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Haematological profile of farmed *Catla catla* from Multan, Pakistan

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Abstract: Haematological parameters are repeatedly used as an important tool to assess the health condition of fish. Although fish haematology acts as a valuable tool, but progress in establishing normal range values for blood parameters has been slow and literature in this area is often incomplete. The purpose of this study was to assess the reference values of some haematological parameters of *Catla catla*, which are mostly cultured in Southern Punjab, Pakistan. Fish were collected during October, 2019 from a fish farm located in Multan, Pakistan. Variation in haematological parameters such as red blood cells (RBCs), white blood cells (WBCs), haemoglobin (HGB) and platelets count (PLT) of fish were observed when compared to the earlier reference values. The RBCs, WBCs, HGB and PLT values in wild *Catla catla* were 2.58±0.53 (106/µL), 83.38±6.51 (106/µL), 11.56±1.44 (g/dl) and 89.87±10.69 (106/µL) respectively. Statistical relationship was studied applying linear regression analysis. RBCs, WBCs, HGB and PLT represented highly significant positive correlation ($p<0.001$) when plotted against length and weight of fish.

Keywords: Haematology, *Catla catla*, Haemoglobin, Fish size

1. INTRODUCTION

By regular monitoring of blood, health status of fish can be determined (De Pedre *et al.*, 2005; Martins *et al.*, 2008) For instance, the level of infection and immune response status can be determined by leukocytes count (Habte-Tsion *et al.*, 2013). Analysis of blood cell characteristics may provide clues of the disease (Anderson, 2004), and due to economic importance and comparative study of fish, knowledge of fish physiology is becoming more imperative (Bhaskar and Rao, 1985). Study of haematological parameters is easiest, cheapest and most reliable methodology to determine fish health (Kumar, 2016).

Major factors which bring major changes in blood chemistry are exogenous as, management, diseases, feeding regime, stocking density and stress due to handling of fish (Cnaani *et al.*, 2004; Chen *et al.*, 2005; Svobodova *et al.*, 2008; Najim *et al.*, 2014).

Blood contains 1.3-7% of fish body weight and ensures gas exchange between organs and the environment. For this reason, fish physiological condition can be depicted by analyzing blood parameters (Belanger *et al.* 2001). The fish blood plasma contains 97% water, minerals, electrolytes, hormones and blood cells (WBCs, RBCs) and platelets similar to other vertebrates (Southamani *et al.*, 2015). A wide range of individual variations in blood parameters is a characteristic of fish (Fazio, *et al.*, 2013).

Gills are the main entry point for pesticides in fish body and transported to various body parts through blood stream. For toxicity study, blood is an ideal medium (Tilak *et al.*, 2007).

Blood vascular system is the main communicating tissue and medium for all the cells of body. Metabolic processes were done in the blood. Physiology and pathology in fishes can be monitored by haematological index. Fish age, health status, species and sexual maturity and influence haematological parameters (Radu, *et al.* 2009; Tanti*et al.*, 2011).

Catla catla is most important fish with maximum market demand along with *Labeo rohita* and *Cirrhinus mrigala* (Krishnaveni *et al.*, 2013). It is a surface feeder and more sensitive to pollutant and environmental stressors (low DO level, thermal stress etc.) (Palaniappan and Karthikeyan, 2009).

Lack of reference values is a major constrain in fish hemograms study, because many factors influence fish blood constituents viz; temperature, habitat, food selection and mode of life, so it is difficult to determine any normal values for whole class (Kumar, 2016).

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Present study was conducted to establish reference values for *Catla catla* under natural conditions.

2. MATERIALS AND METHODS

Samples Collection

A total of 30 fish specimens of *Catla catla* were captured from a Kamran Fish Farm, located near Head Muhammad Wala, Multan, Pakistan, in October, 2019.

Haematological analysis

Fish specimens were anesthetized with buffered MS-222, (30mg/L), weighed with the help of measuring electronic balance (MP-3000 Chyo, Japan) and total length was measured. Subsequently blood was drawn by puncturing the heart directly, using 1 ml hypodermic syringe (*21 gauge*), blood samples were collected in EDTA (ethylene diamine tetra acetic acid, an anticoagulant) vials. Blood samples were delivered immediately to Fisheries Research Laboratory, Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan, for blood analysis. Red blood cells (RBCs), white blood cells (WBCs), haemoglobin (HGB) and platelets (PLT) were recorded using automatic heamatology analyzer (Medonic M-Series).

Data analysis

Mean values with standard deviation for each haematological parameter were determined. Relationships between blood indices and fish size (total length and body weight) and blood indices were also studied by using linear regression.

3. RESULTS

Weight of fish specimens was ranged 1465.00-1995.00 g with mean (±SD) value 1782.17±144.46 g, while total length was ranged 45.2-51.5 cm with mean value 48.89 ±1.69 cm. Means, standard deviations and ranges of the platelets, WBCs, RBCs and HGB in studied *Catla catla* captured from Head Muhammad wala, Multan, Pakistan are summarized in **Table 1.** The platelets, WBCs, RBCs and HGB values were 89.87±10.69 (10^3/µL), 83.38±6.51 (10^3/µL), 2.58±0.53 (10^6/µL) and 11.56±1.44(g/dl) respectively.

Body size and haematological parameters relationship

Linear regression was applied to study the relationship of blood parameters with total length (TL, cm) and wet weight (W, g) and results are showed in **Table 2** and **Table 3** respectively. Platelets (PLT, 10^3/µL), white blood cells (WBCs, 10^3/µL), red blood cells (RBCs, 10^6/µL) and HGB (g/dl) when plot against TL (cm) and W (g) represented highly significant correlations.

Table 1: The haematological parameters of farmed *Catla catla* from Multan

Haematological Parameters	Mean ± SD	Range
PLT(10^3/µL)	89.87±10.69	70.67-110
WBC(10^3/µL)	83.38±6.51	70.5-91
RBC(10^6/µL)	2.58±0.53	0.98-3.02
HGB(g/dl)	11.56±1.44	8-13.7

Table 2: Regression analysis of different blood parameters with respect to total length (TL-cm) of *Catla catla* from Multan (n=30)

Equation	a	b	95% Confidence Interval of a	95% Confidence Interval of b	r (correlation coefficient)	r^2 (coefficient of determination)
PLT=a+bTL	-138.1592	4.66	-219.3766- -56.9418	6.32-3.00	0.736***	0.542
WBCs=a+b TL	-61.2311	2.96	-108.2167- -14.2456	2.00-3.92	0.766***	0.587
RBCs=a+b TL	-7.7680	0.21	-12.2074- -3.3286	0.21-0.30	0.670***	0.449
HGB=a+b TL	-16.4874	0.57	-28.4698- -4.5050	0.33-0.82	0.672***	0.451

Table 3: Regression analysis of different blood parameters with respect to wet weight (W-gm) of *Catla catla* from Multan

Equation	a	b	95% Confidence Interval of a	95% Confidence Interval of b	r (correlation coefficient)	r^2 (coefficient of determination)
PLT=a+bW	-27.6644	0.07	-50.9574- -4.3715	0.05-0.08	0.891***	0.793
WBCs=a+b W	10.7678	0.04	-2.6354-24.1710	0.03-0.05	0.903***	0.816
RBCs=a+b W	-2.8978	0.003	-4.3102- -1.4853	0.002-0.004	0.833***	0.694
HGB=a+b W	-4.2028	0.001	-7.4002- -1.0054	0.007-0.010	0.886***	0.786

***P<0.001

4. DISCUSSION

Ichthyologists from different regions of the world consider hematological indexes as a significant tool for examining pathological and physiological variations. Study of blood parameters is important to find knowledge of fish health status and physiology under adverse conditions and to know the systematic relationship among certain species (Kohanestani *et al.*, 2013; Kumar, 2016).

Kousar *et al.* (2019) had provided reference value of WBCs (42.50-99.40 10^3/µL) in Tilapia (GIFT) fed with 25% dietary protein, which is nearly similar to the present study. Significant correlation between platelets against total length was noted in GIFT similar to the present study. Reference value of RBCs (2.86 10^6/µL) in *Labeo rohita* provided by Arya *et al.* (2018) is found nearly similar to the present study (2.58 10^6/µL).

Hrubec *et al.* (2000) had provided reference values for tilapia hybrid for RBCs (10^6/µL) as 1.91-2.88. The *Catla catla* fish in the present study had more or less similar range to those provided by Hrubec *et al.* (2000). Reference value of Hb (8.5-11.6g/dl) in *C. catla*, was provided by Southamani *et al.* (2015) which is nearly similar to the present study.

Iqbal and Naeem (2016) provided the reference average values of haematological indices in *Labeo rohita* fed upon 25% plant protein diet viz. RBCs (2.1410^6/µL) and PLT (75.17 10^3/µL). Present study findings also observed more or less similar values in above mentioned parameters but showed higher value of HGB as compared to Iqbal and Naeem (2016).

Earlier studies on the haematological parameters of *Catla catla* include the studies of Khan *et al.* (1969) and Rao and Behra (1973). Rao and Behra (1973) reported that the erythrocyte count in the samples of *Catla catla* studied by them having average value of 2.28 10^6/µL which is near to the value of the present study (2.58 10^6/µL). According to Patra *et al.* (2014) hematological parameters of *Catla catla* were found to be influenced by seasonal variations. The total number of erythrocyte ranged from 1.644 x 10^6 and 1.44 x 10^6/mm3, of blood, the data collected, during the present study, on *Catla catla* show that the range of total erythrocyte count in the present sample is higher (0.98 – 3.02; Average =2.58 10^6/µL) and haemoglobin percentage is also higher (8 – 13.7; Average = 11.56 g/dl) in comparison with the earlier studies.

Erythrocytes and leukocytes count are reliable indicator to determine stress conditions of an organism. Mature erythrocytes (RBCs) contain are pigmented protein and iron to transfer oxygen to body tissues (Rehulka, 2002). Low number of RBCs is indicator of the anemic condition in case of stress (Li *et al.*, 2011). Defensive cells of the body are WBCs. Higher levels of WBCs in any species show the ability to fight infection more effectively than other species (Douglass and Jane, 2010).

Platelets represent the morbidity phenomenon, level of resistance to disease and initiate the process of blood clotting. In the present study, higher concentrations of platelets were observed in *Catla catla*. The less number of platelets may be harmful to the blood capillary, spleen and bone marrow (Iqbal and Naeem, 2016). Level of oxygen supply of an organism can be determined by haemoglobin concentration (Southamani *et al.*, 2015), values of haemoglobin noted in the present study suggest sufficient supply of oxygen to fish.

Results of the present study indicated existence of positive correlation between various blood parameters and fish size (Total Length and Weight) in *C. catla* (Table 2 and 3). Iqbal and Naeem (2016) have also found significant influence of fish size (weight and length) on blood parameters in different dietary protein feeds in *Labeo rohita* similar to the present study. Many studies documented that RBC and HGB values increased with the growth of fish (Jawad *et al.*, 2004). Raizada *et al.* (1986) recorded that differences in blood parameters between fish of various sizes are genetically determined, but Chaudhuri *et al.* (1986) suggest that the differences might be due to the higher metabolic rate of bigger fish compared to smaller ones. Active species displayed higher values of haematological parameters compared to less active forms (Svobodova *et al.*, 2008). According to Rambhaskar and Srinivasa Rao (1986), more RBC were related to predaceous nature, high activity and fast movement with streamlined bodies. Understanding relationship between blood parameters and the habitat and the adaptability of the species to the environment becomes easy by considering haematological studies, so there is a need for establishing normal haematological values in different species of fish.

The haematological values studies in the present research provide a contribution of knowledge to standardize the haematological parameters in the *Catla catla* and once the standardized values are obtained, they may be used for immediate reference and necessary precautions may be taken to prevent the disease spread or for the therapy in any area. Present study also concluded significant influence of fish size on blood parameters.

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