

THE INFLUENCE OF STOCKING DENSITIES ON GROWTH PERFORMANCE OF COMMON CARP (*CYPRINUS CARPIO*, LINNE 1758) REARED IN A RECIRCULATING AQUACULTURE SYSTEM

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Abstract

To determine the influence of stocking density on the common carp growth performance and body composition, four variants: 0.9 kg m⁻³ (V₁), 1.8 kg m⁻³ (V₂), 2.6 kg m⁻³ (V₃), and 3.5 kg m⁻³ (V₄) was carried out during 31 days. In this context, 2100 common carp fry, with an initial weight of 1.8 g fish⁻¹, were maintained in twelve rearing units of a recirculating aquaculture system. Fish were fed three times per day with extruded feed containing 50% crude protein and 14% fat. At the end of the experiment, the fish's growth performance was assessed. Better results were obtained for the V₁ variant. Regarding the biochemical composition of fish meat, significant differences ($p < 0.05$) were recorded in the water content, while the ash lipids and protein content showed no significant differences ($p > 0.05$) between the experimental variants. Therefore, it can be concluded that a stocking density of 0.9 kg m⁻³ is optimal for rearing juveniles of common carp without compromising the specific growth rate (SGR), survival, and biochemical composition of fish meat.

Key words: biochemical composition, FCR, SGR, stocking density.

INTRODUCTION

According to FAO, aquaculture is contributing more and more to world production of aquatic food, given that, in the case of the wildest fish stocks, the limits of sustainable exploitation are at present almost touched or even outdated. In 2018, 46% of the total production and 52% from fish for human consumption were assured from aquaculture (FAO, 2020).

In Romania, aquaculture is predominantly freshwater, with the availability of inland waters providing the right conditions for fish farming. According to FAO Fishstat, in 2018, the aquaculture sector from Romania produced 12298 tons.

The most important cultured fish species from our country is common carp (Eurostat, 2017). Usually, it is raised extensively or semi-intensive in polyculture with Asian cyprinids. These growing technologies are based on the natural productivity of the ponds with some additional feed based on local cereals.

Lately, there is also an increasing interest regarding the rearing of carp in intensive conditions, mainly in recirculating aquaculture systems (RAS). Generally, the profitability of

these systems depends on the density at which the fish are stocked (North et al., 2006). Therefore, determining the optimum stocking density is the most important criterion for designing an intensive aquaculture system (Summerfelt & Vinci, 2008).

Higher stocking densities can affect digestion and food absorption (Abdel-Tawwab et al., 2014), reduce fish growth performance, survival, size variation, health, and fish mortality (Ruane et al., 2002; Pouey et al., 2011). Also, higher stocking densities lead to deterioration of water quality because of the metabolic excretion of fish (Çağiltay et al., 2017), increase stress (Aksungur et al., 2007), an aspect which can have consequences in the aggressive behavior of fish. According to a study by Firas et al. (2020), at higher stocking densities, fish spent more time feeding and swimming and less time resting, an aspect that can negatively affect fish growth. On the other hand, lower stocking densities may reduce the overall production (Apu et al., 2012), causing economic losses.

Various studies have been carried out about the effects of stocking density on fish growth performance for different species such as

rainbow trout (Sirakov & Ivancheva, 2008; Mocanu et al., 2011; McKenzie et al., 2012), Atlantic salmon (Wang et al., 2019), African catfish (Van de Nieuwegiessen, 2009) and so on. Positive or negative effects on growth performance have been reported from these studies, and the pattern of this relationship appears to be species-specific. That is why, to maximize the production and profitability of a RAS system, it is important to determine the optimum stocking density suitable for each species and each growing stage.

Although carp is a fish raised worldwide, the information about the optimal stocking density practiced in the RAS is limited or non-existent. Therefore, this study aimed to investigate the effects of stocking densities on the growth performance, survival, and biochemical composition of common carp, with an initial weight of 1.8 g, reared in a recirculating aquaculture system.

MATERIALS AND METHODS

Experimental design. The present study was conducted for 31 days in a recirculating aquaculture system (RAS) at the Faculty of Food Science and Engineering, University Dunărea de Jos, Galați, România. The RAS system is provided with twelve rearing units, with a volume of 0,132 m³ each. The recirculating system was described in detail in the paper of Mocanu et al. (2011).

A total of 2100 fishes with an initial weight of 1.8 g fish⁻¹ were stocked in the rearing units of the RAS system to create four experimental variants: V₁-70 fish, and the initial stocking density of 0.9 kg m⁻³, V₂-140 fish, and the initial stocking density of 1.8 kg m⁻³, V₃-210 fish and the initial stocking density of 2.6 kg m⁻³, and V₄-280 fish and the initial stocking density of 3.5 kg m⁻³. The experiment was conducted in triplicate.

Fish were fed three times per day with a diet containing 50% crude protein and 14% fat at a feeding level of 5% BW day⁻¹ (Table 1). During the experiment, fish were kept under a natural photoperiod of approximately 12/12 h light/dark cycle.

Water quality parameters such as dissolved oxygen, temperature, and pH were recorded daily with the help of Hannah 98194.

Simultaneously, the concentration of nitrogen compounds was measured twice per week with the help of the Spectroquant Nova 400 photometer with Merck kits.

Table 1. Ingredients of the experimental diet

Composition	Quantities
Crude protein	50%
Fat	14%
Crude cellulose	2%
Digestible energy	4100 kcal kg ⁻¹
Lysine	2.5%
Phosphor	1%
Copper	6 mg
Vitamin A	20000 UI kg ⁻¹
Vitamin D3	2000 UI kg ⁻¹
Vitamin E	200 mg kg ⁻¹
Vitamin C	200 mg kg ⁻¹
Ingredients: fish meal, poultry meal, corn gluten, wheat gluten, wheat flour, animal fat, feed yeast, hemoglobin, vitamins, minerals.	

Fish growth performance. At the end of the experiment, the following technological efficiency indicators were calculated: growth rate, food conversion ratio, specific growth rate, and the protein efficiency ratio using the following equations:

- ✓ Weight Gain (W) = Final Weight (W_i) – Initial Weight (W₀) (g);
- ✓ Food Conversion Ratio (FCR) = Total feed (F)/Total weight gain (W) (gg⁻¹);
- ✓ Specific Growth Rate (SGR) (%Body weight day⁻¹) = [(LnW_t–LnW₀)/t] × 100.

Somatic measurements were made at the end of the trial at 50 fish/experimental variant. Total length (TL) and body weight (BW) for each variant were used to determine the relationship $W=a \times L^b$, where “a” is the intercept (the initial growth coefficient), and “b” is the allometric coefficient (Ricker, 1975). The coefficient of variation (CV, %) was calculated as the ratio of the standard deviation to the mean of weight to have a measure of fish dispersion.

Proximate analysis of fish. To determine the biochemical composition of fish, samples were taken both in the initial and final stages of the experiment. The proximate composition of fish was analysed using the AOAC (2000) method. The chemical composition of meat crude protein was analysed according to the Dumas method (N×6.25), and crude lipids were determined by the Soxhlet method, using petroleum ether as a solvent.

Dry matter was determined by drying the samples at $105 \pm 2^\circ\text{C}$ using Jeio Tech Convection Oven, and ash was evaluated by calcification at temperatures of $550 \pm 20^\circ\text{C}$ in a Nabertherm furnace.

The main indicators used for the evaluation of biochemical fish compositions were as follows:

- ✓ Protein efficiency ratio (PER): $\text{PER} = (\text{Bf}-\text{Bi})/(\text{F}\times\text{PB})$, where: F = quantity of administrated fed (kg), PB = amount of fed protein (%);
- ✓ Protein utilization efficiency (PUE): $\text{PUE} = 100\times(\text{W}\times\text{Pf}-\text{Wi}\times\text{Pi})/(\text{F}\times\text{Pb})$ (%), where: Pf - muscle tissue protein at the end of the experimental period (%); Pi - muscle tissue protein at the initial stage of the experimental period (%); Wf - final biomass (kg); Wi - initial biomass (kg); F - total feed quantity consumed (kg); Pb - administrated feed protein concentration (%).

Data analysis. Data were analysed using SPSS 21 for Windows. Data regarding fish growth performance and the biochemical composition were expressed by average and standard deviation (Average \pm SD).

Kolmogorov-Smirnov tests determined the normality of the data used for analysis. One-way ANOVA and Duncan's multiple range tests were used to compare the differences between the experimental groups. Significance was determined at $\alpha = 0.05$.

RESULTS AND DISCUSSIONS

Water quality. It is well known that water quality has a significant impact on the fish's biology and physiology, affecting the health, welfare, and productivity of a fish culture system. Generally, at higher stocking densities, lower growth performance is also determined by an increased production waste production rate.

Water chemical parameters during the experimental period are presented in Table 2. In our recirculating system, all the water parameters were reasonably constant during the experimental period and were not affected by stocking density (ANOVA, $p>0.05$).

Table 2. Synthetic table with the average values (\pm SD) of the main physicochemical parameters of water

Parameters	V ₁	V ₂	V ₃	V ₄
Temperature °C	21.2 \pm 0.18	21.6 \pm 0.11	21.4 \pm 0.12	21.6 \pm 0.14
Dissolved oxygen (mg L ⁻¹)	7.71 \pm 0.12	7.23 \pm 0.09	7.10 \pm 0.12	7.05 \pm 0.08
pH (pH units)	7.62 \pm 0.11	7.32 \pm 0.09	7.41 \pm 0.11	7.29 \pm 0.10
N-NO ₃ ⁻ (mg L ⁻¹)	21.13 \pm 0.20	19.1 \pm 0.7	18.5 \pm 0.36	17.9 \pm 0.65
N-NO ₂ ⁻ (mg L ⁻¹)	0.03 \pm 0.02	0.04 \pm 0.01	0.05 \pm 0.02	0.05 \pm 0.01
N-NH ₄ ⁺ (mg L ⁻¹)	0.17 \pm 0.02	0.22 \pm 0.05	0.19 \pm 0.03	0.19 \pm 0.04
P ₂ O ₅ (mg L ⁻¹)	5.90 \pm 0.19	5.53 \pm 0.35	5.2 \pm 0.43	5.76 \pm 0.32

Note: Data are presented as triplicate mean \pm SD.

Water temperature was around $21.2 \pm 0.18^\circ\text{C}$ in V₁, and $21.6 \pm 0.14^\circ\text{C}$ in V₄, dissolved oxygen content varied between $7.05 \pm 0.08\text{ mg L}^{-1}$ in V₄ and $7.71 \pm 0.12\text{ mg L}^{-1}$ in V₁. Water pH in the recirculation system was kept constant, and the lowest values were recorded in the V₄ variant (7.29 ± 0.10 pH units). Also, the nitrogen compounds were in the optimum interval for the cultivated fish species. The maintenance of these optimum water concentrations was possible by the benefit of optimized technical and technological parameters of the RAS. The fish rearing units

were cleaned daily, and only 10% of fresh water was added to the RAS system. Also, a significant role for the maintenance of optimum water chemical parameters in RAS during the experimental period has the mechanical and biological filters which conditioned the water properly.

Fish growth performance. The mean final weight, mean weight gain, percentage survival rates, FCR, and SGR of fish in all the treatments at the final of the experiment are presented in Table 3.

Table 3. Technological performance indicators obtained at the end of the experimental period

Growth performance	V ₁	V ₂	V ₃	V ₄
Initial biomass (g)	126	252	378	504
Initial biomass (kg m ⁻³)	0.9	1.80	2.60	3.50
The initial number of fish	70	140	210	280
Initial weight (g fish ⁻¹)	1.80±0.00	1.80±0.00	1.80±0.00	1.80±0.00
Final biomass (g)	313.67±6.66	521±12.77	692±20.52	879.67±28.50
Final biomass (kg m ⁻³)	2.20±0.05	3.65±0.09	4.84±0.14	6.16±0.20
The final number of fish	68.00±1.0	122±3	171.67±6.51	221.67±6.03
Final weight (g fish ⁻¹)	4.61±0.03*	4.27±0.08**	4.03±0.15***	3.97±0.02***
Weight gain (g)	187.67±6.66*	269.00±12.77**	314±20.52***	375.67±28.50****
Weight gain (kg m ⁻³)	1.31±0.05*	1.88±0.09**	2.20±0.14***	2.63±0.20****
Individual weight gain (g)	2.81±0.03*	2.47±0.08**	2.23±0.15***	2.17±0.02***
Survival rate (%)	97.14±1.43*	87.14±2.14**	81.75±3.1***	79.17±2.15***
SGR (% day ⁻¹)	2.94±0.07*	2.34±0.08**	1.95±0.10***	1.80±0.10***
FCR(g g ⁻¹)	1.84±0.06*	2.57±0.12**	3.31±0.22***	3.69±0.28****

Note: Data are presented as triplicate mean ± SD.

Despite the fact that all the water parameters were adequately maintained in all treatment groups, in the present study, stocking density affects the growth performance of common carp, being observed a negative correlation between stocking density and growth performance.

The data obtained for each experimental variant regarding the final fish weight showed no deviations from the normal distribution ($p > 0.05$ with Kolmogorov–Smirnov test) that permitted us to apply the parametric tests further. One-way ANOVA used at the end of the experiment showed significant differences between the final weight of fish (ANOVA, $p < 0.05$). So, the mean final weight of fish at the end of the 31-day experimental period was as followed: V₁-4.61±0.03 g, V₂-4.27±0.08 g, V₃-4.03±0.15 g, and V₄-3.97±0.02 g. The post hoc Duncan analysis showed that the final weight of fish from V₁ and V₂ was significantly higher than those of fish from V₃ and V₄.

At the end of the experiment, total length (TL)-weight (W) regressions were plotted (Figures 1-4) to obtain more information about the growth patterns of the fish.

The slope (b) values obtained for all the experimental variants showed a negative allometric growth indicating that the fish length was higher than the body mass. However, in this study, the length-weight relationship was found to be highly correlated, and all values of the coefficient of determination were greater than 0.80.

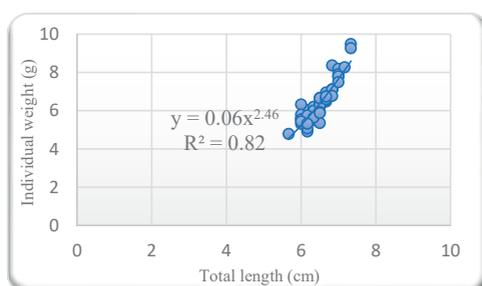


Figure 1. Length-weight regression at the end of the experiment for the V₁ variant (n = 50 fish)

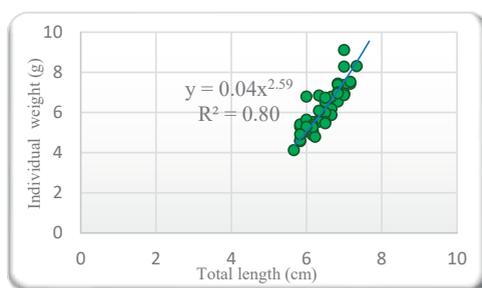


Figure 2. Length-weight regression at the end of the experiment for the V₂ variant (n = 50 fish)

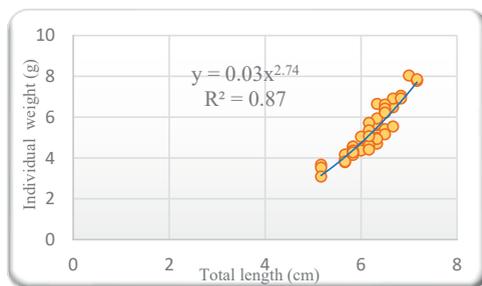


Figure 3. Length-weight regression at the end of the experiment for the V₃ variant (n = 50 fish)

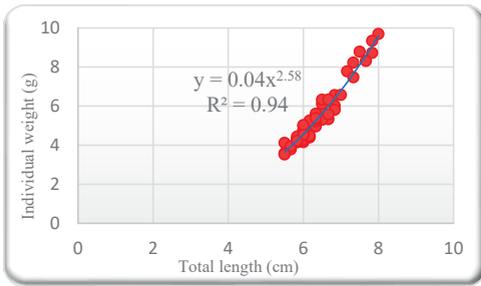


Figure 4. Length-weight regression at the end of the experiment for the V₄ variant (n = 50 fish)

The coefficient of variation (CV) showed higher variability in the V₄ variant ($36.35 \pm 3.88\%$), followed by the V₂ ($32.66 \pm 7.06\%$) and V₃ ($32.53 \pm 3.10\%$). The lowest CV was obtained in V₁ ($CV = 24.47 \pm 3.11\%$).

Results showed that increasing stocking density results in higher variation in individual growth. An increase of the CV over time indicates inter-individual competition within the fish group (Azaza et al., 2013). Obtaining a higher variability at higher stocking density is undesirable in aquaculture, preferable to reduce fish size variations and obtain homogeneous fish size, which facilitates feeding, harvesting, marketing, and processing (Azaza et al., 2010; Azaza et al., 2013).

The individual weight gain of fish was significantly affected by stocking density (ANOVA, $p < 0.05$). Duncan's multiple range tests showed three distinct groups: the individual weight gain of fish from V₁ was significantly different from those of V₂. In contrast, the individual weight gain of fish from V₃ and V₄ was similar.

Survival is a crucial indicator of fish health status (Rey et al., 2019). In our experiment, it can be observed that the survival rate was directly influenced by the stocking density, and significant differences (ANOVA, $p < 0.05$) were obtained. The post hoc analysis showed that the survival rate of fish from the V₁ ($97.14 \pm 1.43\%$) group was significantly higher than that from V₂ ($87.14 \pm 2.14\%$), while no significant differences ($p > 0.05$) were recorded between the V₃ ($81.75 \pm 3.1\%$) and V₄ ($79.17 \pm 2.15\%$) groups.

Also, in SGR and FCR, the best values were obtained in the lowest stocking density (V₁). The average specific growth rate was

significantly higher in V₁ ($2.94 \pm 0.07\% \text{ day}^{-1}$). The post hoc analysis showed that the SGR values from V₁ were higher than V₂ ($2.34 \pm 0.08\% \text{ day}^{-1}$), while in V₃ ($1.95 \pm 0.10\% \text{ day}^{-1}$) and V₄ ($1.80 \pm 0.10\% \text{ day}^{-1}$), the values are similar ($p > 0.05$).

Regarding FCR, Duncan's multiple range test divided the obtained values into four distinct groups, the best values being obtained in V₁. The FCR ranged from 1.84 ± 0.06 in V₁ to 3.69 ± 0.28 in V₄ and increased with an increase in fish stocking density. Therefore, the higher FCR values obtained at the highest stocking densities indicate low food utilization efficiency.

In the present study, a negative correlation was observed between the stocking density and fish growth performance. The effect of stocking density on growth is in line with the results obtained for other cultured fish species. HtayHtay et al. (2019) conducted a study over five months stocking carp as follows (with an individual weight of 0.5-1.6 g, and standard length 2.2-4.9 cm): 5 fish per tank, ten fish per tank, and 15 fish per tank (water volume 40 L/tank). After five months, the best results for fish survival and growth performance were observed in the variant with the lowest stocking density (5 fish/tank). Also, Marandi et al. (2018), reported after 45 days of growing, better values of FCR and SGR for common carp (initial weight of $1.41 \pm 0.5 \text{ g fish}^{-1}$) at lower stocking densities (20 fish/tank, or 0.70 g L^{-1}).

The proximate composition of common carp reared at different stocking densities is presented in Table 4. Generally, fish's biochemical composition is influenced by many factors that depend especially on species, size, age, environmental conditions, and feeding (Cho, 2001).

The results showed significant differences (ANOVA, $p < 0.05$) in fish water content the percentage between the four stocking densities. So, the water content from V₁ and V₂ was significantly different from the water content from V₃ and V₄ variants. A significant increase in water content was observed in V₁, V₃, and V₄ variants compared with the initial moment.

Regarding the protein, lipids, and ash content, no significant differences (ANOVA, $p > 0.05$) were recorded between the four stocking

densities, but significant differences were recorded compared to the initial moment (ANOVA, $p < 0.05$). Thus, there was a

significant increase in protein and lipids' content and a significant decrease in ash content.

Table 4. The proximate composition of common carp meat reared at different stocking densities

Parameters	Experimental variants				
	Initial	V ₁	V ₂	V ₃	V ₄
Water (%)	76.11±0.18	76.48±0.07 ^{ac}	76.33±0.22	75.20±0.22	75.32±0.08
Protein (%)	12.29±0.18	13.15±0.02 ^{bc}	13.26±0.02	13.15±0.16	13.53±0.47
Water/protein	6.19±0.03	5.81±0.05 ^{bc}	5.75±0.02	5.71±0.05	5.57±0.20
Lipids (%)	7.78±0.07	8.80±0.08 ^{bc}	8.71±0.2	8.81±0.21	8.63±0.21
Ash (%)	2.19±0.10	1.42±0.02 ^{bc}	1.39±0.07	1.46±0.03	1.44±0.03

Note: Data are presented as triplicate mean ± SD;

a-significant differences between the experimental variants ($p < 0.05$); b-insignificant differences between the experimental variants ($p > 0.05$).

c-significant differences from the initial moment ($p < 0.05$); d-insignificant differences from the initial moment ($p > 0.05$)

The water to protein ratio is a suitable instrument to detect excessive water, being more precise and reliable than the water content itself (Manthey-Karl et al., 2012). If the value of the Water/Protein ratio is lower, the nutritional value is higher. The applied stocking densities on common carp have no significant differences (ANOVA, $p > 0.05$) in the water to protein ratio. Compared with the initial moment, the nutritional value of fish meat reflected by the water/protein ratio was significantly better (ANOVA, $p < 0.05$). At the beginning of the experiment, the water/ protein ratio was 6.19±0.03, and at the end of the experiment, this ratio decreased for all groups, reaching 5.81±0.05 in the V₁, 5.75±0.02 in V₂, 5.71±0.05 in V₃, and 5.57±0.20 in V₄.

To have more information regarding the fish protein gain, we calculate the protein efficiency ratio (PER) and the productive protein value (PUE) (Figure 5).

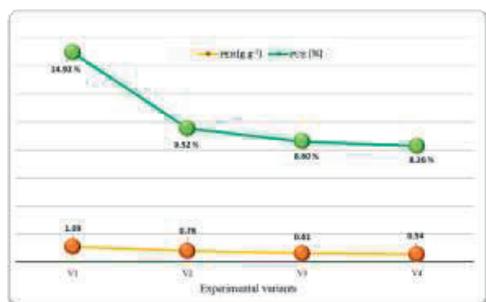


Figure 5. The protein efficiency ratio (PER) and protein utilization efficiency (PUE)

ANOVA test revealed significant differences ($p < 0.05$) in PER and PUE values. Duncan's multiple range tests showed that the PER

values from V₁ were significantly different ($p < 0.05$) from those obtained in V₂, while in V₃ and V₄, no significant differences were obtained in the PER values.

Also, significant differences (ANOVA, $p < 0.05$) were obtained between the values of protein utilization efficiency. The evolution of PUE emphasizes a better protein valorisation inversely proportional to the increase of the stocking density. Duncan's multiple range tests revealed four distinct groups belonging to each tested stocking density.

Our values obtained by us regarding carp carcass's biochemical composition are similar to those obtained from other authors. In a study conducted by Khushwinderjit et al. (2018), the biochemical composition of flesh of the common carp fingerlings (with the weight between 5.41-5.49 g, and length between 6.61-6.74 cm) fed with diets replacing protein of plant origin with animal protein in the form of fish silage at different levels, was as follows: water content ranged between 78.20-81.43%, crude protein 13.90- 16.50%, fat 1.60-2.50%, and ash between 1.06-1.60%.

CONCLUSIONS

The effects of stocking density were evident in the growth of common carp in weight gain and the final weight of fish. The best stocking density concerning growth performance and feed conversion efficiency was at 70 fish per rearing unit, with the initial stocking density of 0.9 kg m⁻³. However, the final results regarding the fish-stocked weight were still low (0.9 kg m⁻³). This study, therefore, recommends further

research with lower stocking densities, which would result in higher final productions.

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