Comparative Biochemistry and Physiology, Part B 201 (2016) 46–52

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part B

journal homepage: www.elsevier.com/locate/cbpb

Effects of dietary phospholipid on lipase activity, antioxidant capacity and lipid metabolism-related gene expression in large yellow croaker larvae (*Larimichthys crocea*)

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ARTICLE INFO

Article history:
Received 28 April 2016
Received in revised form 16 June 2016
Accepted 17 June 2016
Available online 25 June 2016

Keywords:
Large yellow croaker larvae
Phospholipid
Lipid metabolism
Antioxidant capacity

ABSTRACT

It was previously shown that lipid content of the whole larvae body fed the phospholipid (PL)-devoid diet was significantly lower than that of larvae fed PL-supplemented diets ($P < 0.05$) (Feng et al., unpublished results). The mechanisms involved remain unclear and were explored from the perspective of fatty acids delivery, uptake, synthesis and oxidation in the present study. Besides, this study was also designed to investigate the effect of dietary PL on antioxidant capacity in large yellow croaker larvae (*Larimichthys crocea*). Triplicate groups of larvae (initial body weight: 3.86 ± 0.24 mg) were fed three isonitrogenous and isolipidic diets with increasing levels of PL (2.53%, 6.32% and 12.7%) eight times daily for 30 days. Results showed that the specific activities of superoxide dismutase and catalase were significantly higher in 12.7% PL group compared to those in 2.53% PL group ($P < 0.05$), while an opposite trend was observed for MDA content ($P < 0.05$). The specific activity of lipase and the mRNA abundance of fatty acids delivery and uptake-related genes, including lipoprotein lipase, hepatic lipase and fatty acids translocase (cluster of differentiation) were significantly higher in 12.7% PL group than those in 2.52% group ($P < 0.05$). Compared to 2.53% PL group, the transcript levels of fatty acid synthase and stearoyl-CoA desaturase I were significantly lower in 6.32% PL group ($P < 0.05$), while peroxisome proliferators-activated receptor α, carnitine palmitoyl transferase-I and acyl CoA oxidase mRNA expression levels were significantly higher in 12.7% PL group ($P < 0.05$). These results indicated that dietary PL could enhance antioxidant capacity of larvae. Dietary PL might regulate lipid metabolism in large yellow croaker larvae by modulating fatty acids delivery, uptake, synthesis and oxidation at transcriptional level and improved fatty acids delivery and uptake might be responsible for higher body lipid content in 6.32% and 12.7% groups.

1. Introduction

It has been generally accepted that dietary inclusion of intact phospholipid (PL) is essential for fish larvae due to their limited capacity for PL *de novo* synthesis (Tocher et al., 2008). Dietary PL exerts beneficial effects on growth performance, survival and stress resistance in a variety of fish larvae species (Azarm et al., 2013; Gao et al., 2014; Geurden et al., 1995; Hamza et al., 2008; Lu et al., 2008; Niu et al., 2008a; Taylor et al., 2015; Tocher et al., 2008; Zhao et al., 2013). In a recent study, we reinvestigated the phospholipid requirement of large yellow croaker larvae, with survival and growth data confirming a requirement for PL in large yellow croaker larvae (Feng et al., unpublished results). Besides, in accordance with previous research, our study also provided evidence that dietary PL supplementation could enhance lipid content of the whole larvae body (Gao et al., 2014; Niu et al., 2008b; Zhao et al., 2013; Feng, et al., unpublished results). However, the mechanisms involved remain still unclear.

Lipid metabolism in fish is similar to that in mammals (Tocher, 2003). Lipoprotein lipase (LPL) and hepatic lipase (HL) are key enzymes accounting for fatty acids delivery to tissues by hydrolyzing triglyceride (TG) on corresponding lipoproteins (Nilsson-Ehle et al., 1980; Oku et al., 2006). Once free fatty acids are released from TG on lipoproteins, there are several proteins implicating in fatty acids uptake, among which fatty acids translocase (cluster of differentiation, CD36) is the best characterized (Goldberg et al., 2009). In addition, lipid deposition is also related with fatty acids synthesis and oxidation. Fatty acid synthase (FAS) plays a crucial role in *de novo* lipogenesis by catalyzing the conversion of acetyl-CoA and malony-CoA to stearic acid (18:0) (Sargent, 1989), which is then further converted to monounsaturated fatty acids by stearoyl-CoA desaturase (SCD) (Ntambi, 1999). Carnitine palmitoyltransferase I (CPT-1) participates in the conversion of fatty acid-CoAs into fatty acid–carnitines for entry into the mitochondrial

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http://dx.doi.org/10.1016/j.cbpb.2016.06.007
1096-4959/

matrix for oxidation (Kerner and Hoppel, 2000). Acyl-CoA oxidase (ACO) also catalyzes the rate-limiting step in fatty acids β-oxidation (Yan et al., 2015). To our knowledge, no information is available on the mechanisms by which dietary PL regulates lipid metabolism in fish larvae.

Marine fish larvae are considered to be more vulnerable and susceptible to stressors compared to the corresponding juveniles (Kjørsvik et al., 2011). It is well recognized that fish previously exposed to a stressor may show oxidative stress, reflected by overproduction of the reactive oxygen species (ROS) (Lushchak and Bagnyukova, 2006). At low concentration, ROS is critical for defense against microorganisms (Gill and Tuteja, 2010). However, when cellular production of ROS overwhelms a cell's antioxidant capacity, it will damage cellular macromolecules such as lipids, protein, and DNA (Tovar-Ramírez et al., 2010). To protect against the damaging effects of ROS, cells possess several antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Thannickal and Fanburg, 2000). As mentioned above, previous study has demonstrated that dietary PL supplementation could enhance stress resistance in large yellow croaker larvae (Zhao et al., 2013). Whether dietary PL could protect large yellow croaker larvae through enhancing antioxidant capacity against ROS remains unknown.

Large yellow croaker (*Larimichthys crocea*) is a carnivorous marine fish species that has been widely cultured in southeast China due to its delicious taste and commercial value. The aim of the present study was to investigate the underlying mechanisms about how dietary PL influenced lipid metabolism and it was postulated that for fish fed diets with PL, the expression of genes related with fatty acids delivery, synthesis and uptake would be higher, and the expression of genes related with fatty acids oxidation would be lower, resulting into higher lipid content. Besides, the effect of dietary PL on antioxidant capacity was also assessed. This study is expected to gain new insights into the mechanisms underlying the beneficial effects of dietary PL.

2. Materials and methods

2.1. Feed ingredients and diets formulation

The requirement for PL in large yellow croaker larvae was estimated to be approximately 5.72% dry diet based on the previous study of Zhao et al. (2013). Thus, three isonitrogenous (56% crude protein) and isolipidic (19% crude lipid) diets were formulated by adding graded levels of PL (0%, 5%, and 12%), which were regarded as deficient level, sufficient level and excess level, respectively. The analyzed dietary PL concentrations were 2.53%, 6.32%, and 12.7% dry matter (Table 1). Large yellow croaker larvae with initial average weight 3.86 ± 0.12 mg were fed with the experimental diets for 30 days. Other than phospholipid, diets were formulated to meet the dietary requirements of large yellow croaker larvae and soybean oil was used to balance the fatty acids composition of the three experimental diets. Micro-diets have been used and manufactured by micro-bonding technology and the binder used was alginae sodium. The particle size of the formulated diets ranged from 150 to 250 μm for fish from 15 to 25 days after hatch (DAH) and 200 to 350 μm for fish thereafter.

Table 1
Formulation and proximate analysis of the experimental diets for large yellow croaker larvae (% dry matter).

	Dietary phospholipid level (%)		
	2.53	6.32	12.7
Ingredients			
LT-White fish meal[a]	21	21	21
LT-Krill meal[a]	14	14	14
LT-Squid meal[a]	12	12	12
Casein	20	20	20
Hydrolyzed fish meal[a]	4	4	4
Yeast	3	3	3
α-Starch	4	4	4
Alginae sodium	1.5	1.5	1.5
Vitamin premix[b]	1.5	1.5	1.5
Mineral premix[c]	1.5	1.5	1.5
Ascorbyl polyphosphate	0.2	0.2	0.2
Attractant mixture[d]	2	2	2
Mold inhibitor	0.05	0.05	0.05
Antioxidant	0.05	0.05	0.05
Choline chloride	0.2	0.2	0.2
Fish oil	3	3	3
Soybean oil	12	7	0
Soybean lecithin	0	5	12
Proximate composition			
Crude protein	56.35	56.12	56.43
Crude lipid	19.39	18.98	19.44
Phospholipid	2.53	6.32	12.7

[a] Low temperature white fish meal: contained 71.2% crude protein ans 5.3% crude lipid; low temperature krill meal: contained 63.8% crude protein and 13% crude lipid; low temperature squid meal: contained 59.6% crude protein and 2.0% crude lipid; hydrolyzed fish meal: contained 77.1% crude protein and 1.3% crude lipid.

[b] Composition of vitamin premix (IU or g/kg): retinal palmitate, 3,000,000 IU; cholecalciferol, 1,200,000 IU; DL-α-tocopherol acetate, 40.0 g; menadione, 8.0 g; thiamin-HCl, 5.0 g; riboflavin, 5.0 g; D-calcium pantothenate, 16.0 g; pyridoxine-HCl, 4.0 g; meso-inositol, 200.0 g; D-biotin, 8.0 g; folic acid, 1.5 g; para-aminobenzoic acid, 5.0 g; niacin, 20.0 g; cyanocobalamin, 0.01 g; ascorbyl polyphosphate (contained 25% ascorbic acid), 100.0 g.

[c] Composition of mineral premix (g/kg premix): $Ca(H_2PO_4)_2 \cdot H_2O$, 675.0; $CoSO_4 \cdot 4H_2O$, 0.15; $CuSO_4 \cdot 5H_2O$, 5.0; $FeSO_4 \cdot 7H_2O$, 50.0; KCl, 50.0; KI, 0.1; $MgSO_4 \cdot 2H_2O$, 101.7; $MnSO_4 \cdot 4H_2O$, 18.0; NaCl, 80.0; $Na_2SeO_3 \cdot H_2O$, 0.05; $ZnSO_4 \cdot 7H_2O$, 20.0.

[d] Composition of attractant mixture (g/kg premix): betaine, 500 g; glycine, 150 g; alanine, 100 g; argine, 100 g; taurine, 100 g; inosine-5′-monophosphoric acid, 50 g.

2.2. Experimental procedure

Larvae used in this study were obtained and reared at Ningde Fufa Fishery Co., Ltd. (Fujian, China). All larvae in the hatchery were fed with rotifers, *Brachionus plicatilis* ($0.5–1.5 \times 10^4$ individuals/l) from 3 to 8 DAH, *Artemia nauplii* ($1.0–1.5 \times 10^3$ individuals/l) from 6 to 11 DAH, and live copepods and a commercial pellet diet (Marubeni Nisshin Feed Co., Ltd., Japan) from 10 to 14 DAH, and then the larvae were weaned onto the experimental diets. The experiment was carried out in 9 white plastic tanks (water volume 300 l) with a stocking density of 3000 larvae per tank. Three experimental diets were randomly allocated to triplicate groups of larvae. During the rearing period, water temperature ranged from 20 to 24°C, pH from 7.8 to 8.2 and salinity from 21 to 25‰. About 150–300% of the water volume was renewed daily and there was an air stone in each tank. Larvae were reared under 14 h light: 10 h dark dial cycle photoperiod. Light intensity was 8.5 W/m^2 maximum during daytime at the water surface. From 15 to 45 DAH, larvae were manually fed to satiation with the experimental diets eight times (6:00, 7:00, 8:00, 9:00, 13:00, 14:00, 15:00 and 16:00) daily. The feeding experiment has been conducted by Feng et al. (unpublished results).

2.3. Sampling

At the end of the experiment, all larvae were deprived of food to empty their guts for 24 h before sampling. One hundred individuals were randomly collected from each tank and immediately frozen in liquid nitrogen and stored at −80°C for enzyme activities and gene expression analysis.

2.4. Activity of lipase

The whole larvae body of fifty samples were homogenized in nine volumes of ice-cold phosphate buffered saline (PBS) (pH: 7.2–7.4) (Solarbio, China) with a Polytron PT-MR 2100 homogenizer (Kinematica, Switzerland) for 30 s and centrifuged at 4000**g** for 10 min at 4°C. After that, the supernatant fraction was collected

and determined for the concentration of soluble protein by the method of Bradford (1976) using bovine serum albumin (A2153, Sigma, USA) as standard and then stored at −20°C until use. Lipase activity was determined according to the method of Borlongan (1990) using a commercial kit (Nanjing Jiancheng Bio-Engineering Institute, China). Lipase can hydrolyze triglyceride in stabilized emulsion of olive oil, resulting in a decrease in optical density when measured at 420 nm. Using distilled water as blanks. Lipase activity was expressed as units per g protein (U/g protein) and one unit was defined as 1 μmol substrate consumed per min at 37°C.

2.5. MDA content and activities of antioxidant enzymes

MDA was measured using a thiobarbituric acid (TBA) assay kit (Nanjing Jiancheng Bio-Engineering Institute, China). Briefly, 200 μl supernatant fraction of larvae homogenates in each replicate were incubated with 20% tricloracetic acid (TCA) and 0.67% TBA at 95°C for 80 min to produce a pink-coloured material and the intensity was measured at 532 nm. Using distilled water as blanks. Results were converted to nmol MDA per mg protein using a standard sample of 10 nmol/ml malonaldehyde bis (diethyl acetate).

SOD activity was measured using a commercial kit following the manufacture's instruction (Nanjing Jiancheng Bio-Engineering Institute, China). Briefly, SOD activity was determined according to the method of McCord and Fridovich (1969). The reaction mixture consisted of 50 mM-potassium phosphate buffer (pH 7.8), 0.1 mM-EDTA, 0.1 mM-xanthine, 0.013 mM-cytochrome *c* and xanthine oxidase (0.024 IU/ml). The xanthine oxidase was added to start the reaction. The reaction was carried out at 37°C for 40 min. Using distilled water as blanks. SOD activity was expressed as units per mg protein (U/mg protein) and one unit was defined as the amount of enzyme necessary to produce 50% inhibition of the ferricytochrome *c* reduction rate measured at 550 nm.

CAT activity was assayed according to the method of Góth (1991) and Özmen et al. (2002) using a commercial kit (Nanjing Jiancheng Bio-Engineering Institute, China). CAT reacted with H_2O_2 for 1 min and then the reaction was stopped by the addition of ammonium molybdate. The reaction generated a light-yellow complex and the intensity of the complex was measured at 405 nm. Using distilled water as blanks. CAT activity was expressed as units per mg protein (U/mg protein) and one unit was defined as the amount of enzyme necessary to resolve 1 mmol H_2O_2 in 1 s at 37°C.

2.6. RNA extraction, cDNA synthesis and quantitative real-time polymerase chain reaction

Total RNA was extracted from the whole larvae body of fifty individuals using Trizol Reagent (Invitrogen, USA) and electrophoresed on a 1.2% denaturing agarose gel to test the integrity. The RNA concentration and quality were determined on Nanodrop® 2000 (Thermo Fisher Scientific, USA). All samples exhibited high RNA quality with absorbance ratio (260/280) ranging from 1.9 to 2.0. Then, 1 μg total RNA was subjected to reverse transcription using a PrimeScript® RT Reagent Kit with gDNA Eraser (Takara, Japan). About 800 ng/μl first strand cDNA was generated and diluted to 100 ng/μl with sterilized double-distilled water. Quantitative real-time PCR (qRT-PCR) was carried out in a quantitative thermal cycler Mastercycler ep realplex (Eppendorf, Germany). The primers used in the present study for β-actin, FAS, SCD-1, peroxisome proliferators-activated receptor α (PPARα), CPT-1, ACO, LPL, HL and CD36 were synthesized based on the corresponding primer sequences in published papers (Cai et al., 2015; Yan et al., 2015; Xu et al., 2015) and the primers for sterol-regulatory element binding protein-1 (SREBP-1) were designed and synthesized based on the partial cDNA sequence of SREBP-1 from large yellow croaker (GenBank accession no. KP342262). The sequences of these primers were listed in Table 2. 18 s rRNA, β-actin, GAPDH, EF1α and ubiquitin were ranked according to their stability using geNorm (version 3.5) and NormFinder algorithms (Andersen et al., 2004; Vandesompele et al., 2002) and β-actin was employed as the reference gene. Each reaction was performed in a total volume of 25 μl, containing 1 μl of each primer (10 μM), 1 μl of the diluted cDNA, 12.5 μl of 2× SYBR® Premix Ex Taq™II (Takara, Japan) and 9.5 μl of sterilized double-distilled water. QRT-PCR was programmed as follows: 95°C for 2 min, followed by 40 cycles of 95°C for 10 s, 58°C for 10 s and 72°C for 20 s. At the end of each reaction, melting curve analysis was performed to confirm the specificity of production. In addition, standard curves were made with a five point four-fold dilution series of the first strand cDNA (in triplicate) and amplification efficiency of the primers was analyzed according to the following equation $E = 10^{(-1/\text{slope})} - 1$. The generated standard curves showed linearity over the entire quantitation range (The coefficients of linear regression were >0.99). The amplification efficiency was 98.73% for β-actin, 95.74% for FAS, 100.31% for SREBP-1, 100.22% for SCD-1, 98.77% for PPARα, 92.31% for CPT-1, 101.2% for ACO, 94.58% for LPL, 98.67% HL and 101.11% CD36. The ΔCT ($CT_{\text{target genes}}$ - $CT_{\text{reference gene}}$) was further calculated for each cDNA dilution. Then, a plot of the log cDNA dilution versus ΔCT was made. The absolute values of all the slope were <0.1, which indicated that the mRNA expression levels of the target genes could be calculated using $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). The relative mRNA expression of target genes in larvae fed the diet with 2.53% PL was selected as the calibrator.

2.7. Statistical analyses

Software SPSS 17.0 (SPSS Incorporation, USA) was used for all statistical evaluation. All data were subjected to a one-way analysis of variance (ANOVA) and followed by a Duncan's multiple range test. The level of significance was chosen at $P < 0.05$ and the results were presented as means ± standard error of the mean (S.E.M.).

Table 2
Primer pair sequences for real-time PCR.

Target genes	Forward (5′-3′)	Reverse (5′-3′)	References
SREBP-1	TCTCCTTGCAGTCTGAGCCAAC	TCAGCCCTTGGATATGAGCCT	GenBank accession no. KP342262
FAS	CAGCCACAGTGAGGTCATCC	TGAGGACATTGAGCCAGACAC	Yan et al. (2015)
SCD-1	AAAGGACGCAAGCTGGAACT	CTGGGACGAAGTACGACACC	Xu et al. (2015)
PPARα	GTCAAGCAGATCCACGAAGCC	TGGTCTTTCCAGTGAGTATGAGCC	Zuo et al. (2013)
CPT-1	GCTGAGCCTGGTGAAGATGTTC	TCCATTTGGTTGAATTGTTTACTGTCC	Yan et al. (2015)
ACO	AGTGCCCAGATGATCTTGAAGC	CTGCCAGAGGTAACCATTTCCT	Yan et al. (2015)
LPL	GAATTCAACGCGGAAACACAG	ACGCTCATAGAGGGCAGACAC	Yan et al. (2015)
HL	TCCGTCCATCTATTCATTGACTCTC	GCCACTGTGAACCTTCTTGATATTG	Cai et al. (2015)
CD36	GAGCATGATGGAAAATGGTTCAAAG	CTCCAGAAACTCCCTTTCACCTTAG	Yan et al. (2015)
β-Actin	CTACGAGGGTTATGCCCTGCC	TGAAGGAGTAACCGCGCTCTGT	Yan et al. (2015)

SREBP-1, sterol-regulatory element binding protein-1; FAS, fatty acid synthase; SCD-1, stearoyl-CoA desaturase I; PPARα, peroxisome proliferators-activated receptor α; CPT-1, carnitine palmitoyl transferase-I; ACO, acyl-CoA oxidase; LPL, lipoprotein lipase; HL, hepatic lipase; CD36, cluster of differentiation 36.

3. Results

3.1. Survival, growth and body lipid content

Full results of the dietary trial were given in Feng et al. (unpublished results), but were summarized here to provide context for the samples investigated in the current study. Survival rates (SR) and specific growth rates (SGR) of larvae fed diets with 6.32% and 12.7% PL were significantly higher than those of larvae fed the diet with 2.53% PL ($P < 0.05$) (Table 4). The whole body lipid content in 2.53% PL group was significantly lower than that in 6.32% and 12.7% PL groups ($P < 0.05$) (Table 3).

3.2. Specific activity of lipase

The specific activities of lipase in larvae fed diets with 6.32% and 12.7% PL were significantly higher than that in 2.53% PL group ($P < 0.05$, Fig. 1).

3.3. MDA content and specific activities of SOD and CAT

MDA levels were significantly lower in larvae fed diets with 6.32% and 12.7% PL compared to that of larvae fed the diet with 2.53% PL ($P < 0.05$, Fig. 2). The specific activities of SOD in larvae fed diets with 6.32% and 12.7% PL were significantly higher than that in 2.53% PL group ($P < 0.05$, Fig. 2). The specific activity of CAT in larvae fed the diet with 12.7% PL was significantly higher than that in 2.53% PL group ($P < 0.05$, Fig. 2) and the specific activity of CAT in 6.32% PL group was higher than that in 2.53% PL group even though no significant difference was observed ($P > 0.05$, Fig. 2).

3.4. The mRNA levels of lipid metabolism-related genes

The mRNA levels of LPL were significantly increased with the increasing levels of dietary PL ($P < 0.05$, Fig. 3A). The mRNA expression levels of HL were significantly higher in 12.7% and 6.32% PL groups than that in 2.53% PL group ($P < 0.05$, Fig. 3A). The mRNA level of CD36 was significantly higher in 12.7% PL group than those in 2.53% and 6.32% PL groups ($P < 0.05$, Fig. 3A).

The transcript levels of FAS were significantly lower in 6.32% and 12.7% PL groups than that in 2.53% PL group and the transcript level of SCD-1 was significantly lower in 6.32% PL group than that in 2.53% PL group ($P < 0.05$, Fig. 3B). The transcript level of SCD-1 in 12.7% PL group was lower than that in 2.53% group even though no significant difference was observed ($P > 0.05$, Fig. 3B). Also, no significant difference was observed in the mRNA expression of SREBP-1 among dietary treatments ($P > 0.05$, Fig. 3B).

With the increasing levels of dietary PL, the transcript levels of PPAR-α were significantly increased ($P < 0.05$, Fig. 3C). Larvae fed diets supplemented with 6.32% and 12.7% PL had significant higher CPT-1 transcript levels when compare to those in 2.53% PL group ($P < 0.05$, Fig. 3C). The mRNA expression level of ACO was significantly higher in 12.7% PL group than that in 2.53% and 6.32% PL groups ($P < 0.05$, Fig. 3C), while the mRNA levels of ACO were comparable between 2.53% and 6.32% PL groups ($P > 0.05$, Fig. 3C).

Table 3
Effects of dietary phospholipid on growth, survival and lipid content of large yellow croaker larvae for 30 days (mean ± S.E.M., n = 3).

	Dietary phospholipid level (%)		
	2.53	6.32	12.7
Initial weight (mg)	3.86 ± 0.12	3.86 ± 0.12	3.86 ± 0.12
Final weight (mg)	23.75 ± 1.12^a	62.61 ± 2.33^b	70.17 ± 4.43^b
Specific growth rate (% day^{-1})	6.06 ± 0.16^a	9.29 ± 0.12^b	9.67 ± 0.21^b
Survival (%)	12.8 ± 1.31^a	23.3 ± 1.29^b	22 ± 2.11^b
Whole body lipid content (%)	9.61 ± 0.52^a	13.22 ± 0.68^b	14.72 ± 0.97^b

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

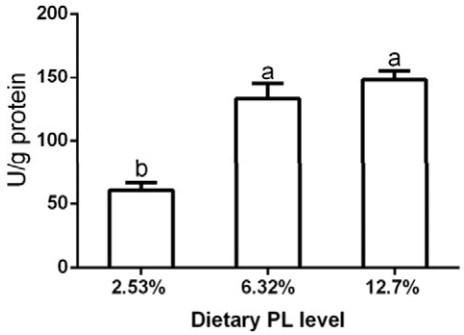

Fig. 1. Effects of dietary phospholipid on activity of lipase in larval large yellow croaker. Data were presented as means ± S.E.M. (n = 3). The enzyme activity of lipase was expressed as specific activity (U/g protein). Different letters above the bars denoted significant difference between experimental groups ($P < 0.05$).

4. Discussion

Marine fish are more vulnerable to oxidative stress during their larval stage due to high requirements for energy and long chain PUFA (polyunsaturated fatty acids). Oxidative stress will give rise to overproduction of ROS, leading to lipid peroxidation. MDA is one of the metabolites derived from lipid peroxidation and is considered to be a principal biomarker of oxidative stress damage (Del Rio et al., 2005). Until now, there is a general lack of knowledge about the effects of dietary PL on status of oxidative stress in large yellow croaker larvae. In the present study, MDA levels in whole larvae body were significantly lower in 12.7% and 6.32% PL groups than that in 2.53% PL group, indicating that oxidative stress damage was greater in larvae fed the no PL-supplemented diet. In order to further investigate how PL exerted beneficial effect on defense against oxidative stress, the specific activities of SOD and CAT were assessed in the present study. In the process of ROS elimination, SOD converts highly reactive superoxide radical to the less reactive H_2O_2, which can subsequently react with CAT and be decomposed into nontoxic H_2O (Liu et al., 2015). Their activities are commonly used as indicators to evaluate antioxidant defense system of fish (Sun et al., 2011). In the present study, the specific activities of both SOD and CAT were significantly higher in 12.7% PL group than those in 2.53% PL group. Thus, the antioxidant capacity-promoting effect of dietary PL might contribute to the increasing defense ability to oxidative stress, resulting into less lipid peroxidation and higher survival rate.

Our recent study has reported that compared to 2.53% PL group the whole body lipid levels were significantly higher in large yellow

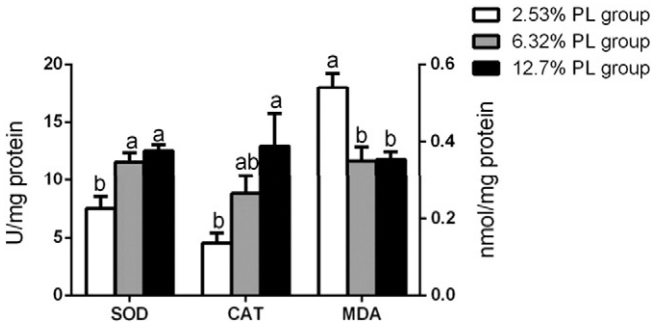

Fig. 2. Effects of dietary phospholipid on activity of SOD and CAT and MDA content in larval large yellow croaker. Data were presented as means ± S.E.M. (n = 3). The enzyme activity of SOD and CAT was expressed as specific activity (U/mg protein). MDA content was expressed as nmol MDA per mg protein. Different letters above the bars denoted significant difference between experimental groups ($P < 0.05$).

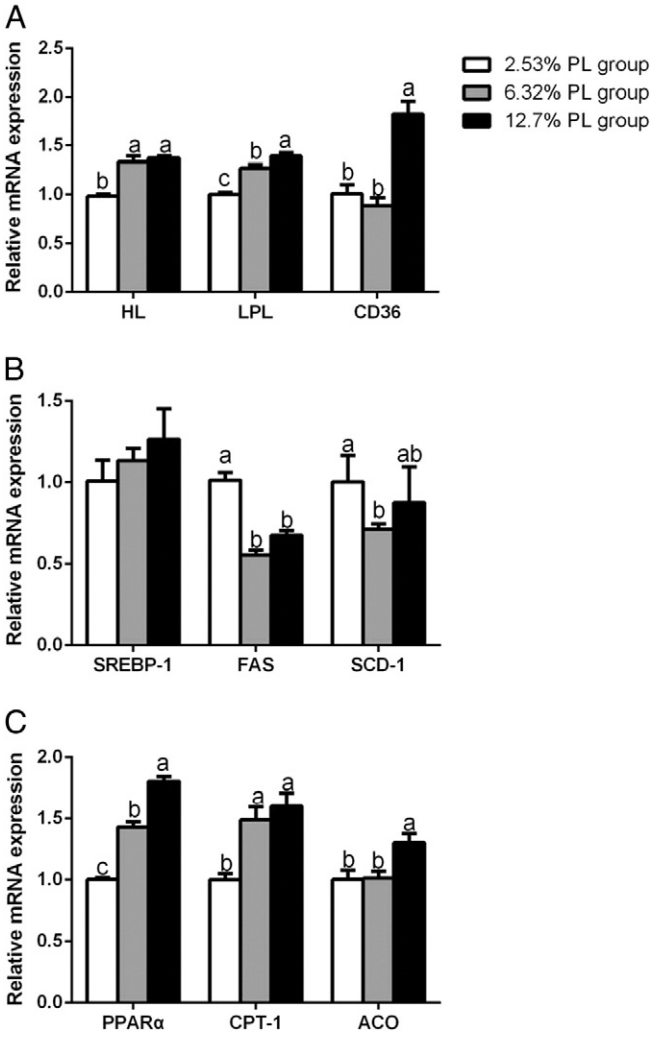

Fig. 3. Effects of dietary phospholipid on relative mRNA expression of genes involved in lipid metabolism in larval large yellow croaker. Data were presented as means ± S.E.M. (n = 3). Different letters above the bars denoted significant difference between experimental groups ($P < 0.05$).

croaker larvae fed diets with 6.53% and 12.7% PL (Feng et al., unpublished results). This was in agreement with some previous studies in which Gao et al. (2014) found that dietary PL supplementation significantly increased fat content of Dojo loach larvae and Geurden et al. (1999) reported that crude lipid content was significantly increased with the increasing levels of dietary PL in common carp larvae. However, the mechanisms involved are still unknown. The ability of larvae to assimilate nutrients depends on their capacity to modulate their digestive enzymes (Cahu and Zambonino-Infante, 2001). In the present study, the specific activities of lipase in PL-supplemented groups were significantly higher compared to the no PL-supplemented group, which might contribute to better lipid digestion and absorption. Inconsistent with the present study, specific activities of lipase in sea bass larvae and Atlantic cod larvae were considered to be influenced by the triacylglycerol level in diets and were higher in low PL-supplemented groups (Cahu et al., 2003; Wold et al., 2007). Specific activity of lipase is not only affected by its substrates. As demonstrated by Gisbert et al. (2005), abnormal lipid accumulation in the intestinal mucosa might suppress the digestive capacity in fish. A number of studies have reported that lack of PL in larval diets could lead to abnormal lipid deposition in intestinal enterocytes due to deficiency in chylomicron production (Daprà et al., 2011; Fontagné et al., 1998; Liu et al., 2002; Olsen et al., 1999; Salhi et al., 1999) and that might account for lower specific activity of lipase in no PL-supplemented group in the present study. In addition, it was reported that activity of pancreatic enzymes was higher in fish exhibiting a good growth (Gisbert et al., 2005).

To our knowledge, no information is available on the mechanisms by which dietary PL regulates lipid metabolism in fish larvae. We hypothesized that for fish fed diets with PL, the expression of genes related with fatty acids delivery, synthesis and uptake would be higher, and the expression of genes related with fatty acids oxidation would be lower, ultimately resulting into higher lipid content. The mRNA levels of LPL and HL were significantly higher in larvae fed diets with PL supply compared to those of larvae fed no PL-supplemented diet. LPL can decrease the plasma triglyceride levels and enhance lipid uptake and deposition by tissues through hydrolyzing TG on chylomicrons and very low-density lipoproteins in mammals (Frayn et al., 1995; Merkel et al., 2002). HL is responsible for TG hydrolysis on small VLDL (Eisenberg and Levy, 1975; Nicoll and Lewis, 1980). In addition, HL also facilitates lipoprotein uptake by cell surface receptors and proteoglycans as a ligand, thereby directly affecting cellular lipid delivery (Santamarina-Fojo et al., 2004). In cobia larvae, Niu et al. (2008a) found that LPL and HL activities were higher in PL-supplemented groups than that in PL-free group, associated with lower TG content in plasma. CD36 is an important cell surface receptor responsible for fatty acids uptake and its deficiency in mice significantly impared fatty acids uptake by heart, skeletal muscle, and adipose tissue (Coburn et al., 2000). The mRNA level of CD36 was markedly higher in 12.7% PL group than that in 2.53% PL group. Taken together, higher LPL, HL and CD36 expression in PL-supplemented group might indicate that more fatty acids were liberated from TG-rich lipoproteins and assimilated by tissues for storage, which consequently resulted into higher whole body lipid content as observed in the present study.

The expression of genes related with lipoprotein TG hydrolysis and fatty acids uptake in response to dietary PL was evaluated above. Further, we detected the mRNA abundance of genes involved in fatty acids synthesis and oxidation in dietary treatments. No information has been published about evaluating the effects of dietary PL levels on mRNA expression of these genes in fish larvae. In the current study, the mRNA expression levels of fatty acids synthesis-related genes, including FAS and SCD-1, were significantly lower in larvae fed diets with PL supplementation. As reported by Perry et al. (2014), a small fraction of intracellular fatty acids supply comes from *de novo* lipogenesis in the cytosol and it is greatly influenced by exogenous fatty acids uptake. As FAS and SCD-1 play important roles in fatty acids endogenous synthesis, lower FAS and SCD-1 expression in PL-supplemented treatments could be partially due to a feedback mechanism in response to higher exogenous fatty acids uptake in larvae. SREBP-1, as a transcription factor, can activate specific genes such as FAS and SCD-1 (DeBose-Boyd et al., 2001). However, no significant difference was observed in SREBP-1 mRNA expression among dietary treatments. Liu et al. (2013) found that in diet-induced-obese mice, the mRNA levels of SREBP-1 were not significantly influenced by dietary soybean phospholipid that was relatively rich in linoleic acid (LNA), whereas it was markedly decreased by dietary eicosapentaenoic acid (EPA)-enriched phospholipid. It appeared that intact PL itself failed to affect the expression of SREBP-1. On the contrary, the mRNA levels of FAS and SCD-1 were significantly decreased by both diets with soybean PL and EPA-enriched PL (Liu et al., 2013), with the effects of EPA-enriched PL superior to that of soybean PL. Thus, the effects of dietary PL with different fatty acids profile on lipid metabolism should be investigated in the future study. At the same time, the mRNA levels of fatty acids oxidation-related genes, including PPARα, CPT-1 and ACO, were significantly higher in PL-supplemented groups than those in no PL-supplemented group. PPARα can induce fatty acids oxidation-related genes expression, such as CPT-1 and ACO, as a transcription factor (Peng et al., 2014). Thus, it was speculated that dietary PL might up-regulate

PPARα mRNA expression and, consequently, increase the gene expression levels of fatty acids oxidation enzymes. In agreement with the present study, studies in mice suggested that dietary PL might decrease the expression of fatty acids synthesis-related genes and enhance the expression of fatty acids oxidation-related genes, eventually leading to less lipid accumulation in the liver (Liu et al., 2013; Rossmeisl et al., 2014). Liu et al. (2016) suggested that PC, as the most abundant PL species in soybean lecithin, could suppress lipid biosynthesis and promote β-oxidation by activating AMPK signal pathway in the liver of rat. However, it should be noticed that the whole larvae body instead of specific organs, such as the liver, was used for gene expression analysis in the present study. An analysis in individual organs will contribute to better understanding the modulating effects of dietary PL on lipid metabolism in fish and the corresponding mechanisms involved.

Although the results obtained with the mRNA abundance of genes involved in fatty acids synthesis and oxidation were contrary to our hypothesis, one should notice that the whole body lipid content of large yellow croaker larvae was still higher in PL-supplemented groups. This might probably be a comprehensive result of dietary fatty acids delivery, uptake, synthesis and oxidation, and greater fatty acids uptake might play a predominate role in the present study.

In summary, the present study showed that dietary PL could enhance antioxidant capacity of larvae. Dietary PL might regulate lipid metabolism in large yellow croaker larvae by modulating fatty acids delivery, uptake, synthesis and oxidation at transcriptional level and improved fatty acids delivery and uptake might responsible for higher body lipid content in PL-supplemented groups.

Conflict of interest

There are no competing interests.

Acknowledgement

This research was supported by the National Natural Science Foundation of China (grant no. 31172425 and 31372541) and the National Basic Research Program of China (973 Program, grant no. 2014CB138600). We thank R.T. Zuo and X.J. Dong for their assistance in the study. There are no conflicts of interest to report.

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