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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*)

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ABSTRACT

A feeding experiment was conducted to determine the effects of fish oil replaced by olive oil (OO) on growth performance, serum biochemical, antioxidant capacity and inflammatory response in large yellow croaker (*Larimichthys crocea*). Four iso-nitrogenous and iso-lipidic diets were formulated by replacing fish oil (FO) with 0% (the control group), 33.3%, 66.7% and 100% OO. Fish fed the diet with 100% OO had the lowest growth performance among dietary treatments. However, there were no significant differences in SGR and FI among fish fed diets with 0% (the control group), 33.3% and 66.7% OO ($P > 0.05$). As to morphological parameters, HSI was significantly increased in fish fed the diet with 100% OO than the control group ($P < 0.05$). Furthermore, the lipid content of the liver in fish fed the diet with 100% OO was significantly higher than the control group ($P < 0.05$). Fish fed the diet with 100% OO had the highest content of C18:1n-9 among dietary treatments. Serum total triglyceride (TG), low-density lipoprotein-cholesterol (LDL-C) levels and activity of serum alanine transaminase (ALT) were significantly increased in fish fed the diet with 100% OO compared with the control group ($P < 0.05$). Meanwhile, dietary OO decreased the activity of superoxide dismutase (SOD) and the total antioxidant capacity (T-AOC) in fish fed diets with increasing dietary OO levels. However, the content of malondialdehyde (MDA) was significantly increased in fish fed the diet with 100% OO compared with the control group ($P < 0.05$). The expression of pro-inflammatory genes, COX-2, IL-1β and TNFα, were significantly increased in the liver of fish fed the diet with 100% OO compared with the control group ($P < 0.05$), which was probably due to the activation of p38 mitogen-activated protein kinase (p38 MAPK) pathways and Jun N-terminal kinase (JNK) as the increased protein ratio of p-p38 MAPK to p38 MAPK and p-JNK to JNK. These results suggested that high level of dietary OO decreased the growth performance and antioxidant capacity but induced inflammation via the activation of p38 MAPK and JNK pathways in large yellow croaker.

1. Introduction

Vegetable oil is widely used in aqua-feed industry for its low price and high production. Oleic acid (OA, C18:1n-9) is a kind of main fatty acid in vegetable oil, especially in olive oil (OO) and rapeseed oil (RO). OA (25 μM) acted as an anti-inflammatory fatty acid to neutralize growth inhibition and cytotoxic effects of stearic acid in aortic endothelial cells [1]. However, OA was more prone to induced hepatic steatosis than palmitic acid through PPARγ and SREBP-1 pathway activation, especially in high levels [2,3]. In studies on fish species, OO or rapeseed oil could partially replace fish oil (FO) without compromising the growth performance in European sea bass (*Dicentrarchus labrax* L.) [4], yellowtail (*Seriola quinqueradiata*) [5], gilthead sea bream (*Sparus aurata* L.) [6]. However, excessive dietary FO replaced by OO or RO often leaded to abnormal lipid deposition, and decreased antioxidant capacity or the non-specific immunity in black carp (*Mylopharyngodon piceus*) [7], Atlantic salmon (*Salmo salar* L.) [8], barramundi (*Lates calcarifer*) [9] and tilapia (*Oreochromis niloticus*) [10]. Progresses were needed to systematically elucidate the mechanism of excessive OA in inducing lipid abnormal deposition and inflammation in fish, which has

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not been fully studied so far.

Mitogen-activated protein kinase (MAPK) families play significant roles in the activity and expression of key inflammatory mediators [11–14]. MAPK is a family of protein kinases, consisting of three different MAPKs: p38 MAPK, Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK) [15–17]. Previous studies have shown that inflammatory mediators could be produced by initiating the MAPK signaling pathways with free fatty acid [18]. OA induced superoxide production, cellular steatosis, lipoapoptosis and the inflammatory cytokines production in bovine neutrophils [19], hepatocyte cell lines [20] and 3T3-L1 cells [21] through JNK and p38 MAPK pathway. Studies have verified that MAPK pathways are highly conservative in vertebrates, and the MAPK families found in fish are homologous to the mammal's [22,23]. In fish, studies of MAPK pathway mainly focus on the hormone induction [24,25], differentiation [26] and proliferation [27], only a few studies are related to the immune response of MAPK pathway [28,29]. Fish oil is able to modify signaling through p38 MAPK that may influences Atlantic salmon's ability to handle infections and stress [29]. However, no information is concerned about the mechanism of OO-induced inflammatory response by MAPK signaling pathway in fish species to date.

Large yellow croaker (*Larimichthys crocea*) has the highest production among marine fish in China, and the use of OO or RO is prevalent in large yellow croaker feeds. The long-term inclusion high level of OO or RO often caused liver abnormal lipid deposition [7] and decreased immunity [9] of fish in the present culture state. Thus, large yellow croaker is an ideal model to systematically evaluate the metabolic effects of OO or RO to prevent lipid abnormal deposition and inflammation in fish. The object of the present study was to elucidate the mechanism of dietary OO on growth, liver lipid deposition and inflammatory responses by MAPK pathways in large yellow croaker, which could provide insight into the inflammatory transduction pathways and find a target to decrease the inflammation in cultured fish.

2. Materials and methods

2.1. Diets formulation

Four iso-nitrogenous (42% crude protein) and iso-lipidic (12% crude lipids) diets were formulated by replacing FO with graded levels of OO levels (0% as the control group, 33.3%, 66.7% and 100%) (Table 1). All ingredients were obtained from Qingdao Great Seven Biotechnology Co., Ltd in Shandong, China. Ingredients were crushed to fine powder and mixed thoroughly to make pellets. The diet preparation and storage were in accordance with specific procedures of Ai et al. [30]. No differences were found in sinking properties or physical quality among the diets. Fatty acid profiles of oil and diets were detected (Table 2).

2.2. Experimental procedures

Experimental fish were purchased from Ningde Fufa Fishery Co., Ltd in Fujian, China. Before the formal trial, large yellow croaker juveniles were raised in floating sea cages (2 × 4 × 2 m) for 2 weeks to acclimate the experimental conditions and diets. After being fasted for 24 h, fish of disease-free and homogenous sizes (average body weight: 15.87 ± 0.14 g) were randomly stocked into 12 sea cages (1 × 1 × 2 m) in triplicate (60 fish per cage). Fish were fed twice daily (05:30 and 17:30) for 10 weeks. During the feeding trial, salinity ranged from 25.0 to 30.0 g/L, and water temperature from 22.0 to 31.0 °C. Dissolved oxygen content was approximately 7.0 mg/L.

This study was conducted in strictly complying with the Management Rule of Laboratory Animals (Chinese Order No. 676 of the State Council, revised 1 March 2017).

Table 1
Formulation and chemical proximate analysis of the experimental diets (% dry weight).

Ingredients	Fish oil replacement level/%			
	0%	33.3%	66.7%	100%
White fish meal[a]	35	35	35	35
Soybean meal[a]	28	28	28	28
Wheat meal[a]	23.8	23.8	23.8	23.8
Soybean lecithin	1.5	1.5	1.5	1.5
Vitamin premix[b]	2	2	2	2
Mineral premix[b]	2	2	2	2
Attractant mixture[c]	0.1	0.1	0.1	0.1
Mould inhibitor[d]	0.1	0.1	0.1	0.1
Fish oil	7.5	5.0	2.5	0
Olive oil	0	2.5	5.0	7.5
Total	100	100	100	100
Proximate analysis (dry matter %)				
Crude protein	42.02	42.12	41.90	42.32
Crude lipid	12.47	12.66	12.02	12.23

[a] The composition of ingredients were determined by Li et al. [35].
[b] The mixture of mineral mixture and vitamin mixture according to Yan et al. [60].
[c] Attractant: the mixture of 50% glycine acid and 50% betaine by weight.
[d] Mould inhibitor: the mixture of 50% calcium propionic acid and 50% fumaric acid by weight.

Table 2
Fatty acid profiles of olive oil and the experimental diets (% total fatty acids)[f].

Fatty acid (% total fatty acids)	Olive oil	Olive oil replacement level/%			
		0%	33.3%	66.7%	100%
14:0	0.04	6.99	4.35	2.53	0.64
16:0	10.94	22.55	19.78	16.89	14.90
18:0	3.19	5.56	4.95	4.08	3.84
20:0	0.37	1.49	1.46	1.31	1.23
ΣSFA[a]	14.54	36.60	30.54	24.81	20.61
16:1n-7	0.75	8.88	6.22	3.90	1.55
18:1n-9	74.98	13.86	27.68	39.95	53.91
ΣMUFA[b]	75.73	22.74	33.91	43.85	55.46
18:2n-6	7.73	11.07	11.83	12.81	13.43
20:4n-6	0.00	1.17	0.83	0.59	0.29
Σn-6PUFA[c]	7.73	12.23	12.65	13.41	13.72
18:3n-3	0.57	1.91	1.27	1.38	1.17
20:5n-3	0.00	6.87	4.70	3.22	1.12
22:6n-3	0.00	5.07	3.75	3.12	1.41
Σn-3PUFA[d]	0.57	13.84	9.72	7.72	3.71
n-3/n-6PUFA	0.07	1.13	0.77	0.58	0.27
Σn-3LC-PUFA[e]	0.00	11.94	8.45	6.34	2.53

[a] SFA: saturated fatty acids.
[b] MUFA: mono-unsaturated fatty acids.
[c] n-6 PUFA: n-6 poly-unsaturated fatty acids.
[d] n-3 PUFA: n-3 poly-unsaturated fatty acids.
[e] LC-PUFA: long chain-polyunsaturated fatty acids.
[f] Some fatty acids, of which the contents are minor, trace amount or not detected, such as C20:0, C22:0, C24:0, C14:1, C20:1n-9, C22:1n-11, C20:2n-6, C18:3n-6, C20:3n-6, C18:4n-3, C22:5n-3, are not listed in the table.

2.3. Sample collection and analysis

At the termination of the feeding trial, all fish were fasted for 24 h and anesthetized with MS222 (1:10 000; Sigma, USA) before harvest. Survival rate (SR) was determined by counting fish in each cage, and all fish were weighted to determine the final weight. Five fish per cage were randomly collected to analyze the body composition, and another six fish per cage were sampled to determine hepatosomatic index (HSI), viscerosomatic index (VSI) and condition factor (CF). After determining morphological parameters, the liver and muscle samples were stored into RNase-free centrifuge tubes and frozen at −20 °C for moisture,

crude lipids analysis and fatty acid profile. Six fish per cage were collected for blood samples from the caudal vasculature used for biochemical analysis. The liver samples of twelve fish per cage were sampled and frozen at −80 °C for the analyses of antioxidant capacity, gene and protein expression. After sampling the liver tissue, the gut and head kidney tissue of twelve fish per cage were sampled and frozen at −80 °C for the analyses of gene expression.

2.4. Composition and fatty acid profiles analysis

The crude lipid and crude protein of ingredients, diets and fish body were determined following the procedures of Association of Official Analytical Chemists (AOAC, 1995) [31]. The moisture content of whole fish samples were determined by drying to a constant weight at 105 °C. The moisture of fish tissue and diets was determined by freezing dried in the lyophilized chamber (Alpha 1–4 LDplus; Christ, Germany), and freeze-dried samples was used for fatty acid profiles analysis. Lipids in the muscle and liver were analyzed by using chloroform/methanol (v/v, 2:1) according to the Folch et al. [32].

The fatty acid profiles were determined according to the method of Metcalfe et al. [33] with minor modifications [34]. Fatty acid were esterified into fatty acid methyl esters (FAME) by KOH–ethanol and methanolic hydrogen chloride according to the procedures previously described by Li et al. [35] and Peng et al. [36]. FAME were separated and measured by a HP6890 gas chromatograph (Agilent Technologies Inc., Santa Clara, USA). Results are expressed as the percentage of each fatty acid to total fatty acid.

2.5. Biochemical and antioxidant capacity analysis

Serum characteristics were determined with matching commercial reagent kits using automatic biochemical analyzer (BS180; Mindray, China). Determined plasma characteristics consisted of the contents of total cholesterol (TC), total triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), activities of serum aspartate aminotransferase (AST) and alanine transaminase (ALT). Total antioxidant capacity (T-AOC), activities of catalase (CAT) and superoxide dismutase (SOD), the content of malondialdehyde (MDA) in the liver were determined by diagnostic reagent kits (Nanjing Jiancheng Bio-engineering Institute, China) according to the instructions.

2.6. cDNA synthesis and real-time quantitative polymerase chain reaction (RT-qPCR)

Total RNA was isolated from the liver of fish using Trizol reagent (Takara, Japan) following the manufacturer's protocols. The quality and concentration of RNA was respectively detected by a 1.2% denaturing agarose gel and a NanoDrop spectrophotometer (Thermo Scientific, USA). Subsequently, 1000 ng of extracted RNA was treated with PrimeScript™ RT reagent Kit (Takara, Japan) to remove DNA contaminant and synthesize cDNA.

Primers (Table 3) for the RT-qPCR were designed based on the nucleotide sequences. Stable expression of the house-keeping genes β-actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were confirmed [37]. The amplification was implemented in a total volume of 25 μL (10 mM each primer: 1 μL; cDNA: 1 μL; sterilized double-distilled water: 9.5 μL; SYBR® Premix Ex Taq™ II: 12.5 μL). The procedure of RT-qPCR program was conducted following to Zuo et al. [34]. Melting curve analysis was used to confirm only one PCR product in the reaction. Standard curves were obtained with four fold serial dilutions (in triplicate) of cDNA, and the amplification efficiency was calculated by the following equation: $E = 10^{(-1/slope)} - 1$. The amplification efficiencies ranged between 0.94 and 1.05. To calculate the gene expression, the comparative Ct method ($2^{-\Delta\Delta Ct}$ method) was adopted.

2.7. Western blot

The liver protein samples were extracted by RIPA reagent (Solarbio, China) with phosphatase and protease inhibitor cocktail (Sigma-Aldrich, USA) as previously described [35]. Protein content was adjusted to the equal protein (20 μg) concentration using a Bradford Protein Assay Kit (Beyotime Biotechnology, China). The protein samples were separated by a 10% SDS-PAGE gel and transferred into a polyvinylidene difluoride (PVDF) membranes (Merck Millipore, Darmstadt, Germany). The PVDF membranes were blocked with 5% skimmed milk in tris-buffered saline with Tween™ (TBST) for 2 h at room temperature and then incubated with primary antibody in TBST overnight in the 4 °C freezer. After washed with TBST, the membranes were incubated with secondary antibody in TBST for 2 h, followed by washed and visualized with a Beyo ECL Plus kit (Beyotime Biotechnology, China).

Polyclonal anti-JNK, anti-phospho-JNK, anti-p38 MAPK, anti-phospho-p38 MAPK, anti-ERK and anti-phospho-ERK antibodies were obtained from Cell Signaling Technology (USA). Anti-GAPDH and HRP-conjugated secondary antibodies were purchased from Golden Bridge Biotechnology (China).

2.8. Calculations and statistical methods

$$\text{Specific growth rate (SGR, \% day}^{-1}) = (\text{Ln } W_t - \text{Ln } W_0) \times 100 / t$$

$$\text{Survival rate (SR\%)} = N_t \times 100 / N_0$$

$$\text{Feed intake (FI, \% day}^{-1}) = F_d \times 100 / [(W_t + W_0) / 2] / t$$

$$\text{Feed efficiency ratio (FER)} = W_g / F_d$$

$$\text{Viscerosomatic index (VSI\%)} = W_v \times 100 / W_t$$

$$\text{Hepatosomatic index (HSI\%)} = W_l \times 100 / W_t$$

$$\text{Condition factor (CF\%)} = 100 \times W_t / (L_t^3)$$

Where W_t and W_0 were final and initial body weight, respectively; N_t and N_0 were final and initial fish number, respectively; F_d was dry feed fed in g; t was duration of experimental days; W_g was wet weight gain in g; W_v was visceral wet weight; W_l was liver wet weight; L_t was final body length.

All data were processed by SPSS 19.0 (IBM, America). Statistics were performed to a one-way analysis of variance (ANOVA) and followed by Tukey's test. Statistics with $P < 0.05$ was regarded to be significant, and the results were shown as means ± S.E.M. (standard error of the mean).

3. Results

3.1. Survival and growth performance

As dietary OO increased, no significant difference was found in SR of fish fed diets with graded levels of OO ($P > 0.05$). SGR significantly decreased from 1.22% day^{-1} to 0.98% day^{-1} with increasing dietary OO levels ($P < 0.05$). FI was significantly decreased in fish fed the diet with 100% OO than the control group ($P < 0.05$). However, there were no significant differences in SGR and FI among fish fed diets with 0% (the control group), 33.3% and 66.7% OO ($P > 0.05$). HSI showed an increasing trend (from 1.88% to 2.33%) with increasing dietary OO levels, and HSI was significantly increased in juvenile fish fed the diet with 100% OO than the control group ($P < 0.05$). However, no significant differences were found in VSI and CF of fish fed diets with increasing dietary OO levels ($P > 0.05$) (Table 4).

Table 3
Sequences of the PCR primers used in this study.

Target gene	Forward primers (5′–3′)	Reverse primers (5′–3′)	Accession number
Nrf2	GATGGAAATGGAGGTGATGC	CATGTTCTTTCTGTCGGTGG	XM 010737768
SOD1	GCGGGACCGTGTTCTTTGAG	GCTACCAGCGTTGCCAGTCTTT	NM 001303360
SOD2	GGCACTGGCAAAGGGAGAC	CACAAGCGGCGATACGAAG	NM 001303364
CAT	CTTCATCAGGGACGCTCTACT	ATGGCATAATCTGGGTTGGT	XM 010735178
COX-2	CTGGAAAGGCAACACAAGC	CGGTGAGAGTCAGGGACAT	KP259877
IL-6	CGACACACCCACTATTTACAAC	TCCCATTTTCTGAACTGCCTCT	KU140675
IFNγ	TCAGACCTCCGCACCATCA	GCAACCATTGTAACGCCACTTA	KM501500
IL-1β	CATAGGGATGGGGACAACGA	AGGGGACGGACACAAGGGTA	KJ459927
TNFα	ACACCTCTCAGCCACAGGAT	CCGTGTCCCACTCCATAGTT	EF165623
IL-10	AGTCGGTTACTTTCTGTGGTG	TGTATGACGCAATATGGTCTG	XM010738826
Arg1	AACCACCCGCAGGATTACG	AAACTCACTGGCATCACCTCA	XM019269015
β-actin	GACCTGACAGACTACCTCATG	AGTTGAAGGTGGTCTCGTGGA	GU584189
GAPDH	GACAACGAGTTCGGATACAGC	CAGTTGATTGGCTTGTTTGG	XM010743420

Nrf2: nuclear factor erythroid 2-related factor 2, SOD: superoxide dismutase, CAT: catalase, COX-2: cyclooxygenase-2, IL-6: interleukin-6, IFNγ: interferon γ, IL-1β: interleukin-1β, TNFα: tumour necrosis factor α, IL-10: interleukin-10, Arg-1: arginase-1, GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

3.2. Body composition analysis

Moisture, protein and lipid contents of fish whole body were not significantly different among dietary treatments ($P > 0.05$). With the increase of dietary OO, the lipid content of the liver showed an increasing trend (from 23.72% to 32.36%). The lipid content of the liver in fish fed the diet with 100% OO was significantly higher than the control group ($P < 0.05$). However, fish fed diets with equal or less than 66.7% OO showed no significant difference in the lipid content of the liver ($P > 0.05$). Dietary OO levels showed no significant effects on the moisture and lipid contents of muscle in fish among dietary treatments (Table 5).

3.3. Fatty acid profiles in the liver

The dietary oleic acid (OA) increased from 13.86% to 53.91% with increasing dietary OO levels (Table 2), and the content of OA in the liver of fish was increased from 21.62% to 49.95% with increasing dietary OO levels (Table 6). Fish fed diets with equal or exceeding 33.3% OO had significantly higher content of OA than the control group ($P < 0.05$). The contents of C16:0, C18:0, C20:4n-6, C20:5n-3 and C22:6n-3 decreased in the liver of fish fed diets with increasing dietary OO levels. Further, the minimum contents of SFA (25.03%), n-3 LC-PUFA (0.7%) and the ratio of n-3 to n-6 PUFA (0.18%) were found in fish fed the diet with 100% OO among dietary treatments ($P < 0.05$) (Table 6).

3.4. Serum biochemical indexes

In the present experiment, increased serum lipid contents were observed in fish fed diets with increasing dietary OO levels. The content of TG significantly increased from 3.15 to 4.80 mmol/L, and the content of LDL-C significantly increased from 0.71 to 1.22 mmol/L with increasing dietary OO levels ($P < 0.05$). However, dietary OO levels showed no significant difference in the content of TC in fish among dietary treatments. The content of HDL-C in fish fed the diet with 100% OO was significantly lower compared with the control group ($P < 0.05$). Activity of serum ALT in fish fed the diet with 100% OO was significantly higher compared with the control group. However, no significant difference in activity of serum AST was found in fish among dietary treatments (Table 7).

Table 5
Body composition analysis of large yellow croaker fed diets with different levels of olive oil (Means ± S.E.M)[a].

Index (wet weight, %)	0%	33.3%	66.7%	100%
Whole body (%)				
Moisture	73.25 ± 0.63	73.96 ± 1.16	73.36 ± 1.02	71.84 ± 1.30
Lipid	7.41 ± 0.24	6.72 ± 0.25	7.05 ± 0.40	6.74 ± 0.29
Protein	15.74 ± 0.20	16.13 ± 0.50	15.95 ± 0.66	16.97 ± 0.33
Liver (%)				
Moisture	58.81 ± 1.35	60.01 ± 1.57	57.49 ± 2.04	59.37 ± 2.45
Lipid	23.72 ± 1.04^{b}	26.72 ± 2.19^{ab}	26.00 ± 2.08^{ab}	32.36 ± 1.12^{a}
Muscle (%)				
Moisture	72.92 ± 0.90	72.70 ± 0.78	73.55 ± 0.83	74.18 ± 1.16
Lipid	7.91 ± 1.00	7.41 ± 1.07	9.09 ± 0.78	8.26 ± 1.22

[a] Data are presented as means ± S.E.M. Means in each row sharing the same superscript letter or absence of superscripts are not significantly different determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

Table 4
Growth, survival and somatic parameters of large yellow croaker fed diets with different levels of olive oil (Means ± S.E.M)[a].

Index	0%	33.3%	66.7%	100%
Initial body weight (IBW, g)	15.89 ± 0.42	15.86 ± 0.27	15.83 ± 0.36	15.89 ± 0.21
Final body weight (FBW, g)	37.31 ± 0.79^{a}	37.08 ± 0.79^{a}	35.44 ± 0.77^{ab}	31.67 ± 0.99^{b}
Specific growth rate (SGR, % day^{-1})	1.22 ± 0.05^{a}	1.21 ± 0.03^{ab}	1.15 ± 0.06^{ab}	0.98 ± 0.06^{b}
Survival rate (SR, %)	90.56 ± 2.42	85.56 ± 1.47	88.33 ± 1.92	84.44 ± 0.56
Feed Intake (FI, %/d)	2.12 ± 0.02^{a}	2.13 ± 0.03^{a}	2.08 ± 0.04^{ab}	1.95 ± 0.04^{b}
Feed efficiency ratio (FER)	0.54 ± 0.02	0.54 ± 0.02	0.52 ± 0.02	0.48 ± 0.03
Viscera-somatic index (VSI, %)	7.14 ± 0.32	7.40 ± 0.37	7.41 ± 0.48	7.33 ± 0.32
Hepato-somatic index (HSI, %)	1.88 ± 0.09^{b}	2.06 ± 0.13^{ab}	2.17 ± 0.12^{ab}	2.33 ± 0.09^{a}
Condition Factor (CF, %)	1.02 ± 0.04	0.94 ± 0.04	0.98 ± 0.04	0.95 ± 0.03

[a] Data are presented as means ± S.E.M. Means in each row sharing the same superscript letter or absence of superscripts are not significantly different determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

Table 6
Fatty acid profiles (% total fatty acids) in the liver of large yellow croaker fed diets with different levels of olive oil (Means ± S.E.M)[f].

Fatty acid (% total fatty acids)	0%	33.3%	66.7%	100%
14:0	4.47 ± 0.20[a]	3.45 ± 0.11[b]	2.78 ± 0.16[c]	1.51 ± 0.09[d]
16:0	22.13 ± 0.75[a]	21.36 ± 1.22[a]	19.49 ± 0.61[ab]	16.87 ± 0.72[b]
18:0	7.23 ± 0.13[a]	6.21 ± 0.24[ab]	5.61 ± 0.59[ab]	5.31 ± 0.44[b]
20:0	1.76 ± 0.15	1.59 ± 0.03	1.66 ± 0.04	1.34 ± 0.11
ΣSFA[a]	35.60 ± 1.02[a]	32.61 ± 1.52[ab]	29.54 ± 1.35[bc]	25.03 ± 1.03[c]
16:1n-7	12.98 ± 0.51[a]	11.38 ± 0.36[a]	9.11 ± 0.67[b]	6.24 ± 0.38[c]
18:1n-9	21.62 ± 1.06[c]	30.80 ± 0.73[b]	36.89 ± 2.71[b]	49.95 ± 0.55[a]
ΣMUFA[b]	34.60 ± 1.30[c]	42.18 ± 0.98[b]	46.01 ± 2.04[b]	56.18 ± 0.31[a]
18:2n-6	7.50 ± 0.10	7.99 ± 0.59	8.36 ± 0.75	9.64 ± 0.74
20:4n-6	0.48 ± 0.09[a]	0.31 ± 0.04[ab]	0.21 ± 0.03[b]	0.16 ± 0.03[b]
Σn-6PUFA[c]	7.98 ± 0.01	8.30 ± 0.62	8.57 ± 0.77	9.79 ± 0.75
18:3n-3	1.64 ± 0.14	1.42 ± 0.22	1.38 ± 0.22	1.10 ± 0.17
20:5n-3(EPA)	3.66 ± 0.54[a]	1.93 ± 0.12[b]	1.46 ± 0.35[bc]	0.31 ± 0.07[c]
22:6n-3(DHA)	2.01 ± 0.18[a]	1.35 ± 0.15[b]	0.47 ± 0.09[c]	0.39 ± 0.09[c]
Σn-3PUFA[d]	7.30 ± 0.50[a]	4.70 ± 0.46[b]	3.32 ± 0.53[bc]	1.79 ± 0.32[c]
n-3/n-6PUFA	0.91 ± 0.06[a]	0.58 ± 0.10[b]	0.38 ± 0.04[bc]	0.18 ± 0.02[c]
Σn-3LC-PUFA[e]	5.67 ± 0.36[a]	3.28 ± 0.25[b]	1.94 ± 0.32[c]	0.70 ± 0.16[c]

[a] SFA: saturated fatty acids.
[b] MUFA: mono-unsaturated fatty acids.
[c] n-6 PUFA: n-6 poly-unsaturated fatty acids.
[d] n-3 PUFA: n-3 poly-unsaturated fatty acids.
[e] LC-PUFA: long chain-polyunsaturated fatty acids.
[f] Some fatty acids, of which the contents are minor, trace amount or not detected, such as C20:0, C22:0, C24:0, C14:1, C20:1n-9, C22:1n-11, C20:2n-6, C18:3n-6, C20:3n-6, C18:4n-3, C22:5n-3, are not listed in the table. Data are presented as means ± S.E.M. Means in each row sharing the same superscript letter or absence of superscripts are not significantly different determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

3.5. Antioxidant capacity and related gene expression

Antioxidant capacity was decreased in the liver of fish fed diets with increasing dietary OO levels. Concentration of hepatic MDA (Fig. 1a) was significantly increased in the liver of fish fed the diet with 100% OO compared with the control group ($P < 0.05$). Fish fed the diet with 100% OO had significantly decreased activity of SOD (Fig. 1b) and decreased capacity T-AOC (Fig. 1c) compared with the control group ($P < 0.05$). However, there were no significant differences in concentration of hepatic MDA, activity of SOD and capacity T-AOC in the liver of fish fed diets with 0% (the control group), 33.3% and 66.7% OO ($P > 0.05$). The activity of CAT showed no remarkable difference among dietary treatments ($P > 0.05$) (Fig. 1d). Transcription of SOD1 and SOD2 was significantly decreased in the liver of fish fed the diet with 100% OO compared with the control group. Further, transcription of nuclear factor erythroid 2-related factor 2 (Nrf2) and CAT decreased in the liver of fish fed diets with increasing dietary OO levels, but no significant difference in the transcription of Nrf2 was observed among fish fed the diet with 100% OO, 66.7% and the control group ($P > 0.05$) (Fig. 2).

3.6. Inflammatory gene expression

Transcription of COX-2, IL-1β and TNFα were significantly increased in the liver (Fig. 3), head kidney (Fig. S1) and gut (Fig. S2) of fish fed the diet with 100% OO than the control group ($P < 0.05$), but no significant differences in transcription of COX-2, IL-1β and TNFα were found in liver (Fig. 3), head kidney (Fig. S1) and gut (Fig. S2) of fish fed diets with 66.7% OO, 33.3% OO and the control group ($P > 0.05$). There were no significant differences in transcription of IL-6 and IFNγ in the liver (Fig. 3) and gut (Fig. S2) among dietary treatments ($P > 0.05$). Transcription of IL-10 was decreased in the liver of fish fed diets with increasing dietary OO levels, and the lowest expression was found in the liver of fish fed the diet with 100% OO among dietary treatments ($P < 0.05$) (Fig. 3). However, transcription of Arg1 showed no significant difference in the liver of fish among dietary treatments ($P > 0.05$) (Fig. 3).

3.7. Western blot for MAPK pathway

To examine the activation status of MAPK pathways in the liver of

Table 7
Serum biochemical indexes and enzyme activities of large yellow croaker fed diets with different levels of olive oil (Means ± S.E.M.)[g].

Plasma biochemical indexes	0% (the control)	33.3%	66.7%	100%
TC[a] (mmol/L)	2.63 ± 0.14	2.79 ± 0.10	2.92 ± 0.16	3.21 ± 0.31
TG[b] (mmol/L)	3.15 ± 0.17[b]	3.44 ± 0.32[ab]	3.98 ± 0.58[ab]	4.80 ± 0.47[a]
HDL-C[c] (mmol/L)	0.77 ± 0.04[a]	0.79 ± 0.03[a]	0.68 ± 0.02[a]	0.41 ± 0.02[b]
LDL-C[d] (mmol/L)	0.71 ± 0.08[b]	0.73 ± 0.07[b]	0.85 ± 0.13[ab]	1.22 ± 0.11[a]
ALT[e] (U/L)	56.99 ± 2.77[b]	60.10 ± 3.94[ab]	64.74 ± 3.32[ab]	73.22 ± 4.76[a]
AST[f] (U/L)	5.64 ± 0.57	6.05 ± 0.57	6.10 ± 0.91	7.25 ± 0.63

[a] TC: Total cholesterol.
[b] TG: Total triglyceride.
[c] HDL-C: High density lipoprotein cholesterol.
[d] LDL-C: Low density lipoprotein cholesterol.
[e] ALT: Alanine transaminase.
[f] AST: Aspartate aminotransferase.
[g] Data are presented as means ± S.E.M. Means in each row sharing the same superscript letter or absence of superscripts are not significantly different determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

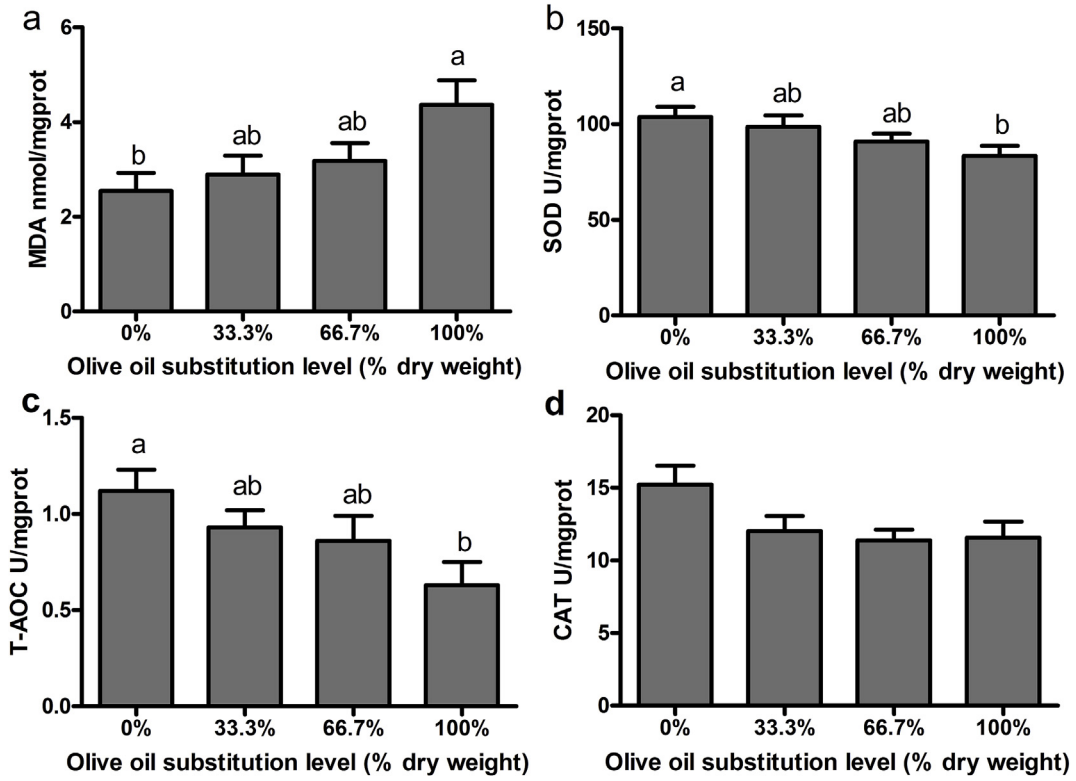

Fig. 1. Concentration of (a) MDA and activities of (b) SOD, (c) T-AOC and (d) CAT in the liver of large yellow croaker. MDA: malondialdehyde, SOD: superoxide dismutase, T-AOC: total antioxidant capacity, CAT: catalase. 0%: control diet with 0% fish oil replaced by olive oil; 33.3%: diet with 33.3% fish oil replaced by olive oil; 66.7%: diet with 66.7% fish oil replaced by olive oil; 100%: diet with fish oil totally replaced by olive oil; Data are presented as means ± S.E.M. Columns sharing the same superscript letter or absence of superscripts are not significantly different determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

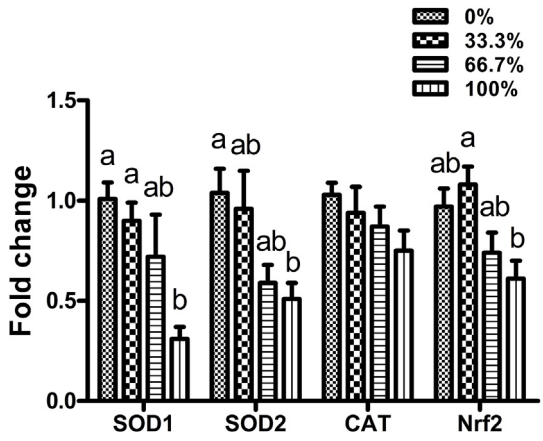

Fig. 2. Antioxidant gene expression in the liver of large yellow croaker. 0%: control diet with 0% fish oil replaced by olive oil; 33.3%: diet with 33.3% fish oil replaced by olive oil; 66.7%: diet with 66.7% fish oil replaced by olive oil; 100%: diet with fish oil totally replaced by olive oil; Data are presented as means ± S.E.M. Columns sharing the same superscript letter or absence of superscripts are not significantly different determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

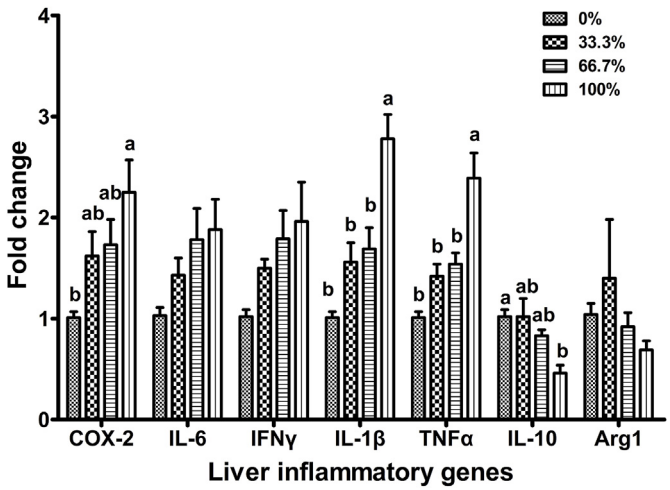

Fig. 3. Inflammatory gene expression in the liver of large yellow croaker. 0%: control diet with 0% fish oil replaced by olive oil; 33.3%: diet with 33.3% fish oil replaced by olive oil; 66.7%: diet with 66.7% fish oil replaced by olive oil; 100%: diet with fish oil totally replaced by olive oil; Data are presented as means ± S.E.M. Columns sharing the same superscript letter or absence of superscripts are not significantly different determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

large yellow croaker fed diets with different dietary OO levels, p-p38 MAPK, p38 MAPK, p-JNK, JNK, p-ERK and ERK protein expression levels were detected. Compared with the control group, the ratios of p-p38 MAPK to p38 MAPK protein (Fig. 4b) and p-JNK to JNK protein (Fig. 4c) were significantly increased in the liver of fish fed the diet with 100% OO ($P < 0.05$). However, there were no significant differences in the ratios of p-p38 MAPK to p38 MAPK protein and p-JNK to JNK protein in the liver of fish fed diets with 0% (the control group), 33.3% and 66.7% OO ($P > 0.05$). No significant difference was detected among the ratio of p-ERK to ERK protein in the liver of fish fed diets with different OO levels ($P > 0.05$) (Fig. 4d). These data indicate that dietary OO induced the activation of MAPK pathway probably via the increasing ratios of p-p38 MAPK to p38 MAPK protein and p-JNK to JNK protein.

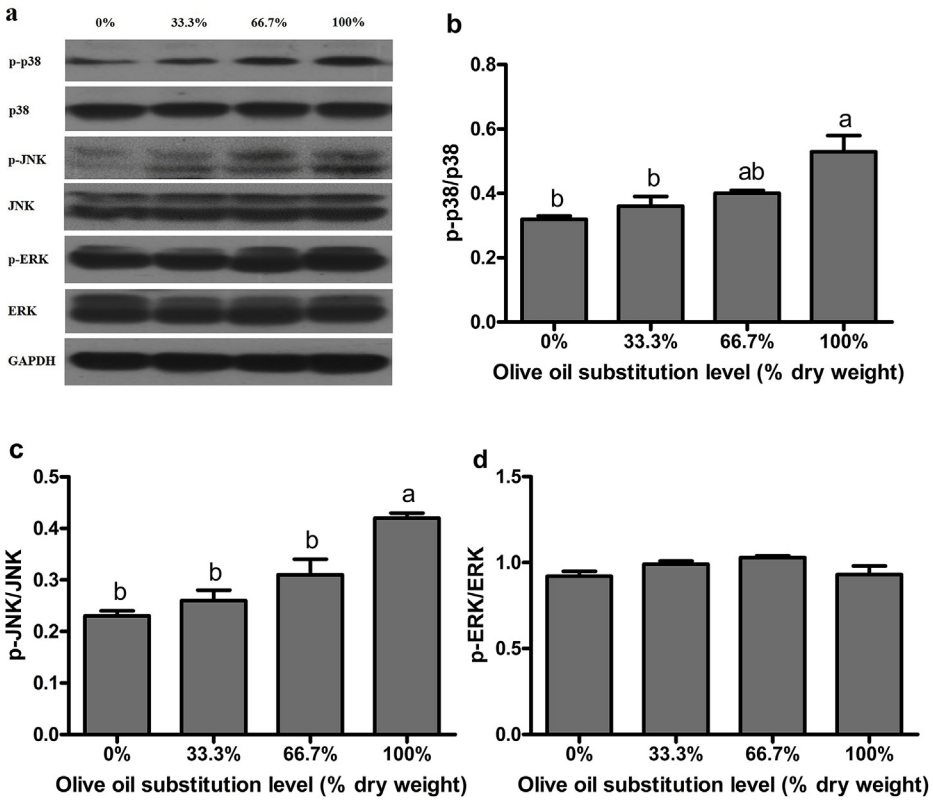

Fig. 4. MAPK pathway in the liver of large yellow croaker. (a) Western blot analysis of proteins involved in MAPK pathways in the liver of large yellow croaker. Data are expressed as A.U. of the western blot and are depicted as a ratio of (b) p-p38 to p38, (c) p-JNK to JNK and (d) p-ERK to ERK. 0%: control diet with 0% fish oil replaced by olive oil; 33.3%: diet with 33.3% fish oil replaced by olive oil; 66.7%: diet with 66.7% fish oil replaced by olive oil; 100%: diet with fish oil totally replaced by olive oil; p-p38: phospho-p38; ERK: extracellular signal-regulated kinase; p-ERK: phospho-extracellular signal-regulated kinase; JNK: Jun N-terminal kinases; p-JNK: phospho-Jun N-terminal kinases; GAPDH: glyceraldehyde-3-phosphate dehydrogenase. Data are presented as means ± S.E.M. Columns sharing the same superscript letter or absence of superscripts are not significantly different determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

4. Discussion

The monounsaturated fatty acids (MUFAs), especially OA play vital roles in affecting growth performance and the lipid deposition of aquatic animals [7,38]. In this study, fish fed the diet with 100% OO displayed a significantly lower SGR (0.98% day^{-1}) than the control group (1.22% day^{-1}), while FER was not affected among dietary treatments. In addition, dietary OO increased HSI and the lipid content of liver in large yellow croaker fed diets with increasing dietary OO levels. In the present study, dietary OA was significantly increased with increasing dietary OO levels, which could be responsible for the decreased growth and abnormal lipid deposition in the liver. Previous studies have shown that OA was more predominant in neutral lipid than stearic acid (18:0) [39], linoleic acid (18:2n-6) [40] and α-linolenic acid (18:3n-3) [41,42]. Further, OA was more prone to promote lipid deposition than palmitic acid in hepatocyte [3,39,43]. This was consistent with the findings in European seabass [4], Atlantic Salmon [38], and yellowtail [5].

Excessive OA inclusion significantly increased the contents of serum TC and LDL-C and decreased the contents of serum HDL-C in fish fed the diet with 100% OO than the control group. Those changes reflected increased serum lipid content in fish fed diets with increasing dietary OO levels. The serum lipid content is a hallmark of lipid metabolism in vertebrate and a kind of indicators evaluating individual physiological state [35,44,45]. The hyperlipidemic status of fish fed the diet with 100% OO was probably due to the increased lipid abnormal deposition induced by the increased OA in the diet. This is consistent with previous findings in yellowtail [5], Japanese seabass (*Lateolabrax japonicus*) [46], blunt snout bream (*Megalobrama amblycephala*) [47]. Liver is a vital organ for metabolism, and activities of the serum ALT and AST are often regarded as general biomarkers of the physiological state of the vertebrate liver [48]. In this study, activity of the serum ALT was significantly increased in fish fed the diet with 100% OO than the control group. In accordance with this result, HepG2 cells incubated in OA showed increased cellular lipid accumulation and followed significant histological damage than the control group [49]. These results indicated that OO was prone to promote lipid deposition and induce liver damage.

The promoted lipid deposition and increased activity of the serum ALT are consistent with the decline in the capacity of hepatic antioxidant defense system. In this study, the MDA concentration in the liver was significantly increased in fish fed the diet with 100% OO compared with the control group. Further, the activity of SOD and T-AOC and transcription of SOD1 and SOD2 were significantly decreased in fish with increasing dietary OO levels. The decreased antioxidant activities and related genes expression clearly demonstrate that high level of dietary OO could reduce antioxidant capacity in fish. This is consistent with previous findings in tilapia [10], yellowtail kingfish (*Seriola lalandi*) [50] and Japanese seabass [51]. The decreased antioxidant defense system was probably due to the abnormal lipid deposition in the liver. Abnormal deposition could result hepatocyte in the metabolic abnormalities and impair the ability of hepatocyte to maintain antioxidant capacity.

The dyslipidemia indexes and decreased antioxidant capacity indicated that fish fed the diet with 100% OO was not in relatively healthy physical condition. As long-term inflammation was associated with poor physiological state of fish [51], we explore whether the poor physiological state of fish was related to the inflammation or not. Previous studies have demonstrated that several transduction signals of the inflammation are regulated by excessive OA, and these pathways seem to converge at the MAPK pathway [52–54]. In this study, the ratio of p-JNK to JNK, p-p38 MAPK to p38 MAPK protein and the COX-2, IL-1β, TNFα mRNA expression were both increased in fish fed the diet with 100% OO. These results mean the activation of JNK and p38 MAPK pathway and the increased inflammation in fish fed the diet with 100% OO compared with the control group. Similar results have been found that the activation of MAPK signaling increase the transcription levels of COX-2 [55], IL-1β [56] and TNFα [57]. The IL-10 is a natural suppressant of inflammatory response, and its transcript level could be influenced by fatty acid [58]. The decreased IL-10 expression could be

the result of high level of olive oil induced abnormal lipid deposition and the inflammatory response in large yellow croaker. However, the ratio of p-ERK to ERK protein had no significant difference in fish among dietary treatments. Studies on fish species have verified that p38 MAPK and JNK are involved in inflammation, while ERK is involved in cell proliferation and survival [23,59]. Similar results were found in our previous finding that high fat diets increased COX-2 expression via intracellular MAPK phosphorylation-dependent NF-κB and AP-1 pathways [28]. Therefore, the activation of p38 MAPK and JNK pathways are involved in high percentage of dietary OO-induced inflammation in large yellow croaker.

5. Conclusion

High level of dietary OO decreased the growth performance, antioxidant capacity and increased liver lipid deposition, the contents of serum TG and LDL-C in large yellow croaker. The inflammatory response in fish fed high level of dietary OO was probably due to the activation of p38 MAPK and JNK pathways.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2019.08.062.

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