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Effect of Microplastic Exposures to The Male Gonad Histology of Catfish (*Clarias gariepinus*)

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Abstract

Aquaculture activities cannot be separated from obstacles that can lead to the failure of aquaculture, including the presence of microplastics. Microplastic polystyrene (Styrofoam) is a type of plastic that is commonly found in aquatic organisms (35%). Microplastics in fish can cause hormonal disturbances and high vacuolation in gonads which can cause apoptosis in gonadal cells. This study aimed to determine the effect of microplastic exposure on catfish *(Clarias gariepinus)* feed on the gonadal histology of male catfish. The research design used an experimental method with a Completely Randomized Design (CRD) which was divided into four treatments and three replications, namely 3% of the feed rate with treatments A (5% microplastics), B (10% microplastics), C (15% microplastics), and K (0% microplastics). Microplastic exposure treatment was given to fish by mixing it with commercial feed for 30 days. The results showed that microplastic exposure had a significant effect on the 15th and 30th days of the study on the Gonad Maturity Level (GML) and Gonado Somatic Index (GSI) of male catfish *(C. gariepinus)* in treatments A, B and C compared to controls (K). Treatment C (Microplastics 15%) had the lowest GML and GSI values, while treatment K (Microplastics 0%) had the highest GML and GSI values. Treatments A, B, and C experienced changes and delays in the development of the gonadal cell structure, while the control (K) developed well. This is presumably because the nutritional needs of control fish (K) were more fulfilled than treatments A, B, and C exposed to microplastics.

INTRODUCTION

The growth of aquaculture is very much needed in the context of food and nutrition security in the future. However, it is also a challenge in terms of managing the impact on the environment because it is prone to contamination by waste both from outside and from within the cultivation environment (Phillips *et al.*, 2016). The aquaculture sector in Indonesia is still dominated by catfish. The type of catfish that is widely cultivated in the African catfish (local name: Dumbo) (*C. gariepinus*) (Dewi and Tahapari, 2017).

This follows the data from the Ministry of Marine Affairs and Fisheries, which published positive achievements for the catfish aquaculture sector, which

increased significantly, namely 13.84% in 2015-2018. Even in 2020 national catfish production reached 347,511.48 tons and is expected to continue to increase (KKP, 2020). Naturally, the female sex ratio is higher than the male, but its production continues to decline, on the other hand, the availability of males is needed for fish spawning and stock availability in hatcheries (Ibrahim *et al.,* 2018).

The process of successful catfish farming is influenced by several things, one of which is water quality. Poor water quality and feed mixed with waste will make fish susceptible to disease and can cause crop failure in aquaculture. One of the reasons is plastic waste. Plastic waste in the aquatic environment will break down into small plastic particles ranging from 0.1 to 5 mm which are known as microplastics (MPs) (Pinheiro *et al.,* 2017). One of the most common types of microplastics is Styrofoam. Styrofoam is a derivative of polystyrene which is more commonly found in the body of organisms than in the water column because of its small size so that it is easily eaten by biota, which is 35% including plankton as natural food for fish (Cera *et al.,* 2020). Styrofoam is a type of polystyrene plastic formed from styrene monomer and is thermoplastic. Excessive use of Styrofoam causes the accumulation of waste because it is difficult to degrade (Satriyatama *et al.,* 2019). Recent records show that freshwater contaminated with microplastics increased with a maximum concentration in water of 1,146,418.36 grains/m^3 (Qiang and Cheng, 2021). Meanwhile, the concentration of harmful microplastics in catfish is 50-500 g/L (Wang *et al.,* 2020).

The accumulation of microplastics in the environment is not good for aquatic organisms. Microplastics in the body of organisms can cause oxidative stress, metabolic disorders, and damage to the liver, kidneys, gills and other vital organs (Lusher *et al.*, 2017). Fish that in the long term consume microplastics will reduce the reproductive ability of fish. In addition, the accumulation of chemicals contained in Styrofoam causes hormonal disturbances and high vacuolation in the gonads which can cause apoptosis in gonadal cells (Driscoll *et al.,* 2020; Sharifinia, *et al.,* 2020). Therefore, this study aimed to determine the effect of exposure to microplastics in catfish (*C. gariepinus*) feed on the histology of male catfish gonads.

METHODOLOGY

Place and Time

The research was conducted from March 2022 to April 2022 at the Fish Cultivation Laboratory, Fish Reproduction Division, Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang.

Research Materials

The material used in this study was male catfish (*C. gariepinus)* size ± 10-15 cm, commercial Comfeed feed 781-2, microplastics (Styrofoam powder) size under 5 mm, formalin 10%, HE (*Hematoxylin Eosin)*. While tools used are aquarium size 50x30x30 cm^3, Blender, microscope (Olympus Cx41), sectio set, analytical balance, 80 mesh sieve, 10 ml volume plastic bottle, object glass, and cover glass.

Research Design

The research method used in this study is an experimental method with a quantitative approach (Samsundari and Wirawan, 2015). The research design used an experimental method with a Completely Randomized Design (CRD) which was divided into four treatments with three replications. The design treatment was carried out randomly on the variables used. Primary data is data that is taken directly through direct observation or experimentation from the object of research. While secondary data is obtained by explaining primary source evidence through various media such as books, scientific journals/articles, websites, or verbal evidence (Larsen *et al.*, 2020). Observation of the main parameters in the form of gonadal

sampling was carried out 3 times during the study for 15 days. The gonads that have been obtained are weighed for Gonado Somatic Index (GSI). The gonads were then placed in a plastic bottle containing 10% formalin for histology and microscopically observed for Gonadal Maturity Level (GML).

Work Procedure

Polystyrene Microplastic from Styrofoam

The microplastic used in this research is polystyrene (Styrofoam). Styrofoam is crushed with a wet blender. The process of crushing polystyrene powder needs to be done up to 2-3 times. After the powder was obtained, it was then dried in the sun and sieved using a flour sieve with a diameter of 22 cm with a mesh of 80 according to SNI No.76222:2011 (Marlisa *et al.*, 2020). The obtained microplastics were then subjected to SEM (Scanning Electron Microscope) test to determine the exact size and shape of the microplastics used.

Animal Test

Animal test in the form of male catfish (*C. gariepinus*) measuring ±10-15 cm was obtained from catfish farmers at the People's Breeding Unit (UPR) Mulyorejo, Malang and then adapted for 7 days at the Fish Cultivation Laboratory, Fish Reproduction Division, Faculty of Fisheries and Marine Sciences Universitas Brawijaya. *C. gariepinus* was reared in an aquarium measuring 50 cm x 30 cm x 30 cm with a water volume of 38 liters and a stocking density of 19 fish in each aquarium. Water quality parameters measured in this study were temperature, pH, dissolved oxygen (DO), ammonia, nitrite and nitrate.

Water quality measurements such as temperature, DO, and pH are carried out every day in the morning (06.00-07.00 WIB) and afternoon (15.00-16.00 WIB). Water quality parameters in the form of ammonia, nitrite and nitrate were carried out once a week. Feed was given twice a day at 07.00-08.00 WIB and 15.00-16.00 WIB. Estimated 3% of test animal biomass with a protein content expected to be 30% of commercial feed. The type of commercial feed used was Comfeed 781-2 catfish feed (Rahma *et al.*, 2015). This study uses an independent variable in the form of exposure to microplastic polystyrene (Styrofoam powder) doses which refers to the study of 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 (microplastic exposure treatment (5%) with a dose of 1.94 grams of microplastics and 36.81 grams of commercial feed), B (microplastic exposure treatment (10%) with a dose of 3.87 grams of microplastics and 34.87 grams of commercial feed), C (microplastic exposure treatment (15%) with a dose of 5.81 grams of microplastics and 32.93 grams of commercial feed) and K (the treatment was not exposed to microplastic in the feed with a commercial feed dose of 38.74 grams). According to Xie *et al.* (2020), gonadal cells (testes) will regenerate and proliferate once every 5-7 days. Thus, the time of rearing catfish with exposure to microplastics was carried out for 30th days with observations for 15th days for histology of the gonads (testes) to see changes in gonadal structure due to exposure to microplastics.

Gonad Histology (Testes)

The gonadal tissue samples were removed quickly, fixed in 10% formalin solution, and routinely processed for paraffin implants. Embedded tissue was cut to 5µm thickness and then stained with Eharlich Hematoxylin and eosin stain (H&E) and mounted on DPX. The slides were observed under a 400x microscope Olympus Cx41 (Jusmaldi *et al.*, 2018).

Data Analysis

Statistical analysis was performed using One-Way ANOVA. If the test values

are significantly different, then proceed with the Duncan Multiple Range Test (DMRT) post-hoc test. DMRT was used to determine and determine which treatment gave the best result at the 0.005 level (95% confidence level) and to determine the differences between treatments.

RESULTS AND DISCUSSION

Styrofoam Testing with SEM (Scanning Electron Microscope)

Samples of Styrofoam powder in the SEM test showed the results of the size and shape of the Styrofoam powder below 5 mm using the wet blender crushing method 1: 2 ratio for water and Styrofoam. This shows that the Styrofoam powder used in this study is still in the category of microplastics with sizes ranging from 197-591 µm (Figure 1). These results are in agreement with Azizi *et al.* (2022), a plastic particle can be categorized as microplastic if the minimum size is 10 µm. Generally, the size of microplastics ranges from 150 µm – 300 µm.

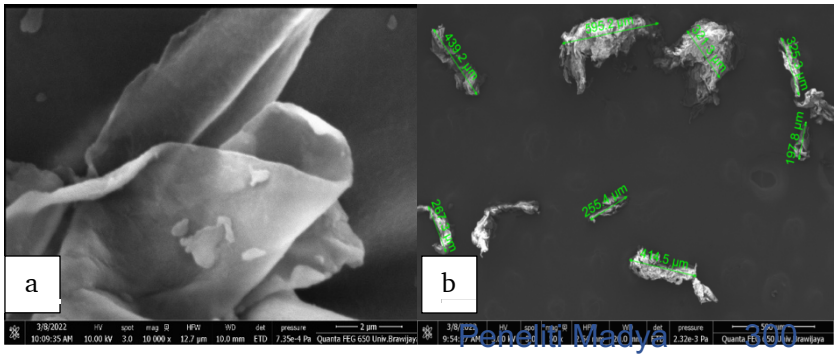

Figure 1. Shapes and sizes of microplastic polystyrene (Styrofoam) in magnification 10.000x (a) and 50x (b).

Gonadal Maturity Level (GML) of Male Catfish

Histological results of the gonads (testes) at the beginning of maintenance still looked good, the spermatogonia (sg), Sertoli cells (sc), and seminiferous tubules (st) were visible and complete. The results of the initial histological observations also showed that the gonads were still immature and still developing (Gonadal Maturity Level I) (Figure 2).

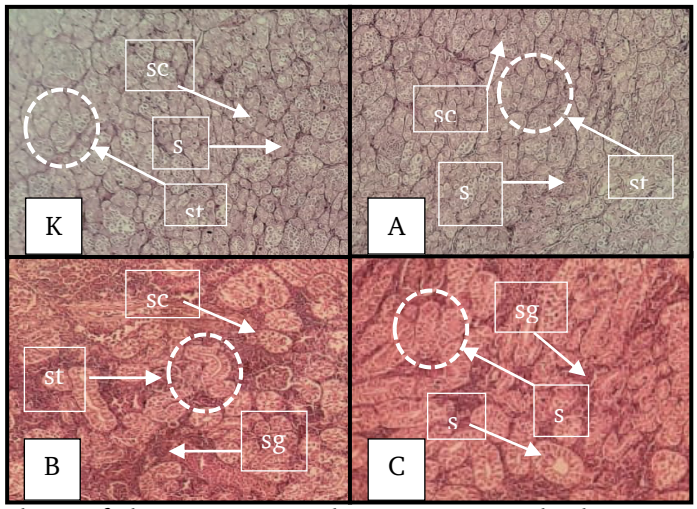

Figure 2. The histology of the testes in each treatment at the beginning of maintenance (treatment K, A, B, and C) spermatogonia (sg), Sertoli cells (sc) and seminiferous tubules (st) were clear and not damaged.

Observation of fish gonads on the 15th day showed a fairly good development of fish gonads in control fish, while in the treatment there was a slowdown in cell development and Sertoli cell vacancies. Treatment A showed the size of the Sertoli cells that began to expand and there was a slight vacuolation. Treatment B showed the development of almost mature spermatozoa (sz) and Sertoli cells (sc) were quite dense and piled up, while treatment C showed quite a lot of voids in Sertoli cells. A large number of empty Sertoli cells causes impaired gonadal development and can reduce fish fertility (Figure 3). The result of Gonadal Maturity Level K is GML II, while treatments A, B and C in GML I.

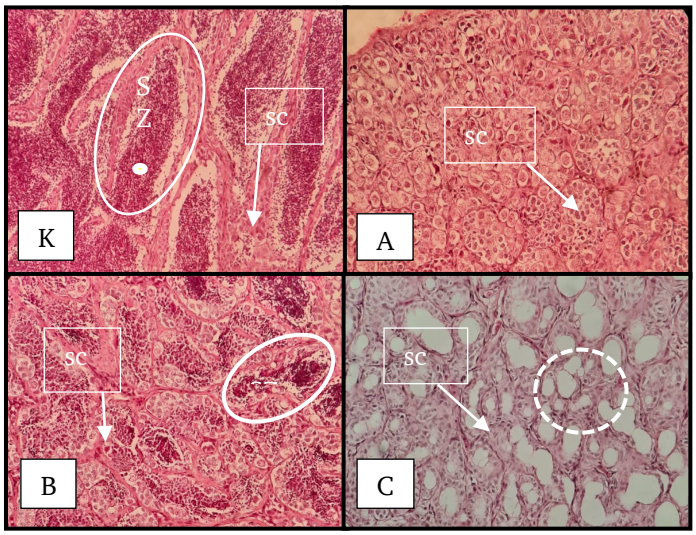

Figure 3. Testicular histology in each treatment on the 15th day of maintenance (treatments K, A, B, and C) with 400x magnification. The results showed that there was a change in gonadal structure and a slowdown in gonadal development in treatments A, B and C when compared to K.

The results of histology on the last day of rearing (day 30th) showed an irregular shape of the seminiferous tubules, large vacuolation and shrinkage of Sertoli cells that caused voids in the seminiferous tubule cells in the gonads. High vacuolation can cause apoptosis in gonads which can trigger cell death while shrinkage of Sertoli cells can cause fertility problems and inhibit sperm cell development. The result of Gonadal Maturity Level K is GML III, while treatments A, B and C in GML II (Figure 4).

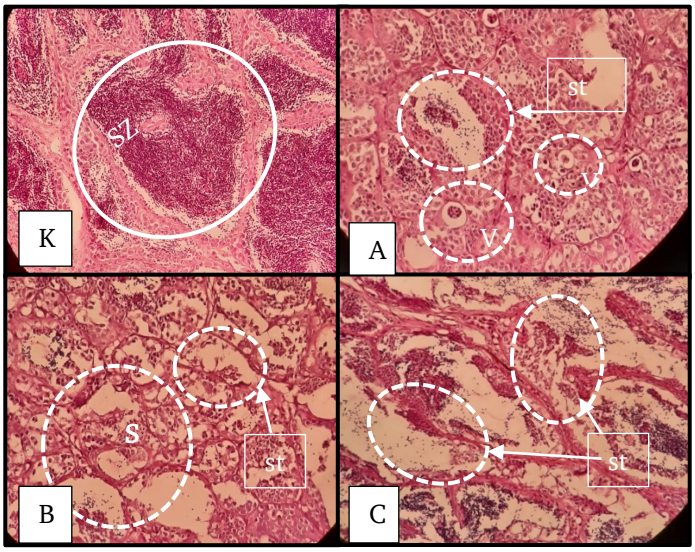

Figure 4. Testicular histology in each treatment on the 30th day of maintenance (treatments K, A, B, and C) with 400x magnification. There was vacuolation in treatment A, and damage to the gonad structure in B and C so that the formation of spermatozoa was disrupted.

Exposure to microplastics containing benzene and styrene as the chemical can alter or damage gonad cells (Kik *et al.*, 2020). This exposure can cause tubular epithelial vacuolization and inflammation of testicular cells, including narrowing of Sertoli cells. Sertoli cells are one of the important cells in the male reproductive organs (testes) where these cells have the function of supporting and providing nutrition for developing spermatozoa and producing the hormone Androgen Binding Protein (ABP). Shrinkage or damage to Sertoli cells can cause the degeneration of spermatogenic cells due to a lack of nutrients provided by Sertoli cells (Adeogun *et al.*, 2018; Berlina *et al.*, 2020).

Gonado Somatic Index (GSI) of Male Catfish

The results of the GSI of male catfish (*C. gariepinus*) can be determined by weighing the gonad weight and body weight of the fish once every 15 days during the study. The GSI of male catfish (*C. gariepinus*) is presented in Table 1. The result on the 0th day of maintenance, the average was almost similar, namely the highest 0.12% (treatments K and A) to the lowest 0.09% (treatments B and C). The next GSI value on the 15th day of maintenance obtained the highest average in the control treatment (K) which was 0.24% and the lowest was in the C treatment of 0.09%. The final GSI value on the 30th day of maintenance also showed the highest percentage value in the control treatment (K) which was 0.39% and the lowest was in treatment C, which was 0.13%.

Table 1. Gonado somatic index (GSI) of male catfish.

Day	Treatments	Subset for alpha = 0.05
15th	C	0.09[a]
	B	0.10[a]
	A	0.12[a]
	K	0.24[b]
30th	C	0.13[a]
	B	0.20[a]
	A	0.33[b]
	K	0.39[b]

The results of statistical analysis using One Way ANOVA (P>0.05) also showed no significant effect at the start of maintenance. Meanwhile, on the 15th and 30th day observations, the results were significantly different. The results of the 5% DMRT test in all treatments where treatment A (5% MPS), B (10% MPS), and C (15% MPS) were significantly different from treatment K (control) on day 15th. While the results on day 30th showed that treatments B (10% MPS) and C (15% MPS) were significantly different from treatments A (5% MPS) and K (control). The results are shown in Table 2.

Table 2. DMRT test results 5% for all treatments on the 15th and 30th days of rearing catfish.

Day	Treatment	Average GSI (%) ± St dev
0	K	0.12 ± 0.06
	A	0.12 ± 0.02
	B	0.09 ± 0.03
	C	0.09 ± 0.02
15	K	0.24 ± 0.03
	A	0.12 ± 0.01
	B	0.10 ± 0.04
	C	0.09 ± 0.03
30	K	0.39 ± 0.12
	A	0.33 ± 0.13
	B	0.20 ± 0.06
	C	0.13 ± 0.09

Catfish that eat feed with a mixture of microplastics in high doses will affect the value of the GSI of fish. The higher the dose of microplastic eaten by fish, the smaller the value of the GSI as the results shown in treatments A, B and C. Catfish that continuously eat feed with a mixture of microplastics will inhibit the fish from producing gonadotropin hormones and will adversely affect their organs. Fish that in the long term consume microplastics will reduce the reproductive ability of fish (Rochman *et al.,* 2014). Another study on the analysis of gonad (testicular) damage due to exposure to polystyrene microplastics in several vertebrates including fish (Japanese Medaka), also showed that the gonads will experience damage at the cellular level (chronic effect) within 21–28 days with a concentration of polystyrene microplastics exposure of 1 mg/l observed with Green fluorescent and Confocal laser scanning microscopy (Assas *et al.,* 2020; Jin *et al.,* 2022). Meanwhile, the length of the maintenance period can cause the bioaccumulation of microplastics in the bodies of exposed organisms. The accumulation of polystyrene microplastics gets a lot of negative responses where most organisms will experience enzymatic decline, lack of energy and inflammation (Chen *et al.*, 2020; Sharifinia *et al.*, 2020).

Water Quality

The results of water quality measurements in this study also showed levels that were still optimal for catfish life where water quality was in the form of temperatures ranging from 25-28.7 °C, pH ranging from 6.6-8.4, and dissolved oxygen ranging from 3.8-6.7. ppm. Water quality levels, such as the optimal temperature for catfish life range from 25-30 °C (Mulia *et al.,* 2022), pH 6,8-8,5 (Nuraisyah and Mukti, 2022), and dissolved oxygen 9 ppm (Samuel *et al.,* 2022). Meanwhile, weekly water quality from the study showed 0 ppm levels of ammonia, nitrite and nitrate. The acceptable content of ammonia and nitrite for fish is <1 mg/l and a good nitrate value for catfish farming is <5 mg/l. Ammonia can kill aquatic organisms, especially fish at a concentration of 0.06 mg/l but catfish can tolerate ammonia up

to 5.70 mg/l (Dhiba *et al.,* 2019; Hartono and Barades, 2022; Putri *et al.,* 2021; Sitio *et al.,* 2017; Sopha *et al.,* 2015).

CONCLUSION

The conclusion of this study was that exposure to microplastics had a significant effect on the 15th and 30th days of the study on the Gonad Maturity Level (GML) and Gonado Somatic Index (GSI) of male catfish *(C. gariepinus)*, namely the higher the dose of microplastics, the more inhibited GML would be and GSI will decrease. Treatment C (Microplastic 15%) had the lowest GML and GSI values, while treatment K (Microplastic 0%) had the highest GML and GSI. Treatments A, B, and C experienced changes and delays in the development of gonadal cell structure while control (K) developed well. This was presumably because the control nutritional needs (K) were more fulfilled than the A, B and C treatment fish exposed to microplastics. The presence of microplastics in the waters even at only 5% has an impact on the disruption of fish reproduction, so that in aquaculture it is necessary to control the quality of water, feed and the environment extra so that the cultivation business carried out is free from exposure to microplastics and obtains maximum results.

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