Fatty acid composition of the catfish -1: *Heterobranchus longifilis* (Pisces: clariidae and *Chrysichthys nigrodigitatus* (Pisces: Bagridae) reared in tropical fresh waters

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Abstract

The relationship of the fatty acid composition of the three developmental stages (fingerling, juvenile and adult) of *Heterobranchus longifilis* (Pisces: Clariidae) and *Chrysichthys nigrodigitatus* (Pisces: Bagridae) have been evaluated by gas liquid chromatography (Glc) on polyethylene glycol-2-nitroterephthalic ester (PEGNTE).

Results indicated that adults of these fishes, accumulated linolenic acid (C18:3w3) above dietary levels at the fingerling stage which was remarkably depleted in the adult fishes. Its essentiality in their nutrition and metabolism is thus suggested.

In the metabolic turnover, C18:3w3, is utilized to build up the store of eicosapentaenoic acid (C20:5w3), in *Heterobranchus longifilis* while the store of eicosapentaenoic acid (C20:5w3), arachidonic acid (C20:4w6), and docosatetraenoic acid (C22:4w6) were improved in *Chrysichthys nigrodigitatus*.

Both catfishes also improved their store of palmitic acid (C16:0), Oleic acid (C18:1w9), and linoleic acid (C18:2w6). Ratios of fatty acids obtained were those which ascribed dominant role to C16 fatty acid, and further deemphasized the role of w6 fatty acids in the metabolism of these catfishes.

Key words: Gas liquid chromatography, polyethylene glycol-2-nitroterephthalic ester, linolenic acid, eicosapentaenoic acid, arachidonic acid, docosatetraenoic acid, palmitic acid, oleic acid.

Resume

Le rapport de la composition d'acide gras de trois stades de developpement (larve, juvenile et adulte) de *Heterobranchus longifilis* (Pisces: Clariidae) et *Chrysichthys nigrodigitatus* (Pisces: Bagridae) a ete evalue par chromatographie a gaz liquide sur ester de la polyethylene glyco-2-nitroterephthalique. Les resultats ont indique que les adultes de ces poissons avaient accumule l'acide linolenique (C18:3w3) au dessus des niveaux alimentaire au stade larvaire. Ceci etait remarquablement reduit dans les poissons adulte. L'importance de l'acide linolenique dans la nutrition et le metabolisme de ces poissons est suggeree. Dans le roulement metabolique, C18:3w3 est utilise pour accumuler la provision en acide eicosapentaenoique (C20:5w3) chez *Heterobranchus longifilis*. Les provisions en acide eicosapentaenoique (C20:5w3), en acide arachidonique (C20:4w6) et en acide docos_tetraenoique (C22:4w6) ont ete ameliorees chez *Chrysichthys nigrodigitatus*. Les deux poissons ont aussi ameliore leur provision en'acide palmitique (C16:j), en acide oleique (C18:1 w9), et en acide linoleique (C18:2w6). Les proportions d'acide gras obtenues ont ete celles qui avaient contribue au role dominant a l'acide gras C16, et plus loin accentuer le role d'acides gras w6 dans le metabolisme de ces poissons.

Mots des: Chromatographie a gaz liquide, ester de polyethylene glycol-2-nitroterephthalique, acide linolenique, acide eicosapentaenoique, acide arachidonique, acide docosatetraenoique, acide palmitique, acide oleique.

Introduction

Catfishes in general have remarkable growth rate. As a result of their ability to adapt to pond conditions, fast growth rate, acceptance of artificial feeds, tolerance of crowded conditions, and the high quality of their flesh, they have gained popularity among connoisseurs of well priced fishes. This perhaps, is responsible for the abundance of information on the nutrition of the catfish, *Clarias gariepinus* (Clay, 1981; Henken *et al.*, 1985; Uys and Hecht, 1985; Machiels and Henken, 1986; Hecht and Appelbaum, 1987; Ayinla, 1988a, b; Ayinla and Akande, 1988), and *Ictalurus punctatus* (Andrew and Page, 1975; Wilson and Poe, 1985).

A lot of nutritional information also exists for the bagrid catfish, *Chrysichthys nigrodigitatus* (Ikusemiju and Olaniyan, 1977; Ezenwa, 1982; Igbinosun and Talabi, 1982; Igbinosun *et al.*, 1982; Igbinosun and Roberts, 1988). It has also been shown to adapt to various culture systems. *C. nigrodigitatus* is therefore being considered to be a promising species for recruitment in the development of fish culture in West Africa (Ezenwa, 1982; Erundu, 1990).

At the African Regional Aquaculture Centre, investigations (Ayinla *et al.*, 1992) have shown that *Heterobranchius longifillius* responds to growth as effectively to a tadpole meal based diet as to a fish meal based diet. This species has been accepted as possessing the potentials for fish culture development in the oil palm belt of Nigeria in particular, and tropical Africa in general.

The main gap created in these studies, is the fact that the fatty acid composition of the developmental stages of these fishes (fingerling, juvenile, adult) have not previously been investigated under rearing condition.

This investigation was therefore undertaken to evaluate the fatty acid composition of the clariid catfish, *Heterobranchius longifillius* and the bagrid catfish, *Chrysichthys nigrodigitatus* for purposes of comparison and to help outline regimes for further nutritional investigation in the future.

Materials and Methods

Fish Samples: Fry of *H. longifillius* were obtained by induced breeding of the adults. Post larval fry obtained by incubation of the fertilized eggs were reared to the fry and fingerling stages by maintaining them on ground ARAC pellets. From the fingerling to the juvenile stage, and from juvenile to the adult stage, fish were fed with the same feed (pellets).

Fingerlings of *C. nigrodigitatus* were obtained by simulation of natural spawning condition in ARAC hatchery. Juveniles and adults were fed with ARAC feed.

Size grading: For the purpose of this work, sizes were graded for the clariid and bagrid catfishes with particular reference to the scheme suggested by Hunter and Dupree (1984).

(i) Fingerlings	2.50 g - 20g;	10 cm-15.9 cm SL
(H) Juveniles	25g - 150 g	16 cm - 19.9 cm
(Hi) Adult	>150 g	SL >20 cmSL

Sample treatment: For fish weighing less than 20g, a number (2-20) whole fish were obtained to give a convenient weight for the extraction. Fish were completely macerated with fine sand and lipid extracted using the solvent system suggested by Folch *et al.* (1957)..

For fish weighing more than 50 g, gut was first removed and a weight (50-200 g) of flesh sufficient enough for the extraction taken from a combined weight. Percentage lipids obtained for the developmental stages of each type of fish is shown on Table 2. The diet used in the synchronized rearing programmes was formulated as shown in Table 1. Analysis showed the diet to contain 31% crude protein and 3106 Kcals/kg diet.

Table 1. Ingredients and proximate composition of the rearing diet (w /w%)

Ingredient	Weight (%) in diet
Yellow maize	36.80
Groundnut cake Fish meal	32.97
Blood meal	9.89
Oil	6.59
Brewer's waste Bone meal	5.00
Oyster shell Vitamix premix Sodium chloride Proximate analysis Crude protein (%) Oil/Fat (%)	2.00
	0.50
	0.60
	0.25
	30.96
	10.20
Crude fibre (%)	3.54
Moisture (%)	13.42
Ash (%)	32.39
ME (Kcal/kg)	3106.00
Caloric/Protein ratio	100.32

Preparation of fatty acid methyl esters (FAME)

Fatty acid methyl esters (FAME) of lipid extract used for the gas liquid chromatography were prepared by the transmethylation method -described by Stoffel *et al.* (1959). The methyl esters were taken up in a little chloroform and stored in a freezer until analysis.

Gas Liquid chromatography (GLC)

Fatty acid methyl esters prepared by the transmethylation method, were analysed on a varian 3400 gas liquid chromatograph equipped with hydrogen flame ionization detector. The analysis was performed on a fused silica column (25 metre x 0.32 mm I.D.) packed with polyethylene glycol-2nitroterephthalic ester (film thickness 0.25 μm) on Permabond FFAP-DP. The column was operated, isothermally at 215°C using helium as a carrier gas at a flow rate of 2ml/min. The detector maintained at 300°C, was connected to a programmable 440 Digital Integrator equipped with a print-out device and a liquid' Crystal display. Size of sample injected was 1.0 μl in petroleum ether. The identification of peaks in gas liquid chromatograph, was performed by comparison of retention time (RT), relative retention time (RRT), and area percent swept to similar parameters obtained for authentic Merck and Sigma standard methyl esters of lauric acid (C12:p, Myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), Oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), eicosanoic acid (C20:0), arachidonic acid (C20:4), and docosatetraenoic acid (C22:4). Methyl esters of myristoleic acid (C14:1) and palmitoleic acid (C16:1) were also available for this comparison.

Proximate analysis

Sample of the dry diet was analysed for crude protein by the modified Kjeldhal distillation procedure suggested by William (1964). 19 aliquot of dr feed was digested with 20 ml concentrated sulphuric acid and: one tablet of catalyst until a clear solution was obtained. Colour developed with an improved Nessler's reagent after dilution was monitored colorimetrically at a wavelength maximum of 520 nm. Ammonium sulphate was used to plot the standard curve from which miligram nitrogen corresponding to an absorbance reading was directly obtained. Crude protein was obtained by multiplying total nitrogen by 6.25. Lipid was extracted by the Soxhlet ether extraction method and ash by incineration at 550°C according to the AOAC, (1980) methods).

Results

Proximate data of the diet is presented on Table 1. Table 2 shows the percentage oil extracted from the graded fresh water fish samples. Fatty. acid composition of the diet is presented on Table 3. Fatty acid data of the fishsample!3 is presented on Table 4. Table 5 shows the ratios of some biologically important fatty acids for purposes of comparison.

Table 2. Lipid extracted from the catfishes *H. longifilis* and *C. nigrodigitatus*

Fish species	Develop- mental stage	Mean live Et. (g)	Wt. (f) Extracted	Lipid (obtained (g)	(W /W)% Lipid
<i>C. nigrodigitatus</i>	Fingerling	3.2	32 $\pm$ 1.23	0.77 $\pm$ 0.04	2.40 $\pm$ 020
	Juvenile	100.0	50 $\pm$ 1.63	1.56 $\pm$ 0.14	3.12 $\pm$ 48.
	Adult	250.0	70 $\pm$ 0.91	2.81 $\pm$ 0.33	4.06 $\pm$ 014
<i>H. longifilis</i>	Fingerling	12.5	27 $\pm$.88	1.65 $\pm$ 0.15	6.10 $\pm$ 0.50
	Juvenile	40.0	601.3.82	0.96 $\pm$ 0.06	1.60 $\pm$ 0.18
	Adult	400.0	10013.42	2.60 $\pm$ 0.22	2.60 $\pm$ 0.19

Values indicated, are average of three (3) deterrninations ($\pm$ standard deviation)

Table 3. Fatty acid composition of the

diet(%) Fatty acid

Composition (%)

Lauric acid: C12:0	1.54
Myristic acid:	2.74
C14:0 Palmitic	17.20
acid: C16:0	1650
Palmitoleic acid: C16:1	1120
Stearic acid: C18:0	20.30
Oleic acid: C18:1	3.21
Linoleic acid: C18:2	2.60
Linolenic acid: C18:3	3.10
Eicosanoic acid: C20:0	1.10
Arachidonic acid: C20:4	2.30
Eicosapentaenoic acid:	2.80
C20:5 Behenic acid:	15.41
C22:0 Unidentified	

Table 4. Fatty acid composition of *H. longifilis* and *C. nigrodigitatus* fed on arac diet

Fatty add	<i>H. longifilis</i>				<i>C. nigrodigitatus</i>		
	RR'r	Fingerling	Juvenile	Adult	Fingerlin g	Juvenile	Adult
C12:0	0.281	0.047	0.013	0.013	-	2.373	1.156
C14:0	0.426	0.125	0.165	0.735	2.31	1.86	1.756
C14:1	0.462	0.065	0.07	0.021	0.44	0.08	7.51
C16:0	0.639	1.69	0.51	23.95	-	6.55	18.691
C16:1	0.684	0.38	0.57	0.26	0.04	0.16	0.73
C18:0	1.00	2.18	0.14	0.21	2.63	8.72	5.95
C18:1	1.063	1.31	2.19	31.44	1.52	4.94	20.97
C18:2w6	1.201	0.93	0.93	7.89	3.61	2.40	3.92
C18:3w3	1.44	84.84	76.95	17.99	15.43	71.29	20.52
C20:0	1.557	0.07	0.08	0.18	1.43	0.09	0.14
C20:4w6	1.853	1.70	3.43	1.11	0.84	0.75	0.96
C20:5w3	2.330	-	0.34	2.87	0.54	0.31	2.10
C22:4w6	2.516	0.28	0.44	0.29	0.34	0.15	1.16
Unidentified		7.29	14.17	13.04	10.87	6.34	15.56
% Monounsaturated		1.76	2.83	31.72	2.0	5.17	21.73
% Polyunsaturated		86.88	82.09	30.15	80.76	71.90	28.66
% Saturation		4.95	0.91	25.09	6.37	13.59	34.05

RR'P=Relative Retention Time to Stearic acid (C18:0) methyl ester=1.0

Table 5. Comparison of ratios of fatty acids of biological interest for *H. longifilis* and *C. nigrodigitatus*

Property compared	<i>H. longifilis</i>			<i>C. nigrodigitatus</i>		
	Fingerling	Juvenile	Adult	Fingerling	Juvenile	Adult
Weight percent saturated	4.95	0.91	25.09	6.37	13.59	34.05
Weight percent monoenoic	1.76	2.83	31.72	2.0	5.17	21.73
Weight % Sat. + monoenoic	6.71	3.74	56.81	8.37	18.76	55.78
Total weight percent dienoic	0.06	0.93	7.89	3.61	2.40	3.92
Total weight percent trienoic	84.84	76.95	17.99	75.43	71.29	20.52
Total weight percent pentaenoic	-	0.34	2.87	0.54	0.31	2.10
Total weight percent polyunsat.	86.88	82.09	30.16	80.76	74.80	28.66
Ratio 16:0/16:1	4.45	0.89	92.00	-	40.94	25.60
Ratio 16:0/16:1+18:1	1.00	0.19	0.74	-	1.28	0.86
Ratio 16:1/18:1	0.20	0.26	0.01	0.03	0.033	0.035
Ratio <u>20:xw6+22:xw6</u>	33	4.16	0.18	0.33	0.38	0.54
18:2w6+ 18:3w6						
Ratio 20:xw3/18:xw3	-	0.004	0.16	0.01	0.004	0.10
Ratio w3/w6	41.59	16.10	2.32	1.75	18.78	14.76

The results presented in Table 1 indicate that the lipid extracted from the developmental stages of these fish are 1.6% to 6.1% for juvenile and fingerling *H. longifilis* respectively. Crude lipid extracted from *C. nigrodigitatus* followed a progressive pattern, rising to a maximum in the adult fish. In *H. longifilis* a higher quantity was extracted in fingerling fish (6.1 %) while

lower quantity (1.6%) was extracted from the juvenile fish.

Contribution to the fatty acid content of the diet is predominantly from palmitic acid (16:0), 17.20%, palmitoleic acid (16:1), 16.50%, and stearic acid (C18:0); 11.20%. The contributions of highly unsaturated fatty acids such as C20:4 and C20:5 were

1.1 % and 2.8% respectively of the total fatty acid content of the experimental diet.

The fatty acid profile of these catfishes indicate the predominance of C18:3w3 and the substantial depletion of this acid in the adult stages of these two catfishes. It could be inferred that this acid has a central role to play in the metabolism of these catfishes. Another observation is that while linolenic acid (C18:3w3) is being efficiently depleted in these two fishes, palmitic acid (C16:0) and oleic acid (C18:1) were being accumulated from relatively low values in the fingerling and juvenile fishes respectively. Side by side with this is the fact that as palmitic acid and oleic acid were being accumulated and linolenic acid was being rapidly turned over, the level of eicosapentaenoic acid (C20:5w3) was gradually rising as indeed was also the level of linolenic acid (C18:2w6) in the adult *H. longifilis* from trace or negligible values in both fingerling and juvenile fishes respectively. While the trend appeared similar, an exception is discernible in *C. nigrodigitatus*. The adult fish is also depositing (but perhaps) marginally, arachidonic acid (C20:4w6) and docosatetraenoic acid (C22:4w6). The level of C22:4w6 deposited in the adult fish is more than thrice the fingerling level. This picture confirms the turnover in the adult fish. Species specific utilization of linolenic acid (C18:3w3) can easily be observed. Although the two fishes at both the fingerling and juvenile stages, demonstrated highly stimulating turnover, in adult *H. longifilis*, it was being utilized to improve the store of eicosapentaenoic acid (C20:5w3) while *C. nigrodigitatus*, it was being utilized to improve the store of eicosapentaenoic acid (C20:5w3), arachidonic acid (C20:4w6), and docosatetraenoic acid (C22:4w6).

Discussion

Results obtained in this investigation showed that fish could either accumulate or deplete relatively higher proportions of unsaturated fatty acids (viz C18:3w3) independent of the dietary levels (Owen *et al.*, 1972). In consonance with this finding, reasons could be advanced that, the pond environment from which these fish samples were obtained could have also provided lipids originating from plants or phytoplankton through the lower i.e. forms eaten by these fish.

Total fatty acid content in fish can be affected to certain limits by varying the fatty acid composition of the food, (Brenner *et al.*, 1963; Reiser *et al.*, 1963; Lovern, 1964). Only a single compounded diet was provided, therefore the difference in (response) the fatty acid composition of the fish sample could be species specific. Linolenic acid was observed to be remarkably depleted by adults of both *H. longifilis* and *C. nigrodigitatus*. The results of this depletion is

observed in *C. nigrodigitatus* to enhance the levels of arachidonic acid (C20:4w6), eicosapentaenoic acid (C20:5w3) and docosatetraenoic acid (C22:3w3). In *H. longifilis*, the depletion of linolenic acid (C18:3w3), brought about enhanced levels of eicosapentaenoic acid (C20:5w3), oleic acid (C18:1w9) and palmitic acid (C16:0), in the adult from low values in the fingerling.

In consideration of all the facts stated above, linolenic acid is a most essential fatty acid in both *H. longifilis* and *C. nigrodigitatus* which is probably utilized above dietary levels (2.60%) in both *H. longifilis* (18%) and *C. nigrodigitatus* (21%) to synthesize high chain polyunsaturated fatty acids such as arachidonic acid, eicosapentaenoic acid, and docosatetraenoic acid. Other acids found to be accumulated may also be playing major role in the inhibitory phenomenon which is known to occur in the metabolism of a fatty acid (Brenner and Peluffo, 1966; Castor *et al.*, 1966; Lindstrom and tinsley, 1966).

Acetate fragments obtained by the breakdown of dietary fats and oil could be utilized in the synthesis of highly unsaturated fatty acid (HUFA) utilizing the two pathways of synthesis (of w3 and w6 unsaturated fatty acids) proposed by Mead *et al.* (1961), and Kayama *et al.* (1963), See Figure 1. It is probable that the unidentified fraction which constituted 13.04% and 15.56% in adult *H. longifilis* and *C. nigrodigitatus* respectively, may contain other proportions of highly unsaturated fatty acids which may be some of the intermediates proposed in the sequence above.

Figure 1. Biosynthetic pathway to the longerchain acids proposed by Mead *et al.* (1961), Kayama *et al.* (1963)

However, the relative proportions of all of the fatty acids are kept in balance in normal fish on a diet as dictated by the species, environmental

conditions, age, stage of sexual development and other factors (Lovem, 1964). In conclusion, therefore, both *H. longifilis* and *C. nigrodigitatlls* require linolenic acid (C18:3w3) as essential fatty acid and cannot synthesize this acid *de novo* (Higashi *et. al.*, 1966). When supplied with these essential fatty acid (levels $\geq 18\%$) these fish can perform chain extension by the usual metabolic pathway to give longer-chain polyunsaturated fatty acids such as C20:4w6, C20:5w3, C22:4w6 and probably C22:5w3. The obligatory conversion of ingested C18:3w3 (which was far in excess of the dietary level), to any or some of the highly unsaturated fatty acids by these two catfishes (which bear no family relationship), is evident from this investigation to inspire further work along the line.

Ratios of the fatty acids are those related to extremely low levels of the highly unsaturated fatty acids having C20 and C22 structure. Ratios of 16:0/16:1, extremely high for *H. longifilis*, confirmed the major role of 16:0 (palmitic) acid in the metabolism of adults. It is also high for juvenile and adult *C. nigrodigitatis* Le. 40.94 and 25.60 respectively. Ratios of w3/ w6 was highest for fingerling *H. longifilis* while the juvenile fish scored an intermediate value (16.10) as did juvenile (18.78), and adult (14.76) of *C. nigrodigitatlls*. Adult *H. longifilis* (2.32) and fingerling *C. nigrodigitatlls* (1.75) scored low values indicative of apparent low levels of w3 acids in adult *H. longifilis* and fingerling *C. nigrodigitatlls* respectively.

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