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Survival Rate and Growth Length of Catfish (*Clarias gariepinus*) Exposed to Microplastics

Diana Permatasari[1,*], Mohamad Fadjar[2] and Ating Yuniarti[2]
[1]Aquaculture Study Program, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya, Ketawanggede, Lowokwaru Sub-District, Malang, East Java 65145, Indonesia
[2]Aquatic Resources Management Department, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya, Ketawanggede, Lowokwaru Sub-District, Malang, East Java 65145, Indonesia

*Correspondence :
dyanapermatasari13@gmail.com

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Abstract

Aquaculture has a land potential of 17.92 million ha consisting of 2.83 million ha of freshwater aquaculture potential, 2.96 million ha of brackish water aquaculture and 12.12 million ha of marine aquaculture. One of the freshwater species that has the potential to be cultivated is catfish (*C. gariepinus*). But now, as a result of the decline in the quality of the freshwater environment, catfish are prone to contamination by plastic waste. If microplastic particles have accumulated in large numbers in the fish's body, they can clog the fish's digestive tract and affect the growth of catfish which will affect the results of aquaculture production. The purpose of this study was to determine the survival rate or survival rate of catfish exposed to microplastics. The research design used in this study was a Completely Randomized Design (CRD) with 1 control variable, namely no mixing of microplastics in commercial feeds and 3 different doses of microplastics, namely A (5%), B (10%), C (15%) mixed with commercial feed, where each treatment was repeated 3 times. The results of the one-way ANOVA analysis showed that the treatment of exposure to microplastics with different doses did not have a significant effect ($P<0.05$) on the survival rate and length growth of catfish. The survival rate ranges from 65- 100%. Survival values that were not significantly different could indicate that there was competition for space and dominance of larger fish in the aquarium which affected fish growth, feed utilization and fish survival. Meanwhile, the length growth value ranged from 21-28cm. The length growth value which was not significantly different could indicate that the dose of microplastic used in the study did not result in muscle and bone cell division which was the cause of the increase in the body length of fish.

INTRODUCTION

Aquaculture is an activity to produce aquatic organisms or in general in controlled containers and conditions (Shafitri and Sujarwo, 2017). Nationally, aquaculture has a land potential of 17.92 million ha consisting of 2.83 million ha of freshwater aquaculture potential, 2.96 million ha of brackish water aquaculture and 12.12 million ha of marine aquaculture (Arrazy and Primadini, 2021). One of the freshwater species that has the potential to be cultivated is catfish (*C. gariepinus*). Besides being rich in essential amino acids, unsaturated fats, and other nutritional components that have a health impact on the body, Negara *et al*. (2015) stated that catfish is also considered a specialty dish, partly because of its delicious meat taste, affordable price, and easy cultivation.

But now, as a result of the decline in the quality of the freshwater environment, catfish are prone to contamination with plastic waste that can endanger the health of humans who consume them. This condition is often ignored by cultivators. Therefore, food safety issues such as polystyrene compounds have long been a concern. This compound was never produced intentionally but formed as a by-product of the manufacture of chemical compounds such as Styrofoam plastic. Styrofoam plastic that is destroyed releases harmful substances in the form of polystyrene compounds that stick to microplastics. According to Mahendradatta *et al*. (2019), the abundance of microplastics in fresh waters in Indonesia is on average 13.33-816.9 particles/L. The concentration of harmful microplastics in catfish is 50-500 g/L (Wang *et al*., 2020).

Polystyrene compounds are toxic to both animals and humans. Microplastics eaten by fish will bioaccumulate and will settle in the fish's body, especially in the gills and stomach (Lu *et al*., 2016). Browne *et al*. (2013) explained several possibilities of carnivorous fish could be contaminated with microplastics through the bottom of the sand substrate containing microplastics being stirred, the prey of carnivorous fish had eaten microplastics before. If microplastic particles have accumulated in large quantities in the fish's body, they can clog the fish's digestive tract and affect the growth of catfish which will affect the results of aquaculture production.

METHODOLOGY

Place and Time

The research was conducted from March to April 2022. The maintenance of test animals and observation of glucose level parameters was carried out at the Fish Cultivation Laboratory, Fish Reproduction Division, Brawijaya University. The SEM EDX test was conducted at the FESEM Facility (FEI Quanta FEG 650) Central Laboratory of Living Sciences (LSIH), Brawijaya University. Molecular biology analysis was carried out at the Bioscience Institute, Brawijaya University, Malang.

Research Materials

The materials used in this study included catfish (*C. gariepinus*) ± 10-15cm in size, Styrofoam, commercial feed Comfeed 781-2 protein 30-32%, freshwater, reagents (ammonia, nitrate, nitrite), aquades, tissue, label, trash bag, black duct tape, zip plastic, porstex, gloves, chlorine, and frosted paper.

Research Design

The research design used in this study was a Completely Randomized Design (CRD). This study used 4 treatments with 3 replications. This study uses an independent variable in the form of exposure to microplastic doses which refers to the study (Ding *et al*. 2018). The calculation of the dose of microplastics was carried out with a median of 10% of microplastics in the feed, so the doses of microplastics used in this study were A (5%), B (10%), C (15%) and K (0%). Meanwhile, the feed dose uses an FR (Feeding Rate) of 3% of the total fish biomass (Arief *et al*., 2014). The stocking density used in this study refers to SNI (2000) so there are 20 catfish in one aquarium.

Work Procedure

Type of Polymer Plastic

The microplastic used for this research is polystyrene. Polystyrene is a polymer with a styrene monomer, polystyrene is usually thermoplastic (Wagner *et al*., 2014). Polystyrene can be solid or foam, while styrene monomer is liquid. There are four types of polystyrene products, namely General purpose polystyrene, High impact polystyrene, Polystyrene foam, and Expanded polystyrene foam. Polystyrene can be used as a carbon source for microorganisms. The chemical formula of polystyrene can be seen in Figure 1. One of the derivative products of polystyrene is Styrofoam. Styrofoam is a type of thermoplastic polymer that is widely used as a vibration barrier and protective material for goods. Styrofoam is generally white and stiff and is often used as food packaging boxes. The flexible, practical, and easy-to-use nature of Styrofoam make its use increase (Satriyatama *et al*., 2019).

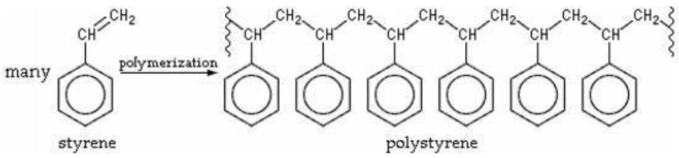

Figure 1. Polystyrene chemical formula (Ho, *et al*., 2018).

Microplastic Manufacturing

Microplastics are obtained by crushing Styrofoam using a wet blender. The ratio of the amount of solvent and Styrofoam is (1:2) and (2:1). This comparison is used to see the size and shape of the smallest microplastic particles during the SEM EDX test. The process of destroying Styrofoam to become microplastic needs to be carried out 3-4 times. After obtaining the crushed microplastic, it was then dried in the sun. After drying, the powder was sieved using a 22cm diameter flour sieve with 80 mesh which when converted to a micrometer size was 177μm. After drying and sifting well, the Styrofoam powder is stored in a plastic jar. In general, the particle size of flour that can be filtered by a sieve is at least 80 mesh. This is in accordance with the provisions of SNI. Diana, *et al*. (2020) stated that the particle size for the flour itself is 80 mesh according to SNI No.76222:2011. The obtained microplastics were then subjected to an SEM EDX test to determine the exact size and shape of the microplastics used.

Manufacturing and Supplementation of Microplastics in Feed

Microplastics for research were obtained by crushing Styrofoam using a wet blender then dried and sieved using an 80-mesh flour sieve. The ratio of the amount of solvent and Styrofoam is (1:2) and (2:1). This comparison is used to see the size and shape of the smallest microplastic particles during the SEM EDX test. Supplementation or exposure to microplastics given in vivo to catfish using microplastic mixing according to a predetermined dose was A (5%), B (10%), C (15%) and K (0%) microplastic.

Maintenance and Calculation of Survival Rate (SR) in Catfish

Catfish were kept for 30 days in an aquarium measuring 50x30x30cm with a water volume of 20L. Each 1L of water is filled with 1 test animal so that in one aquarium there are 20 catfish. Feeding is done ad libitum. The frequency of feeding that has been mixed with microplastics on catfish seeds is given 2 times, namely in the morning at 07.00-09.00 WIB and in the afternoon at 15.00-16.00 WIB (Adrial *et al*., 2018). The number of dead fish was recorded daily in the research logbook from the beginning to the end of the study (Pratama *et al*. 2016). The main parameter observed is survival (SR) based on the formula (Hasniar, 2014):

$$SR = \frac{Nt}{No} \times 100\%$$

Where:
SR = survival rate (%)
Nt = final number of catfish
No = initial number of catfish

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Calculation of Growth Length (Lm) in Catfish

Absolute body length measurements were carried out at the beginning, middle and end of maintenance. The absolute length growth of fish fry was carried out using the formula according to Nazlia and Zulfiadi (2014):

$$L = Lt - Lo$$

Where:

L = absolute length growth (cm)
Lt = final $\bar{x}$ length of individuals (cm)
Lo = initial $\bar{x}$ length of individuals (cm)

Data Analysis

The survival data analysis used was One Way ANOVA (Test F, Shapiro-Wolk) according to the design used in this study, namely a Completely Randomized Design (CRD). This analysis was conducted to determine the effect of treatment (independent variable) on the response of the measured parameters. If the test value is significantly different or very significantly different, then proceed with the LSD (Least Significance Different) test to determine which treatment gives the best results at the 0.05 level (95% confidence interval) and find out the differences between treatments.

RESULTS AND DISCUSSION

SEM EDX (Scanning Electron Microscope-Energy Dispersive X-Ray Spectroscopy) Styrofoam Testing

Styrofoam powder samples were tested by SEM EDX using FESEM set up for EDX High Vacuum. SEM test samples that had previously been crushed with a ratio of 1:2 solvent and Styrofoam showed the results of the shape and size of Styrofoam of 197.8-595.2 m. Meanwhile, samples that have been crushed with a ratio of solvent to Styrofoam 2:1 showed the results of the shape and size of Styrofoam of 448.2-819.1 m. These results indicate that the Styrofoam powder used in the study is still in the category of microplastic particle size, even though the crushed sample in a ratio of 2:1 is larger than the sample that is crushed in a ratio of 1:2. This is in accordance with the opinion of Supusepa *et al*. (2022) that microplastics are plastics measuring less than 5mm (5000μm). A plastic particle can be said to be microplastic if it has a minimum size of 10 m. Generally, the size of microplastics ranges from 150-300 m. Figures 2 and 3 are the results of SEM scanning magnification (a) 50x and (b) 100x.

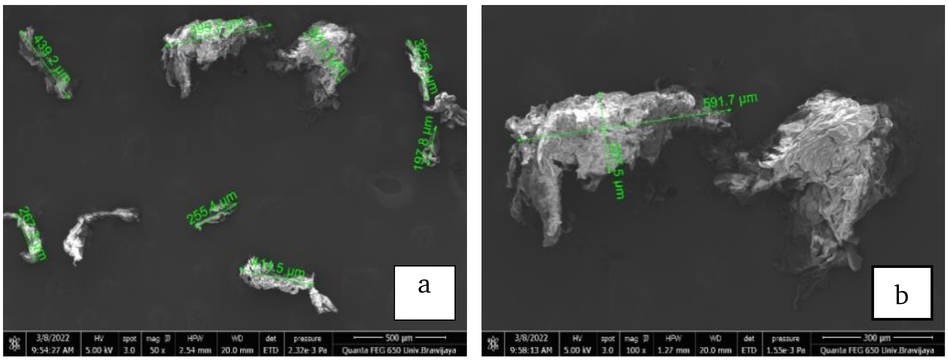

Figure 2. SEM magnification scanning results on Styrofoam powder (comparison of solvent and Styrofoam 1:2).

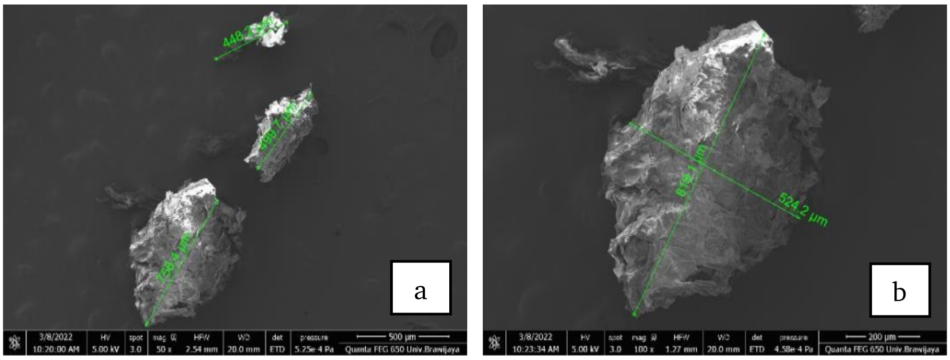

Figure 3. SEM magnification scanning results on Styrofoam powder (comparison of solvent and Styrofoam 2:1).

The results of the EDX test on Styrofoam samples only showed two dominant types of elements making up Styrofoam, such as element C (carbon) and element O (oxygen). Element C in the sample with a ratio of 1:2 solvent and Styrofoam dominates at 81.45%, while element O is 18.55%. Element C in the sample with a solvent ratio of 2:1 Styrofoam also dominates at 80.14%, while element O is 19.86%. Based on the EDX test, it was found that element C with the largest proportion in both samples.

These results show a normal percentage considering that Styrofoam is indeed composed of several chemicals such as styrene and benzene which consists of element C with many functional groups so that it forms poly bonds and composes polystyrene. This is in accordance with the opinion of Yona *et al*. (2021) that Styrofoam microplastic is a type of polystyrene polymer with the highest carbon chain content of about 92%. The characteristics of the elements that make up polystyrene are still dominated by C/O which also plays a role in the particle degradation process because the bonds are easy to decompose. Table 1 is the chemical composition of the EDX test.

Table 1. EDX test chemical composition.

Sample Comparison	Element	Weight (%)	Atom (%)
1:2	C	76.72	81.45
	O	23.28	18.55
2:1	C	75.18	80.14
	O	24.82	19.86

Survival Rate

The survival rates of catfish obtained during the study from each treatment are presented in Table 2 and Figure 4.

Table 2. Average survival rate of catfish (*C. gariepinus*) during the study.

Treatment	Repetition			Total	Average ±
	1	2	3	(%)	STDEV
K (0%)	85	95	100	280	93.33±7.64[a]
A (5%)	85	90	90	265	88.33±2.89[a]
B (10%)	75	70	85	230	76.67±7.64[a]
C (15%)	70	65	85	220	73.33±10.41[a]
Total				995	

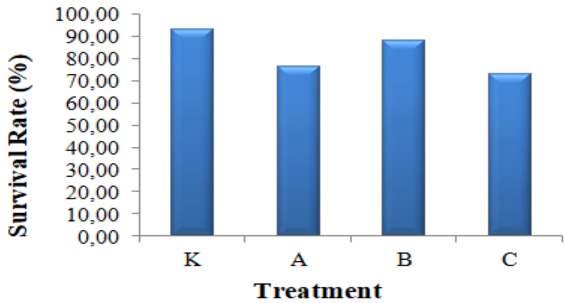

Figure 4. Catfish (*C. gariepinus*) survival bar graph.

The results of one-way ANOVA analysis showed that the treatment of exposure to microplastics with different doses did not have a significant effect (P < 0.05) on the length growth of catfish. The length growth value of African catfish (*C. gariepinus*) in Table 3 ranged from 21-28 cm. It is known that the survival rate in treatment C with a dose of 15% microplastics got the lowest average of 23±2 cm, then in treatment B with a dose of 10% microplastics, the average was 24±1 cm, in treatment A with 5% microplastic dose got an average of 25.67±0.58 cm and the control with 0% microplastic dose got the highest average of 26.67±1.53 cm.

The effect of microplastic exposure on the absolute length growth of African catfish fry (*C. gariepinus*) had no effect, it was indicated that the dose of microplastic used in the study did not result in muscle and bone cell division which was the cause of the increase in body length of the fish. This is also supported by research conducted by Mulqan *et al*. (2017), that growth is influenced by external and internal factors, where the largest part of fish length growth is muscle and bone cell division.

The high absolute length value in the control treatment with a dose of 0% microplastic is due to the water quality of the maintenance media being in good condition because, without exposure to microplastics, this is in accordance with the statement of Monalisa and Minggawati (2010), which states that the live fish media must have properties suitable for fish life. This is because the growth of living things in water is influenced by the medium of life. The low value in treatments A, B, and C was due to the fish rearing media being mixed with microplastics.

CONCLUSION

The exposure to microplastics with different doses did not have a significant effect (P<0.05) on the survival rate and length growth of catfish. The survival rate ranges from 65-100%. It is known that the lowest mean survival value in treatment C with a dose of 15% microplastic was 73.33±10.41% and the highest average value was found in the control with a microplastic dose of 0% at 93.33±7.64%. Survival values that were not significantly different could indicate that there was competition for space and dominance of larger fish in the aquarium which affected fish growth, feed utilization, and fish survival. Meanwhile, the length growth value ranged from 21-28cm. It is known that the length growth in treatment C with a dose of 15% microplastics got the lowest average of 23±2 cm and the highest average value was found in the control with a dose of 0% microplastics of 26.67±1.53 cm. The length growth value which was not significantly different could indicate that the dose of microplastic used in the study did not result in muscle and bone cell division which was the cause of the increase in the body length of fish.

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