



Potential of Basil Seed (*Ocimum basilicum*) Simplicia as a Feed Supplement on the Histomorphometry and Muscle Damage of African Catfish (*Clarias gariepinus*) Exposed to Polystyrene Microplastics

^{1*}Iga Novianti, ²Nurul Suwartiningsih

^{1,2}Department of Biology, Faculty of Applied Science and Technology, Universitas Ahmad Dahlan, Yogyakarta, Indonesia.

*Corresponding Author e-mail: 2200017028@webmail.uad.ac.id

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Abstract: This study aimed to evaluate the potential of basil seed simplicia supplementation in feed to mitigate histomorphometric alterations and muscle damage in African catfish (*Clarias gariepinus*) exposed to polystyrene (PS) microplastics. The experiment employed a Completely Randomized Design (CRD) consisting of five treatments: C (0 mg/L PS microplastics + 0% basil seed simplicia), PC (0 mg/L PS microplastics + 2% basil seed simplicia), NC (100 mg/L PS microplastics + 0% basil seed simplicia), T1 (100 mg/L PS microplastics + 2% basil seed simplicia), and T2 (100 mg/L PS microplastics + 4% basil seed simplicia). A total of 125 catfish were exposed to microplastics for 15 days. The observed parameters included myofiber diameter, myofiber area, muscle degeneration, and necrosis. The results indicated that exposure to PS microplastics significantly decreased myofiber diameter and area while increasing the levels of muscle cell degeneration and necrosis ($P < 0.05$). Supplementation with 2% basil seed simplicia (T1) significantly improved myofiber diameter and area and reduced muscle degeneration and necrosis compared with the negative control (NC), whereas supplementation with 4% basil seed simplicia (T2) showed relatively lower effectiveness. These findings suggest that supplementation of 2% basil seed simplicia in feed has the potential to mitigate histomorphometric alterations and muscle tissue damage in catfish exposed to polystyrene microplastics.

Keywords: Basil seeds; degeneration; microplastics PS; Myofiber; necrosis

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INTRODUCTION

African catfish (*Clarias gariepinus*) is one of the leading freshwater aquaculture commodities in Indonesia, characterized by high market demand that continues to increase annually (Amar et al., 2022). Catfish represent a superior species in aquaculture due to their relatively rapid growth rate and higher tolerance to disease disturbances compared with many other fish species (Yuriana et al., 2017). In Indonesia, catfish production has shown a consistent upward trend, increasing from 1.017.313 tons in 2020 to 1.253.114 tons in 2021 (Ministry of Marine Affairs and Fisheries, 2021). However, catfish productivity may be hindered by environmental pollution in aquatic ecosystems, one of which is microplastic contamination. Microplastic pollution in aquaculture environments can reduce water quality and threaten fish health, ultimately leading to decreased aquaculture productivity (Filho et al., 2023).

Microplastics are plastic particles smaller than 5 mm that originate either from the degradation of larger plastic debris or from plastics intentionally manufactured at microscopic sizes (EFSA, 2016). These particles are widely distributed in global aquatic environments due to poorly managed plastic waste disposal, tire abrasion, and

the release of synthetic fibers during laundry processes (Firnanda et al., 2024). In aquaculture environments, plastic nets, farming equipment, and pond covers represent additional sources of microplastic contamination (Yusuf & Effendi, 2024). Numerous studies have reported the widespread presence of microplastics in freshwater aquaculture systems, including rivers and lakes, where they can potentially accumulate within aquatic organisms (Chen et al., 2021). Wicaksana and Firmani (2025) reported microplastic contamination in pond waters in the villages of Samirplapan and Tambakrejo reaching 41 particles per 250 mL. Nurdhiana (2021) further stated that microplastic contamination in cage-culture waters can facilitate the transfer of toxic particles into fish bodies, thereby affecting their development and aquaculture productivity.

One of the most abundant types of microplastics found in aquatic environments is polystyrene (PS) (Jones et al., 2020), which is known to exert toxic effects on aquatic organisms. Suwartiningsih et al. (2025) reported that PS microplastics at a concentration of 100 mg/L were detected at levels of 2.63 particles/g in the muscle tissue of catfish. Sawalman et al. (2021) indicated that microplastics entering the digestive tract of fish may undergo fragmentation into smaller particles, enabling their translocation into the bloodstream and muscle tissues (Liu et al., 2022). Exposure to PS microplastics can induce oxidative stress and damage fish muscle tissues (Hamed et al., 2021), which play a crucial role in fish growth and development (Kama et al., 2024). Hamed et al. (2021) demonstrated that exposure to microplastics at a dose of 100 mg/L for 15 days resulted in accumulation within the bodies of Nile tilapia, particularly in muscle tissues, leading to histological alterations such as necrosis and muscle fiber degeneration, which indicate structural tissue damage. Similarly, Suwartiningsih et al. (2025) revealed that exposure of Mutiara catfish to microplastics at a dose of 100 mg/L for 28 days significantly reduced myofiber diameter and myofiber area, indicating muscle atrophy. Collectively, PS microplastic exposure in cultured fish can trigger oxidative stress through the increased production of reactive oxygen species (ROS), leading to histomorphometric alterations and histological damage in muscle tissues. Therefore, nutritional interventions using natural ingredients rich in proteins, amino acids, and antioxidant compounds, such as basil seeds, may represent a potential mitigation strategy to reduce histomorphometric alterations and muscle tissue damage while maintaining fish productivity.

The adverse effects of microplastic exposure on catfish productivity and muscle tissue damage may be minimized through the use of natural materials. Basil seeds (*Ocimum basilicum*) are known to contain high protein levels, ranging from approximately 11.4% to 22.5% of their dry weight (Bravo et al., 2021). The protein content of basil seed simplicia has been shown to enhance the productivity of *Sparus aurata* more effectively than basil leaf simplicia (El-Dakar et al., 2015). Basil seeds also contain a high concentration of glutamic acid, accounting for approximately 17.40% of the total amino acid content (Nazir & Wani, 2021). Glutamic acid acts as a primary precursor in the biosynthesis of glutathione (GSH), a major endogenous antioxidant that plays a critical role in neutralizing free radicals (Cai et al., 2025). Increased glutamic acid intake has been shown to elevate GSH levels and antioxidant enzyme activity, thereby reducing oxidative stress caused by exposure to foreign particles such as microplastics (Jiang et al., 2016). Furthermore, Hamed et al. (2020) reported that antioxidant compounds can help restore muscle damage caused by free radical exposure derived from microplastics.

Research on the use of natural materials as mitigation strategies against microplastic exposure in aquaculture remains relatively limited. The urgency of this

issue continues to increase in line with the growing prevalence of microplastic pollution, including PS, in aquaculture waters (Chen et al., 2021; Filho et al., 2023). Exposure to PS microplastics has been reported to induce oxidative stress and tissue damage in fish (Hamed et al., 2020; Mahmud et al., 2024), resulting in histomorphometric changes and muscle tissue damage such as decreased myofiber diameter and area, as well as degeneration and necrosis (Hamed et al., 2021; Suwartiningsih et al., 2025). These conditions ultimately lead to reduced quality and productivity of cultured fish, including catfish (Amar et al., 2022).

Although numerous studies have highlighted the toxic effects of microplastics on fish histological structures, most investigations have primarily focused on toxicity aspects without exploring mitigation strategies based on natural materials (Kama et al., 2024). On the other hand, basil seeds are known to contain nutrients and bioactive compounds with antioxidant activity capable of scavenging free radicals (Bravo et al., 2021; Nazir & Wani, 2021). Supplementation of natural ingredients in fish feed has also been reported to improve tissue quality and physiological resilience in fish (Jiang et al., 2016; Sayed et al., 2023). However, to date, there has been no scientific report specifically examining the effectiveness of basil seed *simplicia* supplementation in fish feed for protecting or restoring the histological structure of fish muscle tissues exposed to PS microplastics. Therefore, this study aims to analyze the effect of basil seed *simplicia* supplementation in feed on myofiber diameter, myofiber area, and the levels of muscle degeneration and necrosis in fish exposed to PS microplastics.

METHOD

This study was conducted between September and December 2025. The rearing and maintenance of African catfish (*Clarias gariepinus*) were carried out in Borongan Village, Tlogo, Prambanan District, Klaten Regency, Central Java, Indonesia. The preparation of basil seed *simplicia*, polystyrene (PS) microplastics, and preliminary observations were conducted at the Laboratory of Plant Structure and Physiology, Ahmad Dahlan University. Fish dissection and data analysis were performed at the Laboratory of Animal Structure and Physiology, Ahmad Dahlan University. The preparation of muscle tissue slides was conducted at the Anatomical Pathology Laboratory, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada.

This study employed an experimental approach designed to evaluate the effectiveness of basil seed *simplicia* incorporated into feed as a protective agent against muscle degeneration in African catfish exposed to PS microplastics. The experimental design used was a Completely Randomized Design (CRD) consisting of five treatments: C (0 mg/L PS microplastics + 0% basil seed *simplicia*), PC (0 mg/L PS microplastics + 2% basil seed *simplicia*), NC (100 mg/L PS microplastics + 0% basil seed *simplicia*), T1 (100 mg/L PS microplastics + 2% basil seed *simplicia*), and T2 (100 mg/L PS microplastics + 4% basil seed *simplicia*). Each treatment was replicated five times, with each replicate consisting of five catfish, resulting in a total of 125 fish. Exposure to PS microplastics was administered through the rearing medium at a concentration of 100 mg/L for 15 days. The primary parameters observed included myofiber diameter, myofiber area, and the occurrence of muscle cell degeneration and necrosis.

Preparation of Basil Seed *Simplicia*

Basil seed *simplicia* were prepared by grinding 300 g of basil seeds using a blender, followed by sieving through a 35-mesh sieve. The *simplicia* were then weighed at 2 g and 4 g according to the treatment levels and subsequently adhered to Tongwei-1 floating pellets using Progol fish-pellet adhesive.

Preparation of PS Microplastic Suspension

PS microplastic particles were prepared by grinding PS plastic using a blender until finely powdered and then sieving the particles through a 35-mesh sieve (Suwartiningsih et al., 2025). The PS microplastics were administered at a concentration of 100 mg/L according to the treatment protocol (Hamed et al., 2021).

Catfish Treatment

The catfish were acclimatized for 7 days (Haetami et al., 2023), after which they were maintained for 15 days under exposure to PS microplastics at a concentration of 100 mg/L (Hamed et al., 2021) and fed diets supplemented with basil seed simplicia at levels of 2% and 4%, depending on the treatment group. The fish were reared in 25 containers (20 L capacity) filled with 10 L of water, with each container housing five fish, resulting in a total of 125 catfish. Feeding was conducted twice daily at 08:00 and 16:00 (WIB), with a feeding rate of 5% of the fish body weight. The rearing medium was replaced every two days using a siphoning method, followed by the re-addition of microplastics according to the treatment concentration after each water replacement (Suwartiningsih et al., 2025).

Preparation of Muscle Tissue Slides

After 15 days of treatment, the fish were fasted for 24 hours prior to dissection. Before dissection, the fish were anesthetized using ice. The abdominal muscle tissue on the right side (approximately 1 cm in length) was then excised. The tissue samples were rinsed with physiological saline (NaCl) and fixed in 10% buffered neutral formalin (BNF) for approximately 24 hours. Subsequently, the tissues were dehydrated using graded ethanol solutions at concentrations of 70%, 80%, 90%, 96%, and absolute ethanol, followed by clearing with toluene for approximately 12 hours. The infiltration process was carried out in an incubator at 55–60°C using a gradual mixture of toluene–paraffin (1:1) and pure paraffin before embedding the tissues in molten paraffin. The hardened paraffin blocks were sectioned using a microtome to obtain slices approximately 4 µm thick, which were then mounted onto glass slides using Mayer's albumin. The tissue sections were subsequently deparaffinized using xylene, rehydrated through graded alcohol solutions, and stained with Hematoxylin–Eosin (HE). Finally, the slides were mounted using entellan and covered with cover slips (Zulfadhli et al., 2016). The prepared slides were observed under a light microscope at 10× magnification across five fields of view and documented using Beta View software. Measurements of myofiber diameter and myofiber area were conducted using Image Raster software (Suwartiningsih et al., 2025).

Data Analysis

The data were analyzed descriptively and presented in the form of tables and figures. Inferential statistical analysis was conducted using Software SPSS. Initially, data normality was tested using the Shapiro–Wilk test. Because the data were not normally distributed ($P < 0.05$), differences among treatments were analyzed using the Kruskal–Wallis test. Post hoc analysis was performed using pairwise comparisons. In addition, regression analysis was conducted to examine the relationship between treatment variables and the observed parameters.

RESULTS AND DISCUSSION

Myofiber Diameter and Area in African Catfish

The results showed that the lowest myofiber diameter in catfish muscle (Table 1) was observed in the NC group ($49.94 \pm 5.87 \mu\text{m}$), whereas the highest value was recorded in the PC group ($110.01 \pm 12.38 \mu\text{m}$). Similarly, the lowest myofiber area was

found in the NC group ($19.86 \pm 47.10 \mu\text{m}$), while the highest value occurred in the PC group ($96.25 \pm 22.98 \mu\text{m}$). Statistical analysis indicated that both myofiber diameter and area differed significantly between the control groups and the treatment groups ($P < 0.05$). However, no significant differences in myofiber diameter and area were observed between the PC and T1 groups ($P > 0.05$).

Regression analysis further demonstrated that the supplementation of basil seed (*Ocimum basilicum*) simplicia in the diet for 15 days significantly influenced the myofiber diameter and area of catfish muscle exposed to PS microplastics ($P < 0.05$). These findings indicate that dietary basil seed simplicia can affect muscle tissue formation, leading to an increase in both myofiber diameter and area in catfish exposed to PS microplastics for 15 days (Figure 1).

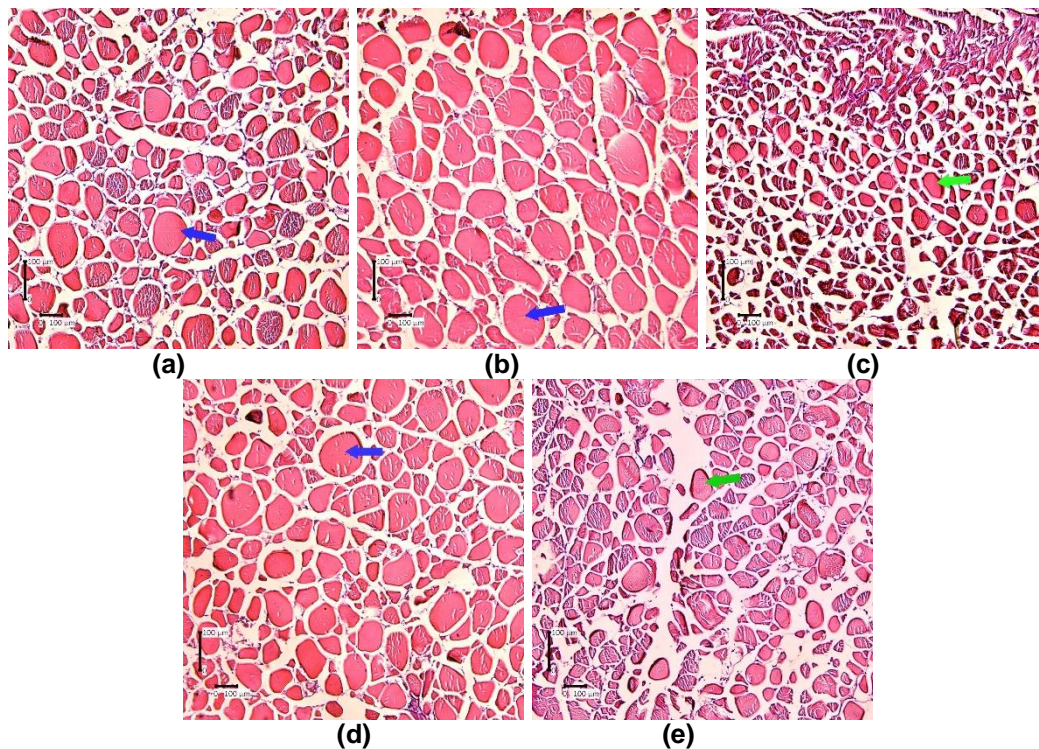


Figure 1. Myofiber diameter and myofiber area of African catfish (*Clarias gariepinus*) after 15 days of administration of basil seed (*Ocimum basilicum*) simplicia and exposure to polystyrene (PS) microplastics: (a) C (0 mg/L PS + 0% basil seed simplicia); (b) PC (0 mg/L PS + 2% basil seed simplicia); (c) NC (100 mg/L PS + 0% basil seed simplicia); (d) T1 (100 mg/L PS + 2% basil seed simplicia); and (e) T2 (100 mg/L PS + 4% basil seed simplicia). The blue arrows indicate normal myofiber diameter, whereas the green arrows indicate reduced (atrophied) myofiber diameter (hematoxylin–eosin staining; scale bar = 100 μm).

Table 1. Myofiber diameter and area of African Catfish (*Clarias gariepinus*)

Treatment	Myofiber Diameter (μm)	Myofiber Area ($\times 10^2 \mu\text{m}^2$)
Control (C)	101.07 ± 10.2^c	81.05 ± 15.63^c
Positive Control (PC)	110.01 ± 12.38^d	96.25 ± 22.98^d
Negative Control (NC)	49.94 ± 5.87^a	19.86 ± 47.10^a
Treatment 1 (T1)	109.01 ± 9.07^d	93.97 ± 15.65^d
Treatment 2 (T2)	81.59 ± 11.89^b	53.38 ± 15.99^b

Note: Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$); identical superscripts indicate no significant difference.

Based on the results presented in Table 1, the myofiber diameter and area differed significantly between the control group (C) and the negative control group (NC) ($P < 0.05$). This finding indicates that exposure to polystyrene (PS) microplastics for 15 days resulted in a significant reduction in myofiber diameter and area in the NC group, which exhibited the lowest values for both parameters. The reduction in myofiber diameter and area reflects the occurrence of muscle atrophy, which is closely associated with impaired myogenesis resulting from oxidative stress induced by PS microplastics (Suwartiningsih et al., 2025). PS microplastics entering the digestive tract of fish may undergo fragmentation into smaller particles, enabling their translocation from the intestine into the bloodstream and subsequent accumulation in muscle tissues (Sawalman et al., 2021; Liu et al., 2022). The accumulation of PS microplastics in fish muscle tissue may release toxic compounds such as phthalates and bisphenol A, which can increase the production of reactive oxygen species and trigger oxidative stress. This oxidative stress can damage cell membranes and structural muscle proteins, ultimately inhibiting myogenesis and the regeneration of myofibers (Hamed et al., 2021; Kama et al., 2024). These findings are consistent with the study by Suwartiningsih et al. (2025), which reported that exposure to PS microplastics at a concentration of 100 mg/L in catfish resulted in decreased myofiber diameter and area, indicating histological alterations in muscle tissue.

The supplementation of basil seed simplicia at 2% in the feed (T1) significantly increased myofiber diameter and area compared with the NC group (Table 1). This effect may be attributed to the protein and amino acid content of basil seeds, particularly glutamic acid. According to Bravo et al. (2021), basil seed simplicia contain high levels of protein, ranging from approximately 11.4% to 22.5% of their dry weight. The protein content in basil seeds supports muscle fiber regeneration by providing amino acids derived from protein digestion as essential substrates for muscle protein synthesis. Amino acids activate the mechanistic target of rapamycin (mTOR) signaling pathway, thereby promoting the synthesis of myofibrillar proteins such as actin and myosin during the repair of damaged muscle fibers and stimulating myofiber hypertrophy (Zhao et al., 2020). An increase in myofiber diameter and area, indicative of hypertrophy, reflects a healthier physiological condition of muscle tissue and is directly associated with improved fish flesh quality (Ziolkowska et al., 2022). In addition, basil seeds are known to contain a high proportion of glutamic acid, accounting for approximately 17.40% of the total amino acid content (Nazir & Wani, 2021). Glutamic acid acts as a protective agent that suppresses oxidative stress, thereby allowing the myogenesis process in muscle tissue to proceed optimally.

However, supplementation with 4% basil seed simplicia in the feed (T2) resulted in lower myofiber diameter and area compared with T1 (Table 1), with a statistically significant difference ($P < 0.05$). This outcome may be attributed to the presence of antinutritional compounds in basil seeds, particularly tannins. Basil seeds are known to contain phenolic compounds, including tannins, which possess bioactive properties. Nazir and Wani (2021) reported that basil seeds have a total phenolic content of 18.24 ± 0.03 mg GAE/g, indicating the presence of polyphenolic compounds including tannins. When administered at high concentrations, tannin-containing basil seed simplicia may reduce feed palatability due to their bitter and astringent taste. Omnes et al. (2017) reported that increasing tannin levels in fish feed, particularly at concentrations of 20–30 g tannin kg^{-1} feed, significantly reduced feed intake as a result of decreased palatability, leading to lower nutrient consumption by fish. Reduced feed palatability consequently limits protein and amino acid intake required to stimulate myofiber regeneration and may disrupt the myogenesis process in fish. In addition to

suppressing appetite, tannins may also reduce digestive efficiency and protein absorption by binding to proteins and interfering with cellular protein synthesis processes (Simanjutak, 2021). Disruption of muscle cell protein synthesis ultimately results in reduced myofiber diameter and area.

Degeneration and Necrosis of Catfish Muscle Cells

The results of the study indicate that the lowest level of muscle cell degeneration in African catfish (*Clarias gariepinus*) (Table 2) was observed in the PC group ($0.53 \pm 0.68\%$), while the highest value was recorded in the NC group ($6.04 \pm 3.55\%$). The lowest level of necrosis was found in the T1 group ($0.03 \pm 0.18\%$), whereas the highest value occurred in the NC group ($2.51 \pm 3.17\%$). Statistical analysis revealed significant differences between the control and treatment groups in terms of reductions in muscle cell degeneration and necrosis ($P < 0.05$). However, no significant differences were observed between the PC and T1 groups regarding muscle cell degeneration and necrosis ($P > 0.05$).

Regression analysis further demonstrated that the administration of basil seed simplicia in the diet for 15 days had a significant effect on reducing muscle cell degeneration and necrosis caused by exposure to polystyrene (PS) microplastics ($P < 0.05$). These findings indicate that the supplementation of 2% basil seed simplicia in the feed can serve as a protective agent against muscle degeneration and necrosis in catfish exposed to PS microplastics for 15 days (Figure 2).

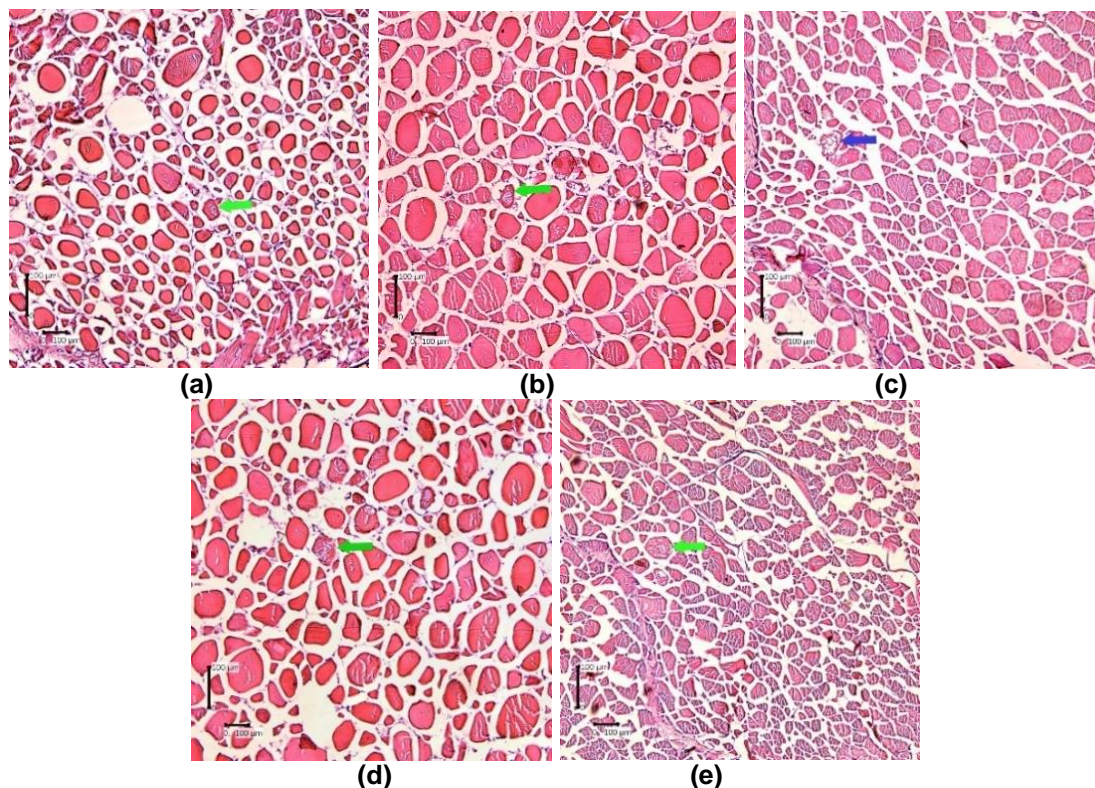


Figure 2. Degeneration and necrosis of catfish muscle cells after 15 days of basil seed (*Ocimum basilicum*) simplicia supplementation and exposure to PS microplastics: (a) C (0 mg/L PS + 0% basil seed simplicia); (b) PC (0 mg/L PS + 2% basil seed simplicia); (c) NC (100 mg/L PS + 0% basil seed simplicia); (d) T1 (100 mg/L PS + 2% basil seed simplicia); and (e) T2 (100 mg/L PS + 4% basil seed simplicia). Green arrows indicate muscle cell degeneration, while blue arrows indicate muscle cell necrosis (Hematoxylin–Eosin staining; scale bar = 100 μm).

Table 2. Degeneration and necrosis of Catfish muscle cells

Perlakuan	Nekrosis (%)	Degenerasi (%)
Control (C)	0.93 ± 0.95 ^{bc}	1.21 ± 1.41 ^a
Positive Control (PC)	0.22 ± 0.52 ^{ab}	0.53 ± 0.68 ^a
Negative Control (NC)	2.51 ± 3.17 ^d	6.04 ± 3.55 ^c
Treatment 1 (T1)	0.03 ± 0.18 ^a	0.61 ± 0.72 ^a
Treatment 2 (T2)	1.50 ± 1.19 ^c	2.24 ± 1.56 ^b

Note: Different superscript letters indicate statistically significant differences ($P < 0.05$), whereas identical superscript letters indicate no significant difference.

Based on the results presented in Table 2, the levels of degeneration and necrosis differed significantly between the control group (C) and the negative control group (NC) ($P < 0.05$). This finding indicates that exposure to PS microplastics significantly increases muscle cell degeneration and necrosis in catfish. PS microplastics entering the fish body act as toxic agents that stimulate the production of reactive oxygen species (ROS), thereby inducing oxidative stress in muscle cells (Hamed et al., 2021). Oxidative stress damages cellular membranes and mitochondria, which play a critical role in cellular energy production, ultimately reducing ATP synthesis required to maintain cellular structure (Mahmud et al., 2024). Reduced energy availability leads to increased membrane permeability and intracellular ionic imbalance, resulting in cellular swelling. Histologically, the cytoplasm appears to contain empty spaces, becomes paler, and forms small vacuoles, while the cells exhibit irregular and loosely arranged structures—hallmarks of cellular degeneration. Degeneration represents an early stage of tissue damage that is still reversible; however, it may progress to necrosis if toxic exposure persists continuously (Hamed et al., 2021). The high level of degeneration observed in the NC group indicates that PS microplastics disrupt the structural stability and metabolic processes of muscle cells.

Continuous exposure to PS microplastics can lead to more severe cellular damage, allowing degeneration to progress into necrosis. Necrosis is an irreversible form of cell death characterized by disruption of the cell membrane, cytoplasmic swelling, and nuclear fragmentation (Alif et al., 2021). The accumulation of ROS due to prolonged PS microplastic exposure not only damages membrane lipids and contractile proteins but also exacerbates structural damage to muscle cells (Mahmud et al., 2024). The high level of necrosis observed in the NC group indicates the inability of muscle cells to repair oxidative damage, resulting in the loss of cellular integrity and permanent cell death. These findings are consistent with the study of Hamed et al. (2021), which reported that exposure to PS microplastics in fish causes histopathological damage in muscle tissues, including muscle cell degeneration and necrosis as a consequence of prolonged oxidative stress.

The supplementation of 2% basil seed simplicia in the diet (T1) significantly reduced the levels of degeneration and necrosis. This effect may be attributed to the amino acid content of basil seeds, particularly glutamic acid. According to Nazir and Wani (2021), basil seeds contain a high level of glutamic acid, accounting for approximately 17.40% of the total amino acid composition. Glutamic acid functions as a primary precursor in glutathione (GSH) synthesis, thereby strengthening the endogenous antioxidant defense system and reducing ROS accumulation in muscle tissues (Cai et al., 2025). The reduction in ROS accumulation prevents lipid peroxidation of cell membranes, damage to contractile proteins, and mitochondrial dysfunction, thereby maintaining the structural stability and metabolic activity of muscle cells. By preserving membrane integrity and cellular energy availability, reversible

degeneration can be suppressed and prevented from progressing into irreversible necrosis. This finding is supported by Sayed et al. (2023), who reported that supplementation with proteins and amino acids in feed can repair histopathological lesions, including muscle degeneration and necrosis, in African catfish exposed to microplastics.

However, supplementation with 4% basil seed *simplicia* in the diet (T2) resulted in higher levels of muscle cell degeneration and necrosis compared with T1 (Table 2), with statistically significant differences ($P < 0.05$). This outcome may be influenced by the presence of tannins in basil seeds at relatively high concentrations, which can exert toxic effects on fish. Nazir & Wani (2021) reported that basil seeds contain total phenolic compounds of 18.24 ± 0.03 mg GAE/g, including tannins. Tannins act as antinutritional compounds because they can bind to proteins, thereby reducing the digestibility and absorption of protein from basil seeds in fish feed. Alif et al. (2021) reported that excessive tannin levels in feed can increase oxidative stress and damage fish organs. High concentrations of tannins in basil seeds may therefore lead to muscle cell damage, as indicated by increased degeneration and necrosis of muscle cells. This observation is supported by Wiranantha et al. (2019), who reported that long-term exposure to tannins at certain concentrations can induce fatty degeneration that may eventually progress to cell death (necrosis).

In addition, basil seeds are also known to contain saponin compounds. According to Marpaung and Romelan (2018), basil seeds contain saponin levels of approximately $3.11 \pm 0.0795\%$, indicating a relatively high saponin content. High concentrations of saponins can exert toxic effects on organisms that consume them. Alif et al. (2021) reported that saponins at high doses—particularly in feed treatments containing approximately $1.7\text{--}1.9$ mg kg⁻¹—can become toxic because they can cause hemolysis of hemoglobin, thereby impairing oxygen transport and ultimately leading to cell death. Consequently, muscle cells that initially experience oxidative stress may more readily progress to degeneration and subsequently to necrosis.

CONCLUSION

Based on the results of this study, it can be concluded that supplementation of basil seed (*Ocimum basilicum*) *simplicia* in the diet of African catfish (*Clarias gariepinus*) exposed to PS microplastics at a concentration of 100 mg/L for 15 days resulted in significant improvements in muscle histomorphometry and reduced muscle tissue damage. These effects were indicated by increased myofiber diameter and myofiber area, accompanied by decreased levels of muscle cell degeneration and necrosis. Dietary supplementation with 2% basil seed *simplicia* (T1) significantly increased myofiber diameter and area and reduced the degree of muscle cell degeneration and necrosis compared with the negative control group (NC) that was exposed only to microplastics. In contrast, supplementation with 4% basil seed *simplicia* (T2) showed lower effectiveness, which may be associated with the presence of tannin and saponin compounds at higher concentrations that potentially affect feed palatability.

RECOMMENDATION

Future research is recommended to focus on alternative processing methods for basil seeds, such as fermentation or solvent-based extraction using universal solvents, in order to enhance their nutritional quality and protective bioactivity. These approaches are expected to produce more effective and safe formulations for application as functional ingredients in fish feed.

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