

Unfavorable Visual Effects of Lactococcosis and Listonellosis on Rainbow Trout Meat Quality

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Abstract: Rainbow trout which is one of the most produced species in Turkey is affected by many bacterial diseases that cause physiological disorders and economically important losses. Especially pathological disorders in the muscle cause heavy loss in fish processing and packaging facilities. In order to determine the cause of physiological disorders such as reddish brown patches and hemorrhages on processed or unprocessed fish products, the diseased fish were defined in ponds and tracked along the processing activity. Following bacterial isolation in every step of the study, biochemical and molecular identification of pathogens were performed and the causative pathogens were determined as *Lactococcus garvieae* and *Listonella anguillarum*. Petechial hemorrhages and ecchymosis that formed on the skin of the fish were observed caused by *Listonella anguillarum* and *Lactococcus garvieae* which were isolated from fish samples showing hemorrhagic lesions, abscess fillet with blood and deep ulcers. It was determined that *L. garvieae* cause more severe hemorrhagic septicemia than *L. anguillarum* and the disease was observed as acute and chronic cases. Hemorrhages in the muscle were observed as red patches in the fillet and after the smoking process, these patches turn into brown marks which are undesirable organoleptic properties in the processing industry and the serving size fish can not be evaluated. Besides, greenish yellow patches on the abdominal walls caused by the overproduction of bile and exploding off the gall bladder which may be related to metabolic disorders or bacterial and parasitic diseases.

Keywords: Bacterial fish disease, economic loss, visual quality of fish.

Lactococcosis ve Listonellosis'in Gökkuşluğu Alabalığı Görsel Et Kalitesi Üzerindeki Olumsuz Etkileri

Öz: Türkiye'de en fazla üretimi yapılan balık türlerinden olan gökkuşluğu alabalığı patolojik bozukluklara neden olan birçok bakteriyel hastalıktan etkilenmekte ve bu hastalıklar önemli ekonomik kayıplara neden olmaktadır. Özellikle kaslardaki patolojik bozukluklar balığın işlenmesinde kayıplara yol açmaktadır. İşlenmemiş ve işlenmiş balıklarda şekillenen kırmızı kahverengi lekeler ve hemorajiler gibi fizyolojik bozuklukların nedenlerinin belirlenmesi amacıyla havuzlarda hasta balıklar tespit edilmiş ve işleme prosesinde takip edilmiştir. Çalışma süresince her adımda bakteriyel izolasyon gerçekleştirilmiş, biyokimyasal ve moleküler yöntemler kullanılarak etken patojenler *Lactococcus garvieae* ve *Listonella anguillarum* olarak tanımlanmıştır. Balıkların derisinde şekillenen peteşiyel hemorajiler ve ekimozların *L. anguillarum*'dan kaynaklandığı gözlenmiştir. Hemorajik lezyonlar, içi kan dolu apseler ve derin ülserler görülen balık örneklerinden *L. garvieae* izole edilmiştir. *L. garvieae*'nin *L. anguillarum*'dan daha şiddetli hemorajik sepsisemiye neden olduğu ve hastalığın akut ve kronik formlarında da belirtilerin görüldüğü tespit edilmiştir. Kaslardaki hemorajiler filetoda kırmızı lekeler olarak görülmekte ve bu lekeler fümeleme işleminden sonra kahverengi lekelerle dönerek işleme endüstrisinde istenmeyen organoleptik nitelikler olarak pazar boyundaki balıkların değerlendirilememesine neden olmaktadır. Ayrıca karın duvarlarındaki yeşilimsi sarı lekelerin metabolik, bakteriyel ve paraziter hastalıklar nedeniyle fazla üretilen safranın safra kesesini patlatmasına bağlı olarak şekillendiği belirlenmiştir.

Anahtar sözcükler: Bakteriyel balık hastalıkları, balığın görsel et kalitesi, ekonomik kayıp.

INTRODUCTION

The bacterial fish disease causes economic losses not only with high mortality rates but also with some post-mortem disorders in fish processing and packaging facilities. Especially, market rate is adversely affecting by the deteriorations which are the results of bacterial diseases (Tavares-Dias & Martins, 2017). The major bacterial pathogens that cause disease in rainbow trout are *Listonella anguillarum*, *Lactococcus garvieae*, *Yersinia ruckeri*, *Aeromonas hydrophila*, *Flavobacterium columnaris*, *Flavobacterium psychrophilum* and *Pseudomonas fluorescens* (Toranzo, 2004; Balta et al., 2010; Balta et al., 2016). Among these pathogens, *Listonella anguillarum* (*L. anguillarum*) and *Lactococcus garvieae* (*L. garvieae*) are the two pathogens that have the most negative effects during the processing of fish.

L. anguillarum, the cause of Listonellosis, has been isolated from many species from salt and freshwater (Balta & Dengiz Balta, 2016). It is also a frequent pathogen in rainbow trout farms. It is a Gram-negative, polar flagellated, comma-shaped rod bacterium which shows an opportunistic character and associated with increased water temperature and clinically characterized by hemorrhage, ulcers and lesions on the ventral region of the body (Actis et al., 1999; Austin & Austin 2007). Pathologically, hemorrhage in the abdominal walls and liver, enteritis and lesions under the skin and muscle are observed (Candan, 1991). In Turkey, Listonellosis is detected frequently from cultured fish species both from salt and freshwater such as sea bass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus mykiss*) (Cagirgan, 1993). *L. anguillarum*, which caused widespread disease in marine fish during the 1990s, was first seen from a terrestrial rainbow trout farm in 2006 (Tanrikul, 2007; Balta, 2016). The reason for the adaptation of this bacterium to an inland farm seems to be using discarded sea fish as a protein source without complying the hygienic principles. After that *L. anguillarum* was spread to other fish farms with contaminated water and porter fish in the same geographical area (Tanrikul, 2007), the disease agent which has increasing pathogenicity shows transmission fish to fish and cause high mortality with severe hemorrhagic septicemia symptoms (Austin & Austin, 2007 Balta & Dengiz Balta, 2017).

Lactococcosis is an emerging disease which is caused by *L. garvieae*. In Turkey, it was firstly isolated from a rainbow trout farm as *Enterococcus* sp. (Cagirgan & Tanrikul, 1995), then Diler et al (2002) identified *L. garvieae* with same biochemical characteristics. In the following years It has been reported from Konya and surrounding (Kav & Erganis, 2007), Black Sea region (Ture & Savas, 2010; Balta & Dengiz Balta, 2019) and Mediterranean region (Ozer et al., 2008) in Turkey.

L. garvieae is a Gram (+), non-motile, facultative anaerobic bacteria that cause septicemia and meningococcalitis in many marine and freshwater fish

species (Eldar et al., 1996; Teixeira et al., 1996; Barnes et al., 2002). It is reported that high water temperature, negative environmental conditions, poor feed quality, and poor oxygen conditions cause the spread of the agent and increase pathogenicity (Fukuda et al., 1997). The typical signs of Lactococcosis are mainly hemorrhagic septicemia, darkness in the skin and with the progression of the disease hemorrhages in the periocular area, head and anal region, exophthalmia, congestion of internal organs and hemorrhagic enteritis (Vendrell et al., 2006). Hemorrhages could be determined at different levels in swim bladder, brain, liver, kidney, heart, intestine and peritoneum.

After the determination of first *L. garvieae* infection from rainbow trout in 1997 from Turkey, it was found almost all of the trout farms in the Aegean, Black Sea and Mediterranean region where the water temperature is usually seen high (Timur et al., 2011). The pathogen, *L. garvieae* is a bacterium that generally causes bovine mastitis as a mesophilic bacterium (Meyburgh et al., 2017). In this study, psychological disorders caused by these important bacterial fish diseases were investigated and the disorders during the processing steps were determined. The economic losses caused by the important quality problems in fish have great impact on industry and determination of the reasons will help to provide the improvement in quality awareness.

MATERIAL and METHODS

Experimental design: Two different methods have been applied with the same isolation and identification techniques in order to determine the cause of the patches on processed and unprocessed products (Figure 1). Firstly, lesions and patches on rainbow trout flesh were determined in every 50 samples from 500 fish (300-350 g approximately) in a rainbow trout processing factory in Mugla, Turkey. The diseases batch was tracked and 20 diseased fish were sampled from the ponds which were harvested before. Secondly, diseased fish were observed and sampled in the rainbow trout farm and the fish harvested from these ponds were tracked through the fish processing factory. The negative effects of the diseases on fillet and smoked products were determined. A healthy fish batch was also observed as a control group during the study.

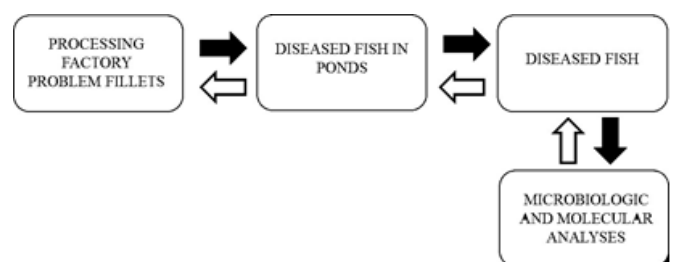


Figure 1. Flow diagram of the research.

Biochemical identification: The bacteria were isolated from spleen, liver and kidney on Tryptic Soy Agar (TSA, Oxoid) and TSA supplemented with 5% defibrinated sheep blood (BTSA, Çağdaş Ltd.). After primer isolation, the representative colonies of *L. anguillarum* were passaged onto thiosulphate citrate bile salts sucrose agar (TCBS, Oxoid) in order to purify colonies. Suspected *L. anguillarum* strains were incubated at 21°C for 48 h and suspected *L. garvieae* strains were incubated at 25°C for 48 h.

The presumptive identification of the strains was performed by motility, Gram staining, catalase and oxidase activity according to standard procedures (Arda, 2015). API 20E for *L. anguillarum* strains and API RAPID ID32 Strep tests for *L. garvieae* strains (BioMerieux S.A., France) were used to determine the biochemical characteristics of the pathogens.

Molecular identification: Molecular identification of the bacteria was conducted; EurX GeneMATRIX Tissue Bacteria DNA (Isolation Kit was used for DNA isolation. Then with Thermo Scientific Nanodrop 2000, density and quality of the isolates were determined. 16SrRNA gene sequence was amplified in order to ensure that the bacteria were *L. anguillarum* and *L. garvieae*. 27F (5' AGAGTTTGATCMTGGCTCAG 3') and 1492R (5' TACGGYTACCTTGTTACGACTT 3') primers were used for PCR amplifications. The strains were obtained from the samples that were isolated from infected rainbow trout during this study. Band screening of the PCR products was observed in the gel electrophoresis. Amplified products of template DNA were sent to the Stab Vida direct sequencing service (Spain) with ABI 3730 XL DNA Analyzer for sequence determination. Then sequences were checked with BLASTN 2.6.1 database.

RESULTS and DISCUSSION

Diseases in rainbow trout farms have constituted a major constraint to the economy and commercial profit by the time. If a bacterial agent is a matter of diseased fish, different pathological disorders are shown depending on the type of disease agent. These pathological disorders are seen as clinical symptoms when the fish are alive and then in fresh products, unsuitable fish can be separated from as a result of the sensory test. However processing of acute or latent diseased fish cause more serious and complicated problems on the product basis.

Diseased rainbow trout samples with *L. anguillarum* commonly showed deep lesions on the surface of the body and hemorrhages (Figure 2). The isolates were found to be Gram-negative comma-shaped rod. API 20E test results identified the bacteria as *L. anguillarum* (the API profile number: 3207726) and the molecular tests were resulted in 100% nucleotide identity with *L. anguillarum* (accession number NR_029103.1).



Figure 2. Rainbow trout with Listonellosis

Petechial hemorrhages and ecchymosis formed on the skin of the fish based on Listonellosis are not demanded on market because of the bloody appearance. Ecchymosis generally causes more impaired skin surface with the appearance of muscles than hemorrhagic lesions. If the infection continues, the skin lose integrity in where ecchymosis are present and cause muscles to contact directly with water (Figure 3). If there is such a lesion in a certain region of the body, fish can be alive. But if the lesions are widespread, it causes death. *L. anguillarum* causes widespread but superficial pathological disorders in rainbow trout. The pathogenic effects of Listonellosis such as petechial or widespread lesions caused by dissociation of blood from vessels to muscular tissues cause dark stain on the fillet (Figure 4). The reddish brown colored patches appeared more significantly on smoked products by the effects of heat, especially when the dissociated blood meets with heat (Figure 4).



Figure 3. A and B: Rainbow trout with Listonellosis, C and D: Fillet of fish that infected by *L. anguillarum*.



Figure 4. Smoked product from fish with Listonellosis.

Pull through the disease caused by *L. anguillarum* acutely or chronically, there isn't any problem about the tissue integrity in the muscle caused by the abscess. The ulcerated fish can be separated visually because of the hemorrhagic septicemia on the surface of the body. However, due to the tearing of the veins in the muscle tissue, spot stains are able to be observed in fillet and smoked products if the blood leaking into the tissue and not absorbed completely.

Another important problem during the processing period is fillets that contaminated with bile. When the gallbladder of fish explodes, a yellowish-green patch is shaped on the abdominal wall. This problem causes undesirable conditions in taste and visual appearance on processed fish. Some applications should be performed in order to reduce this problem. If it is caused by a metabolic disorder, the content of the feed should be changed. But if the problem is related to an infection, the harvest should be done immediately. If there is no time and opportunity to take these precautions, it is necessary to minimize the mechanical trauma during the processing period.

L. garvieae cause more severe hemorrhagic septicemia than *L. anguillarum*. Abscess filled with blood and hemorrhagic lesions are seen on the surface of rainbow trout which have Lactococcosis (Figure 5). Isolated strains were described as Gram-positive, non-motile cocci. API rapid ID 32 STREP V3.0 identification systems resulted in the pathogen as *L. garvieae* with the profile 30021000120. The pathogen resulted in 99% nucleotide identity between the current isolate and *Lactococcus garvieae* (accession number KY486014.1)

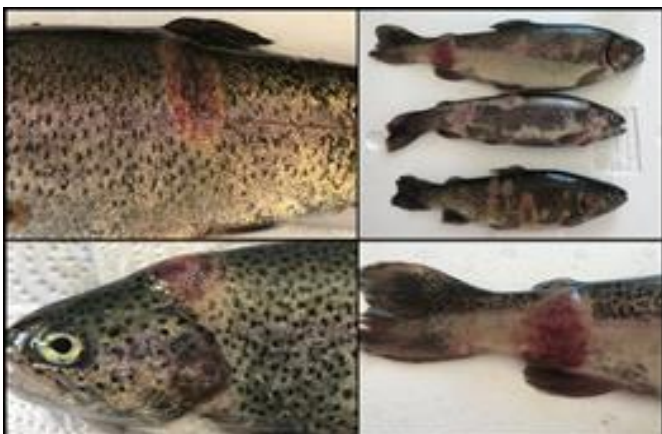


Figure 5. Rainbow trout with Lactococcosis.

The abscesses causing by *L. garvieae* drainage in time and develop deep ulcers in their places (Figure 6). Sometimes these abscesses are formed in the deeper parts of the muscle tissue without absorption and can become encysted. In this type of infected fish, even if the abscess

heals itself, the scars and cicatrized tissue cause deterioration of homogeneous appearance. In some cases, this situation forms cavities in the muscle tissue of infected fish (Figure 6). In addition, just as Listonellosis, undesirable appearance is seen on smoked and filled fish with Lactococcosis. Hemorrhages in the peritoneum and bloody fluid accumulating in the abdominal cavity cause widespread brown patches in infected fish with *L. garvieae* (Figure 7).



Figure 6. A and B: Fish with Lactococcosis, C: Fillet with Lactococcosis, D: Smoked products with Lactococcosis.



Figure 7. Reddish-brown patches on abdominal walls of rainbow trout caused by *L. garvieae*.

Lactococcosis is a bacterial disease that continues throughout the year in the terrestrial ponds and cage cultures where the water temperature does not fall below 12° C in Turkey. The disease caused by *L. garvieae* is one of the most important infectious diseases in trout culture. It causes serious economic losses and observed as acute and chronic cases. The acute form usually occurs in the fishes weighing from 30 to 350 g in spring and the mortality rise, especially in summer and autumn months. The mortality rates decrease when the water temperature is below 13° C but the disease continues as a course of disease. The treatment of the acute form is unlikely in the summer months. At the end of the treatment, the infection may recur acutely several times or turns into a chronic form. It is not possible to evaluate economically from diseased fish whose skin integrity is damaged by abscess after an acute infection. The abscess in the muscle and hemorrhages formed in the abdominal wall are not noticed until the fish fillet is made. These fish can be

separated before serving for consumption. However, the structure of the tissues between the muscles of fish which have a chronic case cannot be clear by sensory testing. The abscess filled with bloody purulent matter are replaced by scar tissue as a result of cicatrization at the time. Some abscesses are also becoming cystic. Smoked products that are made from these fish show grayish or brownish appearance of stains beside of the normal muscle tissue. In the end, the product is not economically evaluated.

The quality of fish meat is closely related to the health of fish as well as to age, gender, health status, harvesting method and pre-harvesting practices (Badonia et al., 1988). The effects of the fish disease have great importance on aquaculture industry, both production and processing facilities, in a negative way. Diseases caused by *L. garvieae* and *L. anguillarum* have been reported several times from rainbow trout farms in Turkey. Tanrikul (2007), identified *L. anguillarum* for the first time from a rainbow trout farm and reported that the infection spreads rapidly in rainbow trout farms. Similarly, *L. garvieae* was isolated from a rainbow trout farm in 2001 (Diler et al., 2002; Balta & Dengiz Balta, 2019) and since then it has been observed in almost every rainbow trout farm in Turkey.

There is another problem which is less frequently observed that greenish yellow patch on the abdominal walls caused by exploding off the gall bladder. The over-production of bile can be related to metabolic disorders or bacterial and parasitic diseases. This type of greenish yellow patch causes bitter in the taste of the areas contaminated with bile and cause problem to put on the market, especially on fillets (Figure 8).

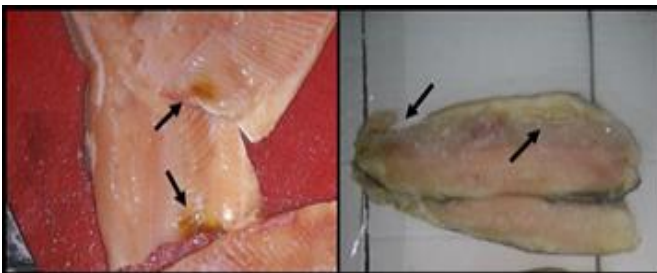


Figure 8. Fillet that contaminated with bile.

The problems mentioned above were not encountered in the control groups. No significant effects on raw material quality were detected during the study (Figure 9).



Figure 9. Control group samples.

In conclusion, bacterial fish diseases such as Listonellosis and Lactococcosis cause great losses, especially in the serving size fish because of these reasons during production and processing steps. It is important to treat the disease immediately with proper methods in order to prevent the chronic forms and continuous mortality. Also, hygienic precautions are necessary to prevent these bacterial pathogens. Besides, the diseased fish should be subjected to visual tests before processing, even if they were treated and it is necessary to evaluate the non-utilizable fish to avoid economic losses.

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