



## Effect of Apricot Kernel Oil on Serum Response of Rainbow Trout (*Oncorhynchus mykiss*)

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### ABSTRACT

Rainbow trout (*Oncorhynchus mykiss*) were examined effects of apricot kernel oil (*Prunus armeniaca* L.) on the immune mechanisms. After Fish weight 40 ±0,03 g was intraperitoneal (i.p.) injection 1% and 10% apricot kernel oil (AKO), blood was taken from the caudal vein of anesthetized (50 ppm, benzocaine) fish. Myeloperoxidase production (MPO), Serum bactericidal activity, protein levels, Lysozyme response (LYS) and total immunoglobulin (Ig) were determined on days 3, 7, 14 and 21. The same strategy was conducted on a control bunch. No contrasts were found within the levels of immunoglobulin between the control and test groups. However, there were considerable increases in bactericidal activity, MPO, LYS, protein levels and important differences were detected between the control and experimental groups. AKO can be utilized to improve the resistant instruments of rainbow trout.

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## Introduction

Chemicals, antibiotics and drugs have been utilized for treating fish disease reasoned by other factors and environmental stress for long time (Sakai, 1999). To control bacterial fish pathogens, infected fish is a general practice feeding with antibiotic medicated feed. But this practice is generally ineffective as disease fish tend to remain off feed and expensive. Also, pathogenic bacteria can develop antibiotic resistance (Davies and Davies, 2010). Hence, eco-friendly disease prevention has been taken into account with the needs to look for alternative techniques.

Essential oils of many medicinal plants and fruits for which an ability to increase immune system and protective effect against diseases has been demonstrated include apricot kernel oil (Kutlu et al., 2009); oregano essential oil (Zheng, et al., 2009); cinnamon (*Cinnamomum verum*) oil (Pongsak and Parichat, 2010); olive oil (Sicuro et al., 2010); *Zataria multiflora* and *Eucalyptus globolus* (Sheikhzadeh et al., 2011); garlic oil (Yılmaz and Ergun, 2012); black cumin seed oil (Awad et al., 2013); thyme and fennel oil (Kucukgul

Gulec et al., 2013); coconut oil (Luo et al., 2014) in several fish species.

The apricot kernel oil contains is is composed of 60% oleic acid (C18:1) and 30% linoleic acid (C18:2) (Al-Khalifa, 1996). Several studies have investigated the potential of oleic and linoleic acid to ameliorate immune response (Kremer et al., 1990; Jeffery et al., 1997; Cook et al., 1999; Sugano et al., 1999; Cook et al., 2003; Puertollano et al., 2007). Recently, apricot and/or apricot kernel oil has become a focus of interest owing to it's on a large scale efficient immune response activity against diseases in human and other animals (Karabulut et al., 2014).

To our knowledge, no previous studies apricot kernel oil related to immune mechanisms of fish have been reported. In our study was implemented to ascertain the impacts of apricot oil on the immune system of rainbow trout. Loss of valuable fish species reasoned by pathogens in fish culture might be forestalled by handling this kind of substrate. There might also be an economic advantage for fish cultivating.

## Materials and Methods

### Experimental Fish

Rainbow trout for the experiments were obtained from a commercial fish farm in Kahramanmaraş province Turkey. This fish was 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 (Granulated feed containing 40% protein and 11% fat) to apparent satiation once daily throughout this period. Fish with average weights of  $40 \pm 0,03$  g were haphazardly selected and stocked at rates of 50 fish/tank for experiments at the end of the acclimation period. The tank water was kept in suitable temperature (10-12°C) and dissolved oxygen (8-10 ppm) with a well aeration and continuously cooling.

### Experimental Design

Essential oil of apricot oil was considered 100% pure. The oil was purchased from a local commercial market, Malatya, Turkey. Four experiments were carried out to measure immune parameters to experimental and control groups fish. Two hundred utilized in each experiment were divided into four groups 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 apricot kernel at a rate of 1 and 10% (groups C and D respectively) (diluted with Sunflower oil). The analysis was performed with seven fish from each group at 3, 7, 14 and 21 days of exposure. No feeding was done on sampling days. Fish are anesthetized with an anesthetic (50 ppm, benzocaine). 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 *Aeromonas hydrophila* 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 calculated following the method described by Zhang et al. (2008) with a slight change. The total protein level was measured 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 serum total Ig Level is difference in protein content after and before depletion.

### Statistical Analysis

Four groups were conducted in triplicate, and 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 analysed using analysis of variance (ANOVA; Minitab Statistical Software Release). Differences between the mean values were considered important when  $P < 0.05$ .

## Results

Serum bactericidal levels, myeloperoxidase and lysozyme resistance, total protein and immunoglobulin levels in the control group and apricot kernel oil injected fish group are given in Figures 1-5.

The important difference was found between AKO treated fish and respective control fish in relation to the serum bactericidal levels ( $P < 0.05$ ). Serum bactericidal levels of AKO treated fish were importantly higher than that of the respective control fish on 14 days of post exposure onwards. Maximum increase in these parameters occurred on 21 days of post exposure to AKO (Figure 1). The Serum bactericidal activity increased by nearly half on 21 days of AKO post-injection compared to the respective controls.

When rainbow trout were injected with AKO, The MPO activity was increased importantly for days 3, 7, 14 and 21 ( $P < 0.05$ ). In myeloperoxidase content observed on the maximum levels of 14 days (Figure 2).

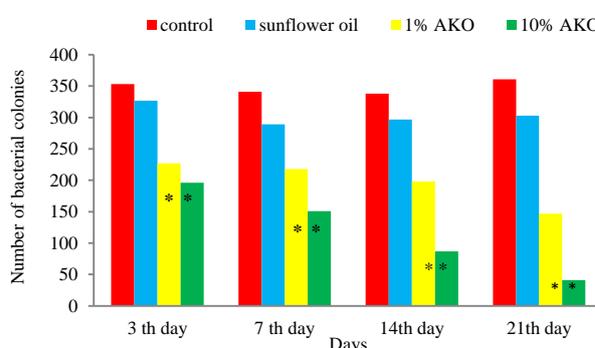


Figure 1. Total serum bactericidal activity during various sampling days.

\*Significant difference from control groups  $P < 0.05$ .

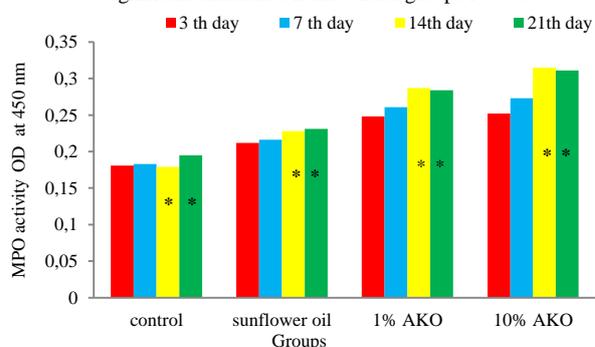


Figure 2. Total Myeloperoxidase activity during various sampling days.

\*Significant difference from control groups  $P < 0.05$ .

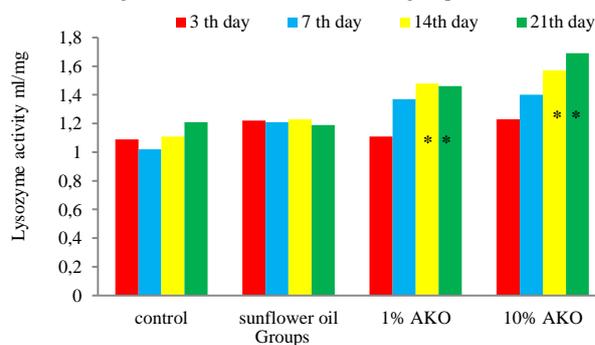


Figure 3. Total Lysozyme activity during various sampling days.

\*Significant difference from control groups  $P < 0.05$ .

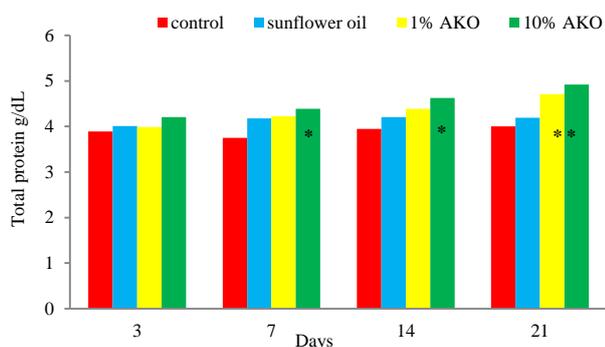


Figure 4. Total serum protein levels during various sampling days.

\*Significant difference from control groups  $P < 0.05$ .

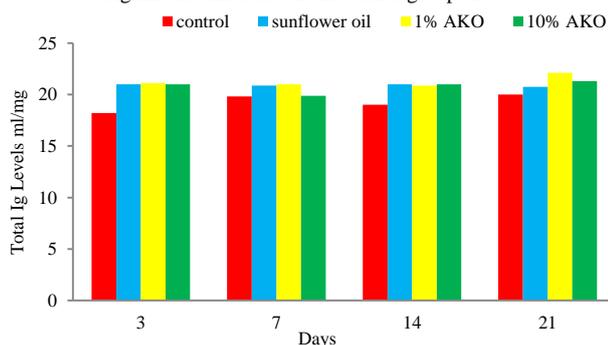


Figure 5. Total Immunoglobulin levels during various sampling days.

Lysozyme activity was similar on days 3 and 7 in the experimental group (Figure 3). Experimental group was an important increase in serum lysozyme activity, compared to the control group ( $P < 0.001$ ). Lysozyme activity in the serum had declined on days 14, but importantly increased in comparison to control values ( $P < 0.05$ ).

In the experimental group are determine 3, 7, 14 and 21 days after the injection total protein levels (Figure 4). The total protein levels were increased importantly when rainbow trout were injected with AKO for days 3, 7, 14 and 21 ( $P < 0.05$ ).

Total Ig level in the AKO-injected group was not importantly different than that in the control group ( $P > 0.05$ ) (Figure 5).

## Discussion

In this research, immune mechanisms of the samples in the experimental groups created by intraperitoneal injection 1 % and 10% AKO to Rainbow Trout were investigated. Lysozyme response, Myeloperoxidase production, Bactericidal activity, total immunoglobulin and protein levels in serum were examined on days 3, 7, 14 and 21.

Mango kernel treated groups increased bactericidal activity according to the control group. As a result, Humoral factors involved in adaptive and/or innate immunities were also increased. Likewise, a fraction from *Quillaja saponaria* Molina, Quil-A, enhanced serum bactericidal activity (Garyson et al., 1987). In the present study, the important difference was found between AKO treated fish and respective control fish in relation to the serum bactericidal levels ( $P < 0.05$ ). Serum bactericidal levels of AKO treated fish were importantly higher than

that of the respective control fish on 14 days of post exposure onwards. Maximum increase in these parameters occurred on 21 days of post exposure to AKO. The Serum bactericidal activity increased by nearly half on 21 days of AKO post-injection compared to the respective controls.

Treatment with apricot diet caused important decrease in Myeloperoxidase production ( $P < 0.005$ ). These results also suggest that, apricot have a protective effect through the inhibition of neutrophil infiltration (Vardi et al., 2008). In our study, when rainbow trout were injected with AKO, The MPO activity was increased importantly for days 3, 7, 14 and 21 ( $P < 0.05$ ). In myeloperoxidase content observed on the maximum levels of 14 days. This result; Vardi et al. (2008) differed from the change she detected.

Lysozyme activity is the first barrier line in innate immune system (Hardie et al., 1996). The lysozyme activity of the mango kernel experimental groups was importantly higher than the control group on days 40, 60 ( $P < 0.05$ ) (Sahu et al., 2007). Lysozyme activity were importantly higher in Mango (*Mangifera indica*) Kernel meal (5 g/kg diet) experimental group when compared with the control group at post-challenge periods ( $P < 0.05$ ) (El-Houseiny et al., 2017). In the present study, Lysozyme activity was similar on days 3 and 7 in the experimental group. Experimental group was an important increase in serum lysozyme activity, compared to the control group ( $P < 0.001$ ). Lysozyme activity in the serum had declined on days 14, but importantly increased in comparison to control values ( $P < 0.05$ ).

After term feeding 20, 40 and 60 days with mango seed, the serum total protein increased compared with the control diets. Total serum protein content in the mango kernel (1 g, 5 g, 10 g) experimental groups was importantly higher compared to control group over the exposure duration ( $P < 0.05$ ) (Sahu et al., 2007). In our study, the total protein levels were increased importantly when rainbow trout were injected with AKO for days 3, 7, 14 and 21 ( $P < 0.05$ ). Increases in serum total protein are thought to be associated with a stronger innate response of fishes (Wiegertjes et al., 1996; Rao et al., 2006; Sahu et al., 2006).

Immunoglobulin M were importantly ( $P < 0.05$ ) higher in experimental groups when compared with the control group at post-challenge periods (El-Houseiny et al., 2017). The impacts of dietary AKO were evaluated in a rat model of cyclophosphamide-induced immunosuppression. The levels of IgA, IgM, IgG in spleen lymphocytes were importantly reduced in experimental groups when compared with the control group. As compared to the healthy control group, the NS-treated group showed extremely important differences in IgA and IgM production ( $P < 0.001$ ) (Tian et al., 2016). In the present study, Total Ig level in the apricot kernel oil injected group was not importantly different than that in the control group ( $P > 0.05$ ). Thus, Apricot kernel oil may prevent against fish diseases.

## Conclusion

Both doses of apricot kernel oil could enhanced myeloperoxidase and lysozyme activity, total protein levels in the serum on 14 - 21 days post-injection to apricot kernel oil. The results revealed that administration of apricot oil (1% and 10%) through injected route is a

potential method in rainbow trout culture for enhancing the resistance of fish. More studies are needed to elucidate the bioavailability of apricot kernel oil and its role in disease resistance and the immune response in fish.

### Acknowledgment

This work was presented as a poster presentation at the International Symposium on Fisheries and Aquatic Science (FABA), 03-05.11. 2016 in Antalya-Turkey.

### Ethical Approval

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

### References

- Al-Khalifa AS. 1996. Physicochemical characteristics, fatty acids composition and lipoxigenase activity of crude pumpkin and melon seed oils. *J. Agric. Food Chem.* 44: 964–966.
- Awad E, Austin D, Lyndon AR. 2013. Effect of black cumin seed oil (*Nigella sativa*) and nettle extract (Quercetin) on enhancement of immunity in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture*. 33: 193-197.
- Cook ME, Jerome DL, Crenshaw TJD, Buege DR, Pariza MW, Albright KJ, Schmidt SP, Scimeca JA, Lofgren PA, Hentges EJ. 1999. Feeding conjugated linoleic acid reduces carcass fat in pigs. *J. Lipids* (submitted).
- Cook MT, Hayball PJ, Birdseye L, Bagley C, Nowak BF, Hayball JD. 2003. Isolation and partial characterization of a pentraxin-like protein with complement-fixing activity from snapper (*Pagrus auratus*, Sparidae) serum. *Develop. and Compar. Immun.* 27: 579–588.
- Davies J, Davies D. 2010. Origins and Evolution of Antibiotic Resistance. *Microbiology and Molecular Biology Reviews*, 74(3): 417–433.
- El-Houseiny W, El-Murr1 A, El-Sayed BM. 2017. Evaluation of Dietary Inclusion of Mango Kernel Meal and Oat Extract on Performance and Immunity of *Oreochromis niloticus*. *Zagazig Veterinary Journal*. 45(S1): 118-125.
- Garyson TH, Williams RJ, Wrathmell AB, Munn CB, Harris JE. 1987. Effect of immunopotentiating agents on the immune response of rainbow trout, *Salmo gairdneri* Richardson, to EMR vaccine. *J. Fish Biol.* 31: 195-202.
- Hardie LJ, Ellis AE, Secombes CJ. 1996. In vitro activation of rainbow trout macrophages stimulates inhibition of *Renibacterium salmoninarum* growth concomitant with augmented generation of respiratory burst products. *Dis. Aquat. Org.* 25: 175-183.
- Ispir U, Gokhan HB, Ozcan M, Dorucu M, Saglam N. 2009. Immune response of rainbow trout (*Oncorhynchus mykiss*) to selected antigens of *Yersinia ruckeri*. *Acta Veterinaria Brn.* 78: 145-150.
- Jeffery NM, Cortina M, Newsholme EA, Calder PC. 1997. Effects of variations in the proportions of saturated, monounsaturated and polyunsaturated fatty acids in the rat diet on spleen lymphocyte functions. *British Journal of Nutrition*, 77: 805-823.
- Karabulut AB, Önal Y, Gül M, Otlu O, Tuzcu M, Gül S, Şahin K. 2014. Nutri-Protection and Mediterranean Diet: Bitter Apricot Kernel and Amygdalin Treatment Effects on a Battery of Oxidative Stress and Apoptosis Biomarkers. *Journal of Plant Physiology & Pathology* 2(3): 1-6.
- Kremer JM, Lawrence DA, Jubiz W, DiGiacomo R, Rynes R, Bartholomew LE, Sherman M. 1990. Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis: Clinical and immunologic effects. *Arthritis and Rheumatism*. 33(6): 810-820.
- Kucukgul Gulec A, Danabas D, Ural M, Seker E, Arslan A, Serdar O. 2013. Effect of mixed use of thyme and fennel oils on biochemical properties and electrolytes in rainbow trout as a response to *Yersinia ruckeri* infection. *Acta Vet. Brno*, 82: 297–302.
- Kutlu T, Durmaz G, Ateş B, Erdoğan A. 2009. Protective effect of dietary apricot kernel oil supplementation on cholesterol levels and antioxidant status of liver in hypercholesteremic rats. *Journal of Food, Agriculture & Environment* 7(3-4): 61 - 65.
- Luo L, Xue M, Vachot C, Geurden I, Kaushik S. 2014. Dietary medium chain fatty acids from coconut oil have little effects on postprandial plasma metabolite profiles in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 420–421: 24–31.
- Pongsak R, Parichat P. 2010. Potential of cinnamon (*Cinnamomum verum*) oil to control *Streptococcus iniae* infection in tilapia (*Oreochromis niloticus*); *Fisheries Science* 76(2): 287-293.
- Puertollano MA, Puertollano E, Alvarez de Cienfuegos G, de Pablo MA. 2007. Significance of olive oil in the host immune resistance to infection. *Br. J. Nutr.* 98 (Suppl. 1): S54–S58.
- Quade MJ, Roth JA. 1997. A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. *Vet. Immunol. Immunop.* 58: 239-248.
- Rao YV, Das BK, Pradhan J, Chakrabarti R. 2006. Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. *Fish Shellfish Immunol.* 20: 263-273.
- Sahoo PK, Kumari J, Mishra BK. 2005. Non-specific immune responses in juveniles of Indian major carps. *J. Appl. Ichthyol.* 21: 151-155.
- Sahu S, Das BK, Mishra BK, Pradhan J, Sarangi N. 2006. Effect of *Allium sativum* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. *J. Appl. Ichthyol.* 22: 1-6.
- Sahu S, Das BK, Pradhan J, Mohapatra BC, Mishra BK, Sarangi N. 2007. Effect of *Magnifera indica* kernel as a feed additive on immunity and resistance to *Aeromonas hydrophila* in *Labeo rohita* fingerlings. *Fish, Shellfish Immunol.* 23: 109-118.
- Sakai M. 1999. Current research status of fish immunostimulants. *Aquaculture*. 172:63-92.
- Sheikhzadeh N, Soltani M, Ebrahimzadeh-Mousavi HA, Shahbazian N, Norouzi M. 2011. Effects of *Zataria multiflora* and *Eucalyptus globulus* essential oils on haematological parameters and respiratory burst activity in *Cyprinus carpio*. *Iranian Journal of Fisheries Sciences* 10(2): 316-323.
- Sicuro B, Dapra` F, Gai F, Palmegiano GB, Schiavone R, Zilli L, Vilella S. 2010. Olive oil by-product as a natural antioxidant in gilthead sea bream (*Sparus aurata*) nutrition. *Aquaculture International*. 18: 511–522.
- Siwicki AK, Anderson DP, Rumsey GL. 1994. Dietary intake of immunostimulants by rainbow trout effects non-specific immunity and protection against furunculosis. *Vet. Immunol. Immunopathol.* 41: 125-139.
- Sugano M, Yamasaki M, Yamada K, Huang YS. 1999. Effect of conjugated linoleic acid on polyunsaturated fatty acid metabolism and immune function. In: Yurawecz MP et al. ed. *Advances in conjugated linoleic acid research*. Vol 1. Champaign, IL: AOCS Press, 327–339.
- Tian H, Yan H, Tan S, Zhan P, Mao X, Wang P, Wang Z. 2016. Apricot Kernel Oil Ameliorates Cyclophosphamide- Associated Immunosuppression in Rats. *Lipids*, 51: 931–939.

- Vardi N, Parlakpınar H, Oztürk F, Ates B, Gul M, Cetin A, Erdogan A, Otlu A. 2008. Potent protective effect of apricot and b-carotene on methotrexate-induced intestinal oxidative damage in rats. *Food and Chemical Toxicol.* 46: 3015–3022.
- Wiegertjes G, Stet RJM, Parmentier HK, Van Muiswinkel WB. 1996. Immunogenetics of disease resistance in fish: a comparable approach. *Dev. Comp. Immunol.*, 20: 365-381.
- Yılmaz S, Ergün S. 2012. Effects of garlic and ginger oils on hematological and biochemical variables of sea bass *Dicentrarchus labrax*. *Journal of Aquatic Animal Health.* 24: 219-224.
- Zhang J, Zou W, Yan Q. 2008. Non-specific immune response of bullfrog *Rana catesbeiana* to intraperitoneal injection of bacterium *Aeromonas hydrophila*. *Chinese Journal of Oceanology and Limnol.*, 26: 248–255.
- Zheng ZL, Tan JYW, Liu HY. 2009. Evaluation of oregano essential oil (*Origanum heracleoticum* L.) on growth, antioxidant effect and resistance against *Aeromonas hydrophila* in channel catfish (*Ictalurus punctatus*). *Aquaculture*, 292: 214– 218.