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Haematological and Biochemical Alterations in Indian major carp, *Labeo rohita* due to Saprolegniasis

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Abstract

The present study was carried out to investigate the haematological and biochemical indices in healthy and *Saprolegnia sp*. infested *Labeo rohita.* The infested fish showed significant reduction (P<0.05) in RBCs, haemoglobin, haematocrit (PCV) eosinophils and lymphocytes. The derived erythrocyte indices, namely, the MCV, MCH and MCHC of infested *Labeo rohita* were also significantly decreased (P < 0.05) from those of normal ones. Whereas, the total leucocyte count (TLC), neutrophils, monocytes and ESR were significantly increased (P<0.05) in infested fishes. The biochemical analysis showed a significant reduction (P<0.05) in bilirubin content, whereas the blood glucose, SGOT and SGPT were significantly increased (P<0.05) in *Labeo rohita* infested with saprolegniasis.

Keywords: Haematology, Anaemia, *Labeo rohita*, Saprolegniasis

Introduction

Fishes provide a protein-rich diet and are also a source of income in developing countries. Fish production is not only important economically but also for food security and social development in many countries, so the widespread disease resulting from an interaction between pathogen, host and environment should be handled to overcome this problem (Muktar *et al.*, 2016). The health of fish can be affected by environmental factors (stress), nutrition as well as pathogens. Stress in fishes may be induced by various abiotic environmental factors such as changes in water temperature, pH, oxygen concentration, water pollutants including pesticides (Meier *et al.*, 1983; Lebelo *et al.*, 2001) petroleum products and heavy metals (Witeska, 2005). Biotic interactions such as predator pressure, parasite invasion or strong competition with other organisms or among the fish in overcrowded areas and by human activities related to fish rearing and harvesting (manipulation and transport) can also be a source of stress to fish (Witeska, 2005).

Fish parasites are of economic importance in that they affect the productivity of fish through mortalities by decreasing growth rate, efficiency of feeding and levels of the total plasma proteins. Due to a fall in absorbed amino acids that are

essential for protein synthesis as well as lowering the quality of the meat (Fraser and Maya, 1986).

Saprolegnia sp. is a freshwater mold and causes the disease, Saprolegniasis. The disease is also known as cotton wool disease due to the appearance of cotton-like whitish and greyish patches on the skin and gills of affected fish. Saprolegniasis causes high mortality in fishes and is widely distributed in freshwater ecosystem, affecting wild and cultured fishes and considered the main cause of economic loss in the fish farming industry (Pottinger and Day, 1999; Hussain *et al.*, 2001). All life stages of fish such as eggs, fry, fingerlings and adult fish may suffer due to saprolegniasis. The fungus can be transmitted through infected fish, eggs, water and equipment (Bruno and Wood, 1999). Handling, poor water quality such as water with slow circulation, low dissolved oxygen, high ammonia content, crowding and decreasing temperature all help the fungus to establish itself (Ali, H. *et al.*, 2011).

Blood is a liquid connective tissue that provides a clear image of all pathological activities occurring in the animals. It is a fluid containing cells and transports oxygen, water, food materials, CO_2 and other products of metabolism as well as internal secretions. Haematological values change depending on the fish species, age, parasitic effect, stress and health condition of the fish (Hrubec *et al.*, 2000). Haematological analysis can provide valuable knowledge for monitoring the health condition of both wild and cultured fishes. Qualitative and quantitative variations in haematological and biochemical parameters including the RBC counts, TLC, DLC, Hb content, size of RBC, WBC, blood glucose, blood urea, bilirubin, SGOT and SGPT are the most significant findings as regards diagnosis.

Labeo rhita is a common Indian major carp and is widely distributed in Indian rivers, canals and ponds. It is a very important food fish and has high-quality palatable flesh that makes it highly demanded and the first choice for fish eaters. Therefore the present study was aimed to investigate the impact of the fungal disease, saprolegniasis on haematological and biochemical parameters of Indian major carp, *Labeo rohita.*

Materials and Methods

Adult healthy and *Saprolegnia sp.* infested *Labeo rohita* were netted with the help of local fishermen from different sites of river Rapti, district Shravasti and transported to the Ichthyology Lab., Department of Zoology, Govt. Degree College, Shravasti and kept separately in a tank of 500 L capacity. The fishes were acclimatized for 24 hours and subjected to parasitological and haematological investigations. Parasitological examination was carried out by detection of *Saprolegnia sp*. on skin and gills. The diseased fishes had cotton-like white to greyish patches, radiating in circular, crescent or whorl patterns on skin and gills.

Twenty blood samples from non-infested and twenty-five blood samples from infested fishes were obtained from the caudal artery and collected in the heparinized tube and then stored in polyethylene cool bags until analysed. RBCs and WBCs were counted by haemocytometer and values were calculated as $10^6/mm^3$ and $10^3/mm^3$ (Wintrobe, 1967). Haemoglobin content in blood was determined by haemoglobin kit. MCV, MCH and MCHC were calculated by using standard formula according to Dacie and Lewis, 1975. Differential leucocyte counts (DLC) were made by using blood smears and films fixed in Leishman's stain. The blood glucose was estimated by standard methods. Bilirubin and blood urea were estimated by the method given by Varley, 1975. The level of SGOT and SGPT in serum was estimated according to Frankel, 1963. All the haematological and biochemical values of healthy and infested fishes were analyzed by using the student 't' test (Biradar, 1988). Differences were considered to be significant at $P<0.05$.

Results

In the present study, twenty apparently healthy and twenty-five diseased *Labeo rohita were* sampled. Statistical analysis showed that the

haematological and biochemical profiles of healthy fishes were significantly different from those of the diseased fishes.

Comparisons of haematological parameters of normal/ healthy and *Saprolegnia sp.* infested *Labeo rohita* are presented in Table 1. The values of RBCs count, Hb, PCV, MCV, MCH and MCHC were 2.35 × 10^6/mm^3, 7.27%, 33.56%, 142.80 µm^3, 30.93 pg and 21.66%, in normal fishes but 1.90×10^6/mm^3, 5.58%, 26.75%, 140.79 µm^3, 29.36 pg and 20.85% in *Saprolegnia sp.* infested fishes, respectively.

The results showed that the primary erythrocyte indices, namely, RBC counts, haematocrit (PCV) levels and haemoglobin concentrations, were significantly decreased ($P < 0.05$) in infected fishes than in the healthy/normal fish. However, derived erythrocyte indices, namely, the MCV, MCH and MCHC of infested fishes were also significantly decreased ($P < 0.05$) from those of healthy/normal fishes.

The ESR, clotting time and leucocyte counts were 2.60 mm/hr, 124.0 seconds and 6.70 × 10^3/mm^3 in normal fishes whereas 3.25 mm/hr, 131.0 seconds and 8.32 ×10^3/mm^3 in infested fishes. The ESR, clotting time and total leucocyte counts of infested fishes were significantly higher ($P < 0.05$) than the normal ones.

The distribution of individual leucocytes (DLC) i.e. neutrophils, monocytes, lymphocytes and eosinophils were 34.50%, 1.08%, 51.40% and 12.92% in normal fishes whereas 38.45, 1.21, 50.06 and 10.28 in infested fishes respectively. There was a significant increase in the percentage of neutrophils and monocytes ($P<0.05$) in infested fishes. The percentage of lymphocytes and eosinophils in the blood of infested fishes was found to be significantly lower ($P < 0.05$) than the healthy/normal fishes.

Table 1: Changes in haematological and biochemical parameters of *Labeo rohita* due to Saprolegniasis.

Parameters of Blood	Normal		Diseased		Deviation (%)	t-test
	Range	Mean ± SD	Range	Mean ± SD		
RBC (×10^6/mm^3)	2.17-2.55	2.35±0.25	1.80- 2.10	1.90±0.42	-19.15%	4.22*
WBC (×10^3/mm^3)	6.25-7.10	6.70±0.25	7.75- 8.92	8.32±0.41	+24.18%	15.49*
Hb. (gm%)	6.27-8.12	7.27±1.43	5.10-6.45	5.58±0.59	-23.25%	5.37*
PCV (%)	30.25-36.80	33.56±2.80	24.50-30.15	26.75±3.85	-20.29%	6.62*
MCV (µm^3)	139.40-144.31	142.80±1.95	136.11-143.57	140.79±2.78	-1.41%	2.73*
MCH (Pg)	28.89-31.84	30.93±1.32	28.33-29.76	29.36±.51	-5.08%	5.47*
MCHC (%)	20.73-22.06	21.66±0.58	20.81-21.39	20.85±.83	-3.74%	3.69*
ESR (mm/h)	2.25-2.75	2.6±0.098	2.95-3.90	3.25±0.25	+25.00%	10.95*
CT (second)	120-127	124±2.30	126-135	131±4.25	+5.64%	6.62*
Lymphocytes %	50.40-52.8	51.40+2.32	50.18-54.18	50.06±0.32	-2.60%	2.86*
Monocytes %	1.0-1.10	1.08±0.05	1.10-1.32	1.21±0.11	+12.03%	4.88*
Neutrophils %	30.0-37.0	34.50±2.12	34.10-40.18	38.45±2.86	+11.45%	5.14*
Eosinophils %	12.75-14.18	12.92±3.11	9.72-11.20	10.28±0.42	-20.43%	4.20*
Bilirubin (mg/ml)	2.40-2.52	2.48±0.18	1.98-2.50	2.12±0.18	-14.51%	6.66*
Blood glucose (mg/ml)	56.72-60.11	58.18±1.85	61.15-65.75	63.35±2.27	+8.89%	8.22*
Blood urea (mg/ml)	5.90-6.70	6.21±0.41	6.02-7.11	6.51±1.32	+4.83%	0.97
SGOT (unit/gm)	55.70-59.18	58.13±0.42	62.14-65.70	63.82±2.11	+9.79%	11.84*
SGPT (unit/gm)	28.11-32.14	30.52±0.31	36.14-39.92	38.12±2.83	+24.90%	11.92*

* = significant ($P < 0.05$)

Comparison of biochemical parameters of normal/healthy and infested *Labeo rohita* are presented in Table 1. The concentration of bilirubin, blood glucose level, blood urea, Serum Glutamic Oxaloacetate Transaminase (SGOT) and Serum Glutamic Pyruvate Transaminase (SGPT) levels was 2.48 mg/ml, 58.18 mg/ml, 6.21, 58.13 unit/gm and 30.52 unit/gm in normal *Labeo rohita* but 2.12 mg/ml, 63.35 mg/ml, 6.51 unit/gm, 63.82 unit/gm and 38.12 unit/gm in infested fish respectively. Thus the bilirubin was significantly decreased ($P<0.05$) in infested fishes. The concentration of Blood Sugar, SGOT and SGPT were significantly increased ($P<0.05$) in infested fishes in comparison to healthy *Labeo rohita*.

Discussion

The results of the present investigation indicate that the Saprolagniasis in *Labeo rohita* have produced alterations in the haematological and biochemical parameters. The results showed a significant reduction in the primary erythrocytic indices, namely, total erythrocyte count, haematocrit (PCV) and haemoglobin concentration, leading to anaemia. Anaemia could be attributed to increasing destruction or loss of erythrocytes and/or suppression of erythropoiesis. The significant reduction in RBC count, Hb. value and PCV in *Saprolegnia sp*. infested *Labeo rohita* is consistent with the previous report on *Saprolegnia* infected *Tinca tinca* (Shah, 2010), *Catla catla* (Ali *et al.*, 2011), *Clarias gariepinus* (Chauhan. *et al.*, 2014), *Labeo rohita* (Debnath *et al.*, 2017) and rainbow trout, *Oncorhynchus mykiss* (Shah *et al.*, 2015). The anaemia was also reported in various parasites infested Bagrids (Omeji *et al.*, 2018), cutaneous ulcerated catfish *Clarias batrachus* (Ali and Ansari, 2023), copepod, *Ergasilus mosulensis* infested *Planiliza abu* (Salem, *et al*., 2023) and Asian cichlid fish, *Etroplus suratensis* (Pathiratne and Rajapakshe 1998).

If the erythrocyte destruction rate is increased without suppression of erythropoiesis, it may affect the proportion of immature and smaller erythrocytes. This should be reflected by a decline in the derived erythrocytes indices, MCV, MCH and MCHC because younger and immature erythrocytes are smaller in size and contain less haemoglobin content (Blaxhell and Daisley, 1973). Thus significant reduction in derived erythrocytic indices of *Saprolegnia sp.* infested fishes in the present study proved that reductions in the primary erythrocyte indices were due to an increased proportion of immature erythrocytes. Kumar et al., (2023) reported decreased values of MCV, MCH, and MCHC with the rise in water temperature in *Labeo rohita*, but Shah *et al.*, (2015) reported a slight increase in values of MCV and MCH in *Saprolegnia sp.* infested rainbow trout, *Oncorhynchus mykiss*. Another reason for the anaemia in infested fish may be due to that the mycelia of *Saprolegnia* penetrate deep causing wounds resulting in the loss of blood (Jauncey and Ross, 1982) and may be due to loss of body fluid from haemorrhagic lesions in severely affected fish (Ali and Ansari, 2023).

Total leucocyte counts of the Labeo rohita infested with saprolegniasis, indicate leucocytosis (i.e. increase in TLC). The percentage distribution of different types of leucocytes (i.e. DLC) in infested fish showed significant changes from the normal distribution pattern. The percentage of neutrophils and monocytes was found to have increased significantly along with a marginal decrease in the percentage of lymphocytes and eosinophils. Shah (2010) reported that infection of various stress-related factors causes tissue damage which leads to an increase in granulocytes. The elevation of TLC in fish is due to a defence mechanism against parasitic infection (Zaki, *et al*., 2008; Movahed,. et al., 2016, Radwan et al., 2021). Neutrophils have also been observed to be capable of phagocytic activity (Finn and Nelson, 1971). The increased percentage of neutrophils in the circulating blood of *Saprolegnia sp. infested Labeo rohita* may be related to their cellular immune function as a response to the local inflammation and increasing damage to the tissues due to deep penetration of fungal hyphae in the skin of infested fish. The marginal decrease in lymphocytes in infested fishes may be

associated with decreased humoral immune response in fish. Similar findings were reported by Shah *et al.,* (2015) in *Saprolegnia* infested rainbow trout, *Oncorhynchus mykiss*, by Jamalzadeh *et al.,* (2009) in *Saprolegnia* infected Capsin Salmon, *Salmo trutta fabrio* and by Chauhan *et al.,* (2014) in *Saprolegnia* infested *Clarias gariepinus*.

Bilirubin is one of the bile pigments released from the liver. It is an excretory catabolic product of haemoglobin and is excreted out in the urine. A decrease in the amount of bilirubin in infested fishes points to a possible hepatodysfunction, which causes less secretion of bilirubin into the blood and leads to hypobilirubinemia. Hypobilirubinemia was reported in several fishes inhabiting naturally as well as experimentally stressed fishes (Ali and Ansari , 2023; Ali *et al.*, 2011; Chaturvedi and Agarwal, 1993).

The increase in blood sugar level in *Saprolegnia sp.* infested *Labeo rohita* may be due to an increase in the breakdown of liver glycogen or due to decreased synthesis of glycogen from glucose. Similar findings were reported by Zaki *et al.* (2008) in *Tilapia nilotica* infested with *Saprolegnia parasitica* and by Yang and Chen (2003) in *Cyprinus carpio*. Hyperglycemic conditions in naturally as well as experimentally stressed fishes may be due to impairment in the hormone level in the blood involved in carbohydrate metabolism (Martin and Black, 1998; Chaturvedi and Agarwa, 1993; Shah *et al.*, 2015 and Omeji, *et al.*, 2018). Plasma glucose is elevated in stressed fish as a consequence of increased blood catecholamine (Wedemeyer *et al.*, 1990; Willoughby and Pickering, 1977; Talash and Gulhan, 2009). Thus hyperglycemia in *Saprolegnia sp.* infested fish seems to be due to reduced insulin secretion, increased corticosteroid and also stimulation of gluconeogenesis. It is due to the metabolization of glycogen deposits in the liver to the site of their active metabolism for the liberation of energy.

The elevated levels of serum transaminases (SGOT and SGPT), which are markers of liver functions, were observed in infested fish. This increased level of serum transaminases is related to disruption of normal metabolism which is due to extensive alterations in the liver cells and indicates liver damage. A significant increase in serum transaminases levels has been recorded in the chicks fed with *Aspergillus terreus* infested feed (Kiran, *et al.*, 2015), *Saprolegnia* infested *Catla catla* (Ali *et al.*, 2011) and cutaneous ulcerated *Clarias batrachus* (Ali and Ansari, 2023).

Conclusion

The present study provides valuable insights into the impact of *saprolgniasis* on the haematological and biochemical parameters of *Labeo rohita.* The infested fish showed significant alterations in their haematological parameters, including reduced RBC counts, Hb. and haematocrit (PCV) values indicating anaemia, while exhibiting an elevated TLC and neutrophils as a defence mechanism against the parasitic infestation. Alterations in biochemical parameters are related to the disruption of normal liver function. So, it can be concluded that saprolegniasis influences the health status of fish, which was reflected by alterations in the haematological and biochemical parameters of fish.

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