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ORIGINAL PAPER

Effect of dietary alternative lipid sources on haematological parameters and serum constituents of *Heterobranchus longifilis* fingerlings

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Abstract The worldwide increase in aquaculture production and the decrease of wild fish stocks has made the replacement of fish oil (FO) in aquafeed industry a priority. Therefore, the use of terrestrial animal fats and vegetable oils, which has lower cost and larger supplies, may be good as substitute for FO. This study investigate the effects of total replacement of FO by two terrestrial animal fats (pork lard and poultry fat) and three vegetable oils (palm kernel oil, sheabutter oil and sunflower oil) on haematological and serum biochemical profile of *Heterobranchus longifilis* over 70 days. FO-diet was used as the control. The haematological parameters were significantly affected by dietary lipid sources. Serum total protein was not influenced by the dietary lipids. However, serum cholesterol was significantly higher in fish fed diet containing sunflower oil. Glucose and activities of liver enzymes in blood serum were significantly reduced in fish fed alternative lipids when compared with the control. These results indicate that FO can be replaced completely with alternative lipids without any serious negative health impacts.

Keywords Fish oil · Alternative lipids · Haematology · *Heterobranchus longifilis* · Serum constituents

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Introduction

Marine fish oils have traditionally been used in diets for cultured fish to provide fish with energy and essential fatty acids. However, these oils are in great demand world-wide and as a result of their limited supply, they are becoming increasingly more costly. To maintain and enhance the economic viability of aquaculture, it has become necessary to find suitable, less expensive alternate plant and/or animal lipid sources that would satisfy the nutritional requirement of fish for growth and health.

Catfish can utilize saturated fats, and several studies have focused on catfish growth rates as a function of dietary lipid (Lim et al. 2001; Shirai et al. 2001; Ng et al. 2003). In these studies, no difference was observed between fish fed fish oil or vegetable oil or animal fats or their combinations. Differences in haematological parameters, immune response and disease resistance as a function of dietary lipid source have also been reported for catfish (Fracalossi and Lovell 1994; Klinger et al. 1996; Ochang et al. 2007),

Atlantic salmon (Balfry et al. 2006) and large mouth bass (Subhadra et al. 2006). However, the influence of alternate dietary lipid sources on *Heterobranchus longifilis* haematological parameters and serum constituents are very scanty. Blood is a good indicator to determine the health of an organism. It also acts as pathological reflector of the status of exposed animal to toxicants (Joshi et al. 2002).

This study was initiated to determine if dietary alternate lipid sources, previously shown to affect haematological parameters of channel catfish, *Clarias gariepinus*, Atlantic salmon and large mouth bass, had any effect on selected haematological parameters and serum constituents of *H. longifilis*. Total red blood cell (RBC) and white blood cell (WBC) counts were used as indicators of hemopoiesis. Measures of mean cell volume (MCV) allowed us to evaluate the physiological state of RBC. The haematological response of *H. longifilis* to dietary alternate lipid sources was evaluated by analyzing these characteristics.

Materials and methods

Experimental diets

The efficiency of five alternative lipid sources: two terrestrial animal fats (pork lard, PL and poultry fat, CF) and three vegetable oils (palm kernel oil, PKO; sheabutter oil, SB and sunflower oil, SFO) were evaluated. Six isonitrogenous (450 gkg^{-1}) and isoenergetic (18 $MJkg^{-1}$) experimental diets were formulated (Table 1), containing 6% of added lipid sources. Fish oil (FO) was used in the control diet, which was completely replaced with the alternative lipid sources in the other five diets. The diets were made into pellet with meat mincer through 2 mm die, sundried, packed in polythene bags, sealed and stored at−20°C until used.

Animal and husbandry

Heterobranchus longifilis fingerlings were obtained from the hatchery of National Institute for Freshwater Fisheries Research (NIFFR), New-Bussa, Nigeria, and maintained in the laboratory for two weeks prior to the experiment. The fish were maintained in circular plastic tanks in a flow-through system during the acclimation and experimental period. The fish were reared under a natural photoperiod of 12D:12L.

During acclimation, fish were fed to apparent satiation twice a day using a commercial catfish diet (45 % CP, 12.5 % crude lipid and supply by NIFFR). Three tanks were randomly assigned to each diet group in 10 weeks experiment. At the start of the experiment, 20 fishes were batch weighed and stocked into each tank. During the feeding period, fish were fed the experimental diet to apparent

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

Ingredients	Diets					
	[1]FO	[2]SB	[3]PKO	[4]SFO	[5]PL	[6]CF
Fish meal (Danish)	398	398	398	398	398	398
Soybean meal	313	313	313	313	313	313
Corn flour (Maize)	177	177	177	177	177	177
Cassava starch	20	20	20	20	20	20
Methionine	10	10	10	10	10	10
[7]Vit./Min. Premix	20	20	20	20	20	20
Salt (NaCl)	1.5	1.5	1.5	1.5	1.5	1.5
Vitamin C	0.5	0.5	0.5	0.5	0.5	0.5
Fish oil	60					
[(2–6)]Alternative lipids		60	60	60	60	60

[1] Fish oil (cod liver oil), [2] Sheabutter oil, [3] Palmkernel oil, [4] Sunflower oil, [5] Pork lard, [6] Poultry fat, [7] Vitamin/mineral premix supplied the following (per kg of diet): calcium, 4500 mg; phosphorus, 4200 mg; potassium, 1700 mg; magnesium, 400 mg; iron, 30 mg; zinc, 30 mg; manganese, 20 mg; copper, 5 mg; iodine, 1 mg; selenium, 0.25 mg; vitamin A, 5000 IU; vitamin D, 2000 IU; DL-α-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 cid, 2 mg; ascorbic acid, 200 mg; inositol, 250 mg; choline, 400 mg; vitamin B_{12}, 0.1 mg and ethoxyquin, 60 mg.

satiation twice a day at 09:00 and 16:00 respectively. One hour, later uneaten feed was siphoned, dried to constant weight at 70°C and reweighed.

Sample collection

At the end of the 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. 1 ml blood sample was collected into bottles containing 0.05 ml EDTA as anticoagulant. Blood samples for serum analysis were collected into bottles without any anticoagulant. Serum was separated by centrifugation at 3500 x g for 5 minutes, and kept frozen at-20°C for the determination of protein, cholesterol, glucose, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities.

Haematological and blood 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). 50 µl haematocrit tube was filled with blood samples, after centrifugation (3500 x g 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 (Hb) concentration estimates were determined as described by Wedemeyer and Yasutake (1977). Measurement of red blood cell count (RBC), haemoglobin concentration (Hb) and PCV enabled the mean cell volume (MCV), mean cellular haemoglobin content (MCH) and mean cell haemoglobin concentration (MCHC) to be calculated according to the following formulas (Dacie and Lewis 2001):

$$\mathrm{MCV(fl)} = \mathrm{PCV}/\mathrm{RBC}\left(10^6 \mu l^{-1}\right)$$

$$\mathrm{MCH(pg)} = \left[\mathrm{Hb}\left(\mathrm{gdl}^{-1}\right) \times 10\right]/\mathrm{RBC}\left(10^6 \mu l^{-1}\right)$$

and

$$\mathrm{MCHC}\left(\mathrm{gl}^{-1}\right) = \left[\mathrm{Hb}\left(\mathrm{gdl}^{-1}\right) \times 10\right]/\mathrm{PCV}$$

The concentrations of total protein, total cholesterol, glucose, AST and ALT activities in plasma were analyzed by using commercial clinical investigation kits (Wako, Osaka, Japan).

Statistical analysis

The data were subjected to analysis of variance (ANOVA) and if significant ($P<0.05$) differences were found, Duncan's multiple range test (Duncan 1955) was used to rank the group using SPSS version 10.0 (SPSS 1997). The data presented as mean ± S.E. M. of three replicate groups.

Results

Haematological profile

The mean value for the haematological parameters of *Heterobranchus longifilis* fingerlings studied are shown in Table 2. Fish fed diets containing SFO and CF had increased PCV, Hb, RBC and WBC contents than those on the other diet groups. Red blood cell and haemoglobin content for fish fed the control (FO), SB and PKO diets were similar.

The blood indices calculated from the mean values of blood parameters shows that MCH and MCHC were significantly reduced in *H. longifilis* fed on diets containing FO, SB, PKO, SFO and PL. While MCV was significantly increased in fish fed diets containing SFO and PL. Percentage of neutrophils and lymphocytes in *H. longifilis* fed FO was significantly different from the other groups. The blood platelets were significantly different among fish fed different lipid sources.

Blood serum biochemical profile

Total protein, total cholesterol and glucose concentrations, and AST and ALT activities in serum of *H. longifilis* at the end of feeding trial are shown in Table 3. Serum total protein of fish was not significantly affected by dietary lipid sources. Serum glucose and total cholesterol were significantly higher in *H. longifilis* fed diets containing SFO and PL.

Serum AST activity increased significantly in *H. longifilis* fed alternative lipid diets compared to the control, except for those fed PKO that had significantly lower value. The highest value was obtained in group fed SB diets. A significantly reduction in ALT

Table 2 Haematological profile of *Heterobranchus longifilis* fingerlings fed diets containing alternative lipid sources for 10 weeks

Parameters	FO	SB	PKO	SFO	PL	CF
PCV (%)	28.00±0.58^{a}	31.00±0.55^{b}	28.00±0.56^{a}	35.00±0.58^{c}	28.00±0.59^{a}	31.00±0.58^{b}
Hb (gdl^{-1})	10.80±0.06^{b}	10.20±0.06^{b}	10.10±0.06^{b}	11.20±0.12 ^{c}	9.00±0.23^{a}	12.50±0.01^{c}
RBC (X 10^{6}µl^{-1})	2.42±0.01^{b}	2.49±0.02^{b}	2.37±0.01^{b}	2.63±0.02^{c}	2.03±0.02^{a}	2.62±0.01^{c}
MCV (fl)	117.33±0.88^{a}	123.00±0.58^{b}	118.00±0.58^{a}	132.00±0.58^{c}	135.00±1.73^{c}	117.00±0.58^{a}
MCH (pg)	41.00±0.58^{a}	42.00±1.51^{a}	43.00±1.73^{a}	43.00±0.58^{a}	44.00±2.31^{ab}	48.00±0.01^{b}
MCHC (gl^{-1})	37.00±1.51^{bc}	35.00±1.73^{ab}	36.00±2.31^{ab}	32.00±0.58^{a}	32.00±0.58^{a}	41.00±0.58^{c}
WBC (X 10^{9}µl^{-1})	220.00±1.15^{a}	244.00±1.15^{b}	222.00±1.15^{a}	250.00±0.58^{c}	220.00±1.15^{a}	249.00±0.58^{c}
Neutrophils (%)	5.00±0.58^{b}	2.00±.057^{a}	2.00±0.59^{a}	2.00±0.58^{a}	3.00±0.58^{a}	3.00±0.56^{a}
Lymphocytes (%)	95.00±0.58^{a}	98.00±0.57^{b}	98.00±0.59^{b}	98.00±0.58^{b}	97.00±0.58^{b}	97.00±0.56^{b}
Platelets	210.00±1.15^{a}	312.00±1.15^{d}	263.00±1.73^{b}	329.00±1.15^{e}	250.00±1.15^{b}	285.00±0.58^{c}

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

activity was recorded in group fed the control diet. The CF diet yielded a significantly higher (P<0.05) ALT than in other dietary groups.

Discussions

Fish haematology is gaining increasing importance in fish culture because of its importance in monitoring the health status of fish (Hrubec et al. 2000). Results of the haematological parameters of *H. longifilis* in this study showed that there were significant differences (P<0.05) among different dietary groups. It has been shown that variables such as age, sex, dietary state and stress alter blood values (Barnhart 1969; McCarthy et al. 1973). Haemoglobin concentration and PCV of fish blood has also been shown to decrease after the capture and transportation (Hattingh and Van Pletzen 1974). The fish used in this study were kept under laboratory condition during the period of feeding with test diets and were tranquilized with MS222 before handling. The effect of stress resulting from handling must have been minimal. Consequently, only the nutritional states of the fish have significantly affected the haematological parameters. However, the PCV values obtained in this study higher than the values obtained for *Clarias gariepinus* (Ochang et al. 2007) and bagrid catfish (Etim et al. 1999).

Similarly, the Hb content of fish in this study exhibited higher values than those obtained by Subhadra et al. (2006) for large mouth bass with diets containing canola oil, chicken oil and menhaden fish oil, which ranged between 3.7–3.9 g dl^{-1}. This shows that the oxygen carrying abilities of the blood of *H. longifilis* higher, and could be attributed to species differences and their ability to utilize n-6 fatty acids present in vegetable oils.

The lower values of RBC and WBC in fish fed FO show clearly that the African catfish cannot effectively utilize n-3 PUFA without dilution with n-6 PUFA. The n-3:n-6 PUFA balance seems critical in the diet of African catfish (Ochang et al. 2007). It is likely that n-3 PUFA in the cod liver oil may have significantly affected the white blood cells and other blood param-

Table 3 Blood serum biochemical profile of *Heterobranchus longifilis* fingerlings fed diets containing alternative lipid sources for 10 weeks

Diets	Serum total protein (g/100 ml)	Serum total cholesterol (mg/100 ml)	Serum glucose (mg/100 ml)	Serum AST (IU/l)	Serum ALT (IU/l)
FO	2.86±0.08	35.47±0.33^{c}	26.21±0.31^{a}	16.11±0.06^{b}	10.11±.05^{a}
SB	2.72±0.11	41.97±0.55^{e}	26.33±0.22^{a}	39.08±0.05^{e}	12.12±0.06^{b}
PKO	2.75±0.08	30.80±0.46^{a}	26.79±0.22^{b}	10.05±0.04^{a}	17.10±0.05^{d}
SFO	2.93±0.09	46.73±0.50^{f}	27.18±0.15^{c}	20.01±0.01^{c}	16.04±0.02^{c}
PL	2.90±0.07	37.37±0.27^{d}	27.29±0.15^{c}	22.03±0.02d^{e}	16.08±0.05^{c}
CF	2.81±0.06	31.83±0.43^{b}	26.20±0.16^{a}	13.07±0.04^{ab}	18.08±0.04^{e}

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

eters, thereby compromising immune system. This may explain lower percent lymphocytes recorded for fish fed FO diet. Lymphocytes are responsible for immune responses and neutrophil is a form of granulocyte that kills and digests microorganisms it has engulfed by phagocytosis. Lall (2000) revealed that fatty acid composition of diet influences production of eicosanoids precursors and immune response in laboratory animals. A diet high in n-6 PUFAs produces relatively high levels of pro-inflammatory 2-series prostaglandins (PGs) and 4-series leucotrienes (LTs) and lopoxins (LXs) derived from arachidonic acid (AA) whereas diets high in n-3 PUFAs produce the anti-inflammatory 3-series PGs and 5-series LTs and LXs derived from eicosapentaenoic acid (EPA). Generally, diets containing n-6 PUFAs enhance the immune response due to the high levels of pro-inflammatory AA-derived eicosanoids, and diet containing n-3 (FO) may be immunosuppressive due to the high levels of EPA-derived anti-inflammatory eicosanoids.

Blood indices (MCV, MCH and MCHC) are particularly important for the diagnosis of anaemia in most animals (Coles 1986). This study showed a significant decrease of MCV and MCH in fish fed FO. So, it is assumed that the decrease or increase of blood indices suggest a possible hemoconcentration, a situation which coincided with the main changes observed in *Prochilodus scrofa* exposed to copper (Mazon et al. 2002).

The reduction in platelets reported in this study for fish fed FO compared with that in fish fed with the alternative lipids may indicate that oil rich in n-3 highly unsaturated fatty acids (HUFAs) (such as FO) reduces the blood clotting tendency and minimises inflammatory responses as suggested by Pepping (1999) and Vauy and Valenzuele (2002).

Blood serum protein is fairly labile biochemical system, reflecting the condition of the organism and the changes happening to it under the influence of internal and external factors. Booke (1964) showed that sex, spawning, food, osmotic pressure, temperature, light, age, hibernation hormones, oxygen depletion and season are factors that demand total serum protein complement in fish. In this study, no significant hyperproteinemia was observed in all fish groups fed various alternative lipids. This shows that the various lipid sources do not lead to osmoregulatory dysfunction, hemodilution or damage of tissues surrounding blood vessels of *H. longifilis*. Hille (1982) attributed high serum protein in rainbow trout to be an indicative of osmoregulatory dysfunction, damage of tissues surrounding blood vessels and hemodilution.

Larsson and Fange (1977) reported that the normal plasma cholesterol values ranging from 86 to 921 mg/100 ml in most marine fish were two to six times higher than that of mammalian plasma. Similarly, low plasma cholesterol concentration has been reported in Japanese flounder fed diet containing lauric acid as sole lipid source (Kim et al. 2002) and starry flounder (Lee et al. 2003).

In the present study, serum glucose concentration reduced significantly in fish fed diets containing cod liver oil, SB and CF. on the other hand the elevated ($P<0.05$) serum glucose in fish fed PKO, SFO and PL, indicate that these lipid sources affects glucose dynamics in *H. longifilis* in order to obtain more energy to withstand and overcome existing stress condition. Plasma or serum glucose levels are often used as an indicator of non-specific stress (Hunn and Greer 1991). Increased blood serum glucose levels might have been due to a glucose shift from tissue to blood or to an impairment of glucose mobilization.

Transamination represents one of the main pathways for synthesis and deamination of amino acids, thereby allowing interplay between carbohydrate and protein metabolism during the fluctuating energy demands of the organism in various adaptive situations. It is also considered to be important in assessing the state of the liver and some other organs (Verma et al. 1981). Therefore attention has been focused on the changes in AST, ALT and alkaline phosphate (ALP) activities, which promotes gluconeogenesis from amino acid, as well as on the changes in aminotransferase activities in the liver (Hilmy et al. 1981; Rashatuar and Ilyas, 1983). Furthermore, AST and ALT might be altered by a variety of chemical, biological and physiological factors or by a disturbance in the Kreb's cycle. Decreased activity of the Kreb's cycle cause a decrease in its intermediates, thereby, ALT and AST compensate by providing α-ketoglutarate (Salah El-Deen and Rogeps 1993). Results of this study showed that serum AST and ALT activities increased significantly in the fish group fed on alternative lipid sources-based diets. The elevated serum AST in *H. longifilis* fed SB and PL is probably due to release of transaminase from cytoplasm due to hepatic cellular damage. It has been reported that higher GOT activities were found in rock fish (Lee 2001), Japanese flounder (Kim et al.

2002) and starry flounder (Lee et al. 2003) fed the n-3 HUFA-deficient diets. However, AST and ALT values found were within the range consistent with good fish health (Sandnes et al. 1988).

Under the experimental conditions described in this study, any of the diet treatment had serious negative health impacts. It is feasible to completely replace FO with alternative lipids in the diets of *H. longifilis* without any negative affects on haematological and serum biochemical profile of the fish.

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