The Effect of Feeding with Different Dietary Protein Levels on Haematological Profile and Leukocytes Population of Juvenile Paddlefish, *Polyodon spathula*

Marian Tiberiu Coadă¹, Neculai Patriche², Victor Cristea¹, Alina Antache¹, Săndiţa (Plăcintă) Ion¹, Mirela (Creţu) Mocanu¹, Ştefan Mihai Petrea¹

¹ “Dunarea de Jos” University of Galaţi, The Department of Aquaculture, Environmental Science and Cadastre, 800149- Galaţi, Dr. Alexandru Carnabel, 61, Romania
² Institute of Research and Development for Aquatic Ecology, Fishing and Aquaculture, 800 211-Galati, Portului, 54, Romania

Abstract

The aim of this paper is to evaluate the haematological parameters and the leukogram of paddlefish juveniles fed with different protein level diets. The experiment was conducted in a recirculating system, populated with 112 days old paddlefish juveniles with a mean weight of 57.93 g. During the experiment, two different types of pellets were tested: Aller Performa (54% crude protein) at variant V1 (B1, B3) and Classic Extra 1P (41% crude protein) at variant V2 (B2,B4). The haematological parameters analyzed were: RBC, Hb, Ht, MCV, MCH and MCHC. In V1 case, RBC, Hb, Ht and MCV registered lower values than those from V2 while MCH and MCHC were higher. By study all the different types of leukocyte found on blood smears (lymphocytes, monocytes and granulocytes), the leukogram was determined. The physiological stress induced by different protein level diets reflected the absolute number of leukocytes. Thus, at V2, the value of 82.05 × 10³ WBC/mm³blood was significantly higher than the one from V1 (50.80 × 10³ WBC/mm³blood). A decrease of lymphocytes percentage was observed in case of V1 (59%), comparing to V2 (71.125%). This shows a decrease of fish immunity, but in exchange, the percentage of neutrophils increased. As a conclusion, in terms of physiological condition, percentage of 41% crude protein (V2) led to a better health condition of paddlefish juveniles.

Keywords: haematological profile, leukograma, *Polyodon spathula*, recirculating aquaculture system

1. Introduction

*Polyodon spathula* (Walbaum, 1792) – paddlefish – Order *Acipenseriformes*, Family *Polyodontidae*, is a fresh water primitive sturgeon, originally from North America, a plankton feeder, mainly of zooplankton, aquatic terrestrial insects [1, 2], chironomidae and great quantities of vegetal scrap [3], but also phytoplankton, organic detritus and plant seeds (Zoology of vertebrates collective – prof. dr. Lotus Mester, Biology Faculty of Bucharest University) [4]. It can reach a total length (TL) of 2.2 m and a body mass of 91 kg, [5], the most common body mass being of 4.5–7 kg [6].

Proteins, the main components of fish pellets, are essential for growth and reproduction of fish species. Numerous studies have highlighted the special role of ensuring and maintaining feed quality in a normal physiological state. Both quantity and proportion of nutrients in fish feed can influence susceptibility to disease. The researches in fish nutrition have emphasized the importance of nutrients in maintaining normal immune functions and resistance to diseases [7]. Any disturbance of environmental condition, extent or duration of certain activities, including
the quantity and quality of food availability, may become a fish stress factor, diagnosed as such, by studying the fish main haematological (index) value [6].

The haematological indices, such as the red blood cells number (RBC – x10^6 cells/µl blood), haemoglobin (Hb – g/dl), hematocrit (Ht-%) and the erythrocyte constants: the mean corpuscular volume (MCV–μm³), the mean corpuscular haemoglobin (MCH–pg), the mean corpuscular haemoglobin concentration (MCHC–g/dl), are important parameters for the evaluation of general physiological condition of the fish and are considered as stress indicators to estimate their response reactions to various environmental conditions.

It is well-known that blood sampling, laboratory techniques, seasonal variations, fish size, genetics patterns, stocking density, food privation, social stress, handling and transport can influence the haematological values [8, 9].

At the Institute of Research and Development for Aquatic Ecology, Fishing and Aquaculture from Nucet, Dambovita, a preliminary haematological study began in 1995, regarding the determination of some biochemical indexes necessary for the establishment of sanguine metabolic profile of *Polyodon spathula* species, being known the fact that the changes, on this level, acts as a high sensitivity sensor, as far as the integrity of environmental conditions are concerned, especially of nutritional status of cultured fishes as well as for wild ones [6]. The changes of the haematological indices depend on fish species, age and cycle of sexual maturity and also on their health condition [10]. General reduction of leukocytes number is the characteristic response to stress of all vertebrates [11, 12].

The present study followed the physiological answer by evaluating leukocytes complex reactions and haematological profile of the paddlefish juveniles fed with different protein level diets.

2. Materials and methods

*Fish biomass and the growing condition*

The experiment was conducted during the period 01/09 to 07/10/2010 in the Pilot Laboratory of Aquaculture, Environmental Sciences and Cadastre Department, Faculty of Food Science and Engineering. Recirculating fish farming system consists in four rearing units with an increase volume of 300 L and the dimensions of 100x80x40 cm. The unit was equipped with mechanical and biological filtration, sterilization unit (UV-C equipment 35,000 Tetra Quiet, power 36 W), aeration (compressor Resun Quiet LP-100 with 100 w power, pressure and flow of 0.045 MPa air 150 L/min.). Water quality in a recirculating system depends largely on the amount of dissolved oxygen, carbon dioxide concentration and nitrogen compounds [13]. Temperature and dissolved oxygen, the main physico-chemical water parameters, were measured daily with a Hach-Lange equipment Sc 1000. Nitrogen compounds (N-NO₂⁻, N-NO₃⁻, N-NH₄⁺) were determined periodically with Spectroquant Nova 400 type spectrophotometer, using kits, compatible, Merk. Recirculating system ensures a flow of 12 L/min/rearing unit.

<table>
<thead>
<tr>
<th>Table 1. Chemical composition of feed used in experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of feed</td>
</tr>
<tr>
<td>Variant</td>
</tr>
<tr>
<td>Composition</td>
</tr>
<tr>
<td>Crude protein</td>
</tr>
<tr>
<td>Fat</td>
</tr>
<tr>
<td>Ash</td>
</tr>
<tr>
<td>Fiber Assay</td>
</tr>
<tr>
<td>P</td>
</tr>
<tr>
<td>Vitamin A</td>
</tr>
<tr>
<td>Vitamin D3</td>
</tr>
<tr>
<td>Vitamin E</td>
</tr>
</tbody>
</table>

The rearing units were populated with juvenile paddlefish aged 112 days, from the Centre of Recherches and Development of Aquaculture Nucet [14]. The popular, juveniles had a mean
weight of 57.93 g and an average total length of 26.13 cm. During the experiment, two different types of pellets were tested: Aller-performance, 54% crude protein, respectively 1P Extra Classic, with 41% crude protein. Thus, two experimental variants were designed, in duplicate, each being assigned to two growth units for each variant (V1 -B1 and B3, V2-B2 and B4). Daily ration during the experiment was 2% and frequency of meals was equal to 6 times per day, using nutritious low-capacity machines. The main chemical characteristics of the two types of feed are presented in Table 1 [15].

Blood sampling and analysis
The blood samples were taken from the caudal vein of the fish using lithium heparin as anticoagulant. Blood was analysed with routine methods used in fish haematology [16]. The red blood cells number (RBC - x 10^6/μl blood) was determined by counting the erythrocytes from 5 small squares of Neubauer hemocytometer using Vulpian diluted solution. The hematocrit (Ht - %) was determined, in duplicate, using heparinised capillary tubes centrifuged for 5 minutes at 12000 rpm in a micro hematocrit centrifuge. Haemoglobin concentration (Hb - g/dl) was estimated by Sahli method. Using standard formulas according Ghergariu, in 1985 [17], the red blood indices were calculated: the mean corpuscular volume (MCV - μm³), the mean corpuscular haemoglobin (MCH - pg) and the mean corpuscular haemoglobin concentration (MCHC - g/dl). For determining the leukogram and absolute number of leukocytes, two blood smears were made for each sample and after that, they were immediately dried fixed and then colored with May-Grünwald Giemsa panoptic method (MGG). The percentage of each type of white blood cells was obtained by microscopic examination of 200 leukocytes on blood smears. The leukocytes type was determined based on identification characters listed by Svobodova Z., in 1991 [18]. Absolute number of peripheral blood leukocytes and thrombocytes were determined in relation to 1000 erythrocytes in haemograms stained with panoptic method MGG and converted to unit blood volume [12].

Statistical analysis
The haematological parameters and the different types of white blood cells (expressed as a percentage and also as a absolute number) from all four experimental groups were expressed by mean and by standard deviation. Resulting values were processed in Microsoft Office Excel.

3. Results and discussion
Because of the dynamism and the functions that are fulfilled in the organism, the blood represents the "mirror" of general physiological condition of the fish. Thus, the haematological modifications of the studied fish were analyzed in collaboration with the technological factors (fed with different protein level) which can influence the metabolic processes. The haematological parameters are presented in Table 2.

Table 2. Haematological parameters of the Polyodon spathula during the experiment

<table>
<thead>
<tr>
<th>Haematological parameters</th>
<th>V1 (54% crude protein)</th>
<th>V2 (41% crude protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (x 10^6cel./μl)</td>
<td>0.69±0.05</td>
<td>0.92±0.17</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>3.68±0.18</td>
<td>4.10±0.10</td>
</tr>
<tr>
<td>Ht (%)</td>
<td>15.42±3.34</td>
<td>25.71±5.85</td>
</tr>
<tr>
<td>MCV (μm³)</td>
<td>227.79±27.32</td>
<td>282.64±21.73</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>55.64±3.98</td>
<td>47.52±6.18</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>23.61±3.01</td>
<td>16.18±3.94</td>
</tr>
</tbody>
</table>

*(mean ± standard deviation)

Following the haematological analysis, a series of changes were observed. The mean number of erythrocytes was 0.69 x 10^6cel./μl blood in variant V1 (feed with 54% crude protein) compared to 0.92 x 10^6cel./μl blood from variant V2. It should be mentioned that in case of sturgeon species (cartilaginous fish), the number of erythrocytes is smaller compared with the one of teleostean fishes, due of their inferior position occupied in systematic class. Thus, even if in the V1 variant, the number of erythrocytes was significantly reduced, it has maintained within the normal limits. For example, at Acipenser baeri species, growth under feeding with low protein level diets
(30%), the number of erythrocytes was 0.57±0.05 x 10⁶ cel./μl blood [12]. At *Acipenser brevirostrum* species, the number of erythrocytes reference interval is situated between: 0.65 to 1.09 x 10⁶ cel./μl blood [19]. Reducing the quantity of haemoglobin in V1 variant is correlated with the reduction of red blood cells number. In case of V1 variant it was recorded a significant decrease of hematocrit value (%) to 15.42±3.34, from 25.71±5.85 as it was in V2 variant. Jarvis et al., found Ht(%) values in shortnose sturgeon (*Acipenser brevirostrum*) ranging from 19.7% to 25% [19]. We can say that a higher percentage of protein in juvenile paddlefish diets can lead to a decrease of red blood cells number, of haemoglobin quantity and also of hematocrit value. In terms of the red blood cells constants, we can say that the reducing of their number, from the variant V1, led to a significant increase of the mean corpuscular haemoglobin concentration (MCHC), also increase the mean corpuscular haemoglobin (MCH) and decrease the mean corpuscular volume (MCV).

**Figure 1.** Morphology of circulating blood cell of the *Polyodon spathula*, 10 oc x 100 ob, MGG staining: a) lymphocytes, b) monocyte, c) basophil (→) and eosinophil (—), d) neutrophil, e) thrombocytes.
At *Acipenser baeri* specie, the mean value of MCV, for a diet containing 30% crude protein, was $362.175 \pm 8.195 \mu m^3$ and in the case of a 46% crude protein diet, the mean value of MCV was $12.41 \pm 295.71 \mu m^3$ [12]. MCHC values for shortnose sturgeon range from 15 to 30 g/dl [19]. In this experiment, the reactions of the leukocytes system were also analyzed, in order to determine the effect of the influence of protein level from diet over the immune system defenses, in case of *Polyodon spathula*.

The leukocyte analysis was realized by microscopic examination of blood smears colored by May-Grünwald Giemsa panoptic method. On the examined blood smears were identified all types of white blood cells: lymphocytes, monocytes, neutrophil granulocytes, eosinophil granulocytes and rare basophil granulocyte.

In this experiment, both relative number and also absolute number of leukocytes (leukograma - %), were determined. The number and the percentage of leukocytes in fish blood are extremely variable even among conspecific individuals grow in similar conditions. From Figure 2 and Figure 3, it results the mean percentage of different types of leukocytes. Therefore, in case of V1 variant, the relative percentage of paddlefish juveniles leukocytes was 59%, comparing with the one from V2 variant, which was 71.125%. It should be mentioned that a higher percentage of lymphocytes have an important role in the defending the body against diseases. The percentage of monocytes, neutrophil granulocytes and eosinophil granulocytes was higher in the variant V1.

![Figure 2. Leukograma to Polyodon spathula / V1](image1)

![Figure 3. Leukograma to Polyodon spathula / V2](image2)

On the analyzed blood smears young forms of the neutrophil granulocytes and the eosinophils were observed, this fact being interpreted as a hyper functional element [6]. The basophils granulocytes percentage was insignificantly, though it seemed equally, both in the variant with 54% crude protein (V1) and also in the variant with 41% crude protein (V2). Palikova (1999), in a quantitative investigation of *Acipenser baeri* leukocyte system, found the following values of the relative number (percentage) of leukocytes: lymphocytes-78.2%, monocytes-0.1%, neutrophils-15.9%, eosinophils-3.4%, 2.4% blasts and basophils granulocytes were absent [21].

For obtaining a eloquent image on the changes produced in the leukocyte system, the percentage of white cells (leukograma) 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 (Table 3).

In the case of fish fed with lower protein pellets (41%), leukopoetically organs were stimulated to synthesize more white blood cells and therefore physiological optimum condition was installed. Knowles et al, in 2006, found leukocyte values in *Acipenser brevirostrum* ranging from 28376 to 90789/μl blood [19]. Primary consequence of reducing the total number of leukocytes is immune suppression and increased susceptibility to diseases [22]. The absolute number of circulating blood lymphocytes from paddlefish juveniles in V1 was reduced significant compared with V2. Inadequate nutrition may be a factor that generates a decreased number of lymphocytes.
Table 3. Changes in absolute values of the white cells of *Polyodon spathula* species

<table>
<thead>
<tr>
<th>White blood cell absolute number (x10^3 cell/μl blood)</th>
<th>V1 (54% crude protein)</th>
<th>V2 (41% crude protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes</td>
<td>50.80±16.52</td>
<td>82.05±3.12</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>29.74±8.27</td>
<td>57.70±3.50</td>
</tr>
<tr>
<td>Monocytes</td>
<td>1.09±0.51</td>
<td>1.55±0.14</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>19.39±8.16</td>
<td>21.92±7.41</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.50±0.50</td>
<td>0.81±0.56</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.08±0.08</td>
<td>0.09±0.09</td>
</tr>
</tbody>
</table>

* (mean ± standard deviation)

Self-feeding systems reduce stress and boost immune defences, as indicated by an increase in anticoagulant titre and lymphocyte counts [23]. It has been observed a reduction in the number of monocytes and neutrophils granulocyte in variant with higher protein content. Mononuclear cells of circulating blood significantly increased in number at tilapia fed with feed containing bacterial-derived 3-1.3 glucan [24]. Klinger and al. (1996) noted that food rich in fat affects platelets number and their function [25]. Thrombocytes were included as a separate category in the cell counts. The absolute number of thrombocytes was of 10.61±3.65 x 10^3 cel./μl blood in V1 variant and in V2 variant was of 7.16±2.85 x 10^3 cel./μl blood. Recent studies have shown that thrombocytes are involved in homeostasis and defense mechanisms, in teleostean fish being produced mainly in the spleen and kidneys [26].

4. Conclusions

The results of these studies have pointed the special role of the protein content of fish pellets in ensuring and maintaining a normal physiological condition. It has been shown that a higher feed protein content generates a decrease of metabolic processes, reflected by significantly reducing of erythrocytes number, haemoglobin quantity, hematocrit value and mean corpuscular volume. This aspect leads the increase of mean corpuscular haemoglobin (MCH) and of mean corpuscular haemoglobin content (MCHC). So we can say that the experimental conditions of our research have led to significant changes of the main haematological indicators.

Due to the significant decrease in the percentage of lymphocytes in blood, in V1 variant the immune defense system of paddlefish juveniles was affected. In terms of growth performance, it was observed a significant increase at V2 variant.

For *Polyodon spathula* species, the consequence of increasing the percentage of protein in feed was the depreciation of physiological condition and a possible influence of the fish sensibility to disease. As a conclusion, in terms of physiological condition, percentage of 41% crude protein (V2) led to a better health condition of paddlefish juveniles.

The results of this study indicates the necessity to effectuate regularly exams, because many nutritional deficiencies can produce diseases, even more serious than those produced by the pathogenic germs and damages (injury) produced by the nutritional disturbances are bigger because a sub clinical evolution is registered, that can cause weak performances of growth and also reproduction problems.

Acknowledgements

The work of Coadă Marian Tiberiu and Ion (Placintă) Sânduţa was supported by Project no. 88/1.5/S “Efficiency of PhD Students Activity in Doctoral Schools no.61445- EFFICIENT and the work of Mocanu (Creţu) Mirela was supported by Project 107/1.5/S The Quality and Continuity of Training as Part of a Doctoral Studies: ID - 76 822 TOP ACADEMIC.

References

gandica.pdf).
14. Coadă, M.T., Patriche ,N., Cristea, V., Costache, M., Bocioc, E., Ĉalin (Sandu), P.G., Sion (Bădălan), C. Preliminary results on the influence of the quality of feed on growth performance species Polyodon spathula (Walbaum 1792) the conditions of a recirculating system. Lucrări Științifice - Seria Zootehnie, 2011, 55
15. Coadă, M.T., Patriche, N., Cristea, V., Costache, M., Bocioc, E., Ĉalin (Sandu), P.G., Sion (Bădălan), C. Preliminary results on the influence of the quality of feed on growth performance species Polyodon spathula (Walbaum 1792) the conditions of a recirculating system. Lucrări Științifice - Seria Zootehnie, 2011, 55