Jurnal Veterinar dan Biomedis
Vol 3, No 2 (2025): 119-123. 10.29244/jvetbiomed.3.1.119-123
https://journal.ipb.ac.id/index.php/jvetbiomed
e-ISSN 2987-2251, p-ISSN 2985-4954

PENELITIAN/RESEARCH OPEN ACCESS

Impact of *Spirulina platensis* Extract as an Immunostimulant on the Histopathological Intestinal of Tilapia Fish (*Oreochromis niloticus*) Infected with *Aeromonas salmonicida*

Maharani Kartika Ramadhan[1], Kurniasih[2], Tri Untari[3], Amaq Fadholly[4*]
[1] Doctoral Program in Animal Biosciences, Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, Bogor, Indonesia,
[2] Department of Pathology, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia,
[3] Department of Microbiology, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia,
[4] Division of Pharmacology and Toxicology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia.

Received: 08/08/2025, Accepted: 20/09/2025, Published Online: 30/09/2025
*Corresponding author: amaqfadholly@apps.ipb.ac.id

ABSTRACT

Furunculosis in fish results in financial detriment to freshwater aquaculture in Indonesia. Furunculosis, an infectious disease produced by *Aeromonas salmonicida*, presents a considerable risk to the large-scale farming of both salmonid and non-salmonid species in freshwater environments. This study sought to evaluate the impact of *Spirulina platensis* extract on the histopatological intestinal health of tilapia infected with *Aeromonas salmonicida*. Thirty tilapia, each around 10 cm in length, were distributed into five groups. Negative control (P0-) without immersion in *Spirulina platensis* extract and free from *Aeromonas salmonicida* infection. Positive control (P0+) not immersed in *Spirulina platensis* extract, infected with A*eromonas salmonicida*. Tilapia subjected to doses of 400 mg/L (P1), 600 mg/L (P2), and 800 mg/L (P3) The extraction of *Spirulina platensis* occurred for three hours on the seventh day (first immersion) and the fourteenth day (second immersion), followed by an evaluation of *Aeromonas salmonicida* infection on the twenty-first day. A necropsy of the fish was performed on day 28. Histopathological analysis revealed that *Spirulina platensis* extract reduced intestinal villous erosion and inflammatory cell density in the intestines of tilapia. Immunohistochemical staining demonstrated a decrease in the presence of *Aeromonas salmonicida* within intestinal tissue at a dosage of 800 mg/L. This work indicates that more research is necessary to examine the histological features of the intestines of tilapia infected with *Aeromonas salmonicida* with those supplemented with *Spirulina platensis* through feed.

Keywords: *Aeromonas salmonicida,* intestine health, *Spirulina platensis*

©2025 Sekolah Kedokteran Hewan dan Biomedis, IPB University
Open Acces Under Creative Commons Attribution-ShareAlike 4.0 International License (CC-BY-SA)

1. Introduction

Since the 1990s, tilapia cultivation has dominated global fisheries, with a commercial production capacity of 2.5 million tons in 2007. In 2008, it was rated fifth and peaked in 2014, over 3.5 million tons, following carp (*Cyprinus carpio*) and salmon (*Salmo salar* L.). The swift expansion has resulted in a rise in the prevalence of illnesses affecting tilapia, attributable to the intense fisheries system employed. High density seeks to enhance production outcomes, resulting in stress-induced immune suppression, thereby rendering fish highly vulnerable to disease infections and elevating the disease transmission rate among farmed fish[1]. The occurrence of furunculosis in fish due to *Aeromonas salmonicida* in Indonesia has led to economic losses in freshwater aquaculture. It is a pathogenic species within the genus Aeromonas, posing significant risks in the intensive farming of salmonid fish. Evidence suggests that non-salmonid fish species inhabiting freshwater, brackish, and marine settings are very vulnerable to this bacterial infection. Pathogenic bacteria in fish can infiltrate the host via three distinct pathways like the skin, gills, and digestive tract. Bacteria can traverse the gastrointestinal barrier through three distinct mechanisms. In intact tissue, bacteria may traverse by transcellular or paracellular routes, potentially compromising the intestinal lining through enzymes or extracellular toxins prior to entry. *A. salmonicida* induces intestinal injury commencing in the anterior intestinal region (foregut)[2].

Mitigating Aeromonas infection can be achieved by enhancing the immune system of fish. Spirulina platensis is a natural substance utilized as an immunostimulant. The hot water extract of *Spirulina platensis* can enhance the immune system in shrimp. The hot water extract of *Spirulina platensis*, administered via injection and immersion techniques, resulted in an elevation of white blood cell counts in shrimp[3]. Investigations have been performed on tilapia, revealing that dosages of 200 mg/L, 400 mg/L, and 600 mg/L resulted in decreased damage to the intestinal villi of gourami fish[4]. The lipopolysaccharide content in *Spirulina platensis* can enhance the immune system and safeguard fish afflicted with Aeromonas[5]. Kozenko and Henson[6] indicated that phycocyanin derived from *Spirulina platensis* promotes hematopoiesis. A study was conducted on the histological and immunohistochemical characteristics of tilapia intestines immersed in *Spirulina platensis* extract and then infected with *Aeromonas salmonicida* bacteria.

2. Materials and Method

2.1. Ethical Approval

Ethical clearance for animal study was obtained from the Faculty of Veterinary Medicine, Gadjah Mada University (Certificate Number: 0023/EC-FKH/Int./2019), ensuring compliance with animal welfare guidelines and ethical standards.

2.2. Study Period and Location

The study was carried out from February to April 2019. The research site and immunohistochemistry analysis of fish intestines were conducted in the Veterinary Pathology Laboratory, Faculty of Veterinary Medicine, Gadjah Mada University. The production of Spirulina platensis hot water extract was conducted at the Joint Laboratory Facility, Faculty of Biology, Gadjah Mada University.

2.3. Preparation of *Spirulina platensis* extract

Forty grams of *Spirulina platensis* flour is combined with 450 milliliters of water and thereafter cooked using a hot plate stirrer for one hour at a temperature of 90°C. The cooled solution is centrifuged at 4°C at 2500 rpm for 15 minutes to separate the sediment from the supernatant. The supernatant is collected and subjected to freeze-drying. The dosage of *Spirulina platensis* hot water extract is determined based on the modified research of Tayag et al. (2010), namely at concentrations of 400 mg/L, 600 mg/L, and 800 mg/L.

2.4. Experimental Design

Thirty tilapia were acclimated for seven days and thereafter partitioned into five groups. The fish groups were P0-, tilapia devoid of Spirulina platensis extract and uninfected by *Aeromonas salmonicida*. Group P0+, tilapia devoid of *Spirulina platensis* extract, infected with *Aeromonas salmonicida*. Group P1 consisted of tilapia immersed in *Spirulina platensis* extract at a concentration of 400 mg/L for 3 hours on the 7th day, followed by a second immersion on the 14th day. Group P2 consisted of tilapia immersed in *Spirulina platensis* extract at a concentration of 600 mg/L, whereas Group P3 was treated with a concentration of 800 mg/L, similar to

©2025 Sekolah Kedokteran Hewan dan Biomedis, IPB University

Group P1. On the 21st day, groups P0+, P1, P2, and P3 were inoculated with *Aeromonas salmonicida* via intraperitoneal injection of 0.1 cc at a concentration of 10^9 cells/ml. All fish underwent an autopsy on the 28th day.

2.5. Histopathological Preparation of Intestinal Organs

The histological tissue process has multiple stages like fixation, dehydration, embedding, blocking, cutting, staining, and mounting. The intestinal organ tissue pieces are stored in 10% phosphate buffered formalin for 24 hours, and trimmed to a size of 0.3 × 0.5 × 0.5 cm. The subsequent procedure employs a tissue processor utilizing ethanol at concentrations of 70%, 80%, and 90%, followed by absolute ethanol three times, each for a duration of 60 minutes. The clarifying procedure involves three washes with xylol, followed by two infiltrations with liquid paraffin, each lasting one hour, and the subsequent sectioning of paraffin-embedded tissue at 5–7 micron using a microtome.

2.6. Data Analysis

Data were acquired by the study of microscopic observations of immunohistochemistry results, antigen-antibody interactions, antigen distribution, and the chromatic quality of binding responses, together with descriptive analysis.

3. Results

Immunohistochemical labeling of tilapia intestines yielded positive results in treatments P0, P1, and P2, but negative controls and treatment P3 exhibited negative immunohistochemistry (**Table 1**). Positive immunohistochemical staining results demonstrated the presence of *Aeromonas salmonicida* antigens, represented by a brown coloration in the tissue (**Figure 1**), with the bacteria uniformly dispersed across the intestinal mucosa. The histopathological examination of tilapia fish intestines treated with varying doses of Spirulina platensis reveals villi erosion also shown in **Figure 2**.

Table 1. The results of immunohistochemical and histopathological examination of intestinal organs

Groups	Number	IHC	HE
P0-	1	-	-
	2	-	-
	3	-	-
	4	-	-
P0+	1	-	-
	2	-	Erosion
	3	+	Erosion
	4	+	Erosion
P1	1	+	Erosion
	2	-	Erosion
	3	+	Inflammation
	4	-	Erosion, Inflammation
P2	1	+	-
	2	-	Erosion
	3	+	Erosion
	4	-	Inflammation
P3	1	-	-
	2	-	Erosion
	3	-	Erosion, Inflammation
	4	-	-

IHC: Immunohistochemistry; HE: Hematoxylin Eosin; +: positive result; - negative results

4. Discussion

Immunohistochemical staining can validate the diagnosis and ascertain the distribution of *Aeromonas salmonicida* in the tissues or organs of the fish, with a positive reaction indicated by the presence of a brown color resulting from the binding of antigens

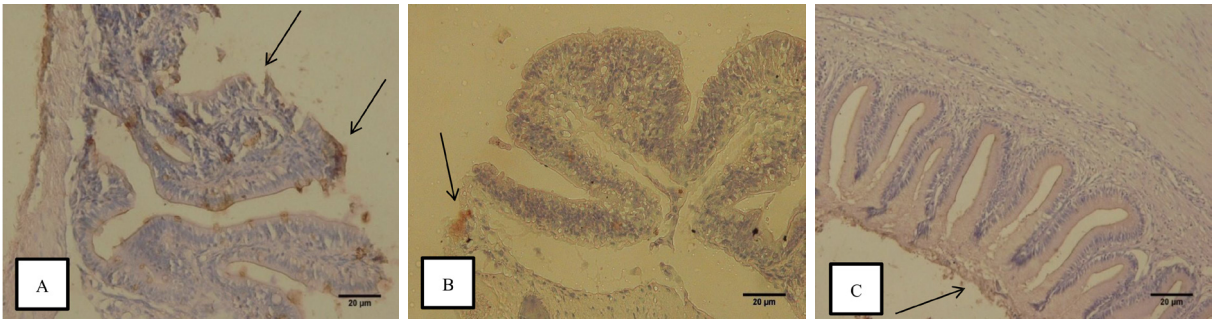

Figure 1. The results of the immunohistochemistry analysis of tilapia intestines indicate positive results, with brown coloration observed. A: P0+; B: P1 at a dosage of 400 mg/L; C: P2 at a dosage of 600 mg/L (↑)

©2025 Sekolah Kedokteran Hewan dan Biomedis, IPB University

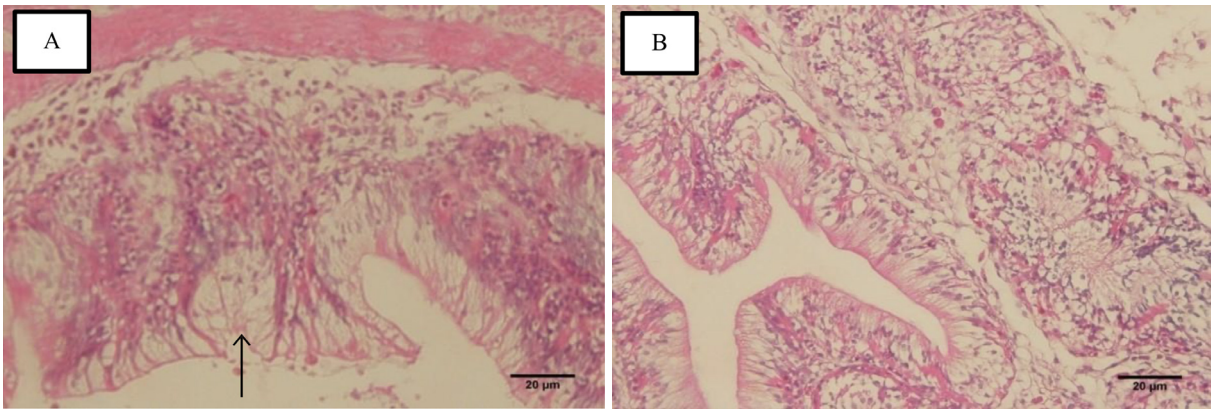

Figure 2. The histopathological examination of tilapia fish intestines treated with varying doses of *Spirulina platensis* reveals villi erosion (↑). A: P1 received a dosage of 400 mg/L; exhibiting an undamaged intestinal image; B: P2 received a dosage of 600 mg/L

and antibodies. Bintari[7] reported that the results of immunohistochemistry for *Aeromonas hydrophila* were consistent with those obtained using rabbit polyclonal serum, revealing brown patches that were either clustered in a specific region or dispersed across the kidney tissue.

The administration of *Spirulina platensis* diminished the proliferation of *Aeromonas salmonicida* within the intestinal tissue of tilapia. Lipopolysaccharide, a constituent of the *Spirulina platensis* cell wall, may act as an immunostimulant. Boajiang[8] asserts that the lipopolysaccharide (LPS) level in *Spirulina platensis* enhances non-specific cellular immunity and particular humoral function. LPS constitutes a component of the cell wall of *Spirulina platensis*. The administration of Spirulina platensis extract as an immunostimulant demonstrated a reduction in villous erosion and inflammatory cell density in the intestines of tilapia infected with *Aeromonas salmonicida*. Fish were immersed in *Spirulina platensis* extract twice, followed by an injection of 10^9 *Aeromonas salmonicida*/ml. The P0 treatment (tilapia devoid of Spirulina platensis extract and *Aeromonas salmonicida* infection) exhibited normal intestinal conditions. Fish in the P0 group exhibited health and were impervious to bacterial infection. The intestinal histology of the P0- group exhibited no alterations. The P0+ group (tilapia devoid of *Spirulina platensis* extract, then inoculated with *Aeromonas salmonicida* via injection) exhibited clinical manifestations of *Aeromonas salmonicida* infection. Bacteria were administered intramuscularly, swiftly entering the bloodstream and subsequently disseminating systemically as bacterial emboli to target organs. These findings align with research indicating the presence of *Aeromonas salmonicida* in several internal organs within 2 to 12 hours post-exposure[9][10].

Reduced activity of *Aeromonas salmonicida* bacteria attributed to immersion in Spirulina platensis. Phycocyanin and lipopolysaccharide derived from Spirulina platensis can enhance the immune system at a concentration of 400 mg/L. The microalga Spirulina platensis, a cyanobacterium, is frequently utilized as a natural feed supplement owing to its varied nutritional composition, which markedly improves biomodulatory and immunomodulatory capabilities[11]. The lipopolysaccharide concentration of Spirulina platensis is recognized for its immunostimulant properties, evidenced by the enhancement of macro- and microglobulin antibody production and a notable increase in macrophages. The polysaccharides in S. platensis can enhance non-specific cellular and humoral immunity[12].

According to the observation results of P1 (tilapia fish immersed in Spirulina platensis extract at a concentration of 400 mg/L for 3 hours on the 7th day, followed by a second immersion on the 14th day), the enhancement of the response remains minimal, indicating that bacterial activity cannot be entirely eradicated. The density of inflammatory cells in P1 indicates that erosion of intestinal villi provokes an inflammatory response characterized by the infiltration of inflammatory cells. Inflammatory cells traverse from blood vessels to the wounded area, characterized by the presence of polymorphonuclear cells and macrophages in the lamina propria. Treatment P2 (600 mg/L) demonstrated superior outcomes compared to P1,

©2025 Sekolah Kedokteran Hewan dan Biomedis, IPB University

as indicated by reduced villi erosion and a less dense presence of inflammatory cells . The P3 therapy (800 mg/L dosage) decreased erosion and inflammatory cell density in the intestines of tilapia. This results from heightened intestinal lymphoid activity and an elevated cell count in the lamina propria, facilitating the direct phagocytosis and elimination of bacteria, hence averting excessive bacterial activity and reducing villous erosion. The reduction in inflammatory cell density led to enhanced intestinal histopathology relative to the prior therapy[13]. Polysaccharides derived from Spirulina platensis stimulate monocytes and neutrophils in tilapia. The elevated monocyte and neutrophil counts in tilapia with Spirulina platensis hot water extract enhanced immune system activation. Neutrophils and macrophages in fish generate reactive oxygen species (ROS), which are linked to the phagocytes' capacity to eliminate infections[14][15].

5. Conclusion

The administration of *Spirulina platensis* extract at a concentration of 800 mg/L through immersion for 3 hours on the 7th and 14th days in tilapia, subsequently infected with *Aeromonas salmonicida*, can diminish the proliferation of *Aeromonas salmonicida* in the intestines of tilapia, as evidenced by immunohistochemistry.

Acknowledgment

The authors express gratitude to the Faculty of Veterinary Medicine at Gadjah Mada University for providing the research facility and to the School of Veterinary Medicine and Biomedical Sciences at IPB University for the collaborative research effort.

References

[1] **Selvaraj V, Sampath K, & Sekar V.** 2008. Administration of lipopolysaccharide increases specific and non-specific immune parameters and survival in carp (*cyprinus carpio*) infected with *Aeromonas hydrophila*. Journal of Aquaculture. 286: 176–183.

[2] **Austin B, & Austin DA.** 2007. *Aeromonadaceae* representatives (*Aeromonas salmonicida*). in: *bacterial fish pathogens*: *diseases in farmed and wild fish*, 4th edition. Praxis Publishing, Chichester. UK: 24–314.

[3] **Castro R, Zarra I, & Lamas J.** 2004. Water-soluble extracts modulate the respiratory burst activity of turbot phagocytes. Aquaculture. 229: 67–78.

[4] **Ramadhan MK, Arimbi & Sarmanu.** 2016. Efek Perendaman ekstrak *spirulina platensis* sebagai imunostimulan terhadap gambaran histopatologi usus ikan gurame (*Osphronemus gouramy*) yang diinfeksi *Aeromonas hydrophila*. Veterina Medika. 9(3): 1–6.

[5] **Tayag CM, Lin YC, Liou CH, & Chen JC.** 2010. Administration of the hot-water extract of *spirulina platensis* enhanced teh immune response of white shrimp litopenaeus vannamei and its resistance against vibrio agnolyticus. Journal Fish and Shellfish Immunology.

[6] **Kozenko R, & Henson RH.** 2010. The Study of Spirulina. Effects on the aids virus. cancer and the immune system. Healthy & Natural Journal. 2.

[7] **Bintari IG.** 2016. Deteksi Aeromonas hydrophila pada ginjal mencit (*Mus musculus*) dengan teknik Imunohistokimia. Surabaya: Universitas Airlangga. 1–56.

[8] **Boajjang G.** 1994. Study on effect and mechanism of polysaccharide of *spirulina platensis* on body immune function improvement. Book of abstracts. Second Asia Pacific Conference on Algal Biotechnology. Page: 24.

[9] **Svendsen YS, Dalmo RA, & Bøgwald J.** 1999. Tissue localization of *Aeromonas salmonicida* in Atlantic salmon, *Salmo salar* L., following experimental challenge. J. Fish Dis. 22: 125–131

[10] **Farto R, Milton DL, Bermúdez M, & Nieto T.** 2011. Colonization of turbot tissues by virulent and avirulent *Aeromonas salmonicida subsp. salmonicida* strains during infection. Dis. Aquat. Org. 95: 167–173.

[11] **Ibrahem MD, & Ibrahim MA.** 2014. The potential Effects of *Spirulina platensis* (*Arthrospira platensis*) on tissue protection of niletilapia (*oreochromis niloticus*) through estimation of P53 level. Cairo Faculty of Veterinary Medicine. Cairo University. page: 133–136.

[12] **Winarni ET.** 2014. Potensi *Spirulina platensis* dalam meningkatkan kekebalan tubuh ikan air tawar. Fakultas Biologi Universitas Jenderal Soedirman. Purwokerto.

[13]**Priyatna R, Indarjulianto S, & Kurniasih.** 2011. Infeksi *Aeromonas salmonicida* dari berbagai wilayah di indonesia pada ikan mas (*Cyprinus carpio*). Biota Vol. 16 (2): 287–297.

[14] **Gallay P, Heumann D, Roy DL, Barras C, & Glauser MP.** 1993. Lipopolysaccharide-binding protein as a major plasma protein responsible for endotoxemic shock. Proceedings of the National Academy of Sciences (USA). 90: 9935–9938.

[15] **Simanjuntak SBI.** 2002. Histologi organ lymphoid ikan patin jambal (*pangasius djambal bleeker*) yang diberi imunostimulan *spirulina* [Thesis]. Program Pascasarjana. Institut Pertanian Bogor. Bogor.

©2025 Sekolah Kedokteran Hewan dan Biomedis, IPB University