TÜBİTAK

Research Article

Turk. J. Vet. Anim. Sci.
2010; 34(4): 313-318
© TÜBİTAK
doi:10.3906/vet-0706-4

Impairment in the haematological parameters of tench (*Tinca tinca*) infected by *Saprolegnia* spp.

Syed Lal SHAH*

Pakistan Science Foundation, G-5/2, Islamabad, PAKISTAN

Received: 07.06.2007

Abstract: Haematological parameters of tench (*Tinca tinca*) infected by *Saprolegnia* spp. were studied. All parameters, except mean corpuscular volume (MCV), decreased in response to infection. A significant ($P < 0.05$) decrease was observed in haemoglobin (Hb) (−26.85%), red blood cell count (RBCc) (−20.94%), leucocrit (Lct) (−24.14%), and mean corpuscular haemoglobin concentration (MCHC) (−9.06%). Haematocrit (Hct), white blood cell count (WBCc), and mean corpuscular haemoglobin (MCH) also decreased (−7.66%, −12.44%, and −6.19%, respectively); however, differences between the infected and control groups were not significant. Among the parameters, only MCV had a non-significant increase (+7.84%) in the infected fish. The study results show that saprolegniasis caused anaemia and immunosuppression, followed by mortality in tench. Mortality was attributed to haemodilution caused by haemorrhaging and the breakdown of osmotic balance due to tissue (skin and muscular layer) destruction via the penetration of the hyphae; however, lethargy was another mortality promoting factor. Mortality among the *Saprolegnia*-infected fish also depended upon the initial site of infection, type of tissue destroyed, growth rate of the fungus, and ability of individual fish to withstand the stress. The present study was conducted to investigate physiological impairment in *Saprolegnia*-infected *Tinca tinca* under laboratory conditions; however, further studies at the population level in natural habitats and under similar stress conditions are needed.

Key words: Haematological parameters, saprolegniasis, *Tinca tinca*

Introduction

Fish are an important source of food and recreation, and are a key unit in many natural food webs. *Tinca tinca* is one of the main freshwater species of commercial interest in some countries, such as Germany (1). It is a resilient fish that can survive in environments where other fish are eliminated. In aquatic environments, almost every freshwater fish is exposed to at least one species of fungus during its lifetime (2). Some changes such as stress and immunosuppression allow infection to develop quickly (3). *Saprolegnia* spp., generally termed water moulds, share common features with both fungi and algae (4). However, they cause fungal disease that appears as cotton-like circular, crescent-shaped, or whorled pattern on the surface of the animal, and not only affects the animal itself but also infects the eggs by penetration of the egg membrane (5). The fish infected with *Saprolegnia* are easily recognised by the cotton-like white to greyish patches on the skin and

* E-mail: drsyed_1@yahoo.com

gills visible to the naked eye (4). It invades epidermal tissues, causing destruction and loss of epithelium integrity due to cellular necrosis by hyphal penetration of the basement membrane (6,7). Lethargy, loss of equilibrium, and death due to haemodilution in *Saprolegnia*-infected fish have also been reported (7,8). The infection progresses very quickly and often results in mortality and can cause huge losses of both fish and ova (4,9). The study of infected fish is essential to make efforts to determine the efficacy of various antifungal treatments (9), and to understand the mode of action of fungal infection and the resistance capability of fish.

Haematology provides an index of physiological status of fish (10) and its study is important for the development of health management for the rapidly growing aquaculture industry (11). The present study was conducted to investigate the physiological impairments in *T. tinca* infected with *Saprolegnia* in laboratory conditions. However, it is just the first step and further work at population level in natural habitats under similar stress conditions is needed.

Materials and methods

Tinca tinca (2- to 3-years-old, average total length 23.22 ± 1.12 cm, average total weight 203.11 ± 5.03 g) were collected from Mogan Lake near Ankara, Turkey, with cast nets and transported to the laboratory of the Department of Biology, Ankara University, Ankara. Fifty-litre capacity water tanks supported with air pumps for oxygen supply were used for the transportation of the fish. The fish were observed for their overall health based on behaviour and development of cotton-like patches on the body. They were provided with commercial pellet food twice a day and maintained at 12 D / 12 L photoperiodicity. Physico-chemical parameters of the laboratory water were as follows: dissolved oxygen 7.68 ± 0.13 mg/L, water temperature 20.67 ± 0.49 °C, pH 7.49 ± 0.9, conductivity 0.29 ± 0.02 mS/cm, bicarbonates 97.6 mg/L, total alkalinity 80 mg/L, chlorine 10.3 mg/L, sulphates 26.1 mg/L, calcium 29.0 mg/L, and magnesium 1.2 mg/L.

A total of 72 specimens of *Tinca tinca* were studied (36 *Saprolegnia*-infected and 36 healthy). The experiments ran for 7-11 days but the fish developed infection generally within 3-5 days. For development of *Saprolegnia* infection, natural stressors such as crowding, transference, handling, water contamination, and temperature were used in laboratory conditions. The severity of infection and susceptibility varied from fish to fish, depending upon the ability of individual fish to withstand the stress. The identification of *Saprolegnia* was performed following Stueland et al. (4), i.e. appearance of cotton-like white to greyish patches on the skin and gills. The identification could not be made at species level, which is only possible by taxonomic analysis of the sexual structure combined with limited morphological characteristics. Fish lesion isolates do not produce any sexual structures under laboratory conditions and could not be identified to species and are therefore grouped in the generic classification, *Saprolegnia* spp. (3,4,6).

The parameters were studied when the fish became lethargic and lost equilibrium. Blood samples were collected within 35-40 s through a cardiac puncture into 2 mL disposable heparinised syringes, with 21-gauge needles after stunning fish by a blow to the head. The syringes were stored at 4 °C until the blood parameter studies were completed. For haematocrit (Hct) determination, three-fourth microhaematocrit capillaries (75 mm ´ 1.1 mm ID, Superior Germany) were filled with blood, sealed at one side by capillary sealer (Marion Feld, Germany), and centrifuged at 11,000 rpm for 6 min in a microhaematocrit centrifuge (Hawksley and Sons, Co. Sussex, UK). The haematocrit (%) was determined by a microhaematocrit reader (12). Haemoglobin (Hb) was determined with a Roach No. 124729 hemoglobin test kit (Roach, GmbH Mannheim, Germany) using the cyanmethaemoglobin method. A 0.02 mL aliquot of blood was mixed with 5 mL of test reagent (potassium hexacyanoferrate 0.6 mmol/L and potassium cyanide 0.75 mmol/L), incubated at room temperature for 10-20 min and absorbance was read at 546 nm using a Shimadzu spectrophotometer (UV-120 IV, Shimadzu Co., Japan). Absorbance values were converted to haemoglobin measurements (g/dL) based on standards included with the test kit. Total numbers of red blood cells (RBCs) and white blood cells (WBCs) were counted under a microscope (Olympus CHK Optical Co. Ltd) at 640´ using an improved Neubauer haemocytometer (Clay, Adams, NY, USA). Blood was diluted 1:200 with Hayem's solution and 1:20 with Turk's diluting fluid for RBCs and WBCs respectively.

Leucocrit (Lct) was determined from the same microhaematocrit capillaries following McLeay and Gordon (13) and values in percentages were calculated as follows: height of greyish-white buffy layer/height of total blood volume ′ 100. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were determined following Gill and Pant (14) as follows: MCV (mm^3) = Hct (%)/RBC (′$10^6/mm^3$) ′ 10, MCH (pg) = Hb (g/dL)/RBC (′$10^6/mm^3$) ′ 10, MCHC (g/dL) = Hb (g/dL)/Hct (%) ′ 100. The data were analysed statistically using Student's t-test and significant difference was established at 0.05 level. Values of blood parameters of *Saprolegnia*-infected tench were compared with those of the control group to determine the significance of the infection effect.

Results

The results reveal that all haematological parameters, except MCV, showed an overall noticeable decrease in *Saprolegnia*-infected *Tinca tinca*. A significant ($P < 0.05$) decrease was observed in Hb (g/dL), RBCs (′$10^6/mm^3$), Lct (%), and MCHC (g/dL). Hct (%), WBCs (′$10^3/mm^3$), and MCH (pg) also decreased but the differences were non-significant ($P > 0.05$) when compared with the controls. Only MCV (mm^3) showed an increase but it was non-significant ($P > 0.05$) when compared with the controls (Table). In addition, 6 of the stressed died fish near the end of experiment on days 10 and 11, whereas the other fish died after the completion of the experiment. No mortality was recorded in the control group.

Table. Haematological parameters of *Saprolegnia*-infected *Tinca tinca*.

Parameters	Control Fish	Infected Fish	Significance	
	Mean ± SE (Range)	Mean ± SE (Range)	P = 0.05	% Change
Hct (%)	24.66 ± 0.56 (18.90-30.20)	22.78 ± 0.88 (12.63-36.84)	P > 0.05	−7.66
Hb (g/dL)	6.89 ± 0.19 (4.80-9.10)	5.04 ± 0.23 (3.10-7.70)	P < 0.05*	−26.85
RBCs (′$10^6/mm^3$)	1.48 ± 0.05 (0.98-2.10)	1.17 ± 0.04 (0.80-1.95)	P < 0.05*	−20.94
MCV (mm^3)	186.79 ± 3.26 (149.90-216.70)	201.45 ± 8.12 (118.03-307.30)	P > 0.05	+ 7.84
MCH (pg)	45.97 ± 0.54 (40.80-50.98)	43.12 ± 2.06 (22.54-72.44)	P > 0.05	−6.19
MCHC (g/dL)	24.80 ± 0.27 (21.90-27.30)	22.55 ± 0.91 (12.69-33.94)	P < 0.05*	−9.06
Lct (%)	0.71 ± 0.01 (0.57-0.91)	0.53 ± 0.03 (0.32-1.07)	P < 0.05*	−24.14
WBCs (′$10^3/mm^3$)	45.65 ± 0.75 (33.20-52.10)	39.97 ± 3.13 (19.20-84.0)	P > 0.05	−12.44

n = 72, *significance, +: % increase, −: % decrease

Discussion

The normal values for *Tinca tinca* blood reported in the present study were not very different from the normal values reported by other researchers for *Tinca tinca*. For example, Eddy (15) reported Hct 24.1 (%), Hb 6.78 (g/dL), RBCs 1.05 (´10^6/mm^3), MCV 244.9 (mm^3), MCH 74.7 (pg), and MCHC 33.1 (g/dL). Collazos et al. (16) reported Hct 56.0 (%), RBCs 1.0 (´10^6/mm^3), and WBCs 38.5 (´10^3/mm^3). Jensen and Weber (17) reported Hct 24.1 (%) and Hb 4.98 (g/dL). In the present study, these were Hct 24.66 (%), Hb 6.89 (g/dL), RBCs 1.48 (´10^6/mm^3), MCV 186.79 (mm^3), MCH 45.97 (pg), MCHC 24.80 (g/dL), WBCs 45.65 (´10^3/mm^3), and Lct 0.71 (%).

Regarding *Saprolegnia* infection, it is evident from the data that saprolegniasis caused anaemia, immunosuppression, haemodilution and osmotic imbalance, mucus release, lethargy, and mortality in *Tinca tinca*. Anaemia in different fish species has been reported to be due to erythrocyte fragility and haemorrhaging (10), inhibition of erythrocyte production or increased erythrocyte destruction due to damage in haematopoietic tissues, haemodilution (18,19), accelerated erythroclasis due to altered membrane permeability and/or increased mechanical fragility (20) and haemolysis (21). The decrease in leucocytes has been reported to be due to increased secretion of corticosteroids and haemodilution (18,21). However, in the present study, the decreases in blood parameters of *Tinca tinca* were attributed to: (i) impairment in gill tissues, (ii) stress-mediated hormonal imbalance, (iii) rupturing of erythrocytes in skin lesions by direct action of the parasite, and (iv) haemodilution caused by haemorrhage. The mortality was caused by the breakdown in osmotic balance when the tissues (skin and muscular layer) were destroyed by the penetration of the hyphae and the lethargy that resulted from excessive energy exerted to overcome infection stress. The increase in mucus thickness on the gills observed in the present study may increase the diffusion distance between water and blood haemoglobin, which may rapidly impair O_2 and CO_2 exchange. The impairment in gas exchange triggers erythropoiesis to increase the number of erythrocytes to maintain haemoglobin at normal levels. However, it is possible that prolonged or continuous stimulation of a system may cause suppression or exhaustion of this capacity, resulting in decreased erythrocytes, as reported for leucocytes in fish (14). The secretion of corticosteroids is a non-specific response to any environmental stress and is a fundamental mechanism in the increased susceptibility of fish to disease (19). These stressors may elicit both neuroendocrine and cellular stress responses, which affect osmoregulation and disease resistance (22). Decreases in erythrocytes and leucocytes because of increased levels of stress hormones have been reported (13,18). The *Saprolegnia*-infected fish were highly stressed because of body lesions and therefore there is a possibility of the triggering of corticosteroids. The accumulation of large amounts of pus on the skin of *Tinca tinca* may be due to death of leucocytes and rupturing of erythrocytes by the direct action of the parasite. A decrease in lymphocyte counts in trout because of fungal infection has been reported (23). The involvement of non-leucocytic cells in the cellular defence against *Saprolegnia* infection in *Salmo trutta* has also been reported (24). A drop in Hct, Hb, and Rbc in sea bass (*Dicentrarchus labrax*) infected with parasites has been reported (25).

In addition to other physiological impairments, the haemodilution caused by haemorrhage and the breakdown in osmotic balance when the tissues (skin and muscular layer) are destroyed by the penetration of the hyphae was recorded as an important factor in the mortality of fish; however, lethargy was found to be another mortality-promoting factor. The infected *Tinca tinca* did not feed, met the high-energy demand to cope with infection stress, and increased energy expenditure on the production and secretion of mucus on the gills and skin, the common sites of parasite infection (11,26) during stressful conditions, resulting in high energy loss causing lethargy and ultimately death. Increased participation of proteins in energy metabolism in response to an increased energy demand to cope with stress and lethargy due to impairments in carbohydrate metabolism has been reported (27,28). Lethargy, loss of equilibrium, and death due to haemodilution in *Saprolegnia*-infected fish have been reported (7,8). Mortality in *Saprolegnia*-infected fish has also been reported to depend upon the initial site of infection, type of tissue destroyed, growth rate of fungus, and ability of individual fish to withstand the stress (6).

In conclusion, the study reveals that saprolegniasis causes anaemia, immunosuppression, haemodilution and osmotic imbalance, mucus release, lethargy, and mortality in tench as evident from the impaired haematological parameters along with additional factors. The purpose of the present study was to investigate the physiological impairments in tench caused by *Saprolegnia* infection in laboratory conditions, which were significant; however, it is the first step of physiological studies of *Saprolegnia*-infected tench, which needs further investigations at population level in natural habitats under similar infection-stressed conditions.

References

1. Grosch, U., Rennert, B., Hilge, V.: Development and use of surface waters and the fate of the related fisheries in the Berlin area of Germany. Fish. Manag. Ecol., 2000; 7: 179-188.
2. Noga, E.J.: Fish Disease: Diagnosis and Treatment. Mosby-Year Book, Inc., St. Louis, MO, 1996; pp: 367.
3. Pickering, A.D.: Factors which predispose salmonid fish to saprolegniosis. In: Mueller, G.J. Ed., Salmon and Saprolegniosis. U.S. Department of Energy, Bonneville Power Administration, Portland, Oregon, 1994; 67-84.
4. Stueland, S., Hatai, K., Skaar, I.: Morphological and physiological characteristics of *Saprolegnia* spp. strains pathogenic to Atlantic salmon, *Salmo salar* L. J. Fish Dis., 2005; 28: 445-453.
5. Willoughby, L.G.: Fungi and Fish Diseases. Pisces Press, Stirling, Scotland, 1994; pp: 57.
6. Pickering, A.D., Willoughby, L.G.: Saprolegnia infections of Salmonid fish. In: Roberts, R.J. Ed., Microbial diseases of fish. Academic Press, London, 1982; 271-297.
7. Bruno, D.W., Poppe, T.T.: A Colour Atlas of Salmonid Diseases. Academic Press, London, 1996; pp. 189.
8. Hatai, K., Hoshiai, G.I.: Pathogenicity of *Saprolegnia parasitica* Coker. In: Mueller, G.J., Ed. Salmon and Saprolegniosis. U.S. Department of Energy, Bonneville Power Administration, Portland, Oregon, 1994; 87-98.
9. Howe, G.E., Stehly, G.R.: Experimental infection of rainbow trout with *Saprolegnia parasitica*. J. Aquat. Anim. Health, 1998; 10: 397-404.
10. Shah, S.L., Altindag, A.: Haematological parameters of tench, (*Tinca tinca* L.) after acute and chronic exposure to lethal and sublethal mercury treatments. Bull. Environ. Contam. Toxicol., 2004; 73: 911-918.
11. Jones, S.R.M.: The occurrence and mechanisms of innate immunity against parasites in fish. Dev. Comp. Immunol., 2001; 25: 841-852.
12. Blaxhall, P.C., Daisley, K.W.: Routine haematological methods for use with fish blood. J. Fish Biol., 1973; 5: 771-781.
13. McLeay, D.J., Gordon, M.R.: Leucocrit: a simple haematological technique for measuring acute stress in salmonid fish, including stressful concentrations of pulp mill effluent. J. Fish. Res. Board Canada, 1977; 34: 2164-2175.
14. Gill, T.S., Pant, J.C.: Erythrocytic and leukocytic responses to cadmium poisoning in a freshwater fish *Puntius conchonius* Ham. Environ. Res., 1985; 36: 327-337.
15. Eddy, F.B.: Oxygen dissociation curves of the blood of the tench, *Tinca tinca*. J. Exp. Biol., 1973; 58: 281-293.
16. Collazos, M.E., Ortega, E., Barriga, C. Rodriguez, A.B.: Seasonal variation in haematological parameters in male and female *Tinca tinca*. Mol. Cell Biochem., 1998; 183: 165-168.
17. Jensen, F.B., Weber, R.E.: Respiratory properties of tench blood and haemoglobin adaptation to hypoxic-hypercapnic water. Mol. Physiol., 1982; 2: 235-250.
18. McLeay, D.J.: Effects of a 12-hr and 25-day exposure to kraft pulp mill effluent on the blood and tissues of juvenile coho salmon, *Oncorhynchus kisutch*. J. Fish. Res. Board Canada, 1973; 30: 395-400.
19. Wepener, V., Van Vuren, J.H.J., Du Preez, H.H.: Effect of manganese and iron at a neutral and acidic pH on the haematology of the banded tilapia (*Tilapia sparrmanii*). Bull. Environ. Contam. Toxicol., 1992; 49: 613-619.
20. Gill, T.S., Epple, A.: Stress-related changes in the haematological profile of the American eel (*Anguilla rostrata*). Ecotoxicol. Environ. Saf., 1993; 25: 227-235.
21. Tort, L., Torres, P., Flos, R.: Effects on dogfish haematology and liver composition after acute copper exposure. Comp. Biochem. Physiol. C, 1987; 87: 349-353.
22. Ackerman, P.A., Forsyth, R.B., Mazur, C.F., Iwama, G.K.: Stress hormones and the cellular stress response in salmonids. Fish Physiol. Biochem., 2000; 23: 327-336.
23. Alvarez, F., Razquin, B., Villena, A. López Fierro, P., Zapata, A.: Alterations in the peripheral lymphoid organs and differential leukocyte counts in *Saprolegnia*-infected brown trout, *Salmo trutta fario*. Vet. Immunol. Immunopath., 1988; 18: 181-193.

24. López-Dóriga, M.V., Martínez, J.L.: Ultrastructure of fish cells involved in cellular defences against *Saprolegnia* infections: evidence of non-leucocytic nature. Dis. Aquat. Organ., 1998; 32: 111-117.

25. Alvarez-Pellitero, P., Pintó, R.M.: Some blood parameters in sea bass, *Dicentrarchus labrax,* infected by bacteria, virus and parasites. J. Fish Biol., 1987; 31: 259-261.

26. Malte, H.: Effects of aluminum in hard, acid water on metabolic rate, blood gas tensions and ionic status in the rainbow trout. J. Fish Biol., 1986; 29: 187-198.

27. De Smet, H., Blust, R.: Stress responses and changes in protein metabolism in carp, *Cyprinus carpio* during cadmium exposure. Ecotoxicol. Environ. Saf., 2001; 48: 255-262.

28. Shah, S.L.: Behavioural abnormalities of *Cyprinion watsoni* on exposure to copper and zinc. Turk. J. Zool., 2002; 26: 137-140.