

Pathogenicity of *Aeromonas hydrophila* on the liver function of African catfish (*Clarias gariepinus*)

MUH MULTAZAM B. H. ABD HAKIM*, HILAL ANSHARY, MUHAMMAD IQBAL DJAWAD, MUHAMMAD REZA PERDIANSYAH

Department of Fisheries, Faculty of Marine Science and Fisheries, Universitas Hasanuddin. Jl. Perintis Kemerdekaan Km. 10, Makassar 90245, South Sulawesi, Indonesia. Tel./fax.: +62-441-586025, *email: azzamj010@gmail.com

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Abstract. Abd Hakim MMBH, Anshary H, Djawad MI, Perdiansyah MR. 2025. Pathogenicity of *Aeromonas hydrophila* on the liver function of African catfish (*Clarias gariepinus*). *Biodiversitas* 26: 5430-5437. *Aeromonas hydrophila* is a major bacterial pathogen in African catfish (*Clarias gariepinus*) aquaculture, responsible for systemic infection, tissue damage, and high mortality. This study investigated the pathogenic effects of graded acute infections on liver function and histopathology of African catfish under controlled laboratory conditions. Seventy-two fish were allocated to three bacterial treatment groups with increasing concentrations and a control group, each with three replicates. After seven days, blood samples were analyzed for ten biochemical indicators of hepatic function, including plasma proteins, transaminases, bilirubin, bile acids, cholesterol, and liver enzymes, while histopathological examinations were conducted on liver tissues. The infection induced significant alterations in albumin, globulin, total protein, aspartate aminotransferase, alanine aminotransferase, and total bilirubin, reflecting impaired plasma protein synthesis, amino acid metabolism, glucose regulation, and bilirubin conjugation. In contrast, gamma-glutamyl transferase, alkaline phosphatase, total bile acids, and cholesterol remained stable, suggesting that the infection predominantly caused hepatocellular rather than cholestatic injury. Histopathological changes were consistent with the biochemical results, showing progressive vacuolar degeneration, inflammatory infiltration, hemorrhage, and hepatocellular necrosis in a dose-dependent manner. Together, these findings provide integrated evidence of liver dysfunction and pathology in *C. gariepinus* following acute *A. hydrophila* infection. The identified biomarkers and histological alterations may serve as reliable indicators of disease progression and offer valuable insights for the development of therapeutic interventions, health prognosis, and sustainable management strategies in catfish aquaculture.

Keywords: Biochemical indicators, experimental infection, fish bacteriosis, hepatic function, histopathology

INTRODUCTION

Catfish is a widely consumed and cultivated source of animal protein in Asia (Needham and Funge-Smith 2014). Especially in Indonesia, the most commonly cultivated species is the African catfish (*Clarias gariepinus* (Burchell, 1822)), which has played a key role in providing dietary protein through freshwater aquaculture for many years (Hastuti and Subandiyono 2018). However, catfish farming faces a major challenge from outbreaks of Motile *Aeromonas* Septicemia (MAS), a rapidly progressing disease that can cause high mortality rates within a short period of time (Zhang et al. 2020). *Aeromonas hydrophila* is the primary pathogen responsible for MAS in catfish aquaculture (Baumgartner et al. 2017) and has caused significant economic losses across Asia and other major aquaculture regions worldwide (Lucas et al. 2019; Abdelrahman et al. 2023; Xu et al. 2023).

Aeromonas hydrophila is part of the normal bacterial flora in both marine and freshwater environments (Tu et al. 2020). However, it is also a well-known opportunistic pathogen capable of invading host tissues and proliferating rapidly (Maloy and Hughes 2013). This Gram-negative bacterium thrives at temperatures between 22-32°C and produces various virulence factors, including α - and β -hemolysins, adhesins, aerolysins, enterotoxins, and lipases

(Pridgeon et al. 2011; Rasmussen-Ivey et al. 2016; Semwal et al. 2023), which contribute to internal organ damage in infected fish (Sellegounder et al. 2018). Notably, poor environmental conditions allow these bacteria to multiply significantly, weaken the host's immune system, and facilitate systemic spread (Abdella et al. 2024).

Aeromonas hydrophila can cause severe internal and external hemorrhage, lesions that may develop into necrotic ulcers, and organ damage in catfish, clinically recognized as hemorrhagic disease (red spot disease) (Rasmussen-Ivey et al. 2016). The liver is one of the vital organs in catfish that is significantly affected by the pathogenic bacterium *A. hydrophila*. This organ plays a crucial role in sustaining life functions, including metabolism (Zheng et al. 2022). Understanding its impact on liver health is essential for supporting the sustainability of *C. gariepinus* aquaculture, particularly for the development of therapeutic approaches, data-driven disease management, health prognosis, and the evaluation of the effectiveness of vaccines, herbal remedies, and probiotics (Elgendy et al. 2024). However, most existing studies have primarily focused on histological and anatomical alterations. At the same time, comprehensive information on functional impairments of the liver caused by *A. hydrophila* in catfish, especially *C. gariepinus*, remains limited.

To assess impaired liver function in the medical field, biochemical parameters in the bloodstream are examined, providing an index that reflects disorders experienced by the liver, such as its ability to work and metabolize, thus indicating abnormalities in liver function and hepatocellular damage (Sellegounder et al. 2018). These biochemical parameters include alanine and aspartate aminotransferase, alkaline phosphatase, and gamma-glutamyltransferase, which are enzymes involved in hepatic metabolic processes (Ramaiah 2007; Kunutsor 2016; Anadón et al. 2019), as well as plasma protein, cholesterol, bile acids, and bilirubin, all of which can serve as indicators of liver damage (Shima et al. 2000; Javed et al. 2017; Hastuti et al. 2019; Chen and Luo 2023).

Despite its importance, comprehensive studies integrating both biochemical and histopathological approaches to assess *A. hydrophila*-induced liver damage in *C. gariepinus* remain scarce. Therefore, this study aimed to evaluate the effects of graded acute *A. hydrophila* infections on liver function and tissue integrity in African catfish by assessing 10 parameters: Albumin (ALB), Globulin (GLOB), Total Protein (TP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Gamma-Glutamyl Transferase (GGT), Total Bilirubin (TB), Total Bile Acids (TBA), and Total Cholesterol (TC). By combining serum biochemical profiling with histopathological assessment, we sought to identify reliable biomarkers of hepatocellular injury and provide evidence for improved disease monitoring, prognosis, and sustainable aquaculture management.

MATERIALS AND METHODS

Ethical approval

All experimental procedures, including the care and handling of animals, were reviewed and approved by the Animal and Research Ethics Committee of the Hasanuddin University Animal Hospital, under approval number 004/UN4.1.RSHUH/B/PP36/2025.

Fish preparation and maintenance

The research was conducted at the Pests and Diseases Laboratory, Faculty of Marine and Fisheries Sciences, Universitas Hasanuddin, Makassar, Indonesia. A total of 72 African catfish (*C. gariepinus*) were used and obtained from the Fish Breeding Center, Parang Tambung, Makassar, Indonesia. Several factors, such as strain and uniform size, were considered to ensure the consistency of results for each individual fish. The fish were healthy, disease-free, and had not undergone any vaccination. The fish used in this study had a length of 23.4 ± 0.27 cm and a weight of 86.34 ± 3.20 g (mean \pm SD). Prior to the core experiment, the fish were acclimatized for seven days in a large aerated aquarium with a water volume of 300 L. At the start of the main experiment, the fish were then distributed into separate aquaria according to the predetermined experimental design. Fish were fed twice daily with commercial floating feed at a rate of 3% of body weight per day during both the seven-

day acclimatization period and the seven-day treatment period.

Preparation of bacterial suspensions

The *A. hydrophila* bacterial isolate used was obtained from the stock of bacterial isolates for research at the Takalar District Brackish Water Aquaculture Center, Indonesia. The bacteria were rejuvenated in liquid tryptic soy broth media for 24 hours, and bacterial colonies were tested using a MALDI-TOF mass spectrometer (bioMérieux, VITEK MS, France) to ensure the authenticity of the *A. hydrophila* bacteria utilized in this study. The concentration was calculated using the standard total plate count method, and the final concentration was 1.4×10^7 CFU/mL. The suspension was diluted with phosphate buffer to obtain the bacterial concentrations used for treatment in this study: 1.4×10^6 CFU/mL as a high concentration, 1.4×10^4 CFU/mL as a medium concentration, and 1.4×10^2 CFU/mL as a low concentration. The bacterial concentrations used were determined based on previous LD₅₀ findings of *A. hydrophila* in *C. gariepinus* reported by Hussein et al. (2017), which established an LD₅₀ value of 1.5×10^7 CFU/fish within 6 days, and the study by Sellegounder et al. (2018), which applied a concentration of 1×10^7 CFU/mL to assess enterotoxic effects in *C. gariepinus* over a 7-day experimental period. Therefore, this study employed lower concentration levels to evaluate milder effects.

Water quality control

Water quality was monitored and maintained twice daily (morning and evening) to ensure that there was no effect of poor water conditions on the test animals by monitoring four parameters, namely temperature in the range of 26-30°C, pH in the range of 6.5-7.5, Dissolved Oxygen (DO) not less than 3 mg/L, and ammonia not more than 0.05 mg/L (Caesar et al. 2021; Nindum et al. 2023; Nhegang et al. 2024), from the acclimatization until the core treatment. Treatment and water replacement were performed when changes occurred that exceeded or fell below the limits of the four parameter ranges. The wastewater produced during the experiment was treated by disinfection.

Experimental design

In this study, four treatment groups were created with *A. hydrophila* bacteria as an intervention with different concentrations of 1.4×10^2 (A), 1.4×10^4 (B), and 1.4×10^6 (C) CFU/mL, and one control group with phosphate buffer without bacteria (K). Each treatment group was replicated three times, resulting in a total of 12 separate treatment aquaria. Each aquarium contained 6 African catfish (*C. gariepinus*) ($n = 72$) with a water volume corresponding to a stocking density of 4 L per fish. The experimental design and sample size determination were based on Federer's (1977) principles and approved by the animal ethics committee, with due consideration of the hazard level and potential risks to both the animals and the environment. A bacterial suspension of *A. hydrophila* was Intra-Muscularly (IM) injected into the epaxial muscle at a volume of 0.1 mL per fish on the first day for each group receiving the bacterial

treatment, according to the predetermined concentration. Meanwhile, the control group was intramuscularly injected with 0.1 mL per fish of phosphate buffer without bacteria. All treatment groups were maintained for seven days post-injection, after seven days of rearing, blood and liver specimens were obtained for biochemical and histopathological testing.

Blood biochemical analysis

Blood samples were randomly collected from three fish per treatment aquarium through the caudal vein using a 23-gauge syringe needle. The blood samples were stored in vacuum tubes containing clot activator (red-top tubes). The collected blood samples were centrifuged at 3000 rpm for 15 minutes to separate the serum and placed in an Eppendorf tube. 100 μ L of serum was added to the blood chemistry rotor. Then the rotor was inserted into the SMT-120VP Veterinary Automatic Biochemical Analyzer (Chengdu Seamaty Technology, China) to test 10 liver function parameters, namely ALB, GLOB, TP, ALT, AST, ALP, GGT, TBA, and TC.

Histopathological examination

Following the trial, three fish were randomly selected from each treatment aquarium unit and euthanized by immersion in an overdose of clove oil (eugenol) at a concentration of 150 mg/L for 20 minutes (Lavalle et al. 2025). Liver samples were fixed in 10% formalin, dehydrated using ascending concentrations of ethanol, cleared with xylene, embedded in paraffin, sectioned, and stained with Hematoxylin and Eosin (H&E) (Jabbar et al. 2021). Image capture and histopathological observation were performed using an advanced optical microscope camera.

Statistical analysis

Data were analyzed using SPSS statistical software. Data normality was first assessed using the Shapiro-Wilk test, as this method is particularly suitable for studies with relatively small sample sizes. Normally distributed data were analyzed using One-Way ANOVA, whereas non-normally distributed data were analyzed using the Kruskal-Wallis

test as an alternative to one-way ANOVA. For variables showing significant differences ($p < 0.05$), post hoc analysis was conducted to identify specific group differences using the LSD test for ANOVA results and the Mann-Whitney U test for Kruskal-Wallis results (McCormick and Salcedo 2017).

RESULTS AND DISCUSSION

Gross lesion

Fish infected with the highest bacterial concentration (1.4×10^6 CFU/mL) exhibited the most severe gross pathological signs after seven days of exposure. External symptoms included reddish lesions around the body surface, hemorrhages at the fin bases, and the appearance of greyish-white skin patches. Internally, the liver showed pale coloration accompanied by peripheral hemorrhages. These findings indicate systemic infection and progressive tissue damage consistent with acute *Aeromonas hydrophila* pathogenicity (Figure 1).

Blood biochemical result

Analysis of serum biochemical parameters revealed significant alterations in several liver function indicators following *A. hydrophila* infection. Albumin levels decreased progressively with increasing bacterial concentrations, while globulin levels increased at lower doses but declined sharply at the highest concentration. Total protein fluctuated in line with these changes, showing the lowest value in severely infected fish. Transaminase activities (AST and ALT) increased significantly in all infected groups, reflecting hepatocellular damage. Total bilirubin also rose in a dose-dependent manner, indicating impaired bilirubin conjugation. In contrast, Gamma-Glutamyl Transferase (GGT), Alkaline Phosphatase (ALP), Total Bile Acids (TBA), and Total Cholesterol (TC) showed no significant differences among groups, suggesting that the infection primarily induced hepatocellular rather than cholestatic injury (Table 1, Table 2).



Figure 1. Gross lesions were observed in *Clarias gariepinus* exposed to the highest concentration of *Aeromonas hydrophila* (1.4×10^6 CFU/mL) after seven days of infection. Reddish lesions on the fin bases (green arrow), red spots on the skin (red arrow), greyish-white skin patches (blue arrow), liver peripheral hemorrhages (black arrow), and pale color (yellow arrow)

Table 1. Blood biochemical parameters of liver function in *Clarias gariepinus* with significant differences among experimental groups after seven days of *Aeromonas hydrophila* infection

Parameters	K (control)	A (1.4×10^2 CFU/mL)	B (1.4×10^4 CFU/mL)	C (1.4×10^6 CFU/mL)
ALB (g/L)*	14.77±0.61 ^a	13.53±0.25 ^{ab}	12.37±0.31 ^b	10.53±0.60 ^c
GLOB (g/L)*	21.40±0.26 ^b	24.37±0.35 ^a	22.40±0.36 ^{ab}	18.60±0.30 ^c
TP (g/L)**	36.17±0.67 ^{ab}	37.90±0.52 ^a	34.77±0.57 ^{bc}	29.13±0.31 ^c
AST (U/L)*	125.33±6.03 ^d	165.00±7.21 ^c	168.33±4.73 ^c	234.33±9.45 ^a
ALT (U/L)*	47.67±2.08 ^b	58.00±2.65 ^{ab}	60.67±3.79 ^a	62.00±3.61 ^a
TB (μmol/L)*	3.13±0.25 ^c	4.03±0.49 ^{bc}	5.18±0.31 ^{ab}	7.40±1.04 ^a

Note: Significance level $p < 0.05$, *One-Way ANOVA, **Kruskal-Wallis

Table 2. Blood biochemical parameters of liver function in *Clarias gariepinus* with no significant differences among experimental groups after seven days of *Aeromonas hydrophila* infection

Parameters	K (control)	A (1.4×10^2 CFU/mL)	B (1.4×10^4 CFU/mL)	C (1.4×10^6 CFU/mL)
GGT (U/L)**	1.99±0.00	1.98±0.01	1.99±0.00	1.99±0.00
ALP (U/L)*	25.67±2.52	25.00±4.58	24.67±3.79	25.67±5.03
TBA (μmol/L)**	0.99±0.00	1.01±0.04	0.99±0.00	0.99±0.00
TC (mmol/L)*	4.70±0.65	4.79±0.67	4.78±0.56	4.80±0.51

Note: Significance level $p > 0.05$, *One-Way ANOVA, **Kruskal-Wallis

Histopathological result

Microscopic examination of liver tissues confirmed progressive damage in fish challenged with *A. hydrophila*. Control fish exhibited normal hepatic architecture with intact hepatocytes and clear sinusoids. In infected groups, lesions increased in severity with higher bacterial concentrations. Mild infections were characterized by vacuolar degeneration of hepatocytes and focal inflammatory cell infiltration. Moderate infections showed more extensive hepatocellular swelling, congestion, and hemorrhages. At the highest bacterial concentration, severe hepatocellular necrosis, massive vacuolation, and widespread hemorrhagic areas were observed, accompanied by infiltration of mononuclear cells. These pathological changes corroborated the biochemical alterations and indicate that *A. hydrophila* infection leads predominantly to hepatocellular injury in a dose-dependent manner (Figure 2).

Discussion

Interpretation of biochemical alterations

The biochemical alterations observed in infected catfish reflected marked impairment of liver function caused by *A. hydrophila*. A significant decrease in albumin and total protein levels indicated reduced synthetic capacity of hepatocytes (Carvalho and Machado 2018; Soeters et al. 2019), consistent with earlier reports that hepatic infections compromise plasma protein synthesis in fish (Abd Allah et al. 2019). Meanwhile, globulin levels initially increased and then decreased, since globulins are primarily involved in the immune response to pathogens (Evans and Casinghino 2018). This immune modulation is thought to underlie the observed globulin fluctuations, as in mild and moderate infections the immune system is still able to respond to pathogens. Similarly, total protein levels also exhibited significant fluctuations, reflecting changes in albumin and globulin, as total protein represents the sum of these two components. Decreased serum albumin and total

protein levels can cause fluid to leak from blood vessels due to reduced osmotic pressure, potentially resulting in edema, and may also impair the delivery of nutrients to tissues in fish, thereby increasing the risk of mortality (Rudneva and Kovyrshina 2011; Nahak and Sahu 2014; Soeters et al. 2019). Elevated activities of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) in all infected groups further confirmed hepatocellular injury (Ramaiah 2007), since leakage of these enzymes into circulation is a widely recognized marker of hepatic cell membrane disruption that impairs the liver's capacity to maintain amino acid metabolism and glucose regulation (Abd Allah et al. 2019; Zhao et al. 2019; Kobayashi et al. 2020). The dose-dependent increase in total bilirubin suggested impairment of bilirubin conjugation and excretion pathways (Chowdhury et al. 2013; Setyawan and Budipramana 2015), a condition commonly associated with hepatocellular necrosis and bile pigment accumulation (López-Velázquez et al. 2014; Nighot et al. 2025). In contrast, Gamma-Glutamyl Transferase (GGT), Alkaline Phosphatase (ALP), Total Bile Acids (TBA), and cholesterol levels remained relatively unchanged, implying that cholestatic injury was not prominent in this study (Shima et al. 2000; Janičko et al. 2013; Masubuchi et al. 2015; Setyawan and Budipramana 2015; Kunutsor 2016). Taken together, these findings demonstrate that acute *A. hydrophila* infection predominantly induces hepatocellular rather than cholestatic damage in *C. gariepinus*.

Correlation with histopathological changes

The histopathological findings provided clear support for the biochemical alterations observed in infected fish. The reduction in albumin and total protein, together with the elevation of AST and ALT, coincided with microscopic evidence of hepatocellular degeneration, necrosis, and inflammatory infiltration. Similar correlations between transaminase elevation and tissue damage have been reported

in other teleosts challenged with bacterial pathogens (Duman et al. 2022). The dose-dependent increase in bilirubin was consistent with the presence of hemorrhages and vacuolar degeneration, which are indicative of impaired hepatocyte function and bilirubin metabolism (Chowdhury et al. 2013; López-Velázquez et al. 2014).

These lesions were most severe in fish exposed to the highest bacterial concentration, confirming that *A. hydrophila* induces progressive and dose-related hepatic injury. Importantly, the absence of changes in GGT, ALP, TBA, and cholesterol was also in line with histological observations, since bile ducts and canaliculi structures did not exhibit prominent lesions. This agreement between biochemical and histological parameters underscores their combined reliability as diagnostic indicators of acute *A. hydrophila* infection in *C. gariepinus*.

Comparison with previous studies

The biochemical and histopathological changes observed in this study are consistent with previous reports on *A. hydrophila* infections in other aquaculture species. Elevated AST and ALT activities, together with reduced serum proteins, have also been documented in Nile tilapia and

hybrid catfish following bacterial challenge (Koeypudsa et al. 2020; Abd Allah et al. 2023), confirming that hepatocellular damage is a common outcome of *Aeromonas* infections (Semwal et al. 2023; Oghenochuko et al. 2024). Asrido et al. (2024) similarly reported that fish with bilirubin-induced jaundice exhibited histological lesions such as necrosis and vacuolation, supporting the present finding that bilirubin can serve as a sensitive indicator of hepatic impairment. Comparable pathological patterns have been noted in common carp and other freshwater species, where *A. hydrophila* triggered degenerative and necrotic changes in liver parenchyma (Abdelhamed et al. 2017; Al Shammari et al. 2025). The stability of GGT and ALP observed here also agrees with studies that distinguish hepatocellular from cholestatic injury (Setyawan and Budipramana 2015), indicating that acute infection of *A. hydrophila* primarily targets hepatocytes rather than bile canaliculi or ducts. Collectively, these comparisons reinforce the generality of our results and highlight the role of biochemical markers as reliable proxies for histological liver damage across fish species exposed to pathogenic *Aeromonas*.

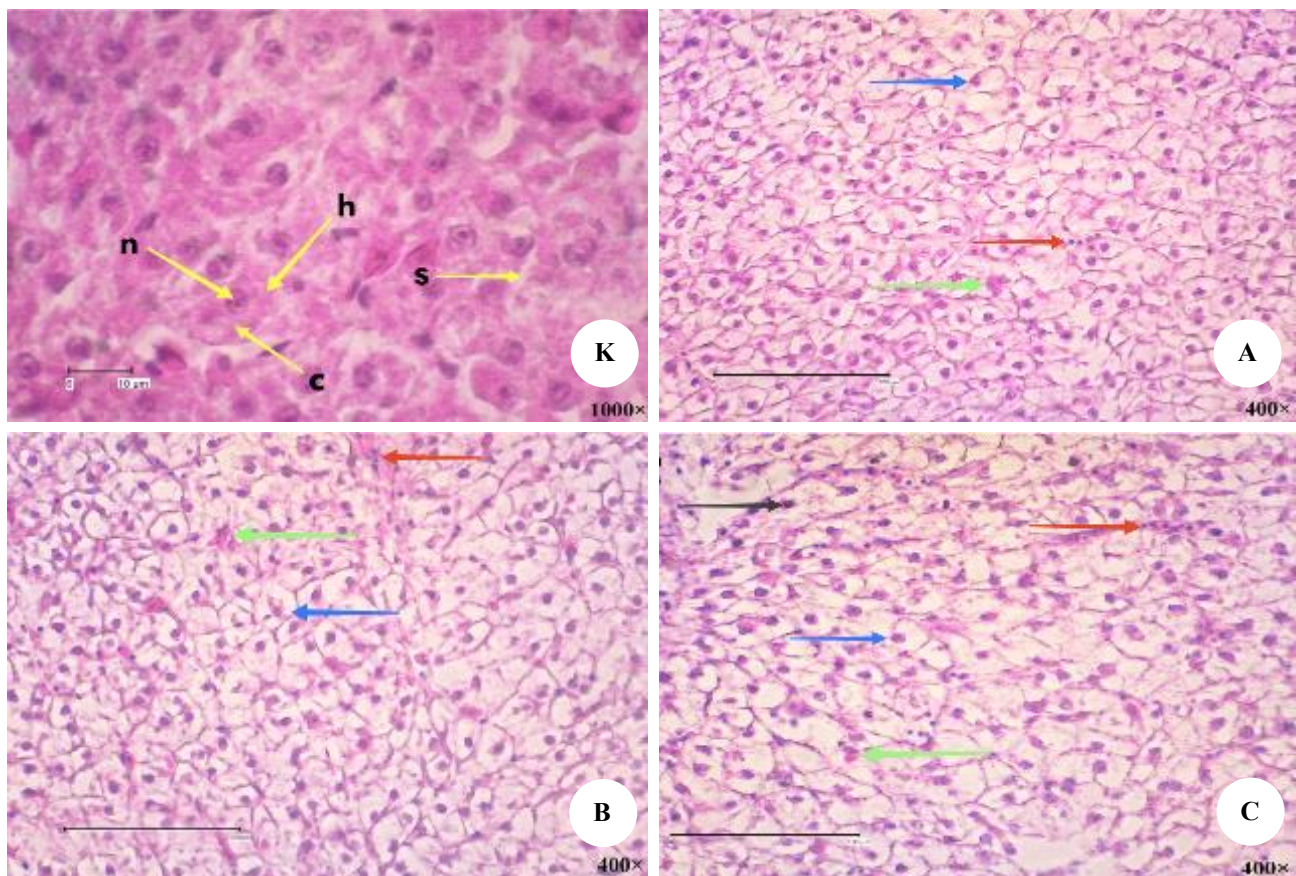


Figure 2. Liver tissue sections of African catfish (*Clarias gariepinus*) from: K. Control, and A, B, C. Infected groups exposed to graded concentrations of *Aeromonas hydrophila* after seven days. Note: h: Hepatocytes, n: Cell nuclei, c: Cytoplasm, s: Sinusoids. Blue arrow: Vacuolar degeneration, Red arrow: Inflammatory cell infiltration, Green arrow: Hemorrhage, Black arrow: Cell necrosis. H&E

Pathogenic mechanisms of *Aeromonas hydrophila*

The hepatocellular damage observed in this study can be explained by the diverse virulence factors produced by *A. hydrophila*. This bacterium is known to secrete extracellular toxins, hemolysins, proteases, and lipases that disrupt cellular membranes and compromise tissue integrity (Jin et al. 2020; Semwal et al. 2023; Oghenochuko et al. 2024). Such toxins increase vascular permeability and contribute to hemorrhages (AlYahya et al. 2017; Fahmi et al. 2019; Kusdarwati et al. 2021), while proteolytic enzymes accelerate hepatocyte necrosis and inflammatory infiltration (Miller and Zachary 2017; Obeng 2021; Semwal et al. 2023). In addition, *A. hydrophila* can stimulate excessive production of reactive oxygen species and pro-inflammatory cytokines in infected fish, further exacerbating hepatic injury (Harikrishnan et al. 2010; Magnadottir 2010; Junior and Baldisserotto 2021). The progressive vacuolar degeneration and necrosis documented here are consistent with the destructive effects of these virulence factors on hepatocytes (AlYahya et al. 2017; Huang et al. 2022). Moreover, the systemic nature of the infection, evidenced by external lesions and internal hemorrhages, reflects the invasive ability of *A. hydrophila* to spread through the bloodstream and colonize vital organs, including the liver (Rashid et al. 2013; Ali et al. 2014; Kartikaningsih et al. 2020; Mohamad et al. 2022). Taken together, these mechanisms highlight the pathogenic potential of *A. hydrophila* and explain the combined biochemical and histological alterations observed in African catfish during acute infection.

Implications for aquaculture management

The integrated biochemical and histopathological evidence obtained in this study provides important insights for disease monitoring and management in African catfish culture. Biomarkers such as albumin, total protein, AST, ALT, and bilirubin can serve as early indicators of hepatic dysfunction, allowing rapid diagnosis of *A. hydrophila* infection before severe mortality occurs. Histopathological confirmation further validates the diagnostic value of these markers, especially in distinguishing hepatocellular from cholestatic injury. Early detection of infection is critical for guiding therapeutic interventions, including the application of antibiotics, probiotics, or immunostimulants to minimize organ damage (Hoseinifar et al. 2018; Torres-Maravilla et al. 2024). Moreover, the dose-dependent responses observed here underscore the importance of managing environmental conditions and stressors that predispose fish to infection, such as poor water quality and overcrowding. By integrating biochemical assays into health monitoring programs, fish farmers can improve prognosis, reduce losses, and promote more sustainable aquaculture practices. These findings, therefore, contribute not only to a better understanding of the pathogenicity of *A. hydrophila* but also to practical strategies for safeguarding the productivity and resilience of African catfish farming systems.

This study demonstrated that acute infection with *A. hydrophila* significantly impairs liver function in African catfish (*C. gariepinus*). The potential impact of this finding on aquaculture systems is significant. Biochemical analyses revealed reduced albumin and total protein, elevated

transaminases (AST and ALT), and increased bilirubin, indicating hepatocellular injury, while cholestatic parameters remained stable. Histopathological evidence of vacuolar degeneration, necrosis, hemorrhage, and inflammatory infiltration further confirmed dose-dependent hepatic damage. Together, these findings highlight that acute *A. hydrophila* infection predominantly induces hepatocellular rather than cholestatic lesions. The integration of biochemical and histological markers provides reliable diagnostic framework for monitoring liver dysfunction and disease progression. Importantly, these indicators can support early detection, guide therapeutic interventions, and improve prognosis in aquaculture systems. By identifying practical biomarkers, this study contributes to the development of effective health management strategies aimed at reducing economic losses and enhancing the sustainability of African catfish farming. The future applications to improve prognosis in aquaculture systems may be significant by implementing vaccine evaluation.

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REFERENCES

- Abd Allah OA, Aly SM, Abd El-Rahman HG, Youssef FMA, Ahmed FK. 2019. Effect of some immunostimulants on clinicopathological findings of African catfish *Clarias gariepinus* infected with motile *Aeromonas septicemia*. *EC Vet Sci* 4 (7): 498-510.
- Abd Allah OA, Aly SM, Abo El-Atta ME, Metwaly SA, Mohamed EH, Fathi M. 2023. Enhancing Nile tilapia health status and immunity against *Aeromonas hydrophila* with a combination of probiotics and immunostimulants (Vimolert®). *Iran J Fish Sci* 22 (5): 940-961. DOI: 10.22092/ijfs.2023.130128.
- Abdelhamed H, Ibrahim I, Baumgartner W, Lawrence ML, Karsi A. 2017. Characterization of histopathological and ultrastructural changes in channel catfish experimentally infected with virulent *Aeromonas hydrophila*. *Front Microbiol* 8: 1519. DOI: 10.3389/fmicb.2017.01519.
- Abdella B, Shokrak NM, Abozahra NA, Elshamy YM, Kadira HI, Mohamed RA. 2024. Aquaculture and *Aeromonas hydrophila*: A complex interplay of environmental factors and virulence. *Aquac Intl* 32: 7671-7681. DOI: 10.1007/s10499-024-01535-y.
- Abdelrahman HA, Hemstreet WG, Roy LA, Hanson TR, Beck BH, Kelly AM. 2023. Epidemiology and economic impact of disease-related losses on commercial catfish farms: A seven-year case study from Alabama, USA. *Aquaculture* 566: 739206. DOI: 10.1016/j.aquaculture.2022.739206.
- Al Shammari NAH, Al-niaem KS, Al Hawash AB, Abomughaid MM, Abdelazim AM, Elumalai P, Eissa ESH, Mahboub HH, Elbaghdady HAM. 2025. Comparative study of mortalities, clinical manifestations, antioxidant-associated genes, and histopathological degeneration upon experiment infection by *Aeromonas hydrophila* in *Cyprinus carpio* and *Oreochromis niloticus*. *Open Vet J* 15 (7): 3012-3023. DOI: 10.5455/OVJ.2025.v15.i7.12.
- Ali MF, Rashid MM, Rahman MM, Haque MN. 2014. Pathogenicity of *Aeromonas hydrophila* in silver carp *Hypophthalmichthys molitrix* and its control trial. *IOSR J Agric Vet Sci* 7 (6): 21-24. DOI: 10.9790/2380-07612124.
- AlYahya SA, Ameen F, Al-Niaem KS, Al-Sa'adi BA, Hadi S, Mostafa AA. 2018. Histopathological studies of experimental *Aeromonas*

- hydrophila* infection in blue tilapia, *Oreochromis aureus*. Saudi J Biol Sci 25 (1): 182-185. DOI: 10.1016/j.sjbs.2017.10.019.
- Anadón A, Martínez-Larrañaga MR, Ares I, Martínez MA. 2019. Biomarkers of drug toxicity and safety evaluation. In: Gupta RC (eds). Biomarkers in Toxicology. 2nd Edition. Academic Press, San Diego. DOI: 10.1016/B978-0-12-814655-2.00038-4.
- Asrido F, Nuryati S, Widanarni W. 2024. Histopathology of liver, kidney, intestine, spleen, and bile of catfish with Jaundice. Jurnal Akuakultur Indonesia 23: 250-261. DOI: 10.19027/jai.23.2.250-261. [Indonesian]
- Baumgartner WA, Ford L, Hanson L. 2017. Lesions caused by virulent *Aeromonas hydrophila* in farmed catfish (*Ictalurus punctatus* and *I. punctatus* × *I. furcatus*) in Mississippi. J Vet Diagn Invest 29 (5): 747-751. DOI: 10.1177/1040638717708584.
- Caesar NR, Yanuhar U, Raharjo DKWP, Junirahma NS. 2021. Monitoring of water quality in the catfish (*Clarias* sp.) farming in Tuban Regency. IOP Conf Ser: Earth Environ Sci 718: 012061. DOI: 10.1088/1755-1315/718/1/012061.
- Carvalho JR, Machado MV. 2018. New insights about albumin and liver disease. Ann Hepatol 17: 547-560. DOI: 10.5604/01.3001.0012.0916.
- Chen H, Luo D. 2023. Application of haematology parameters for health management in fish farms. Rev Aquac 15 (2): 704-737. DOI: 10.1111/raq.12753.
- Chowdhury NR, Chowdhury JR, Avsar Y. 2013. Bile pigment metabolism and its disorders. In: Rimoin D, Peyerit R, Korf B (eds). Emery and Rimoin's Principles and Practice of Medical Genetics, 6th Edition. Academic Press, San Diego. DOI: 10.1016/B978-0-12-383834-6.00073-2.
- Duman M, Altun S, Ozdemir B, Saticioglu IB. 2022. Fatty liver disease and bacterial co-infection in cultured marine fish. Erciyes Univ Vet Fak Derg 19 (1): 1-10. DOI: 10.32707/ercivet.1085205.
- Elgendy MY, Ali SE, Dayem AA, Khalil RH, Moustafa MM, Abdelsalam M. 2024. Alternative therapies recently applied in controlling farmed fish diseases: Mechanisms, challenges, and prospects. Aquac Int 32: 9017-9078. DOI: 10.1007/s10499-024-01603-3.
- Evans EW, Casinghino S. 2018. Clinical Pathology as a Tool to Assess Immunotoxicity. In: McQueen CA (eds). Comprehensive Toxicology 3rd Edition. Elsevier, Amsterdam. DOI: 10.1016/B978-0-12-801238-3.64172-7.
- Fahmi U, Andriani I, Salmah S, Hatta TH, Bin Andi Omar S, Sari DK. 2019. Histopathology of liver and intestine of pangkalan bare fish (*Oryzias matanensis*) polluted by nickel and iron in Lake Matano, South Sulawesi. IOP Conf Ser: Earth Environ Sci 370: 012078. DOI: 10.1088/1755-1315/370/1/012078.
- Federer WT. 1977. Experimental Design: Theory and Application, 3rd ed. Oxford and IBH Publishing Co., Calcutta.
- Harikrishnan R, Kim MC, Kim JS, Han YJ, Jang IS, Balasundaram C, Heo MS. 2010. Immune response and expression analysis of cathepsin K in goldfish during *Aeromonas hydrophila* infection. Fish Shellfish Immunol 28 (4): 511-516. DOI: 10.1016/j.fsi.2009.12.005.
- Hastuti S, Subandiyono S, Windarto S. 2019. Blood performance of jaundice catfish *Clarias gariepinus*. AACL Bioflux 12 (2): 480-489.
- Hastuti S, Subandiyono S. 2018. Haematological parameters of the North African catfish *Clarias gariepinus* farmed using biofloc technology. AACL Bioflux 11 (4): 1415-1424.
- Hoseinifar SH, Sun YZ, Wang A, Zhou Z. 2018. Probiotics as means of diseases control in aquaculture, a review of current knowledge and future perspectives. Front Microbiol 9: 2429. DOI: 10.3389/fmicb.2018.02429.
- Huang X, Liu S, Zhang H, Yao J, Geng Y, Ou Y, Chen D, Yang S, Yin L, Luo W. 2022. Pathological characterization and cause of a novel liver disease in largemouth bass (*Micropterus salmoides*). Aquac Rep 23: 101028. DOI: 10.1016/j.aqrep.2022.101028.
- Hussein MMA, Hassan WH, Kamel MO. 2017. Pathogenicity of *Aeromonas hydrophila* isolated from diseased sharp teeth catfish, *Clarias gariepinus*, with special reference to the lethality of its extracellular products. J Vet Med Res 24 (1): 114-123. DOI: 10.21608/jvmr.2017.43271.
- Jabbar RA, Sari DK, Tahir A. 2021. Histopathology overview of tilapia (*Oreochromis mossambicus*) liver organs contaminated by lead metal (Pb) in Lake Tempe, Wajo Regency. IOP Conf Ser: Earth Environ Sci 870: 012008. DOI: 10.1088/1755-1315/870/1/012008.
- Janičko M, Veseliny E, Leško D, Jarčuška P. 2013. Serum cholesterol is a significant and independent mortality predictor in liver cirrhosis patients. Ann Hepatol 12 (4): 413-419. DOI: 10.1016/S1665-2681(19)31342-0.
- Javed M, Ahmad MI, Usmani N, Ahmad M. 2017. Multiple biomarker responses (serum biochemistry, oxidative stress, genotoxicity, and histopathology) in *Channa punctatus* exposed to heavy metal-loaded wastewater. Sci Rep 7: 1675. DOI: 10.1038/s41598-017-01749-6.
- Jin L, Chen Y, Yang W, Qiao Z, Zhang X. 2020. Complete genome sequence of fish-pathogenic *Aeromonas hydrophila* HX-3 and a comparative analysis: Insights into virulence factors and quorum sensing. Sci Rep 10 (1): 15479. DOI: 10.1038/s41598-020-72484-8.
- Junior GB, Baldisserotto B. 2021. Fish infections associated with the genus *Aeromonas*: A review of the effects on oxidative status. J Appl Microbiol 131 (3): 1083-1101. DOI: 10.1111/jam.14986.
- Kartikaningsih H, Yahya, Rohman FZ, Jaziri AA. 2020. Characteristics of *Aeromonas hydrophila*-infected catfish (*Clarias* sp.). IOP Conf Ser: Earth Environ Sci 493: 012036. DOI: 10.1088/1755-1315/493/1/012036.
- Kobayashi A, Suzuki Y, Sugai S. 2020. Specificity of transaminase activities in the prediction of drug-induced hepatotoxicity. J Toxicol Sci 45 (9): 515-537. DOI: 10.2131/jts.45.515.
- Koepudsa W, Jongjareanjai M, Phalitakul S, Punnarak P. 2020. Comparative blood chemistry of hybrid catfish (*Clarias gariepinus* × *C. macrocephalus*) infected with *Aeromonas hydrophila* to those non-infected with *A. hydrophila*. J Mahanakorn Vet Med 15 (1): 25-42.
- Kunutsor SK. 2016. Gamma-glutamyltransferase-friend or foe within? Liver Intl 36 (12): 1723-1734. DOI: 10.1111/liv.13221.
- Kusdarwati R, Amin M, Wardana AB. 2021. DNase and gelatinase activities of β -hemolysin *Aeromonas hydrophila* isolated from catfish (*Clarias batrachus*). J Aquac Fish Health 10 (3): 331-340. DOI: 10.20473/jafh.v10i3.25918.
- Lavalle C, Clarence S, Khan H, Shires K, Parrott J. 2025. Current research and guidelines for euthanasia in laboratory fish with a focus on fathead minnows. Lab Anim 59 (3): 319-331. DOI: 10.1177/00236772241288146.
- López-Velázquez JA, Chávez-Tapia NC, Ponciano-Rodríguez G, Sánchez-Valle V, Caldwell SH, Uribe M, Méndez-Sánchez N. 2014. Bilirubin alone as a biomarker for short-term mortality in acute-on-chronic liver failure: An important prognostic indicator. Ann Hepatol 13 (1): 98-104. DOI: 10.1016/S1665-2681(19)30910-X.
- Lucas JS, Southgate PC, Tucker CS. 2019. Aquaculture: Farming Aquatic Animals and Plants. 3rd ed. John Wiley & Sons, New Jersey.
- Magnadottir B. 2010. Immunological control of fish diseases. Mar Biotechnol 12 (4): 361-379. DOI: 10.1007/s10126-010-9279-x.
- Maloy S, Hughes K. 2013. Brenner's Encyclopedia of Genetics, 2nd ed. Academic Press, Amsterdam.
- Masubuchi N, Sugihara M, Sugita T, Amano K, Nakano M, Matsuura T. 2015. P1168: Prognostic clinical biomarkers of cholestatic liver injury: Perturbation of bile acid metabolism and reactive oxidative stress marker. J Hepatol 62: S791. DOI: 10.1016/S0168-8278(15)31364-7.
- McCormick K, Salcedo J. 2017. SPSS statistics versus SPSS modeler: Can i be a data miner using SPSS statistics? In: McCormick K, Salcedo J (eds). SPSS Statistics for Data Analysis and Visualization. John Wiley & Sons, Indianapolis. DOI: 10.1002/9781119183426.ch11.
- Miller MA, Zachary JF. 2017. Mechanisms and morphology of cellular injury, adaptation, and death. Pathol Basis Vet Dis 17: 2-43.e19. DOI: 10.1016/B978-0-323-35775-3.00001-1.
- Mohamad NFA, Daud HM, Manaf SR. 2022. Pathogenicity of *Aeromonas hydrophila* in cultured African catfish (*Clarias gariepinus*). J Smart Sci Technol 2 (1): 34-45. DOI: 10.24191/jss.v2i1.25.
- Nahak G, Sahu RK. 2014. Immunomodulatory activity of aqueous leaf extract of *Ocimum basilicum* linn in *Clarias batrachus*. Intl J Pharm Pharm Sci 6 (6): 433-440.
- Nchegang B, Enow TM, Nkongho GO, Tan PV. 2024. Impact of water quality on the growth performance of *Clarias gariepinus* in fish farms within Fako Division, Cameroon. Asian J Fish Aquat Res 26 (7): 98-107. DOI: 10.9734/ajfar/2024/v26i7787.
- Needham S, Funge-Smith SJ. 2014. The consumption of fish and fish products in the Asia-Pacific region based on household surveys. RAP Publication 2015/12. www.fao.org.
- Nightot S, Jadhav A, Deshmukh S. 2025. A clinical case study on jaundice: Causes, diagnosis, treatment, management, and public awareness. Intl J Pharm Sci 3 (5): 2816-2833. DOI: 10.5281/zenodo.1544892.
- Nindum SYN, Laure DTM, Cecile RBT, Adrien MEE, Claude KN, Steve Y-S, Kingsley EA, Moïse N, Serge HZT. 2023. Water quality effects on survival and weight of tank-raised African catfish (*Clarias gariepinus*, Burchell, 1822) fed commercial and farm-made feeds. J Exp Agric Intl 45 (12): 140-154. DOI: 10.9734/jeai/2023/v45i122272.

- Obeng E. 2021. Apoptosis (programmed cell death) and its signals - A review. *Braz J Biol* 81: 1133-1143. DOI: 10.1590/1519-6984.228437.
- Oghenochuko MO, Ola EI, Thomas MR, Daodu OG, Oguntuase GA, Aluko OL, Irokanulo E, Akpor BO. 2024. Effects of single and co-infections of *Proteus mirabilis* and *Aeromonas hydrophila* on baseline hematological, serological, and histological data in cultured *Clarias gariepinus*. *Open Agric* 18: E18743315277346. DOI: 10.2174/0118743315277346231123094611.
- Pridgeon JW, Klesius PH, Mu X, Carter D, Fleming K, Xu D, Srivastava K, Reddy G. 2011. Identification of unique DNA sequences present in highly virulent 2009 Alabama isolates of *Aeromonas hydrophila*. *Vet Microbiol* 152 (1-2): 117-125. DOI: 10.1016/j.vetmic.2011.04.008.
- Ramaiah SK. 2007. A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. *Food Chem Toxicol* 45 (9): 1551-1557. DOI: 10.1016/j.fct.2007.06.007.
- Rashid MM, Hossain MS, Ali MF. 2013. Isolation and identification of *Aeromonas hydrophila* from silver carp and its culture environment from Mymensingh region. *J Bangladesh Agric Univ* 11 (2): 373-376. DOI: 10.3329/jbau.v11i2.19943.
- Rasmussen-Ivey CR, Hossain MJ, Odom SE, Terhune JS, Hemstreet WG, Shoemaker CA, Zhang D, Xu D-H, Griffin MJ, Liu Y-J, Figueras MJ, Santos SR, Newton JC, Liles MR. 2016. Classification of a hypervirulent *Aeromonas hydrophila* pathotype responsible for epidemic outbreaks in warm-water fishes. *Front Microbiol* 7: 1615. DOI: 10.3389/fmicb.2016.01615.
- Rudneva II, Kovyrshina TB. 2011. Comparative study of electrophoretic characteristics of serum albumin of round goby *Neogobius melanostomus* from Black Sea and Azov Sea. *Intl J Adv Biol Res* 1 (1): 131-136.
- Sellegounder D, Gupta YR, Muruganankumar R, Senthilkumaran B. 2018. Enterotoxic effects of *Aeromonas hydrophila* infection in the catfish, *Clarias gariepinus*: Biochemical, histological, and proteome analyses. *Vet Immunol Immunopathol* 204: 1-10. DOI: 10.1016/j.vetimm.2018.08.008.
- Semwal A, Kumar A, Kumar N. 2023. A review on pathogenicity of *Aeromonas hydrophila* and their mitigation through medicinal herbs in aquaculture. *Heliyon* 9: e14088. DOI: 10.1016/j.heliyon.2023.e14088.
- Setyawan N, Budipramana VS. 2015. Correlation between alkaline phosphatase, g-glutamyl transpeptidase, and bilirubin with interleukin-1b level in dogs with obstructive jaundice. *J Med Sci* 47 (4): 154-161. DOI: 10.19106/jmedsci004704201502.
- Shima T, Tada H, Morimoto M, Nakagawa Y, Obata H, Sasaki T, Park H, Nakajo S, Nakashima T, Okanoue T, Kashima K. 2000. Serum total bile acid level as a sensitive indicator of hepatic histological improvement in chronic hepatitis C patients responding to interferon treatment. *J Gastroenterol Hepatol* 15 (3): 294-299. DOI: 10.1046/j.1440-1746.2000.02126.x.
- Soeters PB, Wolfe RR, Shenkin A. 2019. Hypoalbuminemia: Pathogenesis and clinical significance. *J Parenter Enteral Nutr* 43 (2): 181-193. DOI: 10.1002/jpen.1451.
- Torres-Maravilla E, Parra M, Maisey K, Vargas RA, Cabezas-Cruz A, Gonzalez A, Tello M, Bermúdez-Humarán LG. 2024. Importance of probiotics in fish aquaculture: Towards the identification and design of novel probiotics. *Microorganisms* 12 (3): 626. DOI: 10.3390/microorganisms12030626.
- Tu VQ, Nguyen T-T, Tran XTT, Millard AD, Phan HT, Le NP, Dang OTH, Hoang HA. 2020. Complete genome sequence of a novel lytic phage infecting *Aeromonas hydrophila*, an infectious agent in striped catfish (*Pangasianodon hypophthalmus*). *Arch Virol* 165 (12): 2973-2977. DOI: 10.1007/s00705-020-04793-2.
- Xu T, Rasmussen-Ivey CR, Moen FS, et al. 2023. A global survey of hypervirulent *Aeromonas hydrophila* (vAh) identified vAh strains in the lower Mekong River Basin and diverse opportunistic pathogens from farmed fish and other environmental sources. *Microbiol Spectr* 11 (2): e0370522. DOI: 10.1128/spectrum.03705-22.
- Zhang D, Xu DH, Shoemaker CA, Beck BH. 2020. The severity of motile *Aeromonas* septicemia caused by virulent *Aeromonas hydrophila* in channel catfish is influenced by nutrients and microbes in water. *Aquaculture* 519: 734898. DOI: 10.1016/j.aquaculture.2019.734898.
- Zhao H, Luo Y, Wu Z, Zhou Y, Guo D, Wang H, Chen X. 2019. Hepatic lipid metabolism and oxidative stress responses of grass carp (*Ctenopharyngodon idella*) fed diets of two different lipid levels against *Aeromonas hydrophila* infection. *Aquaculture* 509: 149-158. DOI: 10.1016/j.aquaculture.2019.05.029.
- Zheng Y, Shi Y, Yang X, Gao J, Nie Z, Xu G. 2022. Effects of resveratrol on lipid metabolism in liver of red tilapia *Oreochromis niloticus*. *Comp Biochem Physiol C Toxicol Pharmacol* 261: 109408. DOI: 10.1016/j.cbpc.2022.109408.