DOI: http://dx.doi.org/10.15578/iaj.20.1.2025.11-12 *Indonesian Aquaculture Journal, 20 (1), 2025, 11-22*

Available online at: http://ejournal-balitbang.kkp.go.id/index.php/iaj

EFFECT OF DIETARY BANANA PSEUDO-STEM SIMPLICIA, COMMERCIAL VACCINE, AND THEIR COMBINATION ON THE GROWTH, HEALTH STATUS, AND IMMUNITY PERFORMANCE OF NILE TILAPIA AGAINST *Aeromonas hydrophila*

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(Received: March 20, 2025; Final revision: May 2, 2025; Accepted: May 20, 2025)

ABSTRACT

The sustainability of Nile tilapia production faces challenges from motile Aeromonas septicemia (MAS), caused by Aeromonas hydrophila. The use of antibiotics to control MAS has negative impacts on aquatic environments and consumer health. As alternatives, plant-based treatments and vaccination have been increasingly applied to replace antibiotics. This study aimed to evaluate the effects of banana pseudo-stem (BS), a commercial vaccine (CV), and their combination (BS+CV) on the growth, health status, and immune performance of Nile tilapia against A. hydrophila. A completely randomized design was used with five treatments: a negative control (C-), a positive control (C+), BS, CV, and BS+CV—each coated onto feed. Each treatment had three replications. Fish were reared in 36-L aquaria for 42 days to evaluate growth performance, followed by a challenge test with A. hydrophila on day 43. Survival was monitored for 14 days post-challenge. The highest growth performance was observed in the BS treatment compared to the other treatments. Meanwhile, survival rate (SR) and feed conversion ratio (FCR) did not show significant differences (P>0.05) among treatments. After the challenge, survival rates in the BS (96.67%), CV (73.33%), and BS+CV (76.67%) groups were significantly higher (P<0.05) than in the positive control group (50.00%). The BS group did not differ significantly (P>0.05) from the negative control group (100.00%). These findings indicate that BS, CV, and BS+CV enhance the immune response of Nile tilapia against A. hydrophila, with BS being the most effective in improving both growth and disease resistance.

KEYWORDS: *Aeromonas hydrophila*; Banana pseudo-stem simplicia; Immune response, Nile tilapia, vaccine

INTRODUCTION

Aquaculture is one of the fastest-growing food production sectors globally (Sumaila *et al.*, 2022). Various prominent aquaculture commodities have been successfully developed, such as Nile tilapia (*Oreochromis niloticus*) (Lima Verde *et al.*, 2021). According to the Food and Agriculture Organization (FAO), Nile tilapia ranks as the fourth most produced species, reaching 5.3 million tonnes (FAO, 2024). This figure is projected to increase significantly, reaching 7.3 million tonnes by 2030 (Arumugam *et al.*, 2023).

Despite the rapid development of Nile tilapia farming, its sustainability is challenged by several factors, one the most critical being outbreaks of Motile Aeromonas Septicemia (MAS) (Legario *et al.*, 2023). MAS is caused by an infection of the Gram-negative bacterium *A. hydrophila* (Matter *et al.*, 2024). Clinical signs in affected fish include reddened fins, skin discoloration, hemorrhages on the skin, eyes, and fins, abdominal swelling, scale loss, and exophthalmia (protruding eyes) (Korni *et al.*, 2017; Mostafa *et al.*, 2024). Severe infections can lead to mortality rates of up to 80% (Abd El-Gawad *et al.*, 2020).

The prevention of MAS using plant-based materials or vaccination strategies has been increasingly explored (Korni *et al.*, 2020; Sombe *et al.*, 2024). These approaches offer viable alternatives to antibiotics, which are known to negatively impact aquatic ecosystems and human health (Limbu *et al.*, 2021; Abdullah *et al.*, 2024). Among the promising plant-based candidates is banana (Wahjuningrum *et al.*,

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2021), particularly the pseudo-stem, which has demonstrated antibacterial and immunostimulatory properties against *A. hydrophila*. In catfish, immersion in banana pseudo-stem resulted in a higher survival rate after bacterial challenge and led to increased erythrocyte count, hemoglobin levels, leukocyte count, phagocytic activity, lysozyme activity, and a better feed conversion ratio, indicating its ability to improve immune performance and disease resistance (Astria *et al.,* 2017).

The control of *A. hydrophila* infection in Nile tilapia can also be achieved using vaccines. Their application in red hybrid tilapia (*O. niloticus* × *O. mossambicus*) has been shown to enhance immunity and resistance to *A. hydrophila* infection (Monir *et al*., 2020). Moreover, the use of commercial vaccines derived from inactivated *A. hydrophila* strain AHL0905-2 has proven effective in preventing infections in Nile tilapia (Ratulangi *et al*., 2014).

Moreover, the control of *A. hydrophila* infection in Nile tilapia can benefit from the combined effects of plant-based compounds and vaccines. For instance, the combination of garlic (*Allium sativum*) and *Echinacea purpurea* with vaccines has demonstrated improved immunity and resistance against *A. hydrophila* infection (Aly *et al*., 2016; El Tantawy & Ayoub, 2016). The primary goal of combining plant-based compounds with vaccines is to enhance the effectiveness of disease control. Plant-based compounds serve to strengthen the innate immune system of fish, while vaccines provide adaptive immunity specific to the pathogen (Soltani *et al*., 2019).

To date, no studies have evaluated the effectiveness of banana pseudo-stem, commercial vaccines, and their combination on growth performance, health, and resistance against pathogenic bacteria such as *A. hydrophila* in fish. This study aims to evaluate the utilization of banana pseudo-stem, commercial vaccines, and their combination on growth, health status, and immunity performance nile tilapia against *A. hydrophila.*

MATERIALS AND METHODS

Preparation of Containers and Test Fish

The containers used were 15 aquariums with dimensions of 60 × 30 × 30 cm. Each aquarium was equipped with aeration and a heater. The freshwater volume in each aquarium was 36 liters (water height of 20 cm in each aquarium). The freshwater had a temperature range of 28.80–31.40 °C, pH 6.83–7.63, and dissolved oxygen levels of 3.00–6.10 mg L^{-1}, according the rearing requirements based on Indonesian National Standard (SNI 7550:200). The stocking density of Nile tilapia used was 10 fish per aquarium. The Nile tilapia (*O. niloticus*) used were healthy fish with no clinical symptoms of MAS, test fish have body length of 10.17 ± 0.93 cm and body weight of 20.05 ± 5.51 g.

Preparation of Banana Pseudo-Stem Simplicia

The banana pseudo-stem used was obtained from *Musa paradisiaca* (Ambon banana) sourced from local farmers in the Dramaga area, Bogor, West Java. The pseudo-stem was chopped and air-dried for 7 days, then oven-dried at 50°C for 19 hours. The dried material was ground into powder and sieved using a 60-mesh sieve, following the procedure of Mesa *et al*. (2019) with minor modifications. The resulting powder was stored in sealed bottles until use. The banana stem powder was then coated onto commercial feed at a concentration of 3%. This concentration has been reported as optimal for enhancing the immune response of fish against bacterial infections (Pattah *et al*., 2020).

Preparation of Experimental Diets

The experimental diets consisted of four treatments, namely Control Feed (C), Banana Pseudo-Stem Feed (BS), Commercial Vaccine Feed (CV), and Combination Feed (BS + CV). All diets were prepared by coating commercial feed with specific mixtures containing 10% (v/w) distilled water and 2% (v/w) egg white as a binder. For BS, 3% (w/w) banana pseudo-stem powder was added; for CV, 3 mL of commercial vaccine per fish weight (v/w) was included; and for BS + CV, both 3% banana pseudo-stem powder and 3 mL vaccine per fish weight were incorporated. All feeds were homogenized, coated onto the pellets, air-dried, and stored in airtight containers until use.

Preparation of A. Hydrophila

The *A. hydrophila* bacteria were obtained from the isolate collection of the Laboratory of Aquatic Organism Health, Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University. The isolates were tested using the API 20 NE kit to confirm the presence of *A. hydrophila* (Artati *et al*., 2024). The identification results confirmed the presence of *A. hydrophila* (Table 1). Subsequently, an LD50 test was conducted to determine the virulence level of the bacteria by assessing its ability to kill 50% of the population. The bacterial densities tested for LD50 were 10^5 CFU mL^{-1}, 10^6 CFU mL^{-1}, 10^7 CFU mL^{-1}, and 10x CFU mL^{-1}, which were injected intramuscularly. The results showed that the LD50 value of *A. hydrophila* for Nile tilapia was 10^5 CFU mL^{-1}. This dose was used during the challenge test.

Experimental Design and Fish rearing

The experimental design used was a completely randomized design with treatments C-, C+, BS, CV, and BS+CV, each consisting of three replicates. Before testing, a subset of fish was taken from the stock to serve as the initial group on day 0 (without treatment). Feed was administered three times a day (09:00, 14:00, and 18:00 local time) to satiation. The fish rearing period lasted for 42 days, followed by the challenge test on day 43. The *A. hydrophila* challenge test was conducted over 14 days, during which Nile tilapia were fed commercial feed. The complete feeding regime is presented in Table 2.

Observation Parameters

The observed parameters included growth performance parameters such as weight gain (Bunnoy *et al*., 2022), average daily growth (Bunnoy *et al*., 2022), feed conversion rate (Arriaga-Hernández *et al*., 2021), and survival rate (Kari *et al*., 2022); health parameters such as hematological indicators including total erythrocytes (Blaxhall & Daisley, 1973), total leukocytes (Blaxhall & Daisley, 1973), hematocrit levels (Anderson & Siwicki, 1993), and hemoglobin levels (Wedemeyer & Yasutake, 1977); immune responses such as phagocytic activity (Anderson & Siwicki, 1993), respiratory burst activity (Anderson & Siwicki, 1993), lysozyme activity (Ellis *et al*., 2011), and antibody titer (Roberson, 1990); and resistance to *A. hydrophila*. Growth performance parameters were observed from day 0 to day 42. Health parameters were observed on days 0 and 30 before the challenge test, and on days 2, 4, 6, 7, and 14 post-challenge. Resistance to *A. hydrophila* was evaluated after the challenge test by assessing the survival rate of Nile tilapia.

Table 1. Biochemical characteristics API 20 NE of the *A. hydrophila*

Biochemical characteristics	Isolated
Nitrate Reduction (NO3)	+
Tryptophan Deaminase (TRP)	+
Glucose Fermentation (GLU)	+
Arginine Dihydrolase (ADH)	+
Urease (URE)	-
Esculin Hydrolysis (ESC)	+
Gelatin Hydrolysis (GEL)	+
Phosphatase (PNG)	+
Arabinose Utilization (ARA)	+
Mannose Utilization (MNE)	+
Mannitol Utilization (MAN)	-
N-Acetyl-Glucosamine (NAG)	+
Maltose Utilization (MAL)	-
Gluconate Utilization (GNT)	+
Caprate Utilization (CAP)	+
Adipate Utilization (ADI)	-
Malate Utilization (MLT)	+
Citrate Utilization (CIT)	-
Phenylacetic Acid Utilization (PAC)	-
Oxidase (OX)	+
Result	*A. hydrophila* with a percentage of 94%

Data Analysis

Data were tabulated using Microsoft Excel 2021. The normality of each dataset was assessed using the Shapiro-Wilk test, and homogeneity of variances was evaluated using Levene's test, both performed in SPSS software. All variables met the assumptions of normality and homogeneity. Therefore, one-way analysis of variance (ANOVA) was conducted with a 95% confidence interval. When significant differences were detected, Duncan's multiple range test was applied as a post hoc analysis.

Table 2. Research design and maintenance during the study

Treatment	Description
C+	Nile tilapia were given feed C from day 1 to day 42 and injected with *A. hydrophila* bacteria on day 43 at a dose of 10^5 CFU mL^{-1}, 100 µL per fish, intramuscularly.
C-	Nile tilapia were given feed C from day 1 to day 42 and injected with phosphate buffer saline (PBS) on day 43 at a dose of 100 µL per fish, intramuscularly
BS	Nile tilapia were given feed C from day 1 to day 28, then fed BS from day 29 to day 42, and injected with *A. hydrophila* bacteria on day 43 at a dose of 10^5 CFU mL^{-1}, 100 µL per fish, intramuscularly
CV	Nile tilapia were given feed C from day 1 to day 7, then fed CV from day 8 to day 14, after which they were given feed C again from day 15 to day 42, and injected with *A. hydrophila* bacteria on day 43 at a dose of 10^5 CFU mL^{-1}, 100 µL per fish, intramuscularly
BS+CV	Nile tilapia were given feed C from day 1 to day 7, then fed BS+CV from day 8 to day 14, after which they were given feed C again from day 15 to day 42, and injected with *A. hydrophila* bacteria on day 43 at a dose of 10^5 CFU mL^{-1}, 100 µL per fish, intramuscularly

RESULTS AND DISCUSSION

The sustainability of Nile tilapia production faces challenges from motile aeromonas septicemia (MAS), caused by an infection with the Gram-negative bacterium *A. hydrophila* (Legario *et al*., 2023; Matter *et al*., 2024). The use of antibiotics for disease control is known to have negative impacts on aquatic environments and consumer health (Limbu *et al*., 2021; Abdullah *et al*., 2024; Pardede *et al*., 2024). Therefore, using plant-based materials and vaccination has emerged as a promising alternative to antibiotics (Korni *et al*., 2020; Sombe *et al*., 2024).

In aquaculture systems, incorporating plant-based materials has been shown to improve fish growth performance (Wannavijit *et al*., 2024). Observations on growth performance (Table 3) indicated that final weight (W_0), weight gain (ΔW), and average daily growth (ADG) were significantly different among treatments ($P < 0.05$). In general, the highest growth performance values were observed in the BS treatment compared to the other treatments. Meanwhile, survival rate (SR) and feed conversion ratio (FCR) did not show significant differences ($P > 0.05$) among treatments.

Banana pseudo-stem contains phytochemical components such as flavonoids, tannins, saponins, and alkaloids (Nurjanah *et al*., 2018). These bioactive compounds are known to enhance the secretion of growth hormone (GH) and insulin-like growth factor (IGF)-I, which subsequently stimulates fish growth (Chakraborty *et al*., 2014). Additionally, banana pseudo-stem powder also serves as a prebiotic for *Lactobacillus plantarum* and *Pediococcus acidilactici* (Singapurwa *et al*., 2024). Both bacteria act as

Table 3. The initial weight (W_0), final weight (Wt), weight gain (ΔW), average daily growth (ADG), survival rate (SR), and feed conversion ratio (FCR) of Nile tilapia fed with different diets during 42 days rearing

Parameters	C	BS	CV	BS+CV
W0 (g)	202.87 ± 16.65^a	192.67 ± 15.51^a	182.80 ± 8.52^a	211.70 ± 29.55^a
Wt (g)	766.83 ± 147.10^{ab}	838.23 ± 43.43^b	639.16 ± 50.87^a	783.92 ± 101.38^{ab}
ΔW (g)	563.97 ± 131.01^{ab}	645.56 ± 55.78^b	456.36 ± 59.36^a	572.22 ± 86.79^{ab}
ADG (g day^{-1})	13.43 ± 3.12^{ab}	15.37 ± 1.33^b	10.87 ± 1.41^a	13.62 ± 2.07^{ab}
FCR	1.12 ± 0.07^a	1.11 ± 0.02^a	1.22 ± 0.07^a	1.17 ± 0.03^a
SR (%)	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a

Data (Mean ± SD) on the same row followed by different letters indicate significant differences at the 5% test level (Duncan's test). Treatments include: control treatment (C), banana pseudo-stem powder (BS), commercial vaccine (CV), and the combination of banana pseudo-stem powder with commercial vaccine (BS+CV).

probiotics that can enhance enzyme activity, thereby improving fish growth performance (Keereelang *et al*., 2022). Thus, banana pseudo-stem can act as an immunostimulant agent.

However, the CV treatment (commercial vaccine) did not result in increased growth. Similarly, the BS+CV treatment (a combination of banana pseudo-stem and commercial vaccine) showed no significant difference in growth performance compared to the C treatment (control). This finding confirms that the effects of the vaccine on fish growth are secondary outcomes, with the primary focus remaining on improving survival rates through disease protection (Cunningham *et al*., 2016).

The use of plant-based materials, such as banana, along with commercial vaccines, has been shown to prevent MAS infections in fish (Ratulangi *et al*., 2014; Wahjuningrum *et al*., 2021). In Nile tilapia, MAS is characterized by reddened fins, skin discoloration, hemorrhaging on the skin, eyes, and fins, abdominal swelling, scale loss, and exophthalmia (Korni *et al*., 2017; Mostafa *et al*., 2024). MAS infections caused by *A. hydrophila* can lead to mortality rates as high as 80% (Abd El-Gawad *et al*., 2020).

The bacterium *A. hydrophila* produces toxins that contribute to fish mortality. Hemolysin is a toxin that damages red blood cell membranes in the host (Xiong et al., 2021). Consistent with this, studies have shown that *A. hydrophila* injection significantly affects the hematological parameters of fish. Observations on total erythrocyte count (Figure 1) at days D0, D42, and D+2 showed no significant differences ($P > 0.05$)

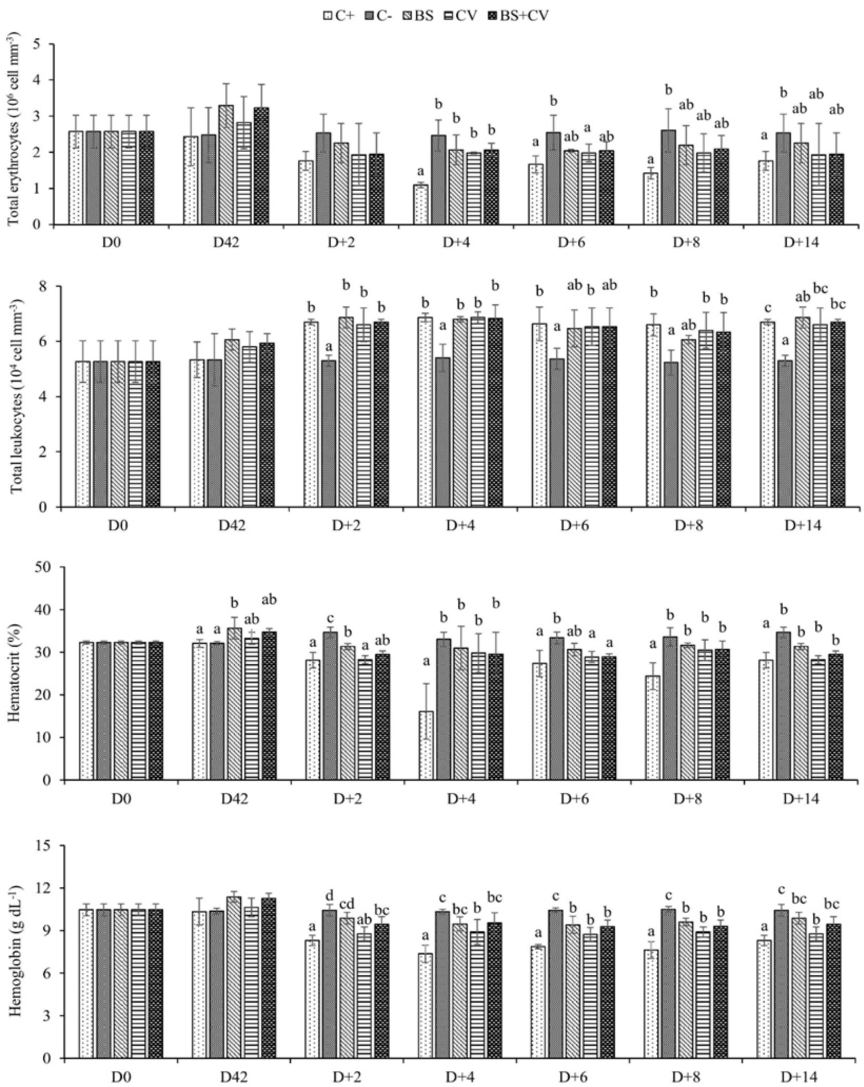

Figure 1. The hematology of Nile tilapia pre-challenge (D0 and D42 pre-challenge) and post-challenge (D+2, D+4, D+6, D+8, and D+14 post-challenge). Data (Mean ± SD) with different letters indicate significant differences at the 5% test level (Duncan's test). Treatments include: positive control (C+), negative control (C-), banana pseudo-stem powder (BS), commercial vaccine (CV), and the combination of banana pseudo-stem powder with commercial vaccine (BS+CV).

among treatments. However, at days D+4, D+6, D+8, and D+14, significant differences were observed (*P* < 0.05). After the challenge test, a decrease in total erythrocytes was noted in all *A. hydrophila*-injected treatments (C+, BS, CV, and BS+CV), which were significantly different (*P* < 0.05) compared to the negative control (C-).

The total erythrocyte count is closely related to hematocrit and hemoglobin levels. A reduction in erythrocytes leads to a decrease in hematocrit, which represents the percentage of red blood cell volume in the blood, ultimately lowering hemoglobin levels. As the primary oxygen-carrying molecule, hemoglobin decreases significantly following erythrocyte reduction (Kishimoto *et al*., 2020). Observations on hematocrit levels (Figure 1) showed no significant differences (*P* > 0.05) among treatments on day D0. However, significant differences (*P* < 0.05) were observed on days D42, D+2, D+4, D+6, D+8, and D+14. After the challenge test, hematocrit levels decreased in all *A. hydrophila*-infected treatments (C+, BS, CV, and BS+CV), with values significantly lower (*P* < 0.05) than the negative control (C-).

Similarly, hemoglobin levels (Figure 1) showed no significant differences (*P* > 0.05) among treatments on days D0 and D42. However, significant reductions (*P* < 0.05) were observed on days D+2, D+4, D+6, D+8, and D+14. After the challenge test, hemoglobin levels decreased in all *A. hydrophila*-infected treatments (C+, BS, CV, and BS+CV), differing significantly (*P* < 0.05) from the negative control (C-). These findings confirm that *A. hydrophila* infection leads to a decline in hematocrit and hemoglobin levels, consistent with the reduction in total erythrocytes. This suggests a direct impact of hemolysin toxin on the hematological parameters of Nile tilapia, aligning with the findings of Kouassi *et al*. (2019) in *A. hydrophila*-challenged Nile tilapia.

Unlike erythrocytes, leukocytes play a crucial role in immune defense, and their numbers typically increase in response to pathogen invasion in fish (Tadese *et al*., 2022). In this study, total leukocyte counts (Figure 2) on days D0 and D42 showed no significant differences (*P* > 0.05) among treatments. However, on days D+2, D+4, D+6, D+8, and D+14, significant increases (*P* < 0.05) were observed. Following the challenge test, total leukocyte counts increased in all *A. hydrophila*-infected treatments (C+, BS, CV, and BS+CV), with values significantly higher (*P* < 0.05) than the negative control (C-). This increase in leukocytes indicates immune system activation, as immune cells are mobilized to the infection site (Jackson *et al*., 2019).

Leukocytes play a crucial role in the immune response against pathogen infections, primarily through phagocytosis. This process is mainly carried out by neutrophils and macrophages, which engulf and neutralize invading pathogens (Bouchery & Harris, 2019). In this study, phagocytic activity (Figure 2) on day D0 showed no significant differences (*P* > 0.05) among treatments. However, on days D42, D+2, D+4, D+6, D+8, and D+14, significant increases were observed (*P* < 0.05). Following the challenge test, phagocytic activity increased in all *A. hydrophila*-infected treatments (C+, BS, CV, and BS+CV), with values significantly higher (*P* < 0.05) than the negative control (C-).

Phagocytosis involves a series of biochemical reactions, one of which is the production of reactive oxygen species (*ROS*). *ROS* are generated by leukocytes through a process known as respiratory burst activity, which enhances pathogen elimination by creating oxidative stress that damages microbial cell membranes (Biller & Takahashi, 2018; Andrés *et al*., 2022). In this study, respiratory burst activity (Figure 2) on days D0 and D+14 showed no significant differences (*P* > 0.05) among treatments. However, on days D42, D+2, D+4, D+6, and D+8, significant increases were observed (*P* < 0.05).

Following the challenge test, respiratory burst activity on days D+2 and D+4 increased significantly in all *A. hydrophila*-infected treatments (C+, BS, CV, and BS+CV) compared to the negative control (C-). On day D+6, respiratory burst activity remained elevated in the C+, BS, and BS+CV treatments, whereas the CV treatment showed a decline. By day D+8, only the C+ treatment exhibited significantly higher activity (*P* < 0.05) than the other treatments. This study confirms that respiratory burst activity plays a crucial role in the oxidative defense mechanism of leukocytes, with significant activation in all infected treatments compared to the negative control.

In addition to respiratory burst activity, leukocytes produce lysozyme enzymes which play a crucial role in bacterial cell wall degradation. Lysozyme functions by lysing the peptidoglycan layer of bacterial cell walls and activating autolytic enzymes within bacterial cells, leading to their destruction (Ragland & Criss, 2017). As phagocytic activity increases, lysozyme activity is also expected to be elevated (Dotta *et al*., 2014). In this study, lysozyme activity on days D0, D42, D+4, D+6, D+8, and D+14 showed no significant differences (*P* > 0.05) among treatments. However, on day D+2, a significant difference was observed (*P* < 0.05). Following the challenge test on day D+2, lysozyme activity increased in the C+, BS, and BS+CV treatments, whereas the

CV treatment exhibited a decrease. On days D+4, D+6, and D+8, although the differences were not statistically significant ($P > 0.05$), lysozyme activity showed an increasing trend before declining again on day D+14.

The decrease in lysozyme activity observed in the CV treatment (commercial vaccine group) contrasts with previous findings in hybrid red tilapia, asian seabass, and pacu, where vaccination significantly increased lysozyme activity (Monir *et al.*, 2020; Farias *et al.*, 2020; Raju *et al.*, 2023). The reduced lysozyme activity in the CV group is likely due to a shift in immune resource allocation toward the adaptive immune system, as the nature of the vaccine tends to direct immune responses along the adaptive rather than the innate pathway (Messina *et al.*, 2019). However, a limitation of this study is that it assessed only antibody titers as an indicator of adaptive immunity, without evaluating other innate immune parameters such as complement activity, cytokine levels, or phagocytic function. Therefore, the interpretation regarding the shift in immune response remains speculative and requires further validation through comprehensive immunological profiling.

The CV vaccination treatment stimulates the production of specific antibodies that directly target pathogens, thereby reducing reliance on nonspecific immune mechanisms such as lysozyme activity. Antibody titer levels (Figure 2) at day 0 (D0) showed no significant differences ($P > 0.05$) among treatments.

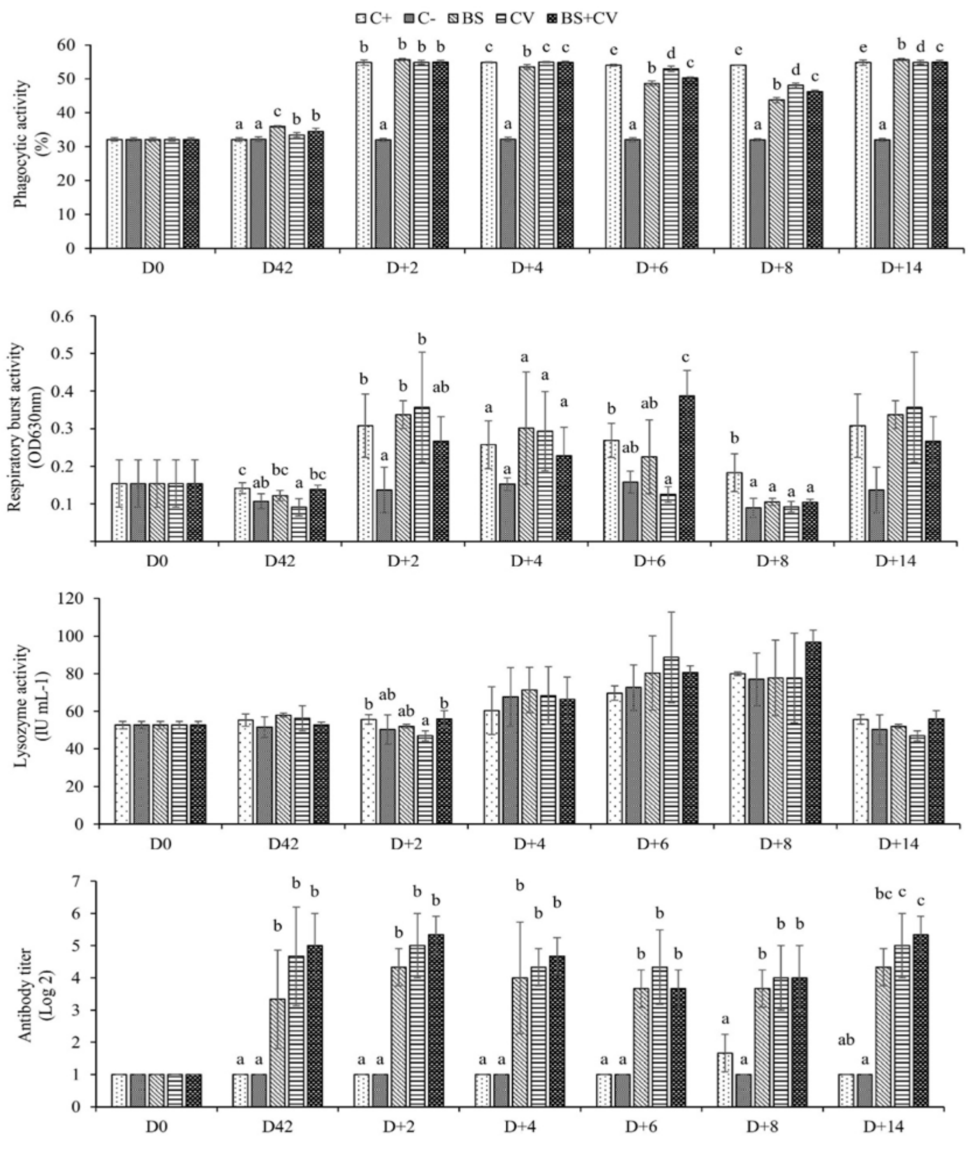

Figure 2. Immune response parameters of Nile tilapia pre-challenge (D0 and D42 pre-challenge) and post-challenge (D+2, D+4, D+6, D+8, and D+14 post-challenge). Data (Mean ± SD) with different letters indicate significant differences at the 5% test level (Duncan's test). Treatments include: positive control (C+), negative control (C-), banana pseudo-stem powder (BS), commercial vaccine (CV), and the combination of banana pseudo-stem powder with commercial vaccine (BS+CV).

However, on days D42, D+2, D+4, D+6, D+8, and D+14, significant differences were observed ($P < 0.05$). Following the challenge test, antibody titers in the BS, CV, and BS+CV treatments increased significantly ($P < 0.05$) compared to those in the C+ and C– groups. Post-challenge observations confirmed a significant rise in antibody titers in the CV-treated group, indicating an effective adaptive immune response. Similar enhancements were also observed in the BS and BS+CV groups. These responses differed significantly from both the positive control (C+) and the negative control (C-), neither of which exhibited a comparable level of adaptive immune activation.

The results of total leukocyte count, phagocytic activity, respiratory burst activity, and antibody titers indicate that the administration of BS, CV, and BS+CV enhances the immune defense against *Aeromonas hydrophila* infection. Banana pseudo-stem supports the immune system through its phytochemical content, including flavonoids, tannins, saponins, and alkaloids (Nurjanah *et al*., 2018). These phytochemicals exhibit immunostimulant properties, acting as antioxidants that protect immune cells from oxidative damage and preserve cell membrane integrity. Consequently, they enhance resistance to pathogen attacks, stimulate antibody production, increase leukocyte phagocytic activity, and inhibit microbial growth (Tiwari *et al*., 2017; Karak *et al*., 2019; Thawabteh *et al*., 2019; Choi & Kim, 2020).

Vaccination strengthens the immune system by stimulating the formation of immunological memory specific to the target pathogen. Vaccine antigens activate B and T lymphocytes, leading to the production of specific antibodies and cellular immune responses (Hong *et al*., 2018). The use of inactivated pathogen-based vaccines has been proven effective in enhancing fish resistance to bacterial infections (Mondal & Thomas, 2022). Furthermore, the combination of banana pseudo-stem and vaccine exerts a synergistic effect in boosting fish immune responses (Aly *et al*., 2016; El Tantawy & Ayoub, 2016).

Phytochemicals primarily enhance the nonspecific immune system, whereas vaccines specifically stimulate adaptive immunity (Soltani *et al*., 2019). Notably, certain phytochemicals such as flavonoids, tannins, and saponins, also function as vaccine adjuvants, further improving immune performance (Tian *et al*., 2021; Cabral-Hipólito *et al*., 2022; Chen *et al*., 2023). Thus, the combination of banana pseudo-stem and vaccine has the potential to optimize fish immune responses by stimulating both innate and adaptive immunity. This effect is evident in the higher antibody titers observed in Nile tilapia treated with the combination compared to those receiving vaccination alone.

These conditions support the mechanism by which BS, CV, and BS+CV induce a more comprehensive immune response, ultimately leading to an increased survival rate. Observations of the survival rate (Figure 3) indicated that the BS, CV, and BS+CV treatments had higher survival rates, which were significantly different ($P<0.05$) compared to the positive control. However, the BS treatment did not show significant differences ($P>0.05$) when compared to the negative control.

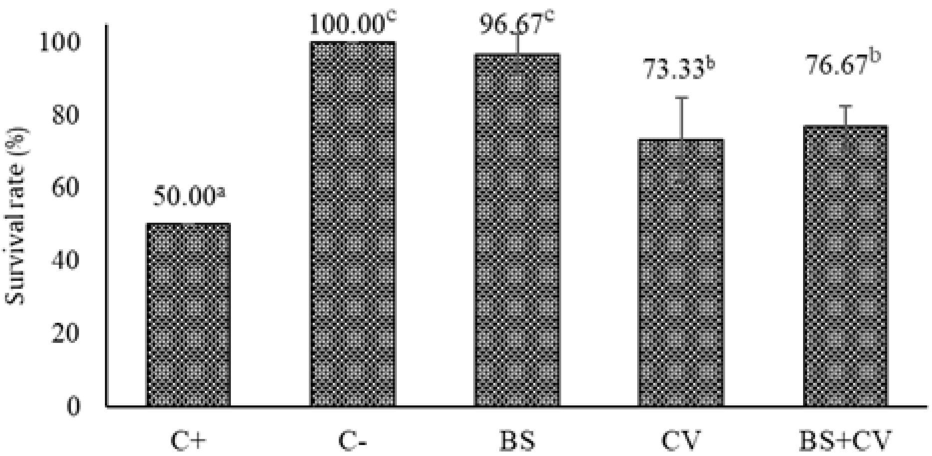

Figure 3. Survival rate of Nile tilapia post A. hydrophila challenge test. Data (Mean ± SD) with different letters indicate statistically significant differences at the 5% level (Duncan's test). Treatments include: positive control (C+), negative control (C-), banana pseudo-stem powder (BS), commercial vaccine (CV), and a combination of banana pseudo-stem powder with commercial vaccine (BS+CV).

The findings related to BS are consistent with the study by Mones & Angeles (2017), which reported that banana plants enhance resistance to *A. hydrophila* infection in Red Tilapia. The results of CV align with those of Ratulangi *et al.* (2014), who demonstrated that commercial vaccines effectively prevent infection in Nile tilapia. Meanwhile, the combination treatment (BS + CV) exhibited a synergistic effect, similar to the findings of Aly *et al.* (2016), who reported that the combination of *Allium sativum* and a vaccine enhanced Nile tilapia resistance against to *A. hydrophila* infection.

CONCLUSION

The administration of banana pseudo-stem powder, commercial vaccine, and their combination can enhance the immune response of Nile tilapia against *A. hydrophila* infection. Overall, banana pseudo-stem was more effective in improving both the growth and immune performance of Nile tilapia compared to the vaccine and combination treatments.

ACKNOWLEDGMENTS

The authors would like to express their sincere gratitude to Adna Sumadikarta and Yanuar Raharja for their great technical help in this study. This research was self-funded by the authors.

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