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Impact of palm oil and extracted fish meal on haematological parameters, serum constituents and histology of African catfish (*Heterobranchus longifilis*) fingerlings

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ABSTRACT

This experiment was carried out to determine the effects of supplementing feed with palm oil and extracted fish meal on the histological, haematological and serum biochemical profile of *Heterobranchus longifilis*. Ten experimental diets were formulated by replacing fish oil with palm oil (PO) at 0, 25, 50, 75 or 100% in extracted and unextracted fishmeal-based diets in a 2 x 5 factorial design. The red blood cell counts (RBC) were significantly affected by the PO levels and highest in fish fed 50% and 75% FO replacement with PO. Extraction of fishmeal significantly affected the haematological parameters. Total protein, glucose concentrations, AST and ALT activities were not significantly ($P > 0.05$) affected by the PO levels. However, serum total cholesterol was significantly ($P < 0.05$) increased in fish fed 50% FO. Similarly HDL was significantly ($P < 0.05$) higher in fish fed 50%, 75% and 100% FO replacement with PO, but HDL of fish fed unextracted fishmeal diets was significantly ($P < 0.05$) lower than those fed the extracted fishmeal diets. Histological examination of the intestines showed no accumulation of lipid vacuoles in the cytoplasm. However, liver from fish fed unextracted fish meal-based diets with 25%, 50%, 75% and 100% FO replacement showed homogenous size hepatocytes with vacuolized cytoplasm, swelling and nuclei displacement were also evident. These results indicate that extraction of fishmeal and use of PO as a substitute for FO in the diets of *H. longifilis* had no noticeable negative health

impacts.

1. Introduction

Aquafeed is the most expenditure in intensive aquaculture operations. The major concern in the industry is the fact that major ingredients (fish meal and fish oil) are scarce and expensive. Much effort is put into the research for substitute feedstuffs in developed and developing countries.

The demand for fish oils in aquafeeds has dramatically increased, outstripping its supply as a result of rapid growth of aquaculture (FAO, 2006). On the contrary, production of global vegetable oil has steadily increased (Bimbo, 1990). Therefore, replacement of fish oil with vegetable oils appears to be a viable option given their availability, low cost and absence of dioxins and pollutants (Caballero *et al.,* 2002; Izquierdo *et al.,* 2003). A key requirement for the replacement of fish oil in aquafeeds is to supply equivalent energy with balanced essential fatty acids. This is necessary in order to sustain high growth and survival, feed conversion efficiency, immune competence, disease resistance, and flesh quality. One potential substitute for fish oil in aquafeeds is palm oil, which is currently the most abundant vegetable oil produced in the world (Ng *et al,* 2006). Crude palm oil (CPO) has high content of carotenoids, phosphatides, sterols, tocopherols and is a rich source of vitamin E (Nesaretnam and Muhammad, 1993). These have shown to be effective against oxidative stress in vitro and in vivo (Aboua et al., 2009). Palm Oil is the richest natural source of β-carotene (500 - 700 mg/l), which is responsible for the characteristic colour of the oil. The carotenoids, together with vitamin E, ascorbic acid, enzymes and proteins, are members of the biological antioxidant network converting highly reactive radicals and free fatty peroxyl radicals to less active species (Peng and Stanley, 2001) thus, protecting against oxidative damage to cells. β-carotene is the most abundant carotenoids which can be converted to vitamin A which plays an important role in the visual process.

Use of vegetable oils in catfish feeds have produced differences in haematological parameters, histology, immune response and disease resistance (Ochang *et al*., 2007; Babalola, *et al*., 2009; Babalola, *et al*., 2011). Palm oil has been showed to elicit increased High Density Lipoprotein Cholesterol and reduced Low Density Lipoprotein Cholesterol in rat (Dauqan, *et al*., 2011). However, the influence of palm oil as an alternative lipid source and extracted fishmeal in the diet of *H. longifilis* on haematological parameters, histology and serum constituents are very scanty.

This study was initiated to determine the health implication of palm oil as lipid source for African catfish *H. longifilis* using an extracted fishmeal–based diet. The influence of this dietary lipid on haematology and cholesterol in blood serum and liver histology were examined.

2. Materials and methods

2.1. Experimental diets

Palm oil was selected and used for this study based on the superior performance exhibited by *H. longifilis* fed palm oil containing diet in the previous study (Babalola and Apata, 2012). Danish fish meal was used as the primary protein source in the test diets because of its prevalent use in diets fed to cultured catfish. Danish fishmeal contains approximately 10% fish oil as purchased, which might provide sufficient HUFA to meet the essential fatty acid requirement of *H. longifilis* and obviate the effects of the lipid of interest. Therefore, the fishmeal was exhaustively extracted with hexane to reduce the endogenous lipid to trace amounts before inclusion in the diets. Ten experimental diets were formulated (Table 1) and used for this experiment, five containing extracted fish meal and five containing unextracted fishmeal. Oil was included at 60 g/kg, with PO contributing 0, 25, 50, 75 or 100% and FO contributing the remainder. This study was carried out using thirty circular bowls capable of holding 62 litres of water in a 2 X 5 factorial design. Each bowl was filled to a depth of 35cm with water from storage reservoir. Six hundred *H. longifilis* were randomly stocked in 30 circular bowls at 20 fingerlings per tank; 60 fingerlings per treatment, experimental diets were fed to triplicate groups of fish twice a day to apparent satiation at 09:00 and 16:00 respectively over a period of twelve weeks. One hour, later uneaten feed was siphoned, dried

to constant weight at 70^0C and reweighed. Total fish weight in each tank was determined every two weeks to check their growth. Feeding was stopped 24 h prior weighing.

2.2. Sample collection and analysis

At the end of the trial, *H. longifilis* that had not been fed for 24 h were anesthetized with 150 mgl^{-1} solution of methane sulphonate (MS222) (Wagner *et al.,* 1997). The caudal peduncles of 4 fish per tank were severed with blade and blood samples were collected from the caudal vein of individual fish into bottles containing 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 for the determination of protein, cholesterol, glucose, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities.

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). 50µl 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 Blaxhail and Drasley (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 formulas proposed by Dacie and Lewis (2001).

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

Liver and intestine samples for histology were collected from one fish per tank. Samples were fixed in 10% buffered formalin, dehydrated in graded ethanol series and embedded in paraffin. Serial 4µm sections were stained with haematoxylin and eosin (H&E) (Martoja and Martoja-Pierson, 1970).

2.3. Statistical analysis

The data were subjected to two way 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 13.0 (SPSS Inc., Chicago, IL, USA) computer program.

3. Results

3.1. Haematological profile

There were no differences ($P > 0.05$) in the PCV, Hb, MCV, MCH, MCHC, WBC or platelets among fish fed diets containing different PO levels (Table 2). The red blood cell counts (RBC) were highest in fish fed 50% and 75% FO replacement with PO followed by those fed 0%, 25% or 100% FO replacement with PO. The percentage of neutrophils was higher in 50% PO fed fish than those fed other dietary groups. Fish on 0% and 75% replacements had similar percentage neutrophils which were significantly lower than those fed other dietary treatments. Conversely, percentage lymphocyte was significantly ($P < 0.05$) increased in fish fed 25%, 50%, 75% and 100% FO replacement with PO and was ($P < 0.05$) significantly decrease in fish fed 0% PO diet compared to fish fed the other dietary treatments.

Fish fed extracted fishmeal diets had significantly ($P < 0.05$) lower RBC, MCV, MCH, WBC, neutrophils and platelets than those fed unextracted fishmeal diets. Significant ($P < 0.05$) interaction effects of fish meal type and PO levels were observed in red blood cell count and MCV. These values were significantly ($P < 0.05$) lower in fish fed unextracted fish meal with 25% PO than in those fed extracted fish meal based diet at 100%. FO replacement with PO. However, RBC and MCV were higher in the fish fed the other diets (Table 2).

3.2. Serum biochemical constituents

Total protein, glucose concentrations, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, total cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol in serum of *H. longifilis* at the end of feeding trial are shown in Table 3. Total protein, glucose concentrations AST and ALT activities of fish were not significantly ($P > 0.05$) affected by the PO levels. Serum total cholesterol was significantly ($P < 0.05$) increased in fish fed 50% FO replacement with PO, the lowest value was obtained in fish fed 75% FO

replacement with PO. Similarly HDL was significantly ($P < 0.05$) higher in fish fed 50%, 75% and 100% than in those fed 0% and 25% FO replacement with PO. On the other hand LDL, of fish fed 0% FO replacement with PO was significantly ($P < 0.05$) higher than those of fish fed other PO levels. Fish fed 75% FO replacement with PO had lower value than other dietary groups. HDL of fish fed unextracted fishmeal diets was significantly ($P < 0.05$) lower than those fed the extracted fishmeal diets.

Table 4 shows the interaction of fish meal type and PO levels on serum total cholesterol, HDL cholesterol and LDL cholesterol. Fish fed unextracted fish meal with 50% PO had the highest total cholesterol content which was not significantly ($P < 0.05$) different from those fish fed extracted fish meal and 0 and 100% FO replacement with PO. Complete fish oil replacement with palm oil together with the extraction of oils from fish meal led to significantly ($P < 0.05$) higher HDL cholesterol in *H. longifilis*. This was not ($P > 0.05$) significantly different in fish fed unextracted fish meal with 50 and 75% PO and those fed extracted fish meal with 0, 50 and 75% FO replacement with PO. The LDL cholesterol content of *H. longifilis* serum decreased with increasing PO level up to 50% in extracted fish meal and 75% FO replacement with PO in the unextracted fish meal-based diet. LDL cholesterol was significantly ($P < 0.05$) increased in fish fed unextracted fish meal with 0% FO replacement with PO, this was not significantly ($P < 0.05$) different from the value for fish fed extracted fish meal with 0% FO replacement with PO and unextracted fishmeal with 100% FO replacement with PO but significantly ($P < 0.05$) different from the other diets.

3.3. Histological examination

3.3.1. Intestine

Fish fed the extracted and unextracted fish meal based diets at all levels of FO replacement with PO showed an epithelium with high columnar enterocytes with small nuclei localised in the mid portion of the cells (Fig. 1). No accumulation of lipid vacuoles was observed in the cytoplasm.

3.3.2. Liver

Liver from fish fed the diet containing unextracted fish meal with 100% FO replacement with PO showed regular-shaped hepatocytes with centrally located nuclei and some lipidic vacuoles in the cytoplasm which did not disturb the hepatocytes size (Fig. 2). Similarly, livers from fish fed diets containing unextracted fish meal with 0%, 25%, 50% and 75% FO replacement with PO showed homogenous size hepatocytes with vacuolized cytoplasm; swelling and nuclei displacement were also evident in the liver (Fig. 3). In contrast, fish fed diets containing extracted fish meal with 25%, 50% and 100% FO replacement with PO showed regular shaped hepatocytes with centrally located nuclei without lipid vacuoles (Fig. 4). However, liver of fish fed extracted fish meal with 0% and 75% FO replacement with PO showed nuclei displacement with varying size lipid vacuoles (Fig. 5).

4. Discussion

Dietary fatty acids also have direct or indirect effects on the immune system (Pablo *et al.,* 2002), either by stimulating the production of cytokines (Yaqoob and Calder, 1995) or by affecting lymphocyte proliferation (Yaqoob *et al.,* 1994). Leukotriene B4 (LTB4), produced from 20:4*n*-6, is the main agent of lymphocyte proliferation. Leukotriene B5 (LTB5), derived from 20:5*n*-3, has a similar but less potent physiological effect (Secombes *et al.,* 1994).

Differential cell counts in *H. longifilis* revealed that the percentage of lymphocytes was highest in *H. longifilis* fed diets with 25% - 100% PO than in *H. longifilis* fed the diet with 0% PO. Fish fed diets with at least 4.41% *n*-3 fatty acids (100% PO), appeared to have a health advantage over those fed the 0% PO diet, because higher lymphocyte counts indicated greater immunocompetence (Pablo *et al.,* 2002). The *n*-3 fatty acids have been associated with both immunosuppressive and immunostimulatory effects in fish (Lall, 2000).

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

	Experimental diets									
	Extracted Fishmeal					**Unextracted Fishmeal**				
	0 PO	**25 PO**	**50 PO**	**75 PO**	**100 PO**	**0 PO**	**25 PO**	**50 PO**	**75 PO**	**100 PO**
Ingredients (g kg $^{-1}$)										
Fish meal (Danish)	398.00	398.00	398.00	398.00	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	313.00	313.00	313.00	313.00
Corn flour (Maize)	172.00	172.00	172.00	172.00	172.00	172.00	172.00	172.00	172.00	172.00
Cassava starch	20.00	20.00	20.00	20.00	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	10.00	10.00	10.00	10.00
Vit./Min. Premix	20.00	20.00	20.00	20.00	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	1.50	1.50	1.50	1.50
Vitamin C	0.50	0.50	0.50	0.50	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	5.00	5.00	5.00	5.00
Oil*	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00
Proximate composition (n=3)										
Moisture (g/kg)	6.20	6.15	6.23	6.10	6.31	6.23	6.20	6.10	6.20	6.12
Crude protein (g/kg)	*45.72*	45.81	45.43	45.56	45.76	45.10	44.97	45.02	44.98	44.98
Lipid (g/kg)	10.67	10.71	10.86	10.64	10.56	11.89	11.92	11.90	11.91	11.89
Ash (g/kg)	8.80	8.70	8.60	8.70	8.81	8.14	8.05	8.10	8.02	8.06
NFE** (g/kg)	34.81	34.78	35.11	35.10	34.87	34.87	35.06	34.98	35.09	35.07
Gross energy*** (MJ/kg)	21.3	21.33	21.36	21.3	21.28	21.65	21.66	21.65	21.67	21.65

* 0 PO = 0% Palm oil and 100% FO, 25 PO = 25 % palm oil and 75% FO, 50 PO = 50% Palm oil and 50% FO, 75 PO = 75% Palm oil and 25% FO, 100 PO = 100% Palm oil and 0% FO. **NFE = Nitrogen free extracts including crude fibre; = 100 – (CP + lipid + ash).
*** calculated from the published compositions of the ingredients used (NRC, 1993).

Table 2
Haematological profile of *Heterobranchus longifilis* fingerlings fed the experimental diets[1] for 12 weeks.

	PCV (%)	Hb (gdl^{-1})	RBC ($x\ 10^{-6}$)	MCV (fl)	MCH (pg)	MCHC (gl^{-1})	WBC ($x\ 10^{-3}$)	Neutrophils (%)	Lymphocytes (%)	Platelets
Dietary treatments										
FO replacement (%)	*	NS	*	*	*	NS	*	*	*	*
0	16.00^{b}	8.37	1.47^{a}	178.50^{a}	93.00^{ab}	57.50	176.80^{a}	11.83^{a}	88.17^{b}	453.17^{ab}
25	14.25^{b}	7.75	1.07^{b}	174.33^{b}	93.50^{ab}	59.00	169.85^{ab}	8.67^{b}	91.33^{a}	402.33^{bc}
50	17.00^{a}	8.43	1.52^{a}	177.17^{ab}	93.17^{ab}	57.50	161.85^{b}	6.33^{b}	93.67^{a}	487.33^{a}
75	15.75^{b}	8.43	1.52^{a}	177.33^{ab}	95.33^{a}	57.33	168.72^{ab}	6.00^{b}	94.00^{a}	369.50^{c}
100	15.50^{b}	8.30	1.36^{a}	177.50^{ab}	92.33^{b}	56.50	169.17^{ab}	8.50^{b}	91.50^{a}	402.17^{bc}
Fishmeal type	NS	NS	NS	NS	*	NS	*	*	*	*
Extracted	16.6	8.28	1.37	175.93	92.60^{b}	57.67	166.13^{b}	6.60^{b}	93.40^{a}	409.13^{b}
Unextracted	15.8	8.23	1.40	178.00	94.33^{a}	57.47	172.43^{a}	9.93^{a}	90.07^{b}	436.67^{a}
FO rep. X FM type	NS	NS	*	*	NS	NS	NS	NS	NS	NS
SEM	0.51	0.18	0.07	1.04	0.71	0.67	2.61	0.72	0.72	16.75

[1]Diets as explained in Table 1
Values in the same column followed by the same letter are not significantly different at $P > 0.05$

Table 3
Serum biochemical constituents of *Heterobranchus longifilis* fed the experimental diets[1] for 12 weeks.

	Total protein (g 100ml^{-1})	Glucose (mg 100ml^{-1})	AST (IU l^{-1})	ALT (IU l^{-1})	Total cholesterol (Mmol/l)	HDL (Mmol/l)	LDL (Mmol/l)
Dietary treatments							
FO replacement (%)	NS	NS	NS	NS	*	*	*
0	33.30	3.37	53.50	29.83	2.88^{ab}	1.35^{b}	0.88^{a}
25	34.00	4.30	42.73	30.33	2.50^{a}	1.33^{b}	0.53^{b}
50	32.33	3.43	45.00	18.17	3.38^{b}	1.68^{a}	0.43^{bc}
75	31.33	3.60	50.50	36.33	2.40^{a}	1.63^{a}	0.28^{c}
100	32.33	3.23	46.17	26.67	2.95^{ab}	1.65^{a}	0.55^{b}
Fishmeal type	NS	NS	NS	NS	NS	*	*
Extracted	32.53	3.66	48.61	32.33	2.81	1.59^{a}	0.45^{b}
Unextracted	32.80	3.51	46.55	24.20	2.83	1.46^{b}	0.61^{a}
FO rep. X FM type	NS	NS	NS	NS	*	*	*
SEM	1.19	0.24	2.18	3.77	0.15	0.06	0.06

[1]Diets as explained in Table 1
Values in the same column followed by the same letter are not significantly different at $P > 0.05$

Table 4
Interactions of fishmeal type and palm oil replacement levels on *Heterobranchus longifilis* serum total cholesterol, HDL and LDL cholesterols.

(A) Total cholesterol

Fish meal type	**Palm oil levels**				
	0%	**25%**	**50%**	**75%**	**100%**
Extracted	3.25^{ab}	2.65^{b}	2.75^{b}	2.30^{b}	3.10^{ab}
Unextracted	2.50^{b}	2.35^{b}	4.00^{a}	2.50^{b}	2.80^{b}
SEM			0.15		

(B) HDL cholesterol

Fish meal type	**Palm oil levels**				
	0%	**25%**	**50%**	**75%**	**100%**
Extracted	1.50^{ab}	1.40^{b}	1.50^{ab}	1.65^{ab}	1.90^{a}
Unextracted	1.20^{b}	1.25^{b}	1.85^{a}	1.60^{ab}	1.40^{b}
SEM			0.06		

(C) LDL cholesterol

Fish meal type	**Palm oil levels**				
	0%	**25%**	**50%**	**75%**	**100%**
Extracted	0.65^{ab}	0.55^{b}	0.30^{c}	0.35^{bc}	0.40^{b}
Unextracted	1.10^{a}	0.50^{b}	0.55^{b}	0.20^{c}	0.70^{ab}
SEM			0.06		

The hematocrits of *H. longifilis* fed the test diets were similar. This findings was consistent with the previous findings in which the hematocrit and lysozyme activity in gilthead sea bream fed diets with fish oil partially replaced with Soyabean oil, rapeseed oil, linseed oil or a mixture of these were similar to those of fish fed a diet with only fish oil for 101 days (Montero *et al.,* 2003). Furthermore, research has shown that increasing dietary lipid (a mixture of fish and corn oils, 50:50 (by weight)) enhanced alternative complement and lysozyme activity in grouper (Lin and Shiau, 2003), indicating an immunostimulatory effect. However, Mourente *et al.,* (2005) observed a reduction in circulating leucocytes and respiratory burst activity - a characteristic of immune suppression when 60% of fish oil in the diet for European seabass was replaced with rapeseed oil, linseed oil and or olive oil.

Haematological values (PCV, RBC and Hb) corresponded to those previously recorded in cod (Lie 1991), *H. longifilis* fingerlings (Babalola *et al.*, 2009) did not appear to indicate poor health status, and are also in line with values reported for the *Clarias gariepinus* (Ochang *et al.,* 2007).

Concentrations of plasma glucose, proteins and enzymes levels were in normal range which, indicate that the animals were apparently healthy throughout the experimental period (Sandnes *et al.,* 1988).

Clinical diagnosis of disease and damage to the structural integrity of liver is commonly assessed by monitoring the status of serum AST and ALT activities (Amin and Hamza, 2005). Higher activities of these enzymes in serum have been found in response to oxidative stress induced by hyperthyroidism (Chattopadhyay *et al.,* 2007; Subudhi *et al.,* 2008). Enzymatic activities of AST and ALT are sensitive serological indicators of metabolic disorder in the liver.

In this study none of these parameters were significantly altered by the experimental diets, suggesting that no critical injury was caused by fish oil substitution with palm oil and does not exert any deleterious effects on the liver.

The transport of lipids and other lipid-soluble components from the intestine to peripheral tissues is predominantly mediated by lipoproteins (Babin and Vernier, 1989). Lipoproteins are classified according to their density into six main classes: chylomicrons (CM), very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL), high density lipoprotein (HDL), and very high density lipoprotein

(VHDL) (= Vitellogenin (VTG)). In fish, it is not yet clearly understood which route the chylomicrons and VLDL take from the enterocytes (Turchini *et al.,* 2009). The LDL is formed from VLDL as the VLDL is stripped of its triacylglycerol (TAG) and certain apolipoproteins (Gjøen and Berg 1993). In contrast to most mammalian species where VLDL and LDL dominate the blood lipids, HDL dominate the lipoprotein content in *H. longifilis* which was in line with the findings of Lie *et al.* (1993) in which HDL dominate the lipoprotein in salmonids, providing a large reservoir for cholesterol and phosphadylcholine. Furthermore, a decrease in plasma and LDL cholesterol has been reported in Atlantic salmon (Jordal *et al.,* 2007) and rainbow trout fed vegetable oil-based diets (Richard *et al.,* 2006), possibly because of the decreased content of dietary cholesterol in the vegetable oil-based diets. A reduction in cholesterol might also result from the content of phytosterols in the dietary plant oils used. Research documented that phytosterols has cholesterol lowering effect; however no evidence of any beneficial effects on cardiovascular disease (CVD) or overall mortality (Weingartner, *et al.*, 2008; Genser, *et al.*, 2012).

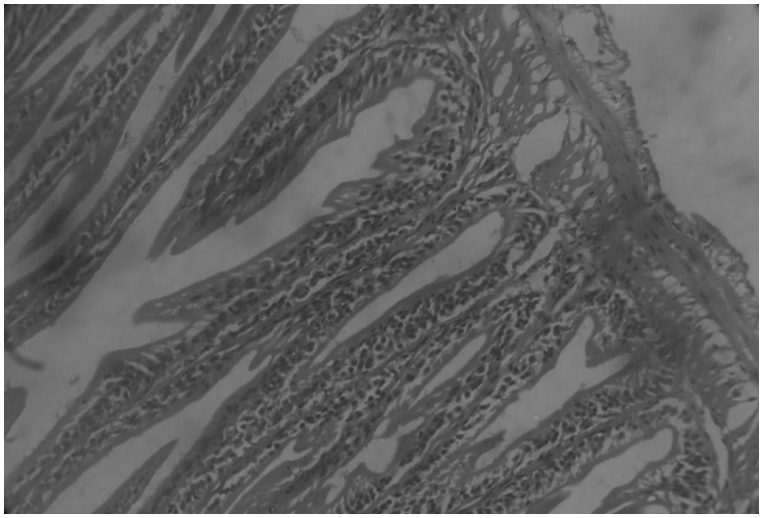


Fig. 1. Intestine of fish fed 100% PO extracted fish meal diet. Lipid vacuoles were not observed in the cytoplasm of the enterocytes (H&E, X 400).

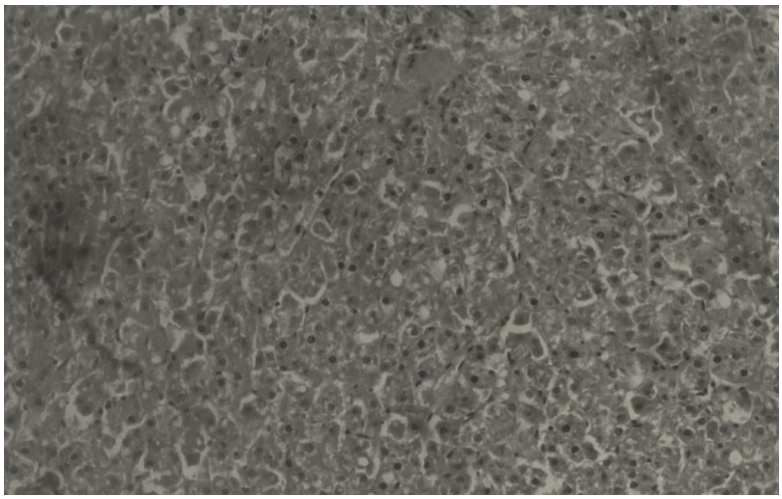


Fig. 2. Liver from fish fed diet containing unextracted fish meal with 100% PO showing regular-shaped hepatocytes with large nuclei and some lipid vacuoles (arrow) in cytoplasm which did not disturb the hepatocytes size (H&E, X 400).

The absence of lipid droplets in enterocytes of *H. longifilis* fed the experimental diets could be due to sufficient synthesis of phospholipids required for the synthesis of lipoprotein by the fish. The accumulation of lipid droplets in the enterocytes may be considered as a temporal storage of lipid due to insufficient lipoprotein synthesis (Deplano *et al.,* 1991). Olsen *et al.* (2000) reported that Arctic charr fed linseed oil showed a tendency to deposit lipid droplets within the cytoplasm. This was not observed in fish fed Soyabean lecithin, suggesting that this accumulation could be due to insufficient synthesis of phospholipids required for lipoprotein synthesis. It has been showed that higher number of absorptive vacuoles in fish gut is probably an indication of a better absorptive capability of the enterocytes (Mourente *et al.,* 2005).

The histological examination in the present study revealed that fish fed the unextracted fish meal with 0%, 25%, 50%, 75% , 100% PO and those fed extracted fish meal with 0% and 75% PO diets were the most compromised with livers being steatotic, while such effect was not evident in the other dietary groups. The lipid accumulation in the livers of these groups suggests a direct effect of dietary lipid on fat metabolism that leads to excessive fat storage.

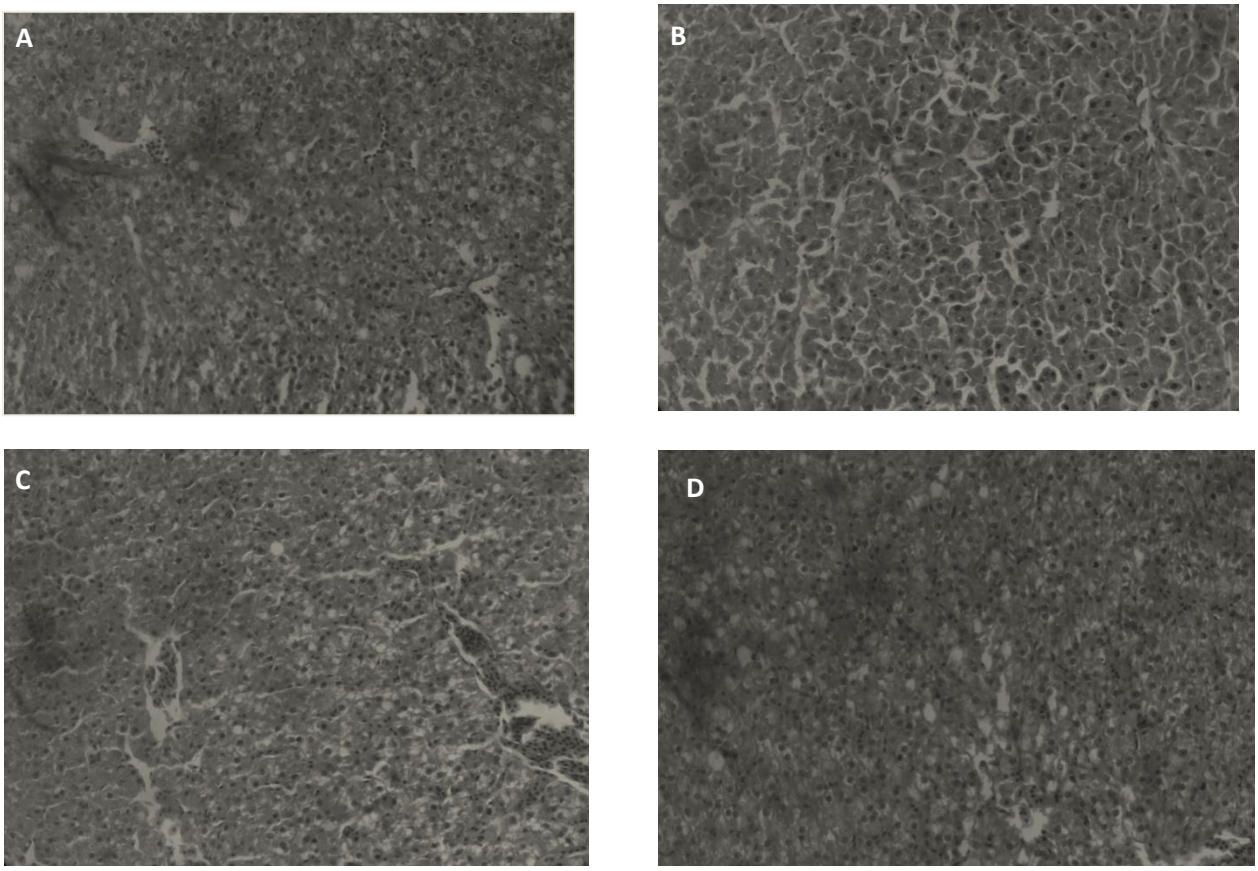


Fig. 3. Liver from fish fed unextracted fish meal with 0% PO (A), 25% PO (B), 50% (C) and 75% PO (D) diets showing homogenous size hepatocytes with lipid accumulation and nuclei displacement (H&E, X 400).

The high dietary content of oleic acid in the diet has been suggested to be responsible for hepatic steatosis in gilthead sea bream fed PO based diets (Fountoulaki *et al.,* 2009). This was also described by Mourente *et al.* (2005) in liver of fish fed rapeseed and linseed oil at a 60% substitution level. In the present study, no such effect was evident in the fish fed the extracted fish meal diet containing 100% PO diet regardless of its high oleic acid content. This findings corroborates the results of Caballero *et al.* (2002) in which palm oil was equally combined with rapeseed oil to replaced 80% fish oil in rainbow trout diets (9 weeks experiment) and histological findings in the

liver were similar to those observed in the present study. Contrary to the lack of intestinal abnormalities seen in the fish fed the experimental diets in the present work, intense accumulation of lipid droplets in enterocytes were observed in a ultrastructural examination performed by Caballero *et al.* (2003) in gilthead sea bream fed a diet containing either rapeseed or soy oil as 60 and 60–80% fish oil replacement respectively, for 12 weeks. The differences in species as well as the diet composition may have led to this discrepancy.

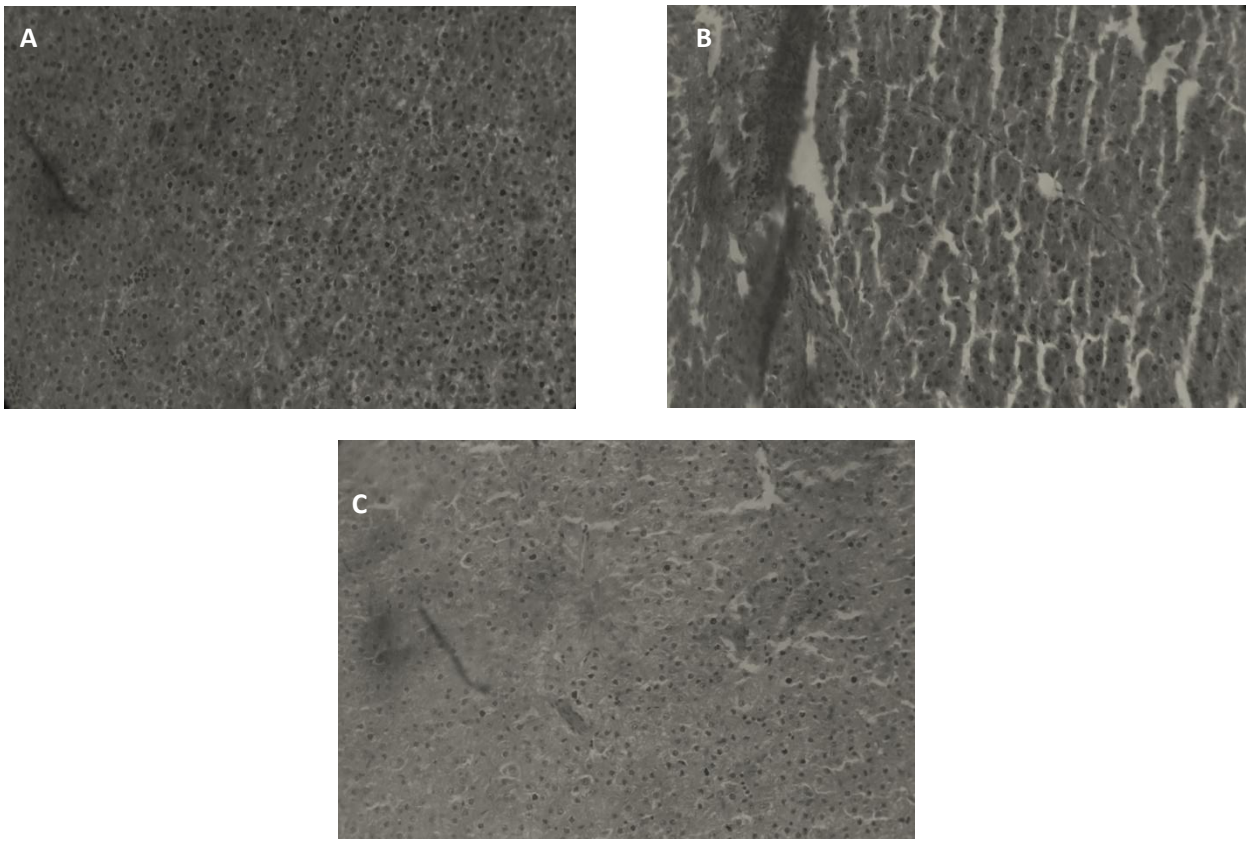
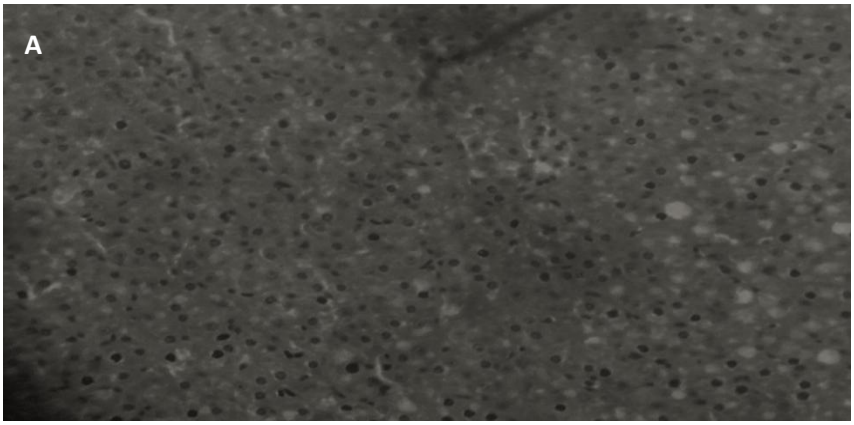


Fig. 4. Liver from fish fed extracted fish meal with 25% PO (A), 50% PO (B) and 100% PO (C) showing regular-shaped hepatocytes with centrally located nuclei (H&E, X 400).



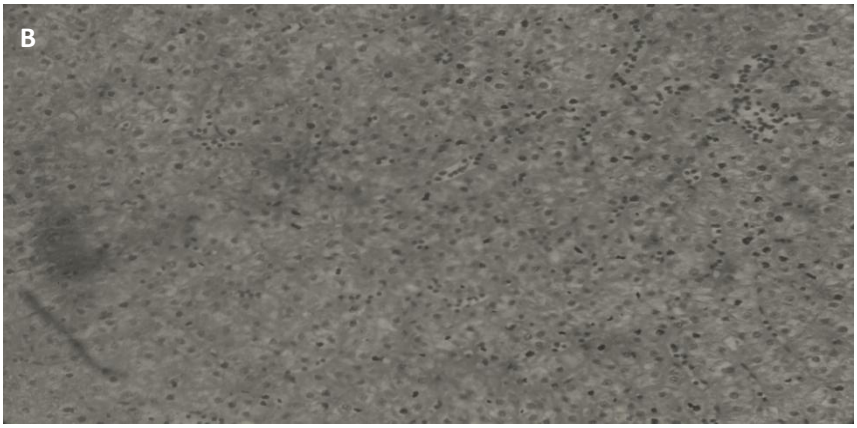


Fig. 5. Liver of fish fed extracted fish meal with 0% PO (A) and 75% PO (B) diets showing nuclei displacement with varying sizes of lipid vacuoles (H&E, X 400).

5. Conclusion

The results of this study suggest that it is possible to completely substitute fish oil with palm oil with extracted fish meal for *H. longifilis*, without affecting haematology and serum biochemical profile negatively.

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