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Research Article

Effect of Dayak Onion (*Eleutherine bulbosa* (Mill.) Urb.) on the Immune Response and Gene Expression of Nile Tilapia (*Oreochromis niloticus*) Infected with *Aeromonas hydrophila*

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Abstract

Dayak onion (*Eleutherine bulbosa* (Mill.) Urb.) has the potential to be an immunostimulant to benefit fish health. This study aimed to evaluate the effectiveness of dietary supplementation with the powder and crude ethanol extracts of Dayak onion on the hematology and immune response parameters of Nile tilapia challenged with *Aeromonas hydrophila*. The research used a completely randomized design with six treatments and three replications. Nile tilapias were fed with Dayak onion (w/v) consisting of powders of 5% (P5), 10% (P10), and 15% (P15), while crude extract was 0.5% (E05) and given during rearing for 30 days. Positive control (C+) and negative control (C-) were used without Dayak onion. Nile tilapia in treatment C+, P5, P10, and P15 were injected with *A. hydrophila* 10^6 CFU mL^{-1}, while that in treatment C- was injected with phosphate buffered saline (PBS) intramuscularly. The results showed that the dietary with 15% powder and 0.5% crude extract significantly improved the hematology and immune response parameters compared to the control after the challenge against *A. hydrophila* ($P<0.05$). Dayak onion supplementation effectively increased the health status based on hematology and immune response parameters of Nile tilapia against *A. hydrophila* infection.

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1. Introduction

Aquaculture is the fastest-growing animal food production sector and contributes to global prosperity (Zahran *et al*., 2014). The Nile tilapia (*Oreochromis niloticus*) commodity has strategic value in the contribution of aquaculture to the regional and national economy. Increasing intensive fish farming can cause stress due to environmental conditions (Yilmaz, 2019) and adversely affect the immune system of fish because of various diseases such as aeromoniasis (Harikrishnan *et al*., 2022). The onset of bacterial diseases causes economic losses in fish farming (Fazio, 2019). *Aeromonas hydrophila* was found to attack tilapia aquaculture. Outbreaks of the pathogen of *A. hydrophila* cause high mortality in farmed fish by inducing internal bleeding with symptoms of skin wounds and chronic necrosis (Derome *et al*., 2016).

Antibiotic use is unaffordable for fish farmers and can harm the environment and human health (Yilmaz, 2019). Overuse of antibacterial drugs in fish farming can lead to antimicrobial bacterial resistance and bioaccumulation of residual compounds in the host (Gauthier, 2015). Fish disease control can be done by administering immunostimulants to enhance the specific or nonspecific defense system. Immunostimulants act as an alternative bioremediation that is biocompatible and environmentally friendly to overcome fish diseases, increase the immune system, and reduce the risk of disease (Harikrishnan *et al*., 2022). Herbs are one of the immunostimulants that have the potential to increase the immune response in fish. Herbal supplementation can trigger the specific and innate immune systems of carp against *A. hydrophila* infection (Harikrishnan *et al*., 2010) and can regulate immune genes expression in red tilapia (Kuswoyo *et al*., 2023). Herbal feed additives have low toxicity, residual effects, are neither drug nor disease resistant, and are easily tolerated by aquatic animals (Zhu, 2020; Zhang *et al.,* 2022).

Dayak onion (*Eleutherine bulbosa* (Mill.) Urb.) has antibacterial potential (Padhi dan Panda, 2015; Munaeni *et al*., 2019; Novaryatiin *et al.,* 2019) and provide natural antioxidant properties (Haerani *et al.,* 2019; Shi *et al*., 2019; Munaeni *et al*., 2020a; Gomes *et al*., 2021). Bioactive compounds of Dayak onion contain naphthalene, naphthoquinone, anthraquinone (Insanu *et al*., 2014), flavonoid, saponin, tannin, steroid, dan triterpenoid (Munaeni *et al*., 2017). Several studies have shown that *E. bulbosa* extract has a potential to be a prebiotic (Munaeni *et al*., 2020a), can affect the microbial diversity in the intestine of shrimp (Munaeni *et al*., 2020b), and can inhibit the growth of *V. harveyi* cell (Munaeni *et al*., 2017) and *V. parahaemolyticus* (Munaeni *et al*., 2019). Based on the characteristics mentioned above, it is known that Dayak onion can be used as an immunostimulant for disease prevention in fish.

Testing Dayak onion on fish is still rarely done, so it is necessary to study the effect of Dayak onions on Nile tilapia infected with *A. hydrophila*. This study aims to investigate the potential of this immunostimulant to enhance fish health by evaluating the effects of dietary Dayak onion on the hematological, immunology, histopathology, and immune gene expression of Nile tilapia against *A. hydrophila* infection.

2. Materials and Methods

2.1 Materials

The fish samples used were Nile tilapias sized 11.78±0.13 cm and 20.17±0.13 g and obtained from a farmer in Bogor, Indonesia. Nile tilapias were acclimatized for two weeks and fed twice a day until satiation. Bacterial isolates of *A. hydrophila* were obtained from the collection of aquatic organism health laboratory and confirmed using the API 20 NE KIT test and polymerase chain reaction (PCR) test. Dayak onions were obtained from Singkawang, West Kalimantan Province, Indonesia. The powder of Dayak onion was extracted using 96% ethanol at a ratio of 1:4 (w/v) and macerated with an orbital shaker for 24 hours. The result of the first maceration was filtered with Whatman filter paper no. 41 and re-macerated with 96% ethanol two times. The filtrate was concentrated using an evaporator vacuum at a temperature of 40°C. Dayak onion was mixed into commercial feed with 2% egg white as a binder.

2.1.1 Ethical approval

This experiment involved fish and was performed in accordance with the principles of animal welfare and was approved by the Ethics Committee on Animal Use of the IPB University (2022/234).

2.2 Method

2.2.1 Experimental design

This study used a completely randomized design (CRD) with six treatments and three replicates, namely negative control of 0% Dayak onion injected with phosphate buffered saline (PBS) (C-), positive control of 0% Dayak onion injected with *A. hydrophila* (C+), 5% of Dayak onion powder (P5) injected with *A. hydrophila*, 10% of Dayak onion powder injected with *A. hydrophila* (P10), 15% of Dayak onion powder

(P15), and 0.5% of Dayak onion extract injected with *A. hydrophila* (E05). Each aquarium contained 10 Nile tilapias with aeration. Water quality conditions during rearing were maintained at a temperature of 27-28°C, pH of 6.9-7.2, dissolved oxygen of 6.2-6.8, and 0 mg L^{-1} of ammonia. Water changes and flushing were done every three days. Feeding was done three times a day at satiation for 30 days.

2.2.2 Challenge test

After a 30-day rearing period, the challenge test was conducted on day 32. Nile tilapia fasted for one day before the challenge test. A 0.1 ml of *A. hydrophila* 10^6 CFU mL^{-1} was injected intramuscularly into Nile tilapia. After the challenge test, Nile tilapia were fed commercial feed and observed for seven days.

2.2.3 Hematological parameters

Blood samples were collected on days 0 and 30 pre-challenge (D) and days 1, 3, 5, and 7 post-challenges (DPC). Blood samples were taken from five tilapia using a 1 ml syringe containing an anticoagulant solution. Red blood cells (RBC) and white blood cells (WBC) were calculated using the method of Blaxhall and Daisley (1973). Hemoglobin (Hb) was measured using a Salinometer with the Sahli method and expressed in g% (Wedemeyer and Yasutake, 1977). Hematocrit (Hct) was calculated by value using the method of Anderson and Siwicki (1995).

2.2.4 Immunological parameters

Respiratory burst activity (RBA) testing used fish blood samples and taken at a volume of 100 µL, to which 100 µL of nitro blue tetrazolium (NBT) solution (0.2%) was added and analyzed with an ELISA reader at a wavelength of 630 nm. Phagocytic activity testing was done by taking 50 µL of blood, putting it on a microplate, and adding 50 µL of Staphylococcus. Phagocytic activity was calculated based on the method proposed by Anderson and Siwicki (1995). A lysozyme activity assay was conducted by adding 25 µL of blood serum and 25 µL of *Micrococcus lysodeikticus* suspension to a microplate. Serum was measured for primary optical density (OD) and final OD at the 30th second and 30th minute at 450 nm. A lysozyme activity was calculated using the method proposed by Nayak (2023).

2.2.5 Histopathological analysis

Histopathological analysis used kidney organs of Nile tilapia from control and treatment groups after the challenge test against *A. hydrophila*. Organ samples were immersed in neutral buffered formalin (NBF) of 10% for 24 hours, then the formalin solution was replaced with a new solution of 10% formalin (Tattiyapong *et al*., 2017). Histopathological analysis was qualitatively analyzed by observing the damage to the structure of kidney tissue. The quantitative observation was done by calculating the percentage of kidney necrosis damage in five different fields of view. Kidneys scoring referred to research by Wolf *et al.* (2015). The percentages and scoring values for organ damage are (0) <20% = minimal; (1) 20%-40% = mild; (2) 40%-60% = moderate; (3) 60%-80% = severe; and (4) >80% = highly severe. The percentage of organ damage was calculated using the method proposed by Izwar *et al.* (2020).

2.2.6 Immune Gene Expression of Nile tilapia

The kidney organs of Nile tilapia were taken from each treatment group before and after the first day of challenge test. Organ samples were added to liquid nitrogen and stored in a freezer at -80ºC. RNA extraction from 50-100 mg of kidney organs was performed by homogenization with 1 ml of RNAxPlus. RNA amount and concentration were evaluated with a spectrophotometer at an absorbance of 260/280 nm. RNA was qualified using 1% agarose gel electrophoresis and ethidium bromide staining (Mehrabi *et al*., 2020).

Table 1. Primer sequences of RT-PCR assay for tilapia

Gen		Sequence	BP	Reference
β-actin	F	TGGTGGGTATGGGTCAGAAAG	171	Chen *et al.* (2016)
	R	GCTCCTCAGGGGCAACTCT		
IL-1β	F	AAGATGAATTGTGGAGCTGTGTT	175	Ren *et al.* (2020)
	R	AAAAGCATCGACAGTATGTGAAAT		
TNF-α	F	GAGGTCGGCGTGCCAAGA	119	Chen *et al.* (2016)
	R	TGGTTTCCGTCCACAGCGT		

RNA primer sequences for the immune genes used were β-actin, interleukin 1 beta (IL-1β), and tumor necrosis factor-alpha (TNF-α) (Table 1). Analysis of immune gene expression was observed quantitatively using reverse-transcription polymerase chain reaction (RT-PCR) and the SensiFAST SYBR Hi-Rox kit. The relative of gene expression was calculated using the $2^{-\Delta\Delta c}{}_{\tau}$ method (Livak and Schmittgen, 2001).

2.2.7 Resistance to A. hydrophila

The survival rate (SR) of Nile tilapia was observed for seven days after the challenge test. The formula for calculating fish survival rate as follows:

$$SR\ (\%) = \frac{\text{Final amount of fish}}{\text{Initial amount of fish}} \times 100 \quad \text{.....(Eq 1)}$$

2.3 Data Analysis

The data were analyzed using Microsoft Excel and one-way analysis of variance (ANOVA) followed by Duncan's test ($P<0.05$) using the SPSS program. Histopathological parameters were examined quantitatively by ANOVA and qualitatively by descriptive analysis. The data were expressed as mean ± standard error (SE). Different letters were used to indicate statistically significant differences.

3. Results and Discussion

3.1 Hematological Parameters

Total RBC, Hb, and Hct activities increased on the fifth day except those in the control group. Total WBC of Nile tilapia increased on the third day after the challenge test except that in the control group. The highest treatment of hematological parameters was produced by E05 treatment, which was significantly different from the positive control ($P<0.05$) (Figure 1, 2, 3, 4). Blood is an important component of the immune system that is susceptible to different factors (Zhang *et al*., 2020). Hematological parameters are used in diagnosing fish diseases in physiological, pathological, and toxicological aspects (Fazio, 2019). *A. hydrophila* caused damage to RBC after the Nile tilapia challenge test. Infection of *A. hydrophila* in fish produces exotoxins that cause erythrocyte lysis. The increase in erythrocytes determines the mechanism of fish resistance against pathogens that affect fish health and increases the role of immunity (Mohammadian *et al*., 2019). The increase of RBC and Hb in Nile tilapia is thought to influence the bioactive content of immunostimulants in Dayak onion, such as flavonoids, saponins, and alkaloids. Previous research showed that giving onion skin to African catfish increased the value of erythrocytes and hemoglobin in the experimental group compared to that in the control group (Aluta *et al*., 2021). Leukocytes act as the main line in the defense system against pathogens or diseases (Mohammadian *et al*., 2019). Feeding Dayak onions for 30 days increased the number of WBC in Nile tilapia. Earlier research also showed that herbal supplementation in the form of *Lycium barbarum* polysaccharides increased the number of leukocytes in Nile tilapia (Zhang *et al*., 2020). The increased number of leukocytes in fish indicates the immune stimulatory properties of forest onions (Aluta *et al*., 2021).

3.2 Immunological Parameters

Respiratory burst and phagocytic activity significantly increased on the third day, whereas lysozyme activity increased on the fifth day after *A. hydrophila* injection compared with those in the control group ($P<0.05$). The treatment that gave a significantly better effect on immunological parameters was E05 treatment (Figure 5, Figure 6, and Figure 7). Dayak onion supplementation modulated immune response parameters in Nile tilapia. Respiratory burst activity is important in limiting the spread of disease in fish (Rodríguez *et al*., 2003). The content of Dayak onion administered for 30 days can increase respiratory burst activity. Phagocytes produce respiratory burst activity to attack pathogens and evaluate host defense capabilities (Harikrishnan *et al*., 2018). Phagocytic activity is employed to evaluate the immune system in fish. The increase in phagocytic activity is caused by the influence of immunostimulants with specific receptors for phagocytic cells that bind to receptor molecules on the surface of circulating and tissue phagocytes in digesting bacteria (Elala and Ragaa, 2015). Lysozyme is a lytic protein in the non-specific defense system (Telli *et al*., 2014). Lysozyme acts as an antibacterial enzyme in activating the complement system, phagocytes that act as opsonin (Amenyogbe *et al*., 2022), and evaluate the bactericidal impact of feed additives (Moustafa *et al*., 2020).

The administration of herbal plants to fish shows the potential for non-specific immunity in fish by activating the immune complement system (Amenyogbe *et al*., 2022). Flavonoid is used as immunostimulant that can increase immune responses in Nile tilapia. The naphthalene-derivate compounds of Dayak onion play a role in antibacterial activity (Munaeni *et al*., 2019). Several studies of herbal plant supplementation have shown an increase in immune response parameters of fish species, for example, 0.15% *Astragalus polysaccharides* supplementation in tilapia (Zahran *et al*., 2014), supplementation of *A. bisporus*

with doses of 5% and 10% on catfish after infection with *Flavobacterium columnare* (Harikrishnan *et al*., 2018), 5 g kg^{-1} of caffeic acid administration to tilapia (Yilmaz, 2019), fenugreek supplementation on tilapia (Moustafa *et al*., 2020), ginger supplementation on carp feed (Mohammadi *et al*., 2020), and supplementation of *Apium graveolens* L. on *Labeo chrysophekadion* (Sutthi *et al*., 2020). The content of alkaloids, tannins, and flavonoids is related to immune and metabolic functions and fish growth performance (Zhu, 2020).

3.3 Histopathological Analysis

The anterior part of the Nile tilapia kidney treated with negative control showed a normal structure. Normal kidney tissue consists of distal tubules and hematopoietic tissue in the anterior part of the kidney. The posterior part of the kidney contains many renal tubules, little interstitial hematopoietic, and lymphoid tissues that play a role in osmoregulation (Hadfield, 2009). Infection with *A. hydrophila* causes damage to target organ tissues, one of which is the kidney.

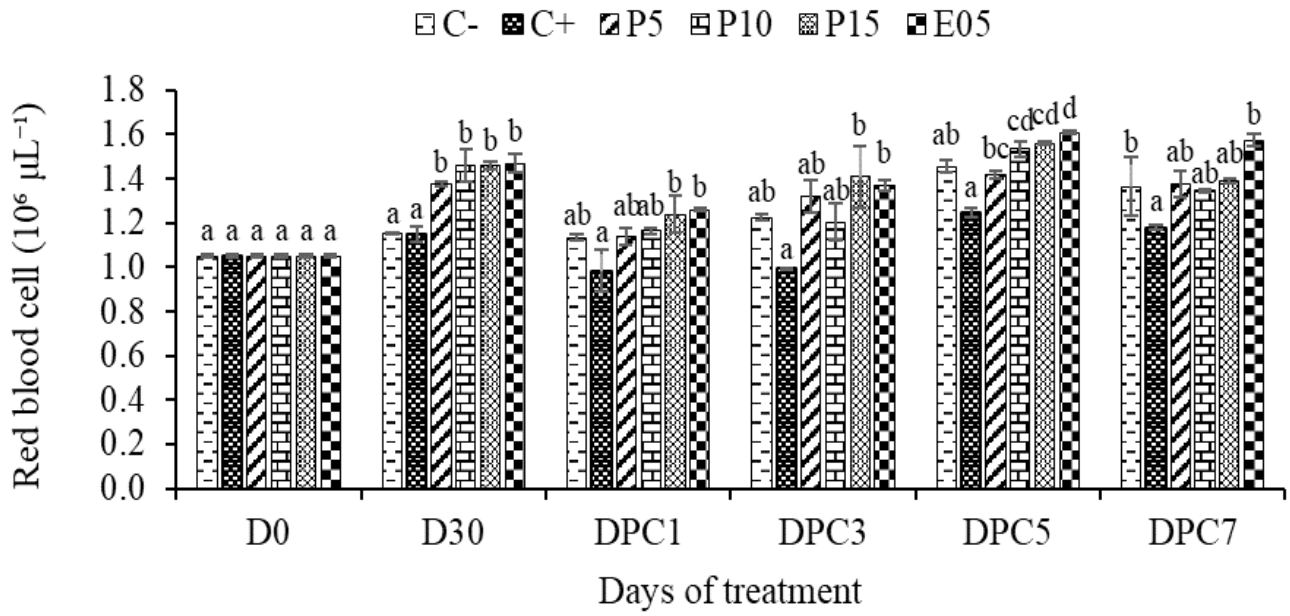

Figure 1. Total red blood cells of Nile tilapia on days 0 and 30 pre-challenge (D); days 1, 3, 5, and 7 post-challenges (DPC) against *A. hydrophila* (mean ± SE). Different letters indicate significantly different treatments (P<0.05).

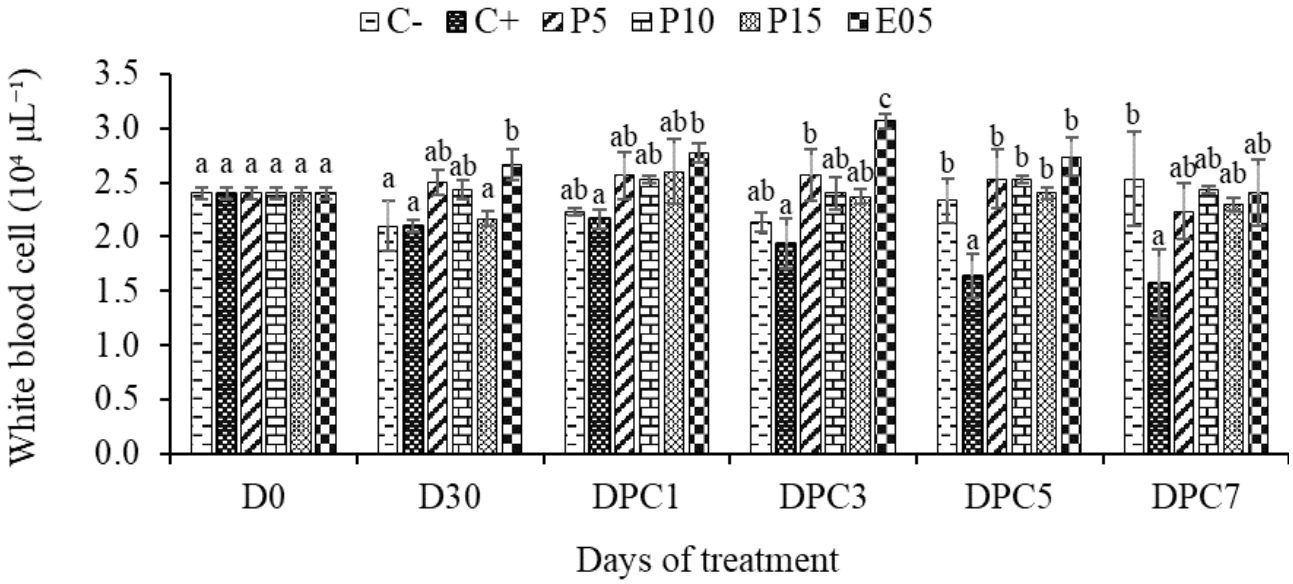

Figure 2. Total white blood cells of Nile tilapia on days 0 and 30 pre-challenge (D); days 1, 3, 5, and 7 post-challenges (DPC) against *A. hydrophila* (mean ± SE). Different letters indicate significantly different treatments (P<0.05).

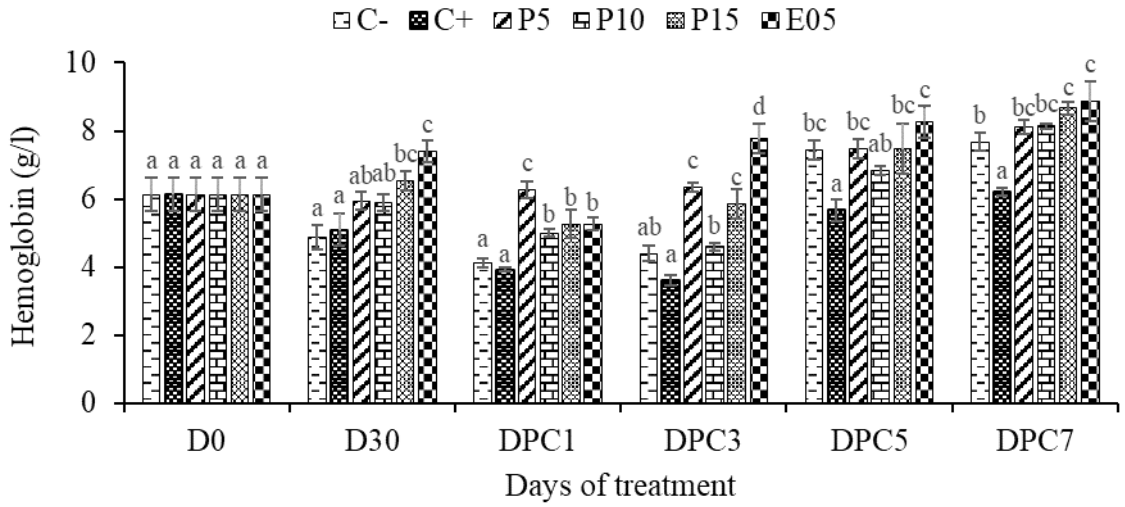

Figure 3. Hemoglobin values of Nile tilapia on days 0 and 30 pre-challenge (D); days 1, 3, 5, and 7 post-challenges (DPC) against *A. hydrophila* (mean ± SE). Different letters indicate significantly different treatments (P<0.05).

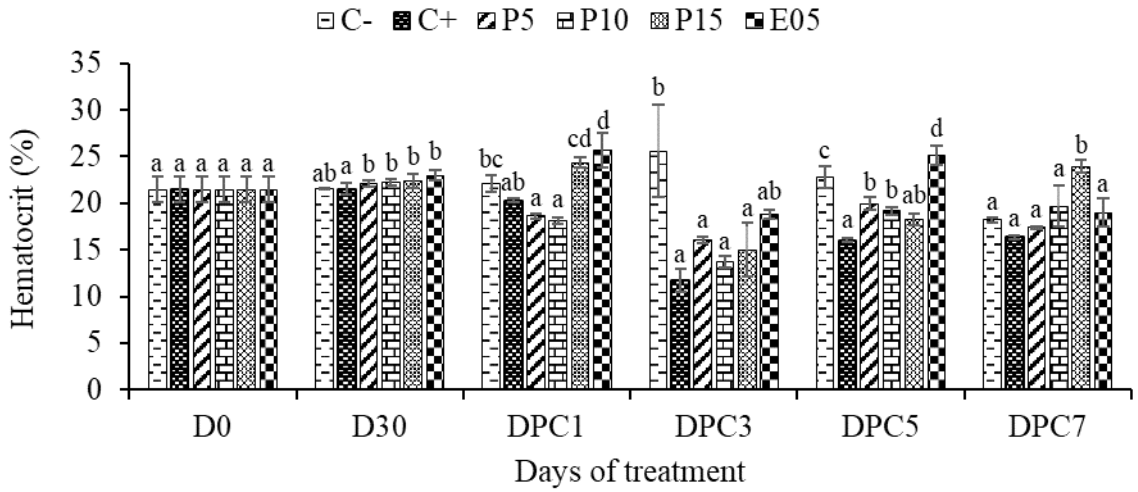

Figure 4. Hematocrit values of Nile tilapia on days 0 and 30 pre-challenge (D); days 1, 3, 5, and 7 post-challenges (DPC) against *A. hydrophila* (mean ± SE). Different letters indicate significantly different treatments (P<0.05).

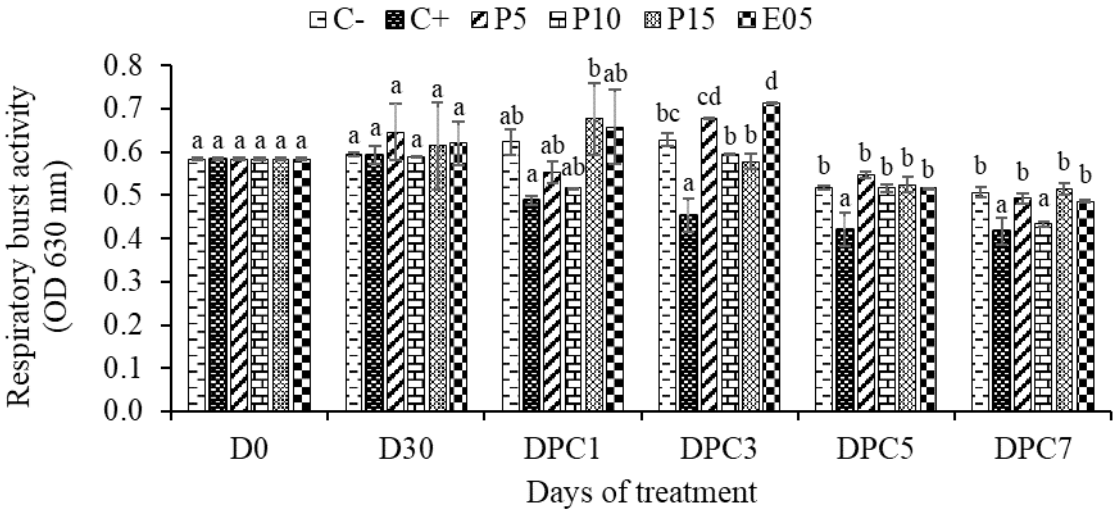

Figure 5. Respiratory burst activity of Nile tilapia on days 0 and 30 pre-challenge (D); days 1, 3, 5, and 7 post-challenges (DPC) against *A. hydrophila* (mean ± SE). Different letters indicate significantly different treatments (P<0.05).

Histopathology results of the Nile tilapia kidney after the challenge test showed symptoms of damage in the form of necrosis (N), hemorrhage (H), and vacuolization in the tubule area (Figure 8). These results are similar to the research of Ren *et al.* (2020) on the histopathology of Nile tilapia's kidney infected with *A. schubertii* that also experienced symptoms of vacuolation and interstitial hemorrhages. The fish affected by the disease showed immunotoxin properties in target tissues and impaired immune function.

The development of fish organs affects fish growth, feed utilization, disease resistance, and stress factors (El-Kady *et al*., 2022). The target organs of *A. hydrophila* infection are gills, spleen, liver, and kidney (Chen *et al*., 2018). Virulence factors include host tissue enhancement, cytotoxic enterotoxins, and hemolysins (Derome *et al*., 2016). Derome *et al.* (2016) added that increased host tissue, cytotoxic enterotoxins and hemolysins cause virulence factors. The level of fish resistance to *A. hydrophila* affects the state of the target organ tissues of infection, including the gills, spleen, liver, and kidneys. The scoring value of kidney damage after a challenge test against *A. hydrophila* in the group of Dayak onion treatment suggested a mild damage compared to that in the group of C+ treatment (Table 2). The bioactive component of Dayak onion can regulate the immune system in Nile tilapia against *A. hydrophila* infection. According to Dawood *et al.* (2020), feeding immunostimulants can increase degenerative changes in the histology of the gills, liver, spleen, and intestines. The results of histopathological analysis of Nile tilapia's kidney tissue revealed immune system alterations caused by *A. hydrophila* exposure.

Table 2. The histopathological test score of the kidney of Nile tilapia

Treatments	Necrosis (%)	Observation
C-	13.85±0.99 ^{a}	Normal
C+	46.06±2.14 ^{d}	Moderate
P5	31.67±1.4 ^{c}	Severe
P10	28.33±0.78 ^{bc}	Severe
P15	25.10±2.03 ^{b}	Severe
E0.5	24.81±1.37 ^{b}	Severe

Data were presented as mean ± SE and different letters indicate significantly different treatments (P<0.05). C- (negative control); C+ (positive control); P5 (5% Dayak onion powder); P10 (10% Dayak onion powder); P15 (15% Dayak onion powder); and E05 (0.5% Dayak onion extract).

3.4 Immune Gene Expression of Nile tilapia

The gene expression levels of IL-1β and TNF-α in tilapia kidney organs were observed before and after the challenge test against *A. hydrophila.* Before the challenge test, the levels of IL-1β and TNF-α gene expression in E05 treatment group were significantly different compared to those in the control groups (P<0.05). After the challenge test, the IL-1β gene expressions of P5 and P15 treatments groups were significantly different from that of the control group (Figure 9). In contrast, the TNF-α gene expressions of the Dayak onion treatment group did not show a significant difference compared to the control treatment group (P<0.05). The IL-1β is a proinflammatory cytokine secreted by macrophages after the activation of T cells and macrophages (Zhang *et al*., 2020). The IL-1β gene partakes in fish immune responses (Amenyogbe *et al*., 2022) as an inflammatory gene that regulates resistance to invading bacteria and keeps balance in fish (Liu *et al*., 2020). The administration of Dayak onion extract significantly increased the regulation of IL-1β gene expression than the control. Upregulation of IL-1β gene expression was shown previously in tilapia after administration of *Lycium barbarum* polysaccharide (Zhang *et al*., 2020). Dayak onion as an immunostimulant contributes to regulating proinflammatory cytokines. Nile tilapia gene expression levels were downregulated as post-infection, which suggests that cytokine genes are involved in inflammation. The transcription of pro-inflammatory cytokine genes takes part in maintaining immunological balance and increasing resistance to pathogenic infections (Moustafa *et al*., 2020). Administration of tea tree oil to yellow catfish liver showed downregulated proinflammatory factors against inflammatory response (Liu *et al*., 2022). Chinese herbs can induce the expression gene of IL-1β in animal immune tissues (Zhang *et al*., 2020).

The TNF-α gene is a proinflammatory cytokine that plays an important role in the innate immune (Liew, 2003) as an antitumor cytokine that inhibits tumor cell proliferation and generates inflammatory responses to pathogen invasion and immunological regulation (MacKenzie *et al*., 2003). The TNF-α gene expression is upregulated in Nile tilapia treated with Dayak onion. After the challenge test against *A. hydrophila*, Nile tilapia gene expression was downregulated (Figure 10). In previous studies, the TNF-α gene after a challenge test against *A. hydrophila* in the spleen and kidney organs of mandarin fish decreased after 72 hours (Chen *et al.* 2018). The TNF-α gene in inflammation and immune response functions as an endocrine that becomes a paracrine facilitator in controlling differentiation and

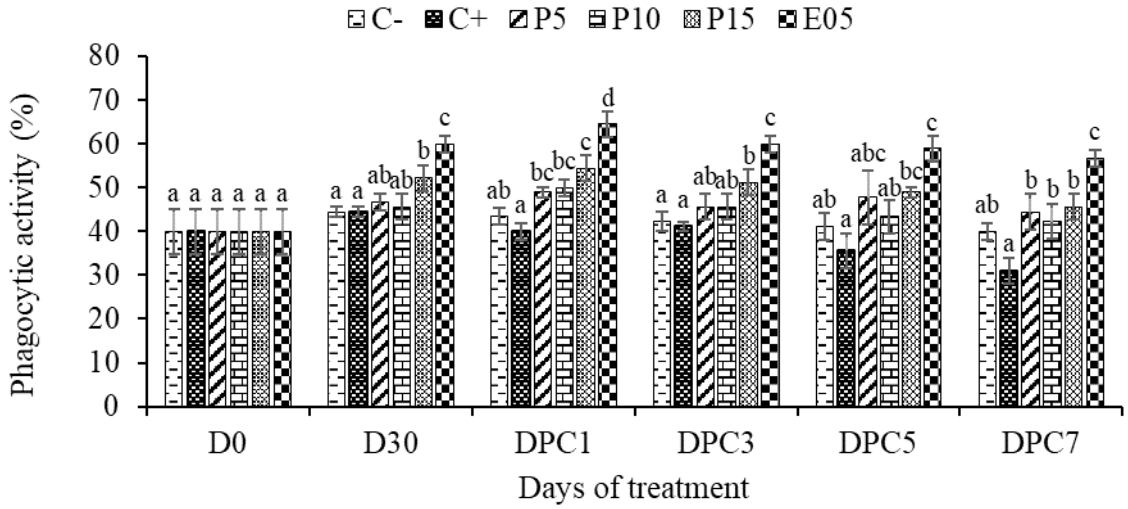

Figure 6. Phagocytic activity of Nile tilapia on days 0 and 30 pre-challenge (D); days 1, 3, 5, and 7 post-challenges (DPC) against *A. hydrophila* (mean ± SE). Different letters indicate significantly different treatments (P<0.05).

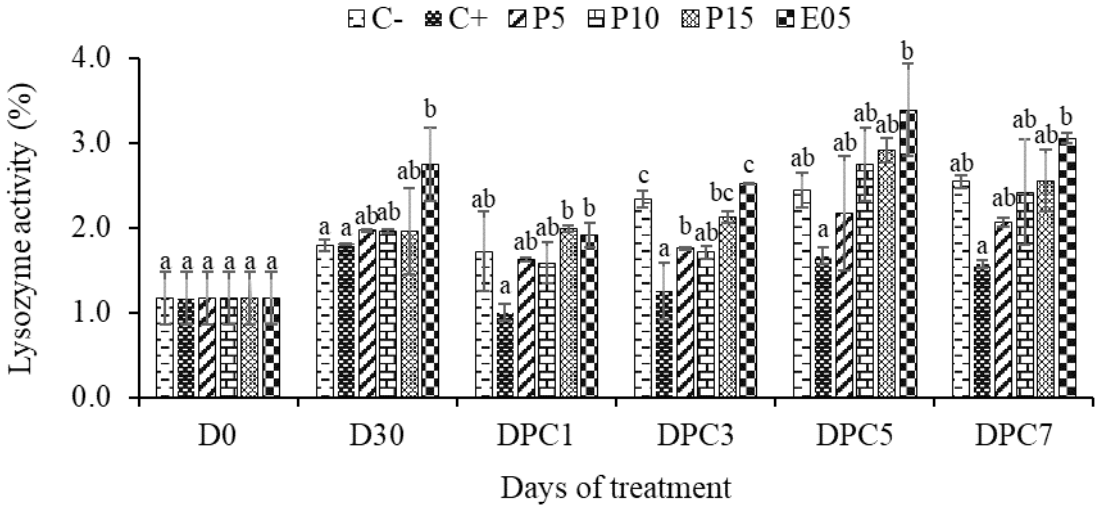

Figure 7. Lysozyme activity of Nile tilapia on days 0 and 30 pre-challenge (D); days 1, 3, 5, and 7 post-challenges (DPC) against *A. hydrophila* (mean ± SE). Different letters in the same period indicate significantly different treatments (P<0.05).

comprehensive cell growth multiplicity (Amenyogbe *et al.*, 2022). Herbal plants act as immunomodulators that increase the expression of pro-inflammatory cytokines in liver and kidney organs after *A. hydrophila* infection (Moustafa *et al.*, 2020). The administration of Dayak onions for 30 days can induce transcription of IL-1β and TNF-α, indicating that Dayak onions can increase the production of anti-inflammatory factors.

3.5 Resistance to A. hydrophila

After the challenge test of *A. hydrophila*, fish mortality occurred in all treatment groups except in the negative control group. The highest survival rate was found in the P15 treatment group and showed a significant difference from that in the positive control, but not significantly different from the P5, P10, and E05 (P<0.05) (Figure 11). Dayak onion supplementation for 30 days resulted in a survival rate above 50% of the number of fish. The immunostimulant effect of Dayak onions is influenced by flavonoids, saponins, alkaloids, steroids, and phenols. Other components contained in forest onions are polyphenols, quinones, and terpenoids (Munaeni *et al.*, 2017). The higher resistance to bacterial infection is due to the restoration of normal physiological functions in the body's immune system and the protective role of lysozyme and peroxidase serum (Sherif *et al.*, 2022). Earlier research into the effects of propolis supplementation and aloe vera on the immunomodulation of tilapia infected with *A. hydrophila* was evaluated based on the survival rate of fish (Dotta *et al.*, 2018).

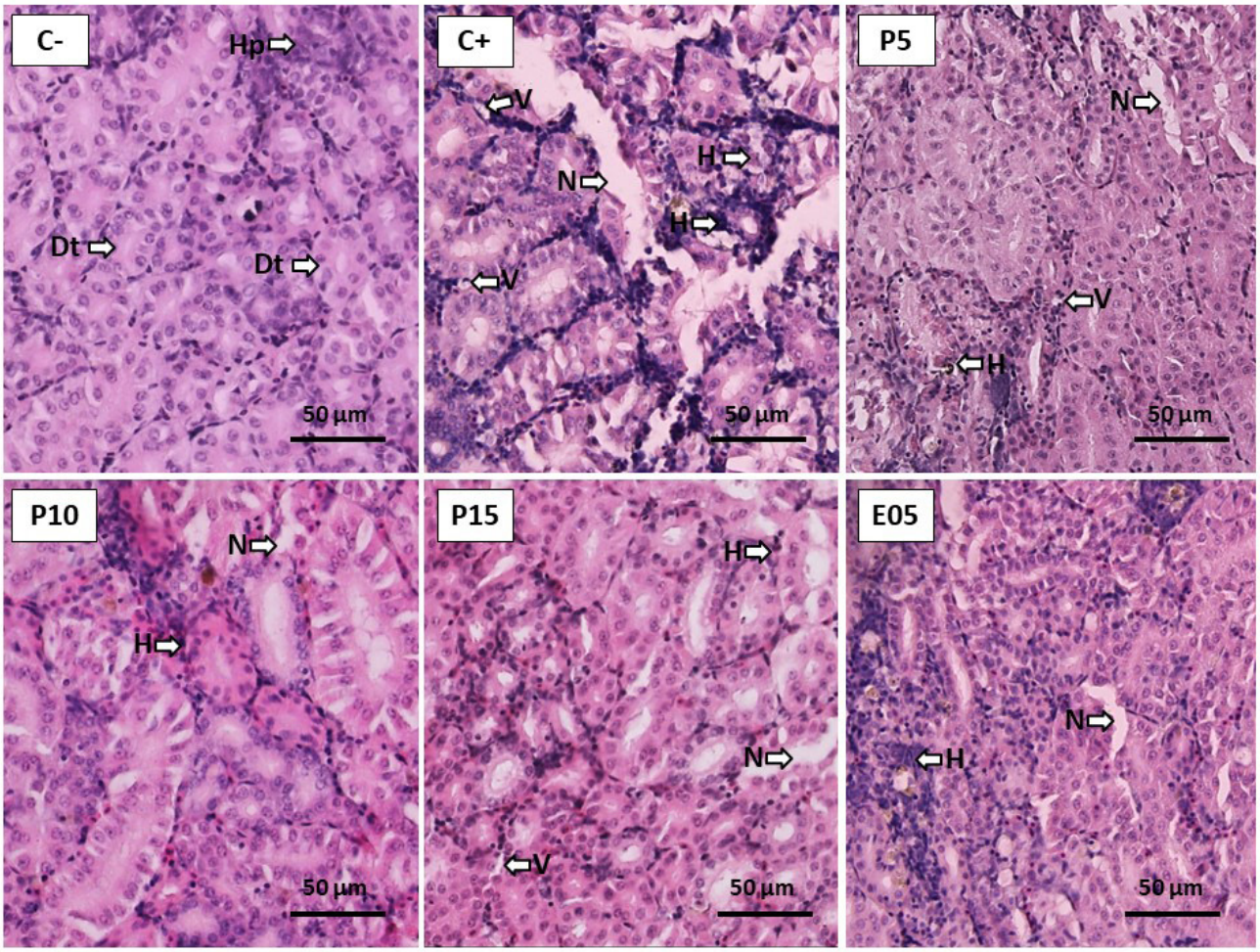

Figure 8. Histopathology of Nile tilapia kidney post-challenge against *A. hydrophila* in different fed diets control and Dayak onion treatment groups during 30-days rearing. Treatment C- showed a normal histopathological structure consisting of distal tubules (Dt) and hematopoietic tissue (Hp); treatment C+, P5, P10, P15, and E05 after *A. hydrophila* challenge showed symptoms of necrosis (N), hemorrhage (H), and vacuolization (V) in the kidney tubule area.

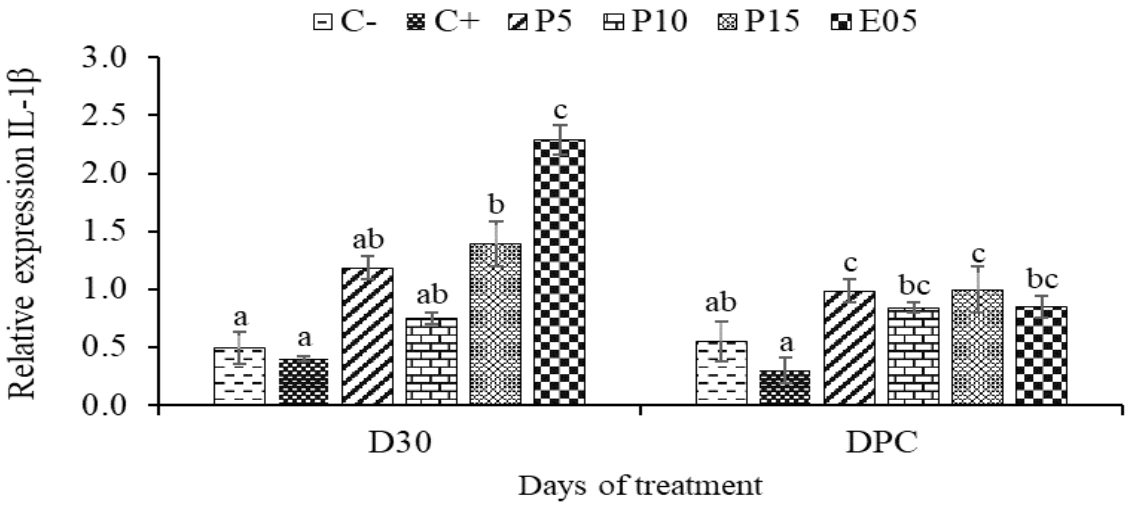

Figure 9. IL-1β gene expression level of Nile tilapia kidney pre-challenge (D30) and day post-challenge (DPC) against *A. hydrophila* (mean ± SE). Different letters indicate significantly different treatments ($P<0.05$).

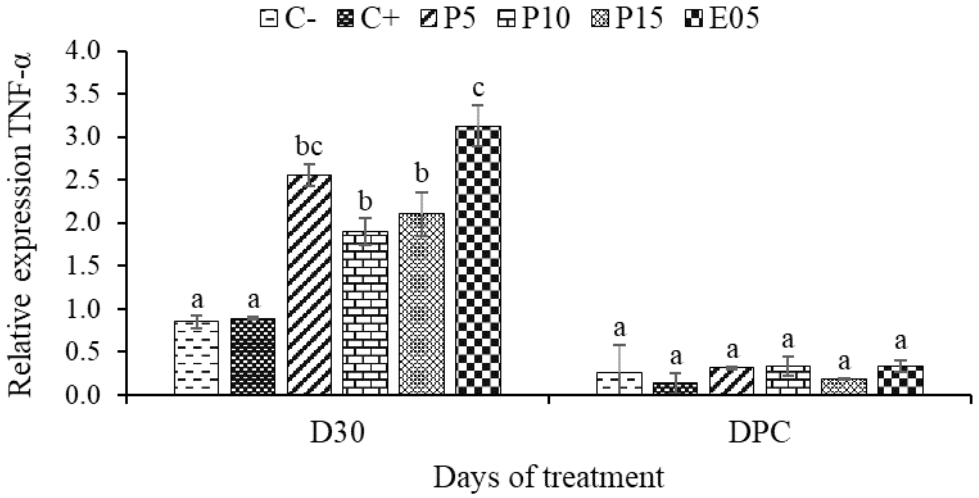

Figure 10. The TNF-α gene expression level of Nile tilapia kidney pre-challenge (D30) and day post-challenge (DPC) against *A. hydrophila* (mean ± SE). Different letters indicate significantly different treatments (P<0.05).

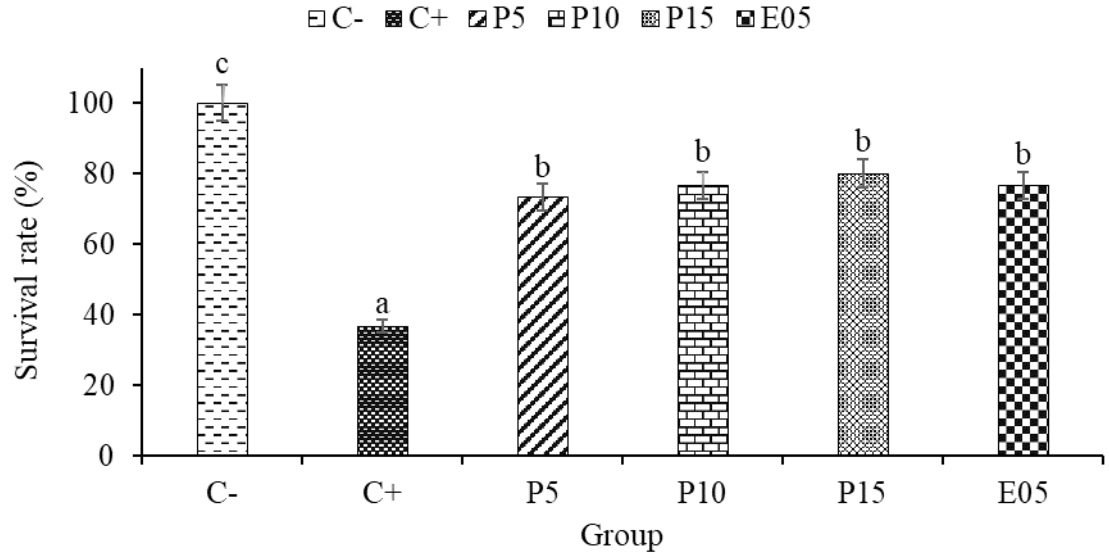

Figure 11. Nile tilapia survival rate post-challenge test against *A. hydrophila* for seven days (mean ± SE). Different letters indicate significantly different treatments (P<0.05).

4. Conclusion

The administration with 15% powder and 0.5% crude extract Dayak onions could improve the hematological levels, immune responses, expression of immunity-related genes, and resistance of the Nile tilapia against *A. hydrophila* infection compared to the control treatments. It is shown that Dayak onion supplementation effectively increased the health status of Nile tilapia against *A. hydrophila* infection.

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Authors' Contributions

The contribution of each author is as follows, ARF; participated in the study design, collected and analyzed data, and wrote the original manuscript. MY; participated in the design analysis and formal analysis and provided the critical revision. W and MS; participated in supervision and verification of data. UA; reviewed and edited the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

All authors declare that they have no conflicts of interest.

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