Matabolism of Amino Acids in Aquatic Animals—III*¹ Indispensable Amino Acids for *Tilapia zillii*

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The indispensable amino acids for *Tilapia (Tilapia zillii)* are reported. Fingerlings were fed with experimental diets deficient in a single amino acid for a period of eight weeks. Growth rates with these diets were compared with that obtained with the basal ration. According to the results, arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine were found to be essential for normal growth of *Tilapia*. In contrast, tyrosine, glycine, alanine, aspartic acid, cystine, glutamic acid, proline, and serine were found to be non-essential for normal growth. It was thus evident that *Tilapia* requires the same ten amino acids reported to be essential for other fishes. The fish showed better growth with casein diet.

Although the essential amino acids for different species of fish have been reported by many researchers¹⁻⁶, research has not established that similar amino acids are essential for all species.

AOE *et al.*⁷ stated that the amino acid test diet failed to maintain the growth of young carp but NOSE *et al.*⁸ succeeded in sustaining the growth of carp to some extent with amino acid test diet sufficient for the determination of essential amino acids and found that the same ten amino acids reported essential for other fish are also essential for carp.

Recently, *Tilapia* has shown a promising future for commercial culture. Knowledge of its nutritional requirements is required for its successful cultivation and for development of an amino acids-enriched commercial feed for maximum production but apparently nothing is known. The present work was undertaken to determine the essential amino acids for *Tilapia* in such an application and to compare the effect of a casein diet on the growth rate against that of an amino acid basal ration.

Materials and Methods

Preparation of Experimental Diets

All the amino acids used were in the L-form.

Table 1 represents the composition of the basal ration. The proportions of amino acids used are shown in the foot-note of the Table. The vitamin and mineral mixture was prepared according to HALVER *et al.*⁸

Table 1. Composition of casein and amino acid basal diets

Constituent	Content (g)	
Casein	50	—
Amino acid mixture ¹	—	50
Cod liver oil	2	2
Soybean oil	5	5
Dextrin	19	19
Cellulose powder +		
Vitamin mixture	10	10
Mineral mixture	4	4
Carboxy methyl cellulose	10	10
Water	100	100

¹ 50 g mixture contains the following proportion of amino acids: arginine HCl 3.5, histidine HCl 1.5, isoleucine 3.0, leucine 4.25, lysine HCl 3.5, methionine 1.25, phenylalanine 2.5, threonine 2.0, tryptophan 0.75, valine 3.0, tyrosine 2.75, glycine 4.0, alanine 3.0, aspartic acid 4.0, cystine 0.5, glutamic acid 5.0, proline 3.5 and serine 2.0.

Deficient diets were prepared by deleting a single amino acid from the basal ration and replacing it with an equal amount of cellulose flour.

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Before mixing with a solidifying agent (C.M.C.), the pH of each diet was adjusted to 7.5 by adding the necessary quantity of 3 N NaOH. The diets were refrigerated at -10°C until used.

Experimental Fish and Feeding Method

Fingerlings were obtained from a local hatchery in two lots during two phases of the experiment. The fish were acclimated for two weeks, first to a commercial diet and then to the experimental one.

Feeding trials were conducted in covered $30 \times 30 \times 100$ cm aquaria equipped with filters. The water was circulated, aerated at 400 ml per min and kept at 25°C . The feeding experiment was carried out for a period of eight weeks. The fish were fed with the experimental diet four times a day following a very rigid schedule, (9 a.m., 12 a.m., 3 p.m. and 6 p.m.) and were weighed weekly. The daily portion of the experimental diet fed to the fish totaled about 10% of their body weight.

Since only twelve aquaria were available, the experiment was conducted in two phases. In the first, the growth rates of the fish on the ten deficient diets were compared to those of fish fed with a complete amino acid basal ration. The basal ration growth rate was also compared to that obtained with a casein diet. Twenty fingerlings averaging about 0.46 g in body weight were released in each aquarium. After four weeks of feeding, the lots of fish exhibiting growth compara-

ble to the control lot were discarded. Each of the groups showing no growth or very little growth when compared to the control was divided into two equal subgroups. One subgroup continued to be fed with the same deficient diet while the other was given a complete basal ration. The experimental feeding was then extended for another four weeks.

In the second phase, the growth rates of the remaining eight deficient groups, which averaged about 0.60 g in body weight, were compared with the control group. The same general feeding procedure described for the first section was followed.

Results and Discussion

The average body weights recorded during the course of experimental feeding in the first section are shown in Table 2. Fish fed with diets deficient in arginine, histidine, isoleucine, leucine or lysine exhibited retarded or, in the case of lysine, minimal growth. These amino acids were thus assumed to be "essential" for normal growth.

Fish fed with diets deficient in tyrosine, glycine, alanine, aspartic acid or cystine, however, exhibited a growth equivalent to the controls. The fish fed with these "non-essential" amino acids were then discarded to provide aquarium space in which the recovery test was conducted. During the recovery test the subgroups in which deficient diets were replaced by the basal ration exhibited growth

Table 2. Average body weight of *Tilapia* on various diets (First Phase)

Diets	Average weight (g)								
	Initial	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
Casein	0.51	0.65	0.76	0.88	0.98	1.08	1.19	1.33	1.48
Basal control	0.51	0.52	0.56	0.60	0.65	0.71	0.77	0.84	0.94
Arginine-deficient	0.46	0.44(1)	0.40	0.39	0.39	0.40	0.42(1)	0.41	0.42
Basal	—	—	—	—	0.39	0.42	0.46	0.50	0.57
Histidine-deficient	0.46	0.45	0.43	0.42	0.41	0.43	0.43	0.46(1)	0.44
Basal	—	—	—	—	0.41	0.46	0.57(1)	0.66	0.78
Isoleucine-deficient	0.29	0.29	0.27	0.26	0.25	0.25	0.24(1)	0.24(1)	0.26(2)
Basal	—	—	—	—	0.25	0.29	0.29	0.34	0.38
Leucine-deficient	0.33	0.32(2)	0.32(2)	0.31	0.31(1)	0.33	0.31	0.32	0.32
Basal	—	—	—	—	0.31	0.32	0.33	0.39	0.45
Lysine-deficient	0.45	0.46	0.43	0.43	0.47(1)	0.44	0.53(1)	0.54(1)	0.55
Basal	—	—	—	—	0.47	0.59	0.65	0.84(1)	0.98
Tyrosine-deficient	—	—	—	—	0.47	0.59	0.65	0.84(1)	0.98
Tyrosine-deficient	0.82	0.80	0.83	0.91	0.98	—	—	—	—
Glycine-deficient	0.38	0.36	0.39	0.43	0.47	—	—	—	—
Alanine-deficient	0.42	0.41	0.44	0.49	0.53	—	—	—	—
Aspartic acid deficient	0.43	0.43	0.46	0.52	0.56	—	—	—	—
Cystine-deficient	0.50	0.48	0.53	0.58	0.62	—	—	—	—

The numbers in parentheses are the mortality during the preceding week.

Fig. 1. Growth of fish on various diets. The arginine-deficient group was divided after 4 weeks. One subgroup was continued on the same deficient diet; the other with the basal diet.

parallel or superior to the control group, thus confirming the essential nature of the amino acids replaced. Although the subgroups which continued to be fed with deficient diets did not show any significant growth, their growth was not arrested. This phenomenon may be due to less competition for food within the reduced population. The positive growth response upon restoration of the missing essential amino acid in the diet has been presented graphically in Fig. 1. Fish on the casein diet grew faster than those on the amino acid test diets (Fig. 1) although they were less active to the diet than the latter.

Deficiency symptoms were most acute in the

leucine-deficient fish. This group exhibited the highest mortality rate (25%) followed by isoleucine (20%), lysine (15%), arginine (10%) and histidine (5%). Fifty percent of the leucine-deficient population developed lordosis as well. Lordosis was also observed to some extent in isoleucine, arginine and histidine-deficient fish. No definite loss in appetite, however, was shown by any of the deficient groups during the entire feeding period. The effects on growth of the basal diet and arginine, histidine, leucine and isoleucine-deficient diets are represented in Fig. 2.

The lordosis disappeared upon replacing the deleted amino acid in the ration as had been established earlier for sockeye salmon²⁾ and rainbow trout.³⁾ However, they found scoliosis and lordosis only in tryptophan-deficient fish. Nose *et al.*,⁶⁾ however, observed lordosis in carp receiving both essential and non-essential amino acid diets as well as those on the control diet. No such specific deficiency symptoms were reported for the eel⁵⁾ or channel catfish.⁴⁾ These results indicate that the development of deficiency symptoms cannot always be attributed to the deficient amino acids, but rather to the individual response of the fish to the diet.

The results of the feeding experiment of the second phase are presented in Table 3. In this phase, methionine, phenylalanine, threonine, tryptophan and valine were found to be essential for normal growth of *Tilapia*. These results were confirmed by the recovery test described earlier. Glutamic acid, proline and serine were found to be non-essential. No definite deficiency symptoms were discernible in this phase. Mortality rates were also lower than in the first phase. It is worthy to note here that none of the fish receiving non-essential amino acid-deficient diets or the basal

Fig. 2. *Tilapia zillii* fed with amino acid control diet and deficient one.

Table 3. Average body weight of *Tilapia* on various diets (Second Phase)

Diets	Average weight (g)								
	Initial	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
Casein	0.65	0.80	0.93	1.10	1.23	1.36	1.53	1.72	1.93
Basal control	0.65	0.62	0.69	0.76	0.83	0.91	0.99	1.07	1.18
Methionine-deficient	0.58	0.56	0.56	0.57	0.57	0.61	0.59	0.59	0.59
Basal	—	—	—	—	0.57	0.65	0.71	0.79	0.89
Phenylalanine-deficient	0.45	0.43	0.43	0.43	0.43	0.43	0.45	0.46	0.48
Basal	—	—	—	—	0.43	0.46	0.49	0.54	0.62
Threonine-deficient	0.71	0.67	0.66	0.69	0.68	0.71	0.70(1)	0.69	0.69
Basal	—	—	—	—	0.68	0.78	0.84	0.91	1.00
Tryptophan-deficient	0.44	0.42	0.41	0.42	0.42(1)	0.43	0.41	0.42	0.43
Basal	—	—	—	—	0.42	0.46	0.50	0.56	0.64
Valine-deficient	0.56	0.53	0.52	0.51	0.51	0.53	0.52	0.52	0.53
Basal	—	—	—	—	0.51	0.59	0.64	0.71	0.82
Glutamic acid-deficient	0.65	0.66	0.69	0.78	0.85	—	—	—	—
Proline-deficient	0.63	0.65	0.65	0.71	0.76	—	—	—	—
Serine-deficient	0.70	0.72	0.75	0.81	0.89	—	—	—	—

The numbers in parentheses are the mortality during the preceding week.

diet died while the essential amino acid-deficient fish showed an average mortality of 10% during the experimental period. The growth rates obtained with proline-deficient diet deviated to some extent from the control one (Table 3). It indicates that proline synthesized by *Tilapia* is not sufficient to sustain its growth. The result obtained by feeding the casein diet in the second phase was similar to that obtained during the first phase. It is thus obvious that a casein diet is more effective than the amino acid basal ration in sustaining growth of *Tilapia*.

Finally, the following conclusions can be drawn from this experiment: first, *Tilapia zilli* requires arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine for normal growth, as do other fish previously reported.¹⁻⁶ In contrast, tyrosine, glycine, alanine, aspartic acid, cystine, glutamic acid, proline and serine are not required for normal growth. Second, on the basis of this experiment and other previous reports, the develop-

ment of deficiency symptoms are dependent upon the species concerned and not always upon an amino acid deficiency in the diet. Third, casein is superior to the amino acid test diet in sustaining growth of *Tilapia*.

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