THE INFLUENCE OF FEEDING FREQUENCY ON THE HAEMATOLOGICAL PROFILE OF *A. STELLATUS* (PALLAS 1771), REARED IN A RECIRCULATING AQUACULTURE SYSTEM

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Abstract

The aim of this study is to assess the physiological status of Acipenser stellatus species (Pallas, 1771) in different feeding frequencies conditions: V1-2 meals/day, respectively V2-4 meals/day. Thus, the physiological status of stellate sturgeon is characterized by hematological indicators, erythrocyte constants, as well as two important blood biochemical parameters (serum protein and serum glucose). At the final of 30 days experiment, both grow performance parameters (FCR, SGR, PER) and hematological parameters analyzed (erythrocyte count, hematocrit, hemoglobin) have almost equal values (statistically insignificant differences $p \geq 0.05$) for both feeding frequencies applied. Among blood biochemical parameters analyzed, glucose values are higher at V1 - 37.53 ± 2.66 mg/dl blood, compared to V2 - 34.26 ± 4.89 mg/dl blood. Regarding serum proteins, the differences between their registered values, for each one of the variants, are statistically insignificant ($p \geq 0.05$). The general conclusion that emerges from this current study shows that in case of six months old stellate sturgeons, reared under recirculating aquaculture system conditions, feeding frequency does not significantly influence their haematological profile.

Key words: stellate sturgeon, feeding frequency, haematological profile

INTRODUCTION

The optimization of nutritional strategies among fish farms can enhance growth and feed efficiency retention [7]. Also, the results of feeding process, applied at an optimal frequency, can lead to tremendous savings in terms of feed costs [5]. Nutritional requirements of sturgeon, reared in intensive recirculating systems were not sufficiently studied. A bad feeding management from the perspective of feeding frequency may influence the welfare of the biological material.

The analysis of biological material haematological profile aims to detect the negative influence of stress. Glucose and serum proteins are also reliable indicators of primary and secondary response of body to stress.

Studies regarding the influence of nutritional management on haematological profile of sturgeons are quite few and they primarily relate to quantitative effect of macro and micro nutrients from feed rations on cultured biomass health [3,6]. However, pathological conditions are mentioned, [12], appeared at fish due to improper feed distribution, from the point of view of feeding period.

The correlation between biological material diet, grown in intensive systems and its haematologic profile is concern of many authors [10, 11, 15]. Thus, this study aims to evaluate the influence of an important aspect of nutritional management, on stellate surgeon grown in a recirculating aquaculture system.

MATERIAL AND METHOD

The current experiment took place during 19.01.2012 and 18.02.2012, at the recirculating aquaculture system pilot station, of Aquaculture, Environmental Science and Cadastre Department, from "Dunarea de Jos" University of Galati, between January-February 2012. The technical description of pilot recirculating aquaculture system is

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presented in detail in other specialized studies (V. Cristea, Euroaliment, I. Vasilean Iasi).

Two variants V1 (B1 and B3) and V2 (B2 and B4) were studied, each experimental rearing unit being populated randomized, with a total number of 13 fish, in order to create homogeneous groups. The only variable that differentiates the two experimental variants was the frequency of feed administration. Thus, the fish from the first experimental variant - V1 received 2 meals/day (at 8:00 and 20:00) while those from the second variant - V2 received 4 meals / day (8:00, 12:00, 16:00, 20:00). The biological material used in this experiment consist of a number of 52 stellate sturgeon, 6 months old, with an initial average individual weight of 97±1.8 g/fish, purchased from Horia sturgeon station, Tulcea - Kaviar House Company. The biological material in question is the result of artificial breeding of captive breeders, first time made in Romanian aquaculture.

Table 1

Initial indicators	Variant 1		Variant 2	
	B_1	B_3	B_2	B_4
Stocking density (kg/m^3)	4,21	4,28	4,09	4,19
Initial biomass (g)	1265	1284	1229	1259
Initial fish number	13	13	13	13
Initial body weight (g)	97	99	95	97

Throughout the experimental period, commercial trout feed (Nutra Pro MP-T) was administrated as extruded mini-granules, with a diameter of 1.7 mm. Biochemical composition of feed is shown in table 2. The feeding intensity used was 2% BW.

Table 2 Chemical composition of feed

Components	Quantity
Protein	50%
Fat	20%
Ash	9%
Cellulose	0,7%
Total P	0,9%
Digestible energy	19,7 MJ/kg
Vitamin A	12000 UI
Vitamin D_3	1800 UI
Vitamin E	180mg
Vitamin C	500 mg

The main physico-chemical parameters (temperature and dissolved oxygen) were monitored daily, with a Hanna HI 9147 portable multi-parameter. Nitrogen compounds ($N\text{-}NO_3^-$, $N\text{-}NO_2^-$, $N\text{-}NH_4^{+}$) - by using spectrophotometer Spectroquant Nova 400 and Merk compatible kits and pH – by using pH meter - model WTW 340, were monitored weekly. The dissolved organic compounds (COD) was determined at the beginning and at the end of the experiment.

Sampling and blood analysis

At the end of the experiment blood was sampled by caudal venous puncture, approximately 1 mL of blood in 20 copies (5 copies for each rearing unit). After sampling, some of the blood was placed in Eppendorf tubes with heparin and the rest in neheparinate tubes. Using the routine methodology from fish hematology [1], haematological indices were measured and analyzed. The number of erythrocytes (RBCc x106/μL) was determined by counting erythrocytes from 5 squares of Neubauer hematocitometer, using Vulpianul as contrast agent. The hematocrit (Ht%) was analyzed using a HETTICH HAEMATOCKIT 210 device for 5 minutes, at 12,000 rotation/ minute. For the accuracy, samples analysis were performed in duplicate. Blood hemoglobin concentration (Hb g / dl) was determined quantitatively, by colorimetric method, with Drabkin reagent, at SPECTROCORD 210 Analytikjena spectrophotometer, at a wavelength of 540 nm.

If for determination of above haematological indices, heparinized blood was used, for measuring biochemical indices (serum glucose and protein) blood without heparin was used. Thus, to obtain blood serum, the blood without anticoagulant was centrifuged 10 minutes, at 3500 rotation / min.

Serum glucose was colorimetrically dosed with o-toluidine at a wavelength λ = 635 nm. Serum proteins were determined spectrophotometrically by Biuret method, at wavelength λ = 546 nm.

After determining hematological indices, using standard formulas [8, 17], erythrocyte constants were calculated (mean corpuscular volume - MCV, mean erythrocyte hemoglobin - MCH, mean erythrocyte hemoglobin concentration - MCHC).

Statistical analysis

Haemalogical parameters were statistically analyzed using Microsoft Excel 2010 statistical computer program, from which we used the following statistical tests: descriptive statistics, parametric t-Student test

RESULTS AND DISCUSSIONS

Haematological examination data, related to eco-technological factors, highlights the physiological state of cultured biomass. Over the experimental period, medial parameters were maintained within the optimal range for stellate sturgeon growth. Thus, in our case, water temperature (20.71 ±0.75°C), dissolved oxygen (6.59± 0.34 mg L^{-1}), pH (7.71±0.37 pH units), nitrite 0.03±0.04 $mg \cdot L^{-1}$, ammonium 0.05±0.04 $mg \cdot L^{-1}$ and organic substance 7.18±3.62 $mg \cdot L^{-1}$ remained approximately constant throughout the experimental period, no significant differences being registered ($p \geq 0.05$). Hematological parameters registered at the end of the experiment are presented in Table 3.

Table 3 The variation of haematological parameters for both experimental variants

Experimental variant		***Haematological indicators***					
		Eryth no. ($x10^6$)	**H_t (%)**	**H_b (g/dL)**	**VEM (μm^3)**	**HEM (pg)**	**CHEM (g/dl)**
V_1	B_1	0,77±0,12	22,2±1,64	7,23±1,61	160,01±31,68	76,92±16,65	46,09±4,05
	B_3	0,73±0,17	21,2±1,1	6,99±0,35	152,75±9,94	60,04±6,39	39,32±3,52
V_2	B_2	0,77±0,13	24,4±3,05	6,76±0,66	148,56±23,21	58,80±9,33	39,99±5,76
	B_4	1,05±0,74	20,2±3,96	6,98±0,34	162,30±31,45	72,37±22,35	43,90±6,31

Both haematological indicators and erythrocyte constants, from table 3, do not show significant variations between the two experimental variants ($p \geq 0.05$).

The average number of erythrocytes is maintained in the normal range of 0.8x106/µL [9]. The low value of erythrocytes, compared to teleostean fish, can be explained by the inferior systematic position of sturgeon. Regarding the two experimental variants, erythrocyte number slightly increases ($p \geq 0.05$) at the variant with 4 meals / day.

The hematocrit shows similar values in both V1 and V2 experimental variants, 21.7±1.42% respectively 22.3±4%, according to insignificant growth trend ($p \geq 0.05$) of average erythrocytes number, from the variant fed with 4 meals/day. The determination of erythrocyte concentration from 100 mL of blood is the most reliable method for assessing the biological material anemia condition. In the literature, the optimum hematocrit value for stellate sturgeon is 47% [9] but, at sturgeon reared in intensive production systems, the hematocrit has lower value. Thus [6], reported hematocrit values of 20-23% for *A. baeri*, [18] and 28-29% for *A. persicus* species. Large fluctuations of hematocrit value indicate, by some authors, stressful conditions or even chronic stress appearance [2].

The amount of hemoglobin in blood decreased insignificant ($p \geq 0.05$) in case of the variant fed with 4 meals/day. Ghittino 1983 [9], reported hemoglobin levels of 11.5g/% at stellate sturgeon but, for sturgeon reared in intensive aquaculture production systems, such as recirculating systems, values of 3.7 - 4.1 g/dL- *P. spathula* [3] and 3.6 - 4.8 g / dL - *A. baeri* [6] were reported.

Erythrocyte constants indicate the qualitative aspect of respiratory function by providing hematological indicators functional information [6].

Thus, the values of erythrocyte constants analysis, calculated at the end of the experiment, keeps a downward trend at the variant fed with 4 meals/day – significant difference ($p \geq 0.05$).

Arithmetic means of mean erythrocytes corpuscular volume (MCV) of V1 - 156.38±22.47 µm, was significantly higher than V2 - 155.43± 7.05 µm. Such a decrease of erythrocytes number from the variant fed twice / day is offset by their volume increase.

Average erythrocyte hemoglobin (HEM) has a value of 66.98±13.96 pg at V1 and 65.59±17.66 pg at V2, also showing a compensation reaction of smaller erythrocytes number by increasing the amount of hemoglobin for each cell.

The most the faithful erythrocyte constant, mean erythrocyte hemoglobin concentration (CHEM) is the ratio of erythrocyte hemoglobin and its volume. In case of both experimental variants, CHEM shows an insignificant increase ($p \geq 0.05$) at the variant fed with 2 meals / day, thereby keeping the trend of other two constants.

Therefore, giving 4 meals/day leads to a better nutrients absorption that creates a better physiological status compared to the variant fed with 2 meals/day.

Determination of glucose and serum proteins represents the most effective and least costly way of stress evaluation [14]. After [16], both glucose and serum proteins are indicators of a poor nutritional conditions caused by starvation, poor content in macro and micronutrients and an inadequate feeding mode.

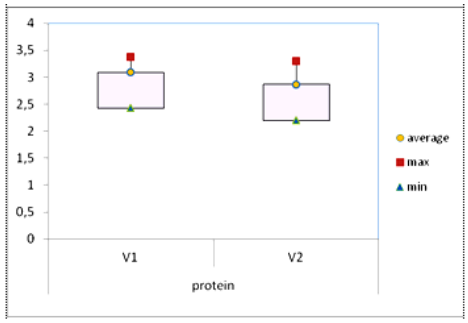

Figure 1 Serum potein variation

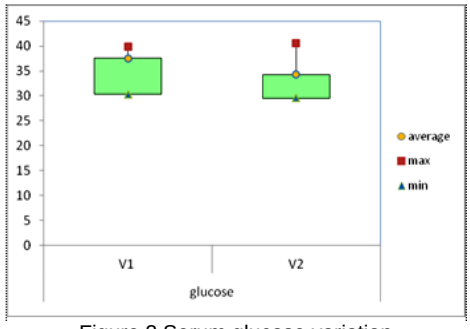

Figure 2 Serum glucose variation

The values of blood biochemical indicators of these are higher at the variant fed with 2 meals / day. Thus, serum proteins registered values of 3.09 ± 0.26 g / dl at V1, respectively 2.87 ± 0.37 g / dl at V2, the differences between the two variants being statistically insignificant - Figure 1. Also, serum protein values for stellate sturgeon, obtained in current study, are similar to others from the literature, 2.75 g/dl [13].

Regarding serum glucose, it remains in the normal range of 30-50 mg / dl [13] - Figure 2. Keeping constant the blood glucose values represents the finest fish homeostatic regulation, involving the liver, extrahepatic tissues and a series of endocrine glands [13].

After the application of comparative statistical analysis, differences between glucose values, at both experimental variants, are significant ($p < 0.05$). Thus, the average serum glucose levels at version fed with 2 meals / day is 2.66 ± 37.53 mg / dl, higher than that from the variant fed with 4 meals / day, 34.29 ± 4.89 mg / dl.

CONCLUSIONS

After the evaluation of biological material growth performance indicators and welfare state, an insignificant influence of feeding frequency can be highlight, in case of stellate sturgeon. Thus, both relative robustness of biological material evaluated at the beginning and at the end of current study and also the interpretation of the values obtained for erythrocyte constants, shows a better performance for growth at the biological material fed with 4 meals / day. Doubling the average individual weight consolidates a higher condition factor and shows a better comfort nutrient absorption in case of the variant fed 4 meals / day, fact that explains also a better welfare state.

The most important indicators for evaluating biological material welfare state, glucose and serum proteins, shows a good adaptation of stellate sturgeon to different feeding frequencies.

The general conclusion that emerges from this study is that for 6 months stellate sturgeon, growth in recirculating aquaculture production systems, feeding frequency did not significantly influence the haematological profile of cultured biomass.

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