PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 9 (7): 724-727, 2010
ISSN 1680-5194

Tissue and Blood Amino Acids Composition of an Ecotype Cichlid 'Wesafu', *Tilapia zillii* and *Oreochromis niloticus* Using Paper Chromatography

A.M. Hammed[1], H.A. Fashina-Bombata[1] and O.O. Fajana[2]
[1]Department of Fisheries, [2]Department of Biochemistry,
Faculty of Science, Lagos State University, P.M.B. 1087, Apapa, Lagos, Nigeria

Abstract: Wesafu is an indigenous ecotype cichlid and a very important part of the fisheries of Epe lagoon in Lagos Nigeria. Investigation of the amino acids composition of tissue and blood samples of Wesafu, *T. zillii* and *O. niloticus* using paper chromatography (Ranjna, 1999) was conducted. Only 14 amino acids (Alanine, Cysteine, Asphatic acid, Phenylalanine, Glycine, Histidine, Isoleucine, Lysine, Leucine, Methionine, Threonine, Valine, Tryptophan, Glutamic acid) were analyzed. In the muscles, 11 amino acids were identified with alanine, guanine and methionine absent in all three fish tissue sampled. Phenylalanine, isoleucine and valine were absent in *O. niloticus* but present in Wesafu and *T. zillii* while Tryptophan and Glutamic acid were present in *O. niloticus* but absent in the tissues of Wesafu and *T. zillii*. However, all 14 AA assayed were present in different proportions in the blood samples of the three species. This report further suggests that the Wesafu is different from either of the two species and warrant species identification at a level of molecular biology.

Key words: Wesafu, *T. zillii*, *O. niloticus*, chromatography, amino acids, tissue, blood

INTRODUCTION

The gradual increase in the population of fish farming over the last sixty years has been linked with advances in aquatic biology and search for alternative food sources. FAO (2003) reported 210 different farmed aquatic animal and plant species to include 131 Finfish species, 42 Mollusca species, 27 Crustacean species, 8 Plant Species and 2 Amphibian and reptile species. Cichlids, commonly called the "Tilapias" of the family Cichlidae are perch-like fish and the family is known to have produced an enormous variety of species in Africa freshwaters. Trewavas (1983) classified cichlid into three genera namely: *Tilapia*, *Oreochromis* and Sarotherodon. These genera are further sub-divided into two groups (Nwadukwe, 1991) based on their breeding habits; they are the guarders and bearers.

New species are genetic resources, within communities and ecosystems and their potentially useful attributes are coded in the genes of these species. Therefore, all concerns with conservation and preservation of new species should be geared toward determination of their identity and potential resource worth for research and science. Domestication for aquaculture is centuries behind crop and livestock breeding. There is a wide array of aquatic species to be accurately identified and their populations characterized for aquaculture potential. Amongst the fin fishes; the tilapias, African fishes of the family Cichlidae, continue to generate high interest for aquaculture around the world.

As tilapia farming progresses and farmed breeds are developed, such characterization will increasingly require descriptors based on molecular genetics. However, given the similarity in appearance of the tilapias (especially in their juvenile stages) and their high propensity to hybridize when transferred for aquaculture or fisheries purposes or when they escape from fish farms. There continues to be the immediate need to characterize new tilapia species and hybrids in natural waters in Nigeria, as a subject for research and aquaculture development studies.

MATERIALS AND METHODS

Experimental fishes: Table sized species of Wesafu, *T. zillii* and *O. niloticus* with average weights and lengths of 1200±52.75 g; 44.55cm±1.26cm, 925±18.12 g; 38±3.45 cm and 765±1.12 g; 36±2.48 cm respectively were used in the study. The fish were obtained from Epe Lagoon and a private fish farm in Badagry, Lagos, Nigeria.

Amino acid profiles: Amino acids profiles in the muscle and blood of Wesafu, *T. zillii* and *O. niloticus* were assayed using paper chromatography to arrive at R_f values of standard amino acids calculated thus:

$$R_f = \frac{\text{Distance traveled by the substance}}{\text{Distance traveled by the solvent front}}$$

(Ranjna, 1999)

Procedure: The following procedure as described by Smith and Feinberg (1973) and Stahl (1969) was followed:

Corresponding Author: H.A. Fashina-Bombata, Department of Fisheries, Faculty of Science, Lagos State University, P.M.B. 1087, Apapa, Lagos, Nigeria

- A line was drawn along the width of Whatman No. 1 chromatography (20 cm x 20 cm) paper about 8 cm, away from the edge
- Small volumes of each of the amino acids solution were applied with the help of capillary tubes to place a small spot
- The chromatogram was inserted in the chromatography chamber which had earlier been equilibrated with solvent
- The chromatogram was run using solvent N-butanol: Acetic acid: Distilled water in the ratio 12:3:5
- After 3 hours, the chromatogram was removed from the chamber and allowed to dry in air
- The dried paper was sprayed with the Ninhydrin in 1% alcohol solution and dried
- The paper was kept suspended for about 8 hours at room temperature (30°C) after which, each amino acid was noticed to have migrated and gave a purple colour spot
- Each spot was marked and R_f values of standard amino acids calculated, and the identification of the amino acids was done in the given solution

R_f = Distance traveled by the substance/Distance traveled by the solvent front (Ranjna, 1999).

Statistical analysis: Data generated were subjected to Analysis of Variance (ANOVA). The significance of difference between means was determined by Duncan's multiple range test ($p<0.05$) using SPSS for windows (Version 11). Values are expressed as means±SE.

RESULTS

Amino acids composition: The results are presented in Fig. 1, 2; Table 1 and 2 respectively.

Table 1 shows the amino acids compositions in the tissues of Wesafu, *T. zillii* and *O. niloticus*.

Table 2 shows the amino acids compositions in the blood of Wesafu, *T. zillii* and *O. niloticus*, also indicated the 14 AA assayed present in different proportions in the blood samples of the three species.

DISCUSSION

The contribution of Nigeria's cichlid aquaculture to world total output is nil, in spite of the potentials and available resources. Tilapia aquaculture in the country is not attractive for several reasons including stunting, low market value, and the lack of developed commercially viable species. Research into the development of a commercial strain has not been conducted and this, in turn, is responsible for the lull in the Cichlid aquaculture industry in the country. Identification of individual species of fish is therefore, very important in aquaculture production as this help in maintaining the purity of discrete stock and introgression of the cultured fishes into the wild (Betiku and Omiogun, 2006).

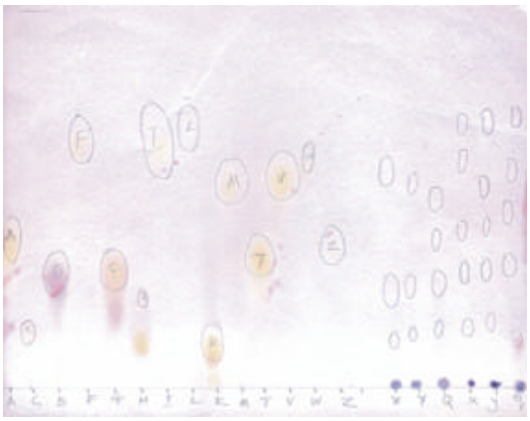

Fig. 1: Amino acids composition in tissue samples of Wesafu, *T. zillii* and *O. niloticus*. A (Alanine), C (Cysteine), D (Asphatic acid), F (Phenylalanine), G (Glycine), H (Histidine), I (Isoleucine), K (Lysine), L (Leucine), M (Methionine), T (Threonine), V (Valine), W (Tryptophan), Z (Glutamic acid), X (Wesafu tissue), Y (*T. zillii* tissue), Q (*O. niloticus* tissue), x (Wesafu blood), y (*T. zillii* blood), q (*O. niloticus* blood)

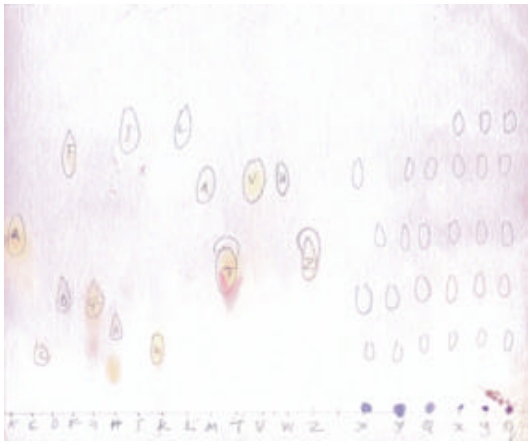

Fig. 2: Amino acids composition in the blood of Wesafu, *T. zillii* and *O. niloticus*. A (Alanine), C (Cysteine), D (Asphatic acid), F (Phenylalanine), G (Glycine), H (Histidine), I (Isoleucine), K (Lysine), L (Leucine), M (Methionine), T (Threonine), V (Valine), W (Tryptophan), Z (Glutamic acid), X (Wesafu tissue), Y (*T. zillii* tissue), Q (*O. niloticus* tissue), x (Wesafu blood), y (*T. zillii* blood), q (*O. niloticus* blood)

Muscle and blood of Wesafu and *O. niloticus* were analyzed for amino acids composition (Table 1 and 2) (10 essential and 4 non essential). The result of assay showed that, 11 amino acids were present in tissue sample of Wesafu, *T. zillii* and *O. niloticus*, with Wesafu

Table 1: Composition of amino acid composition in the tissues of Wesafu, *T. zillii* and *O. niloticus*

		Tissue samples		
Amino acids	Code	Wesafu	*T. zillii*	*O. niloticus*
Alanine	A	-	-	-
Cysteine	C	0.16±0.01[a]	0.18±0.02[a]	0.19±0.01[a]
Asphatic acid	D	0.31±0.02[a]	0.31±0.02[a]	0.33±0.01[a]
Phenylalanine	F	0.61±0.02[a]	0.18±0.01[a]	-
Glycine	G	-	-	-
Histidine	H	0.31±0.00[a]	0.31±0.01[a]	0.33±0.02[a]
Isoleucine	I	0.61±0.02[a]	0.51±0.01[b]	-
Lysine	K	0.16±0.02[a]	0.18±0.01[a]	0.19±0.02[a]
Leucine	L	0.61±0.02[a]	0.51±0.03[b]	-
Methionine	M	-	-	-
Threonine	T	-	-	0.44±0.02[a]
Valine	V	0.61±0.01[a]	0.51±0.02[b]	-
Tryptophan	W	-	-	0.59±0.01[a]
Glutamic acid	Z	-	-	0.44±0.02[a]

Figures in the same horizontal row having the same superscript are not significantly different (p>0.05)

Table 2: Amino acid composition in the blood of Wesafu, *T. zillii* and *O. niloticus*

		Blood samples		
Amino acids	Code	Wesafu	*T. zillii*	*O. niloticus*
Alanine	A	0.45±0.01[a]	0.45±0.01[a]	0.46±0.02[a]
Cysteine	C	0.19±0.01[a]	0.20±0.01[a]	0.18±0.01[a]
Asphatic acid	D	0.33±0.02[a]	0.34±0.01[a]	0.34±0.02[a]
Phenylalanine	F	0.62±0.02[a]	0.74±0.02[b]	0.61±0.03[a]
Glycine	G	0.33±0.01[a]	0.34±0.01[a]	0.34±0.01[a]
Histidine	H	0.33±0.02[a]	0.34±0.01[a]	0.34±0.02[a]
Isoleucine	I	0.72±0.05[a]	0.74±0.04[a]	0.73±0.04[a]
Lysine	K	0.19±0.01[a]	0.20±0.01[a]	0.18±0.02[a]
Leucine	L	0.72±0.02[a]	0.74±0.03[a]	0.73±0.03[a]
Methionine	M	0.45±0.02[a]	0.45±0.03[a]	0.46±0.02[a]
Threonine	T	0.45±0.02[a]	0.45±0.02[a]	0.46±0.03[a]
Valine	V	0.62±0.03[a]	0.74±0.05[b]	0.73±0.04[b]
Tryptophan	W	0.45±0.02[a]	0.45±0.03[a]	0.46±0.03[a]
Glutamic acid	Z	0.45±0.03[a]	0.45±0.03[a]	0.46±0.04[a]

Figures in the same horizontal row having the same superscript are not significantly different (p>0.05)

having the highest value of Phenylalanine, Isoleucine, Leucine and Valine (0.61) followed by *T. zillii* (0.51). These 4 amino acids were completely absent in *O. niloticus* which however had both Tryptophan and Glutamic acid but were absent in Wesafu and *T. zillii* tissues. Alanine, Glycine and Methionine were completely absent in the tissue of all samples analyzed. However, all 14 amino acids analyzed were expressed in the blood samples of the 3 species without significant difference (p>0.05) in individual amino acids. The study suggests that the species are dissimilar and that the ecotype cichlid should not be confused with known species; *T. zillii* and *O. niloticus* buttressing the earlier suggestion of Fashina-Bombata *et al*., 2005a,b, 2006 and 2008 that Wesafu is unidentified cichlid. However, there is the need to establish the identity of Wesafu at a species level by molecular means.

REFERENCES

Betiku, O.C. and O.G. Omiogun, 2006. Genetic diversity of commercial Clarias gariepinuspopulations revealed by SDS-PAGE. In: SA Olakojo, BA Ogunbodede, SR Akande (eds). Proceedings of the 31st Annual Conference of the Genetics Society of Nigeria. NACGRAB, Moor Plantation, Ibadan 6-9 November, 2006. 135-140.

FAO, 2003. Review of the state of World Aquaculture. FAO Fisheries No. 886, Rev. 2. 95pp.

Fashina-Bombata, H.A., R.G. Ajepe and A.M. Hammed, 2008. Age and growth of an ecotype cichlid 'Wesafu' in Epe Lagoon, Lagos, Nigeria. Global J. Agric. Sci., 7: 105-109.

Fashina-Bombata, H.A., A.M. Hammed and R.G. Ajepe, 2006. Food and feeding habits of an ecotype cichlid Wesafu from Epe lagoon, Lagos, Nigeria. World Aquac., 37: 63-66.

Fashina-Bombata, H.A., R.G. Ajepe, A.M. Hammed and A.A. Jimoh, 2005a. Characterization of an ecotype cichlid commonly referred to as Wesafu endemic to Epe-Lagoon, Nigeria. World Aquac., 36: 20-22.

Fashina-Bombata, H.A., A.M. Hammed, R.G. Ajepe and A.A. Jimoh, 2005b. Comparative Organoleptic Assessment of Wesafu (an ecotype Cichlid of Epe Lagoon), *Oreochromis niloticus* and *Tilapia mariae*. Proceedings of the 4th Faculty of Science Annual Conference, pp: 28-29.

Nwadukwe, F., 1991. Tilapia seed production. In: Proceedings of the fish seed propagation course conducted by ARAL 14th-28th August 1991, pp: 40-43.

Ranjna, C., 1999. Practical Clinical Biochemistry: Methods and Interpretations. 2nd Edn., pp: 267.

Smith, L. and J.G. Feinberg, 1973. Paper and thin layer chromatography and Electrophoresis. 2nd Edn., London, Longman.

Stahl, E., 1969. Thin Layer Chromatography: A Laboratory Handbook. 2nd Edn., London, Longman.

Trewavas, E., 1983. Tilapiine fishes of the General Sarotherodon, Oreochromis and Danakilia. British Museum of Natural History, Publ. Numbl. 878. Comstock publishing associates. Ithaca, New York, pp: 583.