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Effect of dietary phospholipid levels on growth, lipid metabolism, and antioxidative status of juvenile hybrid snakehead (*Channa argus*×*Channa maculata*)

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Abstract The study was conducted to evaluate the effect of dietary phospholipids (PLs) on growth, lipid metabolism, and antioxidative status of hybrid snakehead (*Channa argus* × *Channa maculata*). Five isonitrogenous and isolipidic diets with graded levels of PLs (8.5, 19.3, 30.7, 41.5, and 50.8 g kg^{-1}) were fed to triplicate groups of juveniles (initial body weight 12.6 ± 0.23 g) for 8 weeks. Results showed that dietary PL supplementation significantly improved growth of juveniles. The final body weight (FBW) and specific growth rate (SGR) significantly increased with dietary PLs increasing from 8.5 to 41.5 g kg^{-1} ($P < 0.05$). Fish fed with the diet containing 8.5 g kg^{-1} PLs showed higher feed conversion ratio (FCR) compared to the other treatments ($P < 0.05$). Survival rate (SR) was not affected by dietary PL levels ($P > 0.05$). Liver lipid contents, serum triglyceride (TG), and low-density lipoprotein cholesterol (LDL-C) contents significantly decreased with the increasing levels of dietary PLs ($P < 0.05$). However, serum total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) contents and HDL-C/TC and HDL-C/LDL-C value significantly increased with increasing dietary PL levels ($P < 0.05$). The catalase (CAT), superoxide dismutase (SOD), and carnitine palmitoyl transferase I (CPT-1) activities in the liver significantly increased with incremental dietary PL level ($P < 0.05$), while the liver malondialdehyde (MDA) contents and fatty acid synthase (FAS) activity significantly reduced ($P < 0.05$). No significant difference was observed in the glutathione peroxidase (GPx) activity among dietary treatments ($P > 0.05$).These results confirmed that dietary PL supplementation has beneficial effects on growth performance and antioxidant capacity of juvenile hybrid snakehead. Dietary PLs might reduce lipid deposition in the liver of juvenile hybrid snakehead.

Keywords Phospholipid · Snakehead · Growth · Lipid metabolism · Antioxidant ability

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Introduction

The snakehead is an important economic species in China with high market value and demand (Zhao et al. 2016). Recently, the snakehead culture has developed rapidly and widely in Vietnam, Cambodia, Thailand, and other Asian countries. However, aquaculture intensification and expansion of the fish have resulted in the presence of morphological deformities and liver dysfunction, which cause huge economic losses due to

mortalities, reduced growth, or unmarketability of the final product. The causes reported for these morphological anomalies or fatty liver are many and only partially understood. Basically, there are many causative factors including nutritional deficiencies or excesses (Fjelldal et al. 2012). It has long been known that dietary phospholipids (PLs) exert beneficial effects on growth, survival, and stress resistance, and reduce the occurrence of malformation in marine and freshwater species (Rinchard et al. 2007; Daprà et al. 2011; Gao et al. 2014). Most commonly, the levels of phospholipid requirement can vary depending upon fish species and developmental stage (Tocher et al. 2008; Azarm et al. 2013). Generally, dietary phosphatidylcholine (PC) improve growth performance whereas phosphatidylinositol (PI) supplementation has been primarily associated with increased survival and reduced deformities in animal (Geurden et al. 1998; Azarm et al. 2013; Taylor et al. 2015).

The PLs requirement appears to be restricted to early life stages and no requirement has been established in adult fish of any species, although this is largely unstudied (Tocher et al. 2008; Taylor et al. 2015; Feng et al. 2017). Some researchers suggest that the requirement for PLs during larval stage might be due to the limited capacity to de novo synthesize PLs (Tocher et al. 2008; Daprà et al. 2011). Recent studies confirmed that major PL biosynthetic genes displayed lower expression in the intestine during the early developmental stage (fry) of Atlantic salmon (*Salmo salar*) (Carmona-Antoñanzas et al. 2015). However, Cai et al. (2016) showed that dietary PL might regulate lipid metabolism at the transcriptional level in large yellow croaker. This suggested that the molecular mechanism underpinning phospholipid requirement would be located specifically in intestinal or liver tissue/cells. However, many of the regulatory mechanisms of PL biosynthesis are at non-transcriptional levels (Sugimoto et al. 2008). Similarly, the role of phospholipid/PC in stimulating growth in fry was not mediated through major changes in gene expression (De Santis et al. 2015). Therefore, biochemical or enzymological studies are needed to elucidate if larvae could synthesize PLs de novo. Phospholipid is also required for lipoprotein assembly, and thus transport of lipid throughout the body (Tocher et al. 2008). In addition, in commercial diets, the function of dietary PLs is affected by the other ingredients, such as phosphorus, cholesterol, or essential fatty acids (Roy et al. 2006; Li et al. 2016). To date, both the optimal PL level and the composition, as well as the role of dietary PLs remain unclear for most species.

Despite the many studies available denoting the importance of dietary PLs, few have focused on juvenile or larger fish (Tocher et al. 2008). Moreover, PL synthesis is a limiting factor for the development of freshwater fish. Recent studies also found that dietary PLs might reduce lipid deposition in the liver of juvenile large yellow croaker (Feng et al. 2017). To our knowledge, no information has been published evaluating the physiological effects of PLs in diets for juvenile hybrid snakehead (*Channa argus* × *Channa maculata*). Therefore, this study was conducted to determine the effects of supplemental dietary PLs from soybean lecithin added to practical diets on growth, survival, lipid metabolism, and antioxidative status of juvenile hybrid snakehead.

Materials and methods

Experimental diets

Five isonitrogenous (42% crude protein) and isolipidic (8.7% crude lipid) diets were formulated by adding graded levels of PLs (0, 10, 20, 30, and 40 g kg^{-1}) and the final dietary PL concentrations were 8.5, 19.3, 30.7, 41.5, and 50.8 g kg^{-1} (Table 1). Peru steam-treated fish meal, soybean meal, and cottonseed protein were chosen as the main protein sources. Fish oil, soybean oil, and soybean lecithin were chosen as the primary lipid sources. All ingredients were ground through a 320-μm mesh before final mixing and then blended with the oil. The experimental diets were prepared using a cooking extruder (TSE65S, Beijing Modern Yanggong Machinery S&T Development Co., Ltd., China), then air-dried and stored at 4 °C until used.

Experimental procedures

Snakehead (*Channa argus* × *Channa maculata*) used in this experiment were obtained from a commercial farm (Yongchuan, Chongqing, China). Prior to the trial, fish were acclimated and fed a commercial feed (Zhejiang Zhongda Group Feed Co., Ltd., China) for 1 week. The experimental design and procedure were approved by the Animal Care and Use of Committee of Southwest University following the requirements of the Regulations for the Administration of Affairs Concerning

Table 1 Formulation and proximate composition of the experimental diets (g kg^{-1} dry matter)

	Dietary phospholipid levels (g kg^{-1})				
	8.5	19.3	30.7	41.5	50.8
Ingredients					
Fish meal[1]	350	350	350	350	350
Soybean meal[1]	260	260	260	260	260
Cottonseed protein[1]	100	100	100	100	100
Wheat middlings	183	183	183	183	183
Fish oil	20	20	20	20	20
Soybean oil	40	30	20	10	0
Soybean lecithin	0	10	20	30	40
Choline chloride	2	2	2	2	2
Mineral premix [2]	15	15	15	15	15
Vitamin premix [3]	20	20	20	20	20
Monocalcium phosphate	10	10	10	10	10
Proximate composition					
Crude protein	423.7	423.9	424.6	422.8	425.1
Crude lipid	88.3	87.9	87.3	86.7	86.1
Lecithin	8.5	19.3	30.7	41.5	50.8

[1] Fish meal (Peruvian steam-treated): crude protein 675 g kg^{-1}, crude lipid 92 g kg^{-1}; soybean meal (solvent extracted), crude protein 476 g kg^{-1}; cottonseed protein (free gossypol), crude protein 568 g kg^{-1}

[2] Mineral premix (mg kg^{-1} of diet): Na, 30; K, 50; Mg, 100; Cu, 4; Fe, 25; Zn, 35; Mn, 12; I, 1.6; Se, 0.2; Co, 0.8

[3] Vitamin premix (mg kg^{-1} of diet): VA, 18; VD_3, 5; VE, 150; VC (350 g kg^{-1}), 500; VB_1, 16; VB_6, 20; VB_{12}, 6; VK_3, 18; riboflavin, 40; inositol, 320; calcium-D-pantothenate, 60; niacinamide, 80; folic acid, 5; biotin, 2; ethoxyquin, 100

Experimental Animals of China (The State Science and Technology Commission, 1988).

At the start of the experiment, the fish were fasted for 24 h and weighed after being anesthetized with 0.1 g kg^{-1} MS-222 (Sigma, USA). Snakehead (mean initial weight: 12.6 ± 0.23 g) were randomly allocated into 15 cylindrical plastic tanks (capacity: 300 L) with screened covers for the growth trial (30 fish per tank). Each dietary treatment was randomly assigned to three tanks. Fish were fed to apparent satiation by hand three times (08:30, 12:30, and 18:00) daily for 8 weeks. During the experiment, photoperiod was held to a constant 12 h light/12 h dark cycle. The water in each tank was monitored daily for temperature (24.5 ± 2.5 °C), pH (7.4 ± 0.4), dissolved oxygen content (6–7 mg L^{-1}), ammonia nitrogen content (< 0.10 mg L^{-1}), nitrite nitrogen (0.005–0.010 mg L^{-1}), and sulfide (< 0.05 mg L^{-1}).

Sample collection

At the end of the trial, fish were fasted for 24 h before harvest. Total numbers were counted, and mean body weight of fish was measured. Two fish per tank were anesthetized with overdose of MS-222 (Sigma, USA) to assess the whole body composition. Another six fish from each tank were randomly selected and anesthetized with 0.1 g kg^{-1} MS-222 (Sigma, USA), and a blood sample collected from the caudal vein using a 1-mL syringe with a 27-gauge needle and allowed to clot at 4 °C. Following centrifugation (3000 rpm, 10 min) at 4 °C (Sorvall ST 16R, Thermo Fisher Scientific Inc., Germany), serum was separated, immediately frozen in liquid nitrogen, and stored at − 20 °C until analyzed. The bloodless fish were then dissected to obtain viscera, liver, and intraperitoneal fat for calculating morphological parameters. Pooled livers of another three fish per tank were also immediately frozen in liquid nitrogen and stored at − 20 °C until used for malondialdehyde (MDA) determination and enzymatic analysis.

Chemical analysis

All chemical composition analysis of diets and whole body were conducted by standard methods (AOAC 2005). Moisture was determined by oven drying to a constant weight at 105 °C in DHG-9240A (Keelrein instrument Co., Ltd., China). Protein was determined by measuring nitrogen (N × 6.25) using the Kjeldahl method in FOSS Kjeltec 2300 (Foss Analytical Instruments Co., Ltd., Sweden), lipid by ether extraction (without acid hydrolysis) using Soxtec, and ash by combustion at 550 °C for 12 h in a muffle furnace (Shenyang Energy-Saving Electric Furnace Factory, China). Total lipid of the liver was measured following the method of Bligh and Dyer (1959). The PL content of diets was assayed by the method of colorimetric measurement of phosphorus as molybedenum blue according to Li et al. (2005). Biochemical indexes including total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were assayed using automatic biochemical analyzer HITACHI 7100 (ISE)

(Hitachi Ltd., Tokyo, Japan) and attached kit (Sichuan Maker Biotechnology Co., Ltd., Chengdu, China).

Samples of liver were homogenized into 10 volumes (*w*/*v*) of ice-cold, 8.5 g kg^{-1} physiological saline at 10000 r/min for 1 min using fluko superfine homogenizers (FLUKO Equipment Shanghai Co., Ltd. China). Homogenates were centrifuged at 4000 rpm for 10 min at 4 °C (Sorvall ST 16R, Thermo Fisher Scientific Inc., Germany). The supernatant was separated for MDA and enzyme analysis. MDA was measured using a thiobarbituric acid (TBA) assay kit (Nanjing Jiancheng Bioengineering Institute, China). Results were converted to nanomole MDA per milligram protein using a standard sample of 10 nmol/ml malonaldehyde bis (dimethyl acetal). The enzymatic activities of CAT, SOD, GPx, FAS, and CPT-1 were assayed by the commercial kit (Jiancheng Bioengineering Institute, Nanjing, China). CAT activity was expressed as units per milligram 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. SOD activity was expressed as units per milligram 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. GPx activity was expressed as nanomole of oxidized NADPH/min/mg protein. FAS activity was expressed as nanomole oxidized NADPH/min/mg protein. CPT-I activity was expressed as nanomole CoA-SH released/min/mg protein. The protein concentration of the enzyme extracts was determined according to Bradford (1976). All enzyme assays were performed in triplicate.

Statistical analysis

All results are presented as mean ± standard error (SE) and analyzed by using SPSS software version 17.0 (SPSS Inc., Chicago, USA) for Windows. Differences between groups were evaluated by one-way ANOVA followed by Tukey's multiple comparison. The level of significance was set at $P < 0.05$.

Results

Growth performance

The growth performance of snakehead is shown in Table 2. The final body weight (FBW) and specific growth rate (SGR) significantly increased with dietary PLs increasing from 8.5 to 41.5 g kg^{-1} ($P < 0.05$). Fish fed with the diet containing 8.5 g kg^{-1} PLs showed higher feed conversion ratio (FCR) compared to the other treatments ($P < 0.05$). However, there were no significant differences in these parameters (FBW, SGR, and FCR) between the PLs 41.5 and PLs 50.8 groups ($P > 0.05$). Fish fed with the diet containing 8.5 g kg^{-1} PLs and 41.5 g kg^{-1} PLs showed higher feed intake (FI) than both the PLs 19.3 and PLs 30.7 groups ($P < 0.05$). Survival rate (SR) was not affected by dietary PL levels ($P > 0.05$).

Morphological parameters and whole body composition

In the present study, no significant differences were found in the viscera ratio (VR), intraperitoneal fat ratio (IPF), and the whole body moisture, protein, lipid, and ash contents among dietary treatments ($P > 0.05$, Table 3). However, fish fed with the diet containing 8.5 g kg^{-1} PLs showed higher hepatosomatic index (HSI) than both the PLs 30.7 and PLs 41.5 groups ($P < 0.05$). Liver lipid levels significantly decreased with the increasing levels of dietary PLs ($P < 0.05$, Table 3), with juvenile hybrid snakehead fed with the diet with 50.8 g kg^{-1} PLs showing the lowest liver lipid content.

Liver malondialdehyde contents and enzyme activities

The CAT, SOD, and CPT-1 activities significantly increased with incremental dietary PL level ($P < 0.05$), while the liver MDA contents and FAS activity significantly reduced ($P < 0.05$, Table 4). No significant difference was observed in the GPx activity among dietary treatments ($P > 0.05$, Table 4).

Serum lipid metabolism indexes

The contents of TC and HDL-C significantly increased with increasing dietary PL levels ($P < 0.05$, Table 5).The highest TC and HDL-C contents were observed in fish fed with the diets with 50.8 g kg^{-1} PLs. However, the contents of TG and LDL-C significantly decreased with increasing dietary PL levels ($P < 0.05$, Table 5). Fish fed with PL-supplemented diets exhibited higher levels of HDL-C and lower levels of LDL-C than those fed with the control diet (8.5 g kg^{-1} PLs). Fish fed with the diet containing 8.5 g kg^{-1} PLs showed lower HDL-C/TC

Table 2 Effect of different phospholipid levels on growth performance of hybrid snakehead (means ± S.E.M)

Items	Dietary phospholipid levels (g kg^{-1})				
	8.5	19.3	30.7	41.5	50.8
Initial body weight (g)	12.63 ± 0.05	12.70 ± 0.02	12.64 ± 0.03	12.72 ± 0.02	12.79 ± 0.07
Final body weight (g)	56.97 ± 0.82^{c}	57.90 ± 0.63^{c}	65.28 ± 0.52^{b}	72.35 ± 1.59^{a}	69.82 ± 0.66 ^{a}
SGR (% day^{-1})[1]	2.69 ± 0.02^{c}	2.71 ± 0.02^{c}	2.93 ± 0.02^{b}	3.10 ± 0.04 ^{a}	3.03 ± 0.02 ^{ab}
FCR[2]	1.21 ± 0.02^{a}	1.10 ± 0.02^{bc}	1.03 ± 0.01^{c}	1.12 ± 0.02^{b}	1.06 ± 0.01^{bc}
FI (g 100 g^{-1} BW day^{-1})[3]	2.74 ± 0.02^{a}	2.49 ± 0.05^{b}	2.50 ± 0.04^{b}	2.72 ± 0.03^{a}	2.62 ± 0.04^{ab}
Survival (%)	100.00 ± 0.00	97.78 ± 1.11	98.89 ± 1.11	97.8 ± 2.22	100.00 ± 0.00

Values in each row with different superscripts have significant differences ($P < 0.05$)

[1] Specific growth rate (SGR) = [ln (mean final weight) − ln (mean initial weight)/56 days] × 100

[2] Feed conversion ratio (FCR) = total feed intake in dry basis (g) /weight gain (g)

[3] Feed intake (FI) = feed consumption (g) / [(initial weight + final weight) /2 × 56 days]

and HDL-C/LDL-C value compared to the other treatments ($P < 0.05$, Table 5).

Discussion

In the present study, FBW and SGR of juvenile hybrid snakehead were significantly increased with increasing dietary PL levels from 8.5 to 41.5 g kg^{-1}. It is therefore suggested that dietary PLs could improve growth performance of juvenile hybrid snakehead. Similar results have also been observed in juvenile grass carp (*Ctenopharyngodon idella*) (Chen et al. 2015), blunt snout bream fingerlings (*Megalobrama amblycephala*) (Li et al. 2015), juvenile cobia (*Rachycentron canadum*) (Niu et al. 2008), juvenile Japanese flounder (*Paralichthys olivaceus*) (Uyan et al. 2007), juvenile *L. vannamei* (Sánchez et al. 2014), and juvenile

Table 3 Morphological measurements and body composition of snakehead at the end of growth trial (mean ± S.E.M)

Items	Dietary phospholipid levels (g kg^{-1})				
	8.5	19.3	30.7	41.5	50.8
Morphological measurements (*g* kg^{-1})					
VR[1]	93.92 ± 2.39	92.47 ± 1.78	88.90 ± 0.85	89.98 ± 0.57	88.30 ± 0.60
HSI[2]	24.44 ± 0.45^{a}	23.60 ± 0.51^{ab}	20.99 ± 0.91^{b}	21.00 ± 0.69^{b}	21.64 ± 0.45^{ab}
IPF[3]	22.98 ± 0.83	23.32 ± 0.92	20.41 ± 0.53	22.57 ± 0.90	22.31 ± 1.25
Whole body composition (*g* kg^{-1} *wet weight*)					
Moisture	718.23 ± 7.10	726.02 ± 4.49	706.34 ± 3.86	721.07 ± 7.25	717.12 ± 7.61
Crude protein	182.84 ± 1.45	179.22 ± 0.80	178.04 ± 1.62	177.33 ± 0.93	182.85 ± 2.95
Crude lipid	47.21 ± 1.13	51.63 ± 1.29	50.32 ± 0.73	53.31 ± 1.86	52.84 ± 1.49
Crude ash	52.22 ± 1.71	51.97 ± 1.25	53.18 ± 0.74	54.07 ± 1.51	52.80 ± 1.35
Liver lipid content	83.54 ± 2.49 ^{a}	81.93 ± 2.15 ^{a}	70.23 ± 0.87^{b}	68.51 ± 0.55^{bc}	63.37 ± 1.18^{c}

Values in each row with different superscripts have significant differences ($P < 0.05$). Data are presented as means from three replicate tanks (morphological measurements, 6 fish per tank; whole body composition, 2 fish per tank)

[1] Viscera ratio (VR) = 100 × viscera weight (g)/body weight (g)

[2] Hepatosomatic index (HSI) = 100 × hepatic weight (g)/body weight (g)

[3] Intraperitoneal fat ratio (IPF) = 100 × intraperitoneal fat weight (g)/body weight (g)

Table 4 Liver malondialdehyde (MDA) contents and enzyme activities of hybrid snakehead (mean ± S.E.M)

Items	Dietary phospholipid levels (g kg^{-1})				
	8.5	19.3	30.7	41.5	50.8
CAT (U mg $prot^{-1}$)[1]	5.32 ± 0.18^{c}	7.60 ± 0.25^{b}	8.69 ± 0.23^{a}	9.13 ± 0.18^{a}	8.21 ± 0.14^{ab}
SOD (U mg $prot^{-1}$)[2]	457.27 ± 9.42^{c}	505.54 ± 7.56^{b}	537.84 ± 7.14^{ab}	569.76 ± 7.60^{a}	573.10 ± 6.08^{a}
GPx (nmol mg $prot^{-1}$)[3]	13.65 ± 0.30	14.03 ± 0.45	15.62 ± 0.34	16.37 ± 0.75	15.07 ± 0.27
MDA (nmol mg $prot^{-1}$)[4]	0.58 ± 0.06^{a}	0.38 ± 0.05^{b}	0.33 ± 0.03^{bc}	0.21 ± 0.02^{c}	0.23 ± 0.02^{c}
FAS (nmol min^{-1} mg $prot^{-1}$)[5]	6.46 ± 0.32^{a}	4.57 ± 0.23^{b}	3.82 ± 0.17^{b}	2.43 ± 0.27^{c}	2.68 ± 0.16^{c}
CPT-1 (nmol min^{-1} mg $prot^{-1}$)[6]	5.36 ± 0.22^{c}	7.65 ± 0.17^{b}	10.52 ± 0.28^{a}	11.23 ± 0.37^{a}	9.86 ± 0.40^{a}

Values in each row with different superscripts have significant differences ($P < 0.05$). Data are presented as means from three replicate tanks (3 fish per tank)

[1] *CAT* catalase

[2] *SOD* superoxide dismutase

[3] *GPx* glutathione peroxidase

[4] *MDA* malondialdehyde

[5] *FAS* fatty acid synthase

[6] *CPT-1* carnitine palmitoyl transferase I

swimming crab (*Portunus trituberculatus*) (Li et al. 2014). Beneficial effects could be related to an increased lipids transport and lipid mobilization from the intestine to the rest of the body through enhancing intestinal health status (Chen et al. 2015), resulting in enhanced lipid deposition and increased energy availability for growth (Sánchez et al. 2014). However, some results indicated that the beneficial effects of dietary PLs on growth performance were restricted to fish larvae (Daprà et al. 2011; Feng et al. 2017). Similarly, soy lecithin inclusion did not affect survival and growth of juvenile channel catfish (Sink and Lochmann 2014). To date, the precise molecular mechanisms underlying PL requirement in early life stages of fish has not be

Table 5 Serum lipid metabolism indexes of snakehead fed with diets with different PL levels for 8 weeks (mean ± SEM)

Items	Dietary phospholipid levels (g kg^{-1})				
	8.5	19.3	30.7	41.5	50.8
TC (mmol L^{-1})[1]	2.07 ± 0.11^{c}	2.46 ± 0.18^{c}	3.14 ± 0.07^{b}	3.85 ± 0.13^{a}	4.23 ± 0.08^{a}
TG (mmol L^{-1})[2]	0.53 ± 0.05 ^{a}	0.41 ± 0.04^{ab}	0.37 ± 0.03^{b}	0.29 ± 0.03^{b}	0.32 ± 0.04^{b}
HDL-C (mmol L^{-1})[3]	1.24 ± 0.12^{d}	1.97 ± 0.12^{c}	2.26 ± 0.13^{bc}	2.97 ± 0.13 ^{ab}	3.16 ± 0.14 ^{a}
LDL-C (mmol L^{-1})[4]	0.35 ± 0.03^{a}	0.29 ± 0.02^{ab}	0.26 ± 0.03^{ab}	0.21 ± 0.01 ^{b}	0.23 ± 0.02^{b}
HDL-C/TC[5]	0.59 ± 0.02^{b}	0.79 ± 0.05 ^{a}	0.72 ± 0.03 ^{a}	0.76 ± 0.04 ^{a}	0.75 ± 0.03 ^{a}
HDL-C/LDL-C[6]	3.54 ± 0.13^{d}	6.79 ± 0.05^{c}	8.69 ± 0.17^{b}	14.14 ± 0.45 ^{a}	13.74 ± 0.21 ^{a}

Values in each row with different superscripts have significant differences ($P < 0.05$). Data are presented as means from three replicate tanks (6 fish per tank)

[1] *TC* total cholesterol

[2] *TG* triglyceride

[3] *HDL-C* high-density lipoprotein cholesterol

[4] *LDL-C* low-density lipoprotein cholesterol

[5] *HDL-C/TC* high-density lipoprotein cholesterol (mmol L^{-1}) /total cholesterol (mmol L^{-1})

[6] *HDL-C/LDL-C* high-density lipoprotein cholesterol (mmol L^{-1}) /low-density lipoprotein cholesterol (mmol L^{-1})

elucidated. At the same time, we also discovered that additional supplementation above 41.5 g kg^{-1} PLs did not improve the growth of juvenile hybrid snakehead. This finding was in agreement with other studies (Li et al. 2014; Feng et al. 2017), although the reason remains unclear. However, Zhao et al. (2013) found that SGR declined with the increasing levels of PLs (> 5.72%) in large yellow croaker larvae, which may be due to relatively lower content of n-3 HUFA in the highest level of the PL group.

In the current study, lipid content of the liver significantly decreased with increasing dietary PL level, suggesting that dietary PLs could promote liver fat metabolism. This was in accordance with the findings of Feng et al. (2017). Previous studies in mammals have demonstrated that dietary PLs could inhibit hepatic triglyceride synthesis and promote hepatic triglyceride oxidization to reduce lipid content in the liver of rats (Buang et al. 2005; Rossmeisl et al. 2014). Phosphatidylcholine is the most abundant phospholipid in eukaryotic membranes, and is also an important component of lipoproteins. Thus, PC can promote lipid transport from the liver to perihepatic tissues by enhancing the formation of very low-density lipoprotein (Yao and Vance 1988). In contrast, the lipid content of the hepatopancreas increased with PL supplementation in juvenile channel catfish (*Ictalurus punctatus*) (Sink and Lochmann 2014), *L. vannamei* (Gong et al. 2000), and *E. sinensis* (Wu et al. 2010). We further found that FAS activity significantly decreased and CPT-1 activity significantly increased in the liver with increasing dietary PLs levels. Cai et al. (2016) found that PLs could attenuate FAS mRNA expression levels and upregulated mRNA expression of CPT-1 in large yellow croaker larvae (*Larimichthys crocea*). Similar results were also obtained with mice (Liu et al. 2013). On the other hand, dietary PL supplementation also significantly influenced both FAS and CPT-1 activities in this study, two key regulatory enzymes in lipid metabolism, which are also related with fatty acid synthesis and oxidation, respectively. FAS plays a crucial role in de novo lipogenesis, while CPT-1 is involved in fatty acid oxidation in the liver. The comprehensive effects that dietary PL intervention significantly augmented CPT-1 activity, as well as reduced the activity of FAS, may impair fatty acid synthesis. Thus, less substrates are supplied for lipid peroxidation, and the elevated mitochondrial oxidation is sufficiently powerful to prevent triglyceride accumulation and oxidative stress, ultimately reducing lipid accumulation in the liver. In agreement with the present study, studies in mice suggested that dietary PL might decrease the expression of fatty acid synthesis-related genes and enhance the expression of fatty acid oxidation-related genes, eventually leading to less lipid accumulation in the liver (Liu et al. 2013; Rossmeisl et al. 2014). Previous studies demonstrated that PLs could improve emulsification and absorption of lipid in the intestine of the fish larvae (Tocher et al. 2008). However, the specific mechanisms of PL intervention on the formation of hepatic lipid metabolism still remain unclear.

This study indicated that PL-enriched diets resulted in remarkable changes in the plasma lipid profile and lipoprotein metabolism, which also has been observed in other studies (Niu et al. 2008; Hu et al. 2011; Li et al. 2014).The major findings were both a decrease in FAS activity and an increase in CPT-1 activity, and altered lipoprotein and lipid compartmentalization in plasma. However, no significant changes in postprandial plasma lipids were seen in juvenile rainbow trout (Daprà et al. 2011) and juvenile channel catfish (Sink and Lochmann 2014). In this study, the serum TG level was significantly decreased with dietary PL addition, supporting the hypothesis that dietary PLs could enhance lipid and EFA transport for growth. This coincided with the result of Niu et al. (2008). However, some studies have shown that the hemolymph TG level was not affected by dietary PL supplementation (Li et al. 2014, 2016; Sink and Lochmann 2014). On the contrary, Hu et al. (2011) found that the hemolymph TG level increased significantly as PL supplementation increased. This indicates that the modulating effects of dietary PLs on lipid metabolism in fish and crustaceans were complicated and the corresponding mechanisms involved requires further investigation. Many studies have also demonstrated the beneficial effect of PL supplementation, which generally has been attributable to the transport of dietary lipids like TG and cholesterol in the body (Coutteau et al. 1997; Li et al. 2014). This suggested that the addition of PLs significantly increased TC, contributing to TC transport from the liver to the blood (D'Abramo et al. 1982; Hu et al. 2011). Li et al. (2014) further found that the TC level was influenced by dietary phospholipid source. In our experiment, the fish fed with the PL-supplemented diets exhibited higher levels of HDL-C and lower levels of LDL-C than those fed with the PL-deficient controls; simultaneously, the values of HDL-C/TC and HDL-C/LDL-C increased remarkably. The

decline of LDL-C is especially likely since HDL-C, the TC-carrying particle in animals, was significantly increased. This implied that dietary PLs could influence lipid metabolism in snakehead, and perhaps this may help explain the reductions in TG content in the present study. This also indicated that dietary PL deficiency could induce an increase in serum TG content, consequently resulting in an increase in the liver lipid content. Under the present experimental conditions, both lower FAS activity and higher CPT-1 activity was associated with higher TC and HDL-C content and a relatively lower TG content. However, the physiological role of the enzyme is not yet fully established. Therefore, the exact mechanism by which PLs affect FAS or CPT-1 activity needs more detailed investigation.

It has been well documented that over-oxidation involved in hepatic fatty acid metabolism definitely leads to oxidative stress with excessive ROS formation (Lushchak and Bagnyukova 2006). To protect against the damaging effects of ROS, cells possess several antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Fontagne´-Dicharry et al. 2014). Usually, higher levels of SOD, CAT, and GPx activities indicated an increased antioxidant defense in fish (Zhao et al. 2016). In this study, dietary PL supplementation significantly decreased the MDA contents in the liver of juvenile snakehead, suggesting that the liver oxidative damage in juvenile fish were reduced by PLs. In order to further investigate how PLs exerted a beneficial effect on defense against oxidative stress, the specific activities of SOD, CAT, and GPx were assessed in the present study. In this study, the specific activities of both SOD and CAT were significantly higher in the PL-supplemented group than those in PL-deficient controls. The similar result was reported by other studies (Chen et al. 2015; Li et al. 2015; Cai et al. 2016). Thus, the antioxidant capacity-promoting effect of dietary PLs might contribute to the increasing defense ability to oxidative stress, resulting into less lipid peroxidation and higher growth rate (Gao et al. 2014). Interestingly, PL supplementation had no effect on the activity of GPx in this study. No significant change in the GPx activity in the present study may be attributed to the increase in other antioxidant enzymes involved in the elimination of H_2O_2, such as CAT. However, further investigation should be conducted to support our hypothesis.

In summary, this is the first study that evaluated the physiological effects of PLs in diets for juvenile hybrid snakehead. Results showed that supplementing more than 41.5 g kg^{-1} of soybean lecithin in diets could improve growth performance and enhanced antioxidant capacity of juvenile hybrid snakehead. Moreover, dietary PL supplementation decreased fat content in the liver of juvenile hybrid snakehead. However, the specific mechanisms linking phospholipid, epigenetics, and lipid metabolism warrant further investigation. These findings provided a novel insight into the lipotropic role of phospholipid as a vital nutrient of methyl-donation in the intervention of chronic metabolic diseases.

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