

## HEMATOLOGICAL EVALUATION OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) FINGERLINGS FROM DIFFERENT HATCHERIES

RADOSLAV DEKIĆ<sup>1</sup>, ALEKSANDAR IVANČIĆ<sup>2</sup>, NEBOJŠA SAVIĆ<sup>3</sup>,  
MAJA MANOJLOVIĆ<sup>1</sup>, DANIJELA ČETKOVIĆ<sup>1</sup>, SAŠA OBRADOVIĆ<sup>4</sup>

<sup>1</sup>*Faculty of Natural Sciences and Mathematics, University Banja Luka Mladena Stojanovića 2, 78000 Banja Luka, Bosnia and Herzegovina*

<sup>2</sup>*State University of Novi Pazar, Vuka Karadžića bb, 36300 Novi Pazar, Serbia*

<sup>3</sup>*Faculty of Agriculture, Bulevar vojvode Petra Bojovića 1A, University of Banja Luka, 78000 Banja Luka, Bosnia and Herzegovina*

<sup>4</sup>*Faculty of economics and engineering management, Cvećarska 2, 21000 Novi Sad, Serbia*

### HEMATOLOŠKA PROCENA MLADI KALIFORNIJSKE PASTRMKE (*ONCORHYNCHUS MYKISS*) IZ RAZLIČITIH MRESTILIŠTA

#### *Apstrakt*

Za praćenje zdravlja i kondicije riba u prirodnim staništima kao i riba u akvakulturi veliku važnost imaju istraživanja koja se odnose na krv i tjelesne tečnosti. Parametri eritrocitne i leukocitne loze predstavljaju veoma značajne pokazatelje stanja organizma riba i njihove vrijednosti su specifične za svaku vrstu. Osnovne karakteristike normalne krvne slike zdravih jedinki svake vrste moraju biti dostupne, prije njihovog korištenja u procjeni zdravstvenog stanja riba. Svaka vrsta ima karakterističan broj i veličinu eritrocita, koncentraciju hemoglobina, i hematološke indekse. Pored toga, postoje fiziološke varijacije svih parametara koje su pod uticajem pola, reprodukcije ili se pojavljuju tokom različitih faza životnog ciklusa vrste. Dnevne i sezonske oscilacije su takđe veoma dobro izražene. Sve vrijednosti hematoloških parametara kao i njihove varijacije su specifične za vrstu i/ili rasu i varijetet. Iz tog razloga smo u ovom radu ispitali dužičastu pastrmku porijeklom iz pet različitih matičnih jata. Mrijest je obavljen na pet izabраниh ribogojilišta, nakon čega je oplodena ikra dopremljena u mrestilište Klašnik gdje su obezbijedeni isti uslovi sredine tokom embrionalnog razvoja i gajenja za svih pet grupa jedinki. Ukupno je analizirano 50 jedinki koje su podijeljene u 5 grupa. Jedinke su bile raspoređene u bazene, sa protočnom vodom. Hranjene su proporcionalno masi tijela odgovarajućom količinom hrane istog proizvođača. Iz svake grupe za analizu je uzeto po 10 jedinki. Hematološki parametri koji su praćeni u radu predstavljani su parametrima eritrocitne loze: brojem

eritrocita, koncentracijom hemoglobina, hematokritom, srednjom vrijednost zapremine eritrocita (MCV), srednjom vrijednosti količine hemoglobina u eritrocitu (MCH) i srednjom vrijednosti hemoglobina u litri eritrocita (MCHC). Vrijednosti broja eritrocita značajno su niže u drugoj grupi nego kod jedinki iz grupa IV ( $p = 0,024$ ) i V ( $p = 0,020$ ). Vrijednosti hematokrita kod riba prve grupe značajno su više nego kod grupa II ( $p = 0,001$ ), IV ( $p = 0,002$ ) i V ( $p = 0,008$ ). Takođe, vrijednosti kod III grupe riba su signifikantno više nego kod grupa II ( $p < 0,001$ ), IV ( $p < 0,001$ ) i V ( $p = 0,001$ ). Najniže vrijednosti koncentracije hemoglobina konstatovane su kod jedinki iz četvrte grupe i značajno su bile manje u poređenju s ostalim grupama (I  $p = 0,016$ , II  $0,004$ , III  $p = 0,004$ , IV  $p = 0,013$ ). Vrijednosti prosječne zapremine eritrocita značajno su bile manje kod riba prve grupe nego kod jedinki iz grupa II ( $p = 0,007$ ), IV ( $p = 0,002$ ) i V ( $p = 0,005$ ). Takođe, signifikantno više vrijednosti konstatovane su u grupi tri u poređenju sa vrijednostima grupa II ( $p = 0,001$ ), IV ( $p < 0,001$ ) i V ( $p < 0,001$ ). Najmanje vrijednosti MCH utvrđene su kod grupe IV i značajno su bile manje u poređenju sa grupama I ( $p = 0,014$ ), II ( $p = 0,001$ ), III ( $0,003$ ). Vrijednosti MCHC značajno su bile niže kod jedinki iz prve grupe u poređenju sa vrijednostima iz grupa II ( $p = 0,001$ ) i V ( $p = 0,035$ ). Vrijednosti ovog parametra su bile značajno više kod druge grupe u odnosu na grupe III ( $p = 0,001$ ) i IV ( $p = 0,007$ ). Takođe, značajno viša vrijednost zabilježena je kod grupe V u odnosu na grupu III ( $p = 0,028$ ). Uočene razlike mogu se jedino objasniti različitim porijeklom od različitih matičnih jata.

*Ključne riječi: kalifornijska pastrmka, matična jata, mrijest, hematologija*  
*Keywords: rainbow trout, breeding stock, hatching, hematology*

## INTRODUCTION

Fish are in close contact with the environment, so they are very sensitive to physical and chemical environmental changes, which can lead to changes in blood components (Wilson and Taylor, 1993; Adhikari et al., 2004; Maheswaran et al., 2008). In the same time fish hematological parameters represent indicators of the state of the environment (Dekić et al., 2009). Research of blood and body fluids are of a great importance for monitoring the fish health and the conditions in natural habitat and in aquaculture (Ivanc et al., 2005). Changes of environmental conditions lead to specific or unspecific changes in hematological state of certain fish species, and these changes along with seasonal changes affect the blood physiology and biochemistry. Erythrocyte and leukocyte parameters represent significant indicators of fish health, and its values for certain species contribute to the knowledge of the limits of its variations in different phases of life cycle and determination of normal values typical for that species (Ivanc and Miljanović, 2003). According to Rehulka and Adamec (2004), erythrocytes reflect the state of an organism during long period of time, and in the same time are significant and reliable indicators of different stress sources.

The goal of this research was to determine hematological status of *Oncorhynchus mykiss* individuals originating from different breeding stock and different fish farms, but grown under the same conditions in order to access their quality in the production aspect.

## MATERIAL AND METHODS

Individuals of rainbow trout used in this research originate from different breeding stock and different fish farms. Spawn was done in five chosen fish farms, after which

the fertilized roe was transported to Klačnik hatchery where the same environmental conditions for embryonic development and cultivation were provided for all five groups of individuals. Individuals were distributed in pools, with circulating water flow, and proportional to the mass with the same amount of food of the same supplier. From every group 10 individuals were analyzed. Monitored hematological parameters were: number of erythrocytes (RBC), hemoglobin concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

**Hematological analysis.** Blood for hematological analysis was collected by heart puncture using sharp and wide sterile needle (1.0 to 1.2 mm), by applying the rules of the sterile work. Native blood, without an anticoagulant, is used for further analyses. Erythrocyte count was performed in hemocytometer using diluent by Kekić and Ivanc (1982). Hemoglobin concentration (Hb) was determined by hemoglobin cyanide method using Drabkin reagent (Blaxhall and Daisly, 1973). Hematocrit (Hct) was determined by microhematocrit centrifuge. Hematological indices were calculated using values of hematocrit, erythrocyte number and hemoglobin concentration.

$$\text{Mean corpuscular hemoglobin (MCH)} \quad MCH = \frac{Hb / l}{Er.count / l}$$

$$\text{Mean corpuscular hemoglobin concentration (MCHC)} \quad MCHC = \frac{Hb / l}{Hct}$$

**Statistical analysis.** Statistical analyses were done using statistical programs Microsoft Excel 2007 and SPSS 11.5. Comparison was done using ANOVA and LSD test.

## RESULTS AND DISCUSSION

Results of conducted analyses of rainbow trout blood by different groups are present in table 1.

**Table 1.** Mean values of erythrocyte parameters *Oncorhynchus mykiss* from different parent flocks – group I to V

Group		RBC x 10 <sup>12</sup> /l	Hb (g/l)	Hct (l/l)	MCV (fl)	MCH (pg)	MCHC (g/l eryt.)
I	Mean	1.012	82.222 <sup>4</sup>	0.591 <sup>2,4,5</sup>	587.842 <sup>2,4,5</sup>	81.854 <sup>4</sup>	141.987 <sup>2,5</sup>
	Standard deviation	0.078	8.152	0.092	105.472	11.780	25.245
	Coefficient of variation - %	7.750	9.914	15.535	17.942	14.391	17.780
II	Mean	0.978 <sup>4,5</sup>	85.185 <sup>4</sup>	0.449 <sup>1,3</sup>	463.511 <sup>1,3</sup>	88.366 <sup>4</sup>	193.049 <sup>1,3,4</sup>
	Standard deviation	0.100	10.620	0.068	89.603	17.371	33.009
	Coefficient of variation - %	10.257	12.467	15.122	19.331	19.658	17.099

III	Mean	1.000	84.815 <sup>4</sup>	0.622 <sup>2,4,5</sup>	627.039 <sup>2,4,5</sup>	85.572 <sup>4</sup>	140.395 <sup>2,5</sup>
	Standard deviation	0.085	10.966	0.092	109.836	14.396	35.544
	Coefficient of variation - %	8.524	12.930	14.725	17.517	16.823	25.317
IV	Mean	1.063 <sup>2</sup>	68.889 <sup>1,2,3,5</sup>	0.468 <sup>1,3</sup>	445.003 <sup>1,3</sup>	65.381 <sup>1,2,3</sup>	151.079 <sup>2</sup>
	Standard deviation	0.087	8.765	0.093	102.189	10.925	26.904
	Coefficient of variation - %	8.202	12.723	19.817	22.964	16.710	17.808
V	Mean	1.066 <sup>2</sup>	82.593 <sup>4</sup>	0.484 <sup>1,3</sup>	456.079 <sup>1,3</sup>	77.476	174.227 <sup>1,3</sup>
	Standard deviation	0.046	18.064	0.084	85.233	16.249	42.493
	Coefficient of variation - %	4.337	21.872	17.395	18.688	20.974	24.389

Numbers in superscript denotes groups with significantly different mean values of the given parameter ( $p \leq 0.05$ )

Comparison of the results of five examined groups of rainbow trout showed the presence of significant differences in most of the parameters. Values of erythrocyte count were significantly lower in group II comparing with groups IV ( $p = 0.024$ ) and V ( $p = 0.020$ ). PCV values were significantly higher in first group comparing with groups II ( $p = 0.001$ ), IV ( $p = 0.002$ ) and V ( $p = 0.008$ ). Group three had also significantly higher values of PCV comparing with groups II ( $p < 0.001$ ), IV ( $p < 0.001$ ) and V ( $p = 0.001$ ).

The lowest values of hemoglobin concentration were determined in individuals from fourth group and were significantly lower comparing to the other groups (I  $p = 0.016$ , II  $p = 0.004$ , III  $p = 0.004$ , IV  $p = 0.013$ ).

Values of mean corpuscular volume were significantly lower in individuals from first group comparing with those from groups II ( $p = 0.007$ ), IV ( $p = 0.002$ ) and V ( $p = 0.005$ ). Also, significantly lower values were established in group three comparing with values determined in groups II ( $p = 0.001$ ), IV ( $p < 0.001$ ) and V ( $p < 0.001$ ).

The lowest values of MCH were determined in group four and were significantly lower comparing with groups I ( $p = 0.014$ ), II ( $p = 0.001$ ), III ( $p = 0.003$ ). MCHC values were significantly lower in individuals from the first group comparing with those from groups II ( $p = 0.001$ ) and V ( $p = 0.035$ ). Values of second group were significantly higher comparing with values from groups III ( $p = 0.001$ ) and IV ( $p = 0.007$ ). Also, significantly higher values were established in group V comparing with group III ( $p = 0.028$ ).

Looking at the results of monitored parameters it is evident that individuals of rainbow trout from group IV had lower values of hemoglobin concentration, PCV, MCV and MCH.

Given that individuals were under the same conditions and treatment, observed differences can be correlated with fish origin from different breeding stocks. Svobodova et al. (2008) showed similar differences, but among spawners of eight carp breeds, for hemoglobin level (Hb), haematocrit value (PCV), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH).

## CONCLUSIONS

All experimental groups of rainbow trout had been provided with the same conditions for embryonic development and cultivation. Given that individuals were under the same conditions and treatment, observed differences can be correlated with different origins from breeding stock.

## REFERENCES

Adhikari, S., Sarkar, B., Chatterjee, A., Mahapatra, C. T., Ayyappan, S. (2004): Effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recovery in a freshwater teleost, *Labeo rohita* (Ham). *Ecotoxicol. Environ. Saf.* 58: 220–226.

Blaxhall, P.C., Daisley, K.W. (1973): Routine hematological methods for use with fish blood. *J. Fish. Biol.*, 5:771-781.

Dekić, R., Ivanc, A., Bakrač-Bećiraj Azra, Bošković Jelena (2009): Normalne hematološke vrijednosti gajenog lipljena, IV međunarodna konferencija i Sajam tehničkih i tehnoloških dostignuća "Ribarstvo" 27. – 29. Maj, 2009. godine, Zemun – Beograd. 358-364.

Ivanc A., Miljanović, B. (2003): Hidroakumulacije, Multidisciplinovan pristup održivom razvoju, Monografija, Prirodno-matematički fakultet Novi Sad, Ministarstvo za zaštitu prirodnih bogatstava i životne sredine, Zavod za zaštitu zdravlja "Timok" Zaječar, JVP "Vode Srbije", JVP "Vode Vojvodi." Novi Sad.

Ivanc, A., Hasković, E., Jeremić, S., Dekić, R. (2005): Hematological Evaluation of welfare and health of fish, *Praxis veterinaria* 53 (3) 191-202, 2005.

Kekić, H., Ivanc, A. (1982): A new direct method for counting fish blood cells *Ichthyologia*, 14, 1: 55.

Maheswaran R., Devapaul, A., Muralidharan, A., Velmurugan, B., Ignacimuthu, S. (2008): Haematological studies of fresh water fish, *Clarias batrachus* (L.) exposed to mercuric chloride. *International Journal of Integrative Biology*, 2, 1:49-54.

Rehulka, J., Adamec, V. (2004): Red Blood Cell Indices for Rainbow Trout (*Oncorhynchus mykiss* Walbaum) Reared in Cage and Raceway Culture. Department of Zoology, Silesian Museum, 2004.

Svobodova Z, Kroupova H, Modra H, Flajshans M, Randak T, Savina LV, Gela D (2008): Haematological profile of common carp spawners of various breeds. *J Appl Ichthyol* 24:55–59.

Wilson, R.W., Taylor, E.W. (1993): The physiological responses of freshwater rainbow trout, *Oncorhynchus mykiss*, during acute exposure. *J. Comp. Physiol.* 163b: 38- 47.