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Amino Acid Composition of *Heterobranchus longifilis* Fry, Fingerlings, and Broodstock

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Abstract

The whole-body amino acid composition of fry, fingerlings, and brood fish of *Heterobranchus longifilis* was determined using a Technicon Sequential Multisample Auto-Analyser, equipped with a pen recorder for drawing chromatograms. The fish samples consisted of nine essential amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine) and eight nonessential amino acids (alanine, aspartic acid, cystine, glutamic acid, glycine, proline, serine, tyrosine). There were no significant differences among the three stages and all are closely related to those of other fish species.

Introduction

Heterobranchus longifilis (Vallenciennes) is one of the four most cultured clariid catfish in Africa, especially in Nigeria. The other three species are *H. bidorsalis*, *Clarias gariepinus*, and *C. anguillaris*. The culture potential of *H. longifilis* derives from the fact that it is hardy, can survive out of water for several hours, grows to 30 kg in the wild, and accepts a wide variety of food including pelleted diets.

According to NRC (1983), proteins are the major organic material in some animal tissues, comprising 65-75% of their total body weight on a dry basis. Animals must regularly consume protein to obtain a continual supply of amino acids which are used to synthesize new protein and worn-out tissues. If adequate protein is not provided in the diet of cultured animals, including fish, growth will rapidly drop or cease, or there will be a loss of weight. Such weight losses result from withdrawal of protein from tissues to maintain vital functions. If excess protein is supplied in the diet, it is metabolized to produce energy. The amount of protein required in fish diets is directly influenced by the amino acid composition of the diet; a well-balanced mixture of indispensable and dispensable amino acids is needed. Therefore, to determine the amino acid requirements of *H. longifilis*, the amino acid profiles of fry, fingerlings, and brood stock must be determined.

Earlier work reported on the amino acid profiles of *C. gariepinus* and *C. anguillaris* (Fagbenro et al., 2001), *Lates niloticus*, *Synodontis schall*, and *Sarotherodon gallilaeus* in the dry and rainy seasons of Nigeria (Sadiku

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and Oladimeji, 1989), coho salmon *Onchorynchus kisutch* (Arai 1981), cherry salmon *Onchorynchus mason* (Ogata et al., 1983), and Japanese eel, common carp, channel catfish, and chinook salmon (NRC, 1983). Van der Meer et al. (1995) showed that the protein gain at feeding levels below the level for maximum growth in *Colossoma macropomum* was linearly related to the amount of protein administered. They further stated that this leaves little room to economize on feed protein as a decrease in the amount of protein leads to a proportional decrease in protein growth.

This study set out to investigate the amino acid composition of fry, fingerlings, and broodstock of *H. longifilis* and to compare them with other catfish species as a preliminary step towards determining the amino acid requirements of this fish.

Materials and Methods

Heterobranchus longifilis fry (mean wt 0.038±0.001 g), fingerlings (1.44±0.027 g), and adults i.e. broodstock (850 g) were obtained from the hatchery of the National Institute for Freshwater Fisheries Research. They were oven dried at 105°C for 48 h, 96 h, and 144 h, respectively. Each sample was separately defatted by inserting 10 g of the sample into an extraction thimble and extracting the fat with a 2:1 chloroform/methanol mixture using a Soxhlet extraction apparatus (AOAC, 1980). Extraction samples contained 270 pooled fry, seven pooled fingerlings, or a single brood fish and were conducted in triplicate. Extraction lasted 15 h.

Defatted samples were weighed into glass ampoules and 7 ml of 6N HCL was added. Oxygen was expelled by introducing nitrogen into the ampoule to prevent oxidation of amino acids during hydrolysis. The glass ampoule was sealed with a Bunsen burner flame and put into an oven preset at 105±5°C for 22 h. The ampoule was allowed to cool before being broken open at the tip and the contents were filtered. The filtrate was evaporated to dryness at 40°C in a vacuum in a rotatory evaporator. The residue was dissolved with 5 ml of acetate buffer (pH 2.0) and stored in plastic specimen bottles in a freezer.

Ten ml was loaded and dispensed into the cartridge of a TSM analyzer (Technicon Sequential Multisample Amino Acid Analyzer) that separated free acidic, neutral, and basic amino acids of the hydrolysate into chromatograms in 76 min.

At each peak produced by the TSM chart record (each peak represents an amino acid), the half height of the peak was found and the width at the half height was measured. The area of the peak was approximated by multiplying the height of the peak by the width at half height. The norleucine equivalent (NE) for each amino acid was calculated using the formula: NE = area of norleucine peak/area of each amino acid. A constant (S) was calculated for each amino acid in the standard mixture according to the formula: S = NE x mol. wt x UMAA. Finally, the amount of each amino acid in the sample was calculated in g/16 gN or g/100 g protein using the following formula: concentration (g/100 g protein) = NE x width at NE x S x C, where C = dilution/NH x W (nleu).

One-way ANOVA followed by student Newman Keul's test was used to statistically analyze differences in amino acid contents in fish of different stages.

Results

Table 1 shows the essential amino acid compositions of *H. longifilis* fry, fingerlings, and broodstock. There were no significant differences between *H. longifilis* stages and compositions highly correlated with those of other catfishes (Table 2).

Discussion

Supply of a well-balanced mixture of indispensable and dispensable amino acids is required to meet the amino acid needs of fish. Knowledge of the amino acid compositions of various stages of *H. longifilis* serves for setting amino acids levels in nutritional studies of amino acid requirements of fish.

In the amino acid compositions of three fish species in northern Nigeria, methionine was the lowest in both the dry and the rainy seasons (Sadiku and Oladimeji, 1989), as it was for all three stages in our study, although

Table 1. Amino acid composition of fry, fingerlings and broodstock of *Heterobranchus longifilis* and other catfish species (g/100g).

	Heterobranchus longifilis				*Clarias*	*Clarias*	*Channel*
Amino acid	*Fry*	*Fingerling*	*Broodstock*	*Fingerlings*[1]	*anguillaris*[2]	*gariepinus*[2]	*catfish*[3]
Essential							
Arginine	5.00	7.14	8.88	5.86	6.70	6.72	6.67
Histidine	2.58	3.47	3.69	3.16	3.36	3.25	2.17
Isoleucine	3.92	4.19	5.58	3.90	4.68	4.62	4.29
Leucine	6.23	5.11	7.03	7.83	7.48	7.89	7.40
Lysine	5.21	6.22	7.27	9.53	8.32	8.61	8.51
Methionine	2.22	2.65	3.19	2.69	2.42	2.77	2.92
Phenylalanine	4.16	4.36	4.22	4.00	4.19	4.12	4.14
Threonine	3.22	4.19	4.22	6.42	5.08	4.77	4.41
Valine	4.00	4.01	5.06	4.08	4.21	4.38	5.15
Nonessential							
Alanine	3.82	5.00	5.69	5.40	6.42	6.75	6.31
Aspartic acid	9.99	9.43	10.38	8.50	11.40	11.27	9.74
Cystine	0.99	0.79	1.19	1.63	1.05	1.01	8.60
Glutamic acid	17.53	13.18	15.30	16.73	15.42	16.23	14.39
Glycine	4.47	3.97	5.50	5.58	9.16	8.97	8.14
Proline	2.82	4.90	5.14	3.92	6.46	6.30	6.02
Serine	3.25	4.00	4.59	5.72	5.27	5.64	4.89
Tyrosine	3.00	2.86	3.07	3.03	3.33	3.71	3.28

1 Eyo, 2001

2 Fagbenro et al., 2000

3 Wilson and Poe, 1985

Fagbenro et al. (2001) found that tryptophan was lowest. In our study, arginine was the highest essential amino acid (EAA) in fingerlings and adults while leucine was the highest in fry, similar to the findings of Sadiku and Oladimeji (1989) but in contrast to those of Fagbenro et al. (2001) who found that lysine was the highest in *C. gariepinus* and *C. anguillaris*. Glutamic acid was the highest non-EAA in our study, similar to *L . niloticus*, *S. schall*, and *S. gallilaeus* (Sadiku and Oladimeji, 1989), and *C. gariepinus*, *C. anguillaris*, and channel catfish (Eyo, 1999; Fagbenro, 2001). None of the EAA or non-EAA of fingerlings in other studies were identical in quantity with those in this present study. Lysine was highest in the study of Eyo (1999), in contrast to the findings of our study. However, Eyo (1999) analyzed the amino acid content of *H. longifilis* fngerlings with a mean weight of 5.83 g. As the sizes of fish in our study differed from those in Eyo (1999), the studies corroborate one another for the amino acid requirements of *H. longifilis*. In short, our study shows that amino acid levels vary with fish of different sizes. It is also possible that

Table 2. Correlation of amino acid compositions of fry, fingerlings, and broodstock of *Heterobranchus longifilis* in this study and with other catfish species*.

Comparison	*r*
Fry and fingerlings	0.946
Fry and broodstock	0.947
Fingerlings and broodstock	0.987
Fry and fingerlings (Eyo, 2001)	0.927
Fry and *C. anguilaris*	0.897
Fry and *C. gariepinus*	0.92
Fry and channel catfish	0.901
Fingerlings and fingerlings (Eyo, 2001)	0.91
Fingerlings and *C. anguilaris*	0.896
Fingerlings and *C. gariepinus*	0.929
Fingerlings and channel catfish	0.971
Broodstock and fingerlings (Eyo, 2001)	0.916
Broodstock and *C. anguilaris*	0.9
Broodstock and *C. gariepinus*	0.941
Broodstock and channel catfish	0.94

* Sources of compositions for other species as in Table 1.

the feed (Ovie and Ovie, 2005) and time of year (Siddiqui and Siddiqui, 1977) determine some slight variations in EAA and non-EAA levels.

The high correlation observed between whole-body composition in the various stages of *H. longifilis* and other catfishes shows that amino acid requirements may not vary much from one another. Poh (1985) stated that the EAA profile of a particular species is used to formulate diets for the grow-out phase of that species. However, the amino acid profile of eggs has been used to estimate the dietary amino acid requirements of broodstock (Arai, 1981; Ketola, 1982; Ogata et al., 1983). Tacon and Cowey (1985) suggested that each of the dietary EAA must be available at levels equal to or higher than the EAA body level in the cultured fish.

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