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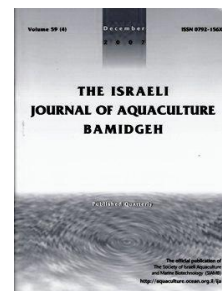
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Hematological Changes in Common Carp (*Cyprinus carpio*) and Rainbow Trout (*Oncorhynchus mykiss*), Infected with *Saprolegnia* spp.

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Key words: hematology, *Cyprinus carpio*, *Oncorhynchus mykiss*, *Saprolegnia*

Abstract

The present study was conducted to investigate the physiological impairment in common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) infected with *Saprolegnia* spp. The following hematological parameters were studied: total erythrocyte count (TEC), total leukocyte count (TLC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Except TLC, which significantly increased in the infected fish, all parameters decreased in response to infection. Decreases in Hb, TEC, and Hct were significant, indicating that saprolegniosis causes anemia and immunosuppression.

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Introduction

Fish are important sources of food and recreation, and key units in many natural food webs. Common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) are important coldwater fish species of commercial interest in many countries. Changes such as stress and immunosuppression allow infection to develop quickly (Pickering, 1994; Frans et al., 2008). *Saprolegnia* spp., generally termed water molds, cause saprolegniosis, a fungal disease in coldwater fish that appears as cotton-like circular, crescent-shaped, or whorled patterns on the surface of the animal, and affects not only the animal itself but also the eggs by penetrating the egg membrane (Willoughby, 1994).

Fish infected with *Saprolegnia* are easily recognized by cotton-like white to grayish patches on the skin and gills that are visible to the naked eye (Stueland et al., 2005). The infection progresses very quickly and often results in mortality, causing huge losses of fish and ova (Howe and Stehly, 1998; Stueland et al., 2005). Study of infected fish is essential to determine the efficacy of antifungal treatments (Howe and Stehly, 1998), and to understand the mode of action of infection and the resistance capability of fish.

Hematology provides an index of the physiological status of fish (Shah and Altindag, 2004). Its study is important for the development of health management in the rapidly growing aquaculture industry. Generally, hematological parameters are measured to determine the effects of toxic substances on fish, evaluate the condition of fish, evaluate the non-specific resistance of fish breeds, strains, and broodfish, assess the suitability of feeds and feed mixture pellets, evaluate the effect of stress, etc. In the present investigation, we evaluated alterations in the following important hematological parameters after fungal infection in coldwater fish: total erythrocyte count (TEC), total leukocyte count (TLC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

Materials and Methods

Adult common carp were collected from the Directorate of Coldwater Fisheries Research (DCFR) farm in Bhimtal; rainbow trout were collected from the state trout farm in Bairangna (Uttarakhand). The fish were fed a balanced ration and provided continuous aeration. Blood samples were taken one, two, and three weeks after the appearance of the infection. Blood of 50 fish was pooled for each sample and hematological parameters of triplicate samples were measured (total 150 healthy and 150 infected fish).

Blood samples were collected from the caudal vein using sterilized disposable 2-ml syringes. Total erythrocyte count (TEC) and total leukocyte count (TLC) were determined in a hemocytometer crystalline chamber using diluting fluids. Sahli's hemoglobinometer was used to estimate hemoglobin (Hb). Packed cell volume (PCV) was estimated using microhematocrit. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Reddy and Bashamohideen (1989). Statistical analysis was done using one-way ANOVA at a 5% level of significance.

Results

Changes in hematological parameters are given in Table 1. Hb and TEC were significantly lower in infected *C. carpio* and *O. mykiss* than in the control fish while TLC was significantly higher.

Discussion

To investigate changes in fish blood factors, these factors must be measured in healthy fish. The quality and quantity of leukocytes are generally used to determine immune reactions and diseases (Cagirgan, 1990). Leukocytes are normally lower in healthy fishes than in infected fish, and can be used as an indicator of infectious disease. All the blood parameters of the control group of *O. mykiss* in our study were similar to values in a control group of the same species, reported by Atamanalp et al. (2008).

Table 1. Changes in hematological parameters (means±SD) of common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) due to *Saprolegnia* infection (n = 50).

	Control ¹	Infected group		
		Week 1	Week 2	Week 3
<i>Common carp</i>				
Hemoglobin (Hb; %g)	5.8±0.24	4.9±0.36	4.5±0.42	4.0±0.38*
Total erythrocyte count (TEC; 10 ⁶ /mm ³)	1.7±0.10	1.5±0.14	1.4±0.12	1.3±0.16*
Total leukocyte count (TLC; 10 ³ /mm ³)	16.2±2.1	17.8±1.8	19.6±1.6	20.4±1.9*
Packed cell volume (PCV; %)	31.70±1.85	27.55±1.85	25.53±1.40	23.04±1.85
Mean corpuscular volume (MCV; µm ³)	186.47±21.2	183.33±19.4	182.14±20.2	176.92±21.7
Mean corpuscular hemoglobin (MCH; pg)	34.11±3.7	32.66±3.1	32.14±3.6	30.76±3.1
Mean corpuscular hemoglobin concentration (MCHC; %)	18.29±1.7	17.78±1.6	17.62±1.4	17.36±1.8
<i>Rainbow trout</i>				
Hemoglobin (Hb; %g)	6.4±0.47	6.1±0.23	5.8±0.32	5.5±0.21*
Total erythrocyte count (TEC; 10 ⁶ /mm ³)	0.78±0.11	0.76±0.17	0.73±0.13	0.70±0.14*
Total leukocyte count (TLC; 10 ³ /mm ³)	73±7.2	75±7.6	78±7.1	82±7.9*
Packed cell volume (PCV; %)	44.00±4.3	42.00±3.9	40.00±4.1	38.00±3.8
Mean corpuscular volume (MCV; µm ³)	564.10±46.2	552.63±49.5	547.94±44.3	542.85±48.9
Mean corpuscular hemoglobin (MCH; pg)	82.05±8.6	80.26±8.1	79.45±7.5	78.57±7.6
Mean corpuscular hemoglobin concentration (MCHC; %)	14.54±1.2	14.52±1.6	14.50±1.4	14.47±1.3

¹ Values for the control were the same in each of the three weeks.

* significantly different from the control, $p < 0.05$

In the present study, the increases in leukocytes in infected fish were accepted as a response of the cellular immune system to fungal infection. The immune systems of fish display a similar response to unfavorable conditions (Palikova and Navratil, 2001) and findings were similar in Caspian salmon (*Salmo trutta caspius*) infected with *Saprolegnia* (Jamalzadeh et al., 2009). Leukocytes are important cells in the immune system because of their main defensive function. Due to the fungal infection, the leukocyte counts were enhanced, indicating that fish can develop a defensive mechanism to overcome the stress caused by infection.

The lower erythrocyte counts in the infected *C. carpio* and *O. mykiss* were similar in Caspian salmon (Jamalzadeh et al., 2009). This reduction can be caused by a lack of sufficient oxygen due to fungal infestation of the gills and can lead to greater destruction of erythrocytes or a lower rate of formation of erythrocytes because of a lack of Hb in the cellular medium (Chen et al., 2004).

The significance of observing PCV is to evaluate the effect of stressors on fish health and to determine the oxygen carrying capacity of the fish blood. MCV, MCH, and MCHC are completely dependent on the levels of PCV, TEC, and Hb in the blood. In the present study, PCV, TEC, and Hb concentrations were altered in the infected fish. Therefore, MCV, MCH, and MCHC were lower in the infected fish than in the control. These measurements could be a useful diagnostic tool for saprolegniosis infection in cultivable coldwater fish species such as common carp and rainbow trout.

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