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Growth performance, lipid deposition and hepatic lipid metabolism related gene expression in juvenile turbot (*Scophthalmus maximus* L.) fed diets with various fish oil substitution levels by soybean oil

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ABSTRACT

A 92-day feeding experiment was conducted to investigate the effects of dietary soybean oil (SO) on growth performance, fatty acid composition, lipid deposition and hepatic lipid metabolism related gene expression in juvenile turbot (*Scophthalmus maximus* L.) (mean initial body weight, 5.88 ± 0.02 g). Three experimental diets were formulated with substitution of 33.3%, 66.7% and 100% fish oil (FO) by SO. Each diet was randomly fed to triplicate tanks, and each tank was stocked with 35 fish. The results showed that specific growth rate of turbot fed diets with 100% SO was significantly lower than that in the 33.3% and 66.7% SO groups. Fatty acid (FA) composition of total lipid in the liver and muscle was closely correlated with those in diets. The lipid content in the liver and muscle significantly increased with increasing dietary SO level. The activity of LPL in the liver of turbot was not significantly affected by dietary SO level. Relative gene expression of LPL, PPARα, FAS and MTP significantly increased, while relative expression of LXR and CPT I significantly decreased with increasing dietary SO level. There was no significant difference in the expression of SREBP-1 among dietary treatments. These results suggested that the growth of turbot fed diets with 100% SO was significantly lower than the low SO group. The increase of lipid deposition in the liver of turbot fed diets with higher dietary SO level would be related to the up-regulation of fatty acid synthesis-related gene (FAS) and the down-regulation of fatty acids oxidation gene (CPT I) expression.

1. Introduction

Due to increasing costs and limited supplies of global fish oil (FO) (Tacon and Metian, 2008), it was currently of great urgency for the aquafeed industry to investigate the possibilities of alternative dietary lipid sources. Vegetable oil (VO) has been taken as a promising candidate for FO replacement, with steadily increasing production, lower concentrations of dioxins and other organic pollutants, high availability and better economic value (Bell et al., 2005; Figueiredo-Silva et al., 2005; Miller et al., 2008). Among VO, soybean oil (SO) has been found to be a possible alternative lipid source for salmonids, freshwater and marine fish since it was rich in poly-unsaturated fatty acids, especially linoleic acid (LA, 18:2n−6) and oleic acid (18:1n−9) (Caballero et al., 2002; Izquierdo et al., 2005; Montero et al., 2005; Mourente and Bell, 2006). Some previous studies showed that growth and feed utilization were not significantly reduced by partially replacing dietary FO with SO in finfish (Bell et al., 1994; Fountoulaki et al., 2009; Menoyo et al., 2004; Regost et al., 2003; Trushenski et al., 2011; Xu et al., 2012), while the LA and lipid contents in the liver had been significantly increased with increasing dietary SO level (Bell et al., 1994; Menoyo et al., 2004). However, the mechanism of lipid deposition in the liver of fish fed diets with higher dietary SO level was still uncertain.

Hepatic lipid deposition was a result of fatty acid (FA) oxidation, synthesis and transport. Following feeding, uptake of FA by the tissues was mediated by lipoprotein lipase (LPL) (Mayes, 1999). FA absorbed by the liver of fish would supply energy through β-oxidation. Peroxisome proliferator-activated receptor α (PPARα) stimulated hepatic β-oxidation by inducing expression of its target genes involved in fatty acid (FA) oxidation including carnitine palmitoyltransferase I (CPT I) (Goto et al., 2011). Accordingly, FA synthesis was regulated by liver X receptor (LXR) and sterol regulatory element-binding protein 1c (SREBP-1c), and SREBP-1c could up-regulate fatty acid synthase (FAS) expression (Fievet and Staels, 2009; Repa et al., 2000; Yoshikawa et al.,

Abbreviations: LPL, lipoprotein lipase; CPT I, carnitine palmitoyltransferase I; PPARα, peroxisome proliferator-activated receptor alpha; LXR, liver X receptor; FAS, fatty acid synthase; SREBP-1, sterol regulatory element-binding protein 1; MTP, microsomal triacylglycerol transfer protein.

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2001). In addition, excessive dietary FA is exported from the liver in the form of very low-density lipoprotein (VLDL). Thus, the secretion of hepatic VLDL was important to hepatic lipid accumulation, which was regulated by microsomal triacylglycerol transfer protein (MTP) (Hirokane et al., 2004; Hussain and Bakillah, 2008). Until now, there are few studies related to gene expression in PPARα, SREBP-1 and MTP in fish fed with higher VO. Therefore, it was essential to investigate the effect of dietary SO level on related gene expression of lipolysis, lipogenesis and lipid transport so as to explain the mechanism about lipid deposition in the liver of fish.

The turbot, with high economic value, delicious meat and rapid growth, is widely cultured in Europe and Asia. To date, some preliminary studies have been conducted on turbot with 100% SO replacement for FO (Bell et al., 1994; Regost et al., 2003). Hepatic lipid level was significantly higher in turbot fed diets with 100% SO in comparison to those fed FO diets (Bell et al., 1994). However, the mechanism of hepatic lipid deposition in turbot induced by higher dietary SO level is still unknown. According to a previous study in our lab, the diet with 33.3% SO showed a relatively better ratio of n−3 to n−6 poly-unsaturated fatty acids (PUFAs), which could be beneficial for growth, feed utilization and FA composition of the muscle in turbot, and no significant difference in hepatic lipid content was found between fish fed diets with 33.3% and 66.7% SO (unpublished results). Thus, the lower, moderate and higher dietary SO levels (with FO substitution by 33.3%, 66.7% and 100% SO, respectively) were designed in this study to evaluate the effect of dietary SO level on the growth, the FA composition, and the mechanism of hepatic lipid deposition in turbot.

2. Materials and methods

2.1. Experimental diets

Using white fish meal, soybean meal, wheat gluten meal and casein as main protein sources, three isonitrogenous (crude protein: 50% of dry matter) and isolipidic (crude fat: 12% of dry matter) practical diets were formulated to contain the lower, moderate and higher SO levels with FO replacement by 33.3%, 66.7% and 100% SO, respectively. Due to the marine FO and phospholipids contained in the fish meal itself, the 33.3%, 66.7% and 100% SO groups contained about 1.27%, 0.82% and 0.37% long-chain poly-unsaturated fatty acids (LC-PUFAs) on a dry matter basis, respectively. Fish meal and soybean meal were ground to pass through a 320-μm sieve before diet preparation. Procedures for diet preparation and storage were as previously described by Ai et al. (2011). No differences were observed in any diets with regard to physical quality or sinking properties. Ingredients and nutrient composition of the three experimental diets are given in detail in Tables 1 and 2.

2.2. Experimental procedure and sample collection

Disease-free juvenile turbot were obtained from a commercial farm (Qingdao City, Shandong Province, China). All fish were conditioned on a commercial diet of turbot (Great Severn Bio-Tech, Qingdao, China) to acclimate to the experimental conditions for 1 week, and were fed three experimental mixed diets (33.3%, 66.7% and 100% SO diets) for 1 week to allow them to become accustomed to the sinking pellets prior to the start of the feeding trial. After being fasted for 24 h, fish of similar sizes (mean initial body weight, 5.88 ± 0.02 g) were randomly distributed into 9 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). Turbot ate up diets within 30–60 s following feeding. The remaining feed and feces were removed by a siphon immediately after feeding. After six weeks, fecal samples were collected using a siphon. For each treatment, shaped fecal samples were collected 5 h after feeding once a day over 5 weeks, then centrifuged (3000 ×*g* at 4 °C for 20 min) and frozen daily at −20 °C. The feces were analyzed for Y_2O_3 and crude lipid to detect the ADC of lipid. All fish were fed in a recycling system for 92 days. Sea water, continuously pumped from the adjacent coast to the experiment station, passed through sand filters, froth separator and biofilter, and finally flowed into each tank at a rate of 2 l min^{-1}. The recycling water was changed 50% volume of

Table 1
Formulation and chemical proximate composition of the experimental diets.

Ingredient (dry weight, %)	Dietary SO replacement level (%)		
	33.3	66.7	100
White fish meal[a]	33.00	33.00	33.00
Wheat gluten meal[a]	10.00	10.00	10.00
Casein[a]	6.00	6.00	6.00
Wheat meal[a]	16.00	16.00	16.00
Soybean meal[a]	18.88	18.88	18.88
Fish oil	5.00	2.50	0.00
Soybean oil	2.50	5.00	7.50
Phospholipid	2.00	2.00	2.00
Mineral premix[b]	2.00	2.00	2.00
Vitamin premix[c]	2.00	2.00	2.00
Choline chloride	0.13	0.13	0.13
Monocalcium phosphate	1.00	1.00	1.00
Calcium propionic acid	0.10	0.10	0.10
Ethoxyquin	0.05	0.05	0.05
Y_2O_3	0.04	0.04	0.04
Phagostimulant	1.30	1.30	1.30
Sodium alginate	0.05	0.05	0.05
Proximate analysis (dry matter, %)			
Crude protein	50.69	50.02	50.47
Crude lipid	11.43	11.76	12.02
Crude ash	12.27	11.35	11.01

[a] White fish meal (dry mater, %): protein 71.18 and crude lipid 6.17; wheat gluten meal (dry mater, %): crude protein 83.09 and crude lipid 0.96; casein (dry mater, %): crude protein 87.91 and crude lipid 1.69; wheat meal (dry mater, %): crude protein 16.10 and crude lipid 1.36; soybean meal (dry mater, %): crude protein 51.53 and crude lipid 1.13. These ingredients are obtained from Great Severn Bio-Tech (Qingdao, China).

[b] 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; zeolite, 15.55 g kg^{-1} diet.

[c] 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; choline chloride, 2500; ethoxyquin, 150; wheat middling, 18.52 g kg^{-1} diet.

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

Fatty acids	Dietary SO replacement level (%)		
	33.3	66.7	100
14:0	3.69	1.85	0.74
16:0	17.34	14.69	13.99
18:0	2.55	2.19	2.69
20:0	3.10	2.82	1.90
∑SFA[b]	26.68	21.55	19.32
16:1	5.51	3.47	1.73
18:1	22.53	23.82	23.48
∑MUFA[c]	28.05	27.29	25.21
18:2n−6	25.37	33.70	43.27
20:4n−6	0.37	0.26	
∑n−6 PUFA[d]	25.74	33.96	43.27
18:3n−3	3.19	5.25	6.39
18:4n−3	1.00	0.76	
20:5n−3	4.99	3.53	2.19
22:6n−3	5.80	4.32	2.38
∑n−3 PUFA[e]	14.97	13.85	10.96
∑SFA/∑PUFA	0.66	0.45	0.36
n−3/n−6 PUFA	0.58	0.41	0.25

[a] 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.

[b] SFAs: saturated fatty acids.

[c] MUFAs: mono-unsaturated fatty acids.

[d] n−6 PUFAs: n−6 poly-unsaturated fatty acids.

[e] n−3 PUFAs: n−3 poly-unsaturated fatty acids.

tank (up to 70% volume of tank in the last 4 weeks) after feeding, with additional aeration provided by a single air-stone. During the experimental period, the water temperature ranged from 17.0 °C to 19.0 °C; salinity ranged from 28.5‰ to 32.0‰; and dissolved oxygen was approximately 7 mg l^{-1}. NH_4–N, NO_3–N and NO_2–N were lower than 100.0 μg l^{-1}, 56.2 μg l^{-1} and 22.5 μg l^{-1}, respectively. At the termination of the experiment, the fish were fasted for 24 h before harvest.

Before the experiment, 20 fish were randomly selected for determination of initial whole-body proximate composition. At the end of the experiment, after being fasted for 24 h, the livers and muscles from three fish in each tank were pooled into 1.5 ml tubes (RNase-Free; Axygen), frozen in liquid N_2 and then stored at −80 °C for later analysis of gene expression related to lipid metabolism. The liver from another six fish in each tank was pooled into 5 ml tubes, frozen in liquid N_2 and then stored at −80 °C for the assay of LPL, 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 moisture, crude protein, crude lipids and crude ash.

2.3. Chemical analyses

2.3.1. Body composition assays

Dry matter, crude protein, crude lipid, and ash were analyzed for ingredients, experimental diets and fish samples. Dry matter was analyzed by drying the samples to constant weight at 105 °C. Crude protein was determined using the Kjeldahl method (AOAC, 1995) and estimated by multiplying nitrogen by 6.25. Crude lipid was measured by ether extraction using Soxhlet method. Ash was examined by combustion in a muffle furnace at 550 °C for 16 h. Duplicate analyses were conducted for each sample.

The lipid of the liver, muscle, diet and feces was assayed according to Folch et al. (1957) with some modification. In brief, about 100.0 mg (dry weight) sample (w0) was added to 4 ml chloroform:methanol (C–M) (2:1, v/v) in tube A, and then tube A was settled at rest for over 24 h (sometimes shaken). Two microliters of C–M was still added to tube A, and tube A was again settled at rest for 4 h. After centrifugation (3000 ×*g* for 10 min at 4 °C) (Hitachi Centrifuge CT15RE), the supernatant was transferred to tube B (constant weight, w1). Two microliters of C–M was added to the residue of tube A, settled for over 2 h, and centrifuged (3000 ×*g* for 10 min at 4 °C) later, and the supernatant was transferred to tube B. 1.2 ml of 1.6% $CaCl_2$ was added to the supernatant of tube B, then mixed, and tube B was settled at rest over 12 h. The upper phase was removed. The lower phase of tube B had been dried under a pure nitrogen steam. Tube A was evaporated at 75 °C and reweighed (w2). Therefore, the lipid content of the sample (%) is (w2 − w1) / w0 × 100. Duplicate analyses were conducted for each sample.

2.3.2. Biochemical analysis

The fatty acid profiles were analyzed using the procedures described by Metcalfe et al. (1966) with some modification. The detail procedures were as previously described by Zuo et al. (2012). Briefly, about 100.0 mg freeze-dried samples were added into a 20 ml volumetric screwed glass tube with plastic cover. Then 3 ml potassium hydroxide methanol (1 mol l^{-1}) was added and heated on a water bath for 25 min at 78 °C. After that, 3 ml HCl–methanol (2 mol l^{-1}) was added and the mixture was heated on a water bath for another 25 min at 78 °C. Previous test had been conducted to make sure that all fatty acids could be esterified following the procedures above. Finally, 1 ml hexane was added into the mixture above, shaken vigorously for 1 min, and then allowed to separate into two layers. Fatty acid methyl esters in the upper layer were separated, and quantified by a HP6890 gas chromatograph (Agilent Technologies Inc., Santa Clara, California, USA) with a fused silica capillary column (007-CW, Hewlett Packard, Palo Alto, CA, USA) and a flame ionization detector. The column temperature was programmed to rise from 150 °C up to 200 °C at a rate of 15 °C min^{-1}, and from 200 °C to 250 °C at a rate of 2 °C min^{-1}. Injector and detector temperature were 250 °C, respectively.

Feces from each replicate were dried for 12 h at 65 °C. Yttrium concentrations in diets and fecal samples were determined with an inductively coupled plasma atomic emission spectrophotometer (ICP-OES; VISTAMPX, VARIAN, Palo Alto, CA, USA) after perchloric acid digestion.

The activity of LPL was measured with frozen (−80 °C) samples as preliminary research showed that it was not affected by freezing. A total of 0.5 g of liver was minced in 0.86% NaCl solution in a ratio of 1:9 (weight/volume), and the supernatant was transferred to a tube after centrifugation (3000 ×*g* for 20 min at 4 °C). The activity of LPL and protein concentration of the liver was assayed with a commercial kit (Nanjing Jiancheng Bioengineering Institute, China). One unit of LPL activity was defined as 1 mmol of non-esterified fatty acid (NEFA) released per hour per gram of liver protein. Duplicate analyses were conducted for each sample.

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

Total RNA was extracted from the liver using TRIzol Reagent (Invitrogen, USA) and electrophoresed on a 1.2% denaturing agarose gel to test the integrity. Then, RNA was treated with RNase-Free DNase (Takara, Japan) to remove DNA contaminant and reverse transcribed to cDNA by PrimeScript™ RT Reagent Kit (Takara, Japan) following the manufacturer's instructions. First strand cDNA was diluted 3 times using sterilized double-distilled water. Real-time RT-PCR was carried out in a quantitative thermal cycle (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 μl of the diluted first strand cDNA product, 12.5 μl of 2× SYBR® Premix Ex Taq™ II (Trans, Japan) 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, 57.5 °C for 10 s, and 72 °C for 20 s. The reaction was carried out with three duplicates for each sample. The primer sequences for reference genes (β-actin), LPL, CPT I, PPARα, FAS, LXR, SREBP-1 and MTP were designed following the published sequences from turbot and were listed 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 amplification efficiency was analyzed according to the following equation $E = 10^{(-1\ /\ \text{slope})} - 1$. The value of CPT I, LPL, PPARα, FAS, LXR, SREBP-1, MTP and β-actin was 1.001, 1.001, 1.001, 0.938, 1.010, 0.883, 1.031 and 0.972, respectively. The absolute ΔC_T (cycle threshold) values between the target genes and inner control gene were all lower than 0.1, which indicates that the $\Delta\Delta C_T$ calculation for the relative quantification of target genes can be used. To calculate the expression of CPT I, LPL, PPARα, FAS, LXR, SREBP-1 and MTP, the comparative CT method ($2^{-\Delta\Delta CT}$ method) was used, and the value stood for n-fold difference relative to the calibrator (Livak and Schmittgen, 2001).

Table 3
Real-time quantitative PCR primers for fatty acid oxidation-, fatty acid 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
MTP	CCAGCAAAGTCTTACGCCA	TACGCAGATGATGACCCAAC	85
β-Actin	GTAGGTGATGAAGCCCAGAGCA	CTGGGTCATCTTCTCCCTGT	204

2.5. Calculations and statistical methods

The following variables were calculated:

$$\text{survival rate}(\text{SR},\%) = \text{Nt} \times 100/\text{No}$$

$$\text{specific growth rate}(\text{SGR},\%/\text{d}) = (\text{LnWt}-\text{LnWo}) \times 100/\text{t}$$

$$\text{feed efficiency}(\text{FE}) = (\text{Wt}-\text{Wo})/\text{dry feed consumed}$$

$$\text{feed intake}(\text{FI},\%/\text{fish/d}) = 100 \times \text{total amount of the feed consumed}(\text{g})/[(\text{Wo}+\text{Wt})/2]/\text{t}$$

$$\text{Apparent Net Protein Utilization}(\text{ANPU}) = [(\text{final carcass protein}-\text{initial carcass protein})/\text{total dry protein consumed}] \times 100$$

$$\text{hepatosomatic index}(\text{HSI},\%) = 100 \times (\text{liver weight}/\text{Wt})$$

$$\text{apparent digestibility coefficient}(\text{ADC})\text{of lipid}(\%) = 100-100 \times (\%\ \text{tracer in diet}/\%\ \text{tracer in feces}) \times (\%\ \text{lipid in feces}/\%\ \text{lipid in diet})$$

where Wt and Wo were the final and initial fish weights, respectively; Nt and No were the final and initial numbers of fish, respectively; and t was the 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 (n = 3), 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), ranging from 99.05% to 100.0%, was independent of dietary SO level ($P > 0.05$) (Table 4). Fish fed diets with low SO level showed higher feed intake (FI) in comparison with the 66.7% and 100% SO groups ($P < 0.05$). Specific growth rate (SGR) of fish fed diets with 100% SO was significantly lower than the 33.3% SO and 66.7% SO groups, and no significant difference was found between the 33.3% SO group and 66.7% SO group ($P > 0.05$). Feed efficiency (FE) in fish fed diets with 33.3% SO was significantly lower than that in the 66.7% and 100% SO groups ($P < 0.05$), though Apparent Net Protein Utilization (ANPU) and apparent digestibility coefficient (ADC) of lipid in fish were not significantly differently among dietary treatments ($P > 0.05$) (Table 4). There were also no significant differences in hepatosomatic index (HSI) of fish among dietary treatments ($P > 0.05$) (Table 4).

Table 4
Effects of dietary SO levels on growth, survival, ADC of lipid and selected body parameters of turbot (*Scophthalmus maximus* L.) for 92 days.

Index	Dietary SO replacement level (%)			ANOVA *P*
	33.3	66.7	100	
SR[a]	99.05 ± 1.65	100.0 ± 0.00	100.0 ± 0.00	0.441
FI[b]	1.83 ± 0.08[b]	1.58 ± 0.04[a]	1.68 ± 0.09[a]	0.019
SGR[c]	2.32 ± 0.07[b]	2.36 ± 0.02[b]	2.15 ± 0.13[a]	0.044
FE[d]	1.04 ± 0.06[a]	1.21 ± 0.03[b]	1.11 ± 0.07[b]	0.018
ANPU[e]	29.29 ± 1.32	32.26 ± 1.18	30.34 ± 0.65	0.093
ADC of lipid[f]	92.63 ± 1.82	89.33 ± 2.87	91.71 ± 2.71	0.332
HSI[g]	1.07 ± 0.03	1.11 ± 0.01	1.12 ± 0.06	0.388

[a,b,c]Mean values (n = 3) within a row with a common superscript letter are not significantly different from the other dietary groups ($P > 0.05$).
[a] SR: survival rate.
[b] FI: feed intake.
[c] SGR: specific growth rate.
[d] FE: feed efficiency.
[e] ANPU: Apparent net protein utilization.
[f] ADC: apparent digestibility coefficient.
[g] HSI: hepatosomatic index.

3.2. Fish tissue composition and apparent digestibility coefficient (ADC) of lipid

There were no significant differences in moisture, crude lipid content, crude protein content, ash content, and ADC of lipid for body composition among dietary treatments ($P > 0.05$) (Table 5). The lipid content of the liver in fish increased with increasing dietary SO level, and was up to the maximum in the 100% SO group ($P < 0.05$). The lipid content of the muscle in the 66.7% and 100% SO groups was higher than that in the 33.3% SO group ($P < 0.05$) (Table 5).

3.3. Fatty acids of the liver and muscle

The FA profiles of the liver and muscle reflected those of diets in this study (Table 6). The content of 18:2n − 6 and 18:3n − 3 in the liver and muscle significantly increased with increasing dietary SO level, while the content of 20:4n − 6, 20:5n − 3 and 22:6n − 3 showed a reverse trend ($P < 0.05$). In addition, as dietary LA increased, 18:2n − 6 became enriched in the muscle and liver lipids at the expense of 20:5n − 3 and 22:6n − 3 in a linear fashion (Fig. 1a, b).

3.4. Activity of LPL and expression of lipid deposition related genes in the liver

The activity of hepatic LPL in fish was not significantly affected by dietary SO level ($P > 0.05$) (Fig. 2), though there was an increasing trend with increasing dietary SO level.

With increasing dietary SO level, relative expression of LPL, PPARα and MTP genes in the liver of turbot significantly increased, and these parameters were higher in the 100% SO group compared with the other groups ($P < 0.05$) (Fig. 3a, c). Relative expression of hepatic CPT I and LXR of turbot significantly decreased with increasing dietary SO level ($P < 0.05$), and was up to the maximum in the 33.3% SO group ($P < 0.05$) (Fig. 3a, b). As dietary SO level increased, there was no significant difference in hepatic SREBP-1 gene expression among dietary treatments ($P > 0.05$). The expression of FAS gene of turbot fed diets with 100% SO was significantly higher than the 33.3% and 66.7% SO

Table 5
Proximate composition (% wet weight) in whole body and the lipid content of the liver and muscle in turbot (*Scophthalmus maximus* L.) fed diets with graded levels of SO for 92 days.

Index	Dietary SO replacement level (%)			ANOVA *P*
	33.3	66.7	100	
Proximate composition of whole body (% wet weight)				
Moisture	76.13 ± 0.66	76.36 ± 1.00	76.69 ± 0.30	0.695
Crude protein	16.63 ± 0.51	16.54 ± 0.83	16.11 ± 0.45	0.653
Crude lipid	3.18 ± 0.12	3.19 ± 0.37	3.43 ± 0.20	0.429
Ash	4.19 ± 0.04	4.18 ± 0.25	4.17 ± 0.11	0.976
Lipid content (% wet weight)				
Liver	7.54 ± 0.55[a]	9.29 ± 0.26[ab]	11.24 ± 1.45[b]	0.013
Muscle	0.91 ± 0.04[a]	1.04 ± 0.05[b]	1.03 ± 0.03[b]	0.024

[a,b,c]Mean values (n = 3) within a row with a common superscript letter are not significantly different from the other dietary groups ($P > 0.05$).

Table 6
Fatty acid composition (% total fatty acids) in the muscle and liver of turbot (*Scophthalmus maximus* L.) fed diets with graded levels of SO for 92 days.[a]

Fatty acid	Dietary SO replacement level (%)			ANOVA *P*
	33.3	66.7	100	
Muscle				
14:0	1.46 ± 0.19[c]	0.99 ± 0.03[b]	0.54 ± 0.01[a]	0.000
16:0	19.97 ± 0.24	20.26 ± 0.81	19.86 ± 0.72	0.210
18:0	5.43 ± 0.18	5.24 ± 0.22	4.81 ± 0.31	0.098
20:0	2.19 ± 0.25[b]	1.68 ± 0.08[a]	1.41 ± 0.05[a]	0.002
∑SFA[b]	29.06 ± 0.33[b]	28.52 ± 0.70[b]	26.62 ± 0.95[a]	0.048
16:1	3.44 ± 0.42	3.30 ± 0.12	3.08 ± 0.49	0.320
18:1	22.00 ± 0.60	21.10 ± 0.90	21.09 ± 0.53	1.000
∑MUFA[c]	25.44 ± 1.19	24.40 ± 0.53	24.17 ± 1.61	0.209
18:2n−6	20.19 ± 0.52[a]	28.63 ± 0.55[b]	38.86 ± 0.91[c]	0.001
20:4n−6	1.14 ± 0.03[c]	0.63 ± 0.02[b]	0.50 ± 0.01[a]	0.000
∑n−6 PUFA[d]	21.33 ± 0.55[a]	29.26 ± 0.55[b]	39.37 ± 0.90[c]	0.000
18:3n−3	1.22 ± 0.11[a]	1.74 ± 0.03[b]	2.60 ± 0.10[c]	0.000
20:5n−3	4.53 ± 0.14[c]	3.21 ± 0.13[b]	2.07 ± 0.09[a]	0.000
22:6n−3	14.74 ± 1.63[c]	10.53 ± 1.02[b]	5.79 ± 0.67[a]	0.000
∑n−3 PUFA[e]	20.49 ± 1.66[c]	15.48 ± 1.13[b]	10.45 ± 0.74[a]	0.000
∑SFA/∑PUFA	0.70 ± 0.03[b]	0.64 ± 0.02[b]	0.53 ± 0.01[a]	0.001
n−3/n−6	0.96 ± 0.09[c]	0.53 ± 0.05[b]	0.27 ± 0.02[a]	0.000
Liver				
14:0	3.56 ± 0.34[c]	2.41 ± 0.22[b]	1.14 ± 0.08[a]	0.000
16:0	16.17 ± 0.65[c]	14.06 ± 0.25[b]	12.23 ± 0.31[a]	0.018
18:0	2.56 ± 0.43	2.35 ± 0.25	2.49 ± 0.44	0.536
20:0	2.34 ± 0.20[b]	1.97 ± 0.22[ab]	1.62 ± 0.02[a]	0.006
∑SFA	23.78 ± 1.34[c]	20.09 ± 0.42[b]	17.47 ± 0.54[a]	0.000
16:1	5.92 ± 0.54[c]	4.11 ± 0.14[b]	2.33 ± 0.03[a]	0.000
18:1	23.98 ± 0.18	24.45 ± 0.50	24.00 ± 0.53	0.265
22:1	1.46 ± 0.17[b]	1.14 ± 0.17[a]	0.87 ± 0.03[a]	0.002
∑MUFA	31.36 ± 0.37[b]	29.71 ± 0.21[a]	27.25 ± 0.48[a]	0.000
18:2n−6	26.69 ± 0.52[a]	35.28 ± 0.59[b]	43.48 ± 1.5[c]	0.000
20:4n−6	0.79 ± 0.28[b]	0.36 ± 0.08[a]	0.44 ± 0.51[a]	0.025
∑n−6 PUFA	27.33 ± 0.13[a]	35.64 ± 0.66[b]	43.92 ± 1.53[c]	0.000
18:3n−3	2.35 ± 0.91[a]	3.30 ± 0.12[ab]	4.43 ± 0.13[b]	0.009
18:4n−3	0.47 ± 0.02[b]	0.41 ± 0.01[b]	0.20 ± 0.02[a]	0.000
20:5n−3	3.16 ± 0.45[b]	1.55 ± 0.17[ab]	0.92 ± 0.12[a]	0.016
22:6n−3	4.81 ± 1.37[b]	2.92 ± 0.43[ab]	1.72 ± 0.30[a]	0.036
∑n−3 PUFA	10.86 ± 1.95[c]	8.08 ± 0.49[b]	7.27 ± 0.39[a]	0.016
∑SFA/∑PUFA	0.36 ± 0.04[b]	0.27 ± 0.01	0.29 ± 0.01[a]	0.001
n−3/n−6	0.45 ± 0.01[b]	0.23 ± 0.01[a]	0.17 ± 0.01[a]	0.000

[a,b,c]Mean values (n = 3) within a row with a common superscript letter are not significantly different from the other dietary groups ($P > 0.05$).

[a] 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.

[b] SFAs: saturated fatty acids.

[c] MUFAs: mono-unsaturated fatty acids.

[d] n−6 PUFAs: n−6 poly-unsaturated fatty acids.

[e] n−3 PUFAs: n−3 poly-unsaturated fatty acids.

groups ($P < 0.05$), and no significant difference could be found between the latter groups (Fig. 3b).

4. Discussion

In the present study, the growth in fish fed diets with 100% SO was significantly lower than the 33.3% and 66.7% SO groups, but no significant difference was found between the 33.3% and 66.7% SO groups after a 92-day feeding ($P < 0.05$), which suggested that FO could be partially replaced by SO in diets of turbot. The result was consistent with some previous studies in other fish (Bell et al., 2005; Caballero et al., 2002; Figueiredo-Silva et al., 2005; Greene and Selivonchick, 1990; Hardy et al., 1987; Izquierdo et al., 2005; Menoyo et al., 2004; Rosenlund et al., 2001; Trushenski et al., 2011; Xu et al., 2012). This would be related to dietary appropriate levels of n3 LC-PUFA. Indeed, the n3 LC-PUFA content in the 66.7% SO and 100% SO groups was 0.82% diet and 0.37% diet, respectively. The n3 LC-PUFA content in the diet with 100% SO was insufficient to meet the EFA requirements of turbot, which is estimated to be 0.8% (Gatesoupe et al., 1977), or even less

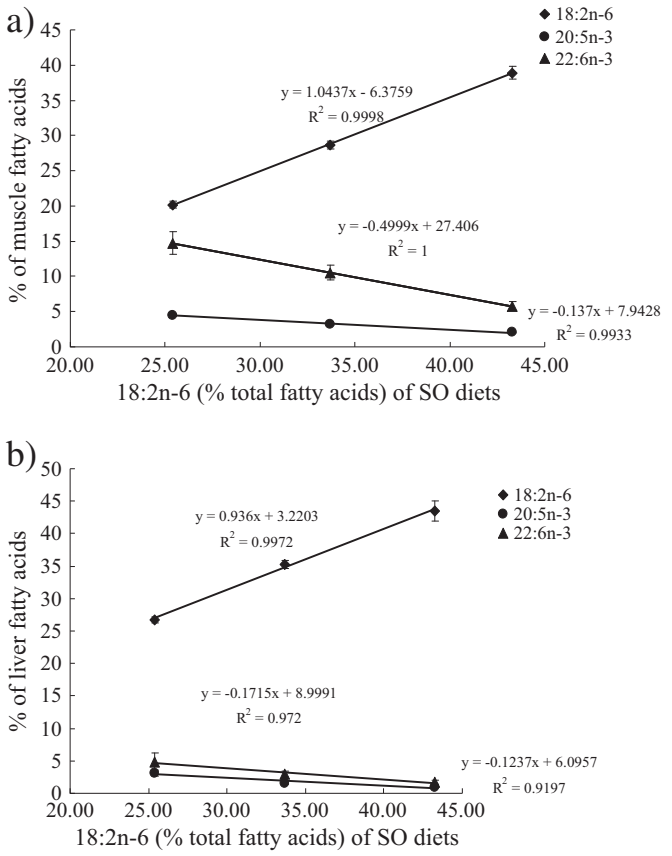

Fig. 1. Effects of graded dietary SO level on 18:2n−6, 20:5n−3 and 22:6n−3 in the muscle (a) and liver (b) of turbot (*Scophthalmus maximus* L.) for 92 days. Relationship between 18:2n−6, 20:5n−3 and 22:6n−3 in the muscle and liver against dietary LA (% total fatty acid) was analyzed by simple linear regression.

(0.6%; Léger et al., 1979). Thus, the lower n3 LC-PUFA content in the 100% SO group would account for the reduction of growth.

The FA profiles of the liver and muscle reflected those of diets in this study, as already shown in other fish studies (Benítez-Dorta et al., 2013; Sun et al., 2011; Tocher et al., 2003; Trushenski et al., 2011; Xu et al., 2012). Compared with the 33.3% SO group, the liver and muscle of fish fed diets with higher SO level were rich in 18:2n−6 and 18:3n−3, but poor in 20:4n−6, EPA and DHA. This was consistent with the findings in turbot of Regost et al. (2003). The significant decrease of EPA and DHA contents in the muscle was found in the 66.7% and 100% SO groups,

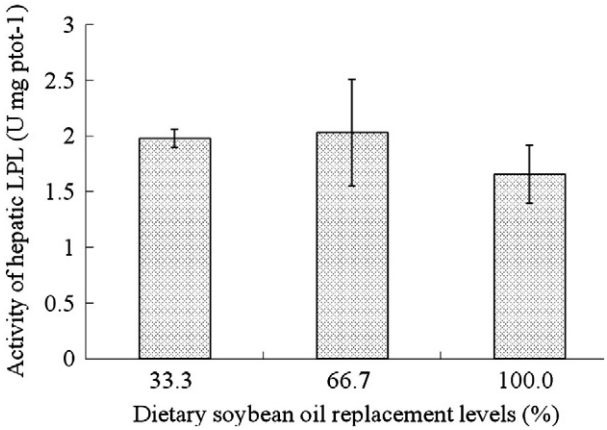

Fig. 2. Activity of hepatic LPL in turbot (*Scophthalmus maximus* L.) fed diets with graded levels of SO for 92 days. Bars bearing the same letters are not significantly different by Tukey's test ($P > 0.05$).

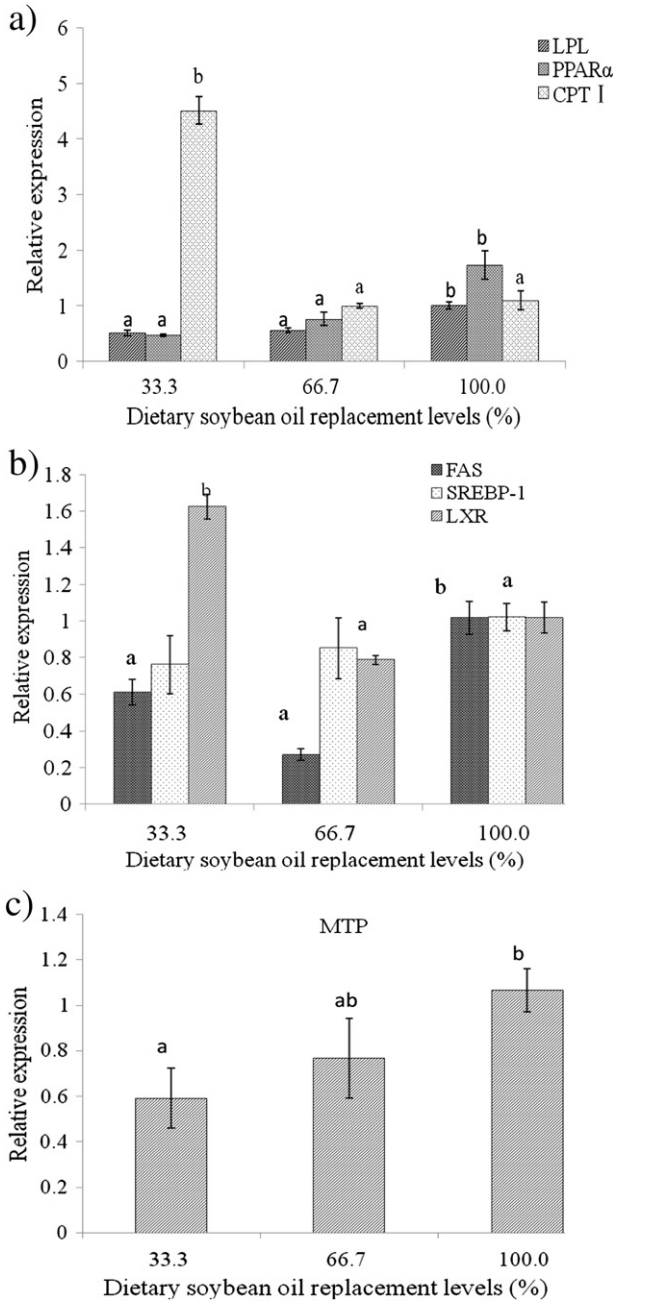

Fig. 3. Effects of graded dietary SO levels on relative mRNA expression of hepatic fatty acid oxidation-related genes (LPL, PPARα and CPT I) (a), lipogenesis-related genes (FAS, LXR and SREBP-1) (b) and lipid transport-related gene (MTP) (c) in turbot (*Scophthalmus maximus* L.) for 92 days. Values are means ($n \geq 2$). Bars bearing the same letters are not significantly different by Tukey's test ($P > 0.05$).

which means that the muscle quality of turbot had been decreased after fed diets with higher SO replacement level (≥66.7%). These results suggested that on the basis of nutritional quality, the SO replacement level in turbot diet should be lower than 66.7%. In addition, it has been well known that the biosynthesis of LC-PUFA was a process of catalysis on C18 substrates, linoleic acid (18:2n−6) or linolenic acid (18:3n−3), through Δ^6 fatty acyl desaturase (Fad), elongase and Δ^5 Fad. In the present study, the content of 20:4n−6, 20:5n−3 and 22:6n−3 in the liver and muscle decreased with increasing dietary 18:2n−6 and 18:3n−3 levels ($P < 0.05$), which suggested that the capacity of turbot to convert 18:2n−6 to 20:4n−6 or 18:3n−3 to EPA and DHA was limited. Because of relatively higher activity of Δ^6 Fad (Ghioni et al., 1999), the limitation of LC-PUFA biosynthesis in turbot would be greatly related to the lower activity of Δ^5 Fad (Owen et al., 1975) and elongase (from C18 to C20) (Ghioni et al., 1999; Tocher et al., 1989).

No effect of dietary SO level was observed in whole body of turbot, which was well in agreement with Regost et al. (2003) in turbot. But, turbot had the ability to store large amounts of lipid in the liver like other flatfish (Nortvedt and Tuene, 1998; Regost et al., 2003). In the present study, the lipid content of the liver (% wet weight) in turbot fed diets with 100% SO (11.24%) was significantly higher than the 33.3% SO group (7.54%). On the other hand, muscle lipid content was low (lower than 2%), confirming earlier report (Regost et al., 2001), but the muscle lipid content of turbot fed diets with 66.7% and 100% SO was significantly higher than the 33.3% SO group. Thus, no significant difference of crude lipid in the whole fish might be related to the reduce lipid of the visceral, except for the liver.

In the present study, significantly higher hepatic lipid deposition in the 100% SO group could be due to lower fatty acid oxidation in the higher SO group. Indeed, a significant down-regulation of CPT I expression (a "rate-limiting" enzyme of β-oxidation) (Eaton, 2002) was found in this study. Dietary n3 LC-PUFA increased mitochondrial fatty acid oxidation by stimulating the activity of CPT I (Madsen et al., 1999). Thus, the lower expression of CPT I could result in lower non-esterified FA (NEFA) delivery to the liver to down-regulate the capacity of β-oxidation and eventually resulted in higher hepatic lipid deposition in the present study. Moreover, low dietary n3 LC-PUFA content in the 100% SO group would be related to higher lipid content in the liver. Previous studies have found that higher dietary n3 LC-PUFA could promote transcription of fatty acid oxidation related genes (Jump, 2004) and mitochondrial proliferation (Vamecq et al., 1993). In addition, it has been reported that EPA can bind to PPARα with higher affinity than saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and n−6 polyunsaturated fatty acid (n−6 PUFA) (Desvergne and Wahli, 1999). PPARα activation by n3 LC-PUFA may induce the expression of lipolytic genes, and increase the activity of enzymes such as CPT I, thus enhanced fatty acid β-oxidation (Baillie et al., 1999; Poudyal et al., 2011). As hepatic n3 LC-PUFA decreased with increasing dietary SO level, the expression of hepatic PPARα gene should decrease with decreasing dietary n−3 PUFA, while it significantly increased in the 100% SO group. This suggested that higher dietary SO (rich in linoleic acid) level would affect other factors, which could up-regulate PPARα gene expression. Indeed, linoleic acid could increase insulin secretion in rat beta-cells (Zhou et al., 2012), and insulin mediated the transcriptional activity of PPARα in rats (Shalev et al., 1996). Moreover, the expression of PPARα gene was down-regulated in higher n−6 PUFA treatment (100% VO) but only in the lean family group of Atlantic salmon (*Salmo salar*) (Morais et al., 2011). Therefore, detailed mechanisms of the effect of higher dietary SO level on hepatic lipolysis should be deeply studied on post-translational regulation.

Hepatic lipid deposition was also related to the uptake of mobilized fatty acids (FAs) from the adipose tissue and hepatic lipogenesis (Vyas et al., 2011). Uptake of fatty acids by tissues is mediated by LPL. In the present study, relative expression of hepatic LPL gene in the 100% SO group was higher than that in the 33.3% and 66.7% SO groups, while hepatic LPL activity was not significantly affected by dietary SO level. No significant difference of hepatic LPL activity in fish fed diets with higher dietary n−6 PUFA level was also confirmed by the findings in rainbow trout (*Oncorhynchus mykiss*) (Richard et al., 2006a) and European seabass (*Dicentrarchus labrax* L.) (Richard et al., 2006b). These findings indicated that the increase in LPL gene expression caused by higher dietary SO level seems to be really too weak to induce modification in the activity of LPL or the regulation of dietary SO level on LPL gene expression could also decrease at a post-transcriptional level. In addition, in mammals, fatty acid synthesis and uptake were induced by LXR agonists through the activation of SREBP-1c gene expression and up-regulation of FAS and LPL (Cha and Repa, 2007; Minghetti et al., 2011; Montanaro et al., 2007; Schoonjans et al., 2000). A significant up-regulation of FAS expression was found in the 100% SO group, which

was in agreement with the results in the liver (Morais et al., 2011) or intestine (Morais et al., 2012) in Atlantic salmon. In both studies, FAS was up-regulated by higher dietary n−6 PUFA (a blend of VO). The results showed that significantly higher hepatic lipid content in the 100% SO group could be due to higher fatty acid synthesis in the liver. However, LXR gene expression in the 33.3% SO group was significantly higher than that in the 66.7% and 100% SO groups, which was consistent with the findings in Atlantic salmon (Cruz-Garcia et al., 2009). In the present study, the reduction of hepatic LXR gene expression caused by higher dietary SO level was not echoed by the increase of FAS gene expression, which indicated that FAS might not be the target gene of LXR in turbot, just as the findings in isolated adipocytes of rainbow trout (*O. mykiss*) (Cruz-Garcia et al., 2012). Thus, higher dietary SO level could account for the higher expression of FAS, which resulted in up-regulation of lipid synthesis and thereby resulted in higher hepatic lipid deposition in the 100% SO group. In addition, no significant difference of SREBP-1 gene expression among dietary treatments was found. Both EPA and DHA had been shown to inhibit SREBP-1 expression through post-translational mechanisms in rat primary hepatocytes (Howell et al., 2009). Thus, the change of SREBP-1 gene expression caused by dietary graded SO level might be at post-translational level.

Besides enhanced FA uptake and lipogenesis, the decrease in very low-density lipoprotein (VLDL) secretion rates could also result in hepatic fat deposition (Nagayoshi et al., 1995). Hepatic VLDL secretion rate was regulated by MTP, which modulated the transport of triglyceride across the endoplasmic reticulum so that it was brought together with apolipoprotein B-100 for assembly of the VLDL particles (Hussain et al., 2008). Relative expression of hepatic MTP gene in turbot increased with increasing dietary SO level, which to a certain extent suggested that the secretion rate of VLDL increased with increasing dietary SO level. Indeed, according to the findings of López-Soldado et al. (2009b), chylomicron remnant-like particles (CRLPs) enriched in n−6 PUFAs (higher content in SO) increased the secretion of VLDL, while CRLPs enriched with n−3 PUFAs could reduce the expression of MTP in the research of López-Soldado et al. (2009a). Thus, the up-regulation of MTP expression was related to the enriching CRLPs induced by higher dietary n−6 PUFAs and relatively lower dietary n−3 PUFA, which resulted in the increase of VLDL secretion to promote excessive dietary fatty acids exported from the liver, and finally reduce the hepatic lipid content.

In conclusion, the growth of turbot fed diets with 100% SO was significantly lower than the low SO group. However, no significant differences were found in the 33.3% and 66.7% SO groups. The increase of hepatic lipid deposition in turbot induced by higher dietary SO level would be related to not only the up-regulation of fatty acid synthesis-related gene (FAS) expression, but also the down-regulation of fatty acid oxidation gene (CPT I) expression. Interestingly, higher dietary SO level would promote the activity of lipid transport in turbot. Future studies are needed to investigate the effects of higher dietary SO level on post-translational regulation of hepatic lipolysis and the regulation mechanism of LXR, SREBP-1 and FAS in turbot.

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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.

AOAC, 1995. Official Methods of analysis of association of official analytical chemists, 16th edn. Arlington, VA. Method. 993, 13.

Baillie, R., Takada, R., Nakamura, M., Clarke, S., 1999. Coordinate induction of peroxisomal acyl-CoA oxidase and UCP-3 by dietary fish oil: a mechanism for decreased body fat deposition. Prostaglandins Leukot. Essent. 60, 351–356.

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., McGhee, F., Dick, J.R., Tocher, D.R., 2005. Dioxin and dioxin-like polychlorinated biphenyls (PCBs) in Scottish farmed salmon (*Salmo salar*): effects of replacement of dietary marine fish oil with vegetable oils. Aquaculture 243, 305–314.

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.

Caballero, M., Obach, A., Rosenlund, G., Montero, D., Gisvold, M., Izquierdo, M., 2002. Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. Aquaculture 214, 253–271.

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*). CBP B Biochem. Mol. Biol. 153, 81–88.

Cruz-Garcia, L., Sánchez-Gurmaches, J., Gutiérrez, J., Navarro, I., 2012. Role of LXR in trout adipocytes: target genes, hormonal regulation, adipocyte differentiation and relation to lipolysis. Comp. Biochem. Phys. A 163, 120–126.

Desvergne, B., Wahli, W., 1999. Peroxisome proliferator-activated receptors: nuclear control of metabolism 1. Endocr. Rev. 20, 649–688.

Eaton, S., 2002. Control of mitochondrial β-oxidation flux. Prog. Lipid Res. 41, 197–239.

Fievet, C., Staels, B., 2009. Liver X receptor modulators: effects on lipid metabolism and potential use in the treatment of atherosclerosis. Biochem. Pharmacol. 77, 1316–1327.

Figueiredo-Silva, A., Rocha, E., Dias, J., Silva, P., Rema, P., Gomes, E., Valente, L., 2005. Partial replacement of fish oil by soybean oil on lipid distribution and liver histology in European sea bass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus mykiss*) juveniles. Aquacult. Nutr. 11, 147–155.

Folch, J., Lees, M., Sloane-Stanley, G., 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., 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.

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. Lipids 1437, 170–181.

Goto, T., Lee, J.Y., Teraminami, A., Kim, Y.I., Hirai, S., Uemura, T., Inoue, H., Takahashi, N., Kawada, T., 2011. Activation of peroxisome proliferator-activated receptor-alpha stimulates both differentiation and fatty acid oxidation in adipocytes. J. Lipid Res. 52, 873–884.

Greene, D.H., Selivonchick, D.P., 1990. Effects of dietary vegetable, animal and marine lipids on muscle lipid and hematology of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 89, 165–182.

Hardy, R.W., Scott, T.M., Harrell, L.W., 1987. Replacement of herring oil with menhaden oil, soybean oil, or tallow in the diets of Atlantic salmon raised in marine net-pens. Aquaculture 65, 267–277.

Hirokane, H., Nakahara, M., Tachibana, S., Shimizu, M., Sato, R., 2004. Bile acid reduces the secretion of very low density lipoprotein by repressing microsomal triglyceride transfer protein gene expression mediated by hepatocyte nuclear factor-4. J. Biol. Chem. 279, 45685–45692.

Howell III, G., Deng, X., Yellaturu, C., Park, E.A., Wilcox, H.G., Raghow, R., Elam, M.B., 2009. N−3 polyunsaturated fatty acids suppress insulin-induced SREBP-1c transcription via reduced trans-activating capacity of LXRα. BBA Mol. Cell Biol. Lipids 1791, 1190–1196.

Hussain, M.M., Bakillah, A., 2008. New approaches to target microsomal triglyceride transfer protein. Curr. Opin. Lipidol. 19, 572.

Hussain, M.M., Rava, P., Pan, X., Dai, K., Dougan, S.K., Iqbal, J., Lazare, F., Khatun, I., 2008. Microsomal triglyceride transfer protein in plasma and cellular lipid metabolism. Curr. Opin. Lipidol. 19, 277–284.

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

Jump, D.B., 2004. Fatty acid regulation of gene transcription. Crit. Rev. Clin. Lab. Sci. 41, 41–78.

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 Comp. Biochem. 64, 345–350.
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.
López-Soldado, I., Avella, M., Botham, K.M., 2009a. Suppression of VLDL secretion by cultured hepatocytes incubated with chylomicron remnants enriched in n−3 poly-unsaturated fatty acids is regulated by hepatic nuclear factor-4α. BBA Mol. Cell Biol. Lipids 1791, 1181–1189.
López-Soldado, I., Avella, M., Botham, K.M., 2009b. Differential influence of different dietary fatty acids on very low-density lipoprotein secretion when delivered to hepatocytes in chylomicron remnants. Metabolism 58, 186–195.
Madsen, L., Rustan, A.C., Vaagenes, H., Berge, K., Dyrøy, E., Berge, R.K., 1999. Eicosapentaenoic and docosahexaenoic acid affect mitochondrial and peroxisomal fatty acid oxidation in relation to substrate preference. Lipids 34, 951–963.
Mayes, P.A., 1999. Lipid transport and storage, Harper's Biochemistry, 25th ed.McGraw Hill Publishers, NY, USA, pp. 268–284.
Menoyo, D., Izquierdo, M., Robaina, L., Ginés, R., Lopez-Bote, C., Bautista, J., 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.
Metcalfe, L., Schmitz, A.A., Pelka, J., 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal. Chem. 38, 514–515.
Miller, M.R., Nichols, P.D., Carter, C.G., 2008. n−3 oil sources for use in aquaculture—alternatives to the unsustainable harvest of wild fish. Nutr. Res. Rev. 21, 85–96.
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 Biol. Lipids 1811, 194–202.
Montanaro, M.A., González, M.S., Bernasconi, A.M., Brenner, R.R., 2007. Role of liver X receptor, insulin and peroxisome proliferator activated receptor α on in vivo desaturase modulation of unsaturated fatty acid biosynthesis. Lipids 42, 197–210.
Montero, D., Robaina, L., Caballero, M., Ginés, R., Izquierdo, M., 2005. Growth, feed utilization and flesh quality of European sea bass (*Dicentrarchus labrax*) fed diets containing vegetable oils: a time-course study on the effect of a re-feeding period with a 100% fish oil diet. Aquaculture 248, 121–134.
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., Taggart, J.B., Bell, J.G., 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.
Mourente, G., Bell, J., 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. Phys. B 145, 389–399.
Nagayoshi, A., Matsuki, N., Saito, H., Tsukamoto, K., Kaneko, K., Wakashima, M., Kinoshita, M., Yamanaka, M., Teramoto, T., 1995. Defect in assembly process of very-low-density lipoprotein in suncus liver: an animal model of fatty liver. J. Cell. Biochem. 117, 787–793.
Nortvedt, R., Tuene, S., 1998. Body composition and sensory assessment of three weight groups of Atlantic halibut (*Hippoglossus hippoglossus*) fed three pellet sizes and three dietary fat levels. Aquaculture 161, 295–313.
Owen, J., Adron, J., Middleton, C., Cowey, C., 1975. Elongation and desaturation of dietary fatty acids in turbot *Scophthalmus maximus* L., and rainbow trout, *Salmo gairdnerii* rich. Lipid 10, 528–531.
Poudyal, H., Panchal, S.K., Diwan, V., Brown, L., 2011. Omega-3 fatty acids and metabolic syndrome: effects and emerging mechanisms of action. Prog. Lipid Res. 50, 372–387.
Regost, C., Arzel, J., Cardinal, M., Robin, J., Laroche, M., Kaushik, S.J., 2001. Dietary lipid level, hepatic lipogenesis and flesh quality in turbot (*Psetta maxima*). Aquaculture 193 (3), 291–309.
Regost, C., Arzel, J., Robin, J., Rosenlund, G., Kaushik, S., 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.
Repa, J.J., Liang, G., Ou, J., Bashmakov, Y., Lobaccaro, J.M., Shimomura, I., Shan, B., Brown, M.S., Goldstein, J.L., Mangelsdorf, D.J., 2000. Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRα and LXRβ. Gene Dev. 14, 2819–2830.
Richard, N., Kaushik, S., Larroquet, L., Panserat, S., Corraze, G., 2006a. Replacing dietary fish oil by vegetable oils has little effect on lipogenesis, lipid transport and tissue lipid uptake in rainbow trout (*Oncorhynchus mykiss*). Brit. J. Nutr. 96, 299–309.
Richard, N., Mourente, G., Kaushik, S., Corraze, G., 2006b. Replacement of a large portion of fish oil by vegetable oils does not affect lipogenesis, lipid transport and tissue lipid uptake in European seabass (*Dicentrarchus labrax* L.). Aquaculture 261, 1077–1087.
Rosenlund, G., Obach, A., Sandberg, M., Standal, H., Tveit, K., 2001. Effect of alternative lipid sources on long-term growth performance and quality of Atlantic salmon (*Salmo salar* L.). Aquacult. Res. 32, 323–328.
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.
Shalev, A., Siegrist-Kaiser, C., Yen, P., Wahli, W., Burger, A., Chin, W., Meier, C., 1996. The peroxisome proliferator-activated receptor alpha is a phosphoprotein: regulation by insulin. Endocrinology 137, 4499–4502.
Sun, K., Kusminski, C.M., Scherer, P.E., 2011. Adipose tissue remodeling and obesity. J. Clin. Invest. 121, 2094.
Tacon, A.G., Metian, M., 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. Aquaculture 285, 146–158.
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. Phys. B 94, 367–374.
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.
Trushenski, J., Schwarz, M., Lewis, H., Laporte, J., Delbos, B., Takeuchi, R., Sampaio, L., 2011. Effect of replacing dietary fish oil with soybean oil on production performance and fillet lipid and fatty acid composition of juvenile cobia *Rachycentron canadum*. Aquacult. Nutr. 17, e437–e447.
Vamecq, J., Vallee, L., de la Porte, P.L., Fontaine, M., De Craemer, D., van den Branden, C., Lafont, H., Grataroli, R., 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 Mol. Cell Bol. Lipids 1170, 151–156.
Vyas, D., Kadegowda, A.K., Erdman, R.A., 2011. Dietary conjugated linoleic acid and hepatic steatosis: species-specific effects on liver and adipose lipid metabolism and gene expression. Int. J. Sport Nutr. Exerc. 2012.
Xu, S., Wang, S., Zhang, L., You, C., Li, Y., 2012. Effects of replacement of dietary fish oil with soybean oil on growth performance and tissue fatty acid composition in marine herbivorous teleost *Siganus canaliculatus*. Aquacult. Res. 43, 1276–1286.
Yoshikawa, T., Shimano, H., Amemiya-Kudo, M., Yahagi, N., Hasty, A.H., Matsuzaka, T., Okazaki, H., Tamura, Y., Iizuka, Y., Ohashi, K., 2001. Identification of liver X receptor–retinoid X receptor as an activator of the sterol regulatory element-binding protein 1c gene promoter. Mol. Cell. Biol. 21, 2991–3000.
Zar, J.H., 1999. Biostatistical Analysis. Prentice-Hall International Editions, London, U.K.
Zhou, Y.-j., Song, Y.-l., Zhou, H., Li, Y., 2012. Linoleic acid activates GPR40/FFA1 and phospholipase C to increase $[Ca^{2+}]_i$ release and insulin secretion in islet beta-cells. Chin. Med. J. Peking 27, 18–23.
Zuo, R.T., Ai, Q.H., Mai, K.S., 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 (*Larimichthys crocea*) following natural infestation of parasites (*Cryptocaryon irritans*). Fish Shellfish Immunol. 32, 249–258.