Effect of feeding with different dietary protein level on leukocytes population in juvenile Siberian sturgeon, *Acipenser baeri* Brandt

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**SUMMARY**

Haematological parameters are important parameters for the evaluation of fish physiological status. The aim of present study was to obtain a basic knowledge of the WBC complex reactions 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/individual, 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 VE46P, respectively ALLER CLASSIC granules with a protein content of 30 % for VE30P (daily ration of 3 %). The sampling of *Acipenser baeri* blood from the four variants before and after the experimental trial allowed determination of white blood cell count. In order to achieve the purpose of the experiment the blood samples were immediately used to make smears which were stained with May-Grundwald Giemsa panoptic method. We also determined the leukogram and the absolute number of leukocytes. The statistical significance was determined using independent Student's t-test. We have identified the following types of white blood cells: lymphocytes, monocytes, neutrophils and eosinophils granulocytes. Basophils granulocytes were absent. Physiological stress induced by maintenance in different technological condition induced by percentage of protein in feed, is reflected in the haematological parameters - white blood cell count (significant decrease, p=<0.05, WBCc). The total number of leukocytes has been registered statistically significant changes: lymphocytes (relative and absolute) have significant reduction with 39.21 %, decrease in monocytes by 39 – 55 %) and neutrophils have been registered a slight reduction (10.6 – 24.7 %). In this experiment, technological condition did affect the immune defence system of the *Acipenser baeri* species.

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**INTRODUCTION**

*Acipenser baeri* is a species that represents a feasible candidate for aquaculture having a good growth rate in different systems of production being capable to reach the sexual maturity in captivity (Ronayi, 1990). The primary results of the physiological researchers of the nutrition were promising (Dabrowski, 1985, Medale, 1989). Proteins, main components of the pellet food for fish are essential for growth, reproduction and maintenance.

Numerous studies have highlighted the special role of ensuring and maintaining quality feed in a normal physiological state. Both quantity and proportion of nutrients in fish feed can influence susceptibility to disease. Fish nutrition researches have emphasized the importance of nutrients in maintaining normal immune function and resistance to disease (Blazer and Wolke, 1984, cited by Maita, 2007).

The number of white cell in circulating blood (WBC) is not constant; it varies with phylogenetic position, age and physiological condition of the organism, the range being rather large: \(20 \times 10^3 - 80 \times 10^3\) cells/µl of blood (Huston, 1990, Nicula, 2004). In general, the average WBC is 100 times lower than the red blood cell (RBC); for comparison the BBC/WBC ratio in mammals is approximately 500 (Wojtaszek et al., 2002).

General reduction of leukocytes number is the characteristic response to stress of all vertebrates (Ellis, 1977). Long lasting deprivation of food results in a decreased haematological indices and the percentage of lymphocytes, eosinophils, monocytes. The neutrophils may develop degenerative changes (Sopinska, A., 1984).

Since the blood, due to the dynamism and functions that it fulfils in the organism, "mirrors" the general physiologic state of the fish, the research from this study has monitored the haematological answer by evaluating the reactions of the leukocyte complex in the Siberian sturgeon under the influence of feed protein level.

**MATERIAL AND METHODS**

*Fish biomass and the growing condition*

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

Table 1. The synthetic table regarding the experimental version

<table>
<thead>
<tr>
<th>Experimental version</th>
<th>VE$_{46P}$</th>
<th>VE$_{30P}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total biomass (g)</td>
<td>2160</td>
<td>2160</td>
</tr>
<tr>
<td>No. individuals</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Individual mean weights (g/fish)</td>
<td>135</td>
<td>135</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>11.25</td>
<td>10.66</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>0.10</td>
<td>0.08</td>
</tr>
</tbody>
</table>

This experiment, that lasted 45 days, followed the influence of two types of feed with different protein content on the physiological status of the species *Acipenser baeri*. Fish were fed with commercial pellets. The two treatments were replicated and noted as follows:

- $VE_{46P}$ – experimental groups of *A. baeri* fed with a commercial fodder containing 46% crude protein
- $VE_{30P}$ – experimental groups of *A. baeri* fed with a commercial fodder containing 30% crude protein

For this experiment, the water quality parameters maintained in the optimum domain: temperature 20 – 22 °C; dissolved oxygen 6.2 – 9.8 mg l$^{-1}$; pH 6.7 – 7.1; unionised ammonia < 0.0003 mg l$^{-1}$, corresponding to the species *A. baeri*. The equipment for the conditioning of the quality of water have succeeded in treating and reusing the technological water, taking into consideration the fact that the water daily loss did not exceeded 10% out of the total volume of the system. No mortalities were recorded during the experiment.

**Blood sampling and analysis**

The 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. At sampling, fish were quickly anesthetized with 2-phenoxyethanol (1:500, Sigma).

Blood was analysed with routine methods used in fish haematology (Blaxhall, Daisley, 1973). For each exemplar two blood smears were immediately dried, fixed and then stained with May-Grünwald Giemsa panoptic method (MGG). Leukogram/leukocyte formula represented the percentage of different types of white blood cells (neutrophils, eosinophils, basophils, monocytes and lymphocytes), determined by counting them in a
stained blood smear under a microscope. Microscopic analysis is done in oil immersion under a strong light, at a 1000- to 1500-fold magnification. The relative proportion (percentage) of each type of white blood cells was obtained by examination of 200 leukocytes on blood smears. The types of leukocytes were determined based on identification characters listed by Svobodova, 1991. Absolute number of circulating blood leukocytes and thrombocytes were determined in relation to 1000 erythrocytes in haemograms stained with panoptic method MGG and converted to unit blood volume.

Statistical analysis
The different types of white blood cells (expressed as a percentage and absolute number) of the four experimental groups were expressed by mean and standard deviation and differences between the values were statistic analysed with t-Student test.

RESULTS AND DISCUSSION
Following this experiment were also analysed the reactions of the leukocyte’s system, in order to determine the effect of the influence of level of protein in feed on the immune system defences and for a fair assessment of physiological changes in Acipenser baeri.

In order to assess the changes in leukocytes we performed both qualitative analysis by observing the morphological particularities of the leukocytes and quantitative analysis to evaluate the relative changes ("leukogram") and the absolute changes (cells/µl blood) of the different types of leukocytes. The microscopic examination of blood smears stained by MGG, did not show morphologic changes in leukocytes.

Almost all types of white blood cells smears were identified on the examined blood samples: small and large lymphocytes (predominating the small lymphocytes), monocytes, granulocytes neutrophils (promyelocytes, metamyelocytes, and neutrophils with un-segmented kernel or with two or four lobs), and rare eosinophils. Basophiles were not found at all.

The white blood cells in Acipenser baeri, compared with other species (cyprinids, salmonids), are fewer in number but larger in volume. Lymphocytes were dominant, both as percentage (70.03 – 77.98 %) and as absolute number (12.16 – 17.15 x 10^3 cell/µl). Lymphocytes have usually been considered the most abundant of the leucocytes in the peripheral blood of healthy fish. On the other hand, neutrophils are scarce, while basophils and eosinophils only occasionally are seen (Alexander, 1980). Our results concerning the two experimental groups confirm these data.
The number and percent composition of leukocytes in circulating fish blood are extremely variable even among conspecific individuals or similar
conditions depending on numerous factors. Palikova (1999), in a quantitative investigation of the leukocyte system of *Acipenser baeri*, finds the following values of the relative number (percentage) of leukocytes: lymphocytes 78.2%, monocytes 0.1%, neutrophils 15.9%, eosinophils 3.4%, blasts 2.4% and basophils granulocytes were absent.

Analysing the variation of the mean percentage of the different types of leukocytes (fig. 1 - 4) we noticed differences both between the two experimental variants and between the initial and final investigation:

- The average percentage of the neutrophils increase in B3f version with 69% from B3i and with 15.5% from B1f. The percentage of neutrophils B1f version is increased by only 16.6% of B1i version.
- The average percentage of eosinophils was reduced by 34.7% in B3f version compared of B3i and with 6% to B1f. The percentage of eosinophils in B1f variant was reduced by 37.3% to B1i version.

In order to obtain a detailed image on the changes produced in the leukocyte system, the number (percentage) relative to white cells (leukogram) was correlated with the absolute number of white blood cells (no. cells / µl blood).
The dynamics of the absolute number of different types of leukocytes showed similar changes observed in the white blood cell counts.

A primary analysis of the absolute number of WBC values and of the different types of white blood cells, presented in Table 2, emphasize a proportional reduction in lower crude protein feed content.

In the case of fish fed with higher protein content pellets (VE₄₆P), the leukopoetic organs were stimulated to synthesize more white blood cells (here was registered a slight increase of 6.63% of the total number of leukocytes) and therefore physiological optimum state was installed.

The absolute number of circulating blood lymphocytes from the species A. baeri in VE₃₀P was reduced significant (p < 0.05) with 34.18 % comparing with initial moment and with the 39.21% comparing with VE₄₆P.

Table 2. Variation of the with blood cell absolute number (x10³ cells/µl blood)

<table>
<thead>
<tr>
<th>Experimental version</th>
<th>Leukocytes (mean ± SD)</th>
<th>Lymphocytes (mean ± SD)</th>
<th>Monocytes (mean ± SD)</th>
<th>Neutrophils (mean ± SD)</th>
<th>Eosinophils (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VE₄₆P initial</td>
<td>19.97±3.2</td>
<td>15.89±2.5</td>
<td>0.31±0.02</td>
<td>3.03±0.5</td>
<td>0.74±0.09</td>
</tr>
<tr>
<td>VE₄₆P final</td>
<td>21.25±4.1</td>
<td>16.42±3.6</td>
<td>0.29±0.06</td>
<td>3.89±0.6</td>
<td>0.65±0.11</td>
</tr>
<tr>
<td>VE₃₀P initial</td>
<td>21.27±3.8</td>
<td>16.74±3.8</td>
<td>0.38±0.25</td>
<td>3.45±1.1</td>
<td>0.70±0.14</td>
</tr>
<tr>
<td>VE₃₀P final</td>
<td>16.03±4.1</td>
<td>12.16±5.1</td>
<td>0.17±0.08</td>
<td>3.12±0.9</td>
<td>0.58±0.21</td>
</tr>
</tbody>
</table>

- a - significant differences (between those two experimental variants) for t-Student test applied to paired variables;  
- b - insignificant differences (between those two experimental variants) for t-Student test applied to paired variables.

Similar results were obtained by other authors: distinctive leukopenia in neutrophils and leukophilia in lymphocytes following dietary protein and fat content increase were observed. It was concluded that within the analysed range of values the increase of dietary protein and lipid content improved the physiological status of sturgeon hybrid fingerlings (Gershanovich, Kiselev, 1993).

The primary consequence of reducing the total number of leukocytes is immune suppression and increased susceptibility to diseases (Wedemeyer and Wood, 1974).

The same trend of reduction was noted also for absolute number of monocytes, but the phenomenon was more pronounced. Thus it was registered a decrease by 55.26% for the VE₃₀P experimental variant in final analyses compared with initial investigations and with 39.29% comparing with VE₄₆P, respectively.

Inadequate nutrition may be a factor that results in a decreased number of lymphocytes. Mononuclear cells of circulating blood significantly increased in number for tilapia fed with the feed containing bacterial-derived 3-1.3
glucans (Cain and al., 2003). Klinger and al. (1996) noted that food rich in fat affects platelets number and their function.

**CONCLUSIONS**

The results from present paper have confirmed the working hypothesis, that feed quality in intensive aquaculture is a crucial factor for ensuring the metabolic health of fish culture.

Disorder affecting metabolic processes affected the immune defence system, this process being emphasized by reducing circulating blood leukocytes of fish in VE30P.

For *Acipenser baeri*, the administration of a fodder containing 30 % proteins had as consequence the depreciation of the physiological status, the fish being exposed to the possible influence of different disease induction factors.

The results of this study indicate the necessity to effectuate regularly of the exams, because many nutritional deficiencies can produce many diseases so serious or more serious than the diseases produced by the pathogenic germs and the damage (injury) produced by the nutritional disturbances are bigger because is it registered a sub clinical evolution, that can cause weak performances in what is concerning the growth and problems of reproduction.

**REFERENCES**


Maita, M., 2007. Fish health assessment in: Dietary supplements for the health and quality of cultured fish


