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Utilization of Amino Acids From Protein by the Growing Rat. Efficiency of Carcass Protein Formation

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The proportion of amino acids, especially the essential ones, in dietary protein is important in the utilization of amino acids for the growth of animals (1). The timing and degree of availability of these amino acids is also important in the efficiency of their utilization for growth (2). If it were not for differences in availability due to structural characteristics of protein, it would be possible to supplement any protein with the amino acids needed for an equal total content of all the amino acids and have the same growth response occur. However, a uniform response does not consistently occur (3,4). Ten Experiment Stations in the northeast region have cooperated in investigating three sources of amino acids (pure amino acids, milk protein and wheat gluten) at two levels (1.2% and 1.6% dietary nitrogen) with different subjects (humans, rats, pigs and protozoa) under different conditions (growth, maintenance and stress) in order to study availability of amino acids in protein. A manual of procedures has been compiled to insure close relationships among the experiments using the same species and different species (5).

Essential amino acids in the FAO provisional pattern (6) were supplied by the following three diets: (a) Pure amino acids; (b) a 5:1 casein–lactalbumin mixture supplemented with pure amino acids; and (c) wheat gluten supplemented with pure amino acids.

Some non-essential amino acids were supplied by the proteins and a mixture of non-essential amino acids in the proportions found in milk was added to each diet to bring to 75% the amount of nitrogen supplied by non-essential amino acids as called for by the FAO provisional pattern. The amino acid patterns of each diet are given by Babcock and Markley (7).

The Maine Experiment Station studied the effect of the above three nitrogen sources containing equal amounts of essential amino acids and nitrogen on the growth of rats over two and four weeks from the starting weight of 62±5 grams.

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Experimental

Male rats of the CFN strain were purchased from Carworth Farms at a weight as close to 50±5 grams as possible. The rats were housed individually in suspended wire cages in a room with constant temperature and humidity. All rats were fed a pre-experimental diet (table 1) for three or more days to bring their weights up to 62±5 grams and to accustom them to eating semi-synthetic diets. The nitrogen of the pre-experimental diet, 1.228%, was furnished by casein (0.9%), the amino acids in the NE-52 pattern described by Babcock and Markley (7) (0.3%) and DL–methionine (0.028%). The other components are listed in table 1.

After the pre-experimental period, the rats were divided into four evenly spaced weight groups between 57 and 67 grams and were randomly assigned to control and test groups of eight rats each. One experimental period was for two weeks and one for four weeks in order to test the reliability of a two-week growth period as an indicator of protein utilization. Thus, there was a total of 14 experimental groups. There were three groups at the 1.2% level of dietary N, three groups at the 1.6% level of dietary N and one control group sacrificed at zero time for each of the two growth periods.

The experimental diets were of the same composition as the pre-experimental diet with the exception that the nitrogen containing components were varied. The sources of nitrogen were as follows:

(a) all crystalline amino acids (AA).

(b) casein–lactalbumin (5:1) which was supplemented with individual amino acids to supply the FAO provisional pattern and a mixture of non-essential L–amino acids in the proportions found in milk (CLAA) to bring the nitrogen provided by non-essential amino acids to 75% as required by the FAO provisional pattern.

(c) wheat gluten supplemented with amino acids (WGAA) as in (b).

The amino acid sources of the nitrogen portions of these diets were presented by Babcock and Markley and are shown in table 2 (7). The final composition of the nitrogen portion of the casein–lactalbumin diet (CLAA) was casein–lactalbumin 49.8% and free amino acids 50.2%. That of the wheat gluten diet (WGAA) was wheat gluten 44.8% and free amino acids 55.2%. The compositions in table 2 are for the 1.2% N diets. For the 1.6% N diet, the amino acid and protein percentages were increased by one third and the carbohydrate portion, 1:1 sucrose–corn starch, decreased a corresponding amount.

The rats and the food were weighed to the nearest tenth of a gram daily. The amount of food for the three groups fed 1.2% N was determined by the lowest consuming group receiving 1.2% N, and the amount of food fed to the groups receiving 1.6% N was controlled by the lowest consuming group receiving 1.6% N.

After two weeks (or four weeks) the rats were killed in a closed jar containing ether and the intestines were removed, washed and placed in the abdominal cavity. The weight of the carcass for analytical purposes was taken at this time. The carcasses were frozen individually in plastic bags and were kept frozen until the carcass analysis was done.

TABLE 1

Composition of the 1.2% Nitrogen Diets

Component	Pre-experimental[1]	Test Diet		
		Amino Acid[2]	Casein–lactalbumin[2]	Wheat Gluten[2]
		Grams per 100 grams of diet		
Casein	6.52	—	—	—
Casein–lactalbumin[3]	—	—	4.54	—
Wheat Gluten[4]	—	—	—	4.24
Amino acids[5]	2.69	9.46	4.69	5.19
Cellulose[6]	2.00	2.00	2.00	2.00
Corn oil[7]	5.00	5.00	5.00	5.00
Mineral mix[8]	4.00	4.00	4.00	4.00
Vitamin mix[9]	2.20	2.20	2.20	2.20
Cornstarch[10]	38.80	38.67	38.78	38.69
Sucrose	38.80	38.67	38.78	38.69
	100.01	100.00	99.99	100.01

[1] Fed ad libitum.

[2] Restricted intake (see text).

[3] A 5:1 mixture of casein and lactalbumin to simulate milk protein.

[4] General Mills Pro-80 Vital Wheat Gluten.

[5] L–amino acids (except DL–methionine) to provide the provisional FAO pattern as described by Babcock and Markley (7). In the pre-experimental diet, approximately three-fourths of the nitrogen was supplied as casein (supplemented with DL–methionine) and one-fourth as amino acids in the proportions specified for the amino acid test diet.

[6] Non nutritive bulk, Alphacel, Nutritional Biochemicals Corporation, Cleveland.

[7] Mazola, Corn Products Company. Englewood Cliffs, N.J.

[8] Jones and Foster mineral mix purchased from Nutritional Biochemicals Corporation.

[9] Vitamin Diet Fortification Mixture in Dextrose, Nutritional Biochemicals Corp., Cleveland, Ohio. Composition per kilo: Vitamin A, 900,000 IU; Vitamin D, 100,000 IU; Alpha tocopherol, 5.0 gm.; Ascorbic acid, 45.0 gm.; Inositol, 5.0 gm.; Choline Chloride, 75.0 gm.; Menadione, 2.25 gm.; p–Aminobenzoic acid, 5.0 gm.; Niacin, 4.5 gm.; Riboflavin, 1.0 gm.; Pyridoxine hydrochloride, 1.0 gm.; Thiamine hydrochloride, 1.0 gm.; Calcium Pantothenate, 3.0 gm.; Biotin, 20 mgm.; Folic acid, 90 mgm.; Vitamin B_{12}, 1.35 mgm; Dextrose to make one kilogram.

[10] Argo, Corn Products Company.

TABLE 2
Amino Acid Sources[1]

	FAO pattern	**Amino acid diet**	Casein-lactalbumin diet		Wheat gluten diet	
		Amino acids[2]	Protein[3]	Amino acids	Protein[3]	Amino acids
	mg/gm dietary nitrogen	mg/gm dietary nitrogen	mg/gm dietary nitrogen		mg/gm dietary nitrogen	
Alanine	—	329	119	197	80	182
Arginine	—	349[4]	115	209[4]	112	193[4]
Aspartic acid	—	697	262	417	96	387
Cystine	126	126	29	97	63	63
Glutamic acid	—	2242	722	1341	1163	1243
Glycine	—	189	60	113	112	105
Histidine	—	269[4]	87	161[4]	72	149[4]
Isoleucine	270	270	187	83	108	162
Leucine	306	306	306	0	217	89
Lysine	270	270[4]	257	13[4]	53	217[4]
Methionine	144	144	85	59	47	97
Phenylalanine	180	180	171	9	180	0
Proline	—	1066	328	637	514	591
Serine	—	563	187	337	148	312
Threonine	180	180	147	33	83	97
Tryptophan	90	90	52	38	30	60
Tyrosine	180	180	166	14	103	77
Valine	270	270	223	47	116	154
Ammonia	—	0	52	0	83	0
Nitrogen content	1000	1000	498	502	448	552

[1] Babcock and Markley, 1967. J. Nutrition, 93:368.

[2] NE–52 amino acid pattern.

[3] Cystine and tryptophan in the proteins were determined microbiologically by Prof. Pela Braucher at the University of Maryland. Other amino acids in the proteins were determined by ion exchange chromatography of acid hydrolysates by Prof. H. H. Williams at Cornell University.

[4] Added as an equivalent amount of the monohydrochloride.

Upon removal from the freezer, the carcasses were allowed to thaw in the plastic bags, cut into pieces with a large pair of scissors and dried to constant weight in a vacuum oven at 80-90°C. The dried carcass was ground in an intermediate size Wiley mill and the ground carcass was extracted in a Soxhlet extractor with ether. The extracted, ground rat tissue was converted to a fine powder with a mortar and pestle. Triplicate analyses for N were done by a microkjeldahl method (8). Student's t–distribution table was used in obtaining probability values.

A number of preliminary experiments were run to perfect techniques and to indicate the range of values to be expected in the data.

Results

Two-Week Growth Period

Weight gains

When the groups fed 1.2% N are considered (table 3), the group fed the CLAA gained significantly more weight than the AA group ($P<0.05$), while the AA and WGAA groups gained about the same amount. At the 1.6% dietary N level, however, the AA and CLAA rations resulted in similar weight gains, while the weight gain from the WGAA ration was significantly less ($P<0.01$) than from the other two.

Carcass water

The water contents of all six groups fed the diets for two weeks were similar. During the two weeks the carcass moisture content went down 6.2 to 7.0% (as was expected with age) ($P<0.001$).

Carcass fat

There was no significant difference in the fat composition among the six groups fed the diets for two weeks. However, the rat carcass composition of all groups collectively was 8% higher in fat after the two weeks. This increase in fat content is nearly the same as the loss in carcass water over the two-week period.

Carcass protein

The WGAA group receiving 1.2% N was significantly higher in carcass protein than the other groups fed 1.2% N and the three groups fed 1.6% N ($P<0.001$). Wheat gluten without supplementation is known to be a poor quality protein (9, 10), but the supplementation improved this diet to a level above the CLAA diet. The AA and CLAA

TABLE 3

Effect of Protein Type and Level on the Growth and Carcass Composition of Rats after Two Weeks

Diet			Carcass Composition		
% N	Protein Source	Weight Gain	H_2O	Wet Wt. Fat	Wet Wt. Protein
		gm	%	%	%
—	Start of experiment	0	70.0±.26	8.43±.2	16.6±.12[1]
1.2	Crystalline amino acids (AA)	29.1±1.20[2,3]	63.1±.46	16.3 ±.3	15.4±.1
1.2	Casein-lactalbumin (CLAA)	33.1±1.23	63.2±.49	16.0 ±.4	15.4±.2
1.2	Wheat gluten (WGAA)	29.6±1.27	63.1±.72	15.6 ±.7	16.6±.1
1.6	Crystalline amino acids (AA)	53.7±1.99	63.0±.85	17.0 ±.6	15.3±.2
1.6	Casein-lactalbumin (CLAA)	54.0±0.87	63.3±.47	16.7 ±.4	15.8±.1
1.6	Wheat gluten (WGAA)	44.5±1.75	63.8±.94	15.6 ±.8	15.9±.1

[1] N x 6.25.
[2] Eight rats per group.
[3] Standard error of the mean.

groups fed 1.2% N and the AA group fed 1.6% N had similar carcass protein levels. The groups fed 1.6% N as CLAA or WGAA had significantly higher carcass protein percentages than the group fed 1.6% N as AA ($P<0.05$).

The sums of the percentages of water, fat and protein for all groups fed the diets for two weeks fell between 94.6% and 95.8%.

Four-Week Growth Period

Weight gain

The higher weight gain of the CLAA group fed 1.2% N was not significant due to the larger standard errors at four weeks (table 4.) The CLAA group fed 1.6% N did make a significantly higher weight gain than the WGAA group ($P<0.001$) at the same nitrogen level.

Carcass water

As was the case when the six diets were fed for two weeks, there was no significant difference in moisture content among the six groups. Over the four weeks the carcass water content went down 8.9 to 11.5%. The decrease in carcass water content was 2.7 to 4.6% during the second two weeks, much less than during the first two weeks.

Carcass fat

In the groups fed the 1.2% N level of dietary protein and in the groups fed the 1.6% N level of dietary protein there was no difference in the fat content of the carcasses. When the same diet is compared at the two dietary N levels, the fat content of the WGAA group receiving 1.2% N was significantly higher than that of the WGAA group fed 1.6% N ($P<0.005$).

Carcass protein

When 1.2% N was fed, the rats receiving the AA had a higher carcass protein level than those receiving WGAA ($P<0.001$). The rats fed CLAA also had a higher carcass protein level than those fed WGAA ($P<0.005$).

At the 1.6% dietary N level the CLAA diet resulted in a higher carcass protein level than the AA diet ($P<0.001$). The AA and WGAA groups had similar levels.

The carcass protein levels after four weeks were higher than the controls at zero time when 1.6% N diets were fed (AA, $P<0.05$; CLAA, $P<0.001$; and WGAA, $P<0.025$). This was not true for the groups fed 1.2% N, nor was this difference observed after two weeks with the groups fed 1.6% N.

TABLE 4

Effect of Protein Type and Level on the Growth and Carcass Composition of Rats after Four Weeks

Diet			Carcass Composition		
% N	Protein Source	Weight Gain	H_2O	Wet Wt. Fat	Wet Wt. Protein
		gm	%	%	%
—	Start of experiment	0	70.9±.23	7.51±.27	16.60±.10[1]
1.2	Crystalline amino acids (AA)	61.8±3.21[2,3]	59.4±.99	19.53±.98	16.75±.12
1.2	Casein–lactalbumin (CLAA)	68.9±2.46	59.8±.77	19.01±.67	16.39±.20
1.2	Wheat gluten (WGAA)	61.8±3.74	59.4±.79	19.42±.69	15.59±.11
1.6	Crystalline amino acids (AA)	94.9±1.37	60.4±.44	17.81±.53	16.97±.11
1.6	Casein–lactalbumin (CLAA)	101.0±2.84	61.0±.71	16.35±.66	17.59±.08
1.6	Wheat gluten (WGAA)	86.9±3.65	62.0±.72	15.82±.69	17.22±.21

[1] N x 6.25.
[2] Eight rats per group.
[3] Standard error of the mean.

The carcass protein levels in all groups but the WGAA group fed 1.2% N were higher after four weeks than after two weeks. The carcass protein level of the WGAA group fed 1.2% N was lower after four weeks than after two weeks on this diet.

Efficiency of dietary N utilization

Table 5 presents the food consumption data, grams gained per gram of food consumed, and the grams of nitrogen gained per gram of nitrogen fed. The rats receiving 1.6% N all consumed more food than those receiving the corresponding 1.2% N diet initially due to a greater acceptability of this dietary nitrogen level and later because of increased size. The rats fed these diets for four weeks consumed slightly over twice as much as the rats fed the corresponding rations for two weeks.

The CLAA diet showed a tendency toward a greater weight gain per gram of food consumed when 1.2% N was fed for two weeks ($P<0.20$) and when 1.2 and 1.6% N were fed for four weeks ($P<0.10$). The AA group showed a high food efficiency when 1.6% N was fed for two weeks, but a low efficiency when 1.2% N was fed for two and four weeks. Wheat gluten resulted in the lowest food efficiency when 1.6% N was fed for two weeks ($P<0.01$) and also showed this tendency at four weeks ($P<0.20$). A comparison of results at different dietary nitrogen levels reveals that the food efficiencies at 1.2% N were the same for two and four weeks, but the two-week efficiencies are greater than the four-week efficiencies at 1.6% N.

The nitrogen efficiency ratios did not parallel the food efficiency ratios as table 5 clearly shows. In evaluating protein utilization the decision must be made as to whether weight gain or actual utilization of the nitrogen for protein formation is paramount. The actual utilization of the dietary nitrogen for protein formation should be considered more important, because weight can be added with lower cost food materials, but protein requires essential amino acids and adequate nitrogen.

After two weeks on a 1.2% N diet the WGAA group retained a greater percentage of dietary N than the AA group ($P<0.005$).

After two weeks at the 1.6% N level of dietary nitrogen, feeding the AA and WGAA diets resulted in a similar nitrogen retention ratio, while the CLAA diet was much more efficiently utilized ($P<0.001$).

Of the six groups fed the diets for two weeks, the AA group fed 1.2% N had the lowest nitrogen utilization ratio and the CLAA group receiving 1.6% N had the highest ratio.

During the four-week period when 1.2% N was fed, the WGAA group was definitely inferior in the retention of nitrogen to the AA and

TABLE 5

Efficiency of Utilization of Dietary N by Rats During Two and Four Weeks

Time in Weeks	N	Diet	Total Food Consumption	$\frac{\text{gm Gained}}{\text{gm of Food Consumed}}$	$\frac{\text{gm N Gained}}{\text{gm N Consumed}}$
	%		gm		
2	1.2	Crystalline amino acids (AA)	161.4±3.4[1,2]	0.180±.006	0.322±.015
2	1.2	Casein–lactalbumin (CLAA)	167.4±1.2	0.197±.007	0.370±.018
2	1.2	Wheat gluten (WGAA)	162.5±2.9	0.181±.007	0.393±.010
2	1.6	Crystalline amino acids (AA)	182.1±0.4	0.295±.011	0.373±.010
2	1.6	Casein–lactalbumin (CLAA)	182.5±1.0	0.295±.005	0.471±.009
2	1.6	Wheat gluten (WGAA)	176.5±2.4	0.252±.008	0.371±.007
4	1.2	Crystalline amino acids (AA)	339.7±6.85	0.181±.0070	0.402±.006
4	1.2	Casein–lactalbumin (CLAA)	350.3±4.68	0.197±.0057	0.418±.011
4	1.2	Wheat gluten (WGAA)	342.3±7.13	0.180±.0077	0.341±.001
4	1.6	Crystalline amino acids (AA)	337.7±3.44	0.251±.0030	0.428±.008
4	1.6	Casein–lactalbumin (CLAA)	381.7±2.16	0.264±.0066	0.470±.011
4	1.6	Wheat gluten (WGAA)	362.9±0.75	0.239±.0065	0.405±.014

[1] Eight rats per group.

CLAA ($P<0.001$) groups. The AA and CLAA groups were of similar efficiencies.

Over the four-week period when 1.6% N was fed, the WGAA was also inferior to the CLAA ($P<0.005$) group in the retention of dietary nitrogen, and the CLAA group was more efficient than the AA group in this function ($P<0.001$).

The efficiency of nitrogen retention for the CLAA groups receiving 1.6% N for both two and four weeks was the same. In the case of the AA and WGAA groups, the retention was greater after four weeks than after two weeks. At the 1.2% dietary nitrogen level the nitrogen retention ratio of the WGAA group decreased during the period from two to four weeks, while the AA and CLAA ratios increased.

Discussion

Our experiments emphasized that there are characteristics inherent in individual proteins which make them different in their utilization for carcass protein formation. The essential amino acid composition and the final nitrogen concentration were the same in each of the levels of dietary N tested. In comparison to the diets containing all free amino acids, the effect may be positive (a greater nitrogen retention efficiency occurred in the groups fed CLAA containing 1.6% N for two and four weeks) or negative (the WGAA group fed 1.2% N for four weeks had a poorer nitrogen efficiency ratio). The effect may be expressed at one time period and not another (WGAA had a higher nitrogen efficiency ratio at two weeks but a lower one at four weeks) and may be expressed at one level of dietary N and not another (the CLAA group had a higher nitrogen efficiency ratio at the 1.6% N level after two weeks than the CLAA group at the 1.2% N level after two weeks).

Babcock and Markley (7) found that pigs gained more weight per gram of nitrogen retained when wheat gluten was fed. With rats, only one of the four groups fed WGAA (1.2% N for four weeks) achieved a greater weight gain per gram of N fed. The higher relative proportion of fat in the pigs due to species differences undoubtedly accounts for this difference. The WGAA diets did not cause a greater deposition of fat in the rat. When 1.6% N was fed for four weeks, the WGAA group had significantly less fat ($P<0.025$) than the AA group. The difference was accounted for by an equivalently greater water content in the WGAA group.

It is difficult to evaluate the possible effects of the amino acid hydrochlorides and the lack of amides in the crystalline AA diets which

Babcock and Markley (7) discussed. The weight gains of the AA group were the same or greater than those of the WGAA group and the nitrogen efficiencies of the AA and WGAA groups were the same in two cases, in one case an AA group was higher, and in another case a WGAA group was higher. Babcock fed the non-nitrogenous portion separately to the pigs and we fed all dietary components together. The immediate buffering effect of the mineral portion of the diet when protein is not fed separately may have some effect here. After metabolism, of course, the net effect would be the same.

If there were an inhibitory factor in wheat gluten or a growth enhancing factor in the casein–lactalbumin, it would manifest itself at both time periods and both nitrogen levels. The WGAA had the lowest nitrogen efficiency ratio in only one case and the CLAA groups receiving 1.6% N over two and four weeks were the only ones showing a significantly superior N retention ratio.

The availability of the amino acids from these diets cannot be the sole factor in the efficiency of nitrogen utilization for tissue formation in these growing rats since the diets containing crystalline amino acids alone would have given maximum growth. Over half of the nitrogen from the CLAA and WGAA diets also came from crystalline amino acids which were used to achieve the FAO pattern and to provide equal non-essential amino acid nitrogen. Yet definite indications of the superiority of the CLAA diet appeared in two cases and the WGAA diet appeared to be inferior in one case.

The possibility of inhibition due to a high concentration of free amino acids must be considered. The highest level of nitrogen, 1.6%, fed for the longest time, four weeks, would show the greatest inhibition if it were a significant factor. Since these groups achieved the highest nitrogen efficiency ratios, it may be concluded that no significant inhibition due to large amounts of free amino acids occurred.

The difference in results between pigs and rats can be attributed to species differences, method of feeding the nitrogen and the fact that it was possible to study the rats under a greater variety of conditions. The rat experiments indicate that a variety of conditions such as N level and time do have an effect. The results after four weeks are the most consistent.

Summary

Three diets—amino acids (AA), casein–lactalbumin (CLAA) and wheat gluten (WGAA)—were made up to the FAO pattern in essential amino acids and to the same total nitrogen (N) concentration. These diets were fed to rats at two levels, 1.2 and 1.6% N, and over

two periods of time, two and four weeks. Variation with time and with dietary nitrogen level was found for weight gains, nitrogen efficiency levels and carcass fat levels.

The ratio of grams nitrogen gained per gram of nitrogen fed was highest for groups fed CLAA at the 1.6% N level after two weeks and four weeks. The WGAA diet resulted in a ratio similar to that from the CLAA diet when 1.2% N was fed for two weeks. After four weeks the WGAA group at each nitrogen level had the lowest nitrogen retention efficiency.

Groups fed CLAA for four weeks had the greatest weight gains at both levels of nitrogen and at the 1.2% level of dietary nitrogen when CLAA was fed for two weeks. The AA and CLAA groups gained similar amounts when fed 1.6% nitrogen for two weeks.

The grams of nitrogen gained per gram of nitrogen fed ratios do *not* parallel the grams of weight gained per gram of food fed ratios.

After four weeks groups fed 1.6% N had a lower fat content than those fed 1.2% N. The fat content of rats fed 1.2% N was greater after four weeks than after two weeks.

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