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Research Article

DNA Damage and Antioxidant Defence Responses in the Brain and Liver Tissues of Rainbow Trout Infected With Different Bacteria

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Abstract

Fish farming provides food and livelihood for many people around the world. The fact that trout is produced in many parts of the world and has a very rich content in terms of nutritional value increases the importance of trout. However, there are many problems in trout production due to bacterial factors, making fish production extremely difficult. Bacterial factors cause significant fish deaths and economic losses such as slowdown in growth. In addition to these, these factors also cause some damage to the DNA of fish and the formation of oxidations by disrupting the balance of free radicals in the tissues. Therefore, in this study; In the trout farms in our province, the trout with the main bacterial agents causing infection were determined and bacterial species (*Staphylococcus epidermidis, Lactococcus garvieae and Bacillius subtilis*) were determined by PCR. The brain and liver tissues of these fish were taken and the changes in antioxidant enzyme levels (SOD, CAT, GSH-Px), lipid peroxidation (MDA) and damage to their DNA (8-OHdG) compared to the control groups were investigated. In the study, it was determined that the antioxidant defense system enzyme levels in all three species of bacteria decreased in tissues, while lipid peroxidation and 8-OHdG levels increased.
As a result; *Staphylococcus epidermidis, Lactococcus garvieae and Bacillius subtilis bacteria* caused changes in antioxidant enzyme levels, lipid peroxidation and 8-OHdG levels in trout brain and liver tissues.

Keywords: DNA Damage, Antioxidants, MDA, Rainbow Trout, Bacterial Diseases.

Farklı Bakterilerle Enfekte Olan Gökkuşağı Alabalıklarının Beyin ve Karaciğer Dokularında Oluşan DNA Hasarı ve Antioksidan Değişimi

Öz

Balık üretimi, dünya çapında birçok insan için yiyecek ve geçim kaynağı sağlamaktadır. Su ürünlerinden alabalığın dünyanın birçok yerinde üretilmesi ve besin değeri bakımından oldukça zengin bir içeriğe sahip olması alabalığın önemini artırmaktadır. Ancak, alabalık üretiminde bakteriyel etkenler sebebi ile birçok sorun yaşanmakta ve balık üretimini son derece zorlaştırmaktadır. Bakteriyel etmenler önemli ölçüde balık ölümlerine ve büyümede yavaşlama gibi ekonomik kayıplar neden olmaktadır. Bunların yanında, bu etmenler balıkların DNA'sında birtakım hasarlara ve dokularda serbest radikal dengesinin bozularak oksidasyonların oluşmasına da neden olmaktadır. Bu nedenle bu çalışmada; ilimizde bulunan alabalık çiftliklerinden enfeksiyon oluşturan başlıca bakteriyel ajanların bulunduğu alabalıklar belirlenerek ve PCR ile bakteri türleri (*Staphylococcus epidermidis, Lactococcus garvieae ve Bacillius subtilis*) tespit edilmiştir. Bu balıkların beyin ve karaciğer dokuları alınarak antioksidan enzim düzeylerindeki (SOD, CAT, GSH-Px) değişimler, lipit peroksidasyonları (MDA) ve DNA'larında meydana gelen hasarın (8-OHdG)) kontrol gruplarına göre değişimleri araştırılmıştır. Çalışmada bakterilerin üç türünde de antioksidan savunma sistemi enzim seviyelerinin genel olarak dokularda azaldığı, lipit perksidasyonunun ve 8-OHdG düzeylerinin ise arttığı tespit edilmiştir.
Sonuç olarak; *Staphylococcus epidermidis, Lactococcus garvieae ve Bacillius subtilis* bakterilerinin, alabalık beyin ve karaciğer dokularında antioksidan enzim düzeylerinde, lipit peroksidasyonununda ve 8-OHdG düzeyinde değişimine sebep olduğu belirlenmiştir.

Anahtar Kelimeler: DNA Hasarı, Antioksidanlar, MDA, Gökkuşağı Alabalığı, Bakteriyel hastalıklar.

1. Introduction

Today, many deaths from viral diseases (Covid-19) are observed all over the world. It is also possible that the same situation is of bacterial origin. Although it is generally thought that bacterial fish zoonosis are mostly caused by Gram negative bacteria, some of the Gram positive bacteria have disease-causing effects in humans (Nemetz 1993; Yeltekin et al. 2018). Fish bacterial zoonosis are transmitted to humans as a result of contact of contaminated fish tissues and water with wounds on the skin or consuming more contaminated fish products as food (Auistin 1999; Öter and Zorer, 2020; Yeltekin and Sağlamer 2019). Bacterial fish zoonosis often results in localized infections under the skin or tissues in humans, mostly symptom-free gastroenteritis. However, they can sometimes cause high mortalities (Nemetz 1993).

Pollution occurring in waters, as in the whole environment, brought with it many problems related to fish farming. Because the source of most of the disease-causing organisms is water contaminated with waste water. Microorganisms need water, energy source, nitrogen source, vitamins and minerals in order to grow and reproduce. Aquatic organisms create a favourable environment for microorganisms (Arda 2000; Yeltekin et al., 2018). Some microorganisms can affect each other with metabolites that have antagonistic effects that they synthesize in the organism in which they settle and cause the death of the living creature on which they live (Güven and Zorba 2013).

In aquaculture, the presence of many fishes together and in close contact compared to free-living fish in nature, causes more diseases to occur. The fact that the environment in which the fish live provides nutritious, physical, chemical, biotic and abiotic optimal living conditions leads to the emergence of many infectious diseases (Arda et al. 2000). Although it was thought that 15-20 bacteria species were pathogenic for the fish, approximately 70 bacteria species were isolated from naturally infected fish (Austin and Austin 1999). It is reported that bacterial diseases cause large economic losses in farms where intensive fish farming is carried out (Roberts and Stepherd 1997).

As in all living things, bacterial diseases in fish affect the immune system and defence mechanisms of living metabolism. In this study; after determining the rainbow trout containing the main bacterial agents causing infection in trout farms, it was aimed to investigate how the antioxidant enzyme levels, lipid peroxidation and DNA damage of these fish varies compared to healthy fish.

2. Material and Method

2.1. Fish

The study was carried out with the decision of the Animal Experiments Local Ethics Committee of Van Yüzüncü Yıl University dated 31.01.2019 and numbered 2019/01. The rainbow trout (*Oncorhynchus mykiss*) used in the study was taken from the companies that produce offspring. For this purpose, a total of 45 fishes, 9 of which were from 5 different enterprises, were purchased. In the sampled fish, fish with symptoms such as slowdown in movements, separate swimming, darkening in colour, symptoms such as bilateral exophthalmos and acid, swimming disorder, fin rot were chosen.

2.2. Identification of bacteria

Real-Time PCR protocol used in the study is given below (Table 1). In trout sampled from farms, 10 *Lactococcus garvieae*, 9 *Bacillus subtilis* and 8 *Staphylococcus epidermidis* agents were isolated.

2.3. Analysis of antioxidant enzyme

Superoxide dismutase (SOD) enzyme activity was measured at 505 nm at 37 ° C in the autoanalyzer with the Randox - Ransod enzyme kit (Xia et al., 1995, Flohe and Ötting, 1984). Catalase (CAT) enzyme activity was determined at 240 nm by UV spectrophotometric method of Aebi (1984) based on the degradation of Hydrogen peroxide (H_2O_2) by catalase. Glutathione peroxidase (GSH-Px) enzyme activity was measured at 37 ºC with Randox-Ransel enzyme kits in the autoanalyzer by 340 nm ultraviolet method (Paglia and Valentine, 1967; Flohe and Gunzler, 1984).

2.4. Analyses of 8-OHdG levels

Homogenates prepared in 8-OHdG analysis were used. The steps of the kit procedure were applied in order for the analysis (ELISA kit, Catalog No: 201-00- 0041/SunRed).

2.5. Analyses of Malondialdehyde (MDA) levels

Homogenates for analysis Mis et al. (2018) prepared according to the modified method Malondialdehyde (MDA) level Placer et al. (1966) was measured by the method based on the spectrophotometric measurement of pink colour absorbance resulting from the thiobarbituric acid (TBA) and MDA reaction.

2.6.Statistical analysis

The values obtained as a result of the analyses are expressed as mean ± standard error. For multiple comparisons, ANOVA and Tukey's test were followed to make a difference. The difference between the values was made according to 0.05.

Table 1. Protocol used in Real-Time PCR phase

Pre-denaturation	95 ºC	10 minute
Denaturation	94 ºC	45 sec
Connecting 45 cycles	56 ºC	30 sec
Elongation	72 ºC	45 sec
Last elongation	72 ºC	7 minute

3. Results

3.1. Real-Time PCR analysis results

DNAs obtained from isolated bacterial agents were used as templates in Real-Time PCR. Real-Time PCR procedure was performed to identify the isolates. Real-Time PCR results performed with Universal (27F-1492R) primers are given below (Figure 1). In line with Real-Time PCR results, it was observed that bacterial DNAs gave positive results in SYBR Green-based fluorescence irradiation by binding with Universal primers.

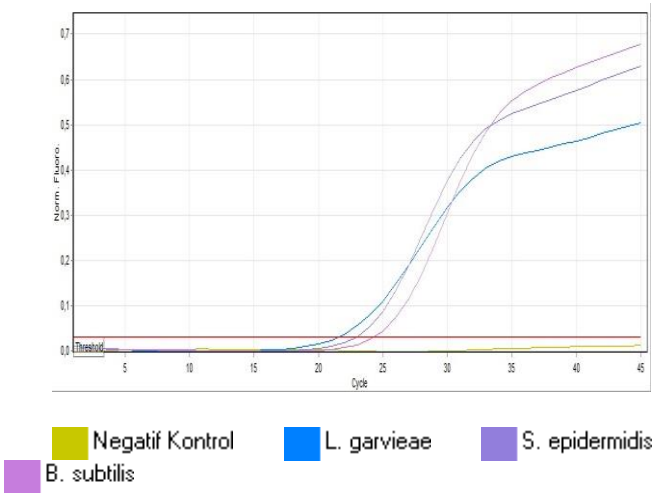

Figure 1. Real-Time PCR image performed with bacterial DNAs isolated in the study (Sigmoidal curves positive, negative control sample below the threshold value).

3.2. Bacterial identification

The nucleic acid sequences obtained after Sanger One-Way sequencing with Real-Time PCR amplicons are given below. As a result of the Blast process of the nucleic acid sequence performed on NCBI and CLC web bases, all of the isolated bacteria had 100% sequence overlap (Figure 2,3,4).

```
   1 GTTCTGCAGG TGATACGTGG CAGCTAGTCG GCTCCAATGG TTACTCCAAC GGCTTCGGGT
  61 GTTACAAACT CTCGTGGTGT GACAGGCGGT GTGTACAAGA CCCGGGAACG TATTCACCGT
 121 AGCATGCTGA TCTACGATTA CTAGCGATTC CAGCTTCATA TAGTCGAGTT GCAGACTACA
 181 ATCCGAACTG AGAACAACTT TATGGGATTT GCTTGACCTC GCGGTTTCGC TGCCCTTTGT
 241 ATTGTCCATT GTAGCACGTG TGTAGCCCAA ATCATAAGGG GCATGATGAT TTGACGTCAT
 301 CCCCACCTTC CTCCGGTTTG TCACCGGCAG TCAACTTAGA GTGCCCAACT TAATGATGGC
 361 AACTAAGCTT AAGGGTTGCG CTCGTTGCGG GACTTAACCC AACATCTCAC GACACGAGCT
 421 GACGACAACC ATGCACCACC TGTCACTCTG TCCCCCGAAG GGGAAAACTC TATCTCTAGA
 481 GGGGTCAGAG GATGTCAAGA TTTGGTAAGG TTCTTCGCGT TGCTTCGAAT TAAACCACAT
 541 GCTCCACCGC TTGTGCGGGT CCCCGTCAAT TCCTTTGAGT TTCAACCTTG CGGTCGTACT
 601 CCCCAGGCGG AGTGCTTAAT GCGTTAGCTG CAGCACTAAG GGGCGGAAAC CCCCTAACAC
 661 TTAGCACTCA TCGTTTACGG CGTGGACTAC CAGGGTATCT AATCCTGTTT GATCCCCACG
 721 CTTTCGCACA TCAGCGTCAG TTACAGACCA GAAAGTCGCC TTCGCCACTG GTGTTCCTCC
 781 ATATCTCTGC GCATTTCACC GCTACACATG GAATTCCACT TTCCTCTTCT GCACTCAAGT
 841 TTTCCAGTTT CCAATGACCC TCCACGGTTG AGCCGTGGGC TTTCACATCA GACTTAAAAA
 901 ACCGCCTACG CGCGCTTTAC GCCCAATAAT TCCGGATAAC GCTTGCCACC TACGTATTAC
 961 CGCGGCTGCT GGCACGTAGT TAGCCGTGGC TTTCTGATTA AGTACCGTCC AGACGTGCAT
1021 AGTTACTTAC ACATTTGTTC TTCCCTAATA ACAAAGTTTT ACGATCCGAG AACTTCATCA
1081 CTCAAGCGGG CGTTGCTCCG TCAAGCTTTC CCCCATTGCG GAAGATTCCT ACTGCTGCCT
1141 CCCGTAGGAA TTGGAAACGG GGGTCAGGTC CCGTGTGCCG AATCCCCCTC CCCGGTCGGC
1201 TACCCACCTT CCCCTGGTTA AGCGTTTCCT TCCAACAACA AAAAGGGGGG GGTCCCCTTA
1261 AAATTAAAGA AACCCCTTTC TTTTTAACCC GGGTGCCAAT TTTTCGTTTA CCCCGTTCCC
1321 CCAATTTCCC CCCCTATGAG GGTTTCTCCG GGTTTTACCC CCCCCCCCCC CCCCCCAAGG
1381 AAGATTTTCA AGGGTGCGTT TTTTTTTTTT TGCGGCGCCC GGGTTGGGGG GCGTGGGGTA
1441 GAAATAAATA AAAAACATAA AAAAAAAAAA AAAAAAAGTT TGTTGCGTGC GCGTCTCCGG
1501 TCCTTTTGCG GTTGCTCTGT GCGTTAAGCT GCTCCGCCGC CCTCTCCC
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Figure 2. Sequence results of *Staphylococcus epidermidis*

```
   1 TTTTTTTTTT CTTTATGGCT CGTCTCGTCG AGCTCCATAG AGGTTACCTC ACCGACTTCG
  61 GGTGTTACAA ACTCTCGTGG TGTGACGGGC GGTGTGTACA AGGCCCGGGA ACGTATTCAC
 121 CGCGGCATGC TGATCCGCGA TTACTAGCGA TTCCAGCTTC ACGCAGTCGA GTTGCAGACT
 181 GCGATCCGAA CTGAGAACAG ATTTGTGGGA TTGGCTTAAC CTCGCGGTTT CGCTGCCCTT
 241 TGTTCTGTCC ATTGTAGCAC GTGTGTAGCC CAGGTCATAA GGGGCATGAT GATTTGACGT
 301 CATCCCCACC TTCCTCCGGT TTGTCACCGG CAGTCACCTT AGAGTGCCCA ACTGAATGCT
 361 GGCAACTAAG ATCAAGGGTT GCGCTCGTTG CGGGACTTAA CCCAACATCT CACGACACGA
 421 GCTGACGACA ACCATGCACC ACCTGTCACT CTGCCCCCGA AGGGGACGTC CTATCTCTAG
 481 GATTGTCAGA GGATGTCAAG ACCTGGTAAG GTTCTTCGCG TTGCTTCGAA TTAAACCACA
 541 TGCTCCACCG CTTGTGCGGG CCCCCGTCAA TTCCTTTGAG TTTCAGTCTT GCGACCGTAC
 601 TCCCCAGGCG GAGTGCTTAA TGCGTTAGCT GCAGCACTAA GGGGCGGAAA CCCCCTAACA
 661 CTTAGCACTC ATCGTTTACG GCGTGGACTA CCAGGGTATC TAATCCTGTT CGCTCCCCAC
 721 GCTTTCGCTC CTCAGCGTCA GTTACAGACC AGAGAGTCGC CTTCGCCACT GGTGTTCCTC
 781 CACATCTCTA CGCATTTCAC CGCTACACGT GGAATTCCAC TCTCCTCTTC TGCACTCAAG
 841 TTCCCCAGTT TCCAATGACC CTCCCCGGTT GAGCCGGGGG CTTTCACATC AGACTTAAGG
 901 AACCGCCTGC GAGCCCTTTA CGCCCAATAA TTCCGGACAA CGCTTGCCAC CTACGTATTA
 961 CCGCGGCTGC TGGCACGTAG TTAGCCGTGG CTTTCTGGTT AGGTACCGTC AAGGTAGCCG
1021 CCCTATTCGA ACGGTACTTG TTCTTCCTTA CAACAAAGCT TTACTATTCG GAAAACATTC
1081 TTCATTCAGC GGCGTTGCTC GTCGAACTTT CTCCATTCGG AAAAATCCCA CTGCTGCCCC
1141 CGTAGAATCG GGGCGGGGGG GGGTCCAAGG TGGCAATCCC CCCTCCGGTG GCACCGCCCC
1201 CTTCCCTTGT TAACCGTGAA CCCCACCAAA GACCAAAAAC CCCGGGGGGC CCCTGAATTT
1261 GTGTAATAAA GGGCCCTCTT TATTGTTTAT AACCTTTGTT AAACAAAGGA AGGGAAGAAG
1321 AACCGCTTTT TCGGGGTACT ACAGTGCCGG AAAGTAAATT CTTGGGGGGC CCCCCCCCCC
1381 CCTCCCCCAG CGCATTATAA AAAGGGAAGT GTGTTTTTTC TCTTTCCGGG TGCGGGGGGA
1441 CGCGAAAAGA AGGTGAGAAA AAAAACAAAA AAAATATAAA AAAATAACAT GGCGTCTCCT
1501 GGCCGTTTGT TCCCTGTTGA TGTTTCGTGC GGCCGCGCGC TGTTTTGTTT GCTTGTGGCC
1561 GTCGCGCCGT TCTGACGGTG TTGGGCTGCG CCCTGGCCCC TGCCGCGCCG CCCCCGGCCC
1621 GCTCTCTGCG TTCGT
```

Figure 3. Sequence results of *Bacillus subtilis*

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   1 TTTTCTGAAT GTAGGATCAG TGTTTCTACG TCGCCCTCCT TGCGGTTAGG CAACCTACTT
  61 TGGGTACTCC CAACTTCCGT GGTGTGACGG GCGGTGTGTA CAAGGCCCGG GAACGTATTC
 121 ACCGCGGCGT GCTGATCCGC GATTACTAGC GATTCCGACT TCATGCAGGC GAGTTGCAGC
 181 CTGAAATCCG AACTGAGAAT GGTTTTAAGA GATTAGCGCA CCCTCGAGGG TTGGCGACTC
 241 GTTGTACCAT CCATTGTAGC ACGTGTGTAG CCCAGGTCAT AAGGGGCATG ATGATTTGAC
 301 GTCATCCCCA CCTTCCTCCG GTTTATCACC GGCAGTCTCA CTAGAGTGCC CAACTTAATG
 361 ATGGCAACTA GTAATAAGGG TTGCGCTCGT TGCGGGACTT AACACAACAT CTCACGACAC
 421 GAGCTGACGA CAACCATGCA CCACCTGTAT CCCGTGTCCC GAAGGAACTC CTTATCTCTA
 481 AGGATAGCAC GAGTATGTCA AGACCTGGTA AGGTTCTTCA CGTTACTTCG AATTAAACCA
 541 CATGCTCCAC CGCTTGTGCG GGCCCCCGTC AATTCCTTTG AGTTTCAACC TTGCGGTCGT
 601 ACTCCCCAGG CGGAGTGCTT AATGCGTTAG CTGCGCTACA GAGAACTTAT AGCTCCCTAC
 661 AGCTAGCACT CATCGTTTAC GGCGTGGACT ACCAGGGTAT CTAATCCTGT TTGCTCCCCA
 721 CGCTTTCGAG CCTCAGTGTC AGTTACAGGC CAGAGAGCCG CTTTCGCCTC CCGTGTTCCT
 781 CCATATATCT ACGCATTTCA CCGCTACACA TGGAAATTCC ACTCTCCTCT CCTGCACTCA
 841 AGTCTCCCAG TTTTCAATGC ACACAATGGT TGAGCCACTG CCTTTTTCAT CAGACTTAAG
 901 AAAACACCTG CGCTCCCTTT ACTCCCACCA AATCCGGACA ACACTTGTGA ACTAACCTAT
 961 TAACTACGGC TGCTGGCAGG GAATTAGCCG TCCCCTTCTT GTTAGAAACG ACCCCCTTAA
1021 GGAAATTTTC TTTTCCACTT AACTTACGTT TTTTCTACAA AATTTTAATT TTACATAAAC
1081 CTTATATCCC TCCCACCGGC GGCCCTTTCA GGGTGGCTGC CTCCCCTTAG TTAGCAAACT
1141 CCTCCCTACC TCTCGCAAAC TAGAACGGGA GGGGGTGTGC TTATCGCCCT GAATCACCCC
1201 TCCCCCCCCT GGAAGGGGGG GCCATTATTT GGTCTTTGGA TGTGCCATAA CAAAACCCCA
1261 CCCCAAGGGG GGGGGGGTTC TATATAGTAA TTATTTATTT TTCTTTTTAA AAAGAAAAAC
1321 CCCTCCCCCG CCTTTATTAA TAGATATTAT ATATTTTTTC CTCGCACCCC CACCGCCCCG
1381 CCCCCACCCC CTTCCGCACC CTCCCCCCCA CCCCACCCCC CCTCCCACCC ACCCGCGGTA
1441 TATGATAAGA ACAAACAAAT TGTGTGTTTT CATACCCAGT GAGTGGTGGT GTGTTTGGCG
1501 CCGCCGCCGC CGATCGCGGC CGTATGAACG AAAAGAAAAC AAACTAAAAA AATATAAAAA
1561 TTTCTGTTTT CTGTTGGGGT GGGCGGGGCG CGTCACGTGT GTGTTGGTTT TATATTTTGA
1621 TTTGGTGATT GCTTGACACT GCAACGGCAG AGTGTAATAT TATAT
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Figure 4. *Sequence results of Lactococcus garvieae*

3.3. Antioxidant Activities

The levels of antioxidant defence system enzymes (SOD, CAT, GSH-Px), which are the first defence system against free radicals, are as follows.

The change of SOD levels of control group fish with other bacterial fish groups was found significant. It was determined that the brain tissue SOD enzyme activity of bacterial fish decreased significantly compared to the control group fish (Figure 5)(p<0.05).

The change in liver tissue SOD levels between the control group fish and bacterial fish groups was found to be statistically significant. It was observed that liver tissue SOD enzyme activity decreased significantly in all three bacterial species. *Bacillus subtilis* bacteria fish were found to have a low level of liver SOD (Figure 5)(p<0.05).

Bacillus subtilis and *Lactococcus garvieae* bacteria were observed to decrease statistically significantly in brain tissue CAT enzyme levels (Figure 5) (p<0.05).

It was determined that the liver tissue CAT enzyme levels of all three bacterial fish groups decreased statistically significantly. In particular, the *Bacillius subtilis* bacterial fish group was observed to be quite low (Figure 5) (p<0.05).

It was found that the brain tissue GSH-Px level of *Staphylococcus epidermidis* and *Lactococcus garvieae* bacteria showed a statistically significant decrease compared to the control group. In the *Bacillus subtilis* bacteria group, it was not

found statistically significant despite the decrease (Figure 5) ($p < 0.05$).

The liver tissue GSH-Px enzyme levels of all three bacterial trout groups were statistically significant compared to the control group. Especially, *Bacillius subtilis* bacteria group showed a more significant decrease (Figure 5) ($p < 0.05$).

3.4. MDA and 8-OHdG results

It was observed that the level of malondialtehyde, which indicates the oxidation rate of lipids, increased in each group. However, it was found that the brain tissue malondialdehyde levels of *Staphylococcus epidermidis* and *Lactococcus garvieae* bacterial trout showed a statistically significant increase (Figure 5) ($p < 0.05$).

The liver MDA level change showed statistically significant increases in all three bacteria groups compared to the control group. Especially in *Lactococcus garvieae* bacterial group, the level of malondialdehyde increased to very high levels (Figure 5) ($p < 0.05$).

In the advanced stages of oxidation, some damage occurs in the DNA chain. 8-OHdG is the most prominent product of these damages. In our study, the brain tissue 8-OHdG level of *Staphylococcus epidermidis* and *Lactococcus garvieae* bacteria showed a statistically significant increase (Figure 5) ($p < 0.05$).

The liver tissue 8-OHdG levels of Staphylococcus epidermidis and *Lactococcus garvieae* bacterial trout groups showed a statistically significant increase compared to the control group. Although there was an increase in *Bacillius subtilis* bacterial trout group, it was not found statistically significant (Figure 5) ($p < 0.05$).

4. Discussion

Fish are constantly in contact with microorganisms due to their environment. Therefore, bacterial diseases, intensive fish farming causes great economic losses in farms (Tanrıkul et al. 1997). SOD and CAT levels of offspring Indian fish *(Cirrhinus mrigala)* infected with *Aeromonas hydrophila* have been found to be significantly reduced (Kumar et al. 2018). Tarnecki et al. (2018) it has been stated that the antioxidant levels of these fish have decreased significantly by detecting Red Drum (*Sciaenops ocellatus*) which contain *Acinetobacter, Bacillus, Corynebacterium*, and *Pseudomonas* bacteria. According to the results of the study, it is stated that bacterial infections in aquaculture negatively affect the fish production efficiency. In our study, it was seen that antioxidant enzyme activity values of all three bacterial groups show parallelism with these studies. It has been stated that many streptococcus species bacteria enter the brain and nervous system of the fish and infect them and cause the decrease of brain tissue antioxidant enzymes. Thus, it has been stated that some changes occurred in the movement of fish as a result of the slowing of brain functions over time (Yanong and Francis-Floyd 2002; Austin and Austin 1999; Eldar et al. 1999). The present study supports this situation in the changes in antioxidant enzymes in the brain tissue and MDA and 8-OHdG levels. When the activity of antioxidant enzymes in the liver is monitored, an intense decrease is observed in all three types of bacteria. However, it has been determined that the antioxidant enzymes of *Bacillus subtilis* bacterial fish are observed at very low levels and MDA and 8-OHdG levels are increased. This can be thought to be caused by toxic substances that *Bacillus subtilis* bacteria produce as a result of their reproduction in the tissue in which they settle. In a study investigating the xanthine oxidase, reactive oxygen species and nitric oxide levels of *Rhamdia quelen* infected with *Streptococcus agalactiae*, it was found that reactive oxygen species increased significantly compared to the control group (Souza et al. 2017). Silver catfish infected with *Aeromonas caviae* bacteria, which cause high mortality in fish, have decreased antioxidant levels and increased malondialdehyde level. In the study, it was found that reactive oxygen species increased significantly in liver tissues (Baldisseraa et al. 2018). In our study, in accordance with these articles, it was observed that it decreased antioxidant enzyme levels and increased MDA and 8-OHdG levels in tissues originating from bacteria. Enzymes such as superoxide dismutase, glutathione peroxidase and catalase are the first and most important steps to defend against stress (Pandey et al. 2003). In our study, it is seen that antioxidant enzyme levels respond differently according to the types of bacteria and the characteristics of the tissues (brain and liver) in which they are located. In the later stages of oxidation in the cells, some damage occurs in the DNA chain. Reactive oxygen types lead to the formation of oxidative damage products in more than 20 base types in the DNA structure (Dizdaroğlu 1998). Among the damaged bases, 8-OHdG is highly sensitive and is the most common oxidative DNA damage marker (De Martinis and Bianchi 2002). A study investigating DNA damage in fish with *Deinococcus grandis* bacteria has been conducted. It has been determined that bacteria in the fish have more DNA damage than the control group (Satoh et al. 2016). In our study, it was determined that all three bacterial species cause significant damage especially on the tissue in which they settle, and as a result, both lipid peroxidation and 8-OHdG levels increase.

5. Conclusions

According to the results of this research; the habitat of the fish, water quality, fish density, water temperature and the characteristics of spring water that feeds ponds can lead to the development of some bacterial species, causing economic losses in enterprises. These bacterial species multiply rapidly in fish tissues, creating oxidative stress. It was observed that antioxidant activity levels decreased, lipid peroxidation and 8-OHdG levels increased with the increase of free radicals as a result of stress. Consumption of these bacterial fish by humans and other creatures in the case of bacteria, it may also spread bacteria and may develop more resistant bacteria species over time. Therefore, with this study, we are of the opinion that it is important to pay attention to the importance of cleaning fish habitats, patient fish isolation, the quality of water sources and feeds in terms of providing less living loss and healthier food production for businesses.

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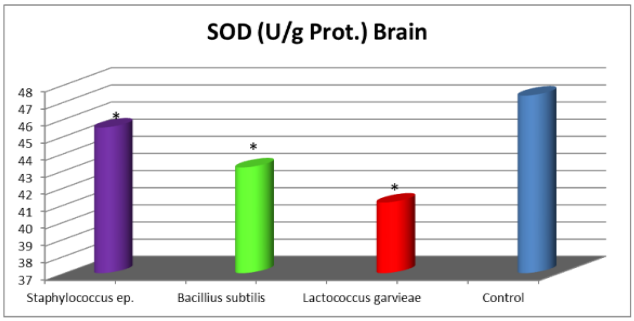

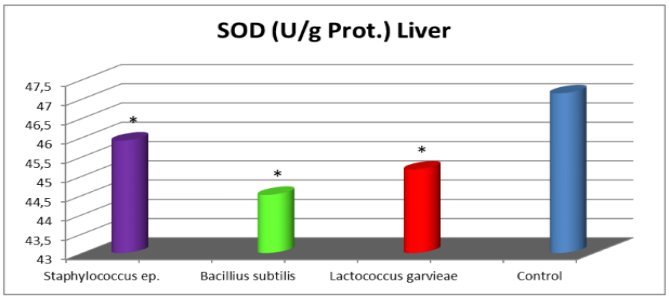

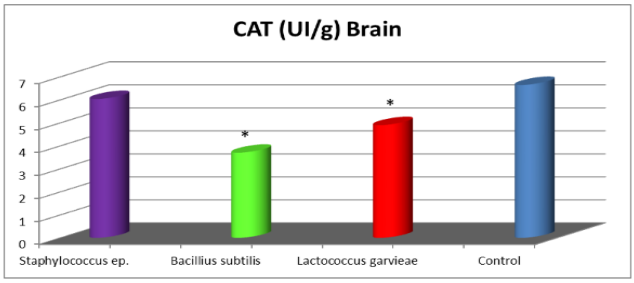

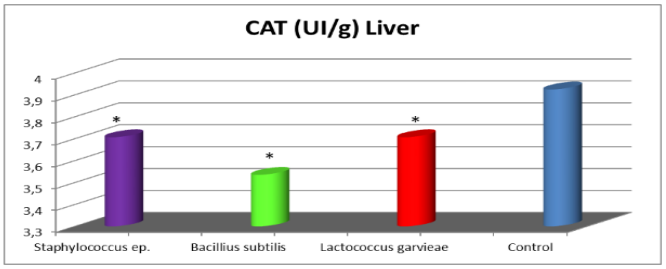

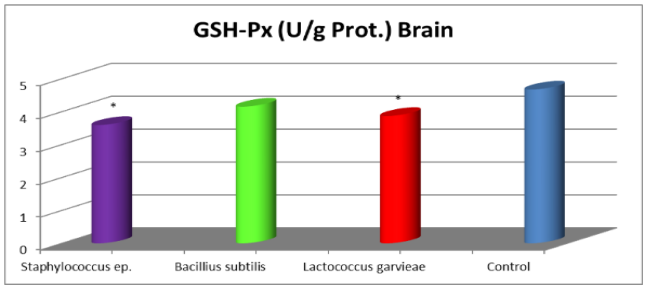

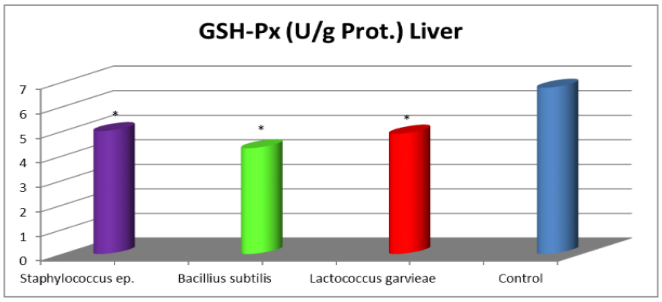

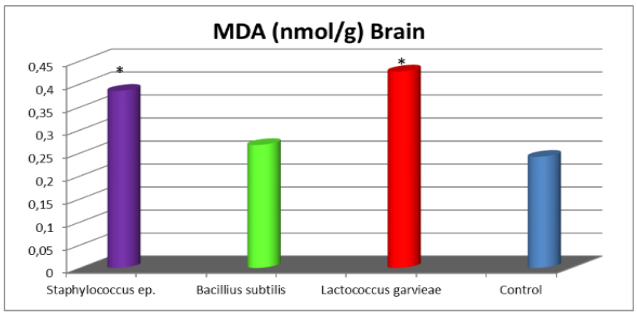

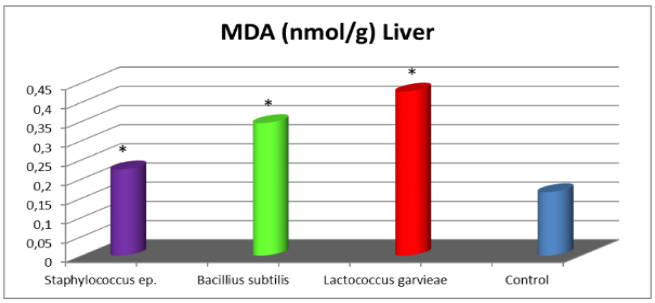

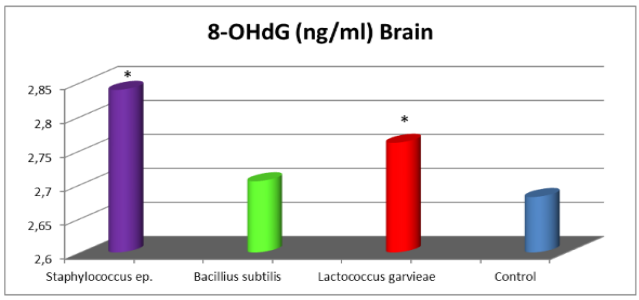

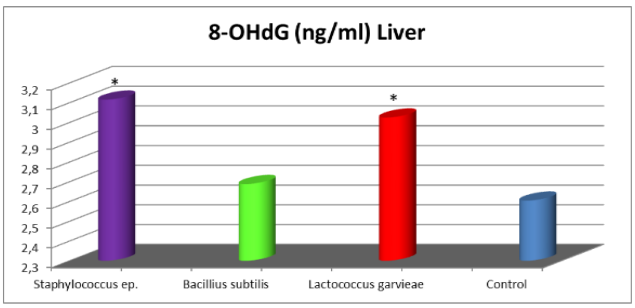

Figure 5. Change of brain and liver tissue antioxidants (SOD, GSH-Px, CAT) activity, MDA and 8-OHdG levels of rainbow trout with different bacteria