HAEMATOLOGICAL STUDIES OF SOME EDIBLE FRESH WATER FISHES OF NCR REGION

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ABSTRACT
The haematological studies have been undertaken to analyses the effect of pollutants on blood parameters. The blood parameters can be considered as a potential bio-indicators in assessing the physiological status of fish and the contained in this regard might also provides substantial on the quality of the water body.

KEYWORD: Channa punctatus, Heteropneustes fossilis, Notopterus notopterus, Erythrocytes, Lucocytes, Monocytes, Haemoglobin.

1. INTRODUCTION
Fish is one of our most valuable source of protein about 25% of animal protein is obtained from fish. Most of pesticides find their way into rivers, lakes and ponds and have been found to be highly toxic not only to fishes but also to the organisms which contribute to the food chain of fishes (Anees, 1975).

NCR has a diverse and important abode of several important fish species of fresh water ecosystem. These natural aquatic resources harbouring variety of fishes exist in the form of rivers, lakes, reservoirs, wetlands ponds and tanks.

The toxicity of sublethal concentrations of effluents from a soap and detergent industry on African catfish Clarias gariepinus using a renewable static bioassay were investigated by Ayandiran et al. (2009). The trend of bioconcentration of metals in the muscle and gut of the test organisms differs significantly (p > 0.05) and it followed the order, gut > muscle. The result revealed that the muscle had the least concentration of manganese at 0.1 x 10^-3 mg/kg and 10.7 x 10^-3 mg/kg recorded for zinc as the highest. While the highest iron concentration of 15.80 x 10^-3 mg/kg was recorded in the gut tissues of C. gariepinus, but
mercury had the least concentration of 1.00 x 10^{-3} \text{ mg/kg}. It was revealed that fish can bioaccumulate heavy metals from a polluted environment, which may result in impairment of natural population size; thus consumption of fish from such polluted environment should be discouraged.

2. Review of Literature

Jordan and speidal (1924) and (1929), first time reported the presence of Neutrophills in the blood of fish. Comparatively little attempt has been made on the morphology of fishes. Gray and Hall (1930), studied blood sugar and the activity of fish. One of the earliest attempts on the morphology of blood in carp and trout was made by Field et al. (1943).

Bell, G.H. have worked on various aspects of protein profile study of fishes. Laemmli (1970) carried out Electrophoresis of proteins in preserve of SDS Band counting method was used as given by Ferguson (1980) described erythrocyte measurements in fishes, Haider (1972), reported haematological observation on rainbow trout *Salmo qairdneri*. Saxena and Sharma (1978) made observation on plasma erythrocyte in *Channa punctatus* and *Notopterus notopterus*.

A review of the literature reveals that the knowledge of fishes blood and protein profile is merge and fragmentary. An attempt therefore has been made to study the Hematology and protein profile study of some fresh water *Channa punctatus*, *Heteropneustes fossilis*, *Notopterus notopterus*.

3. MATERIALS AND METHODS

This section deals in Materials and Methods used is an integrated format for conducting acute and chronic studies, when special situation arises, the standard and basic methods have been modified. Following Fishes were studied *Channa punctatus*, *Heteropneustes fossilis* and *Notopterus notopterus*.

The specimens of live fishes for the present work were collected from different areas like ‘Kalinadi’ of Meerut and ‘Hindan river’ of Ghaziabad and ponds and rivers of the Hastinapur and Delhi/NCR region mainly Hindan & Yamuna river by the help of fisherman.

Fishes were brought to the laboratory for experimentation in plastic bags with proper handling method collection conducted in a way that minimizes habitat disturbance and “Excessive Mortality”. The Fishes were brought to the laboratory were kept in glass aquaria.
to acclimatize the fish with the laboratory conditions. The blood from the fish was collected by taking out the fish from aquaria and made unconscious by stunning. In large size fishes, the blood was collected from the caudal vein while in small fishes; the blood was collected directly from the Heart. The blood was taken with the help of syringe and needle for the total R.B.C and W.B.C counting. Packed cell volume and hemoglobin concentration were analyzed within two hours after collection Red blood cells (R.B.C) and white blood (W.B.C.) were counted by Neubauer's improved haematocytometer using Hayem's and Turk's solution as a diluting fluid respectively, packed cell volume (PCV) were calculated using standard formula. Plastic syringes containing a small amount of anticoagulant such as lithium-ammonium Heparin and Sodium citrate was used to avoid clotting. Study objectives determine the proper selection of type volume and concentration of anticoagulant.

3.1. Erythrocytes
The size of erythrocytes was measured in micrometer on air dried methanol fixed blood film by occulometer. A mean of 60 measurements was taken into consideration.

3.5 Total erythrocytes count
It is the number of RBC per cubic millimeter of blood. D’Amour and Blood (1954) method for TEC was followed along with Hayem’s diluting fluid and Neubauer’s haemocytometer. For the estimation of TEC, the blood was sucked in R.B.C pipette upto 0.5 mark and then it was diluted with Hayem’s diluting fluid (0.5 gm mercuric chloride; 1.0 gm sodium chloride; 5.0 gm sodium sulphate in 200 ml. of distilled water) upto 101 mark and mixed thoroughly by rotating the pipette for about three minutes. In this way the dilution of blood becomes 200 times. After discarding first few drops, the improved Neubauer’s chamber was charged with diluted blood. The erythrocytes were allowed to settle in the counting chambers for 5 - 10 minutes. After the RBCs got settled, their numbers were counted in the 5 squares, 4 at the corners and one at the centre of haemocytometer chamber. The total number of RBCs in 5 squares was multiplies by 10,000 to obtain total number erythrocyte count per cubic millimeter of blood. The same process was repeated for second haemocytometer chamber.

\[
\text{RBC count} = \frac{\text{No. of cells counted} \times \text{dilution} \times \text{depth factor}}{\text{Area counted}}
\]

Where,
\[
\begin{align*}
\text{Dilution} & = 200 \text{ times} \\
\text{Depth of blood film} & = 1/10 \text{ mm} \\
\text{Area count} 80/400 & = 1/5 \text{ sq. mm}
\end{align*}
\]
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= No. of cells counted X 200 X 10 X $5^5$

RBC count = No. of cells counted X 10,000/ mm$^3$

3.6. Leucocytes

For morphology and differential leucocytes counts, blood film was prepared and stained with Leishman’s and Wright’s stain.

3.7. Total Leucocytes Count (TLC)

It is the number of WBC per cubic millimeter of blood. The blood was sucked in WBC pipette up to 0.5 mark and then it was diluted with Turke’s fluid (1.5 ml glacial acetic acid; 1.0 ml aqueous solution of gentian violet (1%) and a pinch of thymol in 98.0 ml distilled water) up to 11 mark and mixed thoroughly by rotating the pipette for about three minutes. In this way, dilution of blood becomes 20 times. After about 2-3 minutes it was again mixed by rotating the pipette for about two minutes. After discarding first few drops, improved Naubauer’s chamber was charged with diluted blood. WBCs were allowed to settle after which, WBCs were counted in large squares of each of the corner. The total number of WBCs counted in 4 large squares was multiplied by 50 to obtain the total leucocytes count per cubic millimeter of blood. The same process was repeated in second haemocytometer chamber.

\[
\text{RBC count} = \frac{\text{No. of cells counted} \times \text{dilution} \times \text{depth factor}}{\text{Area of chamber counted}}
\]

Where,

Dilution = 20 times
Depth of blood film = 1/10 mm
Area of each of its 16 chambers = 4

\[
\text{WBC count} = \frac{\text{Cells counted} \times 20 \times 10}{4} = \frac{\text{cells counted} \times 50 \text{ mm}^3}{4}
\]

3.8. Differential Leucocytes Count

It denotes the percentage count of different types of leucocytes in the blood. After making thin smears, the slides were dried in air. The dried blood smear were fixed in methyl alcohol for 1-2 minutes and put into Wright’s stain for 15-20 minutes washed with distilled water and finally dried in air. Giemsa’s and Leishman’s stains were also tried for comparison. For differential leucocyte count, the stained blood films were examined under oil-immersion lens. Different types of leucocyte were counted in a longitudinal strips from one end to the other to
count 100 cells. The percentage of each type of cell was calculated separately by the following formula:

\[
\text{Percentage of cells} = \frac{\text{No. of type of cells}}{\text{Total No. of WBC}} \times 100
\]

3.9. Packed Cell Volume (PVC)

It represents the percentage of cells present in the blood. Wintrobe’s (1933) method for PCV was adopted by using Wintrobe’s haematocrit tubes. The haematocrit tubes were filled with blood mixed with anticoagulant EDTA up to 100 mm mark with the help of fine glass dropper. Then, the tubes were centrifuged at 3500 rpm. for 15 minutes. The percentage of the height of the column of blood, occupied by the packed red cells, constituted the haematocrit value. Process was repeated 4 or 5 times and mean were taken.

PCV was calculated by using the following formula:

\[
\text{PCV (\%)} = \frac{\text{Length of RBC column}}{\text{Length of whole blood column}} \times 100
\]

3.10. Erythrocyte Sedimentation Rate (ESR)

It represents the time taken for setting of erythrocytes under force of gravity. Wintrobe and Landsbergs’s (1935), method for erythrocyte sedimentation rate was followed. The Wintrobe’s haematocrit tubes were filled with anticoagulated blood up to mark of 100 mm with the help of pipette and were places vertically on a stand. At regular intervals of one hour, the reading of the column, to which the erythrocytes had fallen, were noted. The same experiments were repeated and mean figure was taken.

3.11. Haemoglobin (Hb)

It is the concentration of haemoglobin present in the blood. For estimation of haemoglobin concentration, Sahli’s method was followed.

The graduated haemoglobin tube was first rinsed with distilled water and then with the methylated spirit or 90% alcohol. After drying the tube, it was filled up to 2 gm mark (on percentage side) with deci normal hydrochloride acid. The blood was sucked in haemoglobin pipette upto 20 mm mark. The blood of haemoglobin pipette was then transferred carefully into the graduated tube containing N/10 HCl. After the blood was expelled, the pipette was rinsed twice or thrice with distilled water. Every time the contents of haemoglobin pipette
were expelled into the graduated tube. The blood and N/10 HCl was stirred in the graduated dilution tube with the stirrer and then allowed to stand for about 10 minutes. In this treatment the haemoglobin was changed into acedic haematin and the mixture became dark brown in colour. Now N/10 HCl was added drop by drop and stirred continuously with stirrer till the colour of the content matched with that of the standard glass tube. The reading was noted to denote the concentration of haemoglobin in gms per 100 ml of blood. This process was repeated and mean value was taken.

RESULTS AND DISCUSSION

**Channa punctatus**

**Red Blood Corpuscles (RBC)**

During the present observation the decrease in RBC count was observed in the polluted water fishes. The mean value of RBC count was 2.06 million/cumm in fresh water specimen of *Channa punctatus* and the mean of RBC count was 1.99 Million/cumm in polluted water fishes which were corroborates with the findings of earlier workers like Sachdeva (1994), Singh (1995), Yoshinaga (2001) Joseph John (2007). According to these workers this could be due to haemolysis and haemorrhage and due to invading worms and disturbance in erythropoisis water pollution infection produces macrocytic anemia with decrease RBC Number.

**Total Leucocytes Count (TLC)**

During the present investigation the value of TLC is highly elevated. The value of TLC count was 27,338 in the fresh water fishes and the value of TLC count was 35,983 in the polluted water fishes. This could be due to the immunological responses of fishes against the foreign invader. It might be due to the injuries at various sites, due to the pollutants of water which subsequently stimulates the immune system of fishes and resulted into the increase in the number of TLC. These findings are correlate with the workers like. Omoreg, (1998). Sinha (2000) Shamim and Pandit (2002).

**Packed Cell Volume (PCV)**

Decrease in PCV% was observed in polluted water fishes during the present investigation. The mean of PCV was 11.4% in the fresh water fishes and mean of PCV was 21.5% in the polluted water fishes. These findings corroborates with the Agarwal (1989). Sinha (2000) Abdul et al. (2011). That could be due to infection in fishes caused by the polluted water.
Erythrocyte Sedimentation Rate (ESR)
During the present investigation the ESR level increase in the polluted water fishes. The mean value of ESR in fresh water fishes was 11.1mm/hr in fresh water fishes and the mean value of ESR in polluted water fishes was 21.5mm/hr. These findings were also corroborates with the findings of Singh (1986) Saxena and Chauhan (1993), Joshi, et al. (2002), this could be due to the stress condition.

Haemoglobin (HB)
During the present investigation depletion of Hb concentration in polluted water fishes has been noticed throughout the experiments. The mean value of Hb concentration in fresh water fishes was 9.9gm% and the mean value of Hb concentration in polluted water fishes was 8.1gm%. It corroborates with the earlier findings of. Verdege et al. (1997) Shalaby (2001), Joseph John (2007). According to these workers decreased haemoglobin is due to as acid stress causes increase in erythropoisis, which might have not been followed by haemoglobin synthesis.

Differential Leucocytes Count (DLC)
During the present investigation different type of leucocytes has been studied. Proleucocytes and Lymphocytes percentage has been significantly increased in polluted water. The mean value Proleucocytes in fresh water fishes was 38.5% and the mean value was 52.2% in polluted water fishes. The mean value of lymphocytes was 41.6% and 45.9% in fresh water and polluted water fishes respectively. The percentage of Eosinophils and Monocytes has been dropped in polluted water fishes. The mean value of Eosinophils in fresh water fishes was 4.9% and the men value was 0.16% in the polluted water fish and the mean value of Monocytes was 3% and 2% in fresh water and polluted water fishes respectively which confirms the findings of earlier workers like Goel et al. (1984), Singh (1995).ording to these workers a significant increase in Proleucocytes and lymphocytes and decrease in Eosinophils and Monocytes might be due to immunological reaction to produce more antibodies to cope with toxic effect and the infection induced by the pollutants present in the polluted water.

Heteropneustes fossilis
Red Blood Corpuscles (RBC)
During the present investigation, decrease in erythrocyte count was observed in polluted water fishes. The mean of RBC count in fresh water fishes was 1.95million/cumm and the mean of RBC count in polluted water fishes was 1.14 million/cumm which corroborates with
the findings of earlier workers like. Singh (1986) Murad and Mustafa (1988) Saxena and Seth (2002) and Shalaby (2001). According to these workers this is due to haemolysis and haemorrhage due to invading worms in polluted water.

**Total Leucocytes Count (TLC)**
During the present investigation the TLC count was highly increased in the polluted water fishes. The mean value of TLC count was 27,600/cumm in fresh water and 34,200/cumm in polluted water fishes. Similar findings were observed by earlier workers like Dheer (1988) Devi et al. (2001). Adeyemo (2005). According to these workers this could be due to immunological responses of fishes against the forgine invaders. It is due to the antibodies and antitoxins are present in the fishes.

**Packed Cell Volume (PCV)**
Decrease in PCV% was observed during the present investigation in the polluted water fishes then that of fresh water fishes. The mean PCV% was 26.3% fresh water fishes and the mean PCV was 17% in polluted water fishes. This could be due to the toxicants presents in polluted water influence the Malfunctioning of the haemopoititic system. Therefore the haemopoitetic tissues fail to release the blood cells which subsequently release into the blood stream. Similar type of observation were also reported by the Hussein et al. (2000).

**Erythrocyte Sedimentation Rate (ESR)**
During the present investigation increased in the ESR level was reported. The mean value of ESR in fresh water fishes was 10.9mm/hr and mean value was 56.2 mm/hr in the polluted water fishes. This could be due to the infection produced by the pollutants in the polluted water as discussed by the earlier workers like Joseph John (2007).

**Hameoglobin (HB)**
During the present investigation the regular decrease in haemoglobin was observed throughout the experiments. The mean value of Hb% in fresh water fish was 10.9gm% and 8.5 gm% in polluted water fishes. The decrease in Hb% is due to haemolysis or RBC and subsequent catabolism of Hb as observed by the earlier workers like Tor et al. (1987).

**Differential Leucocytes Count (DLC)**
The use of differential Leucocyte count as a reliable haematological index as suggested by Mohan Gojer and Vijay Sawant (1989). During the present observation different type of
Leucocytes has been studied. Proleucocytes and lymphocytes percentage has been significantly increased in polluted water. The mean value of Proleucocytes in fresh water was 42.9% and the mean value was 46.05% in the polluted water fishes. The mean value of lymphocytes was 44.4% and 47.3% in fresh water and polluted water fishes respectively. Which corroborates with the findings of earlier workers like Goel et al. (1984) Devi et al. (2004) Ramesh and Saravanan (2008). According to these workers a significant increase in proleucytes and lymphocytes and decrese in Eosinophils and Monocytes might be due to immunological reaction to produce more antibodies to cope with toxic effect and the infection induced by the pollutants present in the polluted water. The present study reveals the haematological parameters are more predominantly altered by water pollution. The result also revealed that water pollution infection produce macrocytic anlmia with decreased RBC number and increased in TLC and DLC content in fishes.

*Notopterus notopterus*

**Red Blood Corpuscles (RBC)**

During the present investigation, decrease in RBC count was reported in polluted water fishes. The mean of erythrocyte count in fresh water fishes was 2.24million/cumm and the mean value was 2.17million/cumm in the polluted water fishes which correlate with the findings of earlier workers like Singh (1986) Kumar et al. (1991) Barad and Kulkarni (2010). According to these workers this is due to haemolysis and haemorrhage due to invading worms and disturbances in erythropoiesis.

**Total Leucocytes Count (TLC)**

During the present investigation the TLC count is highly elevated this could be due to the immunological responses of fishes against the foreign invader. The mean value of TLC in fresh water fishes was 25,463/cumm and the mean value of TLC count was 73,129/cumm in the polluted water fishes of genus *Notopterus*. This corroborates with the findings Murad and Mustafa (1988).

**Packed Cell Volume (PCV)**

It was observed that polluted water fishes showed significant depletion in PCV%. The mean value of PCV was 33.4% in fresh water fishes and the mean value of PCV was 31.9% in polluted water fishes. This corroborates with the findings of Kumar and Banerjee (1991), Devi *et. al.* (2004). According the these workers this could be due to the fact that the fall in PCV% is due to the RBC and haemoglobin are also decreasing in the polluted water fishes.
Erythrocyte Sedimentation Rate (ESR)
During the present investigation it was observed that the value of ESR is decreased in polluted water fishes in comparison to fresh water fishes the mean value of ESR in fresh water fishes was 19.7 mm/hr and the mean value of ESR was 68.7 mm/hr in the blood of polluted water fishes. These findings were also corroborated with the findings of Verdegem et al. (1997) Josephjohn (2007) according to these workers this could be due to toxic effect of pollutants present in the polluted water. It is may be due to stress condition.

Haemoglobin (HB)
During the present investigation it was observed that haemoglobin concentration decreases throughout the experiment in the polluted water fishes in comparison to fresh water fishes. The mean value of Hb in the fresh water fishes was 13.1 gm% and the mean value was 10.3 gm% in the blood of polluted water fishes. These findings were also corroborated with the findings of Joshi & Bose (2002) Ramesh and Saravanan (2008) according these workers this could be due to fall in erythrocyte count and due to parasitic infection in polluted water.

Differential Leucocytes Count (DLC)
During the present investigation different type of leucocytes has been studied Proleucocytes and lymphocytes percentage has been significantly increased in polluted water fishes. The mean value of Proleucocytes in fresh water fishes was 33.8% and the mean value was 36.4% in polluted water fishes and the mean value of Lymphocytes was 42.1% and 45.9% in the blood of fresh water and polluted water fishes respectively. The percentage of Eosinophils and Monocytes has been dropped in polluted water fishes in comparison to the fresh water fishes. The mean value of Eosinophils in fresh water fishes was 7.4% and the mean value was 5.7% in the blood of polluted water fishes. The mean value of Monocytes was 4.4% and 1.9% in the blood of fresh water and polluted water fishes respectively which confirms the findings of earlier workers like Adeyemo (2005), Gabriel et al. (2007). According to these worker a significant increase in Proleucocytes and lymphocytes and decrease in Eosinophils and Monocytes might be due to immunological reaction to produce more antibodies to cope with toxic effect and the infection induced by the pollutants present in the polluted water.

The present study reveals that haemotological indices are more predominantly altered by water pollution Yousuf and Shah (1988), Shamim and Pandir (2002). The result revealed that blood values remarkable very in different fishes. The decrease in the number of RBC
probably reflects the physiological functioning of Haemopoisis system which is considered to be the most sensitive indicator towards water pollutants. The water pollution infection produced Macrocytic anemia with decreased RBC number and increased in TLC and DLC content in fishes.

Blood forms a unique compartment between external and internal environment and any agent including toxic substances that cause stress can alter blood composition. Blood parameters are considered good physiological indicators of the whole body conditions and therefore can be used as a diagnosing tool for the structural and functional status of fish exposed to toxicants.

CONCLUSION
The haematological studies have been undertaken to analyse the effect of pollutants on blood parameters. The blood parameters can be considered as a potential bio-indicators in assessing the physiological status of fish and the contained in this regard might also provides substantial on the quality of the water body as such. The review of the literature on haematological studies in fishes indicated that the data obtained from various fish species by various workers around the globe is not uniform. Since the fish are the most sensitive fauna any little change that occurs in their living media might have immediately influenced on their physiology. The result of present study indicates that entry of pollutants in the blood stream of fish promotes their ill effects on various blood parameters the reduction in RBCs and PCV and HB percentage indicates the occurrence of acute anemia such anemia in fishes is known to induced by various toxicant Agarwal et al. (1982). The polluted water fishes suggest the destruction of RBC which results in reduced oxygen carrying capacity of fish and ultimately death of fish. The gills and the body of the polluted fishes become pale whitish and colourless in contrast to the normal individuals in which the gills were dark red. Due to the toxic effect of pollutants present in water. The increased erythrocyte sedimentation rate (ESR) and decreased packed cell volume (PCV) account for the degradation of blood proteins in fish as observed by Bhatt (1985). The increase in TLC count can be correlated with a increase in antibody production which help in survival and recovery of the fishes exposed to the toxicants. Increase number of TLC and DLC values may be associated with the defense mechanism and immunological responses against infectious diseases caused by water pollution.

Under the light of this Haematological study it is concluded that polluted water results in fishes a significant alterations in different haematological parameters and this kind of
physiological change may directly affect the Survivability of these fishes in their natural habitat.

REFERENCES

