

Original Research Article

Effects of dietary vegetable oils on growth, lipogenic enzyme activities and tissue fatty acid composition of *Heterobranchus longifilis*

Theophilus Olayiwola Babalola, John Bunmi Olasunkanmi

Department of Fisheries and Aquaculture, Federal University, Oye-Ekiti, Nigeria

Correspondence to:

T. O. Babalola, Department of Fisheries and Aquaculture, Federal University, Oye-Ekiti, Nigeria;

E-mail: theophilus.babalola@fuoye.edu.g; +2347038733608

Abstract

Feasibility of total replacement of fish oil (FO) with vegetable oils (palm oil (PO) and soybean oil (SO)) in practical diet for *Heterobranchus longifilis* fingerlings was evaluated. Six iso-nitrogenous and iso-caloric diets were prepared. Control diet (A) used FO as the lipid source, diets B–F contained various blends of palm and soybean oils. Fish were fed to apparent satiation twice daily for twelve weeks. No significant ( $P > 0.05$ ) differences were observed for feed intake, weight gain, feed conversion ratio or specific growth rates among dietary treatments. Activities of glucose-6-phosphate dehydrogenase (G6PDH) and malic enzyme (ME) were significantly ( $P < 0.05$ ) higher in fish fed diet C (1.5% PO). Generally, hepatic G6PDH activity was about 3-4 times higher than ME activity in fish fed the experimental diets. The tissue fatty acid (FA) profiles in fillet and liver reflected the dietary FA compositions. However, the concentrations of eicosapentaenoic acid and docosahexaenoic acid were higher in fish tissue (fillet and liver) than in the diets. Results from the present study indicate that replacement of fish oil with vegetable oil resulted in satisfactory growth and body composition characteristics in *Heterobranchus longifilis*. Compared with soybean oil, palm oil is relatively cheap and readily available; therefore, diet F (6% PO) is recommended for use in *H. longifilis* diet.

**Keywords:** fatty acid composition; fish oil; growth performance; *Heterobranchus longifilis*, lipogenic enzyme activities; vegetable oil.

INTRODUCTION

Global production and consumption of freshwater fish reared in captivity systems has increased during the last years. Catfish is the most consumed among the freshwater fish reared because it has good taste and few bones (Babalola, 2010). However, studies show that tissue lipids of fish consuming natural diet are generally rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which exert positive health effects on humans (Leaf and Kang, 1998; Kucska et al., 2006). The ability of individual fish species to modify the dietary fatty acid and the dietary lipid composition are the major factors influencing fatty acid of fish tissues (Bell et al., 1993). Essential fatty acids linoleic (LA) and  $\alpha$ -linolenic acid (LNA) are metabolised by sequential desaturation and elongation enzyme system, which results in the production of long chain of polyunsaturated fatty acids of  $n-3$  and  $n-6$  series. Most freshwater fish are capable of  $\Delta 6$  desaturating  $18:3n-3$  to  $18:4n-3$  and  $18:2n-6$  to  $18:3n-6$ , followed by elongation and  $\Delta 5$ -desaturation to eicosapentaenoic acid (EPA,  $20:5n-3$ ) and arachidonic acid (ArA,  $20:4n-$

$6$ ), which also undergo elongation and  $\Delta 4$ -desaturation to produce docosahexaenoic acid (DHA,  $22:6n-3$ ) and docosapentaenoic acid (DPA,  $22:5n-6$ ) (Henderson, 1996).

Palm oil and soybean oil form the largest vegetable share oil produced worldwide (Shahbandeh, 2019). Palm oil is a rich source of saturated and unsaturated fatty acids. LA and LNA are also present in palm oil. However, the concentration of LA and LNA in soybean oil is higher. Therefore, the blend of these lipid sources at different levels in *H. longifilis* diets will supply the two parent fatty acids – ALA and LA for the synthesis of  $n-3$  and  $n-6$  PUFA, respectively.

The hypothesis of this study is that the inclusion of vegetable oils in the diets of fish could help improve animal performance, product quality in terms of essential fatty acid deposition as well as reducing the cost of fish production. Therefore, this study was designed to investigate the effects of dietary palm oil in combination with soybean oil at different levels on growth, lipogenic enzyme activities and tissue fatty acid composition in *H. longifilis*.

**Table 1.** Composition of experimental diets (g kg<sup>-1</sup>) for fingerling *Heterobranchus longifilis*

Ingredients	Diets*					
	A	B	C	D	E	F
<b>**Basal ingredients</b>	940.00	940.00	940.00	940.00	940.00	940.00
Fish oil	60.00	-	-	-	-	-
Soybean oil	-	60.00	45.00	30.00	15.00	-
Palm oil	-	-	15.00	30.00	45.00	60.00
<b>Analysed proximate composition (n = 3)</b>						
Moisture (g/kg)	63.00	65.00	62.00	61.00	62.00	63.00
Crude protein (g/kg)	456.80	449.50	451.00	450.30	450.10	449.80
Lipid (g/kg)	105.00	112.00	111.10	112.10	112.50	113.20
Ash (g/kg)	83.00	82.00	81.50	81.10	78.90	79.60
<sup>1</sup> Nitrogen free extract (g/kg)	292.20	356.50	356.40	356.50	358.50	357.40
<sup>2</sup> Metabolizable energy (kJ/g)	17.47	17.52	17.51	17.53	17.58	17.58

\*Diets A = FO; B = 0% PO and 6.0% SO; C = 1.5% PO and 4.5% SO; D = 3.0% PO and 3.0% SO; E = 4.5% PO and 1.5% SO; F = 6.0% PO and 0% SO.

\*\*Basal ingredients contain (g kg<sup>-1</sup> of the diet): Fish meal, 398; Soybean meal, 313; cornflour (maize), 177; vitamin/mineral premix, 20; methionine, 10; salt (NaCl), 1.5; vitamin C, 0.5 and cassava starch, 20.

<sup>1</sup> Nitrogen free extracts including fibre = 1000 – (crude protein + crude lipid + ash)

<sup>2</sup> calculated from the published compositions of the ingredients used.

**MATERIALS AND METHODS**

The experiment was carried out at the Nutrition Laboratory of Federal University, Oye-Ekiti, Nigeria with 300 *Heterobranchus longifilis* fingerlings with initial mean weight of 2.34 ± 0.31 g, distributed in 18 65-L circular plastic bowls and submitted to six treatments and three duplications. Fingerlings were fed a control diet with 6% FO. In other diets, i.e. B to F, distinct amounts of soybean oil and palm oil (6:0, 4.5:1.5, 3.0:3.0, 1.5:4.5 or 0:6.0) replaced the FO in the control diet (Table 1). The fish were fed to apparent satiety twice daily at 09.00 and 16.00 h and the amount of feed consumed by each experimental unit was recorded on daily basis. Each tank of fish was weighed every two weeks. After twelve weeks, four fish per tank were slaughtered, filleted and stored in polyethylene packing at -4 °C for fatty acid analysis. Liver samples were weighed for the determination of hepatosomatic index and stored at -4 °C for the determination of hepatic lipogenic enzyme activities and fatty acids.

Fatty acid analysis was performed on three samples of each experimental diet and on two pooled fillet and liver samples for each experimental tank. The extraction of total lipids and preparation of fatty acid methyl esters was performed according to Sukhija and Palmquist (1988). Fatty acid analysis was carried out on a Perkin Elmer gas chromatograph (Model 8700) fitted with an automatic sampler (Model AS 2000B) and FID detector. The conditions used were the following: Omegawax fused silica capillary column (30 m×0.25 mm I.D., 0.25 µm film thickness) (Supelco, Bellefonte, PA), temperature programmed from 100 °C to 250 °C at 3 °C/min, held for 10 min. Carrier gas was helium at 1.0 ml/min, inlet pressure 12 psi. Fatty acids methyl esters were identified in comparison to an

external standard (Supelco™ 37 component FAME Mix). For the lipogenic enzyme assays, liver samples were homogenized in three volumes of ice-cold buffer (0.02 M Tris-HCl, 0.25 M sucrose, 2 mM EDTA, 0.1 M sodium fluoride, 0.5 mM phenylmethylsulfonyl fluoride, 0.01 M β-mercaptoethanol, pH 7.4), and homogenates were centrifuged with Eppendorf 5417R refrigerated centrifuge at 15,000 × g at 4 °C for 20 min. Selected lipogenic enzyme activities of the supernatant were quantified with spectrophotometric procedures: glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) according to Bautista et al. (1988) and malic enzyme (ME, EC 1.1.1.40) according to Ochoa (1955). The enzymatic activity units (IU), defined as micromoles of substrate converted to product per minute at the assay temperature (30 °C) were expressed per gram of liver tissue (wet weight).

Data were subjected to one-way ANOVA using the model for completely randomised design followed by Duncan's multiple range tests for comparisons of the means among different dietary treatments. Analyses were conducted using the SPSS 15.0 software package.

**RESULTS**

**Growth performance and hepatosomatic index**

There was no significant ( $P > 0.05$ ) effect of the dietary treatments on feed intake, weight gain, specific growth rate and feed conversion ratio (Table 3) of *H. longifilis* fed the experimental diets. However, the hepatosomatic index (HSI) of fish fed 6.0% FO-based diet was significantly higher ( $P < 0.05$ ) than in other dietary groups; fish fed 1.50% PO diet had the least HSI.

Table 2. Fatty acid composition of the experimental diets\* (g/100 g of total FA)

Fatty acids	DIETS					
	A	B	C	D	E	F
Lauric acid	1.13	0.58	0.20	0.28	0.24	0.15
Myristic acid	5.98	4.22	2.85	1.98	7.57	6.79
Palmitic acid	18.14	18.77	5.39	23.40	32.97	33.24
Stearic acid	4.53	4.11	6.28	3.89	4.39	4.43
Arachidic acid	0.74	0.66	0.39	0.53	0.18	0.19
Behenic acid	0.20	0.22	0.12	0.14	ND	ND
Lignoceric acid	0.19	0.15	0.08	0.10	ND	ND
Sum saturated FAs	30.91	28.71	15.31	30.32	45.35	44.79
Palmitoleic	5.79	3.70	6.23	4.17	3.70	3.73
Cis-9-hexadecanoic	8.61	6.49	5.43	3.63	0.11	0.11
Oleic acid	9.61	13.95	28.04	23.53	29.18	29.42
Eicosanoic	1.93	1.44	1.50	2.05	2.47	2.50
Nervonic	0.63	0.28	0.22	0.18	0.01	0.01
Sum <i>n</i> -9 FAs	20.77	22.16	35.19	29.39	31.77	32.04
Cetoleic	2.72	1.32	1.50	1.31	0.99	1.00
Linoleic acid	7.55	26.33	26.07	19.09	10.17	10.26
Gamma-linolenic	1.97	1.21	0.58	0.66	0.10	0.10
Eicosadienoic	2.83	0.09	0.13	0.18	0.18	0.18
Dihomo-gamma-linolenic	0.17	ND	ND	ND	0.09	0.09
Arachidonic acid	0.91	0.43	0.45	0.36	0.19	0.19
Sum <i>n</i> -6 FAs	13.42	28.06	27.24	20.30	10.74	10.83
Linolenic acid	1.47	3.19	2.48	1.99	0.11	0.10
Eicosapentaenoic acid	10.95	4.84	5.51	3.66	3.24	3.27
Docosapentaenoic acid (clupanodonic acid)	1.36	0.56	0.49	0.70	0.46	0.47
Docosahexaenoic acid	12.61	7.45	6.06	8.15	3.74	3.77
Sum <i>n</i> -3 FAs	26.39	16.04	14.53	14.50	7.56	7.61
Sum monounsaturated FAs	29.28	27.18	42.92	34.87	36.47	36.77
Sum polyunsaturated FAs	39.81	44.10	41.77	34.80	18.29	18.44
Sum unsaturated FAs	69.09	71.29	84.69	69.68	54.76	55.21
<i>n</i> -3/ <i>n</i> -6	1.97	0.57	0.53	0.71	0.69	0.70

\*Diets A = FO; B = 0% PO and 6.0% SO; C = 1.5% PO and 4.5% SO; D = 3.0% PO and 3.0% SO; E = 4.5% PO and 1.5% SO; F = 6.0% PO and 0% SO.  
 ND = not detected

**Fatty acid composition of the diet, fillet and liver**

Diet FA compositions (Table 2) were different and reflected the changing ratio of soybean oil and palm oil among the diets. In addition, dietary percentages of 18:2*n*-6 were significantly ( $P < 0.05$ ) different in all treatments. Dietary percentage of 18:3*n*-3 concentration was also higher in the 6.0% FO, 6.0% SO, 4.5% SO and 3.0% SO diets than in the other diets. Because of this, the *n*-3/*n*-6 ratios were different in all dietary treatments. The fatty acids content of the fillet are presented in Table 5. The fatty acid composition of the fillet reflected the fatty acids composition of the experimental diets, as the levels of saturated fatty acids, monounsaturated fatty acids (MUFA), *n*-3 HUFA and *n*-6 HUFA reflected the composition of each of the different dietary lipid levels. Fish fed the diet 4.5% PO had the highest

level of MUFA in the fillet, and fish fed FO diet had the highest levels of *n*-3 HUFA. The fatty acid profile in the liver differed between the levels of dietary lipids (Table 6). After 12 weeks, total lipids in the liver of fish fed the control diet (FO) contained 42.70% saturates, 57.23% MUFA and 16.50% polyunsaturated fatty acids (PUFA) with *n*-3 PUFA being the major component (11.77%) (Table 6). In the fish liver, saturated fatty acids and MUFA were highest in fish fed the 6.0% PO and 1.5% PO diets, respectively, whereas *n*-3 and *n*-6 PUFA were higher in fish fed 6.0% FO and 6.0% SO diets, respectively, than in fish fed other diets. The contents of docosahexaenoic acid (22:6*n*-3, DHA) and eicosapentaenoic acid (20:5*n*-3, EPA), and the *n*-3/*n*-6 ratio were significantly reduced by the inclusion of dietary palm oil ( $P < 0.05$ ). Generally, the concentration of EPA (22:5*n*-3) increased in the fillet and reduced

**Table 3.** Growth performance and hepatosomatic index of *H. longifilis* fed diets containing a blend of palm oil and soybean oil for 12 weeks

*Diets	Initial weight(g)	Weight gain (g)	Average Feed intake(g)	FCR	SGR	HIS (%)
A	2.54	29.70	45.05	1.29	2.89	1.10 <sup>a</sup>
B	2.34	29.48	46.14	1.57	2.45	0.44 <sup>cd</sup>
C	2.19	31.70	48.31	1.53	2.48	0.36 <sup>d</sup>
D	2.60	29.66	39.63	1.34	2.34	0.92 <sup>b</sup>
E	2.64	29.08	44.72	1.54	2.30	0.47 <sup>cd</sup>
F	2.58	33.35	42.90	1.29	2.59	0.58 <sup>c</sup>
SEM	0.09	1.10	1.61	0.05	0.07	0.03

FCR = feed conversion ratio (feed intake / weight gain)

SGR = specific growth rate (100 × (ln[final body weight] – ln [initial body weight])/No. days)

HSI = hepatosomatic index (100 × (liver weight / body weight))

\*Diets A = FO; B = 0% PO and 6.0% SO; C = 1.5% PO and 4.5% SO; D = 3.0% PO and 3.0%SO; E = 4.5% PO and 1.5% SO; F = 6.0% PO and 0% SO.

Values in the same column followed by the same letter are not significantly different at  $P > 0.05$

**Table 4.** Hepatic lipogenic enzyme activities of *H. longifilis* fed diets containing a blend of palm oil and soyabean oil for 12 weeks

*Diets	G6PDH (IU/ g liver)	Malic (IU/ g liver)
A	2.00 <sup>b</sup>	0.51 <sup>c</sup>
B	4.21 <sup>a</sup>	0.95 <sup>bc</sup>
C	4.12 <sup>a</sup>	2.94 <sup>a</sup>
D	3.55 <sup>a</sup>	0.71 <sup>bc</sup>
E	3.50 <sup>a</sup>	1.12 <sup>b</sup>
F	3.88 <sup>a</sup>	0.72 <sup>bc</sup>
SEM	0.19	0.09

\*Diets A = FO; B = 0% PO and 6.0% SO; C = 1.5% PO and 4.5% SO; D = 3.0% PO and 3.0% SO; E = 4.5% PO and 1.5% SO; F = 6.0% PO and 0% SO.

Values in the same column followed by the same letter are not significantly different at  $P > 0.05$

**Table 5.** Fillet fatty acid composition (g/100g of total FA) of *H. longifilis* fed diets containing a blend of palm oil and soybean oil for 12 weeks

Fatty acids	*Diets						SEM
	A	B	C	D	E	F	
Sum saturated FAs <sup>1</sup>	29.33 <sup>d</sup>	29.15 <sup>d</sup>	15.39 <sup>c</sup>	30.25 <sup>c</sup>	36.27 <sup>b</sup>	43.15 <sup>a</sup>	0.11
Sum monounsaturated FAs <sup>2</sup>	23.64 <sup>f</sup>	25.17 <sup>c</sup>	43.24 <sup>b</sup>	33.85 <sup>d</sup>	52.08 <sup>a</sup>	37.62 <sup>e</sup>	0.12
Oleic acid	10.09 <sup>c</sup>	16.08 <sup>d</sup>	31.90 <sup>b</sup>	25.56 <sup>c</sup>	50.66 <sup>a</sup>	31.79 <sup>b</sup>	0.15
Sum n-9 FAs <sup>3</sup>	17.38 <sup>c</sup>	21.43 <sup>d</sup>	36.44 <sup>b</sup>	29.39 <sup>c</sup>	51.00 <sup>a</sup>	33.55 <sup>b</sup>	0.10
Linoleic acid	5.60 <sup>d</sup>	20.26 <sup>a</sup>	19.43 <sup>a</sup>	14.11 <sup>b</sup>	10.66 <sup>e</sup>	7.44 <sup>d</sup>	0.07
Arachidonic acid	1.63 <sup>a</sup>	0.81 <sup>b</sup>	0.83 <sup>b</sup>	0.22 <sup>c</sup>	0.30 <sup>c</sup>	0.33 <sup>c</sup>	0.01
Sum n-6 FAs <sup>4</sup>	8.24 <sup>d</sup>	22.03 <sup>a</sup>	21.30 <sup>a</sup>	15.49 <sup>b</sup>	10.89 <sup>c</sup>	7.79 <sup>d</sup>	0.14
Linolenic acid	0.88 <sup>d</sup>	2.02 <sup>a</sup>	1.55 <sup>b</sup>	1.18 <sup>c</sup>	0.65 <sup>c</sup>	0.07 <sup>f</sup>	0.03
Eicosapentaenoic acid	12.63 <sup>a</sup>	5.82 <sup>c</sup>	6.46 <sup>b</sup>	4.48 <sup>d</sup>	3.72 <sup>c</sup>	3.55 <sup>e</sup>	0.08
Docosahexaenoic acid	22.40 <sup>a</sup>	13.99 <sup>b</sup>	11.02 <sup>c</sup>	14.37 <sup>b</sup>	9.34 <sup>d</sup>	6.37 <sup>c</sup>	0.11
Sum n-3 FAs <sup>5</sup>	37.76 <sup>a</sup>	22.97 <sup>b</sup>	21.01 <sup>b</sup>	20.09 <sup>b</sup>	16.64 <sup>c</sup>	11.29 <sup>d</sup>	0.10
n-6/n-3	0.22 <sup>c</sup>	0.96 <sup>b</sup>	1.06 <sup>b</sup>	1.36 <sup>ab</sup>	1.53 <sup>a</sup>	1.50 <sup>a</sup>	0.24
n-3/n-6	4.65 <sup>a</sup>	1.06 <sup>d</sup>	1.01 <sup>d</sup>	1.34 <sup>c</sup>	1.51 <sup>b</sup>	1.46 <sup>bc</sup>	0.30

<sup>1</sup> Contains - lauric, myristic, palmitic, stearic, arachidic, vhenic, and lignoceric acids.

<sup>2</sup> Contains - cis-9-hexadecanoic, palmitoleic, oleic, cis-vassenic, gadoleic, cetoleic andnervonic acids.

<sup>3</sup> Contains - cis-9-hexadecanoic, oleic, and nervoic acids.

<sup>4</sup> Contains - linoleic, gamma-linolenic, eicosadienoic, dihome-gamma-linolenic, arachidonic,docosadienoic, adrenic and docosapentaenoic acids.

<sup>5</sup> Contains - linolenic, stearidonic, eicosatetraenoic, eicosapentaenoic, docosapentaenoic (clupanodonic acid) and docosahexaenoic acids.

\*Diets A = FO; B = 0% PO and 6.0% SO; C = 1.5% PO and 4.5% SO; D = 3.0% PO and 3.0% SO; E = 4.5% PO and 1.5% SO; F = 6.0% PO and 0% SO.

Values in the same row followed by the same letter are not significantly different at  $P > 0.05$

**Table 6.** Liver fatty acid composition (g/100g of total FA) of *H. longifilis* fed diets containing a blend of palm oil and soybean oil for 12 weeks

Fatty acids	Diets						SEM
	A	B	C	D	E	F	
Sum saturated FAs <sup>1</sup>	42.70 <sup>a</sup>	35.85 <sup>b</sup>	21.57 <sup>c</sup>	35.52 <sup>b</sup>	42.30 <sup>a</sup>	42.94 <sup>a</sup>	1.26
Sum monounsaturated FAs <sup>2</sup>	57.23 <sup>b</sup>	42.18 <sup>d</sup>	66.06 <sup>a</sup>	52.15 <sup>c</sup>	51.60 <sup>c</sup>	51.90 <sup>c</sup>	0.31
Oleic acid	21.25 <sup>c</sup>	30.23 <sup>d</sup>	51.42 <sup>a</sup>	41.17 <sup>c</sup>	43.30 <sup>b</sup>	43.32 <sup>b</sup>	0.09
Sum n-9 FAs <sup>3</sup>	24.35 <sup>d</sup>	32.16 <sup>c</sup>	53.00 <sup>a</sup>	42.92 <sup>b</sup>	44.86 <sup>b</sup>	44.62 <sup>b</sup>	0.07
Linoleic acid	3.10 <sup>d</sup>	10.67 <sup>a</sup>	9.01 <sup>b</sup>	6.25 <sup>c</sup>	2.83 <sup>c</sup>	2.82 <sup>c</sup>	0.05
Arachidonic acid	1.16 <sup>a</sup>	0.54 <sup>b</sup>	0.48 <sup>c</sup>	0.38 <sup>d</sup>	0.16 <sup>c</sup>	0.17 <sup>c</sup>	0.04
Sum n-6 FAs <sup>4</sup>	4.76 <sup>d</sup>	11.67 <sup>a</sup>	9.56 <sup>b</sup>	6.82 <sup>c</sup>	3.03 <sup>d</sup>	3.03 <sup>d</sup>	0.01
Linolenic acid	0.38 <sup>c</sup>	0.78 <sup>a</sup>	0.52 <sup>b</sup>	0.40 <sup>c</sup>	0.16 <sup>d</sup>	0.05 <sup>c</sup>	0.03
Eicosapentaenoic acid	3.36 <sup>a</sup>	1.46 <sup>b</sup>	1.40 <sup>b</sup>	0.89 <sup>d</sup>	0.68 <sup>c</sup>	0.67 <sup>c</sup>	0.03
Docosahexaenoic acid	7.20 <sup>a</sup>	4.19 <sup>b</sup>	2.88 <sup>d</sup>	3.68 <sup>c</sup>	1.44 <sup>c</sup>	1.43 <sup>c</sup>	0.03
Sum n-3 FAs <sup>5</sup>	11.77 <sup>a</sup>	6.78 <sup>b</sup>	5.04 <sup>d</sup>	5.32 <sup>c</sup>	2.45 <sup>c</sup>	2.32 <sup>f</sup>	0.01
n-3/n-6	2.45 <sup>a</sup>	0.59 <sup>c</sup>	0.56 <sup>c</sup>	0.71 <sup>b</sup>	0.78 <sup>b</sup>	0.77 <sup>b</sup>	0.02
n-6/n-3	0.41 <sup>c</sup>	1.72 <sup>a</sup>	1.90 <sup>a</sup>	1.28 <sup>b</sup>	1.24 <sup>b</sup>	1.31 <sup>b</sup>	0.06

<sup>1</sup> Contains - lauric, myristic, palmitic, stearic, arachidic, behenic, and lignoceric acids.

<sup>2</sup> Contains - cis-9-hexadecanoic, palmitoleic, oleic, cis-vaccenic, gadoleic, cetoleic and nervonic acids.

<sup>3</sup> Contains - cis-9-hexadecanoic, oleic, and nervonic acids.

<sup>4</sup> Contains - linoleic, gamma-linolenic, eicosadienoic, dihomogamma-linolenic, arachidonic, docosadienoic, adrenic and docosapentaenoic acids.

<sup>5</sup> Contains - linolenic, stearidonic, eicosatetraenoic, eicosapentaenoic, docosapentaenoic (clupanodonic acid) and docosahexaenoic acids.

\*Diets A = FO; B = 0% PO and 6.0% SO; C = 1.5% PO and 4.5% SO; D = 3.0% PO and 3.0% SO; E = 4.5% PO and 1.5% SO; F = 6.0% PO and 0% SO.

Values in the same row followed by the same letter are not significantly different at  $P > 0.05$ .

in the liver when compared to the concentration in the diets. The highest concentration was recorded in fillet of FO fed fish and the lowest concentration was observed in liver of 4.5% and 6.0% PO fed fish. The FAs that were in higher proportion in the fish fillet and liver were EPA and DHA.

The highest accumulation of EPA and DHA was observed in fillet of fish fed diets A, B, C and D and in liver of fish fed diets A and B. MUFA and oleic acid (18:1n-9, OA) significantly reduced in the liver of fish fed 0% PO diet ( $P < 0.05$ ). Linoleic acid (18:2n-6, LA) and linolenic acid (18:3n-3, LNA) increased in fish fed the 6.0% SO diets ( $P < 0.05$ ). Similarly, the LA content in the liver of fish fed 6.0% SO diet was significantly ( $P < 0.05$ ) higher. The replacement of soybean oil in diets reduced the content of n-6 and n-3 polyunsaturated fatty acids ( $P < 0.05$ ). The content of n-3 HUFA in liver of fish fed the 6.0%SO diet was significantly ( $P < 0.05$ ) higher than that of other experimental diets ( $P < 0.05$ ). However, the n-3/n-6 fatty acid ratio in the fillet and liver were significantly ( $P < 0.05$ ) increased in fish fed 3.0%, 4.5% and 6.0% PO diets.

**Lipogenic enzyme activities**

Activities of glucose-6-phosphate dehydrogenase (G6PDH) and malic enzyme (ME) measured in liver of *H. longifilis* fed experimental diets for 12 weeks are presented in Table 4. After 12 weeks, hepatic G6PDH activity increased four folds than ME activity in fish fed

FO, 0%, 3.0% and 6.0% PO diets. However, the G6PDH activity was about 3.0 times higher than ME activity in 4.5% PO fed fish.

**DISCUSSION**

The fatty acid profiles of fish tissues are affected by diet composition, duration of the feeding period, and growth obtained during the experiment (Blanchard et al., 2008). Good growth performance of *H. longifilis* fed diets containing different blends of PO and SO suggested that EFAs were not deficient and that the use of plant oils did not impair growth performances at any levels of substitution. It has been shown that vegetable oils, like linseed oil (LO) or SO, are rich in FA with 18 carbon atoms, especially linoleic and linolenic acid, which are essential for freshwater fish. Therefore, many vegetable oils are good lipid sources in diets for freshwater species such as African catfish (*H. longifilis*, Legendre et al. 1995, Babalola and Apata 2012), common carp (*C. carpio*, Fontagné et al. 1999), Atlantic salmon (*S. salar*, Rosenlund et al., 2000; Tocher et al., 2003) or rainbow trout (*O. mykiss*, Caballero et al., 2002) with replacement rates of at least 40% up to 100%.

The higher HSI in fish fed 3.0% PO diet compared to fish on other diets may be the results of an increased activity of the fatty acyl desaturation and elongation pathways in hepatocytes, as commonly reported in

fish fed vegetable oil compared to those fed fish oil (Zheng et al., 2005; Turchini et al., 2006).

In this study, malic enzyme, which is involved in the fatty acid biosynthesis of converting malate to pyruvate with subsequent generation of extra mitochondrial NADPH, was significantly reduced in liver with diet containing FO, 3.0% and 6.0% PO. According to the report of James (1994), when more fat is absorbed from the intestine the activities of major lipogenic enzymes are reduced. The reduced activity of malic enzyme in this study is an indication of better utilization of dietary lipid in the diets. The G6PDH activity is an excellent model system to analyse the regulation of intracellular metabolism by dietary fat since it participates in multiple metabolic pathways such as lipogenesis, cellular growth and reductive biosynthesis (Kletzien et al., 1994). It also has a direct influence on detoxification reactions (Horton and Fairhurst, 1987). It is reported that dietary PUFA inhibit the expression of the lipogenic enzyme glucose-6-phosphate dehydrogenase (G6PDH) in rat hepatocytes (Stabile et al., 1998), and lipogenesis in rat (Zampelas et al., 1995) and rainbow trout (Alvarez et al., 2000). The diets fed to fish in this study (except the control diet) were devoid of fish oil and this could be the reason for the significant difference in the activities of G6PDH of fish fed various levels of PO than those fed the FO diet.

After twelve weeks of feeding *H. longifilis* with different dietary treatments, the FA composition of target tissues was compared with their corresponding diets. Tissue FA reflecting the FA composition of the diet is a common observation reported by numerous authors (Caballero et al., 2002; Ng et al., 2004; Turchini et al., 2006; Wang et al. 2018). Predominant long chain PUFA of *H. longifilis* tissues were 20:5n-3 and 22:6n-3. Irrespective of the diet used in this study, the accumulation and decreased incorporation of certain dietary FA into fish tissue indicates a selective retention of these FA, with respect to the need and specificity of each tissue. For example, incorporation of SFA and MUFA was less pronounced in the fillet. But at the opposite, fillet tissue mainly accumulated ArA, EPA, and DHA. The n-3 PUFA in the liver, EPA (C20:5n-3) and DHA (C22:6n-3), were significantly reduced in fish, which is like the reports for Atlantic salmon (Bell et al., 2003) and rainbow trout (Drew et al., 2007). These results suggest a higher elongation and desaturation activity in liver than in other tissues and a selective retention of long chain PUFA in fillet tissue, probably reflecting the higher content of phospholipids (Blanchard et al., 2008). According to Henderson and Tocher (1987), DHA is the main component of the phosphoglycerols in fish biomembranes.

A higher liver accumulation of ArA was found in FO than in 0%, 1.5%, 3.0%, 4.5% or 6.0% PO fed fish

this could be an indication that the  $\Delta 6$ - desaturation enzyme may use 18:2n-6 as a substrate when dietary 18:3n-3 content is low. However, this needs to be confirmed with labelled FA in *in vitro* test. The pattern of retention and decreased incorporation of 18:3n-3 in fish tissues according to dietary treatment may indicate that elongation and desaturation activities of 18:3n-3 were occurring in all treatments. In fish fed 3% PO diet, enzyme activities were higher than in fish fed the other diets regarding the percentage of 18:3n-3 in liver and fillet tissues of *H. longifilis*. These results are in line with a previous observation of Xu and Kestemont (2002) in juvenile perch fed 16% olive oil, safflower oil, linseed oil or cod liver oil as the only lipid source in each diet. These authors also indicated an extensive liver elongase and desaturase activity of the 18:3n-3 and 18:2n-6 fatty acids, mainly in fish fed linseed oil. In fish fed safflower oil as the only lipid source, they observed a significant increase of 18:2n-6 products as well as a decrease in DHA and total n-3 FA, indicating that the competition and inhibition between 18:2n-6 and 18:3n-3 for further desaturation and elongation were greatly influenced by the type of dietary lipid and the content of n-3 and n-6 FA in the diet. Similar changes in FA composition and LNA/LA ratios have also been reported for tilapia by Olsen et al. (1990).

## CONCLUSION

The overall results showed that replacement of fish oil with vegetable oil cause improvement in the growth performance and liver health status of *Heterobranchus longifilis*. However, fillet fatty acid compositions were affected but none exhibited n-6/ n-3 fatty acid ratio higher than 1.53 which shows that the fillet contains appreciable concentration of the PUFAs. Thus, the beneficial effects of fish as human food can be maintained in *H. longifilis* when fed diets containing palm oil and soybean oil. Palm oil is readily available and relatively cheaper than soybean oil, therefore diet F (6%PO) is recommended for use in *H. longifilis* diet.

## CONFLICT OF INTEREST

We declare that there is no conflict of interest.

## ETHICAL APPROVAL

We also declare that all applicable international, national and institutional guidelines for the care and use of animals were followed.

## REFERENCES

- Alvarez M. J., Diez A., Lopez-Bote C., Gallego M., Bautista J.M. (2000): Short-term modulation of lipogenesis by macronutrients in rainbow trout (*Oncorhynchus mykiss*) hepatocytes. British Journal of Nutrition 84: 619–628.

- Babalola T. O. (2010): Utilization of dietary animal fats and vegetable oils by African catfish (*Heterobranchus longifilis*) fingerlings. Dissertation, University of Ilorin, Nigeria.
- Babalola T. O., Apata D. F. (2012): Effect of dietary palm oil on growth and carcass composition of *Heterobranchus longifilis* fingerlings. *Journal of Central European Agriculture* 13: 782–791.
- Bautista J. M., Garrido-Pertierra A., Soler G. (1988): Glucose-6-Phosphate dehydrogenase from *Dicentrarchus labrax* iver: kinetic mechanism and kinetics of NADPH inhibition. *Biochimica et Biophysica Acta* 967: 354–363.
- Bell J. G., Dick J. R., Sargent J. R. (1993): Effects of diets rich in linoleic or  $\alpha$ -linolenic acid on phospholipid fatty acid composition and eicosanoid production in Atlantic salmon (*Salmo salar*). *Lipids* 28: 819–826.
- Bell J. G., McGhee F., Campbell P. J., Sargent J. R. (2003): Rapeseed oil as an alternative to marine fish oil in diets of post-smolt Atlantic salmon (*Salmo salar*): changes in flesh fatty acid composition and effectiveness of subsequent fish oil 'wash out'. *Aquaculture* 218: 515–528.
- Blanchard G., Makombu J. G., Kestemont P. (2008): Influence of different dietary 18:3n-3/18:2n-6 ratio on growth performance, fatty acid composition and hepatic ultrastructure in Eurasian perch, *Perca fluviatilis*. *Aquaculture* 284: 144–150.
- Caballero M. J., Obach A., Rosenlund G., Montero D., Gisvold M., Sizquierdo M. (2002): Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 214: 253–271.
- Drew M. D., Ogunkoya A. E., Janz D. M., Van Kessel A. G. (2007): Dietary influence of replacing fish meal and oil with canola protein concentrate and vegetable oils on growth performance, fatty acid composition and organochlorine residues in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 267: 260–268.
- Fontagné S., Pruszyński T., Corraze A-M., Bergot P. (1999): Effect of coconut oil and tricaprilyn vs triolein on survival, growth and fatty acid composition of common carp (*Cyprinus carpio* L.) larvae. *Aquaculture* 179: 241–251.
- Food and Agriculture Organization (FAO)/World Health Organization (WHO) (1994): Fats and oils in human nutrition. Report of a joint FAO/WHO expert consultation. Food and Nutrition Paper 57. Food and Agriculture Organization, Rome
- Gurr M. I. (2009): Lipids in nutrition and health: A reappraisal. [online] Available from: <http://www.oilypress.com>.
- Henderson R. J. (1996): Fatty acid metabolism in freshwater fish with particular reference to polyunsaturated fatty acids. *Archive of Animal Nutrition* 49: 5–22.
- Henderson R. J., Tocher D. R. (1987): The lipid composition and biochemistry of freshwater fish. *Progress in Lipid Research* 26: 281–347.
- Horton A. A., Fairhurst S. (1987): Lipid peroxidation and mechanisms of toxicity. *Critical Reviews in Toxicology* 18: 27–79.
- James D. G. (1994): Fish as Food: Present Utilisation and Future Prospects, *Omega-3 News* 9: 1–4.
- Kletzien R. F., Harris P. K., Foellmi L. A. (1994): Glucose-6-phosphate dehydrogenase: a "house-keeping" enzyme subject to tissue specific regulation by hormones, nutrients and oxidant stress. *The FASEB Journal* 8: 174–181.
- Kucska B., Pál L., Müller T. Bodis M., Bartos A., Wagner L., Husveth F., Bercsenyi M. (2006): Changing of fat content and fatty acid profile of reared pike (*Esox lucius*) fed two different diets. *Aquaculture Research* 37: 96–101.
- Leaf A., Kang J.X. (1998): Omega 3 fatty acids and cardiovascular disease. *World Review of Nutrition and Dietetics* 83: 24–37.
- Legendre M., Kerdchuen N., Corraze G., Bergot P. (1995): Larval rearing of an African catfish, *Heterobranchus longifilis*: effect of dietary lipids on growth, survival and fatty acid composition of fry. *Aquatic Living Resources* 8: 355–363.
- National Research Council (NRC) (1993): Nutrient Requirements of Fish. National Academy Press, Washington D.C., USA.
- Ng W. K., Sigholt T., Bell J. G. (2004): The influence of environmental temperature on the apparent nutrient and fatty acid digestibility in Atlantic salmon (*Salmo salar* L.) fed diets containing different blends of fish oil, rapeseed oil and palm oil. *Aquaculture Research* 35: 1228–1237.
- Ochoa S. (1955): Malic enzyme, in: Colowick S. P., Kaplan N.O. (Eds.): *Methods in Enzymology*, Academic Press Inc, New York, USA, 1955, Vol. 1, pp. 739–753.
- Olsen R. E., Henderson R. J., McAndrew B. J. (1990): The conversion of linoleic acid and linolenic acid to longer chain polyunsaturated fatty acids by tilapia (*Oreochromis niloticus*) in vivo. *Fish Physiology and Biochemistry* 8: 261–270.
- Rosenlund G., Obach A., Gisvold M., Standal H., Tveit K. (2000): Effect of Alternative Lipid Sources on Long Term Growth Performance and Quality of Atlantic salmon (*Salmo salar*). The Ninth International Symposium on Nutrition and Feeding of Fish, May 21–25 Miyazaki, Japan (Abstract).
- Shahbandeh M. (2019): Production of major vegetable oils worldwide from 2012/13 to 2018/2019, by type (in million metric tons). Retrieved from [www.statista.com/statistics/263933/production-of-vegetable-oils-worldwide-since-2000/](http://www.statista.com/statistics/263933/production-of-vegetable-oils-worldwide-since-2000/)

- Stabile L. P., Klautky S. A., Minor S. M., Salati L. M. (1998): Polyunsaturated fatty acids inhibit the expression of the glucose-6-phosphate dehydrogenase gene in primary rat hepatocytes by a nuclear posttranscriptional mechanism. *Journal of Lipid Research* 39: 1951–1963.
- Sukhija P. S., Palmquist D. L. (1988): Rapid method for determination of total fatty acid content and composition of feedstuffs and faeces. *Journal of Agricultural and Food Chemistry* 36: 1202–1206.
- Tocher D. R., Bell J. G., McGhee F. Dick J. R., Fonseca-Madrigal J. (2003): Effects of dietary lipid level and vegetable oil on fatty acid metabolism in Atlantic salmon (*Salmo salar* L.) over the whole production cycle. *Fish Physiology and Biochemistry* 29: 193–209.
- Turchini G. M., Francis D. S., De Silva S. S. (2006): Fatty acid metabolism in the freshwater fish Murray cod (*Maccullochella peeliipeelii*) deduced by the whole-body fatty acid balance method. *Comparative Biochemistry and Physiology* 144B: 110–118.
- Wang S., Liu X., Xu S. Wu Q., You C., Monroig Ó., Tocher D. R., Li Y. (2018): Total replacement of dietary fish oil with a blend of vegetable oils in the marine herbivorous teleost, *Siganus canaliculatus*. *Journal of World Aquaculture Society* 49: 692–702.
- Xu X., Kestemont P. (2002): Lipid metabolism and fatty acid composition of European perch tissues as influenced by dietary fat. *Lipids* 37: 297–304.
- Zampelas A., Morgan L.M., Furlonger N., Williams C. M. (1995): Effects of dietary fatty acid composition on basal and hormone stimulated hepatic lipogenesis and on circulating lipids in the rat. *British Journal of Nutrition* 74: 381–392.
- Zheng X., Torstensen B. E., Tocher D. R., Dick J. R., Henderson R. J., Bell J. G. (2005): Environmental and dietary influences on highly unsaturated fatty acid biosynthesis and expression of fatty acyl desaturase and elongase genes in liver of Atlantic salmon (*Salmo salar*). *Biochimica et Biophysica Acta* 1734: 13–24.

Received: March 13, 2019

Accepted after revisions: December 23, 2019