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

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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 functional 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 derivatives 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 Ovacık 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 beneficial 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 application of 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 Kahramanmaraş 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 4°C. The samples centrifuged for 10 min at 2500 rpm. Serum collected and stored at -20°C.

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).

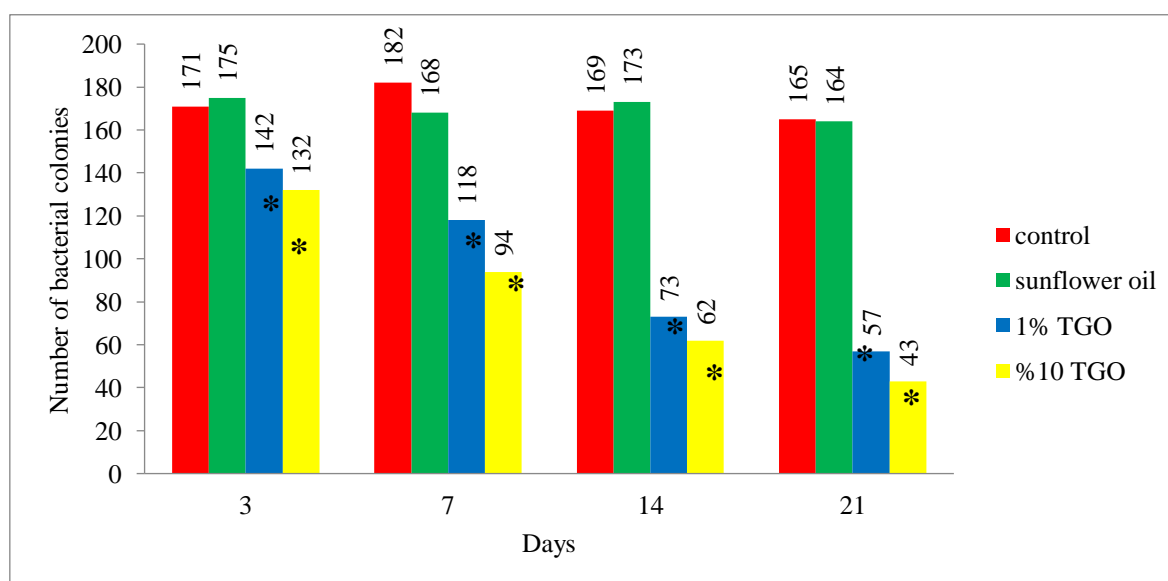


Figure 1. Serum bactericidal activity during various sampling days

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).

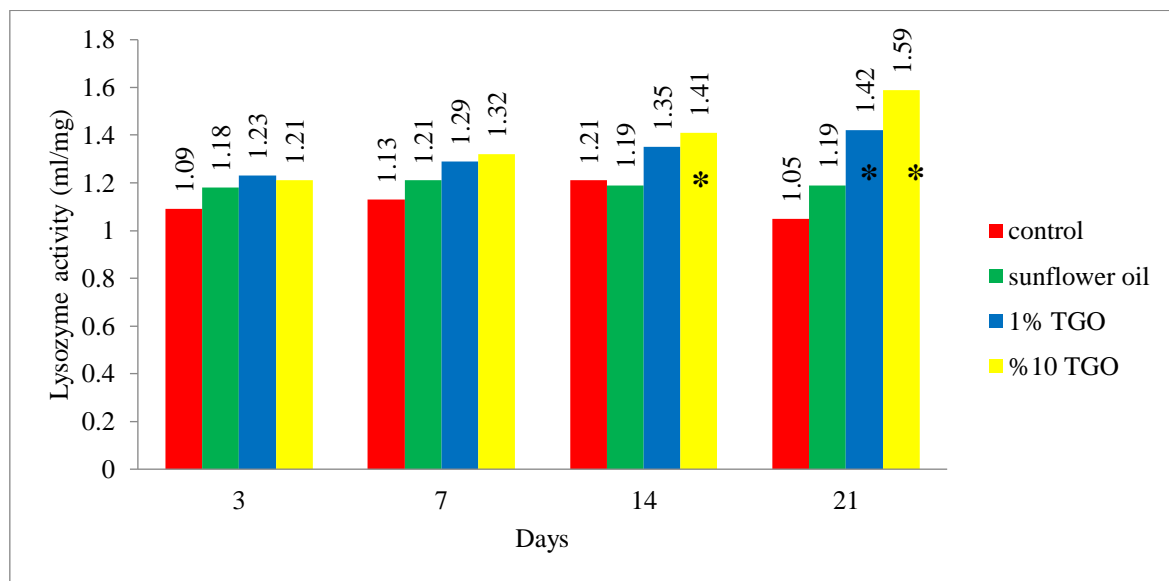


Figure 2. Lysozyme activity during various sampling days

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 ($p<0.05$).

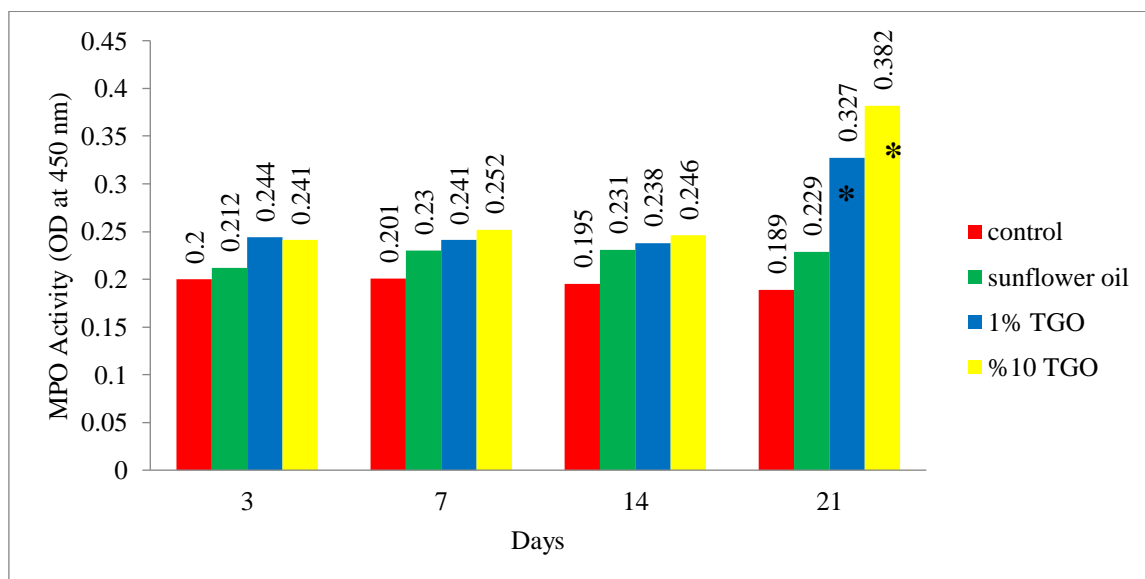


Figure 3. MPO activity during various sampling days

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).

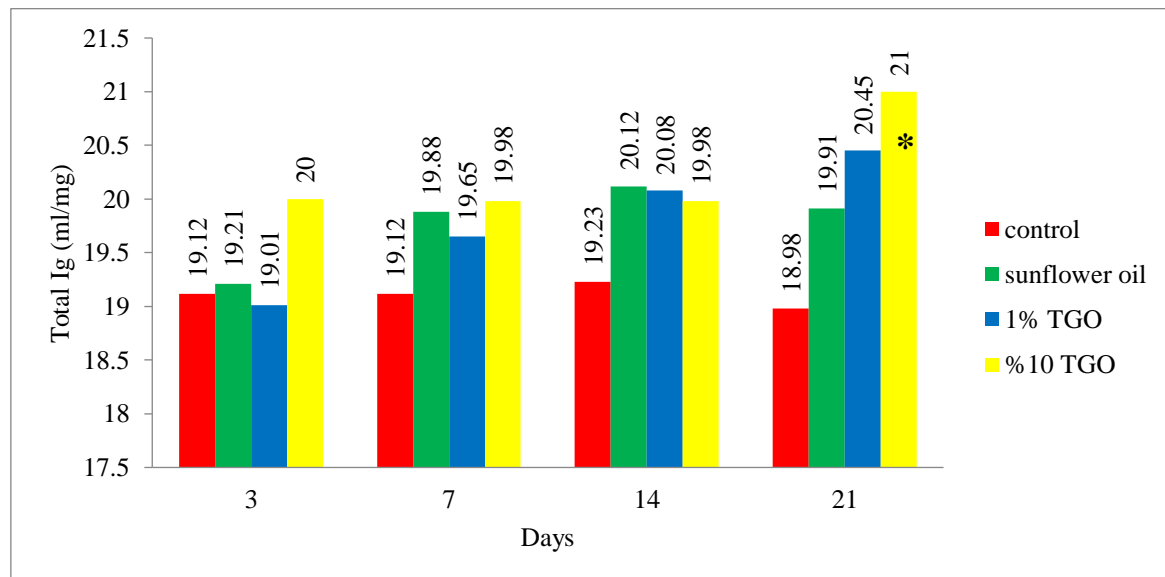


Figure 4. Total Immunoglobulin level during various sampling days

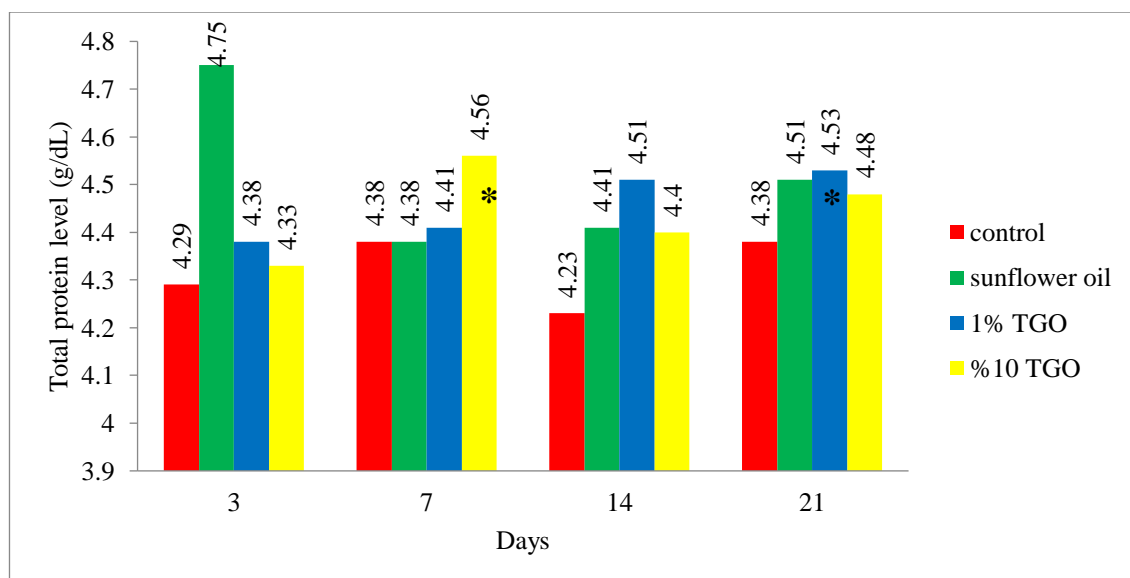


Figure 5. Total protein level during various sampling days

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 additionalled with garlic for 60 days. Then this fish exposed *Aeromonas hydrophila* (1×10^5 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* (1×10^6 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 studies 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 KSÜZİRHADYK and Research Institute (Protocol number: 2016/01).

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Data availability

Not applicable.

Consent for publication

Not applicable.

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