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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.