Assessing the dietary amino acid requirements of tilapia, *Oreochromis niloticus* fingerlings

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Running title: Amino acid requirements of *Oreochromis niloticus*

Keywords: Tilapia, *Oreochromis niloticus*, Amino acid requirements

Abstract

In an earlier study *Oreochromis niloticus* fingerlings (initial weight, 4.31±0.27 g) were fed diets with dietary protein levels, ranging from 7.30% - 44.24% dry matter (dm) for eight weeks. Using growth performance and food conversion ratio, the dietary protein requirement of the fingerlings was estimated at 33.32% dietary protein (dm). In this study the dietary essential amino acid requirements for *O. niloticus* were expressed as the essential amino acid composition of the diet with a protein content of 33.32% dm. This follows the concept that protein requirements is the minimum amount needed to meet amino acid requirements and ensure maximum growth. The essential amino acid requirements, as %dm are: Arginine 1.68, Histidine 0.70, Isoleucine 1.15, Leucine 2.15, Lysine 1.98, Methionine 0.10, Phenylalanine 1.13, Threonine 1.11, Tryptophan, 0.84, and Valine 1.34. This result was verified by comparing fish performance on diets having essential amino acid profile similar to the diet containing 33.32% dietary protein and others mirroring the recommended amino acid requirement for tilapia by Santiago and Lovell (1988). The specific growth rate among different fish groups was not significantly different. Essential amino acid requirements are therefore not absolute values rather an indication of concentration range which must be present in fish diet to enhance adequate performance of fish.

Introduction

With the ever increasing need for cheap sources of protein to meet the world's over-population problem, more attention is focused on fish farming. In the developing countries where the problem is acute, tilapia culture is believed to offer one of the solutions, especially in view of the depletion of the existing fisheries (Balarin, 1979). The relative ease of culture of tilapia and its rapid growth-rate under tropical and semi-topical climates have led to its widespread distribution. Such advantages have given tilapia an important edge over other species. The successful intensification of culture methods for tilapias may be achieved if accurate diets satisfying all of the nutrient requirements are formulated.

Apart from vitamins and essential fatty acids, protein is considered to be a very important component of mixed fish diets (Steffens, 1981). Inadequate protein in the diet results in a reduction or cessation of growth and loss of weight due to withdrawal of protein from less vital tissues to maintain the functions of more vital tissues. On the other hand if excess protein is supplied in the diet, only part of it will be used for protein synthesis and the remainder will be converted to energy (Wilson, 1989), a process which reduces growth as well (Jauncey, 1982; Bowen, 1982) and leads to environmental pollution (Rennert 1994).

The protein and amino acid requirements of many cultured juvenile fish species (trout, salmon, common carp, gold fish, tilapia, Siberian sturgeon) are already estimated (DeLong et al., 1962; Nose, 1979; Dabrowski, 1981; Jackson and Capper, 1982; Santiago and Lovell, 1988; Kaushik et al., 1991; Didier-Fiobè and Kestemont, 1995). Several methods are used for such estimations. They include; the growth and/or biochemical or metabolic responses to graded dietary levels of the concerned amino acid, the whole body tissue EAA patterns or A/E ratios (ratio between individual EAA / sum of EAA × 1000) and the method of daily incrementation.
A critical analysis and assessment of the relationship between protein and amino acid (Wilson, 1985; 1989; Keembiyehetty and Gatlin III, 1992; Tibbets et al., 2000) reveal that: (1) fish like other animals do not have a true protein requirement but have a requirement for a well – balanced mixture of essential or indispensable amino acids; (2) dietary requirement for protein is in fact a requirement for essential amino acids contained in dietary protein; (3) insofar as synthesis of dispensable amino acids requires expenditure of energy, feeding dietary proteins that most nearly meets the needs of fish for both indispensable and dispensable amino acids will result in the most efficient growth by the fish; (4) the gross dietary protein requirement is influenced directly by the amino acid composition of the diet and (5) the concept of balance or pattern of amino acids is basic to protein requirement.

With regards to the above concepts and observations an attempt is made in this study to assess the amino acid requirements of *Oreochromis niloticus* fingerlings based on the dietary protein requirements of the species.

Materials and methods

Diet formulation

In an earlier study, *Oreochromis niloticus* fingerlings (initial weight, 4.31±0.27 g) were fed diets with dietary protein levels, ranging from 7.30% - 44.24% dry matter (dm) for eight weeks (Ogunji and Wirth 2000). Using growth performance and food conversion ratio, the dietary protein requirement of the fingerlings was estimated at 33.32% dietary protein (dm). In this current study, freeze dried and homogenised samples of the diets (1a-8a) with dietary protein levels ranging from 7.30% - 44.24% dry matter (dm) were analysed for amino acid. Three test diets (1b -3b) were then formulated to yield 33.32% dietary protein level which was the diet that resulted to the best performance of the fish (Table 1). Fish meal was the only protein source in diet 1b. Methionine and tryptophan were added to this diet so as to reach the concentration level recommended by Santiago and Lovell (1988) as the requirement for this species (Table 2). Diets 2b and 3b were formulated using alternative protein sources at the level capable of substituting 42% of fish meal in the diet of *Oreochromis niloticus* fingerlings (Ogunji and Wirth 2001). Synthetic amino acid was however, supplemented to reflect the essential amino acid profile of the diet containing 33.32% dietary protein in diet 2b. Amino acid supplementation in diet 3b reflected the already determined amino acid requirement of tilapia *Oreochromis niloticus* by Santiago and Lovell (1988). The dry diet components including vitamin and mineral mixtures were thoroughly mixed with sunflower oil. Water was added and the feed pressed into pellets of 1 mm diameter. The formulated diets were dried at room temperature and stored in a refrigerator (5-7°C) throughout the experiment.

Fish rearing and experimental conditions

Tilapia fingerlings were raised at the facilities of Institute of Freshwater Ecology and Inland Fisheries (Berlin), and reared in a re-circulation system. Fifteen fingerlings (initial weight, 4.45 ± 0.05g) were introduced into six experimental tanks respectively. Each measures 28 × 28 × 51.5 cm and contains 34.61 litres of water. After an adaptation period of two weeks each test diets was assigned to duplicate tanks. The fish were fed at a rate of 5% (wet weight basis) of their total biomass per day in 3 portion for 7 weeks. The Fish was weighed every 2 weeks and quantity of food adjusted accordingly. Experimental tanks were cleaned regularly. Conductivity, pH, oxygen concentration and temperature of water were measured 3 times every week. The water was well aerated and oxygen saturation above 60%. Temperature was maintained at 27±1°C through out the experiment.

Analysis of fish and diet samples

At the end of the experiment all fish in each treatment was weighed. Twenty fish from each treatment group were randomly taken. Their intestine was removed and the carcass homogenised (Kim et al., 1991). Freeze dried samples of fish at the beginning and at the end of the experiments as well as the samples of the test diets were analysed for proximate composition and amino acids. Protein (N × 6.25) was determined by the Kjeltec System (Tecator); crude fat by Soxtec System HT (Tecator) using petroleum ether, and ash by burning in a muffle furnace at 750°C for 4 hours. Oxygen bomb calorimeter (Framo- MK 200) was used for energy determination at two replications per sample. For the amino acid analysis, 5mg of the freeze dried samples were hydrolysed with 6 N HCl at 110 °C for 24 hours. No protecting reagents were added to avoid destruction of sulphur amino acids. Other procedures for the analysis have been reported (Ogunji and Wirth, 2001). All statistical analyses were carried out by the Duncan multiple range method using SPSS for Windows (Version 9). From the experimental data obtained, weight gain, specific growth rate (SGR) and food conversion ratio (FCR) were calculated. $SGR = \ln W_2 - \ln W_1 / t_2 - t_1$

W_1 & W_2 = initial and final weight of fish; t_1 & t_2 = time in days.

FCR = food fed(g)/live weight gain (g).

Results

Amino acid composition of diets (1a-8a) used in earlier experiments, reveal that the amino acids (% dry matter, dm) increased with increasing dietary proteins. It also shows that the experimental diets contain the same amino acid profile regardless of the dietary protein content (Table 3). Fish fed diet 6a containing 33.32% dietary protein, dm recorded higher values of body amino acid composition than the others (Table 4). The increase was however, not linear. Growth data, and feed conversion ratio of the fish fed diets 1b – 3b are presented in Table 5. After 7 weeks, weight gain, SGR and FCR among the fish fed diet 2b and 3b were not significantly different ($P<0.05$). Both treatments differ significantly from treatment 1b only in FCR and weight gain. The protein composition of fish body in all the groups decreased but the fat composition increased (Table 6).

Discussion

Protein requirements is defined as the minimum amount needed to meet requirements for amino acids and to achieve maximum growth (NRC, 1993). Therefore the diet containing 33.32% protein, dm which resulted to the best performance of fish (Ogunji and Wirth 2000) satisfied the protein as well as the amino acid need of *O. niloticus* fingerlings. Almquist (1972) showed a constant relationship between indispensable amino acid requirements and protein intake up to the level required for maximum growth in warm blooded animals. The amino acid composition of the diet with 33.32% dietary protein (dm) based on fish meal diet, which meets the requirement of protein for *Oreochromis niloticus* fingerlings may therefore reflect the optimal dietary amino acid requirements of this species as follows: Arg. 1.68, His. 0.70, Ile 1.15, Leu. 2.15, Lys. 1.98, Met. 0.10, Phe. 1.13, Thr. 1.11, Try. 0.84, and Val. 1.34 (% dm). This conclusion agrees with the report of Tibbets et al. (2000) that, dietary requirement for protein is in fact a requirement for essential amino acids contained in dietary protein. Excluding methionine, the values in this study seem to present a higher requirement of amino acids for *O. niloticus* than were reported by Santiago and Lovell (1988) (Table 2). The variations may be due to different test diets, dietary protein, initial size of fish, and experimental conditions used for the two studies. Kim et al., (1992) attributed the variation among the reported lysine and arginine requirements for rainbow trout to laboratory variances. The methionine concentration in this study (0.10 % dm) may be remarkably low. It is possible that the corresponding cystine concentration is very high. This was not analysed in this study. However, Jackson and Capper (1982) reported, the minimum dietary level of methionine producing satisfactory growth in *Oreochromis (Sarotherodon) mossambicus* at less than 0.53% dm. The level of other sulphur amino acids like cystine is obviously of crucial importance. It seems that the methionine requirement of *O. niloticus* may be lower than reported by Santiago and Lovell (1988). This is evident from the results of this study where supplementation of methionine at the requirement level reported by Santiago and Lovell (1988) (Table 2) did not enhance better performance by the fish with regards to the

SGR and FCR (Table 5). It is also possible that part of the methionine in our samples was oxidised during hydrolysis.

Diet 1b revealed significant difference from diet 2b and 3b in weight gain and FCR but diet 2b and 3b did not differ. These observations suggest that the amino acid composition of the diet containing 33.32% dietary protein dm in our previous study is able to meet the requirements of *Oreochromis niloticus* fingerlings and also ensure maximum growth. Not withstanding the difference in value with the previously reported amino acid requirements for *O. niloticus*, the results of this study has been shown to be reliable. Essential amino acid requirements of fish however, are not absolute values rather an indication of the range of essential amino acid concentrations which must be present in fish diet to enhance adequate performance of fish.

In conclusion the method used here to determine amino acid requirements based on the protein–amino acid balance, may share a lot in common with the classical growth–response method but has more advantages. (1) Protein and amino acid requirements are determined simultaneously. (2) Similar to the biochemical and metabolic methods, it is based on dose–response relationship. It is not laborious but also rapid. (3) The difficulties often encountered by using purified diets are easily over come. The effect of the test diets on nutritional parameters are fully considered.

This method provides the quickest possibility of having a rough idea about the protein and amino acid requirement of a fish species before attempting to substitute dietary fish meal protein with alternative protein sources. This method needs to be tried for other species, to validate its effectiveness and fine tune the processes.

Acknowledgement

We thank Frau A. Tillack and Herr M. Kunow for their technical assistance. A special thanks goes to Herr Michael Nuendel, Institute of Biochemistry, Free University Berlin, for his invaluable advice on the HPLC methods of amino acid analysis.

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Table 1: Formulation and proximate composition (% dry matter) of experimental

diets 1b - 3b

	Diet 1b[1]	2b[2]	3b[3]
Fish Meal	45	26	26
Soy Bean Meal	-	18	18
Blood meal	-	5	5
Wheat Bran	-	10	10
Sunflower Oil	10	6	6
Amino acid	0.9	2.64	1.87
Vitamin Mix[4]	4.7	4.7	4.7
Potato Meal	39.4	27.66	28.43
% Protein Calculated	33.57	33.59	33.59
% Proximate Composition			
Dry Matter	92.38	93.1	92.24
Protein	30.72	32.14	30.46
Fat	15.36	14.70	14.15
NFE + Fibre[5]	39.90	41.94	44.07
Ash	14.02	11.22	11.32
Digestible Energy (kJg^{-1})[6]	11.62	11.91	11.68
Gross Energy (kJg^{-1})[6]	19.76	20.19	19.96

[1]Only methionine and tryptophan were added to make up the recommended profile in Lovel and Santiago (1988). [2] Amino acid profile based on the results of Lovell and Santiago (1988). [3]Amino acid supplemented to reflect diet 6a. [4]Vitamin and Mineral mix (Spezialfutter Neuruppin - VM BM 55/13 Nr. 7310) supplied per 100 g of dry feed : Vitamin A 15000 IU; Vitamin D3 2500 IU; Vitamin E 500 mg; Vitamin K3 23 mg; Vitamin B1 42 mg; Vitamin B2 18 mg; Vitamin B6 21 mg Vitamin B12 59 µg; Nicotinic acid 100 mg; Biotin 544.65 µg; Folic acid 13 mg; Pantothenic acid 123 mg, Inositol 1230 mg; Vitamin C 66.7 mg; Antioxidants (BHT) 121.87 mg; Calcium 20.2%. [5]Nitrogen free extract, (NFE) = 100 - (% protein + % fat + % ash); [6]Gross and digestible energy were calculated according to ADCP (1983)

Table 2: Dietary essential amino acid requirements of *O. niloticus*

	This Study	**Santiago and Lovell (1988)**

	Dietary protein - Type of test diet -	33.32% (dm) Practical diet	Dietary protein - Type of test diet -	28% (dm) Purified diet
	Requirement % Dietary protein	Requirement % Dry matter	Requirement % Dietary protein	Requirement % Dry matter
Arginine	5.04	1.68	4.20	1.18
Histidine	2.11	0.70	1.72	0.48
Isoleucine	3.44	1.15	3.11	0.87
Leucine	6.35	2.15	3.39	0.95
Lysine	5.93	1.98	5.12	1.43
Methionine	0.29	0.10	2.68	0.75
Phenylalanine[1]	3.38	1.13	3.75	1.05
Threonine	3.34	1.11	3.75	1.05
Tryptophan	2.51	0.84	1.00	0.28
Valine	4.02	1.34	2.80	0.78
[1]Tyrosine -	0.24	0.08	1.79	0.5

Table 4: Body amino acid composition (% of dry matter) in *O. niloticus* fingerlings fed diet 1a - 8a

	Diets- 1a	2a	3a	4a	5a	6a	7a	8a
Aspartic acid	3.41	4.20	4.46	3.83	3.97	4.68	3.08	4.26
Glutamic acid	5.08	6.08	6.11	5.52	5.87	6.48	4.63	4.28
Serine	1.50	1.67	1.77	1.59	1.69	1.91	1.23	1.56
Histidine[1]	0.66	0.89	1.05	0.92	0.90	1.15	0.71	1.03
Glysine	3.06	2.69	3.29	2.94	3.06	2.87	2.02	2.36
Threonine[1]	1.54	1.80	2.00	1.75	1.82	2.08	1.37	1.88
Arginine[1]	2.39	2.93	3.19	2.99	2.96	3.38	2.09	2.74
Carnosine	0.13	0.19	0.20	0.18	0.24	0.25	0.14	0.17
Taurine	0.63	0.57	0.74	0.63	0.70	0.76	0.54	0.66
Alanine	2.80	3.00	3.38	3.03	3.18	3.31	2.26	2.86
Tyrosine	0.19	0.24	0.30	0.23	0.24	0.35	0.19	0.26
Tryptophan[1]	0.06	0.17	0.15	0.10	0.12	0.13	0.84	0.11
Methionine[1]	0.12	0.12	0.12	0.11	0.13	0.17	0.10	0.11
Valine[1]	1.45	1.85	2.03	1.78	1.85	2.07	1.38	1.81
Phenylalanine[1]	1.41	1.76	1.00	1.65	1.74	1.95	1.30	1.74
Isoleucine[1]	1.29	1.70	1.85	1.61	1.67	1.91	1.38	1.69
Leucine[1]	2.63	3.26	3.51	3.01	3.26	3.68	2.45	3.25
Ornitine	0.03	0.04	0.06	0.06	0.05	0.07	0.05	0.06
Lysine[1]	2.65	3.26	3.70	2.99	3.32	3.80	2.51	3.50

[1]Essential amino

Table 5: Growth data, food conversion and protein / energy ratio of *O. niloticus* fingerlings fed diet 1b -3b[1]

Diets	% Protein	Initial wt. (g)	Final wt. (g)	Weight gain (g)	Food fed (g/fish)	P / E ratio [2]	SGR[3]	FCR[4]
1b	30.72	4.45±0.05	19.43±0.8[a]	14.53±0.2[a]	21.84	15.54	3.00[a]	1.50 [b]
2b	32.14	4.45±0.05	21.78±0.4[b]	17.33±0.5[b]	22.93	15.92	3.24[a]	1.32[a]
3b	30.46	4.45±0.05	21.70±0.5[b]	17.25±0.6[b]	23.17	15.26	3.23[a]	1.34[a]

[1]Figures in the same row with different superscript letters are significantly different ($P < 0.05$) from each other;

[2]P/E = Protein to energy ratio in mg protein / K J energy; [3]Specific growth rate = $(\text{In}W_2 - \text{In}W_1 / T_2 - T_1) \times 100$;

[4]Food conversion ratio = food fed(g)/live weight gain (g).

Table 6: Proximate composition of experimental fish samples fed diet 1b - 3b.

Fish samples	% Moisture	Ash (% dm)	Fat (% dm)	NFE (% dm)	Protein (% dm)
Initial Status	73.63	14.11	25.88	1.47	58.54
1b	71.78	15.96	26.23	4.51	53.30
2b	72.06	13.82	26.82	5.75	53.61
3b	72.02	14.14	27.27	1.38	57.21