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Effect of dietary fatty acid composition on growth, fatty acids composition and hepatic lipid metabolism in juvenile turbot (*Scophthalmus maximus* L.) fed diets with required n3 LC-PUFAs

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ABSTRACT

A 12-week feeding experiment was conducted to investigate the effects of dietary fatty acids composition on growth performance, lipid deposition and some genes expression of hepatic lipid metabolism in juvenile turbot (*Scophthalmus maximus* L.) (mean initial body weight, 9.49 ± 0.03 g) fed diets with required n3 LC-PUFA. Juvenile turbot were fed diets with palm oil (PO), rapeseed oil (RO), soybean oil (SO) or linseed oil (LO) replacing the same levels of fish oil (FO), respectively. Each diet was randomly fed to triplicate tanks, and each tank was stocked with 35 fish. The results showed that growth performance and feed utilization of turbot fed diets with required n3 LC-PUFA were independent of dietary fatty acids composition. As expected, C16:0, C18:1n, C18:2n-6, C18:3n-3 and n3 LC-PUFAs level in liver were up to the maximum in the PO, RO, SO, LO and FO group, respectively, and significantly higher than that in the other groups ($P < 0.05$). Fish fed the diet with RO had the lowest plasma triglyceride and the highest HDL-c/LDL-c, although no significant difference of plasma triglyceride had been found between the RO group and the LO group. Hepatic lipid content in fish fed diets with SO, RO, and LO was significantly higher compared with PO and FO groups. The higher activities of hepatic *G6PD* and *ME* were found in fish fed diets with PO or FO compared to LO. The relative expression of *LPL*, *LXR*, and *ApoB-100* gene in the RO group and the LO group, *MTP* gene in the LO group, *PPARα* and *FAS* genes in the PO group, and *PPARγ* gene in the SO group were significantly higher than those in the FO group. These results indicated that the increase in hepatic lipid deposition of turbot induced by the SO, RO, and LO diets are associated with up-regulation of some genes involved in lipids biosynthesis metabolism.

1. Introduction

With the costs of fish oil (FO) increasing as global supplies become depleted, vegetable oil (VO) is a promising alternative owing to lower price and concentrations of dioxins, steadily increasing production, availability, and better economic value. Among VOs, palm oil (PO), rapeseed oil (RO), soybean oil (SO), and linseed oil (LO), which are included in the commercial diet of finfish, are rich in saturated fatty acid (SFAs), monounsaturated fatty acid (MUFAs), linoleic acid (LA), and α-linolenic acid (ALA), respectively. Compared with poly-unsaturated fatty acids (PUFAs), SFA and MUFA have traditionally been thought to be good substrates for the β-oxidation (Henderson, 1996). However, all VO have hardly anyn3 LC-PUFAs, especially EPA and DHA (Turchini et al., 2009). Studies have shown that the substitution of FO by VO leads to relatively higher hepatic or whole body lipid content in some fish (Bell et al., 1994, 1999; Lim et al., 2001; Altundag et al., 2014; Li et al., 2016; Torrecillas et al., 2017), and the changes might be related and associated with high level of dietary fatty acids, such as 18:2n-6 in the SO replacement (Li et al., 2016). It is unknown that the different effect of fish fed diets with FO replaced by VO (such as, PO, RO, SO, or LO) when the diet with n3 LC-PUFAs meets to the minimum requirement of fish. Hence, this research provided a better

Abbreviations: LC-PUFA, Long-chain polyunsaturated fatty acid; HDL-c/LDL-c, Ratio of high- to low-density lipoprotein cholesterol; *LPL*, Lipoprotein lipase; *G6PD*, Glucose-6-phosphate dehydrogenase; *ME*, Malic enzyme; *ApoB-100*, Apolipoprotein B-100; *PPARα*, Peroxisome proliferator-activated receptor alpha; *PPARγ*, Peroxisome proliferator-activated receptor gamma; *LXR*, Liver X receptors; *FAS*, Fatty acid synthetase; *MTP*, Microsomal triacylglycerol transfer protein

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understanding of the physiological effects (especially on lipid metabolism) of dietary FO substitution by VO on fish fed with the required n3 LC-PUFAs.

In general, FAs in the diet reach the liver as chylomicron remnants rather than non-esterified FAs (NEFAs). The FA components of triglycerides (TGs) are oxidized for energy, used for synthesis of complex lipids such as phospholipids, or resynthesized into TGs, packaged into very low-density lipoprotein (VLDL), and returned to the blood (Nguyen et al., 2008). Lipid homeostasis in the liver is regulated by transcription factors including peroxisome proliferator-activated receptors (PPARs) (Jump, 2008; Pettinelli et al., 2009), sterol regulatory element-binding protein (SREBP)-1 (Takeuchi et al., 2010; Georgiadi and Kersten, 2012) and hepatocyte nuclear factor 4α (HNF4α) (López-Soldado et al., 2009; Martinez-Jimenez et al., 2010), which stimulate FA β-oxidation, biosynthesis, and lipid transport/VLDL secretion, respectively. In addition, glucose-6-phosphate dehydrogenase (G6PD) and malic enzyme (ME) are key regulators of nicotinamide adenine dinucleotide phosphate (NADPH) production in the liver, which is essential for hepatic FA biosynthesis. Changes in dietary PUFA level affect the activities of G6PDH and ME (Khan et al., 2012; Liu et al., 2013). To date, few studies have examined how substitution of FO by VO in the fish diet affects the expression of genes involved in lipid metabolism, which could in turn explain the observed changes in the lipid deposition.

The turbot, which has delicious meat and grows rapidly, and thus has high economic value, is widely cultured in Europe and Asia. In preliminary studies investigating the replacement of FO in the turbot diet by VOs such as SO (Bell et al., 1994; Regost et al., 2003; Peng et al., 2014), LO (Bell et al., 1994, 1999; Regost et al., 2003), olive oil (Bell et al., 1999), and safflower oil (Altundag et al., 2014), fish consuming high VO diets had a higher level of lipid content in the liver than those fed with a 100% FO diet. We wanted to explore whether VO supplementation will not affect the lipid deposition of liver or not and verify the different effects of fish fed diets with FO replaced by different VO when the diet with n3 LC-PUFAs meets to the minimum requirement of fish. FO substitution by SO, LO, RO, and PO was tested in this study and the effect of dietary FA composition on growth, plasma biochemical parameters, and lipid deposition and the possible mechanism was assessed.

2. Materials and methods

2.1. Experimental diets

White fish meal, casein, soybean meal and wheat gluten meal were used as main protein sources. Five isonitrogenous (digestible protein: 51% dry matter (DM)) and isolipidic (digestible fat: 12% DM) practical diets were formulated to contain different VOs. PO, RO, SO and LO replaced the same FO level in diets, and 100% FO group was set as the control. The ratio of LC-PUFAs (EPA + DHA) to total fatty acids in the lipid of fish oil is 13.3%–25.8% (Li et al., 1996, 20%; Qiu et al., 2017, 16.67%; Turchini et al., 2009, 13.3%–25.8% (except for Capelin oil)). Due to the marine FO and phospholipids contained in the fish meal itself, the basal diet contained > 0.80% LC-PUFAs on a dry matter basis, which had met the requirement of LC-PUFA for turbot (Henderson, 1996; Gatesoupe et al., 1977), and the essential ratio of EPA and DHA for turbot was 0.6–1.3% (Léger et al., 1979). Before the diet preparation, fish meal and soybean meal were smashed to pass through a 320 μm sieve. The procedures for diet preparation and storage were as previously described by Ai et al. (2011). The differences of physical quality or sinking properties in diets had not found. Ingredients and nutrient composition of the five experimental diets are given in detail in Table 1 and Table 2.

Table 1
Formulation and proximate composition of the experimental diets.

Ingredient (dry weight, %)	Dietary oil source				
	FO	PO	RO	SO	LO
White fish meal[a]	27.00	27.00	27.00	27.00	27.00
Wheat gluten meal[a]	12.50	12.50	12.50	12.50	12.50
Casein[a]	9.00	9.00	9.00	9.00	9.00
Wheat flour[a]	19.28	19.28	19.28	19.28	19.28
Soybean meal[a]	18.80	18.80	18.80	18.80	18.80
Fish oil (FO)[b]	7.60	2.80	2.80	2.80	2.80
Palm oil (PO)[b]		4.80			
Rapeseed oil (RO)[b]			4.80		
Soybean oil (SO)[b]				4.80	
Linseed oil (LO)[b]					4.80
Soybean lecithin[c]	2.00	2.00	2.00	2.00	2.00
Mineral premix[d]	0.50	0.50	0.50	0.50	0.50
Vitamin premix[e]	1.00	1.00	1.00	1.00	1.00
Choline chloride	0.13	0.13	0.13	0.13	0.13
Monocalcium phosphate	1.00	1.00	1.00	1.00	1.00
Calcium propionic acid	0.10	0.10	0.10	0.10	0.10
Ethoxyquin	0.05	0.05	0.05	0.05	0.05
Y_2O_3	0.04	0.04	0.04	0.04	0.04
Phagostimulant[f]	1.00	1.00	1.00	1.00	1.00
Proximate analysis (dry matter, %)					
Crude protein	51.43	51.35	51.60	51.92	51.45
Crude lipid	11.82	12.50	12.39	12.39	11.94
Crude ash	8.29	8.46	8.43	8.38	8.51

[a] White fish meal (dry mater, %): crude protein 70.22, crude lipid 6.28; Wheat gluten meal (dry mater, %): crude protein 83.95, crude lipid 1.28; Casein (dry mater, %): crude protein 89.44, crude lipid 1.46; Wheat meal (dry mater, %): crude protein16.03, crude lipid 3.32; Soybean meal (dry mater, %): crude protein 51.36, crude lipid 1.79. These ingredients obtained from Great seven Bio-Tech (Qingdao, China).

[b] FO (fatty acids, %TFA): SFA 23.94, MUFA 23.65, C18:2n-6 4.18, C18:3n-3 1.78, EPA 3.45, DHA 10.57; Palm oil (fatty acids, % TFA): SFA 78.54, MUFA 1.97, C18:2n-6 15.21; RO (fatty acids, %TFA): SFA 12.14, MUFA 51.08, C18:2n-6 15.08, C18:3n-3 6.31, EPA 0.38, DHA 0.21; SO (fatty acids, % TFA): SFA 16.55, MUFA 24.91, C18:2n-6 51.25, C18:3n-3 6.68; LO (fatty acids, % TFA): SFA 16.44, MUFA 11.16, C18:2n-6 16.98, C18:3n-3 54.36, EPA 0.11, DHA 0.05; FO, PO, RO, SO and LO obtained from Great seven Bio-Tech (Qingdao, China), Jin Long Yu (Suzhou, China), Le Nong Pin (Ningbo, China), Jin Long Yu (Suzhou, China), Xin Qi Dian (Beijing, China).

[c] Soybean lecithin (purity > 95%), obtained from Guangzhou Timpol Bio-Tech Co. LTD. (Guangzhou, China).

[d] Mineral premix: (mg kg^{-1} diet): NaF, 2; KI, 0.8; $CoCl_2{\cdot}6H_2O$ (1%), 50; $CuSO_4{\cdot}5H_2O$, 10; $FeSO_4{\cdot}H_2O$, 80; $ZnSO_4{\cdot}H_2O$, 50; $MnSO_4{\cdot}H_2O$, 60; $MgSO_4{\cdot}7H_2O$, 1200; $Ca(H_2PO_3)_2{\cdot}H_2O$, 3000; zoelite, 15.55 g kg^{-1} diet.

[e] Vitamin premix(mg kg^{-1} diet): thiamin, 25; riboflavin, 45; pyridoxine HCl, 20; vitamin B12, 0.1; vitamin K3, 10; inositol, 800; pantothenic acid, 60; niacin acid, 200; folic acid, 20; biotin, 1.20;retinol acetate, 32; cholecalciferol, 5; alpha-tocopherol, 120; ascorbic acid, 2000.

[f] Phagostimulant: taurine: glycine: betaine = 1:3:3, obtained from XianBumper Crop Bio-Tech Co. LTD. (Xian, China), Hebei Yuanchuang Bio-Tech Co. LTD. (Hebei, China), Shenzhen Sendi Bio-Tech Co. LTD. (Shenzhen, China).

2.2. Experimental procedure and sample collection

Disease-free juvenile turbot were obtained from a commercial farm (Yantai City, Shandong, China). Before the formal experiment, turbot were conditioned on a commercial diet of turbot (Great seven Bio-Tech, Qingdao, China) to acclimate to the experimental conditions for 1 week. To become accustomed to the sinking pellets for turbot, another 1 week was set for fish fed the mixed diet of the five experimental diets. After being fasted for 24 h, fish of similar sizes (9.49 ± 0.03 g) were randomly distributed into 15 tanks (300-L) with 35 fish per tank. Each diet was randomly assigned to triplicate tanks. Fish were fed to apparent satiation twice daily (08:00 and 18:00). The remaining feed and feces were removed by a siphon immediately after feeding. All fish were fed in a recycling system for 12 weeks. Seawater, continuously pumped from the adjacent coast to the experiment station, passed through sand filters, froth separator and biofilter, and finally flew into each tank at a rate of 2 L min^{-1}. Additional aeration was provided by a single air-stone, and to keep good water quality, the recycling water

Table 2
Fatty acid composition (% total fatty acids) of the experimental diets.[a]

Fatty acids composition	Dietary oil source				
	FO	PO	RO	SO	LO
16:0	21.10	26.20	12.52	17.31	14.93
18:0	3.50	30.34	33.59	3.19	2.94
20:0	0.43	2.27	5.10	0.33	0.24
ΣSFA[b]	25.04	58.81	51.21	20.84	18.11
14:1	4.90	2.31	1.93	2.52	2.70
16:1	5.63	2.37	2.50	3.08	19.22
18:1	19.28	1.03	33.59	22.73	19.16
ΣMUFA[c]	29.82	5.71	38.03	28.33	41.09
18:2n-6	13.96	16.07	17.58	31.42	19.16
20:4n-6	0.59	0.36	0.36	0.37	0.34
Σ*n*-6 PUFA[d]	14.55	16.43	17.94	31.79	19.50
18:3n-3	2.22	1.97	3.70	3.60	21.64
18:4n-3	1.55	0.67	0.66	0.81	0.79
EPA	5.89	3.50	3.46	3.59	3.36
DHA	7.06	4.21	4.87	3.73	3.67
Σ*n*-3 PUFA[e]	16.72	10.35	12.69	11.73	29.46
Σ*n*-3 LC-PUFA[f]	12.95	7.71	8.33	7.32	7.03
DHA/EPA	1.20	1.20	1.41	1.04	1.09
ΣSFA/ΣPUFA	0.80	2.20	1.67	0.48	0.37
n-3/*n*-6 PUFA	1.15	0.63	0.71	0.37	1.51

[a] Some fatty acids, of which the contents are minor, in trace amounts or not detected (such as 14:0, 22:0, 24:0, 20:1n-9, 22:2n-11, 20:2n-6, 18:3n-6, 20:3n-6 and 22:5n-3), are not listed in the table.
[b] SFA: saturated fatty acids.
[c] MUFA: mono-unsaturated fatty acids.
[d] *n*-6 PUFA: *n*-6 poly-unsaturated fatty acids.
[e] *n*-3 PUFA: *n*-3 poly-unsaturated fatty acids.
[f] *n*-3 LC-PUFA: *n*-3 long chain poly-unsaturated fatty acids.

was changed 50% volume of tank (up to 70% volume of tank in the last 6 weeks) after feeding. During the experimental period, water quality conditions are stable (water temperature, 16.0 °C–17.5 °C; salinity, 28.0‰–31.5‰; dissolved oxygen, 7 mg L^{-1}; NH_4-N, NO_3-N and NO_2-N, < 100.0 μg L^{-1}). The fish were fasted for 24 h before harvest.

15 fish were randomly selected for determination of initial whole-body proximate composition before the experiment. At the end of the experiment, after being fasted for 24 h, fish in each tank were anesthetized with MS-222 (Shanghai Reagent Corp., Shanghai, China), and then individually weighed, counted and sampled. Bloods samples were obtained from the caudal vein of four fish from each tank with 2.5 mL syringes and allowed to clot at room temperature (< 25 °C) for 8 h and then at 4 °C for 4 h. After centrifugation (2500 g; 5 min; 4 °C), the clot was removed and residual blood cells were separated from the plasma. The plasma was frozen in liquid N_2 and then stored at − 80 °C for later analysis of plasma biochemical parameters. Sample size of liver (like a grain of rice) from three fish in each tank were respectively pooled into 1.5 mL tubes (RNAase-Free; Axygen), frozen in liquid N_2 and then stored at − 80 °C for later analysis of some genes expression related to lipid metabolism. Whole liver from another six fish in each tank were pooled into 5 mL tubes, frozen in liquid N_2 and then stored at − 80 °C for the assay of enzyme activity of G6PD and ME, fatty acid composition, moisture and crude lipids. Whole bodies of six fish in each tank were collected into plastic bags and then stored at − 20 °C for the assay of whole fish body composition.

2.3. Chemical analyses

2.3.1. Body composition assays

Dry matter, crude protein, crude lipid, and ash of ingredients, experimental diets and fish samples were analyzed, respectively. The detail was as previously described by Peng et al. (2014). Duplicate analyses were conducted for each sample.

The lipid of liver was assayed according to Folch et al. (1957) with some modification. The detail was as previously described by Peng et al. (2014). Similarly, duplicate analyses were conducted for each sample.

2.3.2. Biochemical analysis

The detail procedures of fatty acid profiles were as previously described by Zuo et al. (2012).

Plasma total protein, triglyceride, cholesterol, high density lipoprotein cholesterol (HDL-c, mmol mg $prot^{-1}$) and low density lipoprotein cholesterol (LDL-c, mmol mg $prot^{-1}$) were quantified with commercially available reagent kits using BS180 automated biochemistry analyzer (Shenzhen Mindry Bio-medical Electronics Co., LTD, Guangzhou, China). Plasma total protein, triglyceride, cholesterol, HDL-c and LDL-c were determined by Biuret, GPO-POD, CHOD-POD, Direct and Direct method, respectively.

For hepatic lipogenic enzyme analysis, liver samples were homogenised in three volumes of ice-cold buffer (0.02 M Tris–HCl, 0.25 M sucrose, 2 mM EDTA, 0.1 M sodium fluoride, 0.5 mM phenyl methyl sulphonyl fluoride, and 0.01 M β-mercaptoethanol, pH 7.4) and centrifuged at 24,000 × g at 4 °C for 20 min. The supernatant was collected separately and immediately used for enzymatic analysis. Soluble protein content of liver homogenates was determined with a commercially kit (Nanjing Jiancheng Bioengineering institutes, China). G6PD was determined by the method of Bautista et al. (1988) in the following mixture: 1 M Tris HCl (pH 7.8), 2 mM $MgCl_2$, 10 mM NADP and 20 mM glucose 6-phospate for G6PD. ME activity was assayed according to Hsu and Lardy (1969) with 50 mM Hepes (pH 7.5), 44 mM L-malate, 10 mM $MgSO_4$ and 1 mM NADP. All two lipogenic enzymes were assayed spectrophotometrically, and the reaction was started by addition of the tissue extract. The activities of lipogenic enzymes were determined by measuring the production of NADPH by reading the change in absorbance at 340 nm per unit of time in the reaction media (monitored at intervals of 15 s for 3 min). One unit of enzyme activity, being defined as the amount of enzyme that produced 1 μmol NADPH min^{-1} at assay temperature (30 °C for G6PD and ME), was expressed as units per milligram of hepatic soluble protein.

2.4. RNA extraction and real-time quantitative polymerase chain reaction (qPCR)

Firstly, total RNA was extracted from liver using Trizol Reagent (Invitrogen, USA) and electrophoresed on a 1.2% denaturing agarose gel to test the integrity. Then, to remove DNA contaminant, RNA was treated with RNA-Free DNase (Takara, Japan). Following the instructions, RNA was reverse-transcribed to cDNA by PrimeScript™ RT reagent Kit (Takara, Japan) and first strand cDNA was diluted by 3 times using sterilized double-distilled water. A quantitative thermal cycle was used in Real-time RT-PCR (Mastercycler® ep realplex; Eppendorf, Germany). The amplification was performed in a total volume of 25 μL, containing 0.5 μL of each primer (10 mM), 1.0 μL of the diluted first strand cDNA product, 12.5 μL of 2 × SYBR® Premix Ex Taq™ II (Trans, China) and 10.5 μL of sterilized double-distilled water. The real-time PCR program was as follows: 95 °C for 2 min, followed by 40 cycles of 95 °C for 10 s, Ta Opt for 10 s (57.5 °C for *LPL*, *PPARα*, *LXR*, *MTP* and *SREBP-1* or 55.0 °C for *FAS*, *HNF 4α*, *PPARγ* and *ApoB-100*), and 72 °C for 20 s. The reaction was carried out with three duplicates of each sample. The primer sequences for reference genes (*β*-actin), *LPL*, *PPARα*, *LXR*, *MTP*, *SREBP-1*, *FAS*, *HNF 4α*, *PPARγ*, and *ApoB-100* were designed following the published sequences from turbot and were list in Table 3. At the end of each PCR reaction, melting curve analysis was performed to confirm that PCR product was present in only one of these reactions. Standard curves were made with six different dilutions (in triplicate) of the cDNA samples and the primer amplification efficiency was analyzed according to the following equation: $E = 10^{(-1/\text{slope})} - 1$. The value of *CPTI*, *LPL*, *PPARα*, *FAS*, *LXR*, *SREBP-1*, *MTP*, *HNF4α*, *PPARγ*, *ApoB-100* and β-actin was 1.001, 1.001, 1.001, 0.938, 1.010, 0.883, 1.031, 1.002, 0.909, 0.969 and 0.972, respectively. The

Table 3
Real-time quantitative PCR primers for fatty acids oxidation-, fatty acids synthesis- and lipid transport-related genes and β-actin of turbot (*Scophthalmus maximus* L.).

Target gene	Forward (5′-3′)	Reverse (5′-3′)	Size (bp)
LPL	CTCCCACGAACGCTCTAT	GCGGACCTTGTTGATGTT	166
PPARα	CGATCAGGTGACCCTGTTAA	TGGAACTTGGGCTCCATC	171
CPT I	GCCTTTCAGTTCACCATCACA	ATGCGGCTGACTCGTTTCTT	113
LXR	GCGTCATCAAGAGTGCCC	ATCTGATTTGCTCCTCCGAG	153
SREBP-1	CGATCCGCACTCCAAGT	CCGCACTGCCCTGAAT	175
FAS	GGCAACAACACGGATGGATAC	CTCGCTTTGATTGACAGAACAC	205
PPARγ	AAGTGACGGAGTTCGCCAAGA	GTTCATCAGAGGTGCCATCA	121
HNF 4α	AGTGCGTGGTGGACAAAGAC	GAGTCGTACTGGCGGTCGTTG	121
ApoB-100	TCTCACCCTCGGTCTCGG	TTCAGGTTTCTCCTCACAACGA	158
MTP	CCAGCAAAGTCTTACGCCA	TACGCAGATGATGACCCAAC	85
β-actin	GTAGGTGATGAAGCCCAGAGCA	CTGGGTCATCTTCTCCCTGT	204

absolute $\triangle C_T$ (cycle threshold) values between the target genes and inner control gene were all lower than 0.1, which indicates that the $\triangle\triangle C_T$ calculation for the relative quantification of target genes can be used. To calculate the expression of *CPTI*, *LPL*, *PPARα*, *FAS*, *LXR*, *SREBP-1*, *MTP*, *HNF 4α*, *PPARγ* and *ApoB-100*, the comparative CT method ($2^{-\triangle\triangle CT}$ method) was used, and the value stood for n-fold difference relative to the calibrator (Livak and Schmittgen, 2001).

2.5. Calculations and statistical methods

The following variables were calculated:

Survival rate (SR,%) = Nt × 100/No.

Specific growth rate (SGR,%d) = (LnWt − LnWo) × 100/t.

Feed efficiency (FE) = (Wt − Wo)/dry feed consumed.

Feed intake (FI,%/d) = 100 × total amount of the feed consumed (g)
/[(Wo + Wt)/2]/t.

Apparent net protein utilization (ANPU)
= (final carcass protein − initial carcass protein)
/total dry protein consumed × 100.

Hepatosomatic index (HSI,%) = 100 × (liver weight/Wt).

Viserosomatic index (VSI,%) = 100 × (Visceral weight/Wt).

where Wt and Wo were final and initial fish weight, respectively; Nt and No were final and initial number of fish, respectively; t was duration of experimental days.

All data were subjected to a one-way ANOVA and differences between the means were tested by Tukey's multiple-range test. The level of significance was set at $P < 0.05$ (Zar, 1999). The results are presented as mean values with their standard errors and all statistical analyses were performed using SPSS 16.0 (SPSS Inc., 2005, USA).

3. Results

3.1. Survival, growth performance and somatic indices

During the experimental period, survival rate (SR), feed intake (FI), specific growth rate (SGR), feed efficiency (FE) and apparent net protein utilization (ANPU) of turbot fed diets with required n3 LC-PUFA were not affected by dietary different oil resource ($P > 0.05$) (Table 4).

Hepatosomatic index (HSI) and Viserosomatic index (VSI) of fish fed diets with required n3 LC-PUFAs also were independent of dietary different oil source ($P > 0.05$) (Table 4).

3.2. Fish tissue composition

The proximate composition of fish whole body (Table 5) showed that there were no significant differences in crude protein content and ash content in fish fed diets with different dietary oil source ($P > 0.05$). The content of moisture of fish fed the diet with PO was significant higher than that in the RO group and LO group ($P < 0.05$). The content of crude lipid in the PO group was significantly lower than that in the LO group ($P < 0.05$).

Lipid content of liver in fish fed diets with PO or FO were significantly lower than those in the SO, RO and LO group ($P < 0.05$). The maximum of hepatic lipid content was found in the LO group (Table 5).

3.3. Fatty acids composition of liver

As expected, C16:0, C18:1n, C18:2n-6, C18:3n-3 and n3 LC-PUFAs proportion in liver were up to the maximum in the PO, RO, SO, LO and FO group, respectively, and significantly higher than the other groups ($P < 0.05$) (Table 6). The highest C20:4n-6 (ARA) percentages of fish fed the diet with higher FO proportion was significantly higher than that in the plant oil group ($P < 0.05$). Fish fed the PO diet had a higher total SFA proportion in liver than those fed the other diets. The lowest total MUFA proportion in liver was observed in fish fed the PO diet, whereas fish fed diets containing FO, RO and LO had higher total MUFA content than those fed the PO and SO diets ($P < 0.05$). The lowest C20:4n-6 (ARA) proportion was found in the LO group, and was significant lower than that in the PO group and the SO group ($P < 0.05$). Although similar EPA and DHA proportions were found in the diets of plant oil group, the DHA proportion of liver in PO group was significantly higher than that in the other plant oil groups ($P < 0.05$), and no significant difference of DHA proportion in liver had been found among the RO, SO, and LO group ($P > 0.05$). On the contrary, the EPA percentages of liver in PO group was significantly lower than that in the RO and SO groups ($P < 0.05$), and had no significant difference with the LO group ($P > 0.05$). The highest ratio of total SFA to total PUFA was found in fish fed the PO diet, and the ratio of total SFA to total PUFA in the RO group was significantly higher than that in the FO group, the SO group and the LO group ($P < 0.05$), no significant difference was found among these three groups ($P > 0.05$). The FO group and LO group have the higher ratio of total n-3 PUFA to total n-6 PUFA than that in the other groups ($P < 0.05$), and the lowest ratio was found in the SO group. In addition, when FO was replaced by VO, DHA and AA proportions in the liver reduced approximately threefold or less than twofold, whereas EPA proportion suffered a fourfold or less than threefold.

3.4. Plasma biochemistry parameters

Plasma TG of fish fed the diet with RO was significantly lower than that of the SO, PO and FO group ($P < 0.05$), which had no significant

Table 4
Effect of dietary fatty acids composition on growth, survival and selected body parameters of turbot (*Scophtalmus maximus* L.) fed the experimental diets for 12 weeks.[a]

Index	Dietary oil source					ANOVA *P*
	FO	PO	RO	SO	LO	
SR[b]	98.10 ± 0.95	98.09 ± 1.90	95.24 ± 3.43	100.00 ± 0.00	100.00 ± 0.00	0.382
FI[c]	1.42 ± 0.04	1.40 ± 0.04	1.40 ± 0.02	1.36 ± 0.03	1.39 ± 0.02	0.381
SGR[d]	2.23 ± 0.07	2.20 ± 0.02	2.21 ± 0.08	2.23 ± 0.03	2.17 ± 0.08	0.757
FE[e]	1.21 ± 0.04	1.23 ± 0.04	1.21 ± 0.02	1.29 ± 0.02	1.24 ± 0.01	0.365
ANPU[f]	38.30 ± 1.30	39.05 ± 0.55	39.22 ± 0.45	40.36 ± 0.60	39.33 ± 1.33	0.651
VSI[g]	0.66 ± 0.04	0.59 ± 0.05	0.61 ± 0.02	0.67 ± 0.06	0.67 ± 0.03	0.562
HSI[h]	3.29 ± 0.08	3.12 ± 0.26	2.77 ± 0.07	3.28 ± 0.19	3.34 ± 0.26	0.284

Where Wt and Wo were final and initial fish weight, respectively; Nt and No were final and initial number of fish, respectively; t was duration of experimental days.
[a] Values in the same row with the same superscript or absence of superscripts are not significant different by Tukey's test ($P > 0.05$).
[b] Survival rate (SR, %) = Nt × 100 / No.
[c] Feed intake (FI, %/d) = 100 × total amount of the feed consumed (g) / [(Wo + Wt) / 2] / t.
[d] Specific growth rate (SGR, %d) = (LnWt − LnWo) × 100 / t.
[e] Feed efficiency (FE) = (Wt − Wo) / dry feed consumed.
[f] Apparent net protein utilization (ANPU) = (final carcass protein − initial carcass protein) / total dry protein consumed × 100.
[g] VSI: Viserosomatic index (VSI, %) = 100 × (visceral weight / Wt).
[h] Hepatosomatic index (HSI, %) = 100 × (liver weight / Wt).

Table 5
Effect of dietary fatty acids composition on whole body proximate composition and lipid content of liver in turbot (*Scophtalmus maximus* L.) fed the experimental diets for 12 weeks [1].

Index (wet weight, %)	Dietary oil source					ANOVA *P*
	FO	PO	RO	SO	LO	
Proximate composition of whole body						
Moisture	76.85 ± 0.20^{ab}	77.96 ± 0.31^{a}	76.69 ± 0.20^{b}	76.96 ± 0.33^{ab}	76.53 ± 0.07^{b}	0.014
Crude protein	15.36 ± 0.05	15.33 ± 0.07	15.71 ± 0.13	15.38 ± 0.13	15.53 ± 0.17	0.202
Crude lipid	3.77 ± 0.19^{ab}	3.01 ± 0.16^{a}	3.80 ± 0.24^{ab}	3.85 ± 0.24^{ab}	4.30 ± 0.01^{b}	0.011
Ash	3.71 ± 0.10	3.64 ± 0.10	3.82 ± 0.11	3.67 ± 0.08	3.61 ± 0.07	0.589
Lipid content of liver	7.52 ± 0.10^{c}	6.61 ± 0.22^{c}	12.07 ± 0.49^{ab}	10.44 ± 0.24^{b}	13.32 ± 0.84^{a}	0.000

[1] Values in the same row with the same superscript or absence of superscripts are not significant different by Tukey's test ($P > 0.05$).

difference with that in the LO group ($P > 0.05$). Plasma HDL-c of fish fed the diet with LO were significantly lower than those in the other groups ($P < 0.05$), and the ratio of HDL-c and LDL-c of fish fed the diet with RO was significantly higher than that in the other groups ($P < 0.05$). There was no significantly difference in cholesterol and LDL-c among dietary treatments ($P > 0.05$) (Table 7).

Table 6
Effects of dietary fatty acids composition on fatty acid composition (% total fatty acids) in the liver of turbot (*Scophthalmus maximus* L.) fed the experimental diets for 12 weeks[1].

Index			Dietary oil source			ANOVA *P*
	FO	PO	RO	SO	LO	
16:0	14.44 ± 1.02 ^{b}	19.41 ± 0.61 ^{a}	12.28 ± 0.96 ^{b}	14.75 ± 1.47 ^{ab}	14.03 ± 0.97 ^{b}	0.008
18:0	1.76 ± 0.33 ^{c}	21.93 ± 0.96 ^{a}	6.99 ± 0.37 ^{b}	3.60 ± 0.63 ^{c}	2.87 ± 0.77 ^{c}	0.000
20:0	1.60 ± 0.28 ^{b}	1.99 ± 0.01 ^{b}	2.86 ± 0.17 ^{a}	0.11 ± 0.00 ^{c}	0.12 ± 0.01 ^{c}	0.000
ΣSFA[2]	17.80 ± 1.00 ^{bc}	43.33 ± 0.84 ^{a}	22.13 ± 1.31 ^{b}	18.46 ± 1.25 ^{bc}	17.01 ± 0.55 ^{c}	0.009
14:1	3.68 ± 0.18 ^{a}	2.49 ± 0.04 ^{b}	2.59 ± 0.15 ^{b}	2.79 ± 0.28 ^{b}	3.20 ± 0.35 ^{a}	0.022
16:1	4.46 ± 0.31	3.24 ± 0.09	3.83 ± 0.32	3.26 ± 0.13	3.50 ± 0.09	0.013
18:1	21.87 ± 0.82 ^{b}	4.59 ± 0.54 ^{d}	25.51 ± 0.39 ^{a}	10.83 ± 0.20 ^{c}	22.97 ± 0.41 ^{b}	0.000
ΣMUFA[3]	30.00 ± 0.88 ^{a}	10.33 ± 0.64 ^{c}	31.93 ± 0.51 ^{a}	16.89 ± 0.38 ^{b}	29.67 ± 0.70 ^{a}	0.000
18:2n-6	17.12 ± 0.04 ^{b}	19.36 ± 0.29 ^{b}	15.89 ± 0.03 ^{b}	33.22 ± 1.65 ^{a}	19.15 ± 0.76 ^{b}	0.000
20:4n-6	1.23 ± 0.01 ^{a}	0.82 ± 0.00 ^{b}	0.37 ± 0.03 ^{cd}	0.42 ± 0.01 ^{c}	0.31 ± 0.02 ^{d}	0.000
Σ*n*-6 PUFA[4]	17.27 ± 1.12 ^{b}	20.18 ± 0.28 ^{b}	16.26 ± 0.01 ^{b}	33.64 ± 1.66 ^{a}	19.46 ± 0.77 ^{b}	0.000
18:3n-3	1.72 ± 0.02 ^{b}	1.45 ± 0.11 ^{b}	2.41 ± 0.09 ^{b}	2.55 ± 0.11 ^{b}	14.31 ± 0.90 ^{a}	0.000
18:4n-3	0.83 ± 0.13 ^{a}	0.38 ± 0.02 ^{b}	0.33 ± 0.03 ^{b}	0.38 ± 0.02 ^{b}	0.55 ± 0.10 ^{ab}	0.003
20:5n-3	4.36 ± 0.08 ^{a}	0.98 ± 0.02 ^{c}	1.66 ± 0.07 ^{b}	1.77 ± 0.55 ^{b}	1.10 ± 0.02 ^{c}	0.000
22:6n-3	9.14 ± 0.35 ^{a}	5.96 ± 0.29 ^{b}	3.08 ± 0.03 ^{c}	3.59 ± 0.04 ^{c}	3.00 ± 0.24 ^{c}	0.000
Σ*n*-3 PUFA[5]	15.49 ± 1.05 ^{b}	8.76 ± 0.19 ^{c}	7.49 ± 0.20 ^{c}	8.29 ± 0.19 ^{c}	18.96 ± 1.16 ^{a}	0.000
ΣSFA/ΣPUFA	0.54 ± 0.04 ^{c}	1.50 ± 0.03 ^{a}	0.93 ± 0.06 ^{b}	0.44 ± 0.03 ^{c}	0.45 ± 0.04 ^{c}	0.000
n-3/*n*-6	0.89 ± 0.02 ^{a}	0.43 ± 0.01 ^{b}	0.46 ± 0.01 ^{b}	0.25 ± 0.01 ^{c}	0.97 ± 0.03 ^{a}	0.000

[1] Some fatty acids, of which the contents are minor, in trace amounts or not detected (such as 22:0, 24:0, 14:1, 20:1n-9, 22:2n-11, 20:2n-6, 18:3n-6, 20:3n-6 and 22:5n-3), are not listed in the table. Values in the same row with the same superscript or absence of superscripts are not significant different by Tukey's test ($P > 0.05$).
[2] SFA: saturated fatty acids.
[3] MUFA: mono-unsaturated fatty acids.
[4] *n*-6 PUFA: *n*-6 poly-unsaturated fatty acids.
[5] *n*-3 PUFA: *n*-3 poly-unsaturated fatty acids.

Table 7
Effects of dietary fatty acids composition on plasma biochemical parameters of turbot (*Scophthalmus maximus* L.) fed the experimental diets for 12 weeks[1].

Index	Dietary oil source					ANOVA *P*
	FO	PO	RO	SO	LO	
TG[2]	4.00 ± 0.39 [a]	3.97 ± 0.43 [a]	2.32 ± 0.56 [b]	4.08 ± 0.20 [a]	2.70 ± 0.42 [ab]	0.049
TC[3]	2.78 ± 0.19	2.51 ± 0.09	2.48 ± 0.01	2.64 ± 0.21	2.21 ± 0.06	0.120
HDL-c[4]	1.93 ± 0.06 [a]	1.69 ± 0.02 [a]	1.77 ± 0.16 [a]	1.65 ± 0.07 [a]	1.15 ± 0.12 [b]	0.003
LDL-c[5]	0.72 ± 0.09	0.66 ± 0.10	0.38 ± 0.09	0.55 ± 0.07	0.38 ± 0.02	0.078
HDL-c/LDL-c	2.77 ± 0.35 [b]	2.68 ± 0.47 [b]	4.70 ± 0.17 [a]	3.09 ± 0.46 [b]	3.02 ± 0.20 [b]	0.013

[1] Values in the same row with the same superscript or absence of superscripts are not significant different by Tukey's test ($P > 0.05$).
[2] TG: Triglyceride.
[3] TC: Total cholesterol.
[4] HDL-c: High density lipoprotein cholesterol.
[5] LDL-c: Low density lipoprotein cholesterol.

3.5. Activity of hepatic G6PD and ME and expression of lipid deposition related genes in liver

The activity of hepatic G6PD of fish fed the diet with FO was significantly higher than that in the RO and LO group ($P < 0.05$), which was significantly lower than that in the PO group ($P < 0.05$). In addition, there was no remarkable difference between the FO group with the SO group ($P > 0.05$). The activity of hepatic G6PD of fish fed the diet with PO was up to the maximum, which was significantly higher than that in the other groups ($P < 0.05$). The activity of hepatic ME of fish fed the diet with LO was significantly lower than that in the RO, PO and FO group ($P < 0.05$), which had no remarkable difference with that in the SO group ($P > 0.05$). Additionally, there was no significant difference of hepatic ME activity among the RO, PO and FO group ($P > 0.05$) (Fig. 1).

Relative expression of hepatic LPL of fish fed the diet with FO was significantly lower than that in the RO, SO and LO group ($P < 0.05$), which had no remarkable difference with that in the PO group ($P > 0.05$) (Fig. 2a). There was no significant difference of hepatic LPL gene expression was found among the SO, RO and LO group ($P < 0.05$). Relative expression of hepatic PPARα of fish fed the diet with FO was significantly lower than that in the PO group ($P < 0.05$), which had no remarkable difference with the other groups ($P > 0.05$). Relative expression of hepatic PPARα of fish fed the diet with RO was significantly lower than that in the PO and SO group, which had no remarkable difference with that in the LO group. Relative expression of hepatic CPTI was independent of dietary different oil source ($P > 0.05$) (Fig. 2a).

Relative expression of hepatic LXR of fish fed the diet with FO was significantly lower than that in the SO, RO and LO group ($P < 0.05$), which had no remarkable difference with that in the PO group ($P > 0.05$) (Fig. 2b). Moreover, no significant difference of hepatic LXR gene expression was found among the SO, RO and LO group ($P > 0.05$). Relative expression of hepatic FAS of fish fed the diet with FO was significantly lower than that in the RO and PO group ($P < 0.05$), which had no remarkable difference with those in the SO and LO group ($P > 0.05$). Relative expression of hepatic SREBP-1 showed a same trend as hepatic FAS, while no significant difference was found among dietary treatments ($P > 0.05$). Relative expression of hepatic PPARγ gene of fish fed the diet with SO was significantly higher than that of the other groups ($P < 0.05$). Although no remarkable difference was found among these groups (except for SO group) ($P > 0.05$), relative expression of hepatic PPARγ gene in the PO group, the RO group, and the LO group was relatively higher than that in the FO group (Fig. 2b).

Relative expression of hepatic HNF4α was independent of dietary different oil source ($P > 0.05$) (Fig. 2c). Relative expression of hepatic ApoB-100 of fish fed the diet with FO was significantly lower than that in the RO and LO group ($P < 0.05$), which had no remarkable difference with that in the SO and PO group ($P > 0.05$). In addition, relative expression of hepatic ApoB of fish fed the diet with RO was significantly higher than that in PO group ($P < 0.05$). Relative expression of hepatic MTP of fish fed diets with FO and SO were significantly lower than those in the LO group ($P < 0.05$), which had no remarkable difference with that of the RO and PO group ($P > 0.05$) (Fig. 2c).

4. Discussion

In the present study, FO being replaced by PO, RO, SO and LO did not significantly affect growth and feed utilization of turbot fed diets with required n3 LC-PUFAs after a 12-week feeding period. This was consistent with the findings of earlier studies of the effects of 100% VO replacement in the turbot diet (Bell et al., 1994, 1999; Regost et al., 2003; Altundag et al., 2014), and suggests that FO can be partially replaced by VO in turbot feed without adversely affecting fish production. This inference was reasonable in the research of Altundag et al. (2014) in turbot, who found the fish fed the diet with fish oil had the lower weight gain compared to the 100% safflower oil group. These results denoted the higher ability of turbot to accept vegetable oils in comparison with other marine fish species such as gilthead seabream (*Sparus aurata*) (Izquierdo et al., 2005), large yellow croaker (*Larimichthys crocea*) (Qiu et al., 2017), which might be related to the n-3 LC-PUFA content of the basal diet, fish species and the feeding time, and so on. There were no differences in HSI or whole body lipid content

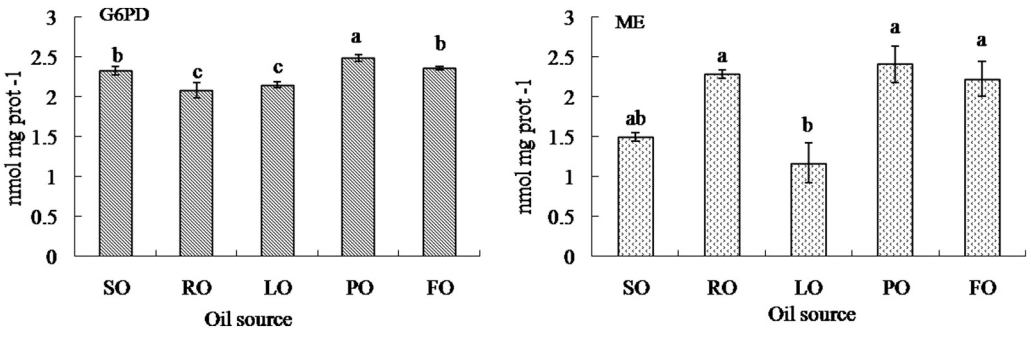

Fig. 1. Effects of dietary fatty acids composition on activities of hepatic G6PD and ME of turbot (*Scophthalmus maximus* L.) fed the experimental diets for 12 weeks. Values are means ($n = 3$), and values bearing the same letter are not significantly different by Tukey's test ($P < 0.05$).

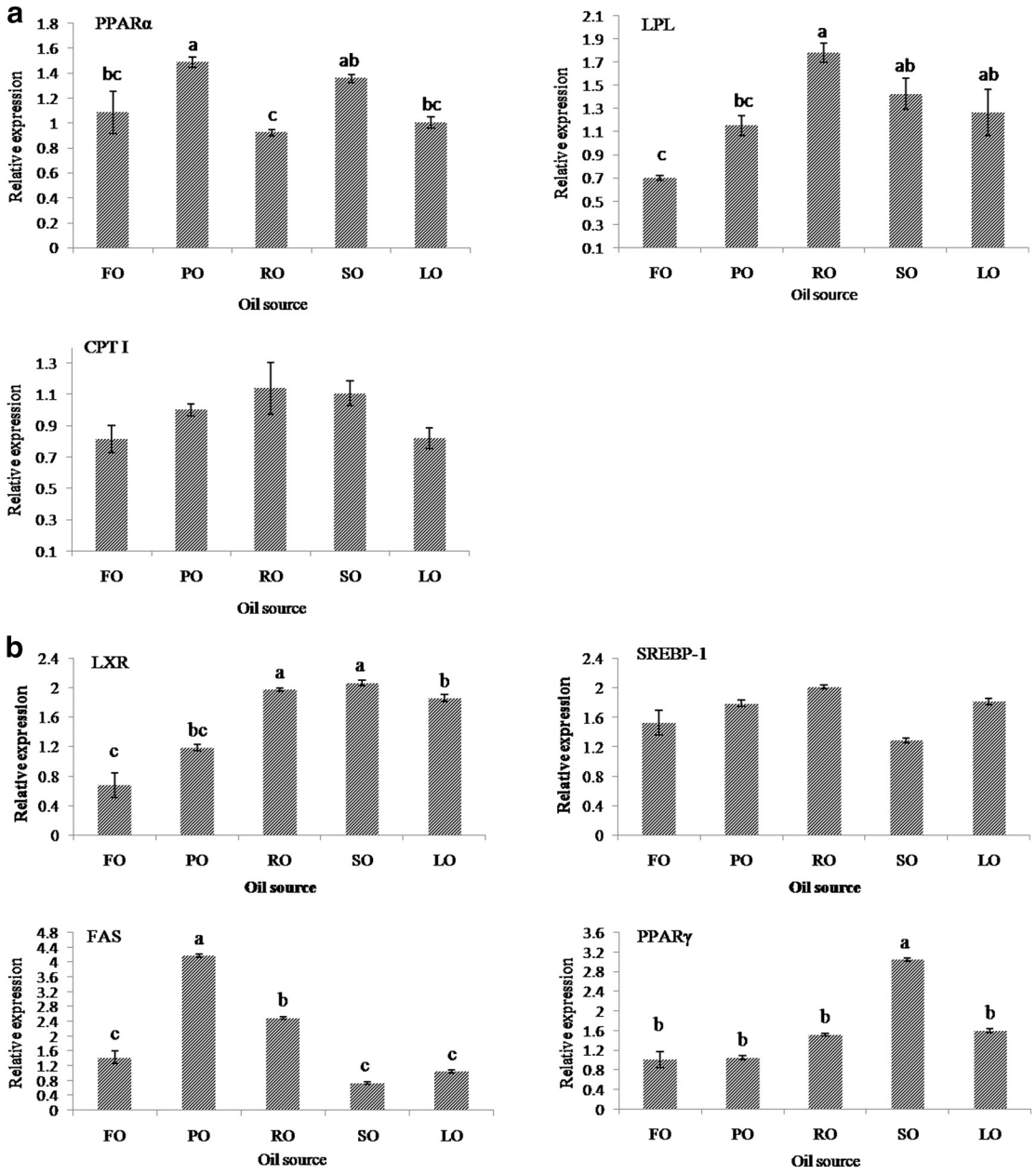

Fig. 2. Effects of dietary fatty acids on relative mRNA expression of hepatic fatty acid oxidation-related genes (LPL, PPARα and CPT I) (a), lipogenesis-related genes (FAS, LXR, SREBP-1 and PPARγ) (b) and lipid transport-related gene (MTP, ApoB-100 and HNF 4α) (c) in turbot (*Scophthalmus maximus* L.) fed the experimental diets for 12 weeks. Values are means ($n = 2 \times 3$), and values bearing the same letter are not significantly different by Tukey's test ($P < 0.05$).

between the FO, SO, and LO groups in the present study, which was in agreement with previous findings in turbot (Regost et al., 2003), European sea bass (*Dicentrarchus labrax* L.) (Mourente and Bell, 2006) and large yellow croaker (Qiu et al., 2017). However, dietary oil source did affect the HSI in sea bream (*Sparus aurata*) (Menoyo et al., 2004) and gilthead sea bream (*Sparus aurata* L.) (Fountoulaki et al., 2009), which might be attributable to an imbalance in the n3 to n6 LC-PUFA ratio (Robaina et al., 1998; Menoyo et al., 2004). In this study, although this ratio differed across diets, the absence of any differences in HSI might be related to the sufficient levels of n3 LC-PUFAs in the diets.

The fatty acid composition of fish is largely determined by the digestible fatty acid intake of the animal, and the relative proportions of these fatty acids are reflected in the fatty acid composition of TAGs deposited. In this research, fatty acid composition of liver lipid had a significant difference among dietary treatments in this research. As expected, maximum values for C16:0, C18:1n, C18:2n-6 (LA), C18:3n-3 (ALA) and n3 LC-PUFAs (EPA and DHA) in liver were observed in the PO, RO, SO, LO, and FO groups, respectively, and in each case, the value was significantly higher than those measured in the other groups. The similar results have been previously observed in Atlantic salmon (*Salmo salar*) (Tocher et al., 2003), Senegalese sole (*Solea senegalensis*) (Benítez-Dorta et al., 2013), large yellow croaker (Wang et al., 2012; Qiu et al., 2017). The biosynthesis of LC-PUFAs results from the catalysis of the C18 substrates LA or ALA through Δ6 and Δ5 fatty acyl desaturase (Fad) and elongase. We could find that dietary total proportion of LA and ALA in the SO or LO groups was higher than the other group (seen in Table 2), but the ARA, and DHA proportions in the liver of the SO group or LO the group was significantly lower than that in the FO group and the PO group. This could be explained by the limited capacity of turbot for converting LA to AA or ALA to EPA and DHA. Indeed, although Δ6 Fad activity of turbot was relatively high (Ghioni et al., 1999), the biosynthesis of LC-PUFA would be limited by the lower activity of Δ5 Fad (Owen et al., 1975) and elongase (from C18 to C20) (Tocher et al., 1989; Ghioni et al., 1999). Inexplicably, although

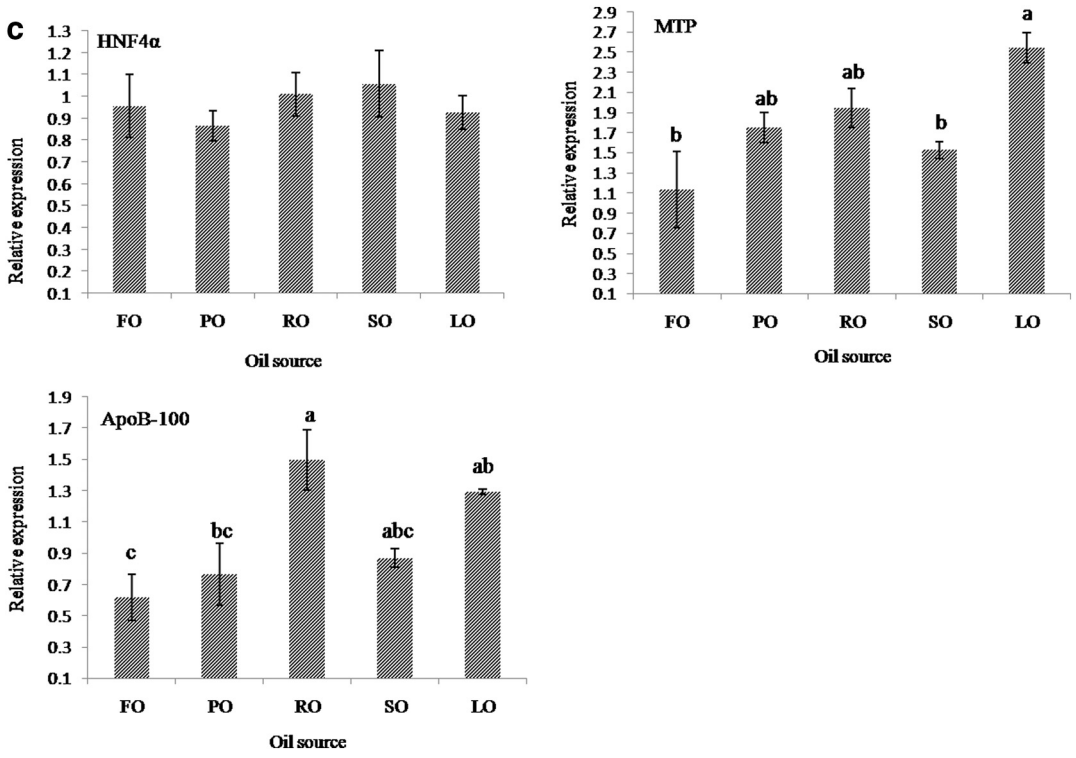

Fig. 2. (*continued*)

similar dietary EPA and DHA proportions were found in the diets of plant oil group, the higher DHA proportions and the lower EPA proportions in liver of fish fed the diet with PO were found relative to the RO group, the SO group, and the LO group. In the research of Qiu et al. (2017), the DHA content in liver of large yellow croaker in the PO group (3.93%) was relatively higher than that in the SO group (3.21%), the LO group (3.66%) and the RO group (3.54%), but no significant difference had been found among these plant oil treatments. The cause of this result (higher DHA proportion in PO group) is still unknown, and need to be explored. Moreover, when FO was replaced by VO, hepatic DHA and AA content were reduced by approximately 3- or < 2-fold, respectively, while EPA proportion decreased by 3- to 4-fold in the liver, indicating a preferential retention of ARA and DHA over EPA (Koven et al., 2001; Menoyo et al., 2004).

Earlier researched had showed that VLDL secretion was increased by SFA, MUFA, and n6 PUFA, and decreased by n3 PUFA (Lin et al., 1995; Pan et al., 2004; Vegusdal et al., 2005; Shearer et al., 2012). As well, different types of fatty acids had been shown to have different effects on HNF 4α, which could regulate hepatic VLDL secretion rates, with SFA enhancing and n3 PUFA inhibiting its activity. Meanwhile, higher circulating VLDL increases plasma cholesterol and TG levels. In the present study, there were no significant differences in plasma cholesterol, TG and LDL levels or *HNF 4α* expression between PO, SO, LO, and FO groups, which could be due to a similar level of n3 LC-PUFAs across treatments, although the content of n3 LC-PUFAs was relatively higher in the FO diet.

HDL partially inhibits the uptake and degradation of LDL, and suppresses LDL-induced changes in sterol level (Carew et al., 1976), as such, HDL levels are inversely correlated with the risk of coronary artery disease (Goldbourt et al., 1997). Interestingly, lower plasma TG and a higher ratio of plasma HDL-c to LDL-c were observed in the RO group than in the FO group. Similar result was found in Atlantic salmon (*Salmo salar* L.), plasma lipid and LDL levels were lower in salmon consuming a VO diet (55% RO, 30% PO, and 15% LO) than those diet with an FO diet (Jordal et al., 2007). This may be due to an increase VLDL clearance and concomitant up-regulation of LPL. Indeed, *MTP* gene expression was relatively higher in the RO group than that in the FO group, although no significant difference had been found between RO group and FO group. Relative expression of *ApoB-100*, and *LPL* gene levels were significant higher in the RO group than these in the FO group. These results suggested that the secretion of hepatic VLDL/TG production and the clearance of plasma VLDL would be increased by higher MUFA/lower n3 PUFA intake, since n3 LC-PUFA decreases plasma TG by reducing hepatic VLDL/TG production. Dietary intake of MUFA improves metabolic syndromes and reduces the risk of cardiovascular disease by modulating blood pressure and lipid levels as well as insulin sensitivity (Schwingshackl et al., 2011; Livingstone et al., 2012). The higher ratio of HDL-c and LDL-c in the RO group compared to the other groups suggests that provided the diet contains adequate n3 LC-PUFA, a higher dietary MUFA would be beneficial for the cardiovascular health of turbot.

The whole fish crude lipid content of fish fed the diet with PO was lower than that in the LO group, owing to the preferential usage of SFA and MUFA over PUFA for energy production (Henderson, 1996). A higher hepatic lipid deposition was detected in the RO, SO, and LO groups than in the PO group and the FO group, possibly due to a higher FA oxidation in the latter. Higher dietary n3 LC-PUFA intake can stimulate the transcription of genes involved in FA oxidation (Jump, 2004) and mitochondrial proliferation (Vamecq et al., 1993). The higher n3 LC-PUFA content of the FO diet should activate *PPARα* expression, which corresponded to an increase in β-oxidation capacity. Qiu et al. (2017) found that relative expression of *lpl* in large yellow croaker fed with FO (with high n3 LC-PUFA content) was lower than that in the RO group (with higher C18:1n content), and speculated that RO could up-regulate the *lpl* gene expression. The similar result had been found in this study, *PPARα* and *lpl* relative expression in the FO group was lower than these in the RO group. Thus, compared to the FO group, the higher hepatic lipid deposition in RO group might be more related to the lipogenesis and lipid transport. Surprisingly, *CPT I* expression was independent of dietary oil source in this research, in contrast to earlier findings that *CPT I* activity was higher in brown trout (Turchini et al., 2003) or sea bream (Menoyo et al., 2004) or large

yellow croaker (Qiu et al., 2017) fed an FO diet than in those consuming MUFA (canola oil) and SFA (olive oil), or ALA (LO) and LA (SO), or MUFA (RO), respectively. Feed intake was similar across treatments, the absence of any changes in *CPT I* expression was likely due to the different diets being isoenergetic and similar in n3 LC-PUFA content.

Hepatic lipid deposition is also linked to the uptake of mobilized FAs from adipose tissue and hepatic lipogenesis (Vyas et al., 2012). G6PD and ME are responsible for the generation of reductor equivalents (i.e., NADPH), and are FAS cofactors. In mammals, FA synthesis and uptake are induced by LXR agonists through up-regulation of *SREBP-1c* expression and stimulation of FAS and LPL activities (Schoonjans et al., 2000; Cha and Repa, 2007; Minghetti et al., 2011). In this research, an increase in *FAS* expression was observed in the RO group and the PO group compared to other groups, and hepatic G6PD and ME activities in the PO group were higher than those in the LO group. There were no significant differences in the expression of the lipid transport-related genes (*HNF4α*, *MTP*, and *ApoB-100*) between the PO group and the LO group. Thus, the excess hepatic and total lipid deposition in the LO group relative to the PO group is primarily due to a down-regulation of genes related to FA oxidation. Indeed, Compared to the PO group, the lower PPARα expression was found in the LO group. This higher FAS expression may be explained by the higher SFA or MUFA levels in the PO and RO diets. FAS activity was stimulated or suppressed by high dietary MUFA (a blend of VO, OA, and RO:PO:LO at 5:3:2) (Morais et al., 2011, 2012) or LA (Cruz-Garcia et al., 2011), respectively. Hepatic FAS activity and abdominal fat deposition in chickens fed a tallow-enriched diet (with higher SFA) were increased relative to those on a sunflower oil diet with higher ALA (Sanz et al., 2000). The primers used for *SREBP-1* amplification corresponded to a region common to *SREBP-1a* and *SREBP-1c*, and therefore the measured *SREBP-1* expression levels included both isoforms. *SREBP-1* expression was similar across dietary treatments; this does not preclude the possibility of differences in *SREBP-1* protein expression. Dietary n3 PUFA suppresses the proteolytic processing of *SREBP-1*, down-regulates the expression of target genes including *FAS*, and promotes the degradation of the nuclear form of the protein (Botolin et al., 2006; Jump, 2008). Additionally, PPARγ is a key mediator of lipogenesis and hepatic steatosis (Matsusue et al., 2008; Morán-Salvador et al., 2011), and its expression in rat was up- or down-regulated by high SFA (pork fat) or high LA or ALA (SO and LO), respectively (Escobar et al., 2009). The hepatic expression of *LXR*, *SREBP-1*, *FAS*, and *PPARγ* was higher in the RO than in the FO group, while the opposite trend was observed for hepatic *G6PD* activities, although no significant difference of relative expression in *SREBP-1* and *PPARγ* gene were found between the RO group and the FO group. These results suggest that the higher hepatic lipid content in fish consuming greater quantities of MUFA (i.e., the RO group) could result from the up-regulation of genes related to FA synthesis. Interestingly, *LXR* expression was up-regulated in the liver by consumption of LA, MUFA, and ALA (SO, RO, and LO groups, respectively), contradicting studies in Atlantic salmon (Cruz-Garcia et al., 2009) and rainbow trout (Cruz-Garcia et al., 2011) that reported a decrease in *LXR* level in fish on a VO diet, which would be attributed to a lower n3 LC-PUFA intake. However, because in the present study the n3 LC-PUFA content was similar across treatments except for the FO group, the increase in *LXR* expression was likely a direct consequence of the type of oil consumed.

In conclusion, growth and feed utilization in turbot were not significantly affected by dietary fatty acids composition when n-3 LC-PUFA requirement are met. The increase in hepatic lipid deposition induced by the SO, RO or LO diets was associated with up-regulation of some genes involved in lipids biosynthesis metabolism. Future studies would investigate the mechanistic basis of increased lipid deposition in muscle of turbot consuming an SO as compared to an RO diet and determine the most cost-effective VO for fish production.

Acknowledgments

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There are no conflicts of interest to report.

References

Ai, Q.H., Xu, H.G., Mai, K.S., Xu, W., Wang, J., Zhang, W.B., 2011. Effects of dietary supplementation of *Bacillus subtilis* and fructooligosaccharide on growth performance, survival, non-specific immune response and disease resistance of juvenile large yellow croaker, *Larimichthys crocea*. Aquaculture 317, 155–161.

Altundag, M.S., Tiril, S.U., Ozdemir, A., 2014. Effects of safflower oil supplementation in diet on growth performance and body fatty acid composition of turbot (*Psetta maxima*). Aquac. Int. 22 (2), 597–605.

Bautista, J., Garrido-Pertierra, A., Soler, G., 1988. Glucose-6-phosphate dehydrogenase from *Dicentrarchus labrax* liver: kinetic mechanism and kinetics of NADPH inhibition. BBA-Gen Subjects 967, 354–363.

Bell, J.G., Tocher, D.R., MacDonald, F.M., Sargent, J.R., 1994. Effects of diets rich in linoleic (18:2n-6) and α-linolenic (18:3n-3) acids on the growth, lipid class and fatty acid compositions and eicosanoid production in juvenile turbot (*Scophthalmus maximus* L.). Fish Physiol. Biochem. 13, 105–118.

Bell, J.G., Tocher, D.R., Farndale, B.M., McVicar, A.H., Sargent, J.R., 1999. Effects of essential fatty acid-deficient diets on growth, mortality, tissue histopathology and fatty acid compositions in juvenile turbot (*Scophthalmus maximus*). Fish Physiol. Biochem. 20, 263–277.

Benítez-Dorta, V., Caballero, M.J., Izquierdo, M., Manchado, M., Infante, C., Zamorano, M.J., Montero, D., 2013. Total substitution of fish oil by vegetable oils in Senegalese sole (*Solea senegalensis*) diets: effects on fish performance, biochemical composition, and expression of some glucocorticoid receptor-related genes. Fish Physiol. Biochem. 39, 335–349.

Botolin, D., Wang, Y., Christian, B., Jump, D.B., 2006. Docosahexaneoic acid (22: 6 n-3) regulates rat hepatocyte SREBP-1 nuclear abundance by Erk-and 26S proteasome-dependent pathways. J. Lipid Res. 47, 181–192.

Carew, T., Hayes, S., Koschinsky, T., Steinberg, D., 1976. A mechanism by which high-density lipoproteins may slow the atherogenic process. Lancet 307, 1315–1317.

Cha, J.Y., Repa, J.J., 2007. The liver X receptor (LXR) and hepatic lipogenesis the carbohydrate-response element-binding protein is a target gene of LXR. J. Biol. Chem. 282, 743–751.

Cruz-Garcia, L., Minghetti, M., Navarro, I., Tocher, D.R., 2009. Molecular cloning, tissue expression and regulation of liver X receptor (LXR) transcription factors of Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. B 153, 81–88.

Cruz-Garcia, L., Sánchez-Gurmaches, J., Gutiérrez, J., Navarro, I., 2011. Regulation of LXR by fatty acids, insulin, growth hormone and tumor necrosis factor-α in rainbow trout myocytes. Comp. Biochem. Physiol. A160, 125–136.

Escobar, E.L., Gomes-Marcondes, M.C.C., Carvalho, H.F., 2009. Dietary fatty acid quality affects AR and PPARγ levels and prostate growth. Prostate 69, 548–558.

Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Fountoulaki, E., Vasilaki, A., Hurtado, R., Grigorakis, K., Karacostas, I., Nengas, I., Rigos, G., Kotzamanis, Y., Venou, B., Alexis, M.N., 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.

Gatesoupe, F.J., Leger, C., Boudon, M., Metailler, R., Luquet, P., 1977. Lipid feeding of turbot (*Scophthalmus maximus* L.): influence on growth of supplementation with methyl esters of linolenic acid and fatty acids of the w-9 series. Ann. Hydrobiol. 8, 247–254.

Georgiadi, A., Kersten, S., 2012. Mechanisms of gene regulation by fatty acids. Adv. Nutr. 3, 127–134.

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*. BBA-Mol. Cell. Biol. L. 1437, 170–181.

Goldbourt, U., Yaari, S., Medalie, J.H., 1997. Isolated low HDL cholesterol as a risk factor for coronary heart disease mortality: a 21-year follow-up of 8000 men. Arterioscler. Thromb. Vasc. Biol. 17, 107–113.

Henderson, R.J., 1996. Fatty acid metabolism in freshwater fish with particular reference to polyunsaturated fatty acids. Arch. Anim. Nutr. 49, 5–22.

Hsu, R.Y., Lardy, H.A., 1969. Malic enzyme. Methods Enzymol. 13, 230–235.

Izquierdo, M.S., Montero, D., Robaina, L., Caballero, M.J., Rosenlund, G., Ginés, R., 2005. Alterations in fillet fatty acid profile and flesh quality in gilthead sea bream (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding. Aquaculture 250 (1), 431–444.

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.). Aquac. Nutr. 13, 114–130.
Jump, D.B., 2004. Fatty acid regulation of gene transcription. Crit. rev. Cl. Lab. Sci. 41, 41–78.
Jump, D.B., 2008. N-3 polyunsaturated fatty acid regulation of hepatic gene transcription. Curr. Opin. Lipidol. 19, 242.
Khan, M.W., Priyamvada, S., Khan, S.A., Khan, S., Naqshbandi, A., Yusufi, A.N.K., 2012. Protective effect of ω-3 polyunsaturated fatty acids (PUFAs) on sodium nitroprusside-induced nephrotoxicity and oxidative damage in rat kidney. Hum. Exp. Toxicol. 31, 1035–1049.
Koven, W., Barr, Y., Lutzky, S., Ben-Atia, I., Weiss, R., Harel, M., Tandler, A., 2001. The effect of dietary arachidonic acid (20: 4n − 6) on growth, survival and resistance to handling stress in gilthead seabream (*Sparus aurata*) larvae. Aquaculture 193, 107–122.
Léger, C., Gatesoupe, F.-J., Metailler, R., Luquet, P., Fremont, L., 1979. Effect of dietary fatty acids differing by chain lengths and ω series on the growth and lipid composition of turbot *Scophthalmus maximus* L. Comp. Biochem. Physiol. B 64, 345–350.
Li, Q.M., Chen, G.F., Gao, S., Wang, L.C., 1996. A method of measuring the amount of EPA and DHA in fish oil by gc-external standard method. J. Shenyang Pharmaceutical University 13 (4), 259–261.
Li, Y., Liang, X., Zhang, Y., Gao, J., 2016. Effects of different dietary soybean oil levels on growth, lipid deposition, tissues fatty acid composition and hepatic lipid metabolism related gene expressions in blunt snout bream (*Megalobrama amblycephala*) juvenile. Aquaculture 451, 16–23.
Lim, P.K., Boey, P.L., Ng, W.K., 2001. Dietary palm oil level affects growth performance, protein retention and tissue vitamin E concentration of African catfish, *Clarias gariepinus*. Aquaculture 202, 101–112.
Lin, Y., Smit, M.J., Havinga, R., Verkade, H.J., Vonk, R.J., Kuipers, F., 1995. Differential effects of eicosapentaenoic acid on glycerolipid and apolipoprotein B metabolism in primary human hepatocytes compared to HepG2 cells and primary rat hepatocytes. Biochim. Biophys. Acta 1256, 88–96.
Liu, X., Xue, Y., Liu, C., Lou, Q., Wang, J., Yanagita, T., Wang, Y., 2013. Eicosapentaenoic acid-enriched phospholipid ameliorates insulin resistance and lipid metabolism in diet-induced-obese mice. Lipids Health Dis. 12, 109.
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.
Livingstone, K.M., Lovegrove, J.A., Givens, D.I., 2012. The impact of substituting SFA in dairy products with MUFA or PUFA on CVD risk: evidence from human intervention studies. Nutr. Res. Rev. 25, 193–206.
López-Soldado, I., Avella, M., Botham, K.M., 2009. Suppression of VLDL secretion by cultured hepatocytes incubated with chylomicron remnants enriched in n3 polyunsaturated fatty acids is regulated by hepatic nuclear factor-4α. BBA-Mol. Cell. Biol. L. 1791, 1181–1189.
Martinez-Jimenez, C.P., Kyrmizi, I., Cardot, P., Gonzalez, F.J., Talianidis, I., 2010. Hepatocyte nuclear factor 4α coordinates a transcription factor network regulating hepatic fatty acid metabolism. Mol. Cell. Biol. 30, 565–577.
Matsusue, K., Kusakabe, T., Noguchi, T., Takiguchi, S., Suzuki, T., Yamano, S., Gonzalez, F.J., 2008. Hepatic steatosis in leptin-deficient mice is promoted by the PPAR gamma target gene Fsp27. Cell Metab. 7, 302–311.
Menoyo, D., Izquierdo, M.S., Robaina, L., Ginés, R., Lopez-Bote, C.J., Bautista, J.M., 2004. Adaptation of lipid metabolism, tissue composition and flesh quality in gilthead sea bream (*Sparus aurata*) to the replacement of dietary fish oil by linseed and soyabean oils. Brit. J. Nutr. 92, 41–52.
Minghetti, M., Leaver, M.J., Tocher, D.R., 2011. Transcriptional control mechanisms of genes of lipid and fatty acid metabolism in the Atlantic salmon (*Salmo salar* L.) established cell line, SHK-1. BBA-Mol Cell. Boil. L. 1811, 194–202.
Morais, S., Pratoomyot, J., Taggart, J.B., Bron, J.E., Guy, D.R., Bell, J.G., Tocher, D.R., 2011. Genotype-specific responses in Atlantic salmon (*Salmo salar*) subject to dietary fish oil replacement by vegetable oil: a liver transcriptomic analysis. BMC Genomics 12, 255.
Morais, S., Silva, T., Cordeiro, O., Rodrigues, P., Guy, D.R., Bron, J.E., Tocher, D.R., 2012. Effects of genotype and dietary fish oil replacement with vegetable oil on the intestinal transcriptome and proteome of Atlantic salmon (*Salmo salar*). BMC Genomics 13, 448.
Morán-Salvador, E., López-Parra, M., García-Alonso, V., Titos, E., Martínez-Clemente, M., González-Périz, A., Clària, J., 2011. Role for PPAR gamma in obesity-induced hepatic steatosis as determined by hepatocyte- and macrophage-specific conditional knockouts. FASEB J. 25, 2538–2550.
Mourente, G., Bell, J.G., 2006. Partial replacement of dietary fish oil with blends of vegetable oils (rapeseed, linseed and palm oils) in diets for European sea bass (*Dicentrarchus labrax* L.) over a long term growth study: effects on muscle and liver fatty acid composition and effectiveness of a fish oil finishing diet. Comp. Biochem. Physiol. B 145, 389–399.
Nguyen, P., Leray, V., Diez, M., Serisier, S., Bloc'h, J.L., Siliart, B., Dumon, H., 2008. Liver lipid metabolism. J. Anim. Physiol. An. N. 92, 272–283.
Owen, J.M., Adron, J.W., Middleton, C., Cowey, C.B., 1975. Elongation and desaturation of dietary fatty acids in turbot *Scophthalmus maximus* L., and rainbow trout, *Salmo gairdneriirich*. Lipids 10, 528–531.
Pan, M., Cederbaum, A.I., Zhang, Y.L., Ginsberg, H.N., Williams, K.J., Fisher, E.A., 2004. Lipid peroxidation and oxidant stress regulate hepatic apolipoprotein B degradation and VLDL production. J. Clin. Invest. 113, 1277–1287.
Peng, M., Xu, W., Mai, K.S., Zhou, H.H., Zhang, Y.J., Liufu, Z.G., Zhang, K.K., Ai, Q.H., 2014. Growth performance, lipid deposition and hepatic lipid metabolism related gene expression in juvenile turbot (*Scophthalmus maximus* L.) fed diets with various fish oil substitution level by soybean oil. Aquaculture 433, 442–449.
Pettinelli, P., Pozo, T., Araya, J., Rodrigo, R., Araya, A.V., Smok, G., Videla, L.A., 2009. Enhancement in liver SREBP-1c/PPAR-α ratio and steatosis in obese patients: correlations with insulin resistance and n-3 long-chain polyunsaturated fatty acid depletion. BBA-Mol Basis Dis. 1792, 1080–1086.
Qiu, H., Jin, M., Li, Y., You, L., Hou, Y.M., Zhou, Q.C., 2017. Dietary lipid sources influence fatty acid composition in tissue of large yellow croaker (*Larmichthys crocea*) by regulating triacylglycerol synthesis and catabolism at the transcriptional level. PLoS One 12 (1), e0169985.
Regost, C., Arzel, J., Robin, J., Rosenlund, G., Kaushik, S.J., 2003. Total replacement of fish oil by soybean or linseed oil with a return to fish oil in turbot (*Psetta maxima*): 1. Growth performance, flesh fatty acid profile, and lipid metabolism. Aquaculture 217, 465–482.
Robaina, L., Izquierdo, M.S., Moyano, F.J., Socorro, J., Vergara, J.M., Montero, D., 1998. Increase of the dietary n − 3/n − 6 fatty acid ratio and addition of phosphorus improves liver histological alterations induced by feeding diets containing soybean meal to gilthead seabream, *Sparus aurata*. Aquaculture 161, 281–293.
Sanz, M., Lopez-Bote, C.J., Menoyo, D., Bautista, J.M., 2000. Abdominal fat deposition and fatty acid synthesis are lower and β-oxidation is higher in broiler chickens fed diets containing unsaturated rather than saturated fat. J. Nutr. 130, 3034–3037.
Schoonjans, K., Gelman, L., Haby, C., Briggs, M., Auwerx, J., 2000. Induction of LPL gene expression by sterols is mediated by a sterol regulatory element and is independent of the presence of multiple E boxes. J. Mol. Biol. 304, 323–334.
Schwingshackl, L., Strasser, B., Hoffmann, G., 2011. Effects of monounsaturated fatty acids on cardiovascular risk factors: a systematic review and meta-analysis. Ann. Nutr. Metab. 59, 176–186.
Shearer, G.C., Savinova, O.V., Harris, W.S., 2012. Fish oil-how does it reduce plasma triglycerides? BBA-Mol Cell. Biol. L. 1821, 843–851.
Takeuchi, Y., Yahagi, N., Izumida, Y., Nishi, M., Kubota, M., Teraoka, Y., Shimano, H., 2010. Polyunsaturated fatty acids selectively suppress sterol regulatory element-binding protein-1 through proteolytic processing and autoloop regulatory circuit. J. Biol. Chem. 285, 11681–11691.
Tocher, D.R., Bell, J.G., Dick, J.R., Crampton, V.O., 2003. Effects of dietary vegetable oil on Atlantic salmon hepatocyte fatty acid desaturation and liver fatty acid compositions. Lipids 38, 723–732.
Tocher, D.R., Carr, J., Sargent, J.R., 1989. Polyunsaturated fatty acid metabolism in fish cells: differential metabolism of (n-3) and (n-6) series acids by cultured cells originating from a freshwater teleost fish and from a marine teleost fish. Comp. Biochem. Physiol. B 94, 367–374.
Torrecillas, S., Mompel, D., Caballero, M.J., Montero, D., Merrifield, D., Rodiles, A., Robaina, L., Zamorano, M.J., Karalazos, V., Kaushik, S., Izquierdo, M., 2017. Effect of fishmeal and fish oil replacement by vegetable meals and oils on gut health of European sea bass (*Dicentrarchus labrax*). Aquaculture 468, 386–398.
Turchini, G.M., Mentasti, T., Frøyland, L., Orban, E., Caprino, F., Moretti, V.M., Valfré, F., 2003. Effects of alternative dietary lipid sources on performance, tissue chemical composition, mitochondrial fatty acid oxidation capabilities and sensory characteristics in brown trout (*Salmo trutta* L.). Aquaculture 225, 251–267.
Turchini, G.M., Torstensen, B.E., Ng, W.K., 2009. Fish oil replacement in finfish nutrition. Rev. Aquac. 1 (1), 10–57.
Vamecq, J., Vallee, L., de la Porte, P.L., Fontaine, M., De Craemer, D., van den Branden, C., Nalbone, G., 1993. Effect of various n-3/n-6 fatty acid ratio contents of high fat diets on rat liver and heart peroxisomal and mitochondrial β-oxidation. BBA-Lipids L. Metabol. 1170, 151–156.
Vegusdal, A., Gjaen, T., Berge, R.K., Thomassen, M.S., Ruyter, B., 2005. Effect of 18:1n-9, 20:5n-3 and 22:6n-3 on lipid accumulation and secretion by Atlantic salmon hepatocytes. Lipids 40, 477–486.
Vyas, D., Kadegowda, A.K.G., Erdman, R.A., 2012. Dietary conjugated linoleic acid and hepatic steatosis: species-specific effects on liver and adipose lipid metabolism and gene expression. J. Nutr. Metabol. 928, 1–14.
Wang, X.X., Li, Y.J., Hou, C.L., Gao, Y., Wang, Y.Z., 2012. Influence of different dietary lipid sources on the growth, tissue fatty acid composition, histological changes and peroxisome proliferator-activated receptor γ gene expression in large yellow croaker (*Pseudosciaena crocea* R.). Aquac. Res. 43 (2), 281–291.
Zar, J.H., 1999. Biostatistical Analysis. Prentice-Hall International Editions, London, U.K.
Zuo, R.T., Ai, Q.H., Mai, K.S., Xu, W., Wang, J., Xu, H.G., Liufu, Z.G., Zhang, Y.J., 2012. Effects of dietary n-3 highly unsaturated fatty acids on growth, nonspecific immunity, expression of some immune related genes and disease resistance of large yellow croaker (*Larmichthys crocea*) following natural infestation of parasites (*Cryptocaryon irritans*). Fish Shellfish Immun. 32, 249–258.