



## Effect of Dietary ImmunoWall® Supplementation on Hepatic Oxidative Status in Juvenile Persian Sturgeon

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### Abstract

Yeast cell wall (YCW) products are a well-established class of prebiotics widely used in aquafeeds. Numerous studies have investigated the effects of YCW on growth performance, feed utilization, and immune responses in sturgeon species. However, limited information is available regarding their effects on oxidative status. Therefore, the present study evaluated the effects of ImmunoWall®, a commercial YCW-based prebiotic, on hepatic oxidative status in juvenile Persian sturgeon (*Acipenser persicus*). Fish were fed diets supplemented with 0% (control), 0.5% (group I), and 1% (group II) ImmunoWall® for eight weeks. At the end of the feeding period, liver samples were collected and analyzed for malondialdehyde (MDA) content as well as superoxide dismutase (SOD) and catalase (CAT) activities. The results showed that MDA levels and the activities of SOD and CAT were significantly higher in both supplemented groups than in the control group ( $p < 0.05$ ). These findings indicate that dietary ImmunoWall® supplementation altered hepatic oxidative status and stimulated antioxidant defense responses in juvenile Persian sturgeon. Further studies involving liver biochemical and histopathological assessments are required to clarify the physiological significance of these changes and to determine the long-term effects of YCW supplementation on liver health.

**Keywords:** Persian sturgeon (*Acipenser persicus*), ImmunoWall®, yeast cell wall, prebiotic, hepatic oxidative status, antioxidant enzymes

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## Introduction

Prebiotics are non digestable food ingredients with beneficial effects on fish health condition and growth performance which have been widely used in aquaculture to reduce side effects of antibiotics including development of antibiotic-resistant bacterial strains and accumulation of residual in edible tissues (Hoseinifar *et al.*, 2015; Jung-Schroers *et al.*, 2016).

ImmunoWall® is a commercial prebiotic constituted mostly by MOS<sup>1</sup> and  $\beta$ -glucans derived from *Saccharomyces cerevisiae* yeast cell wall. Numerous studies have reported different benefits of utilizing food ingredients with MOS and  $\beta$ -glucan (Yarahmadi *et al.*, 2014; Selim and Reda, 2015). As shown previously, through decreasing oxidative damage or increasing antioxidant capacity prebiotics administration positively affects oxidative status in fish (Guerreiro *et al.*, 2018). However, a few studies have focused on prebiotics effects on oxidative stress response in sturgeons as a valuable species. The current study, therefore, aims to determine protective effects of dietary immunowall® on oxidative stress response in Persian sturgeon (*Acipenser persicus*) as an important species in Iran.

## Materials and methods

This study was carried out over 8 weeks at International Sturgeon Research Institute, Guilan Province, Iran. A total number of 153 juvenile Persian

sturgeons (*Acipenser persicus*) with mean initial weight of  $47.78 \pm 0.39$  g (mean $\pm$ SD) were distributed into nine 500-Litr circular tanks (105 $\times$ 102 $\times$ 52 cm) (three treatments with three replicates) at a stock density of 17 fish per each tank. System maintenance consisted of siphoning of solids from each tank and was performed daily. Fish were acclimated to the laboratory conditions for two weeks and over this period were hand fed ad libitum three times a day with basal diet (Biomar™, France).

The experimental diet consisted of basal diet (Biomar®, France) supplemented with 0.5% and 1% prebiotic (Immunowall®, ICC co., Brazil) derived from yeast cell membrane (*Saccharomyces cerevisiae*). The prebiotic was added to basal diet by scattering on pellets and top-dressing with Canola oil (Merrifield *et al.*, 2009).

At the end of trial, three fish were randomly selected from each tank and were then euthanized and their liver was dissected on the ice and then homogenized in 100 mg of the tissue sample in 1 ml of PBS buffer pH 7.4 using a homogenizer (T10 basic, IKA, Staufen, Germany). Cellular debris was removed by centrifugation at 10,000  $\times$ g for 30 min. Then, Supernatant was collected, aliqueted, and stored at  $-80^{\circ}\text{C}$  for further study. The supernatant was used, as follows, in assays to assess the level of malondialdehyde (MDA), superoxide dismutase (SOD) and Catalase activities.

<sup>1</sup> Mannan oligosaccharides

Assessment of MDA level in serum was performed using kit (Zell Bio, GmbH, Germany) based on calorimetric assay and the absorbance was recorded at 530 nm. MDA level is commonly known as an indicator of lipid peroxidation and cell membrane damage (Gaweł *et al.*, 2004). Assay of SOD and catalase was performed using commercial investigation kit (Zell Bio, GmbH, Germany). Superoxide anion was converted to hydrogen peroxide and molecular oxygen enzymatically by SOD. Finally, the product was converted into colored compound with an absorbance of 420 nm. In this assay, CAT activity unit was considered as the amount of the sample that will catalyze decomposition of 1  $\mu$ mole of H<sub>2</sub>O<sub>2</sub> to water and O<sub>2</sub> in one minute.

The obtained results are presented as means $\pm$ SD. All statistical and graphical analyses were performed with SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) and Microsoft Excel version 14.0 (Microsoft Inc., Jones, Chicago, IL, USA).

## Results

The result of antioxidant enzyme activities and the level of MDA in liver are shown in Table 1. The significant elevation of MDA level and activities of SOD and catalase activity were observed in 0.5% and 1% YCW supplemented diet compared to the control group ( $p < 0.05$ ).

**Table 1: Serum oxidative parameters of Persian sturgeon fed diet supplemented with different levels of immunowall® (0.5% and 1%) for 56 days. Different letters indicate significant difference between diet groups ( $p < 0.05$ ).**

	Control	Group I (0.5%)	Group II (1%)
MDA ( $\mu$ M)	13.15 $\pm$ 0.57 <sup>b</sup>	14.63 $\pm$ 0.15 <sup>a</sup>	14.75 $\pm$ 0.27 <sup>a</sup>
SOD (U/ml)	27.76 $\pm$ 1.98 <sup>b</sup>	38.53 $\pm$ 1.5 <sup>a</sup>	40.83 $\pm$ 2.83 <sup>a</sup>
Catalase (U/mL)	3.73 $\pm$ 0.3 <sup>b</sup>	4.81 $\pm$ 0.17 <sup>a</sup>	4.91 $\pm$ 0.1 <sup>a</sup>

## Discussion

By definition, oxidative stress is extra formation and /or inadequate removal of extremely reactive oxygen and nitrogen (Maritim *et al.*, 2003). In aerobic species, oxidative stress is a common phenomenon which prevents oxidative damages. Several antioxidant defense systems including E and C vitamins, uric acid, glutathione, carotenoids and antioxidant enzymes are developed (Martinez-Alvarez *et al.*, 2005). By reducing oxidative damage or increasing

antioxidant capacity, prebiotics have been shown to have positive effects on oxidative status in fish (Guerreiro *et al.*, 2018). For instance, Triangular bream (*Megalobrama terminalis*) fed 0.3% or 0.6% FOS showed lower lipid peroxidation level and increased SOD activity both in liver and plasma (Zhang *et al.*, 2013). Prebiotics such as inulin were reported to have ROS scavenging ability (Stoyanova *et al.*, 2011; Guerreiro *et al.*, 2018). Our results showed that MDA level and activities of SOD and

catalase significantly increased in group I and II compared with those in the control group ( $p < 0.05$ ). MDA is a marker of lipid peroxidation and levels of lipid peroxidation help us get more insight into oxidative stress damage. Higher activity in catalase and SOD is an indicator of natural antioxidant defense system (Kim *et al.*, 2017). According to the observed oxidative responses, it thus seems that dietary high YCW dose leads to oxidative damage in liver of juvenile Persian sturgeon. According to the literature, no previous work has provided evidence for a detrimental effect of prebiotics on oxidative stress response in fish. However, high dose of herbal extract, as shown in some studies, can cause oxidative stress in mammalians (Zhang *et al.*, 2018; Tao *et al.*, 2020). It is necessary, though, to study these side effects in fish, as, to date, very few studies have addressed them in detail. Although most studies on prebiotic supplementation indicate some beneficial effects (Ta'ati *et al.*, 2011; Zhang *et al.*, 2013), possible negative effects should not be disregarded, as these adverse effects could be due to species, fish size, age, environmental conditions, life cycle, dose and kinds of prebiotics. Integration of analyses of oxidative status markers with other important evaluations, such as histopathological examination and assessment of biochemical indices of liver, are needed to learn more about the possible effects of dietary prebiotics on oxidative status physiology of the liver.

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