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Immunomodulatory effect of dietary *Saccharomyces cerevisiae*, β-glucan and laminaran in mercuric chloride treated Nile tilapia (*Oreochromis niloticus*) and experimentally infected with *Aeromonas hydrophila*

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ABSTRACT

Six hundred and forty Nile tilapia (*Oreochromis niloticus*) weighing 80–100 g were randomly allocated into eight equal groups (80 each). The first group acts as control. Groups **S**, **B** and **L** were fed on a ration supplemented with *Saccharomyces cerevisiae*, β-glucans and laminaran, respectively for 21 days. Groups **M**, **MS**, **MB** and **ML** were subjected throughout the experiment to sublethal concentration of mercuric chloride (0.05 ppm). Gps. **MS**, **MB** and **ML** were fed on a ration containing *S. cerevisiae*, β-glucan and laminaran respectively for 21 days. Fish were challenged with *Aeromonas hydrophila* (0.4×10^7 cells mL^{-1}) via intra-peritoneal injection and the mortality rate was recorded up to 10 day post-challenge. The non-specific defense mechanisms, cellular and humoral immunity, beside the total and differential leukocytic count were determined.

Lymphocyte transformation index, phagocytic activity percent, phagocytic index, total lymphocyte count, serum bactericidal activity and nitric oxide as well as the survival rate were insignificantly changed after 21 day in gps. **MS**&**ML**, when compared with mercuric chloride immune depressed group **M**. These parameters as well as the neutrophil adhesion, serum nitric oxide and survival rate were significantly increased in gp. **MB** when compared with gp. **M**. Meanwhile the cellular and humoral immunity beside the survival rate were significantly increased in groups **S**, **B**, **L** when compared with control group.

It could be concluded that the whole yeast *S. cerevisiae*, β-glucan and laminaran can be used as immunostimulants for the farmed Nile tilapia. The β-glucans could be used in farmed Nile tilapia, under immune depressive stressful condition to increase their resistance to diseases.

1. Introduction

Fish are held in aquaculture pens, live in an environment where they are constantly exposed to various stress factors such as handling, crowding, and infection, besides the exposure to pollutants and physiological changes that may lead to immune depression and outbreaks of infections [1]. Water pollution stresses the cultured fish and increases susceptibility to infectious diseases, leading to high mortalities [2].

Mercury (Hg) is a naturally occurring metal in water due to the erosion of earth and volcanoes activity. In addition, contribution from anthropogenic sources is significant. The most common forms of mercury in the environment are elemental mercury, inorganic mercury and organic compounds such as methyl mercury (MeHg) [3]. Fish toxicosis by mercury pollution was reported in several Egyptian lakes [4,5]. The immunotoxicological effect of mercuric chloride on the non-specific and specific immune system of fishes (*Trichogaster trichopterus* and *Dicentrarchus labrax*) has been investigated [1,2].

Immunostimulants are natural and synthetic compounds that counteract the immunosuppressive state of fish by promoting the non-specific immune response, antibody production and/or up-regulation of inflammatory response [6,7]. Natural immunostimulants are biocompatible, biodegradable and safe for the environment and human health [8,9]. The use of natural immunostimulants in aquaculture can improve the immune response of fish [6]. Therefore, the health of fish and enhancement of immunity are of primary concern and worthy of more attention.

β-glucan are widespread in plants, algae, bacteria, yeast and mushrooms. β-glucan from different sources are different in their

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structure and immunomodulatory potencies [10,11]. Oral administration of these compounds could potentiate the general immune response [12,13].

Saccharomyces cerevisiae walls are constructed almost entirely of mannoproteins and glucan (β-1,3-D-glucan and long branched β-1,6-D-glucan) while chitin is present in smaller amounts. Laminaran, a β-1,3-D-glucan and short branched β-1,6-D-glucan, is a major component of sublittoral brown algae (*e.g. phaeophyceae*) and occurs principally in laminariae sp. [14,15]. Laminaran increased survival rate in Atlantic salmon challenged with *Aeromonas salmonicida* [14,15]. On the contrary, dietary laminaran provided non-significant enhancement of resistance to *Aeromonas hydrophila* in tilapia [16]. The use of the whole yeast (*S. cerevisiae*) in fish feeds could be successful as a protein source substituting the expensive fish-meal-protein. The dietary effects of the whole yeast on the immune response of seabream and hybrid tilapia were investigated [13,17]. Enhancement of specific and non-specific immune response after the administration of β-glucan isolated from yeast was documented in yellow croaker [18], Asian catfish [9,19], carp [20] and zebrafish [21]. β-glucan has been found to enhance the resistance against *A. hydrophila* in Asian catfish, carp and zebrafish [19,20,21]. On the other hand, β-glucan has a little prospect in preventing columnaris disease in rainbow trout [22]. However, very little data are available on administering β-glucan, laminaran and whole yeast, as immunostimulants in immune depressed fish.

The objective of this study was to evaluate the immunomodulatory effects of *S. cerevisiae*, β-glucan and laminaran on the immune response in Nile tilapia exposed to mercuric chloride and experimentally infected with *A. hydrophila*.

2. Materials and methods

2.1. Fish

Six hundred and forty Nile tilapia (Oreochromis niloticus) weighing 80–100 g were obtained from the fish farm in Abbassa, Sharkia, Egypt. The fish were acclimatized for two weeks in indoor fiberglass tanks supplied with dechlorinated tap-water with continuous aeration. The pH was 7.1 and total hardness 0.95 mM. The fish were exposed to 12 h dark and 12 h light photoperiod. The fish were randomly stocked at a rate of 10 fish per 120 L aquarium. The temperature was adjusted at 25–27 °C throughout the experiment. Fish were fed twice daily with standard commercially prepared pellets at 2% of their body weight throughout the period of experiment. Fecal matters were siphoned out once daily.

2.2. Rations

A standard commercial ration containing approximately 32% crude protein and 5.6% crude lipid (3% cod liver oil with the remainder coming from other ingredients). The commercial diet vitamins and minerals met the basic dietary requirements of Nile tilapia. The ingredients were mixed mechanically by the horizontal mixer (Hobarts model D300-T, Troy, OH, USA). The pellets were then prepared using a pellet-machine (California Pellet Mill, Roskamp Huller Co.) with 0.5 cm diameter and pellets were left for 24 h for air drying at room temperature (28 °C), broken into small pieces and sieved to obtain the appropriate size. The rations were transferred into plastic bags and stored in a refrigerator at −8 °C until used. Four experimental rations were prepared. The control ration consisted of the standard commercial ration without any treatment. The second ration, contained *S. cerevisiae* (Biosaf®, KW Alternative Feeds, Co., UK) at a concentration of 10 g kg^{-1}, was mixed with the commercial diet [11]. The third and fourth rations were prepared from the commercial ration, treated with β-glucan extracted from *S. cerevisiae* (Hang Zhou Bio-Technology Co., Ltd. China.) and laminaran obtained from *Laminaria japonica* (China Ocean University Organism Project Development Co., Ltd) respectively at a concentration of 0.1%.

2.3. Pathogen

A. hydrophila was previously isolated from naturally infected fish (Oreochromis niloticus) and identified according to the standard bacteriological tests. It was cultured in nutrient broth (Oxoid) for 24 h at 37 °C. The broth culture was centrifuged for 10 min at 3000 × *g*. The supernatant was discarded and the pellets were re-suspended in phosphate buffered saline at pH 7.4 (PBS 7.4) and the optical density (OD) of the solution was adjusted to 0.5 at 456 nM, which correspond to 1×10^7 cells mL^{-1} [23]. This bacterial suspension was serially diluted using standard dilution technique with PBS 7.4 and used for the challenge experiment and bactericidal activity.

2.4. Preparation of mercuric chloride stock solution

Stock solution of concentration in part per million (Cppm) was prepared from standard mercuric chloride. Mesrcury was determined daily throughout the experimental period by using Shimadzu atomic absorption/flame spectrometer model (AA-630-01). The lower limit of detection for Hg using this method was 0.001 ppm. [24].

2.5. Experimental design

Six hundred and forty experimental fish were randomly divided into eight equal groups. Gp.(1) was the control. Gps.(*2,3,4*) were fed on a ration containing *S. cerevisiae*(**S**), β-glucans(**B**) and laminaran (**L**) respectively for 21 days. Group *5* (**M**) and Gps. (6,7,8) were subjected throughout the experiment to sublethal concentration of mercury (0.05 ppm) and Gps.(6,7&8) were fed on a ration containing *S. cerevisiae*(**MS**), β-glucans(**MB**) and laminaran(**ML**) respectively for 21 days. The daily water mercury concentration was estimated throughout the experiment. All groups were challenged with *A. hydrophila* at the end of the experiment.

2.6. Sample collection

Thirty fish from each group (10 fish/replicate) were randomly selected on the 21st day of treatment with *S. cerevisiae*, β-glucans and laminaran. Heparinized blood samples were collected from the caudal vein to study the non-specific defense mechanism (neutrophil adhesion test, lymphocyte transformation test, besides the total and differential leukocytic count). Moreover whole blood was collected, left to coagulate, then centrifuged for 5 min at 3000 × *g* for serum separation to be used for serum lysozyme assay, nitric oxide and bactericidal activity.

2.7. Cellular immunological studies

2.7.1. Neutrophil-glass-adhesion

Within 15 min after blood samples were collected, one drop of blood using heparinized microhematocrit tubes was placed onto a 22 mM square cover slip. The cover slips were placed individually in Petri-dishes-humid-chambers, and incubated for 30 min at room temperature (25 °C) to allow the neutrophils to stick to the glass. After incubation, the cover slips were gently washed with PBS 7.4 and the cover slips were transferred upside down to a microscope slide with a drop (50 μL) of 0.2% filtrated Nitroblue Tetrazolium (NBT) solution. After another 30 min of incubation, the positive,