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HEMATOLOGIC PROFILE AS STRESS INDICATOR IN FISH

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Abstract

Hematologic parameters are considered important indicators of the fish health status, providing useful information for assessing their welfare. Fish response to stressors is similar to higher vertebrates, being represented by the quick release of catecholamines, followed by the corticosteroids release.
The study aims to establish stress level in common carp (Cyprinus carpio) by obtaining an overview of the morphology of blood cells, their number and percentage distribution in peripheral blood. The biological material was represented by a group of 5 fish weighing between 2.5 and 3 kg. From fish there were collected blood samples using heparinized syringes, after which the samples were transferred into Li-heparin containers. Leukocyte count was made on smears with Diff-Quick staining. The study of cells characteristics was performed by optical microscopy. Obtained results were compared with hematologic reference ranges for carp.
Analyzing the results, it was noticed the presence of erythrocytes and platelets and, from the white series, of lymphocytes, monocytes, eosinophils and heterophils. Among leukocytes, 80% were represented by lymphocytes, eosinophils were reduced in number and basophils were not identified, this aspect being in accordance with other authors' findings. The results obtained in this study framed for most parameters within the reference ranges for carp. In fish, according to literature, lymphocytes have a role in specific immune response, neutrophils in the inflammatory response and in phagocytic function, eosinophils have a role in phagocytosis of parasites, monocytes migrate into tissues to become macrophages. Action of stress factors upon the white line cells is manifested as lymphopenia with relative granulocytosis.
Given the results, it can be concluded that there is a low level of stress for the fish in study and they are reared under appropriate, welfare-friendly conditions.

Key words: *fish, stress, hematologic profile, welfare.*

INTRODUCTION

An extensive part of knowledge in the field of vertebrate haematology is based on mammalians′ study. The concept of non mammalian vertebrate blood analysis is relatively new (Claver J.A. and Quaglia A.I.E, 2009), starting to develop successfully as the data concerning the physiology of blood cells and standardized techniques became available (Hrubec T.C. and Smith S.A., 2010).
The above authors (Hrubec T.C. and Smith S.A., 2010) argued that bony fish are "more closely related to other bony vertebrates (e.g. mammals) than they are to their cartilaginous counterparts".
Azevedo et al. (2006) showed that an important health indicator in fish is represented by hematologic profile, which could help the researcher in assessing their welfare.
This approach is also supported by Gabriel et al. (2004), who demonstrated in their study that the environment may have a significant impact upon the haematological parameters in fish.
The environment, especially water quality, can influence the following parameters: packed cell volume (PCV), red blood cells count (RBC), immature erythrocyte count, white blood cells count (WBC) and haemoglobin (Hb).
Leonard J.B.K. and McCormick S.D. (1999) noticed the increasing of the PCV and RBCs number in American shad during upstream migration due to the decreasing of the dissolved oxygen concentration.
El-Sherif et al. (2008) revealed in their study that haemoglobin and PCV decreased in relation with the increasing of the unionized ammonia nitrogen (UIA-N).
Wedemeyer (1996) stated that the exposure to nitrite can affect the thymus by inducing hemorrhagic and necrotic lesions. According to Bowden et al. (2005) the thymus is involved in the development of the adaptive immune system.

Das et al. (2004) described that immature erythrocyte count increased while nitrite − nitrogen concentration increased up to 4 mg/l. The count decreased to zero when nitrite − nitrogen ranged within 8 to 10.4 mg/l, value at which the erythrocytes appeared shrunken and tapered. WBC′s count increased along with the increasing of the exposure period.

Mishra S. and Srivastava A.K. (1979) reported the following haematological changes in a fresh water teleost (*Colisa fasciatus*) exposed to zinc sulphate: decreased RBCs, WBCs count and PCV, with increased erythrocyte sedimentation rate.

Kaoud et al. (2011) found that the exposure of Nile Tilapia (*Oreochromis niloticus*) to cadmium can lead to a significant decrease in PCV, haemoglobin and RBC′s count.

Bozorgnia et al. (2011) studied the effect of temperature on common carp blood parameters and found that the RBCs and WBCs counts increased at 32°C and decreased at 15°C.

The present study aims to analyse the number, percentage distribution and characteristics of the blood cells in order to gain an overall picture of the stress response in carp, with respect to the haematological parameters.

MATERIALS AND METHODS

The biologic material consisted in five individuals of common carps (*Cyprinus carpio*), weighing between 2.5 and 3 kg, collected from a fishpond in Plataresti-Calarasi County. Water temperature was approximately 27°C.

The fish were macroscopically examined for clinical diseases or gross lesions. No visible lesions or gross abnormalities were detected.

Blood was collected from the caudal vessels, as described by Branson. E.J. (2008), using heaprinised sterile syringes with 20-21 gauge needles, depending on fish size. Blood was transferred into Li-heparin containers and refrigerated. Smears were prepared within 4 hours from sampling, air dried and prepared for staining.

Total RBCs count was performed manually using a Burker-Turk chamber and optical microscopy, as described by Dumitru C. Curca. (2005).

The prepared blood smears were stained using Diff-Quick protocol. There were also performed the 100 cell differential count and blood cell morphology examinations.

RESULTS AND DISCUSSIONS

The following cells were identified: erythrocytes, erythrocyte precursors, thrombocytes, and from the white series-lymphocytes, heterophils, monocytes and eosinophils.

The erythrocytes had the higher occurrence in the blood smears. They presented most frequently an oval shape, but round shaped cells were also detected. The nucleus was centrally positioned with an oval to round shape.

The erythrocyte precursors were smaller in size with a round centrally located nucleus. They presented a grater nuclear:cytoplasmic (N:C) ratio than the mature ones.

The thrombocytes were oval, round or spiked cells, smaller than the erythrocytes. They presented centred round to elongated nuclei.

The lymphocytes were the most common detected leukocytes and were classified as small and large ones. The small lymphocytes presented a nucleus that occupied most of the cell space in comparison with the large lymphocytes that presented a higher quantity of cytoplasm. Their shape was round. A small number of lymphocytes with a distorted shape was also found.

The heterophils presented as round shaped cells with a kidney shaped nucleus or having two or three lobes.

The eosinophils were rarely observed in the blood smears. The cells were relatively round with an irregular shaped nucleus and red cytoplasmic granules.

The monocytes were the largest observed white blood cells. They presented an indented nucleus and often vacuolated cytoplasm. A lower nuclear:cytoplasmic ratio and cytoplasmic vacuolas aided in differentiating monocytes from large lymphocytes.

No basophils were detected, but their existence in the peripheral blood is not excluded (Hrubec T.C. and Smith S.A., 2010).

The white blood cells functions and the stress influence on this kind of cells are also described by authors like Hrubec T.C. and Smith S.A., (2010) who stated that the WBC play the following immune roles: lymphocytes participate in innate and specific immune T and B cells response, neutrophils participate in the inflammatory response and possess phagocytic functions, eosinophils protect the fish against parasites being also capable of phagocytosis, monocytes migrate to sites of infection into

tissues and differentiate into macrophages. There are also authors like Clem L.W. et al. (1991) and Ruane N.M et al. (2000) who argue that in fish, the stress response is represented by lymphopenia and granulocytosis.
The percentage distribution and total RBC count is presented in tables 1 and 2.

Table 1. RBC complete count and percentage distribution of white blood cells: samples (S) 1-3

Parameter	S.1	S.2	S.3	Reference values (Hrubec & Smith 2010; Svobodova, Vykusova 1991)
Red blood cells x 10^6/µl	**2.11**	**1.98**	1.69	1.69-1.91
Lymphocytes%	78	85	**73**	76-97.5
Heterophils%	**11**	8	**19**	2-10
Eosinophyls%	1	**2**	1	0-1
Monocytes%	**10**	5	**7**	3-5
Basophils%	0	0	0	0-0.5

Table 2. RBC complete count and percentage distribution of white blood cells: samples (S) 4-5

Parameter	S.4	S.5	Reference values (Hrubec & Smith 2010; Svobodova, Vykusova 1991)
Red blood cells x 10^6/µl	**1.57**	1.73	1.69-1.91
Lymphocytes%	91	86	76-97.5
Heterophils%	7	9	2-10
Eosinophils%	0	1	0-1
Monocytes%	**2**	4	3-5
Basophils%	0	0	0-0.5

Thus, according to the results above, the 5 studied individuals presented a reduced number of parameters slightly exceeding or below the reference ranges.

CONCLUSIONS

The study showed that the fishes presented a low level of stress and that the rearing conditions are adequate in terms of welfare.

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