Hematological Profile of Juvenile European catfish (*Silurus glanis*) Reared under Different Stocking Densities in Recirculating System Conditions

Săndiţa Plăcintă, Victor Cristea, Coadă Marian Tiberiu, Ştefan Mihai Petrea

Dunărea de Jos" University of Galati, Faculty of Food Science and Engineering-Aquaculture, Environmental Science and Cadastre Department, 800008-Galati, Domnească, 47, Romania

Abstract

The aim of present study was to evaluate the hematological parameters and the absolute number of leukocytes of European catfish (*Silurus glanis*) maintained in different technological conditions induced by applied stocking densities. The experiment was conducted over a period of 32 days. A number of 450 European catfish, with an average weight of 158.9±0.26 g, were distributed in four rearing units, in order to create different stocking densities as follows: V1-14.28 kg/m^3, V2–28.59 kg/m^3, V3–42.97 kg/m^3 and V4-57.06 kg/m^3. The following hematological parameters were determined: RBC, Ht, Hb, MCV, MCH and MCHC. A decrease of RBC and higher values of MCH and MCHC were observed at V4, while at V1 an increase in RBC and lower MCV, MCH values can be seen. Regarding the absolute number of leukocytes, no significant statistical differences were registered between the applied stocking densities ($p>0.05$). However, there was a slight increase of leukocyte number with increasing stocking density (240.01 x10^{3}cel./mm^3 at V4, respectively 189.64 x10^{3}cel./mm^3 at V1). It can be concluded from hematological parameters analysis that a stocking density of 60 kg/m^3 seems to be out of the optimal range for catfish growth, in current growth conditions.

Keywords: aquaculture recirculating systems, hematological parameters, *Silurus glanis*, stocking density

1. Introduction

During the fingerlings growth period, the fish dimensions are important criteria, apart from stocking density, administration of suitable feed and water quality, in order to register both a high survival and growth.

Silurus glanis is one of fish species with the longest life cycle and largest size from Romania, being growth in open environment, both in monoculture and polyculture.

Thus, it can be concluded that recirculation aquaculture systems are promising growth technologies, in order to apply high stocking densities, compare to those from natural environment, offering in the same time the opportunity of obtaining high quantity levels of fish productions for market supply all year long. Also, the production is made in controlled conditions that respect the requests imposed by the European standards concerning animal health status.

Currently, the European catfish is growth in recirculating systems until it reach to market size (1.5 kg), during a period of 7–8 months [1, 2].

Some studies on the relationship between growth intensity level and technological efficiency, reported to culture biomass, emphasized the possibility of obtaining maximum production by application of an optimum stocking density in correlated with providing the best environmental conditions and a sufficient amount of feed [3]. For this reason, high stocking densities are considered key factors that may affect fish functions and

* Corresponding author: Plăcintă Săndiţa
Tel: 074621424, Email:sandita_placinta@yahoo.com

growth performances [4], disease resistance, health state [5] and welfare alterations [6]. Also, high stocking densities has a negative impact on some hematological parameters, generating a significant increase in hematocrit and hemoglobin levels [7].

2. Materials and methods

Experimental design. This study used juvenile European catfish reared in the main pilot recirculating system station of Aquaculture, Environmental Science and Engineering Department from Food Science Faculty- „Dunarea de Jos'' University of Galati, described by [8]. The production system mainly consists in 4 rearing units, with a volume of 1 m^3 each and also water quality conditioning units. The experiment was conducted over a period of 32 days, between February–March 2012. A number of 450 juvenile European catfish, with an average weight of 158.9±0.26 g, were distributed in four rearing units, in order to create different stocking densities as follows: V1-14.28 kg/m^3, V2-28.59 kg/m^3, V3-42.97 kg/m^3 and V4-57.06 kg/m^3. The initial individual weight values registered a significant similarity with the normal distribution, for all the variants, by using KS test ($p>0.05$).

The applied feeding intensity was 1.6% of total biomass weight (BW) for the first eight days, respectively 1.8% of BW, distributed in three meals per day, for the rest of the experimental period. As feed, Clasic Extra 3P– extruded pellets was used, with a diameter of 6 mm and with 41% protein content and 12% lipids.

Blood sampling and analysis. The blood samples were taken from the caudal venous of the fish, using lithium heparin as anticoagulant, at the end of the experiment, from 20 fish (5 fish for each rearing unit). Blood was analyzed with routine methods, used in fish haematology [9]. Erythrocytes (RBC) were counted immediately after blood collection in hemocytometer (Improved Neubauer Weber scientific Ltd.), according to [10]. For measuring hematocrit (Ht), ammonium heparinized hematocrit capillary tubes were filled with blood and centrifuged for 5 minutes at 12000 rpm, in a micro hematocrit centrifuge (Haematokrit 24. Hettich). The percentage of hematocrit was determined by using the micro-capillary reader. Hemoglobin (Hb) concentration was estimated as cyanmethemoglobin by adding 20 µl of whole blood to 5 ml of Drabkins solution and by using spectrophotometer (Specord 210-Analytic Jena), at a wavelength of 546 nm, for determining the final values. Using standard formulas the red blood indices were computed: the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC) [11].

MCV (µm³)=Hct/RBC x 10
MCH (pg)=Hb/RBC x 10
MCHC (g/dl)=Hb/Hct x 100

For each exemplar two blood smears were immediately dried, fixed and then colored with May-Grünwald Giemsa panoptic method (MGG), for determining the absolute number of leukocytes. The leukocytes type was determined based on identification characters listed by [12]. Absolute number of peripheral blood leukocytes and thrombocytes were determined in relation to 1000 erythrocytes in hemograms stained with panoptic method MGG and then, converted to unit blood volume.

Besides hematological indices, during the experiment, biotechnological indicators were calculated with the purpose of distinguishing the induced effect stocking density level on the hematological profile of our experimental fish biomass (Table 1).

Table1. Synthetic table of the experimental variants

Technological indicators	V1	V2	V3	V4
Number of fish	45	90	135	180
Initial biomass (g/ex)	158.69	158.83	159.15	158.52
Final biomass (g/ex)	229.20	225.48	221.60	202.69
Initial stocking density (kg/m^3)	14.28	28.59	42.97	57.06
Final stocking density (kg/m^3)	20.62	40.13	59.83	72.97

Water quality. The following technological water quality parameters were monitored daily, within the production system: pH; temperature; dissolved oxygen concentration and also, one time per week,

ammonium, nitrite and nitrate concentrations were measured with Spectroquant Nova 400 photometer. In case of those parameters exceed the critical level the water was exchanged in order to maintain the values within the experimental range.

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 Microsoft Office Excel using Anova test.

3. Results and discussion

The experimental fish hematological modifications were analyzed in correlation with the technological factors (stocking density level, water quality parameters), that can influence the metabolic processes. Debate exists as to whether an improper water quality or increased negative social interaction, that is the primary cause of affecting welfare, are manifested when fish are maintained at higher densities than optimal ones. [13]. Previous studies on fish hematology revealed that blood parameters levels could be affected by water temperature and oxygen concentration variations [14].

In the present research, temperature was kept at a constant value during the whole experiment, the mean value being 21±0.71ºC, while the value of dissolved oxygen (DO) in the four variants was 6.26±0.34 mgl^{-1} (V1), 5.52±0.42 (V2) mgl^{-1}, 5.03±0.62 mgl^{-1} (V3) respectively 4.32±0.55 mgl^{-1} (V4).

In table 2 are presented the hematological parameters of *Silurus glanis* at the end of the experimental period.

Table2. The hematological parameters of catfish (*Silurus glanis*) reared by using all four experimental stocking densities (Mean±standard deviation)

Parameter	V1 (14.28kg m^3)	V2 (28.59kg m^3)	V3 (42.97kg m^3)	V4 (57.06kg m^3)	Bibliographic References[20]
Ht (%)	32.60±3.7[b]	32.65±1.6[b]	32.85 ±1.79[b]	35.75±1.5[b]	22.30 ± 2.7
Hb (g/dl/)	9.08±0.17[b]	9.31±0.49[b]	9.42±0.89[b]	9.66±0.67[b]	7.33 ± 0.88
RBC ($\times 10^6/\mu l$)	2.07±0.13[b]	2.00±0.31[b]	1.94±0.40[b]	1.81±0.29[b]	1.36 ± 0.17
MCV (μm^3)	152.92±12.6[a]	158.96±10.8[b]	170.20±10.5[b]	199.51±11.1[a]	165.66 ± 25.06
MCH (pg)	44.68±6.15[a]	46.55±9.11[b]	48.56±8.34[b]	54.58±8.24[a]	54.43 ± 8.05
MCHC (g/dl)	27.85±3.51[b]	29.13±3.96[b]	28.48±3.26[b]	26.96±4.53[b]	32.97 ± 2.38

„a"-significant differences between density variants ($p<0.05$)
„b"–not significant differences between density variants ($p>0.05$)

Comparing V1 variant (lowest stocking density - 14.28 kg/m^3) with the V4 variant (highest stocking density-57.06 kg/m^3), it can be observed an increase of Ht and Hb that was not statistically significant ($p>0.05$), respectively a significant increase of MCV and MCH ($p<0.05$, $p=0.001$, $p=0.029$).

The effects of stocking densities on blood parameters of juveniles European catfish showed following observations:

• The amount of hemoglobin averaged 9.08 g/dl respectively V1, 9.66 g/dl for V4 variant, but the increase was not statistically significant ($p>0.05$, $p=0.34$), the values being within the normal range [15]. Correlating the results of water quality parameters, it can be state that oxygen concentration decreases with increasing stocking density, which leads to an increase in the concentration of hemoglobin. Environmental or physical stress causes rapid increase of blood hemoglobin due to erythrocytes sampling from spleen and hemoconcentration installed as a result of plasma water loss [16].

• The hematocrit, under stressful effect of density, registered the same upward trend in case of all experimental variants, not significant statistical differences between observed ($p>0.05$, $p=0.52$). The values are within the normal range (32.6% to 35.75%) [17].The packed cell volumes increased with 9.66% in V4, compare with V1. [18] observed significant inceases in hematocrit and plasma cortisol of *O. tshawytscha* by using stocking densities of 32 and 64 kg/m^3, compared with a 8 kg/m^3 stocking density.

In present experiment, the number of erythrocytes in the circulating blood of juveniles catfish recorded values exceeding the optimum range for this species (1.4-1.5 mil. erythrocytes/µl blood)

[19, 20]. The dynamics of erythrocytes number in case of present experimental variants has a significant downward trend ($p<0.05$, $p=0.002$), registering $2.07x10^6$/µl values for V1, respectively $1.81x10^6$/µl for V4, which correlates with hemoglobin and hematocrit values.

By using the hematological indices described above erythrocyte constants of blood juveniles European catfish were calculated (MCV, MCH, MCHC).

• Mean corpuscular volume (MCV), due to increased stocking density, registered a significant increase ($p<0.05$, $p=0.001$) among the experimental variants, maintaining values in normality range as follows: the average value for V1 was 152.92±12.36µm^3, respectively 199.51±11.91 µm^3 for V4.

▪ The MCV is an indication of erythrocytes status or size and reflects an abnormal or normal cell division during erythropoiesis. This response may be a strategy for increasing the oxygen carrying capacity of blood under high energy demand situations such as chronic stress [21].

▪ Mean erythrocyte hemoglobin (MCH), in present experiment, showed a significant increase ($p<0.05$, $p=0.029$), correlating with the values obtained for hemoglobin. The highest value was obtained in juvenile catfish blood from V4 experimental variant with an average of 54.58±8.24 pg.

▪ Mean erythrocyte hemoglobin concentration (MCHC) highlighted the link between erythrocytes number dynamics from circulating blood of juveniles catfish from present experiment and also, hemoglobin dynamics. Mean erythrocyte hemoglobin values slightly decrease ($p>0.05$, $p=0.28$) with increasing stocking density as follows: for V1 an average of 27.85±3.51 g/dl was registered, compare with 26.96±4.53 g/dl for V4.

The reactions of the leukocyte system were analysed, in order to determine the effect of stocking density on the immune system defenses, for obtaining a proper assessment of physiological changes in Europen catfish. On the examined blood smears were identified the following white blood cells: small and large lymphocytes (predominantly the small lymphocytes), neutrophils, thrombocytes and few monocytes (Foto 1).

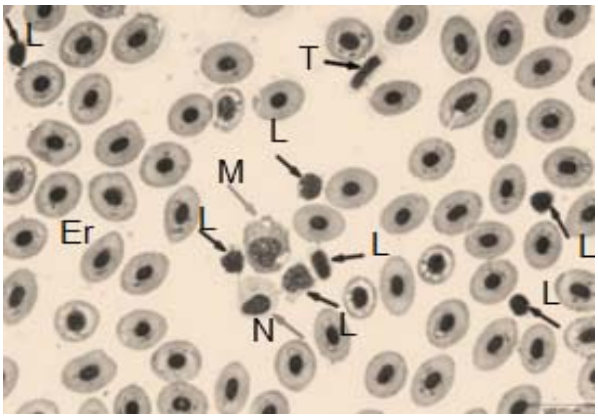

Foto 1–Morphology of circulating blood cell of the European catfish (*Silurus glanis*), 10 oc x 100 ob MGG staining: L–lymphocytes, M–monocyte, N–neutrophils, Er–erythrocytes, T–thrombocytes

Changes in absolute number of lymphocytes, monocytes and neutrophils are presented in Table 3 (mean±SD).

Table3. Variation of the white blood cell absolute number ($x10^3$ cel./µl blood)

Experimental version	With blood cell absolute number ($x10^3$cell/µl blood)(Mean±standard deviation)				
	Leukocytes	Lymphocytes	Monocytes	Neutrophils	Thrombocytes
V1	183.64±1.02^b	180.88±1.06^b	0.91±1.01^b	1.83±1.02^b	23.18±0.97^a
V2	180.74±1.19^b	178.86±1.13^b	0.52±0.49^b	1.95±2.6^b	36.74±1.35^a
V3	174.25±1.17^b	169.83±1.02^b	0.87±1.07^b	4.35±1.07^b	46.75±1.12^a
V4	240.01±1.08^b	234.01±1.1^b	1.29±0.52^b	4.79±1.23^b	57.79±1.17^a
Bibliographic reference [17]	4.36±30.15	73.17±26.37	2.30±0.95	18.82±5.87	14.3±7.1

„a"-significant differences between density variants ($p<0.05$)

„b"-not significant differences between density variants ($p>0.05$)

By analyzing the variation of absolute leukocytes number ($x10^3$cel/µl blood), we observe not significant increase ($p>0.05$, $p=0.18$), with 32.72%, in case of V4 variant, compared with V1. A similar trend was observed in case of absolute number of lymphocytes, without notice any significant differences between the experimental variants ($p>0.05$, $p=0.17$). So, the absolute number of lymphocytes varied from 180.88±1.06

$x10^3$cell/µl blood in V1 to 234.0±1.1$x10^3$cell/µl) blood in V4.

Reported to the whole leukocytes complex, monocytes have a relatively small proportion in the blood stream, when foreign substances are not present [16].

The monocyte reaction showed no significant differences between tested stocking densities ($p>0.05$, $p=0.89$). So, the absolute number of monocytes at V1 variant was 0.91±1.01$x10^3$cel/ µl blood, in being recorded a slight decrease with 5.66% (0.52±0.49 $x10^3$cel/µl blood). In case of V3 variant, the absolute number of monocytes registered a value of 1.29±0.52 $x10^3$cel/µl blood.

The absolute number of neutrophils registered a slight increase with increasing the stocking density, from 1.83±1.02$x10^3$cel./µl blood in variant V1 to 4.79±1.23 $x10^3$cel/µl blood in V4.

Concerning the absolute number of thrombocytes, it can be observed a significant increase ($p<0.05$, $p=0.041$) with the increasing of stocking density, from 23.18±0.97 $x10^3$cel/µl blood in case of V1, to 57.79±1.17 $x10^3$cel/µl blood at V4. Similar results were obtained by [22] for *Silurus glanis* reared at stocking densities between 42.86÷88 kg/m³, in flow-through system conditions. The increased of absolute number of thrombocytes indicate an adaptive response of the body to high stocking densities.

4. Conclusions

Regarding the welfare technological status, characterized clearly by the hematological profile dynamics, it shows the influence of one of the main technological factors (stocking density), on the welfare of the biological material.

Thus, we conclude that in case of catfish juveniles, reared under high stocking densities, the vital space of each individual is decreased, fact that generates neurohormonal changes and stress status, with direct implications on haematological profile.

The general conclusion that emerges from the analysis of experimental results is that catfish juveniles, reared under intensive recirculating system conditions showed a considerable technological plasticity, without serious physiological consequences, fact that allows us to state that stocking density does not significantly compromise the catfish welfare status by applying levels of stocking density up to 60 kg/m^3.

5. References

1. Linhart, O., Štěch, L., Švarc, J., Rodina, M., Audebert, J. P., Grecu J. and Billard, R., The culture of the European catfish, *Silurus glanis*, in the Czech Republic and in France, Aquatic Living Resources, 2002, 15, 139–144.

2. Ulikowski, D., Intensive production of European catfish (*Silurus glanis* L.) in water recirculating systems, Komunikaty Rybackie, 2003, 2, 10–12.

3. Zonneveld, N. and Fadholi, R., Feed intake and growth of red tilapia at different stocking densities in ponds in Indonesia, Aquaculture, 1991, 99, 83-94.

4. Procarione, L. S., Barry, T. P. and Malison, J. A., Effects of high rearing densities and loading tares an the growth and stess response of juvenile rainbow trout, N Am., J. Aquacult, 1999, 61, 91-96.

5. Ortuño, J., Esteban, M. A. and Meseguer, J., Effects of short-term crewding stress on the gilthead seabream (Sparus aurata) innate immune response, Fish Shellfish Immunology, 2001, 11, 187-197.

6. North, B. P., Turnbull, J. F., Ellis, T., Porter, M. J., Miguad, H., Bron, J. and Bromage, N. R., The impact of stockind density on the welfare of rainbow trout (*Oncorhynchus mykiss*), Aquaculture, 2006, 255, 466-479.

7. Trenzado, C. E., Morales, A. E. and Higuera, M., Physiological effects of crowding in rainbow trout, Oncorhynchus mykiss, selected for low and high stress responsiveness, J. Aquaculture, 2006; 258, 583–593.

8. Cristea, V., Ion (Placinta), S., Bocioc, E., Ionescu, T. I., Coada, M. T. and Enache I., "Monitoring the Water Quality in the Aquaculture Recirculating Systems", Journal of Environmental Protection and Ecology (JEPE), 2011, 12(4).

9. Blaxhall, P. C. and Daisley, K. W., Routine haematological methods for use fish blood, Journal of Fish Biology, 1973, 5(6), 771–81.

10. Wintrobe, M. M., Variations of the size and haemoglobin content of erythrocytes in the blood various vertebrate, Folia Hematol., Leipzig; 1934, 51, 32-49.

11. Dacie, J. V. and Lewis, S. M., Practical Haematology, 9th edition. Churchill Livingstone, London, 2001, 633

12. Svobodova, Z., Pravda, D. and Palackova, J., Unified Methods of haematological examination of fish, Research Institute of Fish Culture and Hydrobiology, Vodňany, 1991

13. Ellis, T., North, B., Scott, A. P., Bromage, N. R., Porter, M. and Gadd, D., The relationship between stocking density and welfare in farmed rainbow trout, J. Fish Biol., 2002, 61, 493–531.

14. Jewet, M. G., Behmen, D. J. and Johnson, G. H., Effects of hyperoxic rearing water on blood haemoglobin and haematocrit level of Rainbow trout, J. Aquactic Anim.Health, 1991, 3, 153-160.
15. Fikri Aydın, Hijran Yıldız Yavuzeanı, Serap Pulatsü , A study on some hematological characteristics of healthy European catfish (*Silurus glanis*) and African Catfish (*Clarias lazera*), Veı. Bil. Dt-rg; 1998; 14: 51-53.
16. Nicula, M., Fiziologia organismelor acvatice. I. Fiziologia peştilor. Vol. I. Editura Mirton, Timişoara, 2004, 215.
17. Docan, A. 2010, „Reserches regarding the influence of ecotechnological factors from industrial recirculating aquaculture systems on physiological state and welfare of fish, PhD Thesis, University "Dunărea de Jos" Galati, 2010.
18. Mazur, C. F and Iwama G. K., Effect of handling and stocking density on hematocrit, plasma cortisol, and suvival in wild and hatchery–reared chinook salmon *Oncorhynchus tshawytscha*, Aquaculture 1993, 117,141-147.
19. Munteanu, G. and Cristea V., Blood and parasitological research of *Silurus glanis* from Galati city, International Symposium Volume Aquarom 1995, 95, 316-320.
20. Koprucu, S. S. Koprucu, K. and Urail, M. S., Acute toxicology of synthetic pyrethroid deltamethrin to fingerling European catfish (*Silirus glanis* L.), Bulletin of Environmental Contamination and Toxicology, 2006, 76, 59-65.
21. Srivastava, A. K. and Sahai I., Effects of loading density on carbohydrate metabolism and hematology in the Indian freshwater catfish, J. Aquaculture, 1987, 66, 275-286.
22. Docan, A., Cristea, V., Grecu, I. and Dediu, L., Hematologic response of European catfish, *Silurus glanis* reared in different density in „flow-through" production system, Zootehnica Arhive, 2010; 13, 2, 63-70.