Veterinary Immunology and Immunopathology 162 (2014) 168–173

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Short communication

Dietary fucoidan enhance the non-specific immune response and disease resistance in African catfish, *Clarias gariepinus*, immunosuppressed by cadmium chloride

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ARTICLE INFO

Article history:
Received 23 April 2014
Received in revised form
28 September 2014
Accepted 2 October 2014

Keywords:
Dietary
Fucoidan
Immunomodulatory
Clarias gariepinus
Aeromonas hydrophila
Cadmium chloride

ABSTRACT

Fucoidan is sulfated polysaccharide extracted from seaweed brown algae. This study was designed to evaluate the immunomodulatory effects and disease resistance of dietary fucoidan on catfish, *Clarias gariepinus*, immunosuppressed by cadmium. Three hundred and sixty African catfish, *C. gariepinus*, was allocated into six equal groups. The first group served as a control. Groups (F1 and F2) were fed on fucoidan supplemented ration at concentrations of 4 and 6 g/kg diet respectively for 21 days. Groups (Cd, CdF1 and CdF2) were subjected throughout the experiment to a sub-lethal concentration of 5 ppm cadmium chloride solution and groups (CdF1 and CdF2) were fed on a ration supplemented with fucoidan. Macrophages oxidative burst, phagocytic activity percentages and lymphocytes transformation index were a significant increase in the fucoidan-treated groups (F1 and F2), while serum lysozyme, nitric oxide and bactericidal activity were enhanced only in group (F2) when compared with controls. These parameters as well as absolute lymphocyte count and survival rate were significantly increased in group (CdF2) when compared with cadmium chloride immunosuppressed group (Cd). It could be concluded that the fucoidan can be used as immunostimulant for the farmed African catfish, *C. gariepinus* as it can improve its resistance to immunosuppressive stressful conditions.

1. Introduction

Using of natural immunostimulants in fish for the activation of non-specific immune response is promising to increase disease resistance (Traifalgar et al., 2013). The seaweed algae is wealthy with different minerals, vitamins, amino acids, alginic acid and fucoidan. Fucoidan is seaweed algal sulfated polysaccharide with a wide variety of biological activities including; detoxification of heavy metals (Davis et al., 2003), antiviral, antibacterial and antiparasitic action (Chotigeat et al., 2004; Immanuel et al., 2012; Sharma et al., 2014), antioxidant effect (Wang et al., 2010). Certainly, the importance of fucoidan in disease resistance and immunomodulatory has been highlighted in other farmed aquatic species such as shrimp (Deachamag et al., 2006; Immanuel et al., 2012).

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http://dx.doi.org/10.1016/j.vetimm.2014.10.001
0165-2427/

Unfortunately, natural water reservoirs used for aquaculture are polluted with different environmental pollutants and contaminants (Zikic et al., 2001; Tawari-Fufeyin et al., 2008). Wastes pollutants, including industrial, agricultural and communal wastewater containing high levels of heavy metals like cadmium enter into water reservoirs without their prior treatment (Kumar et al., 2009). It has been proved to be toxic to fish at low concentrations in culture systems (Zikic et al., 2001; Kumar et al., 2009). Cadmium water pollution has been reported in different lakes in Egypt (El-Shehawi et al., 2007). Immunosuppressive effects of cadmium have been reported in *Heteropneustes fossilis*, common carp, catfish and rainbow trout by Radhakrishnan (2010); Basha and Rani (2003); Albergoni and Viola (1995a) and Zelikoff et al. (1995), respectively. The aim of this work was to study the immunomodulatory effect of dietary fucoidan in African catfish, *Clarias gariepinus*, immunosuppressed by cadmium chloride and to evaluate its diseases-resistance after challenge with a virulent strain of *Aeromonas hydrophila.*

2. Materials and methods

2.1. Experimental fish

A total of 360 apparently healthy African catfish, *C. gariepinus* weighing 100–120 g were obtained from the fish farm in Abbassa, Sharkia, Egypt. The fish were maintained in fiberglass tanks supplied with dechlorinated tap water with continuous aeration for one week under observation for acclimatization. The pH ranged from 6.9 to 7.5, pH, 7.6–7.9, total hardness, 115–120 mg/L (as $CaCO_3$), and a temperature of 25–27 °C was kept throughout the experiment.

2.2. Rations

The standard basal ration was formulated to meet the basic dietary requirements of catfish, according to Reis et al. (1989) and it was containing approximately 38% crude protein and crude fat 4%, in brief, 60.0% soybean meal, 26.5% corn grain, 10.5% fish meal with 0.1 and 0.25 vitamins and mineral mixture respectively. Fucoidan, obtained from marine seaweed brown algae, *Laminaria japonica* (Alibaba, Co., Ltd), which is 95% composed of sulfated esters fucose with molecular weight 189 kD. The experimental diets were prepared by thoroughly mixing fucoidan 4 and 6 g/kg of the basal diet in Hobart mixer (D300-T, OH, USA) and extruded out in 1.5 mm diameter pellets.

2.3. Pathogen

A pure culture of virulent strain of *A. hydrophila* isolated from naturally infected catfish, *C. gariepinus* and identified by performing the conventional biochemical tests (API 20E). It was maintained in nutrient broth (Oxoid) for 24 h at 37 °C. The concentration of bacteria was adjusted to 1×10^7 CFU/mL by the optical density of the suspension.

2.4. Experimental design

A total number of 360 African catfish were randomly divided into six equal groups (60 fish). Each group consists of equal three replicate, each replicate was 20 in separate tanks. A control group (**Cont**) was fed on a normal ration. Groups (**F1** and **F2**) were fed on a diet containing fucoidan at concentrations of 4 and 6 g/kg ration respectively for 21 days. Groups (**Cd**, **CdF1** and **CdF2**) were subjected throughout the experiment to a sub-lethal concentration of cadmium chloride 5 ppm solution (CdCl2 H2O, Sigma Co) according to Kumar et al. (2009) methodology, and CdF1 and CdF2 groups were fed on a ration supplemented with fucoidan at concentrations of 4 and 6 g/kg ration respectively.

2.5. Sample collection

Three replicate from each group (10 fish/replicate), were randomly selected on the 21st day of the treatment period. Heparinized blood samples (approximately 3.0 mL) were collected from the caudal vein. One half of each blood sample was immediately used for nitroblue tetrazolium (NBT) testing, lymphocyte-transformation assay, phagocytic activity, in addition to total and differential leukocyte count. The other half was left to coagulate and serum was separated for evaluation of lysozyme, nitric oxide, acid phosphatase and bactericidal activities.

2.6. Culture media

The culture media used in macrophage isolation were prepared according to Miller et al. (1994). Briefly, equal portions of AIM-V (AlbuMAX, Gibco® Life Technologies, USA) and L-15 (Sigma-Chemical, Louis, USA) culture media, 0.05 mM of 2-mercaptoethanol, 8% cell culture grade water (Sigma-Chemical) and 0.09% Na2HCO3 (Adwia Co. Egypt). Antibiotics, gentamicin 100 mg/mL, streptomycin 100 mg/mL and penicillin 100 U/mL were added to the culture medium when required.

2.7. Activities of macrophages and lymphocytes

2.7.1. Isolation of head-kidney macrophage

The head kidney was isolated according to Secombes (1990). In brief, 10 fish was sacrificed by administration of an overdose of anesthetic (3-amino benzoic acid ethyl ester). Head kidneys were removed, pooled, and forced through 100 mm nylon mesh with antibiotic free (af) culture media. Macrophage cells were layered onto 34/51% (v/v) Percoll (Sigma–Aldrich) and centrifuged at 400 × *g* for 25 min at 4 °C. The cell layer at the interface was collected and adjusted to 10^6 cells/mL in af-culture media.

2.7.2. Macrophage respiratory burst activity

The macrophage oxidative burst was assayed according to Rice et al. (1995). In summary, 100 μL macrophage suspended cells were dispensed into 96 well plates. The cells were activated with 100 μL of culture media containing 1 mg/mL NBT and 1 pg/mL Phorbol Myristate Acetate and incubated for 30 min at 27 °C. The formazan dissolved by