**Original Article**



# Effects of the Prebiotic in Reducing Histopathological Changes and Immune Response of *Cyprinuscarpio* after Exposer to Abamectin

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## ABSTRACT

**Background:** To assess the toxicity of environmental pollutants in fish, there are different physiological parameters including histology. This study aimed to investigate the effect of prebiotic dietary supplement on fish immune response after exposure to toxicant.

**Methods:** The study was conducted in the Gorgan University of Agricultural and Natural Resources aquaculture Laboratory in 2017. Common carp species were obtained and exposed to 2 ppm, 3 ppm, 6 ppm Abamectin as a toxicant. Prebiotic isomalto-oligosaccharides added to diet with spray method as 1 g/kg and histopathological examinations were done after 60 d experiment. There were no significant differences between aquariums in water quality during the test and no mortality and injuries were observed during accumulation.

**Results:** The Abamectin caused some lesions such as vacuolization latest, bleeding, necrosis, degeneration of the epithelium, the destruction of the villi in the intestine, destruction of liver cells, ascites, hemorrhage, necrosis and nuclear karyolysis in the liver and lymphocytes penetration and degradation of intestinal epithelium in intestine. Maximum lesions observed in 6-ppm toxicant concentration.

**Conclusion:** The isomalto-oligosaccharides probiotics was not successful in stimulating the immune system and reducing adverse effect of toxicant in common carp, significantly. However, usage of this prebiotic could be useful in some cases.

**Keywords:** Abamectin, Common Carp, Prebiotic Isomalto-Oligosaccharides, Tissue Damage.

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## INTRODUCTION

Prebiotics are a type of non-digestible fiber compound, which has beneficial effects on the host by acting as of health-promoting bacteria in the intestinal tract and stimulating the growth [1].

The mechanism of action is different in various prebiotics. Nevertheless, among the numerous beneficial effects of prebiotics, the most common role of prebiotic in aquaculture is increasing growth rate, improve immune system as well as change the community of bacterial in gastrointestinal track [2]. Isomalto-oligosaccharides (IMOs) are functional oligosaccharides prebiotics that considered as anticariogenic agents and enhancing the resistance to diseases [3-7]. Typically, IMOs are naturally could be found in various fermented foods and produced enzymatically as well [8, 9].

Prebiotics enhance the immune response and can have beneficial effects on physiological status of fish [10, 11] and to date beneficial effects different prebiotics on various fish species have been cited. For instance, dietary of fructooligosaccharide and galactooligosaccharide increased stress resistance, intestinal microbiota and growth performance in *Rutilus rutilus* [12, 13], xylooligosaccharide improved mucosal immunity of *R. frisiikutum* [14] and mannan oligosaccharide promoted growth of *Ctenopharyngodon idella* and increased the non-specific immune systems [15]. However, no scientific information is available on the effects of

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IMOs as prebiotic for fish and shellfish, while the positive effect of IMOs has reported in rats [16] and broilers [17].

Abamectin is a kind of pesticides, widely used in agriculture. Abamectin is a group of fermentation products from a strain of *Streptomyces avermitilis* possessing potent anthelmintic and insecticidal activities [18]. Pesticides (including herbicides, insecticides and fungicides), have immunotoxic effects on fish [19] and several pesticides acute toxicity tests were conducted on fish [20-23]

Common carp (*Cyprinus carpio* Linnaeus, 1758, family: Cyprinidae) is a widespread freshwater fish in Iran. Despite recent advances on the administration of prebiotics on fish species, there is currently no data available on the beneficial effects of IMOs for common carp and basic knowledge about profit usage of IMOs in fishes in a need in aquaculture.

The aim of this study was to determin the effects of IMOs dietary on reduction of histopathological changes and elevating immune response after exposure to Abamectin in common carp.

## MATERIALS AND METHODS

The study was conducted in Gorgan University of Agricultural and Natural Resources aquaculture Laboratory in 2017. Common carp juveniles (average weight of 6.13±0.03 gr) obtained from fish farms were randomly stocked into 200 L aquarium for 1 week to accumulate. During the 60 d experiment, each aquarium contained 21 fish (3 tanks per treatment). There were no significant differences between aquariums in water quality and the following were constant: pH: 7.8±0.07; temperature: 22±1 °C; hardness: 260±6 ppm and dissolved oxygen: 5.9±0.65 and photoperiod set as 12 h dark.

A basal and formulated Common carp diet (Energy 3001) obtained from Mahiran Company, Iran and considered as control. The proximate content of control diet was 41% protein, 6% lipid, 5% fiber and 12% moisture. Experimental diets were prepared by supplementation of the basal with gelatin solution of IMOs prebiotic at rate of 1g per kg. Fish were hand-fed twice a day at rate of 3% of body weight.

Prior to analysis, static acute toxicity test was performed following guideline the Organization for Economic Co-operation and Development OECD standard method [24] and the $LC_{50}$ 96h calculated as 1.24 ppm for Abamectin. Therefore, the following Abamectin sublethal concentrations were selected for experiment: 2, 3 and 6 ppm (with control group which consists of no Abamectin). All treatments were fed with experimental diet.

Fish were sampled from each of the triplicate tanks from each treatment after 60 d of exposure. Five-micron sections were obtained with a microtome (Olympus CUT 4055E, USA) from pieces of gills, liver, and intestine. The tissues were rehydrated in alcohols, stained with hematoxylin-eosin, and then processed following the conventional histological methods for light microscopy. Histological alterations for each organ were evaluated semiquantitatively by the degree of tissue change.

### *Ethical Considerations*

The experiments were performed on fishes complied with the protocols of the Society of Toxicology (code of ethics Jan 31, 1985; Revised Jun 1, 2005; Reviewed and Reaffirmed Sep 14, 2011; Revised Nov 5, 2012) and Canadian Council on Animal Care (CCAC, 1998). All analyses and experiments were performed to minimize suffering. The study was conducted with the minimal number of fishes.

## RESULTS

No mortality and injuries were observed during accumulation. Histopathological examination of gill, liver, and intestine of samples in the control group is represented in Figure. 1. Exposure to Abamectin in caused various injuries in gill, liver, and intestines after 60 d experiment. (Figure. 2-4). Histological examination showed areas of epithelial cells left (intraepithelial "edema"), haemorrhage, necrosis of gill epithelium, lamellar fusion and destruction in gills, degeneration of hepatocytes, cirrhosis, hemosiderin and nuclear karyolysis in liver; and vacuolation, epithelium degeneration, necrosis, villous atrophy and degeneration, lymphocytes penetration and degradation of intestinal epithelium in intestine. The extent of these injuries in different concentrations of Abamectin and with diets of IMOs is represented in Table 1-3. Maximum and minimum injuries in gills, liver, and intestine found in 6 and 2 ppm Abamectin concentration, respectively. Cirrhosis of liver was only observed in 3-ppm Abamectin concentration. Lymphocytes penetration in intestine was maximum in 3-ppm Abamectin concentration.

Table 1-3 represents gill, liver and intestine injuries after exposure to Abamectin without dietary of IMOs. The dietary prebiotic was not successful in reducing the adverse effect of toxicant and increasing the resistance of Common carp from histopathological changes.

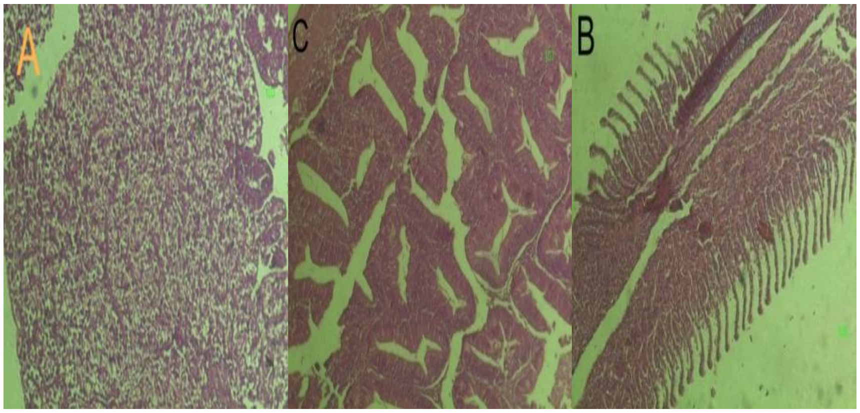


**Figure 1.** Microphotographs of control group histopathological changes in *Cyprinuscarpio.* A: liver tissue, B: gill tissue, C: intestine tissue.

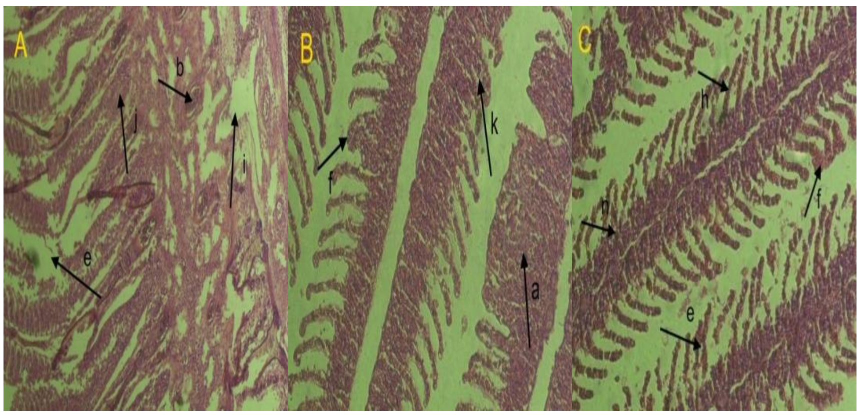


**Figure 2.** Microphotographs of gill sample histopathological changes in Abamectin treatments in *Cyprinuscarpio*fed of dietary isomalto-oligosaccharides. A: 2 ppm concentration, B: 3 ppm concentration, C: 6 ppm concentration. (a): Lamellar fusion, (b): Hemosiderin, (e): Dystrophy in secondary lamellar, (f): Cirrhosis, (h): Degeneration of hepatocytes, (j): Dystrophy in lamellar, (i): Hyperplasia, (n): Necrosis, (k): Lamellar bending.

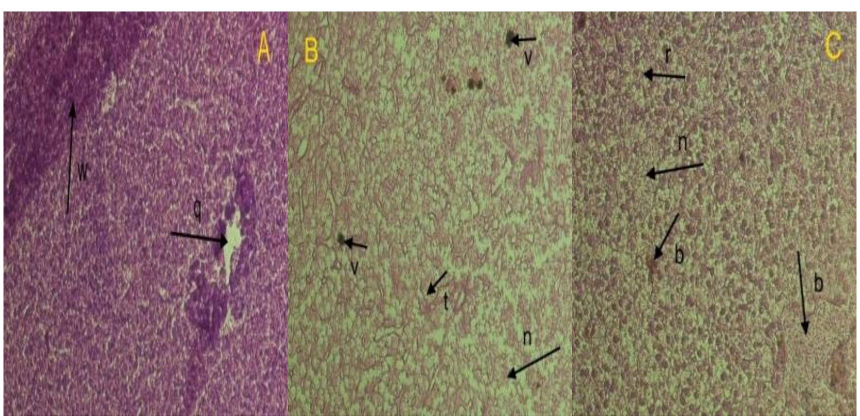


**Figure 3.** Microphotographs of liver sample histopathological changes in Abamectin treatments in *Cyprinuscarpio* fed of dietary isomalto-oligosaccharides. A: 2 ppm concentration, B: 3 ppm concentration, C: 6 ppm concentration. (b): Hemosiderin, (n): Necrosis, (q): Cirrhosis, (r): Degeneration of hepatocytes, (t): Nuclear Karyolysis, (v & w): Primary biliary cirrhosis and swelling.

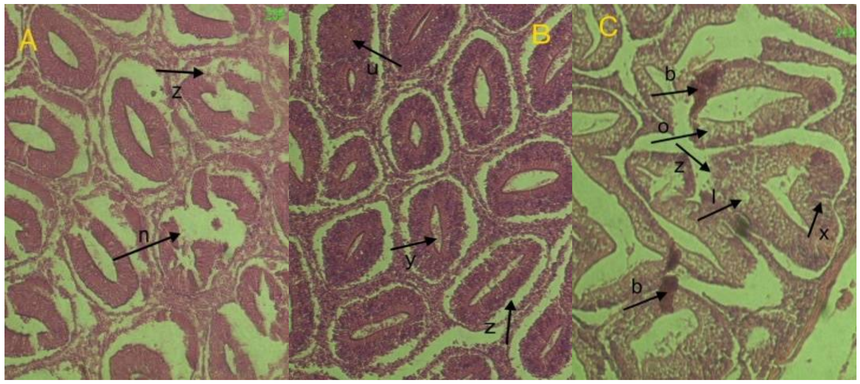


**Figure 4.** Microphotographs of intestine sample histopathological changes in Abamectin treatments in *Cyprinus carpio* fed of dietary isomalto-oligosaccharides. A: 2 ppm concentration, B: 3 ppm concentration, C: 6 ppm concentration. (l) Vacuolation, (o): Epithelium degeneration, (n): Necrosis, (b): Hemosiderin, (z): Villous Atrophy, (y): Lymphocytes penetration, (u): Degradation of intestinal epithelium, (x): Villous degeneration**.**

**Table 1.** Gill lesions after exposure to different concentration of Abamectin in *Cyprinuscarpio*juveniles fed of dietary isomalt-oligosaccharides.

| Gill lesions | Control | 2mg/l | 3 mg/l | 6 mg/l |
|---|---|---|---|---|
| Lamellar fusion | - | + | ++++ | ++++ |
| Hemosiderin | - | ++ | ++++ | ++++ |
| Dystrophy in secondary lamellar | - | ++ | ++++ | ++++ |
| Cirrhosis | - | + | ++++ | ++++ |
| Degeneration of hepatocytes | - | + | ++ | ++++ |
| Dystrophy in lamellar | - | + | ++ | ++++ |
| Hyperplasia | - | ++ | ++++ | ++++ |
| Necrosis | - | + | ++ | ++++ |
| Lamellar bending | - | + | ++++ | ++++ |

-: No observed lesions, +: 1-3 observed lesions, ++: 3-5 observed lesions, +++: 5-11 observed lesions, ++++: 11 and more observed lesions.

**Table 2.** Liver lesions after exposure to different concentration of Abamectin in *Cyprinus carpio* juveniles fed of dietary isomalto-oligosaccharides.

| Liver lesions | Control | 2 mg/l | 3 mg/l | 6 mg/l |
|---|---|---|---|---|
| Hemosiderin | - | + | ++++ | ++++ |
| Cirrhosis | - | ++ | ++ | +++ |
| Degeneration of hepatocytes | - | + | ++ | ++++ |
| Nuclear Karyolysis | - | + | ++++ | ++++ |
| Primary biliary cirrhosis and swelling | - | ++ | ++++ | ++++ |

-: No observed lesions, +: 1-3 observed lesions, ++: 3-5 observed lesions, +++: 5-11 observed lesions, ++++: 11 and more observed lesions.

**Table 3.** Intestine lesions after exposure to different concentration of Abamectin in *Cyprinus carpio* juveniles fed of dietary isomalto-oligosaccharides.

| Intestine lesions | Control | 2 mg/l | 3 mg/l | 6 mg/l |
|---|---|---|---|---|
| Vacuolation | - | ++ | ++ | ++++ |
| Epithelium degeneration | - | + | ++ | ++++ |
| Necrosis | - | +++ | ++ | ++++ |
| Hemosiderin | - | ++ | ++ | +++ |
| Villous Atrophy | - | +++ | ++ | ++++ |
| Lymphocytes penetration | - | + | +++ | + |
| Degradation of intestinal epithelium | - | +++ | ++ | ++++ |
| Villous degeneration | - | +++ | ++++ | ++++ |

-: No observed lesions, +: 1-3 observed lesions, ++: 3-5 observed lesions, +++: 5-11 observed lesions, ++++: 11 and more observed lesions.

## DISCUSSION

Technically, degree of Abamectin toxicity is strongly related to the tolerance level in various fish species [18]. However, commercial brand formulated to cause immediate effect may be more toxic than the active compound. Therefore, we tested the formulated product. Results of this study clearly indicate histopathological alteration of common carp after exposure to Abamectin. The observed injuries in this study were also reported [25] in gill and liver of *Lates calcarifer* after exposure to 5, 10 and 15 ug/L Abamectin concentration.

Upon acute exposure, Abamectin was highly toxic to common carp and fish could not recover after exposure in IMOs diets treatments. The innate immune system is responsible for maintaining cellular and molecular equilibrium during acute phase and inflammatory reactions associated with infections [26]. This is the first attempt to investigate the effects of IMOs as prebiotic on common carp. Prebiotics effectively improve immune response [1,2]. However, in this study, IMOs could not stimulate the immune response of fish through dietary supplements and reduce tissue damage from Abamectin. Toxicant disturb the regulations of cations such as $Na^+$, $K^+$, $Ca^{2+}$ and $Mg^{2+}$ in fish body [27,28]. The reduction in plasma electrolyte may be caused by two important factors; 1) the process of ions uptake is inhibited by the chloride cells of the gills contributing to the negative ions balance of the blood, and 2) increased passive efflux of ions across he gill because of disturbing occurred in branchial permeability leading to haemodilution by enhanced osmotic uptake of water across the gills [25].

Disturbing in the regulations of cations in fish after exposure to Abamectin has been reported [29] in *Orechromis niloticus* and Thanomsit [25] in *Lates calcarifer*. In addition, proteolysis and an increasing in metabolism under Abamectin stress would also cause reduction of protein levels [30]. Isomalto-oligosaccharides may also modulate serum electrolyte concentrations in rats and human while this prebiotic was not successful in modulating common carp serum electrolyte in this study [31, 32]. However, none of serum enzymes, metabolites, and electrolytes of common carp was investigated in this study. Other researchers also reported ineffective role of other prebiotics in fish.

The effects of inulin on *Sparus aurata* were investigated and reported no immunostimulant effect of inulin on the innate immune system of this species [33]. Indeed, inulin does not affect the medium's osmolarity nor the leucocyte viability. Supplement of inulin in *Salvelinus alpinus* diets was caused destructive effect on microvillous organization in the hindgut and enterocytes [34].

## CONCLUSION

Based on the scarce data further studies are mandatory to ascertain whether IMOs disrupts regulations of cations or not. In addition, another effect of IMOs on common carp might be worth to be investigated. IMOs cannot stimulate immune system of common carp to reduce adverse effects of Abamectin.

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