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Growth Performance and Organoleptic Quality of Hybrid Grouper (*Epinephelus fuscogutattus* ♀ × *Epinephelus lanceolatus* ♂) Fed Palm-Oil Based Diets at Grow-Out Stage

(Prestasi Pertumbuhan dan Kualiti Organoleptik Kerapu Hibrid (*Epinephelus fuscoguttatus* ♀ x *Epinephelus lanceolatus* ♂) yang diberi Diet Berasaskan Minyak Sawit pada Peringkat Tumbesaran)

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ABSTRACT

*The performance of crude palm oil (CPO)-based diets in the grow-out stage of hybrid grouper (*Epinephelus fuscoguttatus × Epinephelus lanceolatus*) was examined in a net-seacage culture system. Isoproteic (50% crude protein) and isolipidic (16% crude lipid) experimental diets were formulated to replace fish oil (FO) at 25% increment level; 25CPO, 50CPO, 75CPO, and 100CPO. The FO-based diet was formulated to serve as a control treatment (100FO). Triplicate groups of hybrid grouper containing 30 fish per treatment were stocked in 15 cages and fed once daily. After 4 months of feeding trial, no significant differences (p>0.05) were observed in terms of growth performance, survival, feed utilization efficiency, body indices, fillet yield, and condition factor of fish fed different experimental diets. Except for total cholesterol, all parameters for blood analysis showed no significant differences (p>0.05) among the treatments. Findings from the organoleptic test showed that all fillets were well accepted by the consumers without any significant differences in their scores (p>0.05). In conclusion, CPO is an excellent source of lipid to replace fish oil in the grow-out diet for hybrid grouper,* Epinephelus fuscoguttatus × Epinephelus lanceolatus.

Keywords: Cost-effective feed; grow-out; growth performance; hybrid grouper; palm oil

ABSTRAK

*Prestasi diet berasaskan minyak sawit mentah (CPO) pada peringkat tumbesaran kerapu hibrid (*Epinephelus fuscoguttatus × Epinephelus lanceolatus*) telah diperiksa di dalam sistem pengkulturan sangkar jaring di laut. Diet isoproteik (50% protein mentah) dan isolipidik (16% lipid mentah) telah diformulasi untuk menggantikan minyak ikan (FO) pada tahap 25% kenaikan; 25CPO, 50CPO, 75CPO dan 100CPO. Diet berasaskan FO telah dirumus untuk menjadi diet kawalan (100FO). Kumpulan triplikat kerapu hibrid yang mengandungi 30 ekor ikan per rawatan telah distok di dalam 15 sangkar dan diberi makan sekali sehari. Selepas 4 bulan percubaan pemakanan, tiada perbezaan bererti (p>0.05) yang diperhatikan daripada segi prestasi pertumbuhan, kemandirian, kecekapan penggunaan makanan, indeks badan, hasil filet dan faktor keadaan ikan yang diberi makan diet uji kaji berlainan. Kecuali untuk kolesterol, semua parameter untuk analisis darah menunjukkan tiada perbezaan bererti (p>0.05) dalam rawatan. Penemuan daripada ujian organoleptik menunjukkan bahawa semua filet dapat diterima dengan baik oleh pengguna tanpa mempunyai perbezaan bererti (p>0.0.5) dalam semua rawatan. Secara kesimpulannya, CPO adalah sumber lipid yang cemerlang untuk menggantikan minyak ikan di dalam diet tumbesaran untuk kerapu hibrid,* Epinephelus fuscoguttatus × Epinephelus lanceolatus.

Kata kunci: Kerapu hibrid; makananan berkos-efektif; minyak sawit; prestasi pertumbuhan; tumbesaran

INTRODUCTION

In marine fish farming industry especially in the Southeast Asia and China, hybrid grouper (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*) is gaining more popularity compared to its parent species due to palatability, fast growth, efficient feed conversion, and better survival (Shapawi et al. 2019). Nevertheless, the increasing production of this hybrid fish throughout the years has led to price reduction (Ferdouse 2014). For commercial viability of aquaculture of this fish, a cost-effective approach needs to be applied. In this context, economizing the feeds appears to be a key factor worth consideration.

Fish oil has been the major dietary lipid source used to produce marine fish feed. Recently, there has been a

decline in the production of fish oil and this is causing an increase in fish oil prices (FAO 2014). In order to reduce the production costs, a cheaper alternative to fish oil is a practical solution to the problem. Recent years have seen a growing interest in using vegetable oil to replace the expensive fish oil due to its high availability, steadily increasing production, sustainable supply, and better economic value. Palm oil is one of the potential substitutes for fish oil in marine fish feed. In our recent study on the partial replacement of fish oil with palm oil in a fish meal-based diet using juvenile hybrid grouper of about 11 g, growth of fish fed Refined, Bleached, and deodorized Palm Olein (RBDPO) was significantly better than those fed diets based on either crude palm oil (CPO), crude palm kernel oil (CPKO), corn oil (CO), coconut oil (COCO) or fish oil (FO) (Yong et al. 2019). It would be very interesting to know if the bigger size hybrid grouper would have a better response to diets with alternative oil than the juveniles. Since the grow-out period is the longest period in fish farming, the use of cost-effective diets is extremely important. Considering the lower cost of CPO, more studies are warranted to investigate the full potential of this palm oil product in aquaculture feeds.

Several studies were carried out to demonstrate the effect of various palm oil products on freshwater and marine fish. Investigations conducted on freshwater fish such as tilapia (Bahurmiz & Ng 2007; Ng & Wang 2011; Ng et al. 2013), catfish (Asdari et al. 2011; Ochang et al. 2007), Malaysian mahseer (Kamarudin et al. 2011) and snakehead (Aliyu-Paiko & Hashim 2012) have shown good results that suggest the suitability of replacing fish oil with palm oil in the diets. Meanwhile, substitution of fish oil with palm oil in marine fish such as in humpback grouper (Shapawi et al. 2008), European sea bass (Castro et al. 2015; Mourente & Bell 2006; Richard et al. 2006b), Japanese sea bass (Han et al. 2012), gilthead sea bream (Benedito-Palos et al. 2008; Bouraoui et al. 2011; Fountoulaki et al. 2009), red seabream (Komilus et al. 2008), rainbow trout (Oo et al. 2007; Richard et al. 2006a; Turchini et al. 2013) and salmonids (Bell et al. 2010; Miller et al. 2007; Nanton et al. 2007) also yielded mostly encouraging results in terms of growth and feed efficiency. A detailed review of these published data shows that most of the studies were conducted on juvenile fish on a short-term basis (Shapawi et al. 2019). Thus, the present study was aimed at evaluating the long-term effects of fish oil replacement with crude palm oil on the growth performance, feed efficiency, biological indices, blood parameters and the organoleptic quality in larger hybrid grouper during the grow-out stage.

Materials and Methods

EXPERIMENTAL DIETS

Diets were formulated with 50% crude protein and 16% crude lipid (Shapawi et al. 2014). The dietary fish oil was substituted progressively at 25% with crude palm oil, CPO (25CPO, 50CPO, 75CPO, and 100CPO) and a FO-based diet was used as a control (Table 1). All the dry ingredients were mixed together in a mixer (Okazawa B20N, China) and subsequently added with wet ingredients, eventually forming a moist dough. This moist dough was screw-pressed through a 16 mm die of a meat chopper (Orimas TBS 200, Malaysia), and the strand of feeds were oven-dried in an industrial oven (Hohuki® DO-100A(S), Malaysia) at 38 °C for 6 h. The diets were kept refrigerated at -4 °C until used.

EXPERIMENTAL DESIGN

The experiment was carried out in floating sea net-cages at Terayong Marine Aquaculture Station of the Department of Fisheries, Sabah, Malaysia. The test specimens with average initial body weight of 200 g were randomly distributed into groups of 30 fish in 15 cages (1.5 × 1.5 × 2.0 m). The fish were hand-fed once daily to apparent satiation level in the morning throughout the 4 months of the feeding trial. During the experiment, the values of water parameters including dissolved oxygen ranged from 5.7-6.8 mg L^{-1}, salinity 26-30.8 g L^{-1}, temperature 28.84-31.6 ℃ and pH 7.13-8.22 were recorded.

FISH WEIGHING AND SAMPLING

The fish were starved prior to weighing to ensure that their stomach was without food. Before the measurement, the fish were anaesthetized with alpha-methylquinoline (NIKA) at 0.1 mL L^{-1}. The total body length of the fish was measured to the nearest 0.1 mm and body weight to the nearest 0.05 g. All the fish were individually weighed on a monthly basis.

Ten specimens of the fish from the initial stock and two from each treatment were sampled randomly during the starting and final days of the feeding trial, respectively. These samples were kept at -20 °C for subsequent determination of whole-body composition. In addition, an additional 2 fish per treatment were sampled at the end of feeding trial for the measurement of hepatosomatic index, viscerosomatic index, intraperitoneal fat, and fillet yield. Another 3 fish per treatment were tranquilized for blood collection. Blood samples were obtained from the caudal vein of the fish by using a regular 1 mL syringes and 27-gauge needle and evacuated into blood collection tubes (Vacutainer®; Becton Dickinson, Franklin Lakes, New Jersey, USA) for serum and haematological analysis.

ORGANOLEPTIC TEST

At the end of feeding trial, 3 specimens of the fish from each treatment were randomly chosen and starved for 36 h before being sacrificed by hypothermic treatment in a polystyrene box containing iced seawater. Subsequently, the fish were gutted, filleted and sliced evenly and then kept for 24 h at 4 °C until the analysis. The sliced fish were wrapped in aluminium foil and cooked in a steamer

for 10 min. A panel of tasters comprising 45 untrained people was constituted for conducting the organoleptic test. They were offered the steamed fish fillet that was labelled with alphabet instead of the name of the diet offered to the fish during the feeding trials. The panel members were requested to evaluate 5 attributes of the fish: odour, appearance, flavour, texture, and general acceptance using a rating test based on 5-point hedonic scale (Kappel et al. 1995).

BIOCHEMICAL ANALYSIS

The proximate composition of the diets and body of the fish were analysed following the methods of the Association of Official Analytical Chemists (AOAC 1999). Analyses were carried out in triplicates. The samples were oven-dried and ground into powder prior to proximate analysis. Kjeldahl nitrogen method was used for determination of crude protein by using Kjeltec 2300, Foss, Sweden. Crude lipid analysis was done by extraction using petroleum benzene solvent in Soxtec™ 2043 Foss, Sweden. Crude ash was determined by incineration at 550 °C in a muffle furnace. Moisture content of samples was calculated by loss in weight following drying the samples at 105 °C for at least 24 h until a constant weight was attained.

The extract of crude lipid from the experimental diet was subjected to fatty acid analysis. Following the method of Bligh and Dyer (1959), the extracts were obtained using chloroform: methanol ratio of 1:1 (v/v). The fatty acid methyl esters were prepared by mixing 50 mg of oil in 0.8 mL hexane followed by adding 0.2 mL portion of 1 M solution of sodium methoxide (PORIM 1995), then analysed using Agilent 7890A GC (Agilent Technologies, Singapore) equipped with a polar capillary column HP-88 (0.25 mm internal diameter, 60 m length and 0.2 µm film thickness; Agilent Technologies, Singapore) and an Agilent 5975 MS. The identification of the sample's peak was done with reference to a chromatographic profile containing 37 FAME standards (Supelco, Bellefonte, PA). The percentage of fatty acid was calculated as the ratio of the partial area to the total peaks area.

Blood samples for serum biochemical analysis were allowed to clot for 60 min and centrifuged for 10 min at 30,000 rpm. A Roche reagent kit was used for determination of cholesterol (cat. 03039773) and total protein (cat. 03183734). The analysis was performed on Cobas 6000 analyzer series module C501 (Switzerland). Meanwhile, blood samples for haematology including red blood count and haemoglobin were analysed using XT-1800i Sysmex.

CALCULATION AND STATISTICAL ANALYSIS

Growth performance, survival, feed utilization efficiency and body indices were calculated using the following formulas (Mohamad-Zulkifli et al. 2019):

Weight gain (%) = ((Final body weight-Initial body weight)/
Initial body weight) × 100

Specific growth rate (% day^{-1}) = (Ln (Final weight) – Ln (Initial weight)/Days)) × 100

Survival = (Remaining number of fish in each treatment on the 4th month/Initial number of fish) × 100

Feed conversion ratio = Dry feed consumed (g)/Wet weight gain (g)

Protein efficiency ratio = Wet weight gain (g)/Total protein intake (g)

Net protein utilization = ((Final fish body protein – Initial fish body protein)/Total protein intake) × 100

Hepatosomatic index = (Liver weight (g)/Fish weight (g)) × 100

Viscerosomatic index = (Viscera weight (g)/Fish weight (g)) × 100

Intra-peritoneal fat index = (Intra-peritoneal fat weight (g)/Fish weight (g)) × 100

Fillet yield = (Fish fillets weight (g)/Fish weight (g)) × 100

Condition factor = (Fish weight (g)/(Total length)^3) × 100

All the data were subjected to One-way ANOVA using statistical package IBM SPSS statistics v.20.0 for Windows. Levene's test was used to test the homogeneity of variances. Meanwhile, Tukey HSD post-hoc test was performed for multiple comparisons between the treatments. Differences were considered significant at $p<0.05$.

RESULTS

NUTRIENT COMPOSITION OF DIETS

The observed proximate composition of the experimental diets (Table 2) corresponded with the calculated values of the formulated diet. Crude lipid and protein were at approximately 16% and 50%, respectively. Based on the fatty acid profile of the diet, the palmitic acid (C16:0) level generally increased with the level of CPO inclusion. The total SFA is the highest with 50CPO dietary treatment compared to that of 100CPO diet due to the low level of myristic acid (C14:0) in the latter, whereas the level of MUFA was highest with 100CPO diet. As expected, the control diet had the highest level of EPA (C20:5n3) and DHA (C22:6n3) among the diets. The total PUFA levels in the diet exhibited a decreasing trend as the CPO substitution in the diet increased. EPA and DHA were 8.28 and 7.22%, respectively in 50CPO diet as compared to 12.08 and 10.02%, respectively, in FO diet. The reduction of these fatty acids was not reduced to half even though the replacement of FO with CPO was 50%. However, a complete replacement of FO with CPO caused a remarkable reduction of EPA and DHA values that were 4.35 and 4%, respectively.

GROWTH PERFORMANCE AND FEED UTILIZATION EFFICIENCY

The growth performance and feed utilization efficiency of hybrid grouper provided diets with increasing level of CPO did not produce any significant difference ($p>0.05$) compared with the FO diet (Table 3). The fish fed with 50CPO diet achieved the highest absolute body weight (g), body weight gain (%) and SGR (% d^{-1}) at the end of the feeding trial. The performance of fish with 75CPO and 100CPO diets was comparable with the fish fed with FO diet. The survival rate was above 90% in all the treatments, indicating good health of the fish.

The pattern of growth performance was basically similar to that of the feed utilization efficiency. The best FCR of 0.96 was obtained in 50CPO diet. Interestingly, diets 75CPO and 100CPO had a slightly better FCR values (0.97 and 1.03, respectively) compared to the control diet (1.07). The PER and NPU of the fish ranged from 2.29 (Diet 100CPO) to 2.58 (Diet FO) and 38.59 (Diet 25CPO) to 45.24 (Diet FO), respectively.

BODY INDICES

There was no significant difference ($p>0.05$) detected among the treatments for the body indices and fillet yield of the fish (Table 4). No specific trend was observed for all the parameters except for the VSI of the fish. The VSI of the fish reduced as the replacement with CPO increased. The HSI values ranged from 0.89 to 1.2%. The fish fed with 50CPO diet showed the best value for CF and fillet yield among all the treatments.

PROXIMATE COMPOSITION OF HYBRID GROUPER

The whole body-protein and lipid of the fish were not significantly ($p>0.05$) affected by the dietary lipid supplied (Table 5). However, the whole-body lipid of the fish exhibits a decreasing trend in the fish fed with FO-100CPO diet. The moisture content of the fish fed with 100CPO diet was significantly higher ($p<0.05$) than that in the FO diet but was not significantly different from 25CPO, 50CPO, and 75CPO diets. The whole-body ash content of the fish palm oil-based diets was not significantly different from that in the fish fed with FO diet. However, the fish fed with 75CPO had a significantly higher ($p<0.05$) ash content compared with the fish fed with 50CPO and 100CPO diet.

BLOOD PARAMETERS

Blood analysis results of hybrid grouper presented in Table 6 showed that the dietary lipid supplement significantly affected ($p<0.05$) the total cholesterol in the fish. The total cholesterol in the fish's blood decreased as the supplementation of CPO increased. Significant difference ($p>0.05$) was not observed in the TP, RBC and Hb of the fish. The TP and RBC ranged from 41.67 to 49.00 g L^{-1} and 0.30 to 1.03 × 10^6 mm^3, respectively. Hb ranged from 2.78 to 6.47 g dL^{-1}.

ORGANOLEPTIC TEST

The scores of the test panel are presented in Table 7. There was no significant difference ($p>0.05$) detected among the groups. All the fish received average scores in each attribute and no clear trend were observed.

DISCUSSION

There is a paucity of published data on the use of alternative dietary lipid sources in grouper. Probably, this is the first study on long-term effects of fish oil replacement with palm oil in the diet of hybrid grouper during the grow-out stage. In the present study, no significant differences in growth were found between fish fed the FO diet and CPO diets. The fish fed 50CPO diet produced relatively better results in terms of growth performance, the performance of fish fed 100CPO was comparable to those fed FO. Based on growth and feed utilization efficiency, total substitution of fish oil with crude palm oil for long-term purpose is possible during the grow-out phase of hybrid grouper (200 to 570 g). Some investigations have demonstrated similar observations when fish oil was substituted with vegetable oil in grouper diets. Unfortunately, previous studies on hybrid grouper were mostly conducted on juveniles' fish, making comparison difficult (Jiang et al. 2015; Rahimnejad et al. 2015; Yong et al. 2019). In a recent study on hybrid grouper using wheat germ oil (WGO) to replace fish oil in the diets, the survival rate, weight gain rate, specific growth rate and FCR were also unaffected and this led to the suggestion that 71.8% dietary fish oil can be replaced by WGO (Baoshan et al. 2019). In experiments conducted by Lin et al. (2007) and Shapawi et al. (2008), it was observed that 50% fish oil substitution with vegetable oil (including palm oil) in juveniles, orange-spotted grouper and humpback grouper did not produce any significant effects on growth performance and feed utilization efficiency. This is in contrast with the findings of Niu et al. (2007) that involved using 100% replacement with maize oil in orange-spotted grouper. The difference in the results obtained was probably due to the different life stage of the fish and dietary ingredients used in the feeding trials. In term of fillet yield of the fish, the findings in the present study are comparable to other grouper species (Tucker 1999). In our recent review paper (Shapawi et al. 2019), it is obvious that the hybrid grouper has a higher production potential and resilience based on its performance on different diets.

As expected, the increasing level of fish oil replacement with crude palm oil has resulted in the

increased values of the palmitic acid (C16:0) as well as the linoleic acid (C18:2n6), and decreased amounts of EPA (C20:5n3) and DHA (C22:6n3) in the diet. Nevertheless, even the lower quantities of essential fatty acids in the diets including in 100CPO diet did not seem to undermine the growth rate of the fish. It is important to note that the experimental diets were formulated using fish meal as one of the protein sources which will contribute to the fatty acid composition of the diets. Similar effects on the significant influence of vegetable oil on fatty acid profile of hybrid grouper were observed in our previous studies using the juvenile fish (Yong et al. 2019).

A decreasing trend of whole-body lipid of the fish in the present study is contradicted with the unaffected whole-body composition of hybrid grouper fed with WGO-based diets earlier reported by Baoshan et al. (2019). Concordant views were expressed by Han et al. (2012) based on their research on the Japanese sea bass and by Bell et al. (2002) on Atlantic salmon. It can be concluded that different vegetable oils might have different effects on the adiposity of fish.

The present study showed decreased total cholesterol in the blood as the replacement of crude palm oil in the diet increased, indicating hypocholesterolemic effect. Other studies have presented the same result on black seabream (Peng et al. 2008), African catfish (Babalola et al. 2016) and European sea bass (Richard et al. 2006b). This might be due to the presence of phytosterols in vegetable oils that play a significant role in cholesterol absorption (Gilman et al. 2003).

It was reported that diets containing higher amounts of n−6 fatty acids will increase in the relative amount of n−6-derived volatile aldehydes compounds that are generally reported to negatively affect the odour of fish muscle (Turchini et al. 2007). Interestingly, the organoleptic test of steamed fillet slices showed that there was no detrimental effect on the attributes of the hybrid grouper fillet when fish oil was increasingly replaced with crude palm oil.

TABLE 1. Ingredient composition of experimental diets (g 100 g^{-1} diets)

Ingredients	Experimental diets				
	FO	25CPO	50CPO	75CPO	100CO
Danish fish meal[a]	63.88	63.88	63.88	63.88	63.88
Soybean meal	9.74	9.74	9.74	9.74	9.74
Fish oil[b]	9.38	7.04	4.69	2.34	0.00
Crude palm oil[c]	0.00	2.34	4.69	7.04	9.38
Vitamin premix[d]	3.00	3.00	3.00	3.00	3.00
Mineral premix[e]	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.00	1.00	1.00	1.00	1.00
Carboxymethyl cellulose[f]	1.50	1.50	1.50	1.50	1.50
Tapioca starch[f]	9.51	9.51	9.51	9.51	9.51

[a] Fishmeal 999 Prime Quality, [b] Industrial fish oil, [c] Product of Sawit Kinabalu Sdn. Bhd., [d] Vitamin mixture (g kg^{-1}): Vitamin A, 1.5; Vitamin D3, 0.025; Vitamin E, 50.0; Vitamin B1, 15.0; Vitamin B2, 15.0; Vitamin B6, 12.0; Vitamin B12, 0.025; Biotin, 0.5; Vitamin C, 300.0; Calpan, 25.0; Folic acid, 2.5; Niacin, 50.0; Inositol, 125.0, [e] Mineral mixture (g kg^{-1}): Iron, 25.0; Copper, 0.785; Zinc, 1.9; Iodine; 0.15; Manganese, 0.8; Cobalt, 0.1; Sodium, 0.2; Dcp, 723.0; Sodium chloride,60.0; Anti-caking, 1.0; Potassium chloride, 50.0; Magnesium sulphate, 137.0, [f] Food grade

TABLE 2. Proximate (% dry matter) and fatty acid composition (% total fatty acids) of experimental diets

Proximate composition	Experimental diets				
	FO	25CPO	50CPO	75CPO	100CPO
Moisture	12.38 ± 0.10	13.89 ± 1.47	12.12 ± 0.14	12.82 ± 0.06	13.29 ± 0.04
Ash	13.28 ± 0.07	13.60 ± 0.23	13.39 ± 0.10	12.84 ± 0.05	13.38 ± 0.01
Crude lipid	15.76 ± 0.03	16.30 ± 0.33	15.86 ± 0.36	16.24 ± 0.03	15.90 ± 0.09
Crude protein	51.05 ± 0.24	51.21 ± 0.40	50.86 ± 0.21	50.94 ± 0.34	50.75 ± 0.13
Fatty acid composition					
C14:0	7.14 ± 0.24	7.09 ± 0.74	6.30 ± 1.25	4.85 ± 0.13	3.90 ± 0.01
C16:0	28.89 ± 0.01	33.96 ± 1.50	35.07 ± 0.00	39.61 ± 0.83	41.96 ± 2.23
C16:1	6.33 ± 0.12	6.17 ± 0.43	5.01 ± 0.00	4.06 ± 0.15	2.56 ± 0.00
C18:1n9	24.39 ± 0.22	27.88 ± 0.66	30.18 ± 2.88	32.55 ± 0.02	39.01 ± 1.13
C18:2n6	nd[b]	4.00 ± 0.00	4.91 ± 0.00	6.26 ± 0.06	6.78 ± 0.27
C20:5n3	12.03 ± 0.05	10.74 ± 0.03	8.28 ± 0.27	6.63 ± 0.41	4.35 ± 0.00
C22:6n3	10.19 ± 0.17	8.18 ± 0.86	7.22 ± 0.00	6.04 ± 0.63	4.00 ± 0.03
Total saturates	36.03 ± 0.24	41.06 ± 3.73	41.37 ± 7.08	44.46 ± 0.96	45.86 ± 2.22
Total monoenes	30.72 ± 0.10	34.05 ± 1.09	35.19 ± 0.37	36.61 ± 0.14	41.57 ± 0.15
Total PUFA[a]	22.22 ± 0.12	22.93 ± 2.89	20.41 ± 2.19	18.93 ± 1.10	15.17 ± 0.24
Total n-3	22.22 ± 0.12	18.92 ± 0.89	15.50 ± 3.34	12.68 ± 1.04	8.39 ± 0.04
Total n-6	nd	4.00 ± 0.00	4.91 ± 0.00	6.26 ± 0.06	6.78 ± 0.27
n-3/n-6	-	4.73 ± 0.22	3.16 ± 0.68	2.02 ± 0.15	1.24 ± 0.06

Data express as mean ± standard error (n=3 for proximate analysis; n=2 for fatty acid analysis), [a]PUFA - polyunsaturated fatty acid, [b]nd - not detected

TABLE 3. Growth performance and feed utilization of fish after 4 months of feeding trial

	Experimental diets				
	FO	25CPO	50CPO	75CPO	100CPO
IBW (g)[a]	205.30 ± 16.77	205.40 ± 18.29	205.90 ± 21.10	207.42 ± 17.36	206.83 ± 19.43
FBW (g)[b]	567.03 ± 98.13	558.26 ± 87.77	576.99 ± 101.23	560.94 ± 84.70	561.45 ± 89.60
BWG (%)[c]	176.18 ± 7.99	171.68 ± 3.39	181.63 ± 8.33	170.42 ± 8.33	171.44 ± 14.2
SGR (% day^{-1})[d]	0.85 ± 0.02	0.83 ± 0.02	0.86 ± 0.04	0.83 ± 0.01	0.83 ± 0.04
Survival (%)	97.78 ± 1.11	96.67 ± 0.00	96.67 ± 1.11	98.89 ± 1.92	93.33 ± 1.92
FCR[e]	1.07 ± 0.05	1.03 ± 0.10	0.96 ± 0.04	0.97 ± 0.03	1.03 ± 0.04
PER (%)[f]	2.58 ± 0.07	2.35 ± 0.05	2.36 ± 0.12	2.31 ± 0.06	2.29 ± 0.07
NPU (%)[g]	45.24 ± 2.94	38.59 ± 2.61	40.98 ± 2.58	40.21 ± 4.24	39.39 ± 1.49

Data express as mean ± standard error (n=3), [a]IBW - initial body weight; [b]FBW - final body weight, [c]BWG - body weight gain, [d]SGR - specific growth rate, [e]FCR - feed conversion ratio, [f]PER - protein efficiency ratio, [g]NPU - net protein utilization

TABLE 4. Body indices (% wet weight) of fish after 4 months of feeding trial

	Experimental diets				
	FO	25CPO	50CPO	75CPO	100CPO
HSI (%)[a]	1.09 ± 0.04	0.89 ± 0.10	1.21 ± 0.04	1.15 ± 0.21	0.90 ± 0.06
VSI (%)[b]	10.68 ± 1.04	10.26 ± 0.27	9.76 ± 0.50	9.73 ± 1.36	8.50 ± 0.92
IPF (%)[c]	2.67 ± 0.16	2.75 ± 0.24	2.73 ± 0.53	2.65 ± 0.27	2.40 ± 0.40
CF (%)[d]	2.05 ± 0.05	2.03 ± 0.02	2.11 ± 0.03	2.10 ± 0.02	2.05 ± 0.05
Fillet yield (%)	33.85 ± 0.90	35.75 ± 1.07	38.10 ± 1.14	34.76 ± 2.97	35.92 ± 0.65

Data express as mean ± standard error (n=3), [a] HSI - hepatosomatic index, [b] VSI - viscerosomatic index, [c] IPF – intraperitoneal fat, [d] CF - condition factor

TABLE 5. Whole-body proximate composition (% wet weight) of hybrid grouper fed different levels of crude palm oil

Proximate composition	Experimental diets				
	FO	25CPO	50CPO	75CPO	100CPO
Crude protein	18.40±0.49	17.71±0.28	18.26±0.10	18.34±0.92	18.20±0.30
Crude lipid	9.30 ± 0.27	8.98 ± 0.76	8.49 ± 0.63	8.14 ± 0.51	7.93 ± 0.49
Moisture	65.24±0.20[a]	66.22±0.48[b]	66.98±0.62[b]	66.16±0.72[b]	67.66±0.20[b]
Ash	6.05±0.22[ab]	5.89 ±0.09[ab]	5.27 ±0.10[ab]	6.63 ± 0.24[b]	5.65 ± 0.16[a]

Data express as mean ± standard error (n=3), means within the same row with different uppercase letters are significantly different at $P<0.05$

TABLE 6. Haematological parameters of hybrid grouper after 4 months of feeding trial

	Experimental diets				
	FO	25CPO	50CPO	75CPO	100CPO
TC (mM)[a]	13.47 ± 1.87[a]	10.13 ± 0.20[ab]	12.10 ± 0.20[b]	7.70 ± 0.15[a]	6.40 ± 0.17[b]
TP (g L^{-1})[b]	48.00 ± 3.51	49.00 ± 1.00	42.67 ± 0.33	41.67 ± 2.40	46.00 ± 1.15
RBC (x10^6 mm^3)[c]	0.30 ± 0.06	0.73 ± 0.19	0.70 ± 0.20	0.57 ± 0.17	1.03 ± 0.23
Hb (g dL^{-1})[d]	2.78 ± 1.87	5.27 ± 1.48	6.47 ± 0.67	5.53 ± 0.47	4.83 ± 0.43

Data express as mean ± standard error (n=3), means within the same row with different uppercase letters are significantly different at P<0.05, [a] TC - total cholesterol, [b] TP - total protein, [c] RBC - red blood cell count, [d] Hb - haemoglobin

TABLE 7. Organoleptic quality of fish fillet after 4 months of feeding trial

Attributes	Experimental diets				
	FO	25CPO	50CPO	75CPO	100CPO
Odour	3.59 ± 0.11	3.43 ± 0.14	3.45 ± 0.13	3.41 ± 0.13	3.57 ± 0.14
Appearance	3.64 ± 0.10	3.64 ± 0.10	3.57 ± 0.14	3.66 ± 0.11	3.39 ± 0.14
Flavour	3.57 ± 0.11	3.45 ± 0.11	3.50 ± 0.12	3.45 ± 0.13	3.57 ± 0.13
Texture	3.34 ± 0.12	3.45 ± 0.11	3.64 ± 0.15	3.55 ± 0.14	3.52 ± 0.14
General acceptance	3.61 ± 0.13	3.30 ± 0.18	3.61 ± 0.15	3.68 ± 0.17	3.57 ± 0.17

Data express as mean ± standard error (n=3)

CONCLUSION

This study suggests that crude palm oil is an excellent source of lipid to replace fish oil in the grow-out diet of *Epinephelus fuscoguttatus* × *Epinephelus lanceolatus* hybrid. There were no apparent negative effects on the overall performance of the fish including the sensory attributes of fillet. However, substitution of fish oil with crude palm oil may result in significant changes in the fatty acid profile of the hybrid grouper, which may affect the nutritional advantage of consuming marine fish. Nevertheless, feeding the fish with finishing diets based on fish oil may restore the desired fatty acids. This is the subject of ongoing research activity at our laboratory.

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