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Hematological Parameters and Erythrocyte Osmotic Fragility in Rainbow Trout, *Oncorhynchus mykiss*, Experimentally Infected with *Pseudomonas putida*

[1]S. Bektas and [2]O. Ayik
[1]Department of Fisheries and Aquaculture, Ispir Hamza Polat Vocational School, Atatürk University, Ispir, Erzurum, Turkey
[2]Department of Fisheries and Aquaculture, Faculty of Agriculture, Atatürk University, Erzurum, Turkey

Abstract: In the present study, selected hematological and osmotic fragility values in rainbow trout, following experimental *Pseudomonas putida* infection have been described. Blood samples were collected at day, 1, 3, 7, 14 and 21 post inoculation and examined for the above parameters. Erythrocyte values were significantly decreased at all days. Hematocrit values at day 14 was significantly higher than other days. Hemoglobin values revealed significant decrease from day first onwards. Among red blood cell indices, MCV were found significantly higher from day 1 to 21. Significant decrease in MCHC were also reported. MCH remain unchanged in all days examined. Leukocyte values at day 21 were significantly higher than at day 7. In addition, thrombocytes values at day 21 were significantly higher than at day 7 and 14 . On the other hand, erythrocyte osmotic fragility values were significantly increased at day 14 and 21 post infection. Interpreted hematological data in the present study, can be used to assess an abnormality or disease process in pseudomonas infected rainbow trout.

Key words: Experimental infection, hematology, erythrocyte osmotic fragility, *Pseudomonas putida*, rainbow trout

INTRODUCTION

Generally, the bacterial flora of fish, including *Pseudomonas*, *Cytophaga*, *Flavobacterium*, *Micrococcus* and *Acinetobacter* species and others reflects the microbial population of the aquatic habitat and it is influenced by factors such as bacterial load in the water and salinity. *Pseudomonas* species are widespread and forms a major part of secondary infection in immunocompromised fish (Inglis and Hendrie, 1993). The genus *Pseodomonas* contains three species which, at various times, have been described as etiological agents of diseases in fishes. The species are *P. anguilliseptica*, *P. fluorescens* and *P. chlororaphis.* In addition, there have been some reports of unnamed *Pseudomonas* species, which probably equate with *P. fluorescens* (Austin and Austin, 1987). To date there has been only one report of *P. putida* as a fish pathogen in rainbow trout with a mortality of 35% during a disease outbreak in Turkey (Altınok *et al*., 2006). The diagnostic techniques applied in fish hematology are usually adapted methods developed in human hematology and many of them can be used to assist in providing evidence and possible identification of an abnormality or disease process. For example, hemoglobin estimation, erythrocyte counts and hematocrit value have proved useful in the detection of anemia, total leukocyte and differential leukocyte counts as an indication of the

Corresponding Author: Serdar Bektas, Department of Fisheries and Aquaculture, Ispir Hamza Polat Vocational School, Atatürk University, Ispir, Erzurum, Turkey
Tel: +90(442) 451 2985-129 Fax: +90(442) 451 2959

possible type of an infectious or organic disease (Blaxhall and Daisley, 1973; Walencik and Witeska, 2007). The degree of resistance of red blood cells to lysis as a result of a decrease in the NaCl content of their environment is the basis of the osmotic fragility test. The degree of hemolysis is determined by measurement of hemoglobin release from the cells. The osmotic fragility test is used to determine the extent of red blood haemolysis produced by osmotic stress. Although an old method, the osmotic fragility test is a useful screening assay to detect several pathological conditions and clinically as a diagnostic assay (Orcutt *et al*.,1995; Kafka and Yermiahu, 1998). The following study was performed to determine if there was a significant effect of *Pseudomonas putida* experimental infection in rainbow trout (*Oncorhynchus mykiss*) on some hematological parameters such as erythrocyte, total leukocyte and thrombocyte counts, hematocrit, hemoglobin, Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH) concentration, Mean Cellular Hemoglobin Concentration (MCHC) also we aimed to assess the alterations of Erythrocyte Osmotic Fragility (EOF) during disease. Although, much has been written about the hematological parameters of fish, to our knowledge this is the first study to determine comprehensive hematological and EOF results for *Pseudomonas putida* infection in rainbow trout (*Oncorhynchus mykiss*).

MATERIALS AND METHODS

Fish and Fish Tank Facilities

This study was conducted in Fisheries Department of Agricultural Faculty at Atatürk University (Erzurum/Turkey) from October to December 2002.

A total of 50 rainbow trout (*Oncorhynchus mykiss*) weighting 250±45 g, were obtained from a freshwater farm. Fish were randomly divided into two groups (25 fish in each tank) one for control and the other for experimental infection and kept in two separate 300 L circular fiberglass tanks with a constant water flow of 1.5 L min^{-1} of aerated dechlorinated tap water and held under natural light conditions. During the entire experimental period, the water in the tanks had the following characteristics: temperature 9-11°C; dissolved oxygen 8-9 ppm; pH 7.6-7.8. The fish were acclimated to these conditions for two weeks before the start of the experiment. The fish were fed pelleted dry food (40% protein) at a rate of 1.5% body weight/day. Tanks are cleaned daily by siphoning.

Isolation and Identification of Isolates by FAME-MIS

A disease outbreak was reported from a commercial trout farm in 2002 located in Erzurum (Turkey). A scientific visit was conducted to this farm. Fish were subjected to stressful conditions like poor water quality and high stocking density. Increased mortality rate has been raised and fish were exhibiting characteristic symptoms of a bacterial disease (distended abdomen with ascetic fluid and hemorrhages on the body surface). A total of 10 rainbow trout from this farm were taken to the laboratory. For microbiological examination, materials obtained from liver, kidney, spleen and muscle lesions were inoculated onto Tryptic Soy Agar (TSA) and incubated at 25°C for 48 h. After incubation, isolates were identified by Fatty Acid Methyl-Ester (FAME) gas chromatography analysis using Microbial Identification Systems Software (MIS Delaware, USA) as described by Buyer (2002).

Experimental Infection of Fish

Pseudomonas putida strain, obtained from above mentioned farm used in the experimental infection. Serial tenfold dilutions of bacteria in 0.65% sterile Salt Solution (SS) were prepared for determination of bacterial cell concentration using McFarland's turbidimetric method. After 2 weeks acclimation period fish were injected intramuscularly (i.m) with either 100 μL of SS (controls) or an equivalent volume of SS containing 5×10^5 cells (Berc *et al*., 1999).

Blood Sampling and Hematological Assays

In order to compare daily alterations of the hematological parameters, blood samples were taken 1, 3, 7, 14 and 21 days after the start of the experiment. A total of 10 fish were planed to sampled (5 control and 5 infected) at each day, but 5 of 25 *P. putida* infected fish (20%) died during the experiment, so a total of 20 fish could be sampled from the diseased group. To avoid sampling stress, fish were anaesthetized with tricaine methanesulfanate (MS 222). Blood samples were drawn from each fish, by caudal venous puncture with a heparinized disposable sterile syringe. Hematocrit was determined by spinning the blood sample contained in heparinized capillary tubes in a microhematocrit centrifuge (Carvalho and Fernandes, 2006). Hemoglobin concentration was determined spectrophotometrically (at 540 nm) using the cianmethemoglobin method (20 µL of blood in 5 mL Drabkin reagent) as described by Smith *et al*. (2007). Standard hematological procedures described by Blaxhall and Daisley (1973) were employed in the assessment of various blood parameters. Erythrocytes, total leucocytes and thrombocytes were counted with a Neubauer hemacytometer with Dacies solution as a diluting fluid. The mean cellular volume, mean cell hemoglobin and mean cellular hemoglobin concentration were also calculated by standard formulas (Kang *et al*., 2005). For the determination of erythrocyte osmotic fragility, the method described by Aldrich and Saunders (2001) was used.

Statistical Analysis

The mean values and SD of mean values for each hematological parameter were calculated. Variance analysis was performed to evaluate changes in daily hematological variables between control and infected fish. In cases where a p-value of less than 0.05 was found Duncan's multiple range test was performed for over time changes in blood values. All the calculations were carried out using the statistical package SPSS version 10 for Windows.

RESULTS AND DISCUSSION

Mortalities of *P. putida* injected fish started 3 days after injection 5 of 25 infected fish (20%) died during the experiment (three fish died on days 3, 14, 21 and two fish from the diseased group died on seventh day of the experiment) , so a total of 20 fish could be sampled from the *P. putida* injected group. No mortalities and clinical signs of disease were recorded in the control group. The main pathological findings of *P. putida* injected fish were dark pigmentation of the skin especially at the base of the dorsal fin, pale gills and liver and haemorrhages in the skin in various places, but most frequently at the site of injection, often accompanied by ulcers. Some of the infected fish presented extensive erosion of the pectoral and caudal fins. Large volumes of turbid fluid were noted within the abdominal cavity. No *P. putida* was isolated from any organs of any of the rainbow trout in the SS injected control group. The bacterium was re-isolated from the kidney, spleen and liver of the infected fish. The recovery of the bacteria was highest from the kidney.

In the present study, overall 15 fatty acids with aliphatic chain lengths of 10 to 18 carbon atoms were identified in the bacterial lipid extracts. 16:1 w7c/15 iso 2OH (33.44%), 16:0 (30.60%) and 18:1w7c C(14.21%) are found predominant fatty acids for *P. putida*. The fatty acid compositions of *P. putida* strains were presented in Table 1.

As to the blood parameters, the *P. putida* infected fish had lower erythrocytes and hemoglobin levels from the third day to the end of the experimental infection ranged between 0.58±0.11-0.84±0.09 and 4.80±0.90-6.82±2.25, respectively. Erythrocytes were ranged from 1.13±0.20 to 1.40±0.13 and hemoglobin levels were ranged between 6.80±0.94 and 8.66±0.99 for the healthy fish. Hematocrit values at day 14 were found significantly higher than the other days (Table 2). Fluctuating leukocyte and thrombocyte counts observed in our study. While, the thrombocytes counts at day 21 were significantly higher than at days 7 and 14, leukocyte values obtained from the day 21 of the

Table 1: Composition of fatty acids identified from *P. putida*

Peak name	Percent
10:0 3OH	3.52
12:00	2.18
11:0 ISO 3OH	0.55
12:0 2OH	4.13
12: 3OH	3.81
14:00	0.45
15:0 ISO	0.57
15:0 ANTEISO	1.38
16:1 w7C/15 iso 2OH	33.44
16:00	30.60
17:0 ISO	0.50
17: ANTEISO	0.34
17:0 CYCLO	3.37
18:1w7c	14.21
18:00	0.95

Table 2: Over time alterations of the hematological parameters of control and *P. putida*-infected rainbow trout, *O. mykiss*

	Days				
Parameters	1	3	7	14	21
Erythrocyte (10^6 mm^{-3})					
Control	1.230±0.06	1.290±0.15	1.130±0.20	1.210±0.07	1.400±0.13
Infected	1.120±0.13^{a}	0.840±0.09^{ac}	0.600±0.11^{d}	0.660±0.0.9^{cd}	0.580±0.11^{ac}
Leukocyte (10^3 mm^{-3})					
Control	57.200±17.12	41.600±9.154	41.400±6.18	62.200±19.89	60.800±7.496
Infected	60.600±7.334^{ab}	85.000±25.17^{ab}	53.330±28.44^{a}	56.250±4.645^{ab}	63.000±9.128^{b}
Thrombocyte (10^3 mm^{-3})					
Control	26.000±8.185	19.800±2.683	18.400±3.781	20.800±4.207	29.200±7.563
Infected	27.200±6.685^{ab}	39.000±12.83^{ab}	28.660±14.36^{a}	23.250±4.856^{a}	35.750±8.180^{b}
Hemoglobin (g dL^{-1})					
Control	8.660±0.99	7.520±1.09	7.120±1.22	6.800±0.94	8.460±1.00
Infected	10.360±1.52^{b}	6.820±2.25^{a}	5.460±1.96^{a}	4.800±0.90^{a}	5.270±0.77^{a}
Hematocrit (%)					
Control	39.800±4.38	44.200±2.86	44.000±2.54	48.800±1.30	49.400±4.03
Infected	37.800±3.96^{a}	36.500±1.73^{a}	36.660±4.93^{a}	43.000±3.46^{b}	36.000±1.41^{a}
MCV (μ^3)					
Control	321.400±29.8	343.800±33.9	394.600±60.6	402.400±24.3	354.000±42.7
Infected	337.200±32.5^{a}	437.000±36.1^{ab}	628.660±180.9^{c}	660.000±136.0^{c}	636.250±125.5^{bc}
MCH (μg)					
Control	69.800±9.95	58.000±8.33	65.000±23.39	56.000±8.74	60.000±6.55
Infected	95.600±26.29^{a}	83.000±31.86^{a}	92.330±35.07^{a}	73.500±19.63^{a}	93.500±22.94^{a}
MCHC (%)					
Control	22.000±2.34	16.800±2.16	15.600±03.71	13.600±1.67	17.200±1.64
Infected	27.400±5.63^{c}	18.250±6.29^{b}	14.330±04.04^{ab}	10.750±1.50^{a}	14.750±2.36^{b}
EOF					
Control	0.491±0.03	0.515±0.02	0.514±0.01	0.433±0.01	0.460±0.01
Infected	0.435±0.01^{ab}	0.513±0.04^{c}	0.448±0.06^{b}	0.456±0.06^{a}	0.536±0.03^{c}

Values are expressed as Mean±SD. Different superscript letter(s) indicate statistically significant differences at $p<0.05$

experiment were significantly higher than day 7 results. Within the red blood cell indices, significantly higher MCV and lower MCHC values were reported, however no significant changes in MCH values between days were obtained. Daily alterations in osmotic fragility of fish erythrocytes are shown in Fig. 1. Also, the overtime alterations of the EOF results from the health and *P. putida* infected fish are presented in Table 2. Osmotic fragility curves indicate that erythrocytes of *P. putida* infected fish were more fragile than control group under varying salt concentrations. The EOF values of the infected fish in the last day of the experiment, were significantly higher than the other days, except for the third day results.

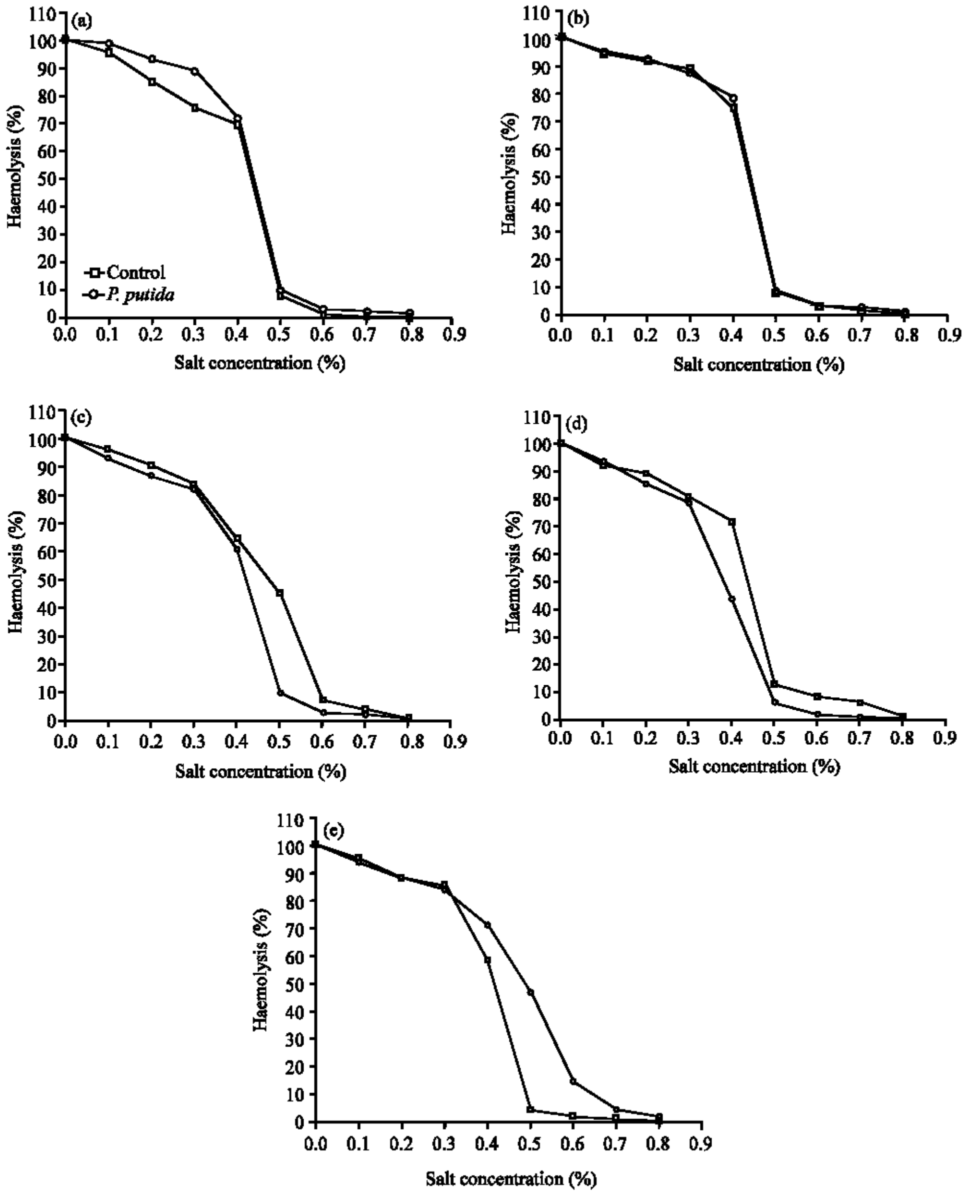

Fig. 1: Daily alterations of erythrocyte osmotic fragility in healthy and *P. putida*-infected rainbow trout (*O. mykiss*). (a) 1st, (b) 3rd © 7th, (d) 14th and (e) 21st day

Fatty acid profile, usually consisting of more than ten different fatty acids is an important feature for the classification of bacteria and often helpful for identification (Busse *et al*., 1996). In the present study,15 fatty acids were detected. Among these, 16:1 w7c/15 iso 2OH (33.44%), 16:0 (30.60%) and 18:1w7c (14.21%) are found predominant fatty acids for *P. putida*. FAME's found to be predominant in the pure cultures tested in *P. putida* were 10:0 3OH, 17:cyclo, 16:1w7c and 18:1w12t/w9t/w7c (Glucksman *et al*., 2000). Bogdan *et al.* (2001) reported predominance of palmitic acid (16:O) amounting to 37.7±1.3%; palmitoleic (16:1), 33.8±3.2% and oleic (18:1), 17.5±2.1% for pseudomonads.

Hematological assays may provide an index of the physiological status of fish, synthesis and analysis of the figures obtained for the individual diseases may provide valuable information on the specific response or the range and nature of the pathological process (Rehulka, 2002).

Erythrocyte count, leukocyte count, hemoglobin and hematocrit are particularly recommended as tests that could be performed on a routine basis in fish farms to monitor the health of the stock (Omoregie and Oyebanji, 2002). Present findings indicate that the erythrocyte values were significantly depressed in all *Pseudomonas putida* infected fish from the third day through the end of the experimental infection. Hematocrit values at day 14 were significantly higher than the other days. Hemoglobin values of the infected fish, presented significant decreases comparing the first day results. Within the red blood cell indices, significantly higher values of MCV were reported, however there were no significant changes in MCH values between days. This picture indicate a macrocytic, hypochromic anemia. No studies were available to compare the alterations of blood values in *pseudomonas putida* infected rainbow trout however, anemic signs of bacterial disease is very common in fish. Rehulka and Minaøik (2007) reported lower RBC and Hb levels, which ranged between 0.36 and 0.79 and between 57.4 and 77, respectively. Also, they reported higher values of MCV, MCH and lower level of MCHC in brook trout *Salvelinus fontinalis* affected by columnaris disease. Microcytic, normochromic, nonregenarative anemia reported in koi (*Cyprinus carpio*) associated with *Flavobacterium columnare* infection. RBC and Hb were found to be significantly lower as were the MCV and MCH while MCHC showed no significant differences between diseased and healthy fish (Tripathi *et al*., 2005). Altun and Diler (1999) studied the effects of *Yersinia ruckeri* infection on hematological parameters of rainbow trout (*Oncorhynchus mykiss*) at different days. They observed microcytic, normochromic anemia on the third day of experimental infection and also they reported macrocytic, normochromic anemia at days 13 and 15. Changes in hematocrit value is one of the most important hematological parameter in detection of anemia. Severe anemia was recorded by Rehulka (2002) with reduced erythrocyte count and lower hematocrit and hemoglobin levels in rainbow trout (*Oncorhynchus mykiss*) infected with *Aeromonas*. Reduced hematocrit was detected in the eel (*Anguilla anguilla*) spontaneously infected with *Aeromonas hydrophila* (Yildiz *et al*., 2005). Total and differential leukocyte counts are important indices of non specific defense activities in fish (Pedro *et al*., 2005). Leucocytes serve two principal functions. They participate in coagulation and thus curtail blood loss from injury sites. In addition they are centrally involved in phagocytic and immune responses to parasitic, bacterial, viral and similar challenges (Houston, 1990). Fluctuating leukocyte counts observed in present study between days in infected group. Leukocyte values obtained at day 21 were significantly higher than 7 day results. Leukocytosis is a condition characterized by an elevated number of white cells in the blood. Leukocytosis due to neutrophila and slight lymphocytosis which was characteristic of an acute infection was reported in nile tilapia *Oreochromis niloticus* infected with *Mycobacterium marinum* (Paiva *et al*., 2004). Increase in total leukocyte in the present study is in agreement with earlier works carried out in other bacterial infections (Altun and Diler, 1999; Yildiz, 1998; Benli and Yildiz, 2004). Thrombocytes are responsible for clot formation and are considered to be distinct, separate and unrelated to lymphocytes (Ellis, 1977). Thrombocytes values at day 21 were significantly higher than at 7 and 14 days. Santarém and Figueras (1995) injected turbot (*Scophthalmus maximus*) with *Vibrio damsela*, *Pasteurella piscicida* O-antigens and phosphate buffered saline (control). They reported a significant increase in thrombocytes of 6 hours sample in all 3 groups of fish. By 12 h post-injection, the proportion of thrombocytes in all 3 groups of fish began to decrease and by 72 h after injection thrombocytes returned to normal values. They claimed that the significant increase in the number of peripheral blood thrombocytes in both immunized and saline injected turbot 6 h after injection may have been due to the lesion effect produced by the injection.

In the present study significant increases in EOF values of the *P. putida* infected group were observed at 14 and 21 days post infection. Fifty percent of hemolysis occurred at a chloride concentration of 0.46% in healthy fish, while in diseased erythrocytes were more fragile and the concentration was 0.53% on the last day of the experiment. When studying the effects of *Streptococcus* and *Aeromonas* infections on erythrocyte fragility in rainbow trout, Barham *et al*. (1980) reported that these infections resulted in more fragile erythrocytes and they assumed that these findings could be caused from production of hemolytic factor by bacteria with a resultant disturbance in the functional state of the blood cell membrane. They claimed that increase in body temperature with the effect of bacterial infection may also affect osmotic fragility of erythrocytes. Erythrocyte count, MCV and osmotic fragility results of the present study indicates that infection with *P. putida* affected not only the count of the erythrocytes but also the physiological state of the erythrocytes.

REFERENCES

Aldrich, K. and D.K. Saunders, 2001. Comparison of erythrocyte osmotic fragility among ectotherms and endotherms at three temperatures. J. Thermal Biol., 26: 179-182.

Altınok, I., S. Kayis and E. Capkin, 2006. *Pseudomonas putido* infection in rainbow trout. Aquaculture, 261: 850-855.

Altun, S. and O. Diler, 1999. Some hematological parameters of the rainbow trouts experimentally infected with *Yersinia ruckeri*. Turk. J. Vet. Anim. Sci., 23: 301-309.

Austin, B. and D.A. Austin, 1987. Bacterial Fish Pathogens. 1st Edn., Ellis Horwood Limited, ISBN: 0-85312-844-8 .

Barham, W.T., G.L. Smit and H.J. Schoonbee, 1980. The effect of bacterial infection on erythrocyte fragility and sedimentation rate of rainbow trout, *Salmo gairdneri Richardson*. J. Fish Biol., 16: 177-180.

Benli, A.C.K. and H.Y. Yildiz, 2004. Blood parameters in nile tilapia (*Oreochromis niloticus* L.) spontaneously infected with *Edwardsiella tarda*. Aquacult. Res., 35: 1388-1390.

Berc, A., Z. Petrinec, E. Matašin and Z. Kozariæ, 1999. *Yersinia ruckeri* septicemia in experimentally infected carp (*Cyprinus carpio* L.) fingerlings. Acta Vet. Hung., 47: 161-172.

Blaxhall, P.C. and K.W. Daisley, 1973. Routine hematological methods for use with fish blood. J. Fish Biol., 5: 771-781.

Bogdan, V.V., L.P. Smirnov and V.S. Sidorov, 2001. Lipids of microorganisims of the family Vibrionaceae, causative agents of fish diseases. Applied Biochem. Micro., 37: 310-313.

Busse, H.J., E.B.M. Denner and W. Lubitz, 1996. Classification and identification of bacteria: Current approaches to an old problem. Overview of methods used in bacterial systematics. J. Biotech., 47: 3-38.

Buyer, J.S., 2002. Rapid sample processing and fast gas chromatography for identification of bacteria by fatty acid analysis. J. Microbiol. Met., 51: 209-215.

Carvalho, C.S. and M.N. Fernandes, 2006. Effect of temperature on copper toxicity and hematological responses in the neotropical fish *Prochilodus scrofa* at low and high pH. Aquaculture, 251: 109-117.

Ellis, A.E., 1977. The leucocytes of fish. J. Fish Biol., 11: 453-491.

Glucksman, A.M., H.D. Skipper, R.L. Brigmon and J.W.S. Domingo, 2000. Use of MIDI-FAME technique to characterize groundwater communities. J. Applied Microbiol., 88: 711-719.

Houston, A.H., 1990. Blood and C irculation. In: Methods for Fish Biology, Schreck. C.B and P.B. Moyle (Eds.). American Fisheries Society, USA., ISBN: 0-913235-58-X pp: 273-334.

Inglis, V. and M.S. Hendrie, 1993. Pseudomonas and Alteromonas Infections. In: Bacterial Diseases of Fish, Inglis, V., R.J. Roberts and N.R. Bromage (Eds.). Blackwell Scientific Publications, Oxford, UK., ISBN: 0-632-03497-1, pp: 169-174.

Kafka, M. and T. Yermiahu, 1998. The effect of EDTA as an anticoagulant on the osmotic fragility of erythrocytes. Clin. Lab. Haematol., 20: 213-216.

Kang, J.C., S.G. Kim and S.W. Jang, 2005. Growth and hematological changes of rockfish, *Sebastes schlegeli* (Hilgendorf) exposed to dietary Cu and Cd. J. World Aquac. Soc., 36: 188-195.

Omoregie, E. and S.M. Oyebanji, 2002. Oxytetracycline-induced blood disorder in juvenile nile tilapia oreochromis niloticus (Trewavas). J. World Aquac. Soc., 33: 377-382.

Orcutt, R.H., T.S. Thurmond and K.E. Ferslew, 1995. Mathematical modeling of the osmotic fragility of rabbit red blood cells. J. Pharmacol. Toxicol. Methods, 34: 169-174.

Paiva, M.J.T.R., C.M. Ishikawa, A.C. Eiras and V.R. Silveira, 2004. Effects of an experimental challenge with *Mycobacterium marinum* on the blood parameters of *Nile tilapia*, *Oreochromis niloticus* (Linnaeus, 1757). Braz. Arch. Biol. Technol., 47: 945-953.

Pedro, N., A.I. Guijarro, M.A.L. Patiòo,, R.M. Álvarez and M.J. Delgado, 2005. Daily and seasonal variations in hematological and blood biochemical parameters in the tench, *Tinca tinca* Linnaeus, 1758. Aquacult. Res., 36: 1185-1196.

Réhulka, J., 2002. *Aeromonas* causes severe skin lesions in rainbow trout (*Oncorhynchus mykiss*): Clinical pathology, haematology and biochemistry. Acta Vet. Brno., 71: 351-360.

Réhulka, J. and B. Minaøík, 2007. Blood parameters in brook trout *Salvelinus fontinalis* (Mitchill, 1815), affected by columnaris disease. Aquacult. Res., 38: 1182-1197.

Santarém, M.M. and A. Figueras, 1995. Leucocyte numbers and phagocytic activity in turbot *Scophthalmus maximus* following immunization with *Vibrio damsela* and *Pasteurella piscida* O-antigen bacterins. Dis. Aquat. Org., 23: 213-220.

Smith, C.J., B.J. Shaw and R.D. Handy, 2007. Toxicity of single walled carbon nanotubes to rainbow trout (*Oncorhynchus mykiss*): Respiratory toxicity, organ pathologies and other physiological effects. Aquat. Toxicol., 82: 94-109.

Tripathi, N.K., K.S. Latimer, C.R. Gregory, B.W. Ritchie, R.E. Wooley and R.L. Walker, 2005. Development and evaluation of an experimental model of cutaneous columnaris disease in koi *Cyprinus carpio*. J. Vet. Diagn. Invest., 17: 45-54.

Walencik, J. and M. Witeska, 2007. The effect of anticoagulants on hematological indices and blood cell morphology of common carp (*Cyprinus carpio* L.). Comp. Biochem. Physiol., 146: 331-335.

Yildiz, H.Y., 1998. Effects of experimental infection with pseudomonas fluorescens on different blood parameters in carp (*Cyprinus carpio* L.). Isr. J. Aquacult. Bamidgeh, 50: 73-81.

Yildiz, H.Y., S. Bekcan, A.C.K. Benli and M. Akan, 2005. Some blood parameters in the eel (*Anguilla anguilla*) spontaneously infected with *Aeromonas hydrophila*. Isr. J. Vet. Med., 60: 91-92.