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EFFICACY OF AMBON BANANA MIDRIB SIMPLICIA ON GROWTH PERFORMANCE, HEMATOLOGICAL, IMMUNITY, AND SURVIVAL OF CATFISH CHALLENGED WITH *Edwardsiella tarda*

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ABSTRACT

Catfish (*Clarias* sp.) is an important aquaculture species, but its production is often constrained by *Edwardsiella tarda* infections that cause significant 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 midrib simplicia on growth performance, hematological parameters, immune responses, and resistance to *E. tarda* infection in catfish. A completely randomized design was applied with five treatments and three replications, consisting of a positive control, a negative control, and diets 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, hemoglobin, hematocrit, dan white blood cell), immune responses (phagocytic and respiratory burst activity), 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 controls. The optimal supplementation dose was 3%, providing the greatest 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 strengthen fish resistance against edwardsiellosis and support sustainable aquaculture.

KEYWORDS: Banana Midrib; *Clarias* sp.; *Edwardsiella tarda;* Growth Performance; Immune Response.

ABSTRAK:

Ikan lele (Clarias sp.) merupakan salah satu spesies 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 sekaligus mengurangi ketergantungan terhadap antibiotik. Penelitian ini bertujuan untuk mengkaji pengaruh simplisia pelepah pisang Ambon terhadap performa pertumbuhan, parameter hematologi, respons imun, dan ketahanan terhadap infeksi E. tarda pada lele. Rancangan acak lengkap digunakan dengan lima perlakuan dan tiga ulangan, terdiri atas kontrol positif, kontrol negatif, serta pakan uji yang diperkaya dengan 2% (B2), 3% (B3), dan 4% (B4) simplisia pelepah pisang. Uji pemberian pakan berlangsung selama 30 hari, kemudian ikan disuntik secara intramuskular dengan E. tarda (10^7 CFU mL^{-1}). Parameter yang diamati meliputi performa pertumbuhan, indikator hematologi (eritrosit, hemoglobin, hamatokrit, dan leukosit), respons imun (aktivitas fagositik dan respiratory burst), serta tingkat kelangsungan hidup. Suplementasi pakan dengan simplisia pelepah pisang terbukti secara signifikan meningkatkan performa pertumbuhan, parameter hematologi, respons imun, dan kelangsungan hidup lele yang diuji tantang dengan E. tarda dibandingkan kontrol. Dosis suplementasi optimal diperoleh pada level 3%, yang

memberikan peningkatan terbesar pada pertumbuhan, imunitas, dan kelangsungan hidup. Temuan ini menegaskan potensi pelepah pisang sebagai aditif pakan fungsional berbasis limbah pertanian untuk memperkuat ketahanan lele terhadap edwardsiellosis dan mendukung akuakultur berkelanjutan.

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 up to 60–100% mortality and economic losses estimated at USD 15.5–45.9 million annually (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, common 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* 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

shown that phytochemicals possess antimicrobial, antioxidant, and immunostimulant properties (Ahmadi *et al*., 2022). Several plants have been tested in aquaculture species and were found to enhance immune responses, reduce pathogen loads, and improve survival rates after disease challenge (Dawood *et al*., 2022). In catfish, plant-based immunostimulants have demonstrated positive effects on growth performance, hematological parameters, immune response, and survival following bacterial infection (Adeshina *et al*., 2021).

Banana is one of the most abundant tropical fruit crops, generating large amounts of agricultural waste such as 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, midrib 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 application of banana midrib as a feed supplement in catfish culture for the prevention of edwardsiellosis has rarely been investigated. Therefore, this study evaluates the effects of Ambon banana midrib simplicia on growth performance, hematological parameters, immune response, and resistance to *Edwardsiella 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 (CRD) 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 *Edwardsiella tarda*, 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 and then followed by *Edwardsiella tarda* challenge.

Table 1. Experimental design of Ambon banana midrib simplicia application in catfish feed.

Treatment	Description
CP	Commercial diet + bacterial challenge
CN	Commercial diet + PBS injection
B2	Commercial diet + 2% banana midrib simplicia + *Edwardsiella tarda* challenge
B3	Commercial diet + 3% banana midrib simplicia + *Edwardsiella tarda* challenge
B4	Commercial diet + 4% banana midrib simplicia + *Edwardsiella tarda* challenge

Preparation of Banana Midrib Simplicia

The midribs used were obtained from Ambon banana (*Musa paradisiaca* var. *sapientum*) in Pelabuhan Ratu, West Java, Indonesia. The preparation followed the method described by Wahjuningrum *et al*. (2021). The midribs were washed thoroughly 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) was 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 binders. 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**

Edwardsiella tarda isolates were obtained from the Freshwater Aquaculture Development Center (Balai Perikanan Budidaya Air Tawar, BPBAT) 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 1400 rpm for 24 hours. The bacterial suspension was centrifuged at 3000 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}), reisolation from infected tissues, and verification using the API 20E KIT. Identification with the API 20E kit confirmed *E. tarda* with 99.9% accuracy (Table 2). Pure isolates were maintained in BHIB for use in the challenge tests.

Table 2. Biochemical characteristics API 20 E of the *Edwardsiella tarda*

Biochemical characteristics	Isolated
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)	+

Biochemical characteristics	Isolated
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	***Edwardsiella tarda* with a percentage of 99.9%**

Determination of LD_{50}

The lethal dose (LD_{50}) of *Edwardsiella 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 for seven days, and LD_{50} values were calculated based on mortality percentages using 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 rinsed thoroughly. 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 oxygen source, 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 (TAN) of 0.06–0.35 mg L^{-1}, in accordance with the rearing requirements specified in the Indonesian National Standard (SNI 6484.4:2014).

Experimental Fish and Challenge Test

Catfish (*Clarias* sp.) 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. The fish were acclimatized for seven days in fiber tanks and fed to satiation three times daily. Stocking density was maintained at 10 fish per aquarium, equivalent to 0.28 fish L^{-1}. The feeding trial lasted for 30 days, after which a bacterial challenge was conducted. Fish length and weight were measured at the beginning and at the end of the feeding period. Water was siphoned every three days to maintain quality. 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 were fed a commercial diet without supplementation for a period of 10 days.

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 following formulas (Ahmed & Ahmad, 2020; Mohammadiazarm *et al.*, 2023):

$$\Delta W\ (g) = Final\ weight\ (g) - Initial\ weight\ (g)$$

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

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

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 rearing 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 following formula:

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

Hemoglobin concentration was measured using the Sahli method (Wedemeyer and Yatsuke, 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 (1993). 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 5000 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 following formula:

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

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 following formula:

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

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 (1993). 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 was calculated as the percentage of phagocytic cells relative to the total observed phagocytes using the following formula:

$$PA\ (\%) = \left(\frac{Number\ of\ phagocytic\ cells}{Total\ phagocytes}\right) \times 100$$

Respiratory burst activity was determined according to the method of Anderson and Siwicki (1993). 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 phosphate-buffered saline (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 rearing period and after the challenge test (post-injection). The survival rate was calculated using the following formula (Amoah *et al*., 2021):

$$SR\ (\%) = \left(\frac{Final\ number\ of\ live\ fish}{Initial\ number\ of\ fish}\right) \times 100$$

Ethical Clearance Statement

All experimental procedures and animal maintenance were conducted in accordance with the guidelines for catfish production based on SNI 01-7246-2006 (Badan Standardisasi Nasional, 2006).

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*., 2021). 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, banana flower flour supplementation 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 (Yossa *et al*., 2018; Liu *et al*., 2021).

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).

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

Parameters	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. 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 powder, respectively + bacterial challenge.

Hematological

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 (Ht), and erythrocyte indices in various aquaculture species (Abdullah *et al*., 2024; Wahjuningrum *et al*., 2024). Similarly, banana-derived products, either in adaptive forms or formulated diets, have shown positive effects on hematological profiles, such as 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 Ht values ($p < 0.05$) compared to the control. Following bacterial challenge (day 32–40), the control group 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 group, 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 banana midrib. Banana stems are rich in iron (Fe), zinc (Zn), and copper (Cu) (Ho *et al*., 2012; Zou *et al*., 2022; Liyadipitiya *et al*., 2025), which are crucial for hematopoiesis (Takahashi, 2022). In addition, banana-derived products act as prebiotics that stabilize intestinal microbiota (Maqsood *et al*., 2025), thereby improving nutrient absorption and supporting blood cell formation (Li *et al*., 2024). These mechanisms likely explain the enhanced hematological profile observed in B2–B4 groups during the rearing period.

During the bacterial challenge, reductions in RBC, Hb, and Ht were primarily associated with *Edwardsiella 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 Ht 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*.

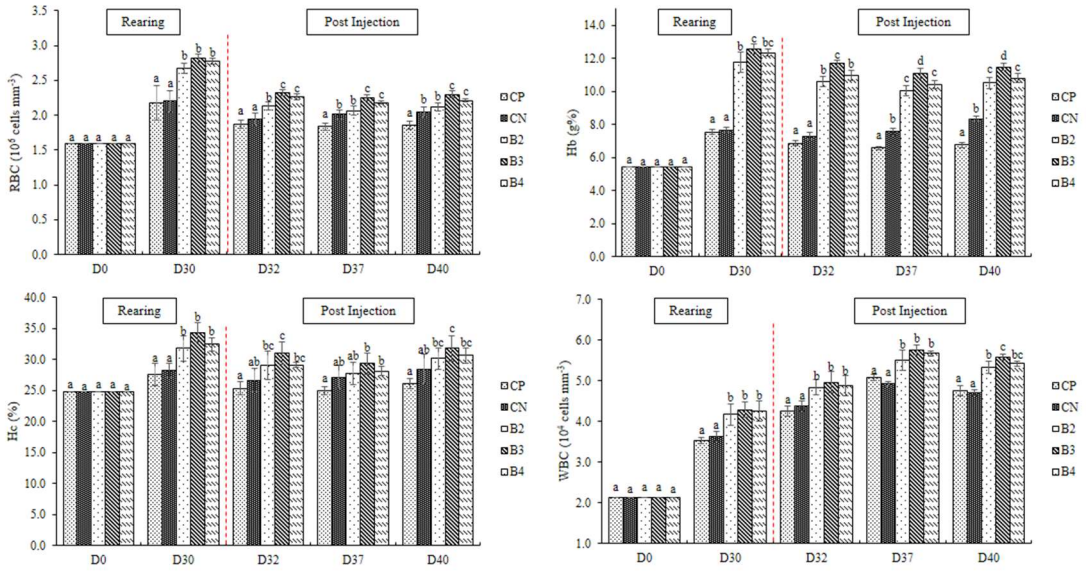

Figure 1. Red blood cell count (RBC), hemoglobin (Hb), hematocrit (Hc), and white blood cell count (WBC) of catfish on days 0, 30, 32, 37 and 40 after challenge with *Edwardsiella tarda*. Different uppercase letters in the same row indicate significantly different results between treatments (Duncan $p<0.05$).

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 *Labeo 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 banana midrib simplicia significantly enhanced the non-specific immune responses of catfish (Figure 2). At the start of rearing (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 until D37–D40, with B2 and B4 showing the highest responses, significantly exceeding CP, CN, and B3 ($p < 0.05$).

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 contribute 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 are in line with 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.

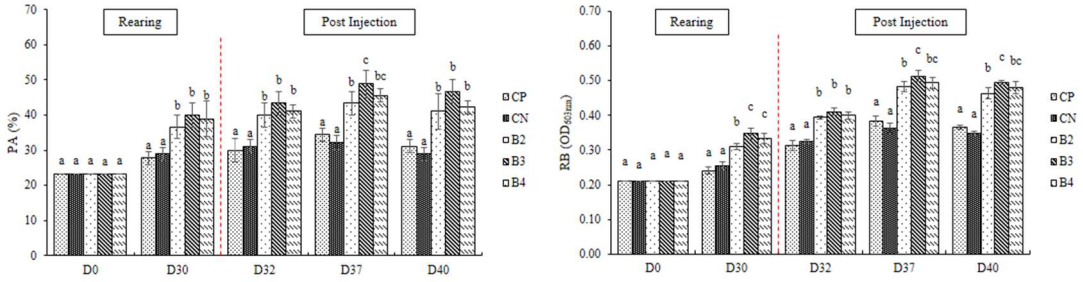

Figure 2. Phagocytic activity (PA) and respiratory burst activity (RB) of catfish on days 0, 30, 32, 37 and 40 after challenge with *Edwardsiella tarda*. Different uppercase letters in the same row indicate significantly different results between treatments (Duncan $p<0.05$).

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 after *E. tarda* challenge, with significant differences among treatments ($p < 0.05$). The negative control (CN) maintained the highest survival, while the positive control (CP) had the lowest. Treatments B2, B3, and B4 showed higher survival than CP and were not significantly different from CN.

Interestingly, *E. tarda* infection has been reported to cause 60–100% mortality 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, 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 gouramy supplemented

with banana midrib against *Aeromonas 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.

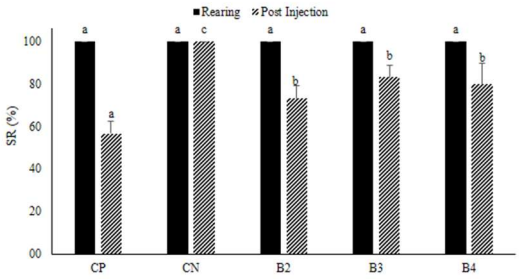

Figure 3. Survival rate (SR) of catfish after challenge with *Edwardsiella tarda*. Different uppercase letters in the same row indicate significantly different results between treatments (Duncan $p<0.05$).

CONCLUSIONS

Dietary supplementation with banana midrib simplicia was proven to enhance 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 support sustainable aquaculture.

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AUTHOR CONTRIBUTION

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

DECLARATION OF COMPETING INTEREST AND USE 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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