International Journal of Food, Agriculture and Veterinary Sciences ISSN: 2277-209X (Online)
An Online International Journal Available at http://www.cibtech.org/jfav.htm
2013 Vol. 3 (1) January-April, pp. 70-75/Kumar and Ramulu
Research Article

HAEMATOLOGICAL CHANGES IN *PANGASIUS HYPOPHTHALMUS* INFECTED WITH *AEROMONAS HYDROPHILA*

***M. Phani Kumar and K. Sree Ramulu**
Department of Zoology, Andhra University, Visakhapatnam- 530 003, Andhra Pradesh, India
**Author for Correspondence*

ABSTRACT
The paper deals with haematological changes in *Pangasius hypophthalmus* infected with *Aeromonas hydrophila*. The hematological parameters included red blood corpuscles (RBCs) count, white blood cells (WBCs) count, packed cell volume (PCV), differential count of WBCs, the derived blood indices of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were studied in the infected and normal fish. In the present study, the mean values of RBC, HB, PCV, MCH, MCHC, Thrombocytes, lymphocyte percentage, eosinophil percentage of the infected fish decreased when compared with the healthy fish but the MCV, WBC, neutrophil percentage, monocyte percentage of the infected fish increased when compared with normal fish.

Key Words: *Pangasius Hypophthalmus, Aeromonas Hydrophila, Infection, Haematology*

INTRODUCTION
The haematological parameters are an important tool of diagnosis that reveals the state of health of fish (Blaxhall, 1972; Rehulka, 2002; Martins *et al.*, 2004). Blood tissue of fish gives clue about physiology and environmental conditions of fish (Ramaway and Reddy, 1978). Knowledge of hematology is very important since it deals with the morphology, physiology and the biochemistry of blood. By analyzing blood cell characteristics, disease status can be identified (Anderson, 2003). Bruno and Munro (1986) have stated that hematological indices aid in the diagnosis and assessment of disease in fish. In fisheries, it is important to find out illness as the source of these causes and may not be generally detectable in early period of the infection. However it is also possible early diagnosis of illnesses in case of evaluating hematological data, particularly blood parameters (Rimsh and Adamova, 1973). Hematological parameters are among one of the important tools for fish disease diagnosis (Ruane *et al*., 2000; Ranzani-Paiva *et al*., 2005; Ghiraldelli *et al*., 2006).
Certain blood parameters serve as reliable indicators of fish health as many parasites can live in a host, causing damage to it. Hematological analysis can provide valuable knowledge for monitoring the health and condition of fish and are important in diagnosing the structural and functional status of the body. Knowledge of hematology is very important since it deals with the morphology, physiology and the biochemistry of blood. Hematological tests and analysis have showed useful information in detection and diagnosis of metabolic disturbance and diseases in fishes. It is known that status of disease are characterized by the installation of some disturbances on the sanguine board level of animal, most times pointed by a general hematological failure (drastic drop of hemoglobin level, hematocrit value, red blood cell counts *etc*.). The evaluation of blood chemistry parameters in animals is a routine and important tool in clinical veterinary medical practice. This simple technique can provide essential information on the physiological status of animals and therefore help the clinician to make proper medical decisions.
Sabina *et al*., (2004) has reported that *A. hydrophila*is one of the important pathogens of fish in freshwater and brackish water. *Aeromonas hydrophila* causes disease in fish known as "Motile Aeromonas Septicemia" (MAS), the disease related to the lesions caused by this bacterium which include septicemia where the bacteria and bacterial toxins are present within numerous organs of the fish, and ulcers of the fish's skin. The disease caused by this bacterium primarily affects freshwater fish such as catfish, several species of bass, and many species of tropical or ornamental fish. The clinical signs of *A.hydrophyla* were ulceration commenced as sloughing off of scales followed by the occurrence of a hemorrhagic spots all

over the body which progress to form an epidermal lesion. The lesion expanded in diameter and depth affecting the internal muscles these results more or less agreed with Sharifuzzaman and Austin (2009).

MATERIALS AND METHODS

24 live catfish (*Pangasius hypophthalmus*) with an average weight of 500 g were collected from private fish farms. Out of which twelve are infected with red spot disease and twelve are normal. The collected fish were transferred alive in polyethylene bags to the laboratory for further investigations. In the present study, during the infection, pH ranged from 8.5 to 8.9. Ammonia level ranged between 0.5 to 1.5 mg/l and the nitrite level ranged between 0.2 to 0.5 mg. /l. High pH and Ammonia was also noticed in the ponds and this clearly indicates the deterioration of water quality in the pond. These water parameters might have induced the bacterial proliferation causing red disease in the catfish, *Pangasius hypopthalmus.*

Blood Samples

Blood samples from each infected and non-infected fish were obtained from caudal artery of anesthetized fish with 150 mg L-1 tricanemethansulphonat (MS 222). Haematological analyses were carried out with the collected blood samples.

Determination of Haematological Parameters

Haematological analyses were carried out by standard methods suggested by Blaxhall and Daisley (1973). Hemoglobin estimation was done by acid-haematin method using sahils haematoglobinometer and the values were expressed in g%. The haematocrit was determined by micro haematocrit tube method (Bull *et al.,* 2000) and packed cell volume (PCV) has been calculated using the following formula:

$$PCV=\frac{\text{height of the RBC column after centrifugation}}{\text{Total height of the blood column}} \times 100$$

Total erythrocyte count (10^6 mm), total leucocyte count (10^4 mm) and total thrombocytes were determined using Neubauers haemocytometer after examining at 40 and 10X magnification using a research microscope. Hendricks solution (Hendricks, 1992) used as the diluting fluid for counting RBC, WBC and thrombocytes. The data was used to calculate the Mean CorposcularVolume (MCV), Mean Corpuscular Haemaglobin (MCHC) suggested by Dacie and Lewis (1984).

For differential leukocyte count, six blood smears per fish were prepared from fresh blood, air-dried, stained with Leishman-Giemsa'sstain and fixed in methanol. In each sample, three visual fields at 1,000 X were identified for the leukocyte count (Harikrishnan *et al.,* 2003).The percentage of neutrophil (NEU), eosinophil (EOS), lymphocyte (LYM) and monocyte (MON) tissues was determined.

Statistical Analysis

A comparison between haematological parameters between normal and infected *Pangasius* was made by student´s –t- test (Snedecor and Cochran, 1967).

RESULTS

In the present study the average mean value of RBC in the normal fish is $4.66(10^6 mm^{-3})$ and in the infected fish it is $4.21(10^6 mm^{-3})$.there is a significant decrease in RBC count (P<.0001) in the infected fish compared to the normal fish . Mean average value of WBC in the normal fish is $3.91(10^4 mm^{-3})$ and in the infected fish the mean value is $6.05(10^4 mm^{-3})$. There is a significant increase in the WBC count (P<.0001) in the infected fish compared to that of normal fish. Thrombocyte count decreased in the infected fish. The average mean value of thrombocyte in normal fish is $15.7(10^6 mm^{-3})$ and in the infected fish it is $12.25(10^6 mm^{-3})$.There is a significant decrease in thrombocytes in the infected fish (P<.0001) compared to that of normal fish.

The average mean gram percentage of haemoglobin in the normal fish is 14.03 where as in the infected fish the mean value is 12.11. There is a significant decrease in haemoglobin percentage (P<.0001) in the infected Fish compared to that of normal fish.

Table-1: Range, Mean and standard deviation difference between normal and *Aeromonad* infected *P.hypophthalmus*

Parameter	Normal	Infected
RBC($X10^6$ mm^{-3})	4.66±0.12 (4.5-4.8)	4.215±0.11 (4.1-4.4)
WBC($X10^4$ mm^{-3})	3.91±0.12 (3.8-4.1)	6.05±0.2 (5.8-6.3)
THROMBOCYTE($X10^4 mm^{-3}$)	15.7±0.54 (15.0-16.4)	12.25±0.37 (11.8-12.7)
HB g%	14.03±0.51 (13.2-14.6)	12.11±0.35 (11.6-12.6)
PCV %	42.79±1.93 (41.2-46.5)	35.16±2.04 (32-38)
MCV(fl)	84.57±3.4 (79.12-88.3)	90.35±2.12 (86.9-92.8)
MCH(pg)	28.56±1.68 (27.4-31.2)	25.51±2.06 (22.5-28.6)
MCHC (%)	33.3±0.67 (32.8-34.2)	30.73±0.86 (29.8-31.7)
NEUTROPHIL %	25.06±1.41 (23-28)	35±1.4 (33-37)
LYMPHOCYTE %	71.08±1.15 (68-72)	60.5±1.5 (58-62)
MONOCYTE %	2.5 (2-3)	3.5 (3-4)
EOSINOPHIL %	2 (1-3)	1 (0-1)

The average mean PCV percentage is 42.79 in normal fish and 35.16 in infected fish. There is a significant decrease in PCV value (P<.0001) in the infected fish compared to that of normal fish.The average mean MCV in normal fish is 84.57 (FL) and in infected fish is 90.35 (FL). There is a significant increase in MCV level in the infected fish (P<.001) compared to that of normal fish.

The average mean MCH in normal fish is 28.56 (pg) and in the infected fish it is 25.51(pg). There is a significant decrease in MCH level in the infected fish (P<.01) compared to that of normal fish .The average mean MCHC percentage in the normal fish is 33.3 and in the infected fish are 30.73.There is a significant decrease in MCHC level in the infected fish (P<.0001) compared to that of normal fish.

In the differential count of WBC, the mean neutrophil percentage in normal fish is 25.06 and in the infected fish are 35. There is a significant increase in neutrophil percentage in the infected fish (P<.0001) compared to that of normal fish. The mean Lymphocyte percentage in the normal fish is 71 and in the infected fish are 60.5. There is a significant decrease in lymphocyte percentage in the infected fish (P<.0001) compared to that of normal fish. The mean Monocyte percentage in the normal fish is 2.5 and in the infected fish it is 3.5. There is a significant increase in monocyte percentage in the infected fish (P<.0001) compared to that of normal fish. The eosinophil percentage in the normal fish is 2 and in the infected fish it is 1. There is a significant decrease in eosinophil percentage in the infected fish (P<.0001) compared to that of normal fish.

In the present study, the mean values of RBC, HB, PCV, MCH, MCHC, Thrombocytes, lymphocyte percentage, eosinophil percentage of the infected fish decreased when compared with the healthy fish but the MCV, WBC, neutrophil percentage, monocyte percentage of the infected fish increased when compared with normal fish (Table 1).

DISCUSSION

The decreased haemoglobin trend may be a result of the swelling of the RBC as well as poor mobilization of haemoglobin from the spleen to other hemopoeitic organs (Scott *et al.,* 1981). The data support the present findings that the significant decrease in RBC and haemoglobin content is possibly due to hypo chromic microcytic anemia caused by *A. hydrophila*. Decreased RBC counts, hematocrit and hemoglobin concentration indicate thatRBCs are being destroyed by the leucocytosis activity in an erythrocytic anemia with subsequent erythroblastosis (Haney *et al*., 1992).

Decreased red blood corpuscles and PCV were found in asian cichlid fish (*Etroplus suratensis*) with epizootic ulcerative syndrome (Pathiratne *et al.,* 1998) in rainbow trout experimentally infected with *Aeromonas sobria* and *A.caviae* (Rehulka, 2002) in carp (*Cyprinuscarpio*) experimentally infected with *A. hydrophila* (Harikrishnan *et al.,* 2003) and in nile tilapia experimentally infected with *Streptococcus iniae* (Chen *et al.,* 2004).In this present study, increase in mean cell volume was observed in fish infected with of *A.hydrophila*, it may be attributed to the swelling of the erythrocytes, resulting in a macrocytic anaemia. An increase in mean cell volume is also linked to the swelling of the RBC as a result of a hypoxic condition or impaired water balance (osmotic stress) or macrocytic anaemia in fishes exposed to stress (Tort *et al.,* 1988) this would increase the affinity for oxygen in the blood (Sovlo *et al.,* 1981). The decreased level of mean cell haemoglobin and mean cell haemoglobin concentration were observed in fish infected with *A.hydrophila* in the present study clearly indicates that the concentration of HB in the RBC was much lower in the infected fishes than in the control fishes, thereby indicating an anemic condition. The mean cell haemoglobin concentration, as a good indicator of RBC swelling is neither influenced by the blood volume nor by the number of cells in the blood, so can be interpreted incorrectly when new cells with different haemoglobin concentration are released into the blood circulation (Soivio *et al.,* 1981). A significant decrease in the mean cell haemoglobin concentration after *A. hydrophila* infection is probably an indication of RBC swelling and or a decrease in haemoglobin synthesis.

The significant decrease in the mean cell haemoglobin concentration in this study was an indication of erythrocytes swelling and/or due to a decrease in hemoglobin synthesis. The increase in mean cell volume and the reduction in mean cell haemoglobin and mean cell haemoglobin concentration in the infested catfish with *Henneguyosis* were similar to the results reported by Lebelo *et al.,* (2001).

Increased number of monocytes and neutrophils and decreased number of eosinophils observed in this study were in agreement with the findings in Pacu, (*Piaractus mesopotamicus)* following infection with *A. hydrophila* (Garcia *et al*., 2007), and in common carp injected with *A. hydrophila* (Selvaraj *et al*., 2004).In the present study, decrease in lymphocytes percentage and increase in those of neutrophils and monocytes were observed.

When the organic defense mechanism isconcerned, the counts of the blood defense cells include leucocytes and thrombocytes (Tavares-Dias *et al*., 1999b, c and Tavares-Dias *et al*., 2000b, c, d). This concept is based on the pathology aspects but not on the physiological ones. Thrombocyte may be considered blood cell of defense and they involved in the organic defense mechanism (Penha Dias*et al*., 1996 and Martins, 2000).

Similar to the present study, the abundance of thrombocytes in blood of healthy fish was observed by several authors (Chondary, 1982; Murray, 1984; Lea-Master *et al*., 1990; Tavares-Dias And Faustino, 1998; Tavares-Dias *et al*., 1999b, c; Tavares-Dias *et al*., 2000b, c, d).

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