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Enhancement of Fish Immunity and Survival Rate Through Dietary Supplementation with *Sargassum* sp. Powder

Peningkatan Imunitas dan Sintasan Ikan dengan Penambahan Serbuk Sargassum sp pada Pakan

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Abstract

Pathogenic bacterial infections in intensive aquaculture systems are among the primary causes of high fish mortality, underscoring the need for safe, sustainable natural antibacterial alternatives. *Sargassum* sp. is known to contain bioactive compounds with potential antibacterial and immunostimulatory properties. This study aimed to evaluate the effects of dietary supplementation with Sargassum sp. powder on the immune response and survival rate of Nile tilapia (*Oreochromis niloticus*). The experiment was conducted using a Completely Randomized Design (CRD) with one factor and five treatments: negative control (Kn), positive control (Kp), and dietary treatments P1 (15 g/kg feed), P2 (30 g/kg feed), and P3 (45 g/kg feed). Observed parameters included hematological profiles (erythrocytes, leukocytes, hemoglobin, hematocrit, and leukocrit) and survival rate during 30 days of rearing. The results showed that dietary supplementation with *Sargassum* sp. powder significantly improved hematological parameters in Nile tilapia compared with the control groups. The highest values were observed in P1, with total erythrocytes (1.66×10^6 cells/mm^3), hemoglobin (8.73%), hematocrit (31.3%), and leukocrit (1.67%). The highest survival rate was also recorded in P1 and Kp (100%). Therefore, the inclusion of *Sargassum* sp. powder at 15–30 g/kg feed is recommended as the optimal dosage to enhance the immunity and survival rate of Nile tilapia.

Keywords: *Sargassum* sp., Immunostimulant, Hematology, Nile tilapia

Abstrak

Serangan bakteri patogen dalam sistem budidaya intensif menjadi salah satu penyebab utama tingginya mortalitas ikan, sehingga diperlukan alternatif antibakteri alami yang aman dan berkelanjutan. *Sargassum* sp. diketahui mengandung senyawa bioaktif yang berpotensi sebagai antibakteri sekaligus imunostimulan alami. Penelitian ini bertujuan untuk mengevaluasi pengaruh penambahan serbuk *Sargassum* sp. dalam pakan terhadap peningkatan imunitas dan sintasan ikan nila (*Oreochromis niloticus*). Riset dilakukan menggunakan Rancangan Acak Lengkap (RAL) satu faktor dengan lima perlakuan: kontrol negatif (Kn), kontrol positif (Kp), serta perlakuan P1 (15 g/kg pakan), P2 (30 g/kg pakan), dan P3 (45 g/kg pakan). Parameter yang diamati meliputi hematologi ikan (eritrosit, leukosit, hemoglobin, hematokrit, dan leukokrit) serta sintasan selama 30 hari pemeliharaan. Hasil penelitian menunjukkan bahwa suplementasi serbuk *Sargassum* sp. mampu meningkatkan parameter hematologi ikan nila

secara signifikan dibanding kontrol. Nilai tertinggi diperoleh pada P1 dengan total eritrosit (1,66 × 10^6 sel/mm^3), hemoglobin (8,73%), hematokrit (31,3%), dan leukokrit (1,67%). Sintasan tertinggi juga diperoleh pada P1 dan Kp, yaitu sebesar 100%. Dengan demikian, penambahan serbuk *Sargassum* sp. sebanyak 15–30 g/kg pakan direkomendasikan sebagai dosis optimal untuk meningkatkan imunitas dan survival rate ikan nila.

Kata kunci: *Sargassum* sp., Imunostimulan, Hematologi, Ikan nila

1. Introduction

The global aquaculture industry experiences significant economic losses each year due to disease outbreaks (Méndez et al., 2024). For several decades, the use of synthetic antibiotics (Nagarajan et al., 2024) has been the primary approach to controlling bacterial infections in aquaculture systems. However, this dependency has led to new problems, including the emergence of antimicrobial resistance, environmental pollution (Etienne et al., 2025), and potential health risks to consumers. Disease outbreaks caused by pathogenic infections remain one of the main threats to the sustainability of aquaculture. Several cases of bacterial infection have been reported in fish farming areas in Aceh, including Aceh Besar (Anggraini et al., 2016). One of the main bacterial pathogens responsible for these outbreaks is *Aeromonas hydrophila*.

Infection by *A. hydrophila* is a common and problematic disease in fish because this Gram-negative bacterium can produce antibiotic-resistant strains (Méndez et al., 2024). It is known as the causative agent of Motile *Aeromonas* Septicemia (MAS) (Fang et al., 2025; Guo et al., 2024; Wang et al., 2025), which is characterized by systemic hemorrhage, tissue damage, and mass mortality. *A. hydrophila* infects fish through the digestive tract and targets vital organs such as the hepatopancreas, kidneys, and brain (Wang et al., 2025). The high mortality rate caused by *A. hydrophila* infection significantly impacts aquaculture productivity (Liang et al., 2024) and the economic stability of fish farmers. Conventional disease management in aquaculture still relies heavily on antibiotics, which in the long term can lead to bacterial resistance and the accumulation of harmful residues in aquatic environments.

These conditions highlight the urgent need to develop natural, environmentally friendly control agents that are both effective and sustainable (Hussaini et al., 2024; Sattanathan et al., 2024). In this context, macroalgae from the genus *Sargassum* have emerged as promising candidates. *Sargassum* species contain a variety of bioactive compounds (Dip et al., 2024), including sulfated polysaccharides, phenolics, flavonoids, and terpenoids, which possess antibacterial (Al-Mur & Alsiary, 2025), antioxidant (Dangariya et al., 2024), and immunostimulant properties. In Indonesia, particularly in the coastal waters of Aceh, *Sargassum* sp. is abundantly available but remains underutilized. Previous studies have shown that the incorporation of *Sargassum* sp. extract into fish feed can enhance the immune response of Nile tilapia (*Oreochromis niloticus*) against *A. hydrophila* infection (Gazali et al., 2024). However, large-scale application of *Sargassum* extracts is often limited by high production costs, which require simpler, more cost-effective processing methods, such as converting the material into powder.

Sargassum sp. powder can be easily incorporated into fish feed to reduce stress levels, enhance immune response, and inhibit pathogen growth (Chellapandian et al., 2023). Therefore, the development of *Sargassum* sp. powder as a natural antibacterial and immunostimulant agent holds great potential for improving fish immunity and survival rates in aquaculture. This study aims to evaluate the effect of dietary supplementation with *Sargassum* sp. powder on fish immunity and survival rate. The improvement in fish immunity was assessed through hematological parameters, including erythrocyte, leukocyte, hemoglobin, and hematocrit levels, while the effectiveness of immune enhancement was evaluated based on survival rates following bacterial challenge tests. Thus, this study is expected to provide an effective natural alternative to improve fish health and support sustainable aquaculture practices without reliance on synthetic antibiotics.

2. Material and Method

2.1. Time and Place

The research was conducted from August to October 2025. The *Sargassum* sp. macroalgae were collected from Lhok Bubon Beach, Samatiga District, West Aceh. The preparation of *Sargassum* powder and haematological analysis of fish blood were carried out at the Laboratory of Environmental Systems and Technology. In contrast, fish rearing was conducted at the Hatchery Laboratory.

2.2. Methods

This study employed an experimental method using a Completely Randomized Design (CRD) with a single factor comprising five treatments and three replications, yielding a total of 15 experimental units. The treatments

were as follows: Kn = Negative Control, Kp = Positive Control, P1 = Feed containing 15 g/kg *Sargassum* sp. powder, P2 = 30 g/kg feed, and P3 = 45 g/kg feed (Effendi et al., 2025). The evaluated parameters consisted of survival rate and hematological parameters.

2.3. Procedures

2.3.1. Preparation of Feed Containing Sargassum sp. Powder

The *Sargassum* sp. used in this study was collected from Lhok Bubon Beach, West Aceh, Indonesia. The *Sargassum* sp. collected from the research site was shade-dried for 7 days without direct sunlight exposure and then ground into a fine powder using a blender. The powder was mixed into commercial feed at doses according to the experimental design. The mixture was pelletized and dried before proximate analysis was conducted to determine its nutritional content (Gazali et al., 2024).

2.3.2. Fish Rearing

The fish size is 3-5 cm. The fish were reared in containers measuring 20 × 40 × 20 cm with a water volume of 10 L and a stocking density of 10 fish per container. The rearing period lasted for 30 days. The fish were fed three times daily at 08:00, 13:00, and 18:00 WIB at a feeding rate of 5% of their body weight (Effendi et al., 2025). Length and weight measurements were taken every 10 days to monitor growth performance.

2.3.3. Blood Sampling

Blood samples were collected twice, once prior to treatment and once after 30 days of rearing. Before blood collection, the fish were anesthetized using clove oil at a concentration of 0.1 mL/L. Syringes and Eppendorf tubes were pre-rinsed with 10% EDTA solution to prevent coagulation. Blood was drawn from the caudal vein along the lateral line using a 1 mL syringe (Effendi et al., 2025). The collected blood was then transferred into Eppendorf tubes and used for haematological analysis. The measured hematological parameters included E = erythrocytes; L = leukocytes; G = blood glucose; Hb = hemoglobin; Hk = hematocrit; and Lk = leukocrit.

2.4. Data Analysis

The data obtained during the experiment, including hematological parameters and survival rate, were tabulated and analyzed descriptively. The results were presented in tables and figures to illustrate differences among treatments.

3. Result and Discussion

3.1. Fish Hematology

Observations on the hematological parameters of Nile tilapia during 30 days of rearing showed variations in erythrocyte (E), leukocyte (L), blood glucose (G), hemoglobin (Hb), hematocrit (Hk), and leukocrite (Lk) values across treatments (Table 1). The erythrocyte count at the beginning of the rearing period was recorded at 1.12 × 10^6 cells/mm^3, which increased after the administration of feed containing *Sargassum* sp. powder. The highest erythrocyte value was observed in P1 (1.66 × 10^6 cells/mm^3), followed by P3 (1.63 × 10^6 cells/mm^3) and P2 (1.62 × 10^6 cells/mm^3), while the lowest value was found in the negative control (Kn) at 1.39 × 10^6 cells/mm^3.

The increase in erythrocyte count indicates an improvement in the physiological condition of the fish due to the positive effect of *Sargassum* sp. supplementation on the hematopoietic system and oxygen-carrying capacity of the blood. According to Royan et al. (2014); Hanum et al. (2021), a higher erythrocyte count reflects enhanced blood capacity to transport oxygen, enabling fish to better adapt to environmental stress. This suggests that the bioactive compounds present in *Sargassum* sp, such as sulfated polysaccharides and fucoxanthin pigments, may act as antioxidants that protect erythrocytes from oxidative damage, thereby supporting improved hematological performance and overall health in tilapia.

Table 1. Hematological Parameters of Nile Tilapia During 30 Days of Rearing

Treatment	RBC (10^6)	WBC (10^4)	G (mg/dL)	Hb (%)	Hk (%)	Lk (%)
Initial Rearing Phase	1.12	1.77	55.33	5.87	25.67	1.33
30 Days of Rearing						
Kn	1.39	1.89	56	7.30	28	1
Kp	1.41	1.88	51	7.40	28.30	1.33
P1	1.66	2.00	74	8.73	31.30	1.67
P2	1.62	1.97	72.33	8.07	30.33	1.33
P3	1.63	1.97	71	8.60	30.67	1.67

Note: RBC: Red Blood concentration; WBC: White Blood Concentration; Kn = Negative control; Kp = Positive control; P1 = feed supplemented with *Sargassum* sp. extract at 20 g/kg; P2 = 30 g/kg feed; P3 = 45 g/kg feed; E = Erythrocytes; L = Leukocytes; G = Blood glucose; Hb = Hemoglobin; Hk = Hematocrit; Lk = Leukocrit

The erythrocyte count of Nile tilapia at the beginning of rearing was 1.12 × 10^6 cells/mm^3 and increased after 30 days of rearing, ranging between 1.39–1.66 × 10^6 cells/mm^3. The highest value was recorded in treatment P1

(1.66×10^6 cells/mm³), while the lowest was found in the negative control (1.39×10^6 cells/mm³). The normal erythrocyte counts in teleost fish ranges from 1.05–3.0 × 10^6/mm³ (Chauhan et al., 2025). The increase in erythrocyte number indicates an enhanced oxygen-carrying capacity of the blood, reflecting improved physiological conditions and red blood cell production influenced by *Sargassum* sp. supplementation. According to Harianto et al. (2020), an elevated erythrocyte count in fish suggests improved respiratory efficiency and greater resistance to environmental stress. The bioactive compounds in *Sargassum* sp., such as sulfated polysaccharides and fucoxanthin pigments, are likely antioxidants that protect erythrocyte cells from oxidative damage.

The leukocyte counts of Nile tilapia increased from 1.77×10^4 cells/mm³ at the beginning to 1.88–1.97×10^4 cells/mm³ after 30 days of rearing. The highest value was observed in treatment P1 (2×10^4 cells/mm³). According to Fauzan et al. (2017), Nile tilapia with leukocyte counts below the normal range of 20,000–150,000 cells/mm³ are more susceptible to bacterial infections. Leukocytes function as non-specific immune cells that play a vital role in the fish's defense system. The increase in leukocyte count indicates an enhanced immune response to pathogenic agents. As stated by Hastuti et al. (2024), an increase in leukocytes in fish fed immunostimulant-supplemented diets reflects the activation of their immune defense mechanisms. The bioactive compounds in *Sargassum*, such as fucoidan and phenolic compounds, are thought to stimulate phagocytic activity of leukocytes, thereby improving the fish's ability to cope with stress and bacterial infection (Muahiddah & Asri, 2024).

The blood glucose levels of Nile tilapia ranged from 51 to 74 mg/dL after 30 days of rearing. The highest glucose level was found in treatment P1 (74 mg/dL), while the lowest was in the positive control (51 mg/dL). Changes in blood glucose levels reflect the physiological stress response of fish. According to Dinar et al. (2023), elevated glucose levels represent a temporary adaptive response to stress or elevated metabolic activity. The normal blood glucose range for Nile tilapia is 46–209 mg/dL. The fact that glucose levels remained within the normal range indicates that *Sargassum* sp. supplementation did not cause excessive stress but instead supported metabolic stability by enhancing enzymatic activity and energy availability.

Hemoglobin levels increased from 5.87% at the start of the experiment to 7.30–8.73% after treatment. The highest hemoglobin concentration was observed in treatment P1 (8.73%), while the lowest was recorded in the negative control (7.30%). According to Siregar et al. (2025), normal hemoglobin levels in Nile tilapia range from 6 to 12.67 g/dL. Hemoglobin plays a crucial role in oxygen binding and transport within the bloodstream (Saraswati, 2021). The increase in hemoglobin concentration demonstrates an improvement in the physiological function of blood because of *Sargassum* sp. supplementation. Clauss et al. (2008) reported that higher hemoglobin levels indicate healthier fish with optimal metabolism (Suriyadin et al., 2023). The presence of minerals such as iron (Fe) and zinc (Zn) in *Sargassum* sp. may contribute to the synthesis of hemoglobin in erythrocytes.

The hematocrit value increased from 25.67% at the beginning to 28–31.3% at the end of rearing, with the highest level observed in P1 (31.3%). Hematocrit represents the proportion of red blood cells in the total blood volume (Mayasari, 2023). The increase indicates a higher erythrocyte concentration and improved oxygen transport efficiency. Elevated hematocrit values indicate good physiological conditions and the absence of anaemia (Maryani et al., 2021). The positive effect of *Sargassum* sp. may be attributed to its sulfated polysaccharides, which enhance metabolism and improve blood circulation.

The leukocrit values of Nile tilapia ranged from 1.00–1.67%, with the highest value observed in treatments P1 and P3 (1.67%). Leukocrit indicates the proportion of leukocytes in the blood and serves as an index of immune system activity (Waruwu, 2023). The increase in leukocrit values in fish fed *Sargassum* sp. powder suggests that this brown seaweed acts as a natural immunostimulant that enhances leukocyte production and activity. Elevated leukocrit levels reflect the stimulation of non-specific defense mechanisms that enable fish to effectively combat pathogenic agents (Hastuti et al., 2024).

Overall, the improvement in haematological parameters demonstrates that *Sargassum* sp. supplementation effectively enhances the blood's capacity for oxygen transport and immune defense (Hanum et al., 2021). This effect is associated with the presence of bioactive compounds such as sulfated polysaccharides, phenolic compounds, and fucoxanthin pigments, which exhibit antioxidant and immunostimulant properties (Rohim, 2019; Maryani et al., 2021; Wahyuni et al., 2024). In conclusion, dietary inclusion of *Sargassum* sp. powder at 20–45 g/kg feed significantly improved various hematological parameters in Nile tilapia, with the optimal results observed in P1 (20 g/kg). This indicates that *Sargassum* sp. has strong potential as a natural immunostimulant for improving the physiological performance and health of cultured fish.

3.2. Survival Rate

Based on observations over 30 days of rearing, the survival rate of Nile tilapia varied among treatments (Figure 1). At the beginning of the experiment, all treatments exhibited 100% survival, indicating that the fish were in good condition and that initial rearing conditions were uniform. After 30 days, the negative control (Kn) group showed a decrease in survival rate to 90%, while the positive control (Kp) and P1 (feed supplemented with *Sargassum* sp. extract at 20 g/kg) achieved the highest survival rate of 100%. P2 (30 g/kg) and P3 (45 g/kg) displayed slightly lower survival rates of 97% and 93%, respectively.

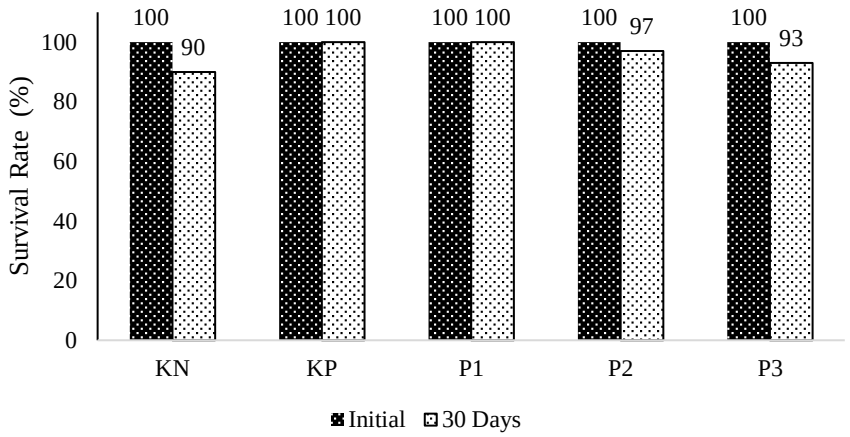

Figure 1. Survival Rate of Nile Tilapia During 30 Days of Rearing
Note: Kn = Negative control; Kp = Positive control; P1 = feed supplemented with *Sargassum* sp. extract at 20 g/kg; P2 = 30 g/kg feed; P3 = 45 g/kg feed

The variation in survival rates indicates that adding *Sargassum* sp. extract to fish feed had a positive effect on survival, particularly at doses of 20–30 g/kg feed. The bioactive compounds present in *Sargassum* sp., such as sulfated polysaccharides, phenolics, flavonoids, and terpenoids, are known for their strong immunostimulant and antioxidant activities (Husein, 2022). These compounds enhance leukocyte phagocytic activity, strengthen the non-specific immune system, and reduce oxidative stress caused by environmental conditions or pathogenic infections (Muahiddah & Diniariwisan, 2024). This explains the high survival rates observed in P1 and P2.

Conversely, the decline in survival rate at the highest dose (P3, 45 g/kg) is likely due to metabolic adaptation or physiological saturation of bioactive compounds within the fish's body (Nugroho & Nur, 2018). Excessive doses may lead to physiological imbalances, interfere with digestive processes, or induce stress due to altered feed flavour and odour, resulting in reduced feed intake. These factors can ultimately compromise immune performance and slightly increase mortality compared to lower-dose treatments.

Overall, these findings demonstrate that moderate supplementation with *Sargassum* sp. extract improves fish survival during the rearing period. Similar phenomena have been reported by Muahiddah & Asri (2024); Tanbiyaskur et al. (2024), who found that supplementation with brown macroalgae enhanced immune responses and survival rates in Nile tilapia infected with *A.hydrophila*. Therefore, dietary inclusion of *Sargassum* sp. extract at 20–30 g/kg feed can be considered the optimal dosage for improving fish survival by enhancing natural immune defense mechanisms.

4. Conclusions

In conclusion, dietary supplementation with *Sargassum* sp. powder significantly improved the hematological parameters and survival rate of Nile tilapia. The optimal response was observed at 15 g/kg feed, indicating that *Sargassum* sp. has strong potential as a natural immunostimulant to support sustainable aquaculture practices.

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