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SHORT COMMUNICATION KISA MAKALE

INVESTIGATION OF CERTAIN BLOOD PARAMETERS IN RAINBOW TROUT (*Oncorhynchus mykiss* Walbaum, 1792) NATURALLY INFECTED WITH *Lactococcus garvieae*

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Abstract: In a commercial rainbow trout farm located in the Aegean region, an outbreak characterized with haemorrhages in the eyes and fins, uni/bi lateral exophthalmia and darkening of skin was observed. Ten samples each were taken from clinically symptomatic fish and from healthy fish kept in a seperate ponds and these fish were grouped and marked as Diseased (D1-10) and Control (C1-10), this was followed by bacteriological examinations. Pathogenic bacteria was not isolated from asymptomatic fish but *Lactococcus garvieae* was isolated and identified with conventional and molecular methods from all clinically symptomatic fish samples. Along with bacteriological examinations, blood samples of both groups were analyzed for certain parameters with a automated blood count device calibrated for fish blood. As a result of the analyses; values for White Blood Cell (WBC), Red Blood Cell (RBC), Haemoglobin (Hb), Platelet Total (PLT), Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) in the diseased group were found to be lower than the control group ($p<0.01$, $p<0.001$)

Keywords: Blood parameters, *Lactococcus garvieae*, Rainbow trout *(Oncorhynchus mykiss* Walbaum, 1792)

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Öz:

Lactoccus garvieae İle Doğal Enfekte Gökkuşağı Alabalıklarında (*Oncorhynchus mykiss* Walbaum, 1792) Bazı Kan Parametrelerinin Araştırılması

Ege bölgesinde ticari gökkuşağı alabalığı yetiştiriciliği yapılan bir işletmede, gözlerde kanama ve uni-bilateral ekzoftalmus, yüzgeçlerde haemoroji ve deride kararma ile karakterize bir hastalık tablosu gözlendi. Klinik olarak hastalık belirtisi gösteren on balık ve farklı bir havuzda yetiştirilen ve herhangi klinik semptom göstermeyen 10 balıktan kan örneği alındı ve bu balıklar Hasta (D 1-10) ve Kontrol (C 1-10) grubu olarak işaretlenerek bakteriyolojik muayeneye alındı. Klinik semptom göstermeyen balıklardan patojen izole edilmezken, klinik semptom gösteren balıkların tamamından *Lactococcus garvieae* izole edilerek konvansiyonel ve moleküler yöntemlerle identifiye edildi. Bakteriyolojik muayenelerle birlikte her iki gruba ait kan örnekleri balık kanına kalibre edilmiş otomatize kan sayım cihazı kullanılarak bazı kan parametreleri yönünden araştırıldı. Sonuç olarak enfekte bireylerdeki Beyaz Kan Hücreleri (WBC), Kırmızı Kan Hücreleri (RBC), Hemoglobin (Hb), Total Trombosit (PLT), Ortalama Trombosit Hacmi (MPV) ve Trombosit Dağılım Aralığı (PDW) kontrol gruba göre düşük bulundu ($p<0.01$, $p<0.001$).

Anahtar Kelimeler: Gökkuşağı alabalığı *(Oncorhynchus mykiss* Walbaum, 1792), Kan parametreleri, *Lactococcus garvieae.*

Introduction

L. garvieae from Lactococcus genus of Streptococcaceae, is Gram positive non-motile lactic acid bacteria which produce α- haemolytic colonies in blood agar. They are non spore-forming, non-acid fast cocci which are catalase and oxidase positive (Ravelo *et al.* 2003; Vendrell *et al.* 2006). Lactococcosis caused by *L. garvieae* is an infectious disease of cultured rainbow trout and outbreaks of this disease generally occur during summer months when water temperatures rise above 16°C (Facklam and Eliot, 1995). Non specific symptoms of haemorrhaging and congestion are seen in *L. garvieae* infections (Kusuda *et al*. 1991; Domenech *et al.* 1996) and the others symptoms are immobility, darkeninig of skin and exophtalmia (Collins *et al*. 1984).

Conventional microbiological methods are often used in *L. garvieae* identification (Austin and Austin, 2007; Koneman *et al*. 1997; Timur and Timur, 2003) but identification with these methods are inefficient and time consuming (Holt *et al*. 1994). Polymerase Chain Reaction (PCR) is reported to be an easier and faster identification method for this agent (Zlotkin *et al*. 1998).

Blood parameters of fish are affected by many factors, among which water quality and infectious diseases rank first. They show significant changes in many septicemic bacterial and viral infections such as Motile Aeromonas Septicemia (MAS), Flavobacteriosis, Vibriosis, Infectious Haematopoietic Necrosis (IHN), Infectious Pancreatic Necrosis (IPN) and Viral Hemorrhagic Septicemia (VHS). These changes in blood parameters is due to losses such as haemorrhagia and the impact of the infectious disease on vital organs such as kidney, spleen, liver and pancreas (Austin and Austin, 2007; Vosyliene, 1996).

The aim of this work is to investigate the impact of Lactococcosis that naturally ocured in rainbow trout on certain blood parameters.

Materials and Methods

Sampling

The outbreak was seen in rainbow trout weighing 200-300 g. in a farm located in the Aegean region of Turkey in June, 2012. Ten diseased (D1-10) and 10 clinically asymptomatic fish (C1-10) were used in this work. For blood sampling, the fish were anesthetized with 2-phenoxyethanol (Sigma) at a concentration of 0.30 ml L^{-1} and blood from tail fins of diseased and control fish were was drawn asceptically into containers with EDTA.

Isolation and identification of bacteria

Liver, spleen and kidney samples of fish were inoculated on Trypticase-soy agar (TSA, LABM), Blood Agar (LABM) and incubations in 25°C for 48 hours were carried out. After incubation, colonies were purified and identified ac-

cording to their physiological, biochemical and enzymatic characteristics (Holt *et al*. 1994).

Genotypic confirmation of isolates

Isolates were confirmed by PCR (Zlotkin *et al*. 1998). DNA Extraction was carried out with boiling method (Çiftçi *et al*. 2009) According to this method colonies grown in TSA were suspended in DPEC-treated water (DNase-RNase free) and and was boiled for 10 min. in 100 °C. This was followed by a centrifuge step in 10.000 rpm for 10 minutes. Supernatan was discarded and remains in the tube were used as the template DNA. In PCR amplification pLG-1 (5'-CATAACAATGAGAATCGC-3') ve pLG-2 (5'-GCACCCTCGCGGGTTG-3') oligonucleotide primers were used. DEPC-treated water, 1XPCR Buffer, 1.5 Mm $MgCl_2$, 0.2 Mm of each d NTP, 1.0 U Taq polymerase, 1 µM of each primer and 5 µl template DNA was used in the PCR mastermix. The amplification consisted of 35 cycles and the steps were an initial denaturation in 94°C, followed by a 1 min. denaturation in 94°C, 1 min of annealing in 55°C, an extension of 1.5 min. in 72°C and 1.5 min.of final extension in 72°C. As a result, 1100 bp long amplification product was considered to be positive. Amplification products were visualized on a 1.5% agarose gel and 100 bp. DNA marker was used. Positive and negative controls were *Lactococcus garvieae* ATCC 43921 and *Enterecoccus faecalis* ATCC 29212, respectively.

Investigation of blood parameters

After aenesthesia, blood from tail fins of diseased and control fish were was drawn asceptically into containers with EDTA. Blood samples were analyzed for with a blood count device calibrated for fish blood (Mindray BC 2800, Turkey). White Blood Cell, (WBC), Red Blood Cell, (RBC), Haemoglobin (Hb), Platelet Total Value, (PLT), Mean Platelet Volume, (MPV) and Platelet Distribution Width (PDW) values were researched.

Statistical analyses of blood parameters

For the statistical analyses of data; SPSS (for Windows Release 11.5 Standart Versiyon Copyright © Spss Inc. 1989-2001) was used. With an independant sampling test, data on blood parameters in these two groups of fish were compared. The $P<0.05$ were accepted significant.

Results and Discussion

Clinical symptoms for Lactoccosis in fish were haemorrhages in different parts of the body, darkening of skin and exophtalmia in some (Figure 1). In necropsy, anemia in liver and splenomegaly was observed. Gram stained slides prepared from internal organs of infected fish revealed Gram positive bacterial colonization (Figure 2) whereas similiar findings were not observed in control group fish.

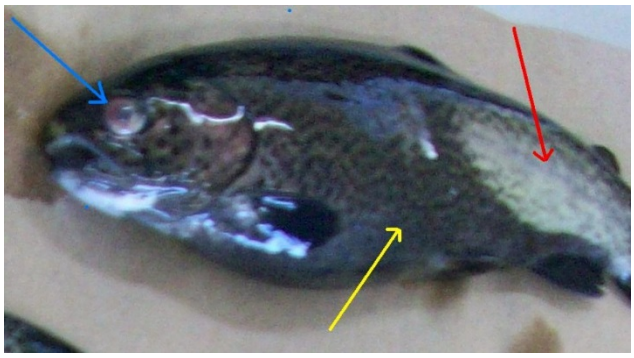

Figure 1. Rainbow trout infected with *L. garvieae*. Red Arrow; ulceration on body, yellow arrow; darkening of skin, blue arrow; exophtalmia

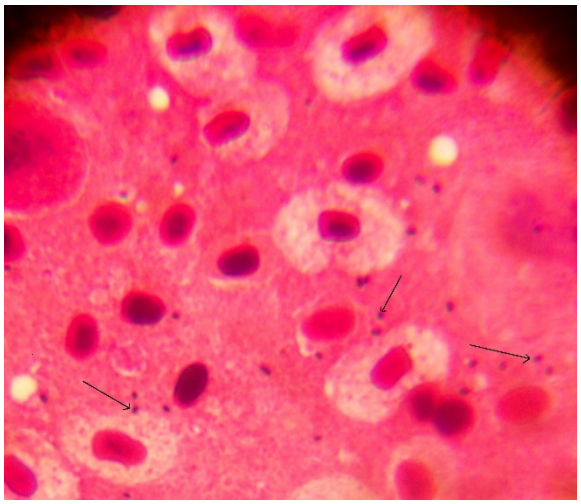

Figure 2. Gram positive cocci in a Gram stained slide prepared from the spleen of an infected fish.

Although *L. garviae* was isolated from all diseased fish, no bacterial pathogens were detected in asymptomatic fish. No differences were observed in phenotypical characterization of isolates and results are summarized (Table 1). Afterwards, PCR confirmation was carried out (Figure 3).

Table 1. Phenotypical characterization of *L. garvieae* isolates

Colony size	<1mm	Growth in % 0 NaCI	+
Bacterial size (Average)	0.4-0.7 µm	Growth in % 6 NaCI	+
Motility	-	Growth in % 8 NaCI	+
Oxidase	-	Lysine Utilization	_
Catalase	-	Ornithin Utilization	_
Pigment production	-	Arginin Utilization	+
Haemolysis in sheep blood	A	Starch	_
Simmon's Citrate Agar	-	Gelatin liquefication	_
Nitrate production	-	Aesculin hydrolysis	+
Methyl red	+	Lactose	_
Voges-Preskauer	+	Glucose	+
Indole	-	Fructose	+
H_2S prodcution	-	Galactose	+
Growth in 4°C'	+	Mannitol	+
Growth in 30 °C'	+	Sucrose	+
Growth in 37 °C'	+	Sorbitol	_
Growth in 45 °C'	+	Rhamnose	_
Growth in Mac-Conkey agar	+	Inositol	_

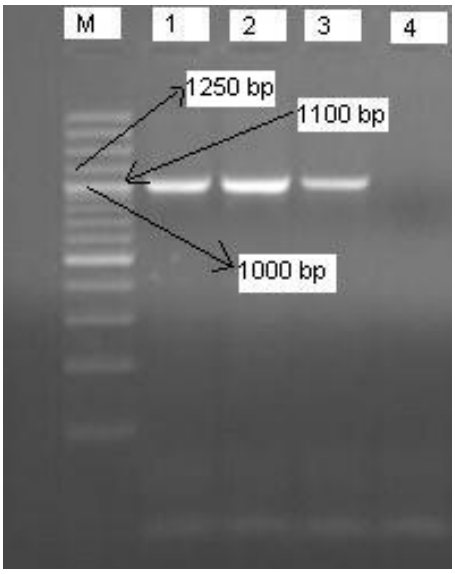

Figure 3. *Lactococcus garvieae* specific PCR, 1100 bp. M; 100-bp DNA ladder, 1: *Lactococcus garvieae* ATCC 43921, 2-3: isolates. 4: *Enterecoccus faecalis* ATCC 29212

Statistical analyses of WBC, RBC, HGB, PLT, MPV ve PDW values obtained from *L. arvieae* infected and control fish (Table 2).

Veterinary haematology is more common in small animal practice; research on blood parameters in fish is limited (Dethlof *et al*. 1999) and analyses are generally carried out manually (Vosyliene, 1996; Cakıcı and Aydın, 2006; Handy *et al*. 1999; Zorriehzahra *et al*. 2010). As fish erythrocytes have nuclei, standard blood count devices record them as leukocytes. Thus, for fast and reliable results with automated devices; the system has to be calibrated with standard (fish) blood. Besides, blood sampling in fish requires special skills as veins are invisible and hard to manipulate. As a result of all these hindrances; determining blood parameters of fish haematology is harder than other animals.

Table 2. Statistical analyses of certain blood parameters of *L. garvieae* infected and control group fish.

Parameters	Control ($X \pm S_{\bar{x}}$) n=10	Infected ($X \pm S_{\bar{x}}$) n=10	P
WBC ($10^3/mm^3$)	29.36 ±0.22	21.88 ±1.12	***
RBC ($10^6/mm^3$)	0.57 ±0.05	0.11 ±0.01	***
HGB (g dL^{-1})	11.30 ±050	5.65 ±0.35	***
PLT (10^9 L^{-1})	75.12 ±4.39	44.77 ±5.11	***
MPV(fL)	5.24 ±0.23	4.36 ±0.06	**
PDW(fL)	17.85 ±0.44	16.30 ±0.06	**

(*P<0.05, **P<0.01, ***P<0.001)

Investigating haematological parameters is an effective way in determining the health status of fish (Blaxhall, 1972; Rehulka, 2002; Martins *et al*. 2008). Erythrocyte (RBC) count in fish naturally infected with *L. garvieae* was found to be significantly lower than the control group. Similar findings of lower RBC values were also reported in coho salmon (*Oncorhynchus kisutch*) infected with *V. anguillarum*, in rainbow trout infected with *Aeromonas sobria, A. caviae, Aeromonas/Streptococcus, Y. ruckeri* and *V. anguillarum*, in tilapia infected with *Streptoccus iniae*, in carp infected with *A. hydrophila* and also in Asian chyclid fish (*Etroplus suratensis*) with epizootic ulcerative syndrome (Harbell *et al*. 1979; Barham *et al*. 1980; Altun and Diler, 1996; Pathiratne and Rajapakshe, 1998; Rehulka, 2002; Harikrishnan *et al*. 2003; Chen *et al*. 2004; Ceylan and Altun, 2010). In Nile tilapia experimentally infected with *Enterococcus sp*., RBC values were reported to be unchanged (Martins *et al*. 2008).

Rainbow trout infected with lactococcosis were also found to have lower WBC values (p<0.001). Although in early stages of experimentally induced Yersiniosis and Vibriosis, leukocyte counts were reported to rise, they were seen to decline as the disease progressed (Altun and Diler, 1996; Ceylan and Altun, 2010). However, Martins *et al*. (2008), have observed increasing WBC values in Nile tilapia experimentally infected with *Enterococcus sp.*

Haemoglobin counts were reported to be significantly lower in rainbow trout infected with *Y. ruckeri, Vibrio anguillarum, A. salmonicida, Aeromonas/Streptococcus*, in Atlantic salmon with cold water Vibriosis, in coho salmon (*Oncorhynchus kisutch*) with *V. anguillarum* and in Asian Chyclid fish (*Etroplus suratensis*) with epizootic ulcerative syndrome (Harbell *et al*. 1979; Barham *et al*. 1980; Altun and Diler, 1999; Pathiratne and Rajapakshe, 1998; Ceylan and Altun, 2010). HGB values obtained in this work were seen to be lower in rainbow trout due to lactococcosis (p<0.001).

Different researchers have noted important increase in platelet counts in experimentally infected with *Y. ruckeri, V. anguillarum, Aeromonas salmonicida* and *Renibacterium salmoninarum* and in Nile tilapia infected with *Enterococcus sp* (Bruno and Munro, 1986; Demirdöğen, 1997; Altun and Diler, 1999; Ceylan and Altun, 2010; Martins *et al*. 2008).. In this research, Lactococcosis infection in rainbow trout was found to lead to a decrease in platelet count

Conclusion

Values for White Blood Cell, (WBC), Red Blood Cell, (RBC), Haemoglobin (Hb), Platelet Total Value, (PLT), Mean Platelet Volume, (MPV) and Platelet Distribution Width (PDW) in rainbow trout naturally infected with *Lactococcus garviae* were found to decrease by Control group (p<0.01, p<0.001).

References

Altun, S., Diler, Ö., (1999). *Yersinia ruckeri* ile infekte edilmis gökkusagı alabalıklarında (*Oncorhynchus mykiss*) Hematolojik

İncelemeler, *Turkish Journal of Veterinary and Animal Sicences*, **23**: 301-309.

Austin, B., Austin, D.A., (2007) Bacterial Fish Pathogens: Disease in Farmed and Wild Fish. 3th ed.(Revised), Praxis Publishing Ltd, Chichester, UK, 457pp.

Barham, W.T., Smit, G.L., Schoonbee, H.J., (1980). The haematological assessment of bacterial infection in rainbow trout, *Salmo gairdneri* Richardson, Journal of Fish Biology, **17**(3): 275-281.

doi: 10.1111/j.1095-8649.1980.tb02761.x

Blaxhall, P.C., (1972). The haematological assessment of the health of freshwater fish. A review of selected literature, *Journal of Fish Biology*, **4**(4):593-604.

doi: 10.1111/j.1095-8649.1972.tb05704.x

Bruno, D.W., Munro, A.L.S., (1986). Haematological assessment of rainbow trout, *Salmo gairdneri* Richardson, and Atlantic salmon, *Salmo salar* L., infected with *Renibacterium salmoninarum*, *Journal of Fish Diseases*, **9**(3): 195-204.

doi: 10.1111/j.1365-2761.1986.tb01004.x

Cakici, H., Aydin S., (2006) Changes in Blood Parameters of Rainbow Trout (Oncorhynchus mykiss Walbaum) after Physical Pollution, *Journal of Applied Animal Research*, **29**(1): 77-80.

doi: 10.1080/09712119.2006.9706576

Ceylan, M., Altun, S.,(2010). Bibrio anguillarum ile infekte edilmiş gökkusağı alabalıklarında (*Oncorhynchus mykiss*) Hematolojik İncelemeler, *Uludağ University Journal of the Faculty of Veterinary Medicine*, **29**(2): 35-42.

Chen, C.Y., Wooster, G.A., Bowser, P.R., (2004). Comparative blood chemistry and histopathology of tilapia infected with *Vibrio vulnificus* or *Streptococcus iniae* or exposed to carbon tetrachloride, gentamicin, or copper sulphate, *Aquaculture*, **239**(1-4): 421-443.

doi: 10.1016/j.aquaculture.2004.05.033

Collins, M.D., Farrow, F.A.E., Phillips, B.A., Kandler, O., (1984) *Streptococcus garvieae* sp nov. and *Streptococcus plantarum* sp. nov, *Journal of General Microbiology*, **129**: 3427-3431

Çiftci, A., Findik, A., Onuk, A.E., Savaşan, S., (2009) Detection of Methicillin resistamce and slime factor as *Staphylococcus aureus* in bovine mastitis, *Brazilian Journal of Microbiology*, **40**: 254-261.

doi: 10.1590/S1517-83822009000200009

Demirdöğen, N., (1997). Deneysel olarak *Aeromonas salmonicida* ile infekte edilen gökkusagı alabalıklarında (*Oncorhynchus mykiss*) bazı hematolojik parametreler, Yüksek Lisans Tezi, *İstanbul Üniversitesi Fen Bilimleri Enstitüsü*, 54s.

Dethloff, G.M., Schlenk D, Khan S., Bailey, H.C., (1999) The Effects of Copper on Blood and Biochemical Parameters of Rainbow Trout (*Oncorhynchus mykiss*), *Archives of Environmental Contamination and Toxicology*, **36**: 415-423.

doi: 10.1007/PL00006614

Domenech, A., Fernandez-Garayzabal, J.F., Pasqual, C., Garcia, J.A., Cutuli, M.T., Moreno, M.A., Collins, M.D., Dominguez. L., (1996) Streptococcosis in cultured turbot, *Scophthalmus maximus* (L.), associated with *Streptococcus parauberis*, *Journal of Fish Diseases*, **19**: 33-38.

doi: 10.1111/j.1365-2761.1996.tb00117.x

Facklam, R.R., Eliot, J.A., (1995). Identification, classification, and clinical relevance of catalase-negative, Gram-positive cocci, excluding the streptococci and enterococci, *Clinical Microbiology Reviews*, **8**: 479-495.

Fihman, V., Raskine, L., Barrou, Z., Kiffel, C., Riahi, J., Bercot, B., Sanson-Le Pors, M.J., (2006). *Lactococcus garvieae* endocarditis: identification by 16S rRNA and sodA sequence analysis, *Journal of Infection*, **52**: 3-6.

doi: 10.1016/j.jinf.2005.04.021

Handy, R.D., Sims, D.W., Giles, A., Campbell, H.A., Musonda, M.M., (1996). Metabolic trade-off between locomotion and detoxification for maintenance of blood chemistry and growth parameters by rainbow trout (*Oncorhynchus mykiss*) during chronic dietary exposure to copper, *Aquatic Toxicology*, **47**: 23-41.

doi: 10.1016/S0166-445X(99)00004-1

Harbell, S.C., Hodgins, H.O., Schiewe, M.H., (1979). Studies on the pathogenesis of vibriosis in coho salmon *Oncorhynchus kisutch* (Walbaum), *Journal of Fish Diseases*, **2**(5): 391-404.

doi: 10.1111/j.1365-2761.1979.tb00391.x

Harikrishnan, R., Nisha Rani, M., Balasundaram, C., (2003). Hematological and biochemical parameters in common carp, *Cyprinus carpio*, following herbal treatment for *Aeromonas hydrophila* infection, *Aquaculture*, **221**(1-4): 41-50.

doi: 10.1016/S0044-8486(03)00023-1

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Williams, S.T., (1994). Bergey's manual of determinative bacteriology. 9th ed. Baltimore, Md: The Williams & Wilkins Co, pp. 527–558.

Koneman, E.W., Allen, S.D., Janda, W.M., Schreckenber, P.C., Winn, W.C., (1997) Color Atlas of Diagnostic Microbiology, 5th ed., Lippincott- Raven Publishers, Philadelphia, USA, pp.121-162.

Kusuda, K., Kawai, K., Salati, F., Banner, C.R., Fryer, J.L., (1991). *Enterococcus seriolicida* sp. nov, a fish pathogen, *International Journal of Systematic Bacteriology*, **41**: 406-409.

doi: 10.1099/00207713-41-3-406

Martins, M.L, Mouriño, J.L.P., Amaral, G.V., Vieira, F.N., Dotta, G., Bezerra, A.J.M., Pedrotti, F.S., Jerônimo, G.T., Buglione-Neto, C.C., Pereira, G. Jr., (2008). Haematological changes in Nile tilapia experimentally infected with *Enterococcus* sp., *B*razilian *Journal of Biology*, **68**: 631-637.

doi: 10.1590/S1519-69842008000300025

Pathiratne, A., Rajapakshe, W., (1998). Hematological changes associated with epizootic ulcerative syndrome in the Asian cichlid fish, *Etroplus suratensis*, *Asian Fisheries Science*, **11**(3-4): 177-316.

Ravelo, C., Magarin˜os, B., Lo´ pez-Romalde, S., Toranzo, A.E., Romalde, J.L., (2003). Molecular fingerprinting of fish-pathogenic *Lactococcus garvieae* strains by random amplified polymorphic DNA Analysis, *Journal of Clinical Microbiology*, **41**: 751-756.

doi: 10.1128/JCM.41.2.751-756.2003

Rehukla, J., (2002). *Aeromonas* causes severe skin lesions in rainbow trout (*Oncorhynchus mykiss*): clinical pathology, haematology and biochemistry, *Acta veterinaria brno*,**71**(3): 351-360.

doi: 10.2754/avb200271030351

Timur, G., Timur, M., (2003). Balık hastalıkları. İstanbul Üniversitesi Su Ürünleri Fakültesi Yayınları No: 5, Dilek Ofset Matbaası, İstanbul, 538s.

Vosyliene, M.Z., (1996). Hematological parameters of rainbow trout (*Oncorhynchus mykiss*) during short-term exposure to copper, *Ekologija/Ehkologiya/Ecology*, **3**: 12-18.

Zorriehzahra, M.J., Hassan, M.D., Gholizadeh, M., Saidi, A.A., (2010). Study of some hematological and biochemical parameters of rainbow trout (*Oncorhynchus mykiss*) fry in western part of Mazandaran province, *Iranian Journal of Fisheries Sciences*, **9**(1): 185-198.