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A Study on Blood Parameters of *Capoeta trutta* (Heckel, 1843)

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Abstract: This study was carried out in order to determine possible seasonal effects on blood parameters of *Capoeta trutta* (Heckel, 1843), from Karakaya Dam Lake (Malatya, Turkey). Age-weight-length values of fish were determined seasonally during a year and the effect of these independent variables on blood parameters were investigated. Comparison of blood parameters was performed according to sex differences. Analysis revealed that the values of blood parameters increase in spring-summer period and decrease in autumn-winter period irrespective of age-weight-length variables. A significant ($p < 0.05$) difference was found between female and male in reproduction period (spring-summer).

Key words: Blood parameters, *Capoeta trutta, Cyprinidae*

Introduction

Blood tissue reflects physical and chemical changes occurring in organism, therefore detailed information can be obtained on general metabolism and physiological status of fish in different groups of age and habitat (Kocabatmaz and Ekingen, 1978). In fisheries, it is important to find out illness and parasites as the source of these causes may not be generally detectable in early period of the infection. However it is also possible early diagnosis of illnesses in case of evaluating hematological data, particularly blood parameters (Rimsh and Adamova, 1973).

Interpretation of fish hematological data is quite difficult due to internal and external variation. It is well known that blood sampling, laboratory techniques, seasonal variations, size and ontogeny of habitat, genetic properties, sex, population density, geographical dispersion, lack of food supply and stress, pH, water and transportation affect hematological data (Wilhem Filho *et al.*, 1992; Hardig and Höglung, 1984; Denton and Yousef, 1975; Ezzat *et al.*, 1973; Van Vuren and Hatting, 1978; Fourtie and Hatting, 1976).

Research on blood of *Tilapia zilli, Salmo gairdneri* and *Cyprinus carpio* revealed that hematological parameters were affected by seasonal variations (Denton and Yousef, 1975; Ezzat *et al.*, 1973; Fourtie and Hatting, 1976). In addition, sex differences also affect blood parameters (Ezzat *et al.*, 1973; Van Vuren and Hatting, 1978; Fourtie and Hatting, 1976). Furthermore, hematological parameters were known to be influenced by age, weight and length of fish (Arda, 1974).

The objective of this study was therefore to analyze and compare blood parameters in relation to seasonal variation, sex and age. Results may be helpful in establishing a hematological data bank for fish including standard values of blood parameters.

Materials and Methods

In this study 335 male and female specimens of *Capoeta trutta* (Heckel, 1843) belonging to *Cyprinidae* family, captured from Karakaya Dam Lake (Malatya, Turkey), were used. Lengths of fish ranged between 15-39 cm, weights between 50-414 g and ages between 1-5 year.

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; Hatting, 1975). Before collecting blood samples no anesthetic was applied to fish as it may affect blood parameters and hemolyzed tissues (Hoffman, 1977; McKnight, 1966). Blood samples were stored in polystyrene cool bag using anticoagulant (EDTA) containing glass tubes until used. Blood analysis were carried out as soon immediately after sampling.

To determine the count of erythrocytes and thrombocytes (per mm^3) blood sample was taken with an erythrocytes pipette and diluted (1/200) with the Hayem solution. One drop of hemolyzed blood was transferred into Thoma lamella and examined in light microscope (Soif, XZS-107B model) with a magnification of 400x (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; De Wilde 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 (20FL) was diluted with Drabkin solution (1mL) 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 (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 12.500 rpm for 5 min and the ratio of 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 100x 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, total nitrogen values of water were measured in monthly periods during a year.

Hematological data were analyzed with SPSS 7.5 for Windows by using one way analysis of variance. Differences between means were determined by using Duncan's multiple range test with 0.05% level of significance.

Results

Water quality criteria of Karakaya Dam Lake between March 1998 and 1999 period (Table 1). Temperature, COD and BOD values were statistically different ($p < 0.05$) at different seasons. The lack of variation in other indices, except temperature, dissolved oxygen concentration level suggest that water chemistry parameters were the main causes for any stress on fish.

Total leukocyte count, eosinophil, neutrophil and monocyte percentages were significantly ($p < 0.05$) higher for both sexes in spring-summer season than those of other seasons. Lymphocyte ratio, also increased significantly ($p < 0.05$) in autumn-winter season (Table 2, 3).

Erythrocyte count, the amount of hemoglobin and hematocrit value increased significantly ($p < 0.05$) in spring-summer season (Table 4, 5). Mean corpuscular volume (MCV) increased significantly ($p < 0.05$) only in spring season. No significant ($p > 0.05$) seasonal variation was observed for mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and thrombocytes (Table 4, 5).

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Table 1: Water chemistry criteria of Karakaya Dam Lake (March 1998-1999)

	Seasons (No. of water sample)			
Parameters	Spring (11)	Summer (12)	Autumn (10)	Winter (10)
Temperature (°C)	18.00 ± 0.10*	23.0 ± 0.40*	11.0 ± 0.30	9.0 ± 0.20
Dissolved Oxygen concentration (mg/L)	17.50 ± 1.65	16.03 ± 2.40	24.03 ± 1.10*	28.50 ± 1.80*
pH	7.43 ± 0.10	7.63 ± 0.08	7.58 ± 0.10	7.46 ± 0.09
Electrical Conductivity (m Siemens)	11.16 ± 1.20	11.00 ± 1.40	12.06 ± 1.80	12.30 ± 0.80
Suspended Solid Matter (ml^{-1})	58.60 ± 3.60	49.00 ± 6.50	55.00 ± 4.70	50.00 ± 3.80
Lead (ml^{-1})	-	Low	-	-
Calcium (ml^{-1})	107.66 ± 2.40	108.60 ± 2.50	112.33 ± 4.10	113.60 ± 1.60
Sodium (ml^{-1})	29.30 ± 2.10	31.00 ± 1.80	28.00 ± 3.20	22.60 ± 4.60
Chloride (ml^{-1})	31.00 ± 0.80	30.00 ± 1.10	29.60 ± 0.90	28.50 ± 0.70
Total Nitrogen (ml^{-1})	9.41 ± 0.40	10.98 ± 0.60	9.80 ± 0.60	10.06 ± 0.30

Table 2: Seasonal variation of total leukocyte and its types in males *Capoeta trutta* (Heckel, 1843)

Seasons	No. of fish	Total leukocyte count ($x10^3/mm^3$)	Neutrophil (%)	Eosinophil (%)	Lymphocyte (%)	Monocyte (%)
Spring	45	13.77 ± 0.30*	26.40 ± 1.03*	1.47 ± 0.02*	61.38 ± 2.46	10.75 ± 0.37
Summer	48	12.51 ± 0.28*	25.40 ± 0.41*	0.96 ± 0.03*	63.55 ± 1.32	10.09 ± 0.78
Autumn	40	9.15 ± 0.50	20.43 ± 0.28	0.39 ± 0.02	72.54 ± 1.24*	6.64 ± 1.57
Winter	38	9.88 ± 0.28	20.06 ± 0.31	0.40 ± 0.04	72.26 ± 0.96*	7.28 ± 1.52

Table 3: Seasonal variation of total leukocyte and its types in females *Capoeta trutta* (Heckel, 1843)

Seasons	No. of fish	Total leukocyte count ($x10^3/mm^3$)	Neutrophil (%)	Eosinophil (%)	Lymphocyte (%)	Monocyte (%)
Spring	42	18.31 ± 0.32*	32.90 ± 0.56*	0.73 ± 0.05*	53.04 ± 2.60	13.33 ± 0.34*
Summer	44	16.76 ± 0.38*	28.46 ± 0.78*	0.65 ± 0.02*	58.25 ± 1.59	12.64 ± 0.30*
Autumn	40	11.68 ± 0.40	24.26 ± 0.33	0.37 ± 0.04	66.80 ± 1.23*	8.57 ± 0.28
Winter	38	10.01 ± 0.49	20.56 ± 0.60	0.48 ± 0.02	70.31 ± 1.05*	8.65 ± 0.34

Table 4: Male dependent seasonal variation in erythrocyte count, hemoglobin density, hematocrit value, erythrocytes indexes (MCV, MCH and MCHC), thrombocytes count of *Capoeta trutta* (Heckel, 1843)

					Erythrocyte index			
Seasons	No. of fish	Erythrocyte count ($x10^6/mm^3$)	Hemoglobin density (g/dl)	Hematocrit value (%)	MCV (μ^3)	MCH ($\mu\mu_g$)	MCHC (%)	Thrombocyte count ($x10^4/mm^3$)
Spring	45	1.929 ± 0.040*	7.92 ± 0.04*	32.93 ± 0.36*	170.7 ± 1.5*	41.0 ± 1.1	24.0 ± 0.2	0.533 ± 0.02
Summer	48	2.316 ± 0.050*	9.35 ± 0.06*	35.98 ± 0.47*	155.3 ± 2.3	40.3 ± 1.2	25.9 ± 0.9	0.505 ± 0.02
Autumn	40	1.830 ± 0.040	7.16 ± 0.05	28.51 ± 0.37	155.7 ± 1.7	39.1 ± 1.6	25.1 ± 2.0	0.446 ± 0.02
Winter	38	1.320 ± 0.010	5.70 ± 0.03	20.35 ± 0.38	154.1 ± 1.5	43.1 ± 3.3	28.0 ± 1.3	0.477 ± 0.03

Table 5: Female dependent seasonal variation in erythrocyte count, hemoglobin density, hematocrit value, erythrocytes indexes (MCV, MCH and MCHC), thrombocytes count of *Capoeta trutta* (Heckel, 1843)

					Erythrocyte index			
Seasons	No. of fish	Erythrocyte count ($x10^6/mm^3$)	Hemoglobin density (g/dl)	Hematocrit value (%)	MCV (μ^3)	MCH ($\mu\mu_g$)	MCHC (%)	Thrombocyte count ($x10^4/mm^3$)
Spring	42	1.502 ± 0.031*	6.15 ± 0.05*	26.06 ± 0.64*	173.5 ± 2.6*	40.9 ± 1.6	23.6 ± 1.1	0.539 ± 0.02
Summer	44	1.827 ± 0.067*	7.50 ± 0.05*	28.20 ± 0.22*	153.3 ± 2.2	41.0 ± 2.9	26.5 ± 1.5	0.492 ± 0.03
Autumn	40	1.317 ± 0.037	5.54 ± 0.06	20.20 ± 0.25	153.3 ± 1.9	42.0 ± 2.8	27.4 ± 2.3	0.468 ± 0.02
Winter	38	1.146 ± 0.025	5.10 ± 0.04	17.85 ± 0.37	155.7 ± 1.6	44.5 ± 2.7	28.5 ± 2.9	0.482 ± 0.03

Table 6: Comparison of blood parameters between male and female *Capoeta trutta* (Heckel, 1843) between March 1998 and 1999

Blood parameters	Males	Females
Total leukocyte count ($10^3/mm^3$)	11.32 ± 1.64	14.19 ± 0.95*
Leukocyt types (%)		
Neutrophil	23.07 ± 1.10	26.54 ± 0.67*
Eosinophil	0.805 ± 0.02	0.557 ± 0.02
Lymphocyte	67.43 ± 1.26*	62.10 ± 2.60
Monocyte	8.69 ± 1.84	10.79 ± 0.86*
Erythrocyte count ($10^6/mm^3$)	1.848 ± 0.05*	1.448 ± 0.03
Hemoglobin density (g/dl)	7.53 ± 0.03*	6.07 ± 0.01
Hematocrit value (%)	29.44 ± 0.67*	23.07 ± 0.26
Erythrocyt indexes		
MCV (μ^3)	158.95 ± 2.36	158.95 ± 2.25
MCH ($\mu\mu_g$)	40.87 ± 2.14	42.10 ± 1.67
MCHC (%)	25.75 ± 1.12	26.50 ± 1.83
Thrombocyte count ($10^4/mm^3$)	0.490 ± 0.04	0.495 ± 0.03

*Significant at $p < 0.05$.

Table 7: Age-length-weight groups dependent frequencies of hematological parameters of *Capoeta trutta* (Heckel, 1843)

	Age (Year)				
parameters	1	2	3	4	5
No. of fish	22	19	24	26	17
Length (cm)	15-19	20-24	25-29	30-34	35-39
Weight (g)	50-124	125-199	200-274	275-349	350-414
Erythrocyte count ($x10^6/mm^3$)	1.264±0.11	1.370±0.09	1.460±0.04	1.740±0.12*	1.960±0.14*
Hemoglobin density (g/dL)	5.830±0.13	6.380±0.43	6.850±0.32	7.900±0.24*	8.760±0.26*
Hematocrit value (%)	18.760±1.39	20.320±1.65	21.700±0.47	26.050±2.38*	29.100±1.13
MCV (μ^3)	148.410±1.39	148.320±1.65	148.600±1.43	149.710±2.20	148.460±1.13
Erythrocyt indexes MCH ($\mu\mu_g$)	46.120±2.40	46.560±3.30	46.910±1.20	45.400±1.80	44.690±0.80
MCHC (%)	31.070±2.40	31.390±1.60	31.560±0.40	30.320±0.80	30.100±0.50
Thromcytes count ($x10^3/mm^3$)	0.718±0.07	0.687±0.04	0.547±0.03	0.691±0.03	0.621±0.07
Total leukocyte count ($x10^3/mm^3$)	24.620±1.26*	23.210±2.14*	24.800±2.03*	17.650±2.15	16.240±1.58
Neutrophil (%)	10.580±1.01	12.280±0.64	13.450±0.53*	14.530±0.49*	18.800±0.86*
Eosinophil (%)	0.420±0.03	0.390±0.01	0.400±0.02	0.430±0.02	0.450±0.02
Lymphocyte (%)	79.760±0.96	76.920±1.37	76.050±0.63	74.260±0.60	64.900±1.99*
Monocyte (%)	9.240±0.21	10.410±0.55	10.100±0.22	10.780±0.30	15.850±0.45*

*Significant at $p < 0.05$.

When blood parameters were compared regarding sex difference; total leukocyte count, neutrophil and monocyte percentages were found to be higher for females then those for males ($p < 0.05$). Erythrocyte count, the level of hemoglobin, hematocrit value and lymphocyte ratios, however, were found to be significantly $p < 0.05$ higher in males than in females. However no statistically significant difference was determined between sexes with respect to erythrocyte indices (MCV, MCH and MCHC) and thrombocyte number (Table 6).

When the values for age, length and weight increased, erythrocyte number, the amount of hemoglobin, hematocrit value, neutrophil and monocyte ratios also increased significantly ($p < 0.05$). Lymphocyte ratios, however, were decreased significantly ($p < 0.05$). Also, there was no significant variation in erythrocyte indices (MCV, MCH and MCHC), eosinophil ratios and thrombocyte number (Table 7).

Discussion

Annual changes of blood parameters showed a clear increase in spring-summer period and a decrease in autumn-winter period (Table 2, 3). Particularly, during reproduction period (March-August) total leukocyte number and neutrophil ratios for female specimen were found to be higher compared to male specimen. Erythrocyte number, hemoglobin level, hematocrit value, neutrophil and monocyte percentages were also higher in the same period. Similar results were reported by other researchers (Denton and Yousef, 1975; Ezzat *et al.*, 1973; Van Vuren and Hatting, 1978; Murachi, 1959; Fourtie and Hatting, 1976; Azizoğlu and Cengizler, 1996). The increase in hematological parameters might have resulted from the difference in water temperature and oxygen concentration (Kocabatmaz and Ekingen, 1978; Denton and Yousef, 1975; Amlacher, 1970).

Sex dependent variation in blood parameters revealed that blood parameters, erythrocyte number, the amount of hemoglobin, hematocrit and lymphocyte values for male fish were significantly higher ($p < 0.05$) than for female fish. Total leukocyte, neutrophil, and monocyte ratios, were determined to be higher in females at reproduction periods, than in males. The results from this study are also in agreement with previous ones (Ezzat *et al.*, 1973; Murray, 1984; Van Vuren and Hatting, 1978; Fourtie and Hatting, 1976; Carthy *et al.*, 1971).

An increase in age-length-weight independent variables was determined to cause an increase in erythrocyte, hemoglobin, hematocrit, neutrophil and monocyte values. However, the lymphocyte ratios were decreased to a moderate level. No difference was observed for erythrocyte indexes (MCV, MCH and MCHC). The effect of age-length-weight variables on blood parameters such as enhanced formation of lymphocytes that is an essential component of immune system in the early stages of growth period has been reported (Aldrin *et al.*, 1984; Macecy and Potter, 1981).

In conclusion, the variation in methodology used for hematological studies, instant changes in physical and chemical properties of microenvironment in which fish lives make difficult comparison of literature and the establishment of limits for hematological data. Therefore, we propose separate data collection and comparison from healthy and unhealthy fish to obtain reliable hematological data. However the result of this study may be helpful in obtaining standard values of blood parameters.

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