Effect of dietary palm oil on growth and carcass composition of *Heterobranchus longifilis* fingerlings

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Abstract

This study investigated the effects of dietary palm oil (PO) on growth performance and carcass composition of *Heterobranchus longifilis* with the goal of replacing dietary fish oil with palm oil. In this study triplicate groups of *H. longifilis* fingerlings were fed the experimental diets for 8 weeks. Five isonitrogenous (45% crude protein), isoenergetic (20 KJg^{-1}) experimental diets were made containing either 6.0% FO and 0% PO, 4.5% FO and 1.5% PO; 3.0% FO and 3.0% PO; 1.5% FO and 4.5% PO; or 0% FO and 6.0% PO using soybean and fish meal as the protein source. Dietary palm oil had no significant effect on growth rate or feed conversion ratio. Similarly, No significant differences were observed between dietary treatments for moisture, protein and ash content in *H. longifilis* fingerlings. However, fillet saturated, monounsaturated fatty acids and liver lipid deposition were significantly ($P<0.05$) higher in fish fed 6.0% PO diet. This study suggests that the replacement of cod liver oil by palm oil as lipid supplement in the diet permitted a clear improvement of growth and FCR of *H. longifilis*. This indicates that PO can effectively replace FO in the diet of the fish without compromising fish growth and feed efficiency.

Key words: carcass composition, cod liver oil, fish oil replacement, growth performance, *Heterobranchus longifilis*, palm oil, polyunsaturated fatty acids

Introduction

Catfish is a fatty fish and is of importance to human health. In addition, catfish are highly resilient, being able to survive poor water quality and can be transported live to the market; they can also be preserved as smoked product (Anetekhai *et al.,* 2004). Presently fish feed alone account for between 60 and 75% of the total cost of fish production (Babalola, 2010). Fish raw materials (fish oil and fish meal) are the most expensive ingredients used in aquafeed production. For successful expansion of the industry, plant oil sources stand out as the most likely candidates to substitute for fish oil in fish feed, because of their relative stability in price and ready availability. Their global production is around 100 times higher than that of fish oils (Bimbo, 1990). Vegetable oils are more abundant and often cheaper than marine fish oils and offer an added advantage of containing fewer organic contaminants (Jacobs *et al.,* 2004; Guruge *et al.,* 2005; Berntssen *et al.,* 2005; Higgs *et al.,* 2006). Therefore, replacement of fish oil with vegetable oil alternatives is necessary and is being practiced.

The use of available lipids such as vegetable oils in the tropics may reduce costs, while producing a comparable or better-quality product compared to non-sustainable marine fish oils. The major drawback with this substitution is a decreased level of the essential long chain *n*-3 fatty acids in the fish diet and in the fish fillet. The available vegetable oil sources

sunflower oil and sesame oil among others. Of these, palm oil is presently the most abundant vegetable oil produced in the world (Turchini *et al.,* 2009), its products such as crude palm oil, refined palm olein or palm fatty acid distillate in the diets of tilapia has been shown to elicited growth and feed utilization efficiency comparable with fish fed equivalent levels of dietary marine fish oils (Ng *et al.,* 2006). Crude palm oil has a deep orange-red colour due to the high content of carotenoids. It is the richest natural sources of β-carotene, tocopherols and tocotrienols which function as natural antioxidants (Nesaretnam and Muhammad, 1993). While most vegetable oils contain almost exclusively tocopherols, palm oil is unique because tocotrienols represent about 70% - 80% of the vitamin E content. These confer beneficial effects to growth and flesh quality when fish are fed high levels of palm oil in their diets (Lim *et al*., 2001, Ng *et al.,* 2004). This would translate to longer shelf life for fish products.

Palm oil has been evaluated in the diets for salmonids (Tortensen *et al*., 2000; Bell *et al*., 2002), catfishes (Legendre *et al*., 1995; Lim *et al*., 2001; Ng *et al*., 2000; Ochang *et al*., 2007a) and tilapias (Ng *et al*., 2001; Ochang *et al*., 2007b). Palm oil can meet the energy requirements of fish by providing easily oxidized fatty acids and at the same time, generate flesh fatty acid composition that are beneficial to the consumer. The use of palm oil, in fish diets has great potential as a practical and cost-effective means of adding value to the flesh quality of aquaculture products. The aim of the present study was to evaluate the influence of partial and/or total fish oil replacement with palm oil on growth and carcass composition of *H. longifilis*.

Materials and methods

Three hundred *H. longifilis* fingerlings were obtained from the hatchery of National Institute for Freshwater Fisheries Research (NIFFR), New-Bussa, Nigeria and used in this study. Prior to the commencement of the experiment, fish were acclimatized to the new environmental condition on a commercial catfish feed for two weeks in a mini-flow-through system.

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. Five *iso*-nitrogenous, *iso*-lipidic, *iso*-caloric experimental diets were formulated with 6% lipid originating from 6% fish oil (FO) and 0% palm oil (PO) (Diet 1); 4.5% FO and 1.5% PO (Diet 2); 3.0% FO and 3.0% PO (Diet 3); 1.5% FO and 4.5% PO (Diet 4) or 0% FO and 6.0% PO (Diet 5) (Table 1). The diets were made into pellet with meat mincer through 2mm die, sundried, packed in polythene bags, sealed and stored at -20^{0}C until used. The diets differ only in their fatty acid compositions (Table 2).

Fish were randomly assigned into groups of twenty per tank. Each dietary treatment had three replications and the experiment was conducted for 8 weeks. The fish were individually weighed at the beginning and at the end of the experiment and bulk-weighed by tank fortnightly in-between. Fortnight bulk weights were used to adjust the daily feed ration for the following week. Fish were offered 50 gkg^{-1} of their body weight per day, sub divided into three equal feeds at 09:00, 13:00 and 17:00 h daily.

Faecal matter was collected once a day at about 8:00 am before feeding commenced during the later part of the experiment. Faeces collection was performed by siphoning materials from the bottom of tank. At the termination of the experiment, five fish were taken from each replication for determination of whole body composition, liver and muscle lipid.

The nutrient composition of the experimental diets, muscle and liver samples was determined by proximate analysis. Moisture was determined by thermal drying to constant weight in an oven at 110℃ for 24 h. Crude protein was determined using the Kjeldhal method (Association of Official Analytical Chemists, 1990), Total lipid of diets, liver and muscle tissue was extracted according to Folch et al. (1957) and quantified gravimetrically.

Fatty acid analysis was performed on three samples of each experimental diet and pooled fillet and liver samples for each experimental treatment. The extraction of total lipids and preparation of fatty acid methyl esters was performed according to Sukhija and Palmquist (1988). Fatty acids methyl esters were separated by GC on a Varian Model 3300 gas chromatograph equipped with flame ionisation detector. A flexible fused silica Megabore column (30 m X 0.32 mm, 1 µm film thickness) with bonded stationary phase of CP-WAX was employed. Helium was used as the carrier gas at 1.0 ml/min, inlet pressure 12 psi. Injector and the detector temperatures were 250 and 300^{0}C, respectively. The column temperature was programmed to be initially 140^{0}C for 5 min and then to increase at a rate of 3^{0}C to a final temperature of 240^{0}C. Fatty acids methyl esters were identified in comparison to an external standard (Supelco^{TM} 37 component FAME Mix).

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

	Diets				
Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Fish meal (Danish)	398	398	398	398	398
Soybean meal	313	313	313	313	313
Corn flour (Maize)	177	177	177	177	177
Cassava starch	20	20	20	20	20
Methionine	10	10	10	10	10
*Vit./Min. Premix	20	20	20	20	20
Salt (NaCl)	1.5	1.5	1.5	1.5	1.5
Vitamin C	0.5	0.5	0.5	0.5	0.5
Fish oil	60	45	30	15	0
Palm oil	0	15	30	45	60
Proximate composition (n=3)					
Dry matter	91.70	92.00	91.91	91.98	91.93
Protein	44.68	44.97	44.70	44.89	44.79
Crude fat	10.50	10.64	10.59	10.57	10.59
Ash	10.30	10.20	10.31	10.33	10.32
Nitrogen free extract	34.52	34.19	34.40	34.21	34.30
Gross energy (MJ/kg of feed)	19.98	19.95	19.89	20.01	19.99

*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-α-tocopherol acetate, 100mg; menadione, 15mg; thiamine hydrochloride, 5mg; riboflavin, 10mg; pyridoxine hydrochloride, 10mg. Panthothenic acid, 35mg; nicotinic acid, 50mg; biotin, 0.5mg; folic cid, 2mg; ascorbic acid, 200mg; inositol, 250mg; choline, 400mg; vitamin B_{12}, 0.1mg and ethoxyquin.

Table 2. Fatty acid composition of the experimental diets (g/100g)

Fatty acid	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
[1]∑ Saturated	32.00 ± 0.01	37.01 ± 0.14	38.06 ± 0.13	43.21 ± 0.21	49.89 ± 0.11
[2]∑ Monounsat.	36.19 ± 0.01	39.67 ± 0.11	37.31 ± 0.03	36.35 ± 0.12	35.60 ± 0.12
[3]∑ n-9	22.13 ± 0.01	27.46 ± 0.05	28.66 ± 0.02	31.40 ± 0.12	31.71 ± 0.04
18:1n-9	17.33 ± 0.04	22.70 ± 0.13	25.38 ± 0.02	28.33 ± 0.10	30.09 ± 0.05
[4]∑ n-6	5.29 ± 0.01	7.14 ± 0.02	7.88 ± 0.01	8.20 ± 0.01	9.35 ± 0.04
18: 2n-6	3.66 ± 0.02	5.72 ± 0.01	6.86 ± 0.05	7.49 ± 0.04	9.13 ± 0.03
20: 4n-6	0.58 ± 0.01	0.49 ± 0.03	0.38 ± 0.04	0.23 ± 0.03	0.09 ± 0.01
[5]∑ n-3	26.52 ± 0.11	18.18 ± 0.08	16.74 ± 0.21	9.89 ± 0.02	5.16 ± 0.02
18: 3n-3	2.13 ± 0.03	1.84 ± 0.01	1.33 ± 0.03	0.87 ± 0.01	0.48 ± 0.01
20: 5n-3	7.75 ± 0.01	5.02 ± 0.02	4.86 ± 0.02	2.71 ± 0.02	1.44 ± 0.02
22: 6n-3	11.82 ± 0.10	10.13 ± 0.06	7.50 ± 0.07	4.39 ± 0.02	2.33 ± 0.02
n-3/n-6	5.01 ± 0.04	2.55 ± 0.02	2.12 ± 0.02	1.21 ± 0.01	0.55 ± 0.01

[1] Contains 12:0, 14:0, 16:0, 18:0, 20:0, 22:0, and 24:0.

[2] Monounsaturated FA, 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

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. The data are presented as mean ± S.E.M. of three replicate groups.

Results

Throughout the feeding trial, no fish died; therefore the survival was 100% among all the dietary treatments. The mean final weights, feed efficiency and body indices of *H. longifilis* fingerling fed all levels of palm oil substitution for fish oil are reported in Table 3. There were no significantly differences in the final weights and feed efficiency among the dietary treatments. Similar trend were evident for other growth parameters (average feed intake (AVFI) and specific growth rates (SGR) (Table 3). The lowest FCR however (1.90) was observed for 25 % PO treatment, while AVFI ranged from 21.02 (25 % PO) to 25.55 (0 % PO). Protein efficiency ratio (PER) ranged from 1.01 (75 % PO) 1.17 (25 % PO) and did not differ significantly between the dietary treatments.

Table 3. Growth performance of *H. longifilis* fed the experimental diets containing increasing levels of palm oil (PO) for 8 weeks.

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Initial weight (g)	5.71 ± 0.17	5.48 ± 0.06	5.44 ± 0.17	5.50 ± 0.10	5.53 ± 0.23
Final weight (g)	12.31 ± 1.21	11.05 ± 0.24	11.50 ± 0.97	11.32 ± 0.28	11.39 ± 0.14
Weight gain (g)	6.70 ± 0.19	5.57 ± 0.08	6.06 ± 0.12	5.80 ± 0.10	5.86 ± 0.11
SGR (% day^{-1})	2.07 ± 0.13	1.97 ± 0.02	2.02 ± 0.14	1.89 ± 0.05	2.00 ± 0.07
Average feed intake (g)	25.55 ± 2.77	21.02 ± 0.31	23.27 ± 0.96	23.01 ± 2.08	22.52 ± 0.79
Feed conversion ratio	2.07 ± 0.08	1.90 ± 0.03	2.04 ± 0.05	2.03 ± 0.06	1.98 ± 0.01
Protein efficiency ratio	1.07 ± 0.03	1.17 ± 0.01	1.10 ± 0.05	1.01 ± 0.07	1.12 ± 0.05

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

Table 4. Proximate composition of muscle and liver lipid concentration of *Heterobranchus longifilis* fed diets containing increasing levels of palm oil (PO) for 8 weeks.

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Moisture	72.37 ± 0.18	72.4 ± 0.12	72.37 ± 0.09	72.30 ± 0.17	72.43 ± 0.18
Lipid	4.43 ± 0.15	4.29 ± 0.02	4.27 ± 0.09	4.27 ± 0.09	4.20 ± 0.06
Protein	17.04 ± 0.22	17.18 ± 0.15	17.12 ± 0.28	17.16 ± 0.21	17.24 ± 0.15
Ash	5.67 ± 0.09	5.67 ± 0.09	5.70 ± 0.10	5.77 ± 0.09	5.70 ± 0.06
Liver lipid	3.31 ± 0.01^{a}	3.34 ± 0.01^{a}	3.50 ± 0.01^{b}	3.61 ± 0.01^{c}	3.84 ± 0.03^{d}

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

Data on the composition of muscle and liver of the experimental fish are reported in Table 4. There were no significant differences observed between dietary treatments for muscle moisture, protein, total lipid and ash content of *H. longifilis* fed the experimental diets. The lipid content of the liver of fish fed 100 % PO was significantly higher than those fed the other diets.

The fatty acid composition of the fillet is shown in Table 5. There were significant differences in the fatty acid composition of fish fillets. Thus 18:3*n*-3, 20:5*n*-3 and 22:6*n*-3 of the fish fed the control (diet 1) were significantly higher than those fed diets in which PO replaced 25, 50, 75 and 100% of the FO in the control diet ($P < 0.05$). Fish fed diets with 6% PO (diet 5) had the lowest concentrations of these fatty acids. However, the concentration of monounsaturated, sum *n*-9 and sum *n*-6 fatty acids were significantly higher in the fillet of fish fed diet in which FO was replaced 100% with PO (diet 5).

Discussion

The result of the present study suggest that PO can be used to completely replace fish oil with minimal adverse effect on *H. longifilis* fingerlings growth, as reported for other fish species (Martino *et al*., 2002; Regost *et al*., 2003; Turchini *et al*., 2003; Francis *et al*., 2006; Ochang *et al*., 2007b). This is evident by the weight gain and feed conversion of fish fed 25%, 50%, 75%, and 100% PO diets which ranged from 5.57 g to 6.70 g and 1.90 to 2.07 respectively, with no significant differences from fish fed the control (FO) diet, and generally comparable to previous results on tropical catfish (Ng *et al*., 2000; Ochang *et al*., 2007a). The non significance differences between SGR and FCR of the experimental diets indicated that fish were able to digest the diets and convert the diets into body tissue with the same degree of efficiency.

Although muscle lipid levels in this study were not significantly different, higher level of lipid was observed in fish fed 0 % PO (100 % FO) compared with fish fed the other diets. Conversely, the muscle protein content was lower in fish fed 0 % PO, the second highest muscle lipid level, found in fish fed 50 % PO, was correlated with the second lowest protein content. This relationship between muscle protein and lipid content has been observed in studies with salmonids (Bell *et al*., 2002). Lipid deposition in the liver was significantly affected by dietary treatment. The fish group fed 100 % PO had the highest liver lipid content. This observation is similar to that of Caballero *et al*., (2004), that the reduction of dietary essential fatty acids due to the inclusion of vegetable oils in the diets tends to promote fat accumulation in the liver of seabream.

Fillet fatty acid composition of total lipids was affected by the palm oil replacement levels. Saturated fatty acids were significantly reduced in the fillet of fish fed FO diet whereas in the muscle of fish fed diet 5, they were significantly increased. Oleic acid increased significantly in the fillet of fish fed diets containing PO, increasing as the levels of PO in the diet increased. The highest increase was observed in fish fed diet 5. Similarly, linoleic acid increased in the fillet of fish fed diet 5; it was 3 times higher than the FO (diet 1) dietary group. Total n-6 fatty acid (FA) were significantly reduced in fillet of fish fed diet 1, largely due to significant lower 18:2n-6 and 20:4n-6 concentrations in the fillet. Total n-3 FA was significantly reduced in the fillet of fish fed various palm oil-based diets. The reduction was more pronounced in the fillet of fish fed diet 5. These differences in individual fatty acid concentrations resulted in respective significant n-3/n-6 reductions. This study showed that specific fatty acids were selectively retained in the fillet of the fish; especially DHA and arachidonic acid (AA). The selective retention of

Table 5. Fatty acid composition of *H. longifilis* fillet under the different experimental diets

Fatty acid	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
[1]∑ Saturated	21.09 ± 0.02[a]	25.79 ± 0.10[b]	28.55 ± 0.02[c]	35.59 ± 0.01[d]	42.93 ± 0.01[e]
[2]∑ Monounsaturated	34.08 ± 0.01[a]	37.27 ± 0.01[c]	36.94 ± 0.01[b]	37.48 ± 0.02[c]	37.81 ± 0.02[d]
[3]∑ n-9	21.09 ± 0.01[a]	25.95 ± 0.02[b]	28.38 ± 0.01[c]	30.89 ± 0.02[d]	34.07 ± 0.01[e]
18:1n-9	15.63 ± 0.03[d]	20.68 ± 0.10[a]	24.29 ± 0.01[b]	29.34 ± 0.00[c]	31.89 ± 0.02
[4]∑ n-6	6.08 ± 0.03[a]	7.70 ± 0.01[b]	8.38 ± 0.00[c]	9.21 ± 0.02[d]	10.21 ± 0.01[e]
18: 2n-6	3.31 ± 0.00[a]	5.24 ± 0.01[b]	6.56 ± 0.03[c]	7.77 ± 0.05[d]	9.69 ± 0.01[e]
20: 4n-6	1.42 ± 0.01[e]	1.21 ± 0.01[d]	0.99 ± 0.01[c]	0.65 ± 0.01[b]	0.26 ± 0.00[a]
[5]∑ n-3	38.75 ± 0.01[e]	29.24 ± 0.02[d]	26.13 ± 0.01[c]	16.57 ± 0.01[b]	9.05 ± 0.01[a]
18: 3n-3	1.82 ± 0.01[e]	1.58 ± 0.01[d]	1.21 ± 0.01[c]	0.85 ± 0.01[b]	0.48 ± 0.01[a]
20: 5n-3	5.15 ± 0.01[e]	4.03 ± 0.01[d]	3.43 ± 0.01[c]	2.07 ± 0.01[b]	1.12 ± 0.01[a]
22: 6n-3	28.43 ± 0.05[e]	24.61 ± 0.10[d]	19.15 ± 0.10[c]	12.12 ± 0.02[b]	6.57 ± 0.03[a]
n-3/n-6	6.38 ± 0.01[e]	3.80 ± 0.01[d]	3.12 ± 0.02[c]	1.80 ± 0.01[b]	0.89 ± 0.01[a]

[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

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

DHA could be related to the higher beta-oxidation of eicosapentaenoic acid (EPA) compared to DHA (Madsen *et al.,* 1998). This probably indicated a selective catabolism of EPA over DHA when dietary levels decreased, possibly to meet the requirement for tissue membrane composition and function. The reduction of EPA in the fillet suggests utilization of this fatty acid. In conclusion, this study showed that FO can be replaced by PO without negative effects on growth performance.

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