HAEMATOLOGICAL AND SERUM BIOCHEMICAL CHANGES ASSOCIATED WITH BACTERIOLOGICAL INFECTION IN *ACIPENSER GUELDENSTAEDTII* REARED IN INTENSIVE CONDITION

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Abstract

Bacterial diseases are among the most important causes of economic losses in cultured fish species. The haematological parameters are an important tool of diagnosis that reveals the state of health of fish species. This study evaluated the haematological changes in Acipenser gueldenstaedtii under effect of the influence of pathogenic bacteria compared to healthy specimens and those treated. Infected specimens of sturgeon reared in recirculating aquaculture system had individual weights of 270 ± 0,08g, 320 ± 0,098 g/ex for treated fish and respectively 450± 0,11 g/ex for healthy specimens. 5 ml of blood was sampling from each fish by caudal venous puncture. Blood was analysed with routine method used in fish haematology. Was determined the following haematological and serum biochemical parameters: plasma glucose (mg/dl) and protein (g/dL), red blood cell counts (RBCc, x 10^6/µl), the haematocrit (PCV, %) and haemoglobin concentrations (gl/dl). Using standard formulas the red blood indices were computed: the mean corpuscular volume (MCV), the mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC). Differences in haematological parameters were statistically analyzed by ANOVA test. Physiological stress, induced by the action of pathogenic bacteria was reflected in the haematological indices: RBC count 0.27 ×10^6cel./mm^3 for infected fish, 0.32×10^6cel./mm^3 for treated fish and 0.45 ×10^6cel./mm^3 for healthy fish, , haemoglobin concentration 4.31, 4.90 and 5.82 g/dl, haematocrit concentration 9, 13 and 15 %, MCV 370.9, 457.2 and 347.29 fL, MCH 172.55, 167.81 and 136.43 pg, MCHC 47.88, 36.89 and 39.72 g/dl. Plasma glucose values recorded 82.9 mg/dL for infected fish, 93.06 mg/dl for treated fish and 91.82 mg/dL for healthy fish. Plasma protein value was 2.56 g/dL for infected fish, 3.19 g/dl for treated fish, respectively 3.37 g/dL for healthy fish.

Key words: sturgeon, pathogenic bacteria, haematological indices, plasma glucose and protein

INTRODUCTION

Acipenser gueldenstaedtii is recorded from the Danube River and the coast of the Black Sea. This species has been successfully bred in aquaculture facilities in Austria, Belgium, Germany, Hungary, the Netherlands, Poland and Romania [4]. But, high stocking densities characteristic of the intensive aquaculture systems, could adversely affect the health status and immune systems of fish species [14]. Bacterial diseases are among the most important causes of economic losses in cultured fish species. Under predisposition factors and stressful condition, the bacteria normally present in the tank water and in the digestive system of the fish found a portal of entry into the fish host and invade their organism [11], [9].In addition to the identification of the bacteria, understanding the haematological changes in infected fish will be helpful in the identification and subsequent control of the disease [16]. The database of the haematological and serum biochemical parameters of fish is an important tool that can be used as an effective and sensitive index to monitor health status, physiological and pathological changes of fishes. Different

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researchers have reported the normal range of various blood parameters in fish physiology and pathology [3], [19], [13], [6], [10]. Evaluation of haematologic and blood chemistry analytes will enhance the culture of fish by facilitating early detection of infectious disease and identification of sublethal conditions affecting production performance. Total protein was considered as a marker for analysis of the health status and nutrition of fish and was used as a diagnostic tool[1 9]. Despite the importance of *Acipenser gueldenstaedtii* as an endangered species, however, little is known about the blood haematology of this species in bacterial infected condition. This study evaluated the haematological changes in *Acipenser gueldenstaedtii* under effect of the influence of pathogenic bacteria compared to healthy specimens and those treated.

MATERIALS AND METHODS

Fish biomass and the growing conditions

Fish used in this study was represented by *Acipenser gueldenstaedtii* specimens aged twelve months raised into a recirculating system of the pilot aquaculture station from the Aquaculture, Environment Science and Cadastre Department. The fish with evident signs of disease underwent a complete pathologic examination: clinical, macroscopic and microscopic.

Biochemical and haematological measurements

10 exemplars of fish sturgeon from each experimental variant were examined, measured and weighed. Approximately 3 mL blood samples were collected from the caudal vein of each fish and transferred to blood collection tubes containing lithium heparin. Blood was analysed with routine method used in fish haematology[2]: the red blood cell counts (RBCc, x 10^6 μL^{-1}) was determined by counting the erythrocytes from 5 small squares of Neubauer haemocytometer slide at a magnification of ×400, using Vulpian diluting solution. The haematocrit (PCV, %) was determined by the microhaematocrit method using heparinised capillary tubes centrifuged for 5 minutes at 12000 rpm, and expressed as percentages. The spectrophotometric cyan haemoglobin method was used for determination of the haemoglobin concentration (Hb, g dL^{-1}) and read the absorbance values at 540 nm. Using standard formulas the red blood indices were calculated: the mean corpuscular volume (MCV), the mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC). To obtain blood serum, the blood without anticoagulant was centrifuged 10 minutes, at 3500 rotation/min. Determination of glucose (SG) and total protein (TP) from serum was performed spectrophotometric by SPECORD 210 AnalytikJena. Dosage of glucose was made by colorimetric method with o-toluidine, readings were made at 635 nm wavelength. Total proteins from serum were determined by Biuret method, the readings was done at a 546 nm wavelength.

Statistical analysis

Data were subjected to statistical analysis using the SPSS software ver. 18. The results were submitted to the variance analysis (ANOVA) ($p<0.05$) and Tukey test used to determine the significant differences between haematological parameters ($p<0.05$).

RESULTS

Infected fish reveal gross lesions on the skin, pale gills, scale erosion and haemorrhagic ulcers, modifications at the internal organs level (with evident degenerative processes) sign of a bacterial invasion. The haematological and some biochemical profiles of the diseased infected *Acipenser gueldenstaedtii* are documented in table 1; various changes were observed in the blood parameters as compared to treated and healthy fish.

Red blood cells count - significant differences between those 3 groups of fish (ANOVA $p<0.05$; $p=0.001721$), Tukey B test revealed that the average value of the healthy fish was significantly higher than in both cases of those infected and who have recovered after treatment. Haematocrit - significant differences between those 3 groups of fish (ANOVA $p<0.05$; $p=0.000002$), Tukey B test shown that the

haematocrit of the infected fish is significantly lower than the treated and healthy fish, and no significant differences between treated and healthy fish groups. Haemoglobin - significant differences between the 3 groups of fish (ANOVA p <0.05; p=0.000099), Tukey B test shown that the mean values of the haemoglobin concentration of the infected and treated fish are significantly lowers than those of the healthy fish, and with no significant differences between the treated and infected specimens. VEM and HEM- significant differences between those 3 groups of fish (ANOVA p>0.05; p=0.159, p=0.209). CHEM- ANOVA test revealed significant differences between the 3 groups of fish (p <0.05, p=0.00027); CHEM values for diseased fish being significantly higher than those for treated and healthy fish.

Table 1. Comparison of the haematological and biochemical parameters of infected, treated and healthy sturgeon

Haematological parameters	Infected fish		Treated fish		Healthy fish	
	Mean	SD	Mean	SD	Mean	SD
RBCc (x 10^6cel/mm^3)	***0.27***	0.08	***0.32***	0.09	***0.45***	0.11
PCV (%)	***9.06***	0.7	***13.44***	1.82	***14.82***	2.73
Hb (g dl^{-1})	***4.31***	0.51	***4.9***	0.37	***5.82***	0.88
MCV (fl)	***370.9***	118.06	***457.2***	141.47	***347.26***	108.92
MCH (pg)	***172.55***	46.17	***167.81***	49.94	***136.43***	40.52
MCHC (g dl^{-1})	***47.88***	6.94	***36.89***	3.12	***39.72***	4.44
Glucose (mg dL^{-1})	***82.89***	2.72	***93.06***	4.57	***91.81***	4.51
Total Protein (g dl^{-1})	***2.56***	0.41	***3.19***	0.44	***3.36***	0.57

RBCc - red blood cell counts, PCV - haematocrit, Hb - haemoglobin concentration, MCV - mean corpuscular volume, MCH - mean corpuscular haemoglobin, MCHC - mean corpuscular haemoglobin concentration

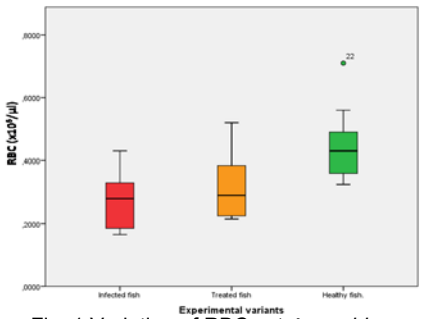

Fig. 1 Variation of RBCc at *A. gueldenstaedtii*

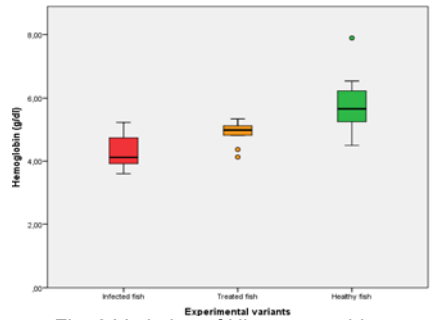

Fig. 2 Variation of Hb at *A. gueldenstaedtii*

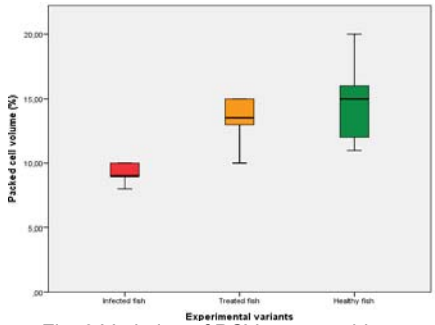

Fig. 3 Variation of PCV at *A. gueldenstaedtii*

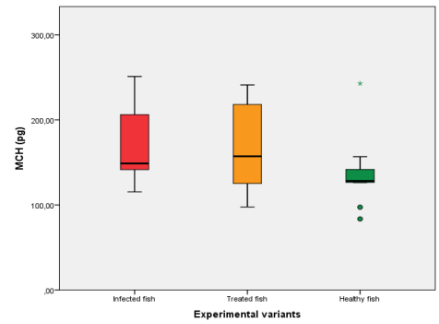

Fig. 4 Variation of MCH at *A. gueldenstaedtii*

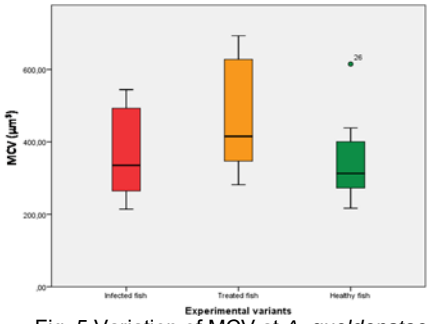

Fig. 5 Variation of MCV at *A. gueldenstaedtii*

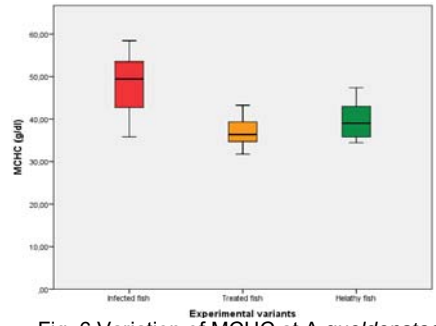

Fig. 6 Variation of MCHC at A.*gueldenstaedtii*

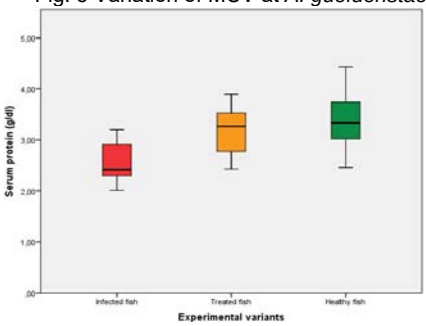

Fig. 7 Variation of SG at *A. gueldenstaedtii*

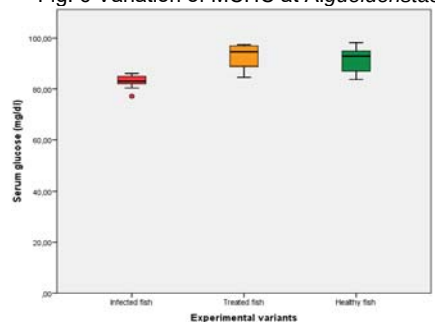

Fig. 8 Variation of TP at *A. gueldenstaedtii*

DISCUSSIONS

Strongly action of pathogenic bacteria on the sturgeon was led to damage of the internal organ leading, implicitly, to severe changes in blood physiology. In our study is evident from the observations that bacterial infection (Grecu I. et al., unpublished data) caused several changes in haematological parameters. Haematological studies are applied to diagnose infectious and non-infectious diseases in aquaculture reflecting the health condition of fish [8]. In the present study decrease in haemoglobin content, haematocrit and red blood cells count showed anaemic characters of the infected sturgeon. Anaemia occurred as a result of depletion of oxygen transported to tissues explaining their lethargic behaviour. Degenerative changes finding at the hematopoietic site (spleen, kidney) correlates with the reduced erythropoiesis. Reduced erythropoiesis is the most distinct feature of the pathogenesis in this case. The decrease of red blood cells in the peripheral blood is not compensated by the production of new cells because of the considerable loss of haemopoietic tissue. Decreasing the value of the main haematological indicators leads to haemodilution and immunosuppression due to which infection grows faster and fish leads to mortality [18]. Anaemia due to bacterial infection in different fish species have been reported by: Rehulka, J. [17] with recorded reduction of Hb and RBCc and increased MCV, MHC in *Salvelinus fontinalis* affected by *Columnar diseases*, Waagbø R. et al.[20]described signs of severe anaemia combined with a reduction of RBCc, Ht and Hb in *Salmo salar* suffering from the cold-water vibriosis, Munteanu G.[12] recorded a reduction of the main blood indicators in *Acipenser stellatus* suffering from the bacterial haemorrhagic septicaemia. Decrease in haemoglobin trend may be a result of swelling of RBCs as well as poor mobilization of haemoglobin from spleen [18].The lower value of RBC in infected sturgeon from this study was in accordance with lower values reported by other researcher at different fish species affected by infection diseases: Martins M.L. et al.[9]. pointed out the erythrocyte amount of *Nile tilapia* experimentally infected with *Enterococcus sp.* was significantly lower in comparison to those in non-infected; results obtained by Koteshwar R. P. et al. [7] reveal

that in comparison with the control (healthy fish) RBC, Hb, PCV values in infected fish (Epizootic Ulcerative Syndrome)were decreased by 63.5%, 23%, 55% respectively. In general, the pattern of change was similar to the observation in bacterial infections. These results indicate that haematopoiesis may be severely affected in bacterial diseases [1]. With the help of the haematological indices the erythrocytes constants (MCV, MCH, MCHC) for the blood of the sturgeon were calculated. Their diagnostic value is very important because they are useful to detect the presence of some physiological lesions in the forming process of haemoglobin and offers information on the size, shape and haemoglobin quantity in erythrocytes [5]. In the present study, there was a significant increase in MCHC value, due to macrocytic anaemia; the adaptation response of blood to the significantly reducing the number of erythrocytes and the haemoglobin content was prompt materialized in increasing HEM and CHEM. The level of glucose and total protein in blood is easily changed under the influence of some external or internal factors. This explains the importance as biochemical indicator of reference in evaluating the degree of normality of the general physiological condition [15].In regard with the biochemical findings in the blood serum of infected *Acipenser gueldenstaedtii*, we found that the total serum protein and glucose concentration were significantly diminished comparing with the non-infected fish maybe due to haemodilution.

CONCLUSION

Therefore the bacteriosis resulted in impaired homeostasis and consequently dynamic balance between production and destruction of erythrocytes which favoured the installation of anaemia with negative consequences. The study also reveals that *A. gueldenstaedtii* are most sensitive to change in their ecosystem which reflects their haematological conditions. The evaluation of haematological and serum biochemical parameters of sturgeon may provide a rapid means for determining the physiological status, which in turn would facilitate the implementation of remedial measures during culture operations.

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