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Comparative study of hematological variation in healthy and fungal infected Kalabans, *Bangana dero* (Hamilton, 1822)

Siddiqui U.¹; Shah R.H.^{2*}; Amrita R.²; Tudu K.²; Kumar S.²; Bisht H.C.S.¹; Pandey N.N.²

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- 1-Department of Zoology, D. S. B. Campus, Kumaon University, Nainital-263002, Uttarakhand, India.
- 2-ICAR-Directorate of Coldwater Fisheries Research. Bhimtal-263136, Uttarakhand, India

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Introduction

Hematological studies have generally been used as an effective and sensitive index to monitor physiological and pathological changes in fishes. Fish blood is being studied increasingly in toxicological research and environmental monitoring as a possible and indicator of physiological pathological changes in fishery management disease investigations (Mulcahy, 1975; Sampath et al., 1993). However, fishes can adapt themselves environmental according the to conditions by changing their physiological activities up to optimum range but in extreme change in water quality i.e. dissolved oxygen level, free carbon dioxide level and hardness, pH, total dissolved solids and presence and virulence of pathogens can change the hematological parameters than normal values or control.

Qualitative and quantitative variations in haematological parameters

including the red blood cell (RBC), haemoglobin (Hb), packed cell volume (PCV), mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), erythrocyte sediment rate(ESR) are significant findings for determination the of anemia, polycythemia, inflammation and infection while total white blood cell numbers (WBC) and differential leukocyte count viz, neutrophils, lymphocyte, eosinophils and monocyte significant findings determination in pathological condition viz. leucocytosis and leucopenia (Eroh et al., 2003; Shah et al., 2009).

The analysis of blood indices has proven to be a valuable approach for analysing the health status of farmed animals these indices provide reliable information on metabolic deficiencies and chronic disorders. stress status before they are present in a clinical setting (Bahmani et al., 2001). Blood biochemistry parameters can be

^{*}Corresponding author's Email: rashadshah91@gmail.com

also used to detect the health of fish (De Pedro et al., 2005). Exogenous factors, such as management (Svobodova et al., 2008), diseases (Chen et al., 2005) and stress (Cnaani et al., 2004), always major changes induce in blood composition. For example, significant fluctuations were detected in concentrations of cortisol. glucose. cholesterol and other basic components in response to handling and hypoxic stress (Skjervold et al., 2001). The levels of cortisol and glucose are considered to be specific indicators of sympathetic activation during stress conditions (Lermen et al., 2004; Tekmedash et al., 2014). Abramis brama orientalis were examined by et (Hayatbakhsh al., 2014) evaluation changes of haematological regarding parameters parasitic infection, physical damages, morphological modifications, growth retardation, hematological changes, immune response disturbance, endocrine disruption, and behavioral changes. Cortisol and glucose are two of the most common stress indicators. Cortisol may be useful only in acute stress experiments monitored and throughout time.

Bangana dero (Hamilton, 1822), a common fish of food value in the Indian Himalayan region was targeted for the study. Saprolegniosis is very common in this species at all life stages under coldwater condition and captive rearing. It was estimated that 12-20% loss has been occurred due to mycosis, directly as mortality and indirectly as growth retardation and stress condition to the fish. This species is commonly

known as 'Kalabans' in India, 'Gardi' and 'Kathalegi' in Nepal, 'Kursa' in Bangladesh and widely distributed all along foot hill regions of Himalayan ranges. The body is ordinarily white and more linear having relatively small head and maximum size is has reported around 750 mm in total length. B. dero bottom feeding herbivorous (Masuda and Karki, 1980) and is a candidate species for aquaculture in mid altitudinal hills. B. dero (Ham.) is of immense economic value in the Uttarakhand state and is found almost throughout the year in the rivers Khoh, Nayar, Bhagirathi and Alaknanda of the Himalayan riverine ecosystems. Though, the faunistic studies biology of this fish has been done by earlier researchers (Hora Mukherjee, 1936; Lal and Chatterjee, 1962; Singh, 1964; Grover, 1971), hematological study is not yet to be reported. Attempt has been done to explore the hematological profile of wild and cultured stock of B. dero and its correlation with the very common saprolegniosis infection.

Materials and methods

40 Healthy and infected specimen of *B. dero* with the average weight of 470±12 g were collected during May-June, 2017 from the Directorate of Coldwater Fisheries Research (DCFR) farm in Bhimtal, Kosi and Ramganga river of Kumaon hills of Uttarakhand in India. Blood samples were taken from 10 healthy and 10 infected fish from each stock and hematological parameters of triplicate samples (3 times, 40 samples in each sampling) were measured.

Blood samples were collected from the caudal vain using sterilized disposable 2-ml syringes. Total erythrocyte count (TEC) and total leukocyte count (TLC) were determined in human hemocytometer crystalline chamber using diluting fluids. Sahli's hemoglobinometer was used to estimate hemoglobin (Hb). Packed cell volume (PCV) was estimated using corpuscular microhematocrit. Mean volume (MCV), mean corpuscular hemoglobin (MCH), and corpuscular hemoglobin concentration (MCHC) were calculated according to (Reddy and Bashamohideen, 1989). Plasma cortisol was measured by RIA. Plasma glucose (mMol/L. serum) was assayed by analysis commercial kit through colorimetric method using an Auto-analyser Technician. Statistical analysis was done using one-way ANOVA at a 5% level of significance.

Result and discussion

Blood samples collected form healthy and 10 infected fish from each wild and cultured stock were analysed in triplicates. The result summarized in the Table 1. The fungal infection was characterized saproligniosis with the observation of elongated tapering zoosporangia formed at the tips of somatic hyphae which were appearing darker and more granular having pear shaped primary zoospores. The data reveal that Hb and were significantly lower infected farmed and wild fish while TLC was significantly higher infected stock in wild as well as in cultured stock. Similarly, there was a significant difference between infected and non-infected B. dero in terms of cortisol, glucose which was measured as stress indicator.

Table 1: Changes in hematological parameters (means \pm SD) of Bangana dero due to Saprolegnia sp infection (n = 20).

Parameters	S 1	S 2	S 3	S 4
Hemoglobin (Hb; %g)	5.65 ±0.22	4.12 ±0.27*	5.24 ±0.08	4.23 ±0.11*
Total erythrocyte count (TEC; 106/mm³)	1.56 ±0.07	1.32 ±0.05*	1.44 ±0.04	1.20±0.073*
Total leukocyte count (TLC; 103/mm ³)	16.3 ± 0.4	18.4 ±0.1*	16.36 ± 0.56	19.2±0.360*
Packed cell volume (PCV; %)	30.63 ± 0.35	24.35 ± 0.39	30.65 ± 0.80	22.34±0.417
Mean corpuscular volume (MCV; μm³)	196.59 ±7.86	183.72 ±7.12	212.98 ±7.92	185.62±12.00
Mean corpuscular hemoglobin (MCH; pg)	36.28 ±2.10	31.07 ±2.32	36.44 ±0.81	35.2±3.014
Mean corpuscular hemoglobin concentration (MCHC; %)	18.44 ±0.54	16.9 ±0.88	17.12 ±0.62	18.93±0.640

^{*} Significantly different from the healthy p < 0.05

^{*}S 1- Wild healthy, S 2- Wild infected, S 3- Farmed healthy, S 4- Farmed infected

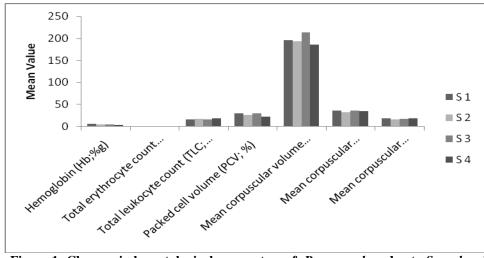


Figure 1: Changes in hematological parameters of *Bangana dero* due to *Saprolegnia* sp infection.

*S 1- Wild healthy, S 2- Wild infected, S 3- Farmed healthy, S 4- Farmed infected

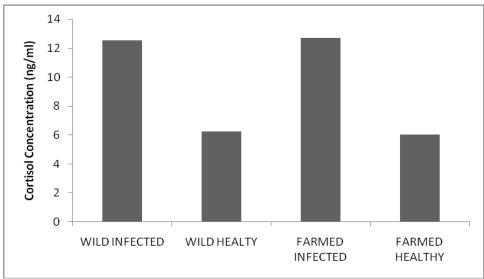


Figure 2: Comparison of cortisol concentration between healthy and infected wild and farmed *Bangana Dero*.

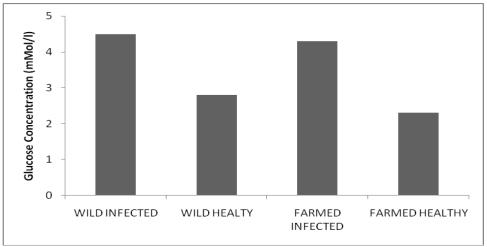


Figure 3: Comparison of glucose concentration between healthy and infected wild and farmed *Bangana dero*.

The quality and quantity of leukocytes are generally used to determine immune reactions and diseases (Cagirgan and Yildirim, 1990). The immune systems of fish display a similar response to unfavorable conditions (Palikova and 2001) Navratil, and findings were similar in Caspian salmon (Salmo trutta caspius) infected with Saprolegnia (Jamalzadeh et al., 2009). Leukocytes are important cells immune system because of their main defensive function. Leukocytes are normally lower in healthy fishes than in infected fish, and can be used as an indicator of infectious disease. In present study, the increasing trend of leukocytes supports previous findings having significant lower values in non infected healthy fish of wild and cultured stocks. Due to the fungal infection, the leukocyte counts were enhanced, indicating that fish can develop a defensive mechanism to overcome the stress caused by Hence, the increase infection. leukocytes from the normal range ie. 16.33±0.45 is an indicator of fungal infection.

In the present study, B. dero blood cells were characterized microscopically and hematological indices were analyzed. The mature erythrocytes of B. dero show an average size and ultrastructural features similar to those described for mature erythrocytes of other fish species (Watson et al., 1963; Hartman and Lessler, 1964; Conroy, 1972; Blaxhall and Daisley, 1973; Javaid and Akhtar, 1977; Nakamura and Shimozawa, 1984; Rowley et al., 1988; Groff and Zinkl,

1999; Esteban et al., 2000; Hrubec et al.. al.. 2000: Ueda etThrombocytes are the most abundant blood cells after erythrocytes, representing more than 50% circulating leucocytes (Ueda et al., 1997). However, some authors do not include thrombocytes within leucocytes (Hrubec et al., 2000, 2001; Orun and Erdemli, 2002; Ranzani-Paiva et al., 2003).

The ranges of serum biochemistry vary from species to species and can be influenced by many biotic and abiotic factors such as water temperature, seasonal pattern, food feed, age and sex of the fish (Jawad et al., 2004). The lower erythrocyte counts in the infected B. dero were similar in Caspian salmon (Jamalzadeh et al., 2009). reduction can be caused by a lack of sufficient oxygen due to 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 higher values of cortisol and glucose were observed in infected fish compared to healthy individuals which indicates the stressful condition to infected fish and are in the conformity of the previous report (Tekmedash *et al.*, 2014).

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 due to fungal infection and calculated values are comparatively lower in the infected fish. These measurements clearly reflect the diseased condition of fish in wild as well as in pond environment. The increasing trend of leukocytes and decrease in other parameters could be a useful diagnostic tool for saprolegniosis infection in cultivable and wild fish species.

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