STUDIES OF EUROPEAN CATFISH (*SILURUS GLANIS* L.) LEUKOCYTES REACTION IN THE CONDITION OF REARING IN "FLOW-THROUGH" AQUACULTURE SYSTEMS

Angelica Docan, V. Cristea, Lorena Dediu, Iulia Grecu

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

Abstract

Researches in this paper have assessed the hematological response of European catfish to the crowding stress induced by high stocking density through the evaluation of WBC complex reactions, in order to determine the effect of the technological factors on the physiological status. The catfish exemplars belonging to the two experimental variants had individual weight of 619.74 ± 216.49 g / ex. for the first system VE 1, respectively 560, 83 ± 193.20 g / ex. for the VE 2. Through the experimental design we have created two stocking densities of 64 kg /mc, respectively 28 kg/mc. In order to achieve the purpose of the experiment the blood samples were immediately used to make smears which were colored with May-Grunewald Giemsa panoptic method. By studying different types of leucocytes was determined leukograma and absolute number of leukocytes (reported in 1000 erythrocytes counted on haemocytometer). We have identified the following types of white blood cells: lymphocytes, monocytes, neutrophils granulocytes and rarely, basophils. Eosinophils were absent. The total number of leukocytes, in general, has not been registered statistically significant changes. Lymphocytes (relative and absolute) have remained relatively constant, without significant variation. Stocking density has triggered a monocytosis phenomenon (significant increase in monocytes by 62 - 85%); neutrophils have been registered a slight reduction. In this experiment, stocking density did not affect the immune defense system of the Silurus glanis specie.

Key words: Silurus glanis, stocking density, leukograma, lymphocytes, monocytes

INTRODUCTION

Silurus glanis, specie reared in our country especially in polyculture technology with cyprinids in the systematic and semi-systematic farms, began to be reared lately in the intensive, semi-closed production systems (such as „flow-through") by providing nutritional requirements.

In teleostean fish, as in higher vertebrates, blood physiology and implicitly hematology indices, represent important parameters to evaluate the general physiological condition of the body, used especially as stress indicators in assessing responsiveness of fish in relation to different environmental conditions [2].

Blood WBC in fish varies with phylogenetic position, sex, season, age, environmental conditions, health status, between 20 and 150 x 103 cel./mm3 blood, which is much lower than the number of erythrocytes [9].

Chronic stress induced by density can lead to inadaptation by compromising immune function. Non-specific immune function is extremely important because it acts as a first line of defense, but can be suppressed as a result of increased blood corticosteroid [8]. Studies undertaken by Ortuñio et al. [10] showed that after exposure to stress density, monocytes and granulocytes are mobilized in the blood, from the top of the kidneys, after 48 hours.

Research in this paper focused on the haematological response of European catfish to the action of stocking density by evaluating leukocyte's complex reactions, in order to determine the influence of the technological factors on their physiological state.

MATERIAL AND METHODS

The rearing experiment of the Silurus glanis specie (aged 2 years) was conducted between 25 January -25 February 2009 in the semi-closed "flow-through" system located at the Department of Aquaculture, Environmental Science and Cadastre, "Lower Danube" University, Galati.

In order to quantify the influence of stocking density on the physiology and health status of the European catfish, the rearing tanks were populated so to create the possibility of experimenting two different stocking densities; thus 92 exemplars with total biomass 55 kg, were randomly distributed in the two experimental tanks (600 l/tank) corresponding to the two flow-through aquaculture systems: first system was populated with 62 individuals weighting 37 kg and the second system with 30 individuals weighting 18 kg. The average individual weight in first system was of 619.79 g/ex, and in the second system 560.83 g/ex. The fish biomass was fed with 4 mm pellets containing 41% crude protein, 1% of biomass given in three portions.

From 10 fish collected from each experimental variant, at the beginning and at the end of the experiment, were collected biological samples of blood (1 ml) by puncture of the caudal vein using lithium heparin as an anticoagulant. For each exemplar two blood smears were immediately dried, fixed and then colored with May-Grünwald Giemsa panoptic method (MGG). The relative proportion (percentage) of each type of white blood cells was obtained by microscopic examination of 200 leukocytes on blood smears. Absolute number of circulating blood leukocytes and thrombocytes was determined in comparison with 1000 erythrocytes counted on haemocytometer, per unit blood volume

In both experiments the differences between different types of white blood cells were statistically analyzed using Student T test. For leukocytes (expressed as a percentage and absolute number) were calculated mean values and standard deviation.

In this study, it was noted with Vi the values of the parameters quantified in the beginning of the expeiment and with Vf1, respectively Vf2 the final values corresponding to the two experimental variants

RESULTS AND DISSCUTIONS

Research presented in this paper is part of a wider study that assessed the degree of normality of physiological status of the specie *Silurus glanis L.*, both qualitatively in terms of haematological indicators (number of erythrocytes - RBC, hematocrit, hemoglobin, MCV, MCH, MCHC) as well as quantitatively through technological indicators.

Following this experiment were also analyzed the reactions of the leukocyte's system, in order to determine the effect of the influence of stocking density on the immune system defenses and for a fair assessment of physiological changes in European catfish. To assess the leukocytemic changes were performed both qualitative analysis by observing the morphological particularities of the leukocytes and quantitative analysis to evaluate the relative changes ("Leukocyte formula") and absolute changes (cells/mm^3 blood) of different types of leukocytes. Microscopic examination of blood smears colored by MGG, did not show morphologic changes in leukocytes.

Regarding the erythrocytes, in the blood smears from catfish exemplars from the first experimental variant (Vf1 - in which stocking density was double) have noted the emergence of a large number of young red blood cells (photo 1).

The emergence of young erythrocytes in peripheral blood is the consequence of stimulated erythropoiesis due to the phenomenon of hypoxia that was installed after decreasing of the amount of dissolved oxygen. Likewise an additional argument of this issue is represented by the significant ($p<0,05$) increase of the amount of hemoglobin found in catfish exemplars in this experimental variant [3]. Hypoxia and anoxia acting through a discharge erythropoietin (protein formed in the kidney) are the most powerful stimuli of erythropoiesis [1].

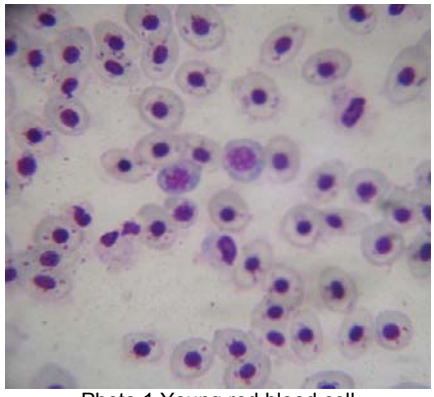

Photo 1 Young red blood cell

Table. 1 Relative values of leukocytes in *Silurus glanis and Clarias gariepinus* in optimum health status condition. (%)

Leukocytes	*Silurus glanis* (100 g)	*Clarias gariepinus*
Lymphocyte	66,2 ± 10,27	58
Monocytes	0,90 ± 1,22	2,6
Neutrophils	29,35 ± 14,98	39,4
Eosinophils	2,80 ± 2,71	0
Basophils	0,75 ± 1,15	0
References	[13]	[6]

Relative changes of the number of different cell types that make up the complex system of leukocytes (lymphocytes, monocytes and neutrophils, eosinophils, basophiles) representing the leucocytes formula, are found in Table 2, where are also indicated statistically significant ($p<0.05$) differences resulted by application of T - Student.

Table 2 . Changes in the leucocytes formula in European catfish during experimental period

LEUCOGRAM (%)			Vi	Vf 1	Vf 2
AGRANULOCYTES	*Lymphocytes*	Min	66,50	71,56	72,15
		Max	84,00	92,75	85,65
		Avg ± SD	76,88 ± 6,02	78,53 ± 6,93 ^{ad}	78,55 ± 4,70^{a}
	Monocytes	Min	1,27	1,55	0,00
		Max	4,00	5,29	3,33
		Avg ± SD	2,50 ± 0,83	3,18 ± 1,23^{ac}	1,71 – 0,96^{a}
GRANULOCYTES	*Neutrophils*	Min	12,00	4,83	14,13
		Max	31,00	23,41	25,11
		Avg ± SD	20,56 ± 6,36	18,00 ± 6,06^{ad}	19,72 ± 3,80^{a}
	Basophils	Min	0,00	0,00	0,00
		Max	0,47	0,49	0,00
		Avg ± SD	0,06 ± 0,15	0,28 ± 0,22^{bc}	0,00^{a}

„a”- unsignificant diferences (comparing with initial values) - T Student test for pair variables
„b”- significant diferences (comparing with initial values)
„c” - significant diferences between density variants
„d” - unsignificant diferences between density variants

Leucocytes formula of the catfish exemplars from this experiment reflects the prevalence of the percentage of lymphocytes reported on all leukocytes, followed by neutrophils and monocytes. The most common granulocyte leukocytes on blood smears were neutrophils, basophils were quite rare while eosinophils were absent.

Table. 3 Changes in absolute values of the leucocyte series during experimental period

Cell type		Vi	Vf 1	Vf 2
Leucocytes (x10^{3}cel./mm^3)	Min	58,87	48,57	73,83
	Max	148,64	144,68	136,52
	Avg ± SD	94,36 ± 30,15	95,89 ± 24,64^{ad}	101,95 ± 22,26^{a}
Lymphocytes (x10^{3}cel./mm^3)	Min	43,82	34,76	57,03
	Max	119,67	134,19	107,96
	Avg ± SD	73,17 ± 26,37	134,19 ± 26,08^{ad}	79,79 ± 16,83^{a}
Monocytes (x10^{3}cel./mm^3)	Min	1,17	1,51	0,00
	Max	3,86	4,82	3,29
	Avg ± SD	2,30 ± 0,95	2,89 ± 1,01^{ac}	1,79 ± 1,07^{a}
Neutrophils (x10^{3}cel./mm^3)	Min	7,06	6,99	12,38
	Max	27,08	24,50	30,19
	Avg ± SD	18,82 ± 5,87	16,10 ± 5,14^{ad}	20,36 ± 6,59^{a}
Basophiles (x10^{3}cel./mm^3)	Min	0,00	0,00	0,00
	Max	0,55	0,45	0,00
	Avg ± SD	0,07 ± 0,18	0,24 ± 0,2^{bc}	0,00^{a}
Thrombocytes (x10^{3}cel./mm^3)	Min	6,17	2,00	3,86
	Max	25,06	16,08	33,00
	Avg ± SD	14,3 ± 7,1	7,01 ± 3,95^{bd}	9,56 ± 9,04^{d}

„a"- unsignificant diferences (comparing with initial values) - T Student test for pair variables
„b"- significant diferences (comparing with initial values)
„c" - significant diferences between density variants
„d" - unsignificant diferences between density variants

For our experiment, the study of variation of absolute number of leukocyte (Table 3) shows a relatively uniform distribution in both experiments, with statistically insignificant differences ($p>0.05$) between mean number found for the two variants at the end of the trial as well as between initial and final values. A decrease in the total number of leukocytes (leukocytopenia) is a bioindicator of immune depression due to acute stress [14].

Analyzing the relative number of lymphocytes and absolute number of cells reported to the total number of leukocytes per blood volume (mm^3) is observed that they are dominant over the other types of leukocytes.

The percentage of lymphocytes remained constant over experimental period with an average of 76.88% at the beginning of the experiment and 78.53%, respectively 78.55% for Vf1 and Vf2 at the end of the experiment. Likewise, in terms of absolute number of lymphocytes is not observed significant changes over time: thus, there were on average 76.67 x 10^3 lymphocytes/mm^3 blood for exemplars from Vf1 variant (double stocking density) and 79,79 x 10^3 lymphocytes/mm^3 blood for exemplars from Vf2 with a slight upward trend from initial number (73.17 x 10^3 lymphocytes /mm^3), but statistically insignificant ($p> 0.05$). The main function of lymphocytes is to play the role of executors of the immune mechanism through production of antibodies, involved in humoral and cellular defense of the organism [4], [9].

Unlike lymphocytic reaction, monocytic reaction of the blood is different in the two experimental variants both at leukogram level (%) and at absolute number reported to the blood volume. In the circulating blood of the catfish from variant Vf1 the relative proportion of monocytes increased significantly stronger ($p = 0.02$) with 85% comparing with exemplars from Vf2 variant. This increase is as pronounced when reporting to the absolute value, observing that were launched in the torrent of blood in average 2.89x10^3 monocytes/mm^3 in Vf1 comparing with 1,79x10^3 cell./mm^3 in Vf2, which corresponds to a statistically significant increase ($p <0.05$) of the number of monocytes by 62%.

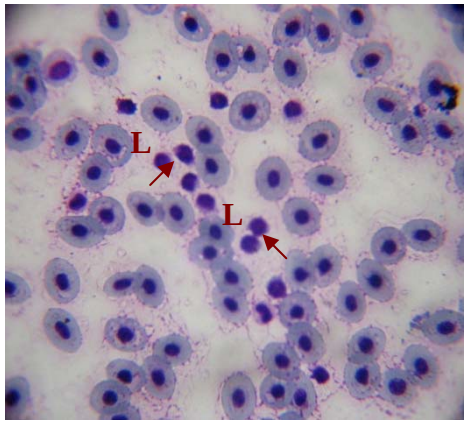

Photo. 2 Small lymphocytes (1000X,MGG)

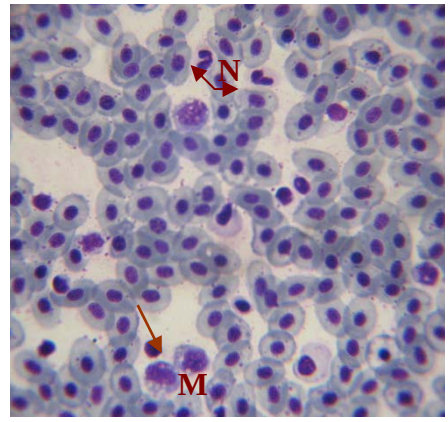

Photo. 3 Monocytes and neutrophyls

Reported to the whole leukocytes complex, monocytes have a relatively small proportion in the bloodstream when foreign substances are not present [9]. Monocytes are cells involved in initiating and regulating immune response [11] but are involved as well in phagocytosis of red damaged cells [1]. In conditions of maintaining of high stocking density from our experiment, this significant increase in monocytes can be explained by an increase in cellular defense mechanisms.

From the total granulocytes, neutrophils are the most numerous; on the examined smears were identified young neutrophils (with rod-shaped unsegmented nucleus with two lobs) as well as metamyelocytes and promyelocytes in a much smaller proportion.

Changes in relative and absolute number of neutrophils, for both variants, shows a relatively uniform response with some small changes but insignificant statistically ($p > 0.05$). Thus, it is noted a slight reduction in the percentage of neutrophils from circulating blood of catfish in Vf1 with 12, 5% comparing with initial values and only 8.7% of Vf2. Changes in absolute neutrophils number follows approximately the same trend, being identified an average of 16.10 x 10^3 neutrophils /mm^3 in the blood of exemplars maintained in high density, Vf1 with 14.4% less compared to Vi (18.8 x 103 cell./mm^3) and with 20% less compared Vf2 (20.36 x 103 cell./mm^3).

Similar results were obtained by Montero, D., [8] in *Sparus aurata* reared in high-density system, which noted that some immune parameters such as agglutination activity and circulating neutrophils number were not affected by stress density. Klinger, H., [7] in the studies on the influence of water quality and stocking density on channel catfish physiology found a lower number of granulocytes.

Eosinophilic granulocytes were not found on any of the blood smears examined. Data from literature shows that eosinophilic granulocytes are rarely found in circulating blood, they being launched in blood torrent especially in parasitic diseases. Total absence of eosinophils in circulating blood leukocytes and in kidney after 7 days of blood collection, is a possible indicator of increased susceptibility to stress [5].

Basophiles counted on blood smears were relatively low in number, in some cases being totally absent. The absolute average number of basophiles in catfish blood determined in the beginning of the experiment was 0,07 x 10^3 cel./mm^3 and increased significantly ($p < 0.05$) in Vf1 where the average was 0.24 x 10^3 cel./mm^3 compared with experimental variant Vf2 where they were absent from circulating blood of examined exemplars.

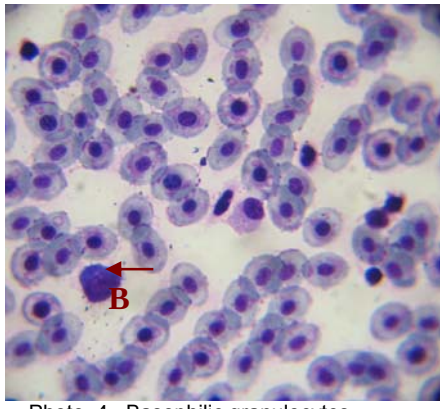

Photo. 4. Basophilic granulocytes

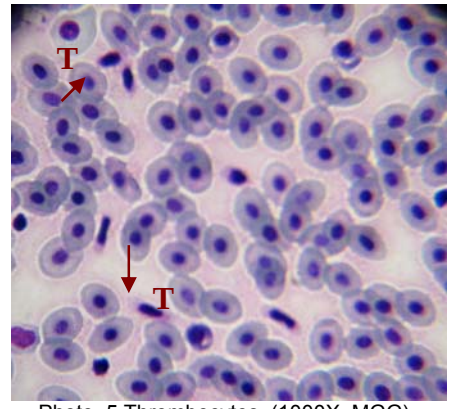

Photo. 5 Thrombocytes (1000X, MGG)

As regards the thrombocytes found in the blood of *Silurus glanis* held in different densities, the number found showed significant variations both in time and between experimental variants. Thus, if in the beginning of experiment the number of thrombocytes was approx. 14.30 x 10^3 thrombocytes /mm^3 blood, they were reduced by 50% in Vf1 which was highly significant ($p = 0.03 < 0.05$) reaching 7 x 10^3 cell./mm^3 and only by 19% in Vf2 (11.56 x 10^3 cell./mm^3), which was statistically insignificant ($p>0,05$). Similarly, the channel catfish grown in recycled high-density system, accorded to Klinger, H., [7] shows a reduction in circulating blood thrombocytes. Recent studies have shown that thrombocytes are involved in homeostasis and defense mechanisms, in teleostean fish being produced mainly in the spleen and kidneys [12].

CONCLUSIONS

High stocking density from Vf1 (88 kg/m^3) was associated with a reduction of dissolved oxygen concentration in the water which led to an increase in feedback metabolic processes, the fish responding adaptively. Thus, through an efficient correction of blood oxygen capacity, which is based on red cell reserves located in erythropoietic organs (kidney and spleen), there was a release of young red blood cells in the circulating blood that provides so-called “emergency adaptive mechanisms”.

From a functional point of view, the complex of leucocytes perform a reactive – adaptive defense system channeled to the destruction/annihilation of foreign components (bacteria, parasites, toxins) and to restore areas with damaged tissue.

On the examined blood smears were identified almost all types of white blood cells: small and large lymphocytes (predominating the small lymphocytes), monocytes, granulocytes neutrophils (promyelocytes, metamyelocytes, and neutrophils with kernel unsegmented or with two or three lobs), and rare basophiles. Eosinophils were not found at all. Lymphocytes were dominant, both as percentage (78.53%) and absolute number (76.67 x 10^3 cell/mm^3), knowing that the fish have a leukocytic lymphocytic system.

The study of leukocytic reactions and reactive changes through morphological analysis but also through analysis of absolute values (cells/mm^3 blood) or differential/relative values (leukocytes formula) made possible the characterization of the physiological status of fish biomass from this experiment where the influence of the stocking density was tested.

The total number of leukocytes, overall, has not registered any changes, which makes us believe that the "general adaptation syndrome" was not seriously affected by stocking density. However, were observed some reactions of the different categories of white blood cells as:

- Lymphocytes, the most important immune system cells involved in protecting the body with antibodies synthesized, recorded a slight increase both in the leukocyte formula and in the absolute number of cells (with 2 to 5%, the increase was insignificant statistically).
- The stocking density influencing as well water quality and metabolic reactions of catfish, has triggered a significant increase (62 - 85%) of monocytes feedback resulting in the of blood concentrations of monocytes (monocytosis). The increased number of monocytes in the Vf1 experimental variant showed regulation of immune response induced by low concentration of dissolved oxygen, aspect emphasized also by the increased amount of hemoglobin in blood of analyzed catfish.
- Relative and absolute number of neutrophils granulocytes in Vf1 decrease slightly, but statistically insignificant ($p>0.05$).
- Eosinophils were missing from circulating blood of analyzed exemplars.
- In Vf1 basophiles significantly increase both from initial moment and compared with Vf2. These granulocytes are important in the production of heparin and histamine, the phagocytic function being very low.

Since the lymphocytes remained relatively constant and the increased number of monocytes released in blood torrent regulated immunological response, we can conclude that in this experiment, stocking density did not affect the immune defense system of the *Silurus glanis* specie.

REFERENCES

[1] Bârză, H.: Ghid de hematologie a animalelor în creşterea intensivă. Editura Ceres. 143 p, 1978

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

[3] Docan, A., Cristea, V., Grecu, I., Dediu, L.: Hematologic response of European catfish, *Silurus glanis* reared in different density in „flow-through" production system. The 8 th International Symposium on *Animal Biology and Nutrition. Bucuresti (recenzată în curs de publicare),* 2009

[4] Fange, F.: Fish blood cells In: Fish physiology. Vol. XII, Part B. The Cardiovascular System. Academic Press, INC. 55 p, 1992

[5] Fijan, N.: Composition of main haematopoietic compartments in normal and bled channel catfish. Journal of Fish Biology, 2002, 60: 1142–1154

[6] Gabriel, U., Ezeru, G.N., Opabunmi, O.: Influence of sex, sources, health status and acclimation on the haematology of *Clarias gariepinus*. African Journal of Biotechnology, 2004,3: 463 – 467

[7] Klinger, H., Deventhal, H. & Hilge, W.: Water quality and stocking density as stressors of channel catfish (*Ictalurus punctatus* Raf.). Aquaculture, 1983, 30, 263–272

[8] Montero, D., Izquierdo, M.S., Tort, L., Robaina, L., Vergara, J.M.: High stocking density produces crowding stress altering some physiological and biochemical parameters in gilthead seabeam, Sparus aurata, juveniles. Fish Physiology and Biochemistry, 1999, 20: 53-60

[9] Nicula, M.: Fiziologia organismelor acvatice. I. Fiziologia peştilor. Vol. I. Editura Mirton, Timişoara, 215 p, 2004

[10] Ortuño J., Esteban M.A. Meseguer J.: Effects of short-term crewding stress on the glthead seabream *(Sparus aurata*) innate immune response. Fish Shellfish Immunology, 2001,11: 187-197

[11] Patriche, T.: Imunitatea la peşti. Ed. Didactica si Pedagogica, Bucureşti, 212 p,2008

[12] Romano, N., Taverine-Thiele, J.J., Maanen, J.C., Rombout, J.H.M.W.: Leucocyte subpopulations in developing carp (*Cyprinus carpio* L.): imunocytochemical studies. Fish & Shellfish Immunology, 1997, 7: 439-453.

[13] Velisek, J., Wlasov, T., Gomulka, P., Svobodova, Z., Novotnny, L.: Effects of 2-phenoxyethalon anaesthesia on sheatfish (*Silurus glanis* L.). Veterinarni Medicina, 2007, 52, (3):103-110

[14] Wedemeyer, G.A., Barton, B.A., McLeay: Stress and acclimation. In: Methods for fish biology. American Fisheries Society,1990: 451-489