Fish Oil Replacement with Palm Oil and Soybean Oil in the Diet of *Heterobranchus longifilis* Affects Liver Biochemical Compositions

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ABSTRACT— ***This study was undertaken to determine the effects of dietary replacement of fish oil with palm and soybean on liver biochemical composition of Heterobranchus longifilis. H. longifilis were fed one of the six diets twice daily for 10 weeks. The test diets were identical in composition, except for the supplemental lipid source which was either 100% FO (fish oil, as control), palm oil (PO), soybean oil (SO), equal blend of fish oil and palm oil (FOPO), equal blend of palm oil and soybean oil (POSO) or equal blend of fish oil, palm oil and soybean oil (FOPOSO). The results shows that the docosahexaenoic acid, eicosapentaenoic acid and the ratio n-3/n-6 were significantly reduced by the inclusion of dietary palm oil and soybean oil (P < 0.05). Similarly, the inclusion of the oils in the diet of H. longifilis reduced thiobarbituric acid-reactive substances. Our results indicated that the inclusion of PO and SO in diet reduced lipid peroxidation in fish and should therefore be used to improve product quality.***

Keywords— Fish oil, soybean oil, palm oil, theobarbituric acid reactive substances, fatty acids, *Heterobranchus longifilis*

1. INTRODUCTION

Wild marine fish are used to produce fish meals and oils that provide essential fatty acids (EFA) and other nutrients in aquafeeds, although the production from these stocks are fully exploited, and is not expected to increase beyond the present level. This has provided the impetus to investigate alternatives to marine ingredients in aquafeeds. Alternatives to fish oil (FO) can be of vegetable origin, as they are often cheaper and more available than marine oils (Naylor, *et al.*, 2000). However, vegetable oils generally contain large quantities of *n*-6 fatty acids in comparison to marine oils. In the past, most studies on fish oil replacement have focused on the growth response to the addition of vegetable oil in various freshwater fish species such as *Clarias geriepinus* (Ochang *et al.*, 2007), *H. longifilis* (Babalola, *et al.*, 2011). It is therefore necessary to investigate the functional response of fish to the supplement of dietary lipids. The liver plays a central role in lipid metabolism including fatty acid synthesis and degradation through enzyme regulations, and it is also a sensitive organ reflecting dietary lipid change in fish (Henderson, 1996). The liver cells can be damaged by lipid peroxidation when the diet contains a high level of unsaturated fatty acids. In contrast, the use of vegetable oils has been reported to reduce lipid peroxidation in mammals (Lopez-Bote, *et al.*, 1997) and fish (Alvarez, *et al.*, 1998). Therefore, the peroxidation in the liver should be considered a physiological indicator when evaluating fish oil replacement by other lipid alternatives. The objective of this study was to investigate the response of *H. longifilis* to partial and total replacement of dietary fish oil with palm oil and soybean oil. Thiobarbituric acid-reactive substances (TBARS) were used to evaluate the possible oxidative and physiological changes of fish subjected to various levels of vegetable oil in the diet.

2. MATERIALS AND METHODS

2.1 Animal and diets

Heterobranchus longifilis fingerlings, used in this study were obtained from National Institute for Freshwater Fisheries Research, New Bussa (NIFFR) hatchery and reared in the laboratory in a flow through system of circular tanks (62 l), and fed a commercial pelleted feed (NIFFR™). The experimental diets (Table 1) were formulated to be isonitrogenous and isolipidic. The six experimental diets were prepared by substituting one of the following oils as the

lipid source; fish oil (FO), palm oil (PO), soybean oil (SO), equal blend of FO and PO (FOPO), equal blend of PO and SO (POSO) or equal blend of FO, PO and SO (FOPOSO) at 60 g^{-1}. The experimental diets were prepared by mixing the dry ingredients with oil and pregelatinized starch and the resulting moist dough was pelleted using a meat mincer through a 2-mm die. The moist pellets were then sun dried and stored under refrigeration in 200 g batches, until used. Samples of the feed were taken for analyses, using standard procedures (AOAC, 1997). The eighteen groups of *Heterobranchus longifilis* were fed the assigned diets (triplicates for each diet) for a period of 10 weeks, after acclimatisation of two weeks.

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

	Experimental diets*					
Ingredients (g kg^{-1})	FO	PO	SO	FOPO	POSO	FOPOSO
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
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
Vit./Min. Premix[1]	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
Oil[2]	60.00	60.00	60.00	60.00	60.00	60.00
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
Proximate composition (n=3)						
Moisture (g/kg)	63.00	60.00	60.90	60.20	60.60	64.10
Crude protein (g/kg)	456.80	452.30	452.00	452.80	454.50	451.60
Lipid (g/kg)	105.00	106.70	105.80	105.90	105.70	106.40
Ash (g/kg)	83.00	82.90	83.20	82.40	82.90	82.10
Carbohydrate(NFE)[3] (g/kg)	292.20	298.10	298.10	298.70	296.30	295.80
Gross energy[4] (MJ/kg)	20.24	20.30	20.26	20.29	20.28	20.23

[1]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 B12, 0.1 mg and ethoxyquin, 60 mg.
[2]FO = fish oil, PO = palm oil, SO – soybean oil, FOPO = fish oil and palm oil (1:1), POSO = palm oil and soybean oil (1:1), FOPOSO = fish oil, palm oil and soybean oil (1:1:1)
[3]NFE = Nitrogen free extracts including crude fibre; = 100 – (CP + lipid + ash)
[4]calculated from the published compositions of the ingredients used (NRC, 2011).

2.2 Sample collection

At the end of the feeding trial, all fish were weighed and six fish were randomly sampled from each tank. The fish were killed by blow on the head, dissected and their livers removed for fatty acid and theobarbituric acid reactive substances determination.

2.3 Sample analysis

Fatty acid analysis was conducted on the liver samples. The sample preparation for fatty acid was done by direct methylation method (Sukhija and Palmquist) with methanolic HCl Fatty acid methyl esters were resolved and analysed by a gas-liquid chromatography (Shimadzu GC-17A). Three aliquots of each of the esterified samples (fatty acid methyl esters) were analysed in a Shimadzu GC 17A, equipped with an Omegawax 250 capillary column (30 m L x 0.32 mm internal diameter), a FID detector and a split injection system (split ratio 50:1). The carrier gas was helium and injector port and detector temperatures were 240^{0}C and 250^{0}C, respectively. The temperature program was 190^{0}C for 5 min, 190–240^{0}C at 2^{0}C min^{-1}, and held at 240^{0}C for 10 min. Fatty acids were identified relative to known external standards and the resulting peaks were quantified using nonadecanoic acid (19:0) as an internal standard.

Thiobarbituric acid-reactive substances (TBARS) were measured using the method of Rueda-Jasso et al. (Rueda-Jasso, *et al.*, 2004) and the results were expressed as µmol of MDA/mg protein.

2.4 Data and statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA). Differences between means were determined by Duncan's multiple range test. Analyses were performed using a SPSS statistical package (SPSS, 1997). Differences were reported as significant if $P < 0.05$.

3. RESULTS AND DISCUSSION

The concentration of total saturated fatty acids in the liver of *H. longifilis* fed the diet with PO, SO, and FOPO were similar but significantly ($P < 0.05$) different from that of fish fed diets with other lipid sources (Table 2). Total *n*-3 fatty acids (FA) were highest in fish fed diet with FO and lowest in fish fed diet with PO. These results are in line with a previous observation of Xu and Kestemont (2002) in juvenile perch fed 16% olive oil, safflower oil, linseed oil or cod liver oil as the only lipid source in diet. Significant increase of 18:2*n*-6 products as well as a decrease in docosahexaenoic acid (DHA) and total *n*-3 FA was observed, indicating that the competition and inhibition between 18:2*n*-6 and 18:3*n*-3 for further desaturation and elongation were greatly influenced by the type of dietary lipid and the content of *n*-3 and *n*-6 FA in the diet.

The oxidative stability of the liver of *H. longifilis* was evaluated by oxidation test (Fig 1). As an index of lipid oxidation, TBARS levels were measured in the liver of *H. longifilis* and it showed significant increase ($P < 0.05$) in the liver of fish fed diet FO than in the liver of fish on the other diets. The least value, which was significantly ($P < 0.05$) lower than the value in the other dietary groups were obtained in the liver of fish fed PO diet. The level of TBARS is one of the most popular and commonly used methods to determine tissue peroxidation (Rosmini, et al., 1996). In the this study, the TBARS values in the fillet of *H. longifilis* fed FO diet was higher than in fish fed vegetable oils or their combinations. This corresponds to earlier observations in grouper (Lin and Shiau, 2007) and mammals (Lopez-Bote, *et al.*, 1997) were the substitution of FO with vegetable oils resulted in a decreased TBARS levels and indicated a reduction in susceptibility to lipid peroxidation. The increased TBARS values in the liver of fish fed FO diet may be due to the oxidative deterioration of long-chain n-3 PUFA (20:5 n-3 and 22:6 n-3) present in the liver.

The lower TBARS values of liver from PO fed fish may suggest that an added protection of lipids was provided by the presence of intrinsic anti-oxidants in PO (tocotrienols). The antioxidant activity of tocotrienol has been reported to be higher than that of α-tocopherol (Kamat *et el.*, 1997). Lipid oxidation is a major cause of food deterioration affecting colour, flavour, texture and nutritional value of animal products. Among vegetable oils, palm oil is the richest natural source of Tocotrienols. Tocotrienols are surprisingly not found in any other vegetable oils like soy bean oil, canola oil, rape seed oil and sunflower oil. (American Palm Oil Council, 2004). Alpha Tocotrienols is 40-60 times more potent than normal Tocopherols making it one of the most powerful lipid soluble anti oxidants available.

Table 2. Liver fatty acid composition of *H. longifilis* fed diets containing fish, palm and soybean oils as replacement for fish oil for 10 weeks (g/100g of total FA).

	*Dietary treatments						
	FO	PO	SO	FOPO	POSO	FOPOSO	SEM
Fatty acids							
∑Saturates[1]	42.70^{a}	41.45^{ab}	40.50^{b}	41.43^{ab}	36.06^{d}	38.51^{c}	0.28
∑monoene [2]	57.23^{e}	58.58^{d}	59.91^{c}	58.84^{d}	64.06^{a}	61.45^{b}	0.15
18:1*n*-9	21.25^{d}	44.49^{a}	29.97^{c}	41.31^{b}	41.07^{b}	40.70^{b}	0.16
∑ *n*-9[3]	24.35^{f}	46.06^{a}	31.89^{e}	44.06^{b}	42.85^{d}	43.27^{c}	0.05
18:2*n*-6	4.76^{c}	3.06^{d}	11.21^{a}	2.52^{e}	6.75^{b}	4.75^{c}	0.03
20:4*n*-6	1.16^{a}	0.16^{d}	0.47^{b}	0.36^{c}	0.38^{c}	0.44^{b}	0.01
∑ *n*-6[4]	3.10^{d}	2.87^{e}	10.48^{a}	2.09^{f}	6.25^{b}	4.12^{c}	0.03
18:3*n*-3	0.38^{d}	0.03^{f}	0.77^{a}	0.25^{e}	0.42^{c}	0.46^{b}	0.003
20:5*n*-3	3.36^{a}	0.68^{e}	1.42^{b}	1.11^{c}	0.89^{d}	1.09^{c}	0.003
22:6*n*-3	7.12^{a}	1.47^{f}	4.15^{b}	3.15^{e}	3.67^{c}	3.46^{d}	0.003
∑ *n*-3[5]	11.77^{a}	2.27^{e}	6.78^{b}	4.81^{d}	5.29^{c}	5.32^{c}	0.02
n-6/*n*-3	0.41^{e}	1.28^{b}	1.65^{a}	0.53^{d}	1.27^{b}	0.87^{c}	0.01
n-3/*n*-6	2.45^{a}	0.77^{d}	0.60^{e}	1.92^{b}	0.79^{d}	1.12^{c}	0.003

*Diets: as explained in Table 1
[1] Contains 12:0, 14:0, 16:0, 18:0, 20:0, 22:0, and 24:0.
[2] 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.
[3] Contains 16:1 *n*-9, 18:1 *n*-9, and 24:1 *n*-9.
[4] 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. [5] 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.
Values in the same row followed by the same letter are not significantly different at $P > 0.05$

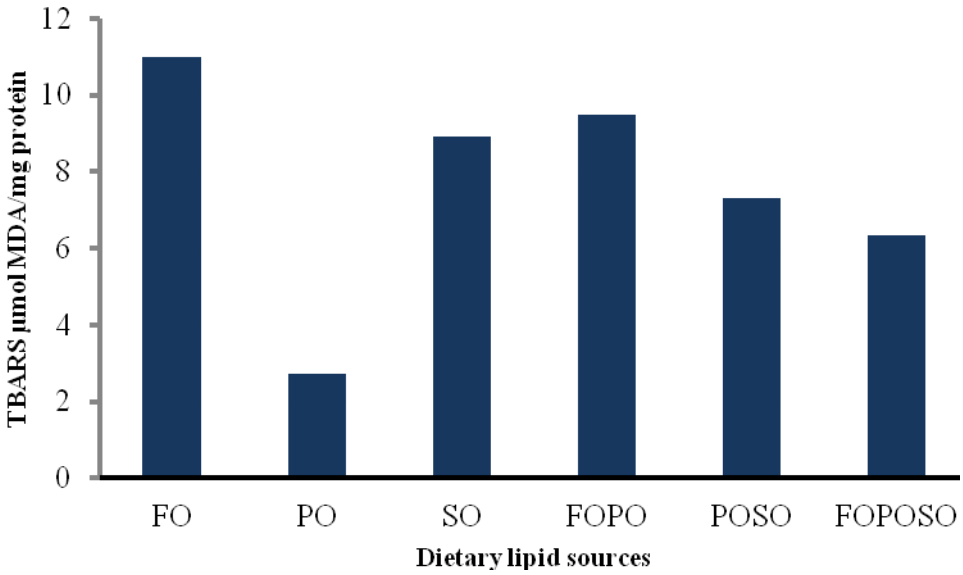

Figure 1. Effects of dietary lipids on thiobarbituric acid reactive substance (TBARS) in *H. longifilis* liver

4. CONCLUSION

The biochemical evidence in this study suggests that the inclusion of PO reduced lipid peroxidation as a result of natural antioxidant present. It should therefore be included in the diet of *H. Longifilis* to improve product quality.

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