Potential of feed supplements on morphometric and gonad weight of fish exposed to microplastics

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Abstract. Microplastic can highly accumulated in fresh water, sea water, sedimentary, and marine life species. Its tiny size and large quantity cause it to become ubiquitous and highly bioavailable in aquatic organism. The potential of probiotic supplement from lactic acid bacteria and Vitamin C has proven effective and beneficial in remedial process of living organisms that exposed to toxic materials in microplastic. This research wants to analyze the potential of feed supplement on morphometric and gonad weight of fish exposed to microplastics. Thirty six tilapias divided into two groups; one group given feed variant (commercial pellet, mixture of commercial pellet and probiotic, and mixture of commercial pellet and Vitamin C) and other group given microplastic concentrate variant (0; 0,1; 1; and 10 mg/L) for 15 days. The result shows changes in morphometry and the weight of fish gonad. A significant microplastic exposure reduce the size of tilapia’s morphometry (body size, standard size, head size, and body width), gonad’s weight, and tilapia’s weight. The addition of probiotics increased the morphometry of tilapia exposed to microplastics. This research will give useful informations for fish farmers in developing fish resources and fish farming.
1. Introduction
The rapid development of industrial and human activities has increased plastic waste disposal in rivers. Plastic waste is very difficult to decompose, thus it will only reduced into tiny particles, called microplastic, if submerged in water for a long time [1]. The existence of microplastic can impair the growth and development of tilapias. Microplastics swallowed by tilapia can cause damage in the gastrointestinal organs [2]. Microplastic exposure can also decrease fertility and harm the fish embryo, even causing death to the fish in the long run [3,4]. Microplastic pollution must be handled immediately so it won’t cause severe effect to tilapias. Microplastics that already enter the tilapia’s body will accumulate, so it needs a remedy to reduce the microplastic contents [5]. One of them is a feed supplement that contains probiotic and Vitamin C that is expected to reduce and absorb the microplastic content inside the tilapia.

Probiotic is a cluster of microbiotic inside the bowel and helps the immune system [6]. The bacteria used in probiotic is usually from the lactic acid bacteria [7] that has the ability to turn toxic material to non-toxic material [8], thus able to reduce environmental pollutant such as microplastic [9]. Study related to the utilization of probiotic on fish feed has been done by quite a few researcher to Javanese mahseer fish (Tor tambra) [10] and King prawn (Litopenaeus vannamei) [11]. The growth of mahseer fish (Tor tambra) increased after being given probiotic dose of 10 ml kg ⁻¹ [10]. Meanwhile, Vitamin C is an antioxidant that can strengthen immune system, reduce stress, free radicals, and repair the growth and development of fish body. Since the body of a fish cannot synthesize vitamin C, they need to get it from the feed. Hence, vitamin C is needed in fish feed [12].

The potential of probiotic supplement from lactic acid bacteria and Vitamin C has proven effective and beneficial in remedial process of living organisms that exposed to toxic materials in microplastic, including fish. A polluted water will affect the biota lives in it. The presence of toxic material like microplastic is harmful for the life sustainability in the polluted water. This study will describe the effect of feed supplements on the morphometry and gonadal weight of tilapia exposed to microplastics.

The study of morphometry and gonadal weight is very important to analyze the development and growth of fish species in a population. Therefore, this information is very important for the development of fish resources and fish farming in the future.

2. Material and methods
2.1 Ethical statement
Fishes used in this research will be treated ethically according to internationally recognized guidelines on the ethical use of experimental animals [13,14]. After the research treatment was completed, the experimental animals were made unconscious by adding 0.1 mL/L of clove oil in the aquarium before the morphometric data was taken [15]. Giving clove oil aims to calm the fish for a few minutes and avoid stress when morphometric measurements are made on the body [13].

2.2 Fish supplement
Feed supplement used in this research has two variants. One is a probiotic of bacterial consortium with the ability to lower and absorb the toxic material content and secrete it together with the fish feces. This probiotic also able to suppress the growth of pathogenic bacteria. The other variant is a Vitamin C feed supplement as an antioxidant that helps increasing immune system. Probiotic supplement was made by mixing commercial fish feed pellet with probiotic suspension (200 mL/kg) with the concentration of 1x10⁸ CFU/mL. After that, the pellet is air dried. Meanwhile, the Vitamin C supplement (100 mg/kg) is mixed evenly on a commercial fish feed pellet.

2.3 Animals
Adult, male tilapias with ripe gonads acquired from Technical Implementation Unit for Freshwater Aquaculture Development in Umbulan, Pasuruan, East Java, Indonesia. Thirty six tilapia fish placed in
a glass aquarium (30×60×50 cm) separately to avoid fish fight. Temperature (27 ± 1.5°C), pH (7.2±0.3), oxygen level, and fish health in the aquarium always monitored during the study. The lighting will use the schedule of 12 hours of illumination and 12 hours of darkness. An aquarium pump (Water pump type WP 103, 25 Watt) is placed inside each aquarium in the corner to circulate water back through the system. The water pumped from the aquarium into the pipes and under the pressure of the pump causes the water to return to the rearing tank again. Commercial pellet feed contains 30% protein, 3% fat and 4% fiber (Takari, Sidoarjo, Indonesia). Feed is given twice a day as much as 2-3% of the fish's weight in the morning and evening. The feeding period is 14 days. The concentration of microplastics (0; 0.1; 1; 10 mg/L) [16], at concentrations of microplastics more than 0.05, it causes oxidative stress or induces protein oxidation.

2.4 Animal experiment
Thirty six tilapias divided into two groups; one group given feed variant (commercial pellet, mixture of commercial pellet and probiotic, and mixture of commercial pellet and Vitamin C) and other group given microplastic concentrate variant (0; 0.1; 1; and 10 mg/L) for 14 days.

2.5 Morphometric analysis
The measurement and calculation of tilapias morphometry was done with a standard method using digital scale and length measuring tool. The measurement of tilapias gonad’s weight and five morphometric characteristics are used to analyze the potential of feed supplement to the reproductive health and the growth of microplastic-exposed tilapias. The gonad and tilapias weight are stated in grams, while the morphometric characters of the tilapias are stated in centimetre (Figure 1).

2.6 Statistical Analysis
All of the data that has been acquired is analyzed using the analysis of variance (ANOVA) and proceeded by Least Significance Different (LSD) to see the different mean between treatments. The accepted level of significance is P<0.05 (SPSS version 21).
3. Results and Discussion
In this research tilapias was given variation of microplastic concentration (0 mg/L; 0.1 mg/L; 1 mg/L; and 10 mg/L) then given three (3) treatments with variation of feeds (control, probiotic, and Vitamin C). The measured parameter in this research consist of morphometry, gonad’s weight, and tilapias weight. The result in this research shows that the probiotic supplement has significantly increase the morphometric body width of tilapias, but didn’t show any significant changes to total body length, body length, and head length of microplastic exposed tilapias. Meanwhile, the Vitamin C supplement has significantly increase the morphometric head length. Other than that, Vitamin C also affect the body width of the microplastic-exposed tilapias at 1 mg/L and 10 mg/L concentration. However, Vitamin C didn’t give any significant effect on morphometric total body length and body length (Figure 2).

![Morphometry](image)

**Figure 2.** Total body length, body length, head length, and body width of tilapias before and after treatment with variation of microplastic concentrate and feed supplement.
Probiotic supplement also significantly increase the gonad weight of microplastic-exposed tilapias at 0.1 mg/L concentration. Aside from that, Vitamin C has significantly increase the gonad weight of the microplastic-exposed tilapias at 1 mg/L and 10 mg/L concentration (Figure 3). Probiotic supplement shows insignificant result on tilapias body weight. Meanwhile, Vitamin C has increase tilapias body weight significantly at 1 mg/L of microplastic exposure (Figure 4).

Microplastic is a toxic substance that can enter the tilapias body through food [17]. Microplastic can enter the tilapias body in three ways: 1) fish mistakenly thinks microplastic as its food; 2) fish accidentaly swallow microplastic while eating; 3) the food itself contains microplastic [18]. Microplastic can increase the mortality rate of tilapias [2], intestinal blockage, reduced predatorial efficiency [19] even liver stress; including severe glycogen depletion, significant changes in estrogen...
receptor-mediated gene expression, single cell necrosis and abnormal proliferation of germ cells in male fish testes [3]. Long term microplastic exposure can change the structure and function of the intestine that cause rapid weight loss [20]. Subsequent effects can result in significant damage to fish development during the early stages of life, thereby affecting reproductive success, fish population size and survival of the fish. Inside the cells, microplastics can cause oxidative stress. The resulting oxidative stress will cause DNA damage, changes in gene expression, and chromosomal aberrations. The emergence of oxidative stress is due to the presence of reactive oxygen species (ROS). ROS will be formed if the microplastic particles are recognized as foreign particles by the body [21]. When ROS levels remain, it will cause cell damage and further cause necrosis or apoptosis.

The addition of various feed supplements succeeded in increasing the morphometry, gonad weight, and body weight of tilapia exposed to microplastics. The probiotics contain consortium of lactic acid bacteria (LAB) which is able to reduce the level of toxic materials by absorbing and excreting them with fish feces. In addition, LAB helps balance healthy bacteria and plays an important role in maintaining the immune and digestive systems of fish [22]. While vitamin C as a source of antioxidants plays a role to help increase the body's resistance that works after being absorbed in the bloodstream. Vitamin C can increase absorption in the intestine which can later help circulate oxygen and play a role in the formation of collagen. In addition, vitamin C also plays a role in the transport of fatty acids to produce energy. Fish bodies that are deficient in vitamin C can reduce energy production and slow bone growth [23].

4. Conclusion

The administration of probiotic supplements had an effect on increasing the morphometry of tilapia, while Vitamin C had an effect on increasing the body weight of tilapia exposed to microplastics at a concentration of 0.1 mg/L.

5. Reference
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