

EFFECTS OF COPPER MICROPARTICLES ON THE GROWTH  
AND SURVIVAL OF JUVENILE RAINBOW TROUT  
(*ONCORHYNCHUS MYKISS* WAL.)

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**Abstract:** During the farming of juvenile rainbow trout (*Oncorhynchus mykiss*), losses can be very high. In aquaculture, various agents are used, such as copper, for the purpose of preventive action and prevention of the appearance of pathogens (ectoparasites, etc.). The aim of this study was to determine the effects of substrate-fixed copper microparticles on the growth and survival of juvenile rainbow trout (*Oncorhynchus mykiss*) up to the age of 5 months. The experiment was conducted in the Laboratory for Aquaculture of the Faculty of Agriculture, University of Banja Luka, for 142 days in flow aquariums with a 65 l/aquarium volume. The experiment was set up in two groups (150 units/group) with three replicates (50 units/replicate), a control group without copper microparticles (K) and a group with copper microparticles (Cu). The initial weight and total body length (mean±SD) of rainbow trout in group K were 0.199±0.008 g and 2.876±0.036 cm, and in group Cu 0.197±0.009 g and 2.893±0.038 cm. The average total length and body weight of individuals from the K and Cu groups were similar ( $p > 0.05$ ). The differences in FCR, CF, SGR and TGC between group K and group Cu were not statistically significant ( $p > 0.05$ ). Survival of juvenile rainbow trout was high and it was 97.33% in group K and 96.00% in group Cu, and no statistically significant difference was found ( $p > 0.05$ ). The growth and survival of juvenile rainbow trout in the K and Cu groups were similar.

**Key words:** microparticles, copper, growth, survival, juvenile, rainbow trout.

### Introduction

The breeding of the youngest age categories of rainbow trout (*Oncorhynchus mykiss*) deserves special attention, as it is the most sensitive stage of cultivation. Taking into account the size of the fry after swimming and the high stocking

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density per unit of production volume, it is necessary to pay special attention to maintaining good conditions of the breeding environment in order to provide optimal conditions for good growth, health and survival of juvenile rainbow trout, especially when it comes to the initial diet of trout, where a significant part of the high-protein feed used in the diet falls to the bottom, which is a good basis for the development of various organisms that can have a negative effect on the water quality and can also lead to diseases in farmed fish. Therefore, in aquaculture, in order to preserve the quality of the breeding environment and good health status of farmed fish, the prescribed technology of breeding different age categories and different assets are applied for the purpose of preventive action and reducing the possibility of pathogens. Heavy metals are toxic and can be very harmful to organisms (Majzlik et al., 2011), but they are still used in a certain form and concentration in aquaculture. Copper is a significant additive in aquaculture for controlling the growth of algae and ectoparasites, and can also affect the microbiological degradation of organic matter, depending on its availability (Noga, 2010; Tom-Petersen et al., 2011). A number of studies have been conducted to investigate the effects of different forms of copper in water on the growth, survival and body organs of rainbow trout. Copper is used in various forms for specific purposes, e.g. copper sulphate is used to control the growth of algae in lakes and ponds, while copper nanoparticles have promising applications, but the availability and toxicity of copper in the form of nanoparticles are largely unknown (Malhotra et al., 2020). Ruparelia et al. (2008) state that the application of copper nanoparticles for use in antimicrobial materials is still being investigated, while Majzlik et al. (2011) state that copper nanoparticles show very good results as antibacterial and antimicrobial agents.

The effects of copper in water on cultivated rainbow trout depend on the form and concentration of copper in the water. The growth rate of rainbow trout larvae reacts sensitively to the chronic toxicity of copper ions, i.e. by increasing the content of copper ions in water, the growth of rainbow trout larvae decreases (Mickėnienė et al., 2007). Marr et al. (1996) state that exposure to copper concentrations in water of  $4.6 \text{ ng l}^{-1}$  resulted in reduced growth of juvenile rainbow trout in the experimental group of about 4.5% compared to the control group, with individuals not recovering and achieving a growth rate of individuals from control groups during the exposure period, i.e. the duration of the experiment. Shaw et al. (2012) state that the mortality of juvenile rainbow trout at exposure to copper sulfate concentration in water of  $100 \text{ } \mu\text{g/l}$  on the fourth day was recorded at 85%, while at exposure to copper nanoparticles at  $100 \text{ } \mu\text{g/l}$ , mortality was significantly lower and was 14%.

In this study, copper microparticles fixed on a substrate placed on the bottom of the aquarium were used, with direct contact with the culture medium, feces and feed deposited on the bottom of the aquarium. The subject of this study was

juvenile rainbow trout from the moment of transition to exogenous feeding with compound feed until five months of age.

The aim of this study was to determine the effects of substrate-fixed copper microparticles on the growth and survival of juvenile rainbow trout (*Oncorhynchus mykiss*).

### Material and Methods

An experiment to examine the effects of copper microparticles on the growth and survival of juvenile rainbow trout (*Oncorhynchus mykiss*) was conducted in the Aquaculture Laboratory of the Faculty of Agriculture, University of Banja Luka. The experiment was carried out during 142 days in flow-through aquariums with an individual volume of 65 l. The experiment was set up in two groups with three replicates, a control group without copper microparticles (K) and a group with copper microparticles (Cu). At the beginning of the experiment, there were a total of 300 individuals (150 individuals/group, 50 individuals/replicate) of rainbow trout. In the group with copper microparticles (Cu), glass (55 x 30 cm) coated with araldit was placed on the bottom of the aquarium, which was used to fix copper microparticles, and the other group was the control (K), without copper microparticles. After coating the entire surface of the glass with araldit, 10 g of copper microparticles with an average size of 60 µm were evenly distributed over the coating. The OMAX A35140U microscope (China) was used to observe copper microparticles, and the size of copper microparticles was determined using the processing and measurement software. Substrates with copper microparticles were placed in three aquariums of the Cu group. The aquarium was cleaned of accumulated feed and feces once a week.

During the experiment, the control of body growth and body length was performed periodically (0, 35, 73, 113 and 142 days). One day before the measurement of body weight and body length, the individuals were not fed. All fish were measured during the checkups, and before the measurement, fish were anesthetized with the anesthetic 2-phenoxyethanol. The initial weight and total body length (mean±SD) of juvenile rainbow trout in the control group (K) were 0.199±0.008 g and 2.876±0.036 cm, and in the group with copper microparticles (Cu) – 0.197±0.009 g and 2.893±0.038 cm. Mortality was registered continuously. During the experiment, the photoperiod was 8:16 (L: D).

Based on the obtained measurement results (body weight and length, water temperature), the feed conversion ratio (FCR) and growth characteristics were determined: condition factor (CF), specific growth rate (SGR), thermal unit growth coefficient (TGC), using the following formula:

$$\text{FCR} = F/G \quad (1)$$

FCR – feed conversion ratio; F – feed consumption (kg); G – weight gain (kg).

$$CF = (BW/L^3) \times 100 \quad (2)$$

CF – condition factor; BW – body weight (g); L – total body length (cm).

$$SGR = [(\ln FBW - \ln IBW)/D] \times 100 \quad (3)$$

SGR – specific growth rate; FBW – final body weight (g); IBW – initial body weight (g); ln – natural logarithm ( $e = 2.7183$ ); D – number of days.

$$TGC = [FBW^{1/3} - IBW^{1/3}]/\Sigma[T \times D] \times 100 \quad (4)$$

TGC – thermal unit growth coefficient; FBW – final body weight (g); IBW – initial body weight (g); T – water temperature (°C); D – days.

The following formula was used to determine the survival of juvenile rainbow trout (%):

$$\text{Survival (\%)} = (N_t/N_0) \times 100 \quad (5)$$

$N_t$  – number of fish at the end of the experiment (n);  $N_0$  – number of fish at the beginning of the experiment (n).

Water temperature (°C) and dissolved oxygen content (mg/l) in water were measured with a digital oximeter WTW (Oxi 330i/SET 2B20 - 0011), and water pH was measured with a digital pH meter WTW (pH 330i/SET 2A20 - 1011). Body weight was measured with a Denver DL - 501 scale, load capacity 0.5 kg (accuracy 0.1 g), and body length was measured with an ichthyometer (accuracy 0.1 cm).

Rainbow trout were fed the same amount and type of feed in all treatments. As the age of juvenile rainbow trout increased, the size and composition of the feed used changed (Table 1).

Table 1. The size and composition of the feed used in the experiment.

Feed composition	Feed size (mm)			
	0.5 mm	0.7 mm	1.5 mm	2.0 mm
Crude protein (%)	55.0	55.0	52.0	48.00
Crude fat (%)	18.0	18.0	20.0	22.00
NFE (%)	8.0	8.0	10.5	-
Ash (%)	10.5	10.5	9.0	7.70
Fiber (%)	0.5	0.5	0.5	0.90
Phosphorus (%)	1.7	1.7	1.5	1.29
Digestible energy (MJ/kg)	19.7	19.7	20.2	-

The obtained data were processed statistically using the statistical program SPSS17 and MS Excel 2016.

## Results and discussion

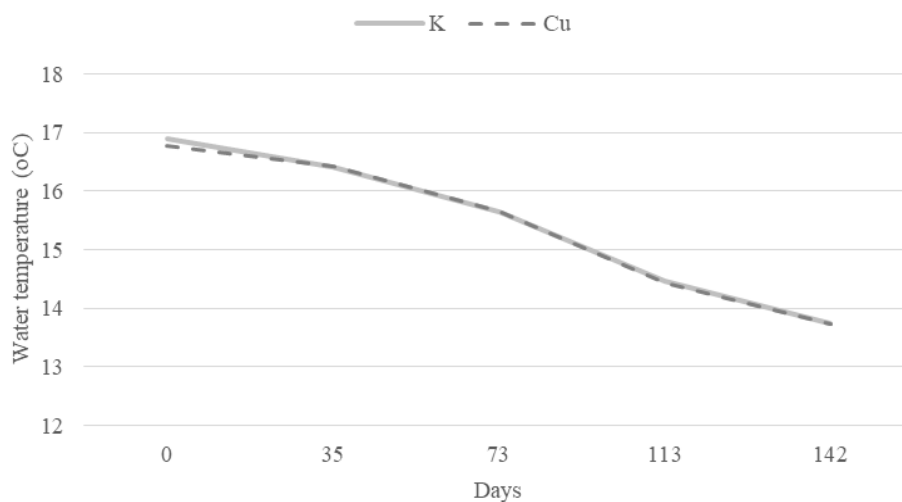
The basic parameters of water quality (Table 2) during the experiment in the observed groups K and Cu were similar.

Table 2. Average values ( $\pm$  SD) and CV of analyzed physical and chemical characteristics of water during the experiment.

Parameter	K		Cu	
	Mean $\pm$ SD	CV	Mean $\pm$ SD	CV
Water temperature ( $^{\circ}$ C)	15.11 $\pm$ 0.02	0.15	15.11 $\pm$ 0.01	0.07
Dissolved oxygen, O <sub>2</sub> (mg/l)	8.16 $\pm$ 0.08	1.01	8.15 $\pm$ 0.04	0.44
Water saturation O <sub>2</sub> (%)	82.17 $\pm$ 0.88	1.08	82.19 $\pm$ 0.49	0.59
Water pH	7.21 $\pm$ 0.05	0.66	7.20 $\pm$ 0.02	0.22

SD – standard deviation; CV – coefficient of variation.

The analysis using the t-test revealed no statistically significant differences ( $p > 0.05$ ) of the observed water quality parameters between the groups. At the beginning of the experiment, the water temperature (Graph 1) was high for rainbow trout that had just swum and averaged 16.8  $^{\circ}$ C (Cu) and 16.9  $^{\circ}$ C (K), but there was no increased mortality.



Graph 1. Average water temperatures during the experiment.

From the beginning to the end of the experiment, there was a constant drop in water temperature, which, in the last period, averaged 13.7  $^{\circ}$ C in the Cu and K

groups. The water temperature was initially very high and was not in the optimal range for the swimming-up of fry rainbow trout, however, no negative effects of high temperature (growth, conversion, survival) were observed.

The average total length and body weight of individuals from the K and Cu groups (Table 3) during the experiment were similar, thus no statistically significant difference was found between the means of the average weight and total body length ( $p > 0.05$ ).

Table 3. Average weight (g), total body length (cm) ( $\pm$ SD) and CV during the experiment.

Days	Parameter	K		Cu	
		Mean $\pm$ SD	CV	Mean $\pm$ SD	CV
0	BW (g)	0.199 $\pm$ 0.008	4.00	0.197 $\pm$ 0.009	4.38
	TL (cm)	2.876 $\pm$ 0.036	1.27	2.893 $\pm$ 0.038	1.30
35	BW (g)	0.91 $\pm$ 0.02	1.99	0.86 $\pm$ 0.03	3.30
	TL (cm)	4.54 $\pm$ 0.05	1.11	4.44 $\pm$ 0.06	1.27
73	BW (g)	3.97 $\pm$ 0.02	0.47	3.89 $\pm$ 0.08	2.17
	TL (cm)	7.32 $\pm$ 0.02	0.23	7.27 $\pm$ 0.04	0.53
113	BW (g)	10.30 $\pm$ 0.12	1.15	10.29 $\pm$ 0.43	4.15
	TL (cm)	9.65 $\pm$ 0.07	0.77	9.67 $\pm$ 0.12	1.29
142	BW (g)	22.17 $\pm$ 0.66	2.97	22.10 $\pm$ 1.20	5.44
	TL (cm)	12.39 $\pm$ 0.07	0.53	12.42 $\pm$ 0.14	1.14

SD – standard deviation; CV – coefficient of variation; BW – body weight; TL – total length.

The average body weights were similar in the K and Cu groups, but it can be seen that SD and the coefficient of variation in the Cu group increased with increasing body weight. FCR and growth characteristic parameters ( $\pm$ SD) by days are shown in Table 4.

The feed coefficient ratio was the lowest in the first 35 days, and then with the increase of weight, the FCR also increased, which is an indicator of good utilization of consumed feed, even with the determined increase. Condition factor (CF) tended to increase in individuals from both groups (K and Cu) from the beginning to the end of the experiment, while SGR had a declining trend (Table 4). SGR is known to decrease with an increasing body weight of fish (Bureau et al., 2000). During the first 35 days, SGR was the highest and was  $4.36 \pm 0.07$  in group K and  $4.21 \pm 0.16$  in group Cu. Uysal & Alpbas (2002) state that the SGR of rainbow trout during the first 30 days was 3.87%, at an average water temperature of 11.90°C, and the faster growth found in our study can be attributed to the higher water temperature which averaged 16.42°C during the first 35 days of the experiment in the K and Cu groups. The analysis of CF and the SGR t-test by controls did not reveal a statistically significant difference between the means ( $p > 0.05$ ).

TGC from the beginning to the end of the experiment had a tendency to increase (Table 4) in individuals from both the control group (K) and the group treated with copper microparticles (Cu). The analysis of the TGC t-test by controls did not reveal a statistically significant difference in the mean ( $p > 0.05$ ).

Table 4. Growth parameters of juvenile rainbow trout (mean  $\pm$  SD) and CV during the experiment.

Parameter	Days	K		Cu	
		Mean $\pm$ SD	CV	Mean $\pm$ SD	CV
CF	0	0.84 $\pm$ 0.027	3.21	0.81 $\pm$ 0.006	0.79
	35	0.98 $\pm$ 0.034	3.46	0.98 $\pm$ 0.005	0.53
	73	1.01 $\pm$ 0.006	0.55	1.01 $\pm$ 0.025	2.43
	113	1.15 $\pm$ 0.030	2.60	1.14 $\pm$ 0.048	4.21
	142	1.17 $\pm$ 0.023	1.98	1.15 $\pm$ 0.027	2.32
FCR	35	0.48 $\pm$ 0.01	1.50	0.52 $\pm$ 0.02	3.25
	73	0.56 $\pm$ 0.01	1.64	0.57 $\pm$ 0.003	0.50
	113	0.62 $\pm$ 0.02	3.09	0.63 $\pm$ 0.04	6.95
	142	0.73 $\pm$ 0.02	2.75	0.68 $\pm$ 0.03	4.94
SGR	35	4.36 $\pm$ 0.07	1.70	4.21 $\pm$ 0.16	3.87
	73	3.97 $\pm$ 0.046	1.17	4.08 $\pm$ 0.043	1.05
	113	2.38 $\pm$ 0.040	1.70	2.43 $\pm$ 0.107	4.39
	142	2.55 $\pm$ 0.079	3.09	2.55 $\pm$ 0.046	1.80
TGC	35	0.067 $\pm$ 0.001	0.91	0.064 $\pm$ 0.002	3.78
	73	0.106 $\pm$ 0.001	1.11	0.107 $\pm$ 0.001	0.76
	113	0.102 $\pm$ 0.002	1.53	0.104 $\pm$ 0.005	5.10
	142	0.154 $\pm$ 0.006	3.61	0.153 $\pm$ 0.005	3.26

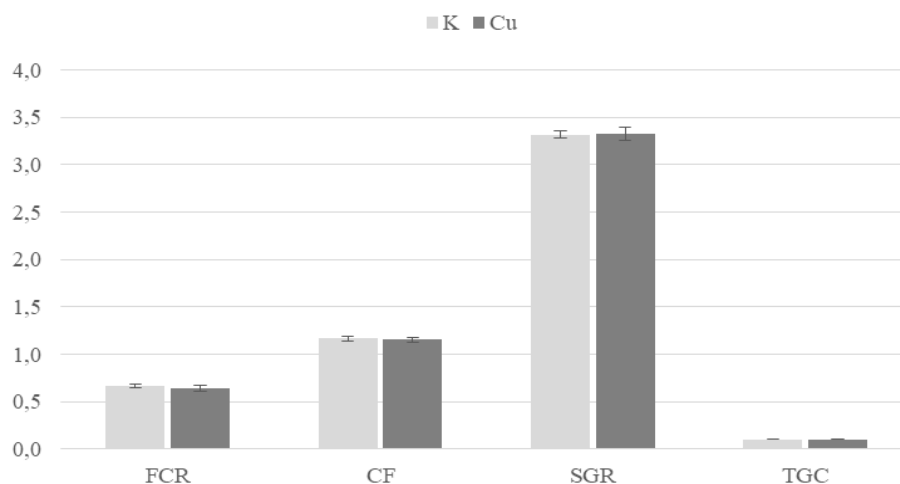
SD – standard deviation; CV – coefficient of variation.

It can be seen from Table 4 that the observed parameters of growth characteristics were similar in individuals from the K and Cu groups, which indicates that juvenile rainbow trout in treatment with copper microparticles had similar growth tendencies.

Graph 2 shows the analyzed parameters, total FCR, CF, SGR and TGC during the experiment.

The coefficient of feed conversion is one of the indicators of the success of fish farming and good breeding conditions. The analysis of the total FCR showed that it was slightly higher in group K ( $0.66 \pm 0.021$ ) in relation to the group Cu ( $0.64 \pm 0.031$ ), and the observed difference in mean was not statistically significant ( $p > 0.05$ ;  $t = 0.937$ ;  $p = 0.402$ ). Shah et al. (2018) state that during the growing period of rainbow trout with an initial weight of 5 g to a final weight of 20 g grown for six months, at average water temperatures of 6 to 9°C, the FCR was 1.9 and 3.1. Based on the total FCR, it can be seen that the feed conversion ratio in the Cu

group was slightly better, i.e. that the individuals used feed somewhat better in the treatment with copper microparticles (Cu).



Graph 2. Total FCR, CF, SGR and TGC ( $\pm$  SD) juvenile rainbow trout in K and Cu groups.

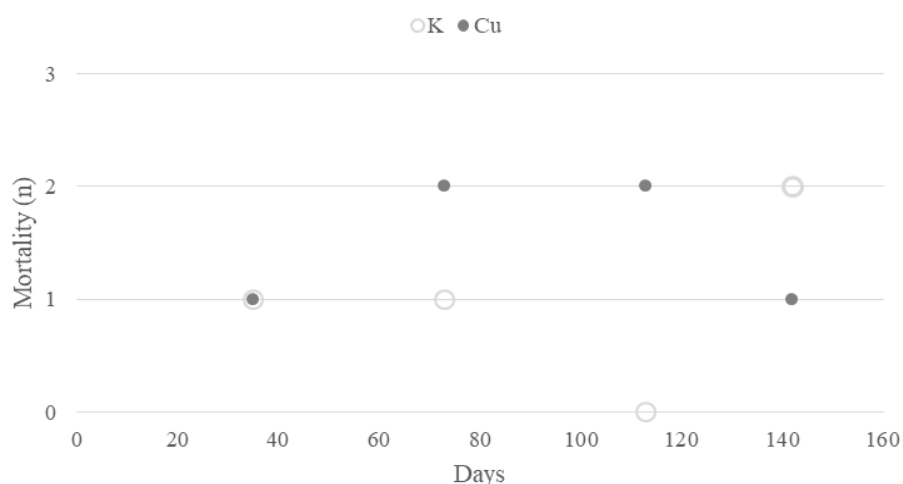
During the breeding process, it is important to monitor the condition of the fish, which indicates the general condition and growth of the rainbow trout. The condition factor of rainbow trout in this experiment was slightly higher in the control group (K) and was  $1.167 \pm 0.03$ , while in the Cu group, it was  $1.153 \pm 0.03$ , and the difference in environments was not statistically significant ( $t = 0.649$ ;  $p = 0.552$ ). Dekić et al. (2016) state that the condition factor of fish is one of the ways to monitor the impact of the breeding environment on fish. The specific growth rate (SGR) of juvenile rainbow trout in the observed period was similar ( $K = 3.317 \pm 0.04$ ;  $Cu = 3.323 \pm 0.07$ ), and no statistically significant difference was found ( $t = 0.145$ ;  $p = 0.892$ ), which means that the individuals had similar growth during the observed period. The thermal unit growth coefficient (TGC) of juvenile rainbow trout was the same in both observed groups ( $K = 0.103 \pm 0.001$ ;  $Cu = 0.103 \pm 0.003$ ), and there was no statistically significant difference ( $t = 0.189$ ;  $p = 0.859$ ).

Observing the total values of CF, SGR and TGC, it is evident that there are slight differences (not statistically significant) between the individuals in the control (K) group and the group treated with copper microparticles (Cu). The observed indicators of the growth characteristics of juvenile rainbow trout indicate very good growth, condition and feed utilization.

Although water temperatures at the beginning of the experiment were high for rearing juvenile rainbow trout, which tended to decline steadily until the end of the



experiment, juvenile survival was high at 97.33% (K) and 96.00% (Cu). Larenas-Uriá et al. (2016) report that, in the conducted experiment of growth and survival of fingerlings, using three different feeds during 8 weeks, survival was 98.5% in the control group and in the treated groups – 98.7%, 99.3% and 99.4%, which was higher compared to the results of this study. These results can be related to the duration of the study, which in our case was 20 weeks. Survival (n) of individuals in the control group averaged  $48.67 \pm 1.53$  individuals, and in group Cu, the average survival of individuals was  $48.00 \pm 1.73$  individuals, and no statistically significant difference ( $t = 0.500$ ;  $p = 0.643$ ) was found between the analyzed groups.



Graph 3. The mortality (n) of juvenile rainbow trout during the experiment.

Although the water temperature in the first half of the experiment was 17–15°C, the mortality of rainbow trout was not high (Graph 3). Mortality was similar in the examined groups (K and Cu), which means that there was no negative effect of copper microparticles on the increase in mortality.

### Conclusion

The analysis of the obtained results showed that the growth of mass and total body length, as well as FCR and the analyzed growth characteristics of CF, SGR and TGC of juvenile rainbow trout (*Oncorhynchus mykiss*) in the control group (K) and the group with copper microparticles (Cu) were similar, without statistically significant differences ( $p > 0.05$ ). The survival of rainbow trout in the control group (K) and the group with copper microparticles (Cu) was high, i.e. mortality was low, without a statistically significant difference between the examined groups ( $p > 0.05$ ). Based on the obtained results, it can be seen that the growth and

survival of individuals in the K (without copper microparticles) and Cu (with copper microparticles) groups were similar, namely good growth and survival of juvenile rainbow trout in the K and Cu groups were achieved.

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EFEKTI MIKROČESTICA BAKRA NA RAST I PREŽIVLJAVANJE  
JUVENILNE DUŽIČASTE PASTRMKE  
(*ONCORHYNCHUS MYKISS* WAL.)

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R e z i m e

Tokom uzgoja juvenilne dužičaste pastrmke (*Oncorhynchus mykiss*) gubici mogu biti veoma visoki. U akvakulturi se primjenjuju različita sredstva, kao što je bakar, radi preventivnog djelovanja i sprečavanja pojave uzročnika bolesti (ekto parazita i drugih). Cilj rada je bio da se utvrde efekti mikročestica bakra fiksiranih za podlogu na rast i preživljavanje juvenilne dužičaste pastrmke (*Oncorhynchus mykiss*) od proplivavanja do starosti od 5 mjeseci. Eksperiment je realizovan u Laboratoriji za akvakulturu Poljoprivrednog fakulteta Univerziteta u Banjoj Luci, tokom 142 dana u protočnim akvarijumima zapremine 65 l/akvarijum. Eksperiment je postavljen u dvije grupe (150 jedinki/grupi) sa tri ponavljanja (50 jedinki/ponavljanju), kontrolna grupa bez mikročestica bakra (K) i grupa sa mikročesticama bakra (Cu). Početna masa i totalna dužina tijela (prosjeck±SD) dužičaste pastrmke u grupi K iznosila je 0,199±0,008 g i 2,876±0,036 cm, a u grupi Cu 0,197±0,009 g i 2,893±0,038 cm. Prosječna totalna dužina i masa tijela jedinki iz K i Cu grupe po kontrolama bile su slične ( $p > 0.05$ ). Razlike između FCR, CF, SGR i TGC između grupe K i grupe Cu nisu bile statistički značajne ( $p > 0.05$ ). Preživljavanje juvenilne dužičaste pastrmke je bilo visoko i iznosilo je u grupi K 97.33% i 96.00% u grupi Cu, te nije utvrđena statistički značajna razlika ( $p > 0.05$ ). Rast i preživljavanje juvenilne dužičaste pastrmke u grupama K i Cu bili su slični.

**Ključne riječi:** mikročestice, bakar, rast, preživljavanje, juvenilna, dužičasta pastrmka.

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