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Enumeration of WBC in the blood of fishes from Ana Sager and Foy Sager Lake

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Abstract
This present study would form a baseline data for assessment of health status of the fish as well as reference point for future comparative study of enumeration of W.B.C. in the blood of fishes from Ana Sager and Foy Sager reservoir of Ajmer. Experimental material incorporated Indian major carps i.e. Rohu (*Labeo rohita*) and Catla (*Catla catla*). A total of 240 fish were incorporated in the study. Out of this, 120 fish type were *Labeo rohita* (Lr) and 120 fish type were *Catla* (Cc). The mean values of WBC number from each site revealed significant differences ($p \leq 0.05$) among themselves. Fish collected from Ana Sager site 4 revealed significantly ($p \leq 0.05$) highest values of WBC number as compared to rest of the other sites. This revealed that maximum stress was developed in the fish collected from Ana Sager site 4. Higher WBC number is an indicative of infection. This site was having maximum pollution as indicated by the water quality parameters.

Keywords: White blood cell, Ana sager and Foy Sager

Introduction
Fishes may be confronted with stress factors such as varied water qualities, pollution, malnutrition and disease. Fishes can adapt themselves to bad environmental conditions by changing their physiological activities. Qualitative and quantitative variations in haematological analytes including the red blood cell (RBC) and white blood cell (WBC) numbers, cell proportions of leukocyte, the amount of haemoglobin (Hb), and the size of RBC and WBC are the most significant findings about diagnosis.
Knowledge of the haematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes. Normal ranges for various blood parameters in fish have been established by different investigators in fish physiology and pathology (Rambhaskar and Srinivasa Rao 1986; Xiaoyun *et al*. 2009) [4, 7]. The analysis of blood indices has proven to be a valuable approach for analyzing the health status of farmed animals as these indices provide reliable information on metabolic disorders, deficiencies and chronic stress status before they are present in a clinical setting (Bahmani *et al*. 2001) [1]. Teixeira (2000) [6] stated that the reference values determined for haematological elements and biochemical tests may not represent precisely those of a certain population or animal species and should, therefore, be carefully interpreted once there was a wide range of physiological variations. According to these authors, these variations might be influenced by environmental conditions, gender, age, origin, breeding system and feeding. Sahan *et al.* (2007) [5] carried out haematological studies in fishes and suggested that higher WBC count along with an increase in neutrophila was suggestive of an infection.
This present study would form a baseline data for assessment of health status of the fish as well as reference point for future comparative study of enumeration of W.B.C. in the blood of fishes from Ana Sagar and Foy Sagar Lakes of Ajmer.

Material & Method
Experimental material incorporated Indian major carps i.e. Rohu (*Labeo rohita*) and Catla (*Catla catla*). A total of 240 fish were incorporated in the study. Out of this, 120 fish type were *Labeo rohita* (Lr) and 120 fish type were *Catla* (Cc). To evaluate the impact of pesticides and their possible damage to aquatic life, water samples and fish were collected from two reservoirs (lakes) of Ajmer city, Rajasthan namely Ana Sagar lake, Ajmer, Rajasthan and Foy Sagar lake, Ajmer, Rajasthan.

Total leucocytic count (TLC) or Enumeration of W.B.C.
The number of white cells in the blood is much smaller. The dilution required is much less. The bulb of WBC pipette has a capacity which is only 10 times that of its stem. The number of white cells is counted in a relatively large amount of space and under low power of the microscope.

1. The column of blood was drawn to the 0.5 mark in WBC diluting pipette.
2. Excess blood from the tip of pipette was removed.
3. While keeping the pipette vertical in position, the diluting fluid was sucked to 11 marks.
4. Blood was mixed with diluting fluid by rolling the pipette between palms.
5. Then discarding of 2-3 drops of fluid from pipette was done and then charging of the haemocytometer as described for RBC was done.
6. Counting was carried out under low power objective of microscope in four large corner squares. Upper Left Square of 16 small squares was counted first, then the upper right, the lower right and last the lower left. The counting followed the same pattern described under RBC counting. Those leucocytes which are half in and half out of the upper and left-hand line are included in the counting but not those halves in and half out of the lower and right-hand lines. The boundary line is the center line of the triple ruling.

Calculation
V = D X Dp X S.A = 1/20 X 1/10 X 4 = 1/50 cu. mm.
V = Volume of blood
D = Dilution of the blood sample 1:20
Dp = Depth of the counting chamber 1/10mm
S.A = Surface area counted Area counted 4 sq. mm.

$$C = N \times \frac{1}{V} \text{ per cu. mm.} = N \times \frac{1}{1/50} = N \times 50$$

C = Cell count per cu. Mm.
N = Number of cells counted in the chamber Sum of the 4 squares
V = Volume of blood (from above formula)

Result & Discussion
WBC counting
Mean ± SEM values of WBC number of male and female fish i.e. *Labeo rohita* (Rohu, LR) and *Catla catla* (Catla, CC) collected from different areas of Ana Sagar lake (site 1, site 2, site 3, site 4 and site 5) and Foy Sagar lake are presented in table 1. In each gender, fish were further grouped as low-weight (LW) and high weight (HW). The data are based on 20 observations each as specified in the section of materials and methods.
The mean values of WBC number from each site revealed significant differences ($p \leq 0.05$) among themselves. Fish collected from Ana Sager site 4 revealed significantly ($p \leq 0.05$) highest values of WBC number as compared to rest of the other sites. This revealed that maximum stress was developed in the fish collected from Ana Sager site 4. Higher WBC number is an indicative of infection. This site was having maximum pollution as indicated by the water quality parameters. A link of WBC number content was observed with the water pH also. Extremely higher or lower pH impinged on WBC number of fish of both genders. This also existed in the values of WBC number of fish collected from different areas.
At each collection site, *Catla catla* fish revealed higher WBC number. This exhibited that CC fish built up greater degree of infection and stress than LR fish. Effect of pollutants in water was more in CC fish. In both the category of fish, females revealed higher WBC number than males. This pointed up that females of both the type of fish had developed higher scale of infection or stress with effect on WBC number. Furthermore, it was found that low-weight male and female fish had larger degree of impact on WBC number than high-weight male and female fish. WBC number was significantly ($p \leq 0.05$) higher in low-weight fish than high-weight fish.
WBC number was associated to be linked with the pH of water samples from where the fish were collected in the investigation. Variations in the pH of water samples indicated greater degree of pollutants in the water. This was also related with greater contents of TSS and TDS in water reservoirs. These features decreased the water quality. It divulged that pollution of the water altered the WBC number of fish of both the types i.e. LR and CC. It appeared that pH of water, presence of pollutants in water, WBC number and antioxidant status of fish were having great relation. Impurities in water can influence water pH. Earlier researchers are of the stance that distant pH values than neutral in a water collection area can affect the development of water animals thereby influencing ecosystem badly and modulating physiology.
The mean values of WBC number obtained in the present investigation showed more or less similar precedent in fish obtained from *Ana Sagar* site 1 and were taken as control values. The average pH of water samples from this site was 7.00. On the basis of available control values, it was concluded that the mean values of WBC NUMBER of both the types of fish divulged stress.
Higher concentration of WBC number in fish indicated the infection in blood. In animals also, lower WBC number is a reflection of effect on health (Kataria and Kataria, 2005). In each area, WBC number was significantly higher in *Catla catla* than in *Labeo rohita.*
Upshot of the present exploration disclosed that stressful condition in water pool could be the motive for changed WBC number values leading to ill effects on health.
At each area the mean values were significantly lower in the blood of LR than in CC. This probably reflected the physiological status of the LR fish as blood is an indicator of physiological condition.
Higher WBC number in the fish probably indicated infection. In the present investigation, the polluted areas were categorized on the basis of variation in pH from neutral pH of water bodies and increase in TSS and TDS values of water bodies. According to these observations, polluted areas were Ana Sagar site 5, Foy Sagar, Ana Sagar site 2, Ana Sagar site 3 and Ana Sagar site 4. WBC number values from fish of Ana Sagar site 1(Neutral pH) were taken as control. The increase in WBC number followed the sequence of polluted areas. Least value of WBC number was observed in the fish from Ana Sagar site 4.
Raised WBC count can be observed in the cases of infection or stress. It is known that leukocyte cells are normally lower in healthy fish and could be used as a significant indicator for infectious diseases. In this study, an increase occurred in leukocyte cells of fish of the area having more pollution. Significant anaemia could be the cause of higher WBC count in polluted areas (Zutshi *et al.* 2010) [9].

Ecological attributes, living conditions and general body conditions are related with higher numbers of WBCs in the blood (Zhou *et al*. 2009) [8]. Juginu *et al.* (2017) [2] measured haematological parameters as a precious tool for assessing fish health. *Labeo rohita* was investigated and exposed to sublethal concentration of plywood effluent. The plywood effluent significantly increased WBC count. It was inferred that the plywood effluent produced haemotoxicity in fish.

Table 1: Mean values of haematological analytes of fishes collected from different areas of Ana Sagar and Foy Sagar lakes

Name of area	Type of fish			Mean ± SEM values WBC, 10^6 L^{-1}
Ana Sagar Site 1	LR Overall value (80)			11.40[b]±0.001
	LR (80)	M (40)	LW (20)	11.43[c]±0.0002
			HW (20)	11.33[d]±0.0001
		F (40)	LW (20)	11.47[c]±0.0002
			HW (20)	11.37[d]±0.0001
	CC Overall value (80)			11.50[c]±0.001
	CC (80)	M (40)	LW (20)	11.52[c]±0.0002
			HW (20)	11.44[d]±0.0001
		F (40)	LW (20)	11.56[c]±0.0002
			HW (20)	11.48[d]±0.0001
Ana Sagar Site 2	LR Overall value (80)			16.60[b]±0.002
	LR (80)	M (40)	LW (20)	16.63[c]±0.0002
			HW (20)	16.53[d]±0.0001
		F (40)	LW (20)	16.67[c]±0.0002
			HW (20)	16.57[d]±0.0001
	CC Overall value (80)			16.70[c]±0.001
	CC (80)	M (40)	LW (20)	16.72[c]±0.0002
			HW (20)	16.64[d]±0.0001
		F (40)	LW (20)	16.76[c]±0.0002
			HW (20)	16.68[d]±0.0001
Ana Sagar Site 3	LR Overall value (80)			17.80[b]±0.002
	LR (80)	M (40)	LW (20)	17.83[c]±0.0002
			HW (20)	17.73[d]±0.0001
		F (40)	LW (20)	17.87[c]±0.0002
			HW (20)	17.77[d]±0.0001
	CC Overall value (80)			17.90[b]±0.001
	CC (80)	M (40)	LW (20)	17.92[c]±0.0002
			HW (20)	17.84[d]±0.0001
		F (40)	LW (20)	17.96[c]±0.0002
			HW (20)	17.88[d]±0.0001
Ana Sagar Site 4	LR Overall value (80)			19.00[b]±0.001
	LR (80)	M (40)	LW (20)	19.05[c]±0.0002
			HW (20)	18.85[d]±0.0001
		F (40)	LW (20)	19.15[c]±0.0002
			HW (20)	18.95[d]±0.0001
	CC Overall value (80)			19.20[b]±0.02
	CC (80)	M (40)	LW (20)	19.25[c]±0.001
			HW (20)	19.05[d]±0.001
		F (40)	LW (20)	19.35[c]±0.001
			HW (20)	19.15[d]±0.001
Ana Sagar Site 5	LR Overall value (80)			14.50[b]±0.001
	LR (80)	M (40)	LW (20)	14.53[c]±0.0002
			HW (20)	14.43[d]±0.0001
		F (40)	LW (20)	14.57[c]±0.0002
			HW (20)	14.47[d]±0.0001
	CC Overall value (80)			14.60[c]±0.001
	CC (80)	M (40)	LW (20)	14.62[c]±0.0002
			HW (20)	14.54[d]±0.0001
		F (40)	LW (20)	14.66[c]±0.0002
			HW (20)	14.58[d]±0.0001
Foy Sagar	LR Overall value (80)			15.56[b]±0.001
	LR (80)	M (40)	LW (20)	15.59[c]±0.0002
			HW (20)	15.49[d]±0.0001
		F (40)	LW (20)	15.63[c]±0.0002
			HW (20)	15.53[d]±0.0001
	CC Overall value (80)			15.66[c]±0.001
	CC (80)	M (40)	LW (20)	15.68[c]±0.0002
			HW (20)	15.60[d]±0.0001
		F (40)	LW (20)	15.72[c]±0.0002
			HW (20)	15.64[d]±0.0001

Figures in the parentheses indicate number of observations in each case
LR = *Labeo rohita* fish
CC = *Catla catla* fish
M = Male
F = Female
WBC = White blood corpuscles
[b] = Significant ($p \leq 0.05$) difference in overall mean values of LR and CC
[c] = Significant ($p \leq 0.05$) difference for LW in a fish type
[d] = Significant ($p \leq 0.05$) difference for HW in a fish type

Reference

1. Bahmani M, Kazemi R, Donskaya P. A comparative study of some hematological features in young reared sturgeons (*Acipenser persicus* and *Huso huso*). Fish Physiol Biochem. 2001; 24:135-140.
2. Juginu MS, Kumari BS, Kumar MM. Impact of plywood effluent on the haematological parameters of fresh water fish, *Labeo rohita*. Inter. J Fisheries and Aquatic Studies. 2017; 5(1):112-115.
3. Kataria AK, Kataria N. Disease associated changes in white blood cell count in the cattle. The Indian cow. 2005; 1(3):25-28.
4. Rambhaskar B, Srinivasa Rao K. Comparative haematology of ten species of marine fish from Visakhapatnam Coast. J Fish Biol. 1986; 30:59-66.
5. Şahan A, Altun T, Çevik F, Cengizler I, Nevşat E, Genç E. Comparative study of some haematological parameters in European Eel (*Anguilla* L. 1758) caught from different regions of Ceyhan River, 2007.
6. Teixeira MA. Haematological and biochemical profiles of rat (*Rattus norvegicu*) kept under micro-environmental ventilation system. Braz. J Vet. Res. Anim. Sci. Sao Paulo, 2000, 37(50).
7. Xiaoyun Z, Mingyun L, Khalid A, Weinmin W. Comparative of haematology and serum biochemistry of cultured and wild Dojo loach *Misgurnus anguillicaudatus*. Fish Physiol Biochem. 2009; 35:435-441.
8. Zhou X, Li M, Abbas K, Wang W. Comparison of haematology and serum biochemistry of cultured and wild Dojo loach *Misgurnus anguillicaudatus*. Fish Physiol. Biochem. 2009; 35(3):435-441.
9. Zutshi B, Prasad SG, Nagaraja R. Alteration in hematology of Labeo rohita under stress of pollution from Lakes of Bangalore, Karnataka, India. Environ. Monit. Assess. 2010; 168(1-4):11-19.