Macedonian Journal of Animal Science, Vol. **6**, No. 2, pp. 95–100 (2016)
220 In print: ISSN 1857 – 6907
Received: July 28, 2016 On line: ISSN 1857 – 7709
Accepted: March 31, 2016 UDC:597.551.2–111

Original scientific paper

COMPARATIVE PHYSIOLOGICAL ASSESSMENT OF COMMON CARP (*CYPRINUS CARPIO*) AND CRUCIAN CARP (*CARASSIUS CARASSIUS*) BASED ON ELECTROLYTE AND HEMATOLOGICAL ANALYSIS

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Studies based on biochemical and hematological parameters in fish are significant in monitoring of health and early detection of pathological conditions. This research obtained values of serum electrolytes and hematological parameters in crucian carp (n = 15) and common carp (n = 13). Significantly different values of K^+, Cl^- and Ca^{2+} concentration were detected. In serum of common carp there were significantly higher concentration of K^+ and Cl^- and lower concentration of Ca^{2+}, compared to the crucian carp. Hematological parameters differ in these two species. In blood of common carp significantly lower PCV, hemoglobin concentration, RBC and MCV and notably higher MCHC and WBC were obtained, compared to crucian carp. This preliminary research is important in establishing referential range of biochemical and hematological parameters in cyprinid fishes.

Key words: common carp; crucian carp, electrolytes; hematological parameters; physiological assessment

СПОРЕДБЕНА ФИЗИОЛОШКА ПРОЦЕНА НА КРАП (CYPRINUS CARPIO) И КАРАС (CARASSIUS CARASSIUS) ВРЗ ОСНОВА НА ЕЛЕКТРОЛИТСКИ И ХЕМАТОЛОШКИ АНАЛИЗИ

Студиите кои се засновани на биохемиски и хематолошки параметри се особено важни при следење на здравјето и откривањето на патолошки состојби кај рибите. Оваа истражување ги опфаќа вредностите на серумските електролити и хематолошките параметри кај карасот (n = 15) и крапот (n = 13). Добиени се значително различни вредности на концентрациите на K+, Cl^- и Ca^{2+}. Во серумот на крапот утврдени се значително повисоки концентрации на K+ и Cl^- и ниски концентрации на Ca^{2+} во споредба со карасот. Хематолошките параметри се разликуваат кај овие два вида. Во крвта на крапот, значително се пониски вредностите на PCV, концентрацијата на хемоглобин, RBC и MCV и видно повисоки MCHC и WBC, во споредба со карасот. Ова прелиминарно истражување е важно при одредување на референтни граници на биохемиските и хематолошките параметри кај ципринидните видови риби.

Клучни зборови: крап; карас; електролити; хематолошки параметри; физиолошка процена

INTRODUCTION

Hematological parameters of fish are an indicator of physiological response on environmental stimuli. Ambiental changes have impact on blood cell number, morphology and distribution (Srivastava & Choudhary, 2010). Analyses of hematological and biochemical parameters in blood are good indicators of environmental and anthropogenic impact; also they are biological indicators of fish health status (Çelik, 2004).

Although their classification has not been well defined due to their great variability, a growing belief has appeared in ichthyology, however controversial, that *Carassius gibelio* (Bloch, 1882) and *Carassius carassius* (Linnaeus, 1758) have the status of separate species (Jiang et al., 2013). Values of hematological parameters oscilate under dif-

ferent impacts of external environment. Variations in the blood count could be the result of internal physiological changes and diversity of ecological factors.

The values of biochemical parameters in serum of crucian carp differ in recent literature. Common carp can survive low oxygen concentration (0.3 – 0.5 mg/l) as well as high oxigen saturation. Temperature variations affect the glucose, urea, uric acid and protein levels in serum of common carp (Yan et al., 2012). Significant increase in blood glucose level was detected after thermal stress exposure of the common carp, while significant fluctuations of this parameter were not observed in crucian carp (Suljević & Mitrašinović, 2009). Fish generally have lower blood volume than other vertebrates. Fish, members of genus *Carassius* (Cypriniformes: Cyprinidae), are more widely used as fish experimental model in various studies (Tsangaris et al., 2011; Falfushynska, et al., 2012; Kreitsberg et al., 2013; Lu et al., 2013). The most research data about biochemical parameters are obtained in *Carassius auratus* (Xuezhen et al., 2007; Zhengxin et al., 2016). Obtained concentration of serum calcium and glucose showed high individual variations in cyprinids, while in other biochemical parameters statistical significance has not been established (Suljević et al., 2015).

The fish blood is very rich in leukocytes, which are divided into granulocytes (usually fish blood contain more than three types of granulocytes) and agranulocytes. Pseudoeosinophils as specific fish granulocytes are known as heterophils in literature. Because fish do not have the bone marrow and lymph nodes, hematopoesis occurs in other organs. The main hematopoietic organs of fish are kidney, spleen and thymus. Additionally, hematopoesis may occur in the gills, the wall of the small intestine and heart. Part of the kidney named pronephros also has a very important role in hematopoiesis because it is the main site where erythropoiesis, granulopoiesis and lymphopoiesis occur. Reticuloendothelial tissue form the stroma where blast cells take place and this tissue composition is similar to the bone marrow in higher vertebrates (Samali et al., 1999). Blood cell differentiation in fish is the subject of the debate because there is no uniform classification or reference interval for both taxonomic ranks, family and genus.

The aim of this study was to determine physiological advantages and disadvantages in two cyprinid species based on comparative analysis of electrolyte concentration and hematological parameters.

MATERIALS AND METHODS

Sampling and experimental design

In this research we analysed biochemical and hematological parameters in two species: *Cyprinus carpio* (Teleostei: Cyprinidae) from Jablanica lake and *Carassius carassius* (Teleostei: Cyprinidae) from Bardača lake. Jablanica lake is an artificial lake and has an area of 13 km^2 (43°41′N 17°51′E). Bardača lake near Srbac town is a natural reservoir with an area of 35 km^2 (45º 4’ – 45º 8’ N, 17º 24’ – 17º 30’E). Fishnets were used for fish sampling. The fish were collected during the winter period (in January). Total number of common carp specimens was 13 and total number of crucian carp specimens was 15. After catching, fish were placed in a 100 l container with water, which was permanently enriched by oxygen by aerator (CHAMPIONCX-0098) and all fish survived transport.

The adaptation of fish took 20 days (Laboratory of Physiology, Faculty of Science, Sarajevo, Bosnia and Herzegovina) with daily monitoring of water oxygen by Winkler method (Vanselow, 1940), ammonia by Nessler method (Carpenter, 1965). During the adaptation period, the fish were placed in two separate aquariums with the 200 L volume. Temperature of water was constantly adjusted at 10°C. Fish were fed with Eco FeedEx C 48/10 (Eco Feed Ltd, Serbia).

Blood sampling and analysis

The puncture was performed with the sterile needles of 1.0 – 1.2 mm (Medoject, Slovak Republic). The collected blood was centrifuged at 3000 rpm for 10 minutes by using Heraeus Sepatech Biofuge model 1217 (Heraeus, Germany) and blood serum was separated for electrolyte analysis. The electrolyte concentration was determined by atomic emission spectroscopy by using Vitros DT 60 (Knauer, Germany).

Hematological parameters included the number of erythrocytes (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and the number of leukocytes (WBC). The number of erythrocytes and leukocytes were determined in a Neubauer chamber (hemocytometer) according to the method of Kekić

& Ivanc (1982). Hb was estimated by Drabkin hemoglobin cyanide method (Blaxhall and Daisly, 1973), and PCV was determined by microhematocritic centrifuges (Hettich Haematokrit 24 zentrifugen, Germany). Hematological values (MCV, MCH and MCHC) were calculated based on the values of PCV, number of erythrocytes and Hb concentration. Euthanasia and all procedures with animals were conducted in accordance to the Directive 2010/63/EU of the the European Parliament and of the the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Statistics

Data are presented as arithmetic mean ±1 SD with minimum and maximum values or range. The Shapiro-Wilk test was used to estimate the normality of data distribution. Student's *t-test* was used to asses the differences between the groups. Statistical analysis were performed by SPSS (Version 20.0, SPSS, Inc., Chicago, IL, USA).

RESULTS

Morphometric features

Figure 1 presents morphometric characteristics of common carp. The average value of weight in common carp was 10.88 ± 1.15 g. Distribution of weight frequency showed that large number of specimens (4) had average weight of approximatelly 10 g. Total body length was 13.06 ± 1.29 cm, while large number of specimens (5) had a length of 13 cm.

Figure 2 presents morphometric characteristics of crucian carp. Average weight value was 69.26 ± 7.30 g. Distribution of weight frequency showed that most of the specimens (5) had average weight value of aproximatelly 75 g. Total length was 16.44 ± 0.943 cm, while the frequency distribution was homogeneous in most specimens.

Electrolyte analysis

Table 1. presents values of electrolyte concentrations in *Cyprinus carpio* and *Carassius carassius.* In serum of common carp were obtained significantly higher Na^+, K^+ i Cl^- and lower Ca^{2+} concentrations than in the serum of crucian carp. Considerably different values of K^+, Cl^- i Ca^{2+} ($p<0.05$) were detected, while the Shapiro-Wilk test showed notable difference in comparison with standard K^+ i Ca^{2+} distribution ($p<0.05$).

Hematological analysis

Hematological values such as erythrocyte count (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and leukocyte count (WBC) in the blood of common carp and the crucian carp are shown in Table 2.

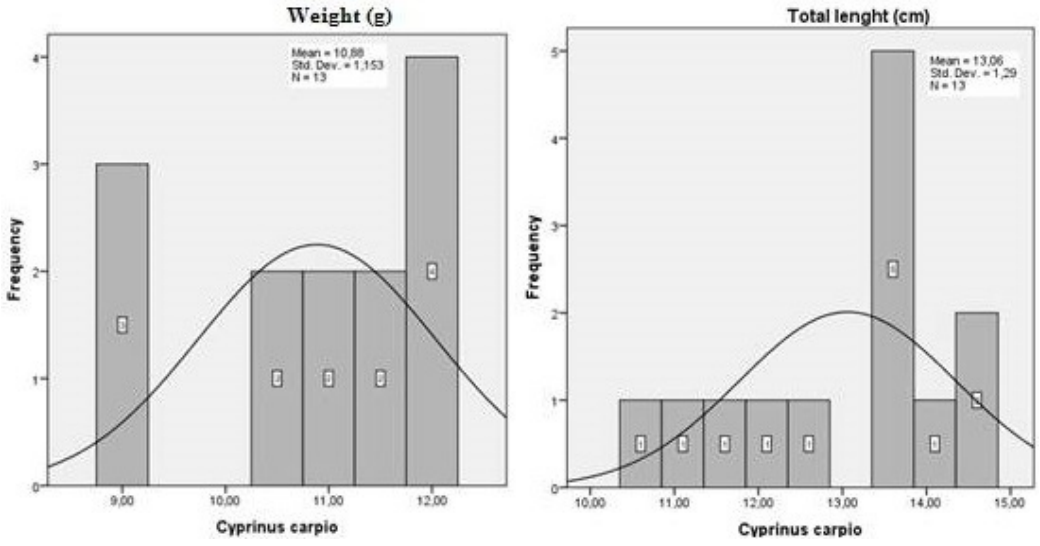

Fig. 1. Average length (cm) and weight (g) of *Cyprinus carpio*

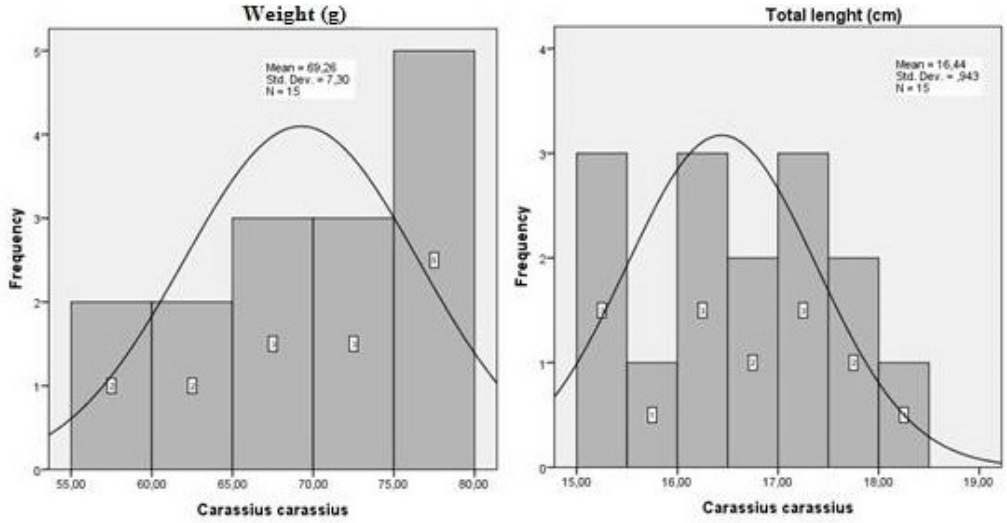

Fig. 2. Average length (cm) and weight (g) of *Carassius carassius*

Table 1

Comparative values of electrolytes in Cyprinus carpio and Carassius carassius serum (mmol/l)

Parameter	*Cyprinus carpio*		*Carassius carassius*		t-test	Shapiro W
	Mean ± SD	Range	Mean ± SD	Range	p-values	
Na^+	136.5 ± 4.52	127 –142	135.20 ± 4.35	126 –142	0.09	0.240
K^+	1.84 ± 0.55	1.30 –2.90	1.14 ± 4.31	0.60 –2.60	0.00	0.00
Cl^-	106.31 ± 4.84	99 –113	101.93 ± 0.54	93 –108	0.00	0.974
Ca^{2+}	1.26 ± 0.26	0.95 –1.78	2.59 ± 0.45	1.64 –3.18	0.00	0.032

Table 2

Comparative values of hematological parameters in Cyprinus carpio and Carassius carassius blood

Parameter	*Cyprinus carpio*		*Carassius carassius*		t-test	Shapiro W
	Mean ± SD	Range	Mean ± SD	Range	p-values	
PCV	0.25 ± 0.04	0.19 –0.31	0.31 ± 0.09	0.25 – 0.58	0.00	0.592
Hb (g/l)	73.215 ± 9.19	56.8 –87.50	79.50 ± 9.81	60.42 –97.66	0.293	0.802
RBC × 10^{12}	1.16 ± 0.07	1.04 –1.25	1.27 ± 0.16	1.01 –1.51	0.00	0.000
MCV (fL)	219.94 ± 33.47	163.6 –269.6	242.54 ± 53.28	165.5 –402	0.001	0.587
MCH (pg)	63.36 ± 9.28	50.27 –79.01	63.26 ± 11.18	40.01 –84.90	0.329	0.094
MCHC (g/dl)	290.5 ± 35.05	224.4 –335.12	267.79 ± 53.99	136.23 –377.07	0.00	0.819
WBC × 10^6	4.50 ± 0.42	4.00 –5.30	3.63 ± 0.90	2.50 –4.75	0.00	0.011

The common carp had higher values of MCHC and WBC ($p<0.05$). There were no notable differences in the average values of MCH ($p>0.05$). The crucian carp had considerably higher values of: PCV, Hb, RBC and MCV ($p<0.05$). We did not establish significant differences in hemoglobin and MCH ($p>0.05$), while other parameters are notably different in both species ($p<0.05$). RBC and WBC ($p<0.05$) considerably deviate from the standard values.

DISCUSSION

There is a growing interest in the study of hematological parameters and structural features of fish blood cells. They are important for aquacultural purposes and comparative physiology. Hematological and biochemical values are important in the evaluation of physiological condition of fish. In order to use blood parameters as biomarkers, it is important to know their standard values and reference interval. This research is particularly valuable for rare and endangered species of fish as shown also in (Dekić et al., 2014). Recent studies have aquired very few hematological data, especially in biochemical values. There are no available data for blood values of these species, that fact makes this research very valuable. Hematological and biochemical values can be compared to other cyprinid species. Although they belong to the same family, the common carp and crucian carp had different concentration of serum minerals. Na^+ and Cl^- are higher in comparison to the tench (Suljević et al., 2015), K^+ concentration in tench was higher in relation to the crucian carp and lower in relation to common carp. The common carp had a lower serum Ca^{2+} than tench and the crucian carp. K^+ values were considerably higher than those of the rainbow trout while the values of Na^+ and Cl^- were lower (Hasković et al., 2011).

Age, habitat, season and nutritional factors have an impact on changes in blood parameters. Generally, it is not possible to establish standard values for fish. Reference interval is hard to determine within the family. Therefore, every species needs to be individually analysed. Normality of blood values distribution is significant indicator of physiological state of analysed fish (Sokal & Rohlf, 1995). RBC values were lower than in *Delminichthys ghetaldii* (Dekić et al., 2014) and *Telestes metohiensis* from the Pribitul stream (Dekić et al., 2011). Some other cyprindis had higher RBC, like *Aulopyge hugeli*, *Leuciscus tursky*, *Chondrostoma phoxinus* (Vuković & Znidaršić-Križek, 1969), *Leuciscus cephalus* (Mitrašinović & Suljević, 2009). RBC values may vary within the same species, under certain physiological limitations. This leads to the different referential data (Dekić, 2010). According to Bogut et al. (2006) RBC values for common carp vary between 1.8-2.2 $\times$ 10^{12}/l. These values are considerably higher than the values obtained by our research. Values of PCV in common carp were considerably lower in comparison to the previous research with the same species (Groff & Zinkl, 1999) and were more similar to the values obtained for crucian carp. According to Hrubec et al. (2000) obtained PCV values and the Hb concentration are in the referential interval while the RBC number was very low.

The crucian carp has higher RBC and Hb concentration. This explains why crucian carp is physiologicaly stronger than the common carp. Crucian carp had higher RBC, which leads to the higher levels of other hematological values. These findings are similar in other research, like those of *Leuciscus cephalus* (Mitrašinović & Suljević, 2009).

Some studies include thrombocytes (Hrubec et al., 2000; Hrubec et al., 2001) in the WBC. Our research indicates that the common carp had higher WBC in comparison to crucian carp. These numbers deviate from the earlier findings (Hrubec et al., 2001) that showed higher WBC number in crucian carp. Hrubec et al. (2000) presented reference WBC values in the interval of 2.15 to 15.47 $\times 10^{12}$/l, while our research showed higher WBC in both observed species in comparison to other Teleostei (Hrubec et al, 2001; Oŕun & Erdemli, 2002; Kori-Siakpere et al., 2005; Rey Vázquez & Guerrero, 2007). Higher WBC number of the common carp may suggest that its immune system is more sensitive to the environmental stress, which makes crucian carp very adaptable to extreme environmental conditions.

The findings in our research contribute to the knowledge about the biochemical and hematological parameters in *Cyprinus carpio* and *Carassius carassius.* These findings may be valuable for the overall health assessment of these and other cyprinids. Hematological evaluation is significant in the early detection of health problems. Hematological values of *Carassius carassius* indicate more efficient energy metabolism and better adaptation abilities than observed for *Cyprinus carpio*. Hematological characteristics of *Carassius carassius* justify its survival abilities in extreme environmental conditions.

Conflict of interest statement: There is no any conflict of interest!

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