International Journal of Agriculture, Environment and Food Sciences
e-ISSN : 2618-5946
www.jaefs.com
JAEFS
edit PUBLISHING

Research Article
Int J Agric Environ Food Sci 6 (1):7-12 (2022)

Immunomodulation function of Tunceli garlic (*Allium tuncelianum*) oil in Rainbow Trout (*Oncorhynchus mykiss*)

Unal Ispir[1] **Mikail Ozcan**[2,*] **Engin Seker**[3]

[1]Malatya Turgut Ozal University, Doğanşehir Vahap Küçük Vocational School, Department of Aquaculture, Malatya, Türkiye
[2]Kahramanmaras Sütçü Imam University, Agriculture Faculty, Department of Fisheries Kahramanmaraş, Türkiye
[3] Munzur University, Fisheries Faculty Tunceli, Türkiye

*Corresponding Author: mikailozcan@ksu.edu.tr

Citation
Ispir, U., Ozcan, M., Seker, E. (2022). Immunomodulation function of Tunceli garlic (*Allium tuncelianum*) oil in Rainbow Trout (*Oncorhynchus mykiss*). International Journal of Agriculture, Environment and Food Sciences, 6 (1), 7-12.
Doi
https://doi.org/10.31015/jaefs.2022.1.2

Received: 28 July 2021
Accepted: 18 January 2022
Published Online: 17 March 2022
Revised: 02 April 2022

Year: 2022
Volume: 6
Issue: 1 (March)
Pages: 7-12

Available online
http://www.jaefs.com
https://dergipark.org.tr/jaefs

Abstract

The effects of injectable Tunceli garlic oil (Allium tuncelianum) on serum response of rainbow trout were examined. Influence of intraperitoneal (i.p.) injection administration of Tunceli garlic oil (TGO) was evaluated on some immunological factors of rainbow trout. Fish weight 40 g were i.p. injected TGO at doses of 1% and 10%. Serum lysozyme activity, bactericidal activity, myeloperoxidase, protein and immunoglobulin levels were measured on days 3, 7, 14 and 21 after the TGO administration. All the immune parameters assayed remained affected when TGO was i.p. administered. Serum bactericidal activity was consistently achieved by immunizing fish with TGO. Levels of serum myeloperoxidase activity were increased in the groups of fish injected with TGO. Myeloperoxidase and bactericidal activities peaked at 14 and 21 days post-injection, respectively. Lysozyme activity was significantly increased throughout experiment in the all groups. Total protein and immunoglobulin levels increased in the 21 days post-injection although no statistically significant differences. In conclusion, these results suggest that there are functionally effects of TGO on the immune mechanisms of rainbow trout.

Keywords

Rainbow trout, Tunceli garlic oil, Immune response, Immunity

Introduction

Fish production is an significant and growing industry worldwide. At the same time, dense fish holding in ponds influences the health of fish. As a result, the physiological condition of productioned fish will be impacted by environmental circumstances. In this way, fish farmers have to application wary husbandry techniques. With the success of plant derivates for the control of bacterial infections, caution has focalized on plant and immunostimulants products which could have a useful impact in disease control (Ispir and Dorucu, 2005).

Allium tuncelianum is essentially named as *Haussk subsp. tuncelianum* Kollmann and *Allium macrochaetum* Boiss (Etoh and Simon, 2002). Even though, it is local to specially at Platos of Munzur Mountains in Ovacık district of Tunceli province Turkey, it naturally grows in the narrow region located among Erzurum and Sivas (Baktır, 2005). Owing to its resemblance to widespread garlic, it is locally called as Ovacik garlic or Tunceli garlic in the region. *A. tuncelianum* generally forms one cloved white bulb, dissimilar garlic which has a a lot cloved bulb. The flower scape of *A. tuncelianum* coils early in its prolongation, which is a original characteristic of some garlic types (Ipek et al., 2008).

Garlic and its extracts has been reported to produce various benefical effects including immunostimulator (Sahu et al., 2007), antimicrobial (Deresse, 2010; Guo et al., 2012; Ranjan et al., 2012) properties and response

against several diseases including bacteria and parasites in fish species (Colorni et al., 1998; Diab et al., 2008; Nya and Austin, 2011; Abd-El Galil and Aboelhalid, 2012; Talpur and Ikhwanuddin, 2012; Williams et al., 2012). But, to our knowledge, no previous studies TGO related to immune mechanisms of fish have been reported. This study was conducted to condition the impacts of TGO on the immune system of *Oncorhynchus mykiss*. By applicationing this kind of substrate, loss of precious fish species reasoned by pathogens in fish production might be prevented and there may also be an economic utility for fish cultivation.

Material and Method

Experimental fish

Rainbow trout for the experiments were obtained from a commercial fish farm in Kahramanmaras province Turkey. This fish were kept in a 225 L fibreglass tank. The fish were acclimated in experimental units for 14 days before each experiment. They were fed a commercial diet to apparent satiation once daily throughout this period. Fish with average weights of 40g were haphazardly selected and stocked at rates of 50 fish/tank for experiments at the end of the acclimation period.

Experimental design

The Tunceli garlic (100 g) was placed in sterilized 2 liter conical flasks, separately with 1 L of corn oil and mixed good. Conical flasks were tightly covered with aluminum foil. They kept for 15 days at room temperature and agitated daily to ensure complete digestion. Then extracts were filtered through sterile muslin cloth.

Four experiments were carried out to measure immune parameters to experimental and control groups fish. Two hundred fish utilized in each experiment were divided into four groups (A, B, C and D) with 50 fish in each group. Each group was non-injected (control group A), injected with sunflower oil (control group B) or the injected with Tunceli garlic at a rate of 1 and 10% (groups C and D respectively). The analysis were performed with ten fish from each groups at 3,7, 14 and 21 days of injection. No feeding was done on sampling days. Blood was collected from the caudal. For serum separation, blood was transferred into serological tubes. The tubes were placed at room temperature for two hours, then overnight at 4ºC. The samples centrifuged for 10 min at 2500 rpm. Serum collected and stored at -20ºC.

Immunology study

Serum antibacterial activity to *Yersinia ruckeri* was determined according to Zhang et al. (2008). According to Sahoo et al., (2005) and Quade and Roth (1997) Total Myeloperoxidase (MPO) activity in serum was measured. Lysozyme activity was determined following the method described by Zhang et al. (2008) with a slight modification. The total protein level was determined through the Biuret method (Siwicki et al., 1994; Ispir et al., 2009). According to the method previously published by Siwicki et al. (1994) The total immunoglobulin level was determined by following. The difference in protein content before and after depletion is the serum total immunoglobulin content.

Statistical analysis

All experiments were conducted in triplicate. Mean values and standard deviations of the data of immune parameters were calculated from the experimental data obtained. Mean significance of immune parameter for experimental groups was analyzed using analysis of variance (ANOVA; Minitab Statistical Software Release). Differences between the mean values were considered significant when $p<0.05$.

Results

There was a statistical difference between groups was at 14d when the serum bactericidal activity levels were significantly higher ($p<0.05$) in the groups of fish injected with sunflower oil and negative control (Figure 1).

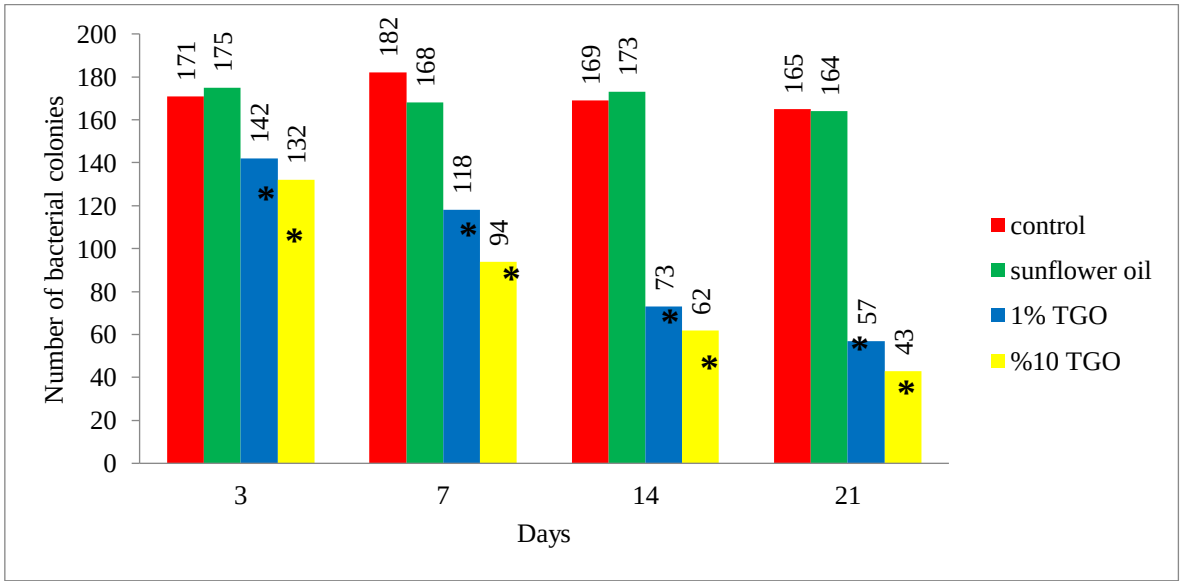

Figure 1. Serum bactericidal activity during various sampling days

The serum lysozyme activity was significantly different ($p<0.05$) between groups after intraperitoneal injection 14 d. The values obtained with serum from experimental groups fish were higher than their corresponding control at 3, 7, 14 and 21d (Figure 2).

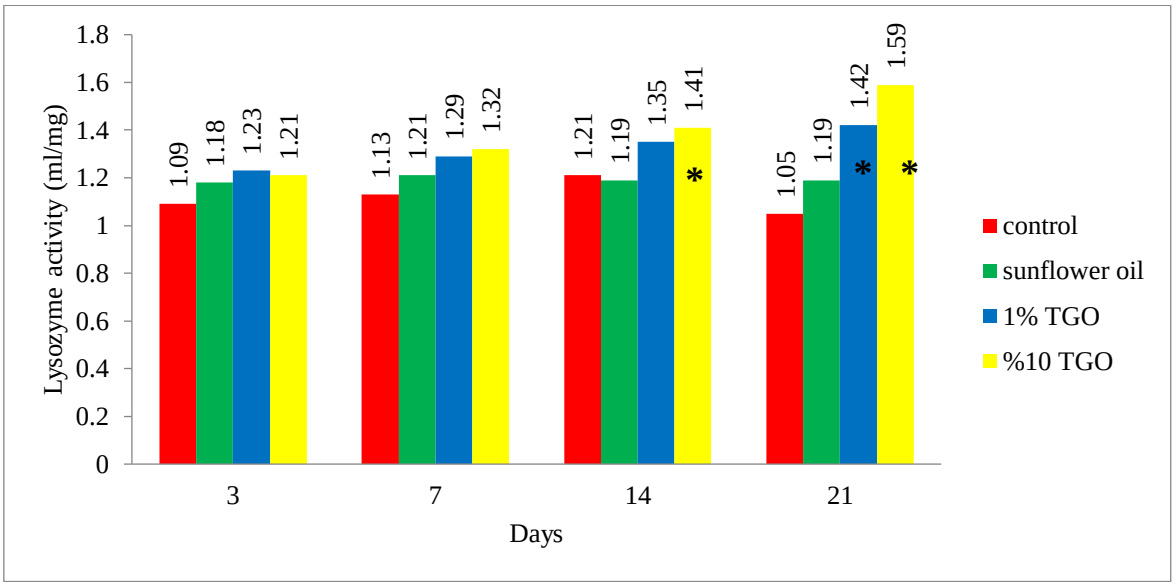

Figure 2. Lysozyme activity during various sampling days

The myeloperoxidase activity was significantly different ($p<0.05$) at 21d, with fish injected with 1.0% and 10.0% TGO having a higher activity than control fish (Figure 3). But, on days 21, MPO activity in the serum had significantly increased in comparison to control values ($p<0.05$).

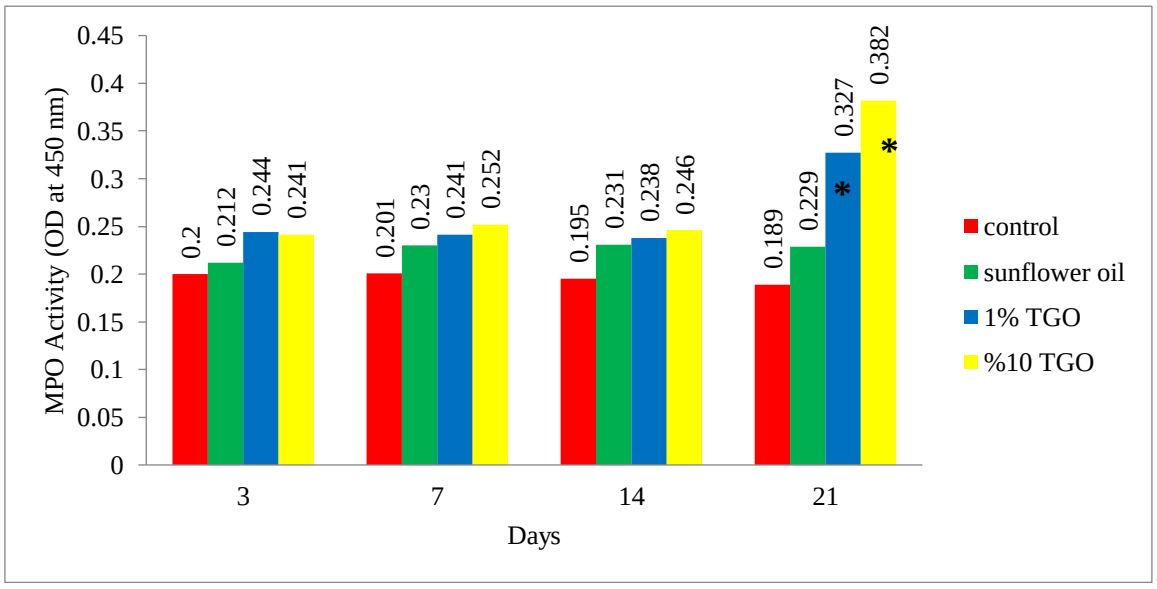

Figure 3. MPO activity during various sampling days

The total immunoglobulin and protein levels in fish injected with TGO were no statistically significant than the control group at 3, 7 and 14d. But, at 21 d post-injection for the groups injected with TGO (Figure 4 and Figure 5).

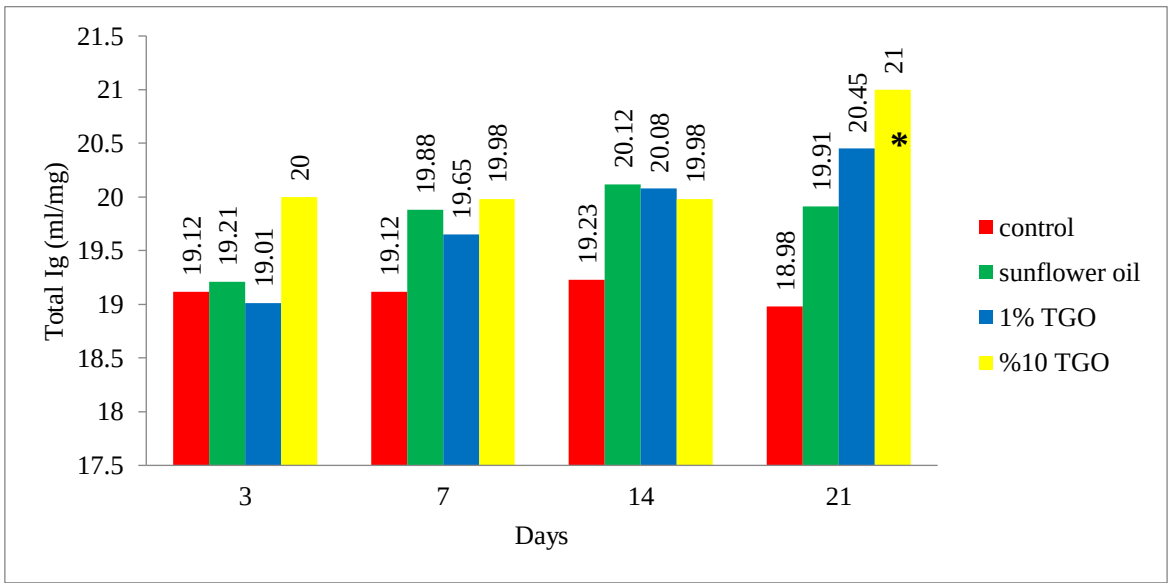

Figure 4. Total Immunoglobulin level during various sampling days

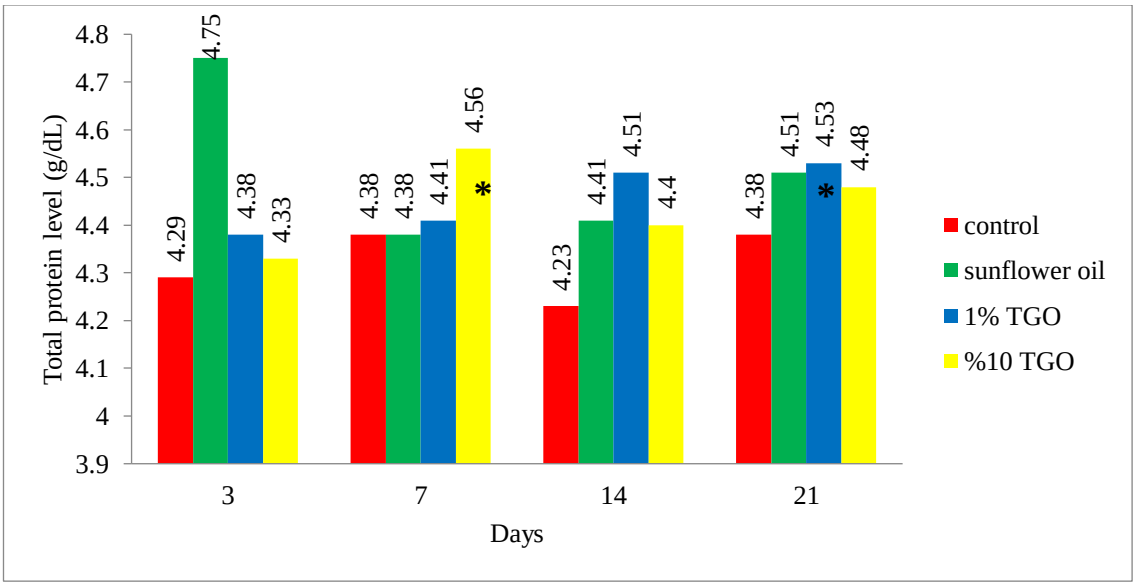

Figure 5. Total protein level during various sampling days

Discussion

Influence of intraperitoneal injection administration of Tunceli garlic oil at doses of 1% and 10% was evaluated on some immunological factors (Bactericidal, Myeloperoxidase and Lysozyme activities; Total immunoglobulin and protein) of rainbow trout.

Rohu (*Labeo rohita*) fingerlings fed a diet additionaled with garlic for 60 days. Then this fish exposed *Aeromonas hydrophila* ($1x10^5$ CFU) by IP injection (Sahu et al., 2007). Rainbow trout (*Oncorhynchus mykiss*) fingerlings fed garlic to groups of for 14 days prior to an intraperitoneal injection challenge with *Aeromonas hydrophila* ($1x10^6$ CFU) per fish (Nya and Austin 2009). It was indicated in both of these studies that fish were fed garlic demonstrated increased serum lysozyme and bactericidal activities, and greater serum total protein. In the present study, There was a statistical difference between groups was at 14d when the serum bactericidal activity levels were significantly higher in the groups of fish injected with sunflower oil and negative control ($P<0.05$). The serum lysozyme activity was significantly different between groups after intraperitoneal injection 14 d ($P<0.05$). The values obtained with serum from experimental groups fish were higher than their corresponding control at 3, 7, 14 and 21d.

In the study by Farahi et al. (2010) was to assess the effect of *Allium sativum* on body compositions in *Oncorhynchus mykiss*. Results of Rainbow trout body compositions demonstrated that crude protein and ash increased importantly with diets containing 30g *Allium sativum*. In this study the total protein levels in fish injected with TGO were no statistically significant than the control group at 3, 7 and 14 d. But, at 21 d post-injection for the groups injected with TGO. This change is similar to the result of their work Tafalla et al. (2009), Thanikachalam et al. (2010) were found different from the changes they detected. These results agree with those obtained by Khattab et al. (2004), Shalaby et al. (2006) and Abdelhamid et al. (2002), who showed that

inclusion of Biogen in the diet increased fish protein content. In this study the total immunoglobulin in fish injected with Tunceli garlic oil were no statistically significant than the control group at 3, 7 and 14d. But, at 21 d post-injection for the groups injected with TGO. This result; Yonar, (2002), Punitha et al. (2008) and Thanikachalam et al. (2010) differed from the change they detected.

Sophora flavescens extract was recorded after administered diet supplemented. Tilapia an increase in myeloperoxidase immune parameters as well as resistance against *Streptococcus agalactiae* (Wu et al., 2013). In the present study, The myeloperoxidase activity was significantly different at 21d ($P<0.05$), with fish injected with 1.0% and 10.0% TGO having a higher activity than control fish. But, on days 21, MPO activity in the serum had significantly increased in comparison to control values ($P<0.05$).

Conclusions

Plant extracts have a primarily potential utilization as an immunostimulant in fish production. As they are not act and expensive against a broad spectrum of pathogens. The preparation of plant extract is inexpensive and much easier. Many plant cultures are used as anti-viral and anti-bacterial materials. The use of plant cultures as immunostimulants in fish production systems might also be of environmental value owing to its biodegradability. In many studies, the usage of plant cultures as immunostimulant has revealed that they increase survival, growth rate and the immune responses of the fish. Owing to the beneficial impact of plant extract as immunostimulants, it can be used in fish farming as alternatives to, antibiotics, chemical drugs and vaccines.

In present study, administration of TGO enhances the some immune response parameters in rainbow trout, which can have a promising role in aquaculture to disease outbreaks and prevent diseases. Additionally, further studys on the immunostimulatory impact of TGO when administered along with feed and the preferred route of administration in the field condition for disease prevention in aquaculture are warranted.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

All animal studies were approved by KSÜZİRHADYEK and Research Institute (Protocol number: 2016/01).

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

This work was presented as a oral presentation at the International Symposium on Fisheries and Aquatic Science (FABA), 03 and 05 November 2016 in Antalya, Turkey.

References

Abd El-Galil, M.A.A. and Aboelhadid, S.M. (2012). Trials for the control of trichodinosis and gyrodactylosis in hatchery reared *Oreochromis niloticus* fries by using garlic, Veterinary Parasitology, 30;185 (2-4): 57-63. Doi: https://doi.org/10.1016/j.vetpar.2011.10.035

Abdelhamid, A.M., Khalil, F. F., El-Barbery, M.I., Zaki V.H. and Husien, H.S. (2002). Feeding Nile tilapia on biogen to detoxify aflatoxin diet. In: Annual scientific conference of animal and fish production, 1, Mansoura. Proceedings. Mansoura University, pp. 207-230.

Baktır, I. (2005). Tunceli Sarımsağı'nın (Allium tuncelianum) in vitro koşullarında çoğaltılması. In: Proceeding of GAP IV. Tarım Kongresi (in Turkish).

Colorni, A., Avtalion, R., Knibb, W., Berger, E., Colorni, B. and Timan, B. (1998). Histopathology of sea bass *Dicentrarchus labrax* experimentally infected with *Mycobacterium marinum* and treated with streptomycin and garlic (*Allium sativum*) extract. Aquac. 190: 1-17. Doi: https://doi.org/10.1016/S0044-8486(97)00234-2

Deresse, D. (2010). Antibacterial effect of garlic (*Allium sativum*) on *Staphylococcus aureus*: An in vitro study, Asian J. Med. Sci. 2 (2): 62–5.

Diab, A.S., Aly, S. M., John, G., Abde-Hadi, Y. and Mohammed, M.F. (2008). Effect of garlic, black seed and bioiogen as immunostimulants on the growth and survival of Nile tilapia, *Oreochromis niloticus* (Teleostei: Cichlidae), and their response to artificial infection with *Pseudomonas fluorescens.* Afr. J. Aquat. Sci. 33 (1): 63-68. Doi: https://doi.org/10.2989/AJAS.2007.33.1.7.391

Etoh, T. and Simon, P. W. (2002). Diversity, fertility and seed production of garlic. In H. D. Rabinowitch, L. Currah (Eds.), *Allium Crop Science: Recent Advances*. CABI Publishing, New York. pp. 101–117.

Farahi, A., Kasiri, M., Sudagar, M., Iraei, M. S. and Shahkolaei, M.D. (2010). Effect of garlic (*Allium sativum*) on growth factors, some hematological parameters and body compositions in rainbow trout (*Oncorhynchus mykiss*). Aquaculture, Aquarium, Conservation and Legislation International Journal of the Bioflux Society, 3 (4): 317-323.

Guo, J.J., Kuo, C.M., Chuang, Y.C., Hong, J.W., Chou, R.L. and Chen, T.I. (2012). The effects of garlic-supplemented diets on antibacterial activity against *Streptococcus iniae* and on growth in orange-spotted grouper, Epinephelus coioides, Aquaculture, 33 (38): 364–365. Doi: https://doi.org/10.1016/j.aquaculture.2012.07.023

Ipek, M., Ipek, A. and Simon, P.W. (2008). Genetic characterization of *Allium tuncelianum*: An endemic edible Allium species with garlic odor. Sci Hortic. 115, 409–415. Doi: https://doi.org/10.1016/j.scienta.2007.11.002

Ispir, U. and Dorucu, M. (2005). A study on the effects of levamisole of immune system Rainbow trout (*Oncorhynchus mykiss*, Walbaum). Turk J. Vet. Anim. Sci. 29: 1169-1176.

Ispir, U., Gokhan, H.B., Ozcan, M., Dorucu, M. and Saglam, N. (2009). Immune response of rainbow trout (*Oncorhynchus mykiss*) to selected antigens of *Yersinia ruckeri*. Acta Veterinaria Brno, 78: 145-150. Doi: Doi: https://doi.org/10.2754/avb200978010145

Khattab, Y.A., Shalaby, A.M.E., Sharaf, S.M., El-Marakby, H.I. and Rizkalla, E.H. (2004). The physiological changes and growth performance of the Nile tilapia *Oreochromis niloticus* after feeding with Biogen as growth promoter. Egypt. J. Aquat. Biol and Fish. 8: 145-158.

Nya, E.J. and Austin, B. (2009). Use of garlic, *Allium sativum*, to control *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish. Dis. 32 (11): 963–970. Doi: https://doi.org/10.1111/j.1365-2761.2009.01100.x

Nya, E.J. and Austin, B. (2011). Development of immunity in rainbow trout (*Oncorhynchus mykiss, Walbaum*) to *Aeromonas hydrophila* after the dietary application of garlic. Fish Shellfish Immunol. 30 (3): 845-850. Doi: https://doi.org/10.1016/j.fsi.2011.01.008

Punitha, S.M.J., Babu, M.M., Sivaram, V., Shankar, V.S., Dhas, S.A., Mahesh, T.C., Immanuel, G. and Citarasu, T. (2008). Immunostimulating influence of herbal biomedicines on nonspecific immunity in Grouper Epinephelus tauvina juvenile against *Vibrio harveyi* infection, Aquaculture Int. 16: 511–523. Doi: https://doi.org/10.1007/s10499-007-9162-6

Quade, M.J. and Roth, J. A. (1997). A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. Vet Immunol Immunop, 58: 239-248. Doi: https://doi.org/10.1016/S0165-2427(97)00048-2

Ranjan, S., Dasgupta, N., Saha, P., Rakshit, M. and Ramalingam, C. (2012). Comparative study of antibacterial activity of garlic and cinnamon at different temperature and its application on preservation of fish, *Adv*. Appl. Sci. Res. 3(1):495-501.

Sahoo, P. K., Kumari, J. and Mishra, B. K. (2005). Non-specific immune responses in juveniles of Indian major carps, J. Appl. Ichthyol. 21: 151-155. Doi: https://doi.org/10.1111/j.1439-0426.2004.00606.x

Sahu, S., Das, B. K., Mishra, B. K., Pradhan, J. and Sarangi, N. (2007). Effect of *Allium sativum* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. J. Appl. Ichthyol. 23(1):80–6. Doi: https://doi.org/10.1111/j.1439-0426.2006.00785.x

Shalaby, A.M., Khattab, Y.A. and Abdel Rahman, A.M. (2006). Effect of garlic (*Allium sativum*) and chloramphenicol on groth performance, physiological parameters and survival of Nile tilapia (*Oreochromis niloticus*). J. Venom Anim. Toxins incl. Trop. Dis. 12 (2):172-201. Doi: https://doi.org/10.1590/S1678-91992006000200003

Siwicki, A.K., Anderson, D.P. and Rumsey, G.L. (1994). Dietary intake of immunostimulants by rainbow trout effects non-specific immunity and protection against furuncluosis. Vet. Immunol. Immunopathol. 41: 125-139. Doi: https://doi.org/10.1016/0165-2427(94)90062-0

Tafalla, C., Novoa, B., Alvarez, J.M. and Figueras, A. (1999). *In vivo* and *in vitro* effect of oxytetracycline treatment on the immune response of turbot, *Scophthalmus maximus*. Journal of Fish Diseases, 22, 271–276. Doi: https://doi.org/10.1046/j.1365-2761.1999.00179.x

Talpur, A.D. and Ikhwanuddin, M. (2012). Dietary effects of garlic (*Allium sativum*) on haemato immunological parameters, survival, growth, and disease resistance against *Vibrio harveyi* infection in Asian sea bass, *Lates calcarifer* (Bloch). Aquac. 364-365:6-12. Doi: https://doi.org/10.1016/j.aquaculture.2012.07.035

Thanikachalam, K., Kasi, M. and Rathinam, X. (2010). Effect of garlic peel on growth, hematological parameters and disease resistance against *Aeromonas hydrophila* in African catfish Clarias gariepinus (Bloch) fingerlings. Asian Pacific Journal of Tropical Medicine, 614-618. Doi: https://doi.org/10.1016/S1995-7645(10)60149-6

Williams, C. F., Lloyda, D., Kolarich, D., Alagesan, K., Duchêne, M., Cablea, J., Williams, D. and Leitsch, D. (2012). Disrupted intracellular redox balance of the diplomonad fish parasite Spironucleus vortens by 5-nitroimidazoles and garlic-derived compounds. Veterinary Parasitology, 190(1–2), 62-73. Doi: https://doi.org/10.1016/j.vetpar.2012.05.011

Wu, Y.R., Gong, Q.F., Fang, H., Liang, W.W., Chen, M. and He, R.J. (2013). Effect of *Sophora flavescens* on non-specific immune response of tilapia (GIFT *Oreochromis niloticus*) and disease resistance against *Streptococcus agalactiae*. Fish. Shellfish Immunol. 34: 220-227. Doi: https://doi.org/10.1016/j.fsi.2012.10.020

Yonar, M.E. (2002). Sülfamerazinin Gökkuşağı alabalığının immun sistemi üzerine etkisinin araştırılması. Fırat Üniversitesi Fen Bilimleri Enstitüsü Su Ürünleri Yetiştiriciliği Anabilim Dalı. Yüksek Lisans Tezi (in Turkish).

Zhang, J., Zou, W. and Yan, Q. (2008). Non-specific immune response of bullfrog *Rana catesbeiana* to intraperitoneal injection of bacterium *Aeromonas hydrophila.* Chinese Journal of Oceanology and Limnology, 26, 248–255. Doi: https://doi.org/1007/s00343-008-0248-4