Effects of Stocking Density on Growth and Hematological Profile of Early Juveniles Stellate Sturgeon (*A. stellatus* Pallas, 1771) Reared in a „Flow-Through" Production System

Maria Desimira Dicu (Stroe)[*], Victor Cristea, Lorena Dediu, Angelica Docan, Iulia Rodica Grecu, Ion Vasilean

"Dunărea de Jos" University of Galati, Faculty of Food Science and Engineering, Aquaculture, Environmental Science and Cadastre Department, 800008-Galati, Domnească Street, 47, Romania

Abstract

The aim of this paper was to evaluate the influence of four different stocking densities, ranging from 18.7 to 28.6 kg m^3, on growth performance and welfare of juvenile stellate sturgeons, reared in an industrial ,,flow-through'' aquaculture system. At the end of 60 days experimental period, a survival rate of 98% and a stocking density that ranged from 32.5 to 39.8 kg m^{-3} were registered. To assess the biological material growth performance, feed conversion ratio (FCR), specific growth rate (SGR) and profile index were calculated. Regarding the growth performance parameters, better values are encountered at B1 (18.7 kg m^{-3}) and B3 (23.3 kg m^{-3}), appreciable value at B2 (20.4 kg m^{-3}) and low value at B4 (28.6 kg m^{-3}). The fish health state was characterized by the values of hematological indices which presented a specific dynamic. Excepting for hematocrit, all the other hematological parameters have shown significant differences from the statistical point of view ($p>0.05$) at the end of experimental period in comparison with the beginning time. Significant differences were registered between the experimental variants for hemoglobin concentration (Hb) and mean erythrocyte hemoglobin concentration (MCHC), also. As conclusion, it can be admitted that the initial stocking density of 28.6 kg m^{-3} is not optimal for rearing juvenile stellate sturgeons in an industrial ,,flow-through'' aquaculture system.

Keywords: "flow-through" aquaculture systems, hematological profile, stellate sturgeon, stocking density

1. Introduction

The sturgeons' culture practice in intensive recirculating systems assumes a good knowledge of the species-specific physiological requirements and a better technological management in the conditions of maintaining the fish biomass welfare. Usually, the intensity of a production system is given by stocking density defined as the quantity of biomass reported to the volume unit. Different studies [1-3] indicate for sturgeon species, as suitable parameter for determining the intensity production, the surface of the rearing unit (in m^2) instead of the rearing unit volume (in m^3) in correlation with their predominant benthic feeding regime. Establishing a suitable stocking density for sturgeons is still a very studied topic and many studies have been reported on this aspect [4-9] etc. The indirect correlation between growth rate and stocking density has been studied and different parameters as fish age, size, water quality, various items related to nutrition and type of culture system were demonstrated that influence these relationship [10]. Usually, low stocking densities are no economical feasible and the higher ones can lead to alterations of the physiological comfort with significant increasing of the incidence of physical injuries [11], [12],

* Corresponding author: Dicu (Stroe) Maria Desimira
desimira.stroe@ugal.ro; desimira_84@yahoo.com

[13] or diseases [14]. Also, for most species, high stocking densities adversely affect growth ([15]-trout, [16]-sea bass) as consequence of the feed intake decrease, but there are species where high stocking densities have a positive influence (e.g. Arctic charr, [17]). The aim of this paper is to evaluate the production potential of industrial flow-through systems, where early juveniles of stellate sturgeon were reared and to assess the optimal stocking density corresponding to a positive correlation between growth rate, health state in relation with their farming conditions.

2. Materials and methods

Experimental design

The experiment was conducted at Horia sturgeon farm, Tulcea, between September 18-November 18, 2012. The biological material was reared in light green conical fiberglass tanks, with the following dimensions (1.3 x 1.2 x 0.4). The volume of water was about 330 liters, with an inlet flow of 6.7 l/min, which ensure a total water exchange in about 45 minutes. The water inlet was made gravitationally, with continuous flow, from the main reservoir of the station supplied from Horia Lake. At the entrance to the sturgeon station, the water passes through a mechanical pre-filter process, with a Crystal filter of 20 m^3, which has the filter material made by 1-1.5 mm silica particles and then passes through a UV filter adapted to a certain used water flow. 340 stellate sturgeons with an average age of 7 months and an individual mean weight of 80±4 g/ex, were divided into four rearing units, obtaining the following stocking densities: B_1-18.69 kg m^{-3}, B_2-20.37 kg m^{-3}, B_3-23.33 kg m^{-3}, B_4-28.57 kg m^{-3} (Table 1), densities that are considered too high for sturgeons [9].

Table 1. Initial technological indicators

Initial technological indicators / *Rearing units*	*B_1*	*B_2*	*B_3*	*B_4*
Initial biomass (g)	5612	6118	7006	8581
Stocking density related of the feeding aria (kg m^{-2})	6.34	6.91	7.92	9.70
Stocking density related of the water volume (kg m^{-3})	18.69	20.37	23.33	28.57
Initial number of fish	70	80	90	100
Mean individual weight (g)	80	76	78	86

Throughout the experimental period, trout feed (Nutra Pro MP-T) with a diameter of 1.7 mm, was administrated four times per day. The biochemical composition of feed is shown in Table 2. The applied feeding intensity was 1% BW. Feed was distributed automatically with electronic fish feeders made by AGK Company from Germany.

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

Water quality

The main physico-chemical parameters (temperature and dissolved oxygen) were monitored daily with EXTECH 407510 portable multi-parameter. Weekly, nitrogen compounds ($N\text{-}NO_3^-$, $N\text{-}NO_2^-$, $N\text{-}NH_4^+$) were determined using Spectroquant Nova 400 spectrophotometer and Merk compatible kits, as well as the water pH was monitored using the WTW 340 pH-meter.

The main water quality physico-chemical parameters registered normal values during the experiment. Thus, if the experiment was started at an optimal sturgeon growth temperature of 18-20°C [18] it decrease towards the end of the period, reaching 11.8°C (Figure 1).

Dissolved oxygen (DO) and pH (Figure 1) were within the optimal range for rearing stellate sturgeon, although small variations were recorded during the experimental period. Thus, the oxygen did not decreased more than 5.9 mg/L, ensuring the saturation rate ranging between 50% and 70%, suitable for biological material feeding process. For juveniles of *A. transmontanus* [19], [20] reported that a high exposure to hypercapnia (increased CO_2 in the blood) under a slightly acidic pH (below 7) did not affect growth. At current experiment, the pH was slightly alkaline, ranging from 7.22 to 8.41 pH units, which provides for fish normal conditions. Nitrogen compounds (Figure 2) that describes the dynamics of metabolic and nitrification products had normal values for industrial aquaculture waters and no

significant differences were observed between variants, throughout the experimental period ($p>0.05$).

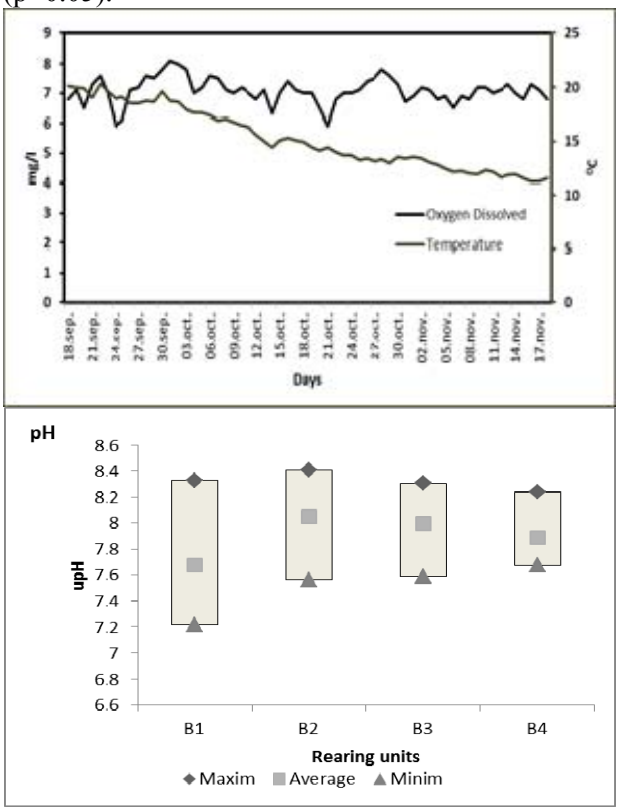

Figure 1.Temperature, DO and pH variation

Growth performance indicators

At the end of the experiment, a series of technological indicators (daily growth rate, feed conversion ratio, specific growth rate and protein efficiency) were calculated using the following formula:

Weight gain (WG)=Final weight (Wt)–Initial weight (W_0) (g);

Food conversion ratio (FCR)=Total feed (F)/Total weight gain (W) (g/g);

Specific growth rate (SGR)=100 x (ln W_t–ln W_0)/t (% BW/day);

Protein efficiency ratio (PER)=Total weight gain (W)/Amount of protein fed;

Also, the correlation between length and weight has been determined by the equation $W=a \cdot L^b$, where W- fish weight (g), L-total length (cm) and "b" the allometric coefficient

Hematological analysis

Blood was sampled by caudal venous puncture at the end of the experiment from 20 fish (5 fish for each rearing unit). After sampling, blood was split in Eppendorf tubes with and without heparin depending by analyze. Using the routine methodology from fish hematology [21], hematological indices were measured and analyzed. The number of erythrocytes (RBCC $x10^6/\mu L$) was determined by counting erythrocytes from 5 squares of Neubauer hematocytometer, using Vulpian solution as diluting agent. The hematocrit (Ht%) was analyzed, in duplicate, using a HETTICH HAEMATOKRIT 210 device for 5 minutes at 12000 rotation/minute. Hemoglobin concentration (Hb g/dl) was determined quantitatively with Drabkin reagent at SPECTROCORD 210 Analytikjena spectrophotometer at a wavelength λ=540 nm.

After determining hematological indices, using standard formulas [22, 23], erythrocyte constants were calculated (mean corpuscular volume-MCV, mean erythrocyte hemoglobin-MCH, mean erythrocyte hemoglobin concentration-MCHC).

For measuring biochemical indices, as serum glucose and protein, blood without heparin was centrifuged 10 minutes at 3500 rotation/min.

Serum glucose was determined using o-toluidine and spectrophtometrically dosed at a wavelength λ=635 nm. Serum proteins were determined by Biuret method at a wavelength λ=546 nm.

Statistical analysis

Technological indicators and hematological parameters were analyzed with Microsoft Excel 2010 applying the following statistical tools: descriptive statistics, parametric t-Student and Anova test ($p=0.05$)

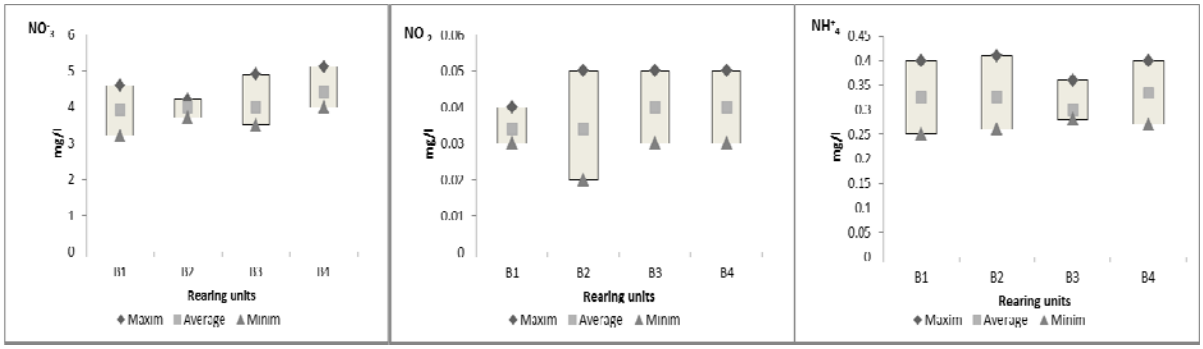

Figure 2. Variation of nitrogen compounds

3. Results and discussion

Fish growth performance

At the end of the experimental period, the first three variants (B_1, B_2, B_3) culture biomass increased by approximately 1.7 times comparing to last variant (B_4) that increased in density by over 1.39 times in conditions of an over 98% survival. Similarly, the stoking density evolution followed the same trend (Figure 4).

It is known that between efficiency of feed utilization factor, expressed by feed conversion ratio (FCR), and the specific growth rate (SGR) is an inverse relationship.

For this experiment we obtained (Figure 3) similar values of FCR's for B_1, B_2, B_3 variants (0.81, 0.92, 0.86), trend that is observed also for SGR. Subunit values of SGR have shown a slower growth rate due to the fish age, similar situation having been reported for *A. baeri* [6], [24], [25]. In the last experimental variant, the values of the two growth performance indicators (FCR, SGR) indicated a decrease of growth performance.

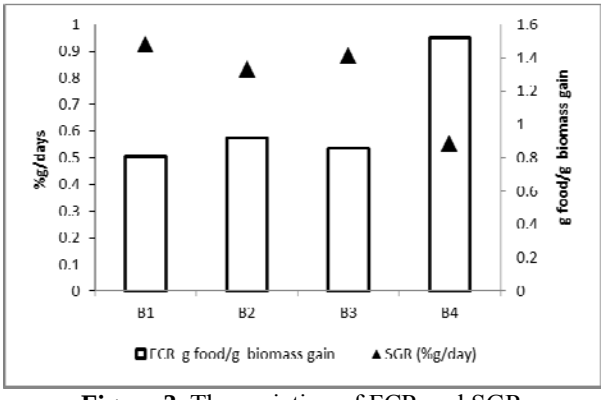

Figure 3. The variation of FCR and SGR

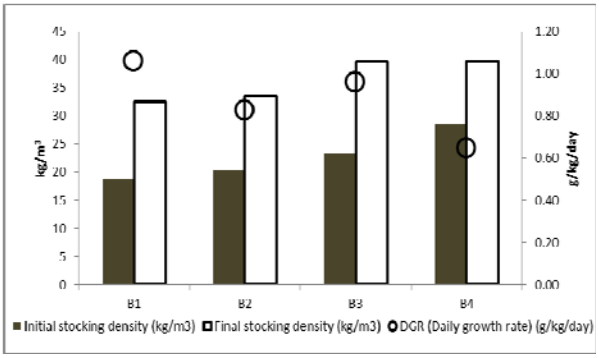

Figure 4.The evolution of stocking density and daily growth rate

Such a decrease in efficiency of feed use may be an indicator of stress caused by high stocking density, fact confirmed for *Huso huso* 93.13±1.04 g [8] reared at a density of 28.64 kg m^{-3}. Also we believe that a downward trend of growth rate with increasing stocking density [26], [27] may be more a consequence of reducing food intake that comes together with crowding stress and less with the strategy of individuals to adapt [8].

Fish condition

A critical analysis of the physiological condition of stellate sturgeon reared under different stocking densities conditions requires calculating, for each experimental group, the relative robustness of fish from different treatments. Thus, length (Lt)-weight (W) regressions were plotted (Figure 5) and fish condition evaluated at the beginning and at end of experiment [28] using index profile/condition factor F ($F=W/L^b$, where "b" is an allometric exponent which has been experimentally determined).

Analyzing the data obtained from processing the individual biometric evaluation, it could be said that no specific trends directly related to the stocking density were observed (Table 3). The

allometric exponent increased for all variants, approaching values closed to an isometric growth at the end of experiments. Index profile thus reveals a higher variability of individual condition in B_2 unit, where the F value dropped from 2.04 to near 0.002, this situation reflecting a length growth detrimental to weight growth. This phenomenon could be explained by a higher genetic variability of the batch. Also, in B_3 unit, the F index increased from 1.83 to 2.14, the fish from this variant showing a more evident robustness comparing with others.

In B_1 and B_4 units the data reflects homogenous groups with relative similar trends regarding weight-length growth. In those cases biomass accumulation was relatively simultaneous with length growth with slightly advantage for the length. This situation is normal for sturgeon sat this stage, when length growth is more evident than the weight growth. The data regarding allometric factor and phenotypic variability of the batches at the end follows the trend of growth performance indicators, registering better values at small stocking densities (Table 3).

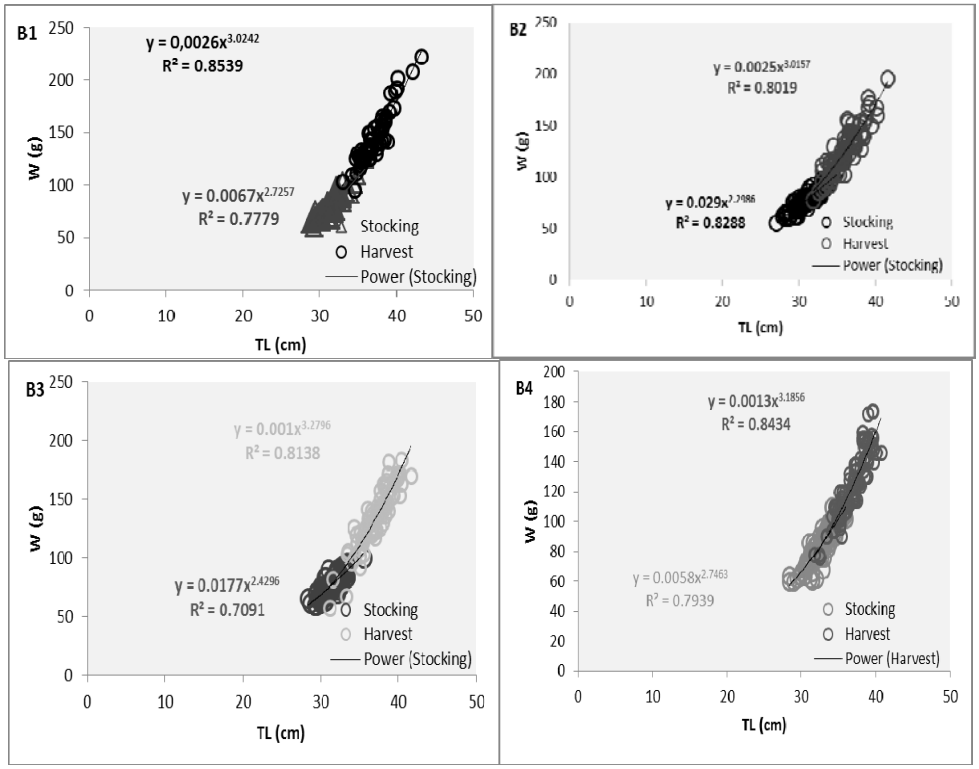

Figure 5. L-W regressions (stocking/harvest) from the each experimental variant

Table 3. The evolution of coefficient of variation for weight; total length and index profile and allometric exponent

Condition indicators	Stocking				Harvest			
	B_1	B_2	B_3	B_4	B_1	B_2	B_3	B_4
Mean weight (g)	80^{a}	76^{b}	78^{ab}	86^{c}	144^{n}	126^{m}	135^{k}	125^{m}
Coefficient of variation weight (%)	14.44	14.04	11.91	15.72	16.7	18.25	17.98	17.57
Mean Total length (cm)	31.32^{m}	30.70^{n}	31.50^{m}	32.69^{p}	37.04^{a}	36.02^{b}	37.05^{a}	36.87^{a}
Coefficient of variation total length (%)	4.47	5.48	4.13	5.15	4.92	5.42	5.41	5.13
Allometric exponent („b")	2.73	2.3	2.42	2.74	3.02	3.02	3.28	3.19
Index profile (F)	0.66^{a}	2.04^{b}	1.83^{c}	0.60^{d}	0.26^{e}	0.002^{f}	2.14^{g}	0.12^{h}
Coefficient of variation index profile (%)	6.43	19.54	6.56	6.89	5.99	53.7	9.17	7.14

The variables with the same letter indicates non-existence of statistical differences ($p>0.05$).

Regarding sturgeon condition, in our case cannot be concluded that stocking density is a factor influencing the growth pattern and robustness. The variability may be associated with other factors such as: initial group structure, genetic variability, dynamic physico-chemical parameters of water quality, the hydrodynamics of rearing units etc.

Fish hematological profile

The data from the hematological examination, related to ones from the eco-technological factors, characterize the physiological state of cultured biomass. The main hematological indicators and derived erythrocyte constants are presented in Table 4. At the beginning, the hematological indices express an anemia, mostly attributed to fish condition variability, life stage and diet. Analyzing at the end of experiment, after applying ANOVA, it was observed that the hematological indicators, except for Hb and MCHC, have shown homogenous values between experimental variants.

Hemoglobin quantity increased significantly in B4 ($p<0.05$), due to the higher density and increased oxygen demand of the fish that caused, in consequence, the erythrocytes to synthesize more hemoglobin in order to maintain the optimum oxygen level in blood. However, hemoglobin values were within the range reported by other authors for sturgeon between 3.6 to 4.8 g/dl [29], [30].

The average number of erythrocytes for all the experimental variants was nearby to the normal value of 0.8 $x10^6/\mu L$ found in sturgeon species [31]. The low number of red blood cells compared to teleost fish, can be explained by the lower position of sturgeons in systematic.

The hematocrit which is an accurate indicator for stressful conditions or distress installation [32], has shown normal values within the range as reported by other authors for sturgeons [29], [33], also. Mean corpuscular volume indicated the highest value in B2 (Table 4). The mean erythrocyte hemoglobin has shown, also, a compensatory reaction of lower number of red blood cells by increasing the amount of hemoglobin for each cell apart. Mean erythrocyte hemoglobin concentration (MCHC) slightly increased with increasing stocking density, apart for the B3 variant.

Determinations of the blood glucose and serum proteins are considered the most effective and less expensive tests in stress evaluation [34]. Thus, in this experiment the serum proteins has shown a constant growth for all four experimental stocking densities tested comparing with initial recorded values (Figure 6). The quantity of serum protein determined at the end of current experiment was nearest the value cited by specialized literature of 2.75 g/dl [35]. Keeping a steady blood glucose control is the finest fish homeostasis involving the liver, extra hepatic tissues and a number of endocrine glands [35].

Table 4. Hematological indicators

Experimental variant		Haematological indicators					
		RBCC ($x10^6$)	PCV (%)	H_b (g/dL)	MCV (μm^3)	MHC (pg)	MCHC (g/dl)
Stocking		0.65 ± 0.04^{a}	23.75 ± 1.71^{c}	2.25 ± 1.71^{e}	368.02 ± 36.04^{g}	34.89 ± 5.46^{i}	9.45 ± 0.71^{m}
Harvest	B_1 -18.7 kg m^{-3}	1.13 ± 0.54^{b}	23.50 ± 4.51^{c}	4.33 ± 0.54^{f}	232.99 ± 75.25^{h}	43.77 ± 15.47^{j}	$18.63\pm1.73^{n*}$
	B_2 -20.4 kg m^{-3}	0.80 ± 0.40^{b}	21.00 ± 2.55^{c}	4.04 ± 0.21^{f}	292.35 ± 79.34^{h}	56.86 ± 16.05^{j}	19.37 ± 1.68^{n}
	B_3 -23.3 kg m^{-3}	0.89 ± 0.11^{b}	23.80 ± 2.59^{c}	4.10 ± 0.41^{f}	270.00 ± 31.1^{h}	46.40 ± 3.90^{j}	$17.20\pm0.90^{p*}$
	B_4 -28.6 kg m^{-3}	0.95 ± 0.16^{b}	24.00 ± 0.89^{c}	5.25 ± 0.68^{i}	257.34 ± 40.59^{h}	55.87 ± 8.42^{j}	21.82 ± 2.15^{q}

The variables with the same letter indicate non-existence of statistical differences ($p>0.05$)

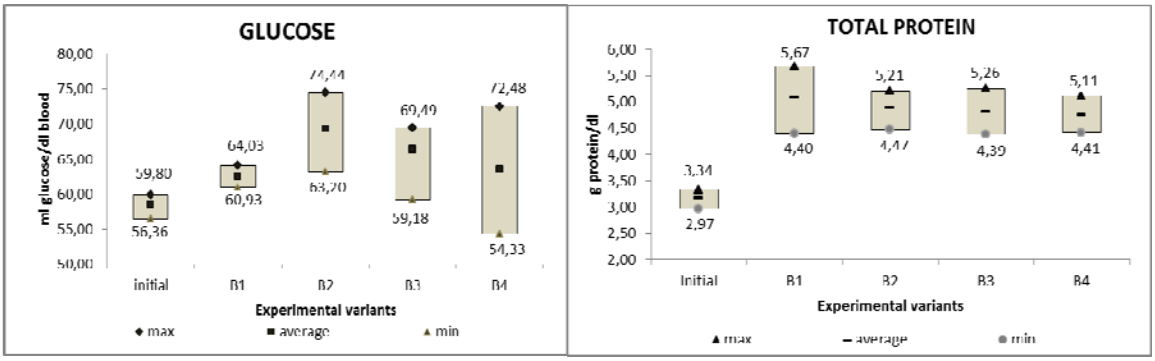

Figure 6 Variation of biochemical indicators of blood serum (glucose- statistical insignificant $p>0.05$ and total protein- statistical significant $p<0.05$ for experimental variants)

4. Conclusions

The results of current experiment have shown that small differences between the tested stocking densities influence the growth condition and welfare of biological material. Thus, insignificant differences were observed between the growth indicators at first three experimental groups (B_1-18, 69 kg m^{-3}, B_2-20.37 kg m^{-3}, B_3-23.33 kg m^{-3}). In terms of growth performance, a decrease was seen at B_4-28.57 kg m^{-3} variant, trend kept also by relative robustness indicator. Regarding sturgeon condition, in our case cannot be concluded that stocking density is a factor influencing the growth pattern and robustness. Increasing heterogeneity into experimental groups is not just an effect of stocking density, other factors as: original group structure, genetic variability, the dynamics of physico-chemical water quality parameters, hydrodynamics at tank level etc., being involved. At the physiological level, from data obtained in our experimental conditions, we can notice a certain stress for the biomass in high stocking density reflected by the level of all correlated hematological indices which can suggest energy-consuming processes to maintain oxygen transfer to tissues. Therefore, in our "flow-through" aquaculture system, densities less than 25 kg m^{-3} are rated as acceptable for growing early juveniles of stellate sturgeon.

Acknowledgements

The work of Desimira Maria DICU was supported by Project SOP HRD – TOP ACADEMIC 76822/2010. The authors are grateful to Dr. Marilena Maereanu, manager of the Kaviar House, for providing the required facilities to conduct this study.

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