Alteration in haemato-biochemical profiles of rainbow trout *Oncorhynchus mykiss* affected by *Saprolegnia* spp- A potential constraint for culture of trout in Kashmir Himalaya

Shah A.F.[1]; Bhat A.S.[2]; Bhat F.A.[3]; Balkhi M.H.[4]; Abubakr A.[4]; Ahmad I.[5]*

Received: April 2013 Accepted: October 2014

Abstract

Haemato-biochemical studies in rainbow trout infected with *Saprolegnia* were carried out under temperate climatic conditions of Kashmir valley to find out the variation in blood parameters. The trial was carried out on 405 cultured rainbow trout fish ranging in length from 47.8 to 69.8 cm and in weight from 1300 to 1920 g. The same experiment was carried out on 2,70000.00 trout fish eggs from November 2010 to April 2011 at a trout fish farm, in Kokernag, India, on account of the susceptibility of eggs to fungal infestation. The infected fish showed signs of lethargy, irritation, loss of appetite, haemorrhages at the base of fins and deep wounds at the sites of severe infection associated with cottony wool like tufts on both the dorsal and ventral sides of the body. The fungi were isolated at high percentages from skin followed by fins and mouth. The haemato-biochemical profile was studied in forty (40) normal and forty (40) infected fish. The haemoglobin content, total erythrocyte count, packed cell volume, lymphocyte percentage, total serum protein, albumin and globulin levels decreased significantly ($p<0.05$) in the *Saprolegnia* infected fish as compared to that in the control. The white blood cells, erythrocyte sedimentation rate, mean corpuscular haemoglobin, mean cell volume, heterophill percentage and total serum glucose showed significant increase in the infected fish irrespective of sex. The infection was more pronounced during the winter season (Temp.<10°C) as compared to that in summer (temp.<17°C). Fungi induced stress leads to haemostatic imbalances in fish reflected in the haemato-biochemical profile and can thus be used as an indicator for *Saprolegnia* induced infection.

Keywords: Saprolegniasis, Fungal infection, Coldwater fish culture, Fish eggs.

1-Div.of Pathology, Faculty of Fisheries,SKUAST-Kashmir-Rangil, Ganderbal, Kashmir-India.
2-Trout Hatchery Farm,Achabal, Anantnag, Kashmir, India.
3- Div. of Fishery Biology, Faculty of Fisheries, SKUAST-KashmirRangil, Ganderbal, Kashmir-India.
4- Div. of Aquatic Environmental Management, Faculty of Fisheries, SKUAST-Kashmir. Rangil,Ganderbal, Kashmir-India.
5-Div of Genetics & Biotechnology, Faculty of Fisheries, SK-University of Agricultural Sciences and Technology of Kashmir-Rangil, Ganderbal, Kashmir-India.
*Corresponding author's email: ahmadirfan@skuastkashmir.ac.in

Introduction

Fungi are pathogens which invade the tissues of fish host rendering them susceptible to infection and other diseases (Allan and Stevenson, 1981; Austin and Austin, 1993). Several climatic factors are involved in the development of fungal infection in fish. Aquatic fungal moulds being opportunistic pathogens become active when the physiochemical parameters of water (Temp, DO, pH, etc.) and availability of susceptible host change which ultimately leads to dermatomycosis. The fungi responsible for dermatomycosis are secondary pathogens and lesions are commonly seen after mishandling and traumatic damage to the skin in overcrowded conditions and in conjugation with pollution, bacterial and viral infection. It is proposed that stress raises the corticosteroid levels in the blood plasma, which suppresses inflammatory reaction and boosts protein catabolism, regulated by the corticosteroids. In the final stage of the disease, protein deficiency leads to atrophy of skeletal muscles and suppression of collagen synthesis. Lack of collagen is reported to lead to poor regeneration of lesions on the skin (Willoughby and Pickering, 1977; Khulbe *et al.*, 1995). Saprolegniasis contributes to heavy mortality among fishes and are widely spread in fresh water ecosystems, affecting wild and cultured fishes and are considered as the single contributing cause of economic loss in aquaculture second only to bacterial diseases in economic importance (Hussain *et al*., 2001).

Saprolegnia belongs to the family Saprolegniaceae and is a typical fungus causing dermatomycosis in cold water fishes. Willoughby (1978) reported that *Saprolegenia* invades epidermal tissue, generally beginning on the head or fins and can spread over the entire surface of body. Mature fishes of both sexes and eggs are prone to fungal infection which persists from November to the end of March when the water temperature ranges between 8.5°C to 11.5°C and the infection vanishes automatically as the temperature rises to above 11.5 °C. These findings were reported in the present study and thus are in coherence with reported values.

Haematological and biochemical analyses provide valuable knowledge to monitor the health status of both wild and cultured fishes. Haematological values change depending on the fish species, age, cycle of sexual maturity and condition of health (Hrubec *et al*., 2000). Haematological tests and analysis of serum constituents have shown useful information in detection and diagnosis of metabolic disturbances and diseases in fishes. Blood chemistry values have been used by fish biologists for a variety of purposes: to detect cellular damage caused by toxicant exposure (Young *et al*., 1994), infection by

pathogenic agent (Brenden and Huizinga, 1986; Grizzle and Kiryu, 1993), traumatic handling (Grizzle *et al*., 1992), to evaluate the effect of diet on liver function (Lemaire *et al*., 1991; Hamre *et al*., 1994; Muruta, 1996) and to evaluate osmoregulatory and ion regulatory functions (Congleton and La Voie, 2001).

Materials and methods

Sample collection site

The Kokernag Fish Farm established in 1984 for the production and culture of the Rainbow trout in Jammu and Kashmir, India, is located at an altitude of 1854 m asl, in south east of Anantnag District about 85 km from Srinagar city and is the second largest Trout Fish Farm of South Asia. The Farm supplies fish seed to almost all other government and private farms of the state and has got the status of mother trout farm of the State.

Collection of fish samples

Dermatomycotic infected fish samples (live and freshly dead) and fungal infected eggs were collected from the Trout Fish Farm in Kokernag, India during the breeding season of rainbow trout. Every effort was made for the safe delivery of infected live fish specimens to the Pathological Laboratory of the Faculty of Fisheries SKUAST-K, Srinagar-India. The live infected fish samples (mean body weight 1565±130.75 gm and mean total length 57.63±4.86 cm) were carried in oxygen packed polyethylene bags in boxes of suitable size (18″×12″×20″). In the laboratory infected fish samples were gross examined and % area of body covered with fungus was noted and gills were thoroughly examined for anaemia. Total body length and weight of the fish were recorded. The morbid trout fish were preserved in 10% freshly prepared formalin.

Preparation of culture media

Wet mounts of mycelium of fungus were taken from the skin of infected live trout fish specimens and infected trout eggs. After thorough rinsing in distilled water, the bits of mycelia were placed on fresh trout eggs in petri-dishes containing 10-15 ml of sterile water. The petri-dishes were kept in the incubator for 24 hours at 30±2°C.

For culture of the fungus Sabourauds dextrose agar of the following composition was used:

Agar: 20g
Peptone water: 10mL
Dextrose: 40g
Water: 1L

The ingredients were properly mixed in a two litre borosil beaker and were heated till the contents boiled to form a viscous mass. The beaker was then allowed to stand for a while to avoid frothing. Sterility of medium was ensured after proper culturing, taking due care to avoid any means of cross infection (Thomas *et al.*, 1991).

Preparation of culture plates

The freshly prepared agar mass was poured in the culture plates which were smoothly shaken to form a uniform semi-solid coat. The spore mass from the freshly prepared colony was removed from the trout eggs and placed in fresh petri dishes and separated into individual units by means of a narrow jet of water. The mass from the petri dishes was removed with the help of a sterile platinum loop of wire and steak inoculated on to the surface of plated semi-solid media. The plates were placed in the incubator at 30±2 ^{o}C for 7 days. After 7 days, the germinated mass was cut from the agar along with the bit of media and transferred surface downwards on to the fresh plate of agar and kept for incubation (Raper, 1937; Tiffney, 1939).

After the appearance of a definite mycelium colony around the inoculation site, a second block of agar about one square centimetre was cut from the edge of the colony and placed on the inoculated surface downwards on to a third plate of agar. The same procedure was repeated five times till bacteria free isolates were obtained. Following the same procedure, 20 isolates from 20 infected fish and eggs were cultured using the same culture media for sexual fruiting and production of oospores. The culture mass was removed from culture plates placed in sterile petri dishes, separated into individual units and used in the preparation of slides without using stains. The slides were also prepared using Lectophenol Cotton Blue, and part of the culture mass was also preserved in 10% formalin. The slides were examined under a research microscope Morphological characters of hyphae and zoospores were recorded (Fig. 2(d) (Thomas *et al*., 1991).

Haematology

Blood was collected from twenty (20) dermatomycotic infected and twenty (20) normal male & twenty (20) female rainbow trout fish, one month after artificial stripping. Blood was collected by stabbing the needle of a 3mL syringe directly into the heart at the base of operculum at an angle of 45°C. The fish specimens were grossly examined. Total body length and weight were recorded. A minimum of 3 mL blood was collected from each fish specimen, respectively.

TRBC (Total Red Blood Corpuscle) and WBC (White Blood Corpuscle) counts were determined by using Newbaurs haematocytometer; the Hayem diluting fluid was used as diluting fluid for RBC and Turks fluid for WBC. Haemaglobin percentage (Hb%) was determined by Drabkins method. Erythrocyte sedimentation rate (ESR) was determined by Wintrobes tube method and results were determined as first hr. reading. Haematocrit (Ht) was determined in heparinised micro haematocrit

capillaries in duplicate, micro haematocrit centrifuge (1500 rpm for 3 min). Mean cell volume (MCV), and mean corpuscular hemoglobin (MCH) were calculated from haematological data. Differential leucocytes count (DLC) and peripheral blood film (PBF) test were performed on thin blood smears fixed in methanol and stained with Leishman's stain.

Blood biochemistry

Blood biochemical autoanalyser (Photometer-5010, Germany) was used for the determination of blood biochemistry using blood biochemical kits (Precision Biotec-India, Ltd) according to manufacturers' instructions.

Results

Physico-chemical parameters

The water temperature of Kokernag spring during the study period ranged from 7.5°C (Jan.) to 11.5°C (Nov.) with a mean value of 9±1.70°C. Conductivity was recorded at a mean value of 412±20.85 µS/cm. Dissolved Oxygen ranged from 10 mg/L to 12 mg/L during November and February, respectively. The pH during the study period was at a minimum (7.2) in the month of February and a maximum (7.6) during November with a mean value of 7.4±0.17. Free CO_2 was present with a mean value of 13.5±0.41 mg/L. Total alkalinity ranged from 92 mg/L (December and January) to 94 mg/L (November) with an average value of 93±0.96 mg/L. Calcium and magnesium concentrations decreased from November to February and their mean values were 33±1.26 mg/L and 4.5±1.26 mg/L, respectively. The mean chloride concentration in the water was 11±0.48 mg/L. The results obtained after comparing the total serum protein, albumin, globulin and glucose of normal and naturally fungal infected rainbow trout using one way ANOVA and Tukey's HSD are presented in Table 1.

Mean values of total serum protein in normal males and fungal infected males were 4.150±0.170 and 3.807±0.163g/L, respectively and differed significantly at $p<0.05$. The mean values of total protein also differed significantly between normal females and infected females and the values were 4.015±0.211 and 3.735±0.228g/L, respectively. The lowest value of total protein was found in infected females and the highest was in normal males (Table 1).

Mean values of albumin between normal males and normal females were 1.990±0.174 and 1.395±0.143, respectively and the differences were non significant at $p>0.05$. However, mean values of albumin content between normal males and infected males and between normal females and infected females varied significantly at $p<0.05$. The mean values of globulin between normal males and infected males were 2.160±0.403 and 1.990±0.751, respectively and the variation

between the values was significant at $p<0.05$. However, the variation between the values of globulin between normal males and normal females (2.160±0.403 and 2.180±0.120, respectively) was not significant at $p>0.05$.

The mean values of serum glucose between normal females and infected females were 97.700±1.250 and 101. 150±1.406, respectively while as in the normal male and infected male the values were 97.225±1.853 and 100.940±1.552, respectively. Statistically the difference between values was significant at $p<0.05$. However, the mean values of glucose showed no significant differences between normal males and normal females (Table 1).

Haematological findings

After comparing the haemoglobin content, Total RBC, ESR, PCV, MCH and MCV of normal and naturally dermatomycotic infected trout during the experimental period using one way ANOVA and Tukey's HSD, results are presented in Table 2(a).

Mean values of haemoglobin were significantly lower ($p>0.05$) in the *Saprolegnia* infected trout as compared to that in the normal fish. Mean values were 5.732±1.014 and 8.108±0.171 in infected and normal females, respectively while in infected males and normal males the values were 6.326±0.631 and 8.247±0.141, respectively. The differences of variation between normal males and normal females were not statistically significant.

In the fungus infected males and fungus infected females the mean values of RBC were 3.583±0.354 and 3.184±0.550, respectively which were significantly different at $p<0.05$. In normal males and normal females the mean values of RBC were 4.703±6.945 and 4.595±9.627, respectively, which were also significantly different at $p<0.05$. Mean values of Total RBC were significantly lower ($p<0.05$) in the fungal infected rainbow trout as compared to that in normal ones (Control).

The fungal infected male and female trout had mean ESR values of 2.68±0.37 and 3.05±0.63 respectively. In case of normal male and normal female the values of ESR were 1.78±0.26 and 1.93±0.18, respectively. Statistically the difference was significant in the mean values of ESR between normal and infected trout fishes at $p<0.05$.

Mean values of PCV were significantly lower at $p<0.05$ in the case of infected male and infected female trout. The lowest value (24.55±4.36) was observed in the infected females and highest value (35.50±0.51) in normal males. The critical difference within the groups was 3.09. The mean values of MCH were significantly different at $p<0.05$ between normal male and normal females. The mean values of MCH were 17.523±0.144 and 17.97±0.196 in normal males and infected females, respectively which were significantly different at $p<0.05$.

Table 1: Biochemical parameters of normal (control) and *Saprolegnia* sp infected rainbow trout values with same superscripts (along the row) do not differ significantly, $p>0.05$

	Normal male	Infected male	Normal female	Infected female	Critical Difference
Total protein (g/dL)	4.150±0.170[a]	3.807±0.163[b]	4.015±0.211[c]	3.735±0.228[d]	0.016
Albumin (g/dL)	1.990±0.17a	1.395±0.143[b]	1.930±0.193[a]	1.730±0.195[c]	0.014
Globulin (g/dL)	2.160±0.403a	1.990±0.751[b]	2.180±8.120[a]	1.850±0.870[c]	0.004
Glucose (g/dL)	97.225±1.853[a]	100.940±1.552[b]	97.700±1.250[a]	101.150±1.406c	1.04

Table 2 (a): Haematological parameters of normal (control) and *Saprolegnia* infected rainbow trout.

	Normal male	Infected male	Normal female	Infected female	Critical Difference
Hb (g/dL)	8.2475±0.1419[a]	6.3265±0.6317[b]	8.1080±0.1710[a]	5.7325±1.0147[c]	0.165
RBC (10^6/mm^3)	4.7035±6.945[a]	3.5830±0.3549[b]	4.5950±9.627[c]	3.1845±0.5509[d]	0.049
ESR (mm)	1.78±0.26[a]	2.68±0.37[b]	1.93±0.18[c]	3.05±0.63[d]	0.070
PCV (%)	35.50±0.51[a]	27.20±2.86[b]	34.65±0.49[a]	24.55±4.36[c]	3.09
MCH (gp)	17.5235± 0.1447[a]	17.6435±0.1918[b]	17.6210± 1.373[c]	17.9745±0.1966[d]	0.010
MCV(μ^3)	96.0750±0.6325[a]	97.2520±4.6145[a]	99.2770±6.944[b]	100.1240±1.1078[b]	0.986

Values with same superscripts (along the row) do not differ significantly, $p> 0.05$

Table 2 (b): Haematological parameters of normal (control) and *Saprolegnia* infected rainbow trout.

	Normal male	Infected male	Normal female	Infected female	Critical Difference
WBC(10^3/mm^3)	38.380±1.588[a]	75.645±3.601[b]	39.105±1.577[a]	79.610±4.226[c]	0.896
Lymphocytes%	73.70±3.01[a]	64.80±2.89[b]	75.95±3.49[a]	62.75±2.71[b]	4.130
Heterophill%	26.30±3.01[a]	35.20±2.89[b]	24.05±3.49[a]	37.45 ± 2.52[b]	4.020

Values with same superscripts (along the row) do not differ significantly, $p >0.05$

The mean values of MCV were 96.075±0.632 and 100.124±1.107 in normal males and infected females, respectively and the differences between the two were significant at $p<0.05$. The mean values in normal males and normal females were 96.075±0.632 and 99.277±6.944, respectively and their variation was also significant at $p<0.05$.

The results obtained after comparing TLC, lymphocyte % and heterophils % between the normal and infected rainbow trout using one way ANOVA and Tukey's HSD are presented in Table 2 (b) and Fig. 1.

Mean values of TLC in normal males and infected males were 38.380±1.588 and 75.645±3.601, respectively and the variations between the two were significant at $p<0.05$. The variations of mean values between normal females and infected females were also significant at $p<0.05$ and the values were 39.105±1.577 and 79.610±4.226 respectively, however, the variation in the mean values of TLC between normal males and normal females was not significant.

The mean values of lymphocyte % in normal and infected males and normal and infected females were 73.70±3.01, 64.80±2.89, 75.95±3.49 and 62.75±2.71, respectively. The variations between the normal and infected sexes were significant at $p<0.05$. However, the variation

between normal males and normal females were not significant.

The mean values of heterophill % in normal males and normal females were 26.30±3.01 and 24.05±3.49, respectively and the variation between the two was not significant. However, the variation in the mean values of heterophill % between normal and infected trout was significant at $p<0.05$.

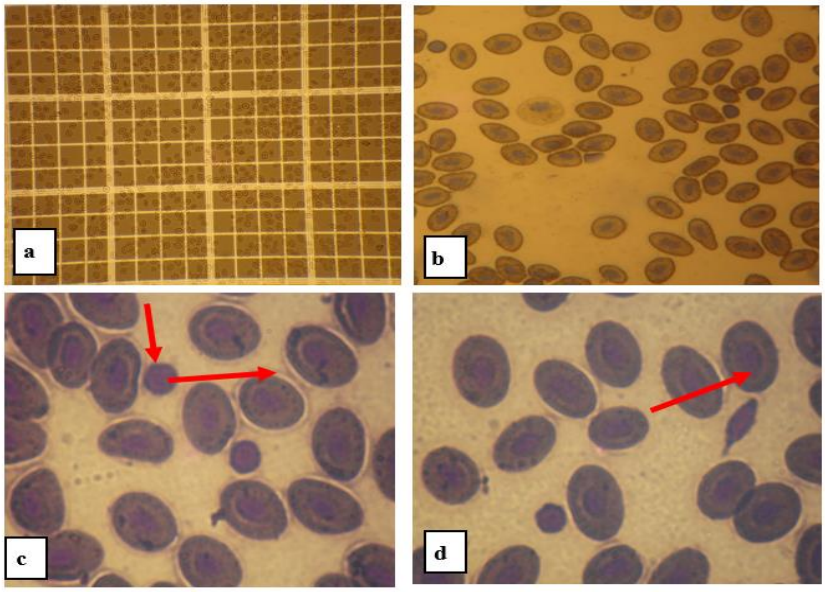

Figure 1: RBC count of infected fish (a), Blood film (PBF) showing anisocytic hypochromic anemia of fungal infected fish (b), Differential Leucocyte Count (c & d).

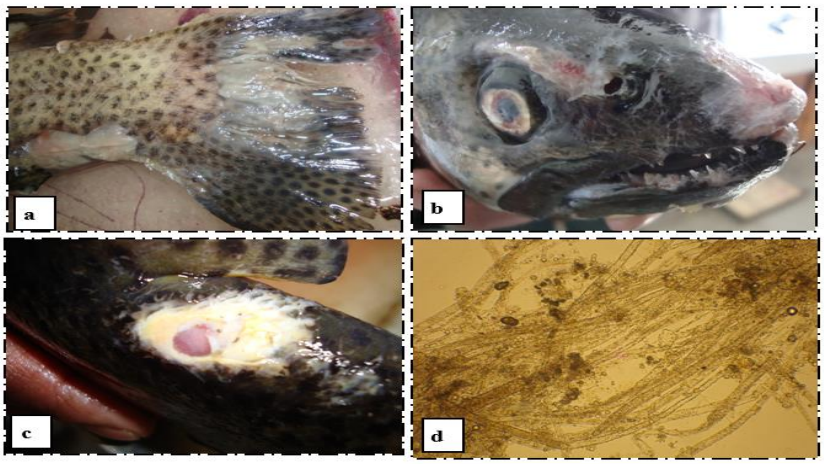

Figure 2: (a) Tail rot due to fungal tufts of *Saprolegnia* sp, (b) advanced form dermatomycosis leading to deformity of jaws and (c) dermatomycosis leading to deep ulcerations on the lateral side of the body. (d) Aceptate hyphae of *Saprolegnia* sp.

Discussion

In the present study the wet preparations from skin lesions and mycelia cultured on SDA at 30 ± 2 °C for seven days showed masses of mature and immature sporangia filled with zoospores. Hyphae appeared profusely branched, aseptate and multinucleate (Fig. 2(d)). Similar results have been reported by Hrubec (2000); Schaperclaus (1954); Marzouk *et al*.(2003); Abu El Atta (2008). This is a characteristic feature of Saprolegnia belonging to the family Saprolegniaceae and is a typical fungus causing dermatomycosis in cold water fishes. It was found in the present study that the fungus attacks any portion of the body where integument is lost by mechanical injury. Similar findings were reported by Willoughby (1978).

Mature fishes of both sexes and eggs are prone to fungal infection which persists from November to the end of March when the water temperature ranges between 8.5°C to 11.5°C and the infection disappears automatically as the temperature rises to above 11.5°C. This was found in the present study and is in agreement with the study of (Hoshima *et al.*,1960), who reported that the *Saprolegnia* of eels ceased when the water temperature rose above 18°C, and in white suckers, fungal infection takes place when water temperature exceeds 10°C (Roth, 1972).

Total erythrocyte count, Hb volume and packed cell volume decreased in the infected group which showed significant differences when compared with that in the normal ones, which may be due to the fact that the mycelia of *Saprolegnia* penetrate deep causing wounds resulting in the loss of blood (Juncey and Ross, 1982). Our findings are in agreement with (Zaki *et al.*, 2008) who reported that *Tilapia nilotica* infected with *Saprolegnia parasitica* resulted in a significant decrease in the total erythrocyte count, Hb and PCV. Similar findings were also reported by (Hatai *et al.*, 1984) on naturally infected Ayu (*Plecoglosus altivelis*) with fungus *Aphanomysis piscicida*. Bruno and Munro (1986) reported that experimental infection of rainbow trout and Atlantic salmon with *Ranibacteriun salmoninarum* resulted in the significant decline of Total erythrocyte count and haemoglobin levels. Similar findings were also reported by (Suzumuto *et al.*, 1977; Aldrin *et al.*, 1978; Zaki *et al*., 2008).

Jamalzadah *et al.* (2009) reported that fungal infected *Salmo trutta fario* showed significant decline in the total erythrocyte count, Hb and PCV as compared to the the values in normal ones and these results are in coherence with the present study. The RBC indices ESR, MCH and MCV in the present study showed significant increase in the *Saprolegnia* infected fish, the increase in the value of RBC indices was more profound in the infected females as compared to the

infected males. Similar results were reported by (Zaki *et al*., 2008) in *Tilapia nilotica* fish, and by Talas and Gulhan (2009) while working on the effects of propolis concentrations on biochemical and haematological parameters on rainbow trout. Atamanap and Yanik (2002) have also reported similar findings while working on the alterations in haematological parameters of rainbow trout which are in agreement with the findings of present study. The overall haemogram of fungal infected fishes showed a general trend of anisocytic hypochromic anaemia which was more pronounced in females. The other types of anaemia which were encountered during the study were, poikilocytic hypochromic anaemia and normocytic hypochromic anaemia, however, microcytic anaemia was not found.

The lymphocyte percentage showed significant decrease and heterophills percentage showed significant increase in the *Saprolgnia* infected rainbow trout in the present study. Similar findings were reported by (Jamalzdah *et al*., 2009) in fungal infected Caspian salmon (*Salmo trutta fario*). A decrease in the percentage of lymphocyctes and an increase in the percentage of Heterophills were seen in European eel (*Anguilla anguilla*) infected with the parasite (Sahan *et al*., 2007).

The serum glucose in the present study showed significant increase (p<0.05) in the fungal infected trout, irrespective of sex as compared to that in normal ones. Similar results were reported by (Zaki *et al.*,2008) in *Tilapia nilotica* infected with *Saprolegnia parasitica* and (Yang and Chen, 2003) in *Cyprinus carpio*. Serum concentrations of glucose are regulated by complex interactions of hormones such as glycogen and cortisol. Environmental stress and diseases cause marked elevations in plasma glucose levels. Plasma glucose is elevated in stressed fish as a consequence of increased blood catecholamine (Wedemeyer *et al*., 1990; Willoughby and Pickering, 1997; Martin and Black, 1998; Talas and Gulhan, 2009). Our findings are also in agreement with the results of Hari Krishnan *et al.* (2003) and those of Ramash and Sarvanan, (2008).

The results of the present study also revealed that the total serum protein content in the *Saprolegnia* infected rainbow trout decreased significantly as compared to that in the normal group. The mean value of total protein was lowest in the infected females as compared to males. Our findings are confirmed by the results of Yang and Chen (2003); Mastan *et al*., (2009). Similar results were obtained by Bruno and Munro (1986) in rainbow trout and Atlantic salmon infected with *Ranibacterium salmonarium* and Harikrishnan *et al.* (2003) in common carp following herbal treatment against the challenge of *Aeromonas hyrdrophila* infection. Decreased

concentration of total protein is common in many diseased conditions and may result from impaired synthesis, reduced absorption or protein loss (Bernet *et al*., 2001). Our results are in confirmation with the results of Shan (2006) and Adeyemo *et al.* (2003).

In the present study the mean albumin content was significantly higher in the control group as compared to *Saprolegnia* infected trout irrespective of sex. Our findings are in agreement with Sahoo and Mukherjee (2000) who reported that albumin content was significantly lower in aflatoxin treated ectothermic species of Indian major carp against the challenge of *Edwardsiella tarda* when compared to that in the normal group. Results demonstrated by Misra *et al*. (2005) who reported that albumin content of the β glucon fed group do not differ significantly as compared to infected group contradict our findings.

Dina Rairakhwada (2007) reported that the albumin content does not differ significantly between the Levan fed group and control group. The mean globulin was significantly lower in the *Saprolegnia* infected rainbow trout as compared with that in the normal group in the findings of present study. The present findings are in agreement with the works of Anderson and Swiciki (1994); Misra *et al*. (2005) and Dina Rairakhwada (2007).

Aquatic environments encompass a wide variety of features virtually all of which are essential for the maintenance of homeostasis in fish, and if altered beyond acceptable limits can cause a variety of diseases in fish. The present study documents that a decrease in albumin and increase in globulin levels of fish can be attributed to activation of humoral immune response against fungal invasion at ambient temperatures. The increase of globulin levels (gamma globulin) generally cause A:G (Albumin: Globulin) ratio reversal in trout fish. In the same way alteration in the haemogram values in infected fish reflects the impact of dermato/systemic myscosis. Hence it can be concluded that stress caused by fungal infection leads to haemostatic imbalances in fish which is reflected in the haemato-biochemical profile of infected fish.

References

Adeyemo, O., Agbede, S.A., Olaniyan, A.O. and Shoaga, O.A., 2003. The haematological response of *Clarias gariepinus* to changes in acclimation temperatures. *African Journal of Biomedical Research*, 6, 105–108.

Aldrin, J.F., Mevel, M., Robert, J.Y., Vigneulle, M. and Baudin, L.F., 1978. Incidence metabolikeus dela corynebacteriose experimetale chezle Saumon coho (*O. kisutch*). *Bulletin de la Societe des Veterinaries et de medicine Comparee de Lyon*, 80, 89–90.

Allan, B.J. and Stevenson, R.M.W., 1981. Extra cellular virulence factors of *Aeromonas hydrophila* infection

in fish. *Canadian Journal of Microbiology*, 27, 11–14.

Anderson, D.P. and Siwicki, A.K., 1994. Duration of protection against *Aeromonas salmonicida* in brook trout immuno stimulated with glucan or chitosin by injection or immersion. *Progressive Fish Culturist*, 56(**4**), 258–261.

Atamanalp, M. and Yanak, T., 2002. Alterations in haematological parameters of Rainbow trout (*Onchorhynchus mykiss*) exposed to mancozeb. *Turkish Journal of Veterinary and Animal Sciences,* 27, 1213–1217.

Austin, B. and Austin, D.A., 1993. Bacterial fish pathogen. Disease of farmed and wild fish. 2nd edition (Simmon and Chuster, Chickester). pp. 111-117.

Bernet, D., Schmidt, H., Wahli, T., Burkhardt. and Holam, P., 2001. Effluent from a sewage treatment works causes changes in the serum chemistry of Brown trout (*Salmo trutta* Linneus). *Ecotoxicology and Environmental Safety,* 48, 140–147.

Brenden, R.A. and Huzinga, H.W., 1986. Pathophysiology of experimental *Aeromonas hydrophila* infection in Gold fish, *Carrasius auratus* L. *Journal of Fish Diseases,* 9, 163–167.

Bruno, D.W. and Munro, A.L.S., 1986. Haematological assessment of Rainbow trout *Salmo giardnari* Richordson and Atlantic salmon, *Salmo salar* L., infected with *Ranibacterium salmonarium*. *Journal of Fish Diseases,* 9, 195–204.

Congelton, J.L. and Levoie, W.J., 2001. Comparison of blood chemistry values for samples collected from juvenile Chinook Salmon by three methods. *Journal of Aquatic Animal Health,* 13, 168–172.

Dina, R., Paul A.K., Bathena, Z.P., Sahu, A. and Mukherjee, S., 2007. Dietary microbial levan enhances cellular non specific immunity and survival of common carp (*Cyprinus carpio* Juveniles). *Fish and Shellfish Immunology,* 22(**5**), 477–486.

Grizzle, J.M., Chen, L., Williams, C. and Spano, J.S., 1992. Skin injuries and serum enzyme activities of channel cat fish (*Ictalurus punctata*) harvested by fish pumpus. *Aquaculture*, 107, 333–346.

Grizzle, J.M. and Kiryu, Y., 1993. Histopathology of gill, liver and pancreas, and serum enzyme levels of channel cat fish (*Ictalurus punctata*) infected with *Aeromonas hydrophila* complex. *Journal of Aquatic Animal Health,* 5, 36–50.

Hamre, K., Hjeltnes, B., Kryvi, H., Sandberg, S., Lorentzen, M. and Lieo, O., 1994. Decreased concentration of haemoglobin, accumulation of lipids oxidation products and unchanged skeletal muscle in Atlantic salmon (*Salmo salar*) fed on cow dietary vitamin E. *Fish Physiology and Biochemistry,* 12, 421–429.

Hari krishnan, R., Rani, M., Nisha and Balasundaram, C., 2003. Haematological and biochemical parameters in common carp, *Cyprinus carpio*, following herbal treatment for *Aeromonas hydrophila* infection. *Aquaculture*, 221(**1-4**), 41 -50.

Hatai, K., Takahashi, S. and Egusa, S., 1984. Studies on the pathogenic fungus of mycotic granulomatosis-IV. Changes of blood constituents in both Aya, *Plecoglossus altivelis* experimentally inoculated and naturally infected with *Aphanomyces Piscicida. Fish Pathology*,19(**1**), 17–23.

Hoshima, T., Sano, T. and Sunayamo, M., 1960. Studies on the *Saprolegniasis* of eel. *Journal of Tokyo University of Fisheries*, 47, 59–79.

Hrubec T.C., Cardinale, J.L. and Smith, S.A., 2000. Haemotology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis hybrid*). *Veterinary Clinical Pathology*, 29(**1**),7–12.

Hussain, M.M., Hatai, A.K. and Nomura., 2001. Saprolegniasis in Salmonids and their eggs in Japan. *Journal of Wildlife Diseases*, 37, 204–207.

Jamalzadeh, H.R., Keyvan, A., Ghomi, M.R. and Gherardi, F., 2009. Camparison of blood indices in healthy and fungal infected Caspian salmon (*Salmo trutta caspius*). *African Journal of Biotechnology*, 8(**2**), 319–322.

Juncey, K. and Ross, B., 1982. A guide to tilapia feed and feeding.* Institute of aquaculture Univ. of Striling, Scotland. 111P.

Khulbe, R.D., Joshi, C. and Bisht, G.S., 1995. Fungal diseases of fish in Nanak Sagar, Nainital, India. *Mycopathologia*, 130(**2**), 71-4.

Lemaire, P., Drai P., Mathieu, A., Lemaire, S., Carriers, S., Juidicelli, J. and Lafaurei, M., 1991. Changes with different diets in plasma enzymes (GOT, GPT, LDH, ALP) and plasma lipids (cholesterol and triglycerides) of sea bass (*Dicentrachus labrax*). *Aquaculture*, 93, 63–75.

Martin, Jr.L.K. and Black, M.C., 1998. Biomarker assessment of the effects of coal-strip mine contamination on channel cat fish. *Ecotoxicology and Environmental Safety*, 41, 307–320.

Marzouk, M.S., Rezeke, M.S., Samira. and Gamal, M.H., 2003. Some mycological investigations on cultured tilapia in Kafr El Sheikh Governorate. Kafr EL Sheikh *Veterinary Medical Journal*, 2(**1**), 97–114.

Mastan, S., Priya, G.I. and Babu, E., 2009. Haematological profile of *Clarias batrachus* exposed to sublethal concentrations of lead nitrate. *The International Journal of Haematology*, 6(**1**), 322–328.

Misra, C.K., Das, B.K., Mukherjee, S.C. and Phalguni, P., 2005. Effect of long term administration of dietary beta glucon on immunity, growth and survival of *Labeo rohita* fingerlings. *Aquaculture*, 255(**1–4**), 82–94.

Ramesh, M. and Sarvanan, M., 2008. Haematological and biochemical responses in a fresh water fish (*Cyprinus carpio*) exposed to Chloropyrephos. *International Journal of Integrative Biology,* 3(**1**), 80–83.

Raper, J.R., 1937. A method of freeing fungi from bacterial contamination. *Science,* 85, 342.

Roth, R.R., 1972. Some factors contributing to the development of fungus infection in freshwater fish. *Journal of Wildlife Diseases,* 8, 24–28.

Sahan, A., Altun, T., Cevik, F., Cengizler, I., Nevsat, A. and Genc, E., 2007. Comparative study of some haematological parameters in European eel (*Anguilla Anguilla* L. 1758) caught from different regions of Ceyhan River (Adana, Turkey). *Canadian Journal of Fisheries and Aquatic Sciences,* 24, 167–171.

Sahoo, P.K. and Mukherjee, S.C., 2000. Immunosuppressive effects of aflotoxin BI in India major carp (*Labeo rohita*). *Comparative Immunology, Microbiology and Infectious Diseases,* 24, 143–149.

Schaperclaus., 1954. *Fish diseases* Volume II, Fishing New Books, Academic Press London, pp. 632 – 635.

Shan, S.L., 2006. Haematological parameters in tench (*Tinca tinca*) after short(s) exposure to lead. *Journal of Applied Toxicology,* 26(**3**), 223–228.

Talas, Z.S. and Gulhan, M.F., 2009. Effects of various propolis concentrations on biochemical and haematological parameters of Rainbow trout (*Onchorhynchus mykiss*). *Ecotoxicology and Environmental Safety,* 72(**7**), 1994–1998.

Thomas, P.A., T. Kuriakose, P. Kirupashankar. and V.S. Maharajan., 1991. Use of lactophenol cotton blue mounts of corneal scrapings as an aid to the diagnosis of mycotic keratitis. *Diagnostic Microbiolgy and Infectious Diseases,* 14, 219-224.

Tiffney, W.N., 1939. The host range of *Saprolegnia parasitica*. *Mycologia,* 31, 310–321.

Wedemeyer, G.A., Barton, B.A. and McLeay D.J., 1990. Stress and acclimation. In: Methods of fish biology. (Edited by C. B. SChreck and P. B. Moyle). American Fisheries Society, Bethesda, USA. pp. 451–490.

Willoughby, L.G. and Pickring, A., 1977. Viable Saprolegniales spores on the epidermis of Salmonid fish (*Salmo trutta* and *Salvilenus alpious*). *Transactions of the British Mycology Society,* 68(**1**), 91-95.

Willoughby, L.G., 1978. *Saporlegniasis* of Salmonid fish in windermire: A critical analysis. *Journal of Fish Diseases,* 1, 151–167.

Young, G.B., Brown, C.L., Nishioka, R.S., Foolmar, L.C., and Andrews, M., Cashman, J.R. and Bern,

H.A., 1994. Histpathology, blood chemistry and physiological status of normal and moribund striped bass (*Morone saxatilis*) involved summer mortality (die off) in the Sacramento – San Joaquin delta of California. *Journal of Fish Biology*, 44, 491–512.

Yang, J.L. and Chen, H.C., 2003. Effects of gallium on Common carp (*Cyprinus carpio*) acute toxicity, serum biochemistry and erythrocyte morphology. *Chemosphere*, 53, 877–922.

Zaki, M.S., Olfat, F.M. and El-Jackey, J., 2008. Pathological and biochemical studies in *Tilapia nilotica* infected with *Saprolegnia parasitica* and treated with potassium permanganate. *American-Eurasian Journal of Agriculture and Environmental Sciences*, 3(**5**), 677–680.