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Effects of berberine on the growth and immune performance in response to ammonia stress and high-fat dietary in blunt snout bream *Megalobrama amblycephala*

Qing-Qing Chen [b], Wen-Bin Liu [b], Man Zhou [b], Yong-Jun Dai [b], Chao Xu [b], Hong-Yan Tian [b], Wei-Na Xu [a, b, *]

[a] Shanghai Key Laboratory for Veterinary and Biotechnology, School of Agriculture and Biology, Shanghai Jiao Tong University, No.800 Dongchuan Road, Shanghai 200240, People's Republic of China
[b] Key Laboratory of Aquatic Nutrition and Feed Science of Jiangsu Province, College of Animal Science and Technology, Nanjing Agricultural University, No.1 Weigang Road, Nanjing 210095, People's Republic of China

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ABSTRACT

This study aimed to figure out the effects of berberine on growth performance, immunity, oxidative stress and hepatocyte apoptosis of blunt snout bream (*Megalobrama amblycephala*) fed with high-fat diet. 320 fish (80.00 ± 0.90 g) were divided randomly into four trial groups (each with four replicates) and fed with 4 diets (normal diet, normal diet with 50 mg/kg berberine, high-fat diet, high-fat diet with 50 mg/kg berberine), respectively. At the end of the feeding trial, ammonia stress test was carried out for 5 days. The result showed the growth performance, immune parameters including plasm acid phosphatase (ACP) activities, lysozyme (LYZ) activities and alternative complement C3 and C4 contents were suppressed in fish fed with high-fat diets but improved in berberine diets compared with control (normal diet). Hepatopancreas oxidative status, the malondialdehyde (MDA), protein carbonyl (PC) and lipid peroxide (LPO) were increased significantly ($P < 0.05$) when fish were fed with high-fat diets. Berberine could slow the progression of the oxidative stress induced by high-fat through increasing superoxide dismutase (SOD) activities and total sulfydryl (T-SH) levels of fish. And the hepatocyte apoptosis in the high-fat group could also be alleviated by berberine. After the ammonia stress test, the accumulative mortality was extremely ($P < 0.05$) low in fish fed high-fat diet with berberine compared to other groups. It was concluded berberine as a functional feed additive significantly inhibited the progression of oxidative stress, reduced the apoptosis and enhanced the immunity of fish fed with high-fat diet.

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1. Introduction

With the development of high-density aquaculture over the years, fish health problems had become more and more serious. Artificial aquaculture stressful circumstance such as inadequate nutrition (e.g. high-fat), water quality, could result in low growth rate and immune ability, as well as high feed conversion rate and mortality [1–3]. As was known to most of the aquarist, raising the dietary fat level appropriately would save protein content, which decreased production costs and nitrogen emissions [4]. The dietary fat could not only provide energy in replacement of protein, but also provide the essential fatty acid that maintained the cell membrane system structure and function for fish [5]. However, our previous studies had found high-fat diets (fat level 10% or 15%) could easily lead to excessive *Megalobrama amblycephala* hepatopancreas fat deposition and seriously impact the fish health [6,7]. It showed that *Megalobrama amblycephala* growth performance was significantly decreased. Significantly higher levels of plasm aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities, triglycerides (TG) and cholesterol were found in *Megalobrama amblycephala* fed with high-fat diet. The hepatic TG secretion rate and plasm very low density lipoprotein (VLDL) amount of *Megalobrama amblycephala* fed high-fat diet were significantly lower compared to those of *Megalobrama amblycephala* fed normal diet (fat level 5%). In the hepatopancreas, muscle and mesenteric fat tissue, the percentage of polyunsaturated fatty acid (PUFA) was

* Corresponding author. School of Agriculture and Biology, Shanghai Jiaotong University, No.800 Dongchuan Road, shanghai 200240, People's Republic of China.
E-mail address: xuweina@sjtu.edu.cn (W.-N. Xu).

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significantly increased, while both saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) percentages decreased in high-fat group. On the whole, high fat intake resulted in hepatocytes fat accumulation and cellular ultrastructural impairments including mitochondria, nucleus and endoplasmic reticulum (ER) [5–7].

Beside inadequate dietary nutrition (e.g. high-fat), the influence caused by aquaculture environment (water ammonia) in the process of breeding could not be ignored. Waterborne ammonia had become a persistent pollutant of aquatic habitats, especially in fish [8]. High environmental ammonia might affect the performance of fish, such as reducing growth rate [9], inducing apoptosis [10], and causing hyperexcitability, coma, convulsions and death. Cheng studied ammonia exposure could induce ROS, interrupt Ca^{2+} homeostasis, and subsequently lead to DNA damage and apoptosis [11].

To solve these problems, appropriate dietary immune-enhancers such as probiotics, functional sugars, Chinese herbal, had been widely studied in aquaculture to improve fish innate immune system without affecting the growth performance in recent years. C.N. Zhang et al. studied that fructooligosaccharide played a role in growth performance, immune response, antioxidant capability and HSP70 and HSP90 expressions of blunt snout bream *(Megalobrama amblycephala)* under high ammonia stress and high heat stress [12,13]. Our lab reported the immunoregulatory effects of emodin (Chinese herb) in *Procambarus clarkii* [14] and *Megalobrama amblycephala*. It was showed that appropriate dietary emodin supplementation could enhance the growth and immune responses and improve fish resistance to infection by bacteria [15,16].

Berberine, a kind of important alkaloids, known as a traditional Chinese medicine had been applied in China for a long time. Multiple pharmacologic effects of berberine have been reported including anti-inflammatory, anti-hypertensive, and anti-proliferative actions [17,18]. Berberine could antagonize pathogenic microorganisms, and was used to treat diarrhea in clinical treatment as over-the-counter drugs. It also had significant effect to anti heart failure, lower cholesterol, improve insulin resistance, anti-inflammatory, and so on [19]. Hydrochloride, natural drug ingredients of berberine were found to have the function of fat control [20], which was used in the treatment of hyperlipidemia and hepatopancreas dysfunction patients [21]. In our previous study, we have studied the effect of different level (50 or 100 mg/kg) of berberine on the growth and hepatopancreas lipid metabolism of *Megalobrama amblycephala*. The results showed that berberine supplemented diets (50 mg/kg level) could attenuate oxidative stress and improve function of mitochondrial respiratory chain via increasing the complex activities. Moreover, the histological results showed that berberine had the potential to repair mitochondrial ultrastructural damage and elevate the density in cells. We firstly studied the CPT-I expression in fish fed with high-fat diet (fat level 15%), and berberine-supplemented diet. The results showed that berberine could increase fat-metabolism gene expression levels and improve fat deposition [22].

Blunt snout bream *Megalobrama amblycephala*, was a berbivorous freshwater carp native to China. K.L. Lu et al. [5–7] had established the nutritional fatty hepatopancreas model of blunt snout bream. Based on these, the present research was conducted to investigate the function of berberine on growth and innate immunity of fish using this model. The data obtained here may prompt some new insights into the immunomodulation of herbs as functional feed additive and its industrial application.

2. Materials and methods

2.1. An ethics statement

All procedures involving animal subjects in this work were received prior approval from Shanghai Jiaotong University Institutional Animal Care and Use Committee.

2.2. Berberine

Berberine (HPLC≥98%) was bought from the spring and autumn biotechnology company, Nanjing,China. And the additive amount of it in this experimental diet was 50 mg/kg [22].

2.3. Fish and the feeding trial

Blunt snout bream were obtained from the Fish Hatchery of Yangzhou (Jiangsu, China). Prior to the experiment, fish were temporary kept in a floating net cages (3 × 3 × 3 m, L:W:H) for 1 weeks to acclimate to the tentative conditions. After the adaptive phase, 320 fish (80.00 ± 0.90 g) were randomly distributed into 16 net cages (2 × 1 × 1 m, L:W:H) in pond (20 fish each cage). Fish were fed with a normal diet (5% fat, ND), normal diet with 50 mg/kg berberine (NDB), high-fat diet (10% fat, HFD) and high-fat diet with 50 mg/kg berberine (HFDB) for 8 weeks [4–7], respectively. Each group had four replicates. This diet was manufactured according to our experimental objective and could also satisfy the nutrient requirements of this species. Fish were fed with utmost care at each meal to minimize feed waste. Formulation and proximate composition of the experimental diet was presented in Table 1. Fish were held under natural photoperiod condition throughout the feeding trail. Water temperature, pH and dissolved oxygen were monitored using YSI 556 MPS multi-probe field meter (Geotech, USA). During the feeding trial, water temperature ranged from 25 to 32 °C, pH fluctuated between 7.0 and 7.6, and dissolved oxygen was maintained above 5.0 mg/L, and total ammonia and nitrite was kept less than 0.2 and 0.005 mg/L, respectively.

Table 1
Formulation and proximate composition of experimental diets.

Ingredients	Normal diet (%)	High-fat diet (%)
Fish meal	5.00	5.00
Soybean meal	26.00	28.00
Rapeseed meal	11.86	11.86
Cottonseed meal	12.58	12.58
Wheat bran	10.00	4.00
Wheat meal	28.50	26.50
Lard oil	1.33	4.42
Soybean oil	1.33	4.42
Calciumbiphosphate	2.00	2.00
[a]Premix	1.00	1.00
Salt	0.40	0.40
Proximate composition (%)		
Moisture	13.90	11.22
Crude protein	29.64	29.38
Crude Lipid	4.77	10.10
Energy (MJ kg^{-1})	17.05	18.80

[a] Premix supplied the following minerals (g kg^{-1} of diet) and vitamins (IU or mg kg^{-1} of diet): $CuSO_4 \cdot 5H_2O$, 2.0 g; $FeSO_4 \cdot 7H_2O$, 25 g; $ZnSO_4 \cdot 7H_2O$, 22 g; $MnSO_4 \cdot 4H_2O$, 7 g; Na_2SeO_3, 0.04 g; KI, 0.026 g; $CoCl_2 \cdot 6H_2O$, 0.1 g; Vitamin A, 900000IU; Vitamin D, 200000IU; Vitamin E, 4500 mg; Vitamin K_3, 220 mg; Vitamin B_1, 320 mg; Vitamin B_2, 1090 mg; Niacin, 2800 mg; Vitamin B_5, 2000 mg; Vitamin B_6, 500 mg; Vitamin B_{12}, 1.6 mg; Vitamin C, 5000 mg; Pantothenate, 1000 mg; Folic acid, 165 mg; Choline, 60000 mg.

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