EFFICACY OF DIETARY SUPPLEMENTATION OF AMBON BANANA MIDRIB SIMPLICIA TO ENHANCE GROWTH PERFORMANCE, HEMATOLOGICAL PROFILE, IMMUNITY, AND SURVIVAL OF CATFISH CHALLENGED WITH *Edwardsiella tarda*

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ABSTRACT

Catfish (*Clarias* sp.) is a significant aquaculture species, but its production is often limited by *Edwardsiella tarda* infections, which cause substantial losses. Plant-derived alternatives such as banana midrib simplicia offer promising solutions to enhance fish health and reduce antibiotic dependence. This study investigated the effects of Ambon banana (*Musa paradisiaca var. sapientum*) midrib simplicia on growth performance, hematological parameters, immune responses, and resistance to *E. tarda* infection in catfish. A completely randomized design was used with five treatments and three replications, consisting of a positive control, a negative control, and commercial feed supplemented with 2% (B2), 3% (B3), and 4% (B4) banana midrib simplicia. The feeding trial lasted 30 days, after which fish were intramuscularly injected with *E. tarda* (10^7 CFU mL^{-1}). Growth performance, hematological indicators (red blood cell count, hemoglobin concentration, hematocrit, and white blood cell count), immune responses (phagocytic and respiratory burst activities), and survival rate were evaluated. Dietary supplementation with banana midrib simplicia significantly improved growth performance, hematological parameters, immune responses, and survival in catfish challenged with *E. tarda* compared to the control groups. The optimal supplementation dose was 3%, providing the most significant improvement in growth, immunity, and survival. These findings highlight the potential of banana midrib as a functional feed additive derived from agricultural by-products to promote fish health and aquaculture productivity.

KEYWORDS: banana midrib; *Clarias* sp.; *Edwardsiella tarda;* growth performance; immune response

ABSTRAK:* *Efikasi Suplementasi Simplisia Pelepah Pisang Ambon pada Pakan untuk Meningkatkan Pertumbuhan, Gambaran Darah, Imunitas, dan Kelangsungan Hidup Ikan Lele yang Diinfeksi* *Edwardsiella tarda

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*Ikan lele (**Clarias** sp.) merupakan salah satu komoditas penting dalam akuakultur, namun produksinya sering terkendala oleh infeksi **Edwardsiella tarda** yang menyebabkan kerugian signifikan. Alternatif berbasis tanaman seperti simplisia pelepah pisang menawarkan solusi potensial untuk meningkatkan kesehatan ikan dan mengurangi ketergantungan terhadap antibiotik. Penelitian ini mengevaluasi pengaruh simplisia pelepah pisang Ambon (**Musa paradisiaca var. sapientum**) terhadap kinerja pertumbuhan, parameter hematologis, respons imun, dan ketahanan terhadap infeksi **E. tarda** pada ikan lele. Penelitian ini menggunakan rancangan acak lengkap dengan lima perlakuan dan tiga ulangan, terdiri atas kontrol positif, kontrol negatif, serta pakan komersial yang disuplementasi simplisia pelepah pisang pada dosis 2% (B2), 3% (B3), dan 4% (B4). Uji pemberian pakan dilakukan selama 30 hari, kemudian ikan diinjeksi intramuskular dengan **E. tarda** (10^7 CFU mL^{-1}). Parameter yang diamati meliputi pertumbuhan, indikator hematologis (jumlah eritrosit, konsentrasi hemoglobin, hematokrit, dan jumlah leukosit), respons imun (aktivitas fagositosis dan respiratory burst), serta tingkat kelangsungan hidup. Suplementasi pakan dengan simplisia pelepah pisang secara signifikan meningkatkan performa pertumbuhan, parameter hematologis, respons imun, dan kelangsungan hidup ikan lele yang diinfeksi **E. tarda** dibanding kelompok kontrol. Dosis optimal diperoleh pada suplementasi 3%, yang memberikan peningkatan terbesar pada pertumbuhan, imunitas, dan kelangsungan hidup. Temuan ini menunjukkan bahwa simplisia pelepah pisang memiliki potensi sebagai aditif pakan fungsional berbasis limbah pertanian untuk meningkatkan kesehatan ikan dan produktivitas akuakultur.*

KATA KUNCI: *Clarias **sp.**; Edwardsiella tarda; **kinerja pertumbuhan**; **pelepah pisang**; **respons imun***

INTRODUCTION

Catfish (*Clarias* sp.) is one of the most important aquaculture species due to its rapid growth, tolerance to environmental fluctuations, and high market demand (Abdel-Hay *et al*., 2019; Barasa & Ouma, 2024). Intensive farming systems of catfish have expanded rapidly; however, their sustainability is often threatened by infectious diseases (Mukaila *et al*., 2023). Among these, edwardsiellosis is considered one of the most serious bacterial diseases, causing mortality rates of up to 60–100% and annual economic losses estimated at USD 15.5–45.9 million (Adikesavalu *et al*., 2016; Kumar *et al*., 2024).

Edwardsiellosis is characterized by lethargy, abnormal swimming, buoyancy disorders, and anorexia. Externally, fish may exhibit exophthalmia, abdominal distension, hemorrhagic spots on the skin and fins, and skin ulcers. Internally, typical findings include serosanguinous fluid in the body cavity, hepatosplenomegaly, and visceral hemorrhage or abscesses (Armwood *et al*., 2022). One of the major causative agents is *Edwardsiella tarda* (Algammal *et al*., 2022).

The use of antibiotics remains the primary strategy for controlling *Edwardsiella* bacterial infections (Batista *et al*., 2025). However, excessive and uncontrolled application has been linked to the emergence of resistant bacterial strains, environmental contamination, and residues in aquaculture products, raising serious concerns for public health and international trade (Chen *et al*., 2020a). Therefore, identifying sustainable and safe alternatives to antibiotics has become a global priority (Bondad-Reantaso *et al*., 2023).

Plant-derived phytopharmaceuticals containing bioactive compounds have emerged as promising candidates for fish health management (Goh *et al*., 2023). Previous studies have demonstrated that phytochemicals exhibit antimicrobial, antioxidant, and immunostimulatory properties (Ahmadi *et al*., 2022). Several plants have been tested in aquaculture species and have been found to

enhance immune responses, reduce pathogen loads, and improve survival rates after disease challenges (Dawood *et al*., 2022). In catfish, plant-based immunostimulants have been shown to have positive effects on growth performance, hematological parameters, immune response, and survival following bacterial infection (Adeshina *et al*., 2021).

Bananas are one of the most abundant tropical fruit crops, generating large amounts of agricultural waste, including peels, leaves, and pseudostems (Emmanuel *et al*., 2025). Recent studies revealed that banana by-products exhibit antibacterial properties against fish pathogens and can stimulate growth and immune parameters (Sulaiman *et al*., 2025). For instance, supplementation of banana pseudostem improved growth, health status, and immunity in Nile tilapia against *Aeromonas hydrophila* (Wahjuningrum *et al*., 2021), while banana peel enhanced growth and feed utilization in striped catfish (Agustina *et al*., 2024).

Among banana by-products, midribs are rich in phytochemicals such as alkaloids, flavonoids, triterpenoids, steroids, tannins, phenolics, and glycosides (Wahjuningrum *et al*., 2021). These compounds are known to improve growth, stimulate immune responses, and enhance disease resistance in fish (Sumana *et al*., 2025). In giant gourami, banana midrib supplementation enhanced hematological profiles, immune response, and resistance to *A. hydrophila* (Wahjuningrum *et al*., 2021). Nevertheless, the use of banana midrib as a feed supplement in catfish culture to prevent edwardsiellosis has rarely been investigated. Therefore, this study evaluates the effects of Ambon banana (*Musa paradisiaca var. sapientum*) midrib simplicia on growth performance, hematological parameters, immune response, and resistance to *E. tarda* infection in catfish.

MATERIALS AND METHODS

Study Site and Duration

The experiment was conducted from December 2024 to February 2025 at the Laboratory of Aquatic Organism Health, Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University, Bogor, Indonesia.

Experimental Design

A completely randomized design was applied with five treatments and three replications (Table 1). The treatments consisted of a positive control (CP): fed with commercial diet and challenged with *E. tarda*, a negative control (CN): fed with commercial diet and injected with phosphate-buffered saline (PBS), and three experimental diets supplemented with 2% (B2), 3% (B3), and 4% (B4) banana midrib simplicia, followed by *E. tarda* challenge.

Preparation of Banana Midrib Simplicia

The midribs used were obtained from Ambon banana tree planted in Pelabuhan Ratu, West Java, Indonesia. The preparation followed the method described by Wahjuningrum *et al*. (2021). The midribs were washed thoroughly

Table 1. Experimental design of Ambon banana midrib simplicia dietary supplementation to prevent *E. tarda* infection in catfish culture

Treatment	Description
CP	Commercial diet + bacterial challenge
CN	Commercial diet + phosphate-buffered saline injection
B2	Commercial diet + 2% banana midrib simplicia + *E. tarda* challenge
B3	Commercial diet + 3% banana midrib simplicia + *E. tarda* challenge
B4	Commercial diet + 4% banana midrib simplicia + *E. tarda* challenge

with running water, cut into small pieces, and shade-dried for seven days to avoid direct sunlight. They were then oven-dried at 50°C for 8 hours, ground into simplicia using a blender, and sieved through a 60-mesh filter to obtain a uniform particle size.

Feed Preparation

The experimental diets were prepared using a commercial catfish feed containing 31–33% crude protein. Banana midrib simplicia at levels of 2% (B2), 3% (B3), and 4% (B4) were coated onto the commercial feed following the methods of Abdullah *et al*. (2024). In addition, 2% egg white and 10% sterile distilled water were added as a binder solution. A total of 100 g of feed was mixed with the appropriate amounts of banana midrib simplicia, egg white, and sterile distilled water, then homogenized, coated, and air-dried. The prepared diets were stored in sterile containers and used during the feeding trials.

Edwardsiella tarda Preparation

E. tarda isolates were obtained from the Freshwater Aquaculture Development Center (*Balai Perikanan Budidaya Air Tawar*) Mandiangin, South Kalimantan, Indonesia. The bacteria were cultured on brain heart infusion agar (BHIA) at 28–30°C for 24 hours and subsequently inoculated into 10 mL of brain heart infusion broth (BHIB) on a shaker at 1,400 rpm for 24 hours. The bacterial suspension was centrifuged at 3,000 rpm for 5 minutes, after which the supernatant was discarded, and the pellet was washed twice with PBS. Pathogenicity was confirmed through intramuscular injection (0.1 mL per fish, 10^7 CFU mL^{-1}), re-isolation from infected tissues, and verification using the API 20E kit (bioMérieux, France). The identification confirmed *E. tarda* with 99.9% accuracy (Table 2). Pure isolates were maintained in BHIB for use in the challenge tests.

Determination of LD_{50}

The lethal dose 50 (LD_{50}) of *E. tarda* was determined following the method of Reed and Muench (1938). Catfish were intramuscularly injected with 0.1 mL of bacterial suspension at concentrations of 10^5, 10^6, 10^7, and 10^8 CFU mL^{-1}. Six fish were used per aquarium for each concentration. Mortality was observed over a seven-day period, and LD_{50} values were calculated based on mortality percentages using a logarithmic analysis. The LD_{50} value was determined to be 10^7 CFU mL^{-1}, which was subsequently used for the bacterial challenge test.

Rearing Containers

Fifteen glass aquaria (60 × 30 × 40 cm) were used, each filled with 45 L of freshwater (water depth 25 cm). The aquaria were cleaned with detergent and then thoroughly rinsed. Freshwater was then added and disinfected with 30 mg L^{-1} $CaOCl_2$, followed by aeration for 24 hours. Sodium thiosulfate at a concentration of 60 mg L^{-1} was added, and the water was re-aerated for another 24 hours to neutralize residual chlorine. Each aquarium was provided with continuous aeration as an oxygenation, and nets were placed above the aquaria to prevent fish from jumping. The freshwater used during the experiment had a temperature of 26.2–27.8°C, pH of 6.6–7.3, dissolved oxygen of 3.5–6.1 mg L^{-1}, and total ammonia nitrogen of 0.06–0.35 mg L^{-1}, in accordance with the rearing requirements specified in the Indonesian National Standard (SNI 6484.4:2014) (Badan Standardisasi Nasional, 2014).

Experimental Fish and Challenge Test

Catfish juveniles with an average length of 8.99 ± 0.08 cm and an average weight of 4.43 ± 0.46 g were obtained from a local farmer in Bogor, West Java, Indonesia. The fish were acclimatized for seven days in fiber tanks and then transferred to aquaria for the feeding

trial. Stocking density was maintained at 10 fish per aquarium, equivalent to 139 fish m^{-3}. The fish were fed to satiation three times daily (08:00, 12:00, and 17:00). The feeding trial lasted for 30 days. Feces and uneaten feed were siphoned every three days to maintain quality. Fish length and weight were measured at the beginning and at the end of the feeding trial period.

After the 30-day feeding trial, fish were intramuscularly injected with an *E. tarda* suspension at a concentration of 10^7 CFU mL^{-1}, with 0.1 mL administered per fish. Following the infection, the fish in all aquaria were fed a commercial diet without any supplementation for 10 days. The fish were fed to satiation three times daily (08:00, 12:00, and 17:00). The water in the aquaria was not replaced during the challenge test period, but continuous aeration was provided.

Blood Sampling Procedure

Blood samples were collected from the caudal vein on days 0, 30, 32, 37, and 40. On day 0, blood was taken from the stock fish prior to distribution into the experimental aquaria. Before sampling, the fish were anesthetized to minimize stress. For each sampling time (except day 0), 2–3 fish were taken from each aquarium. Blood was drawn using a 0.5-mL syringe containing EDTA as an anticoagulant. After sampling, the fish were returned to their respective aquaria for further observation.

Table 2. Biochemical characteristics of *E. tarda* isolate based on bacterial identification using API 20E kit

Biochemical characteristics	**Result**
ONPG (β-galactosidase)	+
Arginine Dihydrolase (ADH)	+
Lysine Decarboxylase (LDC)	+
Ornithine Decarboxylase (ODC)	-
Citrate Utilization (CIT)	+
H_2S Production (H_2S)	+
Urease (URE)	-
Tryptophan Deaminase (TDA)	-
Indole Production (IND)	+
Voges-Proskauer (VP)	-
Gelatin Hydrolysis (GEL)	+
Glucose Fermentation (GLU)	+
Mannitol Fermentation (MAN)	-
Inositol Fermentation (INO)	-
Sorbitol Fermentation (SOR)	-
Rhamnose Fermentation (RHA)	-
Sucrose Fermentation (SAC)	-
Melibiose Fermentation (MEL)	-
Amygdalin Fermentation (AMY)	-
Arabinose Fermentation (ARA)	-
Oxidase (OX)	-
Result	***E. tarda* with a percentage of 99.9%**

Observation of Growth Performance

Growth performance was evaluated based on weight gain (ΔW), specific growth rate (SGR), and feed conversion ratio (FCR) during the 30-day rearing period. The parameters were calculated using the equations (1) to (3) (Ahmed & Ahmad, 2020; Mohammadiazarm *et al*., 2023):

$$\Delta W(g) = \text{Final weight (g)} - \text{Initial weight (g)} \quad \text{.....(1)}$$

$$SGR\ (\%\ day^{-1}) = \left(\frac{\text{ln Final weight (g)} - \text{ln Initial weight (g)}}{\text{Rearing period (days)}}\right) \text{x}100 \text{.....(2)}$$

$$\text{FCR} = \frac{\text{Total feed given (g)}}{\text{Weight gain (g)}} \text{.....(3)}$$

Observation of Hematological Profile

Hematological indices were determined by measuring red blood cell count (RBC), hemoglobin (Hb), hematocrit (Hc), and white blood cell count (WBC) during the feeding trial period and after the challenge test (post-injection). The RBC was measured following the method of Blaxhall and Daisley (1973). Blood was collected using a pipette containing a red mixing bead and drawn to the 1.0 mark. Hayem's solution was then added to the pipette up to the 101 mark. The number of RBC was counted using a hemocytometer under a light microscope at 400× magnification. The RBC count was calculated using the equation (4):

$$RBC\ (cells\ mm^{-3}) = \left(\frac{\sum \text{red blood cells}}{\sum \text{counted squares}}\right) \text{x } 25 \text{ x } \left(\frac{\text{Dilution factor}}{\text{Volume of large square}}\right) \text{(4)}$$

Hemoglobin concentration was measured using the Sahli method (Wedemeyer & Yasutake, 1977). Blood was drawn with a Sahli pipette up to the 0.2 mL mark and transferred into a hemoglobinometer tube pre-filled with 0.1 N HCl solution up to the 10 (red) mark. The sample was mixed using a glass rod for 3–5 minutes. Distilled water was then added drop by drop until the color of the blood sample matched the standard color on the hemoglobinometer. Hemoglobin concentration was expressed in g%, representing grams of hemoglobin per 100 mL of blood.

Hematocrit values were determined using the method of Anderson and Siwicki (1995). Blood samples were filled up to three-quarters of the length of a microhematocrit capillary tube, and the lower end was sealed with crystoceal wax. The tubes were centrifuged at 5,000 rpm for 5 minutes. Hematocrit was calculated as the ratio between the length of packed RBC and the total length of the blood column in the capillary tube using the equation (5):

$$Hc\ (\%) = \left(\frac{\text{Length of packed red blood cells}}{\text{Total blood column length in capillary tube}}\right) \text{x}100 \text{ .....(5)}$$

The WBC was measured following the method of Blaxhall and Daisley (1973). Blood was collected with a Sahli pipette up to the 0.5 mark and mixed with Turk's solution up to the 11 mark. The mixture was homogenized for 3–5 minutes, and the first one to two drops were discarded before loading the hemocytometer. The number of WBC was counted under a light microscope at 400× magnification. The WBC count was calculated using the equation (6):

$$WBC\ (cells\ mm^{-3}) = \left(\frac{\sum \text{White blood cells}}{\sum \text{counted squares}}\right) \text{x } 25 \text{ x } \left(\frac{\text{Dilution factor}}{\text{Volume of large square}}\right) \text{(6)}$$

Observation of Immune Responses

Immune responses were assessed through phagocytic activity (PA) and respiratory burst activity (RB) during the rearing period and after the challenge test (post-injection), following the method of Anderson and Siwicki (1995). For the determination of phagocytic activity, 50 μL of blood was placed in a microtube and mixed with 50 μL of *Staphylococcus aureus* suspension (10^7 CFU mL^{-1}), then homogenized. The mixture was incubated at 28°C for 20 minutes. After incubation, 10 μL of the blood-bacteria mixture was placed on a glass slide

and smeared at a 45° angle using another slide. The smears were air-dried, fixed in absolute methanol for 5 minutes, air-dried again, and then stained with Giemsa solution for 15–30 minutes. The slides were rinsed with distilled water, air-dried, and observed under a light microscope at 400× magnification. Phagocytic activity (PA) was calculated as the percentage of phagocytic cells relative to the total observed phagocytes using equation (7):

$$PA\ (\%) = \left(\frac{Number\ of\ phagocytic\ cells}{Total\ phagocytes}\right) x100 \quad .....(7)$$

Respiratory burst activity was determined using the method described by Anderson and Siwicki (1995). Blood samples (50 μL) were dispensed into a microplate and incubated for 1 hour at 37°C. The supernatant was discarded, and the wells were washed three times with 100 μL of PBS (pH 7.4). Then, 50 μL of 0.2% nitroblue tetrazolium (NBT) solution was added to each well, followed by incubation for 1 hour at 37°C. After incubation, the NBT solution was discarded, and the wells were fixed with 100% methanol for 30 minutes. The methanol was discarded, and the wells were rinsed three times with 100 μL of 30% methanol, with each rinse allowed to stand for 2.5 minutes. Subsequently, 60 μL of 2N KOH and 70 μL of dimethyl sulfoxide (DMSO) were added. The optical density (OD) was measured using a microplate reader (Kayto RT–2100C) at a wavelength of 630 nm.

Observation of Survival Rate

The survival parameter was represented by the survival rate (SR), which was determined during the feeding trial period and after the challenge test (post-injection). The survival rate was calculated using equation (8) (Amoah *et al*., 2021):

$$SR\ (\%) = \left(\frac{Final\ number\ of\ live\ fish}{Initial\ number\ of\ fish}\right) x100 \quad .....(8)$$

Ethical Clearance Statement

All experimental procedures and animal maintenance were conducted in accordance with the guidelines for catfish production, as outlined in SNI 6484.2:2014 (Badan Standardisasi Nasional, 2014).

Data Analysis

Data were analyzed using one-way analysis of variance (ANOVA) at a 95% confidence level. When significant differences ($p < 0.05$) were detected, Duncan's multiple range test was performed. Data were processed using Microsoft Excel and SPSS software version 26.0.

RESULTS AND DISCUSSION

Growth Performance

Banana-based feed ingredients have been demonstrated to improve growth performance in several aquaculture species (Mapanao *et al*., 2022). In *Labeo rohita*, supplementation with banana peel flour enhanced body weight gain, specific growth rate (SGR), and feed efficiency (Giri *et al*., 2016). Similarly, in common carp, diets containing banana peel flour improved absolute weight gain, SGR, feed efficiency, and survival rate (Melanie *et al*., 2024). In Nile tilapia, supplementation with banana flower flour also promoted growth and SGR (Phinyo *et al*., 2024).

In the present study, dietary supplementation with banana midrib simplicia significantly improved the growth performance of catfish. Final weight (Wt), weight gain (ΔW), and SGR were significantly higher ($p < 0.05$) in fish fed diets containing 2–4% supplementation compared to the control groups (CN and CP) (Table 3). Furthermore, the feed conversion ratio (FCR) was significantly lower ($p < 0.05$) in B2, B3, and B4, indicating improved feed utilization efficiency. The growth-promoting effect of banana midrib supplementation may be explained by its bioactive composition. Banana pseudostems, including the midrib,

are rich in cellulose, hemicellulose, and lignin (Díaz *et al*., 2023), which act as prebiotics to support gut health and nutrient absorption (Liu *et al*., 2021; Yossa *et al*., 2018).

In addition, banana midrib contains phytochemicals such as alkaloids, flavonoids, triterpenoids, steroids, tannins, phenolics, and glycosides (Wahjuningrum *et al*., 2021). These compounds are known to enhance growth hormone (GH) and insulin-like growth factor I (IGF-I) concentrations, thereby stimulating protein synthesis and accelerating somatic growth (Chakraborty *et al*., 2014). Together, these mechanisms likely explain the enhanced feed efficiency and growth performance observed in B2–B4 groups. Overall, this study demonstrates that banana midrib simplicia supplementation significantly improves catfish growth performance, consistent with previous findings in hybrid grouper and Nile tilapia (Wahjuningrum *et al*., 2022; Wahjuningrum *et al*., 2025).

Hematological Profile

Hematological parameters are recognized as key indicators of the physiological and immunological status of fish under both normal and stress conditions (Ahmed *et al*., 2020; Chen & Luo, 2023). Plant-based diets have been reported to stimulate hematopoiesis, reflected by increased red blood cell (RBC) counts, hemoglobin (Hb) concentration, hematocrit (Hc), and erythrocyte indices in various aquaculture species (Abdullah *et al*., 2024; Wahjuningrum *et al*., 2024). Similarly, banana-derived products, whether in their natural form or as part of formulated diets, have shown positive effects on hematological profiles, as seen in banana peel flour in Nile tilapia and banana flesh diets in hybrid Nile tilapia (Karaket *et al*., 2021; Susanto & Agustina, 2023).

In the present study, supplementation of banana midrib simplicia significantly improved the hematological profile of catfish (Figure 1). On day 30, treatments B2, B3, and B4 exhibited significantly higher RBC, Hb, and Hc values ($p < 0.05$) compared to the control groups. Following bacterial challenge (day 32–40), the control groups showed a sharp decline in RBC and Hb, while B2, B3, and B4 maintained relatively higher levels. Conversely, WBC counts increased markedly in the control groups, whereas in the supplemented groups, the increase was more regulated, with B3 showing the most balanced immune response.

These improvements may be attributed to the essential mineral content and prebiotic properties of the banana midrib. Banana stems are rich in iron (Fe), zinc (Zn), and copper (Cu) (Ho *et al*., 2012; Liyadipitiya *et al*., 2025; Zou *et al*., 2022), which are crucial for hematopoiesis (Takahashi, 2022). Additionally, banana-derived products function as prebiotics, stabilizing the intestinal microbiota (Maqsood *et al*., 2025),

Table 3. Growth performance of catfish fed diets supplemented with banana midrib simplicia for 30 days

Variable	CP	CN	B2	B3	B4
W0 (g)	4.18 ± 0.21^{a}	4.17 ± 0.21^{a}	4.57 ± 0.20^{a}	4.61 ± 0.17^{a}	4.62 ± 0.13^{a}
Wt (g)	9.61 ± 1.10^{a}	9.83 ± 1.69^{a}	13.62 ± 1.28^{b}	14.83 ± 0.33^{b}	14.50 ± 0.26^{b}
ΔW (g)	5.43 ± 1.12^{a}	5.66 ± 1.70^{a}	9.05 ± 1.30^{b}	10.22 ± 0.37^{b}	9.88 ± 0.30^{b}
SGR (% day^{-1})	2.76 ± 0.53^{a}	2.83 ± 0.53^{a}	3.63 ± 0.25^{b}	3.90 ± 0.15^{b}	3.81 ± 0.11^{b}
FCR	1.51 ± 0.02^{b}	1.49 ± 0.02^{b}	1.38 ± 0.03^{a}	1.35 ± 0.03^{a}	1.37 ± 0.03^{a}

Data are expressed as mean ± SD (n=3). Different superscripts within a row indicate significant differences ($p < 0.05$). CP: Commercial diet + bacterial challenge; CN: Commercial diet + PBS injection; B2, B3, B4: Commercial diet supplemented with 2%, 3%, and 4% banana midrib simplicia, respectively + bacterial challenge.

which in turn improves nutrient absorption and supports blood cell formation (Li *et al*., 2024). These mechanisms likely explain the enhanced hematological profile observed in B2–B4 groups during the feeding trial period.

During the bacterial challenge, reductions in RBC, Hb, and Hc, were primarily associated with *E. tarda* virulence factors, particularly hemolysins and siderophores. Hemolysins contribute to erythrocyte lysis (Hassan *et al*., 2020), while siderophores scavenge iron from host hemoglobin (Khasheii *et al*., 2021). Consequently, declines in RBC, Hb, and Hc were confirmed across infected groups (CP, B2, B3, and B4). Meanwhile, WBC counts increased post-infection, reflecting innate immune activation in response to pathogenic stress (Chen *et al*., 2020b). As central players in host defense, WBCs combat infection, modulate immune responses, and maintain homeostasis (Sayyaf Dezfuli *et al*., 2023). This study confirms that banana midrib simplicia enhances hematological parameters in catfish, which were significantly higher compared with the negative control (CN) that was not exposed to pathogens. These results align with the findings of Kurniawati *et al*. (2025), who reported similar hematological changes in catfish challenged with *E. tarda*.

Immune Responses

Immune responses serve as critical indicators of host defense mechanisms against pathogenic infections. Banana-derived products have previously been reported to enhance immune responses in aquaculture species (Dawood *et al*., 2022). For instance, dietary supplementation of *L. rohita* with banana peel flour improved phagocytic activity and immunoglobulin levels (Giri *et al*., 2016), while giant freshwater prawn fed banana peel hot-water extracts exhibited enhanced respiratory burst, phenoloxidase, and phagocytic activities (Rattanavichai & Cheng, 2015).

In the present study, supplementation of

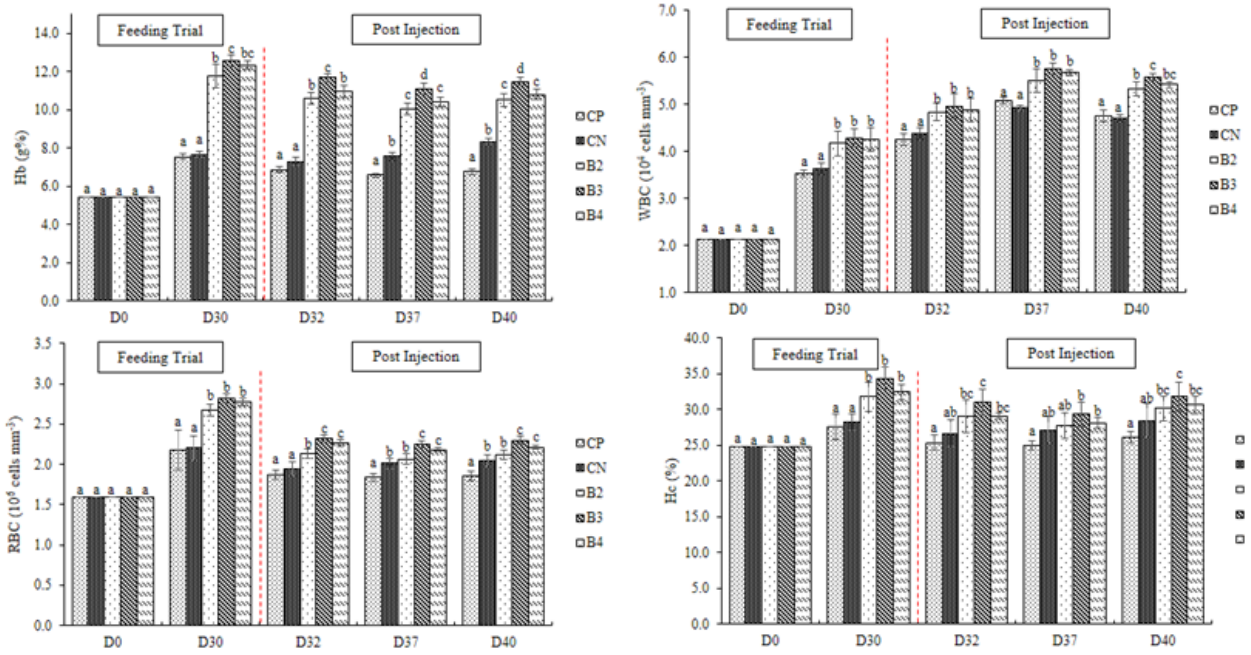

Figure 1. Red blood cell count (RBC), hemoglobin (Hb), hematocrit (Hc), and white blood cell count (WBC) of catfish treated with dietary supplementation of banana midrib simplicial to prevent *E. tarda* infection on days 0, 30, 32, 37, and 40. Different lowercase letters above the bars within the same day indicate significantly different results among treatments (Duncan $p < 0.05$)

banana midrib simplicia significantly enhanced the non-specific immune responses of catfish (Figure 2). At the start of rearing period (D0), phagocytic and respiratory burst activities did not differ among treatments ($p > 0.05$). By days 30 and 32, phagocytic activity in CP was comparable to CN ($p > 0.05$) but significantly lower than B2, B3, and B4 ($p < 0.05$). On days 37 and 40, phagocytic activity in B2, B3, and B4 remained significantly higher compared with CP and CN. A similar trend was observed for respiratory burst activity, where no differences were detected at D0; however, B2, B3, and B4 exhibited significant increases from D30 onward relative to CP and CN ($p < 0.05$). These enhancements persisted from D37 to D40, with B2, B3 and B4 showing the highest responses, significantly exceeding those of CP and CN ($p < 0.05$).

The improved immune responses appear closely associated with elevated WBC counts. Bananas contain diverse bioactive compounds and prebiotic components that support gut health (Liyadipitiya *et al*., 2025; Maqsood *et al*., 2025), thereby promoting hematopoiesis and increasing WBC production. As central components of host defense, WBCs are strongly linked to phagocytic and respiratory burst activities (Wahjuningrum *et al*., 2025), explaining the enhanced responses observed in catfish fed banana midrib simplicia. Additionally, banana midrib is rich in phytochemicals that further strengthen immune functions by stimulating phagocytosis and respiratory burst in fish (Ahmadi *et al*., 2022).

Upon pathogen challenge, host defense mechanisms were activated primarily through neutrophil- and macrophage-mediated phagocytosis (Speirs *et al*., 2024). This process involves a cascade of biochemical reactions, including the generation of reactive oxygen species (ROS) via respiratory burst (Zhu & Su, 2022). ROS contributes to pathogen elimination by damaging microbial cell membranes (Andrés *et al*., 2022). Elevated phagocytic and respiratory burst activities in the banana midrib groups, therefore, represent key indicators of immune competence against infection (Harikrishnan *et al*., 2020). These findings align with those of Bera *et al*. (2020), who observed similar immune enhancements in striped catfish challenged with *E. tarda*. To our knowledge, this study provides the first evidence that banana midrib simplicia can enhance non-specific immune responses in catfish.

Survival of Catfish

In the present study, supplementation with banana midrib simplicia significantly enhanced the survival of catfish (Figure 3). Survival during the rearing period was 100% across all treatments ($p > 0.05$). Mortality occurred only

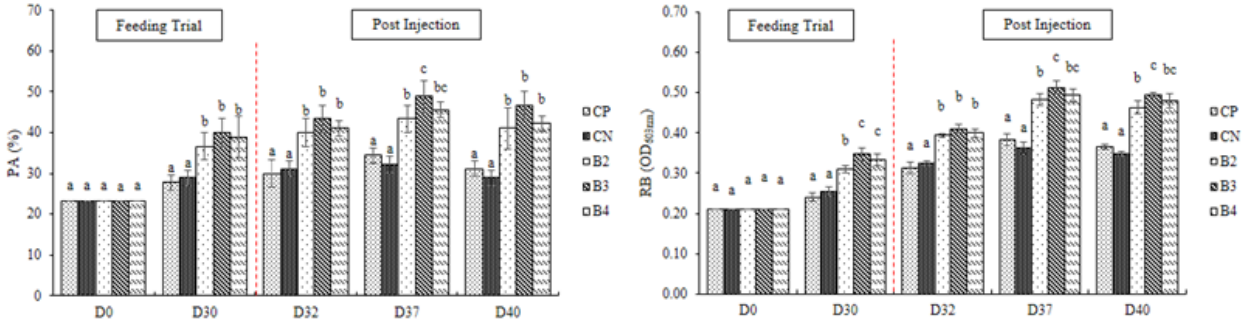

Figure 2. Phagocytic activity (PA) and respiratory burst activity (RB) of catfish treated with dietary supplementation of banana midrib simplicial to prevent *E. tarda* infection on days 0, 30, 32, 37, and 40. Different lowercase letters above the bars within the same day indicate significantly different results among treatments (Duncan $p<0.05$)

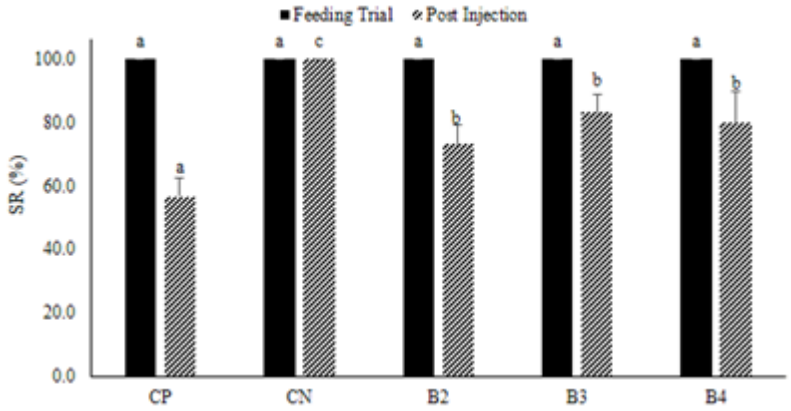

Figure 3. Survival rate (SR) of catfish treated with dietary supplementation of banana midrib simplicia to prevent *E. tarda* infection. Different lowercase letters above the bars within the same period indicate significantly different results among treatments (Duncan $p<0.05$)

after the challenge test against *E. tarda*, with significant differences among treatments ($p < 0.05$). The negative control (CN) maintained the highest survival rate at 100%, while the positive control (CP) had the lowest at 56.7%. Treatments B2, B3, and B4 showed higher survival rates, with B2 reaching 73.3%, B3 reaching 83.3%, and B4 reaching 80.0%, all of which were higher than CP.

Interestingly, *E. tarda* infection has been reported to cause mortality rates of 60–100% in catfish (Adikesavalu *et al*., 2016). The protective effect of banana midrib supplementation can be attributed to its combined immunostimulant, prebiotic, and antioxidant properties. Phytochemicals such as flavonoids, phenolics, and tannins enhance immune defense by stimulating phagocytosis and respiratory burst activity, thereby increasing resistance to infection (Ahmadi *et al*., 2022). Meanwhile, structural fibers (cellulose, hemicellulose, and lignin) function as prebiotics that support gut health and nutrient absorption (Liyadipitiya *et al*., 2025; Maqsood *et al*., 2025). These synergistic effects strengthen host resilience, reduce mortality risk, and ultimately improve fish survival, consistent with findings in gourami supplemented with banana midrib against *A. hydrophila* (Wahjuningrum *et al*., 2021). These findings highlight banana midrib simplicia as a promising natural feed additive to improve disease resistance and survival in catfish culture.

CONCLUSIONS

Dietary supplementation with banana midrib simplicia enhanced growth performance, hematological parameters, immune response, and survival of catfish challenged with *E. tarda* compared to the control. The best outcome was obtained at a supplementation level of 3%. These findings demonstrate the strong potential of banana midrib as a functional feed ingredient derived from agricultural by-products to promote fish health and aquaculture productivity.

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AUTHOR CONTRIBUTION

DW: conceptualization, methodology, supervision, project administration, writing-review, and editing. RN: data curation, investigation, and resources. MY: conceptualization, methodology, supervision, project administration, writing-review, and editing. TC: conceptualization, methodology, supervision, and project administration. TA: formal analysis, validation, visualization, writing-original draft, writing-review, and editing.

DECLARATION OF COMPETING INTEREST AND USE OF GENERATIVE AI

The authors declare no competing interests. The authors did not use generative AI or AI-assisted technologies for writing or editing this manuscript beyond standard spelling and grammar checking.

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