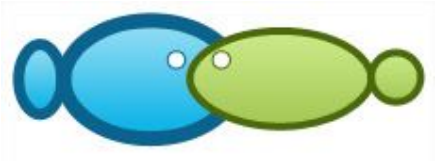

Plasma biochemical responses of hybrid bester juveniles reared at different stocking densities

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Abstract. Serum biochemical parameters are used to monitor physiological and pathological changes and play an important role in the management of endangered species such as some sturgeon. The purpose of this study was to investigate the effect of different stocking densities on the biochemical parameters in bester hybrids, F2 generation, obtained by crossing beluga (*Huso huso*) female and sterlet sturgeon (*Acipenser ruthenus*) male. Bester juveniles (78.02±21.06 g) were stocked at four different desities of 3.19, 4.56, 10.74 and 14.95 kg m^{-3} for 30 days. Serum samplas of 10 specimens from each experimental variant were analysed using commercial kit for blood chemistry autoanalyser (VETTEST 8008 based upon dry chemical technology) for: albumin (g/dL), alanine aminotrasferase (U/L), aspartate aminotransferase, cholesterol (mg/dL), glucose (mg/dL), total protein (g/dL), triglycerides (mg/dL), globulin (g/dL). The results indicated that stocking density had no significant effect (ANOVA, $p>0.05$) of TP, ALB, GLOB, GLU , CHOL, TRIG and ALT in bester. Stocking density had a significant effect only on AST, lowest values of this enzime were observed in 10.74 kg m^{-3} experimental variant. Bester juveniles had low reactions to the stress induced by different stocking densities, suggesting a good capacity for adaptation to this hybrid at the intensive rearing.

Key Words: sturgeon hybrids, stocking density, blood analysis, plasma biochemical indices.

Introduction. Bester is a very common sturgeon hybrid which is a cross between a beluga male (*Huso huso*) and sterlet female (*Acipenser ruthenus*). As many animals species, hybrid sturgeons are bred to get the best characteristics from both parents. Bester hybrid is recommended for aquaculture because it has better growth rates, character inherited from beluga male and early age of sexual maturity like its sterlet female parents (Burtsev 1997). High dietary qualities of bester production continuously provide a great demand on it. The advantages of bester growing in intensive aquaculture system are multiple: easily got used to different rearing conditions and artificial feeds, relatively fast sexual maturity acheaved in controlled systems, valuable caviar (flavour qualities is approached to beluga) grow fast and good resistance to unfavorable factors of aquaculture. The rearing technology of bester hybrid was developed throughout the 1960's (Burtsev 1983).

In Romania, bester is popular among fish farmers and due to production potential, the growth rate and resistance to stress conditions, it is a good candidate for recirculating aquaculture systems. Also, bester is the first hybrid produced in large quantities in a controlled environment (Andrei et al 2016).

Stocking density is an important factor that affects fish growth under intensive condition and it is responsable by assessment the economic viability of the production system. Knowledge of current stocking density practices is importing to appreciate the impact ot the role of any density on economic sustainability. Overcrowding is regarded a chronic stressor factor, ussual in intensive aquaculture systems, which can induce the physiological changes with negative effects at fish biomass. The chronic stress may cause alterations in the physiological parameters such as hematological and biochemical indicators (Ghomi et al 2010; Yarahmadi et al 2015). The results obtained may be used like a instruments to estimate the stress status, but and for optimize the rise conditions of the species (Zarejabad et al 2009). Also, biochemical parameters have been

appreciated as valuable tools to monitoring fish health and are gradually becoming a current practice for assessing their physiological status.

There is little information about the effects of rearing density on bester hybrid sturgeon. Considering the importance of the stocking density in the success of sturgeon production, the present study aimed to evaluate the effects of differnt stocking densities on biochemical parameters in bester hybrid. Therefore, this study was conducted to monitor physiological status of cultured hybrid bester, F2 generation, reared in recirculating aquaculture systems. The results of this research could be very useful and applicable for sturgeon aquaculturists.

Material and Method

Fish biomass and the growing conditions. Fish biomass used in this study was represented by bester with an average weight of 78.02±21.06 g (mean±SD), raised into a recirculating system of the pilot aquaculture station (4 tanks with a volume of 500 L each and water quality treatment equipment). We used 300 juvenile of bester F2 generation, obtained at the of sturgeon farm - Danube Research Consulting, Tulcea county, which was ramdomly distributed into the 4 tanks of the aquaculture recirculating system, such as to create the four different stocking densities SD_135 = 14.95 kg m^{-3} (135 individuals), SD_90 = 10.74 kg m^{-3} (90 individuals), SD_45 = 4.56 kgm^{-3} (45 individuals) and SD_30 = 3.19 kgm^{-3} (30 individuals). Before the initiation of the experiment, fish were acclimatized to experimental condition for two week. Fish were fed manually three times per day (7:00, 13:00, 18:00) at 1.6% per body weight/day, with extruded pellets for sturgeons, 2 mm diameter and protein content of 50%, fat content of 14%, crude cellulose 1.5 %, ash 8.6 %, phosphorus 1.4%, calcium 1.7% and A, C, E, D3 vitamins.

Blood sampling and biochemical analysis. After a 30 days rearing period, ten indivdualsi from each tank were used for blood sampling. To reduce possible dietary influence on metabolic status, fish were not fed on the day before blood collection (Baker et al 2005). Because anaesthesia has been considered as a stressful action and unsuitable for glucose measurement (Papoutsoglou et al 2006) fish were not anesthetized before blood sampling. Blood samples (1 mL) were collected from the caudal vein of individual fish using a heparinized 2 mL syringe. Blood samples were stored in ice and send to laboratory where plasma was separated by centrifugation at 8,000 rpm for 10 min at 4^{o}C. The plasma samples for each specimen were analyzed for the following biochemical parameters: ALB - Albumin (g/dL), ALT - Alanine Aminotrasferase (U/L), AST - Aspartate Aminotransferase, CHOL - Cholesterol (mg/dL), GLU - Glucose (mg/dL), TP - Total Protein (g/dL), TRIG - Triglycerides (mg/dL), GLOB - globulin (g/dL). All analyses were performed using commercial kit for blood chemistry autoanalyser (Model VETTEST 8008) based upon dry chemical technology and colorimetric reaction.

Statistical analysis. The results of data were expressed as mean±SD and subjected to one-way analysis of variance (ANOVA) to determine significant differences among groups; $p<0.05$ was considered as significant.

Results. During the 30 days experimental period of the present study, water quality parameters were within the acceptable range for freshwater sturgeon culture as indicated by Hochleithner & Gessner (1996), making the stocking density as the main variable affecting the hematological and biochemical parameters of the studied species. There was no mortality in the period of experiment (survival rate = 100%).

Changes in some biochemical parameters of bester reared at four different stocking densities are shown in Table 1 and Table 2. In this investigation, biochemical parameters did not considerably differ among rearing densities. The results indicated that density had no significant effect (ANOVA, $p>0.05$) of blood total protein ($p = 0.49$), albumin ($p = 0.56$), globulin ($p = 0.45$), glucose ($p = 0.41$), cholesterol ($p = 0.47$), triglyceride ($P = 0.78$), alanin aminotrasferase ($p = 0.97$) in bester. Stocking density had

a significant effect only on value aspartat aminotrasferase (ANOVA, p = 0.045), lowest values of AST were observed in DS_90 variant.

Table 1

Effect of different stocking densities on plasma glucose, cholesterol, triglycerides and liver indicative parameters of hybrids bester juveniles

Specification	*Glucose (mg/dL)*	*Cholesterol (mg/dL)*	*Triglyceride (mg/dL)*	*ALT (U/L)*	*AST (U/L)*
SD_30	50.4±11.66	68.8±11.25	373±0.91	171±28.14	574.2±100.62
SD_45	47.2±6.69	67.2±19.39	208±0.28	168±15.06	609.4±78.25
SD_90	67±29.11	76±11.93	400±0.56	170±22.3	*491.8±146.11**
SD_135	49.8±7.10	59.75±15.47	374±0.82	194±14.82	590.8±101.74

* Significant difference ($p<0.05$).

Table 2

Effect of different stocking densities on plasma protein content of hybrids bester juveniles

Specification	*Total protein (g/dL)*	*Albumin (A) (g/dL)*	*Globulin (G) (g/dL)*	*A/G ratio*
SD_30	2.04±0.32	0.5±0.1	1.56±0.22	0.32±0.45
SD_45	1.76±0.27	0.42±0.07	1.34±0.22	0.31±0.31
SD_90	1.70±0.80	0.58±0.19	1.63±0.17	0.35±1.11
SD_135	2.13±0.46	0.46±0.19	1.6±0.26	0.28±0.73

Discussion. Some investigation have been carried out to study how stocking density affects the welfare and physiological status of sturgeon species reared in recirculation systems (Hasanalipour et al 2013; Ni et al 2014; Rafatnezhad et al 2008). The rearing density in sturgeon farming is closely related to available oxygen, feeding and the concentration of metabolites (Hochleithner & Gessner 1996). The secondary responses of fish to the stressors can be evaluated by measurement of secondary biochemical indicators, such as changes in hematology and plasma chemistry (Wedemeyer et al 1990). Evaluation of the blood biochemical changes is helpful for estimating the physiological status or of eventual pathological changes that can result as an effect of the organism's defense response.

In the present study, stoking density had no significant effect on serum biochemical parameters of blood in hybrids bester juveniles. It has been known that the levels of plasma glucose fluctuated with glycogenolysis and was significantly elevated in crowding stress in different fish species (Dai et al 2011; Yarahmadi et al 2015). In contrast with other researches, the results obtained in this study shows that the stocking density did not induce physiological changes in glucose metabolism. Glucose levels has recorded similar values as ones found in the other researches: Ni et al (2014) reveal that stocking density (9.3 kg m^{-3}) had no significat effect on glucose concentration, total protein and albumin in juvenile *Acipenser schrenckii*, Rafatnezhad et al (2008) and Hasanalipour et al (2013) show in *Huso huso* juveniles, respectively *Acipenser baerii* kept at high stoking densities that glucose were not altered.

The changes of serum protein level for fish has been evident in some stressful conditions, concentration of this biochemical indicator is affected, in the first instance, by modification in plasma volume.

The protein level was maintained relatively constant, regardless of the experimental variant, reflecting a good health state and resistance to disease.

Serum albumin is the most abundant blood plasma protein and it is produced exclusively in the liver. Albumins level can mirror the metabolic state of fish health or the existence of stressful rearing conditions, because this biochemical parameter is responsable of the transport endogenous and exogenous metabolites, but and in the intravascular hydration (Baker et al 2005).

The decrease in plasma lipid levels was correlated with the action of some stressors on fish species (Svobodová et al 2006). In our case can be also noticed a decrease of the plasma cholesterol levels in the highest density, but with insignificant differences. Cholesterol is one of the structural components of cell membranes as well as the outer layer of plasma lipoproteins and is the precursor of all steroid hormones (Yang & Chen 2003). Triglycerides function primarily in providing cellular energy and can be used as an indicator of nutritional status. A high concentration of blood cholesterol from DS_90, may sugest the dietary lipid imbalance.

The activity of ALT and AST enzymes may be influenced by some stress factors related to cellular energy metabolism (Chatterjee et al 2006). For ALT enzimes no significat differences were observed in their activity, sugesting that this may not serve as good biomarkers of stress in bester hibrid. In experimental condition in this study can be observed a significant decrease ($p<0.05$) of the plasma AST enzymes levels in the DS_90 variant. Under normal rearing condition, AST can be found in soluble cytosol of liver cells, with relatively low activity, and may increase in blood serum when cells are damaged (Ming et al 2012).

Conclusions. In terms of the results obtained in this study the bester, F2 generation, did not seem susceptible to crowding since plasma protein, albumin, glucose, cholesterol, triglyceride and ALT were not affected by different stocking densities. This hybrid (*H. huso* × *A. ruthenus*) has shown a good ability to adapt for intensive growth, it is suitable for aquaculture and can help to reduce current fishing pressure on endangered wild sturgeon stocks. Serum biochemical values reported here will be used as reference for the early identification and monitoring of disease and breeding conditions of bester juveniles hybrids reared in recirculating aquaculture systems.

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