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Effects of different dietary phospholipid levels on growth performance, fatty acid composition, PPAR gene expressions and antioxidant responses of blunt snout bream *Megalobrama amblycephala* fingerlings

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Abstract A 60-day feeding trial was conducted to evaluate the effects of different levels of dietary phospholipid (PL) from soybean lecithin on growth performance, liver fatty acid composition, peroxisome proliferator-activated receptor (PPAR) gene expression levels and antioxidant responses of blunt snout bream fingerlings. Fish (average initial weight 0.35 ± 0.01 g) were fed five experimental diets containing the following inclusion levels of PL: 0, 2, 4, 6 and 8 %. Results showed that final body weight, weight gain and specific growth rate increased significantly ($P < 0.05$) as dietary PL level increased from 0 to 6 %, meanwhile the survival was not affected by dietary PL supplementation. Increasing dietary PL level significantly ($P < 0.05$) increased in 20:4n-6 content in neutral lipid of liver, indicating fish had the capacity to convert C18 to C20 and C22 by elongation and desaturation. The expression levels of *PPAR-α* and *PPAR-γ* and the activities of catalase, superoxide dismutase and glutathione peroxidase in liver were significantly ($P < 0.05$) increased, and liver thiobarbituric acid reactive substances value was decreased with dietary PL supplementation up to 6 % compared with the control. Therefore, it was concluded that supplementation of 6 % (18.8 g kg^{-1}, polar lipid of diet) PL could improve growth performance of blunt snout bream fingerlings.

Keywords *Megalobrama amblycephala* · Fingerlings · Phospholipid · Fatty acids composition · PPAR gene expression · Antioxidant enzyme activities

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Introduction

Phospholipids (PL) are important components for maintaining the structure and function of cellular membranes, emulsifying lipids in gut and improving intestinal absorption of long chain fatty acids (Kanazawa et al. 1983). Most fish have limited capacity to synthesize PL during their early development (Sargent et al. 2002; Geurden et al. 1998). Thus, an exogenous source of PL is required in fish diet. A nutritional requirement for PL during early development stage of fish has been well demonstrated in a number of studies (Kanazawa et al. 1983; Geurden et al. 1995, 1997; Hamza et al. 2008; Zhao et al. 2013). The importance of inclusion of dietary PL on growth has been well documented in a number of fish species including ayu *Plecoglossus altivelus* (Kanazawa et al. 1983), rainbow trout *Oncorhynchus mykiss* (Poston 1990; Rinchard et al. 2007; Daprà et al. 2011; Azarm et al. 2013a, b), common carp *Cyprinus carpio* (Geurden et al. 1995), amberjack

Seriola dumerili (Uyan et al. 2009) and large yellow croaker *Larmichthys crocea* (Zhao et al. 2013). Soybean lecithin, which is rich in linoleic acid (LA, 18:2n-6) and linolenic acid (LNA, 18:3n-3), has been used as an important supply of PL source in aquaculture. Fatty acids are known to play an important role in lipid metabolism in fish (Tocher 2003). Since fish cannot *de novo* synthesize LA and LNA, they require a dietary supply of essential fatty acids (EFAs; Sargent et al. 2002). Furthermore, it is known that freshwater teleosts have an innate capacity to desaturate and elongate LA to 20:4n-6 (arachidonic acid, ARA) and LNA to 20:5n-3 (eicosapentaenoic acid, EPA) and ultimately 22:6n-3 (docosahexaenoic acid, DHA; Sargent et al. 2002). It has been demonstrated that fish larvae use more efficiently n-3 HUFA from the polar lipid fraction than those from the neutral lipid fraction (Cahu et al. 2003; Gisbert et al. 2005). We reported previously that an increase in the percentage of total n-3 fatty acids in neutral lipid fraction was found along with increasing dietary PL levels in loach *Misgurnus anguillicaudatus* larvae (Gao et al. 2014). Moreover, Hamza et al. (2008) demonstrated that ARA, EPA and DHA presented a higher concentration in the polar lipid fraction than in the neutral lipid fraction in pikeperch *Sander lucioperca* fed diet supplemented PL. Therefore, we hypothesized that supplementation of different dietary PL levels would display different fatty acid composition in both neutral and polar lipid of fish.

Peroxisome proliferator-activated receptors (PPARs) have three isotypes, termed *PPAR-α*, *PPAR-β* and *PPAR-γ*. Each isotype is a product of a separate gene, and each one has a distinct tissue distribution (Desvergne and Wahli 1999; Escher et al. 2001; Hihi et al. 2002). PPARs are ligand-dependent transcription factors, which regulate various genes involved in lipid metabolism (Qi et al. 2000; Kliewer et al. 2001; Lee et al. 2003). Ruyter et al. (1997) have for the first time found PPARs in fish and determined their response to peroxisome proliferators and fatty acids. Recent researches have become increasingly focused on PPARs expression with lipid metabolism in aquaculture (Kennedy et al. 2006; Ji et al. 2011; Lu et al. 2013). However, no information has been published about evaluating the effects of dietary PL levels on PPARs mRNA expression in fish.

Fish need an effective antioxidation system to maintain health and prevent oxidation-induced lesions and mortalities (Mourente et al. 1999). In order to protect against free radicals, organisms have also developed a variety of other antioxidant defenses, such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx). The enzymes of SOD, CAT and GPx have been shown to be active in blunt snout bream fingerling (Li et al. 2012). Furthermore, the activities of these antioxidant enzymes have been used as effective biomarkers to evaluate the effects of dietary PL on the biochemical pathways and enzymatic function in Dojo loach *M. anguillicaudatus* larvae (Gao et al. 2014).

The blunt snout bream *Megalobrama amblycephala* is an herbivorous native Chinese freshwater finfish with high potential for aquaculture. This fish is originally produced in Lake Liangzi in Ezhou, Hubei, China, and its main distribution is in the middle reaches of the Yangtze River (Tsao 1960). However, there is no information about the requirement for PL of blunt snout bream. Therefore, this study was conducted to evaluate the effects of different dietary PL levels on growth performance, liver fatty acid composition, PPARs gene expression levels and antioxidant responses of blunt snout bream fingerlings.

Materials and methods

Experimental diets

Ingredients and proximate compositions of the test diets are presented in Table 1. Five experimental diets were formulated to contain the following inclusion levels of PL: 0, 2, 4, 6 and 8 %. The measured polar lipid contents for five treatments were 1.3, 7.2, 13.7, 18.8 and 23.3 g kg^{-1}, respectively. The lipid levels of the diets were balanced by adjusting the level of palm oil. The dried pellet diets were crumbled in an electric motor to make a fine particle and sieved through 400–600 μm mesh sizes. All experimental diets were stored at −20 °C until the time of feeding. Fatty acid compositions of neutral lipid and polar lipid fractions of test diets are detailed in Tables 2 and 3.

Experimental fish and feeding trial

Blunt snout bream fingerlings were obtained from the fish hatchery of subsidiary Haida Co., Ltd. located in

Table 1 Formulation and proximate composition of the experimental diets

Ingredients (%)	Experimental diets				
	PL0	PL2	PL4	PL6	PL8
Defatted fishmeal[a]	20.0	20.0	20.0	20.0	20.0
Soya concentrate	40.0	40.0	40.0	40.0	40.0
CMC[b]	5.0	5.0	5.0	5.0	5.0
α-Starch	10.0	10.0	10.0	10.0	10.0
Dextrin	8.0	8.0	8.0	8.0	8.0
Soybean oil	5.0	5.0	5.0	5.0	5.0
Palm oil	8.0	6.0	4.0	2.0	0.0
Soybean lecithin[c]	0.0	2.0	4.0	6.0	8.0
Minerals mixture[d]	2.0	2.0	2.0	2.0	2.0
Vitamins mixture[e]	2.0	2.0	2.0	2.0	2.0
Proximate composition (%)					
Crude protein (dry mass)	47.7	47.2	48.4	48.4	47.9
Total lipid (dry mass)	9.9	10.0	10.8	10.3	9.8
Neutral lipids (% of total lipid)	98.7	92.8	87.3	81.8	76.1
Polar lipids (% of total lipid)	1.3	7.2	12.7	18.2	23.9
Ash (dry mass)	8.1	8.2	8.3	8.4	8.7
Moisture	11.6	12.3	11.7	11.7	12.9

[a] Fishmeal had been skimmed by 100 % diethyl ether

[b] Carboxy methyl cellulose

[c] Zhejiang BSM Biotechnology Co., Ltd. Phospholipid concentration is 456 g kg^{-1}

[d] Mineral mixture (mg kg^{-1} diet): $MgSO_4$, 3,380 mg; Na_2HPO_4, 2,153.33 mg; K_2HPO_4, 5,913.33 mg; Fe Citrate, 733.33 mg; Ca Lactate, 8,060 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; $CoSO_4$, 26.67 mg

[e] Vitamin mixture (mg kg^{-1} diet): β-carotene, 32.12 mg; vitamin C, 230 mg; Vitamin D_3, 3.24 mg; Menadione $NaHSO_3 . 3H_2O$ (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; Cyanocobalamine (B_{12}), 0.04 mg; d-Biotin, 1.92 mg; Inositol, 1,283.04 mg; Niacin (Nicotinic acid), 256.56 mg; Ca Pantothenate, 89.34 mg; Folic acid, 4.8 mg; Choline chloride, 2,623.12 mg; ρ-Aminobenzoic acid, 127.76 mg

Tuanfeng County, Hubei Province, and transported alive to Laboratory, College of Fisheries, Huazhong Agricultural University, Hubei Province and China. Prior to the feeding experiment, all fish were reared in indoor fiber glass tanks (300 l) for 2 weeks of acclimatization by feeding a commercial feed (35 % crude protein and 10 % crude lipid, Haida Co., Ltd.). At the start of the feeding trial, healthy and homogenous-sized fish (average initial body weight 0.35 ± 0.01 g) were stocked in previously prepared 15 tanks with 30 fish per tank in triplicates per dietary treatment. All fish were fed the respective test diets to the satiation level by hand three times per day (08:00, 14:00 and 20:00 h) for 60 days. Special care was taken to collect uneaten diets by siphon method from the tank every morning, which were freeze dried and finally subtracted from the total amount of supplied test diets to calculate the actual feed intake (FI). Mortalities were removed, and weights recorded when necessary. The experiment was subjected to natural photoperiod (approximately 14 h light/10 h darkness). Water quality parameters were monitored twice a week in the morning. Water temperature ranged from 29 °C to 31 °C, pH fluctuated between 7.0 and 7.5, and dissolved oxygen was maintained approximately at 6.5 mg l^{-1} during the feeding trial.

Sampling collection

At the end of the feeding trial, all fish were fasted for 24 h prior to final sampling. Then fish were anesthetized in an ice-slurry. The fish were then counted, weighed and the length collected from each tank. Five fish were randomly collected from each replicate tank pooled and frozen at −20 °C for subsequent determination of whole body composition. Liver was dissected out from twenty fish in each replicate tank, weighed individually to get hepatosomatic index (HSI), and finally pooled together and kept at −80 °C for subsequent analysis (ten fish for lipid analysis, five fish for thiobarbituric acid reactive substances (TBARS) and five fish for antioxidant enzyme activities). Three fish from each tank were randomly chosen to dissect livers without any excreta and were stored at −80 °C after having been stored in liquid nitrogen for 8 h for the PPAR genes expression-level determination.

Date collection

Body weight gain (BWG), specific growth rate (SGR), survival, feed conversion ratio (FCR), feed intake (FI) and hepatosomatic index (HSI) were calculated as follows:

Table 2 Fatty acid composition (% of total fatty acids) in the neutral lipid fractions of experimental diets

Fatty acids	Experimental diets (dietary polar lipid level, g kg^{-1} dry diet)				
	PL0 (1.3)	PL2 (7.2)	PL4 (13.7)	PL6 (18.8)	PL8 (23.3)
14:0	0.3 ± 0.0^{d}	0.3 ± 0.0^{cd}	0.3 ± 0.0^{bc}	0.1 ± 0.0^{b}	0.1 ± 0.0^{a}
16:0	28.3 ± 0.3^{c}	25.6 ± 0.8^{c}	20.5 ± 0.1^{b}	16.9 ± 1.4^{b}	9.5 ± 2.0^{a}
17:0	–	–	–	–	–
18:0	–	–	–	–	–
Σ saturated	28.7 ± 0.3^{c}	25.9 ± 0.9^{c}	20.8 ± 0.1^{b}	17.1 ± 1.4^{b}	9.6 ± 2.1^{a}
16:1n-7	0.1 ± 0.0	–	0.1 ± 0.0	–	–
18:1n-9	44.9 ± 0.6^{a}	47.0 ± 1.1^{ab}	54.5 ± 2.7^{bc}	58.3 ± 2.2^{c}	63.5 ± 3.0^{d}
18:1n-7	21.8 ± 0.8^{b}	22.0 ± 0.1^{b}	19.4 ± 2.8^{ab}	18.8 ± 0.8^{ab}	15.2 ± 1.6^{a}
20:1n-9	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
22:1n-11	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	–	0.1 ± 0.0
Σ monoenes	67.0 ± 0.3^{a}	69.3 ± 1.3^{b}	74.1 ± 0.2^{c}	77.3 ± 1.5^{d}	78.9 ± 1.6^{e}
18:2n-6 (LA)	3.6 ± 0.1^{a}	3.6 ± 0.1^{a}	4.0 ± 0.3^{a}	5.0 ± 0.2^{b}	7.7 ± 0.1^{c}
20:2n-6	–	–	–	–	–
20:4n-6 (ARA)	0.1 ± 0.0	0.1 ± 0.0	–	–	–
Σ n-6 fatty acids	3.7 ± 0.1^{a}	3.7 ± 0.1^{a}	4.1 ± 0.3^{a}	5.0 ± 0.2^{b}	7.7 ± 0.2^{c}
18:3n-3 (LNA)	–	0.1 ± 0.0	0.1 ± 0.0	–	0.1 ± 0.0
18:4n-3	0.1 ± 0.0	0.1 ± 0.0	–	–	0.1 ± 0.0
20:4n-3	–	–	–	–	–
20:5n-3 (EPA)	0.1 ± 0.0	–	–	–	0.1 ± 0.0
22:5n-3	–	–	–	–	–
22:6n-3 (DHA)	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
Σ n-3 fatty acids	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.5 ± 0.2

Values are expressed as mean from triplicate groups. Values are mean ± SE of three replicates. Means in the same row with different superscripts are significantly different ($P < 0.05$)

LA linoleic acid, *ARA* arachidonic acid, *LNA* linolenic acid, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid

$$\mathrm{BWG}\,(\%) = (\text{Final body weight} - \text{initial body weight}) \times 100/\text{initial body weight}.$$

$$\mathrm{SGR} = (\mathrm{Ln}W_t - \mathrm{Ln}W_0) \times 100/T$$

$$\mathrm{FCR} = \text{total diet fed}\,(\mathrm{g})/\text{total wet weight gain}(\mathrm{g}).$$

$$\mathrm{FI}\,(\mathrm{g/fish/60\,day}) = \text{total feed intake}\,(\mathrm{g})/\text{number of fishes}/T(\mathrm{day})$$

$$\mathrm{CF} = (\text{body weight} \times 100)/\text{total body length}(\mathrm{cm})^3$$

$$\mathrm{HIS}\,(\%) = (\text{liver weight} \times 100)/\text{body weight}.$$

where W_0 and W_t are the initial and final body weight, respectively; T is the culture period.

Proximate and lipid analysis

Proximate compositions of the test diets and whole body samples were analyzed in triplicates for protein, lipid, ash and moisture. Protein ($N \times 6.25$) was determined by using the Kjeldahl method (AOAC 1990), total lipid by Bligh and Dyer (1959) and ash by combustion in muffle furnace at 550 °C for 4 h. Moisture was determined on approximately 1 g of samples by oven-drying at 110 °C to constant weight according to AOAC (1990). Total lipid was separated into neutral and polar lipid fractions by column chromatography (Sep-Pak Silica Cartridges; Waters Corp. Milford, MA) according to Juaneda and Rocquelin (1985). The contents of neutral and polar lipid fractions in total lipid were determined gravimetrically. The fatty acid compositions of neutral lipid and polar lipid fractions in the diets and liver were determined using a gas chromatography (Agilents Technologies Inc., Santa Clara, California, USA; column: OmegawaxTM320) according to the method of Gao et al. (2012). Fatty acid esters (FAMEs) were then produced from neutral lipid and polar lipid fractions aliquots and methylated with boron trifluoride (BF_3) in methanol. The temperatures of the injector and detector (FID) were set at 250 and 260 °C, respectively. The temperature program is 200 °C (40 min) to 240 °C (15 min) at 4 °C min^{-1}. High-purity helium was used as the carrier gas at a flow rate of 1 ml min^{-1}. The samples (1.0 µl) were

Table 3 Fatty acid composition (% of total fatty acids) in the polar lipid fractions of experimental diets

Fatty acids	Experimental diets (dietary polar lipid level, g kg^{-1} dry diet)				
	PL0 (1.3)	PL2 (7.2)	PL4 (13.7)	PL6 (18.8)	PL8 (23.3)
14:0	0.7 ± 0.0^{b}	0.7 ± 0.1^{b}	0.7 ± 0.1^{b}	0.5 ± 0.0^{a}	0.4 ± 0.0^{a}
16:0	31.1 ± 1.2^{e}	24.0 ± 1.6^{d}	21.7 ± 0.9^{c}	18.3 ± 0.2^{b}	16.1 ± 0.3^{a}
17:0	0.4 ± 0.0^{a}	0.3 ± 0.0^{a}	0.4 ± 0.0^{a}	0.5 ± 0.0^{a}	0.7 ± 0.1^{b}
18:0	0.6 ± 0.1^{c}	0.3 ± 0.0^{b}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}
Σ saturated	32.9 ± 1.1^{d}	25.3 ± 0.9^{c}	22.8 ± 1.2^{b}	19.4 ± 0.6^{a}	18.3 ± 0.4^{a}
16:1n-7	4.4 ± 0.2^{d}	2.8 ± 0.3^{c}	2.1 ± 0.2^{b}	1.8 ± 0.2^{b}	1.2 ± 0.1^{a}
18:1n-9	35.3 ± 0.9^{a}	46.8 ± 0.9^{b}	50.0 ± 1.0^{c}	56.1 ± 0.4^{d}	58.0 ± 0.2^{d}
18:1n-7	19.1 ± 0.6^{d}	17.6 ± 1.0^{d}	15.3 ± 0.3^{c}	12.5 ± 0.4^{b}	9.0 ± 0.4^{a}
20:1n-9	0.6 ± 0.0^{ab}	0.7 ± 0.1^{ab}	0.5 ± 0.0^{a}	0.4 ± 0.0^{a}	0.9 ± 0.1^{b}
22:1n-11	0.1 ± 0.0	–	0.1 ± 0.0	–	–
Σ monoenes	59.8 ± 0.3^{a}	67.9 ± 0.6^{b}	68.0 ± 0.8^{b}	70.9 ± 0.7^{d}	69.0 ± 0.4^{c}
18:2n-6 (LA)	2.82 ± 0.3^{a}	5.0 ± 0.2^{b}	6.3 ± 0.4^{c}	8.5 ± 0.3^{d}	10.1 ± 0.5^{e}
20:2n-6	0.2 ± 0.0	–	–	–	0.1 ± 0.0
20:4n-6 (ARA)	0.5 ± 0.0^{c}	0.3 ± 0.0^{b}	0.2 ± 0.0^{b}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}
Σ n-6 fatty acids	3.5 ± 0.3^{a}	5.3 ± 0.3^{b}	6.6 ± 0.2^{c}	8.6 ± 0.1^{d}	10.2 ± 0.1^{e}
18:3n-3 (LNA)	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
18:4n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:4n-3	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
20:5n-3 (EPA)	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
22:5n-3	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	–
22:6n-3 (DHA)	0.9 ± 0.4	0.7 ± 0.2	0.4 ± 0.0	0.2 ± 0.1	0.3 ± 0.2
Σ n-3 fatty acids	1.3 ± 0.2	1.5 ± 0.3	1.3 ± 0.1	0.9 ± 0.1	1.0 ± 0.1

Values are expressed as mean from triplicate groups. Values are mean ± SE of three replicates. Means in the same row with different superscripts are significantly different ($P < 0.05$)

LA linoleic acid, *ARA* arachidonic acid, *LNA* linolenic acid, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid.

manually injected into an injection port, and identified fatty acids were presented as area percentage of total fatty acids.

Determination of hepatic PPARs expression levels

Total RNA was extracted from the liver using RNAiso Plus (Takara Co. Ltd, Japan). RNA samples were treated by RQ1 RNase-Free DNase prior to RT-PCR (Takara Co. Ltd, Japan) to avoid genomic DNA amplification. cDNA was generated from 500 ng DNase-treated RNA using ExScript™ RT-PCR kit (Takara Co. Ltd, Japan). Analyses on gene transcript levels were conducted by real-time PCR method.

Real-time PCR was employed to determine mRNA levels based on the SYBR Green I fluorescence kit. Primers (Table 4) for the real-time PCR analysis were designed using the Primer5 Software, based on the cDNA sequences available in GenBank (*PPAR-α*, HM140628; *PPAR-β*, HM140629; *PPAR-γ*, HM140627). Real-time PCR was performed in a Mini Opticon real-time detector (BIO-RAD, USA). The fluorescent quantitative PCR solution consisted of 10 μl SYBR® premix Ex Taq™ (2×), 0.8 μl PCR forward primer (20 μM), 0.8 μl PCR reverse primer (10 μM), 1.0 μl RT reaction (cDNA solution) and 7.4 μl dH_2O. The reaction conditions were as follows: 95 °C for 30 s followed by 45 cycles consisting of 95 °C for 5 s, 60 °C for 20 s and 72 °C 20 s. The florescent flux was then recorded, and the reaction continued at 72 °C for 3 min. All amplicons were initially separated by agarose gel electrophoresis to ensure that they were of correct size. The gene expression levels were normalized toward the mean of the reference gene (Elongation factor 1 alpha *EF1a*). Normalized gene expressions of the control group were set to 1, and the expression of each target gene for the high-PL group was expressed relative to the control group. The amplification efficiencies of all genes were approximately equal and ranged from 98 to 103 %. Optimized comparative C_t ($2^{-\Delta\Delta C_t}$) value method was used to estimate gene expression levels (Livak and Schmittgen 2001).

Table 4 Nucleotide sequences of the primers used to assay gene expression by real-time PCR

Target gene	Forward (5′–3′)	Reverse (5′–3′)	Annealing temperature (°C)
PPAR-α	GTGCCAATACTGTCGCTTTCAG	CCGCCTTTAACCTCAGCTTCT	60
PPAR-β	CATCCTCACGGGCAAGAC	TGGCAGCGGTAGAAGACA	60
PPAR-γ	AGCTTCAAGCGAATGGTTCTG	AGGCCTCGGGCTTCCA	60
EF1a	CTGGAGGCCAGCTCAAAYAT	ATCAAGAAGAGTARYACCRCTAGCATTWC	60

PPAR peroxisome proliferator-activated receptors, *EF1a* elongation factor 1

Antioxidant enzymes activities and TBARS value

The activities of antioxidant enzymes (CAT, SOD and GPx) of liver were all analyzed spectrophotometrically using diagnostic reagent kits (Nanjing Jiancheng Bioengineering Institute, China). Samples of liver were homogenized in 9 volumes of 6.8 % physiological saline; the homogenates were centrifuged at 3,000 rpm to remove debris. The supernatant was used to determine SOD (EC 1.15.1.1), CAT (EC 1.11.1.6) and GPx (EC 1.11.1.9). SOD activity was assayed according to a SOD kit protocol at 550 nm according to Bayer and Fridovich (1987). CAT activity was assayed according to a CAT kit protocol at 405 nm according to Claiborne (1985). GPx activity was assayed according to a GPx kit protocol at 412 nm according to Wheeler et al. (1990). The measurement of TBARS was carried out using a method adapted from that of Burk et al. (1980).

Statistical analyses

All data were subjected to Levene's test of equality of error variances and one-way ANOVA followed by Tukey's test using SPSS 16.0 (SPSS 16.0, Michigan Avenue, Chicago, IL, USA). Probability values of <0.05 were considered statistically significant.

Results

Growth performance

The results of feeding trial are shown in Table 5. Body weight gain (BWG) and specific growth rate (SGR) were improved with incremental dietary PL level after 60 days. Significantly ($P < 0.05$) improved BWG and SGR were found in PL6 compared with control group, but no significant differences ($P > 0.05$) were found between the PL6 and PL8 groups. Increasing dietary PL significantly increased FI, where FI in PL4, PL6 and PL8 groups were significantly higher ($P < 0.05$) than those in PL0 and PL2 groups. Feed conversion ratio (FCR), condition factor (CF) and survival rates were not affected by different dietary PL levels ($P > 0.05$).

Proximate composition of fish body

Whole body composition of fish is shown in Table 6. Crude protein, crude lipid and ash contents of whole body were significantly affected ($P < 0.05$) by increasing dietary PL levels. The crude protein content significantly increased ($P < 0.05$) with incremental dietary PL levels. The crude protein content in PL8 group was significantly higher ($P < 0.05$) than the control group. On the contrary, the crude lipid content significantly ($P < 0.05$) decreased with PL supplementation. Ash content significantly increased ($P < 0.05$) with incremental dietary PL supplementation up to 6 %. The moisture content of whole body was not affected ($P > 0.05$) by dietary PL level. Contents of total lipid, neutral lipid and polar lipid in liver are also presented in Table 6. Dietary PL significantly reduced neutral lipid content and increased polar lipid content in liver. Meanwhile, the ratio of neutral lipid to polar lipid significantly ($P < 0.05$) decreased with incremental dietary PL level. HSI was significantly decreased by increasing dietary PL level ($P < 0.05$).

Liver fatty acid compositions

Liver fatty acid compositions in neutral lipid and polar lipid fractions were globally reflected by the fatty acid compositions of diets in neutral lipid and polar lipid fractions, respectively (Tables 7, 8). For liver fatty acid composition of the neutral lipid fraction, dietary PL supplementation increased ($P < 0.05$) in the

Table 5 Growth performances of blunt snout bream fingerlings fed diets containing different levels of phospholipid

	Experimental diets (dietary polar lipid level, g kg^{-1} dry diet)				
	PL0 (1.3)	PL2 (7.2)	PL4 (13.7)	PL6 (18.8)	PL8 (23.3)
Initial body weight (g)	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
Final body weight (g)	1.7 ± 0.1^{a}	1.8 ± 0.1^{ab}	1.9 ± 0.0^{ab}	2.0 ± 0.1^{bc}	2.1 ± 0.0^{c}
BWG (%)[1]	392.4 ± 25.8^{a}	414.3 ± 20.0^{ab}	428.6 ± 5.7^{ab}	477.2 ± 33.4^{bc}	501.0 ± 12.8^{c}
SGR[2]	2.7 ± 0.1^{a}	2.7 ± 0.1^{ab}	2.8 ± 0.0^{ab}	2.9 ± 0.1^{bc}	3.0 ± 0.0^{c}
FCR[3]	4.6 ± 0.3	4.4 ± 0.2	4.6 ± 0.1	4.4 ± 0.2	4.1 ± 0.2
FI (g/fish/60 day)[4]	6.3 ± 0.1^{a}	6.3 ± 0.1^{a}	6.8 ± 0.0^{b}	7.2 ± 0.1^{b}	7.2 ± 0.2^{b}
Survival (%)	92.3 ± 0.0	90.2 ± 0.0	90.1 ± 0.0	92.0 ± 0.0	90.8 ± 0.0
CF[5]	1.7 ± 0.1	1.7 ± 0.0	1.7 ± 0.1	1.7 ± 0.1	1.6 ± 0.1

Values are mean ± SE of three replicates (30 fish/tank). Means in the same row with different superscripts are significantly different ($P < 0.05$)

[1] *BWG*, Body weight gain = (Final body weight − initial body weight) × 100/initial body weight

[2] *SGR*, Specific growth rate = $(\mathrm{Ln}W_t - \mathrm{Ln}W_0) \times 100/T$, where W_0 and W_t are the initial and final body weight, respectively; T is the culture period

[3] *FCR*, Feed conversion ratio = Total diet fed (g)/wet weight gain (g)

[4] *FI*, Feed intake = Total feed intake (g)/number of fishes/T (day), where T is the culture period

[5] *CF*, Condition factor) = (Body weight × 100)/total body length $(cm)^3$

Table 6 Whole body composition (%) and liver lipid contents (% of total lipid in liver) of blunt snout bream fingerlings fed diets containing different levels of phospholipid

	Experimental diets (dietary polar lipid level, g kg^{-1} dry diet)				
	PL0 (1.3)	PL2 (7.2)	PL4 (13.7)	PL6 (18.8)	PL8 (23.3)
Whole body composition (dry matter basis)					
Moisture	72.4 ± 0.5	72.4 ± 0.3	72.7 ± 0.2	71.8 ± 0.1	74.0 ± 1.2
Crude protein	53.8 ± 1.0^{a}	54.9 ± 2.5^{ab}	55.6 ± 1.1^{ab}	54.6 ± 0.6^{ab}	59.4 ± 1.5^{b}
Crude lipid	37.2 ± 2.1^{d}	35.8 ± 1.1 ^{cd}	35.3 ± 1.2^{bc}	35.1 ± 1.7^{b}	31.5 ± 1.2^{a}
Ash	7.1 ± 0.4^{a}	7.8 ± 0.5^{ab}	7.8 ± 0.1^{ab}	8.2 ± 0.1^{b}	7.7 ± 0.1^{ab}
Liver lipid contents					
Total lipid	8.3 ± 0.6	7.8 ± 0.9	7.1 ± 0.4	7.3 ± 0.1	7.7 ± 0.3
Neutral lipid	72.3 ± 1.3^{c}	71.3 ± 1.1^{ab}	65.9 ± 2.7^{b}	67.1 ± 2.1^{ab}	59.3 ± 1.1^{a}
Polar lipid	27.7 ± 1.3^{a}	28.7 ± 1.1^{ab}	34.1 ± 2.7^{b}	32.9 ± 2.1^{ab}	40.7 ± 1.1^{c}
Neutral lipid/polar lipid	2.6 ± 0.1^{d}	2.4 ± 0.1^{c}	1.9 ± 0.2^{b}	2.0 ± 0.2^{b}	1.5 ± 0.1^{a}
HSI (%)[1]	1.9 ± 0.1^{b}	1.7 ± 0.1^{ab}	1.5 ± 0.1^{a}	1.6 ± 0.1^{ab}	1.4 ± 0.1^{a}

Values are mean ± SE of three replicates (5 fish/tank). Means in the same row with different superscripts are significantly different ($P < 0.05$)

[1] *HSI*, Hepatosomatic index = (liver weight × 100)/body weight

percentage of ARA and significantly decreased ($P < 0.05$) in the percentages of 16:1n-7, 18:1n-7 and 18:1n-9 fatty acids. In addition, the total n-6 FA was increased significantly ($P < 0.05$) by increasing dietary PL supplementation; however, there were no effects of n-3 fatty acids (Table 7). For liver fatty acid composition of the polar lipid fraction, increasing dietary PL levels significantly increased ($P < 0.05$) in percentages of 16:0, LA and significantly decreased ($P < 0.05$) in percentages of 18:1n-7, 18:1n-9, ARA, EPA, 22:5n-3 and DHA (Table 8). The percentages of main n-3 fatty acids such as EPA, 22:5n-3 and DHA presented a higher concentration in the polar lipid fraction than these in neutral lipid fraction.

Table 7 Liver fatty acid composition (% of total fatty acids) in neutral lipid fraction of blunt snout bream fingerlings fed diets containing different levels of phospholipid

Fatty acids	Experimental diets (dietary polar lipid level, g kg^{-1} dry diet)				
	PL0 (1.3)	PL2 (7.2)	PL4 (13.7)	PL6 (18.8)	PL8 (23.3)
14:0	1.3 ± 0.0^{b}	1.2 ± 0.1^{b}	0.9 ± 0.1^{a}	0.9 ± 0.1^{a}	0.9 ± 0.1^{a}
16:0	15.5 ± 0.5^{a}	17.2 ± 0.6^{ab}	18.3 ± 0.6^{b}	17.4 ± 1.1^{ab}	17.3 ± 0.9^{ab}
17:0	0.3 ± 0.1^{a}	0.3 ± 0.0^{ab}	0.4 ± 0.1^{ab}	0.6 ± 0.2^{b}	0.5 ± 0.1^{ab}
18:0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0
Σ saturated	17.2 ± 0.6^{a}	18.9 ± 0.7^{ab}	19.8 ± 0.5^{b}	19.1 ± 0.9^{ab}	18.9 ± 1.0^{ab}
16:1n-7	4.5 ± 0.6^{c}	4.0 ± 0.1^{bc}	3.2 ± 0.1^{ab}	2.9 ± 0.3^{a}	2.8 ± 0.2^{a}
18:1n-7 + 18:1n-9	65.8 ± 0.6^{c}	64.4 ± 1.0^{bc}	63.3 ± 0.2^{ab}	63.6 ± 0.4^{ab}	62.2 ± 0.9^{a}
20:1n-11	0.6 ± 0.2	0.7 ± 0.1	1.2 ± 0.5	1.2 ± 0.5	1.4 ± 0.3
20:1n-9	0.6 ± 0.3	0.3 ± 0.2	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.0
22:1n-11	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Σ monoenes	71.6 ± 1.4^{c}	69.4 ± 1.3^{bc}	68.0 ± 0.8^{ab}	68.0 ± 0.3^{ab}	66.6 ± 0.9^{a}
18:2n-6 (LA)	7.4 ± 1.0	8.0 ± 0.8	8.4 ± 0.8	8.9 ± 1.0	9.5 ± 0.4
20:2n-6	0.1 ± 0.1^{a}	1.3 ± 0.3^{b}	1.1 ± 0.3^{b}	1.2 ± 0.1^{b}	1.2 ± 0.4^{b}
20:4n-6 (ARA)	0.4 ± 0.1^{a}	0.5 ± 0.0^{ab}	0.9 ± 0.3^{ab}	0.8 ± 0.6^{ab}	1.2 ± 0.1^{b}
Σ n-6 fatty acids	7.9 ± 1.0^{a}	9.8 ± 0.9^{ab}	10.3 ± 0.5^{b}	10.9 ± 1.7^{b}	11.9 ± 0.6^{b}
18:3n-3 (LNA)	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
18:4n-3	0.1 ± 0.1	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.2
20:4n-3	0.2 ± 0.2	0.1 ± 0.2	0.4 ± 0.3	0.2 ± 0.1	0.4 ± 0.4
20:5n-3 (EPA)	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1
22:5n-3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
22:6n-3 (DHA)	0.1 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
Σ n-3 fatty acids	0.6 ± 0.2	0.4 ± 0.3	0.9 ± 0.3	0.6 ± 0.3	0.9 ± 0.2

Values are mean ± SE of three replicates (20 fish/tank). Means in the same row with different superscripts are significantly different ($P < 0.05$)

LA linoleic acid, *ARA* arachidonic acid, *LNA* linolenic acid, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid

PPAR gene expression levels in liver

The PPAR mRNA expressions levels in the liver are shown in Fig. 1. Expression of *PPAR-α* in liver was sixfold higher ($P < 0.05$) in PL6 than control group. However, fish fed PL8 exhibited significantly lower value ($P < 0.05$) than control group. The mRNA expression of *PPAR-β* showed no significant differences ($P > 0.05$) in liver of fish fed the diets contained different dietary PL levels. Expression of *PPAR-γ* in liver was significantly increased ($P < 0.05$) by dietary PL supplementation up to 6 %, while fish fed PL8 exhibited significantly lower expression ($P < 0.05$) than those fed PL6.

Antioxidant enzyme activities and TBARS value

The liver antioxidant enzyme activities are presented in Fig. 2. The activities of CAT and GPx significantly increased ($P < 0.05$) with incremental dietary PL level up to 6 %; then these enzymes decreased ($P > 0.05$) in fish fed PL8. The fingerlings fed diet PL6 showed significantly higher ($P < 0.05$) SOD value than other groups. Liver TBARS values are also shown in Fig. 2. TBARS values were reduced by increasing the dietary PL levels from 0 to 6 %, in which fish fed PL6 diet had a significant lower ($P < 0.05$) value than control group.

Discussion

In the present study, BWG of blunt snout bream fingerlings was significantly increased from 392.4 to 501.0 % with increasing dietary PL levels from 0 to 8 %. It suggested that dietary PL could improve growth performance of blunt snout bream fingerlings. It has been shown that dietary PL induced growth performance and survival of fish including rainbow trout *O. mykiss* fry (Poston 1990; Azarm et al. 2013a), ayu *plecoglossus altivelis* (Kanazawa et al. 1983) and European sea bass *Dicentrarchus labrax* larvae (Cahu

Table 8 Liver fatty acid composition (% of total fatty acids) in polar lipid fraction of blunt snout bream fingerlings fed diets containing different levels of phospholipid

Fatty acids	Experimental diets (dietary polar lipid level, g kg^{-1} dry diet)				
	PL0 (1.3)	PL2 (7.2)	PL4 (13.7)	PL6 (18.8)	PL8 (23.3)
14:0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.0
16:0	17.1 ± 0.5^{a}	19.7 ± 0.4^{ab}	20.7 ± 1.2^{b}	20.2 ± 0.7^{b}	21.3 ± 1.7^{b}
17:0	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.5 ± 0.1
18:0	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.4 ± 0.0
Σ saturated	17.8 ± 0.5^{a}	20.4 ± 0.5^{ab}	22.0 ± 2.2^{ab}	21.2 ± 0.8^{ab}	22.5 ± 1.7^{b}
16:1n-7	0.6 ± 0.1^{b}	0.4 ± 0.1^{ab}	0.4 ± 0.0^{a}	0.4 ± 0.1^{a}	0.4 ± 0.0^{a}
18:1n-7 + 18:1n-9	18.2 ± 0.5^{b}	17.4 ± 0.5^{ab}	17.4 ± 0.2^{ab}	16.9 ± 0.6^{a}	16.4 ± 0.3^{a}
20:1n-11	18.0 ± 0.6	17.0 ± 0.8	16.7 ± 0.8	17.0 ± 0.6	16.8 ± 1.8
20:1n-9	3.9 ± 0.0	3.7 ± 0.3	3.4 ± 0.2	3.3 ± 0.2	3.4 ± 0.5
22:1n-11	12.0 ± 0.4	12.1 ± 1.0	12.5 ± 1.3	12.1 ± 1.2	11.6 ± 1.0
Σ monoenes	52.7 ± 0.7^{b}	50.5 ± 2.1^{ab}	50.3 ± 0.7^{ab}	49.6 ± 0.4^{ab}	48.6 ± 1.3^{a}
18:2n-6 (LA)	13.3 ± 0.4^{a}	15.4 ± 0.6^{b}	16.7 ± 0.5^{bc}	16.9 ± 0.8^{bc}	17.4 ± 0.7^{c}
20:2n-6	0.9 ± 0.1	1.0 ± 0.0	0.7 ± 0.1	1.0 ± 0.2	1.4 ± 0.4
20:4n-6 (ARA)	0.8 ± 0.2^{b}	0.5 ± 0.2^{ab}	0.2 ± 0.2^{a}	0.1 ± 0.1^{a}	0.1 ± 0.0^{a}
Σ n-6 fatty acids	15.0 ± 0.4^{a}	16.9 ± 0.5^{b}	17.5 ± 0.8^{bc}	18.1 ± 0.8^{bc}	18.9 ± 0.7^{c}
18:3n-3 (LNA)	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
18:4n-3	0.1 ± 0.0	0.2 ± 0.3	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
20:4n-3	0.1 ± 0.0	0.4 ± 0.7	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1
20:5n-3 (EPA)	11.3 ± 0.7^{c}	9.5 ± 0.2^{bc}	8.2 ± 0.5^{ab}	8.1 ± 0.6^{a}	6.8 ± 0.5^{a}
22:5n-3	0.9 ± 0.1^{b}	0.8 ± 0.1^{ab}	0.6 ± 0.1^{ab}	0.5 ± 0.2^{ab}	0.4 ± 0.3^{a}
22:6n-3 (DHA)	0.9 ± 0.2^{b}	0.6 ± 0.3^{ab}	0.6 ± 0.3^{ab}	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}
Σ n-3 fatty acids	13.2 ± 0.9^{c}	11.6 ± 1.3^{bc}	9.6 ± 0.6^{ab}	9.0 ± 0.5^{a}	7.6 ± 0.2^{a}

Values are mean ± SE of three replicates (20 fish/tank). Means in the same row with different superscripts are significantly different ($P < 0.05$)

LA linoleic acid, *ARA* arachidonic acid, *LNA* linolenic acid, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid

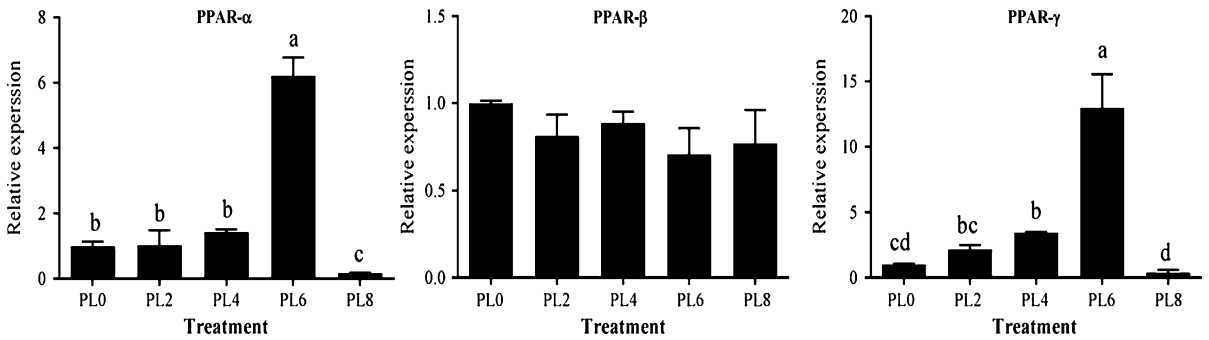

Fig. 1 Relative mRNA expression levels of *PPAR-α*, *PPAR-β* and *PPAR-γ* gene in liver of blunt snout bream fed diets containing different phospholipid levels for 60 days. Values are mean ± SE, $n = 3$ per replicate tanks, three fish were sampled for each tank; *Different letters above the bars* showed there were significant differences among different treatments ($P < 0.05$)

et al. 2003). Therefore, dietary PL is essential for the growth and survival of fish larval and juvenile. The values of FCR in this study ranged from 4.1 to 4.6, which were extremely higher than FCR value (1.5–1.7) from the study of Wu et al. (2013) working on same fish at similar temperature/size. This is probably due to a huge feed wastage in feeding trial, as FI values among all treatments (about 6 g/fish/60 days) were quiet high, but the BWG of fish were only 1.3–1.8 g.

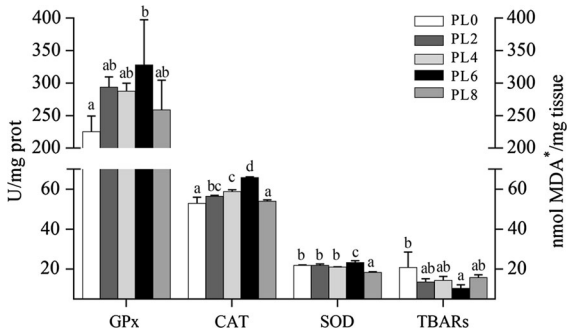

Fig. 2 GPx, CAT and SOD activities (U mg^{-1} prot) and TBARS value (nmol MDA/mg wet tissue) in liver of blunt snout bream fed diets containing different phospholipid levels for 60 days. Values are mean ± SE, $n = 3$ per replicate tanks, five fish were sampled for each tank; *Different letters above the bars* showed there were significant differences among different treatments ($P < 0.05$). **MDA* malondialdehyde

The promote effects of PL on growth in fish larvae have been explained in different ways, providing the essential dietary components such as choline, inositol (Geurden et al. 1995) and essential fatty acid (Kanazawa et al. 1979). The larval digestive system is not completely developed, lacks a functional stomach, and main digestion of ingested food occurs in the intestine (Morais et al. 2006). Therefore, the promote effects of dietary PL on growth most likely related to that the larval fish at first feeding is predisposed to digestion and metabolism of PL and the use of fatty acids from PL for energy (Sargent et al. 1997). Moreover, Tocher et al. (2008) described that the effect of PL on growth may be due to improved transport, assimilation and utilization of dietary lipids from the experimental diets.

In this study, the fish fed PL6 showed significantly higher growth performance than those fed PL0, indicating that fish fed diets containing more than 6 % of soybean lecithin (containing 45 % polar lipid) could induce a higher growth response in blunt snout bream fingerlings. This value met the requirement of PL for most fish species which ranged from 2 to 16 % of the diet, such as 2 % of egg yolk PL (containing 68 % phosphatidylcholine and 28 % phosphatidylethanolamine) for carp *C. carpio* larvae (Geurden et al. 1995), 4 % of soybean lecithin (containing 95 % phosphatidylcholine) for rainbow trout *O. mykiss* fry (Azarm et al. 2013b), 13 % of soybean lecithin (containing 26 % phosphatidylcholine, 20 % phosphatidylethanolamine and 14 % phosphatidylinositol) for pikeperch *S. lucioperca* larvae (Hamza et al. 2008) and 16.7 % of soybean lecithin (containing 62 % PL) for sea bass *D. labrax* larvae (Cahu et al. 2003).

Crude protein and crude lipid contents of whole body were significantly affected by dietary PL levels in this study. This was in agreement with Zhao et al. (2013) who found that crude protein content of the whole body was significantly higher in large yellow croaker *L. crocea* larvae fed containing higher levels of dietary PL. Moreover, Uyan et al. (2009) reported that the dietary PL tended to promote retention of protein in juvenile amberjack *S. dumerili*. These results demonstrated dietary PL could influence protein content of whole body in fish. On the other hand, results from the present study showed that lipid content of whole body decreased with incremental dietary PL level. Similar results were also observed in a number of previous studies (Izquierdo et al. 2000; Saleh et al. 2013a, b). These researchers proposed that dietary PL (soybean lecithin) could induce lipoproteins synthesis in larval gut, promote dietary lipids and proteins transport, and result in reducing lipid droplets accumulation of fish. Generally, HSI value is correlated with the amount of liver fat deposition. In this study, fish fed PL un-supplementation diet had bigger liver than those fed PL supplementation diets. However, the value tended to be lower in fish fed diet with incremental PL levels, suggesting that dietary PL could promote liver fat metabolism. In the percent study, the ratio of neutral to polar lipid in liver decreased with the increase of dietary PL. This indicated a high deposition of polar lipids in liver of the fish fed diet containing the higher PL level. Similar results were also found in other previous studies. Zhao et al. (2013) reported that the ratio of neutral to polar lipid of whole body of large yellow croaker *L. crocea* larvae significantly decreased with the increase of dietary PL. Geurden et al. (1999) also found that dietary PL promoted retention of polar lipid fraction in common carp *C. carpio*.

The experimental diets lacked ARA, EPA and DHA; therefore, the visceral fatty acid compositions were measured to confirm that the fish fed the experimental diets were able to elongate and desaturate the precursors, LA to their respective C20-22 fatty acids. Fatty acid composition of the liver was influenced by the fatty acid composition of the

corresponding test diet. In freshwater fish, it is known that both n-3 and n-6 fatty acid PUFA are important nutrients as LA and LNA can be converted to the long chain n-6 and n-3 fatty acids, respectively. Synthesis of ARA is achieved by Δ6 desaturation of LA. Synthesis of EPA from LNA requires the same enzymes and pathway as for ARA (Li et al. 2010). This might support our observation that the percentages of ARA of neutral lipid fraction in liver increased with increasing dietary PL, because PL of soybean lecithin are rich in LA, which then could convert to ARA as seen in present study. On the other hand, the percentages of EPA, DHA and total n-3 HUFA of polar lipid fraction in liver were decreased by increasing dietary PL from soybean lecithin supplementation. Sargent et al. (2002) reported that there was a competition inhibition between the substrate of n-3 and n-6 fatty acid PUFA because both of them were substrates of elongase and desaturase. These results might also be due to a substrate competition of n-3 HUFA metabolites with n-6 HUFA metabolites for the elongase and desaturase. Similarly, Zhao et al. (2013) stated that the percentages of EPA and DHA were significantly decreased by dietary PL from soybean lecithin supplementation in large yellow croaker *L. larvae*. Hamza et al. (2008) found a decrease in the percentage of n-3 fatty acids in pikeperch *S. lucioperca* larvae fed the diets containing a high level of dietary PL from soybean lecithin.

The functions of the lipid metabolism of PPAR have caused a great deal of attention. To our knowledge, this work was the first study that determined effects of dietary PL on mRNA expression of PPAR genes in fish, which would help provide some information in fish. *PPAR*-α gene plays an important role in the control of lipid metabolism, by regulating the expression of target gene encoding enzymes involved in the peroxisomal and mitochondrial β-oxidation of fatty acids of blunt snout bream (Lu et al. 2013). Our results showed dietary PL supplement from 0 % to 6 % up-regulated *PPAR*-α gene expression in liver, whereas PL6 was sixfold higher than PL0. Supplementation of dietary PL mainly increased in the percentage of LA. Several studies have been reported that LA-conjugated linoleic acid (CLA) could mediate this activation by acting as high-affinity ligands for a number of PPAR isotypes, particularly *PPAR*-α (Moya-Camarena et al. 1999). For instance, Moya-Camarena et al. (1999) demonstrated that CLA was a potent naturally occurring ligand and activator of *PPAR*-α in rat hepatoma FaO cells. Suh et al. (2008) found that linoleic acid increased *PPAR*-α expression in primary cultured chicken hepatocytes. In this study, fish fed diet PL8 down-regulated *PPAR*-α gene expression, which might be correlated with the down-regulation of the β-oxidation-related gene. This suggested that a high dose of PL supplementation might inhibit liver *PPAR*-α gene expression. *PPAR*-γ is considered to play a pivotal role in lipid accumulation and adipocyte differentiation (Walczak and Tontonoz 2002). In this study, the *PPAR*-γ mRNA expression in liver was significantly affected by dietary PL supplementation. The *PPAR*-γ mRNA expression in liver was increased by PL supplement from 0 to 6 %. The result was similar with the liver *PPAR*-α mRNA gene expression. This suggested that the optimum level of PL may dramatically increase the mRNA gene expressions of *PPAR*-α and *PPAR*-γ in liver. The increase in liver *PPAR*-α and *PPAR*-γ activity may suggest a decline tendency of lipid deposition in liver of fish fed PL0-PL6 diets.

SOD, CAT and GPx are the primary enzymes for radicals scavenging, which are involved in protective mechanisms within tissue injury following oxidative processes and phagocytosis (Fontagné-Dicharry et al. 2014). And those activities are commonly used as antioxidant indicators to evaluate antioxidant defense system of fish (Sun et al. 2011). Usually, higher levels of SOD, CAT and GPx activities indicated an increased antioxidant defense in fish (Yang et al. 2010). In this study, liver CAT, SOD and GPx increased with incremental dietary PL level up to 6 %, but the activities of these enzymes reduced in fish fed PL8 diet. These result indicated that appropriate supplementation dietary PL could enhance stress resistance and induce antioxidant responses to protect an organ against oxidative damage (Hamza et al. 2008; Zhao et al. 2013; Gao et al. 2014). Additionally, we found that the liver TBARS value negatively correlated with liver CAT, SOD and GPx activities in fish. The similar result was reported by Bhattacharyya et al. (2014) who demonstrated that the negative correlation was founded between the activities of CAT, SOD and GPx and the TBARS values. Based on these results, we could get a conclusion that fish fed the diet contain 6 % PL (18.9 g kg^{-1}, polar lipid of dry diet) might effectively minimize oxidative damage.

Conclusion

In conclusion, the present study indicated that supplementation of 6 % PL in diet (18.9 g kg^{-1}, polar lipid of dry diet) could improve growth performance of blunt snout bream fingerlings. The fish fed diet containing the higher PL level had higher level of deposition of polar lipids in liver. Our results also found that fish fed diets containing 6 % of PL dramatically up-regulated *PPAR-α* and *PPAR-γ* gene expression in liver. Additionally, a higher levels of liver CAT, SOD and GPx activities and lower TBARS value, which could be considered an effectively minimize oxidative damage, were found in fish fed diet containing 6 % dietary PL.

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