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Effects of dietary oxidized konjac glucomannan sulfates (OKGMS) and acidolysis-oxidized konjac glucomannan (A-OKGM) on the immunity and expression of immune-related genes of *Schizothorax prenanti*

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ABSTRACT

In the present study, konjac glucomannan (KGM) was degraded by H_2O_2, and then used trisulfonated sodium amine and HCl, individually, to obtain two kinds of derivatives: oxidized konjac glucomannan sulfates (OKGMS) and acidolysis-oxidized konjac glucomannan (A-OKGM). The effects of two OKGM modified products on the immune parameters and expressions of toll-like receptor 22 (TLR22), myeloid differentiation factor 88 (MyD88) and interferon regulatory factors 7 (IRF7) genes in *Schizothorax prenanti* were determined. The alternative haemolytic complement (ACH50) activity was found to be significantly increased by the OKGMS diets. The immunoglobulin M (IgM) level was significantly enhanced by the OKGMS diets. The lysozyme activity was significantly increased by both OKGMS and A-OKGM diets. The superoxide dismutase (T-SOD) activity in fish fed with all doses of OKGMS diets was significantly higher than that in fish fed with basal diet. The glutathione peroxidase (GSH-PX) activity in fish fed with 0.8% and 1.6% A-OKGM diets was significantly higher than control group. The malondialdehyde (MDA) level was significantly decreased by both OKGMS and A-OKGM diets. The 0.8% A-OKGM diet significantly up-regulated TLR22 gene expression in the head kidney and spleen. TLR22 gene expression was significantly promoted by all OKGMS diets in the mesonephros and liver. The MyD88 mRNA level in 1.6% A-OKGM group significantly increased in the head kidney. The low dose of OKGMS significantly induced the MyD88 gene expression in the mesonephros, gut and liver, while 0.8% A-OKGM group also showed a significantly enhanced MyD88 mRNA expression in the gut. High dose of OKGMS significantly increased the IRF7 mRNA expression in the mesonephros and spleen. Fish fed with low dose of A-OKGM showed significantly higher expression of IRF7 in the gut and liver. Present study suggested that OKGMS and A-OKGM can act as immunostimulant to improve the immune indexes and up-regulate the immune-related gene expressions.

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1. Introduction

Schizothorax prenanti, also known as 'ya fish' (Cypriniformes, Cyprinidae, Schizothoracinae), is an endemic cold fish in southwest part of China. It mainly reproduces in upper reaches of Yangtze River and Hanjiang River. As the numbers of the wild animal continue to decline, introducing the artificial fish breeding becomes an important step for the conservation and development of *S. Prenanti*. At present, the artificial reproduction technology of *S. prenanti* is almost mature. However, *S. prenanti* populations are often infected with various kinds of diseases, such as motile *aeromonad septicaemia* [1], which threatens the development of *S. prenanti* artificial breeding. Hence, finding a solution to improve the intrinsic immunity of fish is urgent. Meanwhile, many studies have shown that the use of an immunomodulator enhances the intrinsic resistance of fish to infection [2–4].

Konjac glucomannan (KGM) is a natural polysaccharide that is available in the tuber of *Amorphophallus konjac*. KGM consists of β-D-glucose and β-D-mannose monomers mainly connected by β1,4-glycosidic bonds, with some side chains connected through β1,3-glycosidic bonds [5]. KGM possesses good bioactivity, biocompatibility and so forth because of its structure. KGM has

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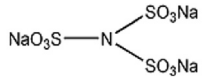
been applied in various fields. For instance, film preparation in food and drug fields [6,7]. It has been designed into a colon-specific carrier to enable the sustained delivery of drugs [8]. However, KGM has a high molecular weight (500–2000 kDa) and the maximum solubility in water is 1%. These properties impede the applications of KGM. Oxidized konjac glucomannan (OKGM), with higher purity and lower viscosity, is an oxidative degradation product of KGM. But its molecular weight is still high. In order to improve its bioactivity, we must decrease its viscosity and molecular weight. So A-OKGM was produced as we described previously [44]. Besides, previous researches have demonstrated that sulfated modification of polysaccharides can improve its bioactivity [9,10]. Moreover, Takemasa et al. [11] proved that a polysaccharide has the highest bioactivity when its molecular weight degraded to 100–200 kDa. So OKGMS was designed as a KGM derivative to obtain the highest bioactivity. OKGMS is a polysaccharide oxidative degraded and then sulfated modified from KGM whose molecular weight is about 160 kDa. So far, few researchers studied the properties and applications of OKGMS and A-OKGM, particularly, their applications in aquaculture field. Furthermore, the effects of OKGMS and A-OKGM on expressions of *S. prenanti* immune-related genes remain unknown.

In this study, we investigated the effects of OKGMS and A-OKGM on expressions of TLR22, MYD88, IRF7 genes in the spleen, head kidney, mesonephros, liver and gut of *S. prenanti*. Besides, the serum also was collected to analyse the immune parameters. These results have profound implication for preventing the infectious diseases in *S. prenanti*.

2. Materials and methods

2.1. Preparation of sulfonating agent (trisulfonated sodium amine)

23.90 g of sodium bisulfite ($NaHSO_3$) was dissolved in 40 ml ultra-pure water and transferred to a 500 ml, three-necked flask, the sodium nitrite ($NaNO_2$) solution (3.73 g of $NaNO_2$ was dissolved in 10 ml ultra-pure water) was dropped one by one into the flask at 4 °C with stirring, then the mixed solution was heated at 90 °C for 90 min. After the reaction, the transparent pale yellow liquid was trisulfonated sodium.

amine solution().

2.2. Preparation of OKGMS

10 g of OKGM which was made through the method described previously [44] was dissolved in 1000 ml distilled water, and added the trisulfonated sodium amine solution (100 ml) into OKGM solution. The mixed solution was heated at 30 °C for 12 h, and then washed with ethanol until the washings became granule, filtrating the samples with vacuum filter. The samples were subsequently dried at 40 °C in the drum wind drying oven, and then we got the OKGMS (Fig. 1).

2.3. Preparation of A-OKGM

The A-OKGM was produced via the method described previously [44].

2.4. Diets

The OKGMS and A-OKGM were tested at three supplemented levels of 4.0, 8.0, 16.0 g kg^{-1}. The diet without OKGMS or A-OKGM was used as control. The diets meet all nutritional requirements for *S. prenanti* and were manufactured through the formulation (Table 1).

2.5. Animals

450 fish were transferred from a local farm (Yuquantown, Tianquan, Yaan, Sichuan, China) to the laboratory of Functional food of Sichuan Agriculture University where they were allowed to adapt to the environment for three weeks in the indoor 20 fiberglass tanks (50 cm × 70 cm × 40 cm), and thereafter 420 healthy and robust fish (initial weight 81.00 ± 2.48 g) were selected for experimental use and they were divided into 7 groups randomly, every group (60 fish) was reared in 3 fiberglass tanks (50 cm × 70 cm × 40 cm) at the temperature of 15–25 °C, under natural photoperiod (12L:12D). Each group was fed with experimental diet (control and diets added 4.0, 8.0, 16.0 g kg^{-1} A-OKGM or 4.0, 8.0, 16.0 g kg^{-1} OKGMS) for 2 months.

2.6. Analyses on raw materials

2.6.1. Intrinsic viscosity [η] measurement

The intrinsic viscosities of OKGMS and A-OKGM were measured through ubbelohde viscometer (0.50–0.60 mm) at 25 ± 0.05 °C, and the solvent is NaCl (0.2 mol l^{-1}). The measurement was conducted in three replicates and the relative viscosity and specific viscosity were calculated through the following equations:

$$\eta r = \frac{t}{t0}$$

$$\eta sp = \eta r - 1$$

$$[\eta] = \lim_{c \to 0} \frac{\eta \mathrm{sp}}{\mathrm{C}} = \lim_{c \to 0} \frac{\ln \eta \mathrm{r}}{\mathrm{C}}$$

Where the t (s) and t0 (s) are the flow time of OKGMS or A-OKGM solution and NaCl solvent, respectively, and C is the concentration of OKGMS or A-OKGM solution.

The viscosity-average molecular weight (Mη) was determined using the Mark-Houwink equation:

$$[\eta] = k \cdot [M\eta]^{\alpha}$$

Where the k and α are the Mark-Houwink constants, $k = 5.96 \times 10^{-2}$, $\alpha = 0.73$ [12].

2.6.2. Determination of the sulfate group substituting degree

The sulfur content of OKGMS was determined by barium chloride-gelatin nephelometry method [13]. The degree of substitution (DS) which indicates the average number of sulfonic groups attached to a glucose unit was calculated according to the following equation [14]:

$$\mathrm{DS} = \frac{162 \times \mathrm{S}\%}{3200 - 102 \times \mathrm{S}\%}$$

Where the S % is the sulfur content (%) of OKGMS.

2.6.3. Infrared spectrum analysis

The infrared spectrometer (IR, BRUKER TENSOR 27, Germany) and attenuated total reflection (ATR) attachment were used to perform this analysis. Sample spectra were collected between 400 and 4000 cm-1. The spectra were subsequently analyzed to