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Regulation of growth, tissue fatty acid composition, biochemical parameters and lipid related genes expression by different dietary lipid sources in juvenile black seabream, *Acanthopagrus schlegelii*

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ABSTRACT

The effects of different dietary lipid sources on physiological characteristics and lipid metabolism relevant genes expression were assessed in juvenile black seabream *Acanthopagrus schlegelii*. 720 black seabream juveniles (initial weight was 5.4 ± 0.0g) were randomly allocated in 18 floating net cages corresponding to triplicate cages of the six dietary treatments, and the 8-week feeding trail was conducted. Five isonitrogenus and isolipidic diets were formulated to contain lipid sources: coconut oil (CO), perilla oil (PO), sunflower oil (SO), arachidonic acid (ARA) and eicosapentaenoic acid combined with docosahexaenoic acid (EPA + DHA), and fish oil (FO) was set as a control group. The results showed that weight gain (WG), specific growth rate (SGR) and feed efficiency (FE) in fish fed diets containing FO and EPA + DHA were significantly higher than CO. In serum, the highest activity of glutathione peroxidase (GSH-PX) and glutathione (GSH) content were recorded in fish fed EPA + DHA. The significantly highest content of malondialdehyde (MDA) was observed in fish fed the EPA + DHA and FO diets. The maximum hepatic content of MDA and the minimum activity of superoxide dismutase (SOD) were all found in fish fed the diet containing EPA + DHA. High contents of cholesterol (CHOL) and triacylglycerol (TAG) were found in fish fed SO, whereas glucose (GLU) content in fish fed EPA + DHA was significantly higher than in the other treatments. Fish fed FO had the highest hemoglobin (HGB) among treatments. The anabolic pathway relevant genes: *acca*, *fas* and *srebp-1* were up-regulated by dietary SO and PO. Whereas, the catabolic pathway relevant genes: *lpl*, *cpt1a*, *atgl*, *hsl* and *ppara* were down-regulated by dietary CO. Furthermore, the long chain polyunsaturated fatty acid (LC-PUFA) biosynthesis relevant genes *elovl5* and *fads2* were significantly up-regulated by dietary SO and PO. The present study focused on comparing the effects of different dietary lipid sources on biochemical characteristics and lipid metabolism relevant genes expression in black seabream. Overall, it demonstrated that physiological-biochemical characteristics as well as lipid metabolism relevant genes expression were significantly regulated by different dietary lipid sources. Moreover, dietary PO and SO regulated some physiological and biochemical indicators. These findings could contribute to optimize aquatic feeds when considering selection of optimal lipid sources or their optimal proportion in the future.

1. Introduction

The short chain polyunsaturated fatty acids (SC-PUFA), such as α-linolenic acid (LNA, 18:3n-3) and linoleic acid (LA, 18:2n-6), are regarded as essential fatty acids (EFA) for vertebrates, because they can be used as the substrate for the synthesis of long chain PUFA (LC-PUFA, $C_{20–24}$) through the action of fatty acyl desaturases (Fads) and elongation of very long-chain fatty acid (Elovl) proteins (Castro et al., 2016; NRC, 2011). However, marine carnivorous fish have limited ability to biosynthesize the physiologically important LC-PUFA, which include eicosapentaenoic acid (EPA, 20:5n-3), arachidonic acid (ARA, 20:4n-6) and docosahexaenoic acid (DHA, 22:6n-3), and consequently

Abbreviations: accα, acetyl-CoA carboxylase alpha; *atgl*, adipose triglyceride lipase; *cpt1a*, carnitine palmitoyltransferase 1A; *elovl5*, elongase 5; *fads2*, fatty acid synthase; *fas*, fatty acid synthase; *g6pd*, glucose 6-phosphate dehydrogenase; *hsl*, hormone-sensitive lipase; *lpl*, lipoprotein lipase; *6pgd*, 6-phosphogluconate dehydrogenase; *srebp-1*, sterol regulatory element-binding protein-1; *ppara*, peroxisome proliferators-activated receptor alpha

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these compounds need to be supplied in the diet to ensure normal growth and development (NRC, 2011). DHA plays important structural roles in biomembranes, especially in neural tissues such as brain and eye, where it is the main component of polar lipids (Seelig and Seelig, 1974; Wassall and Stillwell, 2008). EPA, while mainly being the precursor of highly active compounds such as eicosanoids, itself can partly satisfy the DHA requirements in species with adequate elongase and desaturase activities to convert EPA to DHA (Watanabe, 1993). As a precursor to eicosanoids such as prostaglandins, leukotrienes and thromboxanes, ARA can also affect physical stability of cell membrane, signaling pathways and membrane-associated enzyme activities (Calder, 2009; Waagbø, 2006; Carrier et al., 2011).

As one of the basic dietary ingredients in aquatic feeds for carnivorous marine teleost, fish oil (FO) has high digestibility and sufficient content of EFA. Besides, FO also has suitable amounts of n-3 LC-PUFA, saturated fatty acid (SFA) as well as adequate n3/n6 PUFA ratios (Nasopoulou and Zabetakis, 2012). However, with the limited and high-cost of FO, many terrestrial plant oils have been used to substitute FO (Nasopoulou and Zabetakis, 2012). Different terrestrial plant oils have different and characteristic fatty acid profiles. For instance, coconut oil (CO) is rich in SFA, in particular lauric acid (12:0), representing 40–50% of total fatty acids (TFA) (Luo et al., 2014). A previous study showed that perilla oil (PO) is an important source of n-3 SC-PUFA, containing 55–66% LNA of TFA (Eckert et al., 2010). There have been numerous studies on LNA in fish nutrition (El-Kerdawy and Salama, 1997; Rosenlund, 2001; Izquierdo et al., 2003, 2005; Caballero et al., 2004; Montero et al., 2003, 2005). Sunflower oil (SO) contains a large proportion of LA (approximate 65% of TFA), which have been studied in various fish species (Torstensen et al., 2000; Bransden et al., 2003; Wassef et al., 2009). Besides, LNA and LA are also acting as the precursors of n-3 and n-6 series LC-PUFA, respectively (NRC, 2011; Diwakar et al., 2008; Bransden et al., 2003). Comprehensive described above, in this present study, EPA and DHA were combined (EPA + DHA) as well as ARA were regarded as the fatty acid profiles in diets which could provide n-3 and n-6 LC-PUFA, directly. However, terrestrial plant oils of CO, PO and SO as well as FO were selected as the representative fatty acid profiles in the diets (SFA, LNA and LA) and positive control group, respectively.

Black seabream (*Acanthopagrus schlegelii*) is a very popular and commercially important marine fish species cultured in China, Japan, Korea and other countries in Southeast Asia (Nip et al., 2003; Gonzalez et al., 2008; Ma et al., 2008; Shao et al., 2008; Zhou et al., 2010a, 2010b, 2011). Black seabream has been regarded as an excellent aquaculture species for intensive culture since it exhibits rapid growth, copes well with diseases resistance and has ability to tolerate a wide range of environmental conditions (Shao et al., 2008; Zhou et al., 2010a, 2010b, 2011). To date, there have been a lot of studies focused on FO replacement by various terrestrial plant oils in aquatic feeds, but no study has focused on comparing the effects of different fatty acid profiles characteristic of both VO (SFA, LNA and ALA) and FO (ARA, EPA and DHA) on biochemical parameters as well as the expression of genes related to lipid metabolism. Thus, the objective of present study aimed to clarify the impacts of the different dietary lipid sources (CO, PO, SO, ARA and EPA + DHA) on growth performance, antioxidant capacity, tissue fatty acid profiles and expression of some lipid metabolism relevant genes for juvenile black seabream. Furthermore, this study is also very essential for optimising aquatic feeds when considering selection of optimal lipid sources or their optimal proportion.

2. Materials and methods

2.1. Experimental diets

Six isonitrogenous (about 45% crude protein) and isolipidic (about 14% crude lipid) experimental diets were formulated to contain different lipid sources (Table 1). Casein, defatted fishmeal and soybean meal were used as protein sources, whereas CO (contained 97.4% SFA of TFA), PO (contained 64.3% LNA of TFA), SO (contained 61.17% LA of TFA), ARA (contained 50.04% ARA of TFA), EPA + DHA (the EPA contained 50.13% EPA of TFA; DHA contained 43.77% DHA of TFA) and FO (as a positive control) were used as the main lipid sources (the added amount was approximately 11% dry weight in each diet). The fatty acid profiles of each diet are shown in Table 2. All dry ingredients were ground into fine powder with particle size < 177 μm, micro components such as minerals and vitamins premix were added followed by lipid and distilled water (35%, w/w). The ground ingredients were mixed in a Hobart type mixer and cold-extruded pellets produced (F-26, Machine factory of South China University of Technology) with pellet strands cut into uniform sizes (2 mm and 4 mm diameter pellets were prepared) (G-250, Machine factory of South China University of Technology). Pellets were steamed for 30 min at 90 °C, and then air-dried to approximately 10% moisture, sealed in vacuum-packed bags and stored at − 20 °C until use in the feeding trial.

Table 1
Formulation and composition of the experimental diets (% dry matter).

Ingredient (%)	Dietary lipid sources					FO
	CO	PO	SO	ARA	EPA + DHA	
Casein[a]	16.23	16.23	16.23	16.23	16.23	16.23
Defatted fish meal[a]	18.00	18.00	18.00	18.00	18.00	18.00
Soybean meal[a]	20.00	20.00	20.00	20.00	20.00	20.00
Flour[a]	30.00	30.00	30.00	30.00	30.00	30.00
Coconut oil[b]	11.07	0.00	0.00	0.00	0.00	0.00
Perilla oil[b]	0.00	11.07	0.00	0.00	0.00	0.00
Sunflower oil[b]	0.00	0.00	11.07	0.00	0.00	0.00
ARA-enriched oil[b]	0.00	0.00	0.00	11.07	0.00	0.00
EPA + DHA (enriched oil)[b]	0.00	0.00	0.00	0.00	11.07	0.00
Fish oil[b]	0.00	0.00	0.00	0.00	0.00	11.07
Soy lecithin	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin premix[c]	0.50	0.50	0.50	0.50	0.50	0.50
Mineral premix[d]	1.50	1.50	1.50	1.50	1.50	1.50
Choline chlorid	0.20	0.20	0.20	0.20	0.20	0.20
$Ca(H_2PO_4)_2$	1.50	1.50	1.50	1.50	1.50	1.50
Proximate composition (%)						
Dry matter	92.58	92.82	92.59	92.49	92.62	92.86
Crude protein	44.37	44.28	44.57	44.31	45.51	45.34
Crude lipid	14.87	14.76	14.08	14.20	14.69	13.77
Ash	7.21	6.93	6.79	6.66	6.83	6.57

[a] Casein, crude protein 81.4% DM, crude lipid 0.0% DM; Defatted Fish Meal, crude protein 84.7% DM, crude lipid 2.0% DM; Soybean meal, crude protein 50.0% DM, crude lipid 3.4% DM; Flour, crude protein 12.0% DM, crude lipid 3.0% DM. All of the ingredients were bought from Ningbo Tech-Bank Corp., Ningbo, China.

[b] CO (Coconut oil), SFA 97.4% of TFA, MUFA 6.84% of TFA (Team Asia Co., Ltd., Philip); PO (Perilla seed oil), MUFA 14.7% of TFA, LA (C18:2n-6) 13.1% of TFA, ALA (C18:3n-3) 64.3% of TFA (Huaxiangsiji science and technology Co., Ltd., Changchun, China); SO (Sunflower oil), SFA 6.11% of TFA, MUFA 32.72% of TFA, LA (C18:2n-6) 61.17% of TFA (Dalian kaimei import and export group Co., Ltd., Dalian, China; Imported from Turkey); ARA-enriched oil, ARA 50.04% of TFA (Changsha Kenan Biotechnology Co., Ltd., China); DHA-enriched oil, EPA 1.85% of TFA, DHA 43.77% of TFA (Jiangsu Tiankai Biotechnology Co., Ltd., China); EPA enriched oil: EPA50.13% of TFA, DHA 25.4% of TFA (Hebei Kaiyuankangjian Biological Science and Technology Co., Ltd., China); FO (Fish oil), SFA 28.89% of TFA, MUFA 26.2% of TFA, LA (C18:2n-6) 1.36% of TFA, ALA (C18:3n-3) 0.83% of TFA, ARA 1.89% of TFA, EPA 12.17% of TFA, DHA 16.12% of TFA (Ningbo Tech-Bank Corp., Ningbo, China).

[c] Vitamin premix was based by Zhou et al. (2010a).

[d] Mineral mixture (g/kg premix), $FeC_6H_5O_7$, 11.43; $ZnSO_4{\cdot}7H_2O$, 11.79; $MnSO_4{\cdot}H2O$ (99%), 2.49; $CuSO_4{\cdot}5H2O$ (99%), 1.06; $MgSO_4{\cdot}7H_2O$ (99%), 27.31; KH_2PO_4, 233.2; NaH_2PO_4, 228.39; $C_6H_{10}CaO_6{\cdot}5H_2O$ (98%), 34.09; $CoCl_2{\cdot}6H_2O$ (99%), 0.54. KIO_3 (99%), 0.06; zeolite, 449.66.

2.2. Feeding trial and experimental conditions

Black seabream juveniles (initial weight 5.4 ± 0.0 g) were obtained from a local commercial hatchery at Xiangshan Bay, Ningbo,