Plasma Amino Acid Changes in *Tilapia nilotica* Fed a Casein and a Corresponding Free Amino Acid Diet*¹

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Starved *Tilapia nilotica* were fed a casein diet and an amino acid diet of similar composition. The change in concentrations of plasma-free amino acids of *Tilapia* at 0, 2, 4, 8, 12 and 24 hours after administering the two diets was examined. With respect to the casein diet, all plasma-free essential amino acids attained their maximal values at 4 hours after feeding, and then declined to the fasting levels within 24 hours. With the amino acid diet, all plasma-free essential amino acids peaked at 2 hours, and returned to their fasting levels within 4 to 8 hours. Free non-essential amino acids showed unique patterns of change in the plasma of *Tilapia* after both the casein diet and the free amino acid diet were administered.

The essential amino acids for test fish have been established qualitatively and quantitatively on the basis of growth using the amino acid test diet. MAZID *et al.*¹⁾ determined the optimum dietary protein level for *Tilapia zillii* and also reported that *Tilapia* require the same ten essential amino acids as other fishes that have been studied such as, chinook salmon, rainbow trout, sockeye salmon, eel, channel catfish, carp, red sea bream and plaice. However, MAZID *et al.*²⁾ showed that *Tilapia* grew poorly on a free amino acid diet, a result similar to that found by AOE *et al.*³⁾ for carp. PLAKAS *et al.*⁴⁾ examined changes in the levels of circulating plasma free amino acids in carp after feeding a casein and free amino acid diet, and found some significant differences in plasma free amino acid patterns between the two diets. In an earlier study with rainbow trout,⁵⁾ (a fish which can utilize an amino acid diet nearly as well as a protein diet), we investigated plasma amino acid changes by force-feeding casein and a corresponding amino acid mixture, and compared the results with those obtained by PLAKAS *et al.*⁴⁾ for carp. The results indicated that all dietary free essential amino acids can not be absorbed simultaneously by carp unlike rainbow trout. More recently in our laboratory,⁶⁾ it was shown that a crystalline amino acid diet as well as a casein diet was able to support normal growth

of carp fry, if the number of dialy feedings was increased.

In order to expand the data base in the area of amino acid utilization by fish, the present study was undertaken. It compares the changes in plasma free amino acids in *Tilapia* after feeding a casein and a corresponding amino acid diet, and examines the inability of this species to utilize a free amino acid diet.

Materials and Methods

Tilapia nilotica (mean weight 196.5 g) were acclimated to a commercial diet for one week and then divided into two 400-L tanks with 70 fish per tank. The water temperature was held at $25 \pm 1^\circ\text{C}$ and water quality was maintained by circulation through activated carbon filters (1 l/min), supplemental aeration, periodic aquaria cleaning, and water changes with preconditioned water.

Description and Preparation of Test Diets

The composition of purified diets used in the present study are shown in Table 1. Vitamin free casein (NBC) was purchased from Nakarai Chemicals Ltd. Crystalline amino acids were provided in the L-form by the Kyowa Hakko Kogyo Co., Ltd. The test diets were adjusted

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Table 1. Composition of the experimental diets

Ingredients	Test diets	
	Casein	Amino acid mix.
	(%)	(%)
Casein	50.0	—
Amino acid mix.* ¹	—	50.0
α -Starch	8.0	8.0
Dextrin	20.0	20.0
Cod liver oil	2.0	2.0
Soybean oil	4.0	4.0
α -Cellulose plus vitamin mix.* ²	2.0	2.0
Mineral mix.* ³	4.0	4.0
α -Cellulose	8.0	8.0
Carboxymethylcellulose	2.0	2.0

*¹ in %: Arg-HCl, 4.06; His-HCl, 2.99; Ile, 4.52; Leu, 7.88; Lys-HCl, 8.59; Met, 2.47; Phe, 4.19; Thr, 3.86; Trp, 1.26; Val, 5.40; Ala, 2.61; Asp, 6.80; Cys, 0.91; Glu, 21.85; Gly, 1.83; Pro, 10.52; Ser, 5.24; Tyr, 4.83.

*² An equal mixture of α -cellulose and the vitamin mixture reported by HALVER *et al.*⁽⁷⁾

*³ U.S.P. XII salt mixture No. 2 plus trace minerals reported by HALVER.⁽⁸⁾

with NaOH to give a final pH 6.8, as described by NOSE *et al.*⁽⁹⁾ The wet diets were extruded with plastic syringes and dried in a forced air oven at 40°C overnight to a final moisture content of approximately 10%.

Experimental Procedure

Each tank of fish were fed the experimental diets once daily for one week. After a 4-day fasting period, five fish were removed from each tank for determination of the fasting level of plasma amino acids (0 hour). Two hours later, the remaining fish were fed the casein or the amino acid diet to satiation. Thirty minutes after feeding the casein or the amino acid diet, experimental tanks were cleaned by siphoning to remove any residual diet. At 2, 4, 8, 12 and 24 hours after feeding, five fish per tank were withdrawn, anesthetized in MS-222 (0.01% solution), and blood taken from the caudal peduncle with heparinized syringes. The five blood samples from each tank at each sampling time were immediately centrifuged at 3,000 rpm for 20 minutes to collect plasma. Equal volumes of plasma from five fish within the same group were pooled as one sample for amino acid analysis.

Amino Acid Analysis

Preparation of plasma for free amino acid analysis was performed similar to the method of STEIN and MOORE.⁽¹⁰⁾ To a 1.2 ml-sample of

plasma, 0.5 ml of 1.2 μ M/ml-norleucine solution was added as an internal standard. Deproteinization of the plasma was carried out by the addition of 7 ml of 1%-picric acid solution, after which the suspension was centrifuged at 3,000 rpm for 15 minutes. The supernatant was applied to the top of a column packed with Dowex 2-x8 resin (200-400 mesh, Bio-Rad Laboratories) in the chloride form to remove excess picric acid. The walls of the column and the resin bed were then washed with five 3 ml aliquots of 0.02 N HCl. Next, the clear, colorless effluent and washings were concentrated on a rotary evaporator to dryness, dissolved in 2.5 ml citrate buffer (pH 2.2), and 1.0 ml was injected into Hitachi Liquid Chromatograph (Model 034) in the same manner as described by PLAKAS *et al.*⁽⁴⁾

Results and Discussion

The plasma free amino acid levels of *Tilapia nilotica* before and at various times after feeding the casein and the corresponding amino acid diets, are shown in Table 2 and 3. These results are presented graphically in Fig. 1 through 4.

Fig. 1 shows the change in plasma levels of essential amino acids after feeding the casein diet. Immediately after feeding this diet, all essential amino acids in the plasma began to increase in concentration, attained their maximal values at 4 hours after feeding, and then decreased to the fasting level by 24 hours. In our study, the relative changes in the total levels of plasma free essential amino acids in *Tilapia* were more similar to those of carp⁽⁴⁾ than rainbow trout.⁽⁵⁾ Maximal levels of essential amino acids in the plasma of carp have been found to appear 4 hours after feeding casein. Rainbow trout, however, have demonstrated maximal levels of plasma essential amino acids between 24 and 36 hours after feeding the same diet. This may be attributable to differences in the structure of the two species' digestive tracts, digestive enzyme activities, and/or absorption rates of amino acids liberated from dietary protein. Fig. 2 shows the change in plasma levels of nonessential amino acids after feeding the casein diet. Aspartic acid and glutamic acid were maintained at constant levels after feeding, aspartic acid showing the lowest value of all plasma free amino acids measured. Overall free nonessential amino acids did not show regular patterns of change in concentration in the plasma after feeding. The change in levels

Table 2. Plasma free amino acid levels of *Tilapia nilotica* before and at various intervals after feeding the casein diet

Amino acids	Hours after feeding					
	0	2	4	8	12	24
Aspartic acid	0.012	0.012	0.012	0.011	0.012	0.007
Threonine	0.273	0.543	0.457	0.356	0.336	0.260
Serine	0.077	0.180	0.151	0.119	0.122	0.079
Glutamic acid	0.029	0.025	0.023	0.024	0.023	0.021
Proline	0.157	0.320	0.264	0.298	0.318	0.129
Glycine	0.192	0.178	0.133	0.147	0.127	0.140
Alanine	0.177	0.206	0.222	0.264	0.194	0.184
Valine	0.132	0.254	0.323	0.214	0.214	0.135
Methionine	0.020	0.073	0.085	0.063	0.047	0.021
Isoleucine	0.060	0.139	0.168	0.107	0.094	0.047
Leucine	0.120	0.303	0.384	0.259	0.194	0.106
Tyrosine	0.039	0.143	0.184	0.147	0.141	0.043
Phenylalanine	0.040	0.128	0.143	0.107	0.080	0.030
Lysine	0.177	0.358	0.372	0.218	0.201	0.121
Histidine	0.028	0.074	0.060	0.042	0.039	0.019
Arginine	0.035	0.099	0.097	0.055	0.048	0.033

Table 3. Plasma free amino acid levels of *Tilapia nilotica* before and at various intervals after feeding the amino acid mixture diet

Amino acids	Hours after feeding					
	0	2	4	8	12	24
Aspartic acid	0.010	0.020	0.023	0.011	0.011	0.015
Threonine	0.274	0.651	0.311	0.280	0.254	0.260
Serine	0.065	0.234	0.198	0.100	0.091	0.077
Glutamic acid	0.019	0.022	0.019	0.020	0.024	0.027
Proline	0.023	0.416	0.319	0.115	0.043	0.033
Glycine	0.140	0.173	0.155	0.140	0.173	0.158
Alanine	0.128	0.253	0.238	0.213	0.153	0.134
Valine	0.101	0.458	0.222	0.148	0.113	0.108
Methionine	0.012	0.105	0.035	0.020	0.015	0.014
Isoleucine	0.049	0.224	0.178	0.055	0.050	0.052
Leucine	0.100	0.504	0.344	0.138	0.112	0.123
Tyrosine	0.027	0.241	0.114	0.041	0.037	0.033
Phenylalanine	0.036	0.230	0.099	0.039	0.034	0.035
Lysine	0.167	0.508	0.213	0.188	0.199	0.185
Histidine	0.029	0.098	0.033	0.027	0.030	0.027
Arginine	0.026	0.144	0.040	0.033	0.038	0.040

of plasma essential and nonessential amino acids after feeding the amino acid diet are represented in Figs. 3 and 4, respectively. All essential amino acids showed an increase in value immediately after feeding the amino acid diet and reached peak levels at 2 hours. The plasma concentrations then returned to their fasting levels by 4 to 8 hours. In *Tilapia*, as well as carp and rainbow trout, the time required for plasma essential amino acid levels to reach a maximum, and subsequently

return to fasting levels was more rapid after feeding the amino acid mixture than casein as a dietary protein source.

One report is currently available on the subject of amino acid utilization by *Tilapia*. MAZID *et al.*²¹ have noted that a casein diet is more effective than the amino acid basal ration in sustaining growth of *Tilapia zillii*. This phenomenon is similar to that reported for carp.²¹ In carp, all dietary essential amino acids did not reach peak

Fig. 1. Change in the levels of plasma free essential amino acids of *Tilapia nilotica* at various time intervals after feeding the casein diet.

Fig. 3. Change in the levels of plasma free essential amino acids of *Tilapia nilotica* at various time intervals after feeding the amino acid diet.

Fig. 2. Change in the levels of plasma free non-essential amino acids of *Tilapia nilotica* at various time intervals after feeding the casein diet.

Fig. 4. Change in the levels of plasma free non-essential amino acids of *Tilapia nilotica* at various time intervals after feeding the amino acid diet.

levels, simultaneously in the plasma after feeding an amino acid diet. This may have resulted in an imbalance of amino acids in the body tissues, created by variations in the timing of gastrointestinal absorption of individual essential amino acids. All dietary crystalline essential amino acids, however, appeared to be absorbed simultaneously from the intestine by *Tilapia* in this study (Fig. 3). These results indicate that the inferiority of the amino acid test diet compared to the casein diet in sustaining growth of *Tilapia* may be related solely to the more rapid absorption of dietary free amino acids from the intestine.

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