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Effects of mannan oligosaccharide on the physiological responses, HSP70 gene expression and disease resistance of Allogynogenetic crucian carp (*Carassius auratus gibelio*) under *Aeromonas hydrophila* infection

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ABSTRACT

We evaluated the effect of dietary supplementation with mannan oligosaccharide (MOS) on the resistance to *Aeromonas hydrophi1a* infection in Allogynogenetic crucian carp. The fish were randomly divided into five groups: a control group was fed with basal diet, and four treatment groups fed with basal diet supplemented with 60, 120, 240, 480 mg/kg MOS for 10 weeks, respectively. We then challenged the fish with *A. hydrophi1a* and recorded the mortality and the changes in serum cortisol, T3, T4, lysozyme, alkaline phosphatase (ALP), globin and hepatic total anti-oxidative capacity, superoxide dismutase (SOD), malondialdehyde (MDA) and the relative expression of heat shock protein 70 (HSP70) mRNA for a period of 7 d. Supplementation with 240 mg/kg MOS significantly increased serum ALP activity before infection, 1d and 2d after infection, serum globin concentration prior to infection, 1d and 7d after infection, serum lysozyme activity at 2d after infection, T3 concentration at 2d after infection, hepatic total anti-oxidative capacity prior to infection, hepatic SOD activity at 7d after infection and reduced serum cortisol concentration at 2d after infection, hepatic malondialdehyde content at 1d and 2d after infection. Supplementation with 480 mg/kg MOS significantly increased serum ALP activity before infection, 1d and 2d after infection, T3 content 1d after infection, T4 content prior infection and 7d after infection, serum globin concentration prior to infection, 1d and 7d after infection, serum lysozyme activity prior infection and 1d after infection, serum total anti-oxidative capacity prior to infection and 7d after infection, hepatic SOD activity at 7d after infection and the relative level of hepatic HSP70 mRNA at 2d and 7d after infection, had decreased levels of serum cortisol concentration before the infection, at 2d after infection, T4 concentration at 1d and 2d after infection, hepatic malondialdehyde content at 1d and 2d after infection. Mortality was significantly lower in the group of 240 and 480 mg/kg MOS than the control. Our results suggest that ingestion of a basal diet supplemented with 240–480 mg/kg MOS can enhance resistance against pathogenic infections in Allogynogenetic crucian carp.

1. Introduction

Allogynogenetic crucian carp (*Carassius auratus gibelio*) is a member of the family *Cyprinidae* and is a freshwater fish that inhabits the lakes, rivers and reservoirs in various countries in Asia and Europe, and is the most important cultivated (conventional freshwater fish) species in China. This species (as well as other *Cyprinidae*) is commercially important for its value as a food source. However, Allogynogenetic crucian carp cultured in China as well as in other parts of the world have suffered from serious diseases problem caused by viral infection and bacterial pathogens, which have caused significant economic losses and impeded the sustainable development of the industry throughout the world [1,2]. In recent years, a large amount of antibiotics are used to control outbreaks of fish diseases and thus this leads to the spread of antibiotic-resistant pathogens in cultured species and in the environment [3–5]. Therefore, it is necessary and important to develop non-chemical and natural therapeutics, such as immune-stimulants, probiotics, vaccine and natural therapeutics from plants [6–13].

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Mannan oligosaccharide (MOS) is derived from cell wall of *Saccharomyces cerevisiae*, and is widely used in nutrition as natural dietary supplements to improve gastrointestinal health as well as overall health. Previous studies demonstrated that MOS have proved to be effective in enhancing health and growth performance of the fish [14–18], improving gut morphology [16,19–21], modulating the intestinal micro-biota [16,21], elevating immune and stress resistant ability [14,18,20,22,23] and enhancing the disease resistance [15,18]. However, the effect of MOS on Allogynogenetic crucian carp was reported less.

Aeromonas spp. is ubiquitous bacteria that is native to aquatic environments and consist of two major groups, the psychrophilic group and the mesophilic group [24–26]. In particular, *Aeromonas hydrophila* has been reported as an important pathogen for humans and for lower vertebrates, including amphibians, reptiles and fish [27]. Several strains of *A. hydrophila* are thought to cause a variety of diseases in humans [28–30]. In addition, *A. hydrophila* is responsible for hemorrhagic septicemia and causes high levels of mortality and significant economic loss in freshwater fish [31,32], crustaceans [33,34] and occasionally marine fish [35,36]. The effect of MOS on Allogynogenetic crucian carp physiological responses under *A. hydrophi1a* infection has hardly been found in research reports. Our objective was to evaluate the effect of dietary supplements of MOS on the immune response of Allogynogenetic crucian carp and then we challenged fish with *Aeromonas hydrophi1a* and measured serum metabolites, immune parameters and hepatic oxidization indices and Heat shock protein (HSP70) gene expression. Our results provide insight into the physiological responses and molecular mechanisms underlying the protective effect of MOS on crucian carp under *A. hydrophi1a* infection.

2. Materials and methods

2.1. Fish and diets

We obtained 360 Allogynogenetic crucian carp fingerlings that were of a similar size (mean weight: 16.19 ± 0.03 g) from Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, China. The fingerlings were placed in 15 round fiberglass tanks (φ820 × 700 mm, $N = 24$ fish/tank) being acclimated for 15d. After that, we randomly divided the fingerlings tanks into five groups ($N = 3$ tanks/group): one control and four treatment groups. Triplicate groups of Allogynogenetic crucian carp (3 tanks, 24 individuals per tank) were fed with the basal diet and the basal diet supplied with 60 mg/kg, 120 mg/kg, 240 mg/kg and 480 mg/kg of MOS, respectively (See Table 1).

MOS was obtained from Alltech Bio-products Corporation Limited, China. Various feedstuffs were separately pulverized and screened through 60 mesh size sieve and first mixed calcium dihydrogen phosphate, zeolite powder and additives and then evenly mixed with MOS, and at last evenly mixed bulk feed ingredients. The diets were prepared at the research facilities of Fishery Machinery and Instrument Research Institute with 2.0 mm granular wet pellet. The moisture of feed was about 10% and was kept in refrigerator under −20 °C, ready for use.

2.2. Feeding trial

We cultured the fish in the aquarium with automatic temperature control breeding system. The tanks were supplied with aerated recycled water at a rate of 2 L min^{-1}. The fingerlings were fed by hand (2.0–4.0% body weight) three times a day (8:00–8:30, 12:00–12:30, and 18:00–18:30). During the experiment we measured the water temperature at 8:00 and 16:00 each day and checked the water quality once a week. The mean water quality indices were: water temperature 26.3 ± 1.51 °C, DO > 6 mg L^{-1}, $NH_3 < 0.05$ mg L^{-1}, pH 6.80–7.20. The amount of feed was adjusted every two weeks to account for increasing body weight.

Table 1
Formulation and composition of experimental diet.

Ingredients (%)		Nutrition levels s (%)	
Wheat middling	23.0	Crude protein	31.26
Wheat bran	9.5	Crude fat	4.00
Rice bran	7.0	Phosphorus	1.26
Rapeseed meal	17.0	Calcium	1.01
Soybean meal	19.5	Lys	1.70
Fish meal	8.0	Met	1.01
Cottonseed meal	8.0	Arg	2.00
Fish oil	2.0		
Calcium dihydrogen phosphate	2.0		
Choline chloride	0.5		
Vitamin mix	1.0		
Mineral mix	1.0		
Zeolite power	1.5		

Note: Vitamin premix and mineral premix were provided by Wuxi HANOWE Animal Health Company, China.

2.3. Challenge trial

2.3.1. Effect of MOS on the survival of Allogynogenetic crucian carp

After 10 weeks, the fish from the five groups (3 tanks/group, $N = 5$ fish/tank) were challenged with the bacterial septicemia pathogen *Aeromonas hydrophi1a*, Ah, BSK-10 provided by the Zhejiang Provincial Freshwater Fisheries Research Institute, China. According to the method described by Liu et al. [37] and Ming et al. [38], the strain of *Aeromonas hydrophi1a*, BSK-10 preserved in frozen tube was activated by using agar medium, and purified. The colony was selected and incubated in tube with nutritious broth for 18–24 h. Then the number of bacteria was counted under microscope. *A. hydrophi1a* was diluted by sterile normal saline and the final concentration was set to 1×10^8 cells mL^{-1}. Bacterial suspension (0.5 ml, per 50 body weight) was injected into the abdominal cavity and mortalities were checked 0 h, 12 h, 24 h, 48 h, 72 h, 96 h and 168 h after the challenge.

2.3.2. Effect of MOS on the immune response of Allogynogenetic crucian carp

A second subsample of fish (3 tanks/group, $N = 15$ fish/tank) was also challenged with *A. hydrophi1a*. (5×10^7 cells mL^{-1}, 0.5 ml per 50 g) following the procedure described above. We then collected serum and liver samples from 9 individuals in each group (3 fish/tank) prior to the challenge and at 1, 2 and 7d after the challenge.

2.4. Serum and liver sample collection and measurement

At each sampling point, the fish were rapidly netted and then anesthetized with 150 mg/L MS-222. We collected serum from the caudal vein and stored the samples in a refrigerator at 4 °C for 1–2 h. The serum was then centrifuged at 3000× *g* (4 °C) for 10 min, the supernatant removed and stored at −20 °C. In addition, the liver of each fish was removed, frozen in liquid nitrogen, and stored at −80 °C until further analysis.

2.5. Measurement of serum and liver samples

2.5.1. Serum cortisol, T3 and T4 measurement

We measured serum cortisol, 3,5,3′-triiodothyronine (T3) and thyroxin (T4) by RIA using a test kit (Beijing Beifang Biotech Research Institute, China) and following the method described by Pickering et al. (1983), Xie et al. (2008) and Zhao et al. (2000) [7,39,40].