

Effect of feeding with different dietary protein level on hematological indices of juvenile Siberian sturgeon, *Acipenser baeri* reared under recirculating systems condition

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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 Siberian sturgeon maintained in different technological condition induced by percentage of protein in feed. Specimens belonging to four experimental groups had individual weights of 135 g/fish, aged 5 months. The stocking density was 7.20 kg/m³ for the four variants; the fish biomass have been fed with ADVANCE BE 1 P granules with a protein content of 46 % for VE_{46P}, respectively ALLER CLASSIC granules with a protein content of 30 % for VE_{30P} (daily ration of 3 %). The sampling of *Acipenser baeri* blood from the four variants before and after the experimental trial allowed determination of hematological indices. Red blood cell counts (RBCc), haematocrit values (Hct), 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. Differences in hematological parameters were statistically analyzed by Student *T* test. Physiological stress induced by maintenance in different technological condition induced by percentage of protein in feed, is reflected in the hematological indices (significant decrease, $p < 0.05$, RBCc, Hb, MCV, MCHC) with direct implications at the biotechnological level.

Keywords: Siberian sturgeon, *Acipenser baeri*, recirculating aquaculture systems, red blood cell, haematocrit, haemoglobin concentration.

Rezumat. Indicatorii hematologici sunt parametri importanți pentru evaluarea stării fiziologice a peștilor. Scopul prezentei lucrări a constat în obținerea unor informații privind răspunsul hematologic al speciei *Acipenser baeri* crescută în sistem recirculant de acvacultură în condiții tehnologice diferite induse de procentul de proteine din furaj. Exemplarele din cele patru variante experimentale au avut greutatea individuală de 135 g / ex., în vârstă de 5 luni. Densitatea de stocare a fost aceeași în cele 4 variante - 7,20 kg/m³; biomasa de cultură din VE_{46P} a fost hrănită cu granule ADVANCE BE 1 P cu un conținut proteic de 46 % iar din VE_{30P} cu granule ALLER CLASSIC cu un conținut proteic de 30 % (rația zilnică a fost 3 %). S-au recoltat probe biologice de sânge de la exemplarele de *Acipenser baeri* atât la începutul cât și la sfârșitul perioadei experimentale. Cu ajutorul metodelor utilizate în hematologia peștilor s-a determinat și analizat numărul de eritrocite (Nr. E), hematocritul (Ht), cantitatea de hemoglobină (Hb), volumul eritrocitar mediu (VEM), hemoglobina eritrocitară medie (HEM), concentrația de hemoglobină eritrocitară medie (CHEM). Variația indicatorilor hematologici a fost analizată statistic cu ajutorul testului *T* Student. Stresul fiziologic, indus de condițiile de creștere utilizând strategii diferite de hrănire, s-a reflectat în indicatorii hematologici (reducerea semnificativă, $p < 0,05$, a Nr. E, Hb, VEM, CHEM în VE_{30P}) cu implicații directe la nivel biotehnologic.

Cuvinte cheie: Sturion siberian, *Acipenser baeri*, sistem recirculant de acvacultură, eritrocite, hematocrit, hemoglobină.

Introduction. *Acipenser baeri* represents a feasible candidate for aquaculture having a good growth rate in different production systems, being capable to reach the sexual maturity in captivity (Ronayi et al 1989). The primary results achieved by the physiological nutrition researches were promising (Dabrowski et al 1985; Medale & Kaushik 1989).

Nutrition of the proteins is basically the most studied segment of fish nutrition, since the first attempts to use the combined feed in aquaculture have been tried to reach an optimal protein level in fodder, both quantitatively and qualitatively. Proteins, main components of the pelleted food for fish are essential for growth, reproduction and maintenance. From the physiological perspective, these fulfill important roles in fish body (Oprea & Georgescu 2000), being involved in: oxygen transport, maintaining acid-base balance, metabolic adjustments and immunochemical defense mechanisms.

The feed's quality used in the intensive aquaculture represents an important aspect for the ensurance of the metabolic health of the cultured fish. A well balanced nutrition for each species is linked to normal values of the main hematological indices (Misăilă 1998).

Since hematological parameters reflect the poor condition of fish more quickly than other commonly measured parameters, and since they respond quickly to changes in environmental conditions, they have been widely used for the description of healthy fish (Blaxhall 1972), for monitoring stress responses and for predicting systematic relationships and the physiological adaptations of fish. Some errors of nutrition and also the deficiency of specific proteins, minerals may cause to fish severe anemia states this happens because of the hematocrit reduction and also because of the hemoglobin quantity reduction (Mohamed 2001).

Whereas the blood, because of the dynamism and the functions that are fulfilled in the organism, it represents the general physiologic "mirror" state of the fish (Patriche et al 2011), the research from this study has followed the hematological answer of the Siberian sturgeon under the protein percentage influence from the feed.

Material and Method

Fish biomass and the growing condition. Fish biomass used in this study was represented by *Acipenser baeri* specimens aged five months from the Brates Research and Microproduction Base of I.C.D.E.A.P.A Galați and raised into a recirculating system of the pilot aquaculture station from the Aquaculture, Environment Science and Cadastre Department. The four experimental fish groups had individual mean weights of 135 g/fish; total biomass was 8640 g, equally distributed in the four growth units. The stocking density was 7.20 kg/m³. The daily feeding rate was 3 % of fish body weight (% BW) administrated in three equal meals.

This experiment lasted 30 days, followed the influence of two types of feed with different protein content on the physiological status of the species *Acipenser baeri*:

- ↳ *VE_{46P}* - experimental variant on the influence of feeding 46 % crude protein on growth and physiology of the species *A. baeri*
- ↳ *VE_{30P}* - experimental variant on the influence of feeding 30 % crude protein on growth and physiology of the species *A. baeri*
- ↳ i, f - beginning and the end of experiment

Blood sampling and analysis. 0.5 mL of blood was sampled 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. With routine methods used in fish hematology (Blaxhall and Daisley 1973) were measured and analyzed the hematological indices. The red blood cell counts (RBCC, x 10⁶/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 5 minutes at 12000 rpm in a micro hematocrit centrifuge. The photometrical cyanohaemoglobin method was used for determination of haemoglobin concentration (Hb, g/dL). Using standard formulas according Ghergariu et al (1985) and 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 the growing conditions on the hematological profile of our studied species. **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. *Hematological modifications* of our studied fish were analyzed in corroboration with the technological factors which can influence metabolic processes. Hematological parameters at the beginning and the end of experiment are presented in the following table:

Table 1
Variation of the hematological parameters in *A.baeri* for tested experimental variants

Experim. variant	Hematological indices						
	PCV (%)	Hb (g/dl)	RBCc $\times 10^6/\mu\text{l}$	MCV (μm^3)	MHC (pg)	MCHC (g/dl)	
VE _{46P}	B1i	20±1.2	4.2±0.8	0.695±0.16	287.77±38.1	60.43±18.5	21.0±4.3
	B1f	20±2.1	4.2±0.6	0.706 ±0.19 ^b	283.29±39.6 ^b	59.49±16.2 ^b	21.0±5.1
VE _{46P}	B2i	22±1.7	4.4±0.9	0.708±0.12	310.73±28.5	62.15±15.6	20.0±3.8
	B2f	22±0.8	4.2±0.8 ^b	0.714 ±0.15 ^b	308.12±40.4 ^b	58.82 ±17.5 ^b	19.1±4.4 ^b
VE _{30P}	B3i	23±2.4	4.8±1.1	0.732±0.18	314.21±45.4	65.57±15.2	20.9±4.2
	B3f	22±1.8 ^{bd}	3.7±1.3 ^{ac}	0.594±0.22 ^{ac}	370.37 ±37.3 ^a	62.29±14.6 ^{bd}	16.8±3.8 ^{ac}
VE _{30P}	B4i	21±2.6	4.6±0.7	0.725±0.15	289.66±40.2	63.72 ±18.4	21.9±2.9
	B4f	20±0.9 ^{bd}	3.6±1.1 ^{ac}	0.565±0.21 ^{ac}	353.98 ±34.3 ^a	61.54±22.1 ^{bd}	18.0±3.7 ^{ac}

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

„b”- insignificant differences (comparing with initial values)

„c” - significant differences between the two variants

„d” - insignificant differences between the two variants

Analyses of the blood parameters presented in Table 1 emphasize changes in both comparisons: between experimental variants and between initial and final moments of the experimental period. Analyses targeted the following indicators: red blood cell counts (RBC), hematocrit (PCV, %), hemoglobin (Hb), the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC).

Red blood cell counts (RBC) from VE_{30P}: B3f, B4f counted 0,594 $\times 10^6$ respectively 0,565 $\times 10^6$ registering a significant reduction ($p=0.04<0.05$), by 15.86 % comparing with VE_{46P}: B1f, B2f (0.706 $\times 10^6$ and 0.714 $\times 10^6$ erythrocyte) and 18.85 % comparing with B3i, B4i. After 30 days, in VE_{46P} variant the number of erythrocyte remained constant over initial moment.

Red blood cell counts (RBC) represents a parameter which plays an important role in maintaining the functionality and integrity of the respiratory pigment. Change of the number of red blood cells from the normal values, appropriate for different species and age, shows disturbances at the functional level.

Due to the lower position in systematic classification, the cartilaginous sturgeons have a lower number of erythrocytes compared with teleostean fish.

Although the number erythrocytes from circulating blood of fish fed with feed containing 30% protein was significantly reduced, their value has remained within the normal range of the species *Acipenser baeri*; Ruchin (2007) found in the Siberian sturgeon $0.57 \pm 0.05 \times 10^6$ erythrocytes/mL blood, those being loaded with a quantity of 4.8 g/dL hemoglobin.

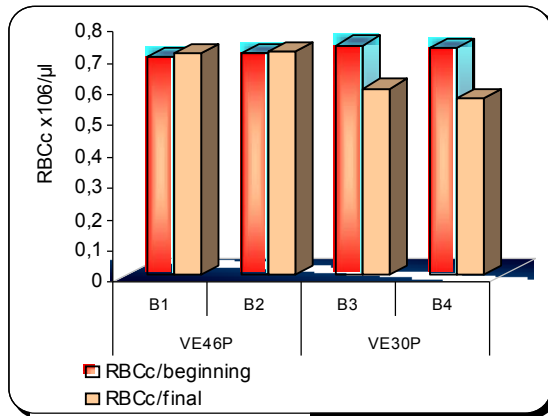


Fig. 1. Red blood cell counts variation.

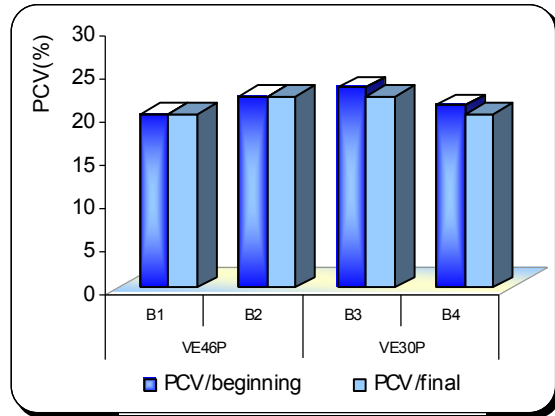


Fig. 2. Hematocrit variation.

Hematocrit values were maintained constant in the experimental variant VE_{46P} , unlike VE_{30P} variant where was registered a slightly, insignificant statistically ($p > 0.05$), reduction comparing with initial values.

The amount of hemoglobin in the circulating blood of fish from VE_{30P} (B3f, B4f) was 3.7 ± 1.3 g/dL blood, respectively, 3.6 ± 1.1 g/dL blood. Those values are significantly ($p = 0.03 < 0.05$) lower (with 11.9%), comparing both with VE_{46P} - B1f, B2f (4.2 g / dL blood) and (by 22.9%) comparing with values from beginning of the experiment - B3i, B4i (4.8 and 4.6 g/dL blood). This reduction, in the amount of hemoglobin, correlates with reduced number of erythrocytes.

Similar results obtains in Misăilă (1998) when rainbow trout and some species of cyprinids (carp, silver carp, grass carp) were subjects for feeding experiments with feed containing different proportions of protein. The author concludes that increased proportion of supplementary feed protein produce, in addition to enhancing growth and improving the physiological state, an increased erythrocyte count and hematocrit and hemoglobin values.

Therefore, the results of hematological examination in the case of Siberian sturgeon from VE_{30P} experimental variant indicate a mild anemia caused by inadequate amount of protein in the feed, necessary to satisfy the nutritional requirements for this stage of development. Anemia may be the consequence of inhibiting the production of red blood cells in specific organs or of increased rate of their destruction. Depriving the body of food (proteins, vitamins, minerals) needed for red blood cell formation leads to anemia installation with all its consequences. Insufficient protein determine perturbation in vitamins and mineral salts assimilation, and generally, a decrease in metabolic processes which reflects in the reduction of plasma proteins and then in the reduction in hemoglobin synthesis, occurring anemia (Bârză 1985).

If the numerical evolution of red blood cells shows the quantitative aspect of the integrity of respiratory function, the qualitative aspect is indicated by erythrocyte constants that provide information concerning functionality of the hematological indices.

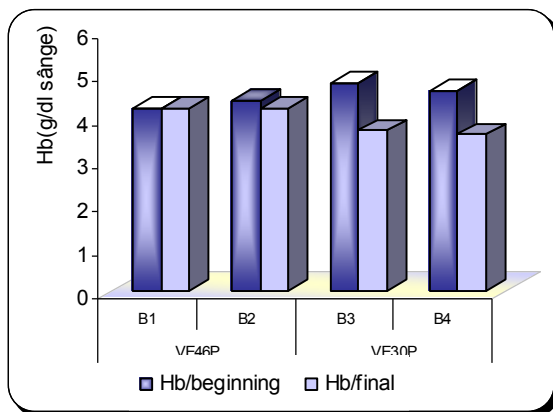


Fig. 3. Hemoglobin variation.

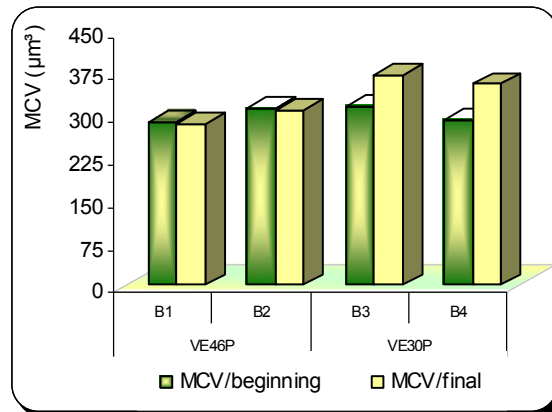


Fig. 4 Mean corpuscular volume variation.

The analysis of the values of erythrocyte's constants, revealed the following:

- ↪ **mean corpuscular volume (MCV)** remained relatively constant in the experimental variant VE_{46P} - B1f, B2f, comparing with start values. In the experimental variant VE_{30P} - B3f, B4f, there was an increase by 20% from start point - B3i, B4i and by 24.7% in comparison with VE_{46P} - B1f, B2f; both tests showing differences statistically significant ($p = 0.04 < 0.05$).
- ↪ **mean corpuscular hemoglobin (MCH)** showed a slightly variation in time for all groups with statistically insignificant ($p > 0.05$) differences when the mean values of the two experimental variants were compared.
- ↪ **mean corpuscular hemoglobin concentration (MCHC)** remained relatively constant in time in the experimental variant VE_{46P} - B1f, B2f. In the experimental variant VE_{30P} - B3f, B4f, there was a reduction of MCHC with 18.4% from initial moment - B3i, B4i and with 19.9% compared with VE_{46P} - B1f, B2f. Both tests showed statistically significant differences ($p = 0.04 < 0.05$).

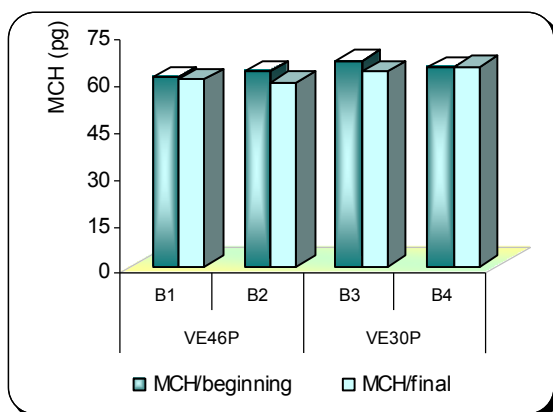


Fig. 5. Mean corpuscular hemoglobin variation.

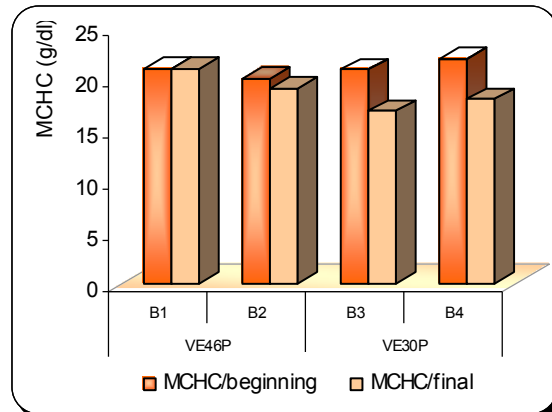


Fig. 6. MCHC variation.

Analysis of the erythrocyte constants reflect an adaptive response of the fish fed with a lower protein feed value. Reducing the erythrocytes number determined, as a reaction of compensation, an increased erythrocyte's volume (MCV) and of the quantity of hemoglobin from each erythrocyte (HEM). However, this reaction was not sufficient, since the red blood cell hemoglobin concentration (MCHC) reported to their volume decreased.

Conclusions. Research results have confirmed the working hypothesis, that the quality of given feed in intensive aquaculture represents a primary factor for metabolic health insurance (the body's physiological steady state) of fish culture.

Haematological examination of *Acipenser baeri* specimens fed with lower protein content (30%) showed major changes in hematological parameters.

Hematological examination results, in case of the Siberian sturgeon VE30P experimental version, indicate a slight anemia caused by inadequate amount of feed protein, to satisfy the nutritional requirements for this stage of development.

This led to an adaptive response of the blood, manifested through an increase of the volume of red blood cells remaining in circulation (MCV) and of the hemoglobin from each erythrocyte (MHC).

In the case of *Acipenser baeri*, the administration of a fodder which contains 30 % proteins had as consequence the depreciation of the physiological estate this leading to some possible influence upon the fish's sensibility to diseases.

The results of this study indicate the necessity to effectuate regularly hematological exams, because many nutritional deficiencies can produce a series of serious diseases, frequently more serious than the diseases produced by the pathogenic germs. The damage (losses) produced by the nutritional disturbances are bigger because they have a sub clinical evolution hard to detect and which can cause poor growth performances and reproduction problems.

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