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Haematological Parameters of Three Cyprinid Fish Species from Karakaya Dam Lake, Turkey

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Abstract: This study was conducted between September 2000 and September 2001 to determine and compare blood parameter levels of ***Alburnoides bipunctatus F.***, ***Chalcalburnus mossulensis*** and ***Cyprinion macrostomus***. Variations in haematological characters of the fish were compared according to species, gender and seasonal differences. Effects of water quality on blood parameters were also determined. The results indicated that blood parameter levels of all species in warm months were significantly different than those measured in cold seasons. The number of total leucocyte, neutrophil and monocyte levels were found to be higher in female fish, especially in reproduction season, than in male fish. Levels of erythrocyte, hemoglobin and hematocrit were high in male fish in an annual period. Result showed there was no gender effects on erythrocyte indexes (MCV, MCH, MCHC) eosinophil and thrombocyte levels. In addition, mean values of blood parameters were found to be similar in ***Chalcalburnus mossulensis*** and ***Cyprinion macrostomus***, but there was an increase in ***Alburnoides bipunctatus F.***

Key words: Cyprinid, haematologic parameters, Karakaya Dam Lake

Introduction

Blood tissue of fish gives clue about physiology and environmental conditions of fish (Ramaway and Reddy, 1978). In recent years, blood parameters have been commonly used to observe and follow fish health (Bhasker and Rao, 1985).

Since variations in blood tissue of fish are caused by environmental stress (Hickey, 1982; Aldrin ***et al.***, 1982), malnutrition (Casillas and Smith, 1977), gender (Siddiquie and Nasim, 1979; Collazos ***et al.***, 1998), fish size (Garcia, 1992), seasonal differences and breeding (Cech and Wohlschlag, 1981) in order to determine haematological values of fish, some characteristics of fish and its environment should be considered. Information about these characteristics help when determining levels of fish blood parameters.

Now a days, values of blood parameters of most fish have already been determined (Blaxhall and Daisley, 1973) but there are some freshwater fish species, of that levels of haematological parameters have not been studied.

Haematological parameters of freshwater fish species in eastern Turkey have not also been studied. Thus, the objective of this study was to provide some valuable information to the field of freshwater fish haematology and to determine blood parameter levels of three freshwater fish species living in natural environments.

Materials and Methods

In this study three fish species ***Alburnoides bipunctatus F.***, ***Chalcalburnus mossulensis*** and ***Cyprinion macrostomus*** belong to Cyprinidae living in Karakaya Dam Lake, Malatya, Turkey were used to determine annual variations in haematological parameters.

Blood was collected from newly dead fish by severing of the caudal peduncle (Ezzat ***et al.***, 1973; Blaxhall and Daisley, 1973; Kocabatmaz and Ekingen, 1982). Before collecting blood samples, no anesthetic was applied to fish as it may affect blood parameters and hemolised tissues (Hoffman, 1977; McKnight, 1966). Blood samples were stored in polystyrene cool bag using anticoagulant (EDTA) containing glass tubes until used. Blood analyses were carried out as soon immediately after sampling.

To determine the count of erythrocytes and thrombocytes (mm^3) blood sample was taken with an erythrocytes pipette and diluted (1/200) with the Hayem solution. One drop of hemolized blood was transferred onto Thoma lamella and examined in an light microscope (Soif, XZS-107B model) with a magnification of 400× (Blaxhall and Daisley, 1973).

Leukocytes counting was performed by transporting blood sample (diluted in Turck solution) with an leukocytes pipette into counting lamella and examined as for erythrocytes (Blaxhall and Daisley, 1973; Blaxhall, 1981; DeWilde and Houston, 1961).

The amount of hemoglobin was determined according to cyanomethemoglobin procedure (Kit 525-A, sigma chemical co.) (Blaxhall and Daisley, 1973). Non-clotted blood (20µL) was diluted with Drabkin solution (1 mL) and left stand for 10 min. The absorbency of the mixture was read at 540 nm and the amount for hemoglobin was calculated from a parelly rumed hemoglobin standard (Blaxhall and Daisley, 1973; Azizoğlu and Cengizler, 1996).

The microhematocrit method was utilized in hematocrit determination (Wilhem Filho ***et al.***, 1992; Jewet ***et al.***, 1991; Amlacher, 1970). Non-clotted blood was transferred into microhematocrit pipette and centrifuged at 12.5 rpm for 5 min and the ratio of shaped blood components in plasma was determined.

Wright staining determined percent leukocyte tissue (Goel ***et al.***, 1981; Atkinson and Judyy, 1978). Stained sample was examined in binocular light microscope (Soif, XZS-107B model) with 100× objective. The percentage of neutrophil, eosinophil, lymphocyte and monocyte tissues was determined temperature, dissolved oxygen concentration, pH, electrical conductivity, suspended solid matter, lead, calcium, sodium, chloride and total nitrogen values of water were measured in monthly periods during a year.

Haematological data were analysed with one-way analysis of variance by using SPSS 7.5 for windows. Differences between means were determined using Duncan's multiple range test (DMRT) ($P<0.05$).

Results

Water quality criteria of Karakaya Dam Lake between September 2000 and September 2001 period (Table 1). Water temperature and dissolved oxygen changed with seasons ($P<0.05$). In order to determine blood parameter levels of each fish species, sex differences and seasonal variations were considered.

Seasonal variations

A statistically significant increase were observed in the levels of total leukocyte, neutrophil and monocyte, especially in spring and summer, but there was a decrease in the level of lymphocyte. No statistically significant variations were found in the level of eosino and basophil between seasons (Table 2, 3, 4). A statistically significant differences were detected in the number of erythrocyte, hemoglobin and hematocrit levels between seasons ($P<0.05$) but this differences did not reflect on erythrocyte indexes (MCV, MCH, MCHC) and thrombocyte numbers.

Differences according to sex

Erythrocyte number, hemoglobin, hematocrit and lymphocyte levels were high in male individuals of all species, but total leukocyte numbers, neutrophil and monocyte levels were found to be high in female fish. There was no statistically significant differences in the levels of eosinophil and basophil, erythrocyte indexes (MCV, MCH, MCHC) and thrombocyte numbers between male and female fish (Table 2, 3, 4).

Table 1: Water quality criteria of Karakaya Dam Lake

	Seasons			
Water quality criteria	Spring	Summer	Autumn	Winter
Temperature (^{0}C)	18.00*	25.00*	12.00	7.00
Dissolved Oxygen concentration (mg l^{-1})	16.50	16.03	22.03*	27.50*
Chemical Oxygen concentration (mg l^{-1})	24.00*	22.60*	17.04	15.50
pH	7.47	7.52	7.54	7.51
Electrical Conductivity (m Siemens)	10.11	10.95	11.06	12.40
Suspended Solid Matter (mg l^{-1})	55.20	48.50	51.30	52.40
Copper (mg l^{-1})	0.01	0.02	0.01	0.02
Lead (mg l^{-1})	0.001	0.001	N.D	N.D
Calcium (mg l^{-1})	104.50	107.20	114.50	113.60
Sodium (mg l^{-1})	30.00	32.00	29.00	26.00
Chloride (mg l^{-1})	30.00	30.00	29.60	28.50
Total Nitrogen (mg l^{-1})	9.60	11.90	10.80	9.10*

Significant at P=0.05, N.D: No Detected

Table 2: Seasonal variations in blood parameters of male and female *Cyprininon macrostomus*

		Seasons			
Haematological	Sex	Spring	Summer	Autumn	Winter
Total Leucocyte Count ($10^3 mm^{-3}$)	Male	3.66±0.12^a	4.04±0.45^a	2.10±0.30^b	1.96±0.15^b
	Female	3.82±0.14^a	4.66±0.70^a	2.40±0.25^b	2.10±0.25^b
Total Types					
Neutrophile (%)	Male	9.82±0.41^a	9.60±0.60^a	6.70±0.35^b	6.50±0.20^b
	Female	10.14±0.56^a	10.50±0.70^a	7.25±0.40^b	7.10±0.35^b
Eosinophile (%)	Male	0.45±0.02	0.50±0.03	0.40±0.09	0.35±0.15
	Female	0.52±0.03	0.55±0.03	0.45±0.09	0.40±0.18
Basophile (%)	Male	0.10±0.02	0.10±0.02	0.09±0.01	0.10±0.05
	Female	0.15±0.03	0.15±0.03	0.09±0.01	0.15±0.07
Lymphocyte (%)	Male	80.43±1.60^b	78.20±1.30^b	84.61±2.14^a	85.55±1.65^a
	Female	74.39±1.20^b	76.10±0.90^b	83.11±1.80^a	84.45±1.25^a
Monocyte (%)	Male	9.20±0.36^a	11.60±0.25^a	8.20±0.20^b	7.50±0.15^b
	Female	14.80±0.40^a	12.70±0.30^a	9.10±0.30^b	7.90±0.20^b
Erythrocyte count ($10^6 mm^{-3}$)	Male	1.570±0.020^b	1.820±0.050^a	1.480±0.020^b	1.250±0.010^c
	Female	1.385±0.010^b	1.540±0.020^a	1.270±0.010^b	1.010±0.05^c
Hemoglobin (g 100 ml)$^{-1}$	Male	8.20±0.20^b	9.60±0.30^a	7.60±0.20^b	6.45±0.32^c
	Female	7.10±0.15^b	8.20±0.25^a	6.50±0.15^b	5.35±0.40^c
Hematocrit (%)	Male	27.10±0.20^b	31.50±0.25^a	25.50±0.15^b	21.50±0.60^c
	Female	23.90±0.40^b	26.50±0.15^a	21.90±0.25^b	18.00±0.50^c
Erythrocyte Indexes					
MCV(μ^3)	Male	171.90±2.60	173.00±3.25	172.29±2.50	172.00±1.25
	Female	172.60±1.50	172.10±1.65	172.44±1.45	171.42±1.35
MCH (μ μg)	Male	52.20±0.50	52.74±0.62	51.35±0.44	51.60±0.60
	Female	51.26±0.45	53.24±0.70	51.18±0.56	52.97±0.70
MCHC (%)	Male	30.25±0.20	30.47±0.30	29.80±0.40	30.00±0.32
	Female	33.66±0.15	30.94±0.55	29.68±0.70	29.72±0.70
Thrombocyte ($10^4 mm^{-3}$)	Male	0.520±0.02	0.540±0.05	0.470±0.09	0.450±0.06
	Female	0.480±0.01	0.510±0.06	0.450±0.04	0.440±0.05

a.b.c: Mean values followed by different letters in the rows are significantly different p=0.05, Averages of a yearly values ± σ_{n-1} of n= 182 males and n= 167 females

Differences according to species

The levels of haematological parameters in both sex of ***Chalcalburnus mossulensis*** and ***Cyprinion macrostomus*** were higher than in ***Alburnoides bipunctatus F.*** However, erythrocyte indexes and thrombocyte numbers did not show any differences between species (Table 5, 6).

Discussion

Previous studies on fish haematology revealed that blood parameter levels could be affected by variations in water temperature and oxygen concentration (Aldrin ***et al.***, 1982; Azizoğlu and Cengizler, 1996; Denton and Yousef, 1975; Hickey, 1982; Houston and Koss, 1984; Jewet ***et al.***, 1991; Martinez et al., 1994). For this reason, water quality of Karakaya Dam Lake (Malatya, Turkey) were determined in this study.

Table 3: Seasonal variations in blood parameters of male and female *Chalcalburnus mossulensis*

		Seasons			
Haematological	Sex	Spring	Summer	Autumn	Winter
Total Leucocyte count ($10^3 mm^{-3}$)	Male	2.66±0.10[a]	2.95±0.17[a]	1.25±0.10[b]	1.35±0.20[b]
	Female	2.82±0.22[a]	3.10±0.32[a]	2.05±0.41[b]	1.95±0.33[b]
Total Types					
Neutrophile (%)	Male	14.50±1.12[a]	14.20±1.85[a]	8.40±0.30[b]	7.65±0.80[b]
	Female	15.50±0.85[a]	18.25±1.75[a]	10.50±0.60[b]	9.80±1.10[b]
Eosinophile (%)	Male	0.80±0.25	1.35±0.40	0.95±0.42	0.60±0.15
	Female	1.12±0.22	1.45±0.60	1.20±0.17	0.95±0.26
Basophile (%)	Male	0.10±0.01	0.15±0.01	0.10±0.01	0.08±0.01
	Female	0.12±0.01	0.15±0.02	0.10±0.01	0.10±0.01
Lymphocyte (%)	Male	70.40±2.80[b]	67.90±1.65[b]	80.05±1.42[a]	83.37±1.24[a]
	Female	63.36±2.13[b]	59.73±2.45[b]	72.46±1.51[a]	75.35±2.80[a]
Monocyte (%)	Male	14.20±3.25[a]	16.40±2.56[a]	10.50±1.85[b]	8.30±1.78[b]
	Female	19.90±2.64[a]	20.42±1.56[a]	15.74±3.21[b]	13.80±2.52[b]
Erythrocyte count ($10^3 mm^{-3}$)	Male	1.650±0.15[b]	1.980±0.24[a]	1.560±0.17[b]	1.250±0.10[c]
	Female	1.440±0.08[b]	1.750±0.30[a]	1.410±0.25[b]	1.080±0.30[c]
Hemoglobin ($g100ml^{-1}$)	Male	8.45±0.80[b]	10.10±0.74[a]	8.15±0.40[b]	6.40±0.65[c]
	Female	7.44±0.20[b]	9.10±0.35[a]	7.35±0.25[b]	5.65±0.20[c]
Hematocrit (%)	Male	28.43±1.14[b]	33.75±2.16[a]	26.50±1.32[b]	21.50±1.20[c]
	Female	24.50±0.70[b]	30.20±0.20[a]	24.30±0.90[b]	18.50±0.40[c]
Erythrocyte Indexes					
MCV(μ^3)	Male	172.30±3.23	170.45±3.20	169.87±1.40	172.00±1.65
	Female	170.13±1.50	172.57±1.30	172.34±0.60	171.29±0.70
MCH(μμg)	Male	51.12±0.36	51.01±0.41	52.24±1.20	51.20±0.60
	Female	51.66±0.50	52.00±0.40	52.12±0.30	52.31±0.40
MCHC(%)	Male	29.72±0.20	29.92±0.50	30.75±0.60	29.76±0.30
	Female	30.36±0.55	30.13±0.70	30.24±0.80	30.54±0.40
Thrombocyte ($10^4 mm^{-3}$)	Male	0.554±0.10	0.551±0.12	0.485±0.15	0.512±0.20
	Female	0.515±0.08	0.504±0.09	0.470±0.12	0.520±0.10

a.b.c: Mean values followed by different letters in the rows are significantly different p=0.05, Averages of a yearly values ± σ_{n-1} of n= 158 males and n= 144 females

The results of this study showed statistically significant differences of water temperature, dissolved oxygen and chemical oxygen between warm season and cold season (Table 1). As a results of that, levels of blood parameters were found to be high in spring and summer (Table 2, 3, 4). This period of year is also reproduction season for fish. Thus, some part of increase in blood parameter levels may be due to high energy demand of fish in this period (Joshi, 1989; Ezzat ***et al.***, 1973; Cech and Wohlschlag, 1981). High erythrocyte number, hemoglobin and hematocrit levels in male individuals of all fish species examined may be caused by high metabolic activity in male fish (Raizada ***et al.***, 1983; Siddiqu and Nasim, 1979; Collasozn ***et al.***, 1998).

Table 4: Seasonal variations in blood parameters of male and female *Alburnoides bipunctatus*

		Seasons			
Haematological	Sex	Spring	Summer	Autumn	Winter
Total Leucocyte (10^3mm^{-3})	Male	3.54±0.20^a	3.85±0.35^a	2.40±0.32^b	1.95±0.25^b
	Female	3.80±0.14^a	4.10±0.20^a	2.75±0.50^b	2.50±0.60^b
Total Types					
Neutrophile (%)	Male	8.50±0.20^a	9.20±0.70^a	6.30±0.40^b	5.90±0.10^b
	Female	9.20±0.40^a	11.50±0.80^a	7.30±0.10^b	6.60±0.20^b
Eosinophile (%)	Male	0.50±0.10	0.75±0.15	0.60±0.20	0.40±0.10
	Female	0.60±0.08	0.90±0.25	0.55±0.10	0.50±0.15
Basophile (%)	Male	0.10±0.01	0.15±0.02	0.10±0.01	0.10±0.02
	Female	0.15±0.02	0.20±0.02	0.15±0.04	0.12±0.03
Lymphocyte (%)	Male	82.40±2.20^b	80.20±1.65^b	86.80±1.50^a	87.95±0.90^a
	Female	80.75±1.35^b	77.20±1.12^b	85.60±1.15^a	86.88±0.65^a
Monocyte (%)	Male	8.50±0.50^a	9.70±0.25^a	6.20±0.10^b	5.65±0.30
	Female	9.30±0.40^a	10.20±0.50^a	6.40±0.15^b	5.90±0.30^b
Erythrocyte (10^6mm^{-3})	Male	1.25±0.010^b	1.353±0.020^a	1.18±0.25^b	1.050±0.32^c
	Female	1.15±0.020^b	1.290±0.010^a	1.09±0.20^b	0.910±0.02^c
Hemoglobin ($g100ml^{-1}$)	Male	6.95±0.15^b	7.70±0.25^a	6.40±0.10^b	5.71±0.40^c
	Female	6.25±0.20^b	7.20±0.30^a	5.80±0.15^b	4.90±0.30^c
Hematocrit (%)	Male	21.40±0.30^b	23.20±0.50^a	20.20±0.26^b	18.05±0.15^c
	Female	19.70±0.20^b	22.00±0.35^a	18.70±0.30^b	15.70±0.40^c
Erythrocyte Indexes					
MCV(μ^3)	Male	171.20±1.30	171.47±1.50	171.18±1.80	171.90±1.30
	Female	171.30±0.50	170.54±0.90	171.55±0.75	172.52±0.50
MCH(μμg)	Male	55.60±0.30	56.10±0.35	54.23±0.20	54.38±0.40
	Female	54.34±0.35	55.81±0.40	53.21±0.40	53.84±0.80
MCHC(%)	Male	32.47±0.80	33.18±0.75	31.68±1.10	31.63±0.55
	Female	31.25±0.70	32.72±0.65	31.01±0.60	31.21±0.40
Thrombocyte (10^4mm^{-3})	Male	0.65±0.20	0.58±0.25	0.55±0.20	0.57±0.30
	Female	0.55±0.35	0.49±0.15	0.51±0.10	0.48±0.25

a.b.c: Mean values followed by different letters in the rows are significantly different p=0.05

Total leucocyte, neutrophil and monocyte were found to be high in female individuals of all species. This can be explained by increase of reproduction activity and defense mechanisms of female fish.

Erythrocyte indexes (MCV, MCH, MCHC) and thrombocyte numbers were not differed between male and female fish. Differences in levels of some haematological parameters could be explained with effects of body size and ecological conditions. But similarity in erythrocyte

Table 5: Comparison annual levels of haematological parameters of male fish

Haematological	Species		
	Cyprininon macrostomus	*Chalcalburnus mossulensis*	*Alburnoides bipunctatus F.*
Total Leucocyte ($10^3 mm^{-3}$)	2.94±0.20	2.05±0.28	2.93±0.51
Leucocyte Types			
Neutrophil (%)	7.66±0.44^b	11.18±1.25^a	7.43±2.14^b
Eosinophil (%)	0.43±0.60^b	0.92±0.40^a	0.56±0.54^b
Basophile (%)	0.09±0.01	0.10±0.01	0.11±0.01
Lymphocyte (%)	82.69±2.16^a	75.43±0.60^b	84.34±0.48^a
Monocyte (%)	9.12±0.80^b	12.35±2.66^a	7.51±2.30^c
Erythrocyte count ($10^6 mm^{-3}$)	1.530±0.01^a	1.610±0.01^a	1.208±0.01^b
Hemoglobin Density (g 100 ml^{-1})	7.96±0.28^a	8.27±0.17^a	6.69±0.10^b
Hematocrit Value (%)	26.40±1.12^a	27.54±0.84^a	20.71±1.10^b
Erythrocyte Indexes			
MCV (μ^3)	172.54±1.13	171.05±1.30	171.44±1.40
MCH (μμg)	52.02±1.20	51.36±1.65	55.38±0.70
MCHC (%)	30.15±0.50^b	30.02±0.60^b	32.30±0.45^a
Thrombocyte count ($10^4 mm^{-3}$)	0.595±0.02	0.525±0.02	0.587±0.03

a.b.c: Mean values followed by different letters in the rows are significantly different p=0.05

Table 6: Comparison annual levels of haematological parameters of female fish

	Species		
Haematological	*Cyprininon macrostomus*	*Chalcalburnus mossulensis*	*Alburnoides bipunctatus F.*
Total Leucocyte (10^3 mm^{-3})	3.25±0.32^a	2.48±0.15^b	3.28±0.10^a
Leucocyte Types			
Neutrophil (%)	8.75±0.30^b	13.51±1.00^a	8.65±2.14^b
Eosinophil (%)	0.48±0.15^b	1.18±0.20^a	0.65±0.10^b
Basophile (%)	0.12±0.02	0.11±0.02	0.15±0.03
Lymphocyte (%)	79.51±2.10^a	67.72±0.35^b	82.60±0.25^a
Monocyte (%)	11.12±0.40^b	17.46±0.80^a	7.95±0.70^c
Erythrocyte count (10^6 mm^{-3})	1.301±0.01^a	1.420±0.01^a	1.110±0.01^b
Hemoglobin Density (g100ml^{-1})	6.78±0.12^a	7.38±0.08^a	6.03±0.10^b
Hematocrit Value (%)	22.57±0.50^a	24.37±0.20^a	19.02±0.30^b
Erythrocyte Indexes			
MCV (μ^3)	173.48±2.20^a	171.61±2.30^b	171.35±2.85^c
MCH ($\mu\mu_g$)	52.11±1.40	51.97±1.10	54.32±1.70
MCHC (%)	30.03±0.60^c	30.28±0.90^b	31.70±1.20^a
Thrombocyte count ($10^4 mm^{-3}$)	0.510±0.03^b	0.502±0.02^c	0.507±0.03^a

a.b.c: Mean values followed by different letters in the rows are significantly different P<0.05

indexes and thrombocyte numbers in all species studied may belong to same family, in consequence of that having same behaviour and stay in same environment.

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