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Supplementation of *Ulva lactuca* in Feed Formulation to Enhance the Non-Specific Immune System of the Nile Tilapia *Oreochromis niloticus*

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ABSTRACT

The Nile tilapia (*Oreochromis niloticus*) aquaculture faces numerous challenges, particularly bacterial infections caused by members of the *Aeromonas* genus. Such infections can result in high mortality rates within tilapia populations, especially when not promptly diagnosed and treated. The incorporation of natural ingredients such as *Ulva lactuca* into fish feed has been explored due to its potential to inhibit *Aeromonas hydrophila*, attributed to its antibacterial properties. *Ulva lactuca* contains ulvan with 12.80–23% sulfate, 12.73–45% rhamnose, 2–12% xylose, and 6.5–25.96% uronic acid, all of which contribute to its antibacterial activity. Therefore, this study aimed to evaluate the effect of dietary supplementation with *Ulva lactuca* on the growth performance of the Nile tilapia and its efficacy in suppressing *Aeromonas hydrophila* infection. The experiment consisted of four treatments with the addition of *Ulva lactuca* to feed formulation (treatment control (0%), A (8%), B (12%) and C (16)), and three replications were performed for each. The results showed that treatment B (12%) yielded the most favorable outcomes supported by a total leukocyte count of 98.000 cells/mm³ and phagocytic activity of 70% following the bacterial challenge. In addition, treatment B exhibited a high lymphocyte percentage on day 14 recorded at 102%.

INTRODUCTION

The Nile tilapia (*Oreochromis niloticus*) is one of the most important freshwater aquaculture species globally due to its rapid growth, adaptability to a wide range of environmental conditions, and high market demand **(Ashour *et al.*, 2020)**. These advantages make tilapia highly sought after in both international and local markets as its flesh is tasty, nutritious and low in fat making it a favorable choice for human consumption. Additionally, the Nile tilapia is recognized as an affordable and sustainable source of animal protein **(Anantasuk *et al.*, 2024)**. However tilapia aquaculture faces several challenges; one of the most significant being bacterial infections, particularly those caused by bacteria from the *Aeromonas* genus.

These bacteria can easily spread through water and affect fish populations extensively particularly in densely stocked aquaculture ponds **(Priyatharshni *et al.*, 2024)**. *Aeromonas hydrophila* infections can lead to a variety of symptoms and pathological conditions in fish, including skin ulcers, hemorrhaging, abdominal swelling and gill damage. These clinical signs not only compromise fish welfare but may also result in mortality if not addressed promptly and effectively **(Maray *et al.*, 2023)**. During bacterial infections, the non-specific immune system in the Nile tilapia provides a faster and more immediate defense compared to the specific immune system, which requires several days to weeks to reach full effectiveness; a delay that may be insufficient to combat rapidly spreading pathogens. This non-specific immune response is therefore critical in initiating early protection against infection particularly in conditions that facilitate the swift dissemination of pathogens **(Abo-Raya *et al.*, 2024)**. The inclusion of natural ingredients such as *Ulva lactuca* in fish feed has been studied for its potential to enhance this non-specific immune response **(Valente *et al.*, 2016)**.

Ulva lactuca contains essential nutrients, such as protein (7.13–28%), carbohydrates (50–61.5%), and ash (11–49.6%) **(Jasmadi *et al.*, 2016)**. This seaweed serves as an excellent nutritional source with varying compositions per 100 grams of dry weight **(Dewi, 2018)**. Specifically *Ulva lactuca* contains approximately 15–26% protein, 0.1–0.7% fat, 46–51% carbohydrates, around 25% fiber, 16–23% ash and 20.9% moisture **(Violle *et al.*, 2018)**. Additionally, *Ulva lactuca* is rich in various bioactive compounds, such as proteins, lipids, and polyphenols, which exhibit antibacterial, antiviral and antimicrobial properties. The high nutritional and bioactive content of *Ulva lactuca* makes it a valuable supplement for enhancing fish health and overall aquaculture productivity **(Alagan *et al.*, 2017)**. The use of *Ulva lactuca* in aquafeed can reduce reliance on antibiotics, however the overuse of which may lead to antibiotic resistance **(Putra, *et al.*, 2024)**. Therefore, *Ulva lactuca* not only supports fish health by enhancing immune function and protecting cells from damage but also contributes to more sustainable and environmentally friendly aquaculture practices **(Natify *et al.*, 2015)**. This dual benefit of improving fish disease resistance and minimizing the need for antibiotics positions *Ulva lactuca* as a valuable additive in the development of eco-friendly aquafeeds.

MATERIALS AND METHODS

Time and location of research

The study was carried out between March to May of 2025 at the Laboratory of Fish Health and Disease as well as the Fish Reproduction Laboratory, Faculty of Fisheries and

Marine Science, Brawijaya University. The feed materials utilized in the experiment were sourced from the BBPBAP in Jepara.

Research preparation

Prior to the commencement of the study, 12 aquarium units each sized 50 × 40 × 40cm were prepared. These aquariums were categorized into four different treatment groups, with each group consisting of three replicates. Each aquarium was filled with 60 liters of water and equipped with continuous aeration to maintain optimal water quality. The experimental fish used in this study were the Nile tilapia (*Oreochromis niloticus*) with an average length of around 12-15cm, and each aquarium was filled with 5 tilapia fish, sourced from the Sumber Pasir Laboratory, Faculty of Fisheries and Marine Science, Brawijaya University. In total, the experiment consisted of four distinct treatments, each replicated three times.

Acclimatization

Before the experiment began, the Nile tilapia underwent a one-week acclimation process. During this time, five fish were placed in each aquarium. They were fed pellet feed twice a day once in the morning and once in the evening to help them adjust to the new environment and feeding regimen. On the day prior to the start of the experiment, the fish were not fed and were weighed to record their baseline body weight. Measurements of fish length and weight were then taken on a weekly basis. The maintenance and observation period lasted for five weeks in total.

Experimental diet

The experimental feed was formulated into four treatment variations, each containing 25% protein. This protein level was chosen based on research by **Karapanagiotidis (2017)**, which showed that the Nile tilapia are capable of achieving optimal growth with dietary protein levels ranging from 20 to 25%. Detailed compositions of each feed treatment are listed in Table (1).

Table 1. Ingredients composition

Material	Treatment			
	K	**A**	**B**	**C**
Fish meal	20.00	20.00	20.00	20.00
Soy flour	22.75	22.75	22.75	22.75
Ulva lactuca flour	0.00	8.00	12.00	16.00
Tapioca flour	11.25	11.25	11.25	11.25
Corn flour	34.00	26.00	22.00	18.00
Fish oil	2.00	2.00	2.00	2.00
Premix	5.00	5.00	5.00	5.00
CMC	5.00	5.00	5.00	5.00
Total material	**100.00**	**100.00**	**100.00**	**100.00**

Dry content (%)	90.26	89.58	89.24	88.90
Protein (%)	24.50	25.07	25.36	25.65
Fat (%)	4.23	3.70	3.43	3.16
Fiber (%)	2.21	2.74	3.00	3.27
Ash (%)	9.09	11.33	12.45	13.57
BETN (%)	59.97	57.16	55.76	54.36
Energy (kkal/g)	3.46	3.34	3.27	3.21

Notes:

Digestible energy values are as follows: 1.0g of protein yields 4 kcal, 1.0g of fat yields 9 kcal and 1.0g of carbohydrate yields 3.5 kcal.

BETN (nitrogen-free extract): calculated using the formula 100 – protein – fat – ash – crude fiber.

- K: Control treatment without the addition of *Ulva lactuca*.
- A: Treatment with the addition of 8% *Ulva lactuca*.
- B: Treatment with the addition of 12% *Ulva lactuca*.
- C: Treatment with the addition of 16% *Ulva lactuca.*

Bacterial culture

Aeromonas hydrophila used in this study was sourced from the BBPBAP in Jepara. The initial bacterial concentration was 10^9 CFU/mL and cultured on agar medium using a zigzag streaking method, followed by incubation at ambient temperature for 24 hours. Distinct and well-separated colonies were then transferred and incubated at 25–28°C for 18 to 48 hours to isolate pure cultures which were characterized by their round shape and yellowish appearance. The verified isolate was cultured in *Trypticase Soy Broth* (TSB) **(Ofek *et al.*, 2023)**.

Bacterial infection

After an acclimation period, the Nile tilapia were relocated to experimental aquaria and exposed to *Aeromonas hydrophila* at a concentration of 10^7 cells/mL the results of LD_{50} test **(Albances & Traifalgar, 2022)**. Each fish received an intraperitoneal injection of 0.1mL of the bacterial suspension. On day 30 following the feeding period with diets supplemented with *Ulva lactuca*, the fish were challenged again by intramuscular injection of 0.1mL of the *Aeromonas hydrophila* suspension per individual **(Lema *et al.*, 2021)**.

Research parameters

Hematocrit and leukocyte levels

The measurement of hematocrit and leukocrit levels followed the method described by **Maftuch (2018)**. Blood samples were drawn into heparinized capillary tubes until filled to the marked volume and sealed with Vitrex. The tubes were then centrifuged at 12.000 rpm for 4 minutes. The hematocrit level was calculated by dividing the length of the red blood cell column by the total length of the blood column in the capillary tube, followed by multiplying by 100. The total leukocyte count was performed using a Neubauer hemocytometer using the method of **Gunanti *et al.* (2019)**. All equipments were cleaned with sodium citrate and dried. Blood was drawn to the 0.5 mark of a pipette and diluted with Turk's solution to the 11.0 mark. After thorough mixing, the first few drops were discarded and the sample was loaded into the hemocytometer. Leukocytes were counted in four large squares under a microscope. Differential leukocyte counts (lymphocytes, monocytes, neutrophils) were performed on Giemsa-stained blood smears examined microscopically.

Phagocytic activity

Phagocytic activity was measured based on the method described by **Purwanto *et al.* (2021)**. A blood sample of 50µL was placed into an Eppendorf tube and mixed with 50µL of *Aeromonas hydrophila* bacterial suspension at a concentration of 10^7 CFU mL^{-1}. The mixture was homogenized and incubated at room temperature for 20 minutes. Subsequently, 10µL of the sample was placed onto a glass slide and fixed with methanol for 8 minutes. The slide was then stained with Giemsa solution for 3–4 minutes rinsed with running water, air-dried and observed under a microscope at 400× magnification.

***Feed conversion ratio* (FCR)**

The success of aquaculture activities was evaluated by calculating the FCR which is the ratio between the amount of feed provided and the weight gain of the cultured organism **(Mengistu *et al.*, 2019)**. A lower FCR value indicates higher feed efficiency. FCR was calculated using the following formula:

$$\mathrm{FCR} = \frac{\mathrm{F}}{\Delta \mathrm{W}}$$

***Survival rate* (SR)**

The SR is a measure that indicates the percentage of organisms that survive relative to the number of fish initially stocked. The SR value is expressed as a percentage (%) **(Kurniawan *et al.*, 2023)**. The SR was calculated using the following formula:

$$\mathrm{SR} = \frac{\mathrm{Nt}}{\mathrm{No}} \times 100\,\%$$

***Sustainable growth rate* (SGR)**

The SGR was calculated as the percentage increase in body weight per unit of time and is usually expressed as a percentage per day. It provides an indication of how rapidly the fish grow relative to their current body weight **(Rahman *et al.*, 2022)**. The SGR was calculated using the following formula:

$$SGR = \frac{\ln W_t - \ln W_0}{t} \times 100\ \%$$

Water quality parameters

During the maintenance period, water quality parameters including temperature, pH and dissolved oxygen (DO) were systematically monitored in both the morning and afternoon. Prior to daily feeding, approximately 50% of the water volume was siphoned and partially replaced each morning to maintain optimal environmental conditions.

Statistical analysis

The observational data were statistically analyzed using the analysis of variance at a 95% confidence level. To identify significant differences among treatment groups, Duncan’s multiple range test was subsequently performed as a post hoc analysis at the same level of confidence.

RESULTS AND DISCUSSION

Hematocrit

Hematocrit measurements in the Nile tilapia blood were conducted on day 1, day 7, day 14 and day 37. Hematocrit values were used to assess the presence of anemia and potential disease as indicated by decreased hematocrit levels. The resulting hematocrit values are presented in Table (2).

Table 2. Level hematocrit

Treatment	Level hematocrit (%)			
	D-0	**D-7**	**D-14**	**D-37**
K (0%)	30.36^{a}	20.55^{a}	16.77^{c}	20.78^{b}
A (8%)	30.28^{a}	25.42^{a}	23.45^{b}	28.25^{ab}
B (12%)	30.53^{a}	23.02^{a}	22.35^{bc}	23.91^{b}
C (16%)	30.17^{a}	25.21^{a}	27.86^{a}	29.07^{a}

Note: Identical letters within the same column indicate no significant difference between treatments ($P > 0.05$).

The hematocrit levels on day 0 ranged from 30.17 to 30.53% (Table 2) which fall within the normal range as reported by **Rini *et al.* (2024)**, who stated that normal hematocrit values in the Nile tilapia range from 23.6 to 37.4%. This finding is further supported by **Osman *et al.* (2018)**, who reported a normal hematocrit range of 20.49 to 38.76% in the Nile tilapia. On day 7, all treatment groups showed a decrease in hematocrit levels with the most substantial reduction observed in the control group (20.55%). Other treatments also experienced a decline though it was less pronounced than in the control treatment.

A further decrease in hematocrit levels was observed on day 14 similar to the trend seen on day 7, except for treatment C which showed an increase of 27.86% compared to day 7. The control group exhibited a more significant decline in hematocrit levels than treatments A and B, with a value of 16.77% on day 14 down from 20.55% on day 7. This decline in hematocrit values may be attributed to stress in the Nile tilapia due to environmental changes and the need for adaptation over the two-week period. **Sebastião *et al.* (2011)** reported that hematocrit levels in the Nile tilapia decrease as a result of stress caused by unstable environmental conditions, which can inhibit hematopoiesis and increase red blood cell damage. On day 37, all treatments showed an increase in hematocrit levels compared to day 14. However, when compared to the values on day 0, the hematocrit levels on day 37 remained lower. This persistent decrease may be attributed to the bacterial challenge test with *Aeromonas hydrophila* administered on day 30.

The results of the least significant difference (LSD) test on day 0 and day 7 indicated no significant differences among all treatments. However, on day 14 a significant difference ($P < 0.05$) was observed between treatment A and control. The observed decrease in hematocrit levels may be associated with physiological stress experienced by the fish, which can lead to a reduction in hematocrit values. Although hematocrit levels decreased following the treatment period, an increase was observed after the challenge test conducted on day 37. Statistical analysis revealed that treatment C differed significantly from both the control and treatment B. A notable reduction in hematocrit level post-challenge was observed in treatment B (23.91%), suggesting a higher level of infection in this group compared to the others. Hematocrit levels in treatment B remained relatively stable from day 7 to day 37. These findings suggest that the immune status of the Nile tilapia improved following dietary administration containing antibacterial properties.

Total leukocyte

Total leukocyte counts were measured on day 0, day 7, day 14 and day 37 of the experiment with the results presented in Table (3).

Table 3. Total leukocyte

Treatment	**Total Leukocyte (cell/mm^3)**			
	D-0	**D-7**	**D-14**	**D-37**
K (0%)	27000^{a}	36000^{a}	78000^{ab}	70000^{b}
A (8%)	23000^{a}	22000^{c}	75000^{b}	46000^{c}
B (12%)	25000^{a}	38000^{a}	83000^{a}	98000^{a}
C (16%)	21000^{a}	26000^{b}	73000^{b}	43000^{c}

Note: Identical letters within the same column indicate no significant difference between treatments ($P > 0.05$).

The total leukocyte counts of the Nile tilapia on days 0, 7, 14, and 37 are presented in Table (3). On day 0, leukocyte values across all treatments ranged from 21.000 to 27.000 cells/mm³, with no statistically significant differences observed among the treatments ($P > 0.05$), indicating a comparable baseline immune status. Leukocyte levels are closely related to hematocrit values, when hematocrit levels are relatively high, total leukocyte counts tend to decrease. **Gazali *et al.* (2023)** reported that elevated hematocrit levels are associated with lower leukocyte counts. Similarly, **Fauzan (2017)** stated that the normal range of leukocyte counts in the Nile tilapia lies between 20.000 and 150.000 cells/mm³.

The LSD test results on day 0 confirmed no significant differences among treatments. However, on day 7, treatment A showed a decrease in total leukocyte count compared to the control, B and C treatments. This reduction may be attributed to environmental changes that induced stress in the fish. **Faizah *et al.* (2024)** reported that total leukocyte counts may decline due to elevated hematocrit values and environmental stress in the Nile tilapia. The LSD test results on day 7 revealed that treatment A was significantly different from the control, B and C. Furthermore, treatment C also differed significantly from all other treatments while the control and treatment B showed no significant difference.

On day 14, all treatments exhibited a significant increase in leukocyte counts compared to day 7. The LSD test results indicated that on day 14, treatment A was highly significantly different from treatment B, while treatment B showed significant differences with both treatments A and C. Following the bacterial challenge on day 37, treatment B demonstrated a substantial increase in leukocyte count reaching 98.000 cells/mm³, which was significantly higher than all other treatments indicating a strong immunological response. In contrast, treatments A and C showed a decrease in leukocyte counts compared to day 14, while the control group also experienced a reduction, though it was not as pronounced.

The LSD test on day 37 revealed that the control group differed significantly from all other treatments. Treatment A was not significantly different from treatment C, while treatment B showed significant differences with all other groups. These results suggest that treatment B was more effective in stimulating the non-specific immune response as evidenced by the increase in leukocyte counts particularly following exposure to *Aeromonas hydrophila*. According to **Gunanti *et al.* (2019)**, an increase in total leukocyte count can enhance the non-specific immune response in fish. This type of immune response is characterized by the migration of leukocytes from blood vessels into infected tissues.

Leukocyte differential

Neutrophil

The neutrophil percentages recorded on days 0, 7, 14, and 37 are presented in Table (4).

Table 4. Neutrophil percentage

Treatment	Neutrophile percentage (%)			
	D-0	**D-7**	**D-14**	**D-37**
K (0%)	22.50^{a}	22.00^{ab}	17.63^{a}	5.55^{a}
A (8%)	13.42^{a}	20.72^{b}	17.00^{a}	6.43^{a}
B (12%)	25.00^{a}	16.35^{c}	11.87^{a}	5.00^{a}
C (16%)	17.57^{a}	24.30^{a}	17.00^{a}	5.43^{a}

Note: Identical letters within the same column indicate no significant difference between treatments (P> 0.05).

The neutrophil values obtained in this study, as presented in Table (4), indicate that on day 0 the percentages ranged from 13.42 to 25%. According to **Olanrewaju *et al.* (2023)**, the average normal neutrophil percentage in the Nile tilapia is approximately 22.73%. Treatment B showed a higher-than-average neutrophil percentage, which may be attributed to increased lymphocyte levels and environmental stress factors. However. the LSD test revealed no statistically significant differences among the treatments on day 0.

On day 7 following dietary treatment administration, only treatments A and C exhibited an increase in neutrophil percentage, while the control group showed a slight decrease, and treatment B experienced a marked decline. The LSD test results showed that treatment A was significantly different from treatments B and C, and treatment C was significantly different from all other treatments. On days 14 and 37, neutrophil percentages declined in all treatments. Nevertheless, no significant differences were observed among treatments at these time points. Despite the decreases, all neutrophil values remained within the normal physiological range for the Nile tilapia.

Osman, *et al.* (2018) stated that neutrophils function to eliminate foreign antigens through the process of phagocytosis. In this study, although a decrease in neutrophil percentage was observed, it was accompanied by an increase in lymphocyte percentage, indicating the activation of the non-specific immune system primarily mediated by lymphocytes. The consistent decline in neutrophil percentages across all observation points is presumed to be due to the initial rapid production of neutrophils following bacterial injection. However by the time of observation on day 37 post-challenge, the percentage of neutrophils had decreased, likely due to their earlier involvement in antigen attack and clearance. According to **Thummabancha *et al.* (2022)** neutrophils become actively engaged when bacteria enter the body, but their activity is relatively short-lived

Lymphocyte

The lymphocyte percentages of the Nile tilapia observed on days 0, 7, 14, and 37 are presented in Table (5).

Table 5. Lymphocyte percentage

Treatment	Lymphocyte percentages (%)			
	D-0	**D-7**	**D-14**	**D-37**
K (0%)	76.00^{a}	78.00^{bc}	92.00^{bc}	86.00^{a}
A (8%)	72.00^{a}	75.00^{c}	78.00^{c}	81.00^{b}
B (12%)	81.00^{a}	83.00^{a}	102.00^{a}	85.00^{a}
C (16%)	80.00^{a}	80.50^{b}	83.00^{c}	82.00^{b}

Note: Identical letters within the same column indicate no significant difference between treatments ($P>$ 0.05).

The lymphocyte percentages presented in Table (5) show that, on day 0 prior to treatment administration, all groups exhibited lymphocyte values within the normal range of 72–81%. This finding aligns with the report by **Neu *et al.* (2016)**, who stated that the normal lymphocyte percentage in the Nile tilapia ranges from 68 to 86%. LSD test results on day 0 indicated no significant differences among treatments. On day 7, an increase in lymphocyte percentages was observed in all treatment groups. LSD test results showed that treatments A and B differed significantly from treatment C, while treatment C was also significantly different from the control group. These increases suggest early immune activation in response to dietary treatments.

By days 14 and 37 lymphocyte percentages continued to rise across all treatments and remained within the reported normal range. However, on day 14, the control and treatment B groups exhibited lymphocyte percentages that slightly exceeded the upper threshold of the normal range indicating a heightened immune response. The LSD test on day 14 revealed that treatment B was significantly different from all other treatments. On day 37, both treatment B and the control group were significantly different from treatments A and C, as determined by the LSD test. The control group which did not receive feed supplemented with *Ulva lactuca*, showed lymphocyte percentages that were comparable to those of treatments A, B and C. This may be attributed to the innate immune defense mechanisms that are naturally present in fish, even in the absence of dietary immunostimulants. Fish possess an inherent immune system that provides baseline protection against pathogenic infections.

As reported by **Zhao *et al.* (2022)**, lymphocytes play a crucial role in antibody production. An increase in lymphocyte percentage indicates the activation of the non-specific immune response to counteract bacterial infections. According to **Wei *et al.* (2019)**, lymphocytes contribute to immune defense by recognizing antigens through specific receptors on their cell membranes. In T lymphocytes the presence of specific antigen receptors is essential, as these cells cannot recognize antigens on their own. The existence of these receptors enables T cells to rapidly identify antigens and initiate an immune response, including the stimulation of B cells to produce antibodies.

Phagocytosis activity

The phagocytic activity observed in each treatment group is presented in Fig. (1).

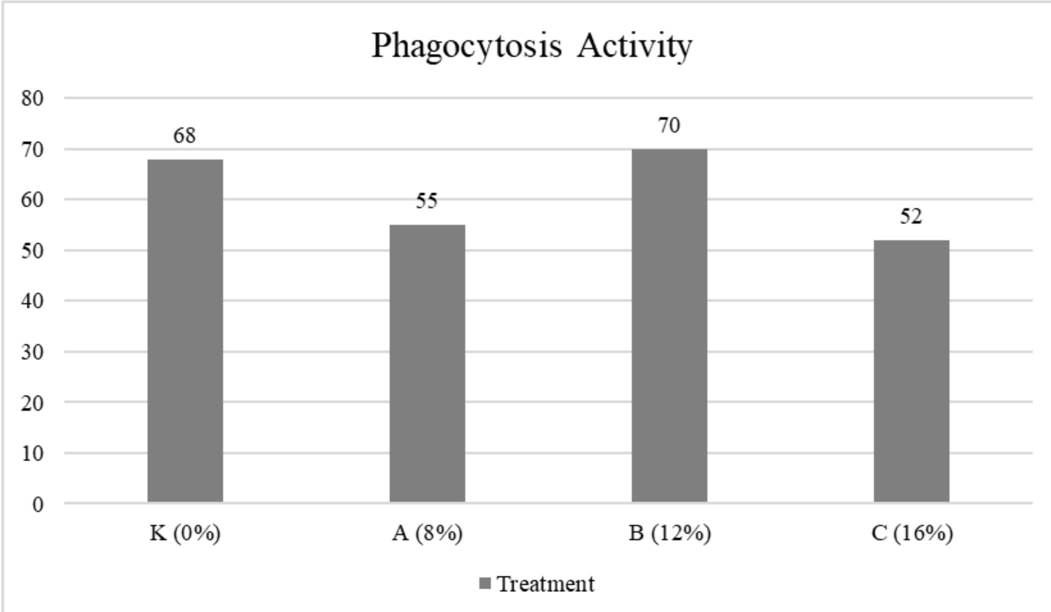

Fig. 1. Phagocytosis activity

The percentage of phagocytic activity in the Nile tilapia following the bacterial challenge is presented in Fig. (1). Based on the observations, the control treatment exhibited a phagocytic activity of 68%, followed by treatment A at 55%, treatment B at 70% and the lowest value was observed in treatment C at 52%. The highest phagocytic activity was recorded in treatment B. Phagocytosis is a component of the non-specific immune response that serves as the first line of defense against pathogen invasion before the activation of the specific immune system **(Datta *et al.*, 2014)**. Higher phagocytic activity indicates a stronger immune capacity in fish to combat bacterial infections. These findings are consistent with those of **Sinaga and Mukti (2022)**, who reported that enhanced phagocytic cell activity can accelerate the elimination of pathogens from the fish body.

Feeding the Nile tilapia with diets supplemented with *Ulva lactuca* was shown to influence phagocytic activity. Treatment B which received a 12% dose of *Ulva lactuca* exhibited the highest phagocytic activity. This finding indicates that dietary supplementation with *Ulva lactuca* can stimulate the innate immune response in the Nile tilapia. This is supported by **Harpeni *et al.* (2015)**, who reported that *Ulva lactuca* significantly enhances immune defense factors including phagocytic activity. The difference in findings between this study and previous research may be attributed to the use of different microorganisms as sources of infection in the Nile tilapia. In the present study, treatment B demonstrated superior phagocytic activity compared to the other treatments highlighting its potential as the most effective dose for immune stimulation.

The findings of this study also suggest that the phagocytic activity observed in treatment B may have been predominantly mediated by lymphocytes. Post challenge observations revealed that treatment B had the highest lymphocyte percentage and the lowest neutrophil percentage. Therefore, it can be inferred that in this study, the cells primarily responsible for the phagocytosis of *Aeromonas hydrophila* were not neutrophils, which are typically known for destroying foreign antigens through phagocytosis but rather lymphocytes.

Growth performance of the Nile tilapia

The results of this study indicate that the inclusion of *Ulva lactuca* in the feed formulation can enhance the growth performance of the Nile tilapia. The detailed findings are presented in Table (6).

Table 6. Growth performance of the Nile tilapia

Parameter	Treatments			
	K	A	B	C
FCR	1.36	1.33	1.42	1.52
SR (%)	100	100	100	100
SGR (%/day)	1.63	2.1	2.4	2.2

The addition of *Ulva lactuca* in the feed formulation for the Nile tilapia resulted in the highest FCR in treatment C (16%) with a value of 1.52. This relatively high FCR suggests that the inclusion of 16% *Ulva lactuca* was not yet sufficient to improve feed acceptability compared to the control treatment without *Ulva lactuca* (1.36) and the treatment with 8% inclusion (1.33). **Harpeni *et al*. (2015)** reported that all treatments supplemented with *Ulva lactuca* were still more efficient in feed utilization and could reduce the waste load entering the aquatic environment compared to an FCR of 1.72. In terms of SR, no fish mortality was observed in any treatment group during the rearing period. According to **Kurniawan *et al.* (2023)**, an SR above 50% is considered good, between 30–50% is moderate, and below 30% is poor. **Yustiati *et al*. (2020)** also noted that environmental conditions, including weather, can influence the growth of the Nile tilapia. The highest SGR was recorded in treatment B (12%) at 2.4%, while the lowest was observed in the control treatment (0%). **Rahman *et al.* (2022)** stated that an SGR of 2% per day indicates optimal use of available space for movement contributing to faster growth in the Nile tilapia. Among all treatments the best overall growth performance was observed in treatment B where the feed formulation included 12% *Ulva lactuca*. This treatment demonstrated the highest SGR and a favorable FCR with no fish mortality recorded throughout the rearing period.

Clinical symptoms

Following the bacterial challenge through injection, clinical symptoms observed in Nile tilapia included the appearance of wounds on the body and the detachment of several fins. Based on these clinical observations, after the administration of the experimental feed, morphological improvements were noted, such as wound contraction and closure in some fish. The response of the Nile tilapia to the feed continued to improve and their swimming behavior gradually returned to normal.

The ability of *Ulva lactuca* incorporated into the feed to promote healing of wounds caused by *Aeromonas hydrophila* infection proved to be highly effective. According to **Mahasu *et al.* (2016)**, the bioactive compounds present in *Ulva lactuca* extract include 4.59% phenolic content and 0.59% flavonoid content. **Putra *et al.* (2024)** further reported that phytochemical analysis of 70% ethanol extracts of *Ulva lactuca* revealed the presence of alkaloids, saponins, flavonoids, triterpenoids and steroids. The bioactive substances contained in *Ulva lactuca*, such as proteins, lipids and polyphenols are known to function as antibacterial, antiviral and antimicrobial agents.

Water quality results

Water quality plays a crucial role in the survival and growth of fish. Poor water quality can hinder fish growth and in severe cases lead to mortality. In this study, the observed water quality parameters during the cultivation of the Nile tilapia included temperature, pH, and DO. The results of these observations are presented in Table (7).

Table 7. Water quality results

Parameter	Treatments				SNI 7550.2009 (BSN. 2009)
	K	A	B	C	
Temperature (^{O}C)	25-29	25-29	25-29	25-29	25-32
pH	6.5-7	6.5-7	6.5-7	6.5-7	6.5-8.5
DO (ppm)	7.3-8.5	7.7-9	7.5-8.7	7.5-9	>3

The temperature measurements during the study ranged from 25 to 29°C, which fall within the optimal range for the Nile tilapia since the ideal temperature for their growth is between 25 and 30°C. Tilapia growth can be inhibited when temperatures fall below 20°C. **Leonard and Skov (2022)** stated that the Nile tilapia are actively foraging during the day with optimal feeding and activity occurring at temperatures above 20°C.

The pH values recorded ranged from 6.5 to 7 which are considered suitable for tilapia cultivation. According to **Martins *et al.* (2019)**, fish can achieve optimal growth within a pH range of 6.5–9. DO levels in this study ranged from 7.3 to 9.0ppm, which are also categorized as suitable for tilapia survival. **Boyd (1982)** noted that DO levels between 1–5ppm can slow fish growth, whereas DO levels above 5ppm are necessary for normal growth.

CONCLUSION

The inclusion of *Ulva lactuca* in the feed formulation for the Nile tilapia was found to enhance growth performance compared to the treatment without *Ulva lactuca*. A dietary inclusion level of 12% *Ulva lactuca* resulted in the most favorable outcomes in terms of FCR, SR and SGR. In addition to growth performance, the inclusion of *Ulva lactuca* also improved non-specific immune responses, as indicated by increased total leukocyte counts, enhanced phagocytic activity and lymphocyte percentage. Notably, the 12% inclusion treatment demonstrated the highest values for these immune parameters. However, following the challenge test, the hematocrit level and neutrophil percentage in the 12% treatment group were recorded as the lowest among the treatments.

REFERENCES

Abo-Raya, M. H.; Shi, Y.; Wang, Y.; Sayed, S. M. and Shukry, M. (2024). Enhancing immune and antioxidant responses in Nile tilapia through dietary supplementation with *Ulva fasciata* extract: a study on gene expression and resistance to *Aeromonas hydrophila*. Journal of Animal Physiology and Animal Nutrition. 108(5): 1415-1429. https://doi.org/10.1111/jpn.13984

Alagan, V.; Valsala, R. N. and Rajesh, K. D. (2017). Bioactive chemical constituent analysis, in vitro antioxidant and antimicrobial activity of whole plant methanol extracts of *Ulva lactuca* Linn. British Journal of Pharmaceutical Research. 15(1): 1-14. https://doi.org/10.9734/BJPR/2017/31818

Albances, J. O. and Traifalgar, R. F. (2022). Probiotic bacteria isolated from saline tilapia green water culture system inhibit gut colonization and prevent infection of *Aeromonas hydrophila* in the juvenile Nile tilapia (*Oreochromis niloticus*). Egyptian Journal of Aquatic Biology and Fisheries. 26(2): 841-857. https://dx.doi.org/10.21608/ejabf.2022.234763

Anantasuk, N.; Phurahong, T.; Pumchan, A.; Hirono, I. and Unajak, S. (2024). Molecular characterization and bivalent vaccine development of *Aeromonas hydrophila* and *Aeromonas veronii* in Nile tilapia (*Oreochromis niloticus*). Aquaculture. 590: 741042. https://doi.org/10.1016/j.aquaculture.2024.741042

Ashour, M.; Mabrouk, M. M.; Ayoub, H. F.; El-Feky, M. M. M. M.; Zaki, S. Z.; Hoseinifar, S. H.; Rossi, W.; Van Doan, H.; El-Haroun, E. and Goda, A. M. A-S. (2020). Effect of dietary seaweed extract supplementation on growth, feed utilization, hematological indices, and non-specific immunity of Nile Tilapia, *Oreochromis niloticus* challenged with *Aeromonas hydrophila*. Journal of Applied Phycology. 32: 3467–3479. https://doi.org/10.1007/s10811-020-02178-1

Boyd. C.E. (1982). Water quality in ponds for aquaculture. International Centre for

Aquaculture Experiment Station. Auburn University. Alabama.

Dewi. N. E. (2018). *Ulva lactuca*. Diponegoro University: Semarang. Indonesia.

Dotta, G.; de Andrade, J. I. A.; Gonçalves, E. L. T.; Brum, A.; Mattos, J. J.; Maraschin, M. and Martins, M. L. (2014). Leukocyte phagocytosis and lysozyme activity in Nile tilapia fed supplemented diet with natural extracts of propolis and Aloe barbadensis. Fish & Shellfish Immunology. 39(2): 280-284. https://doi.org/10.1016/j.fsi.2014.05.020

Faizah, K. N.; ayyadhafif, N. D.; Shafia, N.; Azmi, M. I.; Shabrina, T.; Dzakirah, N.; Mahandaru, M. S. R.; Anggraini, L. N. and Ramadhani, A. Y. (2024). Analysis of EM4 probiotics added in the water on the hematological profile of tilapia (*Oreochromis niloticus*). Journal of Basic Medical Veterinary. 13(2): 49-54. https://doi.org/10.20473/jbmv.v13i2.53038

Fauzan, M. (2017). The influence of the level of lead (Pb) exposure on the blood profiles of tilapia fish (*Oreochromis nilloticus*). Jimvet. 1(4): 702-708.

Gunanti, M.; Wulansari, P. D. and Kinzella, K. (2019). The erythrocyte and leucocyte profile of saline tilapia (*Oreochromis Niloticus*) in a cultivation system with nanobubbles. IOP Conference Series: Earth and Environmental Science. 236(1): 1-7. https://doi.org/10.1088/1755-1315/236/1/012089

Harpeni, E.; Santoso, L.; Sari, R. W. and Purba, O. K. (2015). Study of *Ulva* sp. as feed supplement towards growth performances and non-specific immune response of tilapia (*Oreochromis niloticus*). Maspari Journal. 7(2): 65-84.

Karapanagiotidis, I. T. (2017). Nutrient profiles of tilapia (tilapia in intensive co-culture). World Aquaculture Society. United States.

Kurniawan, A.; Siegers, W. H.; Akbar, M. A. and Nur, I. S. M. (2023). The production model of tilapia (*Oreochromis niloticus*) cultivation with system dynamics approach. Journal of Aquaculture and Fish Health. 12(1): 21-30. https://doi.org/ 10.20473/jafh.v12i1.33282

Kurniawan, A.; Siegers, W. H.; Akbar, M. A. and Nur, I. S. M. (2023). The production model of tilapia (*Oreochromis niloticus*) cultivation with system dynamics approach. Journal of Aquaculture and Fish Health. 12(1): 21-30. http://dx.doi.org/10.20473/jafh.v12i1.33282

Lema, B.; Prabadev, L. and Kassaye, B. W. (2021). *Aeromonas septicemia* infection in cultured nile tilapia, *Oreochromis niloticus* L. Journal of Aquaculture Research & Development. 12(584): 1-5.

Leonard, J. N. and Skov, P. V. (2022). Capacity for thermal adaptation in nile tilapia (*Oreochromis niloticus*): Effects on oxygen uptake and ventilation. Journal of Thermal Biology. 105. 103206. https://doi.org/10.1016/j.jtherbio.2022.103206

Maftuch, M. (2018). Hematological analysis of nile tilapia (*Oreochromis niloticus*) and striped catfish (*Pangasius hypophthalmus*) using hematology analyzer tool software at Fish Breeding Center Jojogan, Tuban, East Java. Research Journal of Life

Science. 5(2): 107-115. https://doi.org/10.21776/ub.rjls.2018.005.02.4

Mahasu, N. H.; Jusadi, D.; Setiawati, M. and Giri, I. N. A. A. (2016). Potential use of *Ulva lactuca* as feed ingredient for tilapia. Jurnal Ilmu dan Teknologi Kelautan Tropis. 8(1): 259-267. https://doi.org/10.29244/jitkt.v8i1.13089

Maray, S. O.; Abdel-Kareem, M. S.; Mabrouk, M. E.; El-Halmouch, Y. and Makhlof, M. E. (2023). In vitro assessment of antiviral, antimicrobial, antioxidant and anticancer activities of ulvan extracted from the green seaweed *Ulva lactuca*. Thalassas: An International Journal of Marine Sciences. 39(2): 779-790. https://doi.org/10.1007/s41208-023-00584-z

Martins, G. B.; da Rosa, C. E.; Tarouco, F. D. M. and Robaldo, R. B. (2019). Growth, water quality and oxidative stress of nile tilapia *Oreochromis niloticus* (L.) in biofloc technology system at different pH. Aquaculture Research. 50(4): 1030-1039. https://doi.org/10.1111/are.13975

Mengistu, S. B.; Mulder, H. A.; Benzie, J. A. and Komen, H. (2020). A systematic literature review of the major factors causing yield gap by affecting growth, feed conversion ratio and survival in Nile tilapia (*Oreochromis niloticus*). Reviews in Aquaculture. 12(2): 524-541. https://doi.org/10.1111/raq.12331

Natify, W.; Droussi, M.; Berday, N.; Araba, A. and Benabid, M. (2015). Effect of the seaweed *Ulva lactuca* as a feed additive on growth performance, feed utilization and body composition of Nile tilapia (*Oreochromis niloticus* L.). International Journal of Agronomy and Agricultural Research. 7(3); 85-92.

Neu, D.; Boscolo, W.; Zaminhan, M.; Almeida, F.; Sary, C. and Furuya, W. (2016) Growth performance, biochemical responses, and skeletal muscle development of juvenile nile tilapia, *Oreochromis niloticus* fed with increasing levels of arginine. Journal of the World Aquaculture Society. 47(2): 248-259. https://doi.org/10.1111/jwas.12262

Ofek, T.; Izhaki, I. and Halpern, M. (2023). *Aeromonas hydrophila* infection in tilapia triggers changes in the microbiota composition of fish internal organs. FEMS Microbiology Ecology. 99(12): 1-11. https://doi.org/10.1093/femsec/fiad137

Olanrewaju, A. N.; Kareem, O. K. and Emikpe, B. O. (2023). Heamatological and biochemical status of cultured and wild blue Tilapia *Oreochromis aureus* from Alau Lake, Northeast Nigeria. Comparative Clinical Pathology. 32(1): 139-146. https://doi.org/10.1007/s00580-022-03423-4

Osman, A. G.; AbouelFadl, K. Y.; Abd El Baset, M.; Mahmoud, U. M.; Kloas, W. and Moustafa, M. A. (2018). Blood biomarkers in Nile tilapia *Oreochromis niloticus* niloticus and African catfish *Clarias gariepinus* to evaluate water quality of the river Nile. Journal of FisheriesSciences. 12(1): 1-15. https://doi.org/10.21767/1307-234X.1000141

Poeloengasih, C. D.; Novianty, H.; Sefrienda, A. R.; Permadi, S.; Suryaningtyas, I. T.; Kumayanjati, B. and Setyono, D. E. D. (2023). Characteristic of *Ulva lactuca* freshness under different temperatures at short-term storage. Journal of Aquaculture & Fish Health. 12(2). 246-258. https://doi.org/10.20473/jafh.v12i2.33710

Priyatharshni, A.; Antony, C.; Ahilan, B.; Uma, A.; Chidambaram, P.; Ruby, P. and Prabu, E. (2024). Effect of dietary seaweed supplementation on growth, feed utilization, digestibility co-efficient, digestive enzyme activity and challenge study against *Aeromonas hydrophila* of nile tilapia *Oreochromis niloticus*. Indian Journal of Animal Research. 58(6): 1-8. http://dx.doi.org/10.18805/IJAR.B-5295

Purwanto, A. K. R.; Larasati, B. S.; Istiqomah, I. and Isnansetyo, A. (2021). Phagocytes response to dietary probiotics SEAL and *Aeromonas hydrophila* infection in red tilapia (*Oreochromis* sp.). IOP Conference Series: Earth and Environmental Science. 718(1): 1-7. https://doi.org/ 10.1088/1755-1315/718/1/012054

Putra, N. R.; Fajriah, S.; Qomariyah, L.; Dewi, A. S.; Rizkiyah, D. N.; Irianto, I. and Arya, N. N. (2024). Exploring the potential of *Ulva Lactuca*: Emerging extraction methods, bioactive compounds, and health applications-a perspective review. South African Journal of Chemical Engineering. 47, 233-245. https://doi.org/10.1016/j.sajce.2023.11.017

Rahman, M. H.; Haque, M. M.; Alam, M. A. and Flura, M. A. (2022). A study on the specific growth rate (SGR) at different stages of Tilapia (*Oreochromis niloticus*) production cycle in tank based aquaculture system. International Journal of Aquaculture and Fishery Sciences. 8(2): 59-65. https://doi.org/10.17352/2455-8400.000079

Rini, R. K.; Aisiah, S. and Olga, L. N. (2024). Hematological and histological analysis of tilapia (*Oreochromis Niloticus*) cultured in floating net cages after disease outbreak. Journal of Research in Scince Education. 10(4): 1787-1793. https://doi.org/10.29303/jppipa.v10i4.5057

Sebastião, F. A.; Nomura, D.; Sakabe, R. and Pilarski, F. (2011). Hematology and productive performance of Nile tilapia (*Oreochromis niloticus*) naturally infected with Flavobacterium columnare. Brazilian Journal of Microbiology. 42(1): 282-289. https://doi.org/10.1590/S1517-83822011000100036

Valente, L. M.; Araújo, M.; Batista, S.; Peixoto, M. J.; Sousa-Pinto, I.; Brotas, V.; Cunha, M. L. and Rema, P. (2016). Carotenoid deposition, flesh quality and immunological response of Nile tilapia fed increasing levels of IMTA-cultivated Ulva spp. Journal of Applied Phycology, 28, 691-701. https://doi.org/10.1007/s10811-015-0590-9

Violle, N.; Rozan, P.; Demais, H.; Nyvall Collen, P. and Bisson, J. F. (2018). Evaluation of the antidepressant-and anxiolytic-like effects of a hydrophilic extract from the green seaweed *Ulva* sp. in rats. Nutritional neuroscience. 21(4): 248-256. https://doi.org/10.1080/1028415X.2016.1276704

Wei, X.; Ai, K.; Li, H.; Zhang, Y.; Li, K. and Yang, J. (2019). Ancestral T cells in fish require mTORC1-coupled immune signals and metabolic programming for proper activation and function. The Journal of Immunology. 203(5): 1172-1188. https://doi.org/10.4049/jimmunol.1900008

Yustiati, A.; Nariswari, S.; Rostini, I. and Suryadi, I. B. B. (2020). Effect of stocking density on survival rate and growth of tilapia (*Oreochromis niloticus Linnaeus*, 1758) in round container with water current combined with venturi aeration system. Asian Journal of Fisheries and Aquatic Research. 8(1): 52-61. https://doi.org/10.9734/ajfar/2020/v8i130132

Zhao, Y.; Wang, Y.; Song, L.; Li, S.; Chen, X.; Tang, S. and Zhao, J. (2022). Effects of chronic alkalinity stress on immune response in nile tilapia (*Oreochromis niloticus*) infected with *Aeromonas hydrophila*. Aquaculture. 561: 738599. https://doi.org/10.1016/j.aquaculture.2022.738599