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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

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ABSTRACT

Soybean oil (SO) is widely used in freshwater aqua-feeds in China. However, little information is available about the effects of dietary SO levels on lipid deposition and hepatic lipid metabolism in fish. This study evaluated effects of different dietary soybean oil (SO) levels on growth performance, lipid deposition, tissues fatty acid compositions and hepatic lipid metabolism related gene expressions in blunt snout bream (*Megalobrama amblycephala*). Fish (average weight 0.34 ± 0.01 g) were fed five experimental diets containing 0% (0%SO), 20% (20%SO), 32% (32%SO), 56% (56%SO) and 100% (100%SO) SO in dietary lipid, and a diet containing 100% fish oil (100%FO). The body weight gain of fish fed 20%SO and 100%FO diets were significantly higher than in the other groups. Hepatic lipid content increased with incremental dietary SO level. The percentages of 18:2n−6 in the liver and muscle significantly increased with increasing dietary SO level. In the fish fed 56%SO and 100%SO diets 20:4n−6 content significantly increased in the liver and muscle suggesting the capacity of blunt snout bream to convert C_{18} fatty acids (PUFAs) to $C_{20/22}$ fatty acids. However, increasing dietary SO level up-regulated acyl-CoA delta-9 desaturase and down-regulated peroxisome proliferator-activated receptors-α and -β, which might be responsible for the high 18:2n−6 content. It is suggested that supplementation of 20% soybean oil (8% lipid in diet) could improve blunt snout bream juvenile growth. However, an excess of 18:2n−6 in SO supplemented diets modified the expressions of lipid metabolism-related genes which induced lipid deposition.

Statement of relevance: The present study was conducted to evaluated the effects of different dietary soybean oil (SO) levels on growth performance, lipid deposition, tissues fatty acid compositions and hepatic lipid metabolism related gene expressions in blunt snout bream (*M. amblycephala*). Fish (average weight 0.34 ± 0.01 g) were fed five experimental diets containing the following inclusion levels of SO: 0% (0%SO), 20% (20%SO), 32% (32%SO), 56% (56%SO) and 100% (100%SO) in dietary lipid, and a diet contained 100% fish oil (100%FO) was also used here. The percentages of 18:2n−6 in the liver and muscle significantly increased with increasing dietary SO level, and the fish fed 56%SO and 100%SO diets significantly increased 20:4n−6 contents in the liver and muscle, suggesting blunt snout bream has the capacity to convert C18 fatty acids (PUFAs) to C20/22 fatty acids. However, increasing dietary SO level up-regulated acyl-CoA delta-9 desaturase and down-regulated peroxisome proliferator-activated receptors-α and -β, which might be related associated with high level of 18:2n−6 in SO. It suggested that supplementation of 20% soybean oil (8% lipid in diet) could improve growth of blunt snout bream juvenile. However, an excess of 18:2n−6 in SO would modify the expressions of lipid metabolism-related genes, which induced lipid deposition in fish.

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1. Introduction

Fatty acids (FAs) are known to play an important role in lipid metabolism in fish (Tocher, 2003). Because fish do not possess the Δ12 and Δ15 desaturase enzymes, they cannot produce 18:2n−6 (linoleic acid, LA) and 18:3n−3 (linolenic acid, LNA) from 18:1n−9 (oleic acid, OA). However, it is well-documented that freshwater fish have an innate capacity to convert LA and LNA to n−6 and n−3 LC-PUFA, respectively (Sargent et al., 2002). Dietary LA has been found to be beneficial for growth performance and to maintain the membranes and eicosanoid metabolism in fish (Bautista and De la Cruz, 1988; Tan et al., 2009).

Fish oil (FO) is traditionally used in aquaculture feeds because of its high n−3 long chain polyunsaturated fatty acids (LC-PUFA) content especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA),

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which are essential to maintain the normal cell membranes function (Sargent et al., 1999). However, the global FO production may not be enough to cover the increasing demand for animal feed. At present, soybean oil (SO) is widely used in freshwater aqua-feeds in China because its production is steadily increasing and its reasonable prices. Furthermore, SO is rich in PUFAs, especially LA which is EFAs for freshwater fish. However, Du et al. (2008) found that the hepatic lipid deposition of grass carp (*Ctenopharyngodon idella*) increased with incremental dietary lipid level. A previous study also found that the fish fed FO and SO group diets had no significant effect on growth performance but the hepatic lipid content was significantly higher in SO fed fish compared to those fed FO (Li et al., 2015b). However, the mechanism of lipid deposition in liver of fish fed diets with higher SO level is still uncertain.

Lipid deposition in liver represents a complex process, including hepatic secretion, oxidation, transport and uptake of lipid (Lu et al., 2013), and many key enzymes and transcription factors are involved in these processes. These enzymes include fatty acid synthase (*FAS*), elongase of very long chain fatty acids-5 (*elovl5*), delta-6 fatty acyl desaturase ($\Delta 6$ *FAD*), acyl-CoA delta-9 desaturase (*SCD*) and lipoprotein lipase (*LPL*) (Jakobsson et al., 2006; Xue et al., 2014; Zhang et al., 2014; Zheng et al., 2014). In addition, several transcription factors, i.e. peroxisome proliferator-activated receptors (*PPAR*-α, *PPAR*-β, *PPAR*-γ) and fatty acid binding protein (*FABP*) play an intermediary role in orchestrating the gene transcription involved in lipid homeostasis (Lu et al., 2014). Although many studies have investigated the effects of higher dietary SO levels on lipid deposition in fish, the underlying molecular processes involved in the alteration of lipid deposition as a response to higher dietary SO levels are not yet known.

Blunt snout bream (*Megalobrama amblycephala*) is an herbivorous native Chinese freshwater finfish with high potential for aquaculture. According to 2013 China Fishery Statistical yearbook, the production of blunt snout bream reached 705,821 tons in China in 2012 (Fisheries Bureau, Ministry of Agriculture of China, 2013). However, little is known about the physiological effects of SO and optimal dietary SO levels for blunt snout bream during their early development stage. Therefore in the present study, the different dietary SO levels were designed to evaluate the effects of dietary SO levels on growth performance, fatty acid composition, and the mechanism of hepatic lipid metabolism in blunt snout bream juvenile.

2. Materials and methods

2.1. Experimental diets

Six iso-nitrogenous (50.0%) and iso-lipidic (8.1%), semi-purified diets were formulated to contain 0, 20, 32, 56, and 100% SO or 100% fish oil as the dietary lipid source (Table 1). The lipid levels of the diets were balanced by adjusting the lard oil concentration. Since lard oil contains high saturated fatty acid and low PUFA. All the ingredients were finely ground and sieved with an 80-mesh sieve, then thoroughly mixed with different SO levels respectively in a feed mixer (SM-168; Muren Corp., Shenzhen, China). An appropriate amount of water was added to produce stiff dough. The dough was then passed through a meat grinder with an appropriate diameter to prepare pellets. The pellets were air dried, broken up and sieved into proper pellet size. All experimental diets were stored at −20 °C until the time of feeding. The fatty acid composition of the test diets are given in Table 2.

2.2. Experimental fish and feeding trial

Blunt snout bream juvenile were obtained from Haida Co., Ltd., Tuanfeng County, Hubei Province, China). Before the experiments, the fish were acclimatized for 2 weeks and healthy, homogenous-sized fish (average initial body weight 0.34 ± 0.01 g) were stocked in 18 tanks with 30 fish per tank in triplicates per dietary treatment. All fish were hand-fed the test diets to the satiation level three times per day (at 08:00, 14:00 and 20:00 h) for 9 weeks. Any dead fish were weighed and removed when necessary. Uneaten feed was collected by siphoning at 0.5 h post-feeding and dried at 60 °C to calculate the exact feed intake. All experiments in the present study were conducted according to the principles of good laboratory animal care, and were approved by the Huazhong Agricultural University Ethical Committee for Laboratory Animals Care and Use.

Table 1
Formulation and proximate composition of the experimental diets.

	Experimental diets (dietary SO level)					
	0%SO	20%SO	32%SO	56%SO	100%SO	100%FO
Ingredients (%)						
Defatted fishmeal[a]	10.0	10.0	10.0	10.0	10.0	10.0
Soya concentrate	35.0	35.0	35.0	35.0	35.0	35.0
Casein	10.0	10.0	10.0	10.0	10.0	10.0
Activated gluten	10.0	10.0	10.0	10.0	10.0	10.0
CMC[b]	5.0	5.0	5.0	5.0	5.0	5.0
α-Starch	10.0	10.0	10.0	10.0	10.0	10.0
Dextrin	8.0	8.0	8.0	8.0	8.0	8.0
Soybean oil	0	1.6	2.6	4.5	8.0	0
Lard oil	8.0	6.4	5.4	3.5	0	0
Fish oil	0	0	0	0	0	8.0
$Ca(H_2PO_4)$	2.0	2.0	2.0	2.0	2.0	2.0
Minerals mixture[c]	1.0	1.0	1.0	1.0	1.0	1.0
Vitamins mixture[d]	1.0	1.0	1.0	1.0	1.0	1.0
Proximate composition (%)						
Crude protein (dry mass)	50.0	50.2	50.1	49.7	50.3	49.8
Ash (dry mass)	7.1	7.2	7.3	7.3	7.1	7.1
Moisture	12.5	12.5	12.3	12.3	11.7	11.7
Total lipid (dry mass)	8.0	8.1	8.2	8.1	8.0	8.1

[a] Fishmeal had been skimmed.
[b] Carboxy methyl cellulose.
[c] Mineral mixture (mg/kg diet): $MgSO_4$, 3380 mg; Na_2HPO_4, 2153.33 mg; K_2HPO_4, 5913.33 mg; Fe citrate, 733.33 mg; Ca lactate, 8060 mg; $Al(OH)_3$, 6.67 mg; $ZnSO_4$, 86.67 mg; $CuSO_4$, 2.67 mg; $MnSO_4$, 20 mg; $Ca(IO_3)_2$, 6.67 mg; and $CoSO_4$, 26.67 mg.
[d] Vitamin mixture (mg/kg diet): β-carotene, 32.12 mg; vitamin C, 230 mg; vitamin D_3, 3.24 mg; menadione $NaHSO_3 \cdot 3H_2O$ (K_3), 15.28 mg; DL-α-tocopherol acetate (E), 12.68 mg; thiamine-nitrate (B_1), 19.24 mg; riboflavin (B_2), 64.12 mg; pyridoxine-HCl (B_6), 15.28 mg; cyanocobalamin (B_{12}), 0.04 mg; d-biotin, 1.92 mg; inositol, 1283.04 mg; niacin (nicotic acid), 256.56 mg; Ca pantothenate, 89.34 mg; folic acid, 4.8 mg; choline chloride, 2623.12 mg; and ρ-aminobenzoic acid, 127.76 mg.

2.3. Sampling collection

At the end of the feeding trial, all fish were fasted for 24 h prior to final sampling and were sacrificed in an ice-slurry. The fish were counted, weighed and the length measured. Five fish per tank were randomly selected and frozen at −80 °C for determination of whole body composition. The liver and muscle from ten fish in each tank were pooled together respectively and then kept at −80 °C for fatty acid compositions. Liver from 5 fish were dissected and frozen in liquid nitrogen and stored at −80 °C for molecular analyses.

2.4. Proximate and lipid analysis

Moisture, crude protein, crude lipid and ash of the experimental diets and whole body samples were determined using standard methods (AOAC, 1995). Total lipid of the diets and tissues were measured following the method of Gao et al. (2012). Fatty acid methyl esters (FAME) were produced from total lipid aliquots and methylated with boron trifluoride (BF_3) in methanol. The fatty acid composition of total lipid in the diets, liver and muscle were determined using gas chromatography (Agilents Technologies Inc., Santa Clara, CA, USA) according to the method of Gao et al. (2012). The temperatures of the injector and detector (FID) were set at 250 °C and 260 °C respectively. The temperature program was 200 °C (40 min) to 240 °C (15 min) at 4 °C/min. High-purity helium was used as the carrier gas at a flow rate of 1 ml/min. The

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