Original article DOI: 10.19027/jai.25.1.129-137

Hematology profile of kissing gourami *Helostoma temminckii* infected with *Aeromonas hydrophila* bacteria

Profil hematologi ikan tambakan *Helostoma temminckii* yang diinfeksi bakteri *Aeromonas hydrophila*

Adna Sumadikarta[1], Sata Yoshida Srie Rahayu[1], Sri Nuryati[2*]

[1]Environmental Management, Post Graduate Study Program, Pakuan University, Central Bogor 16143, West Java, Indonesia
[2]Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University, Dramaga 16680, West Java, Indonesia
*Corresponding author: sri_nuryati@apps.ipb.ac.id

(Received March 11, 2025; Revised April 10, 2025; Accepted August 28, 2025)

ABSTRACT

The kissing gourami (*Helostoma temminckii*) is an important commodity in aquaculture, but its susceptibility to bacterial infections such as *Aeromonas hydrophila* can hinder production. This study aims to investigate the hematological profile of kissing gourami (*Helostoma temminckii*) with an average length of 8.00 ± 1.00 cm and weight of 10.00 ± 1.00 g, following intramuscular injection of *Aeromonas hydrophila* at a dose of 10^6 CFU/mL. Evaluations were conducted on survival rate, hematological parameters such as total erythrocytes, total leukocytes, hemoglobin levels, and hematocrit, as well as phagocytic activity, respiratory burst, and lysozyme activity. Infection with *A. hydrophila* caused a decrease in erythrocytes and hemoglobin, and an increase in leukocytes, phagocytic activity, respiratory burst, and lysozyme activity. The survival rate dropped to 47.5% in the treatment group, while the control group showed 100% survival. The conclusion is, infection of *Aeromonas hydrophila* significantly affects the immune response of kissing gourami, characterized by a decrease in erythrocyte count and hemoglobin levels in the treatment group. However, phagocytic activity, respiratory burst, and lysozyme levels consistently decreased in infected fish.

Keywords: *Aeromonas hydrophila*, hematology, kissing gourami, pathogenicity

ABSTRAK

Ikan tambakan (*Helostoma temminckii*) merupakan komoditas penting dalam budidaya perikanan, namun kerentanannya terhadap infeksi bakteri seperti *Aeromonas hydrophila* dapat menghambat produksi. Penelitian ini bertujuan untuk mengkaji profil hematologi ikan tambakan (*Helostoma temminckii*) dengan panjang rata-rata 8,00 ± 1,00 cm dan bobot 10,00 ± 1,00 g setelah disuntik intramuskular *Aeromonas hydrophila* dengan dosis 10^6 CFU/mL. Evaluasi dilakukan terhadap tingkat kelangsungan hidup, parameter hematologi seperti jumlah eritrosit total, leukosit total, kadar hemoglobin, dan hematokrit, serta aktivitas fagositosis, *respiratory burst*, dan aktivitas lisozim. Infeksi *A. hydrophila* menyebabkan penurunan jumlah eritrosit dan kadar hemoglobin, serta peningkatan jumlah leukosit, aktivitas fagositosis, *respiratory burst*, dan aktivitas lisozim. Tingkat kelangsungan hidup menurun menjadi 47,5% pada kelompok perlakuan, sedangkan kelompok kontrol menunjukkan kelangsungan hidup 100%. Kesimpulannya, infeksi *Aeromonas hydrophila* secara signifikan mempengaruhi respons imun ikan kissing gourami, yang ditandai dengan penurunan jumlah eritrosit dan kadar hemoglobin, serta peningkatan aktivitas fagositosis, *respiratory burst*, dan kadar lisozim pada ikan yang terinfeksi.

Kata kunci: *Aeromonas hydrophila*, hematologi, ikan tambakan, patogenisitas

INTRODUCTION

Global aquaculture production has experienced significant growth, with Indonesia being one of the largest aquaculture producers in Asia. Among the key aquaculture commodities is the kissing gourami (*Helostoma temminckii*), a species known for its remarkable adaptability to extreme environmental conditions, including low oxygen levels and acidic waters (Helmizuryani *et al.,* 2021). These characteristics make *H. temminckii* a preferred species for intensive aquaculture systems, particularly in regions such as Java, Sumatra, and Kalimantan (Sibagariang *et al.,* 2023). However, the intensification of aquaculture through high stocking densities has been associated with an increased risk of pathogenic infections, particularly those caused by *Aeromonas hydrophila* (Indriasari *et al.,* 2020).

Aeromonas hydrophila bacteria is one of the main pathogens that attack *kissing gourami* and cause Motile *Aeromonas* Septicemia (MAS) disease. This disease is characterized by clinical signs such as skin hemorrhages, internal organ damage, loss of appetite, and increased mortality in fish (Rochani *et al.,* 2021). *A. hydrophila* possesses various virulence factors, exotoxins, proteolytic enzymes, and biofilm formation capability, which directly impact fish health and immune resistance to infection (Abdella *et al.,* 2023; El-Hossary *et al.,* 2023). Under intensive aquaculture conditions, this bacterium can spread rapidly, reduce productivity, and cause significant economic losses for fish farmers (Maldonado *et al.,* 2022).

The hematological profile is a crucial parameter for assessing the impact of bacterial infections on kissing gourami. Hematological analysis includes measurements of parameters such as erythrocyte and leukocyte counts, hemoglobin levels, and hematocrit values, as well as phagocytic activity, lysozyme activity, and respiratory burst. Changes in these parameters often serve as indicators of physiological disturbances caused by infection (Docan *et al.,* 2018). *A. hydrophila* infection has been reported to cause significant alterations, such as a decrease in phagocytic activity, an increase in leukocyte count, and changes in hemoglobin levels, reflecting the fish's immune response to the pathogen (El-Bahar *et al.,* 2019).

Investigating the effects of *A. hydrophila* infection on the hematological profile of kissing gouramis is essential for understanding the pathogenicity of this bacterium. The results of this study will provide valuable insights into the mechanisms of the immune response of fish to infection and it will support the development of more effective disease control strategies. Approaches such as vaccination, improved fish health management, and improved environmental quality in aquaculture are critical to preventing disease outbreaks. With proper management, kissing gourami aquaculture can contribute to the sustainability of Indonesia's aquaculture sector while improving the quality and safety of seafood products (Arifin *et al.,* 2017). This study is intended to serve as a scientific reference for future research and the development of sustainable disease prevention strategies in aquaculture.

MATERIALS AND METHODS

Time and place

This research was conducted from August to November 2024 at the Aquatic Organism Health Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University.

Preparation of test fish

Fish were obtained from farmers in Neglasari Village, Dramaga, Bogor, while *A. hydrophila* was sourced from the collection of the Aquatic Organism Health Laboratory, isolated from an infection in tilapia. The test fish were kissing gourami (*H. temminckii*) with a length of 8.00 ± 1.00 cm and a weight of 10.00 ± 1.00 g. Fish were maintained in a 60×30×35 cm^3 aquarium containing 36 L of water at a stocking density of 10 fish per aquarium for the LD_{50} and challenge tests. The challenge test was conducted to evaluate the immune response and resistance of fish to infection with *A. hydrophila* bacteria.

Research design

This study consists of a single treatment, which involves the injection of *Aeromonas hydrophila* (IAh), compared to a control group, with four replications for each group. The IAh group was injected intramuscularly with *A. hydrophila* at a concentration of 10^6 CFU/mL using a dose of 0.1 mL per fish, based on the LD_{50} test results, while the control group was injected with 0.1 mL of PBS solution per fish. The fish were maintained for 14 days, with observations and recordings of changes during the pathogenicity test period.

Procedure

Preparation of bacteria test and a preliminary test

The *A. hydrophila* isolate, obtained from infected tilapia through the Aquatic Organism Health Laboratory, was cultured on tryptic soy agar (TSA), rimler-shotts agar (RS), and tryptic soy broth (TSB), each with a volume of 10 mL, and incubated at 28–30°C for 24 hours in a shaking incubator at a rotation speed of 150 rpm. To confirm that the isolate was *A. hydrophila*, biochemical identification was carried out using tests following standard protocols outlined in Cowan and Steel's Manual for the Identification of Medical Bacteria. Bacterial density was measured by total plate count method and used for LD_{50} test. A preliminary test was conducted by infecting healthy fish with the bacterial isolate and observing disease progression and mortality. These observations were used to determine the effective dose of infection for subsequent studies.

Virulence enhancement of test bacteria

The virulence enhancement of the test bacteria was carried out by Koch's Postulate procedure. Fish were injected intramuscularly with bacteria that had been cultured in TSB media. The stocking density of fish carried out for Koch's Postulate procedure was 10 fish per aquarium for each test bacteria. Fish were observed for 24 hours. If no deaths occur within this period, a bacterial replacement procedure is carried out. This involves re-isolating the injected fish, which are then cultured on agar and TSB media. The re-isolated bacteria are then re-identified to ensure the species of pathogenic bacteria injected. Identification is performed using biochemical tests such as the ones listed in the Cowan and Steel's Manual for the Identification of Medical Bacteria. After ensuring the appropriate bacteria, the re-culture results are reinjected into the fish. This stage involves isolating bacteria from the kidneys and clinical wounds.

Pathogenicity test

A pathogenicity test was conducted by injecting the test fish with each bacterium. The injection was done intramuscularly with a dose of 0.1 mL per fish, containing *A. hydrophila* at a concentration of 10^6 CFU/mL. After infection, hematological profile was observed by taking blood sampling on days 0, 2, 5, 7, and 14. During maintenance, fish were fed commercial feed twice a day using the at satiation method.

Observation parameters

This study observed several parameters, including the hematological profile, such as total erythrocytes and leukocytes, measured by a hemocytometer (Blaxhall & Daisley, 1973), hemoglobin levels determined using a Sahli hemoglobinometer (Wedemeyer & Yasutake, 1977), and hematocrit levels measured by centrifugation in hematocrit tubes (Anderson & Siwicki, 1995). Immune response was assessed by phagocytosis activity and respiratory burst, using particle engulfment and ROS production assays (Anderson & Siwicki, 1995), as well as lysozyme activity through bacterial lysis (Anderson & Siwicki, 1995). Lastly, the survival rate of kissing gourami after *A. hydrophila* challenge was monitored to evaluate the impact of the infection.

Data analysis

Data from the pathogenicity test results of *A. hydrophila* from day 0 to day 14 were tabulated using Microsoft Excel and analyzed using SPSS 20 software to determine significant differences between treatments and controls. If there were only two treatments, the T-test was used to compare the means. For more than two treatments, ANOVA was applied, followed by Duncan's test if the ANOVA results were significantly different ($P<0.05$), with a 95% confidence interval.

RESULTS AND DISCUSSION

RESULTS

Total erythrocytes

Total erythrocytes of kissing gourami on day 0 were recorded at 2.85×10^6 cells/mm³ (Figure 1). After the challenge test, the total erythrocyte counts in the IAh-treated group showed a decrease from day 2 to day 14, with significantly lower values than the control group. In the control group, the total erythrocyte count remained stable in the range of 2.44-3.23 cells/mm³, while in the IAh treatment group, the erythrocyte count decreased to 1.39 cells/mm³ on day 14.

Total leukocytes

The total leukocyte count of kissing gourami in the control and IAh treatment groups is presented in Figure 2. On day 0, the total leukocytes in both groups were 2.20 cells/mm³. However, from day two to day seven, the total leukocytes in the control group increased significantly and peaked on day five. Similarly, the total leukocytes in the IAh-treated group increased significantly after

the challenge test, peaking at 4.23 cells/mm³ on day five. On day 14, total leukocytes showed a decrease in both groups, with values of 2.79 cells/mm³ for the control and 3.97 cells/mm³ for the IAh treatment.

Hemoglobin

Hemoglobin levels of kissing gourami are presented in Figure 3. On day 0, the hemoglobin levels of both groups were the same at 10.98 g/%. After the challenge test (day 2 to day 14), hemoglobin levels in the control group decreased significantly to the lowest value on day 5, before increasing again to 8.18 g/% on day 14. In contrast, the IAh treatment showed a more stable and consistently higher hemoglobin than the control.

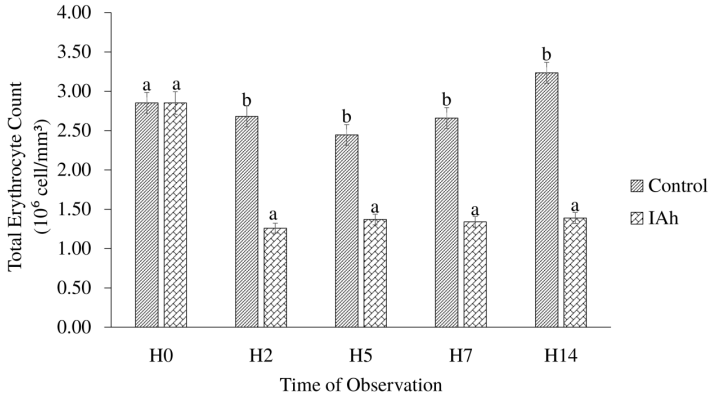

Figure 1. Total erythrocytes of kissing gourami before and after *A. hydrophila* challenge test. Different superscript letters within the same parameter row indicate significant treatment differences (P<0.05).

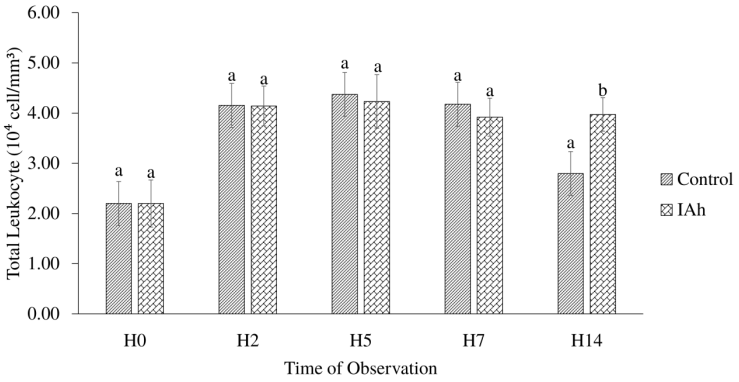

Figure 2. Total leukocytes of kissing gourami before and after *A. hydrophila* challenge test. Different superscript letters within the same parameter row indicate significant differences among treatments (P<0.05).

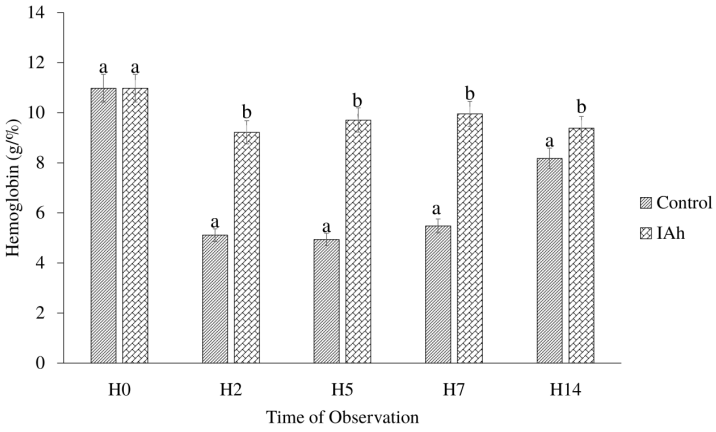

Figure 3. Hemoglobin of kissing gourami before & after *A. hydrophila* challenge test. Different superscript letters within the same parameter row indicate significant differences among treatments (P<0.05).

Hematocrit

The hematocrit of kissing gourami in the control and IAh treatment groups is presented in Figure 4. On day 0, the hematocrit values of both groups were not significantly different. However, after the challenge test (day 2 to day 14), the control group experienced a significant decrease, with the lowest value of 9.88 % on day 5 before slightly increasing to 19.10 % on day 14. In contrast, the IAh treatment group maintained consistently higher hematocrit values than the control.

Phagocytic activity

Phagocytic activity in the control and treatment groups is shown in Figure 5. On day 0, AF was the same in both groups at 42.70%. After the challenge test, the control group showed lower values with AF values ranging from 42.70 to 52.70% throughout the observation period. In contrast, the treatment group showed higher phagocytic activity after bacterial infection, with a peak AF of 52.80% on day 2 (P<0.05).

Respiratory burst

The respiratory burst of kissing gourami is presented in Figure 6. On day 0, the respiratory burst value of both groups was the same at 0.41. After the challenge test, the control group showed a higher value on day 2 until it reached its peak on day 5 with a RB value of 0.48, then decreased until day 14 to 0.42. In contrast, the treatment group showed lower respiratory burst values, because *A. hydrophila* infection can reduce the ability of immune cells to produce specific reactive oxygen, which plays a role in the body's defense response to pathogens.

Lysozyme activity

The lysozyme activity in the control and treatment groups is shown in Figure 7. On day 0, the lysozyme activity values of both groups were the same, amounting to 275.80 U/mL. After the challenge test, the control group showed a significant increase on day 2 until it reached the highest value on day 2 of 397.70 U/mL, then

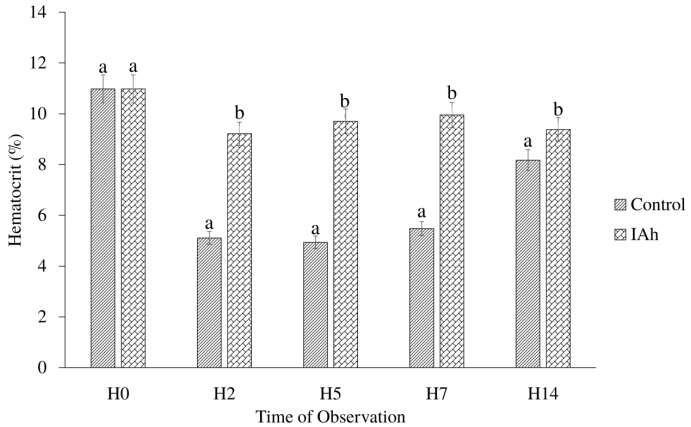

Figure 4. Hematocrit of kissing gourami before & after *A. hydrophila* challenge test. Different superscript letters within the same parameter row indicate significant differences among treatments (P<0.05).

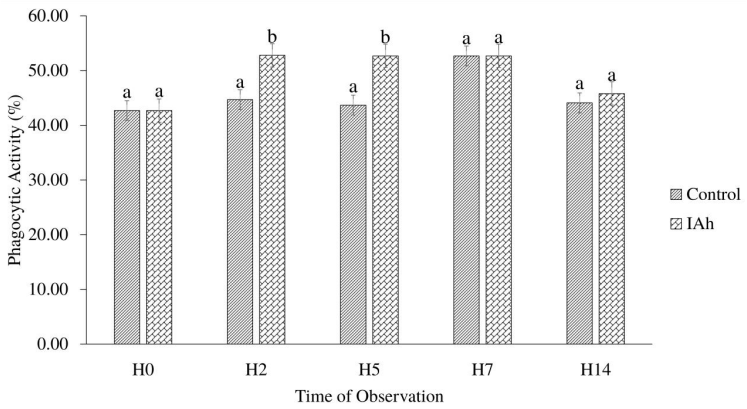

Figure 5. Phagocytic activity of kissing gourami before and after *A. hydrophila* challenge test. Different superscript letters within the same parameter row indicate significant differences among treatments (P<0.05).

gradually decreased until day 14 with a value of 341.50 U/mL. In contrast, the treatment group showed lower and stable lysozyme activity with a range of 313.57 U/mL - 323.80 U/mL during the observation period.

Post-infection survival rate

The post-challenge survival rate showed that the control group had a 100 %, indicating that the kissing gourami could fully survive under untreated conditions. In contrast, the treatment

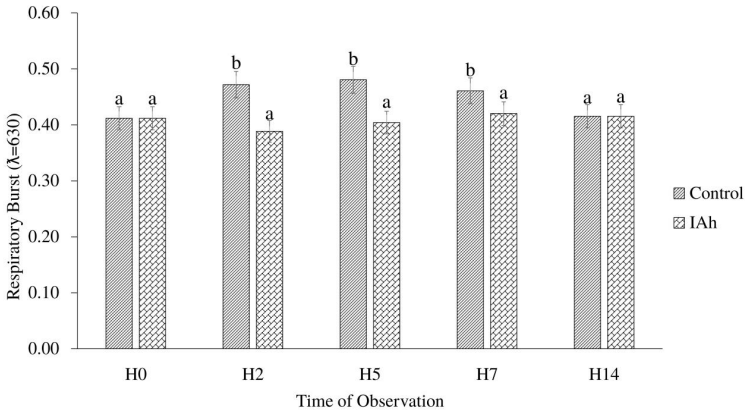

Figure 6. Respiratory burst of kissing gourami before and after *A. hydrophila* challenge test. Different superscript letters within the same parameter row indicate significant differences among treatments (P<0.05).

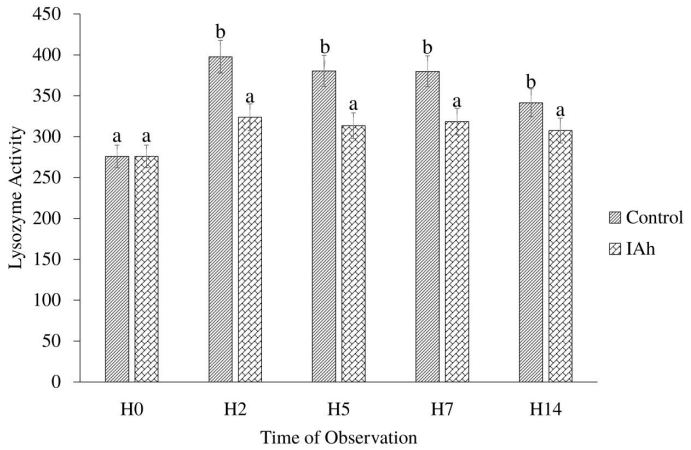

Figure 7. Lysozyme activity of kissing gourami before and after *A. hydrophila* challenge test. Different superscript letters within the same parameter row indicate significant treatment differences (P<0.05).

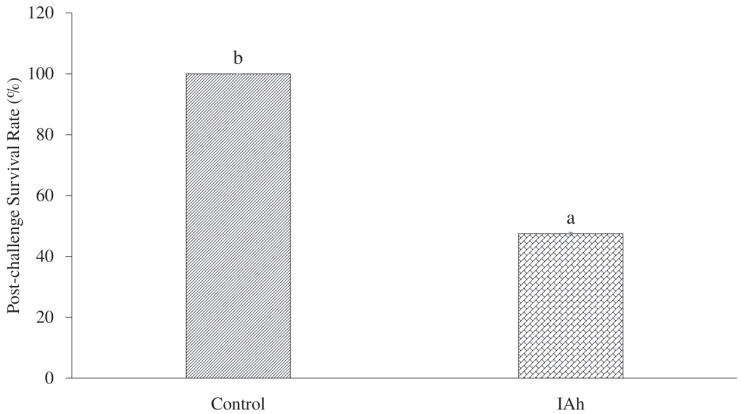

Figure 8. Survival rate of kissing gourami after *A. hydrophila* challenge test. Different superscript letters within the same parameter row indicate significant differences among treatments (P<0.05).

group with *A. hydrophila* injection only achieved a survival rate of 47.5 % reflecting a significant reduction in survival due to the effect of the treatment.

Discussion

Aeromonas hydrophila infection causes hematological changes in fish, affecting parameters such as erythrocyte count, leukocyte count, hemoglobin, hematocrit, phagocytic activity, respiratory burst, and lysozyme activity. In this study, the erythrocyte count in *Helostoma temminckii* showed a significant decrease in the *A. hydrophila* injected (IAh) group compared to the control group. On day 0, the erythrocyte count was recorded at 2.85×10^6 cells/mm³. After the challenge, the IAh-treated group showed a significant decrease in erythrocytes from day 2 to day 14. In contrast, the control group's erythrocyte count remained stable within the range of $2.44\text{-}3.23\times10^6$ cells/mm³ throughout the observation period. The decrease in erythrocytes indicates a hematological response to bacterial infection, likely due to hemolysis from bacterial toxins, increased destruction by the immune system, or disruptions in erythrocyte production in the bone marrow (Cerlina *et al.,* 2021). The normal erythrocyte range for fish is $1.0\text{–}5.0\times10^6$ cells/mm³ (Esmaeili 2021).

Regarding leukocytes, both groups had the same count on day 0 (2.20×10^6 cells/mm³). However, in the IAh-treated group, there was a significant increase in leukocytes from day 2 to day 7, peaking at 4.23×10^6 cells/mm³ on day 5, as an immune response to *A. hydrophila* infection. This increase is related to proinflammatory cytokine production (e.g., IL-1β and TNF-α), which stimulates leukocyte proliferation and migration to the infection site (Mokhtar *et al.,* 2023; Bakry *et al.,* 2024). The increase reflects the activation of an innate immune response to inhibit pathogen development and accelerate healing (Sayyaf *et al.,* 2023). By day 14, leukocyte counts returned to baseline levels, indicating immune recovery. The normal leukocyte range for healthy fish is $2.9\text{–}4.1\times10^4$ cells/mm³ (Pattipeilohy *et al.,* 2020).

Hemoglobin levels showed significant differences between groups. On day 0, both groups had the same hemoglobin level (10.98 g/%). However, the control group showed a significant decrease in hemoglobin, especially on day 5, with a lowest value of 4.94 g/%, reflecting a physiological stress response. Conversely, the IAh-treated group showed an increase in hemoglobin levels (9.21-9.95 g/%) after day 0, indicating an adaptive response to infection. This increase suggests that the fish were able to increase hemoglobin production to support the body's increased oxygen demands during infection. The normal hemoglobin range for fish is 6–11 g/% (Azhari *et al.,* 2020).

Hematocrit levels in the IAh group showed a significant increase (22.66-26.35%) after infection, indicating an adaptive response to the bacterial stress. This increase could reflect the production of red blood cells to enhance oxygen transport during infection. In contrast, the control group showed a decrease in hematocrit due to post-inoculation stress, which affects red blood cell production through stress hormones like cortisol. The normal hematocrit range for healthy fish is 20-30% (Bond, 1979).

Phagocytic activity in the IAh-treated group increased significantly on days 2 and 5 (42.70-52.80%), indicating an immune response to infection. Phagocytosis is activated when immune cells like macrophages and neutrophils recognize pathogen-associated molecular patterns (PAMPs) on the bacteria (Kirchhoff *et al.,* 2025). On day 14, phagocytic activity decreased to 45.77%, suggesting the immune system was reorganizing after the initial response. In contrast, the control group's phagocytic activity remained stable (42.70-52.70%), with a slight increase due to the stress of the injection procedure. For respiratory burst (RB), the control group showed an increase on day 5, peaking at 0.48, indicating a stronger immune response to the PBS injection, which may have triggered a mild inflammatory reaction.

The IAh group showed consistently lower RB values throughout the study (0.39-0.42), indicating a more controlled immune response to infection. This controlled response suggests that the immune system regulated the production of reactive oxygen species (ROS) to avoid tissue damage due to excessive inflammation. Lysozyme activity in the control group significantly increased on day 2 (397.70 U/mL), then gradually decreased to 341.50 U/mL on day 14, showing that the fish responded to the PBS injection by increasing lysozyme production. However, the IAh-treated group showed stable lysozyme activity (313.57-323.80 U/mL), the stability of lysozyme activity in the IAh group after the initial phase indicates a shift in immune priority from the innate response (lysozyme) to the development of specific adaptive immunity. The sustained, significant lysozyme elevation observed in the PBS-injected

control did not occur because IAh is an inactivated antigen that does not induce prolonged infection or pathological stress (Mulia *et al.,* 2022).

Post-challenge survival rates revealed that the control group had a 100% survival rate, indicating that kissing gourami could fully survive without treatment. In contrast, the IAh-treated group experienced a significant survival rate reduction to 47.5%, confirming that *A. hydrophila* has pathogenic properties that decrease the survival rate by causing infections that lead to mortality. Factors that influence *A. hydrophila* pathogenicity include bacterial virulence factors, the fish's immune response, bacterial dose, and environmental conditions (Nhinh *et al.,* 2021). These factors can weaken the fish's immune defenses, making them more susceptible to severe infections. Studies by Bahariyanto *et al.* (2025) also showed that *A. hydrophila* is a highly virulent pathogen that can cause mortality rates 80-100% in farmed fish within 1-2 weeks. This confirms that the bacteria's high virulence is the main cause of the decrease in survival rates of infected fish.

CONCLUSIONS

This study showed that the pathogenicity of *A. hydrophila* bacteria in kissing gourami caused significant changes in the immune response, which were reflected in the hematological profile. Decreases in total erythrocytes, hemoglobin and hematocrites were observed in the group subjected to bacterial injection (IAh), while total leukocytes, phagocytic activity and lysozyme were increased after infection. The survival rate in the IAh treatment (47.5%) was lower than that in the control group (100%). These results confirmed that *A. hydrophila* significantly affected the immune response as well as the survival rate of fish.

REFERENCES

Abdella B, Abozahra NA, Shokrak NM, Mohamed RA, El-Helow ER. 2023. Whole spectrum of *Aeromonas hydrophila* virulence determinants and the identification of novel SNPs using comparative pathogenomics. Scientific Reports 13: 7712.

Arifin OZ, Prakoso VA, Pantjara B. 2017. Ketahanan ikan tambakan (*Helostoma temminkii*) terhadap beberapa parameter kualitas air dalam lingkungan budidaya. Jurnal Riset Akuakultur 12: 241–251. (In Indonesian).

Anderson DP, Siwicki AK. 1995. Basic hematology and serology for fish health programs. Manila, Phillipines: Fish Health Section, Asian Fisheries Society.

Azhari M, Handayani L, Nurhayati N. 2020. Pengaruh penambahan arang aktif tulang ikan pada pakan terhadap gambaran darah ikan nila (*Oreochromis niloticus*). Jurnal Tilapia 1: 19–27.

Bahariyanto DR, Amalo P, Pratiwi R. 2025. Macroscopic and biochemical tests on *Aeromonas hydrophila* as a diagnostic approach for bacterial infection in nile tilapia *Oreochromis niloticus.* Jurnal Megaptera 4: 21–32.

Bakry KA, Emeish WFA, Embark HM, Elkamel AA, Mohammed HH. 2024. Expression profiles of four nile tilapia innate immune genes during early stages of *Aeromonas veronii* infection. Journal of Aquatic Animal Health 36: 164–180

Blaxhall PC, Daisley KW. 1973. Routine haematological methods for use with fish blood. Journal of Fish Biology 5: 771–781.

Bond, C. E. 1979. Biolroyaogy of Fishes. USA: W. B. Saunders Company.

Cerlina M, Riauwaty M, Syawal H. 2021. Gambaran eritrosit ikan lele dumbo (*Clarias gariepinus*) yang terinfeksi *Aeromonas hydrophila* dan diobati dengan larutan daun salam (*Syzygum polyantha*). Jurnal Perikanan dan Kelautan 27: 105–113. (In Indonesian)

Docan A, Grecu I, Dediu L. 2018. Use of hematological parameters as assessment tools in fish health status. Journal of Agrolimentary Processes and Technology 24: 317–324.

El-Bahar HM, Ali NG, Aboyadak IM, Khalil SAES, Ibrahim MS. 2019. Virulence genes contributing to *Aeromonas hydrophila* pathogenicity in *Oreochromis niloticus*. International Microbiology 22: 479–490.

El-Hossary D, Mahdy A, Elariny EYT, Askora A, Merwad AMA, Saber T, Dahshan H, Hakami NY, Ibrahim RA. 2023. Antibiotic resistance, virulence gene detection, and biofilm formation in *Aeromonas* spp. isolated from fish and humans in Egypt. Biology 12: 421.

Esmaeili M. 2021. Blood Performance: A New Formula for Fish Growth and Health. Biology 12: 1-17

Helmizuryani, Suwignyo RA, Hanafiah Z, Faizal M. 2021. Food preference, growth pattern, condition and reproduction factors of kissing

gourami, *Helostoma temminckii* in the peat swamps of South Sumatera, Indonesia. AACL Bioflux 14: 2583–2600.

Indriasari DE, Dewantoro E, Prasetyo E. 2020. Identification of the bacteria Edwardsiella tarda that infects catfish (*Clarias batrachus*) on several farmers in Sungai Raya District Kubu Raya Regency. Jurnal Borneo Akuatika 2: 30–38.

Kirchhoff R, Chromik MA, Schebb NH. 2025. Phagocytosis is differentially regulatedby LPS in M1- and M2- like macrophages via PGE2 formation and EP4 signaling. Prostaglandins & Other Lipid Mediators 178: 106998

Maldonado-Miranda JJ, Castillo-Perez LJ, Ponce-Hernandez A, Carranza-Alvares C. 2022. Summary of economic losses due to bacterial pathogens in aquaculture industry. Bacterial Fish Diseases 19: 399–417

Mokhtar DM, Zaccone G, Alesci A, Kuciel M, Hussein MT, Sayed RKA. 2023. Main component of fish imunity: an overview of the fish immune system. Fishes 8: 1-24

Mulia DS, Utomo T, Isnansetyo A. 2022. The efficacy o f *Aeromonas hydrophila* GPI-04 feed-based vaccine on African catfish (*Clarias gariepinus*). Biodiversitas 23: 1505-1510.

Nhinh DT, Le DV, Van KV, Giang NTH, Dang LT, Hoai TD. 2021. Prevalence, virulence gene distribution and alarming the multidrug resistance of *Aeromonas hydrophila* associated with disease outbreak in fresh aquaculture. Antibiotics 532: 1-17

Pattipeilohy CE, Suprayudi MA, Setiawati M, Ekasari J. 2020. Evaluation of protein sparing effect in nile tilapia *Oreochromis niloticus* fed with organic selenium supplemented diet. Jurnal Akuakultur Indonesia 19: 84–94

Rochani NQS, Sarjito, Desrina. 2021. Pengaruh kombinasi ekstrak daun binahong dan temulawak pada pakan terhadap total eritrosit dan gejala klinis ikan lele (*Clarias gariepinus*) yang diinfeksi *Aeromonas hydrophila*. Jurnal Sains Akuakultur Tropis 5: 128–135. (In Indonesian).

Sayyaf DB, Lorenzoni M, Carosi A, Giari L, Bosi G. 2023. Teleost innate immunity, an intricate game between immune cells and parasites of fish organs: who wins, who loses. Front Immunology. 14: 1-12

Sibagariang RDR, Hendrizal A, Fauzi M. 2023. Growth pattern and condition factor of tambakan fish (*Helostoma* sp.) from Panjang Lake, Riau. Berkala Perikanan Terubuk 51: 1821–1827.

Wedemeyer G, Yasutake WT. 1977. Clinical Methods for Assessment of the Effects of Environmental Stress on Fish Health. USA: Department of the Interior Fish and Wildlife Service.