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Hematological profile in juvenile carp reared under a recirculating system condition

Ionica Enache, Victor Cristea, Angelica Docan, and Adina Popescu

„Dunărea de Jos" University of Galaţi, Faculty of Food Science and Engineering,
Str. Domneasca nr. 47, 800008-Galati, Romania, European Union.
Corresponding author: I. Enache, enaoana@yahoo.com

Abstract. The object of this paper is to investigate the influence the density of population and fish size has on the physiological condition of *Cyprinus carpio* in the light of the hematological coefficient and the erythrocyte constants. The physiology of the blood is an important parameter in order to define the general condition of the organism for all teleostean fish. The investigations of the metabolic profile of the blood aimed at determining the hematologic answer of the carp under the conditions of its exposure to the controlled modification of the following technological factors: size class, stocking density. The low value of the hematocrit (27% in both experiments) suggests a condition of anemia or the dehydration of the organism. The values registered by the hemoglobin were different, according to the size of the fish, varying between 5.22 and 5.34 g/dL blood for fish with size 65-66 g/fish, respectively 6.7-7.02 g/dL blood for fish with size 150-152 g, being with 29.92% higher in the case of fish with a larger size. The average number of red blood cell counts is constant for both experiments and these values are falling into the normal physiological gap. Erythrocyte constants (MCV, MCH and MCHC) vary according to the two size classes: MCH grows by 31.53% and MCHC by 28.60% with the individual growth of the carp. MCV presents values between 186.48 and 199.73 (μm^3), normal value for the studied species. The present study outlines the fact that the main hematological parameters vary according to the size class, the results being related to the results obtained following the investigation of the metabolic profile in the light of the technological parameters.
Key words: carp, hematological parameters, stocking density, size.

Rezumat. Scopul acestei lucrări este acela de a investiga influenţa densităţii de populare şi mărimea peştilor asupra stării fiziologice a speciei *Cyprinus carpio* prin prisma indicatorilor hematologici şi a constantelor eritrocitare. Fiziologia sângelui este considerată un indicator deosebit de important pentru definirea stării generale a organismului la toţi peştii teleosteeni. Investigaţiile profilului metabolic sanguin au vizat determinarea răspunsului hematologic al crapului în condiţiile expunerii la modificarea controlată a următorilor factori tehnologici: clasa de mărime, densitatea de stocare. Valoarea mică a hematocritului (27% în ambele variante experimentale) poate sugera instalarea unei anemii sau deshidratarea organismului. Hemoglobina a înregistrat valori diferite, in funcţie de talia exemplarelor, variind între 5,22 şi 5,34 g/dL sânge la exemplarele cu talia de 65-66 g/exemplar, respectiv 6,7-7,02 g/dL sânge la exemplarele cu talia de 150-152 g, fiind cu 29,92% mai crescută la exemplarele de talie mai mare. Numărul mediu de celule roşii se menţine constant în ambele variante experimentale, valori ce se încadrează în ecartul fiziologic normal. Constantele eritrocitare (VEM, HEM şi CHEM) variază în funcţie de cele două clase de mărime: HEM creşte cu 31,53 % iar CHEM creşte cu 28,60 % odată cu creşterea greutăţii individuale a crapului. VEM are valori cuprinse între 186,48 şi 199,73 (μm^3), valori normale pentru specia studiată. Studiul de faţă evidenţiază că principalii indicatori hematologici variază în funcţie de clasa de mărime, rezultatele corelându-se cu cele obţinute în urma investigării profilului metabolic din punct de vedere cantitativ prin prisma indicatorilor tehnologici.
Cuvinte cheie: crap, parametri hematologici, densitate stocare, clase de mărime.

Introduction. The remarkable eco-technological plasticity of the carp has lead to approach of a variety of experiments concerning its intensive growth in controlled production systems, as it is the case of recirculating systems (Enache et al 2011).

Farmers are interested in breeding fish in higher densities because they can thus reduce the production costs. Nevertheless, it was proved that density affects the health condition of fish (Bakeer & Tharwat 2006) having negative effects on their development and on their immune system (Ruane et al 2002). If the action is of short duration, the stress is acute and allows the organism of fish to respond to stress factors and recover

(to go back to homeostasis /normal physiological balance); if the action is on long term, we then talk about chronic stress with severe effects (Barton et al 2002). The physiology of the blood is considered an important parameter in order to define the general condition of the organism for all teleostean fish. The blood, through its essential functions it carries out in the organism and through its dynamism, offers complex information about the normality degree of the physiological state (Docan et al 2010; Ognean & Barbu 2009). The previous hematological studies on fish have outlined that the interpretation of hematological parameters is difficult, taking into consideration that the dynamics of blood parameters is caused by both internal and external factors (Darvish Bastami 2008; Örün et al 2003). The object of this paper is to investigate the influence of stocking density of population and fish size has on the physiological state of the *Cyprinus carpio* Linnaeus, 1758 species in the light of the hematological coefficient and the erythrocyte constants.

Material and Method. The material base for the experimental measurements was the pilot recirculating system of the Department of Fisheries and Aquaculture, "Dunarea de Jos" University of Galaţi. Experiments were conducted for a period of 30 days, between August and September 2010, in four growth units with a capacity of 500 L/unit.

The biological material used in the experiment is represented by carp sapling, aged 15 months, provided by the Research Institute of Aquatic Ecology and Development, Fisheries and Aquaculture, Galaţi, from the Brates breeding station. Two variants of density were experimented (see Table 1).

Table 1

Experimental variants of *C. carpio* species

Experimental variant	*Experimental variant I*		*Experimental variant II*	
Indicators	*B1*	*B3*	*B2*	*B4*
Stocking density (kg/m^3)	64	64	32	32
Initial biomass (kg)	32.105	32.144	16.239	16.031
Number of fish	491	211	245	107
Initial fish weight (g/ex)	65	152	66	150

The food used for the biomass culture was type MASTER Aller pellets, 4mm grain, 35% protein content (see Table 2) considered optimum for growth at this stage of development specimens (Oprea & Georgescu 2000). The ratio used was 1.7% for body weight.

Table 2

Chemical composition of ALLER MASTER 4mm pellets

Nutrients	Quantity
Crude protein	35%
Crude fat	9%
NFE	36%
Ash	7.8%
Fibres	4.2%
Gross energy	4476/18.7 Kcal/MJ
Convertible energy	3485/14.6 Kcal/MJ
N in dry matter	6.1%
P in dry matter	1.3%
Energy in dry matter	4865/20.3 Kcal/MJ
Vitamin A	2500(IE)
Vitamin D3	500(IE)
Vitamin E	150(mg)

Blood samples (cca 0.5-1 mL blood) were taken from 5 fish/ tank, randomly extracted, through puncture of the caudal vein, using the heparin as anticoagulant.

The quantitative determination of the hemoglobin was done by using the spectrophotometric method. The hematocrit was determined through the microhematocrit method, which presupposes the spin of blood heparinized microhematocrit capillary tubes. The spin is done with the help of HETTICH HAEMATOCKIT 210 device, 12000 rotations/min during 5 minutes. For determination of red blood cells (erythrocytes) used the classical method using Neubauer counting chamber and Vulpian liquid dilution.

The erythrocyte constants were determined with the help hematological parametres: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) according to Ghergariu et al (1985).

Results and Discussion. In order to better evidentiate the health condition of the studied species, we evaluated the hematological response of the carp under the action of eco-technological factors: density population and size classes. Regarding the quality of water, during the experiment the following parameters were daily monitored: temperature (18-26 °C); oxygen (6.8 mg/L); nitrites (7.9-14.4); ammonium (0.4-1.2 mg/L) and pH (7.1-7.8). The investigation of the metabolic blood profile had in mind the determination of the hematological answer of the carp when exposed to the controlled modification of the following parameters: size class, stocking density (see Table 3).

Table 3

Variation of the hematological parameters in *C. carpio* for tested experimental variants

Hematologic parameter	*Experimental variant I*		*Experimental variant II*	
	B1-65 g/ex	*B3-152 g/ex*	*B2-66 g/ex*	*B4-150 g/ex*
Hematocrit (%)	27.2±1.94	27±3.52	27.2±2.04	28.2±2.71
Hemoglobin (g/dL)	5.22±1.05	7.02±1.81	5.34±1.54	6.7±0.82
Red blood cells (x10^6/µL)	1.491±0.24	1.397±0.22	1.404±0.21	1.436±0.22
MCV (µm^3)	186.48±26.1	194.92±22.0	197.96±30.4	199.73±29.5
MCH (pg)	34.94±3.67	50.85±12.85	39.78±14.50	47.43±7.92
CMCHC(g/dL)	19.08±3.16	25.96±5.27	19.62±5.48	23.81±2.28

As it can be noticed form the data shortly presented in Table 3, the main hematological parameters register higher variations according to size classes and less according to stocking density regarding the optimum limits found in literature (see Table 4). According to Ghitino (1983), the number of erythrocytes in *C. carpio* varies between 1.10 and 2.20x10^6µl, hemoglobin (Hb) varies between 6.5 and 10.6 g/dL. Pârvu & Caprarin (1984) (quoted by Radu et al 2009) describes as normal for the hemoglobin the interval between 8.3 and 15.7 g/mL.

Table 4

Normal values of main hematological parameters in *C. carpio* (Ghittino 1983)

Species studied	*Red blood cells (10^6/µL)*	*Hb g/dL*	*Ht (%)*	*MCV (µm^3)*	*MHC (pg)*	*MCHC (g/dL)*
C. carpio	1.10-2.20	6.5-10.6	32-43.8	152-364	50-63	15-25

According to Ghittino (1983), the value of the hematocrit (Ht), 27% for both experiments (see Figure 1) can be considered under the normal physiological limit; but, at the same time, this value can be considered normal if we take into consideration the breeding of the carp in the temperature interval of 18-25^{0}C (according to Örün et al 2003). The low quantity of hematocrit can be the sign of anemia or dehydration at the level of the organism (Bârză 1985). Anemia produced by the significant low quantity of hematocrit can lead to the weakness of the immune system, and thus fish become vulnerable to the action of certain pathogenic agents and their tolerance to the deficit of oxygen in the water decreases. The reduction of hematocrit has as consequence the reduction of the breathing efficiency, respectively of the intensity of metabolic activity of fish.

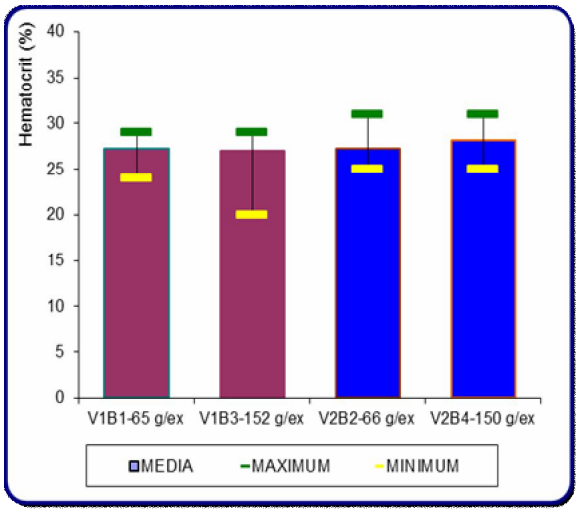

Figure 1. Ht variation in *C. carpio.*

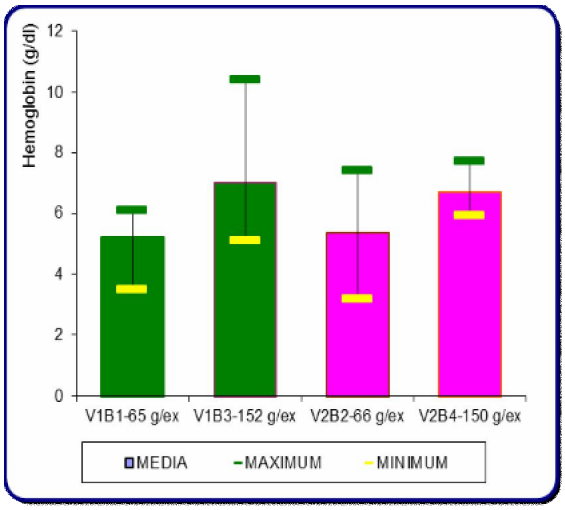

Figure 2. Hb variation in *C. Carpio.*

The general analysis regarding the quantity of Hb (see Figure 2) under the action of technological factors bring out higher values for big-sized fish, irrespective of the population density. Thus, for the specimen of 150-152 g, the quantity of hemoglobin was in the amount of 6.7-7.02 g/dlL blood, approximately 24% higher than in the case of specimen of 65-66 g, for which the hemoglobin registered values 5.22-5.34 g/dL blood. In comparison with the result of the studies carried out by Ghitino, the quantity of hemoglobin in the amount of 5.22-5.34 g/dL, as determined by our study, is lower. Kumar et al (2010) gets similar results regarding the concentration of hemoglobin of approximately 5 g/dL, in an experiment on carp sapling breeding. The results obtained through our experiment, in correlation with the information of the literature, confirms the fact that there is a direct relationship between the concentration of Hb and the size of the biological material. As far as the average number of erythrocytes is concerned (see Figure 3), it can be noticed that it is constant for both experiments, with slight insignificant variations from the statistic point of view. Irrespective of the experiment, the number of erythrocytes remained within the normal physiological limits.

Knowing the importance of the erythrocyte constants (MCV, MHC and MCHC) in establishing the type of anemia, the experiment aimed at determining and interpreting their values regarding different conditions of density and size (see Figure 4, 5 and 6).

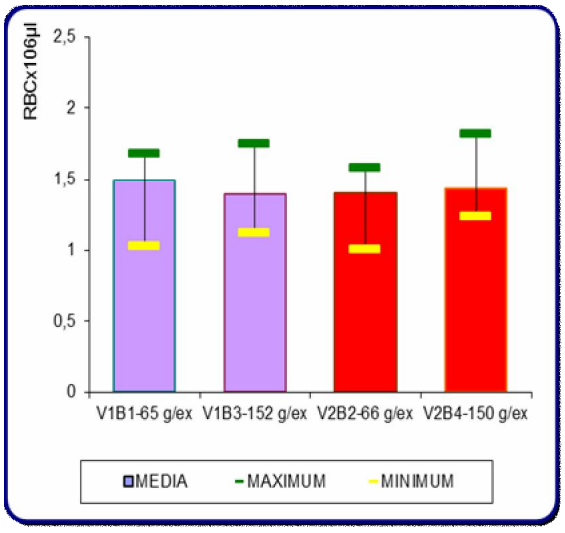

Figure 3. Red blood cells counts variation.

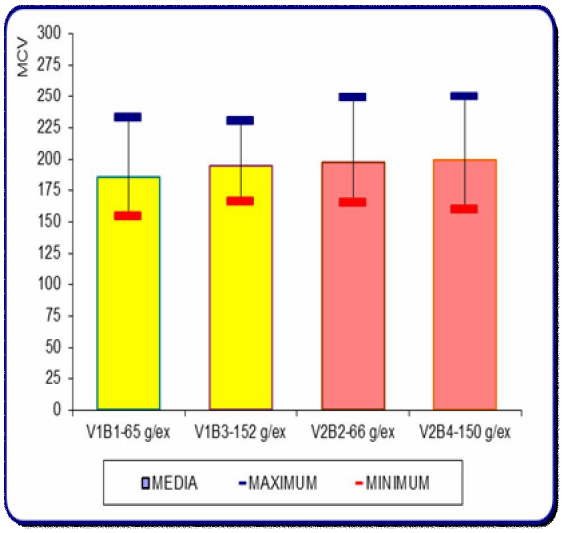

Figure 4. Mean corpuscular volume variation.

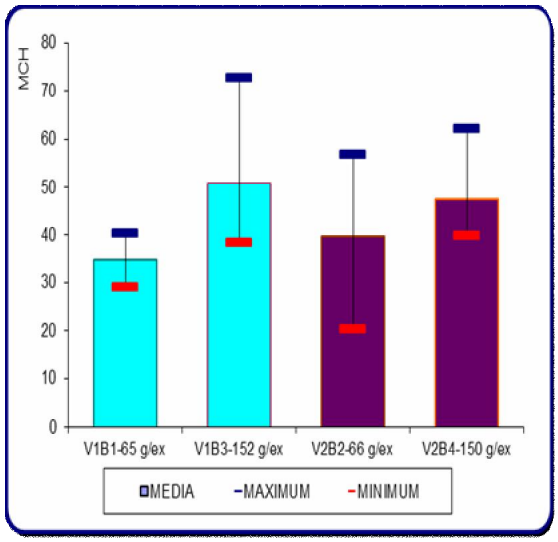

Figure 5. Mean corpuscular hemoglobin variation.

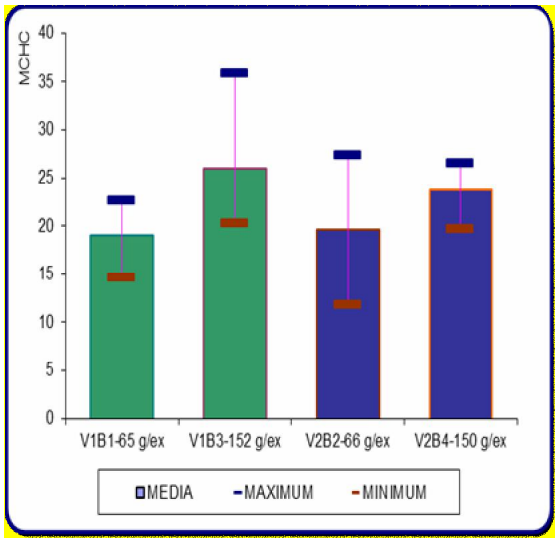

Figure 6. MCHC variation.

The data from Figs 4-6, in correlation to the data from the literature, lead to the conclusion that both the values of the mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) are situated in the optimum limits from the physiological point of view for the species studies. MCV has values ranging between 186.48 and 199.73 (μm^3). An exception to this statement is mean corpuscular hemoglobin (MCH), having values slightly a little under the normal level. A remarkable conclusion is that the Hb depends mainly on the size class and very little on the density of population (Ruane et al 2002).

The higher concentration of Hb in the case of great-sized specimens is illustrated by the MCH constant. Thus, the mean corpuscular hemoglobin of carp specimen of 150-152 g is 49.14 pg, higher by 31.53 % in comparison with the same constant for the sapling of 65-66g/specimen (37.36 pg).

Irrespective of the density of population, the dependency between the mean corpuscular hemoglobin (MCH), respectively the mean corpuscular hemoglobin concentration (MCHC) and the size of the biological material can be noticed. Thus the MCHC constant registers an increase of 28.60% along with the increase of the individual weight (from 19.35 g/dL for specimen of 65-66g to 24.89 g/dL for specimen of 150-152g).

Conclusions. Ghergariu et al (1985) defines the metabolic health as the balanced physiological state of the organism which allows it to develop and offer optimum production, without traceable modifications of the hematological and biological parameters considered normal for the species, age and physiological stage.

The assessment of the physiological condition of fish was done from a quantitative point of view through biotechnological parameters (average mass, daily development rate), as well as from a qualitative point of view through specific hematological parameters.

The hematological examination of crap specimen of different size, grown in different densities of population brought out modifications to main hematological parameters. The modifications were the reduction of the hematocrit in comparison to the normal value, designating a slight anemia.

Because the concentration of hemoglobin in blood is an important synthetic parameter when assessing the degree of physiological integrity, its reduction in the case of specimen with lower individual weight 65–66 g, suggests the beginning of a nutritional disease (Docan et al 2011).

The information from the specialty literature outlines the fact that the reduction of hemoglobin from blood can affect the quantity of oxygen in tissues, leading to a decrease

of the metabolic rate and a lower production of energy. The erythrocyte constants (MCV, MCH and MCHC) vary according to the two size classes: MCH increases with 31.53 % and MCHC increases with 28.60 % along with increase of the individual weight of the carp. It can be noticed from the graphics and the specialty literature that both the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC) are to be found in normal limits for the species studied.

The higher stocking density from our study (64 kg/m^3) did not influence the hematological parameter. Density stress can act upon the hemoglobin synthesis only through hypoxia.

The results obtained following the investigation of the main hematological parameters regarding the crap specimen under technological factors (size classes and stocking density) are correlated to biotechnological parameters (Enache et al 2011) and outline a better physiological condition of fish with a higher average weight (150 – 152 g/ex) irrespective of their stocking density.

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Authors:
Ionica Enache (Bancu), Dunărea de Jos University of Galaţi, Faculty of Food Science and Engineering, Str. Domnească no. 47, 800008-Galaţi, Romania, European Union; e-mail: enaoana@yahoo.com
Victor Cristea, Dunărea de Jos University of Galaţi, Faculty of Food Science and Engineering, Str. Domnească no. 47, 800008-Galaţi, Romania, European Union.
Angelica Docan, Dunărea de Jos University of Galaţi, Faculty of Food Science and Engineering, Str. Domnească no. 47, 800008-Galaţi, Romania, European Union.
Adina Popescu, Dunărea de Jos University of Galaţi, Faculty of Food Science and Engineering, Str. Domnească no. 47, 800008-Galaţi, Romania, European Union.
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