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HEMATOLOGICAL PARAMETERS AS INDICATORS OF TOXIC STRESS PRODUCED BY MYCOTOXIN FOOD CONTAMINATION IN THE EUROPEAN CATFISH (*Silurus glanis* L.)

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Abstract. Hematological indices are important parameters for the evaluation of fish physiological status. The aim of the present study was to obtain a basic knowledge of the hematological response of the European catfish under the action of toxic metabolites (aflatoxin B) secreted by the mold *Aspergillus flavus*, but also the assessment of the physiological changes. The sampling of catfish blood from the two variants (infected and uninfected fish) before and after the experimental trial allowed the determination of the hematological indices. The erythrocyte count (EC) was done in an improved Neubauer haemocytometer, using the Vulpian reagent as dilution, following the method of Svobodova. The microhaematocrit method of Blaxhall and Darsley was employed in the determination of blood haematocrit, assessed through the centrifugation of capillaries to 12 000 rpm. The haemoglobin content of blood samples was determined by the Sahli hemoglobinmeter method. The hematological indices: mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were calculated using the formula of Svobodova. In the infected fish with aflatoxin, a decrease of RBC (–16.24%), PVC (–6%), Hb (–18.2%), MCH (–2.12%) and MCHC (–13.3%) and an increase of MCV (+11.5%) was observed, compared to uninfected fish. The decrease of the number of erythrocytes, the amount of haemoglobin and haematocrit in the blood of infected fish led to the installation of a state of anemia.

Keywords: *Silurus glanis,* hematological indices, aflatoxin, anemia.

AIMS AND BACKGROUND

Silurus glanis, species 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.

Most of the diseases of nutritional nature represent the consequence of nutrition errors, lack of balances or deficiencies in the fodders composition, and also of their contamination with toxic substances[1].

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In teleostean fish, as in higher vertebrates, blood physiology and implicitly haematology 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.

The determination of hematological values of fishes is carried out for a variety of purposes: to establish a 'normal range' of blood parameters[2], to investigate conditions that might lead to alterations of some of these values, such as sampling methods, temperature, sex, maturity, disease condition or nutrition of the fish[3] and to ascertain the effects of some chemical pollutants (e. g. insecticide) and sublethal strength of some toxicants (such as heavy metals, e.g. lead) on blood values[1,4].

The importance of the hematological examination in the fish diseases diagnosis and in the mycotoxins effect evaluation has been widely accepted. The aflatoxins are mycotoxins with hepatotoxic and carcinogenic action, metabolism products of the *Aspergillus flavus* and *A. parasiticus* stems[5].

The aim of the present study was to obtain a basic knowledge of the hematological response of the European catfish under the action of toxic metabolites (aflatoxin B) secreted by the mold *A. flavus*, but also the assessment of the physiological changes.

EXPERIMENTAL

Fish biomass and the growing conditions. The fish biomass used in this study was represented by *Silurus glanis* specimens aged two from the Brates Research and Microproduction Base of I.C.D.E.A.P.A. Galati and raised into a flow-through system of the pilot aquaculture station of the Aquaculture, Environment Science and Cadastre Department. Fish were examined for any sign of infection or disease condition and only considered to be healthy fishes were used for the study.

The two experimental fish groups had individual mean weight of 682 ± 264.49 g/ex. in the first tank (C1), 743 ± 241.79 g/ex. in the second tank (C2), respectively. The stocking density was 74.7 kg/m³ for C1, 74.3 kg/m³ for C2, respectively. The fish from C1 were fed with fodder with 46% protein and those from C2 with fodder with 30 % protein. For both experiments, the settled ration was of 1 % BW per day. The fodder that was given to the fish in C1 was contaminated with *A. flavus.*

Blood collection. 1 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 end of the experimental trial. Some authors consider heparin the most suitable anticoagulant in fish hematology[6,7].

The erythrocyte count (RBCC, $\times 10^6$/mm³) was done in an Improved Neubauer haemocytometer using the Vulpian reagent as dilution way, following the method of Svobodova. The microhaematocrit method of Blaxhall and Darsley[8] was employed in the determination of blood haematocrit (PVC, %), assessed through the

centrifugation of capillaries to 12 000 rpm for 5 min. The haemoglobin content (Hb, g/dl) of blood samples was determined by the Sahli hemoglobinmeter method.

The hematological indices: mean cell volume (MCV, µl), mean cell haemoglobin (MCH, pg) and mean cell haemoglobin concentration (MCHC, g/dl) were calculated using the formula of Svobodova (1991) given below:

– mean corpuscular volume (MCV, µl)= hematocrit (%)/erythrocyte count × 10;

– mean corpuscular hemoglobin (MCH pg)= hemoglobin (g/dl)/erythrocyte count × 10;

– mean corpuscular hemoglobin concentration (MCHC) = hemoglobin (g/dl)/hematocrit (%) × 100.

Statistical analysis. The hematological parameters of the two experimental groups (variants) were expressed by mean and standard deviation (m ± SD) and differences between the values were statistically analysed with the Student *t*-test.

RESULTS AND DISCUSSION

The investigations of the metabolic blood profile aimed at identifying the hematological response of the *S. glanis* in the condition of the toxic metabolites action (aflatoxin B), secreted by the *A. flavus* mould, but also in the evaluation of the occurring physiological changes.

The hematological values used for the physiological status characterisation and for marking evident metabolic dysfunctions, as an answer reaction of the body in the aflatoxin intoxication case, are given in Table 1.

Table 1. Hematological indices of the European catfish during the experiment (mean ± SD)

Hematological indices	Infected fish	Healthy fish
PVC (%)	25.5 ± 1.85[b]	27.1 ± 2.66
Hb (g/dl)	5.58 ± 0.45[a]	6.82 ± 0.73
RBCC (× 10 ^{6}/mm^3)	1.186 ± 0.11[a]	1.416 ± 1.77
MCV (µm^3)	215.83 ± 12.42[a]	194.28 ± 24.4
MCH (pg)	47.18 ± 2.47[b]	48.67 ± 4.66
MCHC (g/dl)	21.89 ± 0.96[a]	25.25 ± 2.5

[a] Significant differences (between 2 experimental variants) for the Student *t*-test applied to paired variables; [b] – insignificant differences (between 2 experimental variants) for the Student *t*-test applied to paired variables.

The analysis of the blood values presented in the table above makes evident the depletion of the majority of these indices, with the VEM exception, as it follows:

– in the case of the healthy fish, the number of erythrocytes varied between minimum 1.055 and maximum 1.775 mil.cel/mm^3 of blood with an average of

1.416, decreasing significantly ($p = 0.01 < 0.05$) with 16–24% at the diseased fish, ranging between 0.95 min. and max. 1.34 mil. cel./mm^3 of blood. This decrease of the erythrocytes number may be due to damage of the erythropoetic function;

– consequently, the haematocrit value in the case of the *S. glanis* with pathological signs has slightly decreased by 5.9%, but in comparison with the values found in healthy fish the differences were statistically insignificant ($p = 0.15 > 0.05$). Thus, the values ranged between 22 to 28% compared to 23–34% in healthy catfish;

– the reducing of the erythrocytes number, of the hemoglobin quantity and haematocrit characterise by anemia conditions, haemodilution, which is produced through the interstitial liquid afflux, through the increase of the plasma volume, respectively;

– the haemoglobin quantity registered a signifiant reduction ($p = 0.0004$) with 18.18%, ranging between 4.8 and 6 g/dl of blood in the intoxicated specimens with mycotoxin, while at the healthy catfish the quantity of haemoglobin ranged between 5.6 and 7.8 g/dl.

Mousa[9] observed the decrease of erythrocytes number of the haematocrit and hemoglobin quantity in the *Clarias gariepinus* blood after the intoxication with ochratoxin – metabolites secreted by the *Aspergillus ochraceus* mold. The reduction of the erythrocytes number, the haematocrit and the hemoglobin value, as a response to the metabolites toxicity secreted by molds, might be attributed to the mature erythrocytes destruction and inhibition of production of new erythrocytes due to the haemoglobin synthesis reduction under the action of the aflatoxin.

As a consequence of the toxic stress caused by the aflatoxin, the erythrocytes constant values change irregularly the reduction of the erythrocytes number at the catfish and of the haematocrit value which determines the apparition of an adaptive reaction of the erythrocytes which remained in circulation, thus increasing its volume *size (so the MCV values increased significantly ($p = 0.02 < 0.05$) by 11.09 %. The mean corpuscular volume (MCV) increase characterises the macrocytic anemia conditions[9,10]. The decrease of the haematocrit, haemoglobin and of the erytrocytes number are signs of anemia[11]. The anemic fish are sensitive to some secondary pathogen agents and present a low tolerance to the oxygen depletion[12].

The haematological indices calculation (MCV, MCH, MCHC) presents a particular importance in the anemia diagnosis at most animals, including fishes[13]. The statistical analysis of the hematological constants revealed significant changes over time. As mycotoxin affects the function of the haemoglobin synthesis this explains the significant reduction of the haemoglobin quantity. This aspect led to the significant decrease ($p = 0.001$), by 13.34% of the mean corpuscular haemoglobin concentration (MCHC) at the catfish.

Thus, the mean concentration of hemoglobin of each erythrocyte (MCHC) varied between 20 and 23.6 g/dl, recording an average of 21.89 ± 0.96 g/dl blood

comparing with the healthy catfish at which the MCHC varied between 22.4 and 28.89 g/dl with an average 25.25 ± 2.5 g/dl blood.

From a physiologic point of view, the fish were strongly affected, the internal homeostasis not being restored, and, though the remaining erythrocytes increased their volume (MCV) the haemoglobin quantity of each erythrocyte (MCH) decreased insignificantly ($p > 0.05$). It is possible that these aspects might have had as a consequence the affection of the respiratory function.

CONCLUSIONS

After the occurrence of this spontaneous mycotoxicosis at the *S. glanis* reared in a semi-closed system, the changes observed at the blood level were an important criterion for diagnosis, but also for estimating the mycotoxin severity.

The erythrocyte series was affected by the erythrocyte number decrease of the haemoglobin and haematocrit quantity which led to the installation of an anemia condition.

The haematological indices reduction, as a response to the metabolites toxicity, secreted by molds, can be attributed to the mature erythrocytes destruction and the inhibition of production of new erythrocytes, caused by the reduction of the haemoglobin synthesis under the action of the aflatoxin.

The statistical analysis of the haematological constants, which have an important role in diagnosing the anemia at fishes, showed significant changes in them: the affection of the haemoglobin synthesis function and the decrease of the mean corpuscular hemoglobin concentration (MCHC).

But although the remaining erythrocytes increased their volume (MCV), the haemoglobin quantity of each erythrocyte (MCH) decreased.

These changes had as a consequence the fishes affection from a physiological point of view, probably installing disorders at the metabolic activity and hematopoetic level, the internal homeostasis not being restored.

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