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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

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ABSTRACT

The aim of this study was to investigate the effects of feeding alternative dietary oils to hybrid grouper fish (♀*Epinephelus fuscoguttatus* × ♂*E. lanceolatu*) on their growth, histological morphology of hepatocytes, disease resistance, immune response, and expression of immune-related genes. Seven experimental fish meal-based isonitrogenous and isolipidic diets were formulated containing 5% fish oil (FO; acting as controls) and 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 (initial mean body weight ± standard error = 15.09 ± 0.01 g) for eight weeks. The results show that 1) alternative dietary oils had no significant effects on weight gain rate, specific growth rate, protein efficiency ratio, and survival rate compared with controls ($P > 0.05$). The weight gain rate (WGR) and specific growth rate (SGR) of the SO group were lower than in the CO and OO groups. 2) These were no differences in morphological indexes among groups; except for the CO group, in which the condition factor and hepatosomatic index were lower than those in other groups. 3) Compared with controls, the whole-body moisture and crude protein contents in the VO groups were higher, while their crude lipid contents were lower. 4) The fatty acid contents in liver and muscle were affected by lipid type, and the contents of eicosapentaenoic acid and docosahexaenoic acid in liver and muscle in the VO groups were markedly lower than in controls. 5) Compared with control group, VO groups damaged the histological morphology of hepatocytes. 6) After a challenge with the *Vibrio parahaemolyticus* bacterium, there were no differences in mortality among groups. However, VO enhanced the activity of non-specific immune enzymes while down-regulating the expression of Nrf2 and inducing the expression of pro-inflammatory factors (IL1β, TNFα, TLR22, and MyD88) in the kidney. It can be concluded that dietary VO substitution does not affect the growth of fish but damaged the histological morphology of hepatocytes and induced the expression of pro-inflammatory factors in tissues. Finally, OO and CO were recommended as the appropriate lipid replacement for FO.

1. Introduction

Fish oil (FO) is the most extensively-used lipid source in aquatic animal feed, owing to its abundant amounts of n-3 long-chain polyunsaturated fatty acids (LC-PUFAs) [1–3], which play essential roles in lipid metabolism, antioxidative status, and immunological function [4]. However, with the rapid development of the aquaculture industry and sharp decline of wild fisheries, FO supplies may become seriously deficient and threaten aquaculture production [5,6]. Therefore, it is imperative to find alternative lipid sources to reduce the dependency on FO [7,8].

The literature reports that the global production of vegetable oils

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Table 1
Ingredient composition and nutrient content of the test diets (%).

Ingredients	Test diets						
	FO	CO	SO	TO	OO	RO	MO
Fish meal	43.00	43.00	43.00	43.00	43.00	43.00	43.00
Soybean meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Casein	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Soybean protein concentrate	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Wheat gluten	13.00	13.00	13.00	13.00	13.00	13.00	13.00
Wheat flour	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Phospholipid	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Fish oil	5.00	–	–	–	–	–	–
Corn oil	–	5.00	–	–	–	–	–
Sunflower oil	–	–	5.00	–	–	–	–
Tea oil	–	–	–	5.00	–	–	–
Olive oil	–	–	–	–	5.00	–	–
Rice oil	–	–	–	–	–	5.00	–
Mixture oil	–	–	–	–	–	–	5.00
Vitamin premix[a]	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Mineral premix[a]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Calcium monophosphate	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Antioxidant	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Attractant	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Sodium carboxymethyl cellulose	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cellulose microcrystalline	1.65	1.65	1.65	1.65	1.65	1.65	1.65
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Proximate composition							
Moisture[b]	9.95	9.93	9.74	9.90	9.88	9.82	9.88
Crude protein[b]	50.75	50.47	50.49	50.67	50.45	50.89	50.56
Crude lipid[b]	9.62	9.81	9.68	9.55	9.56	9.47	9.82

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

(VOs) exceeds that of FO by about 100 times [9]; additionally, VO prices are more stable [10]. At present, a number of studies have reported that VOs can totally or partially replace FO in the feed of different fish species, such as rainbow trout (*Oncorhynchusmykiss*) [11], atlantic salmon (*Salmosalar L.*) [12], beluga sturgeon (Huso huso) [13], amberjack (*Seriola dumerili*) [14], Chinese sucker (*Myxocyprinus asiaticus*) [15], European seabass (*Dicentrarchus labrax*) [16], mullet (*Mugilliza*) [17], and large yellow croaker (*Larimichthys crocea*) [18]. However, VOs have high contents of C_{18} monounsaturated fatty acids (MUFA) and C_{18} polyunsaturated fatty acids (PUFA) while lacking n-3 highly unsaturated fatty acids (HUFA) [19], which would lead to negative effects on non-specific immunity in aquatic animals [20–22]. In the past years, numerous investigations have been elucidated the mechanism of VO-induced negative effects in fish in terms of membrane fluidity, eicosanoid pathways [23], pattern recognition receptor pathways [24], interferon system [25] and antioxidant system [26]. Antioxidant system plays an important role in fish immunity by scavenging reactive oxygen species (ROS) and reactive nitrogen (RNS) [27]. It was reported that the antioxidant defense system includes enzymes, such as superoxide dismutase (SOD), catalase (CAT) and other low molecular weight scavengers in fish [28]. Previous studies have showed that appropriate dietary n-3 LC-PUFA content can improve fish antioxidant capacity or relieve oxidative stress [29,30]. Kobayashi et al. [31] reported that the mechanism of dietary FO in enhancing antioxidant capacity may be related to Nrf2 signaling pathway, which can regulate the transcription of type II detoxifying enzyme genes, such as superoxide dismutase (SOD) [32]. However, the mechanism of lipid sources or fatty acids on antioxidant system remains unclear in aquatic animals.

Groupers are an important aquaculture species in coastal areas of China. The hybrid grouper (♀*Epinephelus fuscoguttatus* × ♂*E. lanceolatu*) is a popular grouper species with high economic and nutritional value and excellent taste. Studies have shown that the hybrid grouper has better growth performance and resistance compared with its parental fish [33]. Meanwhile, it can be transported more easily and, therefore, has become one of the most important species in China aquacultural markets. Currently, there is limited information on juvenile hybrid groupers and their potential alternative dietary oils [34–37]. However, with the development of intensive aquaculture and the deterioration of ecological environment, various diseases caused by *Vibrio* have appeared in the vigorous development of aquaculture, which has caused huge economic losses to the aquaculture industry. *Vibrio parahaemolyticus* is a Gram-negative, halophilic bacterium that occurs naturally in the estuarine environment [38] and it is a virulent pathogen that affects aquaculture [39]. Thus, this study aimed to 1) compare the effects of total replacement of FO by VOs on growth performance, fatty acid composition, lipid metabolism enzymes, disease resistance to *Vibrio parahaemolyticus*, immune response, and expression of immune-related genes in the hybrid grouper and 2) use this information to determine the optimal alternative lipid source for hybrid groupers.

2. Material and methods

2.1. Experimental diets

Seven isonitrogenous and isolipid diets were formulated containing 5% FO, corn oil (CO), sunflower oil (SO), tea oil (TO), olive oil (OO), rice oil (RO), and mixed oil (MO). The FO group was used as a control group. 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 [40]. After homogenization of the feed, virgin FO, CO, SO, TO, OO, RO, and MO were combined with distilled water until moist dough was obtained. 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 at room temperature, then ground and sieved to an appropriate size and stored in ziploc bags at −20 °C until use [41,42]. The ingredients and approximate compositions of the experimental diets are shown in Tables 1–2.

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
ΣSAFA[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
ΣMUFA[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
Σn-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
Σn-3PUFA[d]	29.36	7.63	6.79	6.78	6.98	7.81	10.52
Σn-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.

2.2. Fish and feeding trial

We obtained permission to conduct this study from the ethics review board of the Institutional Animal Care and Use Committee (IACUC) of Guangdong Ocean University. Hybrid groupers (♀*Epinephelus fuscoguttatus* × ♂*E. lanceolatu*) were purchased from a local hatchery at Nansan 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 h, 840 hybrid groupers (mean initial body weight ± standard error = 15.09 ± 0.01 g) were randomly distributed into 21 tanks (1000L; 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 70% of the 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 [43]. Natural illumination, the temperature of the water ranged from 29 to 32 °C, salinity was 28‰, dissolved oxygen was > 7 mg/L, while ammonia and nitrates remained < 0.05 mg/L, which detected by PTF-001B multi parameter water quality detector (WBD Biotechnology Co., Ltd.).

2.3. Sample collection and challenge

At the end of the 8-weeks period, the fish were fasted for 24 h before collecting samples. All fish were collectively weighed and counted in order to calculate the weight gain rate (WGR), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and survival rate (SR). After weighing and counting, three fish were randomly sampled from each tank to collect visceral organs after measuring the body length and weight to calculate a condition factor (CF), hepatosomatic index (HSI), and visceralsomatic indice (VSI). For the histological, studies, two fish of each tank were randomly selected to collecte the liver samples which were kept in Bouin's fluid (pyric acid, saturated aqueous, 75.0 ml; formalin, 25.0 ml, glacial acetic acid, 5.0 ml) for H&E stain. The liver and muscle of three fish were collected randomly to analyze fatty acid composition. Then, another 10 fish from each tank were challenged with a 0.2 ml (4.11×10^9; semi-lethal concentration) live bacterial suspension of *Vibrio parahaemolyticus* from the Key Laboratory of Control for Disease of Aquatic Economic Animals of Guangdong Higher Education Institutes (Zhanjiang, China). Mortalities were recorded and four individuals were sampled from each tank 24 h post-challenge. 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 h. The blood was later centrifuged (4000 rpm for 15 min at 4 °C) and the serum collected and stored at −20 °C for antioxidant and non-specific immunity parameters analysis. Meanwhile, kidneys were immediately separated and loaded in 2 ml enzyme-free centrifuge tubes containing RNA Later, after then stored at −80 °C for subsequent analysis of relative gene expression.

2.4. Methods of analysis

The formula for calculating growth performance and morphological indices were:

Weight gain rate (WGR, %) = 100 × (final weight - initial weight)/ initial weight; Specific growth rate (SGR, %/d) = 100 × ((ln (final weight) – ln (initial weight))/days of experiment; Survival rate (SR, %) = 100% × (total number of fish at termination/total number of fish stocked); Feed conversion ratio (FCR) = feed intake/weight gain; Protein efficiency ratio (PER) = 100 × average weight gain/average protein intake; Condition factor (CF, g/cm3) = weight of fish/length of fish3; Hepatosomatic index (HSI, %) = 100 × (liver weight/body weight); Visceralsomatic index (VSI, %) = 100 × (viscera organ-weight/body weight); Cumulative mortality (%) = 100 × accumulated deaths/total number of challenged fish.

Proximate analysis of the diets and fish samples followed the methods specified by AOAC [44]. Moisture content was determined by drying at 105 °C, crude protein was determined by multiplying nitrogen by 6.25 (Kjeltec™ 8400, Denmark), crude lipid was determined by Soxhlet extraction (using petroleum ether as solvent), and crude ash was determined by calcination at 550 °C in a muffle furnace. The sections were observed with fluorescent inverted microscope (Nikon Eclipse Ti-E).

The activities of superoxide dismutase (SOD), catalase (CAT), alkaline phosphatase (AKP), lysozyme (LYZ) and immunoglobulin M (IgM) were analyzed using commercial ELISA kits (Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China). Fatty acid composition was determined from the total lipid extract. Fatty acid methyl esters were prepared by acid-catalyzed transmethylation of total lipids using boron trifluoride-methanol according to Shantha and Ackman Shantha and Ackman [45] and were analyzed in an gas chromatograph (7890A, Agilent Technologies Inc. US).

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 nm 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™ RTreagent 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).

Table 3
Primers used RT-qPCR.

Primers names	Forward and reverse primers sequence (5′ to 3′)	Genbank accession No.
TGFβ-F/R	CGATGTCACTGACGCCCTGC/AGCCGCGGTCATCACTTATC	GQ205390.1
IL10-F/R	ACACAGCGCTGCTAGACGAG/GGGCAGCACCGTGTTCAGAT	KJ741852.1
IL1β-F/R	CGACATGGTGCGGTTTC/TCTGTAGCGGCTGGTGG	EF582837.1
TNFα-F/R	GTGGCCTACACGACTGCACC/TACAAAGGGCCACAGTGAGA	FJ491411.1
TLR22-F/R	CGAGCCAGGTAAACCCATCA/CTCATCAAACAGGCGGAAGC	JQ965995.1
MyD88-F/R	TGCCTTCATCTGCTACTGCC/TCCGCTTACACCTCTTCTCAAT	GQ202584.1
Nrf2-F/R	GAAGGAGCGTCTGTTGAGTGA/GAAGATGCTGCCGTTAGTTGA	KU892416.1
β-Actin –F/R	ACTGCTGCCTCCTCTTCATC/ACCGCAAGACTCCATACCAA	KU746361.1

TGFβ, transforming growth factor β; IL10, interleukin 10; IL1β, interleukin 1β; TNFα, tumour necrosis factor α; TLR22, toll-like receptor 22; MyD88, myeloid differentiation factor 88; Nrf2, nuclear factor erythroid 2 related factor 2.

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 a10 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 RNse 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 [46].

2.7. Statistical analysis

All data were firstly examined for homogeneity of variance using SPSS version 20.0 (SPSS Inc., USA). The results were subjected to one-way analysis of variance followed by Tukey of tests significant differences among treatment groups, and probability values of $P < 0.05$ were deemed to be statistically significant. The results are presented as means ± standard error (SEM).

3. Results

3.1. Growth performance

The growth performances are shown in Table 4. Alternative dietary oils had no significant effects on the WGR, SGR, PER, and SR compared with the control group ($P > 0.05$). The WGR and SGR of the CO and OO groups were significantly higher than those of the SO group ($P < 0.05$), and there were no significant differences among the other groups ($P > 0.05$). The FCR of the SO and MO groups was significantly higher than that of other groups ($P < 0.05$). The CO group had significantly lower CF and HSI than the other groups ($P < 0.05$) and there were no significant differences among the other groups ($P > 0.05$). The VSI of hybrid groupers was not affected by the lipid type (Table 5).

After the challenge, there were no differences in mortality among the different treatment groups ($P > 0.05$) (Fig. 1.).

Table 5
Morphological indexes of hybrid grouper fed different diets.

Diets	CF (g/cm^3)	HSI (%)	VSI (%)
FO	3.23 ± 0.02^{ab}	2.24 ± 0.16^{b}	9.04 ± 0.45
CO	3.04 ± 0.02^{a}	1.61 ± 0.05^{a}	8.53 ± 0.18
SO	3.34 ± 0.06^{b}	2.25 ± 0.19^{b}	8.77 ± 0.67
TO	3.44 ± 0.02^{b}	2.37 ± 0.07^{b}	9.83 ± 0.10
OO	3.32 ± 0.07^{b}	2.29 ± 0.09^{b}	8.70 ± 0.15
RO	3.33 ± 0.03^{b}	2.41 ± 0.07^{b}	8.78 ± 0.23
MO	3.36 ± 0.07^{b}	2.37 ± 0.12^{b}	9.07 ± 0.43

Note: The results are presentedas the means ± SEM (n = 3). Values with different superscripts in the same column are significantly different ($P < 0.05$). CF, condition factor; HSI, hepatosomatic index; VSI, visceralsomatic index.

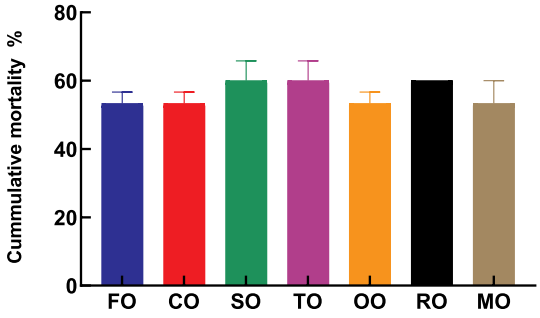

Fig. 1. Cumulative mortality after challenge in different treatments. Values are means ± SEM (n = 3). Dates marked without letters do not have significant differences using Tukey's test ($P > 0.05$).

3.2. Whole-body composition

The whole-body composition results are shown in Table 6. There were no significant differences in whole-body ash among groups ($P > 0.05$). Moisture was lowest in the FO group and was significantly lower than that of other groups ($P < 0.05$). The MO group had the highest moisture content, which was significantly higher than that of

Table 4
Growth performance of hybrid grouper fed different diets.

Diet	WGR (%)	SGR (%/d)	SR (%)	FCR	PER (%)
FO	390.15 ± 8.48^{ab}	2.84 ± 0.03^{ab}	100.00 ± 0.00	0.81 ± 0.01^{a}	2.43 ± 0.02
CO	415.63 ± 20.94^{b}	2.93 ± 0.07^{b}	100.00 ± 0.00	0.83 ± 0.01^{ab}	2.39 ± 0.04
SO	321.51 ± 24.39^{a}	2.56 ± 0.10^{a}	99.17 ± 0.83	0.91 ± 0.03^{b}	2.19 ± 0.07
TO	384.34 ± 20.45^{ab}	2.81 ± 0.07^{ab}	100.00 ± 0.00	0.81 ± 0.01^{a}	2.44 ± 0.02
OO	404.07 ± 11.47^{b}	2.89 ± 0.04^{b}	100.00 ± 0.00	0.86 ± 0.01^{ab}	2.34 ± 0.07
RO	374.89 ± 7.50^{ab}	2.78 ± 0.03^{ab}	99.17 ± 0.83	0.85 ± 0.01^{ab}	2.32 ± 0.02
MO	349.55 ± 20.22^{ab}	2.68 ± 0.08^{ab}	99.17 ± 0.83	0.91 ± 0.04^{b}	2.27 ± 0.08

Note: The results are presentedas the means ± SEM (n = 3). Values with different superscripts in the same column are significantly different ($P < 0.05$). WGR, weight gain rate; SGR, specific growth rate; SR, survival rate; FCR, feed conversion ratio; PER, protein efficiency ratio.

Table 6
Whole body composition of hybrid grouper fed different diets (% dry matter).

Diets	Moisture (%)	Crude protein (%)	Crude lipid (%)	Ash (%)
FO	70.67 ± 0.07^{a}	57.76 ± 0.52^{a}	26.19 ± 0.32^{c}	14.41 ± 0.13
CO	71.78 ± 0.22^{bc}	59.73 ± 0.12^{bc}	24.99 ± 0.43^{bc}	14.69 ± 0.23
SO	71.30 ± 0.02^{b}	59.47 ± 0.24^{bc}	24.63 ± 0.05^{b}	14.63 ± 0.03
TO	71.68 ± 0.09^{bc}	59.01 ± 0.45^{ab}	24.52 ± 0.45^{b}	14.73 ± 0.19
OO	71.72 ± 0.21^{bc}	59.61 ± 0.14^{bc}	23.90 ± 0.23^{ab}	14.75 ± 0.15
RO	71.55 ± 0.16^{b}	58.94 ± 0.28^{ab}	25.26 ± 0.40^{bc}	14.31 ± 0.14
MO	72.32 ± 0.16^{c}	60.51 ± 0.09^{c}	23.02 ± 0.07^{a}	14.71 ± 0.17

Note: The results are presentedas the means ± SEM (n = 3). Values with different superscripts in the same column are significantly different ($P < 0.05$).

the FO, SO, and RO groups ($P < 0.05$). Meanwhile, the other groups showed no significant differences ($P > 0.05$). The crude protein content in the MO group was significantly higher than that in the FO, TO, and RO groups ($P < 0.05$). The crude protein content of the control group was significantly lower than that of alternative groups except the TO and RO groups ($P < 0.05$) and there were no significant differences between the alternative groups except for the MO group ($P > 0.05$). The crude lipid content in the FO group was the highest and was significantly higher than in other groups except for the CO and RO groups ($P < 0.05$), whereas the crude lipid content of the MO group was significantly lower than that of all treatment groups except the OO group ($P < 0.05$).

3.3. Fatty acid composition of liver and muscle

The fatty acid compositions of the liver are shown in Table 7. Total saturated fatty acids (SAFAs) were not significantly different among groups ($P > 0.05$). The OO and TO groups had significantly higher total monounsaturated fatty acid (MUFA) than that the five groups ($P < 0.05$). The SO group had the lowest total MUFA, which was significantly lower than that of the TO, OO, RO, and MO groups ($P < 0.05$). The SO group had significantly higher C18:2n6 contents than most other groups (besides the CO group) and the FO group had significantly more C18:3n3 than other groups ($P < 0.05$). The contents of C20:4n6 (ARA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), sum n-3 polyunsaturated fatty acids (PUFAs), sum n-3 highly unsaturated fatty acids (HUFA), and the n-3/n-6 and the EPA/DHA ratios were significantly higher in the FO group than in all other groups ($P < 0.05$). However, the sum n-6 PUFA content of the FO group was significantly lower than that of the CO, SO, RO, and MO groups ($P < 0.05$).

Fatty acids compositions in muscle are shown in Table 8. The FO group had significantly higher sum SAFA than that of the VOs groups except for the RO group ($P < 0.05$). The sum MUFA contents of the TO and OO groups were significantly higher than those of all other groups ($P < 0.05$). Meanwhile, the sum MUFA contents of the CO and SO groups were significantly lower than those of other groups ($P < 0.05$). The highest levels of C18:2n6 were observed in the SO group and were significantly higher than those of the other groups besides the CO group. The lowest levels of C18:2n6 were observed in the OO group and were significantly lower than those of the CO, SO, RO, and MO groups ($P < 0.05$). The FO group had significantly higher levels of C18:3n3, C20:4n6, EPA, DHA, n-3PUFA and HUFA, and higher n-3/n-6 and EPA/DHA ratios than those of the VOs groups ($P < 0.05$). Fatty acids in the liver and muscle of the MO group were approximately at the mean values observed in the other six groups, as shown in Fig. 2. In addition, as can be seen from Fig. 3, the relative contents of C18:2n6, C18:3n3, EPA, DHA, and n-3 HUFA in muscle were significantly higher than those in liver in all treatments ($P < 0.05$). The ARA contents in muscle were also significantly higher than those in liver in all treatments besides the FO and OO groups ($P < 0.05$).

Table 7
Fatty acid composition in the liver of hybrid grouper fed different diets (% total fatty acids).

Fatty acids	FO	CO	SO	TO	OO	RO	MO
C12:0	0.18 ± 0.04	0.08 ± 0.00	0.05 ± 0.02	0.06 ± 0.03	0.06 ± 0.04	0.04 ± 0.04	0.05 ± 0.02
C14:0	3.09 ± 0.10^{ab}	3.67 ± 0.31^{ab}	3.44 ± 0.17^{ab}	3.87 ± 0.14^{b}	3.01 ± 0.16^{a}	3.33 ± 0.19^{ab}	3.25 ± 0.04^{ab}
C15:0	0.21 ± 0.01^{b}	0.16 ± 0.01^{a}	0.16 ± 0.01^{a}	0.13 ± 0.01^{a}	0.18 ± 0.02^{ab}	0.17 ± 0.01^{ab}	0.17 ± 0.01^{ab}
C16:0	26.35 ± 0.77	28.84 ± 2.52	25.87 ± 0.63	27.80 ± 1.67	22.51 ± 1.15	26.83 ± 1.39	25.23 ± 0.99
C17:0	0.31 ± 0.01^{b}	0.14 ± 0.01^{a}	0.13 ± 0.00^{a}	0.08 ± 0.04^{a}	0.14 ± 0.01^{a}	0.10 ± 0.05^{a}	0.16 ± 0.01^{a}
C18:0	5.64 ± 0.10	2.98 ± 1.51	4.85 ± 0.44	4.34 ± 0.23	3.62 ± 0.27	4.61 ± 0.39	4.31 ± 0.32
C20:0	0.33 ± 0.01^{c}	0.25 ± 0.01^{b}	0.22 ± 0.00^{b}	0.15 ± 0.01^{a}	0.24 ± 0.03^{b}	0.30 ± 0.01^{c}	0.23 ± 0.00^{b}
C22:0	0.14 ± 0.01^{bc}	0.11 ± 0.01^{ab}	0.22 ± 0.01^{c}	0.03 ± 0.00^{a}	0.11 ± 0.01^{ab}	0.08 ± 0.01^{ab}	0.12 ± 0.01^{ab}
C24:0	0	0.12 ± 0.01	0.14 ± 0.00	0	0	0	0.09 ± 0.01
ΣSAFA	36.25 ± 0.94	36.34 ± 2.56	35.09 ± 0.97	36.47 ± 2.00	29.88 ± 1.46	35.55 ± 1.91	33.61 ± 1.33
C16:1n7	5.10 ± 0.05	4.52 ± 0.33	4.64 ± 0.37	5.15 ± 0.21	4.30 ± 0.55	4.55 ± 0.46	4.23 ± 0.03
C17:1n7	0.04 ± 0.00	0.01 ± 0.00	0	0	0.01 ± 0.00	0	0
C18:1n9	27.67 ± 0.07^{a}	27.94 ± 0.37^{a}	26.07 ± 0.62^{a}	39.70 ± 1.42^{c}	43.55 ± 1.17^{d}	32.17 ± 1.28^{b}	33.28 ± 0.39^{b}
C20:1n9	3.46 ± 0.06^{bc}	2.74 ± 0.03^{ab}	2.30 ± 0.14^{a}	3.20 ± 0.18^{bc}	3.50 ± 0.23^{c}	2.74 ± 0.23^{ab}	3.18 ± 0.10^{bc}
C22:1n9	0.55 ± 0.04	0.42 ± 0.03	0.40 ± 0.00	0.50 ± 0.04	0.42 ± 0.05	0.39 ± 0.01	0.44 ± 0.01
C24:1n9	0.43 ± 0.03^{b}	0.31 ± 0.03^{a}	0.30 ± 0.01^{a}	0.29 ± 0.03^{a}	0.25 ± 0.03^{a}	0.30 ± 0.02^{a}	0.30 ± 0.00^{a}
ΣMUFA	37.26 ± 0.73^{ab}	35.94 ± 0.52^{a}	33.71 ± 0.68^{a}	48.84 ± 1.34^{d}	52.04 ± 0.76^{d}	40.15 ± 1.08^{bc}	41.43 ± 0.48^{c}
C18:3n3	1.15 ± 0.04^{d}	0.62 ± 0.05^{ab}	0.58 ± 0.03^{ab}	0.54 ± 0.04^{a}	0.81 ± 0.12^{bc}	0.89 ± 0.00^{c}	0.79 ± 0.03^{abc}
C20:3n3	0.28 ± 0.01^{b}	0.13 ± 0.01^{a}	0.08 ± 0.00^{a}	0.11 ± 0.01^{a}	0.16 ± 0.01^{a}	0.10 ± 0.05^{a}	0.18 ± 0.01^{b}
C20:5n3	4.69 ± 0.09^{d}	0.94 ± 0.09^{a}	0.99 ± 0.06^{a}	1.20 ± 0.01^{ab}	1.30 ± 0.09^{b}	1.22 ± 0.02^{ab}	1.58 ± 0.05^{c}
C22:6n3	7.17 ± 0.30^{c}	1.96 ± 0.21^{a}	2.15 ± 0.06^{ab}	2.26 ± 0.04^{ab}	2.48 ± 0.18^{ab}	2.23 ± 0.18^{ab}	2.93 ± 0.06^{b}
Σn-3PUFA	13.29 ± 0.34^{d}	3.65 ± 0.36^{a}	3.81 ± 0.09^{ab}	4.11 ± 0.08^{ab}	4.73 ± 0.12^{bc}	4.45 ± 0.23^{ab}	5.48 ± 0.13^{c}
C18:2n6	10.66 ± 0.31^{a}	20.72 ± 2.16^{bc}	23.76 ± 1.21^{c}	8.97 ± 0.47^{a}	11.58 ± 0.99^{a}	17.75 ± 0.35^{b}	16.76 ± 0.64^{b}
C18:3n6	0.03 ± 0.00^{ab}	0.09 ± 0.01^{ab}	0.14 ± 0.01^{b}	0.04 ± 0.00^{ab}	0.02 ± 0.00^{a}	0^{a}	0.05 ± 0.00^{ab}
C20:2n6	1.15 ± 0.05^{ab}	2.32 ± 0.15^{c}	2.41 ± 0.32^{c}	0.86 ± 0.08^{a}	1.12 ± 0.11^{ab}	1.49 ± 0.21^{ab}	1.82 ± 0.12^{bc}
C20:3n6	0.33 ± 0.04^{a}	0.34 ± 0.04^{a}	0.50 ± 0.04^{b}	0.27 ± 0.05^{a}	0.22 ± 0.04^{a}	0.32 ± 0.03^{a}	0.30 ± 0.02^{a}
C20:4n6	0.87 ± 0.05^{b}	0.20 ± 0.02^{a}	0.21 ± 0.01^{a}	0.24 ± 0.01^{a}	0.24 ± 0.03^{a}	0.22 ± 0.02^{a}	0.30 ± 0.01^{a}
Σn-6PUFA	13.04 ± 0.33^{a}	23.66 ± 2.35^{bc}	27.02 ± 1.37^{c}	10.40 ± 0.55^{a}	13.18 ± 0.95^{a}	19.78 ± 0.56^{b}	19.22 ± 0.74^{b}
Σn-3HUFA	11.86 ± 0.38^{c}	2.91 ± 0.30^{a}	3.15 ± 0.12^{a}	3.46 ± 0.04^{a}	3.77 ± 0.16^{ab}	3.46 ± 0.19^{a}	4.52 ± 0.10^{b}
n3/n6	1.02 ± 0.02^{d}	0.15 ± 0.00^{a}	0.14 ± 0.01^{a}	0.40 ± 0.01^{c}	0.36 ± 0.03^{c}	0.22 ± 0.01^{b}	0.29 ± 0.01^{b}
EPA/DHA	0.66 ± 0.02^{b}	0.48 ± 0.01^{a}	0.46 ± 0.02^{a}	0.53 ± 0.01^{ab}	0.53 ± 0.06^{ab}	0.55 ± 0.04^{ab}	0.54 ± 0.01^{ab}

Note: The results are presented as the means ± SEM (n = 3). Values with different superscripts in the same line are significantly different ($P < 0.05$).

Table 8
Fatty acid composition in the muscle of hybrid grouper fed different diets (% total fatty acids).

Fatty acids	FO	CO	SO	TO	OO	RO	MO
C14:0	2.27 ± 0.07^{b}	1.97 ± 0.08^{a}	1.93 ± 0.06^{a}	1.95 ± 0.06^{a}	1.88 ± 0.05^{a}	1.97 ± 0.04^{a}	1.86 ± 0.08^{a}
C15:0	0.29 ± 0.01^{b}	0.23 ± 0.01^{a}	0.22 ± 0.01^{a}	0.22 ± 0.01^{a}	0.21 ± 0.00^{a}	0.23 ± 0.00^{a}	0.23 ± 0.01^{a}
C16:0	20.03 ± 0.59^{b}	18.94 ± 0.55^{ab}	17.69 ± 0.43^{a}	18.06 ± 0.26^{a}	18.65 ± 0.38^{ab}	20.32 ± 0.11^{b}	17.98 ± 0.21^{a}
C17:0	0.44 ± 0.01^{c}	0.22 ± 0.01^{a}	0.21 ± 0.01^{a}	0.22 ± 0.01^{a}	0.23 ± 0.01^{a}	0.22 ± 0.00^{a}	0.31 ± 0.02^{b}
C18:0	6.31 ± 0.15^{b}	4.69 ± 0.09^{a}	5.49 ± 0.21^{a}	5.19 ± 0.38^{a}	4.93 ± 0.10^{a}	4.81 ± 0.15^{a}	5.08 ± 0.01^{a}
C20:0	0.44 ± 0.01^{c}	0.37 ± 0.01^{bc}	0.35 ± 0.02^{b}	0.26 ± 0.02^{a}	0.37 ± 0.01^{bc}	0.44 ± 0.01^{c}	0.39 ± 0.00^{bc}
C22:0	0.18 ± 0.00^{ab}	0.11 ± 0.05^{ab}	0.29 ± 0.03^{b}	0.06 ± 0.01^{a}	0.11 ± 0.05^{ab}	0.19 ± 0.01^{ab}	0.20 ± 0.00^{ab}
C24:0	0	0	0.09 ± 0.04	0	0	0.09 ± 0.04	0
ΣSAFA	29.95 ± 0.71^{b}	26.53 ± 0.75^{a}	26.59 ± 0.81^{a}	26.28 ± 0.95^{a}	26.39 ± 0.43^{a}	28.26 ± 0.27^{ab}	26.10 ± 0.40^{a}
C16:1n7	3.80 ± 0.06^{b}	2.52 ± 0.09^{a}	2.47 ± 0.09^{a}	2.52 ± 0.03^{a}	2.89 ± 0.12^{a}	2.65 ± 0.10^{a}	2.61 ± 0.10^{a}
C17:1n7	0.31 ± 0.02^{b}	0.08 ± 0.01^{a}	0.06 ± 0.01^{a}	0^{a}	0^{a}	0.10 ± 0.05^{a}	0.16 ± 0.01^{ab}
C18:1n9	24.51 ± 0.22^{a}	24.49 ± 0.22^{a}	23.09 ± 0.49^{a}	43.92 ± 1.38^{d}	44.20 ± 0.30^{d}	29.67 ± 0.07^{b}	32.15 ± 0.12^{c}
C20:1n9	2.76 ± 0.01^{c}	1.69 ± 0.02^{a}	1.65 ± 0.04^{a}	2.10 ± 0.10^{b}	2.00 ± 0.02^{b}	1.93 ± 0.01^{b}	1.99 ± 0.05^{b}
C22:1n9	0.41 ± 0.01^{c}	0.28 ± 0.00^{a}	0.27 ± 0.01^{a}	0.32 ± 0.01^{b}	0.31 ± 0.01^{ab}	0.32 ± 0.00^{b}	0.31 ± 0.01^{b}
C24:1n9	0.52 ± 0.01^{b}	0.38 ± 0.03^{a}	0.38 ± 0.01^{a}	0.36 ± 0.01^{a}	0.37 ± 0.00^{a}	0.37 ± 0.02^{a}	0.41 ± 0.02^{a}
ΣMUFA	32.30 ± 0.27^{b}	29.44 ± 0.16^{a}	27.93 ± 0.40^{a}	49.22 ± 1.67^{e}	49.77 ± 0.21^{e}	35.04 ± 0.14^{c}	37.65 ± 0.04^{d}
C18:3n3	1.92 ± 0.05^{d}	1.40 ± 0.07^{b}	1.21 ± 0.04^{a}	1.18 ± 0.03^{a}	1.39 ± 0.02^{b}	1.61 ± 0.03^{c}	1.49 ± 0.01^{bc}
C20:3n3	0.21 ± 0.00^{c}	0^{a}	0^{a}	0^{a}	0^{a}	0.05 ± 0.01^{a}	0.13 ± 0.01^{b}
C20:5n3	7.81 ± 0.06^{c}	2.30 ± 0.09^{a}	2.17 ± 0.02^{a}	2.12 ± 0.05^{a}	2.15 ± 0.01^{a}	2.25 ± 0.02^{a}	3.13 ± 0.00^{b}
C22:6n3	9.25 ± 0.26^{c}	4.16 ± 0.17^{a}	4.01 ± 0.16^{a}	3.78 ± 0.15^{a}	3.83 ± 0.06^{a}	4.07 ± 0.07^{a}	5.15 ± 0.06^{b}
Σn-3PUFA	19.19 ± 0.34^{c}	7.86 ± 0.31^{a}	7.39 ± 0.18^{a}	7.07 ± 0.23^{a}	7.37 ± 0.10^{a}	7.98 ± 0.05^{a}	9.91 ± 0.07^{b}
C18:2n6	16.43 ± 0.59^{a}	34.34 ± 0.71^{d}	35.99 ± 0.86^{d}	16.22 ± 0.69^{a}	15.62 ± 0.20^{a}	27.35 ± 0.38^{c}	24.88 ± 0.24^{b}
C20:2n6	0.63 ± 0.02^{a}	1.21 ± 0.02^{c}	1.35 ± 0.07^{c}	0.56 ± 0.03^{a}	0.56 ± 0.02^{a}	1.00 ± 0.02^{b}	0.87 ± 0.04^{b}
C20:3n6	0.25 ± 0.00^{b}	0.19 ± 0.01^{b}	0.20 ± 0.02^{b}	0^{a}	0^{a}	0.05 ± 0.00^{a}	0.16 ± 0.01^{b}
C20:4n6	0.97 ± 0.02^{c}	0.30 ± 0.01^{a}	0.30 ± 0.02^{a}	0.30 ± 0.01^{a}	0.28 ± 0.01^{a}	0.31 ± 0.01^{a}	0.42 ± 0.01^{b}
Σn-6PUFA	18.38 ± 0.65^{a}	36.04 ± 0.70^{d}	37.84 ± 0.78^{d}	17.08 ± 0.65^{a}	16.47 ± 0.21^{a}	28.71 ± 0.35^{c}	26.33 ± 0.29^{b}
Σn-3HUFA	17.06 ± 0.31^{c}	6.46 ± 0.25^{a}	6.18 ± 0.15^{a}	5.90 ± 0.20^{a}	5.98 ± 0.07^{a}	6.32 ± 0.05^{a}	8.29 ± 0.07^{b}
n3/n6	1.06 ± 0.03^{d}	0.22 ± 0.01^{ab}	0.20 ± 0.01^{a}	0.42 ± 0.03^{c}	0.45 ± 0.00^{c}	0.28 ± 0.00^{b}	0.38 ± 0.00^{c}
EPA/DHA	0.85 ± 0.02^{b}	0.55 ± 0.01^{a}	0.54 ± 0.02^{a}	0.56 ± 0.01^{a}	0.56 ± 0.01^{a}	0.55 ± 0.02^{a}	0.61 ± 0.01^{a}

Note: The results are presentedas the means ± SEM (n = 3). Values with different superscripts in the same line are significantly different ($P < 0.05$).

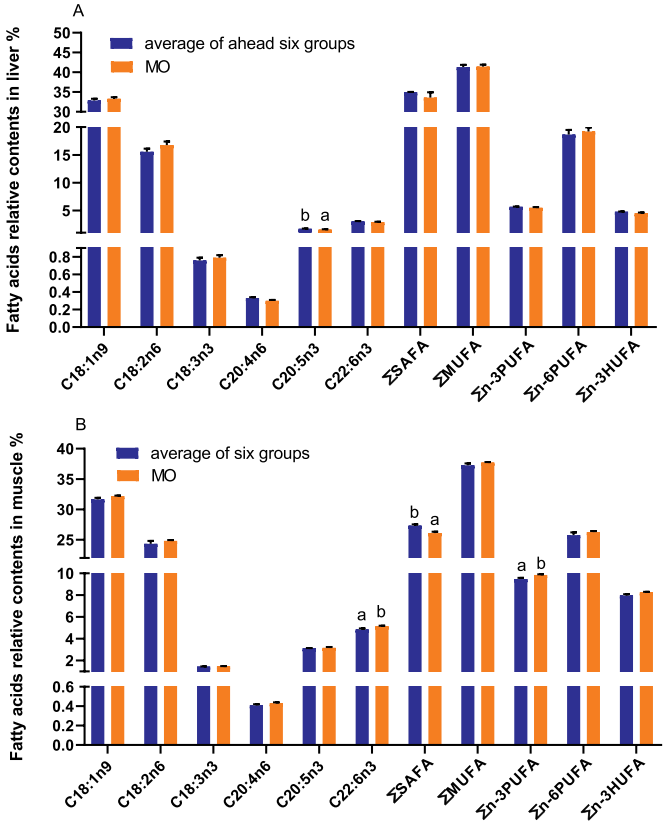

Fig. 2. Fatty acids relative contents in the liver (A) and muscle (B) of the MO group compared with the average of the other six groups. Values are means ± SEM (n = 3). Different letters assigned to the bars represent significant differences using Tukey's test ($P < 0.05$).

3.4. Histological structure of liver

In FO group, the hepatocyte cords of grouper were obvious, the cell morphology was normal, the cell boundary was clear, the nucleus was in the middle of the cell, although there were a few lipid drops in the cytoplasm, but the cell was not swollen, the nucleus was obvious; however, after VOs replaced FO, the hepatocyte appeared obvious histological changes, such as nuclear atrophy or even disappearance, the cell edge was fuzzy, the cell vacuolation was serious, almost all the cells are full of lipid droplets (Fig. 4).

3.5. Antioxidant and non-specific immunity parameters in the serum after challenge

The post-challenge antioxidant and non-specific immunity parameters occurring in the serum of hybrid groupers fed diets with different lipid types are illustrated in Table 9. The SOD and CAT activities for groupers fed VO diets were significantly higher than those of the control group, except for the CO group ($P < 0.05$), while the maximum appeared in the MO group. AKP activities increased significantly after replacement of FO with TO, RO, and MO ($P < 0.05$); however, those of the CO and OO groups decreased significantly ($P < 0.05$). Compared with control the group, the LYZ activities of the SO, TO, and OO groups were not significantly different ($P > 0.05$); however, that of the CO group decreased significantly while those of the RO and MO groups increased significantly ($P < 0.05$). The lowest serum IgM content was obversed in TO group and it was significantly lower than that all other groups ($P < 0.05$).

3.6. Relative expression of immune–related genes in kindeys after challenge

The FO group was selected as the control group. As shown in Fig. 5, some 24 h after the challenge, the highest expressions of anti-inflammatory genes (TGFβ and IL10) were found in the MO group, which were significantly higher than that of controls ($P < 0.05$). Meanwhile,

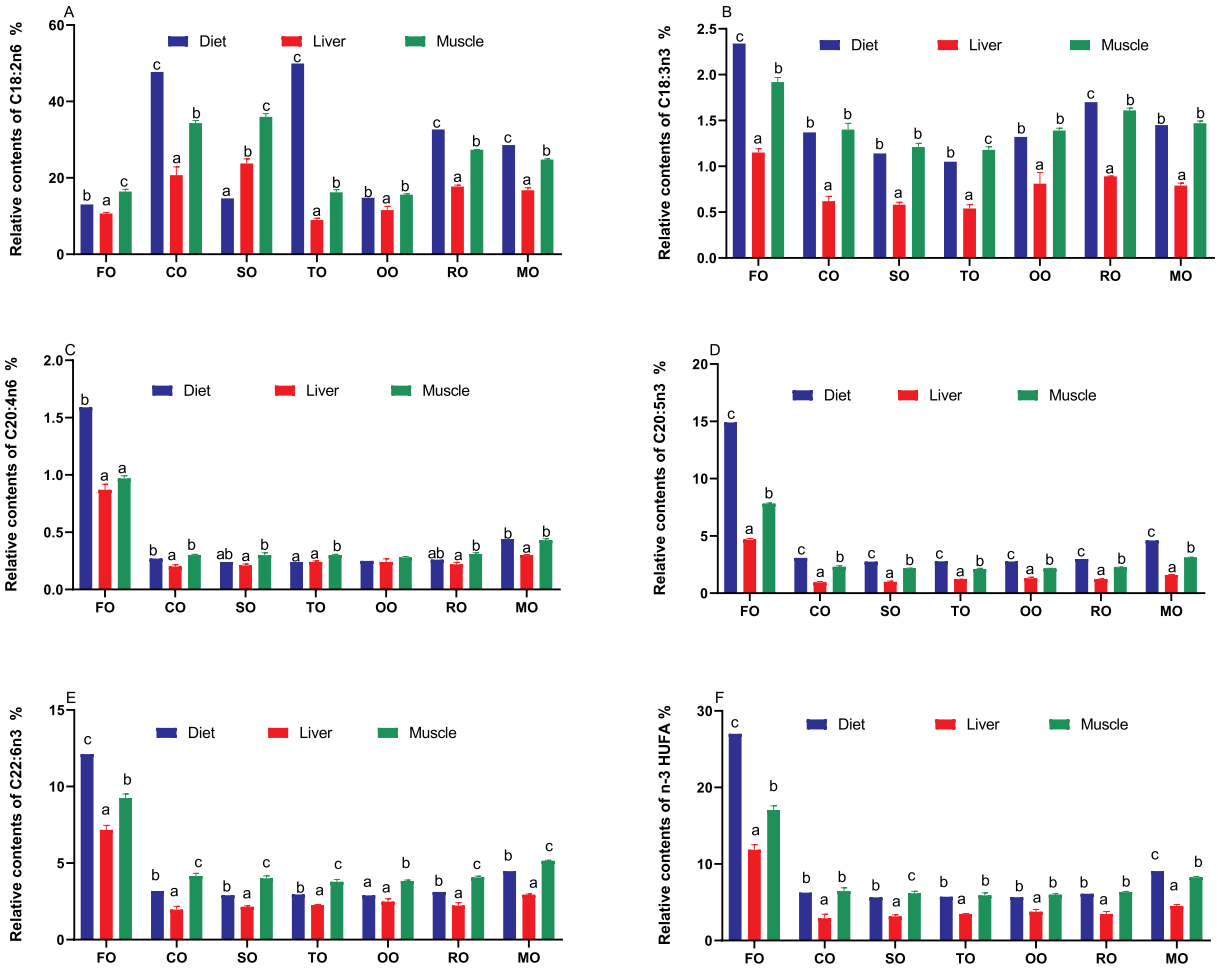

Fig. 3. C18:2n6 (A), C18:3n3 (B), C20:4n6 (C), C20:5n3 (D), C22:6n3 (E) and n-3 HUFA (F) show relative contents in diet, liver and muscle. Values are means ± SEM (n = 3). Different letters assigned to the bars represent significant differences using Tukey's test ($P < 0.05$).

the lowest expression levels of both genes were observed in the control and TO groups, respectively. Compared with the control group, VO groups up-regulated their relative expressions of pro-inflammatory genes IL1β, TLR22 and MyD88, except for the TO group. The expressions of these genes were in the OO group and of the pro-inflammatory genes TNFα and MyD88 in the MO group were significantly higher than in controls ($P < 0.05$). After substitution, the relative expression of Nrf2 was significantly lower in all groups than in the FO group ($P < 0.05$), except for the SO and OO groups.

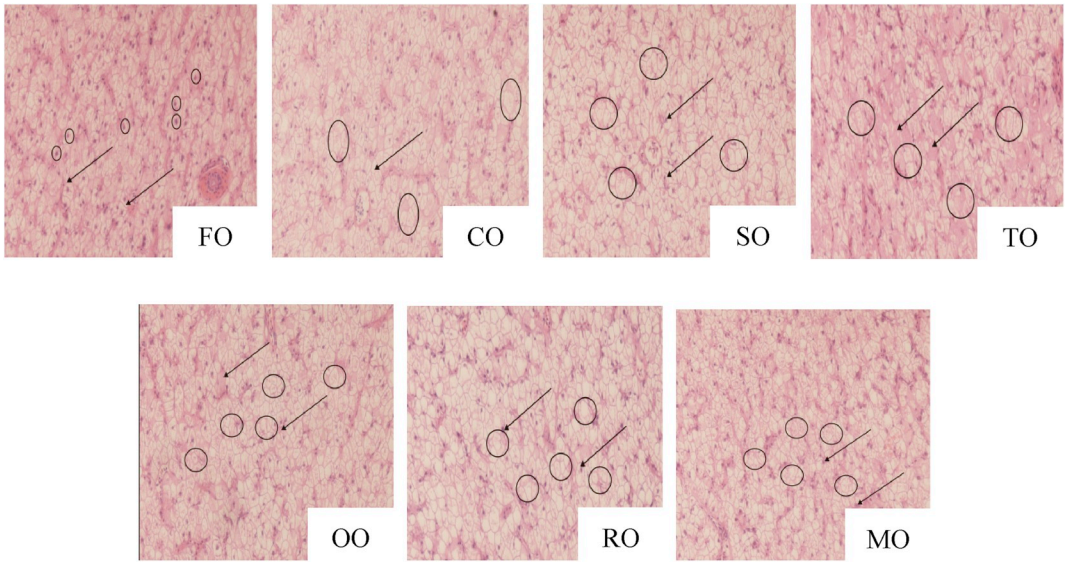

Fig. 4. Liver histological sections of hybrid grouper fed different diets (H & E ×200).

Table 9
Antioxidant and non-specific immunity parameters in serum after challenge of hybrid grouper fed different diets.

Diets	SOD (U/ml)	CAT (U/ml)	AKP (IU/L)	LYZ (U/L)	IgM
FO	61.16 ± 6.15^{ab}	24.21 ± 0.45^{a}	13.44 ± 0.27^{c}	4.77 ± 0.12^{b}	51.86 ± 1.17^{b}
CO	49.16 ± 1.73^{a}	25.72 ± 0.28^{a}	10.85 ± 0.20^{a}	3.36 ± 0.14^{a}	50.25 ± 0.85^{b}
SO	94.05 ± 2.77^{d}	33.84 ± 1.67^{b}	13.86 ± 0.14^{c}	4.99 ± 0.31^{bc}	49.37 ± 0.63^{b}
TO	74.44 ± 4.74^{bc}	34.45 ± 0.65^{b}	16.00 ± 0.08^{d}	4.67 ± 0.11^{b}	41.33 ± 0.51^{a}
OO	104.76 ± 2.48^{d}	33.30 ± 0.57^{b}	12.33 ± 0.15^{b}	5.11 ± 0.39^{bcd}	52.68 ± 1.25^{b}
RO	90.96 ± 2.57^{cd}	31.98 ± 0.56^{b}	17.58 ± 0.33^{e}	6.14 ± 0.18^{d}	52.88 ± 0.35^{b}
MO	129.56 ± 3.39^{e}	43.25 ± 1.20^{c}	15.30 ± 0.21^{d}	5.95 ± 0.04^{cd}	53.42 ± 1.64^{b}

Note: Values are means ± SEM (n = 3). Values with different characters in the same line are significantly different ($P < 0.05$). SOD, superoxide dismutase; CAT, catalase; AKP, alkaline phosphatase; LYZ, lysozyme; IgM, immunoglobulin M.

4. Discussion

The sharply-increasing price and decreasing quality of FO have prompted research into alternative VOs for inclusion in aquaculture diets. A series of investigations have reported that VOs can entirely or partially replace FO in marine fish diets without any adverse effects on growth performance [41,47]. In the present study, the alternate dietary lipid had no effect on SR. All the experimental diets provided good grouper growth performance, which is consistent with previous studies [48]. Studies have shown that the dietary n-3 HUFA requirement for juvenile groupers (*Epinephelus coioides*) is 1.27%–1.42% [49], although the content of n-3 HUFA in VOs is lower than this, however, there was 5.65%–9.07% n-3 HUFA of total fatty acids in the VO groups, which may satisfy the requirements of groupers, as VO group did not exhibit any differences compared with the FO group in terms of growth performance. Similar results have been obtained in Atlantic salmon (*Salmosalar L.*) [50] and grouper (*Epinephelus coioides*) [51]. It is reported that the n-3 HUFA content of diet was higher than the requirement of gilthead seabream but that the high proportion of alternative VOs restrains growth performance [52]. This is similar to the case of the SO group in the present study; owing to them receiving the lowest amounts of n-3HUFA and the highest amounts of MUFA, the FAs could not be utilized efficiently. The best growth performance was obtained in the CO group, because CO contains large quantities of n-6 PUFA, e.g. linoleic acid (18C:2n-6), in comparison to FO [53]. CO provides balanced levels of dietary fatty acids that satisfy the essential fatty acid requirements of fish. Meanwhile, a study of Nile tilapia, *Oreochromisniloticus* [54] suggested that CO is a good candidate for replacing FO.

The morphological indexes of CF, HIS, and VSI, were used as indicators of the nutritional and physiological status of the test subjects [55]. A previous study [56] reported that alternative lipids have no effect on CF and VSI, which is consistent with the present study. The results indicate that this variable had no effect on dietary lipid composition [57]. Meanwhile, the CF and HSI in groups of fish fed a CO diet were significantly lower than those of other groups ($P < 0.05$), which may be attributed to the balanced FAs occurring in the CO diet. In addition, it was reported that there is more abundant Vitamin E (VE) in CO than in the other four VOs [58,59]. VE plays an important role in the prevention of lipid peroxidation, thereby protecting the integrity of fish tissues, and it can improve lipid radical digestion and reduce hepatic lipid deposition [60]. However, dietary VE contents were not measured in this experiment, so its effects on grouper morphological indexes remain to be studied.

Substitution of FO with VOs markedly decreased body lipids and increased body proteins in juvenile hybrid groupers. Yu et al. Yu, Chang, Dong and Liu [15] and Li et alLi, Wang, Huang, Hao, Wang, Huang and Sun [37] reported that VOs replacement of FO has no effect on the body composition of fish. However, when wheat germ oil was used to replace FO, the lipid content decreased in *Cynolossussemilaevis* [61], which is consistent with the present study. VOs are rich in PUFA, which inhibits the expression of fatty acid synthase genes and promotes the expression of lipid oxidative decomposition genes. This inhibits lipid synthesis and accelerates lipid decomposition [61]; thus, the lipid content of whole fish decreased.

It is well documented that the fatty acid make-up of fish depends on dietary fatty acids [62–66], especially C18:1n9, C18:2n6, C18:3n3, C20: 4n6, C20:5n3, and C22:6n3 (Tables 5 and 6), which affects the quality of the fish and their metabolism. Because VOs contain little LC-PUFA when used to replace FO, the LC-PUFA content in fish decreases,

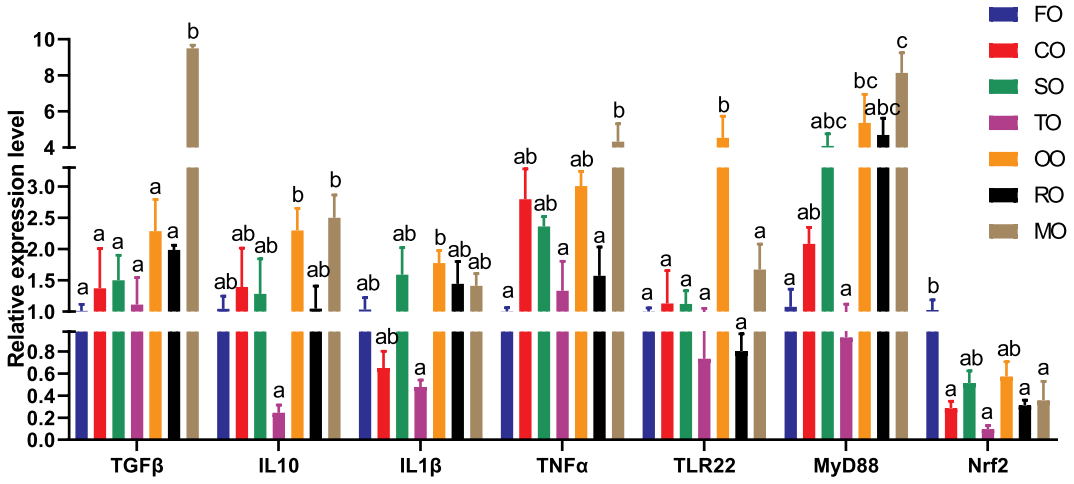

Fig. 5. Relative expression of immune-related genes in the kidney of the hybrid grouper in different treatments after 24 h challenge. Values are means ± SEM (n = 3). Different letters assigned to the bars represent significant differences using Tukey's test ($P < 0.05$). TGFβ, transforming growth factor β; IL10, interleukin 10; IL1β, interleukin 1β; TNFα, tumour necrosis factor α; TLR22, toll-like receptor 22; MyD88, myeloid differentiation factor 88; Nrf2, nuclear factor erythroid 2-related factor 2.

leading to LC-PUFA-related metabolic gene changes [67]. Studies on Atlantic cod, *Gadusmorhua* [68], rainbow trout, *Oncorhynchus mykiss* [69,70] showed that when VOs replace FO, the relative contents of DHA and EPA decrease significantly. Studies on sharpsnout seabream, *Diploduspuntazzo* [71] showed that the substitution of soybean oil and flaxseed oil for FO results in a decrease in the relative content of LC-PUFA in the liver. In the present study, total substitution of dietary FO led to a reduction in tissue LC-PUFAs (C20: 4n6, C20:5n3, and C22:6n3) and tissue fatty acid composition reflected the feed fatty acid composition. The results are consistent with those for seabream and seabass [10], *Epinephelus coioides* [43], rainbow trout [72]. In the present study, it was interested to find that the content of fatty acids in the fish fed the MO diet was approximately the same as the mean of the other six groups. It can be concluded that changes in dietary lipid types lead to changes in lipid deposition and metabolism in tissues [73]. The incorporation of fatty acids in tissues is modulated by various metabolic factors, with the final composition dependent on the initial fatty acids [74]. The relative contents of FAs in liver and muscle within each treatment group were analyzed and it was found that the C18:2n6, C18:3n3, C20:4n6, C20:5n3, C22:6n3, and n-3 HUFA contents in muscle were significantly higher than those in liver ($P < 0.05$), which is consistent with the study of *Epinephelus coioides* [43]. This is because the fatty acid composition of muscle is more sensitive than that of the liver in reflecting dietary fatty acids [57].

The dietary lipid type had no influence on disease resistance, according to the *Vibrio parahaemolyticus* challenge conducted in this study, which is consistent with previous investigations [75–77]. However, it has been reported that replacing fish oil with soybean oil improves the disease resistance and immune system of fish [78]; lipid type influenced the disease-resistance of channel catfish, *Ictalurus punctatus* [79]. These discrepancies can be attributed to differences in fish species and sizes, dietary lipid types and levels, feeding duration, as well as environmental factors [80]. In addition, differences in dietary fatty acid compositions and ratios of n-6/n-3 fatty acids could also be reasons for differences observed in the immunity and disease resistance of fish [81].

The results showed that the substitution of VO for FO in feed had a significant effect on the liver structure of fish [82–84]. LC-PUFA is an important component of lipoprotein phospholipid and VO replacing FO will cause the decrease or loss of LC-PUFA content in feed, long-term ingestion of VO diets will affect the synthesis of fatty acids and lipoproteins, thus reducing the ability of lipid transport, resulting in the accumulation of lipid in liver cells [85–87]. This is consistent with the results of this study. This may be due to the lower ability of grouper to synthesize LC-PUFA by using VO, and the lack of LC-PUFA in fish caused by the substitution of VO for FO, thus affecting the organism structure.

The antioxidant parameters and non-specific immune function of fish plays an important role in defending against pathogenic microorganism invasion. The indicators SOD, CAT, AKP, LYZ and IgM are important for evaluating immunity and health status [88]. The substitution of vegetable oil for fish oil changed the fatty acid composition of the feed, which would affect the non-specific immunity and antioxidant capacity of aquatic animals [27]. Research has reported that there is a higher oxidation priority of n-6 PUFA over n-3 PUFA, which is the reason safflower oil (n-6 PUFA-rich) improved the antioxidant activity of rainbow trout more than linseed oil (n-3 PUFA rich) [26]. This was consistent with the results of the present study, except for the CO group. However, the study [27] pointed out that the conclusion that oxidation has a higher priority for n-6 PUFA may not be persuasive to their present results. In contrast, a large decrease in n-3PUFA in feed will impair antioxidant capacity. In addition, this is also related to the content of reactive oxygen species produced by different fatty acid diets [89]. AKP and LYZ are involved in various metabolic processes and have been identified as indicators of the health and immune status of aquatic animals [90,91]. Low serum AKP activities in the CO and OO groups indicate that oxidative stress might induce a serious imbalance between the production of reactive species and antioxidant defense [92]. High activity of LYS appeared in the SO, OO, RO, and MO groups, indicating that replacing FO with different VOs enhances the defensive capability of the mucosal surface [54]. This may be due to the relative high n-3 PUFA and n-6 PUFA levels, however, the effects of the fatty acid ratio on the antioxidant capacity of fish needs further exploration.

The immune system plays an important role in protecting fish from pathogens and is influenced by nutrition and feed components [93,94]. The lack of LC-PUFA in feed will reduce the disease resistance and immunity of fish [95,96]. LC-PUFA is an important active substance in metabolism. Especially, a series of eicosanoids synthesized with ARA and EPA as precursors play an important role in physiological processes such as immune, inflammatory response and central function, while the derivatives of DHA have anti-inflammatory and lipid-lowering effects [97,98]. Previous studies show that dietary components can alter gene expression [99,100]. RT-qPCR results indicated that VO diets induce inflammation, manifested through increased pro-inflammatory gene expression (the IL1β of SO, OO, RO and MO groups; the TNFα of VO groups; the TLR22 of CO, SO, OO and MO groups; the MyD88 of VO groups except TO group) compared with that in an FO diet. Previous study [101] has also demonstrated that increased dietary VO contributes to the pro-inflammatory response of tissue, which is consistent with the present study. The increase in pro-inflammatory gene expression may be due to decreased n-3 LC-PUFA content in VO diets because the reduction of n-3 fatty acids has been observed to reduce the pro-inflammatory response of the liver in mammalian studies [102]. TGF-β and IL10 are anti-inflammatory cytokines, which are of great significance in improving the disease resistance of tissues [18,103]. The expression of TGF-β observed in this study was not significantly affected by alternative lipid types (except MO), which is consistent with an investigation on juvenile rainbow trout [104]. However, the expressions of TGF-β and IL10 were highly upregulated in the MO group, which indicates that an appropriate fatty acid composition and ratio of n-6/n-3 fatty acids in the diet may be conducive to modifying the immune system of groupers [105]. Nrf2 is an important transcription factor for maintaining antioxidant capacity and plays a role in inhibiting pro-inflammatory responses [106]. Studies on Japanese sea bass, *Lateolabrax japonicus* showed that the potential mechanism may involve FO, rather than VOs, activating the Nrf2 signaling pathway so that the expression of Nrf2 is weakened after FO replacement [27], which is consistent with our observations on groupers. This is also the reason why the expression of pro-inflammatory factors increased after VOs replacement in the present study.

5. Conclusion

Substitution of FO with VOs did not affect the growth performance of hybrid groups. However, the fatty acid compositions of the tissues were significantly influenced by the fatty acid compositions of the dietary lipids. In addition, it was observed that VO could damage the histological morphology of hepatocytes and induce the expression of immune-related genes in the grouper. Therefore, it is suggested that further investigation is needed in these aspects, especially regarding the fatty acid compositions of different lipid types. Their mechanism of immunological function should be validated by conducting lengthy feeding trials and challenges. Finally, OO may be an appropriate alternative to FO as it can improve growth, antioxidant and inflammatory capacity significantly when compared with the FO group, and CO as the second recommended feed lipid source.

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.

Declaration of competing interest

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

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