AMINO ACID REQUIREMENTS AND COMPOSITION OF NILE TILAPIA (*Oreochromis niloticus*)

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ABSTRACT

In one trial 240 Nile tilapia fry received two different feed mixtures contained two different crude protein levels (Diet A, 28 % and B, 36 % crude protein). The average initial live weight was about 0.9 g/fish in each replicate of all treatments. Thirty fish per aquarium were stocked randomly in 8 glass aquaria (100 l each). Fish were fed the experimental diets at a rate of 10-6 % of the body weight daily. The amino acids (AA) content were determined in the whole fish and muscle at the end of the growth period (84 days).

The results indicated that, at the end of the trial Nile tilapia had reached an average live weight of about 5.2 and 8.14 g /fish for diet A and B, respectively. Also, the increase of crude protein (CP) content in the feed up to 36 % caused a significant increase in the content of essential amino acid (EAA) and nonessential amino acid (as % of whole fish and muscle of wet weight). Two relatively EAA methods were subsequently employed to estimate the quantitative EAA requirements of Nile tilapia based on the hypotheses that (a) the dietary requirement pattern of EAA reflects the tissue pattern and (b) the rate of daily deposition in the fish can be equated with the dietary requirement. As percentage of 100 g diet the requirements were Arginine 1.37, Methionine 0.51, Phenylalanine 1.04, Histidine 0.63, Isoleucine 1.12, Leucine 1.68, Lysine 1.63, Threonine 0.99, Valine 1.26 and Tryptophane 0.35.

INTRODUCTION

The essential amino acid requirements for several species of fish have been measured over the past 20 years. The values obtained, when expressed as a proportion of the diet, indicate large variations in the requirements of different species. The question of whether or not these a real differences is considered. Dietary amino acid are needed for growth and maintenance, and the former is quantitatively much the more important in young, rapidly growing fish.

The occurrence of an imbalance of amino acid in fish nutrition is nevertheless possible. Indeed, if protein-sparing feed is provided to fish, the amino acid supply in the feed must be adjusted so as to meet fish needs. Several investigators had varying degrees of success by using practical test diets to determine amino acid requirements (Ogino and Nanri, 1980; Cowey and Tacon, 1983; Wilson and Poe, 1985, and Gatlin, 1987). Diets formulated by using normal feedstuffs will furnish the bulk of the amino acid requirements (Gaber, 1993). The results of various trials have shown that, the smallest possible elimination of endogenous N occurs when feed is used which has an amino acid makeup most close to that of the body protein (Fuller et. al., 1979).

There is a few data available at present concerning the amino acid composition of different fish species under various feeding conditions (Gatline, 1987). Thus in the work presented here, the amino acid content of Nile tilapia was studied in detailed. The protein supply in the feed was scaled over a large range in order to examine simultaneously, the influence of these parameters on the amino acid content and composition of Nile tilapia.

MATERIALS AND METHODS

Feed mixtures were consists of fish meal, meat meal, soybean meal, wheat bran and vegetable oil (Table 1). Two different feed mixtures made by combining two different crude protein levels (Diet A 28 % and Diet B 36 %).

The experiment was conducted in 8-glass aquaria containing 100-liter of dechlorinated tap water in each. About one third of water volume in each aquarium was daily replaced by aerated fresh water after cleaning and removing the accumulated excreta. All aquaria were supplied with compressed air for oxygen requirements. Fish were exposed to the natural lighting conditions. The fish were fed 10 % of the live body weight daily for six weeks then 6 % of the live body weight daily until the end of the experiment. Daily food was divided into two equal feedings (08.00 and 16.00h).

Table 1: Composition and proximate analysis of the experimental diets.

	Diets	
	A (28 % crude protein)	**B (36 % crude protein)**
Ingredients (%):		
Fish meal	10.0	10.0
Meat meal	20.0	20.0
Soybean meal	14.0	34.0
Wheat bran	20.0	20.0
Corn meal	20.0	5.0
Vegetable oil	0.0	10.0
Cellulose	15.0	0.0
Premix[1]	1.0	1.0
Proximate analysis %[2]:		
Moisture	6.4	7.1
Crude protein	28.1	36.2
Crude fat	7.4	14.2
Ash	7.5	11.9
Crude fiber	15.1	5.9
CHO	38.02	23.0
Digestible energy (kcal / kg diet)	3455	4604

[1] Premix supplied the following vitamins and minerals (mg or IU)/ kg of diet, vit. A, 8000 I.U.; vit. D_3, 4000 I.U.; vit. E 50 I.U.; vit. K_3, 19 I.U.; vit. B_2, 25 mg; vit. B_3, 69 mg; Nicotinic acid, 125 mg; Thiamin, 10 mg; Folic acid, 7 mg; Biotin, 7 mg; vit. B_{12}, 75 mg; Cholin, 400 mg; vit. C, 200 mg; Manganese, 350 mg; Zinc, 325 mg; Iron, 30 mg; Iodine, 0.4 mg; Cobalt 2 mg; Copper, 7 mg; Selenium, 0.7 mg and 0.7 mg B.H.T. according to Lovell, 1989).

[2] Values represent the mean of three sample replicates.

Water temperature and dissolved oxygen were measured every other day using a YSI Model 58 oxygen meter. Total ammonia and nitrite were measured twice weekly using a DREL, 2000 spectrophotometer. Total alkalinity and chloride were monitored twice weekly using the titration method, pH was monitored twice weekly using an electronic pH meter(pH pen; Fisher Scientific, Cincinnati, OH). Over the duration of the study, these water-quality parameters averaged (± SD): water temperature, 27.5 ± 0.8 ^{o}C: dissolved oxygen, 6.5 ± 0.5 mg /l : total ammonia, 0.20 ± 0.14 mg /l : nitrite, 0.07 ± 0.05 mg / l : total alkalinity, 180 ± 46 mg / l : chlorides, 573 ± 151 mg / l : pH, 8.5 ± 0.16.

A set of 240 Nile tilapia (*Oreochromis niloticus)* fry average body weight of 0.9 ± 0.02g were taken from the stock of Fish Research Laboratory in Shebin El-Kom, Faculty of Agriculture, Menofiya University and used for the feeding trial. Thirty fish were randomly stocked into each aquarium with four replications per treatment. After stocking, to minimize stress of handling, fish from each aquarium were weighed every 2 weeks and at the end of the feeding trial (84 days). At the end of the feeding trial, a six fish per replicate for each treatment under study were killed and kept in the freezer. For preparation of the samples, the frozen tilapia were slightly thawed, cut into parts, minced and then homogenized in a mixture. The dry matter (D.M.) and crude protein (N x 6.25) were determined from the sample material according to AOAC (1990).

Table 2: Contents of essential and non-essential amino acids in the experimental diets with varying crude protein supply (g / 100g diet).

	Diets	
	A (28 % crude protein)	**B (36 % crude protein**
Essential amino acids %:		
Lysine	1.31	1.79
Methionine	0.34	0.45
Threonine	0.95	1.16
Leucine	1.75	2.23
Isoleucine	1.07	1.34
Histidine	0.57	0.85
Argnine	1.57	1.79
Phenylalanine	1.07	1.50
Valine	1.48	1.79
Tryptophane	0.20	0.30
Non- essential amino acids %:		
Alanine	1.23	1.76
Asparatic acid	3.48	3.45
Cystine	0.05	0.08
Glycine	1.39	1.70
Glutamic acid	3.41	4.67
Proline	1.33	2.01
Serine	1.11	1.42
Tyrosine	0.71	1.06

According to Mason et. al. (1979), three hydrolyses were carried out for each sample , the amino acid composition in each hydrolysate was determined with the help of the automatic amino acid analyzer. The amino acid determination of the feed ration was done in an analogues way to the method for the animal carcasses. Tryptophane was determined according to the AOAC (1990).

The EAA value were evaluated with the help of the SAS program package as one way classification, analysis of variance with application of the student Newman keuls test.

The EAA pattern of whole fish and muscle was then determined by expressing each of the ten EAA as a percentage of the sum of the EAA. This pattern is assumed to be equivalent to requirement pattern. The level at which this pattern is required in the diet of tilapia was calculated by assuming the Lysine requirement to be 1.62 % of diet (Santiago, 1985) and adjusting the levels of the other EAA accordingly.

Ogino (1980) proposed a method for determination of the quantitative EAA requirement of fish based on determination the rate of deposition of each of ten EAA as g/100g fish. Samples of fish fed 36 % protein feed were analyzed for EAA as described before and the rate of deposition calculated. According to feeding rate of the body weight for fish fed on 36 % protein feed with protein digestibility of 90 % were assumed.

RESULTS AND DISCUSSION

The mean contents of essential and non-essential amino acids are given as percentage of wet weight of whole fish and muscle of two treatments and two diets are shown in Tables 2 and 3. The one way statistical evaluation shows that, differing in protein supplie have a significant effect on the crude protein content and thus on all amino acids values in Nile tilapia. The relative amino acids content at the protein level 36 % was higher than at the protein level of 28 %.

The results shown in Tables 2 and 3 demonstrated that changes in the amino acids contents depends up on the protein content in Nile tilapia. The degree to which the amino acids pattern of Nile tilapia protein was effected can be observed when the value of amino acids content per 100 g protein. Thus, the content of essential and non-essential amino acids clearly increased by increasing the crude protein in the feed from 28 % to 36 %. Similar results were also found in carp (Schwarz et. al., 1984 and Zeitler et. al., 1984), in red tilapia (Gaber, 1993) and in tilapia mossambica (Gaber, 1994). The data showed that, a variable dietary protein and energy supply significantly affects the protein content and protein retention of Nile tilapia.

In contrast, the various protein and energy level had no obvious influence on the amino acid pattern of Nile tilapia protein. This result agrees well with results of work done on carp fish in which the amino acid composition of the body protein could not be changed by varying the amount of protein or the protein quality in the diet (Schwarz and Kirchgessner, 1988). Deviations in the amino acid, composition of the body protein are only conceivable under extreme conditions, for example, when a shift in tissue ratio is accompanied by a varying amino acid pattern. In this work, it has been shown Nile tilapia with different live weight (5g to 200 g) have the same amino acid pattern. Similarly, it was demonstrated that, red tilapia (Gaber, 1993) and Nile tilapia (Gunasekera, 1997) with different live weight have the same amino acids composition in their entire bodies.

Table 3 : Influence of varying protein supply on the content essential and non essential amino acids in whole fish and muscle of Nile tilapia (*Oreochromis niloticus*).

	Diets			
	A (28 % crude protein)		**B (36 % crude protein)**	
Live weight (g/fish)	5.2 ± 0.03		8.14 ± 0.43	
Protein supply (%)	28.1		36.2	
Treatments (%):	**Whole fish**	**Muscle**	**Whole fish**	**Muscle**
Protein content (%)	14.5	16.4	16.78	18.65
Moisture content (%)	75.2	76.8	74.1	74.7
Essential amino acid (% wet weight):				
Lysine	1.12	0.98	1.29	1.28
Methionine	0.31	0.32	0.45	0.42
Threonine	0.64	0.60	0.74	0.75
Leucine	0.98	0.93	1.16	1.21
Isoleucine	0.66	0.57	0.87	0.80
Histidine	0.41	0.35	0.41	0.44
Argnine	0.87	0.77	0.93	1.00
Phenylalanine	0.62	0.54	0.66	0.70
Valine	0.84	0.70	0.84	0.83
Tryptophan	0.20	0.20	0.30	0.30
Non-essential amino acid (% wet weight):				
Cystine	0.03	0.04	0.02	0.03
Serine	0.61	0.55	0.68	0.64
Aspartic acid	1.36	1.33	1.76	1.48
Glutamic acid	1.50	1.65	1.99	2.08
Proline	0.57	0.56	0.54	0.64
Glycine	0.71	0.68	0.59	0.68
Alanine	0.91	0.86	0.95	1.02
Tyrosine	0.47	0.43	0.54	0.58

The sparse available data which are concerned with the amino acid composition of protein in the carcasses or edible portions of Cyprinide and Salmonide (Wunsche and Steffens, 1968; Tiews et. al., 1973; Steffens, 1979 and Gatlin, 1987), Tilapia (Gaber, 1993, 1994), striped mullet (Tamara et. al., 1992) and channel catfish (Wilson and Poe, 1985) deexhibit general agreement with the work presented here. In addition, a more recent study done with 12 different salt water fish. species also confirms the quite uniform amino acid pattern (Njaa and Utne, 1982). The studies of Wunsche and Steffens (1968) showed that essential amino acids content in the edible portions of carp and trout were higher as compared to the essential amino acids in whole fish, is in agreement with our present study.

As mentioned earlier, (Fuller et. al., 1979; Gatline, 1987 and Gaber, 1994), Protein (muscle and whole fish) provides clues as to an optimum amino acids composition of feed

protein. It is necessary to consider here the fact that different amino acids can be used in metabolism and serve as building blocks for further body substances (Methionine and Phenylalanine). In addition maintenance, metabolism and the rate of utilization of individual amino acids must be taken into account (Steinhart, 1983). The comparison of the amino acid pattern in the muscle and whole fish with that of the feed protein should at least make manifest any gross amino acid imbalances. In Table 2, possible exceptions are the amino acids Leucine, Argnine and Valine, all of which are present in feed protein in relatively high amounts in comparison to Lysine.

The amino acid pattern which corresponds to the recommendations based upon requirement and adjustment of these amino acids can be also regarded as oriented to the amino acid composition of Nile tilapia protein. Leucine, is clearly deviant, and is regarded as necessary in significantly lower amounts. In addition Methionine and Phenylalanine appear is somewhat lower amount (Table 2). While Lovell (1989) reported that 50 % of Tyrosine can substitute for Phenylalanine and 60 % of Cystine can substitute for Methionine .

Table 4: The quantitative EAA requirement (%) of Nile tilapia (*Oreochromis niloticus*).

Amino acid	**Bassic of whole fish pattern**	**Calculation [a] muscle pattern**	**Daily [b] deposition**	**Recommended EAA for Nile tilapia**	**Required [c] g / 100 g diet**
Lysine	1.62	1.62	1.66	1.63	1.63
Methionine	0.57	0.53	0.42	0.51	1.02
Threonine	0.93	0.95	1.08	0.99	1.15
Leucine	1.46	1.51	2.07	1.68	1.09
Isoleucine	1.09	1.01	1.25	1.12	0.99
Histidine	0.55	0.56	0.79	0.63	0.54
Argnine	0.17	1.27	1.67	1.37	1.33
Phenylalanine	0.83	0.89	1.40	1.04	1.82
Valine	1.06	1.05	1.67	1.26	1.09
Tryptophane	0.38	0.39	0.28	0.35	0.32

[a.] Whole fish or muscle = 1.62 / 1.30 x amino acid % (According to Jauncey et. al., 1983).
[b.] EAA deposition = protein efficiency ratio x amino acid % (According to Ogino, 1980).
[c.] According to Santiago (1985).

Similar conclusions can be drown from the work of Gatlin (1987), to what degree the amino acid composition of Nile tilapia and fish feed protein is actually useful in determining a need-oriented amino acid supply will be the subject of future experiments.

Data in Table 4 shows that, the values of essential amino acids in whole fish and muscle and amino acid deposition of Nile tilapia in relation to Lysine (Lys.= 1.62), is corresponding to the recommendations for minimum amino acid requirements (Nose, 1979 ; Ogino, 1980 ; Wilson and Halver, 1986; Santiago, 1985 and Gaber, 1994). The feed protein in question must be considered especially high-grade based upon its composition and high digestibility of 87-94 % (Zeitler, et. al., 1983). It is obvious that, the amino acid composition of the feed protein corresponds well with the amino acid composition of Nile tilapia. Boorman (1980) showed that for the chick a direct correlation existed between the tissue EAA pattern and dietary requirement pattern. Cowey and Tacon (1981) showed that, this was also true for carp. Generally, the requirements based on the two tissues were close agreement, the main exception being a very low methionine requirement predicted from muscle tissue analysis.

Table 4 shows the results of experiments, where the method of Ogino (1980) equating the deposition rate of each the EAA with requirement was used. The results are in close agreement with those obtained in Table 3. Neither the method of Boorman (1980) or Ogino (1980) take into account the metabolic rate of EAA other than for pattern synthesis is particularly no allowance is made for the maintenance requirement for the AA. However it unlikely that maintenance will change the requirement pattern (Boorman, 1980) and should only have slight influence on the absolute dietary requirements.

In conclusion: composition of table 2 and 3 indicate that, the amino acids Leucine, Argnine and Valine all of which are present in feed protein in relatively high amount in comparison to Lysine. It is obvious that, the amino acid composition of feed protein corresponds well with the amino acid composition of Nile tilapia protein. The EAA requirement pattern of Nile tilapia (*Oreochromis niloticus*) is not dissimilar to that of other fish species. Nevertheless the absolute dietary requirements are sufficiently different as to necessitate formulation of diets on a species specific basis.

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