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The effects of dietary *Lycium barbarum* extract on growth performance, liver health and immune related genes expression in hybrid grouper (*Epinephelus lanceolatus*♂ × *E. fuscoguttatus*♀) fed high lipid diets

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ABSTRACT

Growth performance, hepatic morphology and antioxidant ability, and expressions of antioxidant, inflammatory and apoptosis related genes were investigated in hybrid grouper fed high lipid diets containing 0, 0.5, 1, 2 and 10 g kg^{-1} *Lycium barbarum* extract (LBE) for 8-week feeding. The study showed that dietary LBE significantly increased weight gain rate (WGR) and specific growth rate (SGR) of fish ($P < 0.05$), the highest WGR and SGR were observed in fish fed 10.00 g kg^{-1} LBE diet. Dietary LBE improved liver morphology by decreasing hepatocyte necrosis and inflammatory cell infiltration induced by high lipid diets. Meanwhile, high lipid diets supplemented with 0.5–2 g kg^{-1} LBE improved hepatic antioxidant ability by increasing the expression of antioxidant genes (GPx and CAT) and decreasing Keap1 mRNA levels. Moreover, dietary supplementation with 0.50–2.00 g kg^{-1} LBE significantly decreased IL-8, caspase-3, caspase-8 and caspase-9 mRNA levels and significantly increased IL-10 and TGF-β1 mRNA levels in the liver of fish fed high lipid diets. In conclusion, high lipid diets supplemented with LBE improved growth performance, feed utilization and liver health in hybrid groupers by increasing hepatic antioxidant enzymes activity and its genes expression, as well as inhibition of hepatic inflammatory response and apoptosis.

1. Introduction

The hybrid grouper (*Epinephelus lanceolatus* ♂ × *E. fuscoguttatus* ♀) is widely farmed in the southeastern coastal areas of China due to its fast growth, excellent taste, and high nutritional and economic value [1]. In recent years, due to the rapid increase in the price of protein sources such as fishmeal and soybean meal, the cost of aquatic feed has increased rapidly, which will seriously affect the sustainable development of aquaculture. Therefore, lipids and carbohydrates have been widely added to fish feed as non-protein sources to improve protein deposition in fish, which was based upon the notion that dietary protein could be reduced when more energy was provided by dietary lipids and carbohydrates [2,3]. However, previous studies reported that excessive lipid levels in fish diets often resulted in increased lipid deposition in tissues, especially in the liver, which could induce fatty liver [4,5]. The major harm of fatty liver led to hepatic cell degeneration and necrosis, stress resistance and meat quality decline, especially when the aquaculture environment deteriorated, it was easy to cause fish disease or even death, and ultimately caused huge economic losses [5–8]. A large number of antibiotics and chemicals were used to control fish diseases, which could reduce the risk of disease outbreaks in farmed fish, but it also caused many other problems, such as bacterial resistance, suppression of the fish immune system and pollution of the aquaculture environment [9,10]. Thus, it urgently needs to develop green, non-polluting functional additives to replace antibiotics and chemicals.

Recently, some researchers have reported that dietary supplementation of plant extracts could improve fish growth, immunity and liver morphology in high-fat diet [1,8,11]. Meanwhile, it is reported that many plant extracts can promote growth, stimulate appetite, enhance immunity and resist pathogens in fish farming [12]. Therefore, plant extracts can be added to high-lipid diets to improve growth performance, lipid metabolism and immune ability in fish, which will help

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the application of high lipid feed in aquaculture. *Lycium barbarum*, *Solanaceae* family, has been widely used as well-known traditional medicine and functional food in China for many years [13,14]. Polysaccharides are one of the major active ingredients of *Lycium barbarum*. Many studies have demonstrated that *Lycium barbarum* polysaccharides (LBP) had various bioactivities, such as immunomodulation and antioxidant [15,16], anti-stress [17], hepatoprotective effect [18], improvement of lipid metabolism and oxidative stress [19]. These findings indicated that *Lycium barbarum* extracts might have great potential to be used as a supplement in high lipid diets to improve growth performance and immune ability, and suppress lipid accumulation in fish. However, limited studies have been reported about the effects of dietary *Lycium barbarum* extract on growth performance and immunity in fish fed high lipid diets at present. Therefore, the present study aimed to investigate whether *Lycium barbarum* extract could improve the utilization of dietary lipids and health status of fish, based on examining growth performance, liver health and immune related genes expression in hybrid grouper fed high lipid diets.

2. Materials and methods

2.1. Diet preparation

The five experimental diets were prepared by supplementing the basal diet (Table 1) with 0, 0.50, 1.00, 2.00 and 10.00 g kg^{-1} *Lycium barbarum* extract (LBE, polysaccharides content, 80.0%, Shaanxi Ciyuan biotechnology Co., Ltd., China). The preparation and processing of diets was the same as our previous study [1].

Table 1
Composition and nutrient levels of experimental diets (g kg^{-1}).

Ingredients	Basal diet
Fish meal	450.0
Soybean meal	130.0
Flour	237.4
Beer yeast powder	50.0
Fish oil	50.0
Soybean oil	50.0
Lecithin	10.0
Vitamin premix[a]	2.0
Mineral premix[b]	5.0
Choline chloride (50%)	5.0
Antioxidant	0.1
Vitamin C	0.5
Monocalcium phosphate	10.0
Nutrient levels	
Moisture	5.12
Crude protein	46.63
Crude lipid	14.81
Ash	10.36

[1, 2] Mineral and vitamin premix provided by Guangzhou Fishtech Co., Ltd., China.

[a] Vitamin premix provides the following (mg kg^{-1} diet): vitamin A (500000 IU) 40 mg, vitamin B_1 40 mg, vitamin B_2 93.75 mg, vitamin B_6 20 mg, vitamin B_{12} (1%) 45 mg, vitamin K_3 (50%) 300 mg, inositol 400 mg, calcium pantothenate 250 mg, nicotinic acid 450 mg, folic acid 6 mg, biotin (2%) 10 mg, vitamin D_3 (500000 IU) 15 mg, vitamin E (50%) 300 mg, unite bran 2990.25 mg.

[b] Mineral premix provides the following (mg kg^{-1} diet): Na_2SeO_3 (1%) 20 mg, $CuSO_4{\cdot}5H_2O$ (25%) 24 mg, $FeSO_4{\cdot}H_2O$ (30%) 266.65 mg, $ZnSO_4{\cdot}H_2O$ (34.50%) 100 mg, $MnSO_4{\cdot}H_2O$ (31.80%) 120 mg, Ca $(IO_3)_2$ (5%) 50 mg, $CoSO_4{\cdot}7H_2O$ (5%) 10 mg, zeolite power 4380.55 mg.

2.2. Experimental fish and samples collection

Experimental fish were transported to the experimental condition in cylindrical tanks (500-L) with running water purchased from Marine Fisheries Development Center of Guangdong Province (Huizhou, China). Before the feeding trial, hybrid groupers were fed with the control diet for 2 weeks to adapt to the experimental diets and farming conditions. After fasting for 24 h, total 225 fish of an average weight (121.76 ± 3.21 g) were divided into five experimental groups of 15 each in triplicate and randomly stocked into fifteen cylindrical tanks (500 L, three tanks per treatment). During the feeding trial, the dissolved oxygen > 6.0 mg L^{-1} and total ammonia level < 0.3 mg L^{-1}; temperature, salinity and pH of aquaculture water were 27–31 °C, 30–33 psμ and 7.8–8.25, respectively. Farming conditions, fish management and feeding methods of experimental fish were the same as our previous study [1].

At the end of the feeding trial, experimental fish were fasted for 24 h before sampling and then anesthetized with 100 mg L^{-1} Eugenol (Shanghai Medical Instruments Co., Ltd, Shanghai, China). The three fish livers from each cylindrical tank for genes expression analysis were excised, frozen quickly in liquid nitrogen, and stored at −80 °C until analysis.

2.3. Growth performance

The parameters were calculated as per following formulae:

Weight gain rate (WGR, %) = 100 × (final body weight-initial body weight)/initial body weight;

Specific growth rate (SGR, % day−1) = 100 × (Ln final body weight − Ln initial body weight) / number of days;

Feed efficiency ratio (FER) = wet weight gain (g) / dry feed intake (g);

Survival (%) = 100 × (final number of fish) / (initial number of fish)

2.4. Hepatic antioxidant capacity measurements

Hepatic total antioxidative capacity (T-AOC), catalase (CAT), superoxide dismutase (SOD) activities and malondialdehyde (MDA) were measured according to our previous study [1].

2.5. Liver histological analysis

For histological analysis, three liver samples of per tank were fixed in 4% paraformaldehyde solution for 48 h, then washed in 70% ethanol solution, finally transferred to a 70% ethanol solution for storage until processing into histological slides. Paraffin production process, images collection and samples measurement were determined according to the method described by Torrecillas et al. and Xu et al. [20,21].

2.6. RNA isolation and gene expression analysis

Total RNA from livers were extracted using TRIzol® reagent (Vazyme Biotech Co., Ltd, China). Total RNA quality and integrity are respectively detected by spectrophotometry and agarose gel according to our previous study [22]. The single-stranded cDNA was synthesized from 1 μg total RNA using PrimeScript RT reagent Kit With gDNA Eraser (Takara, Dalian, China) according to the manufacturer's instructions. The cDNA templates were then stored at −80 °C for later analysis.

Primers for this study referred to our previous study [1]. Gene expression of liver samples was measured by ABI 7500 real-time PCR machine (Applied Biosystems, USA) using ChamQTM SYBR® qPCR Master Mix (Vazyme, Nanjing, China) according to our previous study

[1]. Relative gene expression levels were evaluated using $2^{-\Delta\Delta CT}$ method [23].

2.7. Statistical analysis

Results are presented with means ± S.D of three replicates. Before performing one-way analysis of variance (ANOVA), all data were tested for normality distribution (Kolmogorov-Smirnov test) and homogeneity of variances (Levene's test) and then were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. *P*-value < 0.05 was considered significant. All statistical analyses were performed using SPSS 16.0 (SPSS Inc., Michigan Avenue, Chicago, IL, USA) for Windows. The data that did not meet the assumptions of normality and homoscedasticity were converted and then analyzed as described above.

3. Results

3.1. Effects of dietary LBE on growth performance and feed utilization in hybrid grouper fed high lipid diets

In the present study, effects of LBE administration on growth performance and feed utilization of hybrid grouper were shown in Table 2. Dietary LBE significantly improved WGR and SGR of fish ($P < 0.05$), the highest WGR and SGR were observed in fish fed 10.00 g kg^{-1} LBE diet. There was no significant difference in FI, FER and survival among all groups ($P > 0.05$).

3.2. Hepatic antioxidative capacity

The effect of dietary LBE on hepatic oxidative status was shown in Table 3. Dietary supplementation with LBE significantly increased SOD, CAT and T-AOC activity and decreased MDA content in the liver of hybrid grouper fed high lipid diets ($P < 0.05$).

3.3. Liver histology

The liver morphology of hybrid grouper fed high lipid diets was shown in Fig. 1. Hepatocyte surface of fish fed control diet was rough, accompanied by hepatocyte necrosis and inflammatory cell infiltration (Fig. 1-1). Dietary supplementation of LBE improved liver morphology by decreasing hepatocyte necrosis and inflammatory cell infiltration in hybrid grouper fed high lipid diets (Fig. 1-2, Fig. 1-3, Fig. 1-4 and Fig. 1-5).

3.4. Immune- and apoptosis-related genes expression in the liver of hybrid grouper

Expression profiles of antioxidant-related genes were shown in Fig. 2. Dietary LBE significantly improved glutathione peroxidase (GPx) and catalase (CAT) mRNA levels. Transcription levels of Kelch-like-ECH-associated protein 1 (Keap1) showed a trend of decreasing first and then increasing and its expression was significantly down-regulated in the liver of fish fed 0.5 and 1.0 g kg^{-1} LBE diet ($P < 0.05$), compared to that of the control group. There was no significance in GR mRNA levels among all groups ($P > 0.05$).

Transcription levels of interleukin 10 (IL-10), transforming growth factor β1 (TGF-β1), interleukin 8 (IL-8) and target of rapamycin (TOR) in the liver of fish were presented in Fig. 3. Dietary supplementation with LBE significantly increased TGF-β1 and IL-10 mRNA levels, whereas significantly decreased IL-8 mRNA levels ($P < 0.05$).

Apoptosis-related genes mRNA levels in the liver of fish were shown in Fig. 4. The expression of caspase-8 was significantly down-regulated in fish fed dietary supplementation with LBE ($P < 0.05$). The expression of caspase-3 in fish showed a decreasing trend. Caspase-9 mRNA levels in fish were decreased with dietary LBE levels up to 2.00 g kg^{-1} diet, and then increased with levels further increasing ($P < 0.05$).

4. Discussion

Moderate increase in lipid level in fish feeds can save dietary protein, significantly reduce feed costs and improve farming efficiency, especially in grouper aquaculture, in which dietary protein level currently used in production is generally higher than 45%, while the lipid content is less than 10%. Marine fish such as salmon can make good use of dietary lipid which can reach 35% in feed [24]. Previous studies reported that increasing dietary lipid levels could improve protein-sparing effects and growth performance in some fish aquaculture [24,25]. However, it could not promote growth performance but result in fatty liver and immune suppression in many fish species when increasing dietary lipid content [26–29]. Jiang et al. reported dietary lipid level increased from 7% to 13% did not significantly improve growth performance of hybrid groupers, but resulted in reduced feed efficiency and increased fat accumulation [30]. Therefore, simply increasing dietary lipid levels could not improve growth performance and feed efficiency of hybrid grouper.

Long-term feeding of high lipid diets can cause liver dysfunction, which might result in fatty liver and eventually cause to slow down the growth performance of fish. Previous studies had shown that plant extracts might prevent fatty liver by increasing liver lipid metabolism, improving liver health and protein sparing effect of lipids in fish fed high lipid diets [1,11,31]. Dietary silymarin promoted growth

Table 2
Effects of dietary *Lycium barbarum* extract on growth performance and feed utilization in hybrid grouper, *Epinephelus fuscoguttatus* × *E. lanceolatus*.

Diets (g kg^{-1})	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
	0	0.50	1.00	2.00	10.00
IBW (g)	121.92 ± 1.70	118.39 ± 1.03	122.15 ± 4.49	122.29 ± 3.43	124.07 ± 3.96
WGR (%)	165.74 ± 7.02^{a}	179.59 ± 8.83^{ab}	178.13 ± 14.99^{ab}	189.23 ± 1.86^{b}	194.13 ± 7.92^{b}
SGR (%/d)	1.74 ± 0.05^{a}	1.84 ± 0.06^{ab}	1.82 ± 0.09^{ab}	1.90 ± 0.01^{b}	1.93 ± 0.05^{b}
FER	1.01 ± 0.03^{a}	1.05 ± 0.03^{ab}	1.03 ± 0.03^{a}	1.11 ± 0.03^{b}	1.06 ± 0.06^{ab}
FI (g/fish)	199.46 ± 5.34	202.67 ± 13.50	211.58 ± 12.73	208.25 ± 12.49	217.20 ± 10.58
Survival (%)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	97.78 ± 3.85	100.00 ± 0.00
Regressions					
$Y_{WGR} = -100.26x^2 + 125.88x + 168.48$				$R^2 = 0.9231$	$P = 0.048$
$Y_{SGR} = -0.6538x^2 + 0.8165x + 1.7622$				$R^2 = 0.9094$	$P = 0.043$
$Y_{FER} = -0.4983x^2 + 0.549x + 1.009$				$R^2 = 0.7551$	$P = 0.08$
$Y_{FI} = -41.356x^2 + 57.484x + 201.02$				$R^2 = 0.8097$	$P = 0.38$

Values are means ± SD of three replications. Means in the same raw with different superscripts are significantly different ($P < 0.05$).
IBW: initial body weight; WGR: weight gain rate; SGR: specific growth rate; FER: feed efficiency ratio; FI: feed intake.

Table 3
Effect of dietary *Lycium barbarum* extract on hepatic antioxidant capacity in hybrid grouper, *Epinephelus fuscoguttatus* × *E. lanceolatus*.

Diets (g kg^{-1})	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
	0	0.50	1.00	2.00	10.00
SOD activity (U/g)	1444.20 ± 43.16^{a}	1742.20 ± 11.19^{c}	1789.60 ± 41.01^{c}	1707.90 ± 44.74^{bc}	1626.20 ± 3.73^{b}
CAT activity (U/g)	628.42 ± 15.67^{a}	708.22 ± 6.27^{b}	714.87 ± 16.74^{b}	785.80 ± 12.54^{c}	814.62 ± 9.40^{c}
MDA content(nmol/g)	102.36 ± 2.43^{d}	98.71 ± 2.70^{cd}	84.65 ± 6.94^{ab}	80.95 ± 3.28^{a}	90.90 ± 0.98^{bc}
T-AOC activity (U/g)	170.19 ± 8.36^{a}	202.35 ± 12.43^{b}	196.73 ± 13.16^{ab}	203.62 ± 19.98^{b}	206.72 ± 4.87^{b}
Regressions					
$Y_{SOD} = -1358.3x^2 + 1416.9x + 1565.4$				$R^2 = 0.4102$	$P = 0$
$Y_{CAT} = -700.23x^2 + 872.52x + 642.21$				$R^2 = 0.9606$	$P = 0$
$Y_{MDA} = 130.96x2 - 142.43x + 102.4$				$R^2 = 0.8902$	$P = 0.001$
$Y_{T\text{-}AOC} = -140.99x^2 + 167.08x + 180.49$				$R^2 = 0.6356$	$P = 0.051$

Values are means ± SD of three replications. Means in the same raw with different superscripts are significantly different ($P < 0.05$).

performance and improved lipid metabolism and health status by enhancement of lipid metabolism and antioxidant capacity in grass carp fed high-lipid diets [8]. Dietary supplementation of 0.5–10 g kg^{-1} *Panax notoginseng* extract significantly improved growth performance and feed utilization, increased immune ability and improved intestine morphology in hybrid grouper fed high lipid diets [11]. Diets supplemented with 0.50–1.00 g kg^{-1} ginkgo biloba leaf extract improved hepatic antioxidant status and morphology, and increased immune-related genes expression in the head kidney of hybrid grouper fed high lipid diets [1]. In mice fed high-fat diets, dietary *Lycium barbarum* polysaccharides administration improved blood lipid metabolism by increasing serum high density lipoprotein content and decreasing serum low density lipoprotein, total cholesterol and triacylglycerols content [13]. In the present study, dietary LBE not only significantly improved growth performance of hybrid grouper fed high lipid diets than the control group, but also improved feed efficiency ratio, although there was no significant difference. The reason was possible that LBE could improve dietary lipid utilization and protein deposition in hybrid grouper, thereby improving the protein-saving effect of dietary lipids.

Fish liver is the main site of triglyceride synthesis, which is the main cause of fatty liver in fish fed high lipid diets [32]. Hepatic histological morphology is considered to be good indicators to evaluate nutritional status, metabolism and liver health, which is most commonly used morphological parameters: hepatocyte count, hepatocyte surface area and hepatocyte nuclear area [1]. In blunt snout bream (*Megalobrama amblycephala*) fed high-fat diet, excess lipid droplets resulted in nuclear polarization of hepatocyte, extensive swelling of the endoplasmic reticulum and loss of its ribosomes, as well as lost cristae and matrix of hepatic mitochondria, which indicated high lipid diet led to fatty liver of blunt snout bream [33]. However, Zhou et al. reported that high lipid

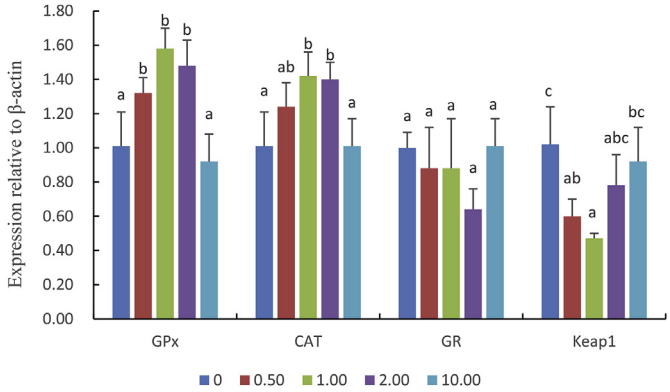

Fig. 2. Effect of dietary *Lycium barbarum* extract on relative expression of antioxidant-related genes in the liver of hybrid grouper fed high-lipid diets. 0, 0.5, 1, 2, 4, 10 represent the fish fed Diet 1, Diet 2, Diet 3, Diet 4 and Diet 5, respectively. Values are means (n = 3), bars bearing the same letters are not significantly different among all treatments ($P > 0.05$). GPx, glutathione peroxidase; CAT, catalase; GR, glutathione reductase; Keap1, Kelch-like- ECH-associated protein 1.

diets supplemented with 50 and 100 mg/kg berberine could attenuate the liver histological abnormalities in blunt snout bream [34]. Previous studies demonstrated that *Lycium barbarum* polysaccharides could improve liver damage caused by carbon tetrachloride in rats [35] and mice [36]. Similarly, dietary LBE decreased hepatocyte necrosis and inflammatory cell infiltration induced by high lipid diets in the present study. These results indicated that dietary supplementation of LBE could improve liver morphology in hybrid groupers fed high lipid diets.

When fish were fed high lipid diets, oxidative pressure occurred in

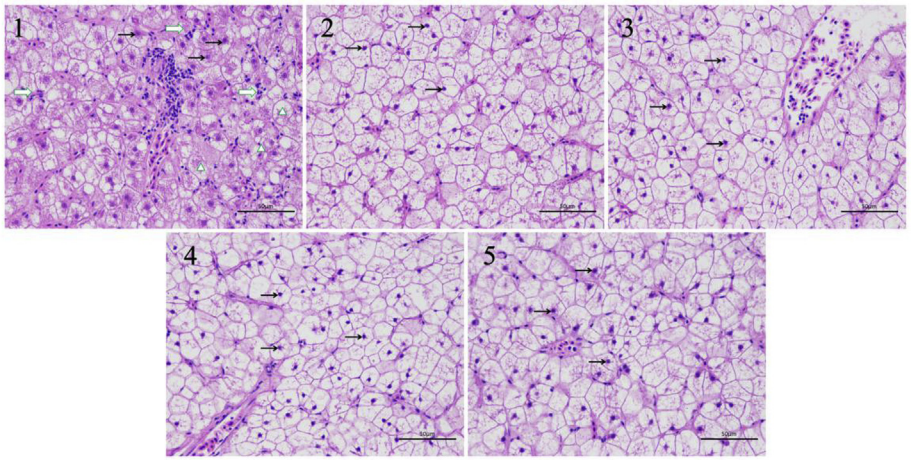

Fig. 1. Liver sections of test fish fed diets supplementation with *Lycium barbarum* extract (LBE) levels (H&E staining 400 ×). 1. Liver from fish fed Diet 1 (0 gkg-1 LBE); 2. Liver from fish fed Diet 2 (0.50 gkg-1 LBE); 3. Liver from fish fed Diet 3 (1.00 gkg-1 LBE); 4. Liver from fish fed Diet 4 (2.00 gkg-1 LBE); 5. Liver from fish fed Diet 5 (10.00 gkg-1 LBE). “ ” nuclei of the hepatocytes; “ ” inflammatory cell infiltration; “ ” hepatocyte necrosis.

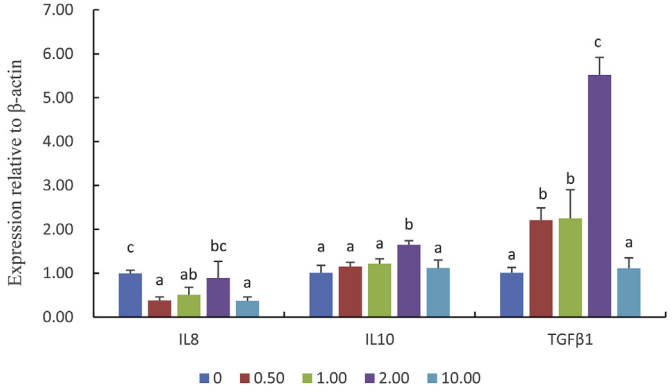

Fig. 3. Effect of dietary *Lycium barbarum* extract on relative expression of interleukin 10 (IL-10), transforming growth factor β1 (TGF-β1) and interleukin 8 (IL-8) in the liver of hybrid grouper fed high-lipid diets.

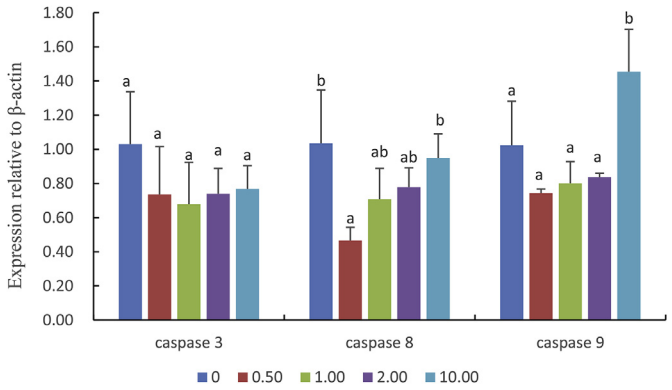

Fig. 4. Effect of dietary *Lycium barbarum* extract on relative expression of apoptosis-related genes in the liver of hybrid grouper fed high-lipid diets.

liver as reactive oxygen species produced exceeded antioxidant defenses, which could result in disruption of normal metabolism and physiology of fish [37]. Reactive oxygen species (ROS) clearance was associated with the enzymatic and non-enzymatic antioxidant defense systems in liver [38]. SOD, CAT, T-AOC and MDA were often used to assess antioxidant capacity in fish. SOD and CAT were important antioxidant enzymes and T-AOC directly reflected the ability of fish to scavenge oxygen free radicals, while MDA content showed the severity of oxidative stress [39,40]. *Lycium barbarum* polysaccharide had strong antioxidant capacity that exhibited inhibition of erythrocyte hemolysis mediated by peroxyl free radicals, 1, 1-diphenyl-2 picrylhydrazyl radical-scavenging and metal chelating activity [41] and inhibition of mitochondrial swelling and erythrocyte hemolysis caused by hydroxyl radicals [42]. Meanwhile, *Lycium barbarum* polysaccharide showed the hepatoprotective and antioxidant effects, which improved carbon tetrachloride-induced liver injury by increasing antioxidant enzymes activity in common carp [43]. Consistent with these studies, our study indicated that dietary LBE significantly improved hepatic antioxidant ability in hybrid groupers fed high lipid diets by increasing activities of hepatic antioxidant enzymes (SOD, T-AOC, CAT) and reducing MDA content. The effects of LBE were consistent with the previous studies that LBE supplementation could effectively inhibit against high-fat diets induced liver oxidative injury by significantly increasing SOD, CAT and T-AOC activities and decreasing MDA content in mice [44] and rat [45]. Previous studies demonstrated that the activities of antioxidant enzymes were closely related to the mRNA levels of antioxidant enzymes. Antioxidant abilities could be improved when antioxidant genes expression increasing and Keap1 expression decreasing [46–48]. In this study, dietary LBE significantly increased the expression of GPx and CAT genes, whereas significantly decreased the mRNA levels of Keap1. These results indicated that dietary LBE improved hepatic antioxidant ability in hybrid groupers fed high lipid diets by increasing the expression of antioxidant genes (GPx and CAT) and decreasing Keap1 mRNA levels.

High lipid diets not only caused oxidative stress in the liver, but also resulted in liver inflammation and apoptosis [49,50]. IL-8 was an important pro-inflammatory cytokine, whereas IL-10 and TGF-β1 were important anti-inflammatory cytokines, which could inhibit inflammatory response [1]. Apoptosis has two general pathways, caspase-8 involved in the extrinsic death receptor pathway and caspase-3 and caspase-9 took part in intrinsic mitochondrial pathway [1]. Previous study reported *Lycium barbarum* polysaccharides could protect rat liver from non-alcoholic steatohepatitis-induced injury by reducing inflammation, oxidative stress and apoptosis [50]. Further, *Lycium barbarum* polysaccharides also improved liver function by reducing hepatic fibrosis, oxidative stress, inflammatory response and apoptosis in non-alcoholic steatohepatitis rats [49]. In the present study, dietary supplementation with 0.50–2.00 g kg $^{-1}$ LBE significantly decreased IL-8, caspase-3, caspase-8 and caspase-9 mRNA levels and significantly increased IL-10 and TGF-β1 mRNA levels in the liver of fish fed high lipid diets, indicating that dietary supplementation of LBE could reduce inflammation and apoptosis in hybrid groupers fed high lipid diets. Consistent with our study, the previous studies reported that the protective effect of *Lycium barbarum* polysaccharides on liver damage might be closely related to its antioxidant, anti-inflammatory and anti-apoptosis effects [49,51,52].

5. Conclusions

In conclusion, the results of our study demonstrated that high lipid diets supplemented with LBE improved growth performance, feed utilization and liver health in hybrid groupers by increasing hepatic antioxidant enzymes activity and its genes expression, as well as inhibition of hepatic inflammatory response and apoptosis. These results provided new insights into the application of plant extracts as feed additives in high lipid diets for fish aquaculture.

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References

[1] X. Tan, Z. Sun, Q. Liu, H. Ye, C. Zou, C. Ye, A. Wang, H. Lin, Effects of dietary ginkgo biloba leaf extract on growth performance, plasma biochemical parameters, fish composition, immune responses, liver histology, and immune and apoptosis-related genes expression of hybrid grouper (*Epinephelus lanceolatus*♂ × *Epinephelus fuscoguttatus*♀) fed high lipid diets, Fish Shellfish Immunol. 72 (2018) 399–409.
[2] J.M. Vergara, M.D.L. Higuera, Protein sparing effect of lipids in diets for fingerlings of gilthead sea bream, Fish. Sci. 62 (1996) 624–628.
[3] T. Boujard, A. Gélineau, D. Covès, G. Corraze, G. Dutto, E. Gasset, S. Kaushik, Regulation of feed intake, growth, nutrient and energy utilisation in European sea bass (*Dicentrarchus labrax*) fed high fat diets, Aquaculture 231 (2004) 529–545.
[4] J. Wang, R. Lu, J. Sun, D. Xie, F. Yang, G. Nie, Differential expression of lipid metabolism-related genes and miRNAs in *Ctenopharyngodon idella* liver in relation to fatty liver induced by high non-protein energy diets, Aquacult. Res. 48 (2017) 4070–4085.
[5] K. Lu, W. Xu, X. Li, W. Liu, L. Wang, C. Zhang, Hepatic triacylglycerol secretion, lipid transport and tissue lipid uptake in blunt snout bream (*Megalobrama amblycephala*) fed high-fat diet, Aquaculture 408–409 (2013) 160–168.
[6] X. Wang, Y. Li, C. Hou, Y. Gao, Y. Wang, Physiological and molecular changes in large yellow croaker (*Pseudosciaena crocea* R.) with high-fat diet-induced fatty liver disease, Aquacult. Res. 46 (2015) 272–282.
[7] C.D. Rocha, S. Domingos, Replacing Fish Oil by Vegetable Oil in a Low Fish Protein Diet: Effects on Visceral Adipose Tissue and Inflammation in Atlantic Salmon, Mastergrad, 2012.

[8] P. Xiao, H. Ji, Y. Ye, B. Zhang, Y. Chen, J. Tian, P. Liu, L. Chen, Z. Du, Dietary silymarin supplementation promotes growth performance and improves lipid metabolism and health status in grass carp (*Ctenopharyngodon idellus*) fed diets with elevated lipid levels, Fish Physiol. Biochem. 43 (2017) 245–263.

[9] Q. Zhang, H. Yu, T. Tong, W. Tong, L. Dong, M. Xu, Z. Wang, Dietary supplementation of *Bacillus subtilis* and fructooligosaccharide enhance the growth, non-specific immunity of juvenile ovate pompano, *Trachinotus ovatus* and its disease resistance against *Vibrio vulnificus*, Fish Shellfish Immunol. 38 (2014) 7–14.

[10] P. Lee, Y. Lin, M. Chen, W. Cheng, Dietary administration of sodium alginate ameliorated stress and promoted immune resistance of grouper *Epinephelus coioides* under cold stress, Fish Shellfish Immunol. 65 (2017) 127–135.

[11] Z. Sun, X. Tan, H. Ye, C. Zou, C. Ye, A. Wang, Effects of dietary Panax notoginseng extract on growth performance, fish composition, immune responses, intestinal histology and immune related genes expression of hybrid grouper (*Epinephelus lanceolatus* ♂× *Epinephelus fuscoguttatus* ♀) fed high lipid diets, Fish Shellfish Immunol. 73 (2018) 234–244.

[12] M. Reverter, N. Bontemps, D. Lecchini, B. Banaigs, P. Sasal, Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives, Aquaculture 433 (2014) 50–61.

[13] M. Ma, G. Liu, Z. Yu, G. Chen, X. Zhang, Effect of the *Lycium barbarum* polysaccharides administration on blood lipid metabolism and oxidative stress of mice fed high-fat diet in vivo, Food Chem. 113 (2009) 872–877.

[14] M. Zhang, F. Wang, R. Liu, X. Tang, Q. Zhang, Z. Zhang, Effects of superfine grinding on physicochemical and antioxidant properties of *Lycium barbarum* polysaccharides, LWT - Food Sci. Technol. 58 (2014) 594–601.

[15] X.M. Li, X.L. Li, A.G. Zhou, Evaluation of antioxidant activity of the polysaccharides extracted from *Lycium barbarum* fruits in vitro, Eur. Polym. J. 43 (2007) 488–497.

[16] H. Wu, X. He, Y. Hong, T. Ma, Y. Xu, H. Li, Chemical characterization of lycium barbarum polysaccharides and its inhibition against liver oxidative injury of high-fat mice, Int. J. Biol. Macromol. 46 (2010) 540–543.

[17] X.M. Li, Protective effect of *Lycium barbarum* polysaccharides on streptozotocin-induced oxidative stress in rats, Int. J. Biol. Macromol. 40 (2007) 461–465.

[18] Y. Liu, L. Cao, J. Du, R. Jia, J. Wang, P. Xu, G. Yin, Protective effects of *Lycium barbarum* polysaccharides against carbon tetrachloride-induced hepatotoxicity in precision-cut liver slices in vitro and in vivo in common carp (*Cyprinus carpio* L.), Comp. Biochem. Physiol. C Toxicol. Pharmacol. 169 (2015) 65–72.

[19] R. Zhao, R. Jin, Y. Chen, F. Han, Hypoglycemic and hypolipidemic effects of *Lycium barbarum* polysaccharide in diabetic rats, Chin. Herb. Med. 7 (2015) 310–315.

[20] S. Torrecillas, A. Makol, M.J. Caballero, D. Montero, L. Robaina, F. Real, J. Sweetman, L. Tort, M.S. Izquierdo, Immune stimulation and improved infection resistance in European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides, Fish Shellfish Immunol. 23 (2007) 969–981.

[21] X.T. Xu, N.S. Chen, Z. Liu, S. Gou, J. Yin, Effects of dietary starch sources and levels on liver histology in largemouth bass, *Micropterus salmoides*, J. Shanghai Ocean Univ. 25 (2016) 61–70.

[22] X. Tan, Z. Sun, C. Zhou, Z. Huang, L. Tan, P. Xun, Q. Huang, H. Lin, C. Ye, A. Wang, Effects of dietary dandelion extract on intestinal morphology, antioxidant status, immune function and physical barrier function of juvenile golden pompano *Trachinotus ovatus*, Fish Shellfish Immunol. 73 (2018) 197–206.

[23] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method, Methods 25 (2001) 402–408.

[24] V. Karalazos, E.Å. Bendiksen, J.G. Bell, Interactive effects of dietary protein/lipid level and oil source on growth, feed utilisation and nutrient and fatty acid digestibility of Atlantic salmon, Aquaculture 311 (2011) 193–200.

[25] Q. Ai, K. Mai, H. Li, C. Zhang, L. Zhang, Q. Duan, B. Tan, W. Xu, H. Ma, W. Zhang, Z. Liufu, Effects of dietary protein to energy ratios on growth and body composition of juvenile Japanese seabass, *Lateolabrax japonicus*, Aquaculture 230 (2004) 507–516.

[26] X. Wang, Y. Li, C. Hou, Y. Gao, Y. Wang, Physiological and molecular changes in large yellow croaker (*Pseudosciaena crocea* R.) with high-fat diet-induced fatty liver disease, Aquacult. Res. 46 (2015) 272–282.

[27] J. Yan, K. Liao, K. Mai, Q. Ai, Dietary lipid levels affect lipoprotein clearance, fatty acid transport, lipogenesis and lipolysis at the transcriptional level in muscle and adipose tissue of large yellow croaker (*Larimichthys crocea*), Aquacult. Res. 48 (2017) 3925–3934.

[28] J. Yan, K. Liao, T. Wang, K. Mai, W. Xu, Q. Ai, Dietary lipid levels influence lipid deposition in the liver of large yellow croaker (*Larimichthys crocea*) by regulating lipoprotein receptors, fatty acid uptake and triacylglycerol synthesis and catabolism at the transcriptional level, PLoS One 10 (2015) e129937.

[29] C. Regost, J. Arzel, M. Cardinal, J. Robin, M. Laroche, S.J. Kaushik, Dietary lipid level, hepatic lipogenesis and flesh quality in turbot (*Psetta maxima*), Aquaculture 193 (2001) 291–309.

[30] S. Jiang, X. Wu, W. Li, M. Wu, Y. Luo, S. Lu, H. Lin, Effects of dietary protein and lipid levels on growth, feed utilization, body and plasma biochemical compositions of hybrid grouper (*Epinephelus lanceolatus* ♂ × *Epinephelus fuscoguttatus* ♀) juveniles, Aquaculture 446 (2015) 148–155.

[31] H. Song, W. Han, F. Yan, D. Xu, Q. Chu, X. Zheng, Dietary *Phaseolus vulgaris* extract alleviated diet-induced obesity, insulin resistance and hepatic steatosis and alters gut microbiota composition in mice, J. Funct. Foods 20 (2016) 236–244.

[32] H.L. Cheng, Fatty liver and regulation of lipids metabolism in fish, Chin. J. Anim. Nutr. 18 (2006) 294–298.

[33] K. Lu, W. Xu, X. Li, W. Liu, L. Wang, C. Zhang, Hepatic triacylglycerol secretion, lipid transport and tissue lipid uptake in blunt snout bream (*Megalobrama amblycephala*) fed high-fat diet, Aquaculture 408–409 (2013) 160–168.

[34] W. Zhou, S. Rahimnejad, K. Lu, L. Wang, W. Liu, Effects of berberine on growth, liver histology, and expression of lipid-related genes in blunt snout bream (*Megalobrama amblycephala*) fed high-fat diets, Fish Physiol. Biochem. 55 (2018) 165–172.

[35] R. Rufchaei, S.H. Hoseinifar, A. Mirzajani, H. Van Doan, Dietary administration of Pontogammarus maeoticus extract affects immune responses, stress resistance, feed intake and growth performance of caspian roach (*Rutilus caspicus*) fingerlings, Fish Shellfish Immunol. 63 (2017) 196–200.

[36] J. Xiao, E.C. Liong, Y.P. Ching, R.C.C. Chang, K.F. So, M.L. Fung, G.L. Tipoe, *Lycium barbarum* polysaccharides protect mice liver from carbon tetrachloride-induced oxidative stress and necroinflammation, J. Ethnopharmacol. 139 (2012) 462–470.

[37] J.Y. Adjoumani, K. Wang, M. Zhou, W. Liu, D. Zhang, Effect of dietary betaine on growth performance, antioxidant capacity and lipid metabolism in blunt snout bream fed a high-fat diet, Fish Physiol. Biochem. 43 (2017) 1733–1745.

[38] J. Zhao, Y. Liu, J. Jiang, P. Wu, W. Jiang, S. Li, L. Tang, S. Kuang, L. Feng, X. Zhou, Effects of dietary isoleucine on the immune response, antioxidant status and gene expression in the head kidney of juvenile Jian carp (*Cyprinus carpio var. Jian*), Fish Shellfish Immunol. 35 (2013) 572–580.

[39] X. Tan, H. Lin, Z. Huang, C. Zhou, A. Wang, C. Qi, S. Zhao, Effects of dietary leucine on growth performance, feed utilization, non-specific immune responses and gut morphology of juvenile golden pompano *Trachinotus ovatus*, Aquaculture 465 (2016) 100–107.

[40] C. Cheng, F. Yang, R. Ling, S. Liao, Y. Miao, C. Ye, A. Wang, Effects of ammonia exposure on apoptosis, oxidative stress and immune response in pufferfish (*Takifugu obscurus*), Aquat. Toxicol. 164 (2015) 61–71.

[41] X.M. Li, X.L. Li, A.G. Zhou, Evaluation of antioxidant activity of the polysaccharides extracted from Lycium barbarum fruits in vitro, Eur. Polym. J. 43 (2007) 488–497.

[42] J.H. Wang, M. Zhang, L. Gan, S.H. Zhang, Study on scavenging effect of *Lycium Barbarum* polysaccharide-2 on the oxidation induced by hydroxyl radicals, Food Sci. (N. Y.) 22 (2001) 11–13.

[43] Y. Liu, L. Cao, J. Du, R. Jia, J. Wang, P. Xu, G. Yin, Protective effects of Lycium barbarum polysaccharides against carbon tetrachloride-induced hepatotoxicity in precision-cut liver slices in vitro and in vivo in common carp (*Cyprinus carpio* L.), Comp. Biochem. Physiol. C Toxicol. Pharmacol. 169 (2015) 65–72.

[44] H. Wu, X. He, Y. Hong, T. Ma, Y. Xu, H. Li, Chemical characterization of lycium barbarum polysaccharides and its inhibition against liver oxidative injury of high-fat mice, Int. J. Biol. Macromol. 46 (2010) 540–543.

[45] C. Bokang, L. Su, L. Xiaojun, W. Jun, L. Shuhong, W. Qibo, L. Shengping, Effects of *Lycium barbarum* aqueous and ethanol extracts on high-fat-diet induced oxidative stress in rat liver tissue, Molecules 16 (2011) 9116–9128.

[46] W. Jiang, R. Tang, Y. Liu, S. Kuang, J. Jiang, P. Wu, J. Zhao, Y. Zhang, L. Tang, W. Tang, X. Zhou, L. Feng, Manganese deficiency or excess caused the depression of intestinal immunity, induction of inflammation and dysfunction of the intestinal physical barrier, as regulated by NF-κB, TOR and Nrf2 signalling, in grass carp (*Ctenopharyngodon idella*), Fish Shellfish Immunol. 46 (2015) 406–416.

[47] L. Li, L. Feng, W. Jiang, J. Jiang, P. Wu, J. Zhao, S. Kuang, L. Tang, W. Tang, Y. Zhang, X. Zhou, Y. Liu, Dietary pantothenic acid depressed the gill immune and physical barrier function via NF-κB, TOR, Nrf2, p38MAPK and MLCK signaling pathways in grass carp (*Ctenopharyngodon idella*), Fish Shellfish Immunol. 47 (2015) 500–510.

[48] L. Chen, L. Feng, W. Jiang, J. Jiang, P. Wu, J. Zhao, S. Kuang, L. Tang, W. Tang, Y. Zhang, X. Zhou, Y. Liu, Intestinal immune function, antioxidant status and tight junction proteins mRNA expression in young grass carp (*Ctenopharyngodon idella*) fed riboflavin deficient diet, Fish Shellfish Immunol. 47 (2015) 470–484.

[49] J. Xiao, F. Xing, J. Huo, M.L. Fung, E.C. Liong, Y.P. Ching, A. Xu, R.C.C. Chang, K.F. So, G.L. Tipoe, *Lycium barbarum* polysaccharides therapeutically improve hepatic functions in non-alcoholic steatohepatitis rats and cellular steatosis model, Sci. Rep. 4 (2015) 5587–5597.

[50] J. Xiao, E.C. Liong, Y.P. Ching, R.C.C. Chang, M.L. Fung, A.M. Xu, K.F. So, G.L. Tipoe, Lycium barbarum polysaccharides protect rat liver from non-alcoholic steatohepatitis-induced injury, Nutr. Diabetes 3 (2013) e81.

[51] F. Gan, Q. Liu, Y. Liu, D. Huang, C. Pan, S. Song, K. Huang, *Lycium barbarum* polysaccharides improve CCl_4 -induced liver fibrosis, inflammatory response and TLRs/NF-kB signaling pathway expression in wistar rats, Life Sci. 192 (2018) 205–212.

[52] L. Chen, W. Li, D. Qi, D. Wang, *Lycium barbarum* polysaccharide protects against LPS-induced ARDS by inhibiting apoptosis, oxidative stress, and inflammation in pulmonary endothelial cells, Free Radic. Res. 52 (2018) 480–490.