Effect of dietary chitosan on immune response and disease resistance in *Cyprinus carpio*

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Abstract:

BACKGROUND: Occurrence of resistance against antibiotics and inadequate efficacy of some vaccines necessitates studies of natural immunostimulators in aquaculture. Shrimps shell derived from Chitosan can be used as immune stimulators in fish. **OBJECTIVES:** In this study, the effects of oral administration of chitosan, derived from shrimp shell, on some immune responses and disease resistance in *Cyprinus carpio* were studied. **METHODS:** Three hundred healthy fish weighing 42.4+8.1 g were divided into 4 equal groups: the first group (G10) was fed with food supplemented with 10 mg kg^{-1} chitosan, the second (G5) and third groups (G2.5) were fed with food supplemented with 5 mg kg^{-1} and 2.5 mg kg^{-1}, respectively. The control group was fed with basal feed (without chitosan). All groups were treated for 60 days. Blood samples were taken on 0, 20, 40, and 60 days post- experiment; In addition, some immunological indices, including serum lysozyme activity, serum bactericidal activity, Nitro Blue Tetrazolium (NBT) reduction activity, serum proteins, white blood cell count (WBC), and differentiated count were measured. At the end of the treatment, fish were challenged with live *Aeromonas hydrophila* and mortality rate was recorded for 14 days. **RESULTS:** Oral administration of chitosan (0.5 and 1%) significantly enhanced NBT reduction activity and resistance to *A. hydrophila* infection ($p=0.012$). Serum lysozyme and bactericidal activity, serum total protein and globulin, WBC and leukocytes ratio showed no significant change among the groups ($p>0.05$). **CONCLUSIONS:** This study indicates that oral administration of shrimp shell chitosan may have a positive effect on some immune parameters and resistance against bacterial infection in *Cyprinus carpio*.

Introduction

Fish culture is an important industry around the world. There is essential attention to improve this industry in closed and small areas. Due to intensive culture, over-crowding leads to poor physiologic conditions and increasing susceptibility to diseases (Sakai, 1999). Vaccination and antibiotics are used for treating and controlling fish diseases; however, there are limited vaccines for fish diseases, and utilization of antibiotics is not safe because of the development of antibiotic-resistant bacteria strain; in addition, these ways are very expensive (Siwicki et

al., 1994; Sakai, 1999; Salisbury et al., 2002). Reducing mortality due to opportunistic pathogens, preventing viral diseases, enhancing efficacy of anti-microbial agents, and vaccines as well as increasing resistance to parasites are benefits of using immune-stimulants (Bricknell and Dalmo, 2005). Enhancing aquatic organism immune status by dietary administration of immune-stimulants is an acceptable practice (Sakai, 1999). Different immune-stimulants have been reported to enhance natural (innate) immunity in fish. These materials include: killed bacteria and bacterial products (Nayak et al., 2007; Aly et al., 2008; Geng et al., 2011), herbal extracts (Dugenci et al., 2003; Selvaraj et al., 2006; Alishahi et al., 2010), some vitamins (Nayak et al., 2007; Cerezuela et al., 2009), Levamisole (Findlay and Munday, 2000; Gopalakannan and Arul, 2006), nucleotides (Low et al., 2003), hormones (Yada et al., 2002), and some biopolymers such as Chitin (Esteban et al., 2000; Cuesta et al., 2003). Recently optimized usage of food industries wastes or by-products as food additives have been increased to better conservation of environment (Esteban et al., 2000). One of these by-products is chitosan.

Chitosan is an amino-oligosaccharide (a linear homo-polymer of ß-(1-4)-2-amino-deoxy-D-glucose) and is obtained with alkaline de-acetylation from Chitin (poly (ß -(1-4)-N-acetyl-D-glucose-amine), obtained from crustaceans exoskeleton, insects cuticle and cell wall of some microbes. Chitosan has biological activities such as immune-modulatory, adjuvant, anti-microbial, wound healing, analgesic, anti-oxidant, anti-tumor, etc. (Seferian and Martinez, 2001; No et al., 2002; Okamoto et al., 2002; Qin et al., 2002; Dutta et al., 2004; Boonyo et al., 2007; Harikrishnan et al., 2012; Ramesh and Maridass, 2010); meanwhile, chitosan has industrial activities such as stimulation of plant growth, preservative, thickener, and stabilizer for sauces and coating of fruit in food technology, seed coating, frost protection in agriculture technology and clarifying water, removal of metal ions and ecological polymers and reducing odor in water treatment (Muzzarelli et al., 1989; Ohta et al., 1999; Rinaudo, 2006). Nowadays, Chitosan is examined to enhance immune status and bacterial and viral diseases protection in aquaculture (Dautremepuits et al., 2004; Gopalakannan and Arul, 2006; Lin et al., 2011; Geng et al., 2011). Seferian and Martinez, (2001) reported immunostimulating and adjuvant effects of Chitosan in intraperitoneal route i. Meanwhile, Anderson and Siwicki (1994) showed immunostimulating effects of chitosan in injection or in immersion routs in rainbow trout.

Common carp cultivates as an important world-wide warm-water fish in earthen pond of cyprinid polyculture system in Iran. Annual production of this species is around 20000 tones. Annual production of farmed shrimp in Iran is about 10000 tons, and around 40 percent of this production is by-products like shell which is left in the environment as waste materials. Therefore, these large amounts of shrimp shell, which nowadays contaminate the environment, can be changed to chitosan and used as a food supplementary material in common carp. Therefore, in this study the effect of oral administration of different levels of chitosan, obtained from farmed shrimp shell, on immune responses of common carp were investigated. To the best of our knowledge, it is the first study which evaluates the effect of chitosan derived from Peneous vanameii as an immunostimulant in common carp.

Materials and Methods

Fish and experimental design: Three hundred healthy common carp, *Cyprinus carpio*, weighing 42.4+8.1 g, were obtained from a fish farm in Ahvaz, Khouzestan province, Iran. They were kept in a 300 l. tank for acclimation for two weeks. Water quality factors were recorded during the experiment: temperature, 25±1ºC; Dissolved oxygen, 8-10 ppm; pH, 7.8±0.2; NO2 <0.01ppm and NH3 <0.1ppm. Water exchange rate was 20% of water volume daily.

Fish were divided into 4 groups in triplicates; groups 1 to 4 were fed with basal diet without chitosan supplementation (as control group), 2.5, 5 and 10 g kg^{-1}chitosan, respectively. Five fish were randomly collected from each group on days 0, 20^{th}, 40^{th}, and 60th of the experiment and anesthetized with 100 ppm MS-222 in de-chlorinated water. Blood samples were taken from caudal vein with a 2cc sterile syringe. Heparinized blood was used for hematological assays. Sera were separated from blood sample via centrifugation, for immunological assays. The sera were stored at -20 ºC until they were used.

Diet preparation: Commercial common carp

food (Beyza feed mill, Shiraz, Iran) was used. Chitosan was grinded by a grinder machine, then was suspended in distilled water, and finally added to diet and mixed completely according to mentioned dosages. After air-drying, the feed were stored at 4 ºC until used (Webster et al., 1997).

Extraction of chitin from shrimp shell: The shrimp shells were washed under running tap water to remove soluble organics, adherent proteins, and other impurities. The shells were then dried at room temperature and grounded. For demineralization of the shells, cold 0.25 M HCl (300 mL) was added to 50.0 g dried and grounded shrimp shells. This extraction was allowed to proceed for 15 min at 4ºC. The suspension was then filtered and additional 300 ml of cold 0.25 M HCl was added to the pellet. After 30 min of cold extraction, the suspension was filtered again. The pellet was washed to neutrality with tap water, rinsed with distilled water, and then oven-dried at 70ºC overnight. Deproteinization of the chitin was carried out using 1.0 M NaOH (15 mL/g) at 70ºC for 20 h. Then, the extract was cooled to room temperature, filtered, and washed with tap water until neutrality was achieved. The pellet was finally washed with ethanol (96%) and dried at 70ºC.

Preparation of chitosan: The conversion of chitin to chitosan involved deacetylation using strong alkaline treatment. The chitin (1 g) was put into 15-20 ml 50% NaOH at 70°C for 20 h. Then, the extract was cooled, filtered, and washed with tap water until neutrality. The pellet was finally washed with ethanol (96%) and dried at 70ºC.

Obtaind: Obtained chitosan characterization: Molecular weight: 580±12 KD, deacetylation rate: % 83.5±%2.7, colure and solubility, white powder soluble in water and PBS (pH=5)

Immunological parameters (Serum lysozyme activity): Serum lysozyme activity was measured following Ellis (1990) and Nayak et al. (2008). Based on turbidometric method, lyophilized and its activity were measured. The lyophilized *Micrococcus lysodeikticus* (Sigma, USA) at a concentration of 0.2 mg mL^{-1} (in 0.02 M sodium citrate buffer) were added to sera ratio of 1:10 v/v in the same buffer. Immediately after adding *M. lysodeikticus*, the first OD was read at 450 nm. The second OD was read 60 minutes later. Lysozyme activity was expressed as units mL^{-1} where one unit is defined as the decrease in absorbance of 0.001 min^{-1}.

Serum bactericidal activity: Serum bactericidal activity was measured according to Kajita et al. 1990 with slight modification. Sera samples from each subgroup were diluted three times with 0.1% gelatin-veronal buffer (GVBC2) (pH 7.5, containing 0.5 mM mL^{-1} Mg2C and 0.15 mM mL^{-1} Ca2C). The bacteria *Yersinia ruckerii* (live, washed cells used earlier) was suspended in the same buffer to make a concentration of 1×10^5 cfu mL^{-1}. The diluted sera and bacteria were mixed at 1:1, incubated for 90 min at 25^{o}C, and shaken. One control group containing bacterial suspension in same buffer was also incubated for 90 min at 25^{o}C. The numbers of viable bacteria was then calculated by counting the colonies from the resultant incubated mixture on TSA plates in duplicate (two plates per sample) after 24 h incubation. The bactericidal activity of test serum was expressed as percentage of colony forming units in test group to that in control group.

Nitroblue-tetrazolium (NBT) reduction: A part of each blood sample was utilized for determining respiratory burst activity that was evaluated by the reduction of nitroblue-tetrazolium (NBT), following Anderson and Siwicki et al. (1994). 1 mL of heparinized blood from fish of each group was mixed with 100 ml of 0.2% NBT (Sigma, USA) solution for 30 min at 25ºC after incubation; 50 ml from the mixture above was added with 1 mL of N-diethyl methyl formamide (Qualigens, India) and then centrifuged at 3000 × g for 5 min. The optical density of the supernatant was measured at 620 nm.

Total serum protein, Albumin and globulin: Total protein of each sample was analyzed following Lowry et al.'s (1951) method. Albumin content was measured using a standard albumin estimation kit (Zistchem Diagnostics, Iran) and the globulin content was estimated by subtracting albumin from total protein.

White blood cell count (WBC), Differential cell count: Leucocyte total count was made in a Neubauer counting chamber. Blood smears were stained with Giemsa, then 100 leucocytes were counted under the microscope (1000X) and the percentage of different types of leucocytes was calculated following Schaperclaus et al. (1991).

Challenge with bacterium: Virulent strain of *A. hydrophila* (isolated from common carp mortality in

Iran) was used for disease resistance assay. Thirty fish from each group were intraperitoneally injected with the bacterial suspension (2.1×10^7 CFU per fish $=LD_{50}$), and the mortality of challenged fish was recorded daily for 14 days. The cause of death was ascertained by re-isolating the infecting organism from kidney and liver of the dead fish. The cumulative daily mortality curve was drawn according to Misra et al.'s method (2006).

Statistical analysis: For statistical analysis of data SPSS software version 13 was used. Analysis of Variance (ANOVA) was used for comparison of means among the groups. Duncan complementary test was used for determining the significant differences among the groups. A p-value of <0.05 was accepted as significant.

Results

The serum lysozyme activity in all groups fed with different level of chitosan is shown in Figure 1. The results showed that lysozyme activity was not significantly affected by feeding chitosan supplemented food ($p=0.087$).

Serum bactericidal activity was not affected by oral administration of different levels of chitosan in common carp; however, slight improvement in bactericidal activity was seen in G1 and G2 (Figure 2).

As showed in Figure 3, although there was a significant enhancement of NBT reduction activity in G10 and G5 at days 20, 40 and 60 of experiment ($p=0.035$), no significant change was induced in G2.5 ($p=0.52$).

Total serum protein, albumin, and globulin of treatments were shown in Table 1. Total protein and immunoglobulin in G10 increased in all sampling period, but not significantly ($p=0.085$).

The results suggested that WBC value and blood Leukocytes ratio showed no significant differences in chitosan treated groups ($p>0.05$) (Table 2).

Mortality percentage of common carp fed on different level of chitosan-supplemented diet and the control diet after challenging with *A. hydrophila* is presented in Figure 4. Mortality in G10 and G5 decreased significantly ($p<0.05$). The mortality percentage was highest (76.7%± 6.7) in the control group and lowest (60%± 4.78) in G5 Chitosan group.

Discussion

Recently, chitin and chitosan have been used to increase the resistance of fish by enhancing the non-specific defense mechanisms (Harikrishnan et al., 2012). In this study, some immune responses of common carp were stimulated following oral administration of chitosan. Although slight increase in lysozyme activity was seen in G10 and G5 in some sampling periods, the changes among treatments were not significant ($p=0.087$).

Lysozyme is an important element of innate immunity of fish. It is a lytic enzyme that destroys peptidoglycan layer of gram-positive bacteria and activates complement system and phagocytes (Ellis, 1990). Similar report by Cha et al. (2008) showed that 1% chitosan-coated diet did not enhance lysozyme activity compared to control in Paralichthys olivaceus. Lin et al. (2011) also reported that dietary 0.2% chitosan (produced by microbial fermentation of the crustaceans shell) in diet did not affect lysozyme activity in *Cyprinus carpio* koi. Geng et al. (2011) showed that supplementation of food with 0.3% commercial chitosan did not affect lysozyme activity in Rachycentron canadum. In spite of these reports, there are some work in which chitosan has the stimulating effect on serum lysozyme activity. Gopalakannan and Arul (2006) reported that using of 1% chitosan in common carp has increasing effect on lysozyme activity. Lin et al. (2012) also found that oral administration of oligo-chitosan has a positive effect on lysozyme activity especially when it used along with probiotic. These contradictory results can be referred to the types of chitosan origin, purification procedure and quality of obtained chitosan or differences in fish species.

Although the serum bactericidal activity was enhanced by oral administration of 5 and 10 mg kg-1 chitosan in food, this enhancement was not significant. Similarly, Maqsood et al. (2010) found that serum bactericidal activity in carp fed on diet supplemented with 2 and 5 percent chitosan increased, but 1% chitosan did not affect serum bactericidal activity. Also, supplementation of food of Viscum album extract (Family Loranthaceae) in common carp enhanced serum bactericidal activity in the study of Alishahi et al. (2012). Divyagnaneswari

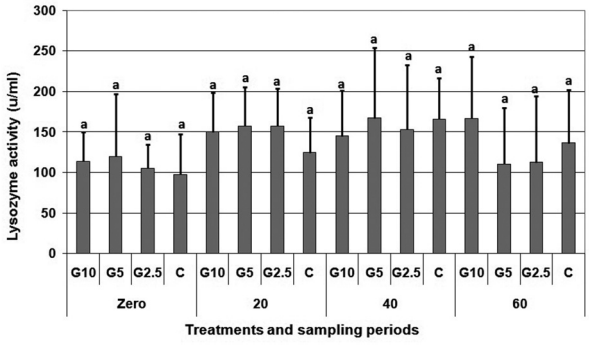

Figure1. The effects of dietary chitosan on serum lysozyme activity (units mL^{-1}) of common carp in each sampling period. Data showed as Mean±SD, n= 15. G10: carp fed with 10g/kg chitosan supplemented food, G5: carp fed with 5 g/kg chitosan supplemented food, G2.5: carp fed with 2.5 g/kg chitosan supplemented feed, C: carp fed with non-supplemented food (Mean±SD, n= 15). Significant differences ($p<0.05$) are marked by different letters.

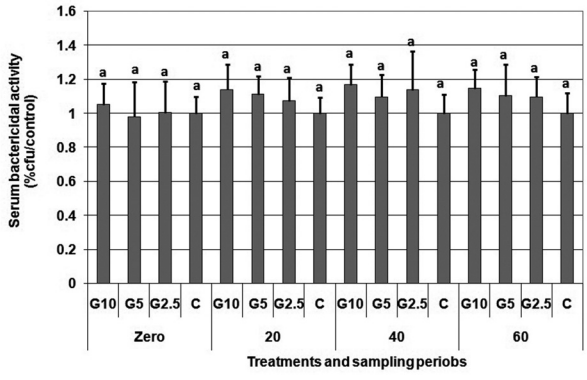

Figure 2. The effects of dietary chitosan on serum bactericidal ativity (as percentage of control group) of common carp in each sampling period. Data showed as Mean±SD, n=15. G10: carp fed with 10g/kg chitosan supplemented food, G5: carp fed with 5 g/kg chitosan supplemented food, G2.5: carp fed with 2.5 g/kg chitosan supplemented feed, C: carp fed with non-supplemented food (Mean±SD, n= 15). Significant differences ($p<0.05$) are marked by different letters.

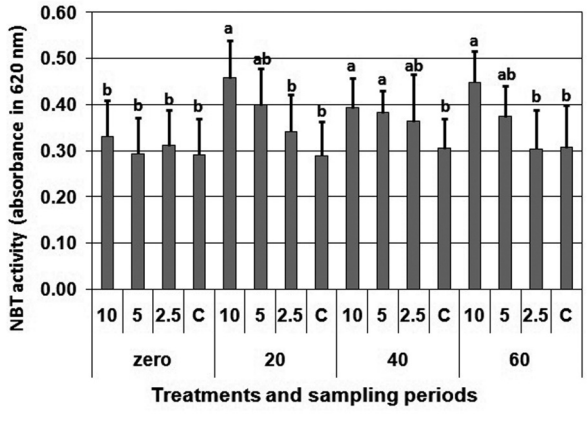

Figure 3. The effects of chitosan enriched diet on NBT activity in common carp in each sampling period. G10: carp fed with 10g/kg hitosan supplemented food, G5: carp fed with 5 g/kg chitosan supplemented food, G2.5: carp fed with 2.5 g/kg chitosan supplemented feed, C: carp fed with non-supplemented food (Mean±SD, n= 15).

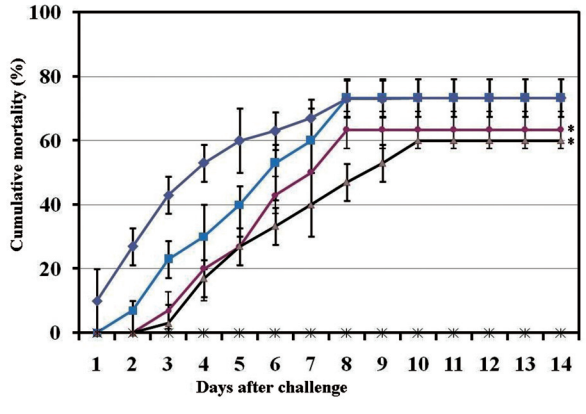

Figure 4. Cumulative mortality of common carp challenged with *A. hydrophila* following oral administration of chitosan-supplemented food. Data showed as Mean±SD, n= 15. G10: carp fed with 10g/kg hitosan supplemented food, G5: carp fed with 5 g/kg chitosan supplemented food, G2.5: carp fed with 2.5 g/kg chitosan supplemented feed, C: carp fed with non-supplemented food (*significant differences with control group, $p< 0.05$).

et al. (2007) in tilapia and Katija et al. (1990) in rainbow trout reported increase of serum bactericidal activity after administration of biological immuno-stimulants. The increased serum bactericidal activity in chitosan treated groups indicates that various humoral factors are involved in innate and/or adaptive immunities which are elevated in the serum to protect the fish effectively from infection (Das et al. 2009). Thus, chitosan proved to be as an effective immunostimulant in preventing the establishment of bacterial infection in common carp.

The NBT activity as an indicator for respiratory burst activity in G10 and G5 groups enhanced significantly compared with the control groups ($p=0.035$). The present result is similar to Siwicki et al. (1994), Lin et al. (2011), Lin et al. (2012), and Gopalakannan and Arul's (2006) reports. Geng et al. (2011) also reported that using of dietary 0.3% and

Table 1. The effect of different level of dietary chitosan of total protein, albumin, and immunoglobulin in common carp in each sampling period. Data showed as Mean±SD, n= 15. G10: carp fed with 10g/kg hitosan supplemented food, G5: carp fed with 5 g/kg chitosan supplemented food, G2.5: carp fed with 2.5 g/kg chitosan supplemented feed, C: carp fed with non-supplemented food. Significant differences ($p<0.05$) are marked by different letters.

Parameters	Treatments	Day zero	days 20	days 40	days 60
Total protein	G10	2.75±0.32 [a]	2.98±0.21 [a]	2.93±0.22 [a]	2.90±0.28 [a]
	G5	2.75±0.32 [a]	2.86±0.36 [a]	2.72± 0.48 [a]	2 a.67±0.26 [a]
	G2.5	2.75±0.32 [a]	2.69±0.46 [a]	2.67±0.47 [a]	2 a.68±0.29 [a]
	C	2.75±0.32 [a]	2.70±0.21 [a]	2.64±0.52 [a]	2.70±0.24 [a]
Albumin	G10	1.27±0.22 [a]	1.28±0.28 [a]	1.19±0.31 [a]	1.29±0.34 [a]
	G5	1.27±0.22 [a]	1.27±0.16 [a]	1.30±0.22 [a]	1.30±0.43 [a]
	G2.5	1.27±0.22 [a]	1.20±0.20 [a]	1.27±0.29 [a]	1.30±0.24 [a]
	C	1.27±0.22 [a]	1.12±0.26 [a]	1.22±0.33 [a]	1.28±0.38 [a]
Immunoglobulin	G10	1.45±0.20 [a]	1.71±0.22 [a]	1.68±0.38 [a]	1.62±0.36 [a]
	G5	1.45±0.20 [a]	1.59±0.28 [a]	1.51±0.25 [a]	1.36±0.37 [a]
	G2.5	1.45±0.20 [a]	1.50±0.36 [a]	1.41±0.29 [a]	1.36±0.35 [a]
	C	1.45±0.20 [a]	1.57±0.35 [a]	1.42±0.29 [a]	1.41±0.27 [a]

Table 2. Leukocyte count and differential count in common carp fed with different level of chitosan. Data showed as Mean±SD, n= 15. G10: carp fed with 10g/kg hitosan supplemented food, G5: carp fed with 5 g/kg chitosan supplemented food, G2.5: carp fed with 2.5 g/kg chitosan supplemented feed, C: carp fed with non-supplemented food. Significant differences ($p<0.05$) are marked by different letters.

Parameters	Treatments	Days zero	days 20	days 40	days 60
WBC	G10	5.29±2.38 [a]	4.73±2.19	5.72±3.18 [a]	4.34±1.20 [a]
	G5	5.19±2.08 [a]	5.67±2.59 [a]	5.77± 2.59 [a]	4.62±1.29 [a]
	G2.5	5.21±1.89 [a]	4.37±1.26 [a]	6.02±3.50 [a]	4.72±1.37 [a]
	Control	5.12±1.81 [a]	4.5±1.84 [a]	5.92±3.65 [a]	4.47±1.43 [a]
Lymphocyte	G10	57.75±6.63 [a]	57.1±5.25 [a]	62.83±6.61 [a]	60.8±6.17 [a]
	G5	56.55±6.12 [a]	59.8±7.90 [a]	62.16±7.49 [a]	60.83±4.30 [a]
	G2.5	59.5±6.13 [a]	58±10.93 [a]	55.83±6.79 [a]	62.33±8.26 [a]
	Control	55.75±7.13 [a]	57.5±2.12 [a]	54±5.2 [a]	58.5±6.36 [a]
Heterophile	G10	24.63±6.12 [a]	27.3±4.68 [a]	21.83±8.61 [a]	26.16±3.37 [a]
	G5	22.24±6.04 [a]	27.67±4.1 [a]	21.83±3.76 [a]	25.83±4.57 [a]
	G2.5	24.1±5.2 [a]	28.5±9.86 [a]	22.5±2.73 [a]	28±4.38 [a]
	Control	25.22±5.77 [a]	26±6.5 [a]	22.5±9.57 [a]	28.2±5.65 [a]
Monocyte	G10	14.66±4.64 [a]	16.17±3.71 [a]	13.5±4.18 [a]	13.4±4.66 [a]
	G5	13.45±4.22 [a]	11.50±4.96 [a]	16.16±4.49 [a]	12.6±4.87 [a]
	G2.5	14.23±4.11 [a]	12.57±6.60 [a]	19±7.21 [a]	15±4.08 [a]
	Control	15.06±3.9 [a]	14.5±2.12 [a]	16.66±2.88 [a]	13.5±2.12 [a]
Heterophile	G10	0.37±0.13 [a]	0.44±0.13 [a]	0.37±0.16 [a]	0.44±0.13 [a]
	G5	0.47±0.45 [a]	0.56±0.37 [a]	0.55±0.27 [a]	0.55±0.28 [a]
	G2.5	0.39±0.13 [a]	0.37±0.11 [a]	0.47±0.16 [a]	0.44±0.13 [a]
	Control	0.37±0.17 [a]	0.57±0.18 [a]	0.41±0.12 [a]	0.37±0.13 [a]
Monocyte	G10	0.71±0.17 [a]	0.61±0.18 [a]	0.67±0.13 [a]	0.63±0.11 [a]
	G5	0.67±0.19 [a]	0.63±0.19 [a]	0.63±0.15 [a]	0.72±0.13 [a]
	G2.5	0.73±0.18 [a]	0.71±0.17 [a]	0.67±0.16 [a]	0.66±0.15 [a]
	Control	0.63±0.23 [a]	0.67±0.18 [a]	0.67±0.15 [a]	0.59±0.18 [a]

0.6% chitosan enhanced the respiratory burst activity in Rachycentron canadum.

Total serum protein and globulin slightly increased in carp fed on diet supplemented with 5 and 10 mg kg-1 chitosan ($p=0.085$). Our result is similar to what Siwicki et al. (1994) reported. They observed no significant change in serum proteins following dietary administration of chitosan in rainbow trout. Besides Dugenci et al. (2003) also showed that 1% Zingiber officinale supplemented diet as an immunostimulant plant in rainbow trout did not increase total plasma protein. On the other hand, Harikrishnan et al. (2012) reported enhancement of total serum protein and globulin following feeding the fish with 1% and 2% chitosan supplemented food in Epinephelus bruneus.

The effect of dietary chitosan on Leukocyte numbers and differential count showed that neither

leukocyte numbers nor leukocytes ratio affected significantly, in other work on chitosan similar results were reported: Gopalakannan and Arul (2006) reported that dietary 1% chitosan in common carp did not enhance Leucocyte numbers. Besides Chang et al. (2006) show total leucocyte numbers were unaffected by dietary 0.5%, 1% and 2% chitosan in Jappanese sea bass. Similarly, Supplementation of rainbow trout diet with chitosan had no effects on total leucocyte numbers (Siwicki et al., 1994). However, the present results contradict with the findings of other studies: Maqsood et al. (2010) reported that 1%, 2%, and 5% dietary chitosan in food can increase WBC in common carp. Lin et al. (2011) also showed that dietary 0.2% chitosan, produced by microbial fermentation of the crustaceans shell, in *Cyprinus carpio* koi had an enhancement effect on WBC count. Meshkini et al. (2012) reported that although 0.25% dietary chitosan enhanced significantly WBC and Leukocyte ratio, 0.50% and 1% chitosan did not enhance these parameters significantly. The contradictory results can be caused by different chitosan extraction procedure and fish species. It is possible that chitosan quality and origin cause these effective different results.

Mortality percentage of common carp fed on chitosan-supplemented diet (5 and 10 mg kg^{-1}) significantly decreased in comparison with the control group ($p<0.05$). This might be due to the enhancement of the non-specific immune system of the fish by chitosan. Maqsood et al. (2010) observed that supplemented diet with 1 and 2 percent chitosan cause decrease in mortality rate following bacterial challenge. Gopalakannan and Arul (2006) also reported that the RPS in the chitosan-supplemented group of common carp challenged with *Aeromonas hydrophila* was significantly higher than the control and chitin supplemented group. Rairakhwada et al. (2007) reported that the highest RPS (100%) was recorded in 0.5% levan fed and the lowest RPS was recorded in 1% levan fed fish. Alishahi et al. (2010) reported that enhancement of protection against *A. hydrophila* infection in common carp fed on 5% Aloe vera extract supplemented diets.

This study indicates that supplementation of food with 0.5 and 1% chitosan induced enhancement of some immune parameters and resistance against bacterial infection in *Cyprinus carpio*. Then, shrimp shell derived chitosan can be used for increasing resistance against bacterial infection and immunostimulation in common carp.

Acknowledgments

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مجله طب دامی ایران، ۱۳۹۳، دوره ۸، شماره ۲، ۱۳۳-۱۲۵

تأثیر تجویز خوراکی کیتوزان بر پاسخ ایمنی و مقاومت در برابر بیماری در ماهی کپور معمولی

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چکیده

زمینـه مطالعه: بروز مقاومت نسبت به آنتی بیوتیک‌ها و ناکارآمدی واکسن‌ها در آبزیان، مطالعات در مورد محرک‌های ایمنی را ضروری می‌نماید. کیتوزان از پوسته میگو بدست می‌آید و به عنوان محرک ایمنی می‌تواند استفاده شود. **هدف:** در این تحقیق اثر تجویز خوراکی کیتوزان بر پاسخ‌های ایمنی و مقاومت با کتریایی ماهی کپور معمولی بررسی شد. **روش کار:** ۳۰۰ ماهی سالم با میانگین وزنی ۸/۱g±۴۲/۴ به ۴ گروه تقسیم و با خوراک حاوی ۱۰g، ۵، ۲/۵ و ۰ کیتوزان در کیلوگرم غذا به مدت ۶۰ روز تغذیه شدند. نمونه‌های خونی از تیمارها، با فاصله‌ی زمانی ۲۰ روز برای اندازه‌گیری برخی فاکتورهای ایمنی (نظیر فعالیت لیزوزیم سرم، فعالیت با کتری‌کشی سرم، قدرت احیای NBT، پروتئین‌های سرم، تعداد کلی و افتراقی سلول‌های سفید) گرفته شد. در انتهای دوره ماهی‌های باقی مانده در هر تیمار با با کتری زنده آئرومونas هیدروفیلا چالش داده شده و تلفات برای ۱۴ روز ثبت شد. **نتایج:** نتایج نشان داد، تجویز خوراکی کیتوزان فعالیت احیای NBT را بهبود بخشید ($p<۰/۰۵$)، اگرچه در فعالیت لیزوزیم و با کتری‌کشی سرم، میزان تام پروتئین و آلبومین سرم، تعداد کلی و نسبت لوکوسیت‌ها تغییر معنی داری در بین گروه‌ها مشاهده نشد ($p>۰/۰۵$). مقاومت به عفونت با کتری آئرومونas هیدروفیلا در گروه‌هایی که با میزان بالای کیتوزان تغذیه شدند نسبت به گروه‌های دیگر تغییر معنی داری را نشان داده است ($p<۰/۰۵$). **نتیجه‌گیری نهایی:** این مطالعه نشان می‌دهد، که تجویز خوراکی کیتوزان، برخی فاکتورهای ایمنی و مقاومت ماهی کپور معمولی به عفونت با کتریایی را بهبود می‌بخشد.

واژه‌های کلیدی: آئرومونas هیدروفیلا، کیتوران، کپور معمولی، پاسخ ایمنی

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