Effects of dietary fish oil replacement by vegetable oil on the serum biochemical and haematological parameters of African catfish (*Heterobranchus longifilis*) fingerlings

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Abstract

The present study aimed to elucidate the impacts of dietary vegetable oil blends as alternative to fish oil on serum biochemical and haematological parameters in *Heterobranchus longifilis* fingerlings. Fish (4.65 ± 0.23g) were fed diets containing fish oil (FO), palm oil (PO), soybean oil (SO), blend of PO and SO (POSO) over a 12-week period. The experiment was carried out in 62-l circular tanks with 10 fish per tank. Fish were fed two times a day until apparent satiation. At the end of the experiment, blood samples were collected from each treatment for investigation of serum biochemical and haematological parameters. The results showed that total protein was not significantly affected by the different dietary lipid sources (p >0.05). However, serum glucose was significantly higher in fish fed the control diet (p<0.05). Activities of liver enzymes (AST, ALT, ALP), total cholesterol, triglyceride, LDL and HDL exhibited a significant increase in the blood of fish fed combinations of the vegetable lipid sources (p<0.05). The PCV, RBC and MCV were not affected by the dietary lipid sources (p>0.05). These results indicate that FO can be replaced with PO, SO or their combinations in the diet of *H. longifilis* fingerlings without any negative health impacts.

Keywords: Fish oil, Vegetable oil, *Heterobranchus longifilis*, Haematology, Serum biochemical

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Introduction

Fish oil derived from wild marine fish populations is becoming increasingly insufficient to meet the demand in the aquafeed industry because the production from these stocks are fully exploited, and is not expected to increase beyond the present level (Ng *et al*., 2003). When demand outstrips supply, cost will go up following the law of demand and supply. The increase in the global demand for fish oil coupled with the high cost of fish oil has created the growing interest in evaluating alternative oils to replace fish oil in fish diets (Rosenlund *et al*., 2001; Cabalero *et al*., 2002; Ochang, 2011; Babalola and Apata, 2011, 2012). On the contrary, the global vegetable oil productions have steadily increased and are readily available at lower cost (Babalola, 2010).

Vegetable oils are rich in C18 PUFAs, being precursors of HUFAs. However, changes in the sources of dietary lipids can affect fish health and disease resistance. Lipids modulate the immune response by influencing the physical properties of immune cell membranes (phospholipids), membrane-associated signalling molecules (eicosanoids) and receptor sites (i.e. protein kinase C) (Montero *et al*., 2004).

Blood is a good indicator to determine the health of an organism. Differences in haematological parameters, immune response and serum biochemical variables as a function of dietary vegetable lipid sources have been reported for catfish (Ochang *et al*., 2007; Babalola *et al*., 2009), Atlantic salmon (Balfry *et al*., 2006), largemouth bass (Subhadra *et al*., 2006) and carp (Yildirim *et al*., 2013). However, the influence of dietary vegetable oil blends as fish oil alternative lipid source on *H. longifilis* haematological and serum biochemical variables are very limited. Thus, the aims of this study were to evaluate the effects of dietary vegetable oils and mixtures composed of soybean oil (SO) and palm oil (PO), the most abundant vegetable oil in the world (Gunstone, 2001) on serum biochemical variables and haematological parameters of *H. longifilis* fingerlings.

Materials and methods

Fish and experimental condition

H. longifilis fingerlings, used in this study were obtained from National Institute for Freshwater Fisheries Research (NIFFR) hatchery. The fish (mean body weight 4.65 ± 0.23g) were randomly allotted into 12 circular tanks (62 L) with 10 fish in each tank. Each of the four treatments was replicated in triplicate. Fish were maintained under 12:12 light: dark regime, with constant aeration and a flow rate of 0.5 L min^{-1}. Water temperature and pH were maintained at 24 ± 1˚C and 6.9 ± 0.2, respectively. Each group was fed one of the four experimental diets, assigned randomly, each diet being assigned to three groups. The *H. longifilis* were acclimatised to the experimental diets for 2 weeks, when the fish were fed to satiation between 0830 to 1030 h and

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1630 to 1830 hours. During the acclimatisation period, tanks were cleaned of feed particles after each feeding and faecal matter siphoned out prior to each feeding.

Feeding trial

The four experimental diets were prepared by substituting one of the following oils as the lipid source; fish oil (FO), palm oil (PO), soybean oil (SO) or equal blend of PO and SO (POSO) at 60 g kg^{-1} (Table 1). The proximate composition of the experimental diets and the dietary fatty acid compositions are shown in Tables 1 and 2, respectively. The experimental diets were prepared by mixing the dry ingredients with oil and pregelatinized starch and the resulting moist dough was pelleted using a locally assembled meat mincer through a 2-mm die. The moist pellets were then sun dried and stored under refrigeration in 200 g batches, until used. The twelve groups of *H. longifilis* were fed daily the assigned diets (triplicated for each diet) to apparent satiation two times daily (between 0830 to 1030 h and 1630 to 1830 hours) for a period of 12 weeks, after acclimatisation.

Sample collection

At the end of the feeding trial, fish were tranquilized with 150 mgL^{-1} solution of methane sulphonate (MS222) (Wagner *et al*., 1997) for blood collection. Blood samples were obtained from the caudal vein of five fish from each tank. One mL blood sample was collected into the bottles containing 0.05 mL EDTA as anticoagulant. Blood for serum analysis were collected into bottles without any anticoagulant. Serum was separated by centrifugation at 7200 rpm for 5 minutes, kept frozen at -20°C for the determination of total protein, cholesterol, glucose, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities.

Haematological and serum biochemical profile

Immediately after sampling, blood smears were prepared, red blood and white blood cell counts were carried out using standard haematological techniques (Dacie and Lewis 2001). Fifty µL haematocrit tube was filled with blood samples, after centrifugation (7200 rpm for 10 min) of each blood sample, packed cell volume (PCV) was determined by the Wintrobe and Westergreen method as described by Blaxhall and Daisley (1973). Haemoglobin levels (Hb in grams per deciliters) were obtained by the cyanomethaemoglobin spectrophotometric method (Dorafshan *et al*., 2008). The blood indices including mean corpuscular volume (MCV in femtoliters), mean corpuscular haemoglobin (MCH in pictograms per cell), and mean corpuscular haemoglobin concentration (MCHC in grams per decilitre) were calculated according to the following formulas (Dacie and Lewis 2001):

$$\text{MCV (fl)} = \frac{\text{PCV (\%)}}{\text{RBC } (10^6\ \mu l^{-1})}$$

$$\text{MCH (pg)} = \frac{[\text{Hb}(gdl^{-1})]}{\text{RBC } (10^6 \mu l^{-1})}$$

and

$$\text{MCHC } (gdl^{-1}) = \frac{[\text{Hb}(gdl^{-1})]}{\text{PCV (\%)}}$$

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Table 1 : Composition of the experimental diets (g kg $^{-1}$) for fingerling *Heterobranchus longifilis*.

	Diets[1]			
	A	**B**	**C**	**D**
Ingredients				
Fish meal (Danish)	398.00	398.00	398.00	398.00
Soybean meal	313.00	313.00	313.00	313.00
Corn flour (Maize)	172.00	172.00	172.00	172.00
Cassava starch	20.00	20.00	20.00	20.00
Methionine	10.00	10.00	10.00	10.00
Vit./Min. premix[2]	20.00	20.00	20.00	20.00
Salt (NaCl)	1.50	1.50	1.50	1.50
Vitamin C	0.50	0.50	0.50	0.50
Chromic oxide	5.00	5.00	5.00	5.00
Fish oil (FO)	60.00	-	-	-
Palm oil (PO)	-	60.00	-	30.00
Soybean oil (SO)	-	-	60.00	30.00
Proximate composition (n=3)				
Moisture (g/kg)	63.00	60.00	60.90	60.60
Crude protein (g/kg)	456.80	452.30	452.00	454.50
Lipid (g/kg)	105.00	106.70	105.80	105.70
Ash (g/kg)	83.00	82.90	83.20	82.90
Crude fibre	22.50	22.34	22.42	22.38
Carbohydrate (NFE) (g/kg)	269.70	275.66	273.58	273.92
Metabolizable energy (kJ/g)	17.47	17.56	17.52	17.53

[1] Diets: A = fish oil, B = palm oil, C = soybean oil, D = palm oil and soybean oil (1:1).

[2] Vitamin/mineral premix supplied the following (per kg of diet): calcium, 4500 mg; phosphorus, 4200 mg; potassium, 1700 mg; magnesium, 400mg; iron, 30mg; zinc, 30 mg; manganese, 20 mg; copper, 5 mg; iodine, 1 mg; selenium, 0.25 mg; vitamin A, 5000 IU; vitamin D, 2000 IU; tocopherol acetate, 100 mg; menadione, 15 mg; thiamine hydrochloride, 5 mg; riboflavin, 10 mg; pyridoxine hydrochloride, 10 mg; panthothenic acid, 35 mg; nicotinic acid, 50 mg; biotin, 0.5 mg; folic acid, 2 mg; ascorbic acid, 200 mg; inositol, 250 mg; choline, 400 mg; vitamin B_{12}, 0.1 mg and ethoxyquin, 60 mg.

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

Fatty acids	A	B	C	D
Lauric acid	1.13	0.15	0.58	0.28
Myristic acid	5.98	0.72	4.22	1.98
Palmitic acid	18.14	35.41	18.77	23.40
Stearic acid	4.53	4.71	4.11	3.89
Arachidic acid	0.74	0.20	0.66	0.53
Behenic acid	0.20	ND	0.22	0.14
Lignoceric acid	0.19	ND	0.15	0.10
Sum saturated FAs	30.91	41.20	28.71	30.32
Palmitoleic acis	5.79	3.97	3.70	4.17
Cis-vassenic acid	ND	ND	ND	ND
Sum n-7	5.79	3.97	3.70	4.17
Cis-9-hexadecanoic acid	8.61	0.12	6.49	3.63
Oleic acid	9.61	31.34	13.95	23.53
Eicosanoic acid	1.93	2.66	1.44	2.05
Nervonic acid	0.63	0.01	0.28	0.18
Sum n-9	20.77	34.12	22.16	29.39
Cetoleic acid	2.72	1.07	1.32	1.31
Sum n-11 FAs	2.72	1.07	1.32	1.31
Linoleic acid	7.55	10.93	26.33	19.09
Gamma-linolenic acid	1.97	0.11	1.21	0.66
Eicosadienoic acid	2.83	0.19	0.09	0.18
Dihomo-gamma-linolenic	0.17	0.10	ND	ND
Arachidonic acid	0.91	0.20	0.43	0.36
Sum n-6	13.42	11.53	28.06	20.30
Linolenic acid	1.47	0.11	3.19	1.99
Stearidonic acid	ND	ND	ND	ND
Eicosatetraenoic	ND	ND	ND	ND
Eicosapentaenoic acid	10.95	3.48	4.84	3.66
Docosapentaenoic acid	1.36	0.50	0.56	0.70
Docosahexaenoic acid	12.61	4.02	7.45	8.15
Sum n-3	26.39	8.11	16.04	14.50
Sum monounsaturated FAs	29.28	39.16	27.18	34.87
Sum polyunsaturated FAs	39.81	19.64	44.10	34.80
Sum unsaturated FAs	69.09	58.80	71.29	69.68
n-3/n-6	1.97	0.70	0.57	0.71
n-6/n-3	0.51	1.42	1.75	1.40

*Diets: A = fish oil, B = palm oil, C = Soybean oil, D = Palm oil and Soybean oil (1:1).
ND = not detected.

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Serum biochemical parameters were analysed using an auto analyser (Tecnicon RA-1000, Technicon Instruments, New York, NY, USA), with commercial clinical investigation kits (Pars Azmoon Kit, Tehran, Iran). Biochemical measurements were carried out for low density lipoprotein (LDL), high density lipoprotein (HDL), total cholesterol, triglyceride (TGL), alanine aminotransferase (ALT), AST, ALP, total protein and glucose.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) to determine the effects of experimental diets on haematological and serum biochemical parameters. When significant differences were observed (p<0.05), the effects of individual treatments were further compared with Duncan's multiple range test. All data were analysed using the SPSS for Windows software, version 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Haematological profile

Haematological parameters of *H. longifilis* fingerlings fed diets containing different n-3 to n-6 fatty acid ratio are shown in Table 3. There were no differences in the PCV, RBC or MCV among fish fed the different diets. The Hb content of fish fed diet C (SO) was significantly (p<0.05) lower than the other dietary treatments. The same dietary treatment consistently had the lowest haematological values which were significantly (p<0.05) different from the other dietary treatments. The MCH and MCHC in *H. longifilis* fed diet A were not significantly (p>0.05) different from those fed diets B, or D. Fish fed diet B had higher values of WBC, lymphocyte and platelets than those fed the other diets.

Serum biochemical profile

The serum biochemical profiles of *H. longifilis* are presented in Table 4. Serum total protein of *H. longifilis* was not significantly (p>0.05) affected by different dietary n-3 to n-6 fatty acids ratio of the diets. Serum glucose was significantly (p<0.05) highest in fish fed diet A (FO) than the group fed diet D and lowest in fish fed diet B (PO). AST, ALT and ALP levels were significantly (p<0.05) different among the dietary groups. Serum AST activity decreased significantly (p<0.05) in fish fed diet D (POSO). Fish fed diet A had the lowest activity of ALT. Similarly, ALP activity was the least in fish fed diet A and highest in fish fed diet D. Serum total cholesterol was significantly higher in fish fed diet C (p<0.05).

Fish fed diet B had the lowest cholesterol concentration. Serum triglyceride, HDL and LDL were significantly (p<0.05) increased in fish fed diet C. The least values for triglyceride and LDL were recorded in fish fed diet B. However, HDL concentration was not significantly (p>0.05) different in fish fed diet A and B.

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Table 3: Haematological profile of *Heterobranchus longifilis* fed diets containing fish oil, palm oil and soybean oil as sources of n-3 and n-6 for 12 weeks.

	PCV (%)	Hb (g dL^{-1})	RBC (× 10^{-6} μL^{-1})	MCV (fl)	MCH (pg $cell^{-1}$)	MCHC (g L^{-1})	WBC (× 10^{-3} μL^{-1})	Neutrophils (%)	Lymphocytes (%)	Platelets
Diets*										
A	30.00	9.37^{a}	3.54	84.00	25.33^{ab}	30.33^{ab}	6.20^{b}	5.67^{a}	94.33^{b}	390.00^{ab}
B	29.00	8.03^{a}	3.72	70.67	21.33^{ab}	29.33^{b}	10.33^{a}	2.00^{c}	98.00^{a}	500.00^{a}
C	24.67	6.97^{b}	3.14	66.33	19.00^{b}	26.33^{c}	7.50^{b}	3.00^{b}	97.00^{a}	370.00^{b}
D	27.00	8.20^{a}	3.03	76.67	27.33^{a}	31.33^{a}	6.27^{b}	3.17^{b}	96.83^{a}	393.30^{ab}
SEM	0.62	0.26	0.09	2.00	0.99	0.56	0.50	0.41	0.41	15.34

*Diets: A = fish oil, B = palm oil, C = Soybean oil, D = Palm oil and Soybean oil (1:1).
Values in the same column followed by the same letter are not significantly different at $p>0.05$.

Table 4: Serum biochemical profile of *Heterobranchus longifilis* fed diets containing fish oil, palm oil and soybean oil as sources of n-3 and n-6 for 12 weeks.

	Total protein (g 100 mL^{-1})	Glucose (mg 100 mL^{-1})	AST (IU L^{-1})	ALT (IU L^{-1})	ALP (IU L^{-1})	Cholesterol (mg 100 mL^{-1})	Triglyceride	HDL	LDL
Diets*									
A	12.20	32.00^{a}	80.20^{b}	5.20^{d}	87.20^{c}	4.16^{b}	3.40^{c}	1.95^{a}	1.19^{c}
B	12.40	20.16^{c}	100.17^{a}	10.16^{b}	139.17^{b}	3.96^{c}	3.16^{c}	1.63^{ab}	2.90^{b}
C	13.35	27.35^{b}	100.35^{a}	8.35^{c}	71.35^{d}	5.25^{a}	5.45^{a}	1.93^{a}	3.85^{a}
D	12.48	29.33^{b}	50.33^{c}	20.33^{a}	189.33^{a}	5.03^{a}	4.33^{b}	1.58^{ab}	3.60^{a}
SEM	0.13	1.32	0.61	0.70	0.39	0.17	0.27	0.05	0.33

*Diets: A = fish oil, B = palm oil, C = soybean oil, D = palm oil and soybean oil (1:1).
Values in the same column followed by the same letter are not significantly different at $p>0.05$.

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Discussion

The absence of variations in PCV, RBC and MCV counts of *H. longifilis* suggests that none of the lipid sources used did induce anaemia in the experimental fish. The values for Hb, MCH, MCHC and total WBC count were significantly different among dietary groups; fish fed diet C (SO) had the lowest values for the same haematological traits. In this study, the consumption of palm oil may not impact negatively on the defensive function of WBC. The platelet counts of the fish fed diets containing palm oil did not indicate significant depreciation when compared with those fed diets A (FO) and D (POSO). An increase in platelet number above normal serves as a marker of vascular disease such as microangiopathy and macroangiopathy (Kwaan, 1992). The Hb concentration value of fish fed diets B and D and those of the diet A (FO) were similar and showed that feeding these lipid sources to *H. longifilis* exert no significant negative influence on Hb concentrations. The Hb concentrations and PCV are basic values revealing the degree of anaemia while the MCHC is a useful index of the average Hb concentration of the red cells (Swash and Mason, 1984). In the present study, *H. longifilis* could be considered to be adequately haemoglobinized as none of the haematological parameters measured are out of the range considered normal for fish (Sandnes *et al*., 1988).

The total protein concentrations of the plasma were close to the reference value of Bonnethead Sharks, *Sphyrna tiburo* (Harms *et al*., 2002). The concentration of plasma protein is a function of the nutritional status, which is one of the factors affecting the state of health of the animal (Igwebuike *et al*., 2008). The normal values indicate nutritional adequacy of the dietary protein. The blood glucose concentrations of the experimental animals were below the reference values for Bonnethead shark (Harms *et al*., 2002). These values indicated a lack of derangement in carbohydrate metabolism.

The AST, ALT and ALP belong to the non-plasma specific enzymes which are localized within tissue cells of liver, heart, gills, kidney, muscle and other organs (Gaudet *et al*., 1975) and in blood plasma they may give specific information about organ dysfunction (Casillas *et al*., 1983). In this study, AST activities were lower than values reported in *Oreochromis niloticus* (Chen *et al*., 2002). The lower activities of ALT ALP and AST in this study imply that the livers of *H. longifilis* were not damaged and transaminases were not released from the cytoplasm. According to Kim *et al*. (2002), elevated AST activity can be associated with the release of transaminase from cytoplasm due to hepatic cellular damage.

ALT activities were similar to those reported for *Salmo trutta*, *Thymallus thymallus*, and *Leuciscus cephalus*

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(Lusková, 1997), but were much lower than values reported for *Oreochromis niloticus* (Chen *et al.*, 2002) and *Chondrorostoma nasus* (Lusková, 1997).

The fish fed the experimental diet containing PO showed decreased serum cholesterol, indicating a hypocholesterolemic effect. Similar results have been reported in other fish. For instance, Peng *et al*. (2008) showed that diets containing soybean oil decreased total plasma cholesterol in black seabream *Acanthopagrus schlegeli*, European seabass (Richard *et al*., 2006a) and rainbow trout *O. mykiss* (Richard *et al*., 2006b). This is probably because diets containing vegetable oils are rich in oleic acid (OA), linoleic acid (LA) and linolenic acid (LNA) and these fatty acids are known to reduce cholesterol (Fernandez and West, 2005). Furthermore, the main hypercholesterolemic fatty acid – palmitic (C16:0) is mostly in the sn-1, 3 configuration. It has been showed that only 17% of palmitic acid in palm oil is in the sn-2 position (Renaud *et al*., 1995) and that fatty acids in the sn-2 position (preferentially absorbed) are able to influence lipaemia since those in the sn-1, 3 positions are released in the intestinal tract and partly excreted in the faeces (Small 1991; Aoyama *et al*., 1996). Another explanation could be the presence of phytosterols in vegetable oil.

Previous study with rat revealed that dietary EPA and DHA reduced hepatic triglyceride concentration by suppressing the activities of enzymes involving fatty acid synthesis in rat liver (Ikeda *et a*l., 1998). In the present study, fish fed diet A (FO, high in n-3 fatty acids) also had decreased serum triglyceride concentration. However, serum triglyceride concentration was not always determined only by fatty acid synthesis in the liver. The activity of lipoprotein lipase has been reported to be a major determinant of serum triglyceride concentration (Tanaka *et al*., 2001). These authors further observed that dietary fish oil increased lipoprotein lipase activity in adipose tissue, suggesting that chylomicron- and VLDL-triglyceride clearance from serum is accelerated in fish oil feeding. Therefore, there is a possibility that the effect of FO on lipoprotein lipase may be different from that of other dietary lipid sources which tend to inhibit or reduce chylomicron- and VLDL-triglyceride clearance from the serum. This has resulted in the elevated triglyceride concentrations recorded for *H. longifilis* fed the other dietary lipid sources that were rich in n-9 and n-6 fatty acids (except PO fed group) in this study. The decreased plasma triglycerides concentration has been attributed to the therapeutic action of omega-3 fatty acids (Rodriguez-Cruz *et al*., 2005, Lai *et al*., 2006, Nambi and Ballantyne 2006) which is due to the up-regulation of enzymes involved in fatty acid β-oxidation and down-regulation of enzymes of fatty acid synthesis (Granlund *et al*., 2005). Plasma triglycerides and cholesterol

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values observed in this experiment are within the values considered as normal levels for rainbow trout and sea bass (Dias *et al.*, 1999).

The results of this study clearly showed the possibility of feeding fish meal-based diets containing PO, SO or blend of PO and SO to *H. longifilis* fingerlings without any negative effects on haematological parameters or serum constituents.

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