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The impact of level of the stocking density on the haematological parameters of rainbow trout (*Oncorhynchus mykiss)* reared in recirculating aquaculture systems

Angelica Docan, Victor Cristea, Lorena Dediu, Mirela Mocanu, and Iulia Grecu

Aquaculture, Environmental Sciences and Cadastre Department,
"Dunarea de Jos" University of Galaţi, Galaţi, Romania, European Union.
Corresponding autor: A. Docan, angelica.docan@ugal.ro

Abstract. Hematological indices are important parameters for the evaluation of fish physiological status. The purpose of this experiment was to evaluate how level of the stocking density influence the blood physiology of of juvenile rainbow trout (*Oncorhynchus mykiss*) reared in a recirculating system. The experiment was conducted over a period of 33 days. A number of 254 rainbow trout with an average weight of 29.51 ± 1.32 g were divided into four rearing units in order to create different stocking densities: in B_1-2.64 kg/m^3, with an average weight of 31.68 g/ex, B_2 – 5.16 kg/m^3 with an average weight of 30.39 g/ex, B_3 – 7.12 kg/m^3 with an average weight of 28.52 g/ex and B_4- 9.42 kg/m^3 with an average weight of 27.46 g. The sampling of *O. mykiss* blood from the four variants before and after the experimental trial allowed determination of hematological indices. Red blood cell counts (RBCc), haematocrit values (Ht), haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were measured and analyzed, with routine methods used in fish hematology (Blaxhall & Daisley 1973; Svobodova 2001). Some hematological indicators (Hb, MCV, MCH) had been influenced by the stocking density. The results of our research provide a contribution to the knowledge of the hematological parameters of the rainbow trout reared in different level of stocking densities, under the recirculating systems condition.
Key words: rainbow trout, recirculating aquaculture system, stocking density, hematological indices.

Rezumat. Indicii hematologici sunt parametri importanţi folosiţi pentru evaluarea stării fiziologice a peştilor. Scopul acestui experiment a constat în evaluarea modului în care densitatea de stocare influenţează fiziologia sângelui puietului de păstrav curcubeu (*Oncorhynchus mykiss*) în condiţiile unui sistem recirculant. Experimentul s-a desfăşurat pe o perioadă de 33 de zile. Un număr de 254 expemplare de păstrav curcubeu cu o masă medie iniţială de 29,51±1,32 g au fost repartizate în patru unităţi de creştere, stocarea materialului biologic făcându-se astfel: în B_1- 2,64 kg/m^3, cu o masă medie inţială de 31,68 g/ex, B_2 – 5,16 kg/m ^{3}cu masă medie de 30,39 g/ex, B_3 – 7,12 kg/m^3 cu masă medie de 28,52 g/ex şi B_4-9,42 kg/m^{3}cu masă medie 27,46 g. În vederea determinării indicatorilor hematologici s-au recoltat probe biologice de sânge de la exemplarele de *O. mykiss* din cele 4 variate experimentale. După metodele folosite în hematologia peştilor (Blaxhall 1972; Svobodova 2001) s-au determinat numărul de eritrocite, hematocritul (Ht), hemoglobina (Hb), volumul eritrocitar mediu (VEM), hemoglobina eritrocitară medie (HEM), concentraţia de hemoglobină eritrocitară medie (CHEM). Unii indicatori hematologici (Hb, VEM, HEM) au fost influenţaţi de densitatea de stocare. Rezultatele din prezentul studiu contribuie la cunoaşterea unor indicatori hematologici ai păstrăvului curcubeu crescut în condiţii diferite de intensivitate într-un sistem recirculant de acvacultură.
Cuvinte cheie: păstrăv curcubeu, sistem recirculant de acvacultură, densitate de stocare, indicatori hematologici.

Introduction. The rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792), compared to the other salmonids (Ognean & Barbu 2011), is the species which is best suited for the intensive rearing, it has a rapid growth rate due to the high assimilation degree of the artificial food, as well as a high resistance to diseases.

The overwhelming importance of the stocking density for the technology of fish breeding was underlined by numerous authors who have studied the hematological changes on different species, as well as for different production systems (Ruane et al 2002; Trenzado et al 2006; Docan et al 2011).

The apparent efficiency of aquaculture systems can be maximized by increasing the rearing densities. High stockind density has been reported to cause decreased growth in salmonids (Vijayan & Leatherland 1988; Procarione et al 1999) and welfare alterations (North et al 2006). Moreover, crowding/high stocking density affects some hematological parameters: significant increase in hematocrit and hemoglobin level (Trenzado et al 2006).

Hematological parameters are closely related to the response of the organism to the environment, an indication that the environment where fishes live could exert some influence on the hematological characteristics (Gabriel et al 2004).

The purpose of this experiment was to evaluate how level of the stocking density influence the blood physiology of of juvenile rainbow trout (*O. mykiss*) reared in a recirculating system.

Material and Method

Fish biomass and the growing condition. Fish biomass used in this study was represented by rainbow trout fingerlings (*O. mykiss*) with an average weight (±SD) of 29.51±1.32 g, raised into a recirculating system of the pilot aquaculture station (4 glass tanks with a total volume of 0.320 m^3 each 40×80×100 cm, and the conditioning unit of the water quality, Fig. 1) from the Aquaculture, Environment Science and Cadastre Department, "Dunărea de Jos" University of Galaţi. The initial individual weight values distribution for all variants showed a significant similarity with the normal distribution ($p > 0.05$ with the test KS) (Table 1).

For this experiment, the feeding intensity was of 3% per body weight per day (for the first six days), respectively 4 % per body weight per day (for the following 27 days of the experiment), in two meals per day. The food used was Nutra PRO MP-T – extruded pellets with the diameter of 1.7 mm and with 50% protein content. During the experiment there was only one case of mortality, registered in variant B_4, with a weight of de 47.41 g. The attention was directed also towards the control of the quality water parameters in order to maintain them in optimum intervals for growth of the species.

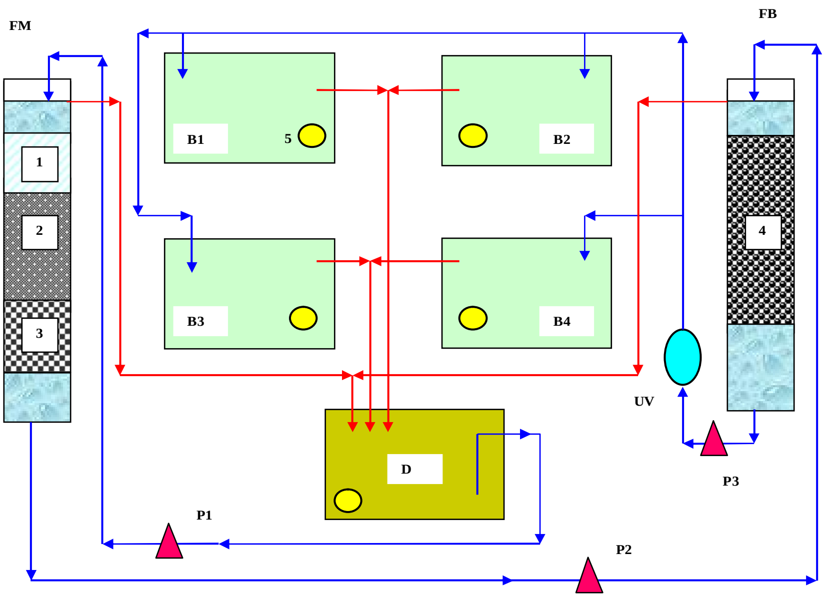

Fig. 1. The scheme of the recirculating aquaculture system (B_1-B_4 – fish rearing units, D– decantation unit; P_1,P_2- pumps, UV- sterilization lamp, FM- mechanical filter, FB- biological filter, 1- sponge, 2- sand, 3- gravel, 4- bactoboll, 5- aeration nozzle).

Blood sampling and analysis. A quantity of 0.3–0.5 mL of blood was taken from 10 fish of each tank by caudal venous puncture using lithium heparin as anticoagulant at the beginning and the ending of the experimental trial. The red blood cell counts (RBCc, x

$10^6/mm^3$) was determined by counting the erythrocytes from 5 small squares of Neubauer hemocytometer using Vulpian diluting solution.

The hematocrit (PCV, %) was determined by duplicate using heparinised capillary tubes centrifuged for 5 minutes at 12000 rpm in a micro hematocrit centrifuge. The photometrical cyanomethhaemoglobin method was used for determination of hemoglobin concentration (Hb). The absorbency of sampling was read at 540 nm and the amount for hemoglobin was calculated from a parelly rumed hemoglobin. Using standard formulas according Blaxhall (1972), Svobodova (2001) the red blood indices were computed: the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC). Besides hematological indices, during the experiment, biotechnological indicators were computed, also, in the purpose to distinguish the induced effect of level of the stocking density on the hematological profile of our studied species. The water quality parameters were also monitored.

Table 1

The synthetic table regarding the experimental version

Technological indicators	DS1- B_1	DS2 - B_2	DS3 - B_3	DS4 - B_4
Number of fish	25	51	75	103
Initial biomass (g)	792	1550	2138.94	2828.20
Mean individual weight (g/fish)	31.68	30.39	28.52	27.46
Initial stocking density (kg/m^3)	2.64	5.16	7.12	9.42
Final numbers of fish	25	50	75	103

Statistical analysis*.* The hematological parameters of the four experimental groups were expressed by mean and standard deviation and differences between the values were statistic analyzed with t-Student test, also.

Results and Discussion. There is growing interest in the study of hematological parameters and structural features of fish blood cells regarded as important for aquaculture purposes. Previous studies on fish hematology have revealed that intrepretation of blood are caused by internal and external factors.

Hematological modifications of our studied fish were analyzed in corroboration with the technological factors (level of stocking density, water quality) which can influence metabolic processes. For this experiment, the water quality parameters maintained in the optimum domain, corresponding to the species *O. mykiss*; the equipment for the conditioning of the quality of water have succeeded in treating and reusing the technological water, taking into consideration the fact that the water daily loss did not exceed 10% out of the total volume of the system.

The knowledge of the hematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fish (Kori-Siakpere et al 2005). Hematological parameters measured in the blood of fish held at different stocking densities are shown in Table 2.

Certain hematological variables were influenced by density, although results were inconsistent. Is observed a slight trend of reducing of the number of erythrocytes with increasing stocking density; the reduction in the erythrocyte number may be due to destruction the red blood cell. As a consequence of reducing the number of erythrocytes in DS3 and DS4 apears a reaction of physiological response, noticing in the increase of mean corpuscular volume (MVC). The MCV gives an indication of the status or size of the erythrocytes and reflects an abnormal or normal cell division during erythropoiesis. Nevertheless, from the physiological point of view, the erythrocytes number register normal values for this species; according to many authors, as a result of hematological studies, rainbow trout has between 0.8 and 1.5 $x10^6$ erythrocytes. Some authors (Srivastava & Sahai 1987) observe at high stocking density a polycythemia reaction (increasing the number of erythrocytes) as a response for demands of cellular oxygen.

Pickering & Pottinger (1987) observed that the number of rainbow trout erythrocytes was not affected if the density increased from 18 to 123 kg / m^3.

Table 2

Haematological parameters of *O. mykiss* held at different stocking densities

Experim. variant	Hematological indices					
	PCV (%)	Hb (g/dL)	RBCc $x10^6/\mu L$	MCV (μm^3)	MHC (pg)	MCHC (g/dL)
DS1	38.00±4.08	6.40±0.30	1.068±010	359.86±6248	60.09±3.64	17.12±2.75
DS2	40.00±2.00	6.50±0.30	1.178±0.05	339.84±22.21	56.86±1.31	16.77±0.77
DS3	39.50±3.85	6.85±0.17	0.886±0.19	454.73±73.85	79.74±14.20	17.85±2.09
DS4	40.00±2.87	7.20±0.62	0.996±0.13	405.30±40.77	73.25±11.76	18.01±1.50

Table 3

Hematology: reference interval of the hematological indices in rainbow trout

References	Hematological indices					
	PCV (%)	Hb (g/dL)	RBCc ($x10^6/\mu L$)	MCV (μm^3)	MHC (pg)	MCHC (g/dL)
Ghittino (1983)	24 - 55	7.6 - 16	0.8 – 1.5	276 - 476	55 - 82	14 - 26
Zorriehzahra et al (2010)	47	9.4	1.33	353	71	20
Saglane-Unal et al (2003)	32.10	7.0	1.49	216.64	47.04	21.83
Farahi et al (2010)	34	47.8	1.18	278.96	32.96	124.82

In present study, hematocrit levels did not differ statistically between density treatments, packed cell volumes were 38, 40, 39.5 and 40% for the DS1, DS2, DS3 and DS4, respectively. The hematocrit value recorded in this study was within the physiological range for this species (24-55%) also raported by some autors (Table 3). Data from the literature (Barton et al 2002) prove that the second level of physiological responses induced by the first level of stress responses (catecholamine secretion, elevation of circulating corticosteroids etc) includes hematological responses that can be used as quantitative indicators of stress: increasing hematocrit value, blood chemistry and even metabolic rhythm etc. Mazur & Iwama (1993) observed signifiant inceases in hematocrit and plasma cortisol of *O. tshawytscha* at densities of 32 or 64 kg/m^3 compared with a 8 kg/ m^3.

The analysis of the variation of haemoglobin quantity (respiratory pigment augments the transportation capacity of oxygen to blood) under the action of rearing density, underlines a magnifying tendency as opposed to the DS1 and DS2 experimental version, for DS3 and DS4 experimental variants (Table 2). Is observed an increase with 6.57% of hemoglobin amount in the experimental variants DS3 and DS4 having values of 6.85 g/dL, respectively 7.40 g/dL. This increase of hemoglobin quantity is correlated with the reduction of the oxygen concentration dissolved in water. The dissolved oxygen (DO) varied according to the population density (mean DO was 6.20±0.58 mgL^{-1}), the lower values being registered in variant DS4 (5.51±0.95 mgL^{-1}), respectively in variant DS3 (5.93±1.01 mgL^{-1}).

For *O. mykiss*, the dissolved oxygen represents the main limitative factor and, for this reason, any deviation from the recommended interval can have serious consequences on the physiological status and technological productivity (Mocanu et al 2011). Zanjani et al (1967) assumed that Hb is related to the oxygen requirement and may act as a control mechanism in erythropoiesis in teleost fish.

Althugh, comparing with the information from literature (Table 3) we observe that in all experimental variants, that the value of the quantity of hemoglobin from the circulant blood is below the normal range for this species. These findings support the hypothesis that hemodilution is a probable cause for decrease in hemoglobin content. Though the results are widely variable and not always conclusive, many authors reported hemoconcentration as a common effect of fish crowding/high stocking densities, manifested as an increase in both hematocrit and hemoglobin values (Mazur & Iwama 1993; Montero et al 1999). This response may be a strategy for increasing the oxygen carrying capacity of blood under high energy demand situations such as chronic stress (Srivastava & Sahai 1987).

The studied red blood constants (MVC, MCH and MCHC) are modifying unequally at the four experimental variants. In this study the fluctuation in the MCV and MCH was observed, but MCHC was unaffected. It was indicated that the concentration of hemoglobin in the red blood cells (MCH) were much higher in *O. mykiss* in the DS3 and DS4 variants group. The MCHC is a superior indicator of erythrocytes swelling (Wepener et al 1992). The MCHC, which is the ratio of blood hemoglobin concentration as opposed to the hematocrit, was not influenced by the blood volume nor by the number of cells in the blood but could be interpreted incorrectly only when new cells, with a different hemoglobin concentration, were released into blood circulation (Sovlo & Nikinmaa 1981).

Conclusions. The purpose of this paper was to evaluate the hematological parameters of rainbow trout reared under different conditions of intensity in the recirculating aquaculture systems. In our experimental conditions, physiological stress induced by maintenance the *O. mykiss* in high stoking density is reflected in the hematological indices. In these conditions, some hematological indicators (Hb, MCV, MCH) had been influenced by the stocking density. A decrease in the concentration of hemoglobin in blood as well as a decrease in oxygen also indicates anaemia or may indicate negative changes occurring in fish. In summary, the results of our research provide a contribution to the knowledge of the hematological parameters of the rainbow trout reared in different level of stocking densities, under the recirculating systems condition. Further experiments are required to study whether high stocking density affects the ability of fish to resist additional stressors, thus being more susceptible to stress and diseases.

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Angelica Docan, Aquaculture, Environmental Sciences and Cadastre Department, “Dunarea de Jos” University of Galaţi, 47 Domnească Street, Galaţi 8000087, Romania, angelica.docan@ugal.ro
Victor Cristea, Aquaculture, Environmental Sciences and Cadastre Department, “Dunarea de Jos” University of Galaţi, 47 Domnească Street, Galaţi 8000087, Romania.
Lorena Dediu, Aquaculture, Environmental Sciences and Cadastre Department, “Dunarea de Jos” University of Galaţi, 47 Domnească Street, Galaţi 8000087, Romania.
Mirela Mocanu (Creţu), Aquaculture, Environmental Sciences and Cadastre Department, “Dunarea de Jos” University of Galaţi, 47 Domnească Street, Galaţi 8000087, Romania.
Iulia Grecu, Aquaculture, Environmental Sciences and Cadastre Department, “Dunarea de Jos” University of Galaţi, 47 Domnească Street, Galaţi 8000087, Romania.
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