HAEMATOLOGICAL PROFILE OF FARMED *CLARIAS BATRACHUS* WITH REFERENCE TO LENGTH AND WEIGHT FROM MULTAN, PAKISTAN

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Abstract
Present study was conducted to elaborate different haematological parameters of Clarias batrachus with reference to its total body length and total body weight (wet and dry). The studied haematological parameters include WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, LYM, RDW and MPV. These all parameters were differentially analyzed in male and female fish samples separately and also with their combined effect. The morphometric relationships of Clarias batrachus was found with all blood parameters which was co related with all these blood parameters. Non-significant ($p>0.05$) relationship was found among the total blood parameters of the fish with body weight of the fish. Total length of the fish was non-significant ($p>0.05$) relationship but only few blood cells were in significant ($p<0.05$ and $p<0.01$) relationship with the total length. There was not found any variation among the blood parameters of the male and female fish samples. This is first reference of this fish in this regard in the region.

Keywords: Haematology, Fish length, Fish weight, Blood parameters, Walking catfish; Clarias batrachus

INTRODUCTION

The local name of *Clarias batrachus* is Magur. This fish is full of protein and is considered as the most delicious and fit to be eaten for many countries especially for the Asian peoples due to the presence of low protein contents in their diet (Maheswaran *et al.*, 2008). This fish species belongs to cyprinidae family and fishes of this family are considered existed almost all over the world but nine species of the cyprinidae family are more common in the Asian countries (Narasimhaiah *et al.,* 2016). *C. batrachus* is selected for this study because of having short period of development, well documented general biology and year-round development (Maheswaran *et al.,* 2008).

The variables in the blood parameters of the fishes are used as source of the information about the working of different organs of the fish. The proper working of fish organs represents the good values of blood parameters. But lower values showed body functioning of the organs is not normal. Similarly, in case of catfish, the values of the blood parameters in the normal situations were found in the best range even when it was given the normal feed comprises of low cast. (Dixie and Ohen, 2006). In many regions of the Pakistan this fish species is commonly known as 'singhi' (Filho *et al.,* 1992).

Haematological studies are important for a species as these studies helps the researcher to collect the knowledge about the physiology of fish and physiological changes occur in them. Blood profile is also used to predict the pathology and pathological changes occur in fish. Blood is composed of different types of cells which include red blood cells, white blood cells and platelets as the major components of blood, which further composed of different types of sub cells (Newman *et al.,* 1997). Many researchers have been made to know about the health of fish, living in natural environment by using different tools. But physiologists are focusing on the blood parameters of the fish as it is used as a major tool for evaluating the health of those peoples who usually feed on fish meat (Jamalzadeh *et al.,* 2008). The structural and functional status of fish depends upon the blood parameters. Blood parameters express the whole physiology of that animal (Adhikari and Sarkar 2004). Oxygen carrying capacity of the blood stream of the fish is dependent over the capacity of blood cell of the fish. If fish is exposed to any environmental pollutant then the levels of protein, glucose, cholesterol and other organic compounds increases or sometimes decreases (depending upon the hazardous factors in that habitat). This brings the physiological

changes in fish but the exact change in the fish body can be measured on the type of toxicant which found in the habitat of that fish (Adewumi and Olaleye, 2011).
The blood parameters of the fish changed with the change of age of fish. These also changed with the biotopes of that aquatic area in which fish is living. So, change in the fish blood parameters is not a single step process while it depends upon many of the variables (Tanti *et al.,* 2011). The blood values of the fish are influenced by the food on which fish is feeding. Its nutritional values can be altered by altering the food products of the fish. However, if fish is feeding on the normal diet then the heamatological parameters will also showed the normal values which can be used to access the health of the fish (Jimoh *et al.,* 2016). This health status can be manipulated with the diet. There are some possible diseases which are attached with the nutritional values, and these diseases directly attached with the health status of the fish (Klinger *et al.,* 1996). There are two parameters of the blood which are used to determine the actual functionality of the oxygen carrying organs of the body. Heamoglobin and red blood cells are the two major parameters of the blood, if the both of these gives the values in normal range then it means that the oxygen carrying capacity is normal in the organisms but in case of any disturbance in the body, the value of these two parameters will below the ideal values(Shah and Altindağ, 2004).
Haematological changes in the body of fish may be due to the change in presence of toxic elements in the habitat of fish. Moreover, the study of such fishes may helpful in knowing about the presence of toxic elements in the fish body and tracking their effects over their users as well (Gabriel *et al.,* 2011). Hematological parameters have turned out to be promising biomarkers in estimating the impacts of concoction toxin in fish. Blood tests can routinely be gotten from test creatures, subsequently permitting the utilization of non-damaging methodology basically evaluation (Akinrotimi *et al.,* 2010). In view that fish are firmly related with the fluid environment, the blood will uncover a few modifications inside the fish rapidly than any physiological evaluation parameters (Nte *et al.,* 2011).
Proper mentoring of fish health can be done by the analysis of blood which includes biochemical and haematological tests. Haematological changes occurs on behalf of different aspects depending on the fish species, the sexual maturity of fish, health conditions of the fish and age of the fish (Hrubec *et al.,* 2000). Fishes are also best source to evaluate the condition of the ecosystem because the environmental pollutants become the part of food chain and their effects can be noted by observing the health of those fishes and these polluted fishes also affects the health of the human beings. Haematological information of an animal is used as indicator of the physiological stress response to exogenous or endogenous changes and not only shows the organized relationship of the animals but also their physiological adaptations (Lermena *et al.,* 2004). The blood composition of the fish is changed with age. The fingerling of fish and mature fish has differentiation in the blood composition (Salman *et al.,* 2010; Lone *et al.,* 2012). Different haematological parameters include red blood cells (RBC) in which dependent parameters i.e, haemoglobin (Hb), packed cell volume (PCV), mean cell volume (MCV), mean cell hemoglobin concentration (MCHC) and erythrocyte sediment rate (ESR) are significant findings for the determination of anemia, polycythemia, inflammation and infection. while white blood cell numbers (WBC) and differential leukocyte count viz, neutrophils, lymphocyte, eiosinophils and monocytes are the significant findings for the determination in pathological conditions viz. leucocytes and leucopenia (Shah *et al.,* 2009).

MATERIALS AND METHOD

Active and healthy *C. batrachus* specimens were collected from the fish farm at Head Muhammad Wala, Muzaffargarh Road, Multan District, Pakistan. This fish source was preferred to obtain the experimental fish as this area was not surrounding by any of the industries or agricultural land. Thirty Fish samples of different length and weight were selected for studying haematological parameters. Samples were started from range of 26.6 cm to 48.2cm in length and 179g to 867g of weight. The fishes were collected very carefully with the help of netting to minimize any stress condition and the collected fish were packed in O_2 filled container with pond water and transported the same day to the Fisheries Biochemistry Research Laboratory of Institute of Pure and Applied Biology (Zoology Division), Bahauddin Zakariya University, Multan, Pakistan, for haematological analysis. The concentration of oxygen was maintained at 9 mg/l in the plastic container during transportation. In the laboratory, the field-collected fish were transferred into a large aquarium and allowed to acclimatize for 5-7 days to the laboratory conditions. The fishes were acclimatized as mixed sex and later for the experiment, they were divided into two separate groups of male and female. Fifteen male samples and fifteen female samples were selected for the experiment. The range of fish weight for the study was 179 - 867g. The study specimens were disinfected by using 1% $KMnO_4$ (Preeti and Seema, 2014). Anesthetic agent was used for blood collection which was composed of solution of benzocaine and acetone. One hundred mg of benzocaine was mixed with 5 ml of acetone, and this mixture was further mixed in 4 -5 ml of water.
Blood samples were collected from live fish by adopting the method of Argungu *et al.,* (2017). The head of the fish was covered by a damp cloth. Caudal vein was punctured to collect a small amount of blood. The blood collection was done in the morning hours to reduce the diurnal variations. The blood was collected by using heparinized 2cc glass syringe (disposable); glass syringes were used because when blood comes in

contact with plastic, its coagulation time increases whereas contact with the glass reduces the coagulation time of blood. These blood samples were shifted to ethylene di-amine tetra acetic acid (EDTA) vials with the help of those syringes. Many researchers have used and recommended EDTA vials, as these are the best anti-coagulant for experimental work on blood of animals (Madhu *et al.,* 2014). The vials containing blood were placed over the mixer to gently mix the anti-coagulant and blood cells. These vials were properly labeled and stored in -80^{0}C until the analysis. All haematological parameters were determined by using standard techniques (Argungu *et al.,* 2017). The haematological parameters included white blood cells (WBC) (the back bone for immune system, neutrophils (NEUT) that-provide assistance to the immune system, lymphocytes (LYM) which identify the antigens of invading cells and produce antibodies, red blood cells (RBC) which transport oxygen inside the body from lungs to all organs, red blood cells distribution width (RDW)-which measures the amount of red blood cells and differentiate between their volume, shape and size, hematocrit (HCT) which measures the ratio between red blood cells and plasma, platelets (PLT)which prevent blood from bleeding, mean corpuscular haemoglobin (MCH)which measures the volume of haemoglobin, mean corpuscular volume (MCV) which-measures the size of red blood cells, hemoglobin (HGB) which transports carbon dioxide and oxygen in the body), mean corpuscular hemoglobin concentration (MCHC)which measures the concentration of haemoglobin. These parameters were observed and counted by using a haemocytometer (Sysmex, KX-21) which was washed after observations of each blood sample.

Results obtained from the readings of haemocytometer were combined and analyzed statistically using ANOVA and regression analysis. The significance level was observed at 95% confidence level.

RESULTS

In this study, mean values and standard error of all the observed parameters of blood were measured separately for male, female and combined samples (Table 3.1). These measured parameters included: WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, LYM, RDW and MPV; their values were analyzed statistically by using ANOVA and regression analysis to determine their significance. This study showed maximum values of WBC's and least values of RBC's in *C. batrachus*. The level of MCV, MCH, MPV, MCHC and RDW was almost similar in all specimens including male and female fishes. Level of three blood parameters, HCT, HGB, and PLT, showed some variations among both sexes, generally, these blood parameters were higher in quantity in male fish as compared to female. It was noticed during the study that male fish were more active and aggressive as compared to female fish. Lymphocytes (LYM) was the only parameter which was quantitatively more in blood of female fish as compared to the male fish (Table 1). The combined values of these parameters are also presented in Table 1.

Haematological parameters values expressed as mean ± S.E in both male and female fish were analyzed with the help of *t*-test. Regressions analysis was performed on total length and wet body weight of the fish, for all blood parameters.

The general ascending order of all blood parameters in male, female, and both sexes combined fish samples analyzed in the present study were as follows (Table 1):

Male: WBC < MCV < LYM < MCH < HCT < MCHC < PLT < RDW < HGB < MPV < RBC

Female: WBC < MCV < LYM < MCH < MCHC < HCT < RDW < PLT < MPV < HGB < RBC

Combined: WBC < MCV < LYM < MCH < MCHC < HCT < PLT < RDW < MPV < HGB < RBC

Quantitatively, similar trend was observed in the blood parameters among the male and female fish except for a few parameters. This showed that the studied blood parameters were not gender dependent.

The relationship between log total length (TL), with the studied blood parameters of *C. batrachus* showed negative allometry for male fish samples (Table 2). Non-significant ($P > 0.05$) relationship was observed between the studied blood parameters and log-transformed values of total length, except for MCHC which had significant ($P < 0.01$) relationship as well as MCH, PLT and RDW values were significant ($P < 0.05$) (Table 2).

All these blood parameters examined as the log-transformed total length in female fish, showed non-significant ($P > 0.05$) relationship (Table 3). Also, these parameters examined in all female and male fish samples, again showed non-significant ($P > 0.005$) relationship, except for MCH showing least significant ($P < 0.05$) correlation with total length in log form (Table 4).

The relationship of body weight (wet) in log form with other blood parameters showed negative Allometry. This negative allomtery was found in male as well a ins female and both sexes combined (Table 5 – 7). The blood parameters showed non- significant ($P > 0.05$) relationship with body length (total) and body weight (wet). Only two blood parameters, MCH and PLT, showed least significant ($P < 0.005$) relationship ship, while other all other studied parameters had non- significant ($P > 0.05$) relationship (Table 5). The same

relationship was found in the study of blood parameters of the female fish samples and all fishes including male and female with log total body weight (Table 6 – 7).
The linear regression results showed non-significance with the coefficient of the determination (r^2) ranging from 0.005 to 0.424 for length correlation and similar results were noted for the weight of the body ranging from 0.00 to 0.349 (Table 5). The regression coefficient “b” had the value less than 3 (*t*-test).
The haematological relationship data between log-transformed total length (TL) values and different blood parameters for ♀ *C. batrachus* are shown in Table 3. Statistically, non-significant ($P > 0.05$) correlation was noted between the length of female *C. batrachus* and different blood parameters, including WBC, RBC, HGB, HCT, MCV, MCHC, PLT, LYM, RDW, MPV. These blood parameters correlated with body weight (BW) of fish revealed that there was no positive correlation among them and male *C. batrachus* and non-significant results ($P > 0.05$) were observed (Table 6). The linear regression results were non-significant with the coefficient of determination (r^2) values ranging from 0.004 to 0.143 for length co relation, which values were much less than the maximum limit of the female fish. Similar results were also found for the body weight ranging from 0.030 to 0.188 (Table 6). The regression coefficient “b” had the value less than 3 (*t*-test).

The linear regression results were non-significant with the coefficient of the determination (r^2) ranging from 0.000 to 0.114 for length correlation. These range limits were less than the range of male and female *C. batrachus*. Similar results were also found for the body weight ranging from 0.001 to 4.53 (Table 7). The regression coefficient “b” had the value less than 3 (*t*-test).

Table 1: Mean ± S.E values of studied blood parameters of *Clarias batrachus*

Variable	(Male) Mean ± S.E	(Female) Mean ± S.E	(Combined) Mean ± S.E
WBC	2.34 ± 0.01	2.29 ± 0.01	2.32 ± 0.01
RBC	0.27 ± 0.02	0.07 ± 0.04	0.17 ± 0.03
HGB	0.97 ± 0.02	0.75 ± 0.06	0.86 ± 0.04
HCT	1.45 ± 0.01	1.27 ± 0.05	1.36 ± 0.03
MCV	2.19 ± 0.01	2.19 ± 0.01	2.19 ± 0.01
MCH	1.59 ± 0.02	1.58 ± 0.02	1.59 ± 0.01
MCHC	1.43 ± 0.02	1.38 ± 0.02	1.40 ± 0.01
PLT	1.42 ± 0.06	1.05 ± 0.09	1.24 ± 0.06
LYM	1.98 ± 0.01	2.13 ± 0.03	2.06 ± 0.02
RDW	1.23 ± 0.03	1.23 ± 0.03	1.23 ± 0.02
MPV	0.92 ± 0.00	0.92 ± 0.00	0.92 ± 0.00

Values are expressed as Log of Mean ± S.E separately for male and female and combined *C. batrachus*

Table 2 Regression analysis data of log-transformed values of total length (TL) and, studied blood parameters of male *C. batrachus* fish, Multan, Pakistan. (n=15)

Sp.	Y = a + b x	a	b	95%CI of a	95%CI of b	r^2	r
Clarias batrachus	WBC = a + b TL	2.39	-0.032	1.99-2.80	-0.29-0.23	0.005	0.071^{ns}
	RBC = a + b TL	0.634	-0.234	-0.054-1.33	-0.67-0.21	0.092	0.303^{ns}
	HGB = a + b TL	0.875	0.061	-0.024-1.77	-0.511-0.63	0.064	0.253^{ns}
	HCT = a + b TL	1.597	-0.092	0.95-2.24	-0.50-0.317	0.133	0.365^{ns}
	MCV = a + b TL	1.95	0.151	1.43-2.47	-0.181-0.483	0.069	0.263^{ns}
	MCH = a + b TL	2.45	-0.545	1.771-3.125	-0.976-(-0.11)	0.364	0.603^{*}
	MCHC = a + b TL	1.975	-0.347	1.27-2.67	-0.791-0.097	0.424	0.651^{**}
	PLT= a + b TL	-1.336	1.759	-3.63-0.957	0.300-3.22	0.343	0.586^{*}
	LYM = a + b TL	2.01	-0.015	1.69-2.33	-0.220-0.189	0.002	0.045^{ns}
	RDW = a + b TL	0.574	0.420	-0.558-1.707	-0.301-1.141	0.330	0.574^{*}
	MPV = a + b TL	0.879	0.028	0.739-1.018	-0.060-0.117	0.189	0.435^{ns}

of samples; Min, Minimum; Max, Maximum; M, Male; F, Female; C, Combined; CI, Confidence l WBC, white blood cells; RBC, red blood cells; HGB, hemoglobin; HCT, hematocrit MCV, corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin tration; PLT, platelets; LYM, Lymphocytes; RDW , Red Blood Cell Distribution Width.

Table 3 Regression analysis data of log-transformed values of total length (TL) and studied blood parameters in female *C. batrachus* fish, Multan, Pakistan. (n=15)

Sp.	Y = a + b x	a	b	95%CI of a	95%CI of b	r^2	r
Clarias batrachus	WBC = a + b TL	2.632	-0.218	1.42-3.84	-0.99-0.55	0.028	0.167^{ns}
	RBC = a + b TL	-0.331	0.259	-3.94-3.28	-2.05-2.57	0.004	0.063^{ns}
	HGB = a + b TL	-3.119	2.481	-7.73-1.49	-0.47-5.43	0.202	0.449^{ns}
	HCT = a + b TL	0.373	0.576	-374-4.48	-2.06-3.21	0.017	0.130^{ns}
	MCV = a + b TL	2.686	-0.315	1.96-3.41	-0.78-0.15	0.143	0.378^{ns}
	MCH = a + b TL	2.258	-0.435	0.54-3.98	-1.53-0.67	0.053	0.230^{ns}
	MCHC = a + b TL	1.328	0.0323	-0.69-3.342	-1.26-1.32	0.0002	0.014^{ns}
	PLT= a + b TL	-0.828	1.206	-8.45-6.79	-3.67-6.09	0.021	0.145^{ns}
	LYM = a + b TL	1.796	0.212	-0.84-4.44	-1.48-1.90	0.005	0.071^{ns}
	RDW = a + b TL	2.425	-0.764	-0.44-5.29	-2.60-1.073	0.058	0.241^{ns}
	MPV = a + b TL	0.882	0.027	0.64-1.13	-0.13-0.18	0.0108	0.104^{ns}

n, No. of samples; Min, Minimum; Max, Maximum; M, Male; F, Female; C, Combined; CI, Confidence Interval WBC, white blood cells; RBC, red blood cells;HGB, hemoglobin; HCT, hematocrit MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelets; LYM, Lymphocytes;RDW , Red Blood Cell Distribution Width.

Table 4 Regression analysis data of log-transformed values of total length (TL) and studied blood parameters of male and female (combined) *C. batrachus* fish, Multan, Pakistan..

	Y = a + b x	a	b	95%CI of a	95%CI of b	r^2	r
Clarias batrachus	WBC = a + b TL	2.383	-0.041	1.89-2.88	-0.36-0.27	0.0514	0.227^{ns}
	RBC = a + b TL	0.197	-0.016	-1.29-1.68	-0.97-0.93	0.000	0^{ns}
	HGB = a + b TL	-0.228	0.696	-2.11-1.65	-0.51-1.90	0.048	0.219^{ns}
	HCT = a + b TL	1.124	0.153	-0.42-2.67	-0.84-1.14	0.003	0.054^{ns}
	MCV = a + b TL	2.114	0.049	1.73-2.50	-0.120-0.29	0.006	0.077^{ns}
	MCH = a + b TL	2.387	-0.511	1.74-3.03	-0.92--0.098	0.187	0.432^{*}
	MCHC = a + b TL	1.773	-0.235	1.002-2.54	-0.73-0.26	0.033	0.181^{ns}
	PLT= a + b TL	-1.648	1.845	-4.77-1.48	-0.15-3.84	0.114	0.337^{ns}
	LYM = a + b TL	2.133	-0.049	1.073-3.19	-0.725-0.63	0.001	0.032^{ns}
	RDW = a + b TL	0.968	0.169	-0.14-2.08	-0.54-0.88	0.008	0.089^{ns}
	MPV = a + b TL	0.882	0.027	0.77-0.99	-0.042-0.096	0.023	0.151^{ns}

Table 5 Regression analysis data of log-transformed values of wet total body weight (BW) and studied blood parameters of male *C. batrachus* fish. Multan, Pakistan.

	Y = a + b x	a	b	95%CI of a	95%CI of b	r^2	r
Clarias batrachus	WBC = a + b BW	2.416	-0.028	2.16-2.67	-0.123-0.071	0.0283	0.168^{ns}
	RBC = a + b BW	0.578	-0.118	0.149-1.01	-0.28-0.046	0.1574	0.397^{ns}
	HGB = a + b BW	0.961	0.004	0.38-1.54	-0.22-0.23	0.000	000^{ns}
	HCT = a + b BW	1.586	-0.051	1.175-2.00	-0.208-0.106	0.0362	0.190^{ns}
	MCV = a + b BW	2.052	0.0524	1.713-2.392	-0.077-0.182	0.055	0.234^{ns}
	MCH = a + b BW	2.133	-0.207	1.69-2.57	-0.376-(-0.038)	0.349	0.591^{*}
	MCHC = a + b BW	1.823	-0.151	1.39-2.261	-0.318-0.016	0.226	0.475^{ns}
	PLT= a + b BW	-0.334	0.674	-1.82-1.154	0.106-1.243	0.336	0.579^{*}
	LYM = a + b BW	2.028	-0.0165	1.822-2.234	-0.0952-0.062	0.0155	0.124^{ns}
	RDW = a + b BW	0.768	0.1784	0.046-1.490	-0.097-0.454	0.130	0.360^{ns}
	MPV = a + b BW	0.891	0.0126	0.801-0.980	-0.021-0.047	0.047	0.216^{ns}

Table 6 Regression analysis data of log-transformed values of wet body weight (BW) and studied blood parameters of female *C. batrachus* fish, Multan, Pakistan.

Y = a + b x	a	b	95%CI of a	95%CI of b	r^2	r

Clarias batrachus	WBC = a + b BW	2.636	-0.132	1.82-3.45	-0.45-0.18	0.060	0.245^{ns}
	RBC = a + b BW	-0.458	0.204	-2.92-2.01	-0.74-1.155	0.0163	0.128^{ns}
	HGB = a + b BW	-1.659	0.929	-4.90-1.58	-0.320-2.18	0.166	0.407^{ns}
	HCT = a + b BW	0.446	0.318	-2.36-3.25	-0.76-1.40	0.030	0.173^{ns}
	MCV = a + b BW	2.509	-0.121	2.005-3.012	-0.32-0.073	0.123	0.351^{ns}
	MCH = a + b BW	2.458	-0.338	1.362-3.55	-0.76-0.084	0.188	0.433^{ns}
	MCHC = a + b BW	2.017	-0.246	0.685-3.350	-0.760-0.267	0.0764	0.276^{ns}
	PLT= a + b BW	-1.323	0.916	-6.429-3.784	-1.050-2.882	0.072	0.268^{ns}
	LYM = a + b BW	1.670	0.176	-0.132-3.47	-0.52-0.870	0.0226	0.150^{ns}
	RDW = a + b BW	0.939	0.113	-1.087-2.966	-0.667-0.	0.00749	0.086^{ns}
	MPV = a + b BW	0.957	-0.012	0.788-1.125	-0.077-0.053	0.0124	0.111^{ns}

n, No. of samples; Min, Minimum; Max, Maximum; M, Male; F, Female; C, Combined; CI, Confidence Interval WBC, white blood cells; RBC, red blood cells; HGB, hemoglobin; HCT, hematocrit MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelets; LYM, Lymphocytes; RDW , Red Blood Cell Distribution Width

Table 7 Regression analysis data of log-transformed values of total wet body weight (BW), and studied blood parameters for Male and Female (combined) *C. batrachus* fish, Multan, Pakistan.

	Y = a + b x	a	b	95%CI of a	95%CI of b	r^2	r
Clarias batrachus	WBC = a + b BW	2.424	-0.041	2.10-2.74	-0.16-0.08	0.0162	0.127^{ns}
	RBC = a + b BW	0.248	-0.029	-0.72-1.22	-0.40-0.34	0.0009	0.03^{ns}
	HGB = a + b BW	0.314	0.210	-0.931-1.56	-0.268-0.687	0.0281	0.167^{ns}
	HCT = a + b BW	1.247	0.045	0.232-2.262	-0.345-0.434	0.002	0.044^{ns}
	MCV = a + b BW	2.143	0.018	1.89-2.395	-0.078-0.115	0.0054	0.073^{ns}
	MCH = a + b BW	2.184	-0.229	1.77-2.59	-0.39- -0.073	0.243	0.493^{ns}
	MCHC = a + b BW	1.824	-0.161	1.34-2.311	-0.348-0.025	0.1007	0.317^{ns}
	PLT= a + b BW	-0.765	0.770	-2.79-1.264	-0.008-1.55	0.128	0.358^{ns}
	LYM = a + b BW	2.055	0.0004	1.36-2.748	-0.266-0.266	4.53	2.128^{ns}
	RDW = a + b BW	0.802	0.166	0.092-1.51	-0.106-0.438	0.053	0.230^{ns}
	MPV = a + b BW	0.904	0.0076	0.83-0.976	-0.020-0.035	0.011	0.105^{ns}

n, No. of samples; Min, Minimum; Max, Maximum; M, Male; F, Female; C, Combined; CI, Confidence Interval WBC, white blood cells; RBC, red blood cells; HGB, hemoglobin; HCT, hematocrit MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelets; LYM, Lymphocytes; RDW , Red Blood Cell Distribution Width

4. DISCUSSION

Fish health can be determined by the haematological analysis, but their hematology gets altered due to the environmental pollutants. Fish plasma has specific albumin concentration which reflects the overall functionality of the fish body (Kovyrshina and Rudneva, 2012). Mean values of RBC, HGB and PCV in *C. batrchus* have previously been reported by Preeti and Seema (2014). The findings of this study are like the earlier report of Samra *et al.,* (2013). White blood cells were the only blood component in *C. batrachus* which were found in maximum concentration as compared to other blood variables in both sexes and similar concentration also occurred in the study of Singh *et al.* (2011). This indicated that the immunity level of *C. batrachus* against the invading viral organisms was good and reflected that the fish had large quantity of different vitamins especially vitamin A, B and C with the addition of calcium and potassium ions including some traces of proteins (Tiger *et al.,* 2011). In this study, the values of MCV were greater and the MCH and MCHC values were lesser in both the sexes. That could have been due to anemic condition in the studied fish. The medical examination is compulsory to determine the exact reason for these lower values. This kind of situation did not exist in the study reported by Preeti and Seema (2014). Preeti (2014) discovered that the decreased value of MCV with increased values of MCH and MCHC observed in the fish blood were due to exposure of fish to alcoholic extract. The finding of low values of red blood cells in the present study was similar to the report of Nilza *et al.,* (2003). The low values could have been due to decreased level of hemoglobin. Usually it is found that the haematological parameters in the control group of catfish fall within the normal range of values of the fish cultured under controlled conditions (Akinrotimi, 2008). The value of MCHC is directly connected with amount of Hb in red blood cells. This study showed ideal values of MCHC which indicated a good level of Hb in the studied *C. batrachus*. The haemoglobin level in fish in the present study was average. There can different reasons for average values of haemoglobin in the studied fish, such as depression in fish or some exhalation of the hemolytic potential in the fish body; also, some toxic chemicals may have been present in the fish habitat that could have cause low Hb levels in the fish blood (Sawhney and Johal, 2000).

Several studies have been undertaken which document the blood profile of fish inhabiting freshwaters exposed to some lethal compounds and different changes have been noted in them, but these studies lack any comparative analysis of the length and weight in relation to different blood parameters. The present study revealed that HGB, MVC MPV and PLT increased with increasing length of fish in both sexes but male fish had higher PLT values than the females. There was no significant change noted in other blood parameters, including RBC, MCV, PCV and MCHC (Kandari and Rauthan, 2015). In the present study, the WBC values were found higher in male fish as compared to female. The reprted maximum value of WBC in different species of *Clarias* has been 19.23 and the highest value of WBC (80.2) has been noted in tilapia (Hamid *et al.,* 2013).

Higher values of RBCs, WBCs, HCT, MCV indicated that the proteins in the feed of the fish were high and the values of MCH, MCHC, and PLT were found to be low when fish were exposed to the low proteins diet. The same values were also noted in the fish with good growth (Iqbal and Naeem., 2016).

Previously, similar (to the present study) haematological values of *C. batrachus* have also been reported by Patnaik and Parta (2006). The reduced values of blood parameters were reported, if the destruction of erythrocytes happens at increased rate it also causes the reduction in blood parameter values (Gabriel *et al.,* 2011). The reduced PCV value has been reported to be due to the reduction in amount of red blood cells and haemoglobin and such results have been supported by Gupta *et al.* (2001) and Saxena *et al.* (2001). High salinity in the fish habitat is also the cause of reduction in values of blood contents and has poor effects on fish growth. This salinity level sometimes can even result in difficulty for fish survival (De-Boeck *et al.,* 2000). Contrary to these, increased values of blood parameters, specially RBC, Hb, and PCV, have been reported in different fishes of *Clarias gariepinus* and *Labeo rohita* when treated with different plant extracts (Kaleeswaran *et al.,* 2012).The basic blood contents especially white blood cells and red blood cells in *C. batrachus* were found to increase when these fishes were treated with plant extracts. Aqueous extract of *Ocimum basilicum* was added in the diet of fish and it was found that the values of blood contents were increased (Nahak and Sahu, 2014). The values of blood parameters in this study agree with the findings of Velisek *et al.,* (2007). The predicted values of RBC were 1.77 (Maheswaran *et al.,* 2008) and 2.09 (Acharya and Mohanty, 2014) for *C. batrachus* which are higher than in the present study and this may be due to change in environment of habitat of the fish. The similar values of erythrocytes have been found in the studies of Abdelmeguid *et al.,* (2002) for *Tilapia zilii*.

The values of all blood parameters, including WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, LYM, RDW and MPV are found to be similar to other catfish studies with minor changes ((Iqbal and Naeem, 2016). However, these values differ from those fish values of other species which confirms that values of the blood parameters may differ if the fish species is changed (George and Akinrotimi, 2017).

The changes in haematology of male and female in a few cases of fish species was due to the difference in behavioral changes but in the present study no remarkable change was noted in both the sexes. This could have been because both sexes of the fish inhabited the same environment and the feed contents for both were the same. The assessment of the impact of sex reaction is vital in the appraisal of fish hematology (Summarwas and Lall, 2013). Different salinity levels can also cause such effect in the fishes. They can even directly influence change in the contents of blood parameters in the body. In case of increase in the concentration of chemical pollutants in the fish habitat, the blood parameters will change with the same ratio but if no huge change occurs then the fish possess the capability to cope with the change in the habitat (George and Akinrotimi, 2017).

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