

## Effect of dietary ImmunoWall® on liver oxidative status in juvenile Persian sturgeon

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### Abstract

Yeast cell wall (YCW) products are a well-known class of prebiotics for use in aqua feeds. Several studies have been conducted to study the effects of yeast cell wall on growth, feed utilization and immune system in sturgeon. To date, however, very few studies have demonstrated the effect of prebiotics on oxidative status in sturgeon. The current study aims to determine the effects of ImmunoWall® as commercial YCW prebiotic on liver oxidative status by measuring Malondialdehyde (MDA), Superoxide dismutase (SOD) and catalase in juvenile Persian sturgeon. For this purpose, fish were fed diets supplemented with 0% (control), 0.5% (I) and 1% (II) ImmunoWall® for 8 weeks. At the end of feeding trails, the level of MDA and activity of SOD and Catalase were determined in liver of test fish. Based on the obtained results, MDA level and activities of SOD and catalase were significantly increased in group I and II compared with those in the control group ( $p < 0.05$ ), suggesting that the dietary dose of YCW can lead to oxidative stress in liver. Histopathological examination and assessment of biochemical indices of liver are needed to further investigate the possible effects of dietary prebiotics on liver.

**Keywords:** Persian sturgeon, ImmunoWall®, Prebiotic, Liver, Oxidative status

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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 β-glucans derived from *Saccharomyces cerevisiae* yeast cell wall. Numerous studies have reported different benefits of utilizing food ingredients with MOS and β-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±SD) were distributed into nine 500-Litr circular tanks (105×102×52 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.

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<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.

#### References

- Gawel, S., Wardas, M., Niedworok, E. and Wardas, P., 2004. Malondialdehyde (MDA) as a lipid peroxidation marker. *Wiadomości Lekarskie*, 57(9-10), 453-455.
- Guerreiro, I., Oliva-Teles, A. and Enes, P., 2018. Prebiotics as functional ingredients: focus on Mediterranean fish aquaculture. *Review in Aquaculture*, 10, 800-823.
- Hoseinifar, S.H., Esteban, M.Á., Cuesta, A. and Sun, Y.Z., 2015. Prebiotics and fish immune response: A review of current knowledge and future perspectives. *Reviews in Fisheries Science & Aquaculture*, 23(4), 315-328.
- Jung-Schroers, V., Adamek, M., Jung, A., Harris, S., Doza, O.S., Baumer, A. and Steinhagen, D., 2016. Feeding of beta-1,3/1,6-glucan increases the diversity of the intestinal microflora of acarp (*Cyprinus carpio*). *Aquaculture Nutrition*, 22, 1026-1039.
- Kim Birnie-Gauvin, K., Costantini, D., Cooke, S.J. and Willmore, W.G., 2017. A comparative and evolutionary approach to oxidative stress in fish: A review. *Fish and Fisheries*, 18, 928-942.
- Maritim, A., Sanders, R.A. and Watkins 3rd, J.B., 2003. Diabetes, oxidative stress, and antioxidants: a review. *Journal of Biochemical and Molecular Toxicology*, 17, 24-38.
- Martinez-Alvarez, R.M., Morales, A.E. and Sanz, A., 2005. Antioxidant defenses in fish: biotic and abiotic factors. *Reviews in Fish Biology and Fisheries*, 15, 75-88.
- Merrifield, D.L., Bradley, G., Harper, G.M., Baker, R.T.M., Munn, C.B.

- and Davies, S.J., 2009.** Assessment of the effects of vegetative and lyophilized *Pediococcus acidilactici* on growth, feed utilization, intestinal colonization and health parameters of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition*, 17, 73-79.
- Selim, K.M. and Reda, R.M., 2015.** Beta-glucans and mannan oligosaccharides enhance Growth and immunity in Nile Tilapia. *North American journal of aquaculture*, 77, 22-30.
- Stoyanova, S., Geuns, J., Hideg, E. and Van Den Ende, W., 2011.** The food additives inulin and stevioside counteract oxidative stress. *International Journal of Food Sciences and Nutrition*, 62, 207–214.
- Ta'ati, R., Soltani, M., Bahmani, M. and Zamini, A., 2011.** Growth performance, carcass composition, and immunophysiological indices in juvenile great sturgeon (*Huso huso*) fed on commercial prebiotic, Immunoster. *Iranian Journal of Fisheries Sciences*, 10, 324-335
- Tao, G., Dagher, F., Moballegh, A. and Ghose. R., 2020.** Role of oxidative stress in the efficacy and toxicity of herbal supplements. *Current Opinion in Toxicology*, 20-21, 36–40.
- Yarahmadi, P., Farahmand, H., Kolangi Miandare, H., Mirvaghefi, A. and Hoseinifar, S.H., 2014.** The effects of dietary Immunogen on innate immune response, immune related genes expression and disease resistance of rainbow trout (*Oncorhynchus mykiss*). *Fish and Shellfish Immunology*, 209-2014.
- Zhang, C.N., Li, X.F., Xu, W.N., Jiang, G.Z., Lu, K.L., Wang, L.N. and Liu, W.B., 2013.** Combined effects of dietary fructooligosaccharide and *Bacillus licheniformis* on innate immunity, antioxidant capability and disease resistance of triangular bream (*Megalobrama terminalis*). *Fish Shellfish Immunology*, 35, 1380-1386.
- Zhang, C., Wang, C, N., Xu, Y., Tan, H.Y., Li, S. and Feng, Y., 2018.** Molecular mechanisms involved in oxidative stress-associated liver injury induced by Chinese herbal medicine: an experimental evidence-based literature review and network pharmacology study. *International Journal of Molecular Sciences*, 19, 2745.