Research Article

THE DETERMINATION OF SOME HEAMATOLOGICAL PARAMETERS AND SPERMATOLOGICAL CHARACTERISTICS OF CRUCIAN CARP (*Carassius carassius* L, 1758) IN ATATÜRK DAM LAKE

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ABSTRACT

In this study, the determination of some heamatological parameters and spermatological properties of crucian carp (*Carassius carassius* L, 1758) were investigated in Atatürk Dam Lake. The live fish (n=20) used in this study that have mean weight 2730.05±203.29 g and mean length 55.16±2.28 cm that captured using gill nets in the Atatürk Dam Lake. Heamatological parameters; PCV (%), WBC (x10^4/mm^3), RBC (x10^6/mm^3), Hb (g/dl), MCV (µm^3), MCH (pg), MCHC (%) were determined as mean % 34.24±0.57; 24.51±1.09 x10^4/mm^3; 1.51±0.22 x10^6/mm^3; 7.78±0.02 g/dl; 233.75±6.19 µm^3; 57.50±0.50 pg 23.33±0.53 % respectively. In collected semen; sperm volume, spermatozoa motility, duration of spermatozoa motility, spermatozoa concentration and sperm pH were determined as mean 1386.50±191.64 µl; 88.42±0.83 %; 118.57±16.30 s; 17.84±3.44 x10^9/ml; 7.42±0.05 respectively.

Keywords: *Carassius carassius*, sperm, blood, Atatürk Dam Lake

ATATÜRK BARAJ GÖLÜ'NDEKİ HAVUZ BALIĞI'NIN (*Carassius carassius* L, 1758) BAZI SPERMATOLOJİK ÖZELLİKLERİNİN VE HEMATOLOJİK PARAMETRELERİNİN BELİRLENMESİ

ÖZET

Bu çalışmada Atatürk Baraj Gölü'ndeki havuz balıklarının (*C. carassius*) balıklarının bazı spermatolojik özellikleri ve hematolojik parametreleri incelendi. Çalışmada solungaç ağları ile yakalanan 2730,05±203,29 gr ağırlığında 55,16±2,28 cm boyunda, 20 adet erkek *C. carassius* balığı kullanıldı. Çalışmada hematolojik özelliklerden; hematokrit (%), lökosit (x10^3/mm^3), eritrosit (x10^6/mm^3) hemoglobin (g/dl), MCV (µm^3), MCH (pg) ve MCHC (%) değerleri sırasıyla % 34,24±0,57; 24,51±1,09 x10^4/mm^3; 1,51±0,22 x10^6/mm^3; 7,78±0,02 g/dl; 233,75±6,19 µm^3; 57,50±0,50 pg % 23,33±0,53 olarak belirlendi. Spermatolojik özelliklerden ise; sperma miktarı (ml), spermatozoa motilitesi (%), motilite süresi (sn), spermatozoa yoğunluğu (x10^9/ml) ve pH sırasıyla; 1386,5± 191,64; 88,42±0,83; 118,57±16,30; 17,84±3,44; 7,42±0,05 olarak belirlendi.

Anahtar Kelimeler: *Carassius carassius*, sperm, kan, Atatürk Baraj Gölü

INTRODUCTION

According to Libosvarsky (1961), *Carassius* spp. are of warm freshwater species and belong to the group of boreal fish (Holopainen et al., 1997). Thus, it has a wide geographic distribution especially in Eastern Europe and its distribution is continuously increasing. Crucian carp, *Carassius carassius* (L, 1758), is one of such species that belongs to family Cyprinidae originating from Asia. The distribution of crucian carp in Turkey is now thought to include both the Thrace (European) region, Marmara Regions, Kızılırmak and Yeşilırmak River Deltas, Çoruh Basin and also it is very common in Europe (Özuluğ et al., 2004). This species fed on herbs in water, bottom animals and fly larvae. Some of the elders of the western countries are producing meat is delicious. The Crucian carp is well known as a deligent fish species for native fish communities, but it is a least concern fish species.

Hematological parameters are closely related to the response of the animal to the environment, an indication that the

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environment where fishes live could exert some influence on the hematological characteristics (Gabriel et al., 2004). These indices have been employed in effectively monitoring the responses of fishes to the stressors and thus their health status under such adverse conditions. They can provide substantial diagnostic information once reference values are established under standardized conditions. Evaluation of the hemogram involves the determination of the total erythrocyte count (RBC), total white blood cell count (WBC), hematocrit (PCV), hemoglobin concentration (Hb), erythrocyte indices (MCV, MCH, MCHC), white blood cell differential count and the evaluation of stained peripheral blood films (Campbell, 2004).

Sperm quality generally refers to the ability of sperm to successfully fertilize an egg (Rurangwa et al., 2004). In fish, sperm quality may depend on several factors including the husbandry practices, environment, feeding regime as well as quality of the feed, broodstock condition and genetic variability, and also the methods utilized for artificial spawning (Rurangwa et al., 2004). Sperm quality can vary between individuals and within an individual of the same species (Rana 1995).

Spermatozoa motility, milt volume and the spermatozoa concentration are good indicators for milt quality (Cabrita et al., 2001, Tekin et al., 2003). The spermatozoa of most fish species are immotile in the testis and seminal plasma. Therefore, motility is induced after the spermatozoa are released into the aqueous environment during natural reproduction or into the diluent during artificial reproduction (Alavi and Cosson, 2006). Milt volume is one of the features reflecting the milt yield and spermatozoa concentration. Likewise, Moon et al. (2003) reported positive correlation between milt volume and spermatozoa concentration in male starry flounder, *Platichtys stellatus*. Spermatozoa are immotile immediately after collection (Morisawa et al., 1988).

Spermatozoa concentration may also influence the rate of fertilization (Aas et al., 1991, Pool and Dillanea, 1998). The milt pH affects the spermatozoa motility and maturation (Billard et al., 1995, Liley et al., 2002).

Although evaluation of milt quality has been studied in many fish species, there are no available data on the crucian carp from Southeastern Turkey. In this study, the determination of some heamatological parameters and spermatological characteristics of crucian carp were investigated in Atatürk Dam Lake.

MATERIAL AND METHOD

Broodstocks and samples preparation

The sample fishes (crucian carp) were caught with gill nets (44-52-60-70 mm) from 15 May to 05 June in Atatürk Dam Lake (37°23′29′′03′′′N, 38°34′38′′05′′′E) in 2012. During the study, physico-chemical parameters of the sampling areas were measured with YSI Environmental (YSI 85). Samples obtained were moved to the laboratories of Harran University Bozova Vocational High School and for age determination, scales and otholiths were examined under a streo microscope (Nikon SMZ 2Tstereo) (Baker and Timmons, 1991).

Heamatological analysis

In this study, the blood samples were taken by puncture caudal vein into 2 ml vacationer tube containing heparin sodium shook for two minutes gently and stored in +4 °C prior to hematological analysis. The indices used to evaluate the hematological profile were included heamatological parameters; Hematocrit (PCV) (%), White Blood Cell (WBC) ($x10^4/mm^3$), Red Blood Cell (RBC) ($x10^6/mm^3$), Hemoglobin (Hb) (g/dl), Mean Corpuscular Volume (MCV) (μm^3), Mean Corpuscular Hemoglobin (MCH) (pg), Mean Corpuscular Hemoglobin Concentration (MCHC) (%) were determined (Houston 1990)

Spermatological parameter measurement

The genital area was cleaned with lake water and dryed to avoid contamination of samples with faeces, urine and lake water. Milt was collected by abdominal massage in male fish. After collections, milt volume was measured by the measuring pipette. It was transported to the laboratory under cold conditions (7–10 8°C). In collected milt; milt volume (µl), spermatozoa motility (%), duration of spermatozoa motility (s), spermatozoa concentration ($x10^9$/ml) and milt pH were determined. Milt motility estimates were determined using a light microscope by placing a 5 µl drop of diluted semen (5 µl of milt was mixed to 500 µl of activating solution) on a slide covered with a glass coverslip (22 mm x 22 mm) at 20 °C, ten seconds after activation (Aas *et al*. 1991). An activation solution, (100µL 50 mM NaCl, 20 mM Tris–HCl, pH 8.0), was used for activation the milt.

The motility percentage was determined by observing the proportion of motile to non-motile spermatozoa, in triplicate. The duration

of sperm motility was visually evaluated as the time elapsed from activation until 5% of the motile spermatoza. Spermatozoa concentration was determined by using hemocytometer and expressed as number of spermatozoa x10^9/ml. Milt was diluted in a 20 ml test tube by adding 10 µl of milt to 9990 µl of a distilled well water and then mixed by pipetting with automatic pipette, and counting the number of spermatozoa in a Thoma chamber (Thoma chamber, American Opticals, Buffalo, NY) viewed with a light microscope at 400x magnification at 20 °C. The pH of milt was measured using pH indicator strips (pH: 0–14; Merck, Germany) after collected milt, in triplicate. The mean of three measurements was used to calculate the actual sperm concentration, motility, duration of motility and pH.

Statistical analysis

Statistical data was conducted using SPSS 10.0.1 (SPSS Inc. 1999). Descriptive analysis was carried out to determine mean and standard error on milt volume, sperm motility percentage, sperm concentration and milt pH. All values are expressed as mean ± standard error (S.E.M.) (SPSS, ver. 10.05; SPSS, Chicago, IL).

RESULTS and DISCUSSION

The live fish (n=20) used in this study that have mean weight 2730.05±203.29 g and mean length 55.16±2.28 cm that captured using gill nets in the Atatürk Dam Lake. The mean values of milt properties and blood parameters in of *C. carassius* in the spawning season are presented in the Table1 and 2.

Heamatological parameters; PCV (%), WBC (x10^4/mm^3), RBC (x10^6/mm^3), Hb (g/dl), MCV (μm^3), MCH (pg), MCHC (%) were determined as mean % 34.24±0.57; 24.51±1.09 x10^4/mm^3; 1.51±0.22 x10^6/mm^3; 7.78±0.02 g/dl; 233.75±6.19 μm^3; 57.50±0.50 pg ve % 23.33±0.53 respectively.

In collected semen; sperm volume, spermatozoa motility, duration of spermatozoa motility, spermatozoa concentration and sperm pH were determined as mean 1386.50±191.64 µl; 88.42±0.83 %; 118.57±16.30 s; 17.84±3.44 x10^9/ml; 7.42±0.05 respectively.

Table 1. Overall mean values of some heamatological parameters in of *C. carassius* in the spawning season (n=20).

Properties	HTC (%)	WBC (x10^4/mm^3)	RBC (x10^6/mm^3)	PCV (g/dl)	MCV (μm^3)	MCH (pg)	MCHC (%)
Values	34.24±0.57	24.51±1.09	1.51±0.22	7.78±0.02	233.75±6.19	57.50±0.50	23.33±0.53

HTC: Heamatoctit, WBC: White Blood Cell, RBC: Red Blood Cell, PCV: Hemoglobin Concentration, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Heamoglobin MCHC: Mean Corpuscular Hemoglobin Concentration

Table 2. Overall mean values of some spermatological characteristics of *C. carassius* (n=20).

Properties	Volume (µl)	Spz. Mot. (%)	Spz. Mot. Dur. (s)	Spz. Con. (x10^9/ml)	pH
Values	1386.50±191.64	88.42±0.83	118.57±16.30	17.84±3.44	7.42±0.05

Spz: Spermatozoa, Mot: Motility, Dur: Duration Con: Concentration

Discussion

Haematocrit (PCV) is the proportion of blood volume that is occupied by RBCs. In this study, obtained PCV values (34.24±0.57 %) were found within the limits. These values rarely goes above 50% (Clarks et al., 1979, Etim et al., 1999, Ikechukwu and Obinnaya, 2010). However the lower PCV values were observed in *Carassius auratus* under infectious condition (Brenden and Huizinga, 1986). Red blood cells perform a task due to their hemoglobin capacity. PCV content is related to growth and volume of RBCs (Houston, 1990). The WBC values of *C. carassius* (24.51±1.09 x10^4/mm^3) were lower than *Carassius auratus* (84.70 x10^3/mm^3). Besides, this values were found higher than *Carassius auratus gibelio* (0.65± 0.03 x10^4 mm^3) (Harikrishnan et al., 2010). This higher number of leucocyt could be related to diffrences of fish species.

A large number of blood cells are erythrocytes. The RBC values were found as 1.51±0.22x10^6/mm^3. Kumar et al. (2013) reported that 0.826±0.017 x 10^6/mm^3 in *Carassius auratus* and in *Carassius auratus gibelio* RBC values reported as 1.05± 0.04 x10^6 mm^3 (Wu et al., 2013). Our results were found higher the former studies. This higher values could be due to the high activity of hematopoeitic blood making organs in this

species. Also, decreased RBC of infected fishes was observed in a previous study, indicating that RBCs are destroyed leading to anemia (Haney et al., 1992).

In this study, Hb values of crussian carp were determined as 7.78±0.02 g/dl. This values were similar to *C. auratus* (5.00±0.25 g/dl) (Harikrishnan et al., 2010). Hb concentration of infected fishes decrease according to destruction of RBC (Scott and Rogers, 1981). In contrast, increasing the physiological fish activity lead to an increase the heamoglobyn concentration (Eisler, 1965; Ikechukwu and Obinnaya, 2010).

In crussian carp blood, MCV (μm^3), MCH (pg) and MCHC (%) values were found mean 233.75±6.19 μm^3; 57.50±0.50 pg and % 23.33±0.53, respectively. In *C. auratus*, these values (MCV, MCH, MCHC) were found as 188 μm^3, 58 pg, 33,3%, respectively (Harikrishnan et al., 2010). Similarly, these values were determined by Kumar et al. (2013) as 188.02 μm^3, 86.66 pg, 4.23 %, respectively. A decrease in MCV may be attributed to a microcytic anaemia (Kumar et al., 2013). The fluctuations in MCH and MCHC value could be seen in infected fish spercies. This situation may be due to changes in the concentration of hemoglobin in red blood cells (Wepener et al., 1992).

In this study, 1386.5 ± 191.64 µl of semen volume were determined. Sperm volume reported by Akcay et al. (2004) for mirror carp, Bozkurt et al. (2008-9) for grass carp were 17.33, 15.43 and 5.25 ml, respectively. Volume of milt in crussian carp is lower than that of others. These differences can be regulated by in climate, the change in day length and food supply. Likewise, it can be altered by environmental conditions, and changed depending on the biological characters as age. Olsén et al. (2006) report that ovulating female fish pheromones affect the efficiency of sperm in male fish. Sperm quality in aquatic species varies depending on various external factors such as water temperature, spawning season and male feeding (Rurangwa et al., 2004).

The determined spermatozoa motility (88.42 ± 0.83 %) in this study is similar to mirror and grass carp values (69.5 to 94.63%) (Akcay et al., 2004, Bozkurt et al., 2008-9, Rahman et al., 2011). Fish sperm energy resources are limited. Motility is a function specific to the male gamete. It is necessary in order to achieve penetration of sperm to the ovum (Islam and Akhter, 2011).

The duration of spermatozoa motility were found as 118.57±16.30 s. These values were similar to that of grass carp (69.50-77.00 s) (Bozkurt et al., 2009), but were lower than that of mirror carp (9:31 min) (Akçay et al., 2004). Several factors such as temperature, pH and ions affect the sperm motility (Alavi et al., 2011; Cosson et al., 1999; Morisawa, 1999). The duration of sperm motility in freshwater fish is a ranged from 30 s to few minutes (Jezierska and Witeska, 1999; Tekin et al., 2003, Bozkurt et al., 2009, Islam and Akhter, 2011).

The spermatozoa concentration of crucian carp was 17.84±3.44 $x10^9$/ml in this study. These values were higher in this study than that of Lubzens et al. (1997) who reported as 14.97±5.30 x 10^9/ml in *C. carpio*. On the other hand, Yuehi and Chang (1997) reported higher values in same species (25–35 x 10^9/ml). This difference may have been due to age, weight and length of fishes, and environmental factors such as daylength, temperature and species of fishes (Bhattacharyya et al., 2005). Hence, these researchers reported that the factors that regulate the spermatozoa concentration could not be determined exactly (Lubzens et al., 1997, Yuehi and Chang, 1997).

Sperm pH of raw milt was 7.42±0.05 which also supports the results of Bozkurt et al. (2009) and Akçay et al. (2004), where they found pH 7.00-8.00 of carp milt pH of milt influences the the initiation and duration of sperm motility (Marian *et al.* 1997). Carp sperm motility can be initiated in media with an external pH of 6.00 - 9.00 (Redondo-Muller et al., 1991, Perchec-Poupard et al., 1997).

In conclusion, observations of heamatological parameters and sperm characteristics determined by manual massage in male *C. carassius* in Atatürk Dam Lake reported in this study are the first. These results represent a valuable baseline dataset and provide background information in these species.

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