INFLUENCE OF THERMAL STRESS ON THE HEMATOLOGICAL PROFILE OF *ONCORHYNCHUS MYKISS* HELD IN DIFFERENT STOCKING DENSITIES IN RECIRCULATING AQUACULTURE SYSTEMS

Angelica Docan, V. Cristea, Lorena Dediu

Aquaculture, Environmental Sciences and Cadastre Department,
"Dunarea de Jos" University of Galati
e-mail: angelica.docan@ugal.ro

Abstract

Hematological indices are important parameters for the evaluation of fish physiological status. The aim of present study was to obtain a basic knowledge of the hematological response of Oncorhynchus mykiss maintained in different technological condition induced by stocking density and temperature. Specimens belonging to two experimental groups had individual weights of 494.34 ± 83.19 g respectively 558.04± 46.51 g and the stocking density was 41 kg/m^3 respectively 28 kg/ m$^{3.}$The sampling of Oncorhynchus mykiss blood from the four variants before and after the experimental trial allowed determination of hematological indices. Red blood cell counts (RBCc), hematocrit values (Hct), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were measured and analyzed, with routine methods used in fish hematology. Differences in hematological parameters were statistically analyzed by Student T test. Physiological stress, induced by maintenance in different technological condition induced by thermal stress, was reflected in the hematological indices (significant increase, p<0.05, RBCc, Hb, Ht, MCV and decrease insignificant, p >0.05 MCH, MCHC) with direct implications at the biotechnological level.

Key words: *Oncorhynchus mykiss*, stocking density, thermal stress, hematological indices

INTRODUCTION

The research carried out at a global level on the species *Oncorhynchus mykiss* have brought up that, unlike other salmonids, it is not very demanding when it comes to environment conditions, it has a high level of fodder assimilation, grows rapidly in captivity, it is resistant to disease (especially to furunculosis). These technological aspects represent an important argument in favour of the breeding in a super intensive recirculation system in order to obtain the trout for consumption purposes.

The high level of colonization can negatively affect a series of biological functions: metabolism, breeding performance, resistance to disease and health condition [1].

The maintaining of temperature at an optimal level is an important aspect for the recirculating aquaculture systems. The recirculating water into a closed system can heat because of the equipment used for the recirculating system as well as because of the heat transfer through pipes and walls. The rainbow trout can survive at temperatures between 0 and 25^{o}C, can feed at a temperature up to 22°C, but numerous studies have shown that the maximum critical temperature is between 24 and 26°C [2]. Other studies have yet identified higher or smaller values of the maximum critical temperature, according to the genetic potential and the environment technological requirements for fish. Being in a permanent dynamic equilibrium with the breeding environment, fish are very sensitive to modifications of physic-chemical parameters and their defence mechanisms are reflected by the figurative elements of the blood [3].

The main purpose of this paper consisted in underlying the technological plasticity of rainbow trout, as well as the potential of this species for intensive aquaculture in mild zones.

MATERIAL AND METHOD

Fish biomass and the growing conditions

Fish biomass used in this study was represented by *Oncorhynchus mykiss* specimens aged twelve months raised into a recirculating system of the pilot aquaculture station from the Aquaculture, Environment Science and Cadastre Department. This experiment lasted 75 days; total biomass was 67263 g, randomly distributed in the four growth units according to data presented in table 1.

Blood sampling and analysis

0,5 ml of blood was sampling 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. Blood was analysed with routine method used in fish hematology [4]. The red blood cell counts (RBCc, x 10^6/µl) 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 4 minutes at 13000 rpm in a micro hematocrit centrifuge. The photometrical cyanohaemiglobin method (Sahli) was used for determination of hemoglobin concentration (Hb, g/dl). Using standard formulas [5], [6] the red blood indices were computed: the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC).

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 DISCUSSIONS

When the colonization was made, the temperature of the water in the breeding system was of 18°C. All along the survey was carried out, the haematological response of fish from the experimental lots form the fish pool farms was analysed, in different conditions of density, under thermal stress because of the increase of the temperature of water to 26°C, value surpassing the optimal limit for the species *Oncorhynchus mykiss*. It is well known the fact that the temperature of water represents a decisive parameter for the developing of metabolic processes.

In order to underline as faithful as possible the physiological response of blood to the stressing action of the environment technological factors (temperature, stock density), there were used the dynamic analysis of the haematological indicators value and eritrocitary constants, at the end of the experiment as against the initial moment, as well as between the two experimental variants.

Table 1. Biometric and statistical data

Experimental version	B1 DS_1	B2 DS_2	B3 DS_2	B4 DS_1
Total biomass (g)	20268	13285	13393	20317
Number individs	41	28	24	41
Average weight (g/ex.)	494,34*	474,46*	558,04*	495,54*
Standard deviation	83.19	53.51	46.51	111.89
Coefficient of variation	0.16	0.10	0.08	0.22

- DS_1 – experimental version of the stocking density was 20 kg/m^3.
- DS_2 - experimental version of the stocking density was 13 kg/m^3.

Table 2. Changes in hematological parameters of rainbow trout during the experiment

Experimental version		Hematological parameters (Average ± standard deviation)					
		Ht (%)	Hb (g/dl)	RBCc x10^6/µl	MCV (µm^3)	MCH (pg)	MCHC (g/dl)
DS1	B1i	40±6,8	7,45±1,00	1,09±0,13	361,20±28,21	68,41±3,13	19,00±0,8
	B1f	52±2,6	8,85±0,38	1,24±0,07	420,64±20,36	71,35±4,74	16,99±1,2
DS1	B4i	35±8,6	7,75±0,75	0,99±0,15	354,88±97,16	79,64±11,27	23,42±4,91
	B4f	50±4,5	9,75±1,59	1,30±0,14	389,40±58,70	76,13±18,26	19,35±1,59
DS2	B2i	33±4,9	8,13±0,40	1,10±0,15	343,33±49,60	77,17±7,42	22,88±3,4
	B2f	55±3,4	11,13±0,26	1,42±0,07	364,81±54,61	78,44±6,46	22,04±4,22
DS2	B3i	33±6,3	7,42±1,39	1,06±0,14	313,40±26,39	69,54±4,93	22,35±2,53
	B3f	50±8,2	11,32±0,88	1,46±0,062	342,93±47,76	77,22±5,23	23,08±4,36

i, f - beginning and the end of experiment

Table 3. Normal values of hematological parameters of salmonid species

Fish species	Hematological parameters					
	Ht (%)	Hb (g/dl)	RBCc x10^6/µl	MCV (µm^3)	MCH (pg)	MCHC (g/dl)
O. mykiss [7]	24-55	7,6-16,0	0,85-1,50	276-476	55-82	14-26
O. mykiss [8]	30-45	6-10	0,80-1,50	350-400	65-75	17-20
O. nerka [7]	41-46	7,7-11,5	1,59-1,64	261-285	48-70	18-24

The analysis of the variation of haemoglobin quantity (respiratory pigment augments the transportation capacity of oxygen to blood) under the action of thermal stress, underlines a magnifying tendency as opposed to the initial moment, for both experimental variants, as it follows:

- ✓ The quantity of Hb for the trout in DS1had an average of 8,85 and 9,75 g/dl, recording a slight increase as opposed to the initial moment, when it was 7,45 and 7,75 g/dl
- ✓ In the case of the second experimental value, DS2, the increase of the quantity of Hb was higher ($p<0,05$), recording an average of 11,13 and 11,32 g/dl, as opposed to the initial moment, when the average was of 8,13 respectively 7,42 g/dl.

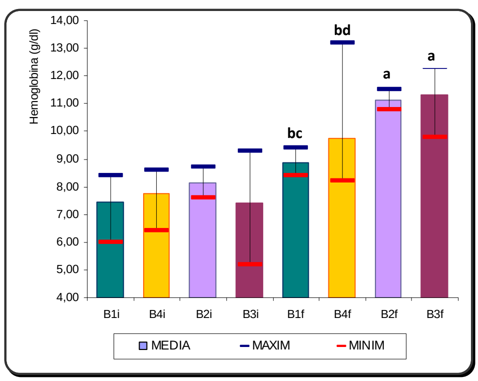

Fig. 1 Hemoglobin variation

„a”- significant diferences (comparing with initial values) - t Student test for pair variables

„b”- unsignificant diferences (comparing with initial values)

„c” - significant diferences between the two variants

„d” - unsignificant diferences between the two variants

The physical or environmental stress causes by the rapid growth of the concentration of Hb, due to the erythrocytes collection from the spleen and the hemoconcentration installed as a consequence of the loss of plasmic water [9].

The quantity of haemoglobin from the blood of trout species in experimental variant B2f, B4f greatly decreased (p<0,05) as opposed to B2f and B3f. The important reduction of haemoglobin can modify the oxygen quantity from tissues and can thus lead to the slow down of the metabolic ratio and to a smaller production of energy [10]. The important decrease of the haemoglobin can also be caused by the increase of the destruction rate of Hb or the reduction of its synthesis rate [11].

The hematocrit or the packed cell volume (Ht), under the stressing effect of temperature, records the same increasing values for both experimental values. As opposed to the initial moment, when there were average values of 40±6,9 and 35±8,6 %, the trout from B1 and B4 registered in average 52±2,7 % and 50±4,6 %, augmenting from a statistic point of view (p<0,05).

In the case of the second variant (DS2), the important growth of Ht (p<0,05) was similar to that in the high density variant (DS1), with an average of 55±3,5 and 50±8,2 %, while at the initial moment it had an average of only 33 ±4,9 %. When the two experimental variants were compared, the value of the hematocrit was constant, with unimportant statistic differences.

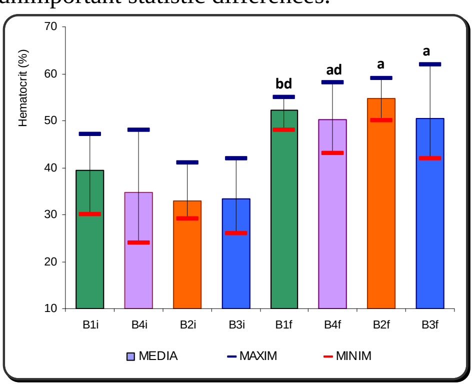

Fig. 2 Hematocrit variation

The increase of the hematocrit is accompanied by the blood viscosity, being considered the superior limit of the quantitative adaptation strategy, due to the additional cardiac effort needed in order to pump more viscous blood [9].

The number of erythrocytes, as it can be observed by analysing the values in Table 2, records values which falls into the normality interval for this species (Table 3). Nevertheless, there were recorded important increases of this parameter in the case of both experimental values as opposed to the initial moment, related to the increase in quantity of haemoglobin and hematocrit.

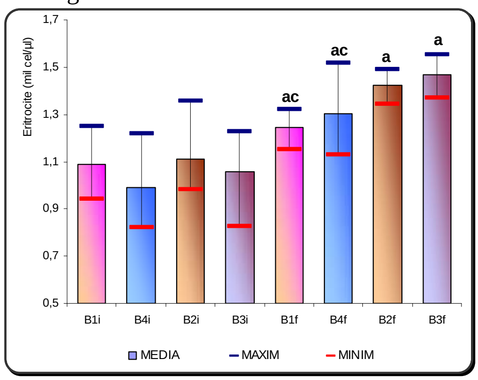

Fig. 3 Red blood cell counts variation

Similar results obtained Valenzuela, A.E. [12] in a research concerning the physical stress, caused by the increase in temperature and the continuous use of light, on the physiology of the blood for *Onchorhynchus mykiss* species. The temperature was an important factor which led to the increase of hematocrit, the haemoglobin concentration and the number of erythrocytes. The continuous exposure to artificial light had as a consequence the contraction of the spleen and the elimination of erythrocytes into blood, thus explaining the increase of hematocrit and the number of erythrocytes. The spleen is considered the depositing place for erythrocytes [13], being able to contract by adrenergic stimuli [14].

When the two experimental variants were compared, there was noticed the important reduction (p=0,01) of the number of erythrocytes in the blood of the trout, under the influence of thermal stress and density, related to the important reduction of haemoglobin quantity. The reduction of the haemoglobin blood concentration has an impact on the cardiac function because the circulating needs and the cardiac rhythm, necessary in order to deliver O_2 to tissues, grow significantly while the hematocrit decreases [9].

With the help of the haematological indices the erythrocytes constants (MCV, MCH, MCHC) for the blood of the trout were calculated. Their diagnostic value is very important because they help to detect the presence of some physiological lesions in the process of formation of haemoglobin and offers information on the size, shape and haemoglobin quantity in erythrocytes [5].

Under the influence of technological factors which acted upon the rainbow trout in the present experiment (the increased temperature of the water from fish pool farms and the stock density), the values of erythrocyte constants recorded the following modifications:

- ✓ MCV significantly increased in both experimental variants as opposed to the initial moment, because of the increased temperature of water over physiological optimal level. For the variant with higher density, MCV varied between 420,64 ± 20,36 μm^3 and 389,4 ± 58,70 μm^3 as opposed to the variant recording a smaller density and with an average of 364,81 ± 54,61 and 342,93 ± 47,76 μm^3);

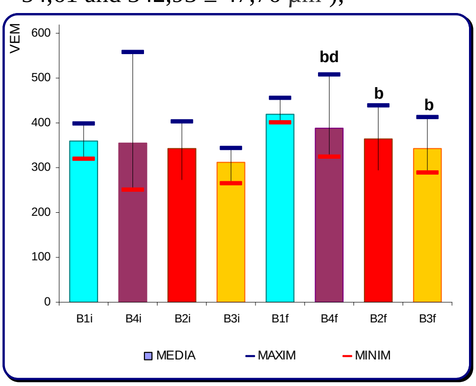

Fig. 4 Mean corpuscular volume variation

- ✓ The value of MCH was relatively constant as opposed to the initial moment, but insignificantly decreased (p>0,05) with approximately 10% in the blood of the trout from the experimental variant regarding a double stock density, arriving at la 71,35 ± 4,74 pg;

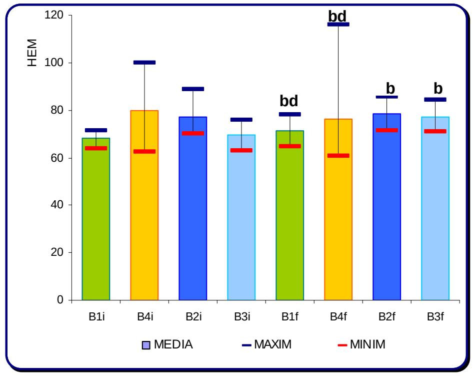

Fig. 5 Mean corpuscular hemoglobin variation

- ✓ MCHC had a similar value to MCH, insignificantly decreasing (p>0,05) with 23% in the blood of the trout from the experimental variant regarding a double stock density, arriving at 71,35 ± 4,74 pg and also related to the important decrease of Hb quantity.

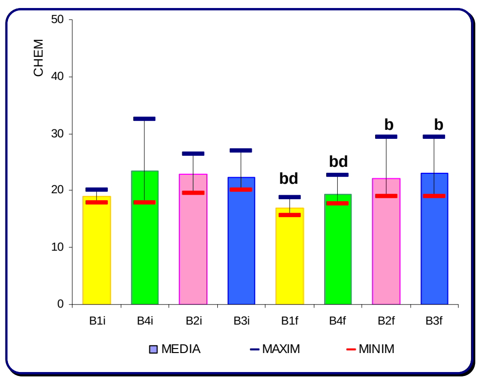

Fig. 6 MCHC variation

Even if the erythrocytes from the blood of the trout in DS1 significantly decrease, the adaptation response of the blood to the stock density action, in the moment of the increase of the temperature of water, was promptly concretized into MCV. But this reaction was not efficient because MCH, as well as MCHC, decreased insignificantly (p>0,05). It is possible that modifications are the result of the stressing effect of stock density, accentuated by the increase of the temperature of technological water over the optimal limit.

These aspects led to a perturbation of the homeostasis of the organism. The thermal stress appears when the temperature of water exceeds the physiological optimal level, aspect which triggers modifications which perturb the normal physiological functions, leading to the waste of energy regarding the answer to stress and even a potential reduction of survival [15].

CONCLUSIONS

The results obtained underline the fact that the blood, by his homeostatic role, is directly involved in metabolic processes and can reflect modifications in the organism of the trout, under the action of some perturbing factors (density and temperature).

The increase of the temperature of the technological water over the optimal physiological limit of the species *O. mykiss*, in the case of both experimental variants compared to the initial moment, determined an ample response at the level of haematological indices. This lead to a significant increase ($p<0,05$) of the number of erythrocytes, of the hematocrit and the quantity of haemoglobin. Because the efficiency of the transport of oxygen to tissues is determined by the characteristics of erythrocytes, their increase in number could be explained an increase of respiratory demands and, thus, of metabolism, once the temperature of water increases. This statement is also sustained by the significant increase ($p<0,05$) of haemoglobin, related to the insignificant increase of MCH. The significant increase of hematocrit is correlated with the increase of MCV, following the cellular hydration.

In the case of the experimental variant where the stock density was double, because of the increase of the temperature of water, important modifications were recorded for the haematological indices, consisting in significantly reducing the number of erythrocytes and the haemoglobin. The adaptation response of blood was prompt and the increase of MCV reduced ($p<0,05$). But this reaction was not efficient because both MCH and MCHC decreased considerably.

Consequently, the stock density in the case of the increase of the temperature of technological water resulted into the reduction of the homeostatic power, favouring the damage of trout physiological response mechanisms.

REFERENCES

Journal article:

[10] Atamanalp M., YanÝk, T., HaliloÛlu I., Aras, M.S.: Alterations in the hematological parameters of rainbow trout (*Oncorhynchus mykiss*) exposed to cypermethrin. Israeli Journal Aquacult.-Bamidgeh. 2002,54: 99-103

[1] Barton B.A., Morgan J.D., Vijayan M.M.: Physiological and condition-related indicators of environmental stress in fish. American Fisheries Society, Bethesda,2002: 111 - 148

[4] Blaxhall, P.C., Daisley, K.W.: Routine haematological methods for use fish blood. Journal of Fish Biology, 1973. 5(6): 771 – 781

[15] Elliot, J.M.: Some aspects of thermal stress on freshwater teleosts. In: Pickering A (ed) Stress and fish. New York, 1981.

[13] Fange, R.: Fish blood cell. In Hoar WS, Randall DJ,Farrell AP (eds) Fish physiology: the cardiovascular system, vol XII, Academic, London, 1992.

[7] Ghittino,P.: Tecnologia e patologia in acquacoltura. Vol.2 – Patologia. Torino, 1985.

[14] Kita, J., Itazawa, Y.: Effects of adrenaline on blood flow through the speen of rainbow trout (*Salmo gairdneri*).Comp biochemistry physiology 95 A. 1990, (4): 591-595.

[2] Molony, B.: Environmental requirements and tolerances of Rainbow trout (*Oncorhynchus mykiss*) and Brown trout (*Salmo trutta*) with special reference to Western Australia. A review. 2001. Fisheries Research Raport No. 130

[11] Reddy, P.M., Bashamohideen, M.: Fenvalerate and cypermethrin induced changes in the haematological parameters of *Cyprinus carpio*. Acta. Hydrochim. Hydrobiol. 1989. 17: 101-107.

[6] Svobodova, Z., Stress in fishes (a review). Bull. VURH Vodnany. 2001. 4: 169-191

[12] Valenzuela, A.E., Silva, V.M., Klempau, A.E.: Effects of different artificial photoperiods and temperatures on haematological parameters of rainbow trout (*Oncorhynchus mykiss*). Fish physiology biochemistry 2008. 34:159-167

[3] Wilson R.W. şi Taylor, E.V.: The physiological responses of fresh-water rainbow trout, *Oncorhynchus mykiss* during acutely lethal copper exposure. Journal of Comparative Physiology, 1993. 163:38 – 47

Book:

[5] Ghergariu, S., Pop A., Kadar, *Ghid de laborator clinic veterinar. Bucureşti,* 1985

[9] Nicula M.: Fizilogia organismelor acvatice. I. Fiziologia peştilor (vol.I). Ed. Mirton Timişoara, 2004.

[8] Svobodová Z., Pravda D., Palácková J.: Unified methods of haematological examination of fish. Research Institute of Fish Culture and Hydrobiology, Vodňany, 1991.