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THE COMBINED EFFECTS OF STOCKING DENSITY, FEEDING REGIME AND INITIAL SIZE ON GROWTH PERFORMANCE OF RAINBOW TROUT FINGERLINGS

Lorena DEDIU, Angelica DOCAN*, Iulia GRECU, Mirela CRETU, Daniela IBĂNESCU, Cristian RAMNICEANU, Victor CRISTEA

Faculty of Food Science and Engineering, "Dunărea de Jos" University of Galaţi,
47 Domnească Street, 800008, Galaţi, Romania

*Corresponding author email: angelica.docan@ugal.ro

Abstract

The main purpose of the present experiment is to evaluate if the growth performance, feed utilization and survival rate of rainbow trout (Oncorhynchus mykiss) fingerlings are influenced by factors such as initial stocking size, stocking density, diet or their interaction. This study was based on a 3x3x2 factorial design with three rearing densities (D1 = 2.61±0.22 kg/m^3, D2 = 3.90 ±0.06 kg/m^3 and D3 = 4.80±0.08 kg/m^3), three size classes (SC1 = 2.22±0.24 g/ex, SC2 = 4.90 ±0.18 g/ex and SC3 = 6.87±0.016 g/ex) and two levels of dietary protein (LP = 45%, HP = 50%). After an experimental period of 70 days, rainbow trout growth performance was evaluated through analysis of various technological indicators: IWG (individual weight gain), FCR (food conversion ratio), SGR (specific growth rate), Relative Growth Rate (RGR), PER (protein efficiency ratio). The statistical analysis of data showed significant differences regarding growth among variants both in terms of density and size class. Diet and fish size factors, and the interaction between these, contributed significantly to the variation in protein efficiency ratio.

***Key words**: feeding regime, rainbow trout, stress density.*

INTRODUCTION

Rainbow trout has become an important cold-water species for world aquaculture due to accessible rearing technology and its adaptability to different production systems. In Romania, trout aquaculture is practiced mainly in intensive flow-through systems and recirculating aquaculture systems. Despite the high demand for trout on the market, the production within the country is still low (2984.28 tons/year).

The main goal of the trout aquaculture sector is to maximize production efficiency to be competitive on the market in terms of both aspects of fish quality and fish price. Optimizing production depends though on several factors such as fish genetics, feed quality and feeding management, the water quality, size and form of the rearing tanks, stocking density, and size of the fish (Bucur et al., 2017; Luna et al., 2020; Arifin et al., 2019; Lhorente et al., 2019; Kok et al., 2020).

Each of the aspects mentioned above has been studied in recent decades, the aspects related to the nutrition of rainbow trout benefiting the most from the attention of the scientific community (Kiron et al., 1995; Murai, 1992; Ma et al., 2019; Kamalam et al., 2020). Therefore, through in-depth research, quantitative data on nutrient requirements for optimal growth and welfare are already available (NRC, 2011). The amount of feed required depends on water temperature and fish size, smaller fish needing more feed relative to their body due to faster metabolic rates.

Given the growing demand for fish, aquaculture technologies have evolved to intensify. Due to space and environmental constraints that limit the development of new farms, most producers have increased the stocking densities of existing facilities, expecting to maintain growth performance (Fornshell, 2002).

In many cultivated fish species, however, growth is inversely related to stocking density and this is mainly attributed to social interactions or poor water quality as a consequence of metabolites accumulation (Costas et al., 2008; Santos et al., 2010; Tolussi et al., 2010; Liu et al., 2014; Hosfeld et al., 2009; North et al., 2006).

Increasing densities keeping the same water flow usually reduces fish swimming activity which leads to decreased fitness (both physical and reproductive), growth, survival, and muscle quality (Alsop et al., 1997; Palstra and Planas, 2013).

In practice, different factors have simultaneous effects on the growth performance and, therefore, there are still many aspects of their interaction pending to be elucidated.

In a farming context, a feeding strategy involving the use of specific purpose feeds during a particular production phase is increasingly gaining importance as a tool to influence product quality and environmental compliance (Fornshell, 2002). The expansion and intensification of rainbow trout farming, like for most farmed animals, primarily depends on nutrition and feeding, and continuous research and development are needed to address new challenges to reduce feed costs from current farm levels and increase profitability.

The main objective of the present study was to assess the main and cumulated effect of stocking density and fish size with protein intake on the growth performance of rainbow trout, *Oncorhynchus mykiss*, cultured in a production flow-through aquaculture system.

MATERIALS AND METHODS

Fish and feeding regime

An experiment was conducted in outdoor raceways with flow-through system at the Gilau Fisheries Research Station, to evaluate the effect of stocking density and protein intake on growth and survival of different size groups of rainbow trout fingerlings. The experiments took place in 36 fry rearing basins, in duplicates allowing the installation of multifactorial experiment of 3 x 3 x 2 variables (stocking density x class size x fodder). The basins with a surface of 2 m^2 and a depth between 0.3 and 0.5 m having a total water volume of 0.85 m^3, were populated with 22128 trout fingerlings. The fish were fed with 2 commercial pellets with different protein content, with a daily ratio of 3% of body weight administered 3 times per day (Table 1). The three class sizes, hereby noted with SC were represented by cohorts with mean individual weight of 2.22±0.24 g/ex (SC1), 4.90±0.18 g/ex (SC2) and 6.87±0.016 g/ex (SC3) respectively. Each of the three size classes were subjected to three stocking densities (D) of 2.61±0.22 kg/m^3 (D1), 3.90±0.06 kg/m^3 (D2) and 4.80±0.08 kg/m^3 (D3). The trial was undertaken for 70 days.

Growth performance assessment

In the end of the experiment the fish were weighed and the growth performance of the fish estimated with the following indexes:

Individual Weight Gain (IWG) = Final Weight (W_t)–Initial Weight (W_0) (g)

Food Conversion Ratio (FCR) = Total feed (F)/Total weight gain (W) (g/g)

Relative Growth Rate (RGR) = (W_t–W_0)/t/BW) (g/kg/d)

Specific Growth Rate (SGR) = 100 x (ln W_t–ln W_0)/t (% BW/d)

Protein efficiency ratio (PER) = Total weight gain (W)/amount of protein fed (g)

Relative Weight Gain (RWG%) = (W_t–W_0) x 100/Wt

Table 1. Biochemical composition of fodder pellets

Chemical composition	LP	HP
Protein	45%	50%
Fat	20 %	20 %
Cellulose	17.9%	0.7%
Ash	7.1 %	9 %
Phosphorus	1.0%	1.3%
Vitamin A	10000 UI/kg	12000 UI/kg
Vitamin D3	2000 UI/kg	1800 UI/kg
Vitamin E	200 mg/kg	180 mg/kg
Vitamin C	280 mg/kg	500 mg/kg
Coper sulfate	10 mg/kg	8,5 mg/kg

Statistical analysis

The data normality was confirmed by Shapiro-Wilk test. All data were analysed with 2-Way ANOVA (size and stocking density as the independent variables). If there was an interaction effect of the independent variables on the measured parameters, the data were subjected to one-Way ANOVA. Duncan's multiple range test was used as a post hoc test to compare between means at $P<0.05$. Data are presented as mean ± S.D. Data analyses were conducted with the SPSS (version 13.0) software.

RESULTS AND DISCUSSIONS

Growth performance of different size rainbow trout, O. mykiss fingerlings, held in different stocking densities and fed diets containing different levels of dietary protein over the 10-week feeding trial is presented in Figures 1 and 2 and Table 2.

Averaged over all treatments, the survival rate was 92.9% being slightly lower, unsignificant ($p<0.05$), when groups from different density variants of the same size group were compared. The highest mortality, however, was correlated with the lowest initial size, for both diet variants, with the highest value for the smallest group fed a low protein diet (Figure 1).

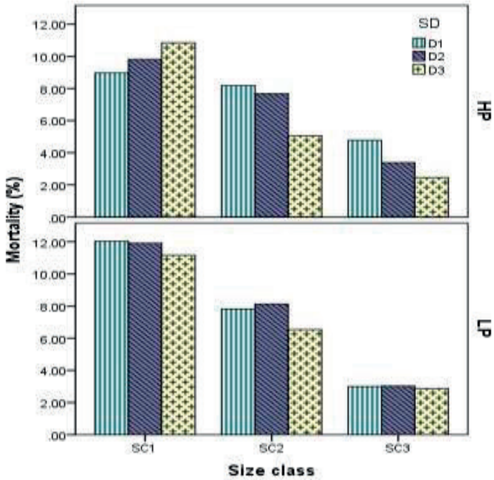

Figure 1. Mean mortality (%) over the experimental trial in all groups

For medium and large-size fish, fed with a high protein diet, slightly higher mortality for the lower densities was observed. This situation could presumably due to the formation of social hierarchies in less crowded fish (North et al., 2006), salmonids having a higher tendency comparing with other species for social dominance (Castanheira et al., 2017), or higher residual nitrogen accumulation and water quality depreciation (Person-Le Ruyet et al., 2008).

In terms of mortalities, the values reached 10.78 % for the smaller fish groups comparing with only 7.23% and 3.25% for the middle size and larger fish. However, for the present study, there was no evidence that higher density induced mortality.

The final mean individual weight did not differ significantly (Anova, $P>0.05$) among density groups but did varied (Anova, $P>0.05$) among size classes (Figure 2).

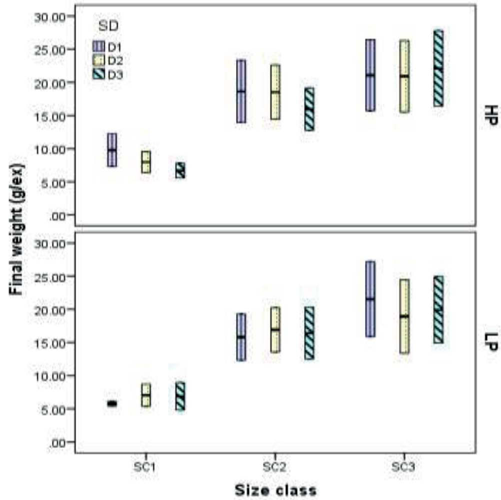

Figure 2. Final individual weight of the trout fingerlings reared in different experimental conditions

At low stocking, densities fish were affected positively in a significant increase in growth in special for smaller fish (Table 2). Thus, mean IWG values over density treatment registered by the SC1 fish fed with high protein were 31 to 35% higher comparing with mean values registered for the fish fed with a low protein diet. Interestingly, for larger fish variants (SC2 and SC3) there were no statistical differences (Duncan test, $P>0.05$) between groups from the same density irrespective of received feed.

Feed is the main input for fish production, feed cost representing almost half of the variable costs during the production cycle (Engle et al., 2020). Feed conversion ratio- FCR is a measure of the feeding efficiency and therefore a lower value is correlated with higher profitability (Cretu et al., 2020). This aspect is not always available since the feed price is also variable, depending mainly on its formulation and ingredients' quality.

Therefore, a small decrease in FCR could not reduce the variable cost if the feed price is high. In this case to have a clear image of the feeding efficiency FCR must be correlated with the cost of the fodder.

The conversion factor values for all experimental groups were within the ranges reported for commercial feed, by other researchers who have conducted similar studies on similar sizes of rainbow trout (Hoseini et al., 2018; Savafi et al., 2019). It was also observed that the feed conversion ratio tends to record higher values with increasing fish weight, registering the best values in small fish groups. Although the mean FCR for various experimental variants differed, no statistically significant differences were found for independent variables represented by density (P>0.05). Nevertheless, FCR values for SC1 increased with density while for SC3 the FCR correlated negatively with stocking density (Table 2). If the negative correlation between density and feeding efficiency was found by various authors (Ellis et al., 2002, Boujard et al., 2002) there are also studies reporting no effects on growth or mortality of density until 30 kg/m^3 (Carbonara et al., 2020), 80 kg/m^3 (North et al. 2006) or even 100 kg/m^3 (Boujard et al. 2002). This was also the case in our trial where no significant impact of stocking density was observed within different sizes of fish regardless of the feeding regime. However, the FCR varied significantly (Anova, P<0.05) among different class sizes and between feeding regimes but no interaction effect of these factors was detected (P>0.05).

Mean specific growth rate (SGR) values over the experimental period ranged between 1.91% BW/day and 2.44% BW/day for the groups fed with a high protein diet and between 1.74%/BW/day and 2.38% BW/day for the groups fed with a low protein diet, varying also with the size of specimens. However, density-linked effects on SGR were detected among size groups rather than for the groups under different feeding regimes (Table 2).

Table 2. Growth performance indices for different sizes of trout fingerlings fed with different diets and reared in open flow-through outdoor system under different densities

			IWG (g/ex)	**RGR - (g/kg/d)**	**SGR (% BW/d)**	**FCR**	**PER**
HP	**SC1**	**D1**	10.86±0.67	0.14±0.07	2.44±1.19	0.68±0.12	2.96±0.11
		D2	7.54±0.77	0.10±0.08	2.08±1.81	0.81±0.13	2.51±0.11
		D3	6.77±0.81	0.08±0.12	1.91±0.9 7	0.82±0.09	2.43±0.13
	SC2	**D1**	18.20±0.23	0.26±0.07	2.39±0.95	0.64±0.02	3.23±0.15
		D2	17.62±0.44	0.25±0.01	2.21±0.53	0.70±0.06	2.85±0.14
		D3	14.17±0.46	0.20±0.08	2.13±0.98	0.71±0.08	2.60±0.14
	SC3	**D1**	20.46±0.55	0.28±0.09	2.08±1.16	0.88±0.05	1.86±0.15
		D2	19.80±0.29	0.27±0.13	2.07±2.72	0.81±0.10	2.14±0.19
		D3	21.73±0.61	0.30±0.23	2.15±0.69	0.77±0.11	2.30±0.25
LP	**SC1**	**D1**	7.17±1.17	0.06±0.08	2.38±0.12	0.77±0.27	2.89±0.17
		D2	5.18±1.12	0.09±0.05	2.11±0.13	0.84±0.35	2.65±0.55
		D3	4.40±2.98	0.10±0.06	1.74±0.09	0.93±0.13	2.41±0.33
	SC2	**D1**	16.86±1.1	0.21±0.08	2.29±0.11	0.77±0.11	3.12±0.17
		D2	15.70±0.88	0.22±0.10	2.20±1.55	0.79±0.12	2.95±0.34
		D3	14.21±0.78	0.22±0.09	2.12±0.89	0.82±0.15	2.71±1.21
	SC3	**D1**	20.15±0.99	0.29±0.11	2.11±1.34	1.12±0.23	2.77±0.87
		D2	18.79±1.45	0.25±0.08	1.95±0.99	1.02±0.19	2.83±0.12
		D3	18.03±1.23	0.26±0.96	2.11±1.3	0.88±0.77	2.96±0.19
2-Way ANOVA		**p values (α = 0.05)**					
Density			0.817	0.987	0.039*	0.96	0.54
Size			0.002*	0.001*	0.401	0.005*	0.026*
Feed			0.046*	0.266	0.506	0.004*	0.035*
Size*Feed			0.631	0.931	0.991	0.432	0.032*
Density*Feed			0.963	0.880	0.073	0.936	0.985
Size*Density			0.484	0.841	0.01	0.286	0.389

Reduced specific growth rate at high rearing density could be attributed to numerous factors as crowding stress, feed intake, or increased energetic cost of feeding (Boujard et al., 2002; Portz et al., 2006; Naderi et al., 2018; Saulnier et al., 2021). In the present study, we did not observe a reduced feed intake in higher densities. More than that, in the larger fish groups (SC3) the density seemed to not have an adverse effect on SGR (Anova one-way, P>0.05). Although an interaction effect of density and size was observed, this was rather associated with SC_1 and SC_2.

The most expensive component in trout feeds is the protein and therefore a reduction in the dietary digestible protein (DP) levels without a negative effect on growth performance could improve protein utilization while reducing nitrogen losses (Hua et al., 2019; Kamalam et al., 2020) this strategy contributing also to a reduction of production cost and increase in profitability.

In our experiment, both factors, diet and fish size, and the interaction between these, contributed significantly to the variation in protein efficiency ratio. However, there were no significant differences among variants held in different stocking density conditions (P>0.05).

In this study, the specific growth rate was influenced by the fish size and density interaction while feeding efficiency by the effect of feed (protein level) and fish size interaction. The short duration of the experiment and relatively low densities may have been a factor, and differences may be more pronounced over time. Nevertheless, the study showed the importance of adapting feeding management to the fish size and stocking density to optimize production costs.

However, to have a clear image of the impact induced by the application of such management on the production cost, carrying out additional research is necessary.

CONCLUSIONS

The present study showed that rearing feeding efficiency of rainbow trout in the rearing conditions tested in this study do not seem to be affected by density but by initial size of the fish and the feed that they receive. Higher densities resulted in significantly lower specific growth rate, in special for smaller fish. The diet and fish size as well as the interaction of these factors induced variation in protein efficiency ratio.

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