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Original Research Article

Growth performance, fatty acid composition, and lipid metabolism are altered in groupers (*Epinephelus coioides*) by dietary fish oil replacement with palm oil

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ABSTRACT

In this study, we conducted a 56-d feeding trial to investigate the effects of replacing the fish oil (FO) with palm oil (PO) on the performance, tissue fatty acid (FA) composition, and mRNA levels of genes related to hepatic lipid metabolism in grouper (*Epinephelus coioides*). Five isolipidic (13% crude lipid) and isonitrogenous (48% CP) diets were formulated by incrementally adding PO to the control diet (25% fish meal and 9% added FO) to replace FO in the control diets. Triplicate groups of 30 groupers (initial weight: 12.6 ± 0.1 g) were fed one of the diets twice daily, to apparent satiety. The replacement of FO with 50% PO revealed maximum growth without affecting the performance and whole-body proximate compositions, and replacing FO with 100% PO revealed a comparable ($P > 0.05$) growth with that of the control diet, suggesting PO as a suitable alternative to FO. The analysis of FA profiles in the dorsal muscle and liver though reflected the FA profile of the diet, PO substitutions above 50% could compromise ($P < 0.05$) the FA profile in the liver and flesh of the fish species in comparison with the control diet. Furthermore, the mRNA levels of *FAS*, *G6PD*, *LPL*, *PPARA*, and *Δ6FAD* genes in the liver had positive linear and/or quadratic responses, but the *SCD*, *HSL*, *ATGL*, *FABP*, *SREBP-1C* and *ELOVL5* had the opposite trend, with increasing dietary PO inclusion levels, whereas the mRNA level of *ACC* was not affected by dietary treatments. The optimal level of PO substitution for FO was estimated to be 47.1% of the feed, based on the regression analysis of percent weight gains against dietary PO inclusion levels; however, it might affect the FA profile in the liver and flesh of the fish species, and further study is required to investigate whether the changes in tissue FA composition will affect the welfare and market value over a production cycle of grouper.

1. Introduction

Lipid is an essential macronutrient for the normal growth of fish because it provides essential fatty acids (EFA) for the metabolism of terrestrial animals and fish to maintain their growth and physiological functions (Turchini et al., 2009). Fish oil (FO) is rich in n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA), such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which makes it a major source of EFA for cultured fish, especially mariculture fish (Fukada et al., 2020). In addition, FO also contains some lipid-soluble substances and flavoring substances, including palatability promoting substances and essential nutrients for fishes (Turchini et al., 2009). As a result, FO has become the first choice of lipid source for fish feed (Nasopoulou and Zabetakis, 2012). However, the global supply of FO is constrained due to decreasing marine catches and cannot match the rapidly growing demand for aquafeed production (Turchini et al., 2011a,b); thus, creating a huge deficit in FO supply (Tocher et al., 2019), leading to high FO prices. Fish oil replacement is another major international research priority in aquafeeds after fish meal replacement (Torstensen et al., 2005). This

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alternative approach to the formulation of aquafeeds offers promising results, but variable successes were recorded by using the vegetable oils in commercial feed formulations for farmed fish, a result generally attributed to the inconsistent nutritional quality and types of oils (Benedito-Palos et al., 2008; Fountoulaki et al., 2009; Abbasi et al., 2020; Alvarez et al., 2020; Chen et al., 2020; Tseng and Lin, 2020). Therefore, it is necessary to find suitable lipid sources to replace FO to promote the sustainable development of aquaculture.

Palm oil (PO) is one of the most commonly used vegetable oils worldwide with a production of around 66 million tons in 2017, present in around 34% to 50% of frequently used food and consumer products (Kadandale et al., 2019; Kushairi et al., 2019). The nutritional health benefits of PO for human consumption have been ascertained and well documented (Sun et al., 2015). Although the oil contains a high level of saturated fatty acids (SFA) compared to other edible vegetable oils (Balbuena-Pecino et al., 2021), it is rich in C16:0 and C18:1n-9 FA, but has relatively low levels of C18:2n-6. Because of its high SFA contents, PO has a higher resistance to becoming rancid, which could produce durable fish feeds (Ng et al., 2007; Riera-Heredia et al., 2020). Therefore, considerable efforts have been made to use PO as a potential replacement for FO in aquafeeds (Bell et al., 2002), and several studies have shown that PO can replace FO in diets at a range between 40% and 100% without adverse effects on the growth performance of fishes (Ng et al., 2004; Fonseca-Madrigal et al., 2005; Balbuena-Pecino et al., 2021). Nevertheless, the fatty acids (FA) profile of the muscle in dietary PO treatment was significantly different from that of FO treatment in different fish species (Bell et al., 2002; Fonseca-Madrigal et al., 2005; Aliyu-Paiko and Hashim, 2012; Ahmad et al., 2013; Ma et al., 2019) when fish were fed a high PO diet, which could affect the quality of fish meat. Therefore, flesh quality assessment may be an important factor in deciding the use of PO in the fish diet (Mock et al., 2019; Gudid et al., 2020; Balbuena-Pecino et al., 2021; Guo et al., 2021).

The grouper is one of the most important maricultured fishes in Southeast Asian countries, including China (Boonyaratpalin, 1997). Its aquaculture production in China has reached 183,100 tons in 2019 (China Fishery Statistics Yearbook, 2020), and in the past decades, the research interests in nutrition and feeds for groupers have garnered increasing interest (Feng et al., 2020; Shapawi et al., 2019). Previous studies on the use of PO as a FO alternative for the fish focused on the effects of dietary PO inclusion on fish growth, feed utilization, and fish flesh quality (Shapawi et al., 2008; Gudid et al., 2020). However, less attention has been paid to the relationship between dietary PO inclusion and fish physiological status and the mechanism of PO utilization in fish. Therefore, in this study, the effects of dietary FO replacement by PO on growth performance, plasma biochemical components, tissue FA composition, and expression levels of genes related to lipid metabolism in grouper *Epinephelus coioides* were investigated, in an attempt to determine whether dietary PO inclusion levels affected growth and feed utilization and directly activated the gene expressions associated with lipid metabolism in groupers.

2. Materials and methods

2.1. Animal ethics

Experimental design and procedures in this study were reviewed and approved by the Animal Ethics Committee of Jimei University, Xiamen, China (Approval number: 2011-58).

2.2. Test feed

A basal diet was formulated to contain 48% CP and 13% crude lipid using a defatted Peru fish meal, soybean meal, wheat gluten, shrimp meal, gelatin and casein as the protein sources and menhaden fish oil (9% FO), palm oil (0% to 9% PO), soy lecithin (2%), fish meal (1% FO), and residue oil (1.3%) from other proteins as the lipid sources (Table 1). The FO was replaced by PO at 0%, 25%, 50%, 75%, and 100% in the basal diets to prepare 5 experimental diets (0% PO, 25% PO, 50% PO, 75% PO, and 100% PO, respectively). The coarse dry feed ingredients were ground in a hammer mill (GH-20B, Jiangyin Kejia Machinery Manufacturing Co., Ltd., Jiangyin, Jiangsu, China) and sifted through a 60-mesh sieve (250 μm particle size), then weighed and homogenized. The liquid ingredients (FO, PO, and soya lecithin) were mixed with the dry feed ingredients, and then freshwater was added (about 40% of the feed weight), and a mash was prepared. This dough was extruded into strands and pelletized through a 2.5-mm die using cold press extrusion (CD4XITS, South China University of Technology, Guangzhou, Guangdong, China). The pellets were dried in a ventilated oven at 55 °C for 24 h until the moisture was reduced to 10% and then sealed in plastic bags and stored in a refrigerator at −20 °C. The FA compositions of the test diets are given in Table 2.

2.3. Feeding management

This experiment was conducted at Fujian Dabeinong Fisheries Technology Company (Zhaoan County, Zhangzhou City, China). Prior

Table 1
Ingredients and composition of experimental diets (as-fed basis, %).

Item	Diets[1]				
	0% PO	25% PO	50% PO	75% PO	100% PO
Ingredients					
Fish meal[2]	25	25	25	25	25
Wheat gluten meal[2]	10	10	10	10	10
Soybean meal[2]	25	25	25	25	25
Gelatin[2]	2	2	2	2	2
Casein[2]	8	8	8	8	8
Shrimp meal[2]	3	3	3	3	3
Corn starch[2]	12.75	12.75	12.75	12.75	12.75
Menhaden fish oil	9	6.75	4.5	2.25	0
Palm oil	0	2.25	4.5	6.75	9
Soy lecithin	2	2	2	2	2
Vitamin premix[3]	0.3	0.3	0.3	0.3	0.3
Mineral premix[4]	0.5	0.5	0.5	0.5	0.5
Stay-C 35%	0.02	0.02	0.02	0.02	0.02
Mold inhibitors[5]	0.1	0.1	0.1	0.1	0.1
Feed antioxidants[6]	0.03	0.03	0.03	0.03	0.03
$Ca(H_2PO_4)_2$	2	2	2	2	2
Choline chloride	0.3	0.3	0.3	0.3	0.3
Proximate composition					
DM	92.96	92.57	92.83	92.70	93.12
CP	48.42	48.50	48.42	48.22	48.26
Crude lipid	13.64	13.46	13.55	13.75	13.51
Ash	8.09	8.23	8.26	8.31	8.30

FO = fish oil; PO = palm oil; 0% PO = control diet without PO addition.
[1] Palm oil replaced 0%, 25%, 50%, 75%, and 100% of FO in the control diet, respectively.
[2] Peru fish meal: CP 68.87% and crude lipid 8.96%; Wheat gluten meal: CP 80.71%; Soybean meal: CP 46.64% and crude lipid 0.62%; Gelatin: CP 88.35% and crude lipid 0.21%; Casein: CP 89.66% and 0.13%; Shrimp meal: CP 58.45% and crude lipid 5.58%; Corn starch: CP 0.41% and crude lipid 0.15%. All the ingredients were obtained from Jiakang Feed Co. Ltd., Xiamen, China. Peru fish meal was further degreased with n-hexane and its crude lipid was reduced to 4.01%.
[3] Vitamin premix (per kilogram diet): retinol acetate, 10 mg; 1,25-dihydroxycholecalciferol, 10 mg; DL-α-tocopherol acetate, 100 mg; menadione sodium bisulfate, 10 mg; thiamin nitrate, 10 mg; riboflavin, 20 mg; pyridoxine hydrochloride, 20 mg; cyanocobalamin, 0.05 mg; nicotinic acid, 50 mg; calcium-D- pantothenate, 100 mg; D-biotin, 1 mg; *meso*-inositol, 500 mg; folic acid, 4 mg.
[4] Mineral premix (per kilogram diet): ferric citrate, 497 mg; $CuSO_4 \cdot 5H_2O$, 24 mg; $ZnSO_4 \cdot 7H_2O$, 176 mg; $MnSO_4 \cdot 4H_2O$, 122 mg; $CoCl_2 \cdot 6H_2O$, 0.18 mg; KIO_3, 0.51 mg; Na_2SeO_3, 0.33 mg.
[5] Mold inhibitors: 50% calcium propionic acid and 50% dimethyl fumarate.
[6] Feed antioxidants: 50% ethoxyquin and 50% butylated hydroxytoluene.

Table 2
Fatty acid composition (mg/g lipid) of lipid sources and experimental diets.

Fatty acids	FO	PO	Diets[1]				
			0% PO	25% PO	50% PO	75% PO	100% PO
C14:0	38.0	7.3	33.7	28.0	21.8	16.1	10.4
C16:0	156.7	286.5	164.8	195.3	213.4	226.4	246.0
C18:0	38.4	26.2	32.3	31.2	29.0	27.6	26.3
C19:0	32.1	20.7	28.1	20.1	23.5	24.2	21.5
C21:0	3.3	0.2	2.8	2.0	1.5	1.0	0.6
C22:0	2.3	0.6	1.9	1.9	1.6	1.5	1.2
ΣSFA	270.4	341.1	263.5	278.0	290.2	297.2	306.4
C16:1	51.9	3.2	44.2	33.8	25.1	16.4	7.8
C17:1	3.0	ND	2.8	2.1	1.6	1.4	0.9
C18:1n-9 (OA)	137.1	349.6	130.1	177.3	226.0	278.7	330.2
C20:1	2.9	ND	1.5	1.2	0.9	0.6	0.2
C24:1	4.1	ND	3.9	3.2	2.8	1.8	1.1
ΣMUFA	199.4	353.2	182.3	218.2	256.7	299.3	340.8
C18:2n-6 (LA)	76.1	96.8	81.0	98.7	112.2	124.2	139.8
C18:3n-6	13.9	ND	14.3	10.7	7.9	4.7	1.6
C20:2n-6	15.3	ND	16.5	12.9	9.6	6.5	3.4
C20:3n-6	4.2	ND	4.2	3.4	2.7	1.8	1.0
C20:4n-6 (ARA)	12.9	ND	6.6	5.2	4.0	2.7	1.4
Σn-6 PUFA	122.2	97.5	122.8	130.6	136.7	140.4	147.6
C18:3n-3 (LNA)	21.6	6.0	18.4	16.0	14.2	9.7	8.0
C20:5n-3 (EPA)	72.6	ND	58.9	45.8	34.3	22.9	11.9
C22:5n-3	13.5	ND	8.2	6.5	5.0	3.3	1.7
C22:6n-3 (DHA)	108.2	ND	91.5	71.0	52.4	33.6	16.3
Σn-3 PUFA	215.6	6.1	177.3	139.6	105.7	69.6	37.3
Σn-3 LC-PUFA	194.2	ND	158.8	123.5	91.9	59.6	29.5
DHA/EPA	1.49	–	1.55	1.55	1.53	1.46	1.37
n-3/n-6 PUFA	1.76	0.06	1.44	1.07	0.78	0.50	0.25

FO = fish oil; PO = palm oil; 0% PO = control diet without PO addition.
SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acid; LC-PUFA = long-chain polyunsaturated fatty acids; OA = oleic acid; LA = linoleic acid; ARA = arachidonic acid; LNA = linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; ND = not detected.
[1] Palm oil replaced 0%, 25%, 50%, 75%, and 100% of FO in the control diet, respectively.

to the start of the experiment, grouper juveniles were kept in a concrete pond and fed with control diet for 2-week acclimatization. The fish were selected for growth trial according to the criteria (the feeding behavior, the color of the fish body, and the microscopic examination of the gills, etc.) of judging whether a fish is healthy. A total of 450 fish initially weighing an average of 12.6 ± 0.1 g/fish (n = 30 fish) were randomly distributed into five groups, each with triplicate floating net cages (length × width × height, 160 cm × 80 cm × 80 cm) at a density of 30 fish per cage within indoor flow-through seawater pools (length × width × height, 300 cm × 200 cm × 150 cm). Each pool was supplied with a continuous flow (15 L/min) of filtered and disinfected seawater. Each floating net cage was anchored using ropes at its four corners. Air was continuously supplied from pipes with holes on the bottom of the pool, placed at 0.8 m intervals. A disc (50 cm in diameter) was placed at the bottom of the cage each to collect uneaten feed. Excess feed was collected 30 min after each feeding, dried at 65 °C and weighed for use in the calculation of feed intake. Each group of fish were hand-fed one of the diets twice daily (6:00 and 18:00) under a natural photoperiod across a feeding period of 8 weeks. As we used natural seawater, the water temperature of the indoor pool generally reflected the change of seawater temperature during the feeding trial. The feeding trial was conducted in the summer to maintain water temperature suitable for the growth of the fish species. The pool was cleaned every 7 d. After cleaning, nearly a third of the pool water was discharged and refilled with fresh seawater until the pool water returned to the original level. Dissolved oxygen and water temperature were measured daily at 15:00 h and nitrite-N was monitored twice a week using a multi-parameter photometer (HI83200; Hanna Instruments, Woonsocket, Rhode Island). Changes of water temperature and dissolved oxygen levels over a 56-d feeding period are shown in Fig. 1. The ammonia nitrogen content was less than 0.22 mg/L.

2.4. Sample collection

At the end of an 8-week feeding, fish were fasted for 24 h, followed by anesthesia with a dose of 100 mg/L solution of MS-222 (tricaine methane sulfonate, Sigma-Aldrich Shanghai Trading Co. Ltd., Shanghai), batch-weighed, and counted by cage to determine weight gain (WG), feeding rate (FR), feed efficiency (FE), and survival. Eighteen fish per treatment (6 fish per cage) were randomly caught and anesthetized with MS 222 (100 mg/L) and weighed individually to calculate the hepatosomatic index (HSI) and condition factor (CF). Blood samples of 6 fish per cage were collected from the caudal vein using a 2-mL heparinized syringe and centrifuged immediately at 1,027 × g at 4 °C for 10 min, and plasma was separated. The samples were pooled by a cage and stored in 1.5-mL Eppendorf tubes at −80 °C for the subsequent biochemical components' analysis. The liver and dorsal muscles of the same set of 6 fish per cage were then aseptically removed and respectively pooled into one tube for each cage, stored at −80 °C for the analysis of biochemical components and gene expression. Another 5 fish in each cage were randomly sampled and pooled in plastic bags and stored at −20 °C for the determination of whole-body composition.

2.5. Plasma component analysis

The glucose (GLU), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) contents in the plasma were determined by using their respective kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following the manufacturer's instructions.

2.6. Proximate composition and fatty acid analysis

Proximate composition of ingredients, diets, and whole-body fish samples were determined according to standard methods (AOAC, 1995). Dry matter was evaluated by drying the samples in an oven at 105 °C to a constant weight. The CP was determined by the Kjeldahl method (N × 6.25) using Kjeltec TM 8400 Auto Sample Systems (Foss Tecator AB, Sweden). Crude lipid was determined by the Soxtec extraction method by using Soxtec Avanti 2050 (Foss Tecator AB). Ash was measured in the residues of samples burned in a muffle furnace at 550 °C for 8 h. The fish samples were autoclaved at 121 °C for 20 min, homogenized, and dried at 65 °C for 24 h prior to compositional analysis.

Total lipids of muscle, liver, and diet samples were extracted by homogenization in chloroform/methanol (2:1, vol/vol) solution according to Folch et al. (1957) and determined gravimetrically after drying a 5 mL aliquot under nitrogen. The freeze-dried samples (approximately 100 mg) were added into a 20 mL volumetric screwed glass tube with a plastic lid. Then 3 mL 2% KOH methanol solution was added and heated on a water bath for 20 min at 75 °C. After cooling, 3 mL 15% boron trifluoride methanol solution was added and the mixture was heated at 80 °C on a water bath for another 20 min. One mL hexane was then added into the mixture above, and shaken vigorously for 1 min. The mixture was allowed to form 2 layers. Fatty acid methyl esters in the upper layer were separated, and determined using gas chromatography (Agilent 7890B-GC, Fairborn Precision Instruments Co., Ltd. Shanghai, China) with C19 alkanoic acid (Sigma-Aldrich Shanghai Trading Co.

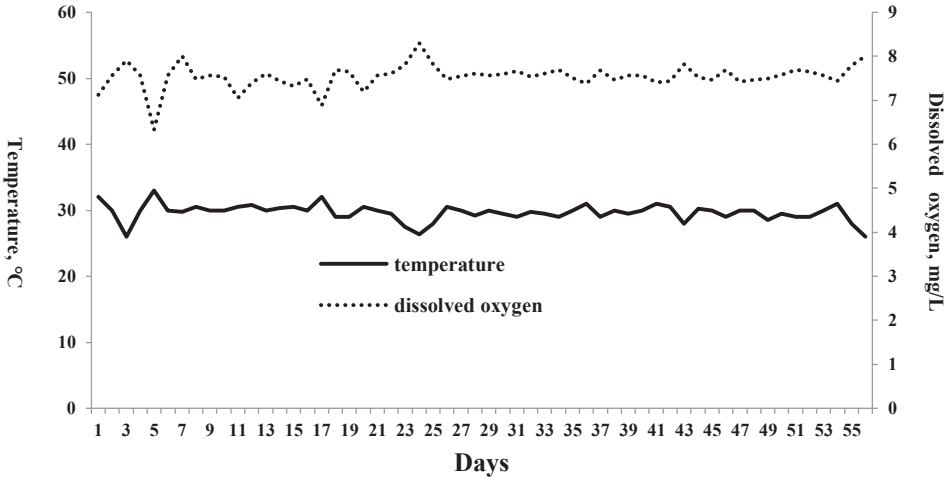

Fig. 1. The changes of dissolved oxygen and water temperature across a feeding period of 56 d.

Ltd., Shanghai) as an internal standard. For GC, an Agilent gas chromatograph equipped with a flame ionization detector (FID) and a HP-88 capillary column (100 m × 0.25 mm × 0.2 μm) was used to inject 1 μL of the methylated sample in the split mode at a 100:1 ratio. The column carrier gas was nitrogen at a constant flow rate of 1.25 mL/min. The injector temperature and the FID temperature were set at 250 and 270 °C, respectively. The oven temperature was programmed from an initial temperature of 100 °C for 13 min, followed by increment of 10 °C/min to 180 °C, 180 °C for 6 min, 1 °C/min increase to 200 °C, 200 °C for 20 min, and then a 5 °C/min increase to a final temperature of 240 °C, 240 °C for 10 min. The FA component was estimated according to the retention time of FA standard and data was collected by peak area normalization with the internal standard.

2.7. RNA extraction and expression analysis

Total RNA was extracted from the liver using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Isolated RNA was quantified using the NanoDrop ND-2000 spectrophotometer, and its integrity was confirmed by agarose gel electrophoresis. The cDNA was generated from 1 μg DNAase-treated RNA and synthesized by a PrimeScript RT Reagent Kit with gDNA Eraser (Perfect realtime) (Takara Co. Ltd., Japan). Real-time PCR was employed to determine mRNA levels based on the TB Green Premix Ex Taq II (Tli RNaseH Plus) (Takara Co. Ltd., Japan) with QuantStudio Real-Time PCR System (ABI, USA) quantitative thermal cycler. The fluorescent quantitative PCR reaction solution consisted of 10 μL TB Green Premix Ex Taq II (Tli RNaseH Plus) (2×), 0.8 μL PCR forward primer (10 μmol/L), 0.8 μL PCR reverse primer (10 μmol/L), 2.0 μL RT reaction (cDNA solution) and 6 μL dH_2O. The thermal program consisted of 30 s at 95 °C, 40 cycles at 95 °C for 5 s and 60 °C for 30 s. The sequences of primers used are given in Table 3. All amplicons were initially separated by agarose gel electrophoresis, and their correct size was ensured. β-actin was used as the internal reference gene to normalize cDNA loading. The qRT-PCR amplification efficiency was calculated according to specific gene standard curves generated from serial dilutions. The gene expression levels of the target genes were analyzed by the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008) after verifying that the primers were amplified with an efficiency of approximately 100%, and data for all treatment groups were compared with the data for the control group.

2.8. Statistical analysis

The results were presented as the mean and standard error of the mean (SEM). The significant differences among treatments were analyzed by analysis of variance (ANOVA) and Student-Neuman-Keuls multiple comparison test after confirming the normality and homogeneity of variance using the Kolmogorov–Smirnov test and Levene's test in SPSS Statistics 22.0 (SPSS, Michigan Avenue, Chicago, IL, USA). Data expressed as percentages or ratios were subjected to data transformation before statistical analysis. Orthogonal polynomial contrasts were used to assess the significance of linear or quadratic models to describe the response of the dependent variable to dietary PO replacement levels. *P*-values < 0.05 were considered statistically significant.

3. Results

3.1. Growth performance

The growth performance of grouper is presented in Table 4. The WG showed an open upward parabola ($P = 0.034$) with increasing dietary PO inclusion levels with a maximum gain at 50% PO inclusion diet. Dietary PO inclusion levels did not influence FR, FE, SR, HSI, and CF. Based upon the polynomial regression analysis of percent weight gains against dietary PO inclusion levels, the optimal dietary PO inclusion levels required to obtain the maximum WG value in groupers was estimated to be 47.1% of the feed (Fig. 2).

3.2. Proximate composition in tissues

As shown in Table 5, whole-body crude protein content showed an open upward parabola with increasing dietary FO replacing level ($P = 0.046$); however, it did not influence the whole-body moisture, crude lipid, and ash content. Moreover, muscular moisture, lipid and protein contents were not affected by incremental dietary PO inclusion levels, whereas the hepatic lipid content showed linear ($P < 0.001$) and an open upward parabola ($P < 0.001$) with increasing dietary PO inclusion levels, and the hepatic moisture content had the opposite trend ($P < 0.001$). Hepatic moisture and lipid contents in diets at 75% PO substitution or higher were significantly lower than that in the control diet ($P < 0.05$).

Table 3
Primers sequences used for real-time PCR.

Genes	Forward primer sequence (5′-3′)	Reverse primer sequence (5′-3′)	KEGG No.
β-Actin	TGCTGTCCCTGTATGCCTCT	CCTTGATGTCACGCACGAT	–
FAS	GGCAAGCCACTCTGGTACAT	GGCTATGTCTGACCGCAGAA	FJ196231
ACC	GGTGGGATACCTACTGGGGT	GGGAACCATACCTGTCCTGC	FJ196229
PPARα	ATGTCCCACAACGCTATCCG	GGCCAGACTCTTTGTGTCCA	FJ849065
LPL	CATCGTGATACACGGCTGGA	CAATCACATTGGCACTGGGC	EU683732
Δ6FAD	CTCATCATTTGGGTCTGGG	GAAGATGTTGGGTTTAGCG	EU715405
HSL	CAGTTAAGGTGAACCGGGCT	ATCTGAACTGGAGCAGTGCC	KF049203
ATGL	TGACAACCTGCCTCAGTACG	TGGATGCTCGTGTTGGTGAA	KY649281
SCD	GCGTGTTTCGTGTATGGTGG	GGAGTTTCCGATGGCCAGAA	NM198815
G6PD	GGCGAACCGTCTCTTCTACC	CCTGTTCCAGCCTTTTGTGC	XM010731710
FABP	CAGAAATCCAGCAGAACGGC	GCTTGACGATGCACTTGAGC	AF254642
SREBP-1c	GGTTCAAACCATGGCACCAC	GTCGTGCTTCAGAGTGGTCA	KT937284
CPT-1	AGGGCCGTTTCTACAAGGTG	GCGGCTAGTTTCTCCTCTCC	HM037343
ELOVL5	GCCTGTGCCAGACAAGGTTA	GCGTCCGGACAATAACCAGA	KU179484

FAS = fatty acid synthase; *ACC* = acetyl-CoA carboxylase; *G6PD* = glucose-6-phosphate dehydrogenase; *SCD* = stearoyl-CoA desaturase; *LPL* = lipoprotein lipase; *HSL* = hormone-sensitive lipase; *PPARα* = peroxisome proliferator activated receptor *α*; *CPT* = carnitine palmitoyltransferase; *ATGL* = adipose triglyceride lipase; *SREBP-1c* = sterol-regulatory element-binding protein-1c; *Δ6FAD* = delta-6 fatty acyl desaturase; *FABP* = fatty acid binding proteins; *ELOVL* = elongase of very long-chain fatty acids.

Table 4
Effects of dietary replacement of fish oil (FO) with palm oil (PO) on growth performance of *Epinephelus coioides* in a 56-d feeding period.[1]

Item	Diets[2]					Pooled SEM	*P*-value		
	0% PO	25% PO	50% PO	75% PO	100% PO		ANOVA	Linear	Quadratic
IBW[3], g/fish	12.58	12.58	12.62	12.59	12.62	0.007	0.120		
FBW[3], g/fish	69.19^{ab}	69.00^{ab}	72.16^{a}	70.54^{ab}	66.96^{b}	0.716	0.033	0.584	0.128
WG[3,5], %	450.1^{ab}	455.3^{ab}	470.2^{a}	460.4^{ab}	441.1^{b}	3.667	0.032	0.635	0.034
FR[3,6], %/d	2.29	2.37	2.40	2.31	2.35	0.015	0.111	0.552	0.284
FE[3,7], %	107	103	104	106	103	0.800	0.355	0.428	0.661
Survival[3,8], %	95.56	96.67	95.56	93.33	96.67	0.840	0.777	0.859	0.894
HSI[4,9], %	2.11	2.05	2.13	2.17	2.18	0.041	0.905	0.404	0.688
CF[4,10], %	2.33	2.40	2.49	2.46	2.49	0.031	0.441	0.083	0.171

FO = fish oil; PO = palm oil; 0% PO = control diet without PO addition; IBW = initial body weight; FBW = final body weight; WG = weight gain; FR = feeding rate; FE = feed efficiency; HSI = hepatosomatic index; CF = condition factor.
[a, b] Values in the same row with different superscripts indicate significant differences ($P < 0.05$).
[1] Statistical analysis was performed by one-way ANOVA, followed by Student-Neuman-Keuls multiple comparison test.
[2] Palm oil replaced 0%, 25%, 50%, 75%, and 100% of FO in the control diet, respectively.
[3] Data were presented as means of 3 triplicates per dietary treatment.
[4] Data were presented as means of 18 fish per dietary treatment.
[5] WG (%) = 100 × (FBW - IBW)/IBW.
[6] FR = 100 × feed intake/[(FBW + IBW)/2 × days].
[7] FE (%) = 100 × (FBW - IBW)/feed intake (as fed basis, g/fish).
[8] Survival (%) = 100 × (final No. of fish)/(initial No. of fish).
[9] HSI (%) = 100 × (liver weight/BW).
[10] CF (%) = 100 × BW/(body length)^3.

3.3. Biochemical components of the plasma

Table 6 shows both plasma TG and TC, which showed an open upward parabola ($P = 0.022$; $P = 0.036$) with increasing dietary PO inclusion levels, and both reached a peak at 50% PO substitution level. The lower plasma TG level in the control diet, TC level in 100% PO substitution diet, and HDL-C level in 75% PO substitution diet were observed in comparison with the 50% PO substitution diet ($P < 0.05$). There were no significant effects of increasing dietary PO inclusion levels on plasma GLU and LDL-C.

3.4. Fatty acid profile in tissues

As shown in Table 7, positive or negative linear and quadratic responses ($P < 0.05$) were observed for all the FA except for C20:1 and C20:3n-6 in the liver, with increasing dietary PO inclusion level, whereas C20:3n-6 remained unaffected. C16:0, C18:1n-9, C18:2n-6 and C18:3n-6 had a positive linear response and an open upward parabola, whereas the rest had negative linear responses and an open downward parabola as a function of dietary PO inclusion level. Both the DHA/EPA ratio and n-3/n-6 PUFA ratio had a negative linear and an open downward parabola ($P < 0.05$) with increasing dietary PO inclusion levels, and were lower ($P < 0.05$) in the 100% PO substitution diet than the control diet.

The FA in the dorsal muscle followed a similar response to that in the liver, with increasing PO inclusion levels (Table 8). The FA, C18:0, C19:0, and C20:1, remained unaffected by dietary PO inclusion levels, whereas C16:0, C21:0, C18:1n-9, and C18:2n-6 had positive linear responses and an open upward parabola, and the remaining FA had negative linear responses and an open downward parabola ($P < 0.05$) as a function of dietary PO inclusion level. The DHA/EPA ratio did not differ across dietary treatments. In contrast, the n-3/n-6 PUFA ratio had negative linear responses and an open downward parabola ($P < 0.05$) with increasing dietary PO inclusion levels, and was significantly ($P < 0.05$) lower in the 100% PO substitution diet than in the control diet.

3.5. Gene expression related to lipid metabolism in the liver

The relative mRNA expressions of the genes related to lipid metabolism in the liver are presented in Table 9. The mRNA levels of FA synthase (*FAS*), glucose-6-phosphate dehydrogenase (*G6PD*),

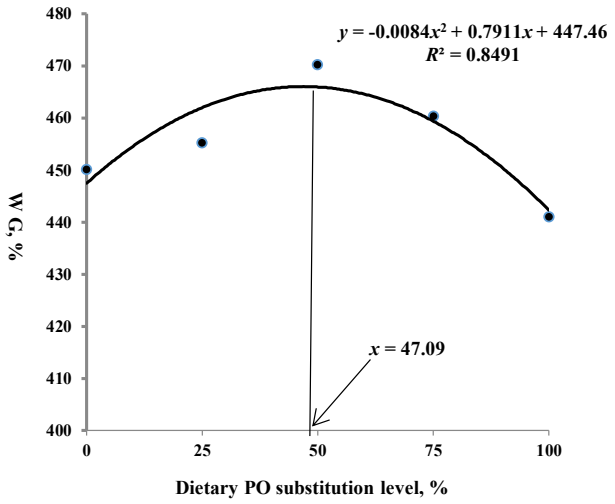

Fig. 2. The relationship between dietary palm oil (PO) substitution levels for marine fish oil and percent weight gain (WG) of groupers (*Epinephelus coioides*) in a 56-d feeding period. Values are means of 3 triplicates per dietary treatment.

lipoprotein lipase (*LPL*), peroxisome proliferator activated receptor (*PPARα*), carnitine palmitoyltransferase-1(*CPT-1*), sterol-regulatory element-binding protein-1c (*SREBP-1c*) and delta-6 fatty acyl desaturase (*Δ6FAD*) had positive linear responses and/or an open upward parabola with increasing dietary PO inclusion levels ($P < 0.05$). In contrast, the mRNA levels of stearoyl-CoA desaturase (*SCD*), hormone-sensitive lipase (*HSL*), adipose triglyceride lipase (*ATGL*), FA binding proteins (*FABP*) and elongase of very long-chain FA-5 (*ELOVL5*) responded to the dietary PO inclusion level in a negative linear and/or an open downward manner ($P < 0.05$). The mRNA level of acetyl-CoA carboxylase (*ACC*) was not affected by the dietary PO inclusion level. The hepatic mRNA levels of *FAS*, *G6PD*, *LPL*, and *CPT-1* genes in fish fed the 100% PO substitution diet were significantly higher ($P < 0.05$) than those in fish fed the 0% to 75% PO substitution diets; the 0% PO and 100% PO substitution diets had higher ($P < 0.05$) *HSL* mRNA levels compared with the 25% to 75% PO substitution diets; *PPARα* mRNA levels in the 50% to 100% PO substitution diets were higher ($P < 0.05$) than those in 0% and 25% PO substitution diets; 75% to 100% PO substitution diets had higher ($P < 0.05$) mRNA levels of *SREBP-1c* and *Δ6FAD* compared to the control diet. However, the mRNA levels of *ATGL*, *FABP*, and *ELOVL5* genes in the control diet were higher ($P < 0.05$) than those in the 100% PO substitution diet.

4. Discussion

4.1. Growth performance

This study showed that fish receiving high PO inclusion diets had a comparable growth performance with those receiving the control diet, suggesting that the diet with 100% PO substitution (9% PO diet) should provide adequate levels of essential FA (DHA and EPA) to support normal growth of juvenile grouper under the present feeding conditions. The DHA and EPA levels in 100% PO substitution diet were provided by 25% fish meal containing 4%

Table 5
Effects of dietary replacement of fish oil (FO) with palm oil (PO) on proximate composition (%:%, wt:wt) of whole-body, muscle and liver of *Epinephelus coioides* in a 56-d feeding period.[1]

Item	Diets[2]					Pooled SEM	*P*-value		
	0% PO	25% PO	50% PO	75% PO	100% PO		ANOVA	Linear	Quadratic
Whole-body									
Moisture	67.48	65.98	66.61	67.01	66.11	0.303	0.549	0.445	0.705
Protein	18.34	19.52	19.39	19.24	19.39	0.151	0.074	0.088	0.046
Lipid	8.10	8.54	8.65	8.24	8.71	0.109	0.332	0.249	0.467
Ash	4.71	4.62	4.61	4.76	4.68	0.055	0.937	0.858	0.911
Muscle									
Moisture	74.48	75.75	74.68	75.49	75.66	0.251	0.404	0.255	0.531
Lipid	1.81	2.07	2.19	2.00	1.96	0.070	0.573	0.669	0.272
Protein	20.27	20.02	21.36	20.78	20.73	0.237	0.101	0.336	0.096
Liver									
Moisture	60.65^{a}	61.56^{a}	60.03^{a}	52.96^{b}	45.27^{b}	1.891	0.003	<0.001	<0.001
Lipid	6.20^{b}	6.07^{b}	7.79^{b}	10.14^{a}	12.28^{a}	0.699	0.001	<0.001	<0.001
Protein	6.99	6.85	6.97	6.93	6.66	0.107	0.904	0.464	0.700

$^{a, b}$ Values in the same row with different superscripts indicate significant differences ($P < 0.05$).
[1] Data are presented as means of 3 replicates per dietary treatment. Statistical analysis was performed by one-way ANOVA, followed by Student-Neuman-Keuls multiple comparison test.
[2] Palm oil replaced 0%, 25%, 50%, 75%, and 100% of FO in the control diet, respectively.

Table 6
Effects of dietary replacement of fish oil (FO) with palm oil (PO) on plasma components (mmol/L) of *Epinephelus coioides* in a 56-d feeding period.[1]

Items	Diets[2]					Pooled SEM	*P*-value		
	0% PO	25% PO	50% PO	75% PO	100% PO		ANOVA	Linear	Quadratic
GLU	4.54	4.17	4.24	4.47	4.34	0.238	0.991	0.954	0.999
TG	1.33^{b}	3.07^{ab}	3.86^{a}	3.27^{ab}	1.90^{ab}	0.362	0.039	0.621	0.022
TC	2.34^{ab}	2.47^{ab}	3.10^{a}	2.17^{ab}	1.83^{b}	0.145	0.042	0.208	0.036
HDL-C	0.48^{ab}	0.47^{ab}	0.62^{a}	0.31^{b}	0.41^{ab}	0.033	0.044	0.207	0.395
LDL-C	2.03	2.36	2.27	2.05	2.18	0.089	0.756	0.988	0.836

GLU = glucose; TG = triglyceride; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol.
$^{a, b}$ Values in the same row with different superscripts indicate significant differences ($P < 0.05$).
[1] The fish were fasted for 24 h before plasma collection. Data are presented as means of 3 replicates per dietary treatment. Statistical analysis was performed by one-way ANOVA, followed by Student-Neuman-Keuls multiple comparison test.
[2] Palm oil replaced 0%, 25%, 50%, 75%, and 100% of FO in the control diet, respectively.

Table 7
Effects of dietary replacement of fish oil (FO) with palm oil (PO) on liver fatty acid composition (mg/g lipid) of *Epinephelus coioides* in a 56-d feeding period.[1]

Fatty acids	Diets[2]					Pooled SEM	*P*-value		
	0% PO	25% PO	50% PO	75% PO	100% PO		ANOVA	Linear	Quadratic
C14:0	14.4^{a}	13.7^{ab}	12.7^{ab}	11.8^{ab}	10.5^{b}	0.476	0.041	0.001	0.002
C16:0	131.4^{d}	139.2^{c}	143.8^{c}	152.4^{b}	163.6^{a}	3.087	<0.001	<0.001	<0.001
C18:0	31.8^{ab}	32.9^{a}	31.7^{a}	30.3^{ab}	26.8^{b}	0.763	0.013	0.077	0.010
C19:0	13.6^{a}	13.4^{a}	7.5^{b}	6.6^{b}	5.8^{b}	1.141	0.025	0.020	0.068
C21:0	3.0^{a}	2.5^{a}	1.6^{b}	1.0^{c}	0.4^{c}	0.245	<0.001	<0.001	<0.001
C22:0	0.9^{a}	0.8^{a}	0.8^{ab}	0.8^{ab}	0.6^{b}	0.028	0.012	<0.001	0.001
ΣSFA	194.5^{b}	202.7^{ab}	198.4^{ab}	203.7^{ab}	208.1^{a}	1.736	0.039	0.027	0.173
C16:1	32.7^{a}	25.8^{b}	23.7^{b}	20.3^{c}	15.3^{d}	1.547	<0.001	<0.001	<0.012
C17:1	3.3^{a}	2.5^{b}	2.6^{b}	2.0^{bc}	1.5^{c}	0.175	<0.001	<0.001	<0.066
C18:1n-9 (OA)	133.6^{e}	161.9^{d}	201.4^{c}	247.7^{b}	273.2^{a}	13.692	<0.001	<0.001	<0.001
C20:1	9.0^{b}	9.2^{a}	10.2^{a}	9.7^{a}	10.5^{a}	0.973	<0.001	0.087	0.209
C24:1	2.9^{a}	2.9^{a}	2.5^{a}	2.4^{a}	1.5^{b}	0.161	0.006	0.023	0.087
ΣMUFA	181.1^{e}	203.5^{d}	241.6^{c}	282.1^{b}	301.2^{a}	12.621	<0.001	<0.001	<0.014
C18:2n-6 (LA)	96.4^{b}	103.4^{ab}	106.9^{ab}	113.2^{ab}	127.7^{a}	4.334	0.008	0.004	0.029
C18:3n-6	0.5^{ab}	0.4^{b}	0.6^{ab}	0.6^{ab}	1.2^{a}	0.043	0.004	0.043	0.097
C20:2n-6	7.8^{a}	6.1^{b}	5.5^{b}	2.2^{c}	3.2^{c}	0.539	<0.001	0.022	0.034
C20:3n-6	1.5^{a}	1.1^{b}	1.2^{ab}	1.3^{ab}	1.2^{ab}	0.074	0.035	0.296	0.373
C20:4n-6 (ARA)	8.5^{a}	8.7^{a}	6.3^{ab}	4.8^{b}	2.6^{c}	0.651	<0.001	0.002	0.002
Σn-6 PUFA	115.6	120.3	120.2	122.8	135.8	3.382	0.448	0.057	0.128
C18:3n-3 (LNA)	15.8^{a}	15.3^{a}	13.1^{b}	12.4^{b}	10.6^{b}	0.546	0.002	<0.001	0.014
C20:5n-3 (EPA)	33.8^{a}	30.5^{a}	24.8^{b}	15.3^{c}	8.7^{d}	7.215	<0.001	<0.001	<0.001
C22:5n-3	21.7^{a}	13.1^{b}	12.3^{b}	8.4^{c}	5.0^{c}	1.547	<0.001	0.009	0.021
C22:6n-3 (DHA)	95.2^{a}	79.0^{b}	58.5^{c}	36.9^{d}	19.3^{e}	2.513	<0.001	<0.001	<0.001
Σn-3 PUFA	166.8^{a}	138.5^{b}	108.3^{c}	71.6^{d}	43.7^{e}	11.543	<0.001	<0.001	<0.001
Σn-3 LC-PUFA	150.6^{a}	122.6^{b}	95.7^{c}	60.8^{d}	32.9^{e}	11.060	<0.001	<0.001	<0.001
DHA/EPA	2.87^{a}	2.63^{a}	2.45^{b}	2.34^{b}	2.19^{b}	0.104	0.021	0.005	0.009
n-3/n-6 PUFA	1.51^{a}	1.14^{b}	0.83^{bc}	0.59^{cd}	0.34^{d}	0.116	<0.001	<0.001	<0.001

FO = fish oil; PO = palm oil; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acid; LC-PUFA = long-chain polyunsaturated fatty acids; OA = oleic acid; LA = linoleic acid; ARA = arachidonic acid; LNA = linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid.
$^{a-e}$ Values in the same row with different superscripts indicate significant differences ($P < 0.05$).
[1] Data are presented as means of 3 replicates per dietary treatment. Statistical analysis was performed by one-way ANOVA, followed by Student-Neuman-Keuls multiple comparison test.
[2] Palm oil replaced 0%, 25%, 50%, 75%, and 100% of FO in the control diet, respectively.

residual FO, which showed that 1% FO in the feed may meet the low EFA requirements of grouper (Wu et al., 2002). Therefore, a reasonable growth performance was observed as fish were fed the diets with PO inclusion. In accordance, several related studies conducted in other marine and freshwater carnivorous fishes have shown no negative effects of FO replacement (Bell et al., 2002; Ng et al., 2004; Fonseca-Madrigal et al., 2005; Shapawi et al., 2008; Aliyu-Paiko and Hashim, 2012; Ahmad et al., 2013; Huang et al., 2016; Sanchez-Moya et al., 2020). However, the WG showed an open upward parabola and reached a peak at the 50% PO substitution diet (4.5% PO diet), as observed with a peak at the diets with 25% (2.25% PO diet) or 50% PO substitution diets in previous studies in rainbow trout (Fonseca-Madrigal et al., 2005), African catfish (Ng et al., 2004), and in grouper (Shapawi et al., 2008; Gudid et al., 2020), indicating a synergistic effect on growth. Although the lowest value was achieved at the 100% PO substitution diet, the value was comparable to that of the diets with 0% PO substitution (9% FO diet = the control diet), which was consistent with the observations by Fonseca-Madrigal et al. (2005), indicating that the replacement of PO in FO diets has no negative effects on fish growth. In contrast, feeding with the diets with 60% PO substitution (6.0% PO; Gao et al., 2012; Huang et al., 2016; Li et al., 2019) and/or 100% PO substitution (6.5% PO; Mu et al., 2020) led to reduced growth, feed utilization, HSI, and CF of large yellow croaker, Chu's croaker, and Japanese sea bass, compared with the control diet. Furthermore, the dietary PO inclusion level of more than 40% has been reported to significantly reduce the growth and feed utilization of Japanese flounder (Han et al., 2015), as a result of lowered digestibilities of FA in PO vs. in FO (Ng et al., 2003). It may be impractical to replace added FO completely when the diet contains a lower level (<40%) of fish meal, due to the low residual FO available in a low fish meal diet (Deng et al., 2014). These findings indicated that the feed utilization abilities of different fishes vary with their species, growth stage and dietary ingredients (Bell et al., 2002; Gudid et al., 2020).

4.2. Tissue proximate composition

Previous studies in different fishes have shown that dietary PO inclusion level did not affect whole-body proximate composition (Shapawi et al., 2008; Aliyu-Paiko and Hashim, 2012; Deng et al., 2014; Huang et al., 2016). On the contrary, in this study, the whole-body protein content showed an open upward parabola and reached a peak at 25% PO substitution level; although the moisture, lipid and ash contents in the whole-body were not affected. The dietary 50% PO substitution level resulted in marginal protein retention in the muscle compared to the control diet, though no difference in protein contents among dietary treatments has been observed in previous studies (Lim et al., 2001; Bell et al., 2002; Turchini et al., 2011a,b). Furthermore, these findings were consistent with the growth of the groupers, suggesting that appropriate PO addition in feed could allow a better utilization of dietary protein for growth due to enhanced protein and energy metabolism by the degree of saturation of FA of lipids as suggested in fishes (NRC, 2011; Lu et al., 2014). This was attributable to the preference for SFA and MUFA over PUFA in fish (Henderson, 1996; Turchini et al., 2011a,b; Rombenso et al., 2018). The increased protein retention was confirmed in African catfish (Lim et al., 2001) and Murray cod (Turchini et al., 2011a,b) receiving the diet with 50% and 100% PO substitution diets (8% PO diet and 13.2% PO diet respectively) over the control diet. The sparing protein of PO for fish growth was explained by providing a suitable FA profile that promotes

Table 8
Effects of dietary replacement of fish oil (FO) with palm oil (PO) on dorsal muscle fatty acid composition (mg/g lipid) of *Epinephelus coioides* in a 56-d feeding period.[1]

Fatty acids	Diets[2]					Pooled SEM	*P*-value		
	0% PO	25% PO	50% PO	75% PO	100% PO		ANOVA	Linear	Quadratic
C14:0	23.4^{a}	20.1^{b}	15.3^{c}	13.4^{cd}	11.0^{d}	1.258	<0.001	<0.001	<0.001
C16:0	132.3^{a}	138.9^{ab}	140.8^{ab}	144.7^{a}	150.5^{a}	1.909	0.011	0.003	0.023
C18:0	41.5	40.7	39.6	40.1	38.9	0.333	0.281	0.244	0.415
C19:0	24.4	23.1	23.6	25.5	25.8	1.132	0.356	0.189	0.307
C21:0	3.5^{ab}	4.6^{a}	3.1^{ab}	2.2^{b}	2.0^{b}	0.296	0.020	0.020	0.034
C22:0	1.3^{c}	1.1^{b}	1.1^{bc}	1.0^{cd}	0.9^{d}	0.030	<0.001	0.009	0.011
ΣSFA	225.9	229.1	225.6	229.2	231.1	1.384	0.267	0.132	0.219
C16:1	35.2^{a}	29.2^{b}	21.8^{c}	17.0^{d}	15.5^{d}	2.028	<0.001	0.002	0.009
C17:1	2.8^{a}	2.4^{ab}	2.1^{bc}	1.9^{bc}	1.6^{c}	0.111	0.002	<0.001	<0.001
C18:1n-9 (OA)	131.8^{c}	165.5^{d}	186.4^{c}	218.8^{b}	237.4^{a}	9.872	<0.001	<0.001	<0.001
C20:1	7.3^{a}	5.4^{b}	6.6^{a}	6.5^{a}	4.4^{b}	2.028	<0.001	0.216	0.319
C24:1	4.6^{a}	4.2^{ab}	4.0^{ab}	3.7^{ab}	3.3^{b}	0.170	0.034	0.007	0.026
ΣMUFA	182.2^{c}	207.3^{b}	219.5^{ab}	247.7^{b}	261.8^{a}	7.474	0.002	0.003	0.010
C18:2n-6 (LA)	101.4^{e}	113.1^{d}	122.2^{c}	133.6^{b}	146.7^{a}	3.331	<0.001	<0.001	0.001
C18:3n-6	8.1^{a}	6.5^{b}	4.3^{c}	3.1^{c}	1.2^{d}	0.651	<0.001	<0.001	<0.001
C20:2n-6	15.4^{a}	13.2^{b}	5.6^{c}	3.9^{d}	2.6^{e}	1.347	<0.001	<0.001	<0.001
C20:3n-6	0.8^{a}	0.7^{ab}	0.7^{ab}	0.7^{ab}	0.6^{b}	0.032	0.028	0.042	0.205
C20:4n-6 (ARA)	6.5^{a}	5.6^{ab}	5.2^{bc}	4.4^{c}	2.7^{d}	0.348	<0.001	<0.001	<0.001
Σn-6 PUFA	132.7^{e}	139.7^{bc}	137.9^{bc}	146.2^{b}	153.8^{a}	2.168	0.001	0.002	0.034
C18:3n-3 (LNA)	17.3^{d}	13.5^{b}	14.6^{c}	11.4^{d}	8.6^{e}	0.725	<0.001	0.003	0.041
C20:5n-3 (EPA)	37.4^{a}	30.6^{b}	23.1^{c}	18.2^{d}	14.6^{e}	2.235	<0.001	<0.001	<0.001
C22:5n-3	11.8^{a}	9.3^{b}	8.3^{b}	6.9^{c}	5.6^{c}	0.562	<0.001	<0.001	<0.001
C22:6n-3 (DHA)	85.0^{a}	69.6^{b}	62.8^{b}	47.5^{c}	37.1^{d}	4.396	<0.001	<0.001	<0.001
Σn-3 PUFA	151.1^{a}	122.7^{b}	106.3^{c}	84.1^{d}	66.4^{e}	7.067	<0.001	<0.001	<0.001
Σn-3 LC-PUFA	132.5^{a}	108.7^{b}	95.4^{c}	72.5^{d}	57.8^{e}	6.956	<0.001	<0.001	<0.001
DHA/EPA ratio	2.27	2.33	2.60	2.61	2.54	0.056	0.191	0.141	0.212
n-3/n-6 PUFA ratio	1.13^{a}	0.86^{b}	0.80^{b}	0.59^{c}	0.47^{d}	0.064	<0.001	<0.001	<0.001

FO = fish oil; PO = palm oil; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acid; LC-PUFA = long-chain polyunsaturated fatty acids; OA = oleic acid; LA = linoleic acid; ARA = arachidonic acid; LNA = linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid.
$^{a-e}$ Values in the same row with different superscripts indicate significant differences ($P < 0.05$).
[1] Data are presented as means of 3 replicates per dietary treatment. Statistical analysis was performed by one-way ANOVA, followed by Student-Neuman-Keuls multiple comparison test.
[2] Palm oil replaced 0%, 25%, 50%, 75%, and 100% of FO in the control diet, respectively.

Table 9
Effects of dietary replacement of fish oil (FO) with palm oil (PO) on relative mRNA expression levels of the genes related to lipid metabolism in the liver of *Epinephelus coioides* in a 56-d feeding period.[1]

Genes	Diets[2]					Pooled SEM	*P*-value		
	0%PO	25%PO	50%PO	75%PO	100% PO		ANOVA	Linear	Quadratic
FAS	0.12^{b}	0.23^{b}	0.17^{b}	0.23^{b}	0.83^{a}	0.041	<0.001	0.002	0.011
ACC	0.22	0.29	0.31	0.26	1.04	0.055	0.057	0.276	0.269
G6PD	0.66^{b}	0.62^{b}	0.57^{b}	0.63^{b}	0.99^{a}	0.038	0.001	0.048	0.132
SCD	1.51	0.93	0.75	0.86	0.83	0.133	0.362	0.009	0.033
LPL	0.37^{d}	0.79^{c}	0.40^{d}	1.15^{b}	2.23^{a}	0.108	<0.001	0.001	<0.001
HSL	1.24^{a}	0.37^{c}	0.51^{c}	0.77^{b}	1.11^{a}	0.063	<0.001	0.083	<0.001
PPARα	0.71^{b}	0.64^{b}	1.04^{a}	1.06^{a}	1.58^{a}	0.069	<0.001	0.006	0.012
CPT-1	0.95^{b}	0.64^{b}	0.49^{b}	0.61^{b}	1.75^{a}	0.072	<0.001	0.313	0.028
ATGL	1.20^{a}	0.30^{b}	0.23^{b}	0.18^{b}	0.18^{b}	0.072	<0.001	<0.001	<0.001
SREBP-1c	0.56^{b}	0.80^{ab}	0.86^{ab}	1.33^{a}	2.01^{a}	0.182	<0.001	0.030	0.070
Δ6FAD	0.87^{b}	2.48^{b}	0.98^{b}	2.30^{b}	12.98^{a}	0.657	<0.001	0.029	0.020
FABP	1.75^{a}	1.81^{a}	1.19^{b}	0.75^{b}	0.75^{b}	0.100	<0.001	<0.001	<0.001
ELOVL5	1.21^{a}	1.01^{ab}	0.73^{ab}	1.43^{a}	0.34^{b}	0.112	0.014	0.323	0.029

FO = fish oil; PO = palm oil; *FAS* = fatty acid synthase; *ACC* = acetyl-CoA carboxylase; *G6PD* = glucose-6-phosphate dehydrogenase; *SCD* = stearoyl-CoA desaturase; *LPL* = lipoprotein lipase; *HSL* = hormone-sensitive lipase; *PPARα* = peroxisome proliferator activated receptor α; *CPT* = carnitine palmitoyl transferase; *ATGL* = adipose triglyceride lipase; *SREBP-1c* = sterol-regulatory element-binding protein-1c; *Δ6FAD* = delta-6 fatty acyl desaturase; *FABP* = fatty acid binding proteins; *ELOVL* = elongase of very long-chain fatty acids.
$^{a-c}$ Values in the same row with different superscripts indicate significant differences ($P < 0.05$).
[1] Data are presented as means of 3 replicates per dietary treatment. Statistical analysis was performed by one-way ANOVA, followed by Student-Neuman-Keuls multiple comparison test.
[2] Palm oil replaced 0%, 25%, 50%, 75%, and 100% of FO in the control diet, respectively.

mitochondrial β-oxidation for more energy production when fish are fed a 100% PO substitution diet (13.2% PO diet; Lim et al., 2001), which may be attributed to the synergistic effect of mixing FAs. There still is a certain amount of FO in the 100% PO substitution diet, which is provided by the residual FO in the fish meal. On the other hand, we observed that the dietary oleic acid (OA) and linoleic acid (LA) levels varied significantly (ranging from 16.1% to 40.8% and 12.1% to 19.5% respectively) in response to dietary PO inclusion levels, on account of the higher proportion of OA and LA in PO than FO. This results in increased liver lipid accumulation at high dietary

PO substitution levels (75% and 100% PO substitution diets, respectively) in this study and previous studies with fish and rats (Aliyu-Paiko and Hashim, 2012; Muhlhausler and Ailhaud, 2013; Huang et al., 2016; Mu et al., 2020). The high lipid accumulation may be attributed to an imbalance between hepatic lipid synthesis, β-oxidation, and transport in tissue cells (Posti and Girard, 2008; Musso et al., 2009; Viscarra and Sul, 2020), leading to the lipid metabolic syndrome of the liver (Fountoulaki et al., 2009; Marchix et al., 2020). On the contrary, lipid retention in the liver has been shown to be unaffected by PO inclusion levels in a few studies (Bell et al., 2002; Li et al., 2019). These findings collectively suggest that PO supplementation could have different effects on lipid retention in the liver of different fishes.

4.3. Plasma biochemical components

The plasma biochemical components are the intermediary products of some metabolic processes of fish and strongly reflect the nutritional status of fish across a feeding period (Ye et al., 2011). Gudid et al. (2020) reported a hypocholesterolemic effect on the same fish species when the dietary plant oil substitution for FO increased. This response occurred in other fish in a study with soybean oil substitution for FO (Peng et al., 2008). Inconsistent with those observations, in the present study, we observed the plasma TG and TC level exhibited an open upward parabola with increasing dietary PO substitution level and reached a peak at 50% PO substitution level, and the changes were paralleled with the growth of grouper. Although there were no linear and quadratic responses of plasma HDL-C level to increasing PO substitution level, the 50% PO substitution diet maintained a relatively high HDL-C level compared with other dietary treatments. The higher plasma levels of TG, TC, and HDL-C in fish receiving the 50% PO substitution diet may reflect a relatively balanced state of hepatic lipid metabolism of the fish species, in comparison with other diets. The improved FA utilization in feed may be one of the factors contributing to the enhancement of growth and feed utilization due to the improved and balanced FA profile caused by a proper blend of PO and FO (Monge-Ortiz et al., 2018; Riera-Heredia et al., 2020). The lipid-promoting effect on fish fed a moderate PO substitution diet was also observed in studies in other fishes (Lim et al., 2001; Huang et al., 2016). Furthermore, the high PO substitution levels did not affect the grouper's growth performance compared to the control diet, though it showed enhanced growth at 50% PO substitution level. These results indicated moderate alterations in lipid homeostasis by replacing FO with a relatively high level of vegetable oils (13.7% PO and other vegetable oils blend) without adverse effects on growth performance (Fukada et al., 2020; Sanchez-Moya et al., 2020), supporting the dietary use of vegetable oils, including PO for the sustainable production of important farmed marine fish species including grouper.

4.4. Tissue fatty acid profile

The FA compositions of lipids in the fish fillet are easily influenced by the FA composition of lipids in the feed (Bell et al., 2002). In this study, increasing PO inclusion level in the feed elevated the contents of C16:0, C18:1n-9 and C18:2n-6 both in the muscle and liver, but the contents of other FA were decreased. In accordance, several studies have reported similar effects on FA contents (Bell et al., 2002; Fonseca-Madrigal et al., 2005; Ng et al., 2007) using either vegetable oils alone or their blend (Emre et al., 2016; Monge-Ortiz et al., 2018). Interestingly, the changing trend of n-3/n-6 PUFA ratio in the dorsal muscle and liver was paralleled with that in the diets in response to the incremental dietary PO level, reflecting the FA profile of the diet. The n-3/n-6 PUFA ratio (0.82-1.0) in feed is also a vital reference index for consideration of feed formulations (Jin et al., 2019; Ma et al., 2019), as the imbalance of n-3 and n-6 PUFA could cause a series of adverse physiological consequences (Bagga et al., 2003; Berge et al., 2009; Jin et al., 2019; Lima et al., 2019; Ma et al., 2019; Sanchez-Moya et al., 2020), including hepatic lipid deposition (Lemaire et al., 1991; Robaina et al., 1998). Here, we report that 50% PO substitution level resulted in the highest growth with an n-3/n-6 PUFA ratio of 0.68 in feed for proper growth of grouper, which was in accordance with that reported for black seabream (Jin et al., 2019). In addition, a diet at 100% PO substitution level (lowest n-3/n-6 PUFA ratio) diet had comparable growth to the control diet (highest n-3/n-6 PUFA ratio), indicating that even the lowest n-3/n-6 PUFA ratio diet could maintain relatively good growth. On the contrary, dietary n-3/n-6 PUFA ratio was found to be negatively correlated with liver lipid content ($R = -0.996$; $P = 0.008$), as observed in Chu's croaker (Huang et al., 2016). A lower dietary n-3/n-6 PUFA ratio may trigger LA and linolenic acid (LNA) transport to the intermediary metabolism for energy production but stimulate DHA deposition in the liver and muscle (Bandarra et al., 2011; Turchini et al., 2011a,b). Thus, it is understandable that the lowest n-3/n-6 PUFA ratio in 100% PO diet contributed to the maximum lipid accumulation in the liver (Bell et al., 2003; Bandarra et al., 2011; Jin et al., 2019). Therefore, high PO substitution levels could reduce the flesh quality and fillet nutritional value from the perspective of the edible value of fish (Yildiz et al., 2018; Yu et al., 2019; Álvarez et al., 2020). In this regard, the potential effect of long-term use of PO in feed on fish flesh quality needs to be evaluated in the future studies.

The individual DHA and EPA in feed play various important physiological roles for most marine fishes (Trushenski et al., 2012; Tocher et al., 2019), as does the DHA/EPA ratio in feed (Ibeas et al., 1997; Zhang et al., 2019). This significant property of the DHA/EPA ratio is usually considered in feed formulations of farmed marine fish. The dietary DHA/EPA ratio ranged between 1 and 2 for most marine fish (NRC, 2011; Zuo et al., 2012; Ma et al., 2014; Xu et al., 2016; Chen et al., 2017; Jin et al., 2017; Xu et al., 2018; Zhang et al., 2019). In this study, the dietary DHA/EPA ratios (1.37–1.55) are almost identical for the test diets regardless of PO substitution level and are within the aforementioned range. The contents of DHA and EPA were decreased with increasing dietary PO inclusion levels, and the hepatic DHA/EPA ratio showed a declining trend as well, possibly because of their different utilization and deposition in the fish (Turchini et al., 2011a,b).

4.5. Hepatic gene expression related to lipid metabolism

Lipid metabolism in vertebrates is mainly divided into two parts: FA synthesis and β-oxidation. The enzymes involved in FA synthesis include FAS, ACC, G6PD, and SCD, whereas the lipolytic enzymes include LPL, ATGL, and HSL in adipose tissues (Musso et al., 2009; Saponaro et al., 2015). The CPT-1 is a rate-limiting enzyme that mediates the transport of long-chain FA into the mitochondria for subsequent β-oxidation (Musso et al., 2009; Lu et al., 2014). The FABP mainly bind and transport FA (Torstensen et al., 2009). The lipid-activated transcription factor, PPARα, is considered to be primarily involved in regulating glucose and lipid homeostasis (Saponaro et al., 2015). The *PPARα* activation by n-3 LC-PUFA may induce lipolytic genes expression and subsequently increase CPT-1 activity, thus enhancing FA β-oxidation (Poudyal et al., 2011). The sterol-regulatory element-binding protein (SREBP) is a member of the membrane-bound transcription factors that control sterol and FA biosynthesis through the regulation of genes required for hepatic triglyceride synthesis, including *ACC*, *FAS*, and *SCD* in animal cells (Treeprasertsuk et al., 2011). Dietary supplementation of EPA or DHA reduced the gene expression of

Δ6FAD and *FAS*, *ACC*, and *G6PD* in Atlantic salmon, gilthead sea bream, and rainbow trout, which could be the result of repressed *SREBP-1* expression by n-3 PUFA (Alvarez et al., 2000; Minghetti et al., 2011; Magalhaes et al., 2020). In the present study, we observed an upregulated expression of *PPARα* and *CPT-1* in a 100% PO substitution diet compared to the control diet, which was in synchrony with Vestergren et al. (2013), who reported an upregulation of *PPARα* and *CPT-1* genes in rainbow trout fed a 100% linseed oil substitution diet (20.0% linseed oil diet) vs. FO diet (19.3% FO diet). We observed an upregulation *SREBP-1c* gene in the 100% PO substitution diet but a restricted expression in fish fed the control diet, which further confirmed that high levels of n-3 PUFA could lead to a repression of the *SREBP-1c* gene. Therefore, a high mRNA level of *SREBP-1c* in a 100% PO substitution diet due to low n-3 PUFA supply could enhance the hepatic lipid deposition. Increasing dietary PO inclusion level led to increased dietary levels of Σn-6 PUFA and decreased levels of Σn-3 PUFA, Σn-3 LC-PUFA, and n-3/n-6 PUFA ratio. The upregulation of *FAS, G6PD, LPL*, and *PPARα* genes with increasing dietary PO inclusion level, was in synchrony with the increase in Σn-6 PUFA, and the decrease either in hepatic and muscular Σn-3 PUFA in the present study and in previous studies (Jin et al., 2017), indicating an enhanced lipogenesis induced by the upregulation of these genes due to the decrease of dietary n-3 PUFA level (Jin et al., 2017). Meanwhile, the mRNA levels of *SCD*, *ATGL*, and *FABP* responded to the dietary PO inclusion level in a negative linear and/or quadratic manner, which can be linked to the reduced n-3 LC-PUFA deposition in the liver in response to increasing dietary PO inclusion. In this case, a decreased β-oxidation and eventually declined hepatic lipid consumption in the higher PO groups could have triggered an enhanced accumulation of lipid in the hepatocytes (Torstensen et al., 2009).

Hepatic lipid deposition is associated with FA uptake and lipogenesis, and LPL mediates uptake of FA by tissues (Musso et al., 2009; Lu et al., 2014; Saponaro et al., 2015). In the present study, the upregulated expression of hepatic *LPL* gene in the 100% PO fed fish was comparable to 100% FO fed fish. This was in contrast to what has been shown in rainbow trout and European seabass: dietary 60% vegetable oils inclusion did not affect hepatic lipogenesis and activity of *LPL* in the liver and adipose tissues (Richard et al., 2006a, 2006b). The elongation process is catalyzed by elongases of very-long-chain FA (ELOVL) that act as the first step of the elongation pathway of FAs, among which ELOVL5 displays high elongation activity towards LC-PUFA (Morais et al., 2009). The Δ6FAD is a rate-limiting enzyme in the biosynthetic pathway of highly unsaturated FA (HUFA) that converts polyunsaturated FA (PUFA) such as LA and LNA into HUFA (Izquierdo et al., 2008; Zou et al., 2019; Torres et al., 2020). Dietary 60% vegetable oils (soybean oil, rapeseed oil, linseed oil and their blend) substitution for FO stimulated Δ6FAD activity in gilthead seabream and seabass (Izquierdo et al., 2003). Likewise, a significantly upregulated expression of *Δ6FAD* and/or *ELOVL5* genes was observed in the liver of Atlantic cod (Tocher et al., 2006; Xue et al., 2014) and Atlantic salmon (Bell et al., 2002) when they are fed 100% plant oil (camelina oil or PO) substitution diets (9.7% camelina oil and 24.1% PO diets for Atlantic cod and Atlantic salmon, respectively), in comparison with those fed the control diets (8.8% and 28.0% FO diets for Atlantic cod and Atlantic salmon, respectively), which has been shown to be associated with high 18:3n-3 and/or low n-3 LC-PUFA levels in the plant oil-rich diets (Tocher et al., 2006; Xue et al., 2014). Similarly, enhanced expression of hepatic *Δ6FAD* gene in groupers fed 100% PO substitution diet vs. the control diet was observed in our current study. By contrast, there was a restricted expression of the *ELOVL5* gene in the 100% PO substitution-fed fish vs. other treatments. These inconsistent results indicate that the repertoire and function of genes encoding FAD and ELOVL varies among fish species, thus determining the extent to which various fish species can biosynthesize LC-PUFA such as EPA and DHA from C18 PUFA. Our current results provide useful information to better understand the difference of vegetable oil utilization by fish and the reasons behind it.

5. Conclusion

This study showed that the growth rate, proximate composition in tissue, and plasma TG and TC levels showed positive linear responses and/or an open upward parabola with increasing dietary PO inclusion level. Feeding a 50% PO substitution diet (4.5% PO diet) resulted in maximum growth, possibly due to the synergistic effect of FO and PO mixed in an appropriate proportion, and dietary PO substitution levels at above 50% had a growth comparable to the control diet. However, increasing dietary PO inclusion level decreased the tissue contents of n-3 PUFA, EPA, DHA and n-3/n-6 PUFA and affected the expression of FA metabolism-related genes. These results indicate that all the alterations in the genes related to FA metabolism reflected an enhancement of the bioconversion pathway of PUFA to LC-PUFA in the case of a deficiency of n-3 LC-PUFA. Therefore, maintaining an optimum dietary ratio of LC-PUFA will be relevant in the plant oil-rich fish feed.

Author contributions

Yingmei Qin: Methodology, Data curation, Writing-Original draft preparation; **Lingyun He:** Investigation, Data curation, Writing-Original draft preparation; **Yanfei Wang:** Validation, Investigation; **Dong Li:** Investigation, Visualization; **Weijun Chen:** Investigation, Resources; **Jidan Ye:** Conceptualization, Writing-Reviewing and Editing, Supervision, Project administration.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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