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Research Article

Effect of oxytetracycline on serum response of rainbow trout, *Oncorhynchus mykiss*

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Abstract: In this study, the effect of oxytetracycline (OTC), which is widely used in the treatment of fish diseases, on serum response was investigated. We conducted two experiments, the first with intraperitoneal (IP) injection and the second with immersion (IM) bath administration of OTC. For this purpose, fish was injected with IP 20 mg/kg fish weight dose of OTC every day for 4 days. After 24 hours the last treatment serum was collected. In trial second, fish were exposed to 50 mg/L concentration of OTC for 48 hours. The several immune parameters (lysozyme, myeloperoxidase, and total protein activities) and bactericidal activity against *Yersinia ruckeri* were studied. The present results show that immune parameters (lysozyme, myeloperoxidase and total protein activities) were significantly reduced after injection administration of OTC. However, in the immersion administration of OTC in fish, while the difference in lysosome and total protein activities was not significant, the decrease in myeloperoxidase activities was found to be significant compared to the control group. The serum bactericidal activity of all groups showed a significant increase in comparison with the control untreated group.

Keywords: Antibiotics, Immune parameters, Oxytetracycline, Rainbow trout, *Yersinia ruckeri*

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1. INTRODUCTION

Over the past ten years, aquaculture has expanded significantly and grown to become a significant economic sector. It has the most potential to satisfy the rising demand for aquatic food and is now the industry with the fastest rate of growth in the world of food production. Aquaculture is growing, increasing, and diversifying on a global scale. Increasing production efficiency to maximize revenue is a recurring objective in aquaculture worldwide. The use of various pesticides and antibiotics has become necessary to maximize output in order to accomplish this goal. However, there are issues with food safety and human health, and the use of pesticides and antibacterial materials in fish farms does not always have beneficial results. The development of antibiotic resistance in fish germs as a result of antibiotic use is a bigger worry. Antibiotic-resistant infections in people can arise from these antibiotic usage because they can also cause antibiotic resistance in non-pathogenic bacteria, whose resistance genes can then be passed on to disease-causing bacteria (Subasinghe et al., 2009).

Numerous investigations have revealed interactions between antibiotics and fish immune systems. Antibacterials and other medications have a wide range of immunomodulatory effects, and the effects of each medicine examined have varied. It has been demonstrated that certain medications increase the immune system's defenses (Ellis, 2001; Harikrishnan et al., 2011). For instance, when administered in conjunction with immunization, the antihelmintic medication levamisole stimulated both the rainbow trout's particular and non-specific defense systems, increasing their resistance to disease (Wiegertjes et al., 1996; Sakai, 1999). In contrast, some researchers (Rijkers et al., 1981; Grondel et al., 1987; Siwicki et al., 1989; Serezli et al., 2005; Mog et al., 2021) have shown that oxytetracycline (OTC) suppresses the immune functions in carp and rainbow trout.

The aim of the present study was the examine if OTC would influence some immune response parameters of rainbow trout.

2. MATERIAL AND METHOD

2.1. Fish

Rainbow trout (*Oncorynchus mykiss*), body weight 168.42 ± 13.5 g, were used in the experimental trials. These fish were reared in tanks at Fırat University, Fisheries Facultuy Research Laboratory. The fish were moved to a tank with aerated well water before each trial, and they were allowed to acclimate for at least two weeks. Throughout the trials, fish were given a commercial feed (Ecobio, Türkiye) on an as-needed basis. Aeration, moving water, and biological filtration all contributed to the preservation of water quality. American Public Health Association was used to determine the water quality characteristics in the experimental units. The water used to prepare the test solutions had the following average quality parameters: pH 7.4–7.6, temperature 15–16°C, and dissolved oxygen 8.1–8.4 mg/L.

2.2. Adminisration Procedure of OTC

Trial I consisted of 6 tanks (3 tank control and 3 tank experimental groups) with 8 fish per tank. These fish were divided into two groups (control and experimental) and IP injected with 20 mg/kg fish weight dose of OTC for 4 days. After 24 hours the last treatment serum were collected.

Six tanks (3 tank conrol and 3 tank experimental groups) with 8 fish per tank were assigned for trial II. These fish were divided equally into two groups (three tanks per group). Fish were exposed to 50 mg/L OTC for 48 hours.

2.3. Serum Collection

The caudal vein was used for blood collection. Blood was moved into serological tubes in order to separate the serum. After two hours at room temperature, the tubes were kept overnight at 4ºC. The samples were centrifuged at 2500 rpm for 10 minutes. Serum was obtained and kept at -20°C (İspir & Özcan, 2021; İspir et al., 2022).

2.4. Immunology Study

Serum antibacterial activity to *Yersinia ruckeri* was determined according to Zhang et al. (2008). Total Myeloperoxidase activity in serum was measured according to Quade & Roth (1997), and Sahoo et al. (2005). 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).

2.5. Statistical Analysis

Every experiment was carried out in triplicate, and the experimental data was used to compute the mean values and standard deviations of the immunological parameter data. Analysis of variance (ANOVA; Minitab Statistical Software Release) was used to determine the mean significance of the immunological parameter for the experimental groups. Significant differences between the mean values were defined as $p < 0.05$.

3. RESULTS

The results of lysozyme and myeloperoxidase contents are shown in Figure 1 and 2. The lysozyme activity also showed variation among control and OTC injected group. In this study demonstrate that lysozyme and myeloperoxidase activities slightly decreased after the injection administration of OTC. But, lysozyme and myeloperoxidase levels in the OTC-injected group was significantly different ($p < 0.05$) than that in the control group. The immersion administration of OTC in fish had no effect on this parameters.

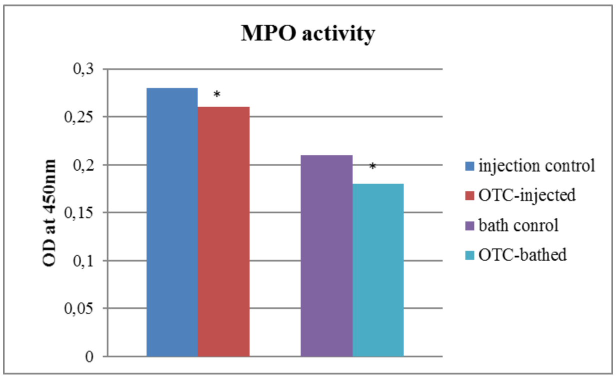

Figure 1. Effect of OTC on the myeloperoxidase activity of rainbow trout (*Significant difference from control ($p < 0.05$))

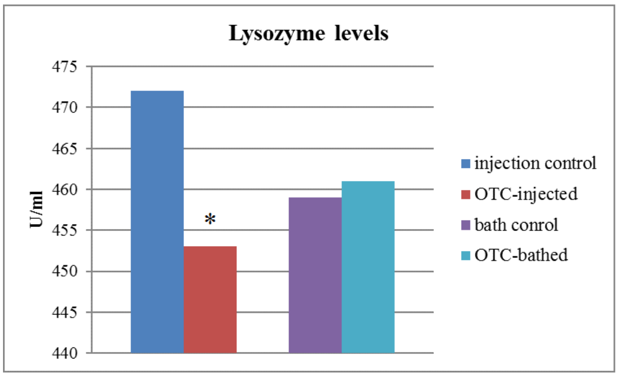

Figure 2. Effect of OTC on the lysozyme levels of rainbow trout (*Significant difference from control ($p < 0.05$).

Serum protein activity recorded in the samples of controls, OTC-injected and OTC-bathed group is depicted in Figure 3. Comparatively, (4.82 ± 0.14 g/dl) protein activity was observed in control groups of injection administration; whereas it was higher (4.12 ± 0.09 g/dl) in OTC-injected group. But, protein activity of OTC-bathed administration and control groups was observed as 4.42 ± 0.11 and 4.39 ± 0.10 ($p < 0.05$), respectively.

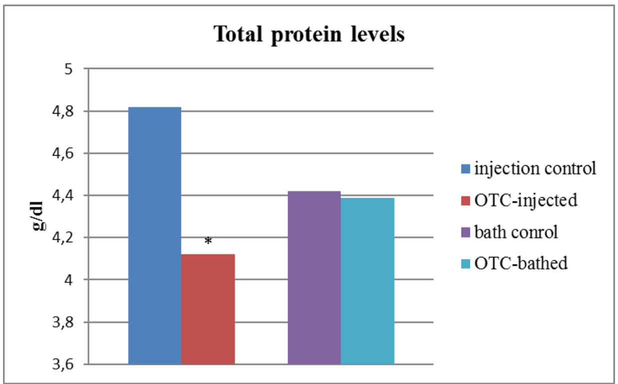

Figure 3. Effect of OTC on the total protein levels of rainbow trout (*Significant difference from control ($p < 0.05$))

Serum bactericidal activity of fish OTC-injected and OTC-bath group and control groups is shown in Figure 4. Serum bactericidal activity of OTC-injected and OTC-bathed group was always higher than the control groups ($p < 0.05$).

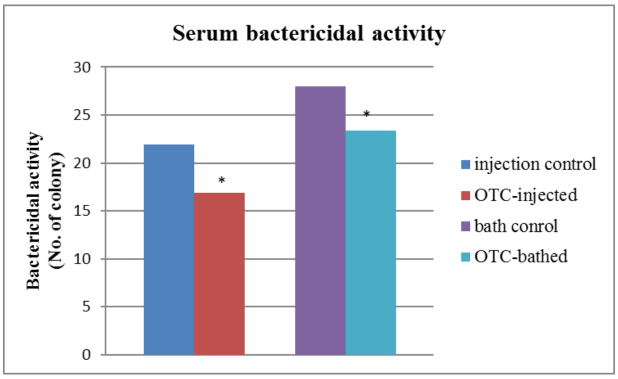

Figure 4. Effect of OTC on the serum bactericidal activity of rainbow trout (*Significant difference from control ($p < 0.05$))

4. DISCUSSION AND CONCLUSION

Various chemotherapeutic agents such as tetracyclines, sulfonamides and nitrofurans have been used for a long time both for the treatment of infections that cause significant economic losses in fish and for preventive purposes (Michel et al., 1990; Uno et al., 1993; Sakai, 1999). Among the tetracyclines, there are many tetracycline derivative antibiotics such as OTC, methacycline, doxycycline, rolitetracycline, chlortetracycline. Although resistant microorganisms have developed over time against these drugs, which have a very wide spectrum of action, they are the most commonly used drugs (Kayaalp, 1984). Although OTC is the most effectively used drug in the treatment of bacterial fish diseases, the use of these drugs is limited due to significant side effects such as degeneration in some organs, accumulation in muscles and reaching people, and bacteria becoming resistant to these drugs. Other important side effects of OTC include causing oxidative stress in fish and its immunosuppressive effect (Grondel et al., 1987; Björklund et al., 1991; Inglis et al., 1996; Sağlam & Yonar, 2009; Yonar et al., 2011; Yonar, 2012; Safi & Mişe Yonar, 2022).

Defense mechanisms, or immunity, in a very general sense means protecting the body against infectious diseases caused by pathogenic microorganisms. The entirety of the body's reactions to foreign agents is called the immune response (Iwama & Nakanishi, 1996). The immune system in fish is divided into two as specific and nonspecific. Lysozyme enzyme is one of the most important humoral factors of the nonspecific immune system. Lysozyme activity is an important parameter that expresses the immune system, such as neutrophil, complement and phagocytic activity. Lysozyme is found in blood serum, mucus, spleen, kidney, liver, skin, gills, muscle, digestive tract, ovary and eggs, phagocytic cells, leukocytes and macrophages in fish (Balfry & Iwama, 2004). In addition, thanks to lysozyme activity, the cell walls of bacteria are broken down, thus increasing resistance to diseases (Murray et al., 2003). Lysozyme activity varies depending on the fish species, health status, stress, gender, season, temperature and sexual maturity (Caruso et al., 2002). In this study, a significant decrease in lysozyme activity was determined after OTC injection application, while no significant difference was detected between lysozyme activity in OTC immersion application. Similarly, Soltanian & Fereidouni (2017) applied cypermethrin at doses of 0.042, 0.085 and 0.17 µg/L to carp for 21 days. As a result of the study, there was no significant difference in serum lysozyme activity between the groups on the 7th day, while a significant decrease was detected on the 14th and 21st days. There are studies in the literature reporting that different pesticides cause significant decreases in lysozyme activity in fish (Sopinska & Guz, 1998; Nayak et al., 2004; Jee et al., 2005; Shelley et al., 2009; Siwicki et al., 2010). The decrease in serum lysozyme activity observed in this study indicates the immunostimulatory effects of OTC.

Myeloperoxidase, which catalyzes chloride ions and hydrogen peroxide to form hypochlorous acid, damages the invading microorganisms. In other words, serum myeloperoxidase activity is an effective enzyme in killing and removing pathogens in fish (Klebanoff, 1968). In this study, it was determined that there was a significant decrease in myeloperoxidase activity after OTC injection and immersion applications. Similarly, Kaya et al. (2013) applied 0.50, 2.5 and 5 mg/L lead doses to *Oreochromis mossambicus* fish for 14 days. As a result of the study, they stated that the doses decreased fish serum myeloperoxidase activities on the 7th and 14th days. However, Siwicki (1987) determined that in carp (*C. carpio*) injected with 5, 10, 15 and 20 mg/kg body weight levamisole, myeloperoxidase activity started to increase in the second week in the 5 mg/kg and 10 mg/kg groups and decreased after the sixth week, but remained higher than the other levamisole groups until the end of the study (12 weeks). In addition, Sarıhan (2005) examined myeloperoxidase activity in blood samples taken on the 7th, 14th, 21st and 28th days of the experiment after 5, 7.5 and 12.5 µg/ml levamisole was applied to *Oreochromis niloticus* fish by bathing for 24 hours. They stated that myeloperoxidase activity values were higher than the control group on the 7th, 14th and 21st days in the 5 µg/ml and 12.5 µg/ml levamisole groups, and on the 7th, 14th and 28th days in the 7.5 µg/ml group.

Total protein is an important indicator of humoral defense system and health status used to monitor the course of diseases in immune disorders, liver dysfunctions and impaired kidney activities (Mochida et al., 1994). Increased concentrations of total protein in fish may cause excessive energy expenditure and impaired control of blood flow balance at low environmental pH. Increased total protein amount is observed as a result of hunger and low or impaired absorption, and decreases as a result of dehydration. Serum protein amount is a parameter related to the quantity and quality of nutrition of fish individuals and indicates a situation related to inadequate nutrition of fish for any reason (Çelik & Bilgin, 2007). Many studies in the literature have addressed the negative effects of different pollutants and pesticides on total protein levels in fish species (Yamawaki et al., 1986; Oruç & Uner, 1998; Handy et al., 1999; Lusková et al., 2002; Das & Mukherjee, 2003; Martinez et al., 2004; Banaee et al., 2011). For example, it was found that total plasma protein levels in turbot (*Scophthalmus maximus*) decreased with OTC application (Tafalla et al., 1999). Dobšíková et al. (2013) stated that OTC increased the total protein levels in carp on some days of the experiment and decreased them on other days. Öntaş (2017) stated that oxyteracycline administered orally at a dose of 75 mg/kg fish had no statistically significant effect on serum total protein levels in sea bass. After 4 weeks of application of Danitol (Fenpropathrin), a synthetic pyrethroid, in Chinese grass carp (*Ctenopharyngodon idella*), serum total protein was found to decrease (Ahmad et al., 1995). Soltanian & Fereidouni (2017) applied cypermethrin at doses of 0.042, 0.085 and 0.17 µg/L to carp fish for 21 days. As a result of the study, they stated that total protein levels significantly decreased in fish exposed to 0.085 and 0.17 µg/L cypermethrin after 21 days of exposure. Similar results were found in rainbow trout after exposure to deltamethrin (Siwicki et al., 2010). In addition, plasma total protein concentration was significantly decreased in cypermethrin-treated Korean rockfish (Jee et al., 2005) and in Nile tilapia with deltamethrin poisoning (El-Sayed & Saad, 2007). The decrease in serum protein content may be partly due to the decrease in the number of WBCs, which are the main sources of serum protein production such as immunoglobulin, lysozyme, complement factors and bactericidal peptides (Misra et al., 2006a, 2006b). In parallel with the findings obtained from the above-mentioned studies, in this study, it was found that total protein activity decreased in the group administered OTC by injection. However, in the immersion application of OTC in fish, the difference in total protein activities was not significant when compared to the control group.

Teleost fish's immune system contains defense systems against germs that use protective proteins in different tissues. Serum bactericidal activity, a crucial instrument for immune system analysis, can be used to measure the protein effect (Biller-Takahashi et al., 2013). In the present study, a statistically significant increase was detected when the serum bactericidal levels of fish treated with OTC administered both IP and IM were compared with those of the control group ($p < 0.05$). Similarly, Biller-Takahashi et al. (2013) in their study challenged *Piaractus mesopotamicus* fish with *Aeromonas hydrophila* for a short time to assess serum bactericidal activity and serum samples were taken one week after the challenge. The study's findings demonstrated that bacterial infection raised serum bactericidal activity, decreased colony-forming unit values, and increased the quantity of protective proteins. A rise in serum protective proteins, which typically happens following a natural infection like disease outbreaks or following an artificial infection like vaccination and challenge, is indicated by increased serum bactericidal activity found after reading. These proteins are referred to as acute-phase inflammatory proteins. Hepatic, neuroendocrine, and immune system alterations may also result in elevated acute phase proteins right after infection or damage (Magnadottir et al., 2011).

Recent years have seen significant advancements in our understanding of the immunological regulation of fish illnesses, which has benefited the expanding aquaculture sector globally and improved our comprehension of certain fundamental immunological phenomena. The immune parameters analyzed in this study, such as lysozyme, myeloperoxidase and total protein levels, constitute the stages of a sequential and mutually dependent mechanism. Therefore, the decrease in the amounts of lysozyme, myeloperoxidase and total protein levels as a result of OTC treatment is an indicator that the health of the fish is affected. Under these conditions, the risk of developing bacterial diseases in fish or their resistance to other environmental factors will increase. This is due to the negative effects of xenobiotics on the immune system of fish; many of them have an "immunotoxic" effect on fish. In conclusion, the present results show that OTC does significantly influence the immune parameters studied even if a slight effect on the myeloperoxidase and lysozyme function is indicated. When compared to previous studies, the effects of OTC on the immune parameters studied are more pronounced than those demonstrated for florfenicol and oxolinic acid.

Authors' Contributions

MEY: Manuscript design, Field sampling, Laboratory experiments, Draft checking, Writing, Reading, Editing.
Üİ: Manuscript design, Field sampling, Laboratory experiments, Draft checking, Writing, Reading, Editing, Statistical analyses.
MK: Manuscript design, Field sampling, Laboratory experiments, Draft checking, Writing, Reading, Editing, Statistical analyses.
SMY: Manuscript design, Laboratory experiments, Draft checking, Writing, Reading, Editing.
All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Statement on the Welfare of Animals

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Data Availability Statements

The authors confirm that the data supporting the findings of this study are available within the article.

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