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Research Article

Effects of Pantothenic Acid on Growth Performance, Oxidative Stress, and Lipid Content in High-Fat-Fed *Megalobrama amblycephala*

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This study was to elucidate the effects of dietary pantothenic acid (PA) on growth, lipid metabolism, and antioxidant status in juvenile blunt snout bream *Megalobrama amblycephala*. Fish were fed with four experimental diets containing two lipid levels (5% and 11%) and two PA supplementation levels (24 mg kg^{-1} and 240 mg kg^{-1}) for eight weeks. The results showed that diets containing 11% lipid significantly increased condition factor, mesenteric fat index, triacylglycerol (TG), and cholesterol (TCH) in the liver as well as total lipid content and TCH in the muscle ($P < 0.05$), but significantly decreased specific growth rate and whole-body protein ($P < 0.05$). As the dietary lipid level increased, hepatic antioxidant enzyme activities were significantly increased ($P < 0.05$) as well as malondialdehyde content ($P < 0.05$). Besides, cytosolic cytochrome *c* concentration was also significantly ($P < 0.05$) increased, while mitochondrial protein, cytochrome *c*, and Na^+-K^+-ATPase activities were significantly decreased ($P < 0.05$). There were no significant changes on growth performance and body composition of PA supplementation. Then, significantly negative ($P < 0.05$) interactions were observed between dietary lipid and PA supplementation on EPA, DHA, and EPA + DHA content of the muscle. Saturated fatty acid (SFA) and polyunsaturated fatty acid (PUFA) contents in the liver were significantly increased ($P < 0.05$) with dietary lipid level increased, while liver monounsaturated fatty acid (MUFA) was significantly decreased ($P < 0.05$). Muscle SFA, DHA, and EPA + DHA contents were significantly decreased ($P < 0.05$) when dietary fat level increased. Meanwhile, EPA, DHA, and EPA + DHA content was significantly decreased ($P < 0.05$) with dietary high PA supplementation. On the whole, a high PA supplementation level of 240 mg kg^{-1} could alleviate lipid content accumulated and oxidative damage induced by a high-fat diet.

1. Introduction

The high utility of fat-rich feeds is a current trend in the modern aquaculture industry due to its protein sparing and growth promoting effects [1, 2]. However, most freshwater fish cannot tolerate high dietary lipids, and excess lipid may lead to fatty liver and cause dyslipidemia [3, 4]. According to our previous studies, the liver of blunt snout bream *Megalobrama amblycephala* showed a healthy status when fed with a 5% fat diet [4]. When given a 7% fat diet, blunt snout bream showed best weight gain and protein utilization [5]. However, when the dietary lipid level reached 11%, fat accumulation occurred in the liver of blunt snout bream [3]. Under normal physiological conditions, there is a balance between lipid deposition and decomposition to maintain lipid homeostasis *in vivo*. When blunt snout bream cannot tolerate high dietary lipid, the excess lipid may cause multiple adverse consequences, including retarded growth, fatty liver, fat deposition, and oxidative damage [4]. Thus, it is necessary to find an effective way to alleviate these problems.

Dietary manipulation has been proven to be a useful approach for alleviating the adverse effects. Pantothenic acid (PA) is an essential water-soluble vitamin for fish. PA functions as a component of coenzyme A (CoA) which plays a core

role in numerous intermediary metabolic reactions in carbohydrates and lipid metabolism [6]. CoA forms a thioester bond with acyl carbons and thus mediates acyl transfer reaction of fatty acid synthesis, fatty acid oxidation, and cholesterol synthesis in diverse pathways [7]. Besides, PA also donates a 4′-phosphopantetheine moiety to acyl-carrier protein (ACP), which carries the growing acyl chain from one enzyme to another [8]. Previous studies indicated that dietary PA deficiency in grass carp (*Ctenopharyngodon idella*) and grouper (*Epinephelus malabaricus*) would result in fish hepatic fat deposition and lipid metabolic dysfunction [9, 10]. In a study of blunt snout bream, increased dietary PA levels enhanced the expressions of various genes involved in liver fatty acid synthesis, resulting in the enhanced liver SFA synthesis [11]. However, whether dietary PA has a positive regulatory function on the lipid metabolism in dyslipidemia fish is still unknown. Considering biological functions of PA in lipid metabolism, the investigations of PA on alleviating the adverse effect induced by a high-fat diet are worthwhile.

In order to maintain the body redox equilibrium, the antioxidant systems of the SOD, CAT, and GPX enzymes play important roles, while MDA content represents direct evidence of lipid peroxidation caused by free radicals or disordered lipid metabolism [11]. Besides, mitochondria which play a vital role in cellular homeostasis are considered to be the most critical subcellular organelles for mediating oxidative stress [12, 13]. Meanwhile, mitochondria are also recognized as a significant source of reactive oxygen species (ROS) production and endogenous oxidative stress [14]. So far, studies have extensively investigated the effects of nutritional factors on the antioxidant defenses of fish. Recently, PA and its related compounds have been reported to protect the cell membrane against damage caused by lipid peroxidation in an *in vitro* study [15], indicating the potential correlation between dietary PA levels and body antioxidant capability. Our previous study showed the PA could increase liver CAT activities and decrease liver MDA content [11]. PA supplementation also increased hepatic GSH levels in Jurkat cells that was attributed to increased ATP production as a result of increased mitochondrial CoA levels [16]. Pantothenic supplementation also increased hepatic GSH and liver resistance to irradiation, suggesting that GSH peroxidase and phospholipid-hydroperoxide GSH peroxidase may have detoxified any hydroperoxides formed. Pantothenic acid also prevented the collapse of mitochondrial membrane potential and restored ATP synthesis levels as well as the activity of antioxidant enzymes, such as catalase, GSH peroxidase, GSH reductase, and the NADPH forming malic enzyme in vitro and in vivo [17]. However, little information is given about how dietary PA works on liver antioxidant capacity and how the mitochondrial respiration chain functions in dyslipidemia fish.

Bearing these in mind, the present study was conducted to elucidate the effects of dietary PA supplementation on growth, lipid metabolism, and antioxidant status of juvenile blunt snout bream (*Megalobrama amblycephala*), an economically important herbivorous freshwater fish widely cultured in China. The data can provide further information about nutritional modulation on antistress mechanisms and dyslipidemia in freshwater fish.

2. Material and Methods

2.1. Diets. A 2×2 factorial design with three replicates was used in the experiment. Four semipurified diets were formulated to contain two lipid levels (5 and 11%) and two PA supplementation levels (24 and 240 mg kg^{-1}). Those four experimental diets were named as 5/24, 5/240, 11/24, and 11/240, representing the lipid level with PA supplementation representatively. Formulation and proximate composition of the basal diet were presented in Table 1. The experimental diets were formulated to meet the optimal dietary protein, lipid, and pantothenic acid requirements of blunt snout bream [5, 11]. PA supplied in diets was detected to be 26.30 mg kg^{-1} and 249.70 mg kg^{-1} via high-performance liquid chromatography. All diets were prepared in the laboratory as detailed by Qian et al. [11].

2.2. Fish and Feeding Trial. Blunt snout bream juveniles were obtained from the Fish Hatchery of Yangzhou (Jiangsu, China). The feeding trial was performed in an indoor recirculating aquaculture system. Fish were reared in several plastic tanks ($3 \times 0.8 \times 0.8$ m, $L : W : H$) for two weeks to acclimate to the experimental conditions before the experiment. During the acclimation period, fish were fed 5/24 experimental diets. Then, fish (25.97 ± 1.01 g) were randomly divided into 12 plastic tanks ($3 \times 0.8 \times 0.8$ m, $L : W : H$) and were fed with experimental diets to apparent satiation thrice daily (8:00, 12:00, and 16:00 h) for eight weeks.

2.3. Sample Collection. At the end of the feeding trial, fish were deprived of feed for 24 h. The fish were then anaesthetized in diluted MS-222 (1 : 10000), counted, and weighed. Six fish per tank were collected and stored at -70°C for body composition analysis. A total of 8 fish per tank were sacrificed for blood, liver, and muscle collection. Then, the blood samples were allowed to clot at 4°C overnight and centrifuged at 5000×g at 4°C for 20 min to collect serum. Samples of the individual liver were quickly collected and stored at -70°C for determination of oxidative stress parameters and mitochondrial status analysis.

2.4. Proximate Composition Analysis. The proximate composition of diets and fish were analyzed following the detailed methods [18]. Moisture was determined by drying to constant weight at 105°C. Crude protein (nitrogen × 6.25) was determined by using the Kjeltec Analyzer Unit (2300, FOSS Tector, Hoganas, Sweden). Crude lipid was determined by ether extraction method using the Soxtec Auto Extraction Unit (2050, Sweden). Ash content was measured by combustion at 550°C for 4 h.

2.5. Determination of Oxidative Stress Parameters in the Liver. For analyzing oxidative stress in liver, we determined the concentration of total superoxide dismutase (T-SOD), catalase (CAT), glutathione peroxidase (GPX) activities, glutathione (GSH), and malondialdehyde (MDA) following the methods described by Lygren et al. [19]. All the measurements were operated by the commercial kits (Nanjing Jiancheng Bioengineering Institute, China).

Table 1: Formulation and proximate composition (% air-dry basis) of the diets.

Ingredient ($g\,kg^{-1}$)	5/24	5/240	11/24	11/240
Fish meal[1]	50	50	50	50
Soybean meal[2]	100	100	100	100
Casein[3]	270	270	270	270
Soybean oil[2]	24	24	54	54
Fish oil[2]	24	24	54	54
Corn starch[2]	250	250	250	250
α-Starch	50	50	50	50
Cellulose[4]	200	200	140	140
Premix without pantothenic acid[5, 6]	10	10	10	10
Calcium biphosphate[7]	18	18	18	18
Sodium chloride	4	4	4	4
Pantothenic acid	24×10^{-3}	240×10^{-3}	24×10^{-3}	240×10^{-3}
Proximate composition ($g\,kg^{-1}$)				
Crude protein	306.9	306.1	301.4	305.2
Crude lipid	52.6	52.8	113.3	112.7
Ash	68.3	68.2	68.6	67.8
Crude fiber	223.3	222.9	155.5	157.1

[1]Fishmeal mainly made by *Engraulis japonicus*, crude protein 63%, provided by Tech-bank Co., Ltd. (Ningbo, China). [2]Soybean meal provided by Zhengchang Feed Industry Co., Ltd. (Jiangsu, China), crude protein 43%. [3]Provided by Hulunbeier Sanyuan Milk Co., Ltd. (Inner Mongolia, China). [4]Provided by Yifeng Food Additives Co., Ltd. (Shanghai, China). [5]Provided by Huamu Animal Science Research Institute (Nanjing, China). [6]Premix provided the following minerals and/or vitamins (per kg premix): FeSO4·7H2O 25.00 g, copper-chelated peptide 10.00 g, zinc-chelated peptide 80.00 g, MnSO4·4H2O 7.00 g, Na2SeO3 0.04 g, KI 0.026 g, CoCl2·6H2O 0.10 g, vitamin A 900,000.00 IU, vitamin D 200,000.00 IU, vitamin E 4500.00 mg, vitamin K_3 220.00 mg, vitamin B_1 320.00 mg, vitamin B_2 1090.00 mg, vitamin B_6 500.00 mg, vitamin B_{12} 1.60 mg, vitamin C 10,000.00 mg, folic acid 165.00 mg, choline 120,000.00 mg, niacin 2500.00 mg, and myoinositol 15,000.00 mg. [7]Provided by Sinopharm Chemical Reagent Co., Ltd. (Beijing, China).

2.6. Isolation of Liver Mitochondrial and Cytoplasmic Fractions. The isolation of liver mitochondria was followed by the methods of Suarez and Hochachka [20]. Briefly, liver tissue (0.1-0.2 g) was excised and homogenized ($w/v = 1 : 10$), then the homogenate was centrifuged twice at 800×g for 10 min at 4°C. The superficial lipid layer was removed. After that, the remaining supernatant was then centrifuged at 15,000×g for 15 min at 4°C. The supernatant and the residual pellet were considered to be the cytoplasmic fraction and the mitochondrial fraction, respectively. The obtained mitochondrial fraction was washed three times in medium and was then resuspended in the medium with $1\,mg\,ml^{-1}$ fatty acid-free bovine serum albumin.

Cytochrome *c* contents in mitochondrial and cytosol fractions were measured according to the method of Sun et al. [21]. For enzymatic analysis, the mitochondrial pellets were suspended in the isolation medium. Succinate dehydrogenase (SDH) was estimated with sodium succinate as substrate according to the method of Philip et al. [22]. The Na^+- K^+-ATPase (3.6.3.9) activity was measured according to McCormick and Björnsson [23]. Mitochondrial protein concentration was determined by using the method of Lowry et al. [24]. Enzymatic activities were expressed in units (U) per milligram of mitochondrial protein. All the measurements were operated by the commercial kits (Nanjing Jiancheng Bioengineering Institute, China).

2.7. Tissue Lipid Concentration and Fatty Acid Composition. Total lipid content in liver and muscle was determined as described by Folch et al. [25] using chloroform : methanol (2 : 1, *v*/*v*) to extract the total lipid. Total triacylglycerol (TG) and total cholesterol (TCH) concentrations were measured with commercial kits (Nanjing Jiancheng Bioengineering Institute, China). According to the method of Xu et al. [26], the relative quantity of each FA of lipid was determined by measuring the area under the chromatograph peak.

2.8. Total RNA Extraction, Reverse Transcription, and Real-Time PCR. According to the method of Xu et al. [26], the relative expression of *COX1*, *COX2*, *ND1*, *CYTB*, and *ATP6* were tested with real-time PCR analysis. Briefly, total RNA in mitochondrial of juvenile blunt snout bream was extracted using Trizol reagent (Invitrogen, USA) and treated with RQ1 RNase-free DNase (Promega, USA) to eliminate genomic DNA contamination. Quantity and purity of RNA was determined by absorbance measures (A260/280), and its integrity was tested by electrophoresis in 1.0% formaldehyde denaturing agarose gels. cDNA was generated from 500 ng DNase-treated RNA using ExScript™ RT-PCR kit (Takara, Japan). The resulting first-strand cDNA from each tissue was then diluted and used as template for PCRs. The set of primers for *COX1*, *COX2*, *ND1 CYTB*, and *ATP6* genes were designed by Primer 5 according to the codifying sequences obtained in our laboratory (Table 2). Melting curve analysis of amplification products was performed at the end of each PCR reaction to confirm that the specific products were obtained. Each sample was run in triplicate. PCR reactions without the addition of the template were

Table 2: Nucleotide sequences of the primers used to assay gene expression by real-time PCR.

Target gene	GenBank accession number	Forward (5′-3′)	Reverse (5′-3′)	Length (bp)
COX1	NC010341	CATACTTTACATCCGCAACA	TCCTGTCAATCCACCCAC	158
COX2	NC010341	AACCCAGGACCTTACACCC	CCCGCAGATTTCAGAACA	232
ND1	NC010341	CTGACCACTAGCCGCAATA	GGAAGAAGAGGGCGAAGG	140
CYTB	NC010341	CATACACTATACCTCCGACAT	TCTACTGAGAAGCCACCT	357
ATP6	NC010341	TGCTGTGCCACTATGACT	ATTATTGCCACTGCGACT	315
β-Actin	AY170122.2	TCGTCCACCGCAAATGCTTCTA	CCGTCACCTTCACCGTTCCAGT	152

Note: *COX1*: cytochrome-c oxidase subunit 1; *COX2*: cytochrome-c oxidase subunit 2; *ND1*: NADH dehydrogenase subunit 1; *CYTB*: cytochrome b; *ATP6*: ATP synthase subunit 6.

Table 3: Effects of dietary pantothenic acid and lipid levels on growth performance and body parameters of juvenile blunt snout bream.

Diets Lipid (%)	Pantothenic acid (mg kg^{-1})	FBW	SGR	CF	VBR	HSI	DP	MFI
5	24	53.41	1.29	2.12	8.78	1.20	72.40	3.00
	240	54.33	1.32	2.10	8.88	1.14	72.32	2.80
11	24	44.88	0.98	2.21	8.76	1.36	73.05	3.74
	240	51.79	1.22	2.17	8.61	1.25	72.75	3.60
Pooled SEM		3.70	0.12	0.05	0.55	0.17	1.13	0.34
Means of main effect								
Lipid levels								
5		53.87^{a}	1.30^{a}	2.11^{b}	8.83	1.17	72.36	2.90^{b}
11		48.33^{b}	1.10^{b}	2.19^{a}	8.68	1.31	72.90	3.67^{a}
Pantothenic acid levels								
24		49.15	1.13	2.17	8.77	1.28	72.72	3.37
240		53.06	1.27	2.14	8.74	1.20	72.53	3.20
Two-way ANOVA								
Lipid		0.040	0.030	0.030	0.672	0.225	0.461	0.006
Pantothenic acid		0.122	0.106	0.360	0.939	0.462	0.792	0.441
Interaction		0.223	0.194	0.754	0.728	0.806	0.882	0.886

Note: means and pooled SEM are presented for each parameter. Means in the same row with different superscripts are significantly different ($P < 0.05$). SGR: specific growth rate (%day^{-1}) = (Ln W_t − Ln W_0) × 100/day. CF: condition factor (%) = body weight (g) × 100/body length (cm)^3. VBR: viscera/body ratio (%) = viscera weight (g) × 100/body weight (g). HSI: hepatosomatic index (%) = liver weight (g) × 100/body weight (g). DP: dressout percentage (%) = carcass (with head and viscera removed) weight (g) × 100/body weight (g). MFI: mesenteric fat index (%) = mesenteric fat weight (g) × 100/body weight (g).

used as negative controls. At the end of the reaction, the fluorescent data were converted into Ct values. Each gene expression level was normalized to β-actin using the $2^{-\Delta\Delta CT}$ method [27].

2.9. Statistical Analysis. According to the method of Xu et al. [26], data were subjected to two-way analysis of variance (ANOVA) using the SPSS statistical package version 20.0 (SPSS Inc., Chicago Avenue, IL, USA) to test the effects of dietary lipid, PA (main effect), and interaction between lipid and pantothenic acid after testing the homogeneity of variances with the Levene test. If an interaction between dietary lipid and pantothenic acid was detected ($P < 0.05$), Duncan's multiple range test was used to rank the means. A significant ($P < 0.05$) main effect and no interaction allowed data to be pooled before mean separation (main effect means for dietary lipid or pantothenic acid) by Duncan's multiple range test. All data were presented as means ± S.E.M (standard error of the mean) of three replicates.

3. Results

3.1. Growth Performance. The growth performance was determined in juvenile blunt snout bream fed with experimental diets containing different lipid level and PA supplementation. The SGR of fish was reduced ($P < 0.05$) significantly as the dietary lipid levels increased from 5% to 11%, whereas CF and MFI were significantly increased ($P < 0.05$) (Table 3). VBR, HSI, and DP were not markedly influenced by dietary lipid and PA supplementation ($P > 0.05$) (Table 3). There was no significant interaction effect on above mentioned growth performance parameters between PA supplementation and lipid contents ($P > 0.05$).

3.2. Whole-Body Composition. For analyzing proximate composition of juvenile blunt snout bream, we determined the proportion of moisture, protein, lipid, and ash. As the dietary lipid content increased from 5% to 11%, the lipid content of fish increased markedly ($P < 0.05$), whereas

Table 4: Effects of different dietary pantothenic acid and lipid contents on whole-body composition of juvenile blunt snout bream.

Diets Lipid (%)	Pantothenic acid (mg kg^{-1})	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
5	24	73.45	15.65	5.78	3.73
	240	73.83	15.59	5.88	3.69
11	24	71.73	14.58	8.62	3.83
	240	72.67	14.48	8.05	3.65
Pooled SEM		1.07	0.42	1.01	0.13
Means of main effect					
Lipid levels					
5		73.64	15.62^{a}	5.83^{b}	3.71
11		72.20	14.53^{b}	8.34^{a}	3.74
Pantothenic acid levels					
24		72.59	15.12	7.20	3.78
240		73.25	15.04	6.97	3.67
Two-way ANOVA					
Dietary lipid		0.059	0.003	0.004	0.693
Pantothenic acid supplementation		0.344	0.771	0.712	0.197
Interaction		0.679	0.958	0.610	0.432

Note: means and pooled SEM are presented for each parameter. Means in the same row with different superscripts are significantly different ($P < 0.05$).

protein content showed a significant decrease ($P < 0.05$) (Table 4). No significant effects were observed on whole-body ash and moisture contents of juvenile blunt snout bream ($P > 0.05$). There was no significant interaction effect on whole-body composition between PA supplementation and lipid contents ($P > 0.05$).

3.3. Transmission Electron Microscope Images of Hepatocyte, Nucleus, and Endoplasmic Reticulum (ER) Ultrastructure in Blunt Snout Bream. As shown in Figures 1(a) and 1(b), fish fed with diets containing 5% lipid demonstrated a normal hepatocyte ultrastructure. Each hepatocyte had a large, round nucleus centrally located within a moderate cytoplasm (Figure 1(a)). The nucleus was ovoid and contained a prominent nucleus (Figure 1(b)). The endoplasmic reticulum was well developed and often adjacent to the nucleus (Figure 1(a)). Hepatocytes displayed dark, slender mitochondria with well-developed cristae and matrix (Figure 1(b)). No significant differences were found between the 5/24 and 5/240 groups. However, some abnormalities were observed in livers of fish fed with diets containing 11% lipid. Hepatocytes exhibited many large, electron-dense fat droplets (Figure 1(c)). These extensive intracellular lipid droplets resulted in the displacement of the nucleus to the cell margin and a loss of cytoplasm (Figure 1(d)). The nucleus was atrophic and presented as a polygon (Figure 1(c)). The endoplasmic reticulum developed poorly and lost most of its ribosomes (Figure 1(d)). Disruption was observed in all of the cellular membranes, including plasma, mitochondria, and nuclear membranes. The mitochondria exhibited a loss of cristae and matrix, as well as great hydropic changes of matrix density (Figure 1(f)).

3.4. Lipid Levels in Tissues and Plasma. To investigate the lipid levels in the liver, muscle, and plasma of blunt snout bream, we detected the total lipid content and the TG and TCH concentration (Table 5). No significant ($P > 0.05$) interaction effects between dietary lipid and PA were observed. In the liver, we observed significantly higher levels of total lipid, TG, and TCH in fish fed with 11% dietary lipid ($P < 0.05$). The significantly higher levels of total lipid and TCH were also found in the muscle. Plasma TG was significantly lower ($P < 0.05$). What is more, dietary PA supplementation showed no significant ($P > 0.05$) effects on body lipid level.

3.5. Fatty Acid Composition. The fatty acid composition of the liver and muscle was presented in Table 6 and Table 7. Fish fed with high-fat diets exhibited significantly ($P < 0.05$) higher saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) than those fed with low-fat diets in the liver, including C18:0, C20: 0, C18: 2n-6, and C20: 5n-3(EPA) contents. On the contrary, monounsaturated fatty acids (MFA) were significantly decreased ($P < 0.05$). Though insignificant, PA supplementation showed a positive effect on fatty acid synthesis ($P > 0.05$). Significant ($P < 0.05$) interactions between dietary lipid and PA supplementation were found on liver C18: 3n-6 and C22:5n-3 of blunt snout bream.

In muscle, SFA and MUFA of C16: 1n-9 and C18: 1n-9 were significantly ($P < 0.05$) reduced when dietary lipid level increased from 5% to 11%. Meanwhile, PUFA was significantly increased due to C18: 2n-6, C18: 3n-3, and C18: 3n-6 ($P < 0.05$), rather than DHA ($P < 0.05$). However, DHA and EPA + DHA content was significantly decreased regardless of increased lipid level or PA supplementation. Significant ($P < 0.05$) interactions between dietary lipid and PA supplementation were found on muscle EPA, DHA, C22: 5n-3, and EPA + DHA of blunt snout bream, which also showed negative effect with PA supplementation increased.

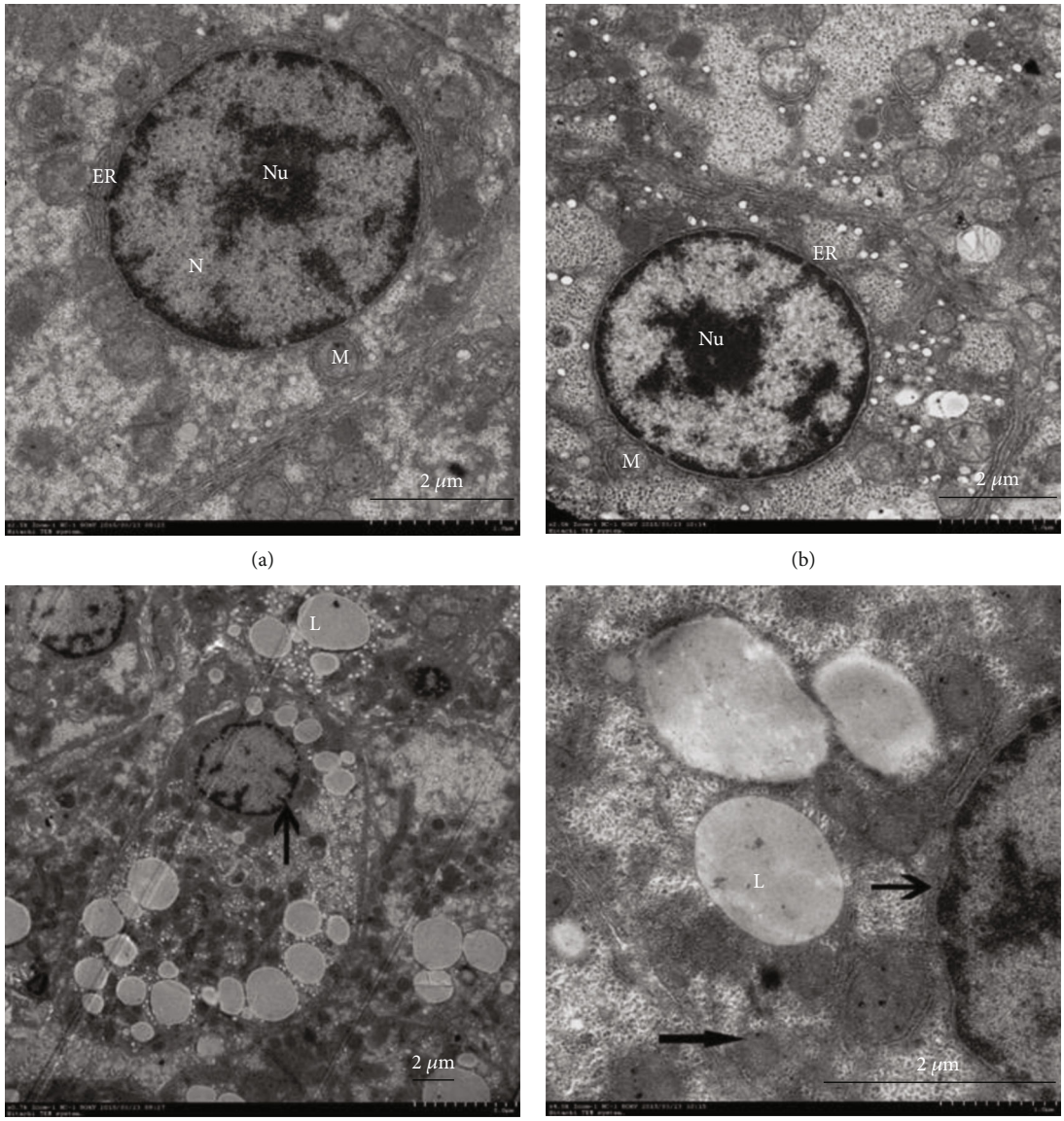
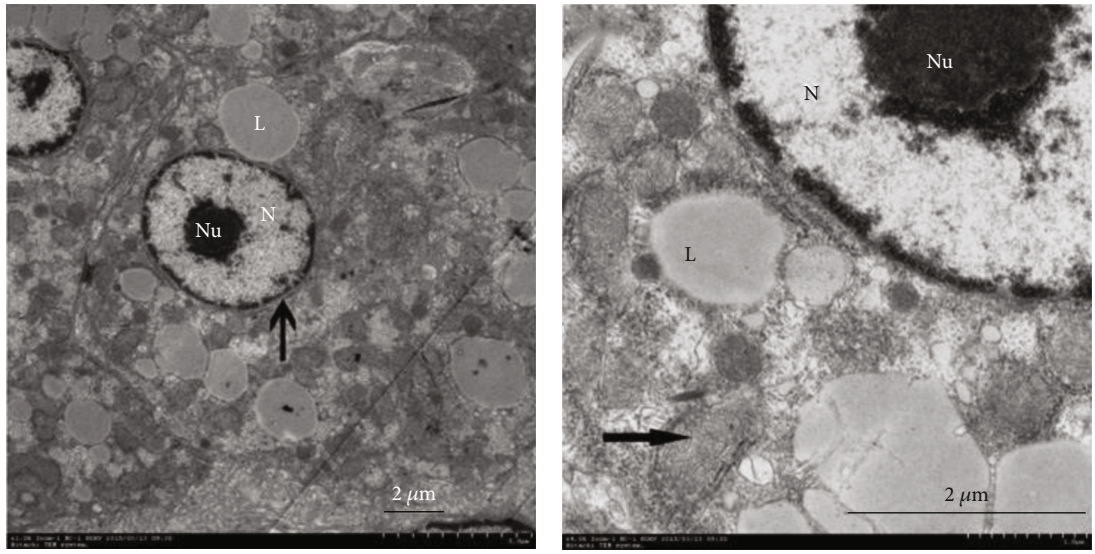


Figure 1: Continued.



(e) (f)

Figure 1: Transmission electron microscope images of blunt snout bream hepatocyte. Note: nucleus, mitochondria, and ER ultrastructure: N (nucleus), L (lipid droplet), M (mitochondrion), ER (endoplasmic reticulum). (a) Hepatocytes of fish in 5/24 group with normal structure; (b) hepatocytes of fish in 5/240 group with normal structure; (c, d) hepatocytes of fish in 11/24 group; (e, f) hepatocytes of fish in 11/240 group; bar = 2 μm.

Table 5: Effects of different dietary pantothenic acid and lipid contents on tissues and plasma lipid levels of juvenile blunt snout bream.

Diets		Liver		Muscle		Plasma	
Lipid (%)	Pantothenic acid (mg kg^{-1})	TG (mg g^{-1})	TCH (mg g$^{-1)}$	TG (mg g^{-1})	TCH (mg g^{-1})	TG (mmol L^{-1})	TCH (mmol L^{-1})
5	24	33.21	1.85	0.42	0.27	7.14	2.49
	240	34.41	2.13	0.45	0.27	7.09	2.22
11	24	45.59	3.43	0.5	0.35	5.65	2.08
	240	48.06	3.06	0.49	0.32	5.58	2.16
Pooled SEM		6.22	0.27	0.10	0.03	0.89	0.32
Means of main effect							
Lipid levels							
5		33.81^{b}	1.99^{b}	0.44	0.27^{b}	7.11^{a}	2.36
11		46.83^{a}	3.24^{a}	0.49	0.34^{a}	5.62^{b}	2.12
Pantothenic acid levels							
24		39.4	2.64	0.46	0.31	6.39	2.29
240		41.23	2.59	0.47	0.30	6.34	2.19
Two-way ANOVA							
Dietary		0.009	0.001	0.399	0.013	0.026	0.268
Pantothenic acid		0.644	0.805	0.892	0.556	0.921	0.631
Interaction		0.871	0.084	0.776	0.516	0.992	0.404

Note: means and pooled SEM are presented for each parameter. Means in the same row with different superscripts are significantly different ($P < 0.05$).

3.6. Liver Antioxidant Status. Oxidative stress parameters in the liver were remarkably affected by the high-fat diet. As the dietary lipid level increased from 5% to 11%, we found a significant increase in the concentration of MDA, T-SOD, CAT, and GSH-Px, whereas a decrease in the concentration of GSH ($P < 0.05$) (Table 8). As dietary PA supplementation increased, GSH-Px activity of the liver was significantly decreased ($P < 0.05$), whereas the concentration of GSH significantly increased. T-SOD, CAT, and MDA concentrations showed a decreased tendency but without significance ($P > 0.05$). No significant interaction effects were found on *antioxidant status* between dietary lipid levels and PA supplementation ($P > 0.05$).

3.7. Mitochondrial Status. Liver mitochondrial status of blunt snout bream was affected by dietary lipid levels and PA supplementation in Table 9. Mitochondrial protein, cytochrome *c*, and NA^{+}-K^{+}-ATPase activity decreased significantly ($P < 0.05$) as the dietary lipid levels increased, whereas cytosolic cytochrome *c* showed an opposite

Table 6: Effects of different dietary pantothenic acid and lipid levels on liver fatty acid composition (% of total fatty acids) of juvenile blunt snout bream.

Fatty acid composition (% of total fatty acids)	Diets	5/24	5/240	11/24	11/240	Pooled SEM	Lipid (%)		Pantothenic acid (mg kg^{-1})		Two-way ANOVA		
							5	11	24	240	Lipid	Pantothenic acid	Interaction
C14:0	0.307	0.68	0.66	0.55	0.52	0.07	0.67^{a}	0.54^{b}	0.61	0.59	0.021	0.601	0.897
C16:0	12.481	14.04	13.94	14.62	14.43	0.66	13.99	14.52	14.33	14.19	0.223	0.736	0.911
C18:0	3.937	12.37	13.44	14.29	14.03	0.69	12.91^{b}	14.16^{a}	13.33	13.73	0.019	0.370	0.156
C20:0	0.922	0.11	0.11	0.14	0.15	0.02	0.11^{b}	0.15^{a}	0.13	0.13	0.004	0.842	0.654
ΣSFA[1]	17.744	27.43	28.49	29.90	29.41	1.15	27.67^{b}	29.36^{a}	28.40	28.64	0.040	0.743	0.342
C16:1n-9	0.640	2.83	2.71	1.85	1.84	0.27	2.77^{a}	1.84^{b}	2.34	2.28	0.001	0.723	0.750
C18:1n-9	23.462	41.22	40.24	32.95	34.66	1.97	40.73^{a}	33.81^{b}	37.08	37.45	0.001	0.769	0.298
C20:1n-9	0.073	1.32	1.23	1.01	1.07	0.07	1.27^{a}	1.04^{b}	1.17	1.15	0.001	0.674	0.134
ΣMUFA[2]	24.175	45.36	44.18	35.81	37.57	2.17	44.77^{a}	36.69^{b}	40.59	40.88	0.001	0.834	0.299
C18:2n-6	49.517	10.69	10.29	13.46	12.85	0.70	10.49^{b}	13.16^{a}	12.08	11.57	0.001	0.268	0.809
C18:3n-3	2.824	0.46	0.39	0.63	0.60	0.09	0.43^{b}	0.62^{a}	0.55	0.50	0.009	0.396	0.682
C18:3n-6	2.853	0.20^{a}	0.23^{ab}	0.27^{c}	0.25^{bc}	0.02	0.22	0.26	0.24	0.24	0.005	0.733	0.042
C20:5n-3 (EPA)	3.148	0.25	0.24	0.42	0.45	0.06	0.24^{b}	0.44^{a}	0.34	0.34	0.001	0.822	0.478
C22:5n-3	0.024	0.19^{a}	0.17^{a}	0.28^{b}	0.35^{c}	0.03	0.18	0.32	0.23	0.26	0.001	0.146	0.041
C22:6n-3 (DHA)	1.198	4.64	5.30	5.95	5.66	0.95	4.97	5.81	5.29	5.48	0.189	0.755	0.439
ΣPUFA[3]	55.565	16.43	16.62	21.01	20.17	1.01	16.53^{b}	20.59^{a}	18.72	18.39	0.001	0.611	0.432
EPA + DHA	4.347	4.89	5.54	6.37	6.12	0.99	5.21	6.24	5.63	5.83	0.128	0.755	0.480

Note: means and pooled SEM are presented for each parameter. Means in the same row with different superscripts are significantly different ($P < 0.05$). [1]Saturated fatty acids. [2]Monounsaturated fatty acids. [3]Polyunsaturated fatty acids.

Table 7: Effects of different dietary pantothenic acid and lipid levels on muscle fatty acids composition (% of total fatty acids) of juvenile blunt snout bream.

Fatty acid composition (% of total fatty acids)	Diets	5/24	5/240	11/24	11/240	Pooled SEM	Lipid (%)		Pantothenic acid (mg kg^{-1})		Two-way ANOVA		
							5	11	24	240	Lipid	Pantothenic acid	Interaction
C14:0	0.307	0.50	0.52	0.48	0.46	0.03	0.51	0.47	0.49	0.49	0.076	0.788	0.400
C16:0	12.481	17.53	17.37	16.14	17.05	0.67	17.45	16.59	16.84	17.21	0.068	0.384	0.224
C18:0	3.937	7.40	6.41	6.23	6.50	0.66	6.91	6.37	6.82	6.46	0.221	0.400	0.158
C20:0	0.922	0.12	0.11	0.14	0.13	0.03	0.12	0.13	0.13	0.12	0.324	0.566	0.888
ΣSFA[1]	17.744	25.84	24.65	23.24	24.38	0.90	24.98^{a}	23.57^{b}	24.27	24.28	0.030	0.966	0.071
C16:1n-9	0.640	2.48	3.04	1.88	1.93	0.26	2.76^{a}	1.90^{b}	2.18	2.49	0.001	0.088	0.143
C18:1n-9	23.462	28.76	33.07	28.20	28.22	2.32	29.25^{a}	28.21^{b}	28.48	28.98	0.031	0.736	0.742
C20:1n-9	0.073	0.75	0.83	0.69	0.71	0.13	0.79	0.70	0.72	0.77	0.294	0.538	0.718
ΣMUFA[2]	24.175	31.99	33.62	30.77	30.85	2.35	32.80	30.81	31.38	32.23	0.204	0.568	0.606
C18:2n-6	49.517	18.92	21.39	26.76	25.59	1.34	20.16^{b}	26.17^{a}	22.84	23.49	0.001	0.452	0.057
C18:3n-3	2.824	0.96	1.14	1.35	1.50	0.07	1.05^{b}	1.42^{a}	1.15^{b}	1.32^{a}	0.001	0.004	0.742
C18:3n-6	2.853	0.27	0.27	0.36	0.33	0.03	0.27^{b}	0.35^{a}	0.32	0.30	0.009	0.534	0.391
C20:5n-3 (EPA)	0.148	0.73^{a}	0.50^{c}	0.60^{b}	0.64^{b}	0.03	0.62	0.62	0.66	0.57	0.824	0.001	0.001
C22:5n-3	0.024	0.51^{a}	0.35^{b}	0.47^{ba}	0.44^{a}	0.04	0.43	0.46	0.49	0.40	0.405	0.007	0.044
C22:6n-3 (DHA)	0.198	7.59^{a}	5.52^{b}	5.44^{b}	5.13^{b}	0.53	6.55	5.29	6.51	5.32	0.005	0.007	0.028
ΣPUFA[3]	55.565	28.97	29.18	34.98	33.62	1.83	29.07^{b}	34.30^{a}	31.98	31.40	0.002	0.619	0.506
EPA + DHA	0.347	8.31^{a}	6.02^{b}	6.04^{b}	5.77^{b}	0.54	7.17	5.91	7.18	5.90	0.005	0.005	0.016

Note: means and pooled SEM are presented for each parameter. Means in the same row with different superscripts are significantly different ($P < 0.05$). [1]Saturated fatty acids. [2]Monounsaturated fatty acids. [3]Polyunsaturated fatty acids.

Table 8: Effects of different dietary pantothenic acid and lipid contents on hepatic antioxidant status of juvenile blunt snout bream.

Diets		T-SOD (U mgPro^{-1})	CAT (U mgPro^{-1})	GSH (nmol mgPro^{-1})	GSH-Px (U mgPro^{-1})	MDA (nmol mgPro^{-1})
Lipid (%)	Pantothenic acid (mg kg^{-1})					
5	24	96.86	48.69	10.00	33.86	8.50
	240	102.925	46.95	11.26	26.86	7.36
11	24	127.81	65.02	6.97	47.20	16.66
	240	121.25	60.55	8.51	41.79	14.62
Pooled SEM		13.07	5.11	0.74	4.83	1.15
Means of main effect						
Lipid levels						
5		99.89^{b}	47.82^{b}	10.63^{a}	30.36^{b}	7.93^{b}
11		129.53^{a}	62.79^{a}	7.74^{b}	44.50^{a}	15.64^{a}
Pantothenic acid levels						
24		112.33	56.86	8.48^{r}	40.53^{q}	12.58
240		117.08	53.75	9.89^{q}	34.33^{r}	10.99
Two-way ANOVA						
Lipid		0.008	0.002	0.001	0.001	0.001
Pantothenic acid		0.587	0.364	0.022	0.027	0.311
Interaction		0.880	0.684	0.781	0.737	0.767

Note: means and pooled SEM are presented for each parameter. Means in the same row with different superscripts are significantly different ($P < 0.05$).

Table 9: Effects of different dietary pantothenic acid and lipid contents on mitochondrial status of juvenile blunt snout bream.

Diets		Mitochondrial protein (mg g^{-1} wet tissue)	SDH[1] (U mg^{-1} prot)	NA^{+}-K^{+}-ATPase (U mg^{-1} prot)	Mitochondrial cytochrome c (μg g^{-1} prot)	Cytosolic cytochrome c (μg g^{-1} prot)
Lipid (%)	Pantothenic acid (mg kg^{-1})					
5	24	4.09	0.13	9.18	44.43	13.73^{b}
	240	3.77	0.13	9.77	48.13	13.68^{b}
11	24	2.32	0.12	6.91	24.89	15.93^{a}
	240	2.40	0.12	8.83	32.48	14.12^{b}
Pooled SEM		0.43	0.02	0.65	5.93	0.56
Means of main effect						
Lipid levels						
5		3.93^{a}	0.13	9.48^{a}	46.28^{a}	13.70
11		2.36^{b}	0.12	7.87^{b}	28.68^{b}	15.02
Pantothenic acid levels						
24		3.20	0.12	8.05^{r}	34.66	14.83
240		3.08	0.12	9.30^{q}	40.30	13.90
Two-way ANOVA						
Lipid		0.001	0.459	0.005	0.004	0.004
Pantothenic acid		0.674	0.989	0.018	0.232	0.022
Interaction		0.490	0.925	0.157	0.667	0.028

Note: means and pooled SEM are presented for each parameter. Means in the same row with different superscripts are significantly different ($P < 0.05$). [1]SDH: succinate dehydrogenase.

variation trend ($P < 0.05$). Moreover, NA^{+}-K^{+}-ATPase activity was increased significantly with increased dietary PA supplementation ($P < 0.05$). No significant differences were observed in mitochondrial SDH activity ($P > 0.05$). There was a significant interaction between dietary lipid and PA supplementation on cytosolic cytochrome c ($P < 0.05$).

3.8. Liver Mitochondrial-Related Gene Expression Levels. Results of liver mitochondrial COX1, COX2, ND1, CYTB, and ATP6 gene expression levels were presented in Table 10. There were no significant differences on mitochondrial genes COX1, COX2, ND1, CYTB, and ATP6 among groups ($P > 0.05$). No significant interaction effects were

Table 10: Liver mitochondrial gene relative mRNA expression of different dietary pantothenic acid and lipid levels of juvenile blunt snout bream.

Diets Lipid %	Pantothenic acid mg kg^{-1}	*COX1*	*COX2*	*ND1*	*CYT*	*ATP6*
5	24	1.10	1.04	1.06	1.04	1.02
	240	1.05	1.02	1.89	1.41	1.24
11	24	1.37	1.11	2.89	0.91	0.81
	240	1.14	0.95	1.89	1.51	0.90
Pooled SEM		0.41	0.37	0.83	0.39	0.26
Means of main effect						
Lipid levels						
5		1.07	1.03	1.48	1.22	1.13
11		1.26	1.03	2.39	1.21	0.85
PA levels						
24		1.24	1.08	1.98	0.97	0.91
240		1.09	0.99	1.89	1.46	1.07
Two-way ANOVA						
Dietary lipid		0.586	0.701	0.864	0.077	0.351
Pantothenic acid supplementation		0.482	0.997	0.112	0.950	0.128
Interaction		0.733	0.743	0.111	0.648	0.702

Note: *COX1*: cytochrome-c oxidase subunit 1; *COX2*: cytochrome-c oxidase subunit 2; *ND1*: NADH dehydrogenase subunit 1; *CYTB*: cytochrome b; *ATP6*: ATP synthase subunit 6.

found on gene expression levels between dietary lipid levels and PA supplementation ($P > 0.05$).

4. Discussion

The present study showed that high PA supplementation level of 240 mg kg^{-1} could improve lipid content accumulated and oxidative damage induced by high dietary fat. Lipids, as essential nutrients including phospholipids and essential fatty acids, play an essential role in the growth and development of various creatures [28]. The proper dietary lipid and protein ratio supports higher growth rates, partly based on protein-sparing in many species, including blunt snout bream [2]. However, the present study showed that dietary 11% lipid significantly suppress the specific growth rate of fish, which is similar to results obtained in blunt snout bream, golden pompano (*Trachinotus ovatus*), and largemouth bass (*Micropterus salmoides*) fed with 11%, 18%, and 18% fat diet, respectively [3, 26, 29, 30]. From the perspective of energy utilization, a possible reason may be that excessively high, dietary fat-driven energy intake results in the imbalance of metabolism and uptake of other nonlipid nutrients of fish [31, 32]. Higher condition factor, mesenteric fat index, and whole-body crude lipid of fish fed with 11% fat dietary also confirmed the explanation. According to our previous study, the growth performance came to a plateau when the dietary PA supplementation level reached an optimal point of 24 mg kg^{-1} at 5% fat and 31% protein level diet of blunt snout bream, and no adverse effect was found between high-PA dietary (~64 mg/kg) and growth performance [11]. Though insignificance, the present study still showed a better growth performance of fish fed with high PA supplementation than that of fish fed with low PA supplementation at 11% fat level, which might be due to improved digestive enzyme activities caused by PA supplementation [11]. In addition, grass carp and Bloch (*Channa punctatus*) fed 38 and 28 mg kg^{-1} PA diet, respectively, have the highest growth performance, which decreased as dietary PA supplementation increased [9, 33]. In this light, despite that the better growth performance of blunt snout bream was observed in the present study (that is, just tiny positive trend), the dose of 240 mg kg^{-1} PA supplementation is still higher for blunt snout bream fed with 11% fat diet. Considering different sensitivity among species to pantothenic acid, the optimal PA supplementation diet on blunt snout bream fed with an 11% diet needs further study.

As is well known, dietary lipid level is closely associated with fish whole-body lipid content, and an excessive lipid diet is generally accompanied with increased fat deposition in the liver, viscera, and muscle [29, 34, 35]. The lipid homeostasis of the liver is maintained by the uptake, secretion, and transport of lipid. Previous studies suggested that the blocking of hepatic lipid secretion makes the imbalance between the secretion and uptake of lipid responsible for hepatic lipid accumulation in rats and blunt snout bream, respectively [4, 36]. In this study, the liver lipid content was significantly higher in fish fed with a high-fat diet than fish fed with a low-fat diet. These results indicated that the ability to transport lipid out of the liver is attenuated, resulting in higher liver TG and TCH content after the intake of a high-fat diet [4]. As lipids are absorbed from the gut and transported to the liver for further processing, plasma TG and TCH concentrations would be elevated after high-fat feeding [4, 37]. Thus, the reduced plasma TG and TCH further indicated that the capacity of endogenous lipid transport out of the liver in fish fed with high-fat diets is

weakened, resulting in liver fat deposition. Recently, many studies have suggested that PA supplementation and its derivatives can reduce plasma TG and TCH, but the effect showed a dose specificity which is manifested as excess PA supplementation inhibiting the lipid elimination [38–40]. In agreement with these data, the lipid content in the present study was increased when dietary 240 mg/kg PA supplementation is at 5% fat level, while lipid content was reduced when dietary 240 mg/kg PA supplementation is at 11% fat level. In addition, increased liver DHA and EPA content may be another factor to explain the attenuated synthesis and secretion of triglycerides [41]. These results indicated that dietary pantothenic acid can regulate lipid metabolism in blunt snout bream fed with high-fat diet.

Generally, a high-fat diet is easier to generate more reactive oxygen species (ROS) that cause oxidative stress and oxidative destruction of cellular membranes [29, 42]. Fortunately, fish have the enzymatic and nonenzymatic antioxidant system, including SOD, CAT, glutathione peroxidase (GSH-Px), and GSH, which have been confirmed to protect cells and tissues against ROS [43]. However, the imbalance between generation and elimination of ROS would lead to the accumulation of MDA and other lipid peroxides. Therefore, MDA content is considered as an indicator of lipid peroxidation [44]. In the present study, fish fed with a high-fat diet showed higher liver antioxidant enzyme activities and MDA content that indicated oxidant stress does occur and activate the antioxidant system. On the contrary, regardless of dietary lipid level, dietary high-PA supplementation reduces liver oxidative damage and improves liver health, as evidenced by lower MDA content. Consistently, dietary PA supplementation enhanced the antioxidant defense capability of grass carp and white Pekin ducks, thereby reducing liver oxidative damage [9, 45]. This beneficial effect of PA, as an antioxidant, is attributed to its ability to protect the cell by increasing glutathione level, promote cellular repair mechanisms through CoA by potentiating synthesis of membrane phospholipids, and directly scavenge ROS generation [11, 15, 33, 45, 46]. Moreover, the increased ability of antioxidation and the decreased oxidation damage may be another explanation for the improved growth performance. As the antioxidant system is energy-consuming, liver mitochondria, where most cellular ROS is generated, play a critical role in the process [47]. However, long-term feeding of a high-fat diet caused lipid deposition and nuclear polarization, resulting in damaging the functionality of the liver and mitochondria, which agreed with previous research finding in mice fed with HFD [48]. As the consequence of structural and functional damages of cells and mitochondria, a large amount of cytochrome *c* is released into the cytoplasm and eventually leads to mitochondrial dysfunction and cell apoptosis which is manifested as ATP deficiency, ROS stress exacerbation, and Na^+/K^+-ATPase activity inhibition [12, 47, 49–51]. Dietary PA deficiency might cause oxidative stress of juvenile blunt snout bream. Optimal PA level could alleviate the cellular and/or molecular damages caused by oxidative stress [11, 52].

Slyshenkov [53] studied that PA alleviated the ultraviolet-induced decrease of glutathione content, diminished lipid peroxidation, and partly protected the cells against apoptosis produced by ultraviolet irradiation. In the present study, dietary high-PA supplementation decreased lipid droplet accumulation in the liver and alleviated mitochondria structural and functional damages caused by a high-fat diet, which may be explained by higher CoA content synthesized from PA [11]. Hence, sufficient ATP produced by oxidative phosphorylation in mitochondria of the liver cell may support greater antioxidant status, which at least is manifested as higher GSH content, less oxidative damage, higher mitochondrial cytochrome *c*, and lower cytosolic cytochrome *c*. Dietary PA may decrease lipid peroxidation caused by high-fat diet to maintain body lipid content.

In conclusion, blunt snout bream fed with high-fat diets showed poor growth performance, severe oxidant stress, and enhanced lipid accumulation in tissues, while dietary PA supplementation increased from 24 mg kg^{-1} to 240 mg kg^{-1} could alleviate these negative consequences by reducing lipid deposition and improving energy utilization and antioxidant ability. Though high dose showed a positive effect on alleviating adverse effects caused by a high-fat diet, the optimal dose of PA supplementation in an 11% fat level diet and its mechanism still need further study.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors have no conflicts of interest to declare. All coauthors have seen and agree with the contents of the manuscript, and there is no financial interest to report.

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