The influence of different stocking densities on growth performances of hybrid bester (*Huso huso* ♂ x *Acipenser ruthenus* ♀) in a recirculating aquaculture system

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Abstract. The aim of this paper was to evaluate the influence of different stocking densities on growth performance of the hybrid bester sturgeons (*Huso huso* ♂ x *Acipenser ruthenus* ♀), reared in a recirculating aquaculture system. A number of 300 hybrid bester with an average weight of 76.6±5.36 g were randomly divided into four rearing units in order to create different stocking densities: V1 - 14.95 kg m^{-3}; V2 - 10.74 kg m^{-3}; V3 - 4.56 kg m^{-3} and V4 - 3.19 kg m^{-3}. Researches were conducted between October 20th, 2015 and November 18th, 2015, in the recirculating system of the Aquaculture, Environmental Science and Cadastre Department, University of Galati. The fish were fed with commercial STECO PRE GROWER-14–extruded pellets for sturgeons with a protein content of 50% and a fat content of 14%, the feeding intensity being 1.6% from body weight (BW) per day. At the end of the experiment, specific growth rate (SGR) calculated in V1 variant was 1.58% g day^{-1}, in V2 - 1.56% g day^{-1}, V3 - 1.40% g day^{-1} and V4 - 1.55% g day^{-1}. The best body weight was obtained in the group V_2 followed by V1, where was the highest stocking density. The fish from these groups achieved an individual weight gain of 46.73 g/ind and 42.97 g/ind. Mortality was positively correlated with stocking density.
Key Words: bester, stocking density, recirculating aquaculture system, technological performance indicators, feed conversion ratio.

Introduction. Increasing customer demand for aquaculture products, together with increasing environmental constraints and also the costs associated with land and water, have determined producers to develop their technological facilities or to implement new engineering solutions to assure the practice of high stocking densities, thus obtaining enough fish supplies to cover the production costs and equally, to meet the market demands (Mocanu 2013).

In recent years, the intensive and super-intensive aquaculture has developed particularly among recirculating systems, where environmental conditions are permanently monitored and controlled through mechanic and biologic water filtration processes. Moreover, the practice of the last decades indicates that recirculating aquaculture systems production is a feasible alternative to traditional pond aquaculture. Although these technologies are expensive, the possibilities of obtaining a continuous cycle biomass production throughout the year and also close positioned to market, are important considerations for using recirculating aquaculture systems (Cristea et al 2002).

Rearing sturgeon in intensive aquaculture systems requires a good knowledge of the limiting factors that influence growth and development of a certain species. The stocking density was defined as total biomass per unit of volume (Cristea et al 2002). Stocking density is one of the important factors that affect fish growth and it have being confirmed that this factor plays a key role in the rearing of sturgeon (Mohler 2000). Generally, higher stocking density affects growth performances (Dicu et al 2013; Ni et al

2014), physiology and fish behavior (Docan et al 2011; Hasanalipour et al 2013) and fish survival (Fajfer et al 1999).

Hybrid sturgeons are bred to get the best technological characteristics from both parental species. Bester sturgeon is a hybrid between beluga, *Huso huso* ♂ and sterlet, *Acipenser ruthenus* ♀. This hybrid was created in 1952 (Nikoljukin 1964) and the rearing technology was developed throughout the 1960s (Burtzev 1983). This hybrid acquired the growth of the beluga and the early age of sexual maturity from the sterlet and it leaves behind the bad, the beluga's cannibalistic tendencies and the slow growth and poor caviar of the sterlet. Bester ensure a great potential for intensive aquaculture technology, it easily get used to various conditions of the maintenance and artificial feeds, grows fast and presents high stability to adverse negative factors of aquaculture. Also, bester is the first hybrid produced in large quantities in a controlled environment.

The aim of this study was to determine the growth rate, survival rate and behavior of bester juveniles reared in a recirculating aquaculture system, at four different stocking densities.

Material and Method

Experimental design. The experiment lasted for 30 days, between 20 October and 18 November 2015 and was carried out at the pilot recirculating system located in a laboratory of „Aquaculture, Environmental Science and Cadastre" Department, "Dunărea de Jos" University of Galaţi. This system is provided with 4 rearing units with a volume of 700 L each. The pilot recirculating grow system used for these experiments has been described, constructively speaking, in other study (Enache et al 2011), reason for which this paper no longer contains its description.

The biological material used in the present experiment consisted of 300 bester juveniles, 5 months old, with a mean body mass of 76.6±5.36 g, provided from Kaviar House farm, Tulcea region. Prior to the start of the trial, fishes had been investigated in terms of ichtiopathology and formalin prophylactic baths were applied. The bath concentration was 0.25 mL formalin solution/liter of water, for a short time, 30 minutes (Schäperclaus 1992).

Fish were divided into four rearing units, obtaining the following stocking densities: V1 = 14.95 kg m^{-3} (135 fish), V2 = 10.74 kg m^{-3} (90 fish), V3 = 4.56 kgm^{-3} (45 fish) and V4 = 3.19 kgm^{-3} (30 fish). Fish were fed manually three times per day (7:00, 13:00, 18:00) at 1.6% per body weight/day. The food used was STECO PRE GROWER, extruded pellets for sturgeons, with the diameter of 2 mm and protein content of 50%, fat content of 14% (Table 1).

Table 1

Ingredients of the experimental diet

Parameters	*Quantity*
Crude protein (%)	50
Crude fat (%)	14
Crude cellulose (%)	1.5
Ash (%)	8.6
Phosphorus (%)	1.4
Calcium (%)	1.7
Sodium (%)	0.3
Vitamin A (IU)	10.000
Vitamin C (mg Kg^{-1})	500
Vitamin E (mg Kg^{-1})	200
Vitamin D3 (IU)	1.410
Ingredients: fish meal, soybean extracts, maize gluten, rape oil, hemoglobin, wheat gluten, blood meal.	

The temperature (°C), dissolved oxygen (DO; mg L^{-1}) and pH (pH units) were monitored daily with a multi-parameter WTW Multi 3410, respectively with WTW 340 pH-meter. Weekly, the nitrogen compounds, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$, $N\text{-}NH_4^+$ (mg L^{-1}) were determined using Spectroquant Nova 400 spectrophotometer compatible with Merk kits.

Calculations. After the experimental period fish were weighed individually (W±1 g) and their growth performance was calculated: weight gain (WG) = final weight (Wt_1) - initial weight (Wt_0) (g), feed conversion ratio (FCR) = total feed (F)/total weight gain (W), specific growth rate (SGR (%body weight day^{-1})) = $[(\ln Wt_1 - \ln Wt_0)/t] \times 100$, coefficient of variation (%), protein efficiency ratio (PER) = total weight gain (W)/amount of protein fed (g), thermal-unit growth coefficient (TGC) which, according to Cho (1992) express fish growth in relation with temperature, as follows TGC = $(Wt_1^{1/3} - Wt_0^{1/3})/\Sigma$ water temperature (°C x days) x 100. For each stocking density, total length (TL) and body weight (W) were used to determine the relationship $W = aL^b$, where a is the intercept (the initial growth coefficient) and b is the allometric coefficient (Ricker 1975).

At the beginning and at the end of the experiment, all fish from each rearing unit were measured to determine their body weight W (to the nearest 0.1 g), total length - TL, fork length - FL; standard length - SL (to the nearest 0.1 cm). For that, fish were anaesthetized with 2-phenoxyethanol in order to reduce handling stress.

Statistical data processing. Data was analyzed using SPSS version 21. One-way Analysis of Variance (ANOVA) was used to detect differences between the water quality parameters and stocking densities. Homogeneity of variances was tested with Levene's test. When the differences were significant at $p < 0.05$ level, post-hoc Tukey's B test was used to compare the means between the experimental variants.

Results and Discussion

Water quality. During the experiment temperature registered similar results in all variants, with an average mean of 20.33°C, without significant differences ($p > 0.05$).

Regarding the values of dissolved oxygen (DO) it varied according to the population density, the lower values being registered in variant V_1 and V_2, while in V_3 and V_4 the mean value of DO was higher (Table 2).

Table 2

Synthetic table with the average values (±SD) of the main physico-chemical parameters of water

Parameter	*V1*	*V2*	*V2*	*V4*
Temperature (°C)	20.33±1.15	20.33±1.15	20.33±1.16	20.33±1.17
Dissolved oxygen (mg L^{-1})	7.40±0.35	7.72±0.34	8.10±0.28	8.32±0.34
pH (pH units)	7.91±0.15	7.84±0.18	7.94 ±0.17	7.79±0.15
$N\text{-}NO_2^-$ (mg L^{-1})	0.04±0.01	0.04±0.01	0.02±0.01	0.01±0.01
$N\text{-}NH_4^+$ (mg L^{-1})	0.13±0.06	0.11±0.04	0.09±0.03	0.07±0.01
$N\text{-}NO_3^-$ (mg L^{-1})	39.95±0.15	39.37±0.14	39.38±0.12	39.25±0.12

The statistical comparison between the values of the dissolved oxygen outlined significant differences ($p < 0.05$). In fact, the post-hoc analysis (Tukey B) grouped the DO values corresponding to the four rearing units in four distinctive subsets of values.

The pH values ranged from 7.52 to 8.22 unit pH, with an average of 7.87±0.16 pH units), $N\text{-}NO_2^-$ fluctuated from 0.01 to 0.04 mgL^{-1}, $N\text{-}NO_3^-$ from a minimum of 16.2 mgL^{-1} at the beginning of the experiment to 71 mgL^{-1} at the end of the experiment (average of 39.48±0.13 mg L^{-1}) and $N\text{-}NH_4^+$ ranged from 0.05 mg L^{-1} to 0.24 mg L^{-1} (average of 0.10±0.03 mg L^{-1}).

Although, throughout the experiment period, the water quality in all the treatments remained within the range required for sturgeons (Order 161/2006), it can be observed a slight increase of nitrogen compounds with the increase of stocking density. Similar results were obtained by Rafatnezhad & Falahatkar (2011) in the case of beluga growing at densities from 1 kg m^{-3} to 8 kg m^{-3}, suggesting that the high stocking density represents due to the metabolic excrement and uneaten feed, the major source of N input in the growing systems.

Fish growth performances. Although the average initial weight was higher in variant V_2 then others experimental variants (V1, V3 and V4), statistical analysis revealed no significant differences for the 95% confidence interval ($p > 0.05$) (Table 3). The weight (W) homogeneity of the tested groups was verified and confirmed by the Levene's test ($p > 0.05$). Also, regarding the final length, no significant differences were registered between the four experimental groups ($p > 0.05$) (Table 3).

Table 3

Somatic characteristics of experimental biological material

Experimental period	*Exp. variants*	*W (g)*	*TL (cm)*	*FL (cm)*	*SL (cm)*
Initial	V1	77.50±21.23	27.83±2.49	24.24±2.07	21.21±1.94
	V2	79.15±22.58	28.66±2.75	24.92±2.24	21.74±2.10
	V3	72.82±15.06	27.34±1.83	23.74±1.63	20.79±1.55
	V4	74.46±18.64	27.27±2.13	23.68±1.97	20.82±1.73
Final	V1	122.40±40.23	31.06±3.16	27.05±2.65	24.28±2.51
	V2	130.28±44.24	31.75±3.53	27.84±3.00	25.31±2.80
	V3	111.44±31.03	30.30±2.72	26.40±2.44	23.58±3.90
	V4	114.90±30.52	30.22±2.62	26.42±2.29	24.36±2.70

Where: W - average individual weight; TL - total length; FL - fork length; SL - standard length.

The growth performance of bester is presented in Table 4. The final mean weight was 122.40±40.23 g in V_1, 130.28±44.24 g in V_2, 111.44±31.03 g in V_3 and 114.90±30.52 g for V_4, without significant differences between the experimental variants ($p > 0.05$).

Table 4

Technological performance indicators obtained at the end of the experimental period

Growth parameters	*Experimental variants*			
	V1	*V2*	*V3*	*V4*
Initial biomass (g)	10462	7520	3191	2234
Number of fish	135	90	45	30
Initial weight (g ex^{-1})	77.49	83.55	70.91	74.46
Initial stocking density (kg m^{-3})	14.95	10.74	4.56	3.19
Final biomass (g)	15178	11204	4510	3332
Final number of fish	126	86	43	29
Final weight (g)	122.40	130.28	111.44	114.90
Final stocking density (kg m^{-3})	21.68	16.01	6.44	4.76
Survival rate (%)	93.33	95.56	95.56	96.67
Individual weight gain (g ex^{-1})	42.97	46.73	33.97	40.44
Total weight gain (g)	4716	3684	1319	1098
Specific growth rate (% day^{-1})	1.58	1.59	1.40	1.55
Thermal-unit growth coefficient	1.19	1.18	1.07	1.11
Daily growth rate (g day^{-1})	1.53	1.67	1.21	1.44
Feed conversion ratio	1.04	0.95	1.13	0.95
Protein efficiency ratio (g/g)	1.93	2.10	1.78	2.11

Distributions of body weight values and final length are emphasized in Figures 1 and 2. The highest weight and total length were obtained in highest stocking densities (V1 and V2).

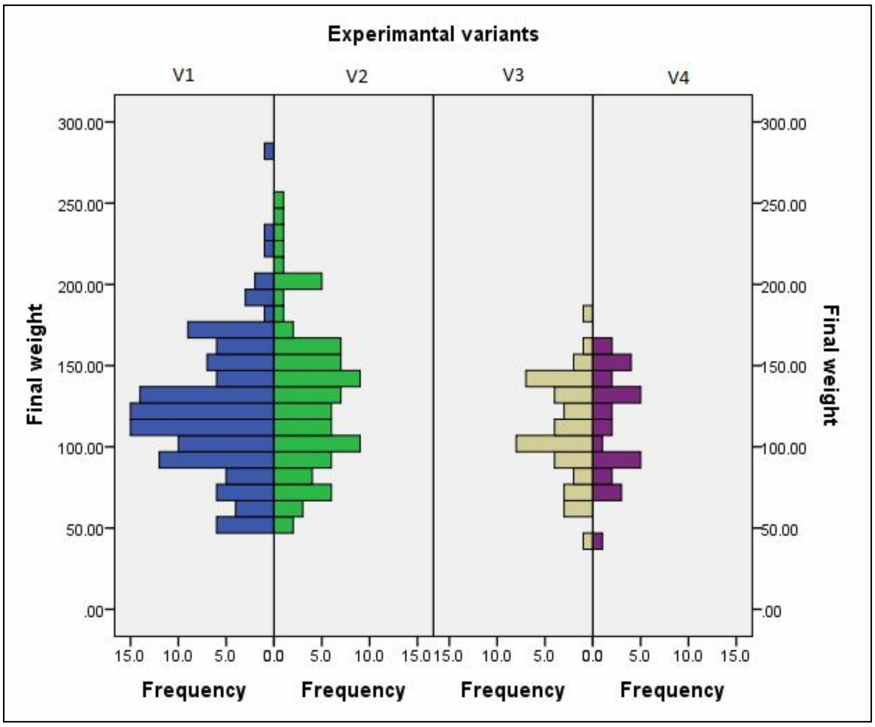

Figure 1. Weight classes histogram.

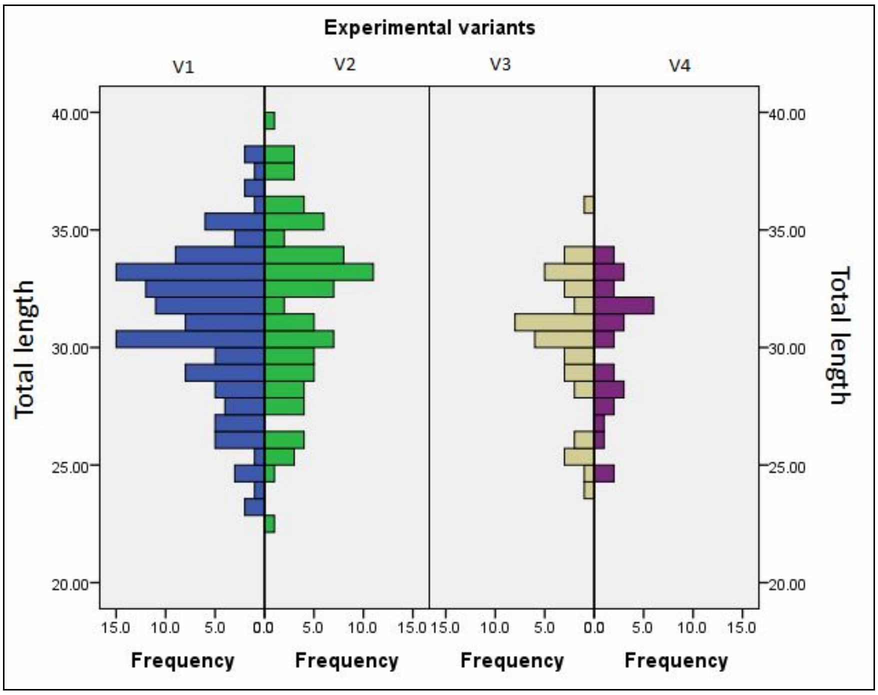

Figure 2. Total final length classes histogram.

Although in all four stocking densities the biomass gain was significant, analyzing the results in terms of economic efficiency, it can be concluded that for bester sturgeons, the higher economic efficiency were recorded at the higher stocking densities (V1 and V2). At the end of the experiment, the best stocking density was recorded in case of V1 and V2 (21.68 kg m^{-3} in V_1, 16.01 kg m^{-3} in V_2).

Survival is a key indicator of health status. Although, in our experiment, the fish survival was high, ranging between 93.33 and 96.67%, it can be observed that the survival rate was directly influenced by the stocking density.

SGR, which is the parameter that indicates most accurately the dynamic of individual growth, registered similar values in V2 (1.59 % day^{-1}) and V1 (1.58 % day^{-1}).

The best FCR was obtained in the case of V2 and V4 (0.95), at high density, followed by V1 (1.04) and V3 (1.13). Our results were similar to those of the study of Dediu et al (2012), who reported for bester (128-134 g) a FCR of 1.01-1.25 and a SGR of 1.38-1.66% day^{-1}. Also Dediu et al (2011) reported for bester (102.91±26.34 g) reared at densities between 4.16-5.29 kg m^{-3}, a higher FCR (1.53-2.76)

Usually, TGC can be used to predict biomass growth in other thermal conditions (Dumas et al 2007) and can be a useful tool for aquaculture management (Mayer et al 2012). According to Kaushik (1998) and Bureau et al (2000) the TGC is less affected by fish size and time interval between weightings than other growth rate estimates such as SGR and thus offer a simple model for growth rate comparisons.

In our experiment, the calculated TGC for bester ranged from 1.19 for V1 to 1.07 in V3. By plotting values of TGC and stocking density it can be observed a positive correlation (Pearson Correlation = 0.918) (Figure 3). The figure showed that the highest TGC was obtained in the case of V1 and V2 highlighting a slight decrease with the decreasing of stocking density.

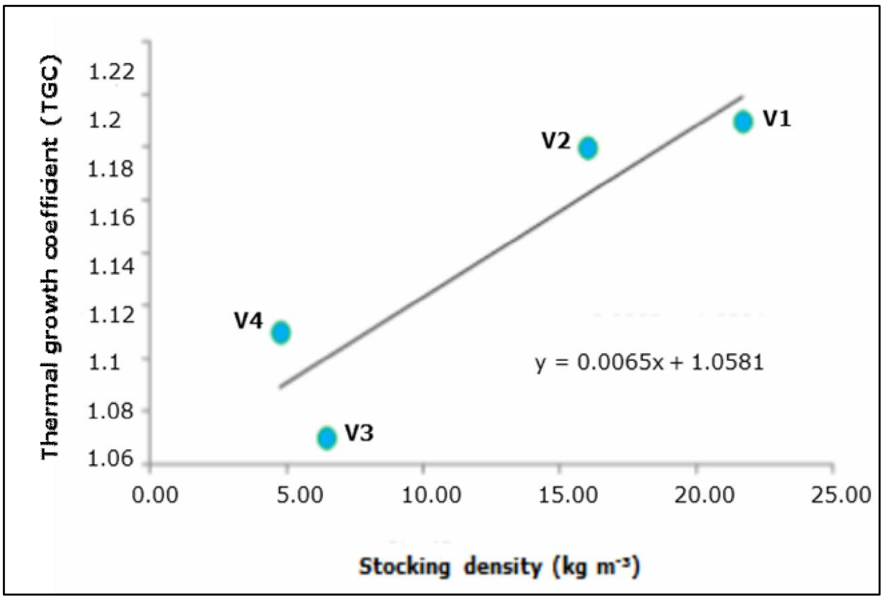

Figure 3. Thermal-unit growth coefficient (TGC) as a function of stocking density.

The determination of the correlation between the total length (TL) and body weight (W) was carried out with the data obtained from the biometric measurements from the beginning and from the end of the experiment. In this regard, the obtained data were processed to develop a growth model for bester. Analyzing the allometric coefficients obtained at the initial moment it can be observed a negative allometry ($b < 3$) and weaker condition of bester (Figure 4), the allometric factor registering the lower value for the highest stocking density (2.35 for V1 and 2.55 for V2). At the end of the experiment, it can be observed an improvement of relative robustness of the fish in all stocking densities.

The highest allometric factor was registered for V1 (3.14) and for V3 (3.06). Based on the experimental data, we can conclude that the condition of the fish from these variants is correlated with the technological indicators obtained in these densities, whose value represents the best growth performance. Also, comparing the initial coefficient of determination (R^2) it can be observed a stronger correlation between weight and length at the end of the experiment in all four densities (densities (0.90 in V1, 0.90 in V2, 0.85 in V3 and 0.81 in V4).

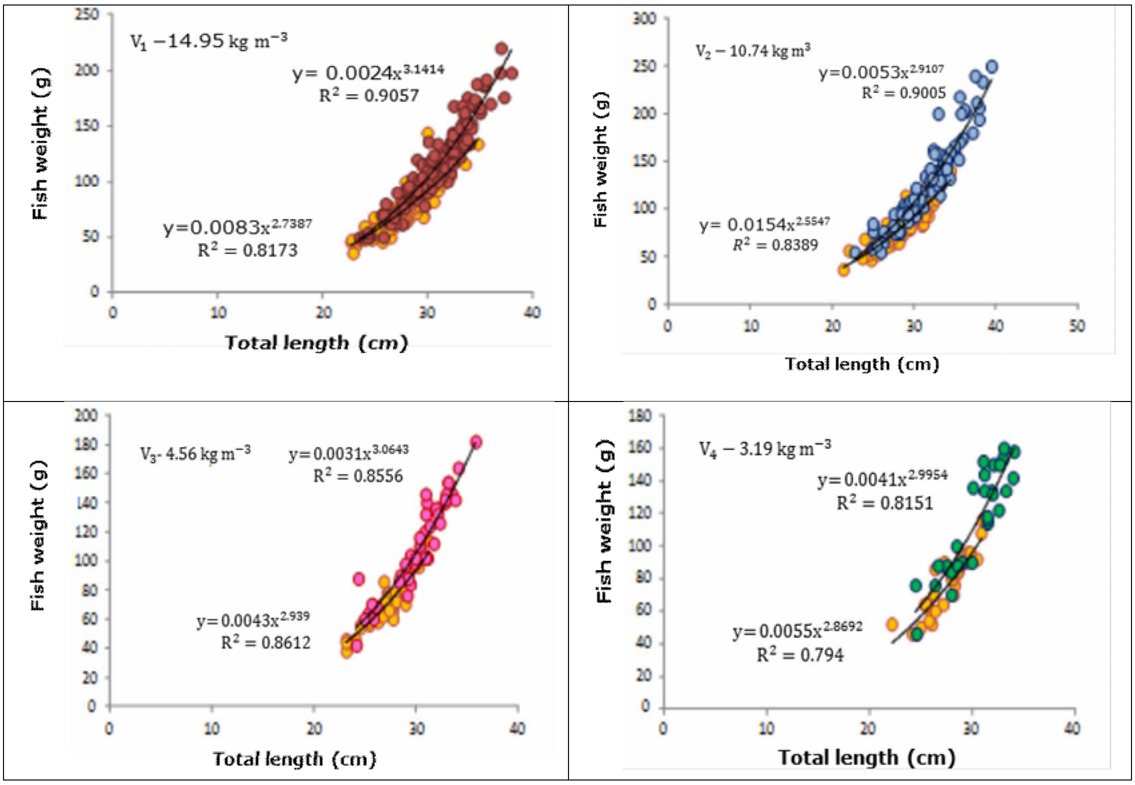

Figure 4. Length-weight regression of all four experimental variants, at the initial moment and at end of the experiment.

According to Ako et al (2005) and Aksungur et al (2007) stocking density represents an important factor which determines the economic viability of production systems. Generally, the mechanisms by which stocking density affects the growth and feed efficiency are controversial, being influenced by the fish species (Ellis et al 2002), age and size, their behavior under conditions of high density, intensity of feeding and water quality (Rafatnezhad & Falahatkar 2011; Luo et al 2013; Diatin et al 2015). The results of the present study indicated that stocking density of 14.95 kg m^{-3} and 10.74 kg m^{-3} improved the bester growth and feed utilization compared to stocking density of 4.56 kg m^{-3} and 3.19 kg m^{-3}. The results obtained by us are in contradictory with those reported by literature which emphasizes growth reduction in high stocking densities (Szczepkowski et al 2011; Dicu et al 2013; Ni et al 2014).

Conclusions. The results of our experiment in terms of growth performance and feed conversion efficiency indicate that bester sturgeon can be growth successfully at densities between 10.74 kg m^{-3} and 14.95 kg m^{-3}, being a good candidate for sturgeons aquaculture. However, our experiment covers only a few aspects regarding the bester growing in recirculating systems and further experiment should be conducted at higher rearing densities in order to determine the upper limits of density at which growth may be affected.

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