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PARAMETERS OF THE RED BLOOD CELL COUNT IN THREE SPECIES OF CARP FISHES

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Abstract

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The study was conducted under laboratory conditions during the years 2007-2008. We examined 11 parameters of the red blood cell count in three carp species: *Carassius gibelio* (L.), *Alburnus alburnus* (L.) and *Scardinius erythrophthalmus* (L.). The results were compared for individual species, as well as with data for other freshwater fishes. The survey can be used as a basis for determining bio-constants of the red blood cell count of the three species.

Key words: Cyprinidae, haematological parameters, erythrocyte, *ex-situ*

Introduction

The determination of haematological parameters of freshwater fishes gives an idea of their physiological status and the influence of various environmental factors (Ramaway and Reddy, 1978). The determination of blood parameters and in particular those of the red blood cell count are used for assessing fish health (Bhaskar and Rao, 1985). Variations in blood tissue in fish depends on the stress effects of environmental factors (Orun, 2003; Aldrin et al., 1982; Hickey, 1982; Gbore et al., 2006; Arnaudov et al.,); on biological peculiarities of species as well (Siddiquie and Nasim, 1979; Collazos et al., 1998); and what also has significance is the size (Garcia, 1992), seasons (Orun et al., 2003) and the way of their breeding (Cech and Wohlschlag, 1981). For this reason, there is few data on normal haematological values, including these on carps, some of which was described long ago (Blaxhall and Daisley, 1973). Some studies were made for the fish inhabiting natural and artificial lakes (Orun, et al., 2003; Gabriel et al., 2004; Svobodova et al., 2006; Latifi et al., 2007; Zexia Gao, 2007). This directed us to conduct this study ex-situ. The aim was to obtain data on the parameters of the red blood cell count in three carp species: *Carassius gibelio, Alburnus alburnus* and *Scardinius erythrophthalmus*, placed under controllable conditions for the factors: t^0C, PH and amount of dissolved O2.

Purpose and objectives

This article can serve as a basis, as well as for

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future studies in order to produce bio-constants of the red blood cell count to the investigated species. To achieve this objective, we set the following tasks – to determine the number of erythrocytes, hemoglobin content, hematocrit, MCV, MCH and MCHC, and the metric parameters of erythrocytes as well (small and large diameter of the cell, and small and large diameter of the nucleus).

Material and Methods

Carassius gibelio: 9.5 – 12.0 cm
Alburnus alburnus: 8 - 12 cm
Scardinius erythrophthalmus: 12 - 18 cm

Experimental animals

For the purposes of the experiment we used 10 specimens of the fish species: *Carassius gibelio; Alburnus alburnus* and *Scardinius erythrophthalmus*, collected in each season of the years 2007-2008. All specimens were of one and the same age-size group, respectively: *Carassius gibelio*: 9.5 – 12.0 cm, *Alburnus alburnus*: 8 - 12 cm, *Scardinius erythrophthalmus*: 12 - 18 cm . Fish were taken from ponds with no anthropogenic pollution; *Scardinius erythrophthalmus* and *Alburnus albu*rnus - from the dam lake Enchets (near Kardzhali), and *Carassius gibelio* - from a reservoir near the town of Brezovo. Then the three fish species were placed in an aquarium with an aerator and of volume 25 l of stagnant tap water. Physic-chemical characteristics of the water were: t - 19-20^{0}C, Ph - 7-7.5 and water hardness of 9.5 dH. Fish stayed for 96 hours in the water without being fed.

Obtaining blood for testing

Blood was obtained by cardiac puncture. To prevent haemo-coagulation we used anticoagulant EDTA.

Haematological tests

We tested the main parameters of the red blood cell count through the methodology described by Angelov et al. (1999). The following parameters were determined: - Number of erythrocytes – through the chamber method - with Burker's camera. - Content of hemoglobin – through the cyan-haemoglobin method - at a wavelength of 540nm. - Hematocrit - by centrifugation in capillary tubes. Centrifuging was conducted at 12 5 rpm for 5 min.

On the basis of the values of the above parameters we also calculated erythrocyte indices - mean corpuscular volume (MCV), mean hemoglobin content (MCH) and mean hemoglobin concentration in the cell (MCHC) as described by the formulas of Penev & Dukova-Peneva (2007). Erythrocyte-metric parameters were also determined - large and small diameter of the cell (Dc and dc), and large and small diameter of the nucleus (Dn and dn) – through ocular-micrometer of the microscope Olimpus CX21.

Mathematical processing of the results

We processed the data with the variation-statistical methods described by Sepetliev (1986). We determined the mean arithmetic quantity (X), standard deviation (s) and dispersion (y2). The presented results were based on the tested individuals during the four seasons by using the same methodology. We conducted comparison of the data by determining the $\chi 2$ (statistics 7).

Results and Discussion

As a result of the investigations carried out, we received the following results shown in Tables 1 and 2.

We found by mathematical analysis that for three parameters (number of erythrocytes, content of hemoglobin and hematocrit) there are no reliable differences ($p > 0.05$) between the tested species and that used as a benchmark species *Cyprinus carpio;* the values of the *Prussian carp* are the closest to its. We received reliable differences ($p < 0.05$) for the parameters MCV, MSN, MSNS for the same two species. In our opinion, this indicates that the cells of the species we studied, despite their less volume, have similar hemoglobin content in a single cell and in erythrocyte mass as a whole.

The results obtained in this study for the quantita-

Table 1

Quantitative characteristics of the red blood cell count of three species of carp

Parameters	Unit of measurement	Bleak (*Alburnus alburnus*)			Rudd (*Scardinius erythrophthalmus*)			Prussian carp (*Carassius gibelio*)		
		$\bar{X}$	σ	σ2	$\bar{X}$	σ	σ2	$\bar{X}$	σ	σ2
Number of	xl-12	1.17	0.06	0.0036	1.15	0.21	0.0441	1.5	0.54	0.2916
Hemoglobin	mmolxl-1	5.21	0.37	0.1369	5.77	0.44	0.1936	5.65	0.42	0.1764
Hematocrit	l/l	0.268	0.02	0.0004	0.278	0.03	0.0009	0.227	0.01	0.0001
MCV	fl	229.06	17.6	309.76	241	15.8	249.64	165.31	34.5	1190.25
MCH	pg	71.8	5.7	32.49	80.9	6.8	46.24	49.5	10.1	102.01
MCHC	%	31.34	1.75	3.0625	33.45	1.21	1.4641	32.26	2.05	4.2025
Cell division	%	1.4	0.1	0.01	1.2	0.08	0.0064	0.9	0.07	0.0049

Table 2

Metric characteristics of the red blood cell count of three species of carp

Parameters	Unit	Bleak (*Alburnus alburnus*)			Rudd (*Scardinius erythrophthalmus*)			Prussian carp (*Carassius gibelio*)		
		$\bar{X}$	σ	σ2	$\bar{X}$	σ	σ2	$\bar{X}$	σ	σ2
Dc	μm	9.16	0.4	0.16	9.52	0.5	0.25	13.3	0.3	0.09
dc	μm	6.27	0.3	0.09	5.2	0.4	0.16	7.8	0.5	0.25
Dn	μm	5.18	0.2	0.04	3.86	0.4	0.16	7.8	0.4	0.16
dn	μm	3.01	0.3	0.09	2.06	0.2	0.04	3.71	0.6	0.36

tive and size parameters of erythrocytes of the Prussian carp are close to the data presented by Tomova et al. (2008), Arnaudov et al. (2009) and Arnaudov et al. (2008) for control groups in ex-situ study the influence of zinc and copper on red blood parameters of the individuals caught from the same water basins. We think this is due to the similar conditions in which the tested fish are placed.

The quantitative parameters of erythrocytes of Scardinius erythrophthalmus and Alburnus alburnus were compared to the data of Arnaudova et al. (2008) for individuals of the same species from the dam lake "Kardzhali". Our results show that the studied parameters were comparable and close to these obtained by the author ($p > 0.05$).

We think this is probably due to the similar life conditions of the studied species such as their nutrition and habitat type.

This study may serve as a baseline for future studies on determining bio-constants in carp fishes.

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