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The Effect of Exposure to Microplastic Polyvinyl Chloride (PVC) in Feed on the Growth and Survival of Tilapia (*Oreochromis niloticus*)

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ABSTRACT

The presence of microplastics in the waters is due to plastic garbage that is difficult to decompose. Tilapia that live in the water has a high tolerance for polluted environments, thus making them vulnerable to microplastic contamination. The most common type of microplastic found in water is polyvinyl chloride. The study aims to investigate the effects of microplastic exposure on Tilapia growth and survival. The research plan used a complete random design (CRD) with four treatments and three repetitions. Exposure to microplastics in fish is carried out through feeding, with doses: without microplastic addition; addition of 0.01 mg per 0.75 g of feed; addition of 0.1 mg for 0.75 g of feeding; and addition of 1 mg for every 0.75 grams of feed. The mixed microplastic feed was given three times a day at a dose of 5% of the fish's body weight. Growth and survival data are analyzed using Anova; if there is any real impact, then further testing is done using Duncan. The study's findings showed that adding microplastics to specific weight growth, absolute weight, absolute length, feed conversion ratio, and survival at a dose of 1 mg per 0.75 g of feed was significantly different from treating the animals without adding microplastics. Therefore, we can conclude that a dose of 1 mg per 0.75 g of feed will impact the growth and survival of tilapia.

INTRODUCTION

Plastic is a material that we frequently see or encounter in our daily lives. Human activities almost always involve the use of plastic. After China, Indonesia ranks second in waste production, generating 1.29 million tons annually. In line with the increase in plastic waste in Indonesia, the level of plastic consumption for daily needs is also increasing. Data from the Indonesian Plastics Industry Association (INAPLAS) and the Central Statistics Agency (BPS) shows that plastic waste in Indonesia reaches 64 million tons per year. The sea receives 3.2 million metric tons of plastic waste annually (Rahmi & Selvi, 2021). This alarming rate of plastic waste accumulation poses significant environmental and health challenges.

Because plastic waste is difficult to decompose in water, it will degrade into small particles called microplastics. Microplastics with high human exposure can cause the human immune system to decline and increase the risk of cancer (Aulia *et al.*, 2023). These tiny particles can persist in the environment for hundreds of years, making their way into various ecosystems, including marine and freshwater habitats. The pervasive nature of microplastics has raised concerns about their potential impacts on human health and the environment, necessitating comprehensive studies and effective waste management strategies.

Aquatic organisms, both in marine waters and fresh waters, also experience exposure to microplastics. Exposure to microplastics in aquatic biota can cause various problems for biota in these waters because microplastics can be toxic to fish, both physically and chemically (Sandra & Radityaningrum, 2021). Fish and other marine life ingest microplastics, mistaking them for food, which can lead to physical blockages in their digestive systems, internal injuries, and even death. Additionally, chemicals associated with microplastics can leach into the bodies of these organisms, causing further toxicological effects that can disrupt their growth, reproduction, and overall health.

Microplastics easily contaminate tilapia, which live in water and have a high tolerance for unfavorable environments. Polyvinyl chloride (PVC) is a commonly found type of microplastic in water. Not only in waters, microplastics have also been found in feed, which is a source of protein for fish to support fish growth and survival. The presence of microplastics in fish feed is particularly concerning because they have a direct impact on the food chain. Microplastics can accumulate in the tissues of fish that consume contaminated feed, thereby posing a significant risk to food safety and public health.

Therefore, this research is crucial to determine how microplastics in feed affect tilapia growth and survival. Understanding the extent to which microplastics affect tilapia can assist in formulating guidelines for aquaculture practices and feed production. This research aims to provide critical insights into the mechanisms through which microplastics affect aquatic life and to develop strategies to mitigate these impacts. Addressing this issue can contribute to aquaculture's sustainability and protect both environmental and human health from the negative effects of plastic pollution.

METHODS

The Fish Production and Reproduction Laboratory, Aquaculture Study Program, Department of Fisheries and Marine Sciences, Faculty of Agriculture, University of Mataram, hosted this research for 45 days, from January 13–February 26, 2024. The tools and materials used are aerators, 45-liter containers, Do meters, pH meters, digital scales, trays, rulers, tilapia, microplastics, and feed.

We conducted this research using various microplastic dosages: P1 included no microplastic addition (control), P2 included 0.01 mg/0.75 g of microplastics in the feed, P3 included 0.1 mg/0.75 g of microplastics in the feed, and P3 included 1 mg/0.75 g of microplastics in the feed. For maintenance purposes, a 45-liter container serves as the laboratory scale. We fill each container with 30 liters of water, each containing 2 liters of tilapia fry, resulting in a total of 15 tilapia fry per container. We expose fish to microplastics by reprinting and mixing commercial feed with them. During maintenance time, the fish will receive microplastic-mixed feed three times a day, specifically in the morning, afternoon, and evening hours. We carried out weight sampling to observe fish growth at the beginning, middle, and end of rearing. In the meantime, we observe survival from the start of maintenance to its conclusion.

We will test this research using a completely randomized design (CRD). We will analyze the obtained data using the F-test (ANOVA). We conducted this test to determine the impact of the treatment, an independent variable, on the measured parameters' responses. If the test values are significantly different, then we will continue using the Duncan test to determine which treatment gives the best results at a level of 0.05 (95% degree of confidence).

RESULTS

Specific Growth Rate (SGR)

Figure 1 displays the specific growth rate (SGR). According to the Annova test results, adding microplastics to feed at different doses affects ($p<0.05$) the specific growth rate. We obtained the highest specific growth rate value at P1, which was 1.31%/day, and the lowest value at P4, which was 0.76%/day. We conducted a further test, the Duncan test, to determine the real difference between each treatment. The Duncan test results revealed a significant difference between the values of P4, P1, P2, and P3. Meanwhile, P1, P2, and P3 are not significantly different.

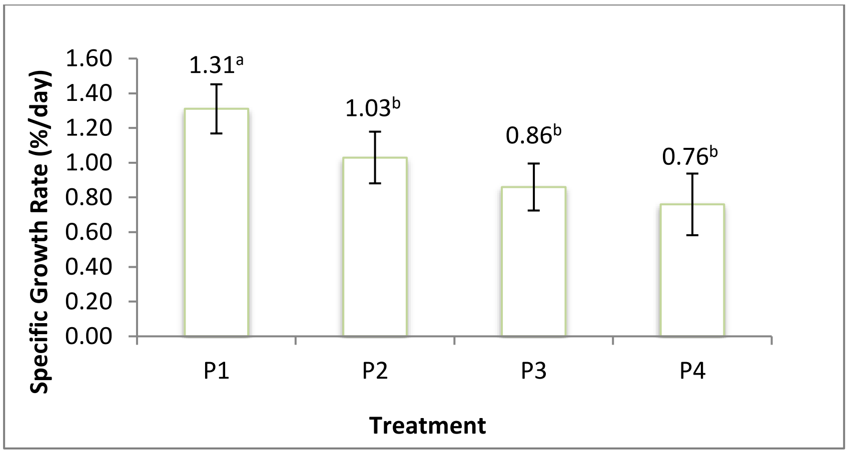

Figure 1. Specific Growth Rate (SGR). Note: P1 Included No Microplastic Addition (Control), P2 Included 0.01 mg/0.75 g of Microplastics in the Feed, P3 Included 0.1 mg/0.75 g of Microplastics in the Feed, and P3 Included 1 mg/0.75 g of Microplastics in the Feed.

Absolute Weight Growth

Figure 2 displays the absolute weight growth. According to the results of the ANOVA test, adding microplastics to feed at different doses had an effect ($p<0.05$) on absolute weight growth. P1 yielded the highest absolute weight growth at 59.13 g, while P4 yielded the lowest weight at 34.00 g. We conducted a further test, the Duncan test, to determine the true differences between each treatment. The Duncan test results revealed a significant difference between the values of P1, P2, P3, and P4. Meanwhile, the values of P2, P3, and P4 are not significantly different.

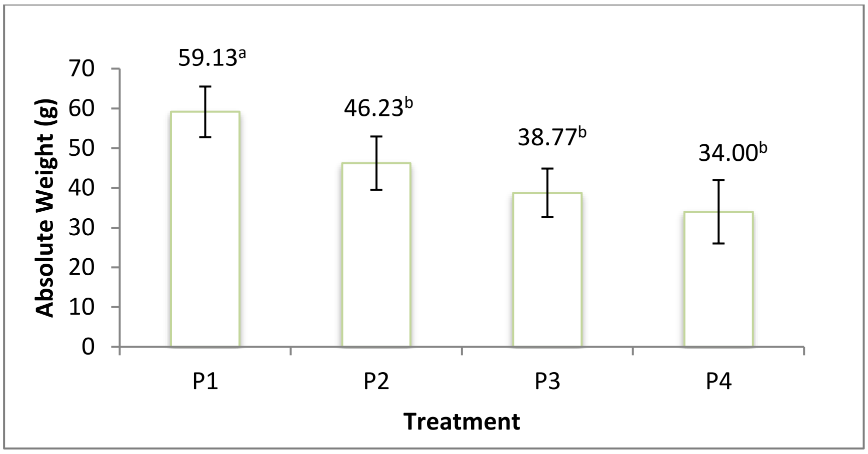

Figure 2. Absolute Weight Growth. Note: P1 Included No Microplastic Addition (Control), P2 Included 0.01 mg/0.75 g of Microplastics in the Feed, P3 Included 0.1 mg/0.75 g of Microplastics in the Feed, and P3 Included 1 mg/0.75 g of Microplastics in the Feed.

Absolute Length Growth

Figure 3 shows the absolute length increase. According to the results of the ANOVA test, adding microplastics to feed at different doses had an effect ($p<0.05$) on absolute length growth. We obtained the highest absolute length growth at P1, measuring 4.77 cm, and the highest absolute length growth at P4 and P3, measuring 3.17 cm. We conducted a further test, the Duncan test, to determine the true difference between each treatment. The Duncan test revealed that the value of P1 did not significantly differ from the value of P2. The value of P1 was significantly different from the values of P3 and P4, while the values of P2, P3, and P4 were not significantly different.

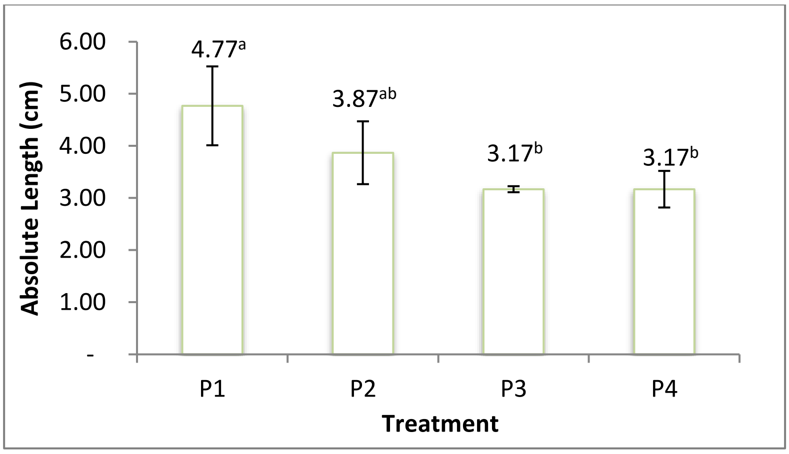

Figure 3. Absolute Length Growth. Note: P1 Included No Microplastic Addition (Control), P2 Included 0.01 mg/0.75 g of Microplastics in the Feed, P3 Included 0.1 mg/0.75 g of Microplastics in the Feed, and P3 Included 1 mg/0.75 g of Microplastics in the Feed.

Feed Convention Ratio (FCR)

Figure 4 displays the Feed Convention Ratio, or FCR. The results of the ANOVA test revealed that the administration of microplastics to feed at different doses had an effect ($p<0.05$) on the feed convention ratio (FCR). We find the highest feed convention ratio at P4, 2.6, and the lowest at P1, 1.2. We conducted a further test, the Duncan test, to determine the real difference between each treatment. The Duncan test revealed that P1 did not significantly differ from P2, but it did significantly differ from the values of P3 and P4. The value of P2 is significantly different from the values of P3 and P4. Meanwhile, P3's value is not significantly different from P4's.

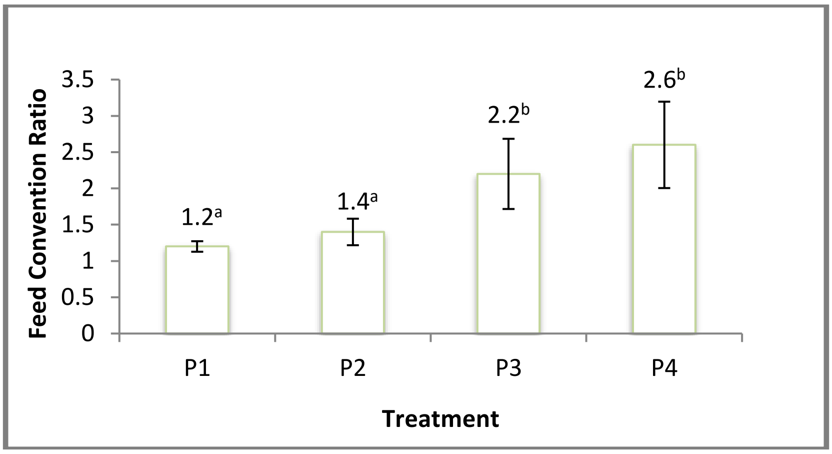

Figure 4. Feed Convention Ratio (FCR). Note: P1 Included No Microplastic Addition (Control), P2 Included 0.01 mg/0.75 g of Microplastics in the Feed, P3 Included 0.1 mg/0.75 g of Microplastics in the Feed, and P3 Included 1 mg/0.75 g of Microplastics in the Feed.

Survival Rate

Figure 5 displays the survival rate. The results of the ANOVA test revealed that administration of microplastics in feed at different doses had an effect ($p<0.05$) on the survival rate. We obtained the highest survival rate at P1, specifically 80%, and the lowest at P4, specifically 51%. We carried out a further test, the Duncan test, to see the real differences between each treatment. The Duncan test results revealed that P1 did not significantly differ from P2, but it did significantly differ from P3 and P4. The P2 value is not significantly different from the P3 value. The value of P2 is significantly different from the value of P4. Meanwhile, the value of P4 is significantly different from the values of P1, P2, and P3.

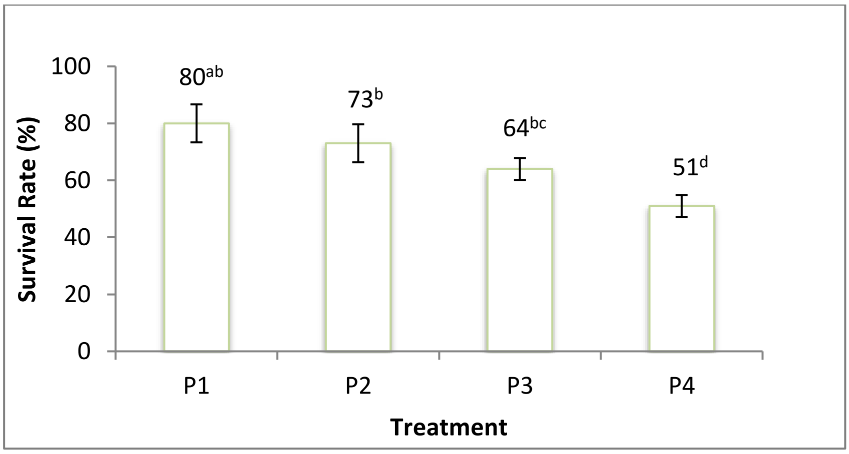

Figure 5. Survival Rate (SR). Note: P1 Included No Microplastic Addition (Control), P2 Included 0.01 mg/0.75 g of Microplastics in the Feed, P3 Included 0.1 mg/0.75 g of Microplastics in the Feed, and P3 Included 1 mg/0.75 g of Microplastics in the Feed.

Water Quality

Water quality parameters in this study include temperature, pH, DO, ammonia, nitrate, and nitrite. During maintenance, we checked the water quality and found it to be within the normal range for water in a cultivation environment. Table 1 displays the results of our water quality check.

Table 1. Water Quality Parameters During Maintenance

Parameter	Unit	Range Value	Standard
Temperature	^{O}C	25.3-32.7	24-32°C (Mina, 2016)
Acidity (pH)	-	6.8,-8.5	6.5-8.5 (Pradhana *et al.,* 2021)
Dissolved oxygen (DO)	mg/l	4.4-6.9	3.2-6 mg/l (Djaelani *et al.,* 2023)
Ammonia	mg/l	0.15-0.5	0.01-0.5 mg/l (Arifin, 2016)
Nitrate	mg/l	10-25	10-30 mg/l (Ombong & Salindeho, 2016)
Nitrite	mg/l	0.1-0.25	0.1-1 mg/l (Karimah, 2018)

DISCUSSION

Specific Growth Rate

During the rearing period, cultivated fish use the SGR-specific growth rate as a parameter to measure their daily weight growth. The addition of microplastics to the feed is believed to be the cause of the low P4 value (0.76). This blockage in the digestive tract prevents the fish from optimally digesting the feed, leading to a low SGR in the P4 biota. Al-Fatih (2022) asserts that the introduction of microplastics into the fish's digestive system can lead to detrimental effects for the fish, including the obstruction of food filtering and the accumulation of microplastics in the intestines. Of course, disrupting the fish's digestive system can also disrupt its metabolic processes, making it difficult for the fish to properly digest the nutritional content of the provided fish food. Research by Hermawan *et al*. (2022) further reinforces this, stating that the accumulation of microplastics in the digestive tract can potentially injure and block the fish's digestive tract. Additionally, the polymer compounds in microplastics are physiologically toxic and can harm tilapia, particularly when exposed to them over an extended period. Microplastics can cause significant intestinal changes, leading to both structural and functional changes in the fish's intestine. Researchers D'Avignon *et al*. (2023) observed a significant decrease in specific weight growth values when they added microplastics to biota, resulting in a 0.18% reduction in daily mass.

Absolute Weight

Absolute weight is used to track biota growth throughout maintenance. The addition of microplastics to the feed allegedly disrupted the digestive system of the fish under cultivation, resulting in the lowest P4 value. The inclusion of microplastics in the feed undoubtedly exposes the fish under cultivation to these microplastic particles. Long-term exposure to microplastics can damage the tissue in the fish's digestive system. These microplastics contain dangerous chemicals that can cause tissue damage and blockage of the digestive tract, leading to false satiety and decreased appetite. Therefore, issues in the digestive system of the residing biota impede the proper or regular digestion of the provided feed. Wildan *et al*. (2023) assert that the long-term accumulation of non-hydrolyzable microplastics in the fish's digestive tract can pose a significant risk. Unhydrolyzed microplastics can clog the digestive tract, leading to a false sense of fullness. False satiety can reduce the fish's appetite and cause physical damage to the villi, or epithelium, of the digestive tract. Over a long time, continuous exposure to microplastic chemicals can cause stressors that affect the activity of digestive enzymes. Research by Doncel *et al*. (2022), which asserts that exposure to microplastics in feed influences growth, further reinforces the influence of microplastic exposure on absolute weight growth. The results of research conducted during 150 days of rearing without exposure to microplastics obtained a growth value of 200 grams. Those who received microplastics experienced an absolute weight gain of 150 grams.

Absolute Length

The growth or change in the average length of biota during maintenance, specifically from the start to the end, is known as absolute length. Adding microplastics to P4 resulted in the lowest score. Microplastics in feed are believed to affect the fish's digestive tract and muscle function. Consequently, the fish's absolute length growth is slow. This follows Simanjuntak *et al*. (2024) statement, which stated that the accumulation of microplastics in muscle tissue could disrupt muscle function. Microplastic-induced muscle dysfunction can lead to a decrease in creatinine production, which in turn affects serum creatinine levels, making it impossible to assess kidney function through serum creatinine index analysis. This is also confirmed by Permatasari *et al.* (2023), who found that 55-day fish rearing produced different absolute length growth values between treatments exposed to microplastics and treatments not exposed to microplastics.

Feed Convention Ratio (FCR)

The Feed Convention Ratio (FCR) measures the amount of feed required to generate one kilogram of meat for the fish under care. It is believed that the addition of microplastics to the feed in P4 contributes to the high FCR, exposing the fish to these microplastics. These microplastics are believed to hinder optimal digestion of the food that P4 fish receive. The fish's body uses most of the provided feed to supply energy to fend off attacks from existing pathogens triggered by microplastic exposure. This is in line with Dias *et al*. (2016) statement. Fish consume microplastics from feed, which build up in their bodies and inflict physical and chemical harm, including internal organ damage that disrupts the metabolic and immune systems. Microplastics, found in fish, can cause blockages in the digestive tract due to the chemicals they contain. Wildan *et al*. (2022) reinforce this statement, asserting that microplastics pose a significant threat to living organisms in marine waters, brackish waters, and fresh waters. Lu *et al*. (2022) conducted similar research and found significant results in the feed conversion ratio when exposed to microplastics at 1%, 4%, 6%, and 8%. The results showed a significant difference between treatments with 1% and 8% microplastic exposure.

Survival Rate (SR)

The survival rate refers to a fish's ability to survive during the rearing period. Experts attribute the low survival rate in the P4 treatment to the addition of microplastics, which disrupt several internal organs in the fish, leading to a blocked digestive tract and potentially death. This is in line with Nurdhiana (2022), which states that microplastics have an impact on fish because they can absorb hydrophobic compounds such as persistent organic pollutants and contaminants. Organisms that swallow large plastics have the potential to choke, experience internal or external wounds, ulceration, blockage of the digestive tract, impaired eating capacity, hunger, lack of energy, and even death. This is also consistent with Yu *et al*. (2020) research, which indicates that exposure to microplastics affects the aquatic biota. Fish exposed to microplastics had a survival value of 70%, whereas biota reared without microplastics had a survival value of 85%.

Water Quality

Water quality is an important component in fish farming because excellent water quality indicates that the waters are excellent for supporting the survival and growth of cultivated biota. The water quality parameters used in this research include temperature, pH, DO, ammonia, nitrate, and nitrite. Table 1 displays the values for the water quality data. The values obtained are still within the normal range for fish growth and survival. This aligns with the assertion (Shofura *et al*., 2018) that the water quality during rearing significantly impacts the growth and survival of the fish. The findings of Aryani *et al*. (2021), who conducted trials on microplastic exposure in fish, further strengthen the obtained range. Water quality data included pH, temperature, and DO. The average water quality values obtained were pH 7.51, temperature 27.42^{o}C, and DO 7.73 mg/l.

CONCLUSION

According to the research data, the best treatment, P1, without the addition of microplastics, showed the highest growth in absolute weight, absolute length, specific weight of FCR, and survival rate. On the other hand, P4, which added microplastics to the feed at the highest dose (1 mg/0.75 g feed), had poor values in each parameter. Therefore, exposure to microplastics negatively affects the survival and growth of tilapia. The fish's survival rate and growth will deteriorate as their exposure to microplastics increases.

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