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Investigations of *Serratia liquefaciens* Infection in Rainbow Trout (*Oncorhynchus mykiss* Walbaum)

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Abstract: This study was conducted to investigate *Serratia liquefaciens* infection in juvenile cultured rainbow trout (*Oncorhynchus mykiss* Walbaum) during June and July in 1996 about 20 days after flooding in three farms in Erzurum. Bacterial isolates were identified from each farm and tested to determine sensitivity against 18 chemotherapeutants. In addition to minimum bactericidal concentrations (MBC) of chloramine-T and potassium permanganate ($KMnO_4$) were exposures of 17.07-20 mg/l for 1 h and 23.27 mg/l for 10 min. Infections caused fallen scales, bloody and swollen kidney, hyperaemic and pale regions in the liver, haemorrhagic spots in the gills and bloody exudate in the intestine. Histopathological examination demonstrated pathological changes in the liver, spleen and kidney. Naturally infected fish were examined for chemical parameters of blood and compared with healthy fish. Amylase and glutamate oxalacetate transaminase (GOT) enzymes, triglyceride (TG), cholesterol (CHOL), albumin (ALB), albumin/globulin (A/G), iron (Fe) levels, total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC) and saturation (ST) in the blood serum of infected fish were significantly increased. Glutamate pyruvate transaminase (GPT) and L-lactate dehydrogenase (LDH) enzymes, bilirubin (BIL), glucose (GLC), creatinine (CRE) and total protein (TP) values were not significantly different between the naturally infected fish and the healthy fish. In the therapeutic applications, oral applications of potentiated sulphonamide after disinfections with chloramine-T controlled the infections.

Key Words: Rainbow trout, disease, *Serratia liquefaciens*, blood, histopathology

Gökkuşağı Alabalığında (*Oncorhynchus mykiss* Walbaum) Görülen *Serratia liquefaciens* Enfeksiyonunun Araştırılması

Özet: Erzurum çevresinde yetiştiricilik yapılan üç işletmedeki gökkuşağı alabalıklarında (*Oncorhynchus mykiss* Walbaum) 1996 yılının Haziran- Temmuz aylarında ve sel baskınlarından 20 gün kadar sonra ortaya çıkan *Serratia liquefaciens* enfeksiyonu araştırıldı. Her işletmedeki balıklardan izole edilen bakteri izolatlarının 18 kemoterapotiğe karşı hassasiyeti test edildi. Kloramin-T'in izolatlara karşı minimum bakterisidal konsantrasyonu (MBC); bir saatlik bir muamele sonunda 17,07- 20 mg/l arasında, potasyum per manganat'ın ($KMnO_4$) MBC'u ise 10 dakikalık muamele süresinde 23,27 mg/l olarak bulundu. Enfekte balıklarda klinik olarak; pul dökülmesi, böbrekte şisme ve kanama, karaciğerde hiperemik ve solgun bölgeler, solungaçlarda nokta kanamalar ve bağırsakta kanlı eksudat gibi belirtiler gözlendi. Karaciğer, dalak ve böbrekte histopatolojik bulgular elde edildi. Hasta balıklarla sağlıklı balıkların kanının kimyasal özellikleri, karşılaştırmalı olarak incelendi; serumda amilaz ve glutamat oxalasetat transaminaz (GOT) enzimleri, trigliserit (TG), kolesterol (CHOL), albumin (ALB), albumin/globulin (A/G), demir (Fe) seviyeleri ile, kanın toplam demir bağlama kapasitesi (TIBC), doymamış demir bağlama kapasitesi (UIBC) ve saturasyonunun (ST) enfekte balıklarda önemli derecede yükseldiği görüldü. Glutamat piruvat transaminaz (GPT) ve L- laktat dehidrojenaz (LDH) enzimleri ile, bilirubin (BIL), glukoz (GLC), kreatinin (CRE) ve toplam protein (TP) seviyelerinde ise önemsiz değişmeler gözlendi. Kloromin- T banyosu ile birlikte oral olarak potansiyel sulfonamid uygulaması hastalığın tedavisinde etkili oldu.

Anahtar Sözcükler: Gökkuşağı alabalığı, hastalık, *Serratia liquefaciens*, kan, histopatoloji

Introduction

The control and prevention aspects of fish diseases pose serious problems due to the living environment of fish. Infected terrestrial animals could possibly be isolated from a contamined area while this is not possible for aquatic animals.

Fish diseases have been gaining a great deal of importance with the rapid expansions in the aquaculture industry. Until recently, only 15-20 bacteria species were known to be pathogenic for fish, whereas today approximately 70 species of bacteria have been identified from naturally infected fish (1).

Species of *Serratia* genus exist in normal microbial flora of soil, water (1, 2), and the organs and intestines of fish (3). One of the bacteria of this genus, *Serratia liquefaciens*, is considered a pathogenic bacterium of fish, and it causes infection leading to heavy mortalities in Atlantic salmon populations (4). *Serratia liquefaciens* infections must be taken into consideration in the aquaculture industry due to the fact that the disease can cause economic losses however low the number of infection cases is (4).

The objectives of this study were as follows: 1) to investigate the chemical parameters of blood and the histopathological effects of natural *Serratia liquefaciens* infection in rainbow trout (*Oncorhynchus mykiss* Walbaum) raised in farms and 2) to develop adequate quarantine and treatment techniques for this disease.

Materials and Methods

Farms: In the study and rearing facility in three farms in Erzurum in Turkey, rainbow trout are produced intensively. Infection was observed during June and July in 1996 in juvenile (1.5-year-old) rainbow trout (*Oncorhynchus mykiss* Walbaum) about 20 days after flooding. The mean individual weight of naturally infected fish was 152±54 g.

The behaviour of diseased fish, the mortalities and the results of examinations were recorded daily. Infected fish samples were described using their gross external clinical signs and internal gross pathology, and some organs were examined bacteriologically (90 fish) and histopathologically (30 fish). In addition, chemical examinations of the blood were conducted to compare the blood parameters of 10 healthy fish and 10 naturally infected fish.

Microbiological investigation: Microbiological investigations were carried out on naturally infected rainbow trout, during infection cases at each farm. The infected fish were killed by anaesthesia and the gross clinical signs were recorded during necropsy. For bacterial isolation, inocula were aseptically obtained from each homogenate of the gills, kidney, liver, spleen and muscle of naturally infected fish and passaged on tryptone soya (TS) agar (Oxoid, UK), deoxycholate hydrogen sulphide lactose (DHL) agar, Sakazaki (Merck, Germany), GSP (*Aeromonas* and *Pseudomonas* selective) agar (Merck), blood agar (Merck), *Cytophaga* agar (Merck), MacConkey agar (Merck), thiosulphate citrate bile sucrose (TCBS) agar (Merck) and Baird-Parker agar. After incubation at 22ºC for 48 h, individual colonies were enriched in MacConkey broth (Merck) and tryptone soya (TS) broth (Merck) at 22ºC for 24 h, and re-streaked on MacConkey agar. Individual colonies were used in the identification tests (1, 5, 6). After general biochemical identification tests, identification of the bacteria was reconfirmed by the API 20 E test system (7). The API strips were inoculated with the bacteria and incubated at 35ºC for 24 h and analyzed with APILAB software.

***In vitro* efficacy of some disinfectants and antibiotics:** After completion of the identification procedure, pure-culture bacteria was centrifuged, and standardized with a spectrophotometer to a 30% transmittance (525nm) with sterile phosphate buffer. These aliquots (0.5 ml) of isolate ($9x10^8$ viable cell/ml content) were used to test the sensitivity of bacteria to antimicrobial compounds. Sensitivities of isolated bacteria to chemotherapeutants were tested by the agar disc diffusion method (8) in Antibiotic Medium (Merck). In addition, *in vitro* assays were conducted to determine minimum bactericidal concentrations (MBCs) of chloramine-T and potassium permanganate ($KMnO_4$) to three isolates of *Serratia liquefaciens*, and aliquots were used to inoculate serial dilutions in arithmetic progression from 0.4 to 325 µg/ml of chloramine-T and $KMnO_4$ in 5 ml of phosphate buffers. Tubes containing only phosphate buffer or TS broth were inoculated to ensure culture viability. After 1 h for chloramine-T and 10 min for $KMnO_4$ postinoculation, in order to prevent contact between the organism and disinfectants, each dilution was washed with sterile physiological saline, and centrifuged. Each dilution was cultured in MacConkey broth, and incubated for 7 days at 22ºC and visually examined for survival and growth.

Chemotherapy: In order to effect the recovery of infected fish in two farms a, 50 mg/kg fish/day dosage of potentiated sulphonamide (sulphamethoxazole / trimethoprim) was given orally for 5 days after a single

application of chloramine-T (20 mg/l of water, as a bath for 1 h). One farmer did not apply any treatment even though we had recommended it.

Histopathological examination: Tissues of naturally infected fish were excised and placed in Bouin's fixative and processed for light microscopy by routine methods (9). Then they were embedded in paraffin wax and 5 µm sections were cut and the histological sections were prepared and stained with haemotoxylin-eosin (H&E), eosin and Brown & Brenn Gram stains.

Sampling and analytical procedure of blood: After measuring the body weight, 3-4 ml blood was drawn from the caudal vein by puncture and immediately transferred into silicone-coated vacutainer tubes. Blood was centrifuged promptly at 5000 rpm for 10 min. Serum was removed with a disposable transfer pipette. Amylase, glutamate pyruvate transaminase (GPT), L-lactate dehydrogenase (LDH) and glutamate oxalacetate transaminase (GOT) enzymes, triglyceride (TG), cholesterol (CHOL), albumin (ALB), albumin/globulin (A/G), bilirubin (BIL), glucose (GLC), creatinine (CRE), total protein (TP), iron (Fe) levels, total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC) and saturation (ST) were determined by enzymatic methods with a Hitachi-717 auto-analyze after 1 h at 25ºC.

Statistical analysis: The data obtained from blood analysis was subjected to the Mann-Whitney test for two independent samples using the Minitab-User Guide program (10).

Results

Infection cases and identification of the bacteria: All of the 90 bacterial isolates were identified as *Serratia liquefaciens* (Table 1). This result was reconfirmed with the API 20 E rapid identification tests. Infection cases were obtained about 20 days after flooding.

Sensitivity of the bacteria to antimicrobial compounds: The results of antibiogram tests for the isolates from each farm are shown in Table 2. The National Committee for Clinical Laboratory Standards (11) was used as a reference in the evaluation of antibiogram tests.

In addition, minimum bactericidal concentrations (MBCs) were 17.07, 18 and 20 mg chloramine-T/l for a 60-minute exposure, respectively. The MBC of potassium

Table 1. Biological and biochemical characteristics of bacteria isolated from diseased rainbow trout in Turkey.

Characteristic	Response		
	Isolate 1	Isolate 2	Isolate 3
Gram stain	-	-	-
Motility (Room temperature)	+	+	+
Oxidase	-	-	+ w
Catalase	+	+	+
Lipase	+	+	+
Esterase	+	+	+
Arginine dihydrolase	-	-	-
Lysine decarboxylase	+	+	+
Ornithine decarboxylase	+	+	+
DNA'ase	+	+	+
Tryptophan deaminase	-	-	-
Methyl-Red	-	-	-
Voges-Proskauer	-	-	-
Simmon's citrate	+	+	+
Urease	-	-	-
H_2S production	-	-	-
Growth at KCN	+	+	+
Indole	-	-	-
Nitrate reduction	-	-	-
Esculin hydrolysis	+	+	+
Starch hydrolysis	-	-	-
Gelatin hydrolysis	+	+	+
Lecithin hydrolysis	+	+	+
B-Galactosidase	+	+	+
Phenylalanine deaminase	-	-	-
Casein hydrolysis	+	+	+
Sodium citrate	+	+	+
Haemolysis (human blood)	+	+	+
Production of acid from			
Glucose	+	+	+
Mannitol	+	+	+
Lactose	-	-	-
Fructose	+	+	+
Inositol	+	+	+
Sorbitol	+	+	+
Rhamnose	-	-	-
Sucrose	+	+	+
Melibiose	+	+	+
Amygdaline	+	+	+
Dulcitol	-	-	-
Maltose	+	+	+
Raffinose	+	+	+
Arabinose	+	+	+
Salicin	+	+	+
Xylose	+	+	+
Trehalose	+	+	+
Cellobiose	+	+	+
Galactose	+	+	+
Glycerole	+	+	+
Adonitol	-	-	-
Gas production from glucose	+	+	+
O/F	F	F	F

w= weak

Table 2. Results of susceptibility test of *Serratia liquefaciens* isolates to chemotherapeutants.

Antibiotic (disc=mg)	Sensitivity			Reference[1] (zone diameter=mm)		
	Isolate 1	Isolate 2	Isolate 3	R	MS	S
Norfloxacin (10)	S	MS	S	≤12	13-16	≥17
Sulp./Trim. (23.75/1.25)	S	S	S	≤10	11-15	≥16
Ofloxacin (5)	S	S	S	≤12	13-15	≥16
Tetracycline (30)	S	R	R	≤14	15-18	≥19
Netilmycin (30)	S	S	S	≤12	13-14	≥15
Kanamycin (30)	MS	R	R	≤13	14-17	≥18
Vancomycin (30)	R	R	R	≤9	10-11	≥12
Cefaclor (30)	R	R	R	≤14	15-17	≥18
Chloramphenicol (30)	S	R	R	≤12	13-17	≥18
Gentamicin (10)	S	S	S	≤12	13-14	≥15
Rifampycin (5)	R	R	R	≤16	17-19	≥20
Oxytetracycline (30)	MS	MS	MS	≤14	15-18	≥19
Imipenem (10)	R	R	R	≤13	14-15	≥16
Cephoperazone (75)	R	R	R	≤15	16-20	≥21
Cefixime (5)	NA	R	R	≤15	16-18	≥19
Ampicillin (10)	NA	R	R	≤13	14-16	≥17
Mezlocillin (75)	NA	R	R	≤17	18-20	≥21
Clarithromycin (15)	NA	MS	MS	≤13	14-17	≥18
Amp./Sulb. (10/10)	R	NA	NA	≤11	12-14	≥15
Penicillin G (10 unit.)	R	NA	NA	≤11	12-21	≥22

[1] = NCCLS (1992) R = resistant S = sensitive MS = moderate sensitive NA = not applied
Sulp./Trim.= Sulphamethoxazole/Trimethoprim Amp./Sulb.= Ampicillin/Sulbactam

permanganate ($KMnO_4$) to three bacterial isolates was 23.27 mg/ml.

Medical treatments and effects of infections: About 50-150 fish were dying per day when the infection initially appeared in farms. Mortalities of the fish treated with chloramine-T and potentiated sulphonamide remarkably slowed, and then stopped in farms. Mortality rates of fish treated in farms were observed to be 8.4% and 12.3%. However, the mortality rate was about 42% in the farm where the fish were not treated.

Clinical observations: Infected fish had behavioural abnormalities such as slow movements and anorexia. Fallen scales, bloody and swollen kidney, hyperaemic and pale regions in the liver, haemorrhagic spots in the gills and bloody exudate in the intestine were observed.

Histopathological observations: There were no apparent histopathological changes in the skin, muscle, gill or air-bladder in the cross-section of these tissues. Mononuclear inflammatory cell infiltration, necrotic hepatocytes, and sinusoidal and intravascular congestion were present in the liver tissue (Figure 1). Intravasculalar congestion and bacterial colonies were observed in the spleen. There were tubular necrosis, interstitial lymphocyte infiltration, haemorrhage (Figure 2) and bacterial colonies in the kidney.

Blood analysis

Blood analysis was conducted on 10 fish from both the healthy (non-infected) group and the naturally infected group and the results for the two groups were statistically compared. The purpose of this test was to determine the changes in blood components in response to natural infection and to investigate the possibility of diagnosing the disease using blood criteria, as summarized in Table 3.

Serum enzyme levels: There were statistically significant increases ($p<0.05$) in serum levels of amylase and GOT in fish naturally infected with *Serratia liquefaciens* compared with healthy fish (Table 3). No significant variations were detected ($p>0.05$) between the treatments for activities of two serum enzymes and

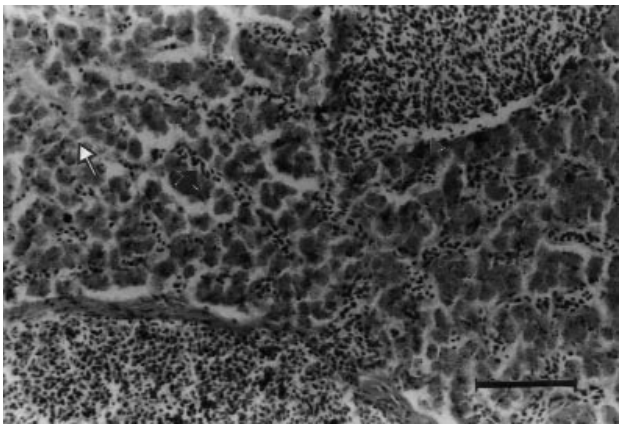


Figure 1. Necrotic hepatocytes (white arrow), sinusoidal (thick arrow) and intravascular congestion (thin arrow) in the liver of rainbow trout with natural infection of *S. liquefaciens* (H&E x200). Bar = 80 µm.

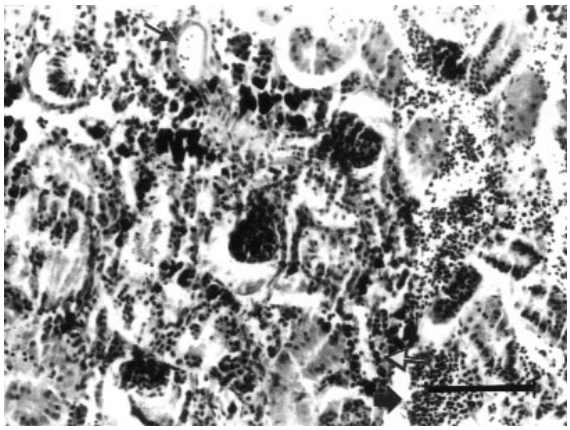


Figure 2. Tubular necrosis (thin arrow), haemorrhage (white arrow) and interstitial mononuclear inflammatory cell infiltration (thick arrow) in kidney tissue of rainbow trout naturally infected with *S. liquefaciens* (H&E x200). Bar = 80 µm.

Table 3. Mann-Whitney test for two independent samples results on blood biochemical parameters findings of naturally infected and healthy juvenile fish groups (minimum and maximum values in parentheses).

Test	Naturally Infected Fish[1] (n=10)	Healthy Fish (n=10)
Amylase (iu/l)	4403 (3327-5326)*	2352 (1046-3910)
GOT (u/l)	1665 (1094-1752)*	822 (443-1237)
GPT (u/l)	272 (214-313)	192 (84-310)
LDH (u/l)	927 (910-972)	1152 (735-1434)
TG (mg/dl)	874 (490-924)*	346 (327-385)
CHOL (mg/dl)	365 (357-380)*	300 (114-359)
GLC (mg/dl)	32 (13-101)	78 (74-93)
CRE (mg/dl)	0.70 (0.5-1)	0.50 (0.3-0.8)
TP (g/dl)	3.20 (2.9-4.6)	3.90 (3.4-4.1)
ALB (g/dl)	2.50 (2.2-2.6)*	1.80 (1.6-2.3)
A/G	3.80 (3.4-4.1)*	0.90 (0.6-1.1)
BIL (mg/dl)	0.40 (0.2-0.7)	0.40 (0.2-0.8)
Fe (mg/dl)	581 (503-624)*	46 (41-173)
TIBC (mg/dl)	1000 (872-1200)*	317 (84-502)
UIBC (mg/dl)	423 (402-438)*	271 (81-302)
ST	58 (51-63)*	15 (4-24)

*p<0.05, [1]= the fish showing clinical signs

there was an increase of GPT and a decrease of LDH in fish with infection (Table 3).

Glucose values: GLC levels of the infected fish group were lower than those of healthy fish but no statistically significant differences were observed (p>0.05) between the treatments (Table 3).

Triglyceride values: The serum TG level of fish naturally infected with *S. liquefaciens* was significantly higher (p<0.05) than that of healthy fish (Table 3).

Cholesterol levels: The CHOL levels were also significantly higher (p<0.05) in infected fish compared with the other treatment (Table 3).

Creatinine values: The CRE level of the infected group was higher than that of healthy fish (Table 3), although none of them were statistically significantly different from each other (p>0.05).

Total protein levels: There was a decrease in the mean serum level of TP in fish with *S. liquefaciens* infection but no statistically significant difference was observed (p>0.05) between the treatments (Table 3).

Albumin values: The mean ALB level was significantly higher (p<0.05) in infected fish compared with the healthy fish (Table 3).

Albumin/Globulin rates: The mean serum A/G rate of naturally infected fish with *S. liquefaciens* was significantly higher (p<0.05) than that of healthy fish (Table 3).

Bilirubin: As seen in Table 3, there was no difference between two treatments in terms of mean serum BIL levels.

Iron levels: The Fe level of serum had also dramatically increased (p<0.05) in infected fish compared with healthy fish (Table 3).

Total iron binding capacity and unsaturated iron binding capacity: Both the TIBC and UIBC of blood of the diseased fish group were significantly higher ($p<0.05$) than those of the healthy fish group (Table 3). The increase in these parameters may come from the iron increase and haemolytic condition under the stress caused by infection.

Saturation: The mean of ST was significantly higher ($p<0.05$) in blood of infected fish compared with the healthy fish (Table 3).

Discussion

The identification test results of bacterial isolates were in agreement with previous information on *Serratia liquefaciens* (1-4). This bacterium is a part of the normal microbial flora of water (1-3), and is a potential disease agent for fish (1,4). During the present study, this bacterium caused natural epizootic outbreaks in juvenile rainbow trout. The form of *Serratia liquefaciens* infection in our study was almost identical with previous reports. McIntosh and Austin (4), and Austin and Austin (1) reported that outbreaks caused heavy mortalities in Atlantic salmon populations. Infection appeared about 20 days after flooding possibly due to the reduced resistance of the fish and the increases in microbial activity after physical water pollution and incubation of micro-organisms in 20 days.

Antimicrobial sensitivity of isolates was not significantly different among the farms. In agreement with McIntosh and Austin (4), isolates of *S. liquefaciens* were sensitive to oxytetracycline, but this antibiotic was not recommended for the treatment of fish in farms. Bacterial isolates were resistant to tetracycline (two isolates), penicillin and amphicillin, and sensitive to chloramphenicol (two isolates), as McIntosh and Austin (4) reported. According to the results of our study, the quinolone group (norfloxacin, ofloxacin), gentamycine, netilmycine and potentiated sulphonamide (=sulphamethoxazole / trimethoprim) could be recommended to treat fish infected with *S. liquefaciens*. Potentiated sulphonamide was used for the medical treatment due to its availability and low cost. Oxolinic acid was not used for control of *S. liquefaciens* infections during our research, in contrast to previous reports (4). For treatment, chloramine-T was recommended to farmers, but, according to Austin and Austin (1), the MBC of $KMnO_4$ was higher than could be tolerated by rainbow trout.

The difference in mortality rates between treated and non-treated fish in farms showed that treatments could be effective. The mortality rate of the non-treated group was higher than 30 % reported in Atlantic salmon populations infected with *S. liquefaciens* (4).

Clinical signs were also similar to previous findings (4). Histopathological changes would be expected in some organs (liver, spleen, kidney) when the gross clinical and pathological signs of this of disease occur (1, 12). Bacterial colonies observed in tissues would be proof of the presence of bacterial infection.

The increases in serum levels of amylase and GOT in fish naturally infected with *S. liquefaciens* may be a result of the infection. However, previous researchers have not reported the effect on the activity of these enzymes of *S. liquefaciens* infection in fish (4). The enzyme-abundant tissues contribute to the aspect of the circulating enzyme pattern in the serum. When damage occurs in enzyme-abundant tissue, some enzymes leak from injured cells and the activities of serum enzymes will change. In this study, the increase in serum GOT activity may be an indication of considerable clinical damage and histopathological changes caused by the infection in the liver. This is because GOT, LDH and GPT activities in fish serum are known to be very useful as an index for diagnosis of liver function (13-17). The increase in amylase activity may be as a result of the infection due to an abnormality in digestive tract activity. In our study, the amylase level of healthy rainbow trout was higher than that of *Salmo trutta* reported by Studnicka and Siwicki (18). Levels of serum enzymes of somewhat higher value may have come from the effects of geographical difference (19), species of fish (20), environmental conditions (21,22), and nutritional (17,23) as well as genetic factors.

The lack of significant differences observed between GLC levels of treatments may partially be due to the relatively large statistical variation determined in the diseased group (24). The variation in this group and low GLC may come from hypoglycaemia due to the decreased hepatic LDH activity and the increased GOT, GPT activity under stress given by the infection. In addition, the decrease in blood GLC may account for the degeneration of muscular tissue, although histopathological changes have not been observed in muscle tissue of diseased fish.

Blood GLC level in fish is known to be very useful as a criterion for diagnosis of liver and muscle tissue functions (25). These observations show no disagreement with the literature values (26-31).

S. liquefaciens infection could cause the increase in serum TG values of rainbow trout. The TG levels in healthy fish seem to be within the upper normal limits (32). A wide range of variation in the blood TG values of fish was commonly observed (33).

The significant difference between CHOL levels of naturally infected and healthy fish groups could be correlated with the effects of the disease because both infected and healthy fish groups were grown in identical conditions even if the values of the infected fish were within normal limits (100-500 mg/dl). Although the CHOL value may increase in July (34), it can also dramatically increase in infections as was the case in our study. Researchers have stated that the blood CHOL of healthy rainbow trout can also show considerable variation (32-34). In the present study, a correlation was observed between changes in serum TG and CHOL levels. It is known that CHOL, TG and lipoproteins values are connected with the metabolism of lipids and functions of the liver and kidney in mammals (35), but further studies are needed to explain these characteristics in fish.

The mean serum TP value of healthy fish was within the normal limits given for rainbow trout (26,32,34,36, 37). The increase of ALB level in infected fish may be as a result of the infection. The mean serum level of ALB in the healthy fish group was within the normal limits indicated in rainbow trout (32). The increase in A/G rate showed that globulin could decrease, and infection could inhibit immune responses.

The BIL values reported in rainbow trout (38) show no disagreement with the serum BIL levels of healthy rainbow trout in the present study.

Although infection may cause a haemolytic condition, further research is needed to clarify the relationship between infection, haematological parameters and serum Fe levels. The increases in TIBC and UIBC may also come from the Fe increase and haemolytic condition under the stress caused by infection.

To our knowledge, no information is currently available regarding the effect of bacterial infections on CRE value, UIBC, TIBC and ST of blood. Further studies are needed to explain the biochemical characteristics of fish.

In agreement with McIntosh and Austin (4) and Austin and Austin (1), *Serratia liquefaciens* must be considered to be a potential bacterial pathogen for fish. Infection caused histopathological changes in the organ tissues of diseased fish. Another point of this study is that *S. liquefaciens* infection could cause an increase in amylase and GOT enzymes, TG, CHOL, ALB, A/G, Fe levels, TIBC, UIBC and ST in the blood of rainbow trout while the other tested serum parameters (GPT and LDH enzymes, BIL, GLC, CRE, TP values) showed no significant alterations from those of healthy fish. The chemotherapy with potentiated sulphonamide after chloramine-T bath provided complete recovery of the naturally infected fish in farms.

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