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Effects of dietary lipid source on growth, digestibility and tissue fatty acid composition of *Heterobranchus longifilis* fingerlings

Theophilus Olayiwola Babalola [a,*], David Friday Apata [b]

[a] *Department of Animal Science, Landmark University, Omu Aran, Nigeria*
[b] *Department of Animal Production, University of Ilorin, Nigeria*

Abstract

One of the major problems facing aquaculture is the inadequate supply of fish oil mostly used for fish feed manufacturing. The continued growth in aquaculture production cannot depend on this finite feed resources, therefore, it is imperative that cheap and readily available substitutes that do not compromise fish growth and fillet quality be found. To achieve this, a 12-week feeding trial with *Heterobranchus longifilis* fed diets differing in lipid source was conducted. Diets were supplemented with 6 % lipid as fish oil, soybean oil, palm oil, coconut oil, groundnut oil and melon seed oil. Triplicate groups of 20 *H. longifilis* were fed the experimental diets two times a day to apparent satiation, over 84 days. Growth, digestibility, and muscle fatty acid profile were measured to assess diet effects. At the end of the study, survival, feed intake and hepatosomatic index were similar for fish fed experimental diets. However, weight gain, SGR and FCR of fish fed soybean oil-based diet was significantly reduced. Apparent nutrient digestibility coefficients were significantly lower in fish fed soybean, coconut and groundnut oil-based diets. Fillet and hepatic fatty acid compositions differed and reflected the fatty acid compositions of the diets. Docosahexaenoic acid (22:6n-3), 20:5n-3 and 20:4n-6 were conserved in vegetable oils-based diets fed fish possibly due to synthesis of HUFA from 18:3n-3 and 18:4n-6. Palm oil diet was the least expensive, and had the best economic conversion ratio. The use of vegetable oils in the diets had positive effect on growth and fillet composition of *H. longifilis*.

Keywords: digestibility, growth performance, fatty acids, fish oil, *Heterobranchus longifilis*, vegetable oil

1 Introduction

Heterobranchus longifilis is a promising fish with many qualities that make it an excellent species for culture (Babalola & Apata, 2006). It is a clarid fish, having omnivorous feeding habit, rapid growth, disease resistance, high yield potential, high fecundity, air breathing, and good market potentials. Most studies that addressed *H. longifilis* nutrition and feeding have focused on general requirements, but more specific information is needed to produce cost-effective practical diet formulations for commercial *H. longifilis*. Fagbenro *et al.* (1992) conducted studies on the protein requirement of *H. bidorsalis*, growth and body composition of *H. longifilis* fingerlings in response to dietary protein and lipid levels (Babalola & Adebayo, 2007) have also been investigated. Ibiyo *et al.* (2006) determined the response of *H. longifilis* to dietary supplementation of vitamin C. However, the effect of dietary lipid sources has not been fully addressed in *H. longifilis*.

One of the major component of fish feed is fish oil, mainly because of its high content of n-3 HUFA (highly unsaturated fatty acids), which are considered essential fatty acids for fish (Sargent & Tacon, 1999). However, it has been projected that in a few years, global fish oil production may not be enough to cover the increasing demand for animal feed. On the contrary, global vegetable oil production has increased in recent years, reaching volumes 100 times that of fish oil (Bimbo, 1990). As a result, prices for vegetable oils have been more stable and have even decreased in some markets, with some vegetable oils becoming less expensive than

* Corresponding author
Phone: +2348053521463
Email: tobabalola@yahoo.com

fish oil. Some vegetable oils, such as soybean, palm, canola, and linseed oils, are considered good alternative lipid sources for fish oil in animal feed (Bell *et al.*, 2001; Rosenlund *et al.*, 2001; Caballero *et al.*, 2002; Ng *et al.*, 2004). Total and partial fish oil replacement has been successfully achieved in various fish species (Regost *et al.*, 2003-04; Izquierdo *et al.*, 2005; Montero *et al.*, 2005; Mourente *et al.*, 2005; Babalola, 2010). One side effect of including vegetable oils is that it alters the n-3/n-6 fatty acid ratio and interferes with eicosanoid synthesis (Sargent *et al.*, 2002; Babalola, 2010). In general, replacement of fish oil with vegetable oil has resulted in a lower level of long chain n-3 fatty acids and higher levels of the 18C fatty acids in tissues of several fish species (Izquierdo *et al.*, 2005; Montero *et al.*, 2005; Almaida-Pagán *et al.*, 2007; Martínez-Llorens *et al.*, 2007).

Some freshwater fish can elongate and desaturate fatty acids with 18 carbons, specifically linoleic and linolenic acid to PUFA with 20–22 carbons of the n-3 and n-6 series (Babalola, 2010). The ability of fishes to convert the linoleic and linolenic acids (abundant in many vegetable oils) into functionally active HUFA can be modulated by diet. If *H. longifilis* can elongate linoleic and linolenic acids to their respective HUFAs, then a variety of less expensive vegetable oils might be effective dietary lipid sources. Therefore, successful replacement of fish oil with vegetable oils would reduce both the absolute dependence on this ingredient and associated costs. The aim of this study was to assess the effects of complete replacement of dietary fish oil by soybean, palm, coconut, groundnut or melon seed oils on *H. longifilis* growth, nutrient digestibility, and muscle fatty acid profile, as well as to estimate the economic impact of such replacement.

2 Materials and methods

2.1 Experimental diets

Six isonitrogenous, isocaloric and isolipidic diets (Table 1) were formulated to contain either 6 % fish oil (FO), Soybean oil (SO), palm oil (PO), coconut oil (CO), groundnut oil (GNO) or melon seed oil (MSO). The fatty acid composition of the diet is shown in Table 2. The vegetable oils (SO, PO, CO, GNO and MSO) were less costly and more available than fish oil. Fish oil was used in the control diet and was completely replaced with different lipid sources in the other five diets. Chromic oxide was added to each of the experimental diets at 0.05 % of the diet as a non-digestible marker for nutrient digestibility determination. The diets were made into pellet with meat mincer through 2 mm die, sundried, packed in polythene bags, sealed and stored at –10 °C until used.

2.2 Culture system, experimental design and sampling

Heterobranchus longifilis fingerlings were obtained from the hatchery of National Institute for Freshwater Fisheries Research, New-Bussa, Nigeria. Upon arrival fish were stocked in 60-l plastic tanks in a flow-through system and acclimated to laboratory conditions for two weeks. During acclimation, fish were fed to apparent satiation twice a day using a commercial catfish feed. 20 fishes were batch weighed and stocked into each of eighteen 60-l plastic tanks, which in turn were randomly assigned to each diet group, three replicate per treatment. Water quality was maintained by continuous aeration and a flow rate of 1l per minute per tank. Dissolved oxygen and ammonia levels were within the normal range recommended for catfish (Viveen *et al.*, 1985). The ranges were dissolved oxygen 4.7–6.8 mg/l and ammonia 0.05–0.54 mg/l. A diurnal light/dark cycle of 12:12 h was maintained during the feeding trial. Fish were fed to apparent satiation twice daily (09:00 and 16:00) for twelve weeks. Uneaten feed was collected by siphoning from each tank, filtered and sundried. Feed intake was recorded daily by subtracting uneaten feed from total feed fed. During the trial, fish in each tank were counted and weighed every two weeks and mortalities were recorded daily. Ten days to the end of the feeding trial, tanks were evacuated of any uneaten feed daily 30 minutes after the 16:00 h feed in preparation for faeces collection the following morning. In the morning the faeces were siphoned out, sundried daily and stored at –10 °C until analysed.

On completion of the feeding trial, all fish were starved for 48 h to empty the digestive tract, 5 fish from each tank were killed by blow to the head, and weighed. Liver (minus gall bladder) and muscle tissues (fillet) (without the skin) were removed, pooled and stored frozen at –10 °C for subsequent analysis.

2.3 Proximate composition and fatty acid analysis

The diets and faecal samples were analysed for proximate composition using standard methods (AOAC, 1997). Moisture was determined gravimetrically after drying in an oven at 105 °C for 24 h; ash by incineration in a muffle furnace at 450 °C for 16 h; protein (N x 6.25) by the Kjeldahl method after acid digestion; total lipids were extracted and quantified from fillet and liver (Folch *et al.*, 1957) and then saponified and methylated for fatty acid quantification according to method described by Morrison & Smith (1964). Chromic oxide of diet and faeces was measured by acid digestion according to Furukawa & Tsukahara (1966).

Fatty acid methyl esters (FAME) were analysed using a Perkin Elmer gas chromatograph (Model 8700)

Table 1: *Composition of the experimental diets (g /kg)*

	Diets					
	Cod liver oil	*Soybean oil*	*Palm oil*	*Coconut oil*	*Groundnut oil*	*Melon seed oil*
Ingredients (g/kg)						
Fish meal (Danish)	398.00	398.00	398.00	398.00	398.00	398.00
Soybean meal	313.00	313.00	313.00	313.00	313.00	313.00
Corn flour (Maize)	172.00	172.00	172.00	172.00	172.00	172.00
Oil	60.00	60.00	60.00	60.00	60.00	60.00
Cassava starch	20.00	20.00	20.00	20.00	20.00	20.00
Methionine	10.00	10.00	10.00	10.00	10.00	10.00
Vitamin/Mineral Premix *	20.00	20.00	20.00	20.00	20.00	20.00
Salt (NaCl)	1.50	1.50	1.50	1.50	1.50	1.50
Vitamin C	0.50	0.50	0.50	0.50	0.50	0.50
Chromic Oxide	5.00	5.00	5.00	5.00	5.00	5.00
Proximate composition (n 3)						
Moisture (g/kg)	125.20	125.00	125.00	124.70	124.90	124.60
Crude protein (g/kg)	449.00	451.00	450.00	448.00	450.20	451.00
Lipid (g/kg)	107.40	109.20	109.90	103.50	105.60	105.10
Ash (g/kg)	87.20	88.40	79.50	89.40	90.30	86.70
Metabolizable energy (kJ/g) **	17.50	17.52	17.68	17.38	17.41	17.46

* Vitamin/mineral premix supplied the following (per kg of diet): calcium, 4500mg; phosphorus, 4200mg; potassium, 1700mg; magnesium, 400mg; iron, 30mg; zinc, 30mg; manganese, 20mg; copper, 5mg; iodine, 1mg; selenium, 0.25mg; vitamin A, 5000IU; vitamin D, 2000IU; DL-2-tocopherol acetate, 100mg; menadione, 15mg; thiamine hydrochloride, 5mg; riboflavin, 10mg; pyridoxine hydrochloride, 10mg. Panthothenic acid, 35mg; nicotinic acid, 50mg; biotin, 0.5mg; folic acid, 2mg; ascorbic acid, 200mg; inositol, 250mg; choline, 400mg; vitamin B12, 0.1mg and ethoxyquin, 60mg.

** Calculated based on 16.70, 16.70, 37.70 kJ/g for carbohydrate, protein and lipid, respectively.

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, Bellafonte, PA), temperature programmed from 100 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). The results of the individual fatty acids were expressed as g $100g^{-1}$ of total identified FAMEs.

2.4 Economic conversion estimates

Based on the price of each raw materials and the amount that were required to make different diets, the cost for one kilogram of each diet was calculated. The raw material prices used were the average prices in 2010. The economic conversion ratio (ECR) was determined using the following equation:

$$ECR = \text{Cost of diet} \times \text{Feed Conversion Ratio (FCR)}$$

(Piedecausa *et al.*, 2007).

2.5 Calculations

The following formulae were applied to the data: Specific growth rate (SGR) was calculated as % daily growth increase:

$$SGR = \frac{\ln W_1 - \ln W_0}{\text{days of trial}} \times 100$$

Where W_0 and W_1 are the initial and final body weights, respectively.

Feed conversion ratio (FCR) was calculated as the amount of dry diet consumed and the total biomass gain:

$$FCR = \frac{\text{feed intake, g}}{\text{wet weight gain, g}}$$

Hepatosomatic index:

$$HSI\,\% = 100 \times \frac{\text{liver weight, g}}{W, \text{g}}$$

Apparent digestibility:

$$AD\,\% = 100 - 100 \times \frac{\%\,Cr_2O_3 \text{ in feed}}{\%\,Cr_2O_3 \text{ in faeces}} \times \frac{\%\text{ nutrient in faeces}}{\%\text{ nutrient in feed}}$$

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

Fatty acids	*Diets*					
	Cod liver oil (n 3)	*Soybean oil (n 3)*	*Palm oil (n 3)*	*Coconut oil (n 3)*	*Groundnut oil (n 3)*	*Melon seed oil (n 3)*
14:0	6.05	4.24	0.72	5.21	7.78	1.70
16:0	18.35	18.88	35.41	15.99	26.37	22.55
18:0	4.58	4.13	4.71	1.40	3.60	4.74
16:1 *n*-7	5.85	3.72	3.97	2.17	0.27	1.68
18:1 *n*-9	9.72	14.03	31.34	4.90	1.09	27.62
18:2 *n*-6	7.63	26.49	10.93	0.88	35.68	33.20
20:4 *n*-6	0.92	0.43	0.20	0.61	0.67	0.06
18:3 *n*-3	1.49	3.20	0.11	0.11	3.92	0.55
20:5 *n*-3	11.08	4.87	3.48	5.08	4.74	0.38
22:6 *n*-3	12.75	7.50	4.02	4.40	2.60	0.21
∑ Saturates *	30.12	28.30	41.20	75.66	40.56	30.48
∑ monoenes †	29.61	27.34	58.80	10.82	59.44	69.52
∑ *n*-9 ‡	21.01	22.29	34.12	5.38	1.60	27.97
∑ *n*-6 §	13.57	28.23	11.53	2.09	37.19	35.17
∑ *n*-3 ¶	26.69	16.14	8.11	11.43	16.16	3.62
n-3/*n*-6	1.97	0.57	0.70	5.46	0.43	0.10

* Contains 12:0, 14:0, 16:0, 18:0, 20:0, 22:0, and 24:0.
† Contains 16:1 *n*-9, 16:1 *n*-7, 18:1 *n*-9, 18:1 *n*-7, 20:1 *n*-11, 22:1 *n*-11 and 24:1 *n*-9.
‡ Contains 16:1 *n*-9, 18:1 *n*-9, and 24:1 *n*-9.
§ Contains18:2 *n*-6, 18:3 *n*-6, 18:4 *n*-6, 20:2 *n*-6, 20:3 *n*-6, 20:4 *n*-6, 22:2 *n*-6, 22:3 *n*-6, 22:4 *n*-6 and 22:5 *n*-6.
¶ Contains 18:3 *n*-3, 18:4 *n*-3, 20:4 *n*-3, 20:5 *n*-3, 22:5 *n*-3 and 22:6 *n*-3.

2.6 Statistical analysis

Each of the six dietary treatments was assigned to three plastic tanks in a completely randomised design. Weight gain, feed intake, FCR, SGR, hepatosomatic index, dry matter, protein and lipid digestibility as well as the fatty acid composition of fish fillet and liver data were subjected to one-way Analysis of Variance (ANOVA). When significant differences among treatments were found ($P < 0.05$), Duncan's' multiple range test (Duncan, 1955) was used to compare the treatment means using the software SPSS 13.0.

3 Results

3.1 Growth, survival and feed utilization

Survival was 100 % in all treatments. Weight gain of *H. longifilis* fed the test diets ranged from 12.99 to 20.10 g. SBO fed fish had the least weight gain, weight gain of fish in the other dietary treatments were similar. Feed intake and hepatosomatic index did not differ among dietary treatments (Table 3). Weight gain, FCR and SGR of *H. longifilis* fed PO was higher than that of fish on the other diets, but fish fed diet containing SO had the lowest values for these parameters. The HSI was not influenced by the diet, averaging 0.80 ± 0.07 (Table 3).

3.2 Apparent nutrient digestibility

The mean apparent digestibility of dry matter (% ADM), protein (% PD), and lipid (% LD) of the experimental diets in *H. longifilis* is given in Table 4. It is evident that the ADM (75.73–79.30 %) and nutrient digestibility of all the diets were high (PD and LD ranged from 74.27 to 78.23 and 81.10 to 87.53, respectively), and that significant ($P < 0.05$) differences among diets were observed for ADM, PD and LD among the dietary groups. Apparent DM, PD and LD were highest in the case of the PO diet, and differed ($P < 0.05$) from the other diets.

3.3 Fatty acid profile of Heterobranchus longifilis fillet and liver

The different diet treatments resulted in significant differences in the fillet fatty acid composition of *H. longifilis* and mirrored the dietary fatty acid composition (Table 5). The concentration of palmitic acid (16:0) was generally high in muscle lipid irrespective of diet.

Table 3: *Growth performance and economic parameters of H. longifilis fed diets containing different lipid sources for 12 weeks.*

	Lipid type					
Variable	*Cod liver oil*	*Soybean oil*	*Palm oil*	*Coconut oil*	*Groundnut oil*	*Melon seed oil*
Initial weight (g)	2.22 ± 0.08	2.43 ± 0.14	2.29 ± 0.09	2.29 ± 0.12	2.22 ± 0.08	2.43 ± 0.20
Weight gain (g)	15.82 ± 1.99 ^{ab}	12.99 ± 0.03 ^{a}	20.03 ± 0.12 ^{b}	15.31 ± 0.01 ^{ab}	18.34 ± 2.19 ^{b}	20.10 ± 0.03 ^{b}
Feed intake (g)	16.44 ± 2.31	16.51 ± 2.46	18.43 ± 1.96	17.31± 2.50	20.36 ± 2.50	22.12 ± 2.29
FCR (%)	1.04 ± 0.04 ^{ab}	1.27 ± 0.02 ^{b}	0.92 ± 0.04 ^{a}	1.13 ± 0.10 ^{b}	1.11 ± 0.03 ^{ab}	1.10 ± 0.10 ^{ab}
SGR (% per day)	2.99 ± 0.04 ^{b}	2.64 ± 0.04 ^{a}	3.50 ± 0.02 ^{c}	2.97 ± 0.09 ^{b}	3.04 ± 0.02 ^{b}	3.18 ± 0.04 ^{b}
Survival (%)	98.88 ± 0.14	97.78 ± 2.10	98.00 ± 0.82	98.12 ± 0.64	99.00 ± 0.28	97.85 ± 0.62
HSI (%)	0.69 ± 0.08	0.65 ± 0.01	0.89 ± 0.23	0.66 ± 0.03	0.99 ± 0.10	0.89 ± 0.03

Data are mean ± S.E.M. (n = 3): means with different superscripts are significantly different ($P < 0.05$).
FCR = feed conversion ratio
SGR = specific growth rates
HSI = hepatosomatic index

Table 4: *Apparent nutrient digestibility of H. longifilis fed diets containing different lipid sources for 12 weeks.*

	Lipid type					
Variable	*Cod liver oil*	*Soybean oil*	*Palm oil*	*Coconut oil*	*Groundnut oil*	*Melon seed oil*
Protein digestibility	85.60 ± 0.26 ^{ab}	88.03 ± 0.30 ^{b}	88.23 ± 0.35 ^{b}	85.97 ± 0.12 ^{ab}	86.40 ± 0.29 ^{ab}	84.67 ± 1.53 ^{a}
Lipid digestibility	82.63 ± 0.27 ^{a}	81.10 ± 1.58 ^{a}	87.53 ± 0.12 ^{b}	83.00 ± 0.11 ^{a}	83.33 ± 0.13 ^{a}	82.67 ± 1.00 ^{a}
Dry matter digestibility	77.25 ± 0.14 ^{ab}	77.83 ± 0.12 ^{bc}	79.30 ± 0.40 ^{c}	76.67 ± 0.18 ^{ab}	75.73 ± 0.67 ^{a}	77.67 ± 0.12 ^{b}

Data are mean ± S.E.M. (n = 3): means with different superscripts are significantly different ($P < 0.05$).

Table 5: *Fillet lipid content and fatty acid composition of H. longifilis fed diets containing different lipid sources for 12 weeks (g/100g of total FA).*

	Lipid type					
Lipid content/Fatty acid	*Cod liver oil*	*Soybean oil*	*Palm oil*	*Coconut oil*	*Groundnut oil*	*Melon seed oil*
Lipid content	4.30 ± 0.13	4.40 ± 0.09	4.36 ± 0.12	4.43 ± 0.07	4.49 ± 0.06	4.53 ± 0.06
14:0	3.53 ± 0.66^{b}	2.82 ± 0.01^{b}	0.42 ± 0.03^{a}	10.24 ± 0.01^{d}	5.11 ± 0.01^{c}	1.13 ± 0.01^{a}
16:0	20.50 ± 0.03^{b}	20.40 ± 0.03^{b}	29.36 ± 0.01^{d}	18.51 ± 0.05^{a}	24.12 ± 0.06^{c}	16.10 ± 0.06^{a}
18:0	6.33 ± 0.01^{c}	5.29 ±0.01^{b}	2.98 ± 0.01^{a}	6.01 ± 0.01^{bc}	2.55 ± 0.05^{a}	6.77 ± 0.01^{c}
16:1(n-7)	5.91 ± 0.01^{d}	3.84 ±0.01^{c}	5.07 ± 0.01^{d}	5.08 ± 0.06^{d}	0.37 ± 0.11^{a}	2.37 ± 0.01^{b}
18:1(n-9) (oleic acid)	13.75 ± 0.02^{b}	16.23 ± 0.01^{c}	30.83 ± 0.01^{f}	22.53 ± 0.01^{d}	1.23 ± 0.01^{a}	29.99 ± 0.02^{e}
18:2(n-6) (linoleic acid)	8.03 ± 0.01^{a}	20.69 ± 0.06^{d}	10.75 ± 0.02^{b}	12.74 ± 0.06^{c}	38.70 ± 0.06^{f}	36.10 ± 0.03^{e}
18:3(n-3) (linolenic acid)	1.14 ± 0.01^{c}	2.06 ± 0.01^{e}	1.34 ± 0.01^{d}	0.14 ± 0.01^{a}	4.08 ± 0.01^{f}	0.57 ± 0.01^{b}
20:4(n-6) (arachidonic acid)	1.46 ± 0.01^{e}	0.71 ± 0.01^{d}	0.54 ± 0.01^{c}	0.08 ± 0.01^{a}	1.97 ± 0.01^{f}	0.19 ± 0.01^{b}
20:5(n-3) (eicosapentaenoic acid)	8.78 ± 0.01^{f}	5.92 ± 0.01^{e}	2.52 ± 0.01^{c}	0.88 ± 0.01^{b}	3.76 ± 0.01^{d}	0.29 ± 0.01^{a}
22:6(n-3) docosahexaenoic acid	18.30 ± 0.03^{f}	13.96 ± 0.01^{e}	9.27 ± 0.06^{d}	3.89 ± 0.06^{b}	7.49 ± 0.01^{c}	0.61 ± 0.01^{a}
Saturates *	31.96 ± 0.01^{c}	29.43 ± 0.01^{b}	32.92 ± 0.01^{d}	51.61 ± 0.01^{f}	33.02 ± 0.01^{e}	24.52 ± 0.01^{a}
Monoenes †	27.45 ± 0.01^{c}	25.38 ± 0.01^{b}	40.74 ± 0.01^{f}	30.66 ± 0.01^{d}	5.97 ± 0.01^{a}	33.54 ± 0.01^{e}
∑ n-9 ‡	20.75 ± 0.01^{b}	21.44 ± 0.01^{c}	34.59 ± 0.01^{f}	24.89 ± 0.01^{d}	1.91 ± 0.01^{a}	30.50 ± 0.01^{e}
∑ n-6 §	10.56 ± 0.02^{a}	22.17 ± 0.01^{d}	12.15 ± 0.01^{b}	12.81 ± 0.01^{c}	42.58 ± 0.06^{f}	39.17 ± 0.01^{e}
∑ n-3 ¶	30.03 ± 0.01^{f}	23.02 ± 0.01^{e}	14.19 ± 0.01^{c}	4.91 ± 0.01^{b}	18.43 ± 0.01^{d}	2.76 ± 0.01^{a}
n-3:n-6	2.84 ± 0.01^{f}	1.04 ± 0.001^{d}	1.17 ± 0.01^{e}	0.38 ± 0.01^{b}	0.43 ± 0.001^{c}	0.07 ± 0.001^{a}

Data are mean ± S.E.M. (n=3): means with different superscripts are significantly different ($P < 0.05$).

* Contains 12:0, 14:0, 16:0, 18:0, 20:0, 22:0, and 24:0.

† Contains 16:1 *n*-9, 16:1 *n*-7, 18:1 *n*-9, 18:1 *n*-7, 20:1 *n*-11, 22:1 *n*-11 and 24:1 *n*-9.

‡ Contains 16:1 *n*-9, 18:1 *n*-9, and 24:1 *n*-9.

§ Contains18:2 *n*-6, 18:3 *n*-6, 18:4 *n*-6, 20:2 *n*-6, 20:3 *n*-6, 20:4 *n*-6, 22:2 *n*-6, 22:3 *n*-6, 22:4 *n*-6 and 22:5 *n*-6.

¶ Contains 18:3 *n*-3, 18:4 *n*-3, 20:4 *n*-3, 20:5 *n*-3, 22:5 *n*-3 and 22:6 *n*-3.

Table 6: *Liver lipid content and fatty acid composition of H. longifilis fed diets containing different lipid sources for 12 weeks (g/100g of total FA).*

	Lipid type					
Lipid content/Fatty acid	*Cod liver oil*	*Soybean oil*	*Palm oil*	*Coconut oil*	*Groundnut oil*	*Melon seed oil*
Lipid content	6.19 ± 0.06	6.13 ± 0.39	6.11 ± 0.51	6.06 ± 0.57	6.12 ± 0.05	6.17 ± 0.08
14:0	3.39 ± 0.05^{c}	2.85 ± 0.02^{b}	0.22 ± 0.01^{a}	5.96 ± 0.01^{d}	5.40 ± 0.01^{d}	0.66 ± 0.01^{a}
16:0	29.75 ± 0.01^{b}	28.76 ± 0.01^{b}	33.01 ± 0.05^{c}	18.66 ± 0.61^{a}	52.94 ± 0.11^{d}	19.55 ± 0.07^{a}
18:0	8.06 ± 0.06^{b}	7.37 ± 0.01^{b}	4.78 ± 0.06^{a}	4.39 ± 0.06^{a}	7.87 ± 0.02^{b}	11.79 ± 0.06^{c}
16:1(n-7)	13.10 ± 0.03^{f}	9.66 ± 0.01^{e}	5.12 ± 0.01^{c}	7.01 ± 0.01^{d}	0.72 ± 0.01^{a}	2.58 ± 0.05^{b}
18:1(n-9) (oleic acid)	27.32 ± 0.01^{b}	30.21 ± 0.01^{c}	50.12 ± 0.06^{e}	35.85 ± 0.03^{d}	3.72 ± 0.01^{a}	53.42 ± 0.01^{f}
18:2(n-6) (linoleic acid)	3.53 ± 0.01^{b}	10.57 ± 0.02^{d}	2.90 ± 0.01^{a}	6.00 ± 0.12^{c}	20.54 ± 0.02^{e}	10.56 ± 0.02^{d}
18:3(n-3) (linolenic acid)	0.54 ± 0.01^{c}	0.78 ± 0.006^{e}	0.59 ± 0.003^{a}	0.02 ± 0.006^{d}	1.81 ± 0.01^{f}	0.12 ± 0.003^{b}
20:4(n-6) (arachidonic acid)	0.99 ± 0.006^{e}	0.47 ± 0.001^{c}	0.13 ± 0.001^{b}	0.11 ± 0.005^{b}	0.90 ± 0.01^{d}	0.04 ± 0.001^{a}
20:5(n-3) (eicosapentaenoic acid)	2.31 ± 0.006^{f}	1.43 ± 0.006^{e}	0.42 ± 0.01^{c}	0.22 ± 0.001^{b}	1.21 ± 0.006^{d}	0.04 ± 0.001^{a}
22:6(n-3) docosahexaenoic acid	5.33 ± 0.06^{f}	4.18 ± 0.05^{e}	0.98 ± 0.01^{c}	0.51 ± 0.01^{b}	1.39 ± 0.01^{d}	0.07 ± 0.001^{a}
Saturates *	42.52 ± 0.11^{d}	39.58 ± 0.58^{c}	38.16 ± 0.01^{b}	47.60 ± 0.57^{e}	67.80 ± 0.58^{f}	32.67 ± 0.06^{a}
Monoenes †	47.02 ± 3.32^{b}	42.29 ± 0.58^{b}	57.23 ± 0.17^{c}	44.95 ± 0.57^{b}	6.05 ± 0.29^{a}	56.25 ± 0.03^{c}
∑ n-9 ‡	29.77 ± 0.06^{b}	32.20 ± 0.12^{c}	51.93 ± 0.17^{e}	37.54 ± 0.23^{d}	4.39 ± 0.01^{a}	53.63 ± 0.12^{f}
∑ n-6 §	4.92 ± 0.01^{b}	11.31 ± 0.01^{e}	3.05 ± 0.01^{a}	6.11 ± 0.57^{c}	21.60 ± 0.03^{f}	10.85 ± 0.29^{d}
∑ n-3 ¶	8.86 ± 0.06^{f}	6.83 ± 0.05^{e}	2.13 ± 0.01^{c}	0.76 ± 0.01^{b}	4.54 ± 0.05^{d}	0.23 ± 0.01^{a}
n-3:n-6	1.80 ± 0.01^{e}	0.60 ± 0.001^{c}	0.70 ± 0.01^{d}	0.12 ± 0.01^{b}	0.21 ± 0.001^{b}	0.02 ± 0.001^{a}

Data are mean ± S.E.M. (n=3): means with different superscripts are significantly different ($P < 0.05$).

* Contains 12:0, 14:0, 16:0, 18:0, 20:0, 22:0, and 24:0.

† Contains 16:1 *n*-9, 16:1 *n*-7, 18:1 *n*-9, 18:1 *n*-7, 20:1 *n*-11, 22:1 *n*-11 and 24:1 *n*-9.

‡ Contains 16:1 *n*-9, 18:1 *n*-9, and 24:1 *n*-9.

§ Contains18:2 *n*-6, 18:3 *n*-6, 18:4 *n*-6, 20:2 *n*-6, 20:3 *n*-6, 20:4 *n*-6, 22:2 *n*-6, 22:3 *n*-6, 22:4 *n*-6 and 22:5 *n*-6.

¶ Contains 18:3 *n*-3, 18:4 *n*-3, 20:4 *n*-3, 20:5 *n*-3, 22:5 *n*-3 and 22:6 *n*-3.

Palmitic acid was present at high concentrations in the PO and GNO diets (35.41 % and 26.37 %, respectively) (Table 2), but its concentration in muscle remained in the range of 16.10 to 29.36 % in fillet (Table 5) and 18.66–52.94 % in the liver (Table 6). Fillet of fish fed MSO contained the lowest percentage of saturated FA and PO contained the highest percentage of monounsaturated fatty acids. Oleic acid increased significantly ($P < 0.05$) in the fillet of fish fed alternative lipids (except fish fed GNO diet). The highest increase was observed in fish fed PO diet ($P < 0.05$). Linoleic acid increased in the fillet of GNO fed fish, being 4.8 times higher than the FO dietary group. Total n-6 fatty acids (FA) were significantly reduced ($P < 0.05$) in fillet of fish fed FO diet. Total n-3 FA was significantly reduced ($P < 0.05$) in the fillet of fish fed the alternative lipid sources diets. The reduction was more pronounced for EPA than for DHA. These differences in individual fatty acid concentrations resulted in respective significant n-3/n-6 reduction.

The liver FA compositions of *H. longifilis* fed the experimental feed over 84 days are presented in Table 6. Liver of fish fed GNO was characterised with higher concentration of total saturated fatty acids (67.80 %) majorly of palmitic acid (52.94 %) and lowest concentration of total monoenes, total n-9 fatty acids and 18:1n-9 in particular. Total saturated fatty acids ranged from 32.67 % in MSO fed fish to 67.80 % in GNO fed fish. The total monoenes fatty acids concentrations were higher in PO and MSO fed fish than others. Liver tissue of fish fed FO diet was highest in total n-3, 20:5n-3 and 22:6n-3; those fed GNO diet were highest in 18:3n-3 and total n-6, while fish fed diet containing PO had the lowest total n-6 fatty acids in the liver. Total n-3 fatty acids in liver were highest in fish fed diet containing FO and lowest in MSO fed fish. The ratio of n-3 to n-6 fatty acids in liver was highest in fish fed FO diet, lowest in fish fed MSO and intermediate in fish fed the other diets.

3.4 Economic estimates

The economic estimates (Table 7) show that the PO diet was the least expensive and the one having the best economic conversion ratio (ECR). For an average fish farm with a production of 100 tons per year, feeding cost would amount to ₦56,029,000.00 with fish oil, ₦19,872,000.00 with PO, ₦20,460,000.00 with PKO and ₦27,241,000.00 with SO. Using the less expensive PO would reduce the feeding cost by ₦36,157,000.00, which means a savings of 64.53 % with respect to fish oil.

Table 7: *Economic parameter of the experimental diets.*

	FCR	*Feed cost (₦/kg)*	*Economic conversion ratio (₦/kg)*
FO	1.04	538.74	560.29
SO	1.28	212.82	272.41
PO	0.92	194.82	179.23
CO	1.13	196.62	222.18
GNO	1.11	193.32	214.59
MSO	1.10	209.82	230.80

₦ = Naira; 1 USD ≙ ₦ 150.00

4 Discussion

This study has shown that vegetable oils have considerable potential as replacements of fish oil in diets for *Heterobranchus longifilis*. However, the usefulness of these plant oil resources, as gauged by growth and composition variation, does vary according to plant sources of the oil. It is noteworthy that the vegetable oils in this study have no significant deleterious effects on the growth performance relative to that obtained from fish fed diets with fish oil as the sole added lipid source. The growth of *H. longifilis* fingerlings over 12 weeks was not compromised by feeding diets in which vegetable oil was the main added lipid. This suggests that *H. longifilis* can effectively utilize these lipid sources. However, there were no significant differences in feed intake between the test treatments of the study. Despite this, the combined influences of both feed intake and growth, when revealed as FCR, did show some significant differences between treatments. Notable was the deteriorating FCR value with the inclusion of soybean oil. Our results are similar to the observations on coho salmon, (Dosanjh *et al.*, 1984); rainbow trout, (Greene & Selivonchick, 1990); brown trout, (Arzel *et al.*, 1994) and Atlantic salmon, (Hardy *et al.*, 1987; Thomassen & Røsjø, 1989; Polvi & Ackman, 1992; Koshio *et al.*, 1994). In contrast, similar growth and FCR was observed in Atlantic salmon when fed diets in which FO was partially replaced with rapeseed oil or other vegetable oils (Bell & Waagbø, 2008; Glencross *et al.*, 2003; Torstensen *et al.*, 2005; Turchini *et al.*, 2009). Nevertheless, the positive effect of PO on growth as observed in this study corroborate previous observations in *Clarias gariepinus* and Atlantic salmon (Ng *et al.*, 2003, 2004).

The apparent nutrient digestibilities reported in this study were high and in agreement with those obtained for Murray cod (Francis *et al.*, 2006, 2007). The slight but significant apparent protein and dry matter digestibility observed could be as a result of dietary modification due to FO substitution with vegetable oils as previously reported for different species (De Silva & Anderson, 1995). The high apparent lipid digestibility values fell within the range of other studies (Caballero *et al.*, 2002; Bendiksen *et al.*, 2003), supporting the statement by Olsen & Ringø (1997), that lipids are generally well digested by fish.

Although the EFA needs of *H. longifilis* have not been determined, signs of EFA deficiencies i.e., stunted growth, high mortality, fin rot and elevation in 20:3n-9 levels in the body lipids were absent in this study. Hence, the requirements for the EFA might be low and that it has been satisfied by the residual lipid in the fish meal and/or the abundance of their precursors in the vegetable oil-based diets.

The dietary lipid generally affected the fillet and liver fatty acid of *H. longifilis*. Supplementing diets with vegetable oils resulted in lowered SFA in all of the *H. longifilis* body parts analyzed. Similar results was obtained in studies with pikeperch (Schulz *et al.*, 2005; Kowalska *et al.*, 2010) and rainbow trout (*Oncorhynchus mykiss*) (Caballero *et al.*, 2002). Palmitic acid is an important component of phospholipid, especially at the sn-1 position of phosphatidylcholine and to a lesser extent, phosphatidylethanolamine. The presence of dietary phospholipids is reported to greatly affect lipid digestibility, absorption and transport (Caballero *et al.*, 2003; Morais *et al.*, 2005). According to Olsen *et al.* (1999, 2000) a diet containing phospholipids have higher apparent lipid digestibility than diets containing high amount of triacylglycerols. This supports observation in this study that fish on PO, PL and PKO had higher apparent lipid digestibility than those from the other group, because of their higher concentrations of palmitic acid which is a component of phospholipids. Vegetable oils contains higher concentrations of saturated and monounsaturated fatty acids, these provides suitable dietary fatty acids that induces β-oxidation which supply enhanced energy through lipid oxidation, this resulted in improved growth of fish by sparing protein for fish growth (Lim *et al.*, 2001). In vitro studies done on mitochondrial β-oxidation in fish suggest that there exist a substrate preference for saturated and monounsaturated fatty acids over polyunsaturated fatty acids (Henderson, 1996).

The principal site of fatty acid metabolism is the liver, and the quantity of HUFA in fish bodies can influence the bioconversion capacity (Tocher *et al.*, 2000; Sargent *et al.*, 2002; Menoyo *et al.*, 2007). A total replacement of fish oil with vegetable oils in this study shows specific fatty acids such as 18:1n-9, 18:3n-3, 18:2n-6 and 20:5n-3 to be readily oxidized in fish fillet and liver tissues, whereas essential fatty acid 20:4n-6 and 22:6n-3 were selectively retained in the fillet of the fish. DHA and arachidonic acid (AA) concentrations in the fillet

and liver were higher than the dietary concentrations in all dietary groups. Similarly, high levels of DHA and AA were noted in the tissues of rainbow trout, Atlantic salmon, and brown trout (*Salmo trutta*), gilthead sea bream (*Sparus aurata* L.), European sea bass, rainbow trout, turbot and brook trout (*Salvelinus fontinalis*) (Boggio *et al.*, 1985; Greene & Selivonchick, 1990; Arzel *et al.*, 1994; Caballero *et al.*, 2002; Regost *et al.*, 2003-04; Montero *et al.*, 2005; Mourente & Bell, 2006; Fountoulaki *et al.*, 2009), when these species were fed with vegetable oil-based diets. The reduction of EPA in both fillet and liver at a lesser extent than its respective dietary concentration in this study suggest utilization of this fatty acid.

The presence of 20:4n-6 and 22:6n-3 in tissues of *H. longifilis* fed diets containing lower concentrations (SO, PO, GNO and MSO) indicates that *H. longifilis* possess both the Elongase and desaturase enzymes necessary for synthesising these HUFA. Thus, improved growth performance of *H. longifilis* fed PO, GNO and MSO diets above or similar those fed FO diet suggest the presence of optimal essential fatty acids (EFA) in these diets and shows that EFA requirements of *H. longifilis* could be satisfied by 18:2n-6 and/or 18:3n-3. The low rate of deposition of 22:6n-3 (43.53 % above dietary content) in the fillet of fish fed FO diet compared to the concentrations of this FA in PO, GNO, and MSO (161.69 %, 188.08 %, and 190.48 % above dietary contents respectively), indicates that bioconversion and selective retention of this FA increases in *H. longifilis* when 0.11 % to 0.55 % (as in the case of PO, GNO, and MSO) of the precursor, LNA (18:3n-3) is present in the diet. Thus, unlike marine fish, they may not require pre-formed 20:4n-6, 20:5n-3 or 22:6n-3 in their diet.

The results of the economic performance in this study show that PO diet is the least expensive diet and the one that had the best economic conversion ratio. The use of FO on the other hand increased the feeding cost. The reduced cost of PO based diet could be attributed to the readily availability of the oil (Bimbo, 1990), coupled with the stability of the price in many tropical countries where aquaculture is being practiced.

In conclusion, the results of this study suggest that cheap and readily available vegetable oils can be used to replace expensive and scarce FO, with no negative effect on the growth, FCR and nutrient digestibility of *H. longifilis*. It is therefore recommended that vegetable oils (PO, GNO, SO and MSO) be added to the diet of *H. longifilis* for improved nutrient utilization and growth performance. Moreover, further study aimed at increasing the concentration of the essential fatty acids (especially the long chain polyunsaturated fatty acid) in the fillet is needed.

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