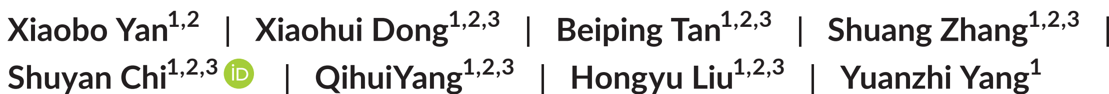
Received: 27 May 2020 | Revised: 1 September 2020 | Accepted: 6 September 2020

DOI: 10.1111/anu.13168

ORIGINAL ARTICLE

Aquaculture Nutrition WILEY

# Effects of alternative dietary oils on lipid metabolism and related gene expression in hybrid grouper (♀*Epinephelus fuscoguttatus* × ♂*E. lanceolatu*)



Xiaobo Yan[1,2] | Xiaohui Dong[1,2,3] | Beiping Tan[1,2,3] | Shuang Zhang[1,2,3] | Shuyan Chi[1,2,3] | QihuiYang[1,2,3] | Hongyu Liu[1,2,3] | Yuanzhi Yang[1]

[1]Laboratory of Aquatic Nutrition and Feed, College of Fisheries, Guangdong Ocean University, Zhanjiang, China

[2]Aquatic Animals Precision Nutrition and High Efficiency Feed Engineering Research Center of Guangdong Province, Zhanjiang, China

[3]Key Laboratory of Aquatic, Livestock and Poultry Feed Science and Technology in South China, Ministry of Agriculture, Zhanjiang, China

**Correspondence**
Xiaohui Dong, Beiping Tan, Laboratory of Aquatic Animal Nutrition and Feed, College of Fisheries, Guangdong Ocean University, Zhanjiang 524088, China.
Emails: dongxiaohui2003@163.com, bptan@126.com

**Funding information**
National Key R&D Program of China, Grant/Award Number: 2019YFD0900201; National Natural Science Foundation of China, Grant/Award Number: NSFC31972808; Postgraduate Education Innovation Project of Guangdong Ocean University, Grant/Award Number: 202028; Agriculture Research System of China, Grant/Award Number: CARS-47; "Chong Yi Liu" project of Guangdong Ocean University, Grant/Award Number: 231419011

## Abstract

An 8-week growth trial was conducted to evaluate effects of dietary oil sources on growth, enzymes activity and genes expression levels related to lipid metabolism of hybrid grouper (♀*Epinephelus fuscoguttatus* × ♂*E. lanceolatu*) juveniles. Seven iso-lipid (97 g/kg of dry matter) and iso-protein (503.5 g/kg of dry matter) experimental diets were formulated containing 50 g/kg fish oil (FO; acting as controls) or various vegetable oils (VOs): corn oil (CO), sunflower oil (SO), tea oil (TO), olive oil (OO), rice oil (RO) and mixed oil (MO; comprising equal amounts of these oils). Each diet was fed to triplicate groups of 40 fish for per repetition (15.09 ± 0.01 g) for 56 days. The results show that (a) alternative dietary oils had no significant effects on final weight compared with control group ($p > .05$); (b) compared with FO group, VOs significantly changed the contents of serum lipoproteins, cholesterol, triglycerides and the activity of liver lipid-metabolizing enzymes ($p < .05$); (c) CO group had the least effect on the serum lipoproteins, triglycerides and cholesterol of grouper compared with control; the activity of liver lipid-metabolizing enzymes in RO and control group was the closest; (d) the mRNA levels of Δ6 Fatty acid desaturase (Δ6Fad), hormone-sensitive lipase (HSL) and lipoprotein lipase (LPL) were not significantly effected by lipid sources, but CO, TO, OO and MO significantly down-regulated the expression of fatty acid synthetase (FAS) mRNA level in liver, while RO opposite ($p < .05$); (e) vegetable oil significantly up-regulated peroxisome proliferator-activated receptor α (PPARα) and peroxisome proliferator-activated receptor β (PPARβ) mRNA levels, while TO and RO down-regulated peroxisome proliferator-activated receptor γ (PPARγ) mRNA levels ($p < .05$); and 6) MO significantly increased the mRNA levels of heart-type fatty acid-binding protein (H-FABP) and adipocyte-type fatty acid-binding protein (A-FABP) ($p < .05$), while other VOs had no effect on them ($p > .05$). In conclusion, dietary substitution of FO by VO in diet affected lipid metabolism of grouper, which may be regulated by PPARs.

**KEYWORDS**

fish oil, hybrid grouper, lipid metabolism, vegetable oil

 © 2020 John Wiley & Sons Ltd 

## 1 | INTRODUCTION

Fresh water fish can convert C18-unsaturated FAs into long-chain polyunsaturated FAs (LC-PUFA), while sea water fish lack of this transformation ability (Ghioni et al., 1999; Tocher & Ghioni, 1999); therefore, a certain amount of LC-PUFA must be added to the diets of sea water fish (Bostock et al., 2010; Tidwell & Allan, 2015). Fish oil (FO) is an important high-quality lipid source for aquatic animals due to its high level of n-3 high unsaturated fatty acid (HUFA) (Calder, 2012). However, the demand for FO is increasing with the rapid development of the aquaculture industry, and the price of FO is rising gradually, which makes the cost of aquaculture keep rising. It has become one of the hotspots in the field of aquatic animal nutrition and feed science to find the substitute of FO. Vegetable oils (VO) have the advantages of high yield, wide source and low price, which make it become the preferred source substitute for FO. At present, corn oil (Lin & Shiau, 2007), sunflower oil (Bransden et al., 2003), tea seed oil (Han et al., 2013), olive oil (Li, et al., 2019) and rice oil (Gandomkar et al., 2013) have been used in aquatic animal feed production. Nevertheless, due to the large amount of linoleic acid (c18:2n-6) and linolenic acid (c18:3n-3) in VO, and the relative lack of LC-PUFA, replacing FO with VO will lead to the decrease of the content of LC-PUFA in diets (Yıldız et al., 2018), which will affect the lipid metabolism of fish, and further affect the antioxidant and anti-stress ability of fish (Peng et al., 2016).

The genome-wide expression analysis showed that peroxisome proliferator-activated receptors (PPARs) is a ligand-dependent transcription factor regulating genes related to lipid metabolism (Cunha et al., 2015). And LC-FA including n-3 HUFA are ligands of PPARs (Schoonjans et al., 1996). The liver is the most important site for vertebrates to metabolic FAs (FAs) from food, and it is also the main site for the enhancement of β-oxidation of FAs (Lock et al., 1989). Fatty acid-binding protein (FABP) can transport FAs to different sites for metabolism or storage. FABP is widely distributed in tissues and has a high affinity for fatty acids. It can transport FA from cell membrane to intracellular utilization site and plays an important role in FA metabolism (Di Pietro & Santomé, 2001). However, there are few studies on the effects of lipid sources on lipid metabolism and PPARs or FABP family genes in fish (Sarameh et al., 2019).

Groupers are an important aquaculture species in coastal areas of China. The hybrid grouper (♀*Epinephelus fuscoguttatus* × ♂*E. lanceolatu*) is a marine carnivorous fish which has the high economic and nutritional value and excellent taste (Wu et al., 2017). Studies have shown that the hybrid grouper has better growth performance and resistance compared with its parental fish (Faudzi et al., 2018). Meanwhile, the convenience of transportation makes it one of the most important species in China aquaculture markets. At present, there are many researches on the nutrition of juvenile hybrid grouper (Jiang et al., 2016; Wu et al., 2017; Yin et al., 2018), but the research on the relationship between lipid source and lipid metabolism is limited (Li, et al., 2019). Therefore, the present study aimed to compare effects of VO substitute for FO on liver enzymes activity and gene expression related to lipid metabolism of juvenile hybrid grouper (♀*Epinephelus fuscoguttatus* × ♂*E. lanceolatu*).

## 2 | MATERIAL AND METHODS

### 2.1 | Experimental diets

Seven iso-lipid and iso-protein diets were formulated containing 50 g/kg FO (acting as controls), corn oil (CO), sunflower oil (SO), tea oil (TO), olive oil (OO), rice oil (RO) and mixed oil (MO; comprising equal amounts of these oils), respectively. All ingredients were crushed and sieved through a sixty mesh sieve, then thoroughly mixed using the progressive enlargement method, as described by Ayisi and Zhao (2017). Then added oil and lecithin, rubbed them manually, putted them into V-type vertical mixer after sieving and mixed them evenly, and then added distilled water (300–400 ml/kg) to mix them evenly. The diets were processed into 2.0 mm and 2.5 mm diameter pellets by a twin screw extruder (F–26, South China University of Technology, Guangdong Province, China), air-dried to about 100 g/kg of feed moisture content at room temperature, then ground and sieved to an appropriate size and stored in ziploc bags at −20°C until use. The ingredients and approximate compositions of the trial diets were shown in Tables 1 and 2.

### 2.2 | Fish and feeding trial

All animal experiments were conducted strictly based on the recommendations in the "Guide for the Care and Use of Laboratory Animals" set by the National Institutes of Health. The animal protocols were approved by the Animal Ethics Committee of Guangdong Ocean University (Zhanjiang, China). Hybrid groupers (♀*Epinephelus fuscoguttatus* × ♂*E. lanceolatu*) were purchased from a local hatchery at Nanshan Island (Zhanjiang China) and acclimatized to the experimental conditions for one week while being fed with a commercial diet at the Donghai Island Breeding base of Guangdong Ocean University (Zhanjiang China). After fasting for 24 hr, 840 hybrid groupers (mean initial body weight ± standard error = 15.09 ± 0.01 g) were randomly distributed into 21 tanks (1,000 L; 0.8 m in water depth). Each type of experimental feed was fed to triplicate groups of fish twice daily (08:00 and 16:00) until apparent satiation was observed. The amount of food ingestion was recorded for 8 weeks. About 500 L water was exchanged to maintain water quality every day. Each tank was provided with one piece of polyvinylchloride (PVC) pipe of 20.0 cm (diameter) × 30.0 cm (length) as shelter for the fish (Lin et al., 2007). The temperature of the water ranged from 29–32°C, salinity was 28, dissolved oxygen was >7 mg/L, while nitrates remained <0.03 mg/L.

**TABLE 1** Composition and nutrients levels of the test diets (air dry matter g/kg)

| Ingredients | Test diets | | | | | | |
|---|---|---|---|---|---|---|---|
| | FO | CO | SO | TO | OO | RO | MO |
| Fish meal | 430.0 | 430.0 | 430.0 | 430.0 | 430.0 | 430.0 | 430.0 |
| Soybean meal | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 |
| Soybean protein concentrate | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 |
| Casein | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 |
| Wheat gluten | 130.0 | 130.0 | 130.0 | 130.0 | 130.0 | 130.0 | 130.0 |
| Wheat flour | 150.0 | 150.0 | 150.0 | 150.0 | 150.0 | 150.0 | 150.0 |
| Phospholipid | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 |
| Fish oil | 50.0 | – | – | – | – | – | – |
| Corn oil | – | 50.0 | – | – | – | – | – |
| Sunflower oil | – | – | 50.0 | – | – | – | – |
| Tea oil | – | – | – | 50.0 | – | – | – |
| Olive oil | – | – | – | – | 50.0 | – | – |
| Rice oil | – | – | – | – | – | 50.0 | – |
| Mixture oil | – | – | – | – | – | – | 50.0 |
| Vitamin premix[a] | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Mineral premix[a] | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Calcium monophosphate | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 |
| Antioxidant | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Attractant | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Choline chloride | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Sodium carboxymethyl cellulose | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 |
| Cellulose microcrystalline | 16.5 | 16.5 | 16.5 | 16.5 | 16.5 | 16.5 | 16.5 |
| Total | 1,000.0 | 1,000.0 | 1,000.0 | 1,000.0 | 1,000.0 | 1,000.0 | 1,000.0 |
| Proximate composition | | | | | | | |
| Moisture[b] | 99.5 | 99.3 | 97.4 | 99.0 | 98.8 | 98.2 | 98.8 |
| Crude protein[b] | 507.5 | 504.7 | 504.9 | 506.7 | 504.5 | 508.9 | 505.6 |
| Crude lipid[b] | 96.2 | 98.1 | 96.8 | 95.5 | 95.6 | 94.7 | 98.2 |

[a]Vitamin and mineral premix were obtained from Qingdao Master Biotechnology Co, Ltd (Qingdao, China).
[b]Measured value.

## 2.3 | Sample collection

At the end of the 8-week period, the fish were fasted for 24 hr before collecting samples. All fish were collectively weighed and record final weight. After weighing, four fish from each tank were randomly selected for blood collection by 1-ml sterile syringes. Blood was placed in 1.5-ml microcentrifuge tubes and stored at 4°C for 12 hr. The blood was later centrifuged (4 000 rpm for 15 min at 4°C) and the serum collected and stored at −20°C for enzyme activity analysis. The liver and intestine of another three fish were collected randomly to analyse enzyme activity. Then, liver of another three fish from each tank were immediately separated and loaded in 2-ml enzyme-free centrifuge tubes containing RNAlater, after then stored at −80°C for subsequent analysis of relative gene expression.

## 2.4 | Methods of analysis

The contents of total cholesterol (TC), triglyceride (TG), very low-density lipoprotein (VLDL) and high-density lipoprotein (HDL) and the activities of FA decompose enzyme (FADE), lipoprotein lipase (LPL), hepatic lipase (HL), carnitine acyltransferase 1 (CACT1), adipose triglyceride lipase (ATGL) and FA synthetase (FAS) were analysed using commercial ELISA kits (Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China). FA composition of diets was determined from the total lipid extract. FA methyl esters were prepared by acid-catalysed transmethylation of total lipids using boron trifluoride-methanol according to Shantha and Ackman (1990) and were analysed in a gas chromatograph (7890A, Agilent Technologies Inc. US).

**TABLE 2** Fatty acid composition of the experimental diets (% total fatty acids)

| Fatty acids | FO | CO | SO | TO | OO | RO | MO |
|---|---|---|---|---|---|---|---|
| C14:0 | 1.61 | 0.96 | 0.94 | 0.95 | 0.92 | 1.07 | 1.06 |
| C15:0 | 0.22 | 0.1 | 0.09 | 0.14 | 0.09 | 0.11 | 0.12 |
| C16:0 | 14.47 | 14.37 | 12.20 | 10.63 | 13.86 | 16.90 | 13.61 |
| C17:0 | 0.57 | 0.25 | 0.24 | 0.13 | 0.09 | 0.16 | 0.32 |
| C18:0 | 5.15 | 2.72 | 2.89 | 4.01 | 3.63 | 2.61 | 3.35 |
| C20:0 | 0.54 | 0.41 | 0.16 | 0.28 | 0.43 | 0.50 | 0.37 |
| C22:0 | 0.21 | 0.17 | 0.10 | 0.57 | 0.17 | 0.22 | 0.24 |
| C24:0 | 0.00 | 0.15 | 0.00 | 0.21 | 0.10 | 0.24 | 0.14 |
| ∑SAFA[a] | 22.77 | 19.13 | 16.62 | 16.92 | 19.29 | 21.81 | 19.21 |
| C16:1n7 | 3.58 | 1.55 | 1.54 | 1.51 | 1.91 | 1.61 | 1.86 |
| C17:1n7 | 0.31 | 0.09 | 0.10 | 0.09 | 0.08 | 0.08 | 0.12 |
| C18:1n9 | 23.75 | 25.48 | 58.00 | 22.60 | 54.70 | 33.29 | 36.76 |
| C20:1n9 | 3.72 | 1.55 | 1.56 | 1.44 | 1.49 | 1.78 | 1.85 |
| C22:1n9 | 0.49 | 0.27 | 0.28 | 0.26 | 0.25 | 0.29 | 0.30 |
| C24:1n9 | 0.47 | 0.22 | 0.25 | 0.21 | 0.20 | 0.22 | 0.26 |
| ∑MUFA[b] | 32.32 | 29.16 | 61.73 | 26.11 | 58.63 | 37.27 | 41.15 |
| C18:2n6 | 13.05 | 43.73 | 14.62 | 49.94 | 14.81 | 32.68 | 28.59 |
| C18:3n6 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.13 | 0.06 |
| C20:4n6 | 1.59 | 0.27 | 0.24 | 0.24 | 0.25 | 0.26 | 0.44 |
| ∑*n*−6PUFA[c] | 14.84 | 44 | 14.86 | 50.18 | 15.06 | 33.07 | 29.09 |
| C18:3n3 | 2.34 | 1.37 | 1.14 | 1.05 | 1.32 | 1.70 | 1.45 |
| C20:5n3 | 14.90 | 3.08 | 2.75 | 2.78 | 2.77 | 2.99 | 4.60 |
| C22:6n3 | 12.12 | 3.18 | 2.90 | 2.95 | 2.89 | 3.12 | 4.47 |
| ∑*n*−3PUFA[d] | 29.36 | 7.63 | 6.79 | 6.78 | 6.98 | 7.81 | 10.52 |
| ∑*n*−3HUFA[e] | 27.02 | 6.26 | 5.65 | 5.73 | 5.66 | 6.11 | 9.07 |

[a]Saturated fatty acids.
[b]Monounsaturated fatty acids.
[c]18:2n-6, 18:3n-6 and 20:4n-6.
[d]18:3n-3, 20:5n-3 and 22:6n-3.
[e]20:5n-3 and 22:6n-3.

## 2.5 | RNA extraction and cDNA synthesis

TransZol UP (TransGen Biotech, Beijing, China) of 1 ml was added to the samples, and the total RNA was extracted according to the manufacturer's protocol. The quantity and quality of isolated RNA were detected at 260 and 280 nm using a NanoDrop 2000 spectrophotometer (Gene Company Limited, Guangzhou, China) and by electrophoresis in 1% agarose gel, respectively. The first-strand cDNA was synthesized using PrimeScript™ RT Reagent Kits with cDNA Eraser (Takara, Japan) according to the manufacturer's instructions. The cDNA was stored at −20°C for real-time quantitative polymerase chain reaction (RT-qPCR).

## 2.6 | Real-time quantitative polymerase chain reaction (RT-qPCR)

Real-time quantitative polymerase chain reaction (RT-qPCR) was performed in a 384-well plate with a 10 μl reaction volume containing 5 μl of SYBR® Green Real-time PCR Master Mix, 0.8 μl of each primer, 1 μl of cDNA sample and 3.2 μl of RNase Free $dH_2O$. The PCR conditions were set using a thermal programmer at 95°C for 30 s, 40 cycles of 95°C for 5 s and 60°C for 34 s. Each sample was tested in triplicate. Primers of the reference gene (β-actin) and target gene were designed according to published sequences of groupers (Table 3). Threshold cycle (Ct) values were collected from each sample after finishing the process. The relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001).

## 2.7 | Statistical analysis

All data were firstly examined for homogeneity of variance using SPSS version 20.0 (SPSS Inc., USA). The results were analysed by one-way ANOVA and Tukey test, and $p < .05$ was deemed to be statistically significant. The results are presented as means ± standard error (*SEM*).

**TABLE 3** Primers used RT-qPCR

| Primers names | Forward and reverse primers sequence (5' to 3') | Genbank accession No. |
|---|---|---|
| Δ6Fad-F/R | CCTTTGTTCTTCTGCCTCC/ AAGCGTCAGTATCCAGTTAGTT | KP295471.1 |
| FAS-F/R | GGCGGCATTGTAGGCATTA/ CAATCAAAGTGTAGCCTCGGTAG | FJ196231.1 |
| HSL-F/R | TGCTCCGAGATTTCCGACAG/AGGATGCCTCCGAAATGC | KF049203.1 |
| LPL-F/R | CCACCTGTTCATCGACTCCC/ TCGGACGGACCTTGTTGAT | EU683732.1 |
| PPARα-F/R | AAAAGCACGGCTCATACTCAC/ GCTCTGGCAGCAGTGGAA | FJ196235.1 |
| PPARβ-F/R | GCTACAGAGCAGCACGACA/CTCCTCATCTTCGCTTTCC | DQ232867.1 |
| PPARγ-F/R | ACCGCAGCACGAAGAACAAC/ TGGACGCCATAGTGAAACCC | KM052849.1 |
| L-FABP | TCCCAGGAGAACTACGAGG/ TGCCGATGGTAAAGGAGTTG | GU982566.1 |
| H-FABP | GTGGATGGCGACAAGGTGAC/ TTCCGTTGACTTCCCTGACC | MF034870.1 |
| A-FABP | ACTTCAAACTGGGCGAGGAG/ GCCTTCTCATAGTGGCGTGT | EU042103.1 |
| β-Actin -F/R | ACTGCTGCCTCCTCTTCATC/ ACCGCAAGACTCCATACCAA | KU746361.1 |

Abbreviations: A-FABP, adipocyte-type fatty acid-binding protein; FAS, fatty acid synthetase; H-FABP, heart-type fatty acid-binding protein; Δ6Fad, Δ6 Fatty acid desaturase; HSL, hormone-sensitive lipase; L-FABP, liver-type fatty acid-binding protein; LPL, lipoprotein lipase; PPARα, peroxisome proliferator-activated receptor α; PPARβ, peroxisome proliferator-activated receptor β; PPARγ, peroxisome proliferator-activated receptor γ.

**TABLE 4** Growth performance of hybrid grouper fed different diets

| Diet | FO | CO | SO | TO | OO | RO | MO |
|---|---|---|---|---|---|---|---|
| IW (g) | 15.09 ± 0.01 | 15.08 ± 0.01 | 15.10 ± 0.01 | 15.09 ± 0.01 | 15.09 ± 0.01 | 15.10 ± 0.01 | 15.10 ± 0.04 |
| FW (g) | 73.97 ± 1.22 | 77.80 ± 3.11 | 69.68 ± 3.70 | 73.13 ± 3.06 | 76.05 ± 1.72 | 71.71 ± 1.14 | 70.86 ± 3.66 |

*Note:* Values are means ± *SE*(*n* = 3).
Abbreviations: FW, final weight; IW, initial weight.

## 3 | RESULTS

### 3.1 | Growth performance

The initial weight and final weight are shown in Table 4. As we can see, alternative dietary oils had no significant effects on the final weight compared with the control group ($p > .05$).

### 3.2 | Biochemical indices and lipid metabolism enzyme activities in serum

The results for these parameters are shown in Table 5. Compared with the control group, FADE was significantly higher except the SO and TO groups ($p < .05$); furthermore, the maximum value appeared in the MO group and was significantly higher than that of other groups ($p < .05$). The LPL value of the RO and MO groups was not significantly different ($p > .05$), but was significantly higher than that of other groups ($p < .05$). The LPL of the CO and SO groups was not significantly different compared with the control group ($p > .05$). Compared with the control group, the TC of the SO and TO groups was significantly higher ($p < .05$), while that of the OO and MO groups were significantly lower ($p < .05$). The TG and VLDL levels decreased significantly after alternative feeding, except in the CO group ($p < .05$). In contrast, the HDL level increased significantly after alternative feeding, except in the CO group ($p < .05$).

### 3.3 | Lipid metabolism enzyme activities in liver and intestine

The lipid metabolism enzyme activities in the liver are shown in Table 6. It was observed that, compared with the control group, the liver LPL content of the SO and MO groups was significantly higher ($p < .05$), with the MO group having the highest value. However, the CO group had a significantly lower value than control ($p < .05$).

**TABLE 5** Biochemical indices and lipid metabolism enzymes activities in serum of hybrid grouper fed different diets

| Diets | FADE U/L | LPL U/L | TC nmol/L | TG nmol/L | VLDL nmol/L | HDL mg/dL |
|---|---|---|---|---|---|---|
| FO | $476.97 \pm 5.48^{a}$ | $269.70 \pm 11.78^{a}$ | $6.81 \pm 0.31^{c}$ | $7.34 \pm 0.12^{c}$ | $12.78 \pm 0.10^{d}$ | $33.04 \pm 1.42^{ab}$ |
| CO | $576.55 \pm 2.57^{b}$ | $248.26 \pm 11.53^{a}$ | $7.01 \pm 0.02^{cd}$ | $7.60 \pm 0.14^{c}$ | $13.05 \pm 0.07^{d}$ | $31.05 \pm 0.27^{a}$ |
| SO | $473.48 \pm 9.56^{a}$ | $267.87 \pm 2.92^{a}$ | $7.69 \pm 0.10^{e}$ | $5.42 \pm 0.03^{ab}$ | $11.90 \pm 0.10^{c}$ | $42.67 \pm 0.34^{c}$ |
| TO | $473.81 \pm 6.62^{a}$ | $421.64 \pm 3.78^{c}$ | $7.51 \pm 0.06^{de}$ | $5.11 \pm 0.10^{ab}$ | $10.82 \pm 0.13^{b}$ | $51.43 \pm 0.56^{d}$ |
| OO | $571.02 \pm 4.14^{b}$ | $356.28 \pm 23.32^{b}$ | $5.31 \pm 0.06^{b}$ | $5.15 \pm 0.36^{ab}$ | $10.14 \pm 0.20^{a}$ | $37.28 \pm 0.88^{bc}$ |
| RO | $585.56 \pm 1.71^{b}$ | $527.53 \pm 10.25^{d}$ | $6.92 \pm 0.01^{cd}$ | $4.62 \pm 0.18^{a}$ | $10.19 \pm 0.08^{a}$ | $57.65 \pm 0.80^{e}$ |
| MO | $706.97 \pm 6.15^{c}$ | $516.22 \pm 17.78^{d}$ | $4.44 \pm 0.12^{a}$ | $5.84 \pm 0.39^{b}$ | $9.84 \pm 0.10^{a}$ | $53.06 \pm 2.72^{de}$ |

*Note:* Values are means ± *SE* ($n = 3$). Values with different superscripts in the same column are significantly different ($p < .05$).
Abbreviations: FADE, fatty acid decompose enzyme; HDL, high-density lipoprotein; LPL, lipoprotein lipase; TC, total cholesterol; TG, triglyceride; VLDL, very low-density lipoprotein.

**TABLE 6** Lipid metabolism enzymes activities in liver of hybrid grouper fed different diets

| Diets | LPL (U/mg.pro) | ATGL (mIU/mg.pro) | CACT1 (U/mg.pro) | FAS (U/mg.pro) | HL (U/mg.pro) |
|---|---|---|---|---|---|
| FO | $0.35 \pm 0.02^{b}$ | $423.09 \pm 4.32^{d}$ | $1.02 \pm 0.02^{b}$ | $2.35 \pm 0.03^{d}$ | $35.68 \pm 0.94^{a}$ |
| CO | $0.24 \pm 0.01^{a}$ | $352.74 \pm 5.48^{bc}$ | $0.66 \pm 0.04^{a}$ | $1.71 \pm 0.13^{abc}$ | $34.48 \pm 0.14^{a}$ |
| SO | $0.56 \pm 0.02^{c}$ | $373.91 \pm 10.61^{c}$ | $1.03 \pm 0^{b}$ | $2.19 \pm 0.07^{d}$ | $53.64 \pm 1.60^{cd}$ |
| TO | $0.34 \pm 0.03^{b}$ | $316.74 \pm 6.43^{a}$ | $1.09 \pm 0.01^{bc}$ | $1.49 \pm 0.03^{ab}$ | $49.42 \pm 0.33^{c}$ |
| OO | $0.35 \pm 0.02^{b}$ | $325.48 \pm 5.50^{ab}$ | $1.21 \pm 0.04^{c}$ | $1.72 \pm 0.02^{bc}$ | $57.42 \pm 1.52^{d}$ |
| RO | $0.40 \pm 0.02^{b}$ | $361.31 \pm 3.46^{c}$ | $0.95 \pm 0.04^{b}$ | $2.05 \pm 0.12^{cd}$ | $43.57 \pm 0.51^{b}$ |
| MO | $0.65 \pm 0.02^{c}$ | $340.57 \pm 11.47^{abc}$ | $0.73 \pm 0.02^{a}$ | $1.36 \pm 0.01^{a}$ | $50.86 \pm 0.25^{c}$ |

*Note:* Values are means ± *SE* ($n = 3$). Values with different superscripts in the same column are significantly different ($p < .05$).
Abbreviations: ATGL, adipose triglyceride lipase; CACT1, carnitine acyltransferase; FAS, fatty acid synthetase; HL, hepatic lipase; LPL, lipoprotein lipase.

ATGL and FAS levels were significantly lower in all groups than those in controls, except for the FAS of the SO and RO groups ($p < .05$). However, HL levels were significantly higher, except in the CO group ($p < .05$). Compared with the control group, the CACT1 level of the OO group were higher ($p < .05$), but that of the CO and MO groups were lower ($p < .05$), there were no significant differences among other groups ($p > .05$).

The effects of the experimental diets on intestinal FADE activity are shown in Figure 1. Compared with the control group, the CO, SO, TO and OO groups were not significantly different ($p > .05$), while the RO and MO groups had higher FADE activity ($p < .05$).

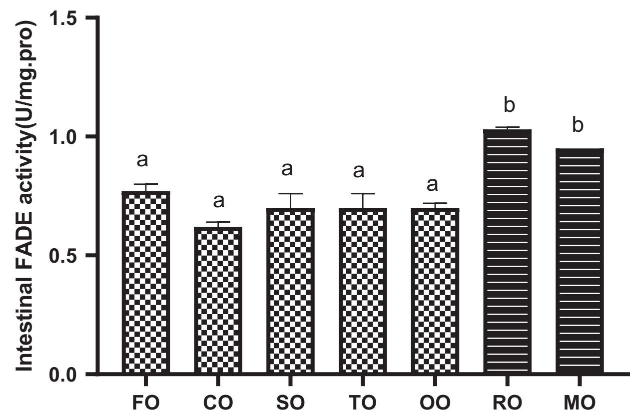



**FIGURE 1** Intestinal FADE activity of hybrid grouper in different treatments. Values are means ± *SEM*($n = 3$). Different letters assigned to the bars represent significant differences using Tukey's test ($p < .05$). FADE, fatty acid decompose enzyme

## 3.4 | Gene expression related to lipid metabolism

Relative gene expression of lipid metabolism-related genes for liver are presented in Figure 2. Compared with control, CO significantly up-regulated Δ6Fad mRNA expression level ($p < .05$) and there is no significant difference among the other groups ($p > .05$). Compared with control, CO, TO, OO and MO groups significantly down-regulated FAS mRNA expression level ($p < .05$) and RO group was opposite. MO group significantly up-regulated HSL mRNA expression level, and OO group significantly up-regulated LPL mRNA expression level ($p < .05$); there were no significant differences in the other groups for HSL and LPL mRNA expression levels ($p > .05$). Compared with control, CO, OO and RO groups significantly up-regulated the PPARαmRNA expression levels ($p < .05$); CO, SO and RO groups significantly up-regulated the PPARβ mRNA expression levels ($p < .05$); however, TO and RO groups significantly down-regulated the PPARγ mRNA expression levels ($p < .05$). Meanwhile, CO,

TO and RO groups significantly down-regulated the L-FABP mRNA expression levels ($p < .05$); MO group significantly up-regulated the H-FABP and A-FABP mRNA expression levels compared with control ($p < .05$). There were no significant differences among the other groups for FABP family genes ($p > .05$).

## 4 | DISCUSSION

The results showed that dietary lipid source did not affect the FW of grouper after 8 weeks. A large number of studies have shown that VOs can completely or partially replace FO on the basis of meeting the essential FAs of marine fish (Fountoulaki et al., 2009; Yıldız et al., 2018). In this paper, 430 g/kg fish meal was added to the feed formula design, so that the fatty acids in the feed can meet the needs of grouper juveniles. This was consistent with previous study (Huang et al., 2016; Larbi et al., 2018; Peng et al., 2016).

In the study of fish oil substitution, increasing attention has been paid to changes in FAs (Fountoulaki et al., 2009; Jordal et al., 2007), because they also play essential roles in the digestion, absorption and synthesis of oils and lipids (Li et al., 2016; Monge-Ortiz et al., 2017). The primary pathways of lipid metabolism usually include digestion and absorption, lipid transport, lipid production and β-oxidation (NRC, 2011). Fish blood is closely related to metabolism, nutritional status and disease. When the fish body is affected by external factors and changes in physiology or pathology, this is primarily reflected in blood indicators. Blood lipid content is only a tiny fraction of the total body lipid content but it is very active in metabolism (Nakagawa, 1978). Exogenous food lipids absorbed by the intestinal tract, endogenous lipids synthesized by the liver, and mobilized adipose tissue must first pass through the blood to other tissues, and the transportation of fish lipid mainly depends on plasma (Hiraoka et al., 1979). Thus, blood lipid levels can reflect whole-body lipid metabolism. In studies on *Dicentrachus labrax* and *Oncorhynchus mykiss* (Figueiredo-Silva et al., 2015) and hybrid sturgeon (Liu et al., 2018), TG content decreased and TC content increased when FO was replaced with soybean or linseed oils. Previous studies on trout show that total replacement of FO with VOs leads to a decrease in plasma cholesterol (Luo et al., 2014; Richard et al., 2006), which is consistent with the case of the OO and MO groups in the present study. It is reported that HDL and VLDL levels indicate metabolic transport activity involving lipids (Ma et al., 2015). In this study, VLDL was significantly decreased and HDL was significantly increased by using VOs diet, except CO diet. Elevation of HDL may clean up excessive cholesterol.

Dietary lipid types not only affect the serum biochemical parameters of grouper, but also significantly affect the activities of LPL, HL, ATGL, CACT1 and FAS in liver. The liver is the main organ of FA β-oxidation and lipid synthesis in fish. Lipase mainly includes LPL and HL, collectively known as total lipase. LPL can catalyse the hydrolysis of TG carried by VLDL and chyle particles into glycerol and FAs for tissue storage and utilization (Feoli-Fonseca et al., 1998; Huang et al., 2017). HL has a variety of lipase activities and can be used as ligands to promote the entry of LDL and chyle particles into hepatocytes and directly participate in the reversal of HDL cholesterol and the decomposition of HDL cholesterol. It is also one of the enzymes related to endogenous TG metabolism in blood circulation, which has similar functions to LPL. It mainly acts on small granular lipoproteins and represents a major determinant of plasma HDL concentration (Cohen et al., 1999; Zambon et al., 1993). The activity of HL influences HDL cholesterol and generates small dense LDL cholesterol, which implies a role for HL in atherosclerosis (Schiekofer et al., 2017). ATGL is a rate-limiting enzyme for hydrolysis of TC (Smirnova et al., 2006), and lack of ATGL will lead to hepatic steatosis. CACT plays an important role in transporting FAs through the mitochondrial inner membrane to produce energy (Vitoria

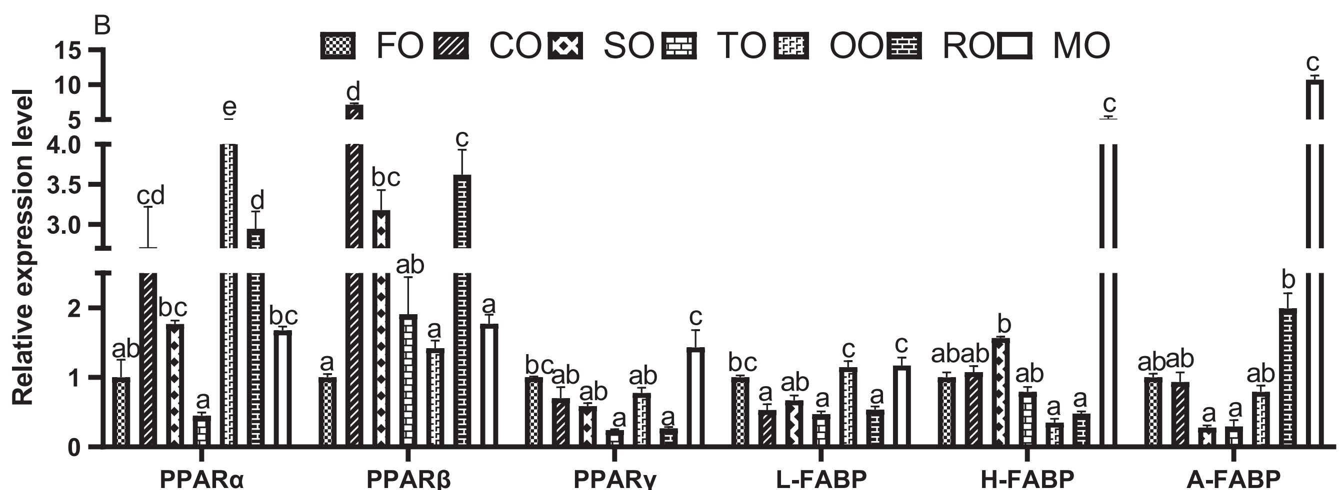



**FIGURE 2** Relative expression of lipid metabolism-related genes for liver of hybrid grouper in different treatments. Values are means ± *SEM*($n = 3$). Different letters assigned to the bars represent significant differences using Tukey's test ($p < .05$). Δ6Fad, Δ6 Fatty acid desaturase; FAS, fatty acid synthetase; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; PPARα, peroxisome proliferator-activated receptor α; PPARβ, peroxisome proliferator-activated receptor β; PPARγ, peroxisome proliferator-activated receptor γ; L-FABP, liver-type fatty acid-binding protein; H-FABP, heart-type fatty acid-binding protein; A-FABP, adipocyte-type fatty acid-binding protein

et al., 2014). FAS is a key enzyme involved in the ab initio synthesis of FAs in animals. In teleosts, FAS exhibits a ubiquitous tissue-specific expression pattern, being higher in liver and adipose tissues (Liu et al., 2010). The results of the present study show that HL levels increased after dietary VO replacement (except in the CO group). This may be due to the fact that VO substitution increased the content of ∑MUFA in feed, and the fish can digest and absorb SAFA and MUFA preferentially (Rainuzzo et al., 1994), which increased the digestible FA content of the diet and promoted HL activities. However, ATGL and FAS levels were lower after replacement, which was related to the lower TG content of the VO groups and the inability to synthesize LC-PUFA from scratch. In addition, the results of LPL and CACT1 were different when FO was replaced by different VO, so enzyme activity may not be a sensitive evaluation index. Thus, the effect of lipid types on the lipid metabolism mechanism of groupers requires further study.

The regulation of lipid tissue synthesis and catabolism in animals is mainly through the regulation of the content and activity of key enzymes in lipid metabolism, and the level of gene expression of key enzymes determines the amount and activity of enzyme proteins (Yue et al., 2012). FAS activity and gene expression determine the speed of FA synthesis. It is generally believed that the determination of HSL activity in lipid and the concentration of FAs in blood can reflect the situation of lipid decomposition, and the expression and activity of HSL gene are affected by energy level, lipid type and fasting (Awad & Chattopadhyay, 1986; Stich et al., 1997; Sztalryd & Kraemer, 1994). LPL can catalyse the decomposition of triglycerides linked to proteins into glycerol and free FA, which play a key role in the metabolism of triglycerides (Yue et al., 2012). In this study, the substitution of vegetable oil for fish oil had no significant effect on HSL and LPL, but MO and OO significantly up-regulated the HSL and LPL gene expression level, respectively. These indicted that MO FAs was more balanced after various oils was mixed, which was conducive to the decomposition of lipid, while OO may be rich in some factors (antioxidant squalene) that promote the decomposition of lipid and TC. In addition, CO, TO, OO and MO significantly inhibited the FAS gene expression. However, Blake and Clarke (1990), Xu et al. (1999) indicated that PUFA can inhibit the expression of FAS mRNA in mouse liver, and the inhibition effect of n-3 PUFA was stronger than that of n-6 PUFA (Clarke et al., 1990). This was contrary to some results of this experiment, which may be due to the lipid deposition in liver after vegetable oil instead of fish oil (Yan et al., 2020).

PPARs family is an important transcription factor regulating metabolic balance, glucose, lipid and energy metabolism, insulin sensitivity, etc (Gross et al., 2017). In present study, differences in PPARs expressions were also affected among treatments, which likely resulted in different efficiency of lipid metabolism (Sarameh et al., 2019). This was indicted that PPARs genes were highly influenced by dietary lipid sources. After replacing FO with VOs, the expression levels of PPARα in CO, OO and RO groups and PPARβ in CO, SO and RO groups were significantly up-regulated. On the one hand, this may be related to the fact that the VOs up-regulated the expression of pro-inflammatory factors in grouper tissues in our another paper (Yan et al., 2020), which was one of the targets of PPARs (Derosa et al., 2018). After PPARs is activated, the production of chemokines, cytokines and adhesion molecules will be reduced and anti-inflammatory effect will be produced by acting on inflammation-related transcription factors, so as to reduce tissue damage (Palomer et al., 2013). On the other hand, the reduction of dietary ∑n-3PUFA, ∑n-3/∑n-6PUFA and EPA/DHA, resulting from the substitution of dietary FO with VOs, may also be the reason for changing PPARs mRNA levels in grouper. Intracellular FAs and their metabolites can coordinate physiological processes through transcription factors, which are activated or inhibited by different FAS, thus controlling energy metabolism (Desvergne et al., 2006). However, the PPARγ expression levels in TO and RO groups significantly down-regulated, which probable owing to different PPAR subtypes play different roles in adipocyte differentiation (Oku & Umino, 2008). PPARα mainly regulates lipid metabolism by regulating FA metabolism-related genes, including FA β-oxidation and transport process, so as to reduce TG, free FA and VLDL synthesis. PPARβ-regulated gene expression is involved in cell energy metabolism, lipid and glucose utilization, maintaining energy balance. PPARγ regulates the transcription of multiple genes involved in the differentiation of lipid precursor cells, regulates the glucose uptake of insulin-mediated peripheral tissues and increases insulin sensitivity (Ren et al., 2018). At the same time, PPARs expression also has tissue specificity (Shi et al., 2002) and plays different roles in lipid and energy dynamics and has different expression patterns (Sarameh et al., 2019). In addition, the concentration and proportion of FAs in feedstuffs from different oil sources were different in present study, which had different effects on various PPAR subtypes. However, the effects of different concentrations and proportions of FAs on PPARs transcription in fish need further study.

FABPs can increase the solubility of FAs, transport FAs from lipid membrane to FA oxidation site, to triglyceride or phospholipid site, and enter into the nucleus to play its regulatory role (Di Pietro & Santomé, 2001). L-FABP mainly promotes the absorption, diffusion and metabolism of FA (Her et al., 2003); H-FABP mainly promotes FA binding and transport (Binas et al., 2003); A-FABP mainly regulates the oxidative energy supply of FAs and the metabolism of phospholipids and triglycerides (Chmurzyńska, 2006). In this study, CO, TO and RO significantly down-regulated the L-FABP, while MO significantly up-regulated the H-FABP and A-FABP. However, there was study shown that the expression of FABP3 (H-FABP) and FABP11 (the function is similar to A-FABP) in the white muscle of Atlantic salmon was significantly reduced after replacing FO with mixed VO (55 g/kg rapeseed oil, 30 g/kg palm oil and 15 g/kg linseed oil) (Torstensen et al., 2009), this is due to that the total replacement of FO with pure VOs reduced the absorption and transport of FAs in fish, while the MO group in this study was composed of FO and 5 kinds VOs to made its FAs complete, balanced and rich in special substances such as tea polyphenols, squalene and β-sitosterol, which are easy to lipid metabolism. Meanwhile, MO had no significant effect on L-FABP, but the ratio of MO to other group was the

largest. Under the mediation of these three genes, the serum TC and TG contents in MO group were significantly reduced compared with FO group.

## 5 | CONCLUSION

In summary, VOs changed serum biochemistry, lipoprotein level and liver lipid-metabolizing enzyme activity of grouper and also affected PPARs mRNA expression levels. We speculate that VOs may affect lipid metabolism of grouper by activating or inhibiting PPARs, and the concentration and/or proportion of FAs may be the activator or inhibitor of lipid metabolism process. The regulation of lipid metabolism pathway by FAs on PPARs needs to be further explored.

### ACKNOWLEDGEMENTS

This study was supported financially by the National Key R&D Program of China (2019YFD0900201), National Natural Science Foundation of China (NSFC31972808), Postgraduate Education Innovation Project of Guangdong Ocean University (202028), Agriculture Research System of China (CARS-47) and "Chong Yi Liu" project of Guangdong Ocean University (231419011).

### CONFLICT OF INTEREST

The authors declare that there are no potential conflicts or competing of interest.

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

### ORCID

*Shuyan Chi* https://orcid.org/0000-0002-8753-8318

### REFERENCES

Awad, A. B., & Chattopadhyay, J. P. (1986). Effect of dietary saturated fatty acids on intracellular free fatty acids and kinetic properties of hormone-sensitive lipase of rat adipocytes. *The Journal of Nutrition*, *116*, 1095–1100. https://doi.org/10.1093/jn/116.6.1095

Ayisi, C. L., & Zhao, J. L. (2017). Fatty acid composition, lipogenic enzyme activities and mRNA expression of genes involved in the lipid metabolism of Nile tilapia fed with palm oil. *Turkish Journal of Aquatic Sciences*, *17*, 405–415. https://doi.org/10.4194/1303-2712-v17_2_20

Binas, B., Han, X.-X., Erol, E., Luiken, J. J., Glatz, J. F., Dyck, D. J., Motazavi, R., Adihetty, P. J., Hood, D. A., & Bonen, A. (2003). A null mutation in H-FABP only partially inhibits skeletal muscle fatty acid metabolism. *American Journal of Physiology-Endocrinology and Metabolism*, *285*, E481–E489. https://doi.org/10.1152/ajpendo.00060.2003

Blake, W. L., & Clarke, S. D. (1990). Suppression of rat hepatic fatty acid synthase and S14 gene transcription by dietary polyunsaturated fat. *The Journal of Nutrition*, *120*, 1727–1729. https://doi.org/10.1093/jn/120.12.1727

Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L., Handisyde, N., Gatward, I., & Corner, R. (2010). Aquaculture: Global status and trends. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*, 2897–2912. https://doi.org/10.1098/rstb.2010.0170

Bransden, M. P., Carter, C. G., & Nichols, P. D. (2003). Replacement of fish oil with sunflower oil in feeds for Atlantic salmon (*Salmo salar* L.): Effect on growth performance, tissue fatty acid composition and disease resistance. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *135*, 611–625. https://doi.org/10.1016/S1096-4959(03)00143-X

Calder, P. C. (2012). Mechanisms of action of (n-3) fatty acids. *The Journal of Nutrition*, *142*, 592S–599S. https://doi.org/10.3945/jn.111.155259

Chmurzyńska, A. (2006). The multigene family of fatty acid-binding proteins (FABPs): Function, structure and polymorphism. *Journal of Applied Genetics*, *47*, 39–48. https://doi.org/10.1007/BF03194597

Clarke, S. D., Armstrong, M. K., & Jump, D. B. (1990). Nutritional control of rat liver fatty acid synthase and S14 mRNA abundance. *The Journal of Nutrition*, *120*, 218–224. https://doi.org/10.1093/jn/120.2.218

Cohen, J. C., Vega, G. L., & Grundy, S. M. (1999). Hepatic lipase: New insights from genetic and metabolic studies. *Current Opinion in Lipidology*, *10*, 259–267. https://doi.org/10.1097/00041433-199906000-00008

Cunha, I., Galante-Oliveira, S., Rocha, E., Urbatzka, R., & Castro, L. (2015). Expression of intercellular lipid transport and cholesterol metabolism genes in eggs and early larvae stages of turbot, *Scophthalmus maximus*, a marine aquaculture species. *Marine Biology*, *162*, 1673–1683. https://doi.org/10.1007/s00227-015-2706-9

Derosa, G., Sahebkar, A., & Maffioli, P. (2018). The role of various peroxisome proliferator-activated receptors and their ligands in clinical practice. *Journal of Cellular Physiology*, *233*, 153–161. https://doi.org/10.1002/jcp.25804

Desvergne, B., Michalik, L., & Wahli, W. (2006). Transcriptional regulation of metabolism. *Physiological Reviews*, *86*, 465–514. https://doi.org/10.1152/physrev.00025.2005

Di Pietro, S. M., & Santomé, J. A. (2001). Structural and biochemical characterization of the lungfish (*Lepidosiren paradoxa*) liver basic fatty acid binding protein. *Archives of Biochemistry and Biophysics*, *388*, 81–90. https://doi.org/10.1006/abbi.2001.2277

Faudzi, N. M., Yong, A. S. K., Shapawi, R., Senoo, S., Biswas, A., & Takii, K. (2018). Soy protein concentrate as an alternative in replacement of fish meal in the feeds of hybrid grouper, brownarbled grouper (*Epinephelus fuscoguttatus*) × giant grouper (*E. lanceolatus*) juvenile. *Aquaculture Research*, *49*, 431–441. https://doi.org/10.1111/are.13474

Feoli-Fonseca, J. C., Lévy, E., Godard, M., & Lambert, M. (1998). Familial lipoprotein lipase deficiency in infancy: Clinical, biochemical, and molecular study. *Journal of Pediatrics*, *133*, 417–423. https://doi.org/10.1016/s0022-3476(98)70280-x

Figueiredo-Silva, A., Rocha, E., Dias, J., Silva, P., Rema, P., Gomes, E., & Valente, L. M. P. (2015). Partial replacement of fish oil by soybean oil on lipid distribution and liver histology in European sea bass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus mykiss*) juveniles. *Aquaculture Nutrition*, *11*, 147–155. https://doi.org/10.1049/ic.2011.0195

Fountoulaki, E., Vasilaki, A., Hurtado, R., Grigorakis, K., Karacostas, I., Nengas, I., Rigos, G., Kotzamanis, Y., Venou, B., & Alexis, M. (2009). Fish oil substitution by vegetable oils in commercial diets for gilthead sea bream (*Sparus aurata* L.); effects on growth performance, flesh quality and fillet fatty acid profile: Recovery of fatty acid profiles by a fish oil finishing diet under fluctuating water temperatures. *Aquaculture*, *289*, 317–326. https://doi.org/10.1016/j.aquaculture.2009.01.023

Gandomkar, H., Javaheri Baboli, M., Gorjipoor, E., & Moradyan, H. (2013). Investigation on replacement of fish oil by vegetable oils (Rice bran, soybean and linseed) in diet of rainbow trout (*Oncorhynchus mykiss*). *ISFJ*, *22*, 99–112. https://doi.org/10.22092/ISFJ.2017.110123

Ghioni, C., Tocher, D. R., Bell, M. V., Dick, J. R., & Sargent, J. R. (1999). Low C18 to C20 fatty acid elongase activity and limited conversion of stearidonic acid, 18: 4 (n–3), to eicosapentaenoic acid, 20: 5 (n–3), in a cell line from the turbot, *Scophthalmus maximus*. *Biochimica Et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, *1437*(2), 170–181. https://doi.org/10.1016/S1388-1981(99)00010-4

Gross, B., Pawlak, M., Lefebvre, P., & Staels, B. (2017). PPARs in obesity-induced T2DM, dyslipidaemia and NAFLD. *Nature Reviews Endocrinology*, *13*, 36. https://doi.org/10.1038/nrendo.2016.135

Han, C.-Y., Zheng, Q.-M., & Feng, L.-N. (2013). Effects of total replacement of dietary fish oil on growth performance and fatty acid compositions of hybrid tilapia (*Oreochromis niloticus*× O. *aureus*). *Aquaculture International*, *21*, 1209–1217. https://doi.org/10.1007/s10499-013-9624-y

Her, G. M., Chiang, C.-C., Chen, W.-Y., & Wu, J.-L. (2003). In vivo studies of liver-type fatty acid binding protein (L-FABP) gene expression in liver of transgenic zebrafish (*Danio rerio*). *FEBS Letters*, *538*, 125–133. https://doi.org/10.1016/S0014-5793(03)00157-1

Hiraoka, Y., Nakagawa, H., & Murachi, S. (1979). Blood properties of rainbow trout in acute hepatotoxity(sic) by carbon tetrachloride. *Bulletin of the Japanese Society for the Science of Fish*, *45*, 527–532.

Huang, H. L., Zhang, Y., Cao, M. Y., Xue, L. Y., & Shen, W. L. (2017). Effects of fasting on the activities and mRNA expression levels of lipoprotein lipase (LPL), hormone-sensitive lipase (HSL) and fatty acid synthetase (FAS) in spotted seabass *Lateolabrax maculatus*. *Fish Physiology & Biochemistry*, *44*, 387–400. https://doi.org/10.1007/s10695-017-0442-4

Huang, Y., Wen, X., Li, S., Li, W., & Zhu, D. (2016). Effects of dietary fish oil replacement with palm oil on the growth, feed utilization, biochemical composition, and antioxidant status of juvenile Chu's Croaker, *Nibea coibor*. *Journal of the World Aquaculture Society*, *47*, 786–797. https://doi.org/10.1111/jwas.12307

Jiang, S., Wu, X., Luo, Y., Wu, M., Lu, S., Jin, Z., & Yao, W. (2016). Optimal dietary protein level and protein to energy ratio for hybrid grouper (*Epinephelus fuscoguttatus* ♀×*Epinephelus lanceolatus* ♂) juveniles. *Aquaculture*, *465*, 28–36. https://doi.org/10.1016/j.aquaculture.2016.08.030

Jordal, A.-E., Lie, Ø., & Torstensen, B. E. (2007). Complete replacement of dietary fish oil with a vegetable oil blend affect liver lipid and plasma lipoprotein levels in Atlantic salmon (*Salmo salar* L.). *Aquaculture Nutrition*, *13*(2), 114–130.

Larbi, A. C., Zhao, J., & Wu, J. W. (2018). Replacement of fish oil with palm oil: Effects on growth performance, innate immune response, antioxidant capacity and disease resistance in Nile tilapia (*Oreochromis niloticus*). *PLoS One*, *13*, e0196100. https://doi.org/10.1371/journal.pone.0196100

Li, B. S., Wang, J. Y., Huang, Y., Hao, T. T., Wang, S. X., Huang, B. S., & Sun, Y. Z. (2019). Effects of replacing fish oil with wheat germ oil on growth, fat deposition, serum biochemical indices and lipid metabolic enzyme of juvenile hybrid grouper (*Epinephelus fuscoguttatus*♀× *Epinephelus lanceolatus*♂). *Aquaculture*, *505*, 54–62. https://doi.org/10.1016/j.aquaculture.2019.02.037

Li, S. L., Mai, K. S., Xu, W., Yuan, Y. H., Zhang, Y. J., Zhou, H. H., & Ai, H. Q. (2016). Effects of dietary lipid level on growth, fatty acid composition, digestive enzymes and expression of some lipid metabolism related genes of orange-spotted grouper larvae (*Epinephelus coioides* H.). *Aquaculture Research*, *47*, 2481–2495. https://doi.org/10.1111/are.12697

Li, X., Cui, K., Fang, W., Chen, Q., Xu, D., Mai, K., Zhang, Y., & Ai, Q. (2019). High level of dietary olive oil decreased growth, increased liver lipid deposition and induced inflammation by activating the p38 MAPK and JNK pathways in large yellow croaker (*Larimichthys crocea*). *Fish & Shellfish Immunology*, *94*, 157–165. https://doi.org/10.1016/j.fsi.2019.08.062

Lin, H. Z., Liu, Y. J., He, J. G., Zheng, W. H., & Tian, L. X. (2007). Alternative vegetable lipid sources in diets for grouper, *Epinephelus coioides* (Hamilton): Effects on growth, and muscle and liver fatty acid composition. *Aquaculture Research*, *38*, 1605–1611. https://doi.org/10.1111/j.1365-2109.2007.01811.x

Lin, Y., & Shiau, S. (2007). Effects of dietary blend of fish oil with corn oil on growth and non-specific immune responses of grouper, *Epinephelus malabaricus*. *Aquaculture Nutrition*, *13*, 137–144. https://doi.org/10.1111/j.1365-2095.2007.00458.x

Liu, C., Wang, J., Ma, Z., Li, T., Xing, W., Jiang, N., Li, W., Li, C., & Luo, L. (2018). Effects of totally replacing dietary fish oil by linseed oil or soybean oil on juvenile hybrid sturgeon, *Acipenser baeri Brandt*♀× A. *schrenckii Brandt*♂. *Aquaculture Nutrition*, *24*, 184–194. https://doi.org/10.1111/anu.12546

Liu, Y., Tian, W. X., Ma, X. F., & Ding, W. J. (2010). Evaluation of inhibition of fatty acid synthase by ursolic acid: Positive cooperation mechanism. *Biochemical & Biophysical Research Communications*, *392*, 386–390. https://doi.org/10.1016/j.bbrc.2010.01.031

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, *25*, 402–408. https://doi.org/10.1006/meth.2001.1262

Lock, E. A., Mitchell, A. M., & Elcombe, C. R. (1989). Biochemical mechanisms of induction of hepatic peroxisome proliferation. *Annual Review of Pharmacology and Toxicology*, *29*, 145–163. https://doi.org/10.1146/annurev.pa.29.040189.001045

Luo, L., Xue, M., Vachot, C., Geurden, I., & Kaushik, S. (2014). Dietary medium chain fatty acids from coconut oil have little effects on postprandial plasma metabolite profiles in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, *420*, 24–31. https://doi.org/10.1016/j.aquaculture.2013.10.024

Ma, F., Li, X. Q., Li, B. A., & Leng, X. J. (2015). Effects of extruded and pelleted diets with differing protein levels on growth and nutrient retention of tilapia, *Oreochromis niloticus* × *O. aureus*. *Aquaculture International*, *23*, 1341–1356. https://doi.org/10.1007/s10499-015-9888-5

Monge-Ortiz, R., Tomás-Vidal, A., Rodriguez-Barreto, D., Martínez-Llorens, S., & Lorenzo, A. (2017). Replacement of fish oil with vegetable oil blends in feeds for greater amberjack (*Seriola dumerili*) juveniles: Effect on growth performance, feed efficiency, tissue fatty acid composition and flesh nutritional value. *Aquaculture Nutrition*, *24*, 605–615. https://doi.org/10.1111/anu.12595

Nakagawa, H. (1978). Classification of albumin and globulin in yellow tail plasma. *Nippon Suisan Gakkaishi*, *44*, 251–257. https://doi.org/10.2331/suisan.44.251

NRC (2011). *Nutrient requirements of fish and shrimp*. NRC.

Oku, H., & Umino, T. (2008). Molecular characterization of peroxisome proliferator-activated receptors (PPARs) and their gene expression in the differentiating adipocytes of red sea bream Pagrus major. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *151*, 268–277. https://doi.org/10.1016/j.cbpb.2008.07.007

Palomer, X., Salvadó, L., Barroso, E., & Vázquez-Carrera, M. (2013). An overview of the crosstalk between inflammatory processes and metabolic dysregulation during diabetic cardiomyopathy. *International Journal of Cardiology*, *168*, 3160–3172. https://doi.org/10.1016/j.ijcard.2013.07.150

Peng, X., Li, F., Lin, S., & Chen, Y. (2016). Effects of total replacement of fish oil on growth performance, lipid metabolism and antioxidant capacity in tilapia (*Oreochromis niloticus*). *Aquaculture International*, *24*, 145–156. https://doi.org/10.1007/s10499-015-9914-7

Rainuzzo, J. R., Reitan, K. I., Jørgensen, L., & Olsen, Y. (1994). Lipid composition in turbot larvae fed live feed cultured by emulsions

of different lipid classes. *Comparative Biochemistry and Physiology Part A: Physiology*, *107*, 699–710. https://doi.org/10.1016/0300-9629(94)90372-7

Ren, K., Xue, L., Huang, B., Pan, W., Wu, K., & Jiang, Q. (2018). PPARs signaling pathway is involved in diabetic hepatopathy in mice. *Chinese Journal of Pathophysiology*, *34*, 555–560.

Richard, N., Kaushik, S. L., Panserat, S., & Corraze, G. (2006). Replacing dietary fish oil by vegetable oils has little effect on lipogenesis, lipid transport and tissue lipid uptake in rainbow trout (*Oncorhynchus mykiss*). *British Journal of Nutrition*, *96*, 299–309. https://doi.org/10.1079/BJN20061821

Sarameh, S. P., Bahri, A. H., Salarzadeh, A., & Falahatkar, B. (2019). Effects of fish oil replacement with vegetable oil in diet of sterlet sturgeon (*Acipenser ruthenus*) broodstock on expression of lipid metabolism related genes in eggs. *Aquaculture*, *505*, 441–449. https://doi.org/10.1016/j.aquaculture.2019.02.031

Schiekofer, S., Kleber, M. E., Maerz, W., Rasche, F. M., & Schneider, J. G. (2017). The Proline 7 Substitution in the Preproneuropeptide Y Is Associated with Higher Hepatic Lipase Activity In Vivo. *International Journal of Endocrinology*, *2017*, 1–7. https://doi.org/10.1155/2017/2869090

Schoonjans, K., Staels, B., & Auwerx, J. (1996). Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *Journal of Lipid Research*, *37*, 907–925. https://doi.org/10.1016/0929-7855(95)00062-3

Shantha, N. C., & Ackman, R. G. (1990). Nervonic acid versus tricosanoic acid as internal standards in quantitative gas chromatographic analyses of fish oil longerchain n-3 polyunsaturated fatty acid methyl esters. *Journal of Chromatography B Biomedical Sciences & Applications*, *533*, 1–10. https://doi.org/10.1016/S0378-4347(00)82182-9

Shi, Y., Hon, M., & Evans, R. M. (2002). The peroxisome proliferator-activated receptor δ, an integrator of transcriptional repression and nuclear receptor signaling. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 2613–2618. https://doi.org/10.1073/pnas.052707099

Smirnova, E., Goldberg, E. B., Makarova, K. S., Lin, L., Brown, W. J., & Jackson, C. L. (2006). ATGL has a key role in lipid droplet/adiposome degradation in mammalian cells. *Embo Reports*, *7*, 106–113. https://doi.org/10.1038/sj.embor.7400559

Stich, V., Harant, I., Glisezinski, I. D., Crampes, F., Berlan, M., Kunesova, M., Hainer, V., Dauzats, M., Rivière, D., & Garrigues, M. (1997). Adipose tissue lipolysis and hormone-sensitive lipase expression during very-low-calorie diet in obese female identical twins. *The Journal of Clinical Endocrinology & Metabolism*, *82*, 739–744. https://doi.org/10.1210/jcem.82.3.3793

Sztalryd, C., & Kraemer, F. B. (1994). Regulation of hormone-sensitive lipase during fasting. *American Journal of Physiology-Endocrinology and Metabolism*, *266*, E179–E185. https://doi.org/10.1152/ajpendo.1994.266.2.E179

Tidwell, J. H., & Allan, G. L. (2015). *Ecological and economic impacts and contributions of fish farming and capture fisheries*. *Federal University Dutsin-Ma Institutional Repository*. Retrieved from http://hdl.handle.net/123456789/364

Tocher, D. R., & Ghioni, C. (1999). Fatty acid metabolism in marine fish: Low activity of fatty acyl Δ5 desaturation in gilthead sea bream (*Sparus aurata*) cells. *Lipids*, *34*, 433–440. https://doi.org/10.1007/s11745-999-0382-8

Torstensen, B., Nanton, D., Olsvik, P., Sundvold, H., & Stubhaug, I. (2009). Gene expression of fatty acid-binding proteins, fatty acid transport proteins (cd36 and FATP) and β-oxidation-related genes in Atlantic salmon (*Salmo salar* L.) fed fish oil or vegetable oil. *Aquaculture Nutrition*, *15*, 440–451. https://doi.org/10.1111/j.1365-2095.2008.00609.x

Vitoria, I., Martín-Hernández, E., Peña-Quintana, L., Bueno, M., Quijada-Fraile, P., Dalmau, J., Molina-Marrero, S., Pérez, B., & Merinero, B. (2014). Carnitine-acylcarnitine translocase deficiency: Experience with four cases in Spain and review of the literature. *JIMD Reports*, *20*, 11–20. https://xs.scihub.ltd/https://doi.org/10.1007/8904_2014_382

Wu, M., Lu, S., Wu, X., Jiang, S., Luo, Y., Yao, W., & Jin, Z. (2017). Effects of dietary amino acid patterns on growth, feed utilization and hepatic IGF-I, TOR gene expression levels of hybrid grouper (*Epinephelus fuscoguttatus*♀× *Epinephelus lanceolatus*♂) juveniles. *Aquaculture*, *468*, 508–514. https://doi.org/10.1016/j.aquaculture.2016.11.019

Xu, J., Nakamura, M. T., Cho, H. P., & Clarke, S. D. (1999). Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acids a mechanism for the coordinate suppression of lipogenic genes by polyunsaturated fats. *Journal of Biological Chemistry*, *274*, 23577–23583. https://doi.org/10.1074/jbc.274.33.23577

Yan, X., Dong, X., Tan, B., Zhang, S., Chi, S., Liu, H., & Yang, Y. (2020). Influence of different oil sources on growth, disease resistance, immune response and immune-related gene expression on the hybrid grouper (♀ *Epinephelus fuscoguttatus*×♂ *E. lanceolatu*), to *Vibrio parahaemolyticus* challenge. *Fish & Shellfish Immunology*, *99*, 310–321. https://doi.org/10.1016/j.fsi.2020.02.025

Yıldız, M., Eroldoğan, T. O., Ofori-Mensah, S., Engin, K., & Baltacı, M. A. (2018). The effects of fish oil replacement by vegetable oils on growth performance and fatty acid profile of rainbow trout: Re-feeding with fish oil finishing diet improved the fatty acid composition. *Aquaculture*, *488*, 123–133. https://doi.org/10.1016/j.aquaculture.2017.12.030

Yin, B., Liu, H. Y., Tan, B. P., Dong, X. H., Chi, S. Y., Yang, Q. H., Zhang, S., & Chen, L. Q. (2018). Cottonseed protein concentrate (CPC)suppresses immune function in different intestinal segments of hybrid grouper ♀*Epinephelusfuscoguttatus*×♂*Epinephelus. lanceolatu* via TLR-2/MyD88 signaling pathways. *Fish & Shellfish Immunology*, *81*, 318–328. https://doi.org/10.1016/j.fsi.2018.07.038

Yue, Y., Liu, G., Zhang, A., Zhang, H., Wang, X., Li, T., & Lu, Z. (2012). Modulating the expression of genes regulating key enzymes for lipid metabolism in growing animals. *Chinese Journal of Animal Nutrition*, *24*, 232–238.

Zambon, A., Austin, M. A., Brown, B. G., Hokanson, J. E., & Brunzell, J. D. (1993). Effect of hepatic lipase on LDL in normal men and those with coronary artery disease. *Arteriosclerosis and Thrombosis*, *13*, 147–153. https://doi.org/10.1161/01.atv.13.2.147

**How to cite this article:** Yan X, Dong X, Tan B, et al. Effects of alternative dietary oils on lipid metabolism and related gene expression in hybrid grouper (♀*Epinephelus fuscoguttatus* × ♂*E. lanceolatu*). *Aquacult. Nutr.* 2021;27:105–115. https://doi.org/10.1111/anu.13168