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The Effect of Exposure to Polystyrene Microplastics in Feed on the Growth of Tilapia (*Oreochromis niloticus*)

Bagus Dwi Hari Setyono[1*], Baiq Triska Saomadia[1], Rangga Idris Affandi[1]

[1] Aquaculture, Faculty of Agriculture, University of Mataram, Mataram, Indonesia.

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Corresponding Author:
Bagus Dwi Hari Setyono
bagus.setyono@unram.ac.id

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Abstract: Indonesia is a country that is rich in natural resources and has various potentials that can be developed, especially in the field of aquaculture. Aquaculture activity that is often carried out by Indonesian people is the cultivation of tilapia (*Oreochromis niloticus*). Apart from that, Indonesia is known as the second largest contributor of plastic waste in the world after China. The scattered plastic waste will be degraded into microplastics which can then spread to tilapia farming locations. The aim of this research is to determine the effect of exposure to polystyrene microplastics given to tilapia feed on their growth. The research method used was a completely randomized design with 4 treatments and 3 replications with doses P1 = 0 mg/0.75 g feed (control), P2 = 0.01 mg/0.75 g feed, P3 = 0.1 mg/ 0.75 g feed, and P4 = 1 mg/0.75 g feed. The parameters tested were absolute weight growth, absolute length growth, spesific growth rate (SGR), feed conversion ratio (FCR) and water quality. The results obtained were that the effect of exposure to polystyrene microplastics in feed was significantly different on absolute weight growth, SGR, and FCR in tilapia (*Oreochromis niloticus*), but not significantly different on absolute length growth.

Keywords: Feed; Growth; Microplastics; Polystyrene; Tilapia

Introduction

Indonesia is a country that is rich in natural resources and has various potentials that can be developed, especially in the field of fisheries cultivation. Many people in Indonesia take advantage of the existing potential by carrying out cultivation activities such as aquaculture. Wicaksono (2022) stated that aquaculture activities are one of the fisheries sectors that are developing globally. Aquaculture itself can be defined as the activity of maintaining or breeding aquatic organisms in a controlled environment. Along with the increase in the world's population, the global demand for fishery commodities has also increased. Based on data released by FAO, the average global fisheries production per year can grow by 3.1% with a total production reaching 178 million tons in 2018. Of the large fisheries production, around 46% are commodities originating from aquaculture activities. The value of global aquaculture commodity production has also grown with an average growth rate of 5.6% per year in the last two decades. Indonesia itself has aquaculture production of more than 15 million tons in 2017, in terms of volume exceeding capture fisheries production with a production of only 7 million tons. This shows the great prospects for aquaculture activities in fulfilling food sources for the community.

One of the biota that is often cultivated by the community is tilapia because it is easy to do and the production costs are affordable for the community. In the cultivation process, there are many factors that must be considered, especially environmental factors and feeding. Feed is an important aspect that must be considered because it is a supporting factor in the growth of tilapia (*Oreocromis niloticus*). Tilapia is a type of fish that is very popular with the community, because of its fast growth, easy to obtain feed, and can be kept in all places (Nurhalisa et al., 2023). Vidiastuti et al. (2023)

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added that tilapia is one of the freshwater fish commodities that is widely cultivated in Indonesia as a food fish and is in great demand by the world market because it has the ability to reproduce well, and has a high survival rate and economic value.

Apart from being a country rich in natural resources, according to Sincihu et al. (2022), Indonesia is the second largest contributor of plastic waste in the world after China. The scattered rubbish is not only in land areas but has also entered water areas with various types of rubbish, starting from leftover plastic food packaging and other packaging. As time goes by, the waste contained in the waters will continue to decompose into very small plastic waste which is then spread throughout the waters which are usually called microplastics. Microplastics are plastic components that are small in size, namely <5 mm (Khalis et al., 2024; Yuranda et al., 2024), most of which come from the decomposition of large plastics (Yona et al., 2020).

Microplastics found in the environment have the potential to have a negative impact on the aquaculture environment (Dhea et al., 2023), one of which is tilapia. According to Aliyansyah et al. (2024), microplastics can contaminate fish through fish organs such as gills and digestive tracts. Gills are organs that have great potential for contamination because these organs function as a place for water to enter and exit for respiration, while the digestive tract can also potentially be contaminated because these organs are organs that function for fish to obtain food. Rochman et al. (2015) added that exposure to microplastics has had a negative impact on the lives of aquatic organisms which include pathological stress, oxidative stress, decreased reproductive performance and growth performance. Tilapia often consume microplastics because their shape and appearance are almost the same as food often found in nature and are easy to consume. One type of microplastic that can pollute the tilapia cultivation environment and be ingested by tilapia was Polystyrene (PS). According to Azis (2017) that polystyrene, known as styrofoam, is often used as a packaging material for food and has several properties such as dissolving quickly when exposed to oil. David et al. (2021) added that polystyrene (PS) is a synthetic hydrophobic polymer with a high molecular weight that is included in the thermoplastic type. Polystyrene can be recycled but is difficult to biodegrade, requiring a minimum of 500 years to degrade naturally. The long degradation time of polystyrene and the negative impact of microplastics on the aquaculture environment encourage the need to investigate the effects of polystyrene on aquaculture biota, especially tilapia.

Based on the impact and potential dangers of microplastics on aquatic organisms, especially tilapia, it is necessary to conduct research on the effects of polystyrene (PS) microplastic exposure on tilapia because no research has been conducted. The purpose of this study was to determine the effect of polystyrene microplastic exposure on tilapia growth performance, so that it is hoped that a solution will emerge that can overcome this microplastic problem in the tilapia cultivation environment.

Method

Time and Location

This research activity was carried out for 45 days, starting from January, 2024 - February, 2024 at the Fish Production and Reproduction Laboratory, Aquaculture Study Program, Department of Fisheries and Marine Sciences, Faculty of Agriculture, University of Mataram.

Research Design

The design used was a completely randomized design (CRD) with the following treatments based on previous research by Suwartiningsih et al. (2023):

P1 : Without administration of polystyrene microplastics (control)
P2 : Administration polystyrene microplastics of 0.01 mg/0.75 g feed
P3 : Administration polystyrene microplastics of 0.1 mg/0.75 g feed
P4 : Administration polystyrene microplastics of 1 mg/0.75 g feed

The feed used in this research is H-Pro-vite 781 -2 feed which will then be mixed with microplastics. The microplastic that will be used in this research is polystyrene, commonly known as styrofoam. Before styrofoam is given to test animals, it needs to be crushed first using a blender to micro size. After that, it is dried and filtered using a flour sieve. The filtered microplastics are stored in zip plastic. The sifted polystyrene microplastics are mixed into the feed according to the dose to be used for each treatment. The dosage of polystyrene mixed is P1 without microplastic mixing, P2 with a dose of 0.01 mg/0.75 g feed, P3 with a dose of 0.1 mg/0.75 g feed, and P4 with a dose of 1 mg/0.75 g feed. After that, repellet the feed.

In this study, tilapia with a size of 10-12 cm and a weight of 16.8-48.1 g were used. The test animals used were obtained from the Lingsar Fish Seed Center, Lingsar District, West Lombok Regency. Before stocking, acclimatization is carried out first so that the tilapia used do not experience stress in the new environment so they can survive. The tilapia used in containers is 1 fish per 2 L of water so that the water used in 1 container is 30 liters with 15 tilapia. During activities, tilapia are fed 3 times a day with a feeding rate of 5%.The parameters measured in this research are absolute weight growth, absolute length growth, specific growth rate (SGR), feed conversion ratio (FCR) and water quality.

Absolute Weight Growth

The absolute weight of tilapia can be determined by knowing the biomass of tilapia at the end of rearing which is then calculated using the formula according to Puspitasari et al. (2024); Setyono et al. (2023) as follows:

$$Wm = Wt - Wo \quad (1)$$

Note:
Wm : Absolute weight growth (g)
Wt : Final biomass weight (g)
Wo : Initial biomass weight (g)

Absolute Length Growth

The absolute length of tilapia can be determined by calculating the difference between the final length of tilapia and the initial length of tilapia. The absolute length can be calculated using the formula according to Scabra et al. (2022) as follows:

$$Pm = Lt - Lo \quad (2)$$

Note:
Pm : Absolute length growth (cm)
Lt : Final average length (cm)
Lo : Initial average length (cm)

Specific Growth Rate (SGR)

Specific growth rate of tilapia can be calculated using the formula according to Rahmawati et al. (2024); Serdiati et al. (2023) as follows:

$$\mathrm{SGR} = \frac{\ln \mathrm{Wt} - \ln \mathrm{Wo}}{\mathrm{t}} \times 100\% \quad (3)$$

Note:
SGR : Specific growth rate (%/day)
Wo : Average initial maintenance weight (g)
Wt : Average weight at the end of rearing (g)
t : Length of maintenance (days)

Feed Conversion Ratio (FCR)

FCR or feed conversion ratio is the value of a feed conversion used during rearing to produce the weight of the fish being kept. Calculating the FCR value can use the formula according to Fahrizal & Nasir (2017); Inayah et al. (2023) which is as follows:

$$\mathrm{FCR} = \frac{\Sigma\ \mathrm{Feed}}{\Delta\ \mathrm{Biomass}} \quad (4)$$

Note:
FCR : Feed Conversion Ratio
Σ Feed : Amount of feed given during the study (g)
Δ Biomass : Difference between biomass at the start of the study and the end of the study (g)

Water Quality

Water quality measurements were carried out 4 times during the 45 days of research. The water quality parameters measured in this research activity are temperature, pH, ammonia, and DO *(Dissolved oxygen)*.

Data Analysis

The research data will be tested using a completely randomized design (CRD) which will then be analyzed using the F test (ANOVA) using the SPSS application. This test was carried out to determine the effect of treatment (independent variable) on the response of the measured parameters. If the test values are significantly different then it will be continued by using the Duncan test to determine which treatment gives the best results at a level of 0.05 (95% degree of confidence).

Result and Discussion

Absolute Weight Growth

The absolute weight growth of tilapia (*Orechromis niloticus*) can be done by weighing the fish at the beginning of rearing and at the end of rearing. The results of measuring the absolute weight of tilapia can be seen in Figure 1.

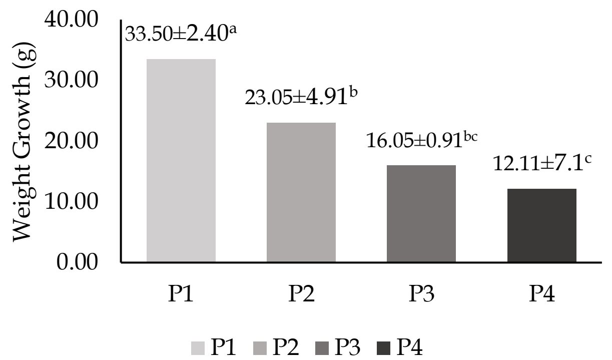

Figure 1. Weight growth

Based on the results of research that has been carried out, the absolute weight growth value of tilapia obtained ranges from 33.50 g – 12.11 g. The highest absolute weight growth value was found in P1, namely with an average weight of 33.50 g, then followed by P2 with an average weight of 23.05 g, then P3 with an average weight of 16.05 g and the lowest absolute weight growth was in P4, namely with an average weight of 12.11 g. Based on the results of the ANOVA test with a level of 0.05, it shows that there is an influence of giving microplastics on absolute weight growth. The highest absolute weight growth of tilapia was in P1 and the lowest was in P4 because in P1 there was no exposure to microplastics in the feed, whereas in P4 they were given exposure to microplastics at the highest dose, causing differences in growth in the tilapia tested.

Exposure to microplastics given to feed can affect the quality of the feed itself so that fish that consume this feed do not have good weight growth. Weight growth in tilapia can be influenced by the feed factor itself.. According to Efendi et al. (2023) said that feed containing microplastics can cause fish to experience false satiety which results in low weight growth of tilapia. The false immunity experienced by tilapia is caused by exposure to microplastics consumed through feed that block the tilapia's digestive tract. Nair & Perumal (2024) stated that the intake of microplastic may cause a loss of appetite or a feeling like a satiated stomach in fish. Mbugani et al. (2022) added that small intestines microplastic-induced damage impaired digestion and absorption processes in fish. This led to malnutrition and subsequent energy and nutrients deficit, resulting in retardation in length and decline in final weight and weight gain of the exposed fish.

Feed that is exposed to microplastics will cause water quality to get worse due to the presence of feed remains because feed utilization is not optimal. This is in line with Abd El-Hack et al. (2022) which states that the low absolute weight growth value of tilapia is caused by the large amount of leftover food and fish feces contained in the waters. Poor water quality causes fish to experience stress which can disrupt the growth of the tilapia. So the feed eaten by tilapia is used for metabolic activities to maintain good body condition but is not used for the weight growth needs of tilapia.

Absolute Length Growth

The absolute length growth of tilapia (*Orechromis niloticus*) was carried out by measuring the length of the fish at the beginning of rearing and at the end of rearing. The results of measuring the absolute length of tilapia can be seen in Figure 2.

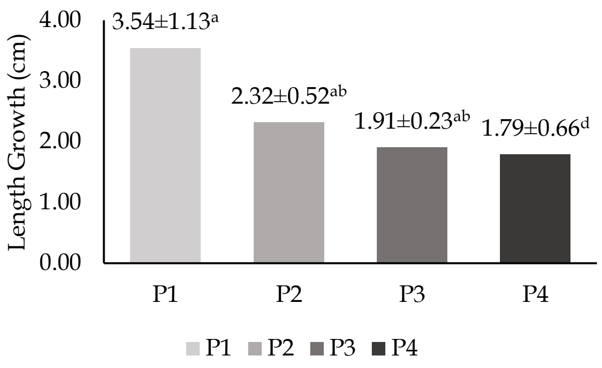

Figure 2. Length growth

Based on research, the absolute length growth value of tilapia obtained ranged from 1.79 cm - 3.54 cm. The highest absolute length growth value was found in P1, namely with an average length of 3.54 cm, followed by P2 with an average length of 2.32 cm, then P3 with an average length of 1.91 and the lowest length growth was in P4 namely with an average length of 1.79 cm. Based on the results of the ANOVA test with a level of 0.05, it shows that exposure to polystyrene microplastics has no significant effect on the absolute length growth of tilapia. For the absolute length of tilapia, the highest value is at P1 and the lowest absolute length is at P4. The highest absolute length in P1 was due to no exposure to microplastics given to the feed, while P4 had the lowest absolute length value due to exposure to microplastics in the feed at the highest dose (1 mg/0.75 g feed).

There are several factors that influence the growth in length of tilapia, namely the ability to eat and environmental conditions such as water quality. Providing exposure to microplastics in feed can affect the growth in length of tilapia because feed is one of the requirements for growth so that exposure to microplastics affects the quality of the feed consumed, causing tilapia to have slow growth in length. Kardgar et al. (2024) stated that microplastic contamination including oxidative stress, disruption in growth, reproduction, and mortality of the fish resulting in detriments in the economy of aquacultures and adverse effects on human health by consumption of aquaculture products. Akhtar et al. (2024) added that fish and other aquatic organisms can ingest these microplastics which can cause toxic diseases and even death to them. Microplastics have also been found to affect the growth of fish body length. Moreover, a delayed maturation of the gonads and decreased fecundity were reported in fish exposed to 10 µm polystyrene particles, while hatching rate and offspring body length were also affected (El-Naggar et al., 2024).

In addition, feed given exposure to microplastics can cause environmental conditions to become worse so that the quality of the water in the rearing media becomes poor. Water quality that is not optimal can also affect the length of growth, one of which is the long growth of tilapia. This is in line with the statement from Pane et al. (2024) that poor water quality can slow down the growth of tilapia. Poor water quality will affect the tilapia's appetite, causing the tilapia's growth rate to be lower. This is in line with Arifin (2016) which states that water quality can influence the level of appetite and thus influence the growth of tilapia.

Specific Grow Rate

The results of the Specific Grow Rate calculation for tilapia can be seen in Figure 3.

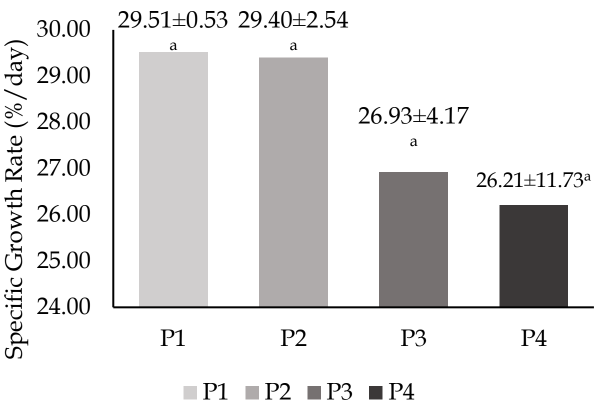

Figure 3. Specific grow rate

Based on the results of the specific Grow Rate (SGR) analysis, the values obtained ranged from 26.21-29.51%/day. The highest SGR value is found in P1 with an SGR value of 29.51, while the lowest SGR value is found in P4 with a value of 26.21%/day. Based on the results of the Anova test that has been carried out, it shows that exposure to polystyrene microplastics has a significant difference in the specific weight growth of tilapia. The results of further tests carried out using the Duncan test on P1 were significantly different from P3 and P4, but not significantly different from P2 because the dose in P2 was relatively low. The difference between P1 and P2, P3, and P4 is because in P1 there was no exposure to microplastics so it was not able to influence the appetite level of the tilapia. Meanwhile, P4 is not significantly different from P2 and P3 due to exposure to microplastics which can influence the specific weight growth of tilapia.

Microplastics can cause fish to experience a lack of appetite, which can affect feed consumption levels. Lack of fish feed consumption can certainly have an impact on the specific growth of tilapia. Apart from that, microplastics contained in the cultivation media can enter fish organs such as the gills and digestive tract, which can disrupt the growth of tilapia. According to Hermawan et al. (2022) stated that microplastics that enter the fish's body can cause damage. If damage occurs, the food consumed by the fish can be used as energy, but not for fish growth because the fish will use more food for energy needs to stay alive because the organs are not functioning properly. Lu et al. (2021) added that the exposure of microplastic could inhibit the growth of fish weight and reduce the health status of fish, which may be due to the accumulation of microplastic in the intestines, disrupting the metabolic balance and causing respiratory and metabolic disorders. Exposure to microplastic could reduce the thickness of the fish intestinal muscle layer. This phenomenon was due to the thinning of muscle cells and loose connections, which would further affect the absorption of nutrients and ultimately put the fish in an energy-depleted status. Khan et al. (2024) said that microplastic deposit in fish and have a wide range of negative impacts i.e decreased feeding activity, impeded growth, energy interruption, oxidative stress, and even genotoxicity. Microplastic particles are easily ingested by fish in unintended ways due to their small size and similarity to natural food items. Microplastic hinder fish metabolism by lowering the amount of energy needed for growth and delaying ovulation.

Feed Conversion Ratio (FCR)

The results of measuring the Feed Conversion Ratio of tilapia can be seen in Figure 4.

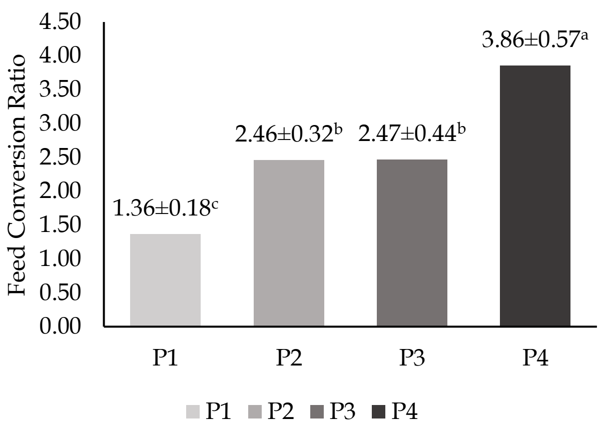

Figure 4. Feed conversion ratio

Based on the results of FCR measurements of tilapia exposed to polystyrene microplastics. The FCR values obtained ranged from 1.36 - 3.86 and the highest FCR was in P4 while the lowest was in P4. Based on the ANOVA test, it was found that there was an influence of exposure to microplastics on the FCR value of tilapia. The highest FCR value is found in P4 with a value of 3.86 and is significantly different from P1, which has a low

FCR value of 1.36. Meanwhile, P2 and P3 FCR values are not significantly different.

This is due to exposure to microplastics because in treatment 4 the feed dose given to microplastics was 1 mg/0.75 g feed which affected the quality of the feed which could cause the fish in P4 to experience false satiety so that the tilapia would reject large amounts of feed for consumption. so feed conversion is fairly high. According to Utomo et al. (2022) stated that food that is exposed to foreign objects such as microplastics, if consumed by fish in excess, can entser the digestion of tilapia, causing the fish to experience stress so that the fish's appetite is disturbed, causing the fish to die. In this study, feed utilization decreased due to microplastic with increase in FCR. In line with the statement of Rashid et al. (2023) which states that it has been demonstrated that the consumption of microplastic causes an increase in energy demand of organisms as well as energy losses via lipid catalysis. Once microplastic enter the body, they do not leave but accumulate in fish's body and cause functional and anatomical changes in cells.

Feed has a very important role in fish farming activities. Most fish production requires 60-70% just for feed needs. This is in line with the statement from Agustini et al. (2019) who said that in cultivation activities the costs incurred for procuring feed reach 60-70%. The frequency of feeding in cultivation activities needs to be considered so that the feed given is neither excessive nor insufficient. Fish will grow optimally when feeding is appropriate because feeding requires attention to nutritional intake. According to Arzad et al. (2019) states that a FCR value for tilapia ranges between 1.7 - 1.73 and FCR of fish generally ranges between 1.5 - 2.5. So from this statement it is stated that normal FCR is in treatment P1.

Water Quality

The water quality parameters observed in this research include the physical and chemical parameters of water, namely temperature, DO, pH and ammonia. Water quality measurements are carried out every 15 days during the 45 day maintenance period. The results of water quality measurements carried out for 45 days showed normal results and there were no significant fluctuations for the parameters temperature, DO, pH and Ammonia. The results of measuring the water quality of tilapia can be seen in Table 1.

Table 1. Water Quality during Research

Parameter	Treatment				References
	P1	P2	P3	P4	
Temperature (°C)	29.3-31.3	28.7-31.4	29-31.2	29.1-31.1	25-30 (Indriati & Hafiludin, 2022)
pH	6.8-8.4	6.7-8.6	6.7-8.5	6.6-8.5	6.5-8.5 (Salim & Edidas, 2023)
Ammonia (ppm)	0.25-1.0	0.25-0.5	0.25-0.5	0.25-0.5	<1 (Yudiana et al., 2022)
DO (ppm)	4.9-6.3	5.4-6.4	5.0-6.4	4.5-5.6	5-7 (De Breving & Rompas, 2013)

Based on the results of water quality measurements in the tilapia rearing container, the temperature obtained ranged from 29-31.1°C. The results obtained in the rearing water were considered optimal because they were in line with the statement from Indriati et al. (2022) because the optimal temperature in tilapia cultivation activities is 25-32°C. Temperatures that are not optimal will affect the life expectancy of tilapia because high temperatures will cause a decrease in oxygen levels in the rearing container so that tilapia will experience stress even to experience death. Meanwhile, when the temperature in the rearing container is optimal, it will increase the appetite of the tilapia so that it can support the growth of the tilapia well.

The degree of acidity obtained during the maintenance period ranges from 6.6-8.6. The pH value obtained can be said to be optimal because it is still within the optimal limit of pH content when rearing tilapia. This is in line with the statement from Salim et al. (2023) that the optimal pH value for raising tilapia is between 6.5-8.5. If the pH value fluctuates, it will cause problems with water quality because if the pH value is too low it can disrupt the growth rate of tilapia, whereas if the pH value is too high it can cause an increase in ammonia levels in the water.

Ammonia levels obtained during maintenance activities range from 0.25-0.5 ppm. The ammonia level obtained is still considered optimal because the optimal level of ammonia content when rearing tilapia is <1. According to Yudiana et al. (2022) the optimal ammonia level for tilapia is <1 ppm. The ammonia obtained in the rearing container is caused by the presence of tilapia feces. Apart from that, ammonia can be caused by the presence of uneaten food residue which causes the remaining food to settle in the rearing container. High ammonia will cause fish to experience stress because they are unable to adapt to a bad environment.

The dissolved oxygen levels obtained in tilapia rearing containers ranged from 4.5-6.6 mg/L. The dissolved oxygen value obtained was still within the optimal DO limit for raising tilapia. This is in line with the statement De Breving et al. (2013) which states that the optimal DO value for tilapia is 5-7 ppm. Tilapia really need oxygen levels in the water because if the dissolved oxygen levels are low it will inhibit the growth of the tilapia, besides that the tilapia will experience stress and even die.

Conclusion

Based on the research that has been carried out, it can be concluded that the effect of exposure to polystyrene microplastics in feed at doses of 0.01 mg/0.75 g, 0.1 mg/0.75 g, and 1 mg/0.75 g was significantly different on absolute weight growth, specific grow rate (SGR), and feed conversion rate (FCR) in tilapia (*Oreochromis niloticus*), but not significantly different on absolute length growth.

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Author Contributions
Conceptualization, B. D. H. S., B. T. S., and R. I. A.; data curation, B. T. S.; formal analysis, R. I. A.; funding acquisition, B. D. H. S.; investigation, R. I. A.; methodology, B. D. H. S. and B. T. S.; project administration, B. D. H. S.; resources, B. D. H. S.; software, B. T. S.; supervision, B. D. H. S. and R. I. A.; validation, B. D. H. S., B. T. S., and R. I. A.; visualization, B. T. S. and R. I. A.; writing – original draft, B. T. S. and R. I. A.; writing – review & editing, B. T. S. and R. I. A. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
The authors declare no conflict of interest.

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