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EFFECT OF *Chaetomorpha* sp. EXTRACT ON THE HEMATOLOGY OF TILAPIA (*Oreochromis niloticus*) INFECTED WITH *Aeromonas hydrophila*

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ABSTRACT

Chaetomorpha sp. is a green macroalgae that contains various bioactive compounds with antibacterial and anti-inflammatory activities, as well as the potential to improve the physiological health of fish. This research investigates how incorporating *Chaetomorpha* sp. extract into the diet influences the hematological parameters of Tilapia (*Oreochromis niloticus*) challenged with *Aeromonas hydrophila*. The study used a completely randomized design (CRD) with four levels of extract supplementation (0, 25, 50, and 75 mL/kg of feed), with each treatment replicated three times. The hematological parameters analyzed included total erythrocytes, hematocrit, hemoglobin, and blood glucose. The findings indicated that adding *Chaetomorpha* sp. extract to the diet significantly ($p<0.05$) improved all hematological indicators relative to the control, with 50 mL/kg of feed as the most effective dosage. The highest erythrocyte count 1.89 $x10^6$ cells/mm^3, hematocrit value 35.67%, and hemoglobin concentration 6.87 g/dL were observed in fish fed 50 mL/kg extract. This increase in hematological values indicates improved oxygen transport capacity, physiological condition, and metabolic response in fish. Bioactive constituents, including sulfated polysaccharides and flavonoids, are believed to enhance the hematological condition of fish. These findings confirm the potential of *Chaetomorpha* sp. as a natural supplement to support Tilapia health in sustainable aquaculture systems.

Keywords: *Chaetomorpha* sp, Hematology, *Oreochromis niloticus, Aeromonas hydrophila*

1. INTRODUCTION

In recent decades, the aquaculture sector has expanded rapidly worldwide to meet the increasing demand for sustainable protein sources. Among cultured species, Tilapia (*Oreochromis niloticus*) is economically important due to its broad environmental tolerance, efficient feed conversion, and high consumer acceptance[1]. However, intensification of aquaculture systems often leads to environmental stress and deteriorating water quality, increasing susceptibility to bacterial diseases, particularly infections caused by *Aeromonas hydrophila*[2]. This pathogen is the primary etiological agent of *Motile Aeromonas Septicemia* (MAS), which is characterized by hemorrhagic lesions, tissue necrosis, and high mortality in Tilapia and other freshwater fish species[3].

Hematological parameters represent reliable physiological indicators of fish health during bacterial infection. Alterations in erythrocyte count, hemoglobin

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concentration, and hematocrit reflect changes in oxygen transport capacity and hematopoietic function. At the same time, blood glucose serves as a sensitive biomarker of metabolic stress and immune activation in response to pathogenic or environmental challenges[4,5].

To reduce dependence on antibiotics and support sustainable aquaculture, bioactive compounds from marine resources, particularly macroalgae, have gained increasing attention as natural immunostimulants and antimicrobial agents[6,7]. *Chaetomorpha* sp., a filamentous green macroalga commonly found in coastal and estuarine environments, has been reported to exhibit antioxidant, antibacterial, and immunomodulatory activities[8]. These effects are largely attributed to bioactive compounds such as phenolics and sulfated polysaccharides, which enhance immune responses and mitigate oxidative stress in aquatic organisms[9].

Several studies have demonstrated the positive effects of *Chaetomorpha* sp. supplementation on fish physiology and disease resistance. For example, *Chaetomorpha* sp. extracts have been shown to improve hematopoietic and immunological parameters in Catfish (*Clarias batrachus*) challenged with *A. hydrophila* and in Tilapia infected with *Edwardsiella* tarda[10,11].

Despite increasing evidence of Chaetomorpha sp.'s immunostimulatory potential, information on hematological responses of *O. niloticus* to *A. hydrophila* challenge remains limited. In particular, studies systematically evaluating the dose-dependent effects of *Chaetomorpha* sp. extract on key hematological indicators during bacterial infection are scarce.

Therefore, this study provides an integrated evaluation of the dose-response effects of dietary *Chaetomorpha* sp. extract on key hematological parameters (erythrocytes, hemoglobin, hematocrit, and blood glucose) in *O. niloticus* experimentally challenged with *A. hydrophila*. By combining hematological profiling with a controlled bacterial challenge model, this study provides new insights into the physiological mechanisms underlying *Chaetomorpha* sp.'s protective role as a natural feed additive.

Accordingly, this study aimed to investigate the effects of different dietary doses of *Chaetomorpha* sp. extract on the hematological responses of *O. niloticus* challenged with *A. hydrophila*, and to identify an optimal supplementation level to support fish health in sustainable aquaculture systems.

2. RESEARCH METHOD

Time and Place

This study was carried out between August and December 2023 at the Marine Microbiology Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Riau.

Method

An experimental approach was employed using a completely randomized design (CRD) consisting of four treatments, each replicated three times. The treatment dose refers to Wahyuni et al.[12], namely:

T0 : Commercial pellets
T1 : *Chaetomorpha* sp extract dose of 25 mL/kg feed
T2 : *Chaetomorpha* sp extract dose of 50 mL/kg feed
T3 : *Chaetomorpha* sp extract dose of 75 mL/kg feed

Procedures

Preparation of *Chaemtomorpha* sp. Extract and Feed

The *Chaetomorpha* sp. macroalgae used in this study were collected from Lhok Bubon Beach, located in Samatiga District, West Aceh Regency. A total of 20 g of samples were washed with running water to remove impurities, then extracted with warm water (60°C) at a ratio of 1:5 (b/v) for 30 minutes. Warm water was selected as the extraction solvent because it is effective for extracting water-soluble bioactive compounds, particularly sulfated

polysaccharides and phenolic compounds, while avoiding the residual toxicity associated with organic solvents[13]. The extraction temperature of 60°C was chosen to enhance extraction efficiency without causing thermal degradation of heat-sensitive bioactive compounds[14]. The extract was filtered using filter paper to obtain a brown aqueous extract, which was subsequently used for feed supplementation.

The commercial pellets were FF-999 (PT. Central Proteina Prima Tbk, Deli Serdang, Indonesia). Commercial pellets were weighed at 1 kg per treatment, and the extract solution was applied by spray at 25, 50, and 75 mL/kg feed. The feed was then dried and ready for use.

Fish Adaptation and Maintenance

Tilapia were sourced from farmers in Kampar Regency, Riau, Indonesia. The test fish weighed 2.63±0.26g and 5.87±0.07 cm in length. Before use, the fish were acclimated for 7 days in 2 x 1 m fiber tanks with water quality parameters of 28-30 ˚C, pH 5.5-6.8, and DO 4-5 mg/L.

After the acclimation period, fish were randomly allocated to experimental tanks and dietary treatments using a random assignment procedure to ensure equal probability of distribution and to minimize selection bias, in accordance with the principles of a completely randomized design (CRD). During the rearing phase, fish were maintained for 60 days in black tanks measuring 60 × 30 × 30 cm with a volume of 80 L, equipped with a recirculating filtration system. Each replicate consisted of 20 fish per tank at a stocking density of 1 fish per 4 L, resulting in 60 fish per treatment and a total of 240 fish used in the experiment. Fish were fed three times daily at 08:00 AM, 01:00 PM, and 05:00 PM at a feeding rate of 5% of their body weight[12].

Challenge Test with *A. hydrophila*

The challenge test was conducted following the 60-day rearing period. The *A. hydrophila* isolate used originated from the culture collection of the Marine Microbiology Laboratory at the Faculty of Fisheries and Marine Sciences, Universitas Riau. Fish were injected intramuscularly with 0.1 mL of the bacterial suspension at a concentration of 10^8 CFU/mL. This concentration was selected based on previous studies demonstrating that 10^8 CFU/mL is sufficient to induce clinical infection and hematological alterations in Tilapia without causing excessive acute mortality, thereby enabling reliable assessment of physiological and immune responses[3,11]. After infection, the fish were maintained for an additional 14 days, during which clinical signs and mortality were monitored.

Collecting Fish Blood

Blood samples were collected at three time points: at the start of the experiment (day 1), after the rearing period (day 60), and after the challenge test (day 75). Prior to sampling, the fish were anesthetized using cold temperature (±8 °C) as described by Effendi et al.[15]. Blood was then withdrawn from the caudal vein using a 1 mL syringe and placed into Eppendorf tubes for analysis of total erythrocyte count, hematocrit, hemoglobin, and blood glucose levels. After sampling, the fish were transferred to an aerated recovery container until they regained normal movement, then returned to their original tanks.

Total Erythrocytes

To measure total erythrocytes, blood treated with an anticoagulant was drawn into a hemocytometer pipette (marked with red beads for erythrocytes) up to the 0.5 mark. Hayem's solution was then added until the pipette reached the 101 mark. The mixture was homogenized by gently shaking the pipette in a figure eight motion for 3–5 minutes. Before counting, the first two drops were discarded to eliminate air bubbles. A drop of diluted blood was then placed on the hemocytometer chamber, covered with a cover glass, and examined under a microscope at 10×10 magnification. The total erythrocyte count was obtained from

five small squares of the hemocytometer using the formula provided by Blaxhall & Daisley[16].

Number of Erythrocytes =∑n x 10^6 cells/mm^3

n: number of the calculated erythrocyte

Hematocrit

The blood sample is placed in a hematocrit capillary tube until approximately four-fifths of the tube is filled. The capillary tube is sealed at its end with a special stopper or a crystoseal, and the capillary is placed in a microhematocrit centrifuge. The microhematocrit tubes were then centrifuged at 3000 rpm for 5 minutes, with equal volume tubes positioned opposite each other to maintain balanced rotation. The hematocrit value is then measured. The hematocrit value is expressed as a percentage of the blood cell volume[17]. The hematocrit value is then read on a microhematocrit reader.

Hemoglobin

Hemoglobin concentration was determined using the Sahli method. The procedure began by adding 0.1 N HCl to a Sahli tube up to the zero mark (bottom scale). The tube was then positioned between two standard color tubes. Blood from the fish was drawn using a Sahli pipette, transferred into the Sahli tube, and allowed to react for 3 minutes after the pipette tip was cleaned. Distilled water was subsequently added dropwise with a dropper while stirring with a glass rod until the resulting color matched the reference standard. Hemoglobin values were recorded in g/dL[18].

Blood Glucose

Blood glucose levels were assessed using a GlucoDr (Allmedicus) device with a measurement range of 20–600 mg/dL. The glucose analysis was performed in the morning prior to feeding the fish[19].

Data Analysis

Data from total erythrocyte, hematocrit, hemoglobin, and blood glucose measurements were collected and tabulated in the Table. Subsequently, the data were statistically evaluated using SPSS version 26 and analyzed using a One-Way ANOVA. If the analysis results indicated an influence, a further test was carried out using Student-Newman-Keuls (SNK).

3. RESULT AND DISCUSSION

The results showed that the addition of *Chaetomorpha* sp. extract to the feed resulted in significant differences between treatments ($p<0.05$) in the hematological parameters of Tilapia during 60 day of maintenance and after the challenge test with *A. hydrophila* on day 75, in line with previous findings that hematological parameters are sensitive indicators for assessing fish health during bacterial infection and when given natural immunostimulants[20].

Based on Table 1, after 60 days of feeding, total erythrocyte counts increased in all treatments receiving *Chaetomorpha* sp. extract compared to the control. The highest value was observed in treatment T2 50 mL/kg, reaching 1.88 x10^6 cells/mm^3, which was significantly higher than the control T0 1.62 x10^6 cells/mm^3. This increase may indicate enhanced erythropoietic activity or improved erythrocyte stability, potentially associated with the presence of antioxidant and immunostimulatory compounds in *Chaetomorpha* sp., such as phenolics and sulfated polysaccharides[21,22].

Over the 60-day maintenance period, treatment T2 showed the highest hematocrit value of 35.67%, which was significantly higher than that of the control (32.00%). Elevated hematocrit levels may reflect improved oxygen transport capacity and overall physiological condition in supplemented fish[23]. This response may be associated with the presence of bioactive compounds in *Chaetomorpha* sp., including phenolics, flavonoids, and sulfated polysaccharides, which have been reported to exert antioxidant and immunostimulatory effects that support erythrocyte stability[24]. Similar improvements in hematocrit have

been reported in Tilapia fed plant- or algae-derived additives, where enhanced antioxidant status was linked to reduced physiological stress[25].

Table 1. Hematological Profile of Tilapia (*O. niloticus*)

Parameters	Treatment (mL/kg feed)			
	T0 (control)	T1 (25)	T2 (50)	T3 (75)
60 day				
Total Erythrocytes (x10^6 cells/mm^3)	1.62±0.01^a	1.78±0.01^b	1.88±0.05^d	1.82±0.01^c
Hematocrit (%)	32.00±1.00^a	33.33±0.58^a	35.67±0.58^b	33.33±0.58^a
Hemoglobin (g/dL)	6.47±0.11^a	6.67±0.11^a	6.87±0.11^b	6.53±0.11^a
Blood glucose (mg/dL)	55.67±5.13^a	53.33±2.51^a	56.00±2.64^a	53.67±2.51^a
Post challenge test (75 days)				
Total Erythrocytes (x10^6 cells/mm^3)	-	1.79±0.05^b	1.89±0.05^d	1.83±0.05^c
Hematocrit (%)	-	33.67±0.58^b	35.67±0.58^c	33.67±0.58^b
Hemoglobin (g/dL)	-	6.67±0.11^b	6.87±0.11^b	6.67±0.11^b
Blood glucose (mg/dL)	-	84.00±5.56^b	90.00±1.00^b	87.33±1.52^b

Notes: Different *superscript* letters in the same column indicate significant differences between treatments ($P<0.05$)

After the challenge test on day 75, hematocrit values in treatment T2 remained higher 35.67% than in the other treatments. This sustained response suggests that *Chaetomorpha* sp. extract may help maintain blood homeostasis during bacterial infection. Such effects may be related to the role of sulfated polysaccharides in enhancing non-specific immune responses and mitigating tissue damage, thereby reducing hemodilution commonly observed under pathological stress conditions[26].

Hemoglobin concentration is a key indicator of respiratory efficiency and metabolic capacity in fish. After 60 days of feeding, treatment T2 showed the highest hemoglobin level 6.87 g/dL, which was significantly higher than that of the control. This increase may reflect improved erythrocyte function and oxygen-carrying capacity rather than direct stimulation of erythropoiesis. A similar hemoglobin enhancement has been reported in Tilapia fed algal or plant-based immunostimulants[10,27]. The observed response may be associated with bioactive compounds in green algae, including sulfated polysaccharides, flavonoids, and phenolic compounds, which have been reported to support hematopoietic function and maintain hemoglobin levels by modulating erythropoietic pathways [28].

Following the challenge test on day 75, hemoglobin levels in treatment T2 remained the highest 6.87 g/dL, indicating a sustained hematological response during *A. hydrophila* infection. This finding suggests that dietary supplementation with *Chaetomorpha* sp. extract may help maintain hemoglobin stability under pathogenic stress. While bacterial infections are often associated with hemoglobin reduction due to hemolysis and increased metabolic oxygen demand, fish receiving the extract exhibited a more stable hemoglobin profile, consistent with previous Tilapia studies reporting protective effects of algal supplementation during pathogen exposure[10,21].

Blood glucose is a sensitive physiological indicator of stress and energy allocation in fish. During the maintenance period on day 60, glucose levels ranged from 53 to 56 mg/dL across all treatments and did not differ significantly, suggesting that dietary supplementation with Chaetomorpha sp. extract did not induce metabolic stress or activate the hypothalamic-pituitary-*interrenal* (HPI) axis under normal rearing conditions[29]. This indicates that the supplemented feed was metabolically safe during the pre challenge phase.

In contrast, glucose levels increased markedly after the *A. hydrophila* challenge on day 75, reflecting a typical stress

response associated with bacterial infection. This elevation may be linked to glucocorticoid mediated mobilization of hepatic glycogen to meet the increased energy demand of innate immune responses[30]. Treatment T2 exhibited the highest glucose level 90 mg/dL. However, this value remained within the physiological range reported for Tilapia during acute infection, suggesting an adaptive rather than pathological stress response. The higher glucose level in T2 may be associated with enhanced immune activation supported by bioactive compounds in *Chaetomorpha* sp., including sulfated polysaccharides and flavonoids, which have been reported to stimulate innate immune activity and increase metabolic energy requirements[31]. Overall, the post challenge glucose response likely reflects increased immune related energy allocation rather than metabolic dysfunction.

4. CONCLUSION

Dietary supplementation with *Chaetomorpha* sp. extract improved hematological parameters in Tilapia, with the optimal response observed at 50 mL/kg feed, indicating its potential as a natural functional additive for sustainable aquaculture. However, as this study employed a crude hot water extract and focused on hematological responses, further research is needed to identify active compounds and evaluate long term effects under different culture conditions.

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