Seasonal variations in serum lipids, lipoproteins and some haematological parameters of chub (Leuciscus cephalus)

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ABSTRACT

The purpose of the present investigation was to characterize monthly fluctuations in haematology and serum biochemical data in wild chub (Leuciscus cephalus) by measuring red blood cells (RBC), white blood cell counts (WBC), haemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentrations (MCHC), triglycerides (TG), cholesterol (CHOL), high density lipoprotein (HDL), light density lipoprotein (LDL) and very low density lipoproteins (VLDL). TG, CHOL, HDL and VLDL amounts were found to reach the highest values in the pre-spawning period (May and June). LDL values in cold months were higher than in the rest of the year. The highest RBC, Hct, MCV and WBC values were determined in May. The minimum values were obtained in cold months for RBC, Hct, MCV and WBC. The highest and the lowest values for Hb, MCH and MCHC were found in January and in warm months, respectively. Consequently, it was concluded that all the studied parameters were affected by many endogenous and exogenous factors such as reproductive cycle, water temperature, and metabolic rate.

Key words: Haematological parameters, Leuciscus cephalus, Seasonal variations, Serum lipids, Lipoproteins.

RIASSUNTO

VARIAZIONI STAGIONALI DI ALCUNI PARAMETRI EMATOLOGICI E DI LIPIDI E LIPOPROTEINE NEL SIERO DI CAVEDANI (LEUCISCUS CEPHALUS)

Scopo del presente studio è stato determinare le fluttuazioni mensili di alcuni parametri biochimici nel sangue e nel siero di cavedani, misurando: eritrociti (RBC), leucociti (WBC), emoglobina (Hb), valore ematocrito (Hct), volume corpuscolare medio (MCV), contenuto emoglobinico corpuscolare medio (MCH), trigliceridi (TG), colesterolo (CHOL), lipoproteine ad alta densità (HDL), lipoproteine a bassa densità (LDL), lipoproteine a densità molto bassa (VLDL). I valori più alti di TG, CHOL, HDL e VLDL sono stati...
raggiunti nel periodo antecedente all’ovodeposizione (Maggio e Giugno), mentre la concentrazione di LDL è stata maggiore nei mesi più freddi. I valori più alti di RBC, Hct, MCV e WBC si sono registrati in Maggio. I valori minimi di RBC, Hct, MCV e WBC si sono registrati nei mesi più freddi. I valori massimi di Hb, MCH e MCHC sono stati rilevati nel mese di Gennaio, quelli minimi nei mesi più caldi. Conseguentemente è possibile concludere che tutti i parametri studiati sono stati influenzati da diversi fattori sia endogeni che esogeni, come il ciclo riproduttivo, la temperatura dell’acqua e il ritmo metabolico.

Parole chiave: Parametri ematologici, Leuciscus cephalus, Variazioni stagionali, Lipidi sierici, Lipoproteine.

Introduction

The chub (Leuciscus cephalus) is a fish species living in almost every freshwater body in Turkey (Geldiay and Balik, 1996) and a total of 738 tons of chub were captured in 2003 (Anonymous, 2005).

Fish use lipids rather than carbohydrates as the main energy source like other poikilothermic organisms (Henderson and Torcher, 1987). In all living organisms, lipids are transported throughout the circulation bound to proteins in specific macromolecular complexes called lipoproteins (Fried et al., 1968). Lipoproteins can be classified according to hydrated density, ultracentrifugation flotation velocity, and electrophoretic mobility on agarose and apolipoprotein composition and these classes are: chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL) (Babin and Vernier, 1989).

Haematological parameters are valuable tools for the monitoring of fish health (Jawad et al., 2004) and they are affected by many endogenous and exogenous factors such as water temperature, reproduction cycle and metabolic rate (Martinez et al., 1994; Svoboda et al., 2001; Kavadias et al., 2003; Bayir, 2005). Establishing reference intervals for various haematological parameters of fish is important for evaluating the effects of various environmental changes on the health of populations in the wild. Very few data on chub blood parameters have been published. Thus, this study was performed to obtain basic information by describing the monthly pattern in serum lipids, lipoproteins and some haematological parameters in Leuciscus cephalus.

Material and methods

Study area and data collection
Chub were caught in the Hinis Stream in Erzurum, Turkey (039° 18’041”N, 041° 46’02”E) (Figure 1). The sampling period was from February 2004 to January 2005. Every month, a total of 10 fish were captured by means of cast nets and capturing time was between 10.00 and 13.00. Water temperature values were measured with a digital thermometer. After capturing, fish were transferred to laboratory using a fibreglass tank. In the laboratory, the fish were held in a 600 L water volume fibreglass tank. Water temperature was regulated to natural water temperature by heaters in summer and ice masses in winter and 3-4 days later approximately 1 ml blood was taken from caudal vein of each fish using a 2-ml heparinized syringe for haematological analyses and nonheparinized syringe for biochemical analyses.

Haematological and biochemical analysis
Red blood cell (RBC) and white blood cell (WBC) counts were determined by the method of Klinger et al. (1996), haemoglobin (Hb) and haematocrit (Hct) values were determined as described by Blaxhall and Daisley (1973), and finally erythrocyte indices were determined according to Schreck and Moyle’s method (1990).
For biochemical analysis the blood samples were transferred from syringe to tubes and plasma was obtained after centrifugation at 4000 rpm for 10 min at 4°C. Biochemical analyses were made by means of an automated system (Olympus® AU 2700).

**Determination of gonadosomatic index**

Gonadosomatic index (GSI) was calculated as 
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GSI = \frac{\text{Gonad Weight}}{\text{Fish Weight}} \times 100
\]

**Statistical analysis**

The statistical analysis was performed with SPSS version 10.0 for Windows (SPSS, 1996). Data were presented as mean ± standard deviation (SD) of the mean. Data were analyzed by one-way analysis of variance (ANOVA). The significant means were compared by Duncan’s multiple range tests at \(\alpha=0.05\) level. The correlation coefficients \((r)\) were also calculated between all examined parameters and water temperature or GSI changes using SPSS package.

**Results and discussion**

**Gonadosomatic index and temperature changes**

In the prespawning period (May and June), GSI reached maximum value (6.30±0.62 and 6.09±0.79, respectively) and in July it rapidly diminished to the lowest value (0.79±0.09) as spawning was completed (Figure 2). The highest and the lowest temperature values were found in August (23.8°C) and January (0.1°C) (Figure 3), respectively.

**Seasonal changes in serum lipids and lipoproteins**

Plasma triglyceride (TG), cholesterol (CHOL) and VLDL concentrations exhibited an increasing trend from February to May, with the greatest concentrations occurring in May (prespawning period) as 440.60±30.53 mg/dL, 393.60±16.41 mg/dL and 88.12±6.11 mg/dL, respectively. Al-
though the annual cycle of HDL exhibited a similarity with TG, CHOL and VLDL changes, HDL reached to the highest values in the prespawning period (June) as 222.60±16.91 mg/dL. LDL reached peak values in February (134.28±18.11 mg/dL) and LDL values in the winter months were higher than other seasons (Table 1). These parameters were statistically different from each other (P< 0.05).

Monthly changes of TG, HDL and VLDL were highly correlated with seasonal temperature and GSI changes. The CHOL was highly correlated with GSI, not with temperature, and LDL was highly correlated with temperature not with GSI. Correlation

Figure 2. Seasonal changes of gonadosomatic index values of *Leuciscus cephalus* (Values are means ±SD).

Figure 3. Seasonal temperature changes in Hinis Stream.
| Months | TG (mg/dL) | CHOL (mg/dL) | HDL (mg/dL) | LDL (mg/dL) | VLDL (mg/dL) |
|--------|------------|--------------|-------------|-------------|---------------|
| February | 146.6±15.55f | 298.40±22.50d | 134.80±11.73d | 134.28±18.11a | 29.32±3.11f |
| March | 156.40±18.94f | 300.60±23.77d | 141.80±8.67cd | 127.52±22.43ab | 31.28±3.79f |
| April | 269.00±32.23c | 308.80±11.01cd | 165.20±6.98b | 90.00±20.51de | 53.80±6.45c |
| May | 440.60±30.53a | 393.60±16.41a | 210.80±16.78a | 94.68±26.19cd | 88.12±6.11a |
| June | 410.00±20.58b | 362.20±12.46b | 222.60±16.91a | 57.60±11.88f | 82.00±4.12b |
| July | 230.20±19.23d | 258.80±22.33e | 153.00±15.82bcd | 59.76±4.77f | 46.04±3.85d |
| August | 243.60±20.70cd | 297.20±17.02d | 157.00±17.96bcd | 91.48±10.70cde | 48.72±4.14cd |
| September | 254.20±18.16cd | 327.60±14.12c | 166.80±8.73b | 109.96±917bc | 50.84±3.63cd |
| October | 201.40±12.38e | 260.60±16.94e | 148.40±14.84bcd | 71.92±8.44df | 40.28±2.48e |
| November | 136.40±7.37f | 266.60±17.87e | 153.20±9.42bcd | 86.12±11.96de | 27.28±1.46f |
| December | 139.40±23.67f | 301.40±10.88d | 155.60±7.83bcd | 117.92±16.65ab | 27.88±4.74f |
| January | 154.60±20.02f | 304.00±21.76cd | 161.20±34.08bc | 111.88±17.50bc | 30.92±4.00f |
| Average | 231.87±100.46 | 306.65±41.55 | 164.20±29.11 | 96.09±28.21 | 46.37±20.09 |

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a, b, c, d, e, f: means in a column with identical letters are not significantly different.

Values given as mean ±SD; *: P<0.05.
n=5 for each month, a total of 60 samples.

TG: triglyceride; CHOL: cholesterol; HDL: high density lipoprotein; LDL: light density lipoprotein; VLDL: very low density lipoproteins.
coefficients (r) for these parameters were shown in Table 2.

Seasonal changes in some haematological parameters

Seasonal changes of some haematological parameters were presented in Table 3. As seen in this table, the differences in haematological parameters were significant at P<0.05 level among months. Maximum RBC, Hct, mean corpuscular volume (MCV) and WBC values were determined in the prespawning months (May and June) as 1.49±0.14 10^6/mm³, 51.80±3.11%, 766.58±31.94 μ³ and 2.72±0.43 10^4/mm³, respectively. The minimum values were in November for RBC (0.75±0.11 10^6/mm³), in January for Hct (31.00±2.35%) and MCV (238.28±35.90 μ³) and in December for WBC (1.38±0.45 10^4/mm³). The highest values for Hb (12.02±0.77 mg/dL), mean corpuscular haemoglobin (MCH) (158.52±21.75 μg) and mean corpuscular haemoglobin concentration (MCHC) (38.84±2.20%) were found in January and the lowest values for Hb and MCHC were in September (8.50±0.98 mg/dL and 17.80±2.78%), and for MCH in May (64.23±9.62 μg).

At the end of the present study, it was found that monthly changes of RBC, Hct, MCV and WBC were positively affected by seasonal temperature and GSI changes. Hb, MCH and MCHC were negatively correlated with temperature, not with GSI. The correlation coefficient values for these parameters were given in Table 4.

It is known that lipids are the principal substrates in the energy metabolism in fish (Patton et al., 1970) and they are transported from one tissue to the other by macromolecular protein-containing complexes: lipoproteins (Fried et al., 1989). The concentration of circulating lipids and lipoproteins is under the complex control of genetic and environmental factors (Chapman, 1980, 1986).

Many researchers have suggested that lipids and lipoproteins in fish showed seasonal fluctuations, especially when dealing with temperature changes and reproductive cycle (Patton et al., 1970; Erdoğan et al., 2002; Kavadias et al., 2003). For this investigation, the spawning period started in June and it was completed in July (Figure 2). Serum lipids (TG and CHOL) reached top levels during the prespawning period and fell to the lowest values during the postspawning period (Table 1). Kavadias et al. (2003) showed that plasma TG concentrations in Dicentrarchus labrax during the postspawning period were about 50% lower than during the prespawning period. Similarly, Bayir (2005) indicated that the highest serum TG and CHOL amounts were found during the prespawning period and the lowest amounts were found during the postspawning period in Capoeta capoeta umbla within the same stream.

| Table 2. Correlation coefficients of serum lipids and lipoproteins in Leuciscus cephalus. |
|---------------------------------|-------|-------|-------|-------|-------|
|                                | TG    | CHOL  | HDL   | LDL   | VLDL  |
| Temperature                    | 0.631** | 0.207 | 0.400** | 0.557** | 0.631** |
| GSI                            | 0.748** | 0.711** | 0.709** | -0.217 | 0.748** |

**: P<0.01.
TG: triglyceride; CHOL: cholesterol; HDL: high density lipoprotein; LDL: light density lipoprotein; VLDL: very low density lipoproteins.
GSI: Gonadosomatic Index.
Table 3. Seasonal changes in some haematological parameters of *Leuciscus cephalus*.

| Months  | RBC (10⁶/mm³) | Hb (g/dL) | Hct (%) | MCV (µ³) | MCH (µg) | MCHC (%) | WBC (10⁴/mm³) |
|---------|---------------|-----------|---------|----------|----------|----------|--------------|
| February| 0.99±0.27de   | 11.14±1.23ab | 31.40±2.30f | 346.12±103.28de | 121.31±46.31bc | 32.14±4.38bc | 2.06±0.42c   |
| March   | 1.01±0.17de   | 10.90±0.91bc | 34.80±1.92e | 319.62±80.52def | 110.14±16.73c | 34.83±3.59b  | 2.16±0.21bc  |
| April   | 1.08±0.17d    | 10.08±0.95c  | 37.80±2.49de | 404.86±55.08d  | 95.38±16.43cde | 26.80±3.63d  | 2.26±0.43abc |
| May     | 1.49±0.14a    | 9.54±1.13c   | 51.80±3.11a  | 766.58±31.94a  | 64.23±9.62e   | 18.47±2.31e  | 2.72±0.43a   |
| June    | 1.38±0.24abcd | 9.58±0.82d   | 48.20±2.59b  | 662.28±96.52b  | 70.71±10.77a  | 19.98±2.67e  | 2.58±0.30abc |
| July    | 1.13±0.24bcd  | 8.72±1.12d   | 42.40±2.88c  | 479.76±100.78c | 79.15±15.17de | 20.52±1.48e  | 2.34±0.30abc |
| August  | 1.27±0.14abc  | 8.60±0.50d   | 45.40±2.97c  | 578.04±67.33e  | 68.25±9.52e   | 19.02±1.91e  | 2.40±0.27abc |
| September| 1.34±0.27ab   | 8.50±0.98d   | 48.00±2.12b  | 644.20±137.93b | 65.45±15.84a  | 17.80±2.78e  | 2.38±0.25abc |
| October | 0.89±0.09de   | 9.32±0.99c   | 42.60±2.30c  | 379.60±38.20de | 106.06±20.05cd| 21.88±2.07e  | 1.52±0.30d   |
| November| 0.75±0.11e    | 10.48±0.72c  | 39.00±1.58d  | 291.56±42.61ed | 142.46±22.19ab| 26.95±2.78e  | 1.46±0.11d   |
| December| 0.76±0.14e    | 11.08±0.83b  | 36.80±3.42de | 277.12±32.78fd | 149.94±30.71ab| 30.31±3.42ed | 1.38±0.45d   |
| January | 0.77±0.09c    | 12.02±0.77a  | 31.00±2.35f  | 238.28±35.90f  | 158.52±21.75a | 38.84±2.20e  | 1.40±0.38d   |
| Average | 1.07±0.30     | 10.00±1.38   | 40.77±6.92   | 449.00±181.98  | 102.67±38.65  | 25.63±7.34  | 2.06±0.56    |

a, b, c, d, e, f: means in a column with identical letters are not significantly different.

Values given as mean±SD; *: P<0.05.
n=5 for each month, a total of 60 samples.

RBC: red blood cell; Hb: haemoglobin; Hct: haematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentrations; WBC: white blood cell counts.
Reproduction is one of the important factors affecting fish physiology. Lipid stores represent major energy reserves in fish (Wallaert and Babin, 1994), and during sexual maturation they are transferred from previously stored tissues to gonads for gonadal development (Bayir, 2005) because gonads require supplies of constituents such as phospholipids for membranes and cholesterol as substrate for steroid production (Babin, 1986; Loir, 1990). As seen in Table 2, serum TG and CHOL amounts showed a clear positive correlation with monthly GSI changes. An increase in serum lipids during the prespawning period was probably associated with the utilization of lipids as energy source for reproduction. These results of this recent study agree with those of various authors (Wallaert and Babin, 1994; Svoboda et al., 2001; Kavadias et al., 2003).

The main function of lipoproteins in living organisms is lipid transport (Babin and Vernier, 1989). Therefore, monthly lipoprotein changes closely correspond with serum lipid changes. In this study, it was also found that seasonal HDL and VLDL changes were highly correlated with seasonal GSI changes, but seasonal LDL changes did not correlate with GSI (Table 2). Furthermore, monthly TG and CHOL fluctuations highly correlated with GSI changes, and seasonal CHOL variations were not affected by water temperature changes (Table 2). Svoboda et al. (2001) suggested that TG and CHOL concentrations in blood serum of Tinca tinca were higher in June (22°C) than in April (10.3°C) and this alteration related to different metabolic rates. In fish, metabolic rate and feeding activity increase with an increase in temperature. Many researchers reported that because of high metabolic rate and feeding activity, serum lipids and lipoproteins in plasma were higher during warm seasons than cold seasons (Svoboda et al., 2001; Kavadias et al., 2003; Bayir, 2005).

Consequently, our study has been supported by previous studies and higher plasma lipid and lipoprotein concentrations in warm seasons (Table 1) were associated with high metabolic rate and feeding activity in these seasons.

Determination of haematological parameters of native fish is helpful in obtaining population properties and in describing properties of pollutants (Hickey, 1976). In this work, significant changes in all studied haematological parameters were observed throughout the year. The highest values in RBC, Hct, MCV and WBC were found in May (prespawning period) and summer and the lowest in cold seasons (autumn and winter). Therefore, they were very positively affected by water temperature and GSI fluctuations (Table 4). These results agreed with some previous studies (Martínez et al., 1994; Frangioni et al., 1997; Collazos et al., 1998; Guijarro et al., 2003). Guijarro et al. (2003) reported that owing to high temperature, oxygen availability was reduced during summer in tench. Martínez et al. (1994) suggested that there was a clear correlation among RBC, Hb, Hct and environmental factors and water temperature was the most effective one. It was suggested that WBC was affected by some factors such as water temperature and reproduction period (Bayir, 2005). Hence, in this investigation, the high RBC, Hct, MCV and WBC during prespawning period and warm months were explained with increasing energy needs for reproduction, and decreasing oxygen amounts.

Contrary to other haematological parameters; Hb, MCH and MCHC were quite negatively correlated with temperature changes and these parameters had no correlation with GSI (Table 4). It was reported that the lowest Hb was found within summer in tench (Guijarro et al., 2003) and mirror carp (Sahan (Azizoglu), 2000). The highest and
the lowest Hb concentrations were observed in January (cold season) and in September (warm season), respectively. The highest and the lowest values of MCH and MCHC were also found in these seasons (Table 3). Study area was covered with ice about 3 months and fish could not be fed and metabolic rate was at minimum level during this period. Denton and Yousef (1975) suggested that decreases in haematological parameters within cold seasons were associated with lack of food. Therefore, the high Hb, MCH and MCHC values during cold seasons were probably related to lack of food and/or adaptation to cold environment.

**Conclusions**

At the end of present study, it was found that serum lipids, lipoproteins and haematological parameters in *Leuciscus cephalus* were affected by many endogenous and exogenous factors such as the reproduction cycle, water temperature and metabolic rate.

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