EFFECT OF CHITOSAN ON HAEMATOLOGICAL PARAMETERS, BIOCHEMICAL PROFILE AND GROWTH RATE OF *Cyprinus carpio* L. INFECTED WITH *Aeromonas hydrophila*

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ABSTRACT

This study was carried out to investigate the effect of chitosan on some haematological parameters, biochemical profile, growth rate and survival rate of *Cyprinus carpio* L. challenged with *Aeromonas hydrophila*. A total of 100 common carp were divided into five dietary groups: The control group fed on chitosan-free diet; T1 was fed on diet supplemented with 0.75% chitosan; T2 was fed on diet supplemented with 1.5% chitosan; T3 was fed on diet supplemented with 2% chitosan, and T4 was fed on diet supplemented with probiotics for 45 days. Fish fed diets were challenged intramuscularly with 0.2 ml *A. hydrophila* at a concentration $1.2x10^7$ CFU ml^{-1} on day 45. After 45 day of feeding trail and post challenge, Blood samples were takan to conduct the haematological parameters and biochemical profile. WBCs count revealed significantly increase ($p \leq 0.05$) in chitosan-feeding group of fish (T1, T2 and T3) as compared with probiotic (T4) and control groups pre challenge and post challenge. Albumin, globulin levels and the ratio of albumin globulin observed a significant increase ($p \leq 0.05$) in all chitosan contaired trials at 45 and 60 days as compared with the control and probiotic groups. SGR (%) of the *C. carpio* were significantly affected ($p<0.05$) by dietary containing chitosan and probiotic. Post challenge with *A. hydrophila* the survival rate showed an increase in T3 and T2; 100 and 75% as compared with T1, and T4 and the control groups. Based on the obtained results it concluded 2% chitosan as fed additives could be used as prophylactic in common carp to improve protection against possible infection by *Aeromonas hydrophila* which that is beneficial for application in fish culture.

INTRODUCTION

Fish culture productions are exposed to infectious diseases and decline of the environmental conditions which might cause serious economic losses. Recently, fish are protected from infectious diseases by vaccination or chemotherapeutic agent. However, due to extensive use of the chemotherapeutic agents, the development of antimicrobial resistance among pathogens and the related environmental threats have been well recognized. Hence, several alternatives pathways such as using immune stimulants have been suggested, the use of immune-stimulants such as probiotics could suppress fish pathogens and several deleterious environmental conditions (27).

Indication of the valuable effects of probiotics gave generate the idea of prebiotics (15 and 28) which are categorized as complex low molecular weight oligosaccharides and commonly cannot be digested by the fish but are metabolized by promoting healthy bacteria within the fish gut. Consistently, the beneficial effects of some oligosaccharides have also been documented on fish (19 and 24). Chitooligosaccharide (COS) is a type of oligosaccharides, which is obtained by chemical and enzymatic hydrolysis of chitosan (18). In this regard, Chitosan is commercially produced by de-acetylation of chitin, which is the

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structural element in the exoskeleton of crustaceans and cell wall of fungi. Several studies revealed that Chitosan have immune stimulating and growth characteristics in various aquatic animals (23) with solubility than chitin in water and other polar solvents (14). Many studies have been carried out in different species of animals (26, 18, 7 and 4). But still there are little information's related to prebiotics in fish. Hence, this study was undertaken to determine the effects of dietary chitosan on some haematological indices, biochemical profile and specific growth rate in *C.carpio* challenged with *Aeromonas hydophila.*

MATERIALS AND METHODS

DIET PREPARATION

Five experimental diets were formulated to be supplemented for fish which including: wheat flour (45%), soybean meal (32%), corn (11%), animal protein (10%), premix (1%) and di-calcium phosphate (1%). Control diet as it was not supplemented with chitosan, whereas Chitosan diets comprised the same ingredients of control diet but were supplemented with Chitosan at concentration of 0.75, 1.5 and 2%. All the ingredients were properly were ground and singly in an electric dicer and systematically mixed and the water was supplement in adequate quantity (2).

EXPERIMENTAL DESIGN

***C. carpio* L., were obtained from a local fish farm (Babylon, Fish Farm, Iraq) and transported to the aquarium facilities. Fish were acclimated for two weeks. After that, 100 fish (average weight 36.4 g; average length 12.3 cm) were randomly distributed to fish tank (10 fish tank^{-1}). Each treatment was achieved in duplicate and were fed daily for three times at a rate of 3% of average body weight for 60 days according to their respective treatments as follows: control group (no added chitosan); Treatment 1 (T1), fed basal diet supplemented with 0.75% chitosan; Treatment 2 (T2), fish fed basal diet contained 1.5% chitosan; Treatment 3 (T3) fish fed diet contained 2% chitosan; Treatment 4 (T4) fish fed diet contained probiotic (Poultry star me 0.05%). Each group was placed in a fully prepared aquarium containing de-chlorinated tap water. To estimate SGR (%) the experimental fish were weighed every two weeks and within this period feed input was changed weekly depending on weight gain. The average of water temperature was 22± 1.50^{o}C, dissolved oxygen (DO) was 4.8±1.60 mg l^{-1} and the pH was 7.17±1.80 using YSI D.O. meter Model 55 and pen-type.**

CHALLENGE STUDY

Fish fed diets supplemented with and without chitosan were challenged intramuscularly with *A. hydrophila* on day 45. A virulent strain of *A. hydrophila* was received in Tryptose Soy Broth (TSB) from Al-Kendi Company. Sub cultures were made on Tryptose Soy Agar (TSA) at 25 ^{o}C. At the day seven LD_{50} was detected by intramuscular injection of 48 fish with graded doses of *A. hydrophila* (10^6, 10^7, 10^8, 10^9 and 10^{10} CFU ml^{-1}) at 25°C. The result showed that the LD_{50} on day 7 was 10^8 CFU/ml. The fish were injected "intramuscularly" (im) with 0.2 ml of *A. hydrophila* at a concentration 1.2x10^7 CFU ml^{-1} in phosphate buffer saline (PBS) as a medium. After that, all the fish were observed for their response against injection.

HAEMATOLOGICAL PARAMETERS AND BLOOD CHEMISTRY

At the end of the feeding trial and post challenge, six fish from each tank were sampled to determine the hematological parameters and blood chemistry.

Blood was collected from the caudal vein after euthanasia by immersion in solution containing clove powder; 50 mg L^{-1}. The blood samples were divided into two aliquots; one aliquot was mixed with heparin and used for counting white blood cells (WBCs). All blood cells were counted under a light microscope using Neubauer hemocytometer after dilution with Dacie's fluid. Differential leukocyte counts (neutrophil, lymphocyte, and monocyte) were determined using Giemsa staining method of blood smears under a light microscope. Cells were identified on the basis of morphology and cell ultra-structure Dacie and Lewis (6). The other aliquot was transferred into an Eppendrof tube and allowed to clot at room temperature for 1 h. Then, the serum were separated by centrifugation (1500 *g* for 5 min at 4 °C). The serum samples were used for assaying the albumin, globulin levels using commercial kit according to Dumas and Biggs (8).

Growth and survival rate

Specific growth rate and survival rate were estimated for both control and experimental groups according to the following equations:

$$\text{SGR (\%)} = 100 \times [\ln \text{final weight} - \ln \text{initial weight}] \div \text{duration of the experiment}$$

$$\text{Survival (\%)} = [\text{final number of fish\_ initial number of fish}] \times 100.$$

Statistical analysis

Statistical analysis was performed using Statgraphics v5.1 software (StatSoft, USA). Data were presented as average mean ± S.E. was analysed using multifactor analysis of variance (two way ANOVA). Significant ANOVA was followed by a multiple comparison test. Level of significance was taken as $P \leq 0.05$.

RESULTS AND DISCUSSION

Haematological parameters

WBCs count

WBCs count revealed a significantly increase *(*$P \leq 0.05$) in chitosan-feeding group of fish (T1, T2 and T3) as compared with probiotic (T4) and control group at 45 days (pre challenge) and 60 days (after challenge). The highest mean value was observed in T3 at 45th day in comparison with T2, T1 and T4 respectively) and the control group WBC counts showed increased significantly ($P \leq 0.05$) in treated groups post-challenged than the pre-challenged, the highest value were recorded in T3, followed by treatment T2, T1 and T4 respectively (Figure 1).

WBCs are one of the most affecting factors in immunity of fish and WBC counts have been used as bio-marker of health of fish (9). Increase in number of WBC in diseased fish could be serving as a protective barrier towards any infection (30). These results are compatible with the data obtained by Ritesh *et al.* (25), who recorded significant increase in leukocytes count in Asian seabass fish, *Latescal calcifer* fed diet supplemented with chitosan 0.5, 1 and 2 % diet for 60 days as compared with the control group. Meshkini *et al.* (22) also observed an increase in WBC count in rainbow trout, *Oncorhynchus mykiss* fed supplemented diet with chitosan in 0.25, 0.5 and 1% concentration for 11 weeks as compared with the control group.

Al-Saphar (2) found that there was an increase in WBC of treated groups with probiotic at 0.05, 0.25, 0.5, 0.75 and 4 % as compared with the control group. These results indicated that stimulation of non-specific immune response in *C. carpio* groups supplemented diets with chitosan as manifested by increased

number of white blood cells, by affecting to release factors that stimulate hematopoietic organs to produce leukocytes (30).

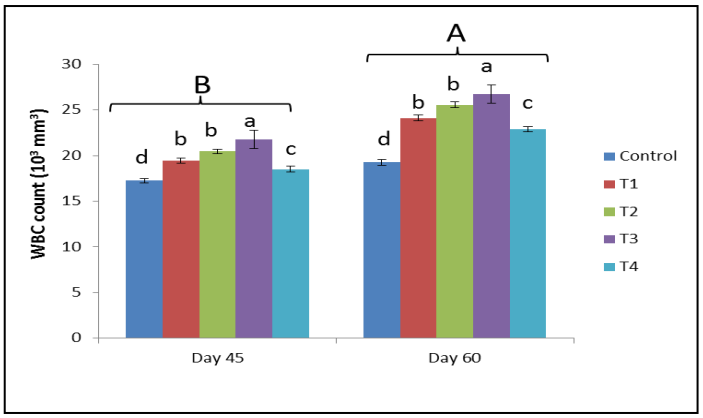

Figure 1:Total WBC count (10^3 mm^3) of *C. carpio* fed different levels of chitosan and probiotic and control diet in pre challenge (day 45) and post challenge (day 60). Values are expressed as mean ±SE. Different alphabetic letters indicated significantly different at ($p \leq 0.05$); capital letters indicated differences between day 45 and day 60, small letters indicated differences between treatment groups.

Differential leukocyte count

Data for differential leucocyte count are presented in Figure 2 A-D. Lymphocyte revealed a significant decrease ($p<0.05$) in fish groups (T1, T2 and T3) as compared with the control diet group and probiotic group at 45 and 60 day. Also, there was a significant differences among pre challenge and post challenge groups (Figure 2). Neutrophils and monocytes (%) showed a significant increase in chitosan groups as compared with the control and probiotic groups. All treatment groups were significantly increased ($p<0.05$) for post-challenged (day 60) than the pre-challenged groups (45 day). The highest mean values were recorded in T 3 followed by T2, T1 and T4 respectively as compared with control group. Neutrophil showed a significant increase ($p<0.05$) in treated group as compared with the control diet group, the highest mean value was observed in T3 followed by T2, T1 and T4 and control group. In contrast, eosinophil revealed a significant decrease ($p<0.05$) as compared with probiotic and to control groups. All treatment groups were significantly increased ($p<0.05$) post-challenged (day 60) than the pre-challenged groups (day 45).

These results are in agreement with those obtained by Shimei *et al.* (29), who found that both of chitosan oligosaccharides (COS) and *Bacillus coagulants* significantly influence on head kidney macrophages through effect on respiratory burst activity and phagocytic capacity as compared with control group in *C. carpio* koi during feeding for eight weeks. Similarly, Gopalakannan and Arul (14) observed that diet contained chitosan supplementation at 1% significantly increase respiratory burst activity and lysozyme activity compared to normal diet group during 30 days. Also, Esteban *et al.* (13) observed that the innate immune responses of sea bream, *Sparus aurata* stimulated through stimulate phagocytic activity, respiratory burst and also cytotoxic by dietary chitin supplemented when given after six weeks.

Debtanu *et al.* (11), suggested at mode of action for - Glucans, Chitin and Chitosan that activate the immune systems of organisms against invading pathogens by macrophage activator. The obtained results could be attributed to

(chitosan) polysaccharides that simulate to fungal or bacterial gram-negative therefore can be recognized as foreign agents for the fish immune system and therefore the fish's system produces an inflammatory response, as it would against a pathogen that provides short term protection (20). Additional theory that chitosan bound specifically to the proteins receptor on cell surface of lymphocytes or phagocytes to stimulate the production of immune response through helping by cytokines to activate the non-specific immunity for animals (16). Also, chitosan (Polysaccharides) can stimulate the immune system through stimulated the immune cells such as granulocytes, macrophages, monocytes and naturel killer cells to start the secretion of cytokines that will stimulate the immunity (32).

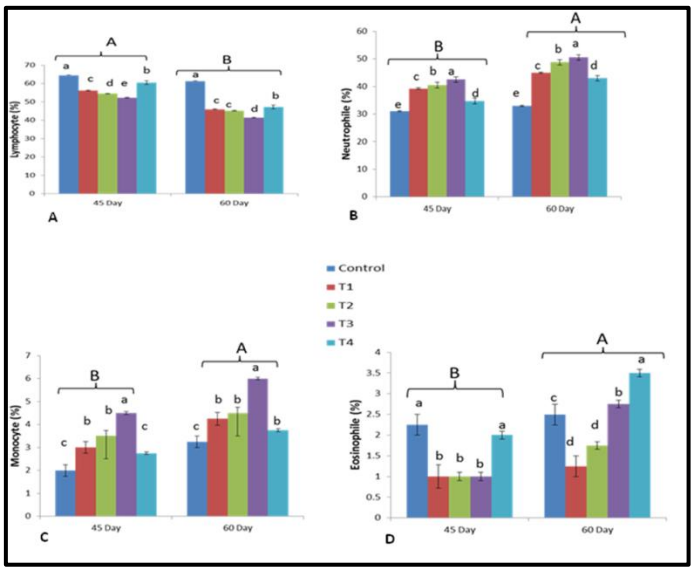

Figure 2: Differential leukocyte count (%) A: lymphocyte, B: neutrophil, C: monocyte, D: eosinophil of *C. carpio* fed different level of chitosan and probiotic and control diet in pre challenge (day 45) and post challenge (day 60). Values are expressed as mean ± SE. Different alphabetic letters indicated significant difference at P ≤ 0.05. Capital letters indicated differences between day 45 and day 60, small letters indicated differences between treatment groups.

Biochemical profile (albumin, globulin and albumin /globulin ratio)

Albumin, globulin contents and the ratio of albumin globulin exhibited a significant increase (P≤ 0.05) in chitosan groups (T1, T2 and T3) at 45 and 60 days as compared with the control and probiotic groups. The highest mean value was observed in T3 followed by T2 and T1 respectively. Albumin, globulin contents and albumin/ globulin ratio revealed no significant differences in all treatment groups pre and post challenge (45 and 60 days) (Figure 3: A-C).

Albumin and globulin is one of the major proteins in the serum proteins, which have an essential role in immune response (33). Globulin is considered very important for keeping good immunity and also has all the immune globulins in blood. The increase in albumin and globulin contents gives evidence as strong non-specific immune response in fish (33). In this study, proteins levels increased in all treated groups, that indicates improvement of innate immunity for fish. These results are in line with Ritesh *et al*. (25) in Asian seabass, *Latescal calcifer*. Similar findings recorded in olive flounder, *Paralichthys olivaceus* (5), *C. carpio* (10) and *E. bruneus*. On contrary, Bagni and Archetti (3) showed no significant

differences in the serum proteins in seabass, *Dicentrarchus labrax* when used diet containing β-glucan, ascorbic acid and α-tocopherol. This could be due to different immune-stimulants that used in diet and the change in their feeding formula.

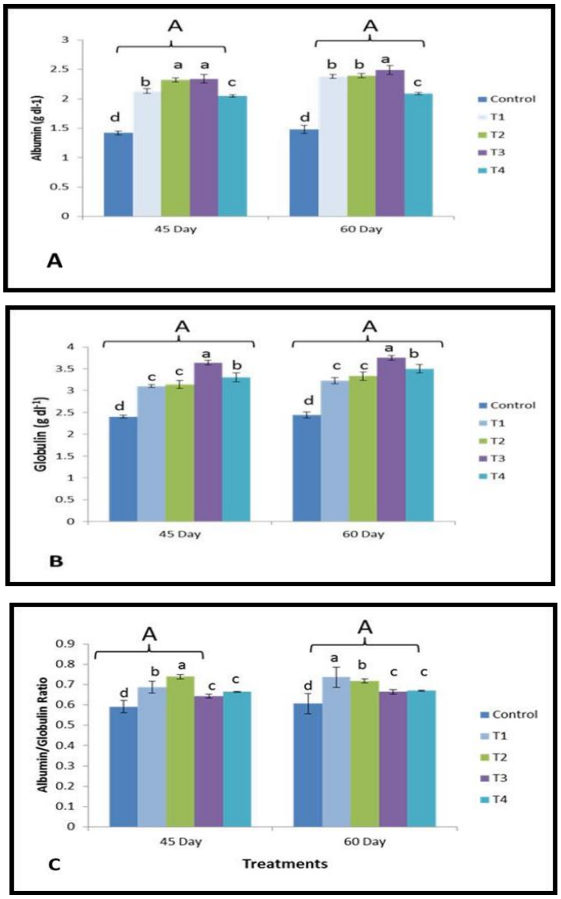

Figure 3: A: Albumin g/dl, B: Globulin g/dl, C: Albumin/globulin ratio of *C. carpio* groups after 45 and 60 days (pre and post challenge) supplemented with different concentrations of dietary Chitosan. Data are mean ± SE. Different small alphabetic litters indicate significant differences among treatment groups at P < 0.05. Capital letters indicated differences among day 45 and day 60, small letters indicated differences among treatment groups.

Growth and survival rate

SGR (%) of the *C. carpio* significantly affected (p<0.05) by dietary containing chitosan and probiotic. The maximum enhancements in SGR (%) were in the treatment contained probiotic (T4) that increased significantly (p<0.05) in comparison with chitosan groups (T1, T2, T3) and the control group. Also, T3 and T2 revealed a significant difference compared to T1 and to control group (Figure 4).

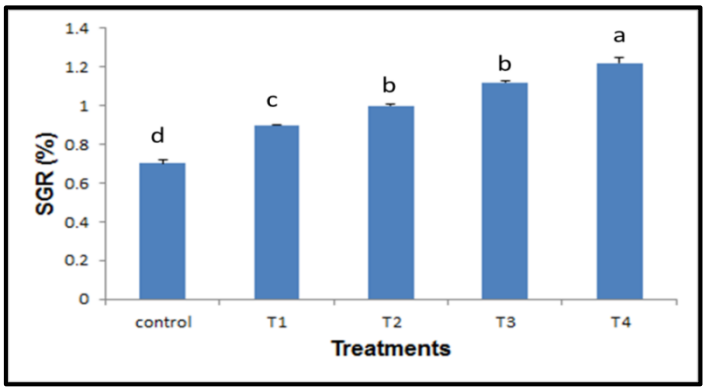

Figure 4: SGR (%) of *C. carpio* feed with different concentration of chitosan, probiotic and supplemented control diet for 45 days. Data are mean ± SE. different alphabetic letters indicated significantly different at *P*<0.05; n=6.

This result is in accordance with Lara-Flores *et al.* (17) who confirmed that the use two types of probiotics (yeast and lactic acid bacteria) in *Oreochramis niloticus* have beneficial effects on the feed efficiency and growth performance. The positive effects of probiotic could be attributed to the ability to increase the digestibility of organic matter and protein through stimulate and/or produce digestive enzymes (protease, amylase and lipase) that when attach in the intestine after transit via the stomach, so probiotics could stimulate appetite and improve nutrition (12). Xu *et al.* (34) recorded that the improvement of growth performance by chitosan probably due to increase of apparent digestibility of major nutrients (dry matter, crude protein, Ca and P). Another study by Tang *et al.* (31) confirmed that levels of growth hormone (GH) and IGF- I in serum, could be enhanced by dietary supplementation of chitosan. Thereby, fish feed with diets supplemented with high concentrations of chitosan might increase serum GH level.

After challenge test with *A. hydrophila* (after 45 days of the experimental period) the percentage of survival of *C. carpio* were measured. The results showed an increase in the survival rate in T3 and T2; (100 and 75 %) respectively, as compared with T1, T4 and the control groups (50, 50 and zero % respectively (Figure 5).

These results are in agreement with Maqsood *et al.* (21), who reported that when *C. carpio* feed chitosan at 1%, 2% and 5% and after challenged intra-peritoneal by *A. hydrophila*. Also, Aathi *et al.* (1) found highest survival rate in Indian major carp rohu *Labeo rohita* that fed different concentration of chitosan as compared on group without chitosan. This could be through improved fish nonspecific immunity through assisting the function of phagocytosis and increasing bactericidal activity.

In conclusion, the dietary feed of 2% chitosan exhibited increased protection against the infection with *A. hydrophila* and higher survival rate which indicate increased non-specific-immune responses and disease resistance of carp fish.

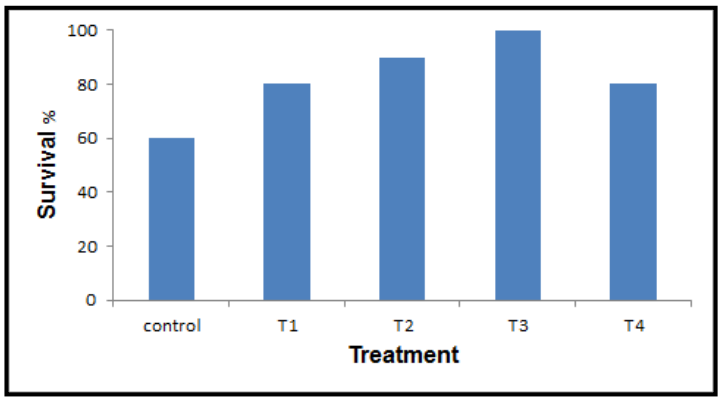

Figure 5: Effect of dietary chitosan on percentage of survival of *C.carpio* post challenge with *A. hydrophila.*

REFERENCES

1-Aathi, K.; V. Ramasubramanian; V. Venkatachalm; V. Uyhayakumar and S. Munirasu (2013). Effect of chitosan supplement diet on survival, growth, hematological, biochemical and immunological response of Indian major carp (*Labeo rohita*). Interna. Res. h J. Pharm., 4: 141-147.

2- Al-Saphar, S. A .N. (2012). Production of a local probiotic and its effect on growth of common carp *Cyprinus carpio* L. and resistance to pathogenic bacteria *Aeromonas hydrophila* . M.Sc. thesis , Coll. of Vet. Med., Univ. of Baghdad, p:102.

3- Bagni, M. and L. Archetti (2000). Effect of long-term oral administration of an immunostimulant diet on innate immunity Sea bass (*Dicentrarchus labrax*). J. Vet. Med., 47B: 745–751.

4- Bielecka, M.; E. Biedrzycka and A. Majkowska (2002). Selection of probiotics and prebiotics for synbiotics and confirmation of their in vivo effectiveness. Food Res. Interna., 35: 125–131.

5- Cha, S. H.; J. S. Lee; C. B. Song; K. L. Lee and Y. J. Jeon (2008). Effects of chitosan-coated diet on improving water quality and innate immunity in the olive flounder (*Paralichthys olivaceus*). Aquaculture, 278: 110–118.

6- Dacie, J. V. and S. M. Lewis (1995). Practical haematology, 8th ed. Churchill Livingstone, Edinburgh; New York, p:609.

7-Daniels, C. L.; D. L. Merrifield; D. P. Boothroyd; S. J. Davies; J. R. Factor and K. E. Arnold (2010). Effect of dietary *Bacillus spp*. and mannan oligosaccharides (MOS) on European lobster (*Homarus gammarus* L.) larvae growth performance, gut morphology and gut microbiota. Aquaculture, 304:49–57.

8-Dumas, B. T. and H.G. Biggs (1972). Standard Methods of clinical chemistry . Ed., Academic press, New York.

9-Duncan, P.L. and P.H. Klesius (1996 . Dietary Immunostimulants enhance nonspecific immune responces in channel catfish but not resistance to *Edwardsiella ictaluri* . J. Aquat. Anim. Health, 8:241-248.

10-Dautremepuits, C.; S. Paris-Palacios; S. Betoulle and G. Vernet (2004). Modulation in hepatic and head kidney parameters of carp (*Cyprinus carpio* L.) induced by copper and chitosan. Comp. Biochem. Physiol., 137 C: 325–333.

11-Debtanu, B.; N. Phanna; C. Sagar and K. Vikash (2013). Immunostimulants for Aquaculture Health Management. Mar. Sci. Res. Develop., 3: 3-4.

12-El-Haroun, E. R.; M. A. Goda; and M. A. Kabir (2006). Effect of dietary probiotic Biogens supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia, *Oreochromis niloticus*. Aqua. Res., 37: 1473-1480.

13-Esteban, M. A.; V. Mulero; A. Cuesta; O. J. Ortun and J. Meseguer (2000). Effects of injecting chitin particles on the innate immune response of gilthead seabream (*Sparus aurata* L.). Fish and Shellfish Immunol., 10: 543-548.

14-Gopalakannan, A. and V. Arul (2006). Immunomodulatory effects of dietary intake of chitin, chitosan and levamisole and immune system of *Cyprinus carpio* and control of *Aeromonas hydrophyla* infection in pond. Aquaculture, 255: 179-187.

15-Gibson, G. R. and M. B. Roberfroid (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nut., 125: 1401–1412.

16-Lambrecht, B.; M. Gonze; D. Morales; G. Meulemans and T. P. Van den Berg (1999). Comparison of biological activities of natural and recombinant chicken interferon-gamma. Vet. Immunol. Immunopathol.,70: 257-267.

17-Lara-Flores, M.; M. A. Olvera-Novoa; B. E. Guzman-Mendez and W. Lopez-Madrid (2003). Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus,* and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia *(Oreochromis niloticus*) Aquaculture, 216: 193-201

18-Li, X. J.; X. S. Piao; S.W. Kim; P. Liu; L. Wang; Y.B. Shen; S.C. Jung and H.S. Lee (2007). Effects of chito-oligosaccharide supplementation on performance, nutrient digestibility, and serum composition in broiler chickens. Poultry Sci., 86: 1107–1114.

19-Luo, L.; X. F. Cai; C. He; M. Xue; X.F. Wu and H. N. Cao (2009). Immune response, stress resistance and bacterial challenge in juvenile rainbow trouts *Oncorhynchus mykiss* fed diets containing chitosan-oligosaccharides. Current Zool., 55: 416–422.

20-Magnadóttir, B. (2006). Innate immunity of fish (overview). Fish Shellfish Immun., 20: 137-151.

21-Maqsood, S.; P. Singh; M. H. Samoon and A. K. Balange (2010). Effect of dietary chitosan on non-specific immune response and growth of *Cyprinus carpio* challenged with *Aeromonas hydrophila*. Int. Aquac. Res., 2: 77-85.

22-Meshkini, S.; A. Tafy; A. Tukmechi and F.F. Pajuh (2012). Effects of chitosan on hematological parameters and stress resistance in rainbow trout (*Oncorhynchus mykiss*). Vet. Res. Forum, 3:49–54.

23-Pariser, E. R. and D. P. Lombardi (1989).Chitin sourcebook: a guide to the research literature. New York: Wiley-Interscience: 560p.

24-Ringo, E.; R. E. Olsen; T.O. Gifstad; R. A. Dalmo; H. Amlund and G. I. Hemre (2010). Prebiotics in aquaculture: a review. Aquac. Nut.,16: 117–136.

25-Ritesh, R.; P. Kurcheti; T. Vani and K. Rajesh (2014). Effect of dietary chitosan on haematology, innate immunity and disease resistance of Asian seabass *Lates calcarifer* (Bloch). Aquac. Res., 45: 983–993.

26-Roberfroid, M. B. (2000). Prebiotics and probiotics: are they functional food? American J.Clin. Nutr., 71: 1682–1687.

27-Sakai, M. (1999). Current research status of fish immune-stimulants. Aquaculture, 172: 63-92.

28-Teitelbaum, J. E. and W. A. Walker (2002). Nutritional impact of pre- and probiotics as protective gastrointestinal organisms. Annual Rev. Nut., 22: 107–138.

29-Shimei, L.; M. Shuhong; G. Yong; L. Lin; L. Li and P. Yu (2012). Effects of dietary chitosan oligosaccharides and Bacillus coagulans on the growth, innate immunity and resistance of koi (*Cyprinus carpio* koi). Aquaculture, 36: 342-343.

30-Talpur, A. D. and M. Ikhwanuddin (2013). *Azadirachta indica* (neem) leaf dietary effects on the immunity response and disease resistance of Asian seabass, *Lates calcarifer* challenged with *Vibrio harveyi*. Fish Shellfish Immunol., 34: 254–264.

31-Tang, Z. R.; Y. L. Yin; C. M. Nyachoti; R. L. Huang; T. J. Li; C. B. Yang; X. J. Yang; J. Gong; J. Peng; D. S. Qi; J. J. Xing; Z. H. Sun and M. Z. Fan (2005). Effect of dietary supplementation of chitosan and galacto-mannan-oligosaccharide on serum parameters and the insulin-like growth factor-I mRNA expression in early-weaned piglets. Domest. Anim. Endocrinol., 28: 430–441.

32-Togola, A.; M. Inngjerdingen; D. Diallo; H. Barsett; B. Rolstad and T.E. Michaelsen (2008). Polysaccharides with complement fixing and macrophage stimulation activity from *Opilia celtidifolia,* isolation and partial characterisation. J. Ethnopharmacol., 115(3): 423–31.

33-Wiegertjes, G. F.; R. J. M. Stet; H. K. Parmentier and W. B. Van Muiswinkel (1996). Immunogenetics of disease resistance in fish, a comparative approach. Develop. Comp. Immunol., 20: 365–381.

34- Xu, Y.; B. Shi; S. Yan; J. Li; T. Li; Y. Guo and X. Guo. (2014). Effects of chitosan supplementation on the growth performance, nutrient digestibility, and digestive enzyme activity in weaned pigs. Anim. Sci., 59 (4): 156–163.

تأثير الكيتوسان في المعايير الدمية والصفات الكيموحيوية ومعدل النمو في أسماك الكارب الشائع *Cypriuns carpio* المصابة ببكتريا *Aeromonas hydrophila*

جمال خلف عطية الفراجي* زياد عارف صالح**

الملخص

أجريت هذه الدراسة لمعرفة مدى تأثير الكيتوسان في بعض المعايير الدمية والصفات الكيموحيوية ومعدل النمو النوعي المطلق (٪) ودراسة معدل البقاء في اسماك الكارب الشائع ***Cyprinus carpio*** بعد إجراء فحص التحدي مع بكتريا ***Aeromonas hydrophila***. أُجريت الدراسة على **100** من اسماك الكارب الشائع (**36.4±0.73 g**) قسمت إلى خمس مجموعات غذائية:

المجموعة الأولى (**T1**): هي مجموعة السيطرة تمت تغذيتها على نظام غذائي خالي من الكيتوسان؛ المجموعة الثانية (**T2**): تمت تغذيتها على عليقة بنسبة **0.75%** من الكيتوسان من وزن العليقة؛ المجموعة الثالثة (**T3**): غذيت على عليقة بنسبة **1.5%** من الكيتوسان من وزن العليقة؛ المجموعة الرابعة (**T4**): غذيت على عليقة تضم نسبة **2%** من الكيتوسان من وزن العليقة؛ المجموعة الخامسة: غذيت على عليقة مع المعزز الحيوي **probiotic**.

بعد **45** يوماً تم إجراء فحص التحدي ببكتريا ***Aeromonas hydrophila*** عن طريق الحقن في العضلة وبعد التحدي أخذت عينات من الدم لغرض إجراء الفحوص الدمية والفحوص الكيمو حيوية (الألبومين والكلوبيولين ونسبة الكلوبيولين الى الألبومين). أظهرت النتائج وجود زيادة معنوية (**$p\leq0.05$**) في مجاميع الكيتوسان في الأسماك (**T1** و **T2** و **T3**) بالمقارنة مع مجموعة المعزز الحيوي (**T4**) ومجموعات السيطرة قبل وبعد فحص التحدي. كما لوحظ زيادة معنوية (**$p<0.05$**) في مستوى الالبومين والكلوبيولين ونسبة الالبومين الى الكلوبيولين في مجاميع الكيتوسان في **45** و**60** يوماً بالمقارنة مع المجموعتين السيطرة والمعزز الحيوي. أظهرت نتائج معدل النمو النوعي **SGR** تأثرت معنوياً (**$p<0.05$**) في المجاميع المعاملة بالكيتوسان. أظهر معدل البقاء زيادة في **T3** و **T2** بالمقارنة مع **T1** و**T4** ومجموعة السيطرة. استناداً إلى النتائج التي تم الحصول عليها تبين ان **2%** من الكيتوسان كإضافات غذائية يمكن أن تستعمل كوقاية في اسماك الكارب الشائع لتحسين الوقاية ضد أي عدوى محتملة من قبل بكتريا ***Aeromonas hydrophila*** الذي يعد مفيداً للتطبيق في مجال تربية الأسماك.

جزء من رسالة ماجستير للباحث الثاني.

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