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Original Article

# Protective effects of prebiotic in zebrafish, *Danio rerio*, under experimental exposure to Chlorpyrifos

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**Abstract:** The current study estimated the immunotoxicological effects of the herbicide chlorpyrifos at sub-lethal concentration and the potential ameliorative effects of galactooligosaccharide (GOS) in *Danio rerio*. Fish was sampled after a 56-days feeding with GOS and then exposed to 15 mg/l chlorpyrifos for 7-days to assess the non-specific immune responses (total protein, immunoglobulin and ALP activity). The results revealed that feeding zebrafish with 1% dietary GOS increased total protein levels ($P<0.05$), but no significant effect was noticed between groups fed 0.5 and 2% GOS and control ($P>0.05$). There were significant difference between total immunoglobulin levels 1% and control group ($P<0.05$). Furthermore, in case of ALP activity no significant alteration was noticed between GOS fed fish and control ($P>0.05$). The present findings revealed that dietary supplementation with GOS could be useful for modulation of the immunity in response to chlorpyrifos exposure, thereby presenting a promising feed additive in aquaculture.

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## Introduction

Aquatic ecosystems that run through agricultural areas have probability of contamination by several chemicals such as pesticides that are used extensively, which on entails multiple change to the aquatic ecosystem, resulting in multiple changes in organism, such as altering the growth rate, patterns of behavior, nutritional value and etc. Aquaculture is a major part of the world's food production, therefore it is essential to maintain fish health (Tripathi et al., 2002). Obviously, water contaminated with chemical pesticides and stressful conditions may increase the incidence of infectious diseases by opportunist bacteria (Moraes et al., 2007; Rudneva, 2006).

Chlorpyrifos (O,)-diethyl-O3,5,6-trichlor-2pyridyl phosphorothioate (CPF) is located in an expansive group of organophosphorus insecticides that is commercially used to control foliar insects and affects agricultural products (Rusyniak and Nanagas, 2004) and subterranean termites (Venkateswara Rao et al., 2005). It was first introduced in 1965 and used to control insects in agriculturally and in the home. Organophosphorus compounds (OP) are acetylcholin-esterase (AChE) inhibitors generally used to control snails. Annually more than 3 million cases of acute poisoning whit organophosphorus compounds are reported, resulting deaths of 300,000 people (Bertolote, 2006). Exposure to OP prevents AChE activity leading to the accumulation of the neurotransmitter acetylcholine (Ach), activation of Ach receptors, and high stimulation of cholinergic neurons and seizures. It has been observed that vitamins and minerals could ameliorate pyrethroids effects on chicks (Aslam et al., 2010). Although, there is no report available on fish. Lately, much attention has been paid to approaches for decreasing damaging effects of pesticides on fish.

Galactooligosaccharide (GOS) is a prebiotic, produced through the enzymatic conversion of lactose and mainly consists of galactose and glucose molecules (Sako et al., 1999). In recent years, zebrafish, *Danio rerio*, raised a model for quick analysis of genes function and biological activities of organic molecules. In this way, possibility of compounds test will be performed (Crawford et al., 2008).



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Using prebiotics can be a convenient and cost effective way to reduce the inevitable intake of pesticides in humans and wildlife. Despite limited works on potential of prebiotics to reduce stress, several studies have reported beneficial effects on prebiotics and algae. Those stress agents are transportation, temperature stress, environmental pollution and other stressful situations (Carvalho et al., 2009; Liu et al., 2010). Dietary administration of probiotics improved fish immune parameters under stressful conditions (Taoka et al., 2006; Gomes et al, 2009). By inversely regulating the gut colonization of the probiotic bacterial strain and body cortisol level (Carnevali et al., 2006). Perhaps the mechanism of action of prebiotics is like probiotic. The use of probiotic Alchem Poseidon (containing a mix of *Bacillus subtilis, Lactobacillus acidophilus, Clostridium butyricum* and *Saccharomyces cerevisiae*) increased stress tolerance capacity in *Paralichthys olivaceus* (Taoka et al., 2006). In addition, *Spirulina platensis* is used to combat organ toxicants by heavy meals, including lead and cadmium (Taoka et al., 2006). Despite the potential of prebiotics, there is very limited available information about possible ameliorative effects of prebiotics when fish exposed to pesticides. Thus, the present study was performed to elucidate possible ameliorating effects of dietary prebiotic galactooligosaccharide immune-logical stress *Danio rerio* as a result of Chlorpyrifos exposure.

## Materials and Methods

**Fish and experimental condition:** The current study was conducted at Aquaculture Lab of Gorgan University of Agricultural Science and Natural Resources. Four hundred and twenty zebra fish fry were purchased from a private of ornamental fish center (Golestan Orovince, Iran). Fish (45±0.1 mg) were adapted for two weeks to experimental condition then randomly released into 12 aquarium (60 L), at a density of 35 fish per aquarium. Treatments were under static aerated water conditions with 50% change every day during rearing.

**Experimental diets and feeding trial:** Vivinal-GOS® was kindly supplied by friesland foods Domo Company (Zwolle, The Netherlands). A commercial diet (Biomar Fish Foods, protein 54%, fat 18%, moisture 10%, Digestible energy 4 cal/gr) were supplemented by different levels of GOS (0, 0.5, 1 and 2%). The prepared diets were kept 24 hours in room temperature, dried and stored in plastic bag at -4$^{o}$C. Fish were hand-fed three times a day(09:00-11:00-13:00) for 8 weeks to satiation. At the end of feeding trial, prebiotic fed fish were exposed to a pre-determined $LC_{50}$ of chlorpyrifos (15 mg/l) for one week.

**Evaluation of immune parameters**

**Samples collection:** Six fish were randomly sampled under sterile conditions after anesthetized with clove solution with dose of 2000 ppm were cut head and fins, whole body were taken as sample according to Holbech et al. (2001). Then, they were homogenized and transferred to 1.5 ml sterile tubes, centrifuged (2000rpm for 10 min at 4°C) and the obtained supernatant were stored at -80°C until analyses.

**Whole body total protein**: Whole body total protein levels were measured by biuret method using a commercial kit (Pars Azmun co, Ltd., Tehran, Iran) according to company protocol and their absorption was at 560 nm.

**Whole body total Ig**: The total protein was measured according to the method explained above and 12% polyethylene glycol solution was added to supernatant. After centrifugation immunoglobulin molecules were precipitated and total protein levels were measured again.

**Whole body Alkaline phosphatase activity (ALP)**: ALP activity of the whole body was determined using a commercial kit (Pars Azmun co, Ltd., Tehran, Iran) (Hoseinifar, 2015). Samples were prepared following company protocol and their absorption was read at 405 nm.

**Statistical analysis:** After checking the normality of the data, statistical analysis was performed using One way ANOVA followed by Duncan's multiple-range test. The significant difference levels was considered when $P<0.05$. The statistical analysis was performed by SPSS software 19 (SPSS, USA).

## Results

The results of total immunoglobulin of GOS fed zebrafish exposed to the chlorpyrifos are shown in Figure 1. There were significant different between total immunoglobulin levels of 1% GOS fed zebrafish and control group (*P*<0.05). However, no significant difference was noticed between 0.5 or 2% GOS and control group (*P*>0.05).

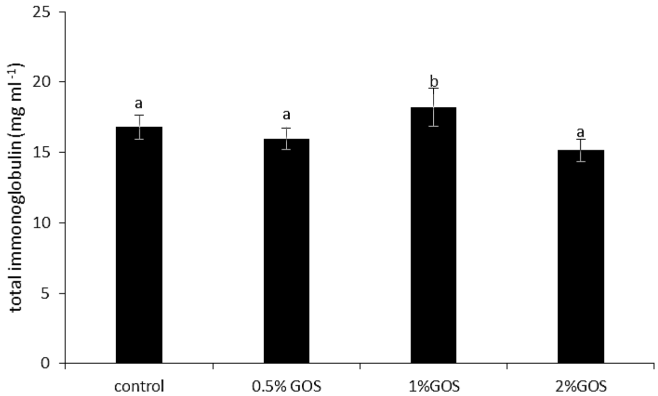


Figure 1. The total immunoglobulin levels of GOS fed zebrafish exposed to chlorpyrifos (Values are presented as the mean±S.D.; the bars assigned with different letter denote significant difference between treatments (*P*<0.05)).

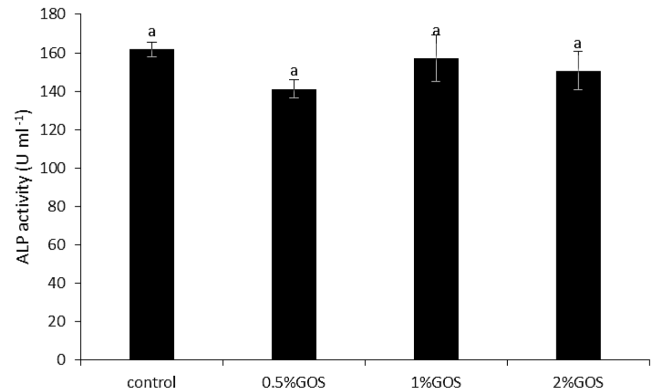


Figure 2. The ALP activity of GOS fed zebrafish exposed to chlorpyrifos (Values are presented as the mean±S.D.; the bars assigned with different letter denote significant difference between treatments (*P*<0.05)).

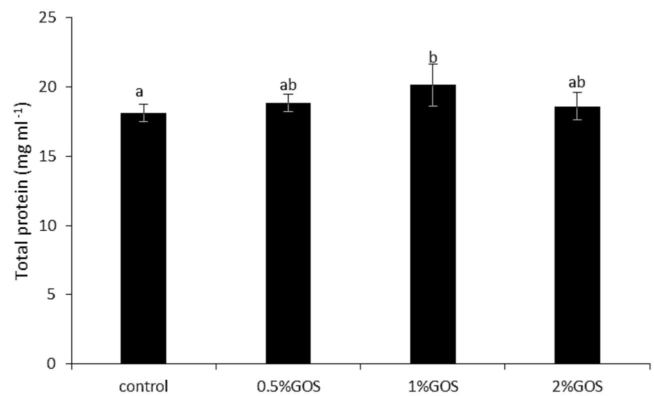


Figure 3. The total protein levels of GOS fed zebrafish exposed to chlorpyrifos. Values are presented as the mean±S.D.; the bars assigned with different letter denote significant difference between treatments (*P*<0.05)).

Following pesticide exposure, in case of ALP activity no significant was noticed between GOS fed and control group (*P*>0.05) (Fig. 2). Although, ALP levels were slightly decreased in GOS fed fish. Furthermore, feeding zebrafish on 1% dietary GOS increased total protein levels (*P*<0.05), but no significant difference was noticed between groups feeding on 0.5 and 2% GOS fed and control (*P*>0.05) (Fig. 3).



## Discussion

Fish are constantly exposed to chemicals released into aquatic system and therefore are organisms that are always exposed to disruption of innate and adaptive immune reasons (Watzke et al., 2007). Pesticides are considered as one of the main stressors in the environment which in living organisms cause induce oxidative, metabolic, immunological stress and growth retardation (Slaninova et al., 2009; Sahar et al., 2011). In fact, serum immune parameters are very good indicator of immune system (Nayak, 2010). The immune response is a protective response against infective agents enter the body. Hence it is host resistance challenge test determines the immune system function with highest level of communication biological relevance, because it measures an integrated immune reasons at the level of the whole organisms (Köllner et al., 2002). Immunoglobulins are natural antibodies that are produced in the absence of stimulant external antigens (Magnadottir et al., 2011). In the present study, the total protein and immunoglobulin was found increased in group fed 1% GOS and pesticide exposed. However, in case of ALP activity, no significant was noticed between GOS fed fish and control. However, being fed with prebiotic supplemented diets, the values increased considerably. This might suggest the positive role of the prebiotic in enhancing the immune status of the pesticide exposed fish. Although there are several reports of the effect of prebiotics on the immune system, so that reported increased immune response administration of GOS in Rainbow trout (Hoseinifar et al., 2015) and goldfish (Miandare et al., 2016) diet significantly increased level mucus total protein. However, there is a limited studies on protective effects of prebiotic in pesticide exposed fish. Jayantha Rao et al. (1984) reported that *Tilapia mossambica,* treated with phosphamidon, an organophosphate insecticide, showed a higher serum bilirubin level and severe liver damage. Also, Mohapatra et al. (2012) reported that values of total protein and albumin were decreased in the fenvalerate exposed *Labeo rohita* as to the probiotic supplemented fish. Also, it has been reported that fenvalerate exposed fish in comparison to the non-exposed fish high level of serum ALP and ACP. The improved immune system caused using GOS can be due to the production of short chain fatty acids (SCFAs) (Guerreiro et al., 2015) that is result of GOS fermentation by beneficial intestinal microbiota. SCFAs binding to receptors GPR43 which is modulated innate immune response and inflammatory cells (Maslowski et al., 2015). Furthermore, the effects of prebiotics on immune response attributed to modulation of intestinal microbiota and increase in lactic acid bacteria and *Bacillus* which their cell walls (lipopolysaccharide) function as immunostimulant (Song et al., 2014; Hoseinifar et al., 2012).

This study indicate the impacts of chlorpyrifos on fish. In addition, the role of GOS in protecting immune cells against chlorpyrifos induced immunotoxicity by improving innate immune system to defend against infections through stimulation of innate immune responses, which underlines the important role of dietary GOS supplements in aquaculture. Further research using different concentrations of GOS supplementation should be performed to provide a complete protection and help on understanding the role of GOS in defending against chlorpyrifos induced immune-toxic impact.

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## References

Abdelkhalek N.K.M., Ghazy E.M., Abdel-Daim M.M. (2015). Pharmacodynamic interaction of *Spirulina platensis* and deltamethrin in freshwater fish Nile tilapia, *Oreochromis niloticus*: impact on lipid peroxidation and oxidative stress. Environmental Science and Pollution Research, 22: 3023-31.

Aslam F., Khan A., Khan M.Z., Sharaf S., Gul S.T., Saleemi M.K. (2010). Toxico-pathological changes induced by cypermethrin in broiler chicks: their attenuation with Vitamin E and selenium. Experimental and Toxicologic Pathology, 62(4): 441-50

Bertolote J.M. (2006). Deaths from pesticide poisoning: a global response. The British Journal of Psychiatry, 189: 201-203.

Brookes P.S. (2004). Calcium, ATP, and ROS: a mitochondrial lovehate triangle. American Journal of Physiology-Cell Physiology, 287: C817-C833.

Burr G., Hume M., Ricke S., Nisbet D., Gatlin D. (2010). In vitro and in vivo evaluation of the prebiotics GroBiotic a, inulin, mannanoligosaccharide, and galactooligosaccharide on the digestive microbiota and performance of hybrid striped bass (*Morone chrysops Morone saxatilis*). Microbial Ecology, 59: 187-98.

Carnevali O., DeVivo L., Sulpizio R., Gioacchini G., Olivotto I., Silvi S., Cresci A. (2006). Growth improvement by probiotic in European sea bass juveniles (*Dicentrarchus labrax*, L.), with particular attention to IGF-1, myostatin and cortisol gene expression. Aquaculture, 258: 430-438.

Carvalho E.S., Gomes L.C., Brandao F.R., Crescencio R, Chagas E.C., Anselmo A.A.S. (2009). The use of probiotic Efinol during transportation of tambaqui (*Colossoma macropomum*). Arquivo Brasilerio de Medicina Veterinaria e Zootecnia, 61: 1322-1327.

Crawford A.D., Esguerra C.V., de Witte P.A. (2008). Fishing for drugs from nature: zebrafish as a technology platform for natural product discovery. Journal of Planta Medica, 74(06): 624-632.

Gomes L.C., Brinn P.R., Marcon J.L., Dantas L.A, Brandao F.R., De Abreu J.S., Lemos P.E.M., McComb D.M., Baldisserotto B. (2009). Benefits of using the probiotic Efinol during transportation of cardinal tetra, *Paracheirodon axelrodi* (Schultz), in the Amazon. Aquaculture Research, 40: 157-165.

Grisdale-Helland B., Helland S.J., Gatlin III D.M. (2008). The effects of dietary supplementation with mannanoligosaccharide, fructooligosaccharide or galactooligosaccharide on the growth and feed utilization of Atlantic salmon (*Salmo salar*). Aquaculture, 283: 163-7.

Guerreiro I., Couto A., Machado M., Castro C., Pousao-Ferreira P., Oliva-Teles A. (2015). Prebiotics effect on immune and hepatic oxidative status and gut morphology of white sea bream (*Diplodus sargus*), Fish and Shellfish Immunology, 50: 168-174.

Holbech H., Andersen L., Petersen G.I., Korsgaard B., Pedersen K.L., Bjerregaard P. (2001). Development of an ELISA for vitellogenin in whole body homogenate of zebrafish (*Danio rerio*). Journal of Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology, 130(1): 119-131.

Hoseinifar S.H., Esteban M.A, Cuesta A., Sun Y.Z. (2015). Prebiotics and fish immune response: a review of current knowledge and future perspectives. Review in Fisheries Science and Aquaculture, 23: 315-328.

Hoseinifar S.H., Esteban M.Á., Cuesta A., Sun Y.Z. (2015). Prebiotics and fish immune response: a review of current knowledge and future perspectives. Journal of Reviews in Fisheries Science and Aquaculture, 23(4): 315-328.

Hoseinifar S.H., Roosta Z., Hajimoradloo A., Vakili F. (2015). The effects of Lactobacillus acidophilus as feed supplement on skin mucosal immune parameters, intestinal microbiota, stress resistance and growth performance of black swordtail (*Xiphophorus helleri*), Fish & Shellfish Immunology, 42: 533-538.

Hoseinifar S.H., Mirvaghefi A., Amoozegar M.A., Sharifian M., Esteban M.Á. (2015). Modulation of innate immune response, mucosal parameters and disease resistance in rainbow trout (*Oncorhynchus mykiss*) upon synbiotic feeding. Fish and Shellfish Immunology, 45(1): 27-32.

Jayantha Rao K., Azhar Jr B., Ramamurthy K. (1984). Effect of a systemic pesticide phosphamidon on some aspects of freshwater fish, *Tilapia mossambica*, Indian Journal of Environmental Health, 26: 60-64.

Magnadottir B. (2006). Innate immunity of fish (overview). Fish and Shellfish Immunology, 20(2): 137-51.

Miandare H.K., Farvardin S., Shabani A., Hoseinifar S.H., Ramezanpour S.S. (2016). The effects of galactooligosaccharide on systemic and mucosal immune response, growth performance and appetite related gene transcript in goldfish (*Carassius auratus gibelio*). Fish and Shellfish Immunology, 55: 479-483.

Köllner B., Wasserrab B., Kotterba G., Fischer U. (2002). Evaluation of immune functions of rainbow trout (*Oncorhynchus mykiss*) how can environmental influences be detected? Toxicology Letter, 131: 83-95.

Maslowski K.M., Mackay C.R. (2010). Diet, gut microbiota and immune responses. Nature Immunology, 12: 5-9.

Moraes B.S., Loro V.L., Glusczak L. (2007). Effects of four rice herbicides on some metabolic and toxicology parameters of teleost fish (*Leporinus obtusidens*). Chemosphere, 68: 1597-601.

Nayak S.K. (2010). Fish Probiotics and immunity: a fish perspective. Fish and Shellfish Immunology, 29(1): 2-14.

Roosta Z., Hajimoradloo A., Ghorbani A., Hoseinifar S.H. (2014). The effects of dietary vitamin C on mucosal immune responses and growth performance in Caspian roach (*Rutilus rutilus caspicus*) fry. Fish Physiology and Biochemistry, 40: 1601-1607.

Rudneva I.I. (2007). Agricultural aspects of aquatic environmental toxicology (a review of literature). Gigiena i sanitariia, (2): 24-28.

Rusyniak D.E., Nanagas K.A. (2004). Organophosphate poisoning. Seminars in Neurology, 24: 197-204.

Sahar E., Magd E.L., Abou M.E.S. Laila A., Shoukry A. (2011). Pyrethroid toxic effects on some hormonal profile and biochemical markers among workers in pyrethroid insecticides company. Life Science Journal, 8(1): 311-322.

Sako T., Matsumoto K., Tanaka R. (1999). Recent progress on research and applications of non-digestible galactooligosaccharides. International Dairy Journal, 9: 69-80.

Shephard K.L. (1994). Functions for fish mucus. Reviews in Fish Biology and Fisheries, 4: 401-29.

Simsek N., Karadeniz A., Kalkan Y., Keles O.N., Unal B. (2009). *Spirulina platensis* feeding inhibited the anemia- and leucopenia-induced lead and cadmium in rats. Journal Hazardous Materials, 164: 1304-9.

Slaninova A., Smutna M., Modra H., Svobodova Z. (2001). review: oxidative stress in fish induced by pesticide. Neuroendocrinology Endocrinology Letters, 30(1): 2-12.

Song S.K., Beck B.R., Kim D., Park J., Kim J., Kim H.D. Ringø E. (2014). Prebiotics as immunostimulants in aquaculture: a review. Fish & Shellfish Immunology, 40(1): 40-48.

Taoka Y., Maeda H., Jo J.Y., Jeon M.N., Bai S.C., Lee W.J., Yuge K., Koshio S. (2006). Growth, stress tolerance and non-specific immune response of Japanese flounder, *Paralichthys olivaceus* to probiotics in a closed recirculating system. Fisheries Science, 72: 310-321.

Taoka Y., Maeda H., Jo J.Y., Jeon M.N., Bai S.C., Lee W.J, Yuge K., Koshio S. (2006). Growth, stress tolerance and non-specific immune response of Japanese flounder, Paralichthys olivaceus to probiotics in a closed recirculating system. Fisheries Science, 72: 310-321.

Tripathi G., Harsh S. (2002). Fenvalerate-induced macromolecular changes in the catfish, *Clarias batrachus.* Journal of Environmental Biology*,* 23: 143-146.

Van Hai N., Fotedar R. (2009). Comparison of the effects of the prebiotics (Bio-Mos and b-1, 3-D-glucan) and the customised probiotics (*Pseudomonas synxantha* and *P. aeruginosa*) on the culture of juvenile western king prawns (*Penaeus latisulcatus* Kishinouye, 1896). Aquaculture, 289: 310-6.

Venkateswara Rao J., Parvati K., Kavitha P., Jakka N.M., Pallela R. (2005). Effect of chlorpyrifos and monocrotophos on locomotor behaviour and acetylcholinesterase activity of subterranean termites, *Odontotermes obesus*. Pesticide Management Science, 61: 417-421.

Watzke J., Schirmer K., Scholz S. (2007). Bacterial lipopolysaccharides induce genes involved in the innate immune response in embryos of the zebrafish (*Danio rerio*). Fish and Shellfish Immunology, 23: 901-5.

Yang S.T., Silva E.M. (1995). Novel products and new technologies for use of a familiar carbohydrate, milk lactose. Journal of Dairy Science, 78: 2541-62.

Zhou Q.C., Buentello J.A., Gatlin III D.M. (2010). Effects of dietary prebiotics on growth performance, immune response and intestinal morphology of red drum (*Sciaenops ocellatus*). Aquaculture, 309: 253-7.

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چکیده فارسی

# اثرات حفاظتی پربیوتیک بر ماهی زبرا (*Danio rerio*) در مواجه با سم کلرپیریفوس

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**چکیده:**
در مطالعه حاضر اثرات زیست ایمنی علف‌کش کلرپیریفوس در غلظت تحت کشنده و اثرات حفاظتی پربیوتیک گالاکتوالیگوساکارید در ماهی زبرا (*Danio rerio*) مورد بررسی قرار گرفت. بعد از ۵۶ روز تغذیه با پربیوتیک و یک هفته مواجه با سم جهت بررسی پاسخ ایمنی غیر اختصاصی (پروتئین کل، ایمونوگلوبولین کل و فعالیت آلکالین فسفاتاز) از کل بدن ماهی نمونه‌برداری انجام شد. نتایج نشان دهنده این بود که پروتئین کل در گروه تغذیه شده با سطح ۱ درصد گالاکتوالیگوساکارید افزایش یافته بود ($P$<۰/۰۵). در حالی‌که بین سطوح ۰/۵ و ۲ درصد با گروه شاهد اختلاف معنی‌داری وجود نداشت ($P$<۰/۰۵). در میزان ایمونوگلوبولین کل در گروه تغذیه شده با سطح ۱ درصد در مقایسه با گروه شاهد اختلاف معنی‌داری وجود داشت ($P$<۰/۰۵). در خصوص میزان فعالیت آلکالین فسفاتاز نیز اختلاف معناداری بین گروه‌های تغذیه شده با پربیوتیک و گروه شاهد مشاهده نشد ($P$>۰/۰۵). بنابراین استفاده از پربیوتیک گلاکتوالیگوساکارید در جیره تأثیر مثبتی بر فاکتورهای ایمنی در ماهی زبرا در مواجه با سم کلرپیریفوس داشت. در نتیجه می‌تواند نوان یک افزودنی با نتایج امیدوار کننده در صنعت آبزی‌پروری معرفی شود.
**کلمات کلیدی:** کلرپیریفوس، پربیوتیک، ایمونوگلوبولین، پاسخ ایمنی.