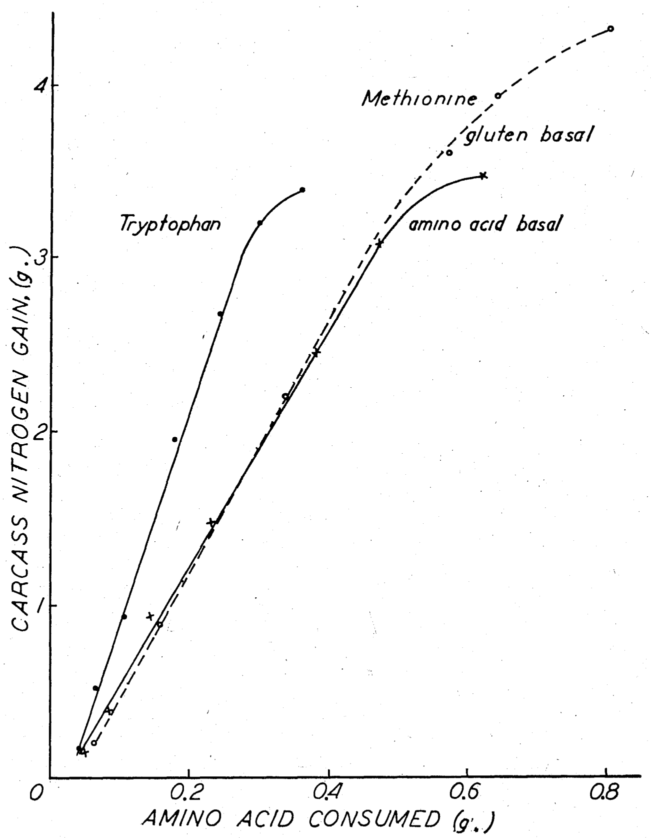

Fig. 1. Increase in total carcass nitrogen as a function of amount of limiting amino acid consumed: methionine (gluten basal and amino acid basal) and tryptophan.

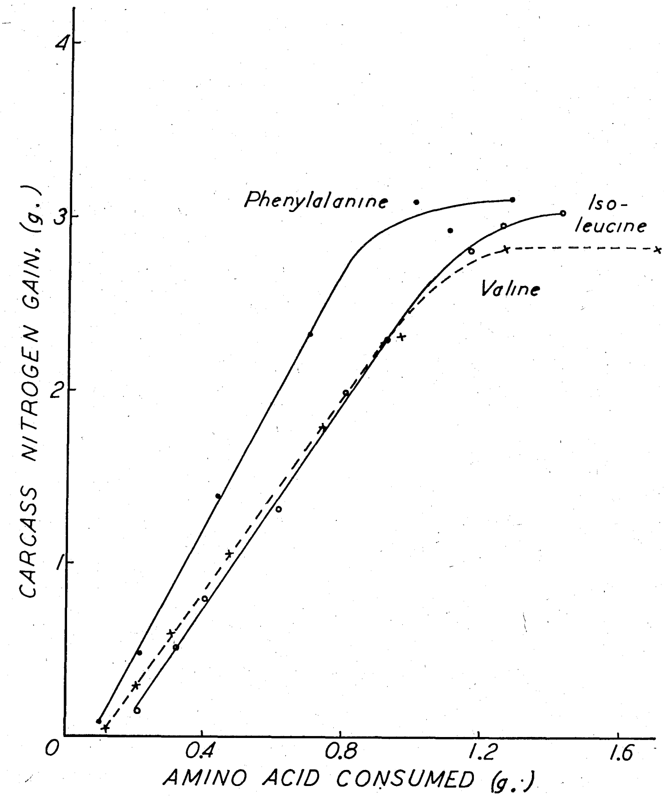

Fig. 2. Increase in total carcass nitrogen as a function of amount of limiting amino acid consumed: phenylalanine, isoleucine, and valine.

available methionine consumed. Crystalline amino acids were assumed to be completely available. The standard plots show that carcass nitrogen increased in direct proportion to the amount of available amino acid consumed for each essential amino acid tested. The concordance of the two methionine curves shows that this relationship was not altered by the presence of gluten, and permits the inference that it would not be affected by the protein content of added sample.

Calculations were made by referring the values for nitrogen gain to the appropriate standard curve to obtain the corresponding amount of available amino acid. From the latter figure was subtracted the amount of available amino acid consumed from the basal diet, and the difference, representing the available amino acid arising from the sample, was compared to the amount consumed from the sample as determined by microbiological analysis, expressed as percent. Calculations and results by this method are grouped in Tables III, IV, and V

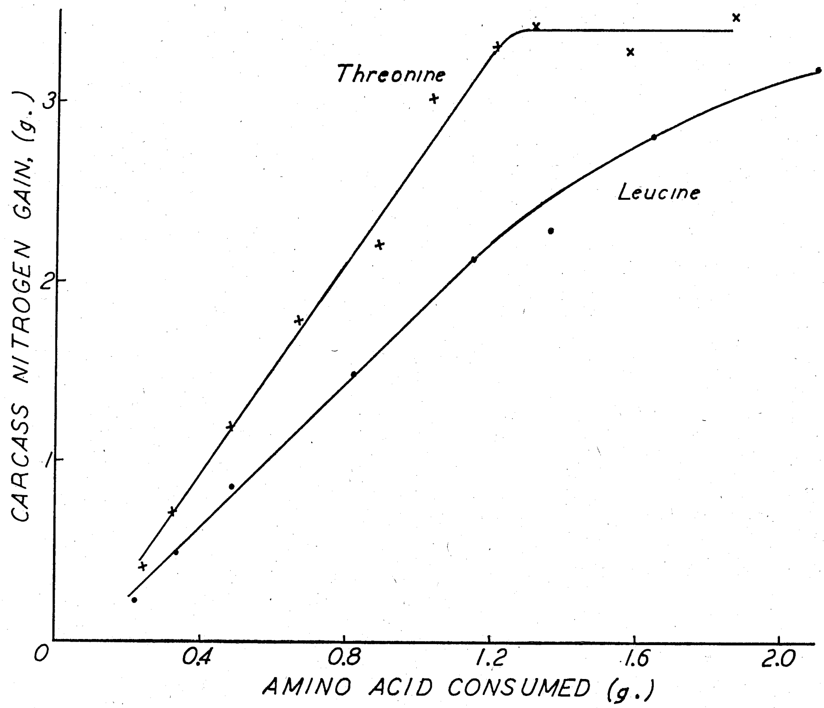

Fig. 3. Increase in total carcass nitrogen as a function of amount of limiting amino acid consumed: threonine and leucine.

for convenience in referring to the corresponding standard curves in Figs. 1, 2, and 3, respectively.

Generally good agreement was found between sample levels for each amino acid. The notable exception is with isoleucine (Table IV), for which lower values were obtained with the lower sample concentrations of wheat, bread, and gluten than with the higher levels. In Table III no result is shown for the 16% gluten level fed with the gluten basal diet, because the observed nitrogen gain exceeded the expected response and could not be read from the standard curve. The values for valine (Table IV) showed the lowest availability for all samples tested. It is possible that the utilization of isoleucine and valine is antagonized by the amounts of leucine present in the basal diet and in the samples. Values for phenylalanine (Table IV) in wheat, flour, and bread were lower than those for the remaining amino acids in these samples. Except for wheat and flour on the gluten basal, the availability of methionine, tryptophan (Table III), and threonine (Table V) was 90% or greater for all samples. Phenylalanine in gluten

TABLE III

AVAILABILITY OF METHIONINE AND TRYPTOPHAN AS CALCULATED BY INCREASE IN CARCASS NITROGEN

SAMPLE	DIET LEVEL	FOOD INTAKE	AMINO ACID CONSUMED		CARCASS N GAIN	AVAILABLE AMINO ACID		AVAIL-ABILITY
			From Basal	From Sample		Total [a]	Sample [b]	
	%	g.	mg.	mg.	g.	mg.	mg.	%
			Methionine, gluten basal					
Wheat	35	181	130	158	1.60	255	125	79
	70	273	197	478	3.69	577	380	79
Flour	35	151	109	129	1.34	219	110	85
	70	243	175	417	3.34	503	328	79
Bread	35	170	122	154	1.75	275	153	99
	70	276	199	498	4.13	704	505	101
Gluten	8	173	125	173	1.88	293	168	97
	16	284	204	568	4.52	...	...	...
			Methionine, amino acid basal					
Wheat	22.5	128	64	72	0.76	133	69	96
	45	215	108	242	2.06	322	214	88
Flour	25	133	67	81	0.84	145	78	96
	50	214	107	262	2.29	355	248	95
Bread	22.5	127	63	74	0.80	138	75	101
	45	196	98	228	1.98	311	213	93
Gluten	5	135	67	84	0.86	148	81	96
	10	232	116	290	2.61	402	286	99
			Tryptophan					
Wheat	25	168	67	74	1.29	132	65	88
	40	244	98	173	2.82	255	157	91
Flour	25	161	64	61	1.12	118	54	88
	40	189	76	115	1.81	175	99	86
Bread	25	167	67	60	1.20	125	58	97
	40	216	86	124	2.31	214	128	103
Gluten	5	162	65	55	1.24	128	63	114
	8	220	88	119	2.27	211	123	103

[a] Obtained from standard curve (Fig. 1) for corresponding nitrogen gain.
[b] Total available amino acid found minus amino acid consumed from basal.

was utilized to the same high degree, but its values for wheat, flour, and bread were lower (Table IV).

Availability by Fecal Analysis. The extent to which amino acids are not absorbed from ingested food (and thus remain unavailable to the animal) has been employed as a means of determining the availability of amino acids in various foods. In the lysine study this method gave results in varying agreement with those obtained by nitrogen gain, but the comparison was considered to be doubtful because the collection period was limited to 3 days and the results may not have represented conditions over the entire feeding experiment. In the present studies fecal collections were made over the last 19 days.

TABLE IV
AVAILABILITY OF ISOLEUCINE, PHENYLALANINE, AND VALINE AS CALCULATED BY INCREASE IN CARCASS NITROGEN

Sample	Diet Level	Food Intake	Amino Acid Consumed		Carcass N Gain	Available Amino Acid		Availability
			From Basal	From Sample		Total[a]	Sample[b]	
	%	g.	mg.	mg.	g.	mg.	mg.	%
				Isoleucine				
Wheat	20	174	348	208	1.05	500	152	73
	35	235	470	493	2.29	917	447	91
Flour	20	180	360	217	1.22	558	198	91
	35	220	440	464	2.05	837	397	86
Bread	20	188	376	244	1.19	548	172	70
	35	218	436	494	2.27	910	474	96
Gluten	4	185	370	237	1.14	533	163	69
	7	246	492	551	2.61	1,030	538	98
				Phenylalanine				
Wheat	15	124	149	133	0.64	252	101	76
	25	160	192	286	1.21	402	210	73
Flour	15	123	148	131	0.70	266	118	90
	25	174	209	309	1.41	454	245	79
Bread	15	142	170	169	0.83	300	130	77
	25	170	204	337	1.58	498	294	87
Gluten	3	135	162	156	0.80	292	130	83
	5	180	216	346	1.92	588	372	107
				Valine				
Wheat	25	123	172	223	0.68	341	169	76
	40	174	244	505	1.43	606	362	72
Flour	25	135	189	223	0.74	362	173	78
	40	174	244	461	1.47	620	376	82
Bread	25	115	161	218	0.64	326	165	76
	40	174	244	527	1.55	650	406	77
Gluten	5	130	182	213	0.70	350	168	79
	8	170	238	447	1.47	620	382	85

[a] Obtained from standard curve (Fig. 2) for corresponding nitrogen gain.
[b] Total available amino acid found minus amino acid consumed from basal.

The method is based upon the observation that the amount of amino acid excreted in the feces is directly proportional to the amount of food consumed for a given diet. Calculations presume that the same proportion found when no sample is present in the diet will result from diets containing added sample, and that this amount can be subtracted from the total fecal content to yield a measure of the amino acid arising solely from the sample.

The percentage of sample amino acid found in the feces, subtracted from 100, is an expression of "percent availability." The experimental data and results are given in Tables VI–VIII, in the same order of grouping as in Tables III–V for ease of comparison.

TABLE V

AVAILABILITY OF LEUCINE AND THREONINE AS CALCULATED BY INCREASE IN CARCASS NITROGEN

Sample	Diet Level	Intake	Amino Acid Consumed		Carcass N Gain	Available Amino Acid		Availability
			From Basal	From Sample		Total[a]	Sample[b]	
	%	g.	mg.	mg.	g.	mg.	mg.	%
				Leucine				
Wheat	25	151	378	370	1.29	726	348	94
	40	196	490	769	2.21	1,190	700	91
Flour	25	153	382	376	1.17	665	283	75
	40	188	470	740	2.20	1,185	715	97
Bread	25	159	398	420	1.50	830	432	103
	40	200	500	846	2.54	1,400	900	106
Gluten	5	150	375	390	1.36	760	385	99
	7.5	186	465	725	2.23	1,200	735	101
				Threonine				
Wheat	30	206	412	269	1.70	655	243	90
	45	215	430	422	2.32	855	425	101
Flour	30	172	344	191	1.36	539	195	102
	45	205	410	341	1.87	710	304	88
Bread	30	204	408	267	1.90	716	308	115
	45	236	472	463	2.56	932	460	99
Gluten	6	167	334	188	1.34	534	200	106
	9	229	458	386	2.41	881	423	109

[a] Obtained from standard curve (Fig. 3) for corresponding nitrogen gain.
[b] Total available amino acid found minus amino acid consumed from basal.

Availability values determined by fecal analysis show good agreement between sample levels. For each amino acid the results for gluten were highest (nearly 100%) and those for wheat were lowest (approximately 80% or less), probably reflecting the differences in digestibility between products. The same degree of difference between basal diets in the values for wheat and flour are evident in Table IV as in Table III. These data support the similar findings with lysine (1) and may imply that the presence of gluten in the basal diet depresses the utilization of amino acids by its influence on digestion or absorption, or both.

Excretion availabilities tend to be higher in general for valine, phenylalanine, and isoleucine than those determined by nitrogen gain. For these amino acids some factor other than digestion or absorption may be limiting. The previous suggestion that leucine antagonism could explain the reduced utilization of isoleucine and valine is an example. Differences in which excretion values are lower than those obtained by nitrogen gain are observed for threonine, tryptophan, and leucine. Although the cause is unknown, it is probable that these arise from increased excretion of the amino acids on the sample diets rather

TABLE VI

AVAILABILITY OF METHIONINE AND TRYPTOPHAN AS DETERMINED BY FECAL ANALYSIS

SAMPLE	DIET LEVEL	AMINO ACID CONSUMED IN SAMPLE	TOTAL AMINO ACID IN FECES	FECAL AMINO ACID, NOT FROM SAMPLE [a]	SAMPLE AMINO ACID IN FECES [b]	UNABSORBED SAMPLE AMINO ACID	AVAILABILITY
	%	mg.	mg.	mg.	mg.	%	%
				Methionine, gluten basal diet			
Wheat	35	149	71	16	55	37	63
	70	453	121	24	97	21	79
Flour	35	122	38	13	25	20	80
	70	397	91	21	70	18	82
Bread	35	144	39	15	24	17	83
	70	472	76	24	52	11	89
Gluten	8	162	23	15	8	5	95
	16	538	45	25	20	4	96
				Methionine, amino acid basal diet			
Wheat	22.5	66	20	9	11	17	83
	45	223	60	15	45	20	80
Flour	25	74	15	9	6	8	92
	50	245	34	15	19	8	92
Bread	22.5	67	17	8	9	13	87
	45	206	34	13	21	10	90
Gluten	5	77	10	9	1	1	99
	10	274	20	16	4	1	99
				Tryptophan			
Wheat	25	339	142	42	100	29	71
	40	814	178	63	115	14	86
Flour	25	280	79	40	39	14	86
	40	537	142	49	93	17	83
Bread	25	276	92	42	50	18	82
	40	578	118	55	63	11	89
Gluten	5	252	52	41	11	4	96
	8	556	63	57	6	1	99

[a] Food consumed on sample diet x amino acid excreted per g. of diet containing no sample (as determined for each experiment in mg./g. food intake: methionine, gluten basal, 0.092; methionine, amino acid basal, 0.074; tryptophan, 0.055).

[b] Total amount of amino acid in feces minus amount not from sample.

than to increased efficiency of utilization of the amino acids for protein formation.

Few other studies have been published on the availability of amino acids in wheat products. Kuiken and Lyman (4) obtained values in excess of 90% for all essential amino acids in wheat, as determined by a fecal excretion method. Steele *et al.* (5) reported that the sulfur-containing amino acids of wheat flour were as well utilized as the crystalline amino acids for maintaining nitrogen balance of human subjects. Schweigert and Guthneck (6) found the methionine of wheat germ to be 66% available by growth studies, but only 10% was recovered in examination of the feces. A number of studies investigating other cereals illustrate differences apparently attributable to the

TABLE VII
AVAILABILITY OF ISOLEUCINE, PHENYLALANINE, AND VALINE AS DETERMINED BY FECAL ANALYSIS

Sample	Diet Level	Amino Acid Consumed in Sample	Total Amino Acid in Feces	Fecal Amino Acid, Not from Sample [a]	Sample Amino Acid in Feces [b]	Unabsorbed Sample Amino Acid	Availability
	%	mg.	mg.	mg.	mg.	%	%
				Isoleucine			
Wheat	20	188	77	28	49	26	74
	35	450	115	38	77	17	83
Flour	20	199	35	29	6	3	97
	35	433	89	36	53	12	88
Bread	20	215	42	29	13	6	94
	35	454	64	35	29	6	94
Gluten	4	212	32	29	3	1	99
	7	510	44	40	4	1	99
				Phenylalanine			
Wheat	15	121	41	19	22	18	82
	25	264	61	25	36	14	86
Flour	15	120	31	19	12	10	90
	25	289	49	28	21	7	93
Bread	15	157	26	22	4	3	97
	25	313	52	27	25	8	92
Gluten	3	144	28	21	7	5	95
	5	322	30	29	1	0	100
				Valine			
Wheat	25	206	63	21	42	20	80
	40	466	134	32	102	22	78
Flour	25	205	40	25	15	7	93
	40	428	59	32	27	6	94
Bread	25	199	41	21	20	10	90
	40	479	70	31	39	8	92
Gluten	5	193	27	23	4	2	98
	8	414	40	31	9	2	98

[a] Food consumed on sample diet x amino acid excreted per g. of diet containing no sample (as determined for each experiment, in mg./g. food intake: isoleucine, 0.176; phenylalanine, 0.170; valine, 0.198).
[b] Total amount of amino acid in feces minus amount not from sample.

method, but opinion has been divided as to their interpretation. Ousterhout *et al.* (7) describe the unknown action of intestinal microflora and the possible effect of diet on secretions into the intestinal tract as two important disadvantages of the fecal excretion method. On the other hand, De Muelenaere and Feldman (8) support the latter method because of the possible influence imposed by proteins of nonbalanced amino acid composition on growth rate. Evidence for both points of view can be seen in the data presented here and serves to emphasize the need for caution in interpretation of results. In the writers' opinion, the relation between amino acid intake and deposition of nitrogen is the more fundamental test and should be given the greatest consideration. Determination of the net unabsorbed amino

TABLE VIII
AVAILABILITY OF LEUCINE AND THREONINE AS DETERMINED BY FECAL ANALYSIS

Sample	Diet Level	Amino Acid Consumed in Sample	Total Amino Acid in Feces	Fecal Amino Acid, Not from Sample [a]	Sample Amino Acid in Feces [b]	Unabsorbed Sample Amino Acid	Availability
	%	*mg.*	*mg.*	*mg.*	*mg.*	%	%
				Leucine			
Wheat	25	348	92	31	61	18	82
	40	725	172	40	132	18	82
Flour	25	354	51	31	20	6	94
	40	698	86	39	47	7	93
Bread	25	395	51	33	18	5	95
	40	786	74	40	34	4	96
Gluten	5	364	35	31	4	1	99
	7.5	678	43	38	5	1	99
				Threonine			
Wheat	30	252	130	36	94	37	63
	45	394	168	37	131	33	67
Flour	30	176	77	29	48	27	73
	45	316	119	35	84	27	73
Bread	30	247	50	35	15	6	94
	45	428	87	40	47	11	89
Gluten	6	171	28	28	0	0	100
	9	360	38	39	−1	0	100

[a] Food consumed on sample diet x amino acid excreted per g. of diet containing no sample (as determined for each experiment in mg./g. food intake: leucine, 0.218; threonine, 0.185).
[b] Total amount of amino acid in feces minus amount not from sample.

acid by analysis of feces provides valuable accessory information but may not be sufficient in itself to evaluate the amino acid contribution of a food.

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