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Supplementary Feed Potential on Histology and Immune Response of Tilapia (*Oreochromis niloticus* L.) Exposed to Microplastics

(Potensi Makanan Tambahan ke atas Histologi dan Tindak Balas Imun Tilapia (*Oreochromis niloticus* L.) Terdedah kepada Mikroplastik)

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ABSTRACT

Polyester microplastics (PS) are toxic and hazardous chemicals in the ecosystem that can induce stress. Disposing PS articles into the environment can negatively impact health of aquatic biota, including fish. This study aimed to investigate the potential of probiotics or vitamin C supplementation in improving the histological structure of organs and cytokine secretion in tilapia fish exposed to PS. Thirty-six tilapia were divided into 12 groups consisting of treatment groups (four PS concentration variations: 0, 0.1, 1, and 10 mg/L). Each treatment was given three types of feed: Commercial feed alone, commercial feed containing probiotics (200 mL/kg), and commercial feed containing vitamin C (100 mg/kg). The study had a sample size of n=3. After treatment was completed, all parameters were measured. The result showed that the addition of probiotics and vitamin C could decrease TNF-α levels and increase IFN-γ levels. Probiotics and vitamin C prevent healthy cells to be damaged by pro-inflammatory cytokines. The percentage of normal hepatocytes increased significantly in all treatment groups with the addition of probiotics or vitamin C. Furthermore, the percentage of hepatocytes with swelling and necrosis decreased significantly in treatment groups ($p < 0.05$). Additionally, villi height, lamina propria width, submucosa height, and the number of goblet cells all increased significantly in all treatment groups with the administration of probiotics or vitamin C ($p < 0.05$). Overall, both probiotics and vitamin C supplements have the potential to maintain fish health. Vitamin C exhibits a greater potential than probiotics in regulating immune responses. Meanwhile, both probiotics and vitamin C supplements have potential to inhibit damage to the hepatic and intestine structures of fish exposed to PS.

Keywords: Fish; fisheries; freshwater; polystyrene; probiotics

ABSTRAK

Mikroplastik poliester (PS) adalah bahan kimia toksik dan berbahaya dalam ekosistem yang boleh menyebabkan tekanan. Penyingkiran artikel PS ke alam sekitar boleh memberi kesan negatif kepada kesihatan biota akuatik, termasuk ikan. Penyelidikan ini bertujuan untuk mengkaji potensi probiotik atau suplemen vitamin C dalam memperbaiki struktur histologi organ dan rembesan sitokin pada ikan tilapia yang terdedah kepada PS. Tiga puluh enam tilapia dibahagikan kepada 12 kumpulan yang terdiri daripada kumpulan rawatan (empat variasi kepekatan PS: 0, 0.1, 1 dan 10 mg/L). Setiap rawatan diberi tiga jenis makanan: Makanan komersial sahaja, makanan komersial yang mengandungi probiotik (200 mL/kg) dan makanan komersial yang mengandungi vitamin C (100 mg/kg). Kajian ini mempunyai saiz sampel n=3. Selepas rawatan selesai, semua parameter diukur. Hasil kajian menunjukkan bahawa penambahan probiotik dan vitamin C boleh mengurangkan tahap TNF-α dan meningkatkan tahap IFN-γ. Probiotik dan vitamin C menghalang sel yang sihat daripada rosak oleh sitokin pro-radang. Peratusan hepatosit normal meningkat dengan ketara dalam semua kumpulan rawatan dengan penambahan probiotik atau vitamin C. Tambahan pula, peratusan hepatosit dengan bengkak dan nekrosis menurun dengan ketara dalam kumpulan rawatan ($p < 0.05$). Selain itu, ketinggian vili, lebar lamina propria, ketinggian submukosa dan bilangan sel goblet semuanya meningkat dengan ketara dalam semua kumpulan rawatan dengan pemberian probiotik atau vitamin C ($p <0.05$). Secara keseluruhan, kedua-dua probiotik dan suplemen vitamin C berpotensi untuk mengekalkan kesihatan ikan. Vitamin C menunjukkan potensi yang lebih besar daripada probiotik dalam mengawal tindak balas imun. Sementara itu, kedua-dua probiotik dan suplemen vitamin C berpotensi untuk menghalang kerosakan pada struktur hati dan usus ikan yang terdedah kepada PS.

Kata kunci: Air tawar; ikan; perikanan; polistirena; probiotik

Introduction

Polystyrene is a common plastic that is produced to meet the demands of modern life (Cheng et al. 2022). The characteristics of plastic are persistent, strong, anti-corrosion, and relatively cheap material that causes high use of plastic in human life (Frias & Nash 2019). Poor plastic waste management causes terrestrial and aquatic pollution. This situation gave ecotoxicological effects on the health of organisms in ecosystems (Hayati et al. 2021). Plastic is widely recognized as a global threat to aquatic life, including freshwater, brackish, and marine. Plastics have high durability, so they can accumulate in various environments, from aquatic environments to soil environments (Zhu et al. 2018). Plastic waste damages aquatic habitats. It can be carried by water current, accumulated, or coated on the surface of the sediment (Barria et al. 2020; Lönnstedt & Eklöv 2016). Furthermore, plastic waste can also harm aquatic life, including fish, by causing entanglement or being ingested (UNEP 2021).

Plastic waste does not decompose easily but will break into smaller pieces due to degradation through mechanical abrasion, oxidation, weathering, and ultraviolet radiation (Thompson et al. 2009). The structure of plastic fragments becomes simplified, and the chemical bonds of multiple branched polymer structures become susceptible to breaking. When polystyrene plastic particles become smaller than 5 millimetres in size, it is called microplastics (Hartmann et al. 2019). The plastic particles can easily enter the body of fish through their gills, leading to increased toxicity effects on their gill lamellae. Plastic particles and fibers, which are derived from household waste were found in the gastrointestinal tracts of shellfish and fish (Rochman 2015).

Due to their small sizes, plastic particles become food particles sizes, which aquatic organisms normally ingest. Fish usually eat plastic particles accidentally because they cannot distinguish between plastic particle and their prey. Plastic particles tend to have attractive shapes and colors (Wang et al. 2017). Microplastics enter the fish's body through the chains of food and are accidentally consumed by various aquatic organisms (Cole et al. 2013). The consumption of these microplastics has raised concerns that they can cause physical damage and interfere with the function of the digestive tract, liver, gonads, and other organs, thereby inhibiting growth (Nabila & Mufti 2021).

Filter feeders such as whale sharks, basking sharks, rays, and many other fish are highly vulnerable to consuming microplastics in the wild because they can directly feed on and selectively swallow them (Graham & Thompson 2009). The absorption of MPs (<80 μm) by mussels has been shown to occur both through the gill surface and through the transfer of MPs along the gill pathway to the mouth and digestive gland, where translocation into cells can occur. Microplastics have been found to appear in the digestive lumen through ciliary movement, which moves particles into the stomach and digestive tract. Histologically, microplastics were found in the intestine, and many particle aggregates were observed in the lumens of primary and secondary tracts and in the digestive gland (Avio et al. 2015).

Plastic particles also induce the release of pro-inflammatory cytokines (e.g., tumor necrosis factor-alpha TNF-α and interferon-gamma IFN-γ) that cause inflammatory effects. Interleukin and tumor necrosis factor secretion will be stimulated by microplastic particles (Annkatrin et al. 2022). A recent study conducted by Cheng et al. (2022) reported that exposure to polystyrene plastic led to an increase in liver inflammation and the number of neutrophils in zebrafish, with a higher concentration of plastic particles resulting in a greater effect.

Increased cell and tissue damage due to the toxicity of polystyrene plastic particles could be solved by using probiotic bacteria (Bozkurt et al. 2022). Probiotic bacteria will precipitate microplastics and plastic-related chemicals (Dong et al. 2022). Administration of vitamin C as an antioxidant agent will reduce the toxicity of polystyrene particles that enter the fish's body (Notash 2012). Some studies have been conducted in Indonesia to investigate the impact of plastic waste on tilapia fish. However, few studies have explored the effects of probiotics and vitamin C on tilapia fish that have been exposed to polystyrene plastic waste.

Therefore, the objective of this research was to investigate the potential of probiotics and vitamin C supplementation in fish feed to restore the histological structure of the liver and intestine, as well as the secretion of cytokines (TNF-α and IFN-γ) on tilapia fish that have been exposed to polystyrene plastics.

Materials and Methods

RESEARCH PERIOD AND LOCATION

The experimental study was conducted at the Animal Laboratory and Histology Laboratory, Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, East Java, Indonesia. This research was carried out from January to July 2022.

CHEMICAL MATERIALS

For this study, the PS particle powder was procured from CV. Central Plastics Indonesia. Plastic powder size of 0.1-50 µm was obtained using a sieve size of 200 mesh. The particle size was verified using OPTILAB Advance Digital Microscope Camera. All other chemicals and solvents used in the experiments were of analytical grade. IFN- γ and TNF-α ELISA kits were obtained from Bioassay Technology Laboratory (Shanghai Korain Biotech, Shanghai, China).

PROBIOTICS AND VITAMIN C

Four strains of bacteria were cultured in de Man, Rosaga, and Sharpe (MRS) broth for 24 h at 37 °C. Cells precipitation was carried out by centrifugation of culture broth from four strains at 8,000 g, 4 °C for 15 min. The bacteria cells were then diluted with phosphate buffer saline to a final concentration of 1×10^8 CFU/mL. The probiotics consortium (1:1:1:1) consists of Lactobacillus fermentum (ME3), Lactobacillus casei (DSM 20011), Lactobacillus bulgaricus (NBRC13953), and Lactobacillus buchneri (DSM 20057). The probiotic was sprayed onto fish feed (25 mL/kg) and then air-dried. Vitamin C (Ascorbic Acid) was obtained from Weisheng Pharmaceutical (Shijiazuang, China).

ANIMAL

Healthy male tilapia fish (*Oreochromis niloticus*) with matured gonads were procured from Pandaan Aquaculture Management Unit, East Java Province, Indonesia. Thirty-six male fish (the number of group animals is determined by the formula according to Federer, 1967) weighed 200±20 g and aged 4-5 months were prepared for this study. They were housed in a glass rectangular tank (90 × 60 × 50 cm) specifically designed for fish-rearing, with a water recirculation system comprising of an aquatic pump (WP-4880, 45 Watt) located within the tank. The fish were fed twice daily (morning and afternoon) with commercial pellets (Takari, Sidoarjo, Indonesia), at a rate of 2-3% of body weight. The pH was maintained at 7.5 ± 0.03, and the lighting was set at a 12-hour dark and 12-hour light cycle.

ANIMAL EXPERIMENT

The fish were acclimated for 14 days. Thirty-six tilapias were divided into twelve groups, with each group consisting of three fish. The control group was administrated with commercial feeds only, negative control groups were administrated with commercial feed and exposed to different concentrations of polystyrene plastics (0.1; 1; and 10 mg/L), four treatment groups were exposed to various concentrations of polystyrene plastics (0.1; 1; and 10 mg/L) and administrated with probiotic diet (200 mL/kg, 1 × 108 CFU/mL), four treatment groups were exposed to various concentration of polystyrene plastics (0.1; 1; and 10 mg/L) and administrated with vitamin C (100 mg/kg). Treatment was conducted for two weeks.

MEASUREMENT OF INTERFERON IFN-γ AND TUMOR NECROSIS FACTOR TNF-α

Serum was collected after centrifugation of whole blood (3,000 rpm, 10 min). The level of IFN-γ and TNF-α were measured by ELISA method according to the kit's instruction and read at 450 nm using a microplate reader.

HISTOLOGY OF LIVER AND INTESTINE

For the fixation of liver and intestine tissues, 10% neutral buffered formalin was used. Subsequently, tissues were processed and sectioned to a thickness of 4 µm. Standard operating procedures for histology were followed. Alcohol dehydration, clearing, and paraffin embedding were carried out. Hematoxylin and Eosin (HE) were used for staining the organ. We observed the number of normal hepatocytes, hepatocytes with swelling, hepatocytes with necrosis, villi height, submucosa height, lamina propria width, and the number of goblet cells using the light microscope (Olympus) and photographed camera microscope (Optilab, Miconos, Indonesia).

STATISTICAL ANALYSIS

Data was analyzed statistically using Windows Statistical Package for the Social Sciences software v.24 (IBM Corp., New York, USA). We used a two-way analysis of variance (Two-Way ANOVA). A *p*-values less than 0.05 was considered statistically significant. The results were presented as mean ± standard deviation (SD).

RESULTS

LEVEL OF TNF-A IN FISH SERUM

TNF-α levels increased in all treatment groups (fed with commercial feed and exposed to various concentrations of PS (0.1, 1, and 10 mg/L) (Figure 1, blue color box). At concentrations of 1 and 10 mg/L of PS, there were significant differences ($p < 0.05$) compared to the normal control (commercial feed, PS concentration of 0 mg/L). This indicates that exposure to PS can affect the level of TNF-α when PS concentration is increased from 0.1 mg/L to 1 and 10 mg/L. There was a significant decrease in TNF-α levels after probiotics or vitamin C supplement administration in exposures to 1 and 10 mg/L PS. The research data showed that the potential of vitamin C is similar to probiotics in reducing TNF-α levels during exposure to 1 mg/L PS.

LEVEL OF IFN-γ IN FISH SERUM

The IFN-γ levels significantly increased in treatment groups (only fed with commercial feed and exposed to various concentrations of 0.1, 1, and 10 mg/L PS) (Figure 2, blue color box). Furthermore, there were significant differences ($p < 0.05$) in IFN-γ levels between the groups with and without supplementation, except for probiotic supplementation after exposure to 1 mg/L PS (Figure 2, comparison of blue, orange, and gray boxes at different PS concentrations). Fish given probiotic or vitamin C supplements showed a significant increase in IFN levels after exposure to 0.1 and 10 mg/L PS (Figure 2, comparison of blue, orange, and gray boxes at PS concentrations of 0.1 and 10 mg/L). The potential of probiotic or vitamin C supplements at PS concentrations of 0.1 and 10 mg/L showed similar potential in increasing IFN-γ levels.

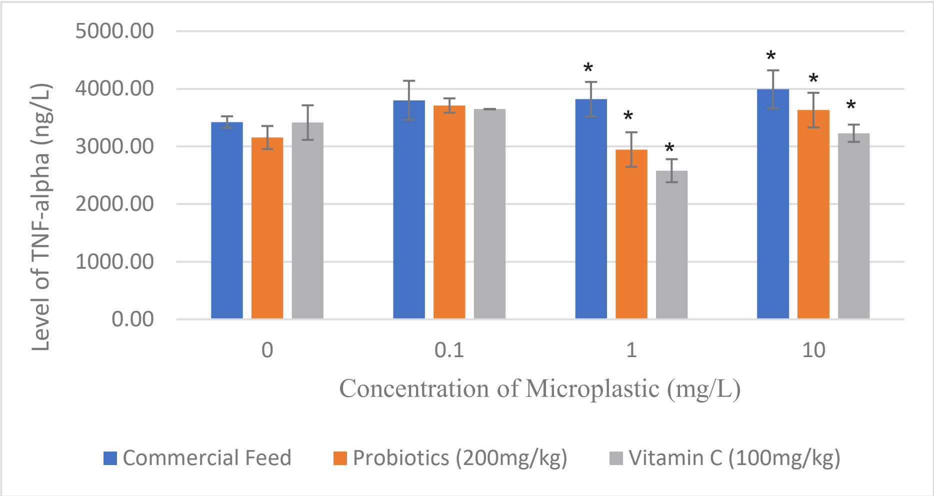

FIGURE 1. Effect of probiotics or vitamin C on the level of TNF-α after microplastic exposure. Blue bar: exposure to various concentrations of microplastic, orange bar: exposure to various concentrations of microplastic and administrated with different concentrations of probiotics diet, and grey bar: exposure to various concentrations of microplastic and administrated with different concentrations of vitamin C diet. Each bar presents means ± SD (n = 3). * $p < 0.05$ compared to the control group

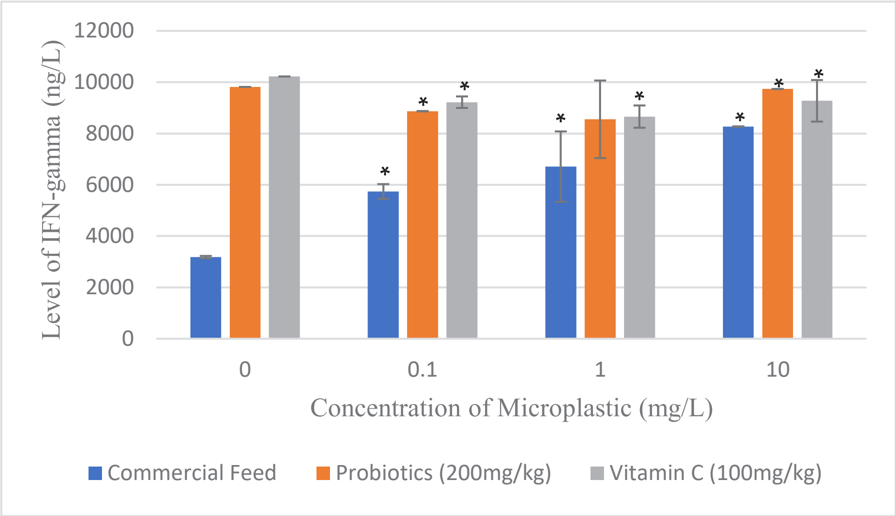

FIGURE 2. Effect of probiotics or vitamin C on the level of IFN-γ after microplastic exposure. Blue bar: exposure to various concentrations of microplastic, orange bar: exposure to various concentrations of microplastic and administrated with different concentrations of probiotics diet, and grey bar: exposure to various concentrations of microplastic and administrated with different concentrations of vitamin C diet

HISTOLOGY OF LIVER

Histological observations were carried out by counting the normal hepatocytes, hepatocytes swelling, and hepatocytes with necrosis. The results of the histopathology of the fish liver after being exposed to PS and administrated various concentrations of probiotics or vitamin C are presented in Figure 3.

According to Figure 4, exposure to PS (0.1, 1, and 10 mg/L) significantly ($p < 0.05$) (Figure 4(A)). In the groups exposed to 1 and 10 mg/L PS, administration of probiotic or vitamin C supplements can significantly increase the percentage of normal hepatocytes.

The percentage of hepatocyte swelling increased significantly with the increase of PS concentrations (1 and 10 mg/L) (Figure 4(B)). Administration of 200 mg/kg probiotic supplement or 100 mg/kg vitamin C significantly reduced the percentage of hepatocyte swelling after exposure to 10 mg/L PS ($p < 0.05$).

The same trend was observed in the percentage of hepatocyte necrosis. The percentage of necrosis increased with the increase in PS concentration, while the administration of supplements reduced hepatocyte necrosis (Figure 4(C)). Administration of 200 mg/kg probiotic or 100 mg/kg vitamin C significantly reduced the percentage of necrosis after exposure to 1 and 10 mg/L PS ($p < 0.05$).

On the other hand, there were no significant changes in the diameter of the portal vein (Figure 4(D)), either in the groups exposed to PS or in those given probiotic or vitamin C supplements.

HISTOLOGY OF INTESTINE

Histological observations were carried out by counting the number of goblet cells, the height of the villi, the width of lamina propria, and the height of the submucosa. The results of the histopathology of fish intestines are presented in Figure 5.

Exposure to PS without supplementation significantly decreased the number of goblet cells, villi height, lamina propria width, and submucosa height, except for submucosa height at 0.1 mg/L PS exposure (Figure 6(A)-6(D)).

There was an increase in the number of goblet cells in all treatment groups with probiotic or vitamin C supplementation ($p < 0.05$) (Figure 6(A)). This indicates that probiotic or vitamin C supplementation can inhibit goblet cell damage and death. Likewise, villi height, lamina propria width, and submucosa

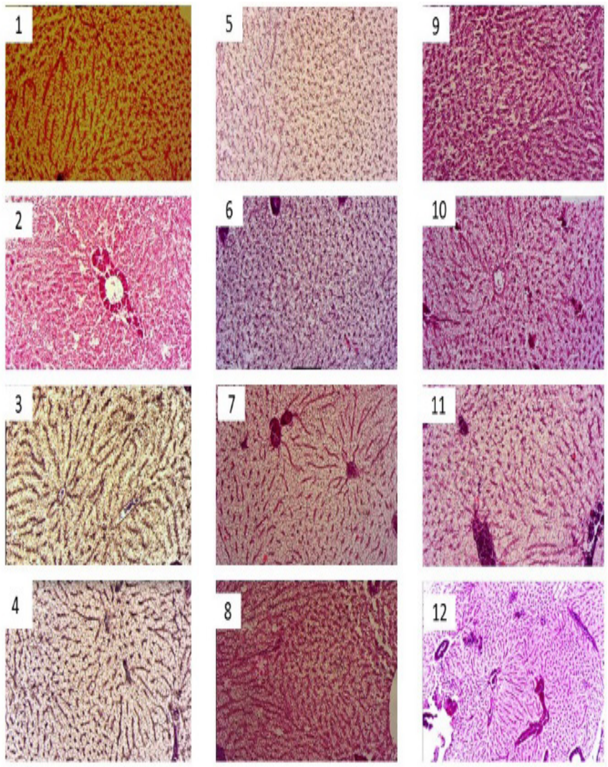

FIGURE 3. Effect of probiotics and vitamin C on histology of fish liver after microplastic exposure. 400× magnification. 1: control group (commercial feed); 2: first negative control (microplastic 0.1 mg/kg); 3: second negative control (microplastic 1 mg/kg); 4: third negative control (microplastic 10 mg/kg); 5: first positive control (probiotics 200 mg/kg); 6: treatment group (microplastic 0,1 mg/kg + probiotics 200 mg/kg); 7: treatment group (microplastic 1 mg/kg + probiotics 200 mg/kg); 8: treatment group (microplastic 10 mg/kg + probiotics 200 mg/kg); 9: second positive control group (vitamin C 100 mg/kg); 10: treatment group (microplastic 0.1 mg/kg + vitamin C 100 mg/kg); 11: treatment group (microplastic 1 mg/kg + vitamin C 100 mg/kg); 12: treatment group (microplastic 10 mg/kg + vitamin C 100 mg/kg)

height also decreased with increasing PS concentration. When supplements were added to fish feed, villi, lamina propria, and submucosa size increased ($p < 0.05$) (Figure 6), except for submucosa height probiotic supplementation at 0.1 mg/L PS exposure. According to the data, both supplements have the same potential to inhibit cell damage due to PS exposure.

DISCUSSIONS

Microplastic can be considered as an antigen that disturb the normal function of a fish's body by increasing oxidative stress. The present result showed raising IFN-γ and TNF-α levels in negative control group fish after exposure to microplastic (0.1; 1; and 10 mg/kg). As the concentration of microplastic exposure increased, the levels of IFN-γ and TNF-α also increased. IFN-γ and TNF-α are cytokines that are produced by macrophages and T helper in response to non-self components such as microplastics, leading to immune system activation. All treatment groups with probiotics and vitamin C showed an increased IFN-γ level. In line with our study, Cocci et al. (2022) observed that mRNA and protein levels of IFN-γ elevated in the zebrafish intestine after exposure to polystyrene microplastics. Huang et al. (2020) also reported that the protein of IFN-γ, TLR4, and IL-6 elevated significantly in the intestine of guppy when exposure to

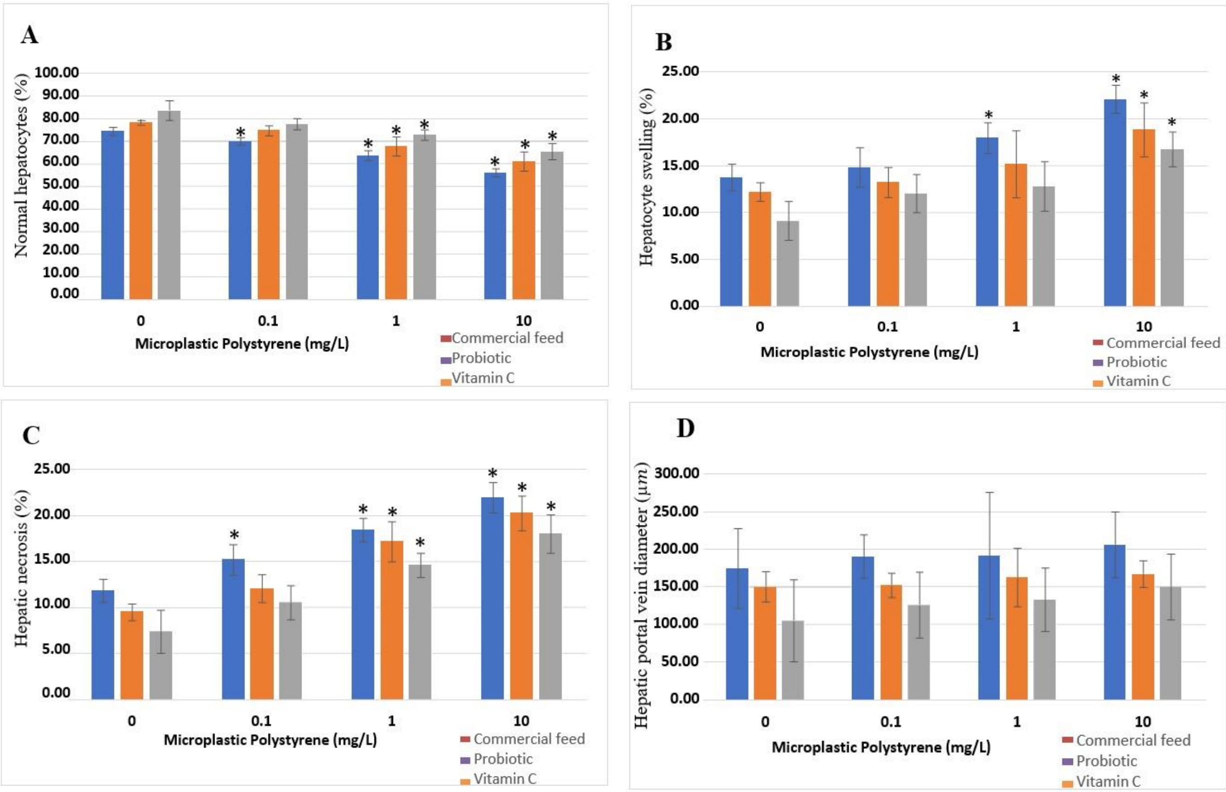

FIGURE 4. Effect of probiotics or vitamin C on the A: percentage of normal hepatocytes; B: percentage of hepatocyte swelling; C: percentage hepatic necrosis; D: hepatic portal vein diameter after microplastic exposure. Blue bar: exposed to various concentrations of microplastic, orange bar: exposed to various concentrations of microplastic and administrated with different concentrations of probiotics diet, and grey bar: exposed to various concentrations of microplastic and administrated with different concentrations of vitamin C diet. Each bar presents means ± SD (n = 3). * $p < 0.05$ compared to the control group

100 and 1000 µg/L microplastics was performed for 28 days. In fish, cytokines secretion can be considered an effective biomarker of the inflammatory response (Jin et al. 2015).

Microplastic as an antigen can modulate increasing cytokine secretion to regulate the immune response (Hu & Palic 2020). The toxicity of microplastic is usually derived from ROS accumulation causing oxidative stress and induction of inflammation (Chiang et al. 2003). In contrast with the result of IFN-γ, the present result showed a decreased TNF-α level in all treatment groups with probiotics and vitamin C. The excessive production of TNF-α is associated with the development of various diseases (Hu & Palic 2020). In this study, supplementation of probiotics and vitamin C in fish feed increased serum levels of IFN-γ and controlled serum levels of TNF-α possibly to prevent healthy cells from becoming damaged. Healthy cells could be damaged when inflammatory cytokines such as IFN-γ and TNF-α were overproduced. Both IFN-γ and TNF-α could cause excessive inflammation (Annkatrin et al. 2022).

The liver receives a significant proportion of its blood supply (around 70%) from the intestines, indicating a strong interaction between the microbiota and toxic substances in these organs (Cedervall et al. 2012). When the liver is exposed to plastic particles, it will elevate ROS levels and the liver will be in a state of cellular oxidative stress. The present study found that microplastic exposure had an impact on the liver histological structure of fish, with a decrease in the percentage of normal hepatocytes observed in all negative control groups exposed to PS (0.1, 1, and 10 mg/kg). Administration of

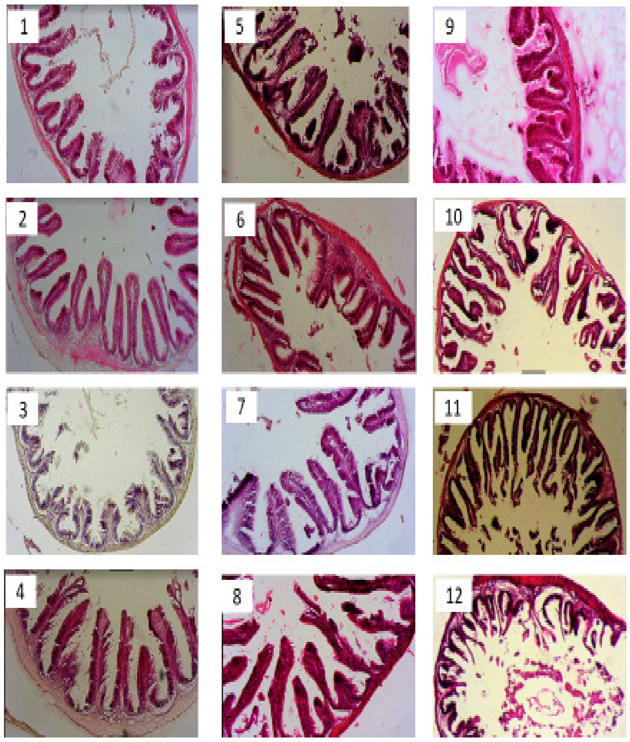

FIGURE 5. Effect of probiotics and vitamin C on histology of fish liver after microplastic exposure. 100× magnification. 1: control (commercial feed only); 2: first negative control (microplastic 0.1 mg/kg); 3: second negative control (microplastic 1 mg/kg); 4: third negative control (microplastic 10 mg/kg); 5: first positive control (probiotics 200 mg/kg); 6: treatment group (microplastic 0.1 mg/kg + probiotics 200 mg/kg); 7: treatment group (microplastic 1 mg/kg + probiotics 200 mg/kg); 8: treatment group (microplastic 10 mg/kg + probiotics 200 mg/kg); 9: second positive control group (vitamin C 100 mg/kg); 10: treatment group (microplastic 0.1 mg/kg + vitamin C 100 mg/kg); 11: treatment group (microplastic 1 mg/kg + vitamin C 100 mg/kg); 12: treatment group (microplastic 10 mg/kg + vitamin C 100 mg/kg)

200 mg/kg probiotics or 100 mg/kg vitamin C decreased the percentage of hepatocytes with necrosis and the percentage of hepatocytes with swelling, while also restoring the number of normal hepatocytes.

Probiotic bacteria in the digestive tract can interact to increase and activate immune cells, which can enter the bloodstream and be distributed to body tissues to enhance the immune response of fish. Oxidative stress arises due to an imbalance of free radicals and antioxidants in the body. Oxidative stress can be overcome with vitamin C as an antioxidant. While the fish body already contains antioxidant enzymes called endogenous antioxidants, the number of plastic particles that enter the fish's body can overwhelm these defenses and require supplementation with exogenous antioxidants from outside the body (Sylviana et al. 2017). Vitamin C is an effective exogenous antioxidant for preventing lipid peroxidation due to ROS accumulation (Kumar et al. 2015). Vitamin C can also provide an electron pair to complement the free radicals produced by the fish's body (Popovic et al. 2015).

Microplastics that accumulate in the intestines can cause blockages and are not easily degraded. The current study also found that exposure to microplastics (0.1, 1, and 10 mg/kg) led to a decrease in the number of goblet cells, villi height, lamina propria width, and submucosa height in all negative control groups. Research carried out by Ahrendt et al. (2020) also showed that polystyrene microplastic exposure caused leukocyte infiltration, hyperemia. and damage of villi in *Girella*

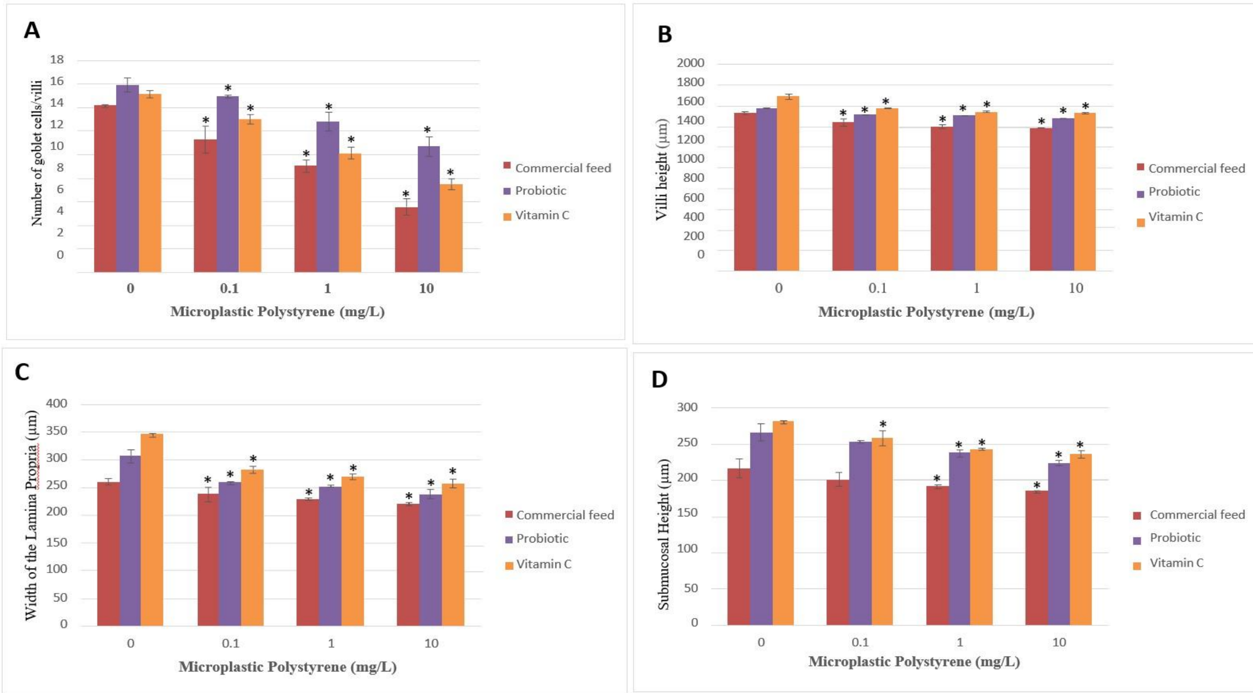

FIGURE 6. Effect of probiotics or vitamin C on the A: number of goblet cells; B: villi height; C: width of lamina propria; D: submucosa height after microplastic exposure. Red bar: exposure to various concentrations of microplastic, purple bar: exposure to various concentrations of microplastic and administrated with different concentrations of probiotics diet, and orange bar: exposure to various concentrations of microplastic and administrated with different concentrations of vitamin C diet. Each bar presents means ± SD (n = 3). * $p < 0.05$ compared to the control group

laevifrons fish. In another research conducted by Mbugani et al. (2015), a decrease in villi width and villi height occurred when tilapia fish were exposed to 10 PE of microplastics.

This study found that the addition of vitamin C or probiotics significantly increased the number of goblet cells, villi height, lamina propria width, and submucosa height in all treatment groups compared to the negative control group. Probiotics consist of a consortium of microbes including *Lactobacillus plantarum, Bacillus subtilis, B. meganterium, B. licheniformis, Sphingomonas, Nitrosomonas, Nitrobacter,* and *L. fermentum*. Probiotics could lower pathological problems occurred in the intestine. Probiotics have been shown to reduce pathological problems in the intestine, modulate immune response, maintain fish health, produce anti-pathogen factors, and secrete antioxidant enzymes to reduce inflammation. Vitamin C supplementation in fish feed can also reduce stress levels, elevate immune response, and accelerate the wound-healing process in fish (Ahrendt et al. 2020).

Conclusions

We concluded that probiotics and vitamin C supplementation in fish feed could not significantly modulate the immune response against polystyrene microplastic exposure. However, there was decreasing level of TNF-α and an increasing level of IFN-γ to prevent overexpression of pro-inflammatory cytokine and improve better immune response against microplastic particles. Both probiotics and vitamin C supplements have equal potential in inhibiting damage to the hepatocyte and intestinal structure of fish exposed to PS. Therefore, this study suggests that probiotic and vitamin C supplements in fish feed can act as alternative biofilters and antioxidants to maintain fish health and reduce the toxicity of polystyrene particles that enter their bodies.

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