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The Effects of Suplementation of *Ulva reticulata* Extract on Growth-Related Parameters and Hematological Profile of the Red Tilapia (*Oreochromis* sp.)

Agus Oman Sudrajat[1*], Nurbety Tarigan[1, 2], Harton Arfah[1], Alimuddin Alimuddin[1], Dinamella Wahjuningrum[1]

[1]Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University, West Java 16680, Indonesia
[2]Study Program of Fishery Products Technology, Faculty of Science and Technology, Universitas Kristen Wira Wacana Sumba -East Nusa Tenggara 87113, Indonesia
[*]**Corresponding Author: agusom@apps.ipb.ac.id**

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ABSTRACT

Ulva reticulata extract contains phytochemical components that have the potential to be utilized in the field of environmentally friendly aquaculture. *U. reticulata* extract acts as a growth agent and health status enhancer in fish. This study aimed to evaluate the effects of different doses of *U. reticulata* extract on the growth of the red tilapia (*Oreochromis* sp.) fry. This study used a completely randomized design consisting of five doses of *U. reticulata* extract (0; 50; 150; 150; 200mg kg^{-1}) feed with four replicates given to the red tilapia fry measuring 5,2±0.09cm total length and 3,65±0.17g total weight on average during 30 days of rearing. The parameters evaluated were the growth rate, feed conversion ratio, blood hematology, and growth gene expression of the red tilapia fry. The results showed that *U. reticulata* extract significantly improved growth performance, evidenced by the increased expression of GH and IGF-1 growth genes, the decreased feed conversion ratio, and the improved blood biochemical profile of red tilapia, especially at the treatment dose of 200mg kg^{-1} feed ($P<0.05$). Feeding *U. reticulata* extract at 200mg kg^{-1} significantly increased the final individual weight (21.07g) by increasing the gene expression of GH by 4-fold and IGF by 2.5-fold, and decreasing the feed conversion ratio (1.25%). In addition, fish fry had the highest hemoglobin (10.54%), phagocytic activity (50.75%), differential leukocytes with lymphocytes (73%), neutrophils (19%), and lower monocytes (14%) than the control. In conclusion, *U. reticulata* extract 200mg kg^{-1} improved the growth and health of the red tilapia fry and could be an alternative to aquaculture fish hatchery productivity.

INTRODUCTION

Aquaculture activities have significantly increased, along with an increase in the world population **(FA0, 2018)**. This is because aquaculture products provide benefits that meet consumer nutritional needs **(Ashour *et al*., 2021)**. In an effort to increase aquaculture products, there are several obstacles, such as feed quality, quality of seed supply, and fish diseases, that have a negative impact on financial returns (**Henriksson *et al.,* 2017**). Several strategies have been developed to resolve these obstacles, such as the use of antimicrobials and vaccinations during the fish production phase **(Ashour *et al.*, 2020)**. However, the continued overuse of synthetic antimicrobial agents poses a significant threat to the sustainability of aquaculture, the environment, and human health **(Butt *et al.*, 2021)**. These threats must be addressed through sustainable and environmentally friendly practices to ensure the long-term sustainability of aquaculture **(Anshour *et al.*, 2024)**. Therefore, efforts are needed to use natural materials as an environmentally friendly strategy in aquaculture development **(Klongklaew *et al.*, 2021)**. One type of natural material that can be used as a substitute for synthetic chemicals in aquaculture is seaweed **(Cunha & Grenha, 2016)**.

Seaweed is a marine plant with several diverse sources of bioactive compounds with antimicrobial properties **(Klongklaew *et al.*, 2021)**. Bioactive compounds in seaweeds have been widely utilized in aquaculture **(Abo-Raya *et al.*, 2021)**. The extract form of seaweed can be used in feed to increase growth and immune responses in fish **(Pradhan *et al.*, 2023)**. *Ulva reticulata* is a seaweed that can be used. It is a green seaweed (Phylum: *Chlorophyta*; Class: *Chlorophyceae*) that is abundant in several waters in Indonesia. Furthermore, *U. reticulata* has bioactive compound components such as alkaloids, flavonoids, phenols, and steroids, as well as high antioxidant activity **(Tarigan *et al.*, 2023)**. The bioactive compound components of *U. reticulata* work synergistically as immunomodulatory, antiviral, and antioxidant agents. Bioactive compounds stimulate antioxidant enzymes to suppress oxidative stress, thus affecting the growth and health of fish **(Alagawany *et al.*, 2020; Hosseni *et al.*, 2021)**. The components of the bioactive compounds obtained vary depending on the type of extraction solvent and extraction method used **(Sarangi *et al.*, 2023)**. Solvents using ethanol showed maximum activity in absorbing bioactive compounds in *U. reticulata* compared to other solvents **(Tarigan *et al.*, 2023)**. Antioxidant compounds found in *U. reticulata* have anti-inflammatory properties and can neutralize free radicals during the cell proliferation process, thus affecting growth **(Jimenez-Lopez *et al.*, 2021)**. In addition, antioxidant compounds can accelerate the reendothelialization process by upregulating adhesion molecules and stimulating rapid cell growth. Antioxidant compounds such as flavonoids and alkaloids in seaweed extracts help reduce TNF-α-mediated ROS formation at the endothelial level, thereby maintaining a balance between cell proliferation and differentiation during fish growth **(Chakraborty *et al.*, 2014)**. Furthermore, *U. reticulata* extract has bioactive compound components that work synergistically to act as immunomodulatory agents to improve the innate immune system in fish **(Pradhan *et al.*, 2023)**.

Several studies have reported that the use of *Ulva* spp. is effective in improving the growth and health of fish. Increased fish growth can be evaluated through the expression levels of growth genes, such as growth hormone (GH), insulin-like growth factor 1 (IGF-1) and blood **(Vazirzadeh *et al.*, 2022)**. The addition of *Ulva* sp. seaweed extract at a concentration of 50 g kg^{-1} can improve the growth and health of *Argyrosomus japonicus* **(Madibana *et al.*, 2017)**. Utilization of *U. fasciata* extract at 100mg kg^{-1} can increase growth gene expression and improve the health status and antioxidants in tilapia **(Abo-Raya *et al*., 2021)**. Using 9% *Gracilaria pulviana* and *Sargassum ilicifolium* seaweed extracts can increase growth genes and the immune system in the grouper fish **(Seyedalhosseini *et al*., 2024)**. Therefore, *U. reticulata* extract has the potential to be utilized as a natural substitute for synthetic chemicals to improve fish growth and health. To date, the use of *U. reticulata* extract for fish fry growth has not yet been reported.

The Nile tilapia seeds were used in this study. The red tilapia fry is one of the superior fish species cultivated in Indonesia which has several advantages, namely easy to maintain and has a short production cycle and is an omnivorous fish that is able to consume plant material in feed **(Abaho *et al*., 2022)**. In aquaculture, the sustainability of fish production is highly dependent on the quality of the fish fry. Superior fish seeds contribute to genetic diversity and disease resistance, thereby supporting sustainable aquaculture. To date, the utilization of *U. reticulata* to improve the growth performance and health of the red tilapia fry has not been reported. The purpose of this study was to evaluate the effects of different doses of *U. reticulata* extract in feed on the growth and health of the red tilapia (*Oreochromis* sp.) fry.

MATERIALS AND METHODS

1. Preparation of *U. reticulata* extract

U. reticulata Forsskål, 1775 from Moudolung waters, East Sumba, NTT, Indonesia, was morphologically identified in the Integrated Laboratory of Oceanographic Research of the National Research and Innovation Agency (BRIN), Ancol-Jakarta. Extraction was carried out using the maceration method with 96% ethanol solvent at a ratio of 1:15 **(Tarigan *et al*., 2023)**. Subsequently, simplisia up to 1000g was dried, mashed, and placed into a dark glass jar container soaked in 15 liters of 96% ethanol. The maceration process was carried out for 5 days (5 × 24h) using ethanol at room temperature under dark conditions. The product obtained was filtered using Whatman 0.2 paper and *U. reticulata* extract was concentrated using a rotary evaporator at 40^0C.

2. Feed formulation

The test feed used was a commercial feed with a protein content of 28%, and *U. reticulata* extract was added at a predetermined dose. *U. reticulata* extract was mixed by a coating method using carboxymethyl cellulose (CMC) as an adhesive. The process of making 1kg of feed was performed by adding 1% CMC as an adhesive, stirring until evenly mixed, then adding 100mL of water to the feed, and stirring until the entire feed was evenly distributed. The feed that had been mixed was air-dried for 1h, and was then ready for use.

3. Fish and experimental design

The containers used were 7 units of outdoor concrete containers, each of which was blocked using 3 nets with a mesh size of 4 x 4 mm having a size of 100 x 70 x 100 cm, and each container was stocked with 10 fish with an average total length of 5.2 ± 0.09

cm, and an average total weight of 3.65 ± 0.17 grams. The dose of *U. reticulata* extract used was modified from **Abo-Raya *et al.* (2021)**: 0 mg kg^{-1} (control), 50 mg kg^{-1}, 100 mg kg^{-1}, 150 mg kg^{-1}, and 200 mg kg^{-1} . Feeding was carried out three times a day at satiation at 08.00, 12.00, and 16.00 WIB, with the length of fish rearing carried out for 30 days. All maintenance and experimental procedures were approved by the IPB University Animal Care and Use Committee (no. 251-2022 IPB).

4. The experimental measurements

4.1 Final weight of fish

The final weight of each fish was measured at the end of the study using the following formula:

The final weight of fish = final average weight of fish (g) x number of individuals

4.2 Spesific growth rate

Specific growth rate of fish was determined at the end of the study using a formula based on **Pratiwi *et al.* (2016)**.

$$\text{SGR}\ (\%) = \left[\sqrt[t]{\frac{Wt}{Wo}} - 1\right] \text{x}\ 100$$

Description: SGR = Spesific growth rate (%/day)
Wt = Final weight of fish (g)
Wo = Initial weight of fish (g)

4.3 Feed conversion ratio

The feed conversion ratio was calculated using a formula based on **Jayant *et al.* (2018)**

$$\text{FCR} = \frac{F}{Bt - Bo + BM}$$

Description : Bt = Final biomass of fish (g)
Bo = Initial biomass of fish (g)
Bm = Dead biomass of fish (g)
F = Total amount of feed during rearing (g)

4.4 Fish hematology analysis

Blood parameters, including hemoglobin levels, hematocrit levels, phagocytic activity, and red and white blood cell counts, were observed at the end of the study. Blood samples were collected from six fish in each treatment group. Blood samples were collected from the caudalis fin using a syringe that had been administered with anticoagulants. Blood samples were stored in Eppendorf tubes for laboratory observation. Hemoglobin levels were analyzed using the **Wedemeyer and Yasutake (1977)** method. The number of red and white blood cells, phagocytic activity, and hematocrit were determined using the method described by **Anderson and Siwicki (1995)**.

4.4 Growth hormone (GH) and Insulin-Like Growth Factor 1 (IGF-1) gene analysis

Growth gene analysis of IGF-1 and GH was performed at the end of the study by taking six randomly selected fish from each treatment. Samples were collected in the form of hypophysis and liver organs. Moreover, fish were soaked in 1mL of 3.8% sodium citrate, stabilized using GENEzol™ RNA Reagent (Geneaid, Taiwan), and stored at -80°C for total RNA extraction. Total RNA was extracted using GENEzol™ reagent, according to the manufacturer's instructions. GH and IGF-1 gene expression was analyzed quantitatively using real-time PCR (RT-qPCR) with a Rotor-Gene 6000 machine (Corbett, USA). Amplification was performed using a KAPA SYBR® FAST qPCR kit (KAPA, USA). The PCR was run at 95°C pre-denaturation for 2min, followed by 40 cycles of 95ºC denaturation for 15 seconds, 60ºC annealing for 15 seconds, 72ºC extension for 10s. The melting curve was analyzed at 75-95ºC to evaluate the primer specifications. The gene expression ratio was determined using the relative gene expression $2^{-\Delta\Delta ct}$ method with normalization using the β-actin gene. The primer sequences used to identify GH and IGF-1 gene expression are listed in Table (1).

Tabel 1. Primer sequences used for identification of GH dan IGF-1 in the red tilapia fry (*Oreochromis* sp.)

Primer	Sequence (5'- 3')	TM (⁰C)	Reference
OnGH-F	GTTGTGTGTTTGGGCGTCTC		
OnGH-R	CAGGTGCGTGACTCTGTTGA		
		57	**Abo - Raya *et al*. 2021**
OnIGF-F	TCCTGTAGCCACACCCTCTC		
On IGF-R	ACAGCTTTGGAAGCAGCACT		
ACTB-F	CCACACAGTGCCCATCTACGA	65	
ACTB-R	CCACGCTCTGTCAGGATCTTCA		

5. Statistical analysis

Research data in the form of daily growth rate, final biomass, feed conversion ratio, blood picture, and gene expression of GH and IGF-1 were analyzed using ANOVA with SPSS version 2.5.0 to determine significant differences in all treatments with a 95% confidence interval. All treatments that were significantly different (P< 0.05) were further analyzed using Duncan's multiple range test (DMRT).

RESULTS

1. Growth rate

The results of the observations on the growth of the red tilapia fry during 30 days of rearing are shown in Table (1).

Table 1. Growth of red tilapia fry during 30 days of rearing

Parameter analysis	Dose of *U. reticulata* extract (mg kg^{-1} feed)				
	0 (control)	50	100	150	200
Final weight (g)	10,56±0,6[a]	19,75±0,40[b]	19,91±1,08[b]	19,49±0,58[b]	21,07±1,65[b]
Final length (cm)	8,64±0,22[a]	10,28±0,10[a]	10,28±0,24[a]	10,53±0,08[a]	10,35±0,22[a]
Growth rate (%/days)	3,81±0,54[a]	5,74±0,27[b]	5,80±0,21[b]	5,81±0,25[b]	5,94±0,23[b]
Feed conversion rasio (FCR)	2,17±0,29[b]	1,41±0,08[a]	1,36±0,11[a]	1,29±0,08[a]	1,25±0,05[a]

Note: Data are presented as means ± standard deviation. Different superscript letters in the same row indicate significant differences (P< 0.05).

Dosing *U. reticulata* extract at 200mg kg^{-1} feed significantly increased the red tilapia fry's growth and length (P< 0.05) compared to the control treatment. In addition, the *U. reticulata* extract administered to the red tilapia fry had significantly higher final weight and daily growth rate than the control treatment during rearing (P< 0.05). In contrast, the feed conversion ratio was significantly lower in the *U. reticulata* extract-treated group than in the control group (P< 0.05).

2. Fish hematology analysis

The dosing of *U. reticulata* extract in the feed resulted in blood picture results that were not significantly different for all treatments presented in Table (2).

Table 2. Hematology analysis of red tilapia fry at the end of the study

Parameter analysis	Dose of *U. reticulata* extract (mg kg^{-1} feed)				
	0 (control)	50	100	150	200
Erythrocytes (10^6 cells/mm^3)	1,68±0,32[a]	1,98±0,19[a]	1,98±0,20[a]	2,00±0,21[a]	2,02±0,17[a]
Hemoglobin (%)	8,27±1,09[a]	9,85±0,26[bc]	9,00±0,82[ab]	10,25±0,91[bc]	10,54±0,70[c]
Hematocrit (%)	22,6±2,36[a]	28,26±4,61[a]	24,27±1,00[a]	29,64±5,12 [a]	33,67±10,70[a]
Phagocytic activity (%)	47,25±1,0[a]	50,50± 1,00[b]	50,25±1,00[b]	50,75± 2,00[b]	50,75± 2,00[b]

Note: Data are presented as means ± standard deviation. Different superscript letters in the same row indicate significant differences (P<0.05).

However, the parameters of phagocyte activity and hemoglobin showed significant differences compared with the control treatment. Treatment with 200mg kg^{-1} *U. reticulata* extract resulted in significantly higher results than the other treatments. Furthermore, the differential leukocytes (lymphocytes) of the red tilapia fry were significantly higher in the *U. reticulata* extract treatment group than in the control group. Neutrophil and monocyte counts were not significantly different among the treatments (Fig. 1).

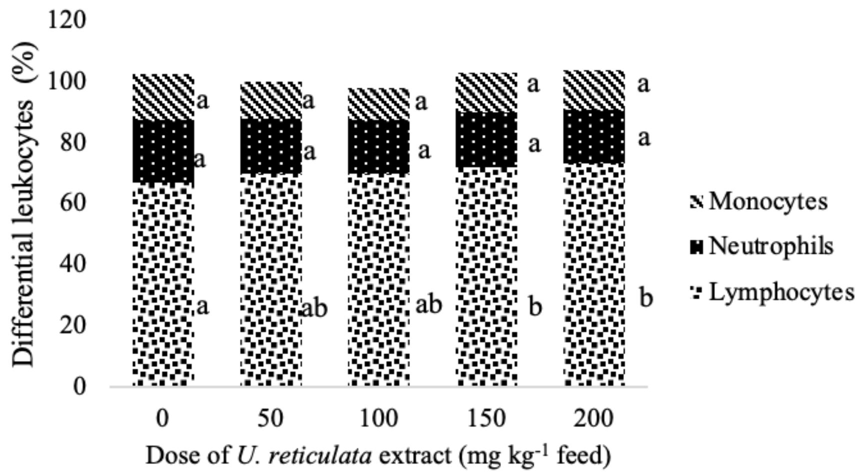

Fig. 1. Differential leukocytes (lymphocytes, neutrophils, and monocytes) of the Nile tilapia fry treated with *U. reticulata* extract. Letters above different bars at different doses indicate significant differences (*P*< 0.05).

3. GH dan IGF-1 gene analysis

Data from the measurement of GH expression in the red tilapia treated with *U. reticulata* extract are presented in Fig. (2).

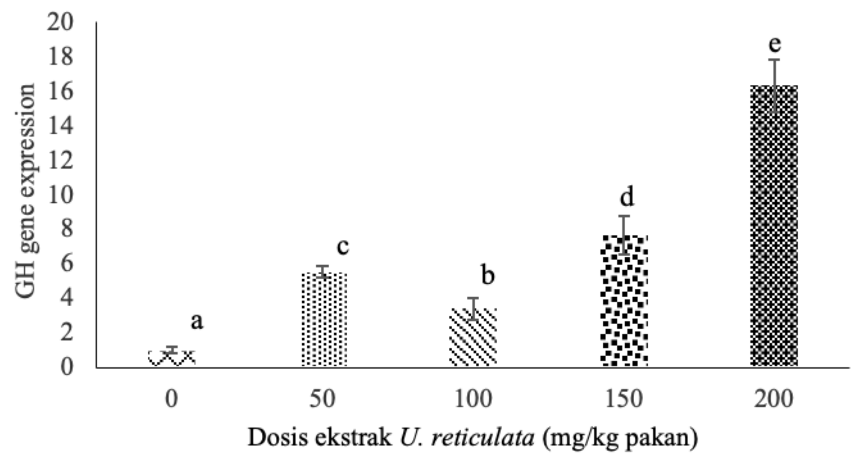

Fig. 2. GH gene expression levels in female red tilapia at the end of the study by dosing with *U. reticulata* ethanol extract. Letters above different bars in different diagrams indicate significant differences (*P*< 0.05).

The level of GH gene expression in the female red tilapia at the end of the study was significantly higher (*P*< 0.05) with the treatment of *U. reticulata* extract doses than with the control treatment. The highest level of GH gene expression was observed in the dose treatment with *U. reticulata* extract at 200mg kg^{-1} feed. Furthermore, observation of IGF-1 gene expression in this study also showed an increase in the treatment with *U. reticulata* extract, which was significant compared to the control treatment at the end of maintenance (Fig. 3). The highest IGF-1 gene expression in the red tilapia fry was shown in the treatment of giving a dose of *U. reticulata* extract of the 200mg kg^{-1} Nile tilapia fry feed at the end of maintenance.

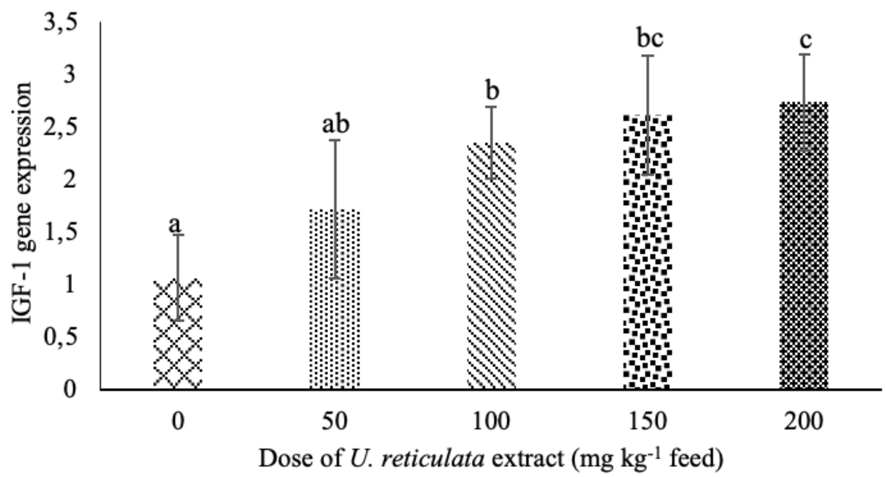

Fig. 3. IGF-1 gene expression level in the red tilapia at the end of the study by dosing with *U. reticulata* ethanol extract. Letters above different bars in different diagrams indicate significant differences (*P*< 0.05).

DISCUSSION

The use of plant extracts is one of the environmentally friendly strategies used in the field of aquaculture **(Klongklaew *et al.*, 2021)**. One of the marine plant extracts that can be used as a substitute for synthetic chemicals in aquaculture is seaweed **(Pradhan *et al.*, 2022)**. Seaweed extract can be used as a supplement with natural bioactive components that can be used in the field of aquaculture **(Thépot *et al.*, 2022)**. The application of seaweed extracts in the field of aquaculture can be included in feed because it can act as a growth promoter, antioxidant, and immunostimulant **(Abo-Raya *et al.*, 2021; Pradhan *et al.*, 2022)**. Plant extract is one of the strategies used in the field of aquaculture that is environmentally friendly **(Klongklaew *et al.*, 2021)**. One of the marine plant extracts that can be used as a substitute for synthetic chemicals in aquaculture is seaweed **(Pradhan *et al.*, 2022)**. Seaweed extract can be used as a supplement that has natural bioactive components that can be used in the field of aquaculture **(Thépot *et al.*, 2022)**. The application of seaweed extract in the field of aquaculture can be included in feed because it can act as a growth promoter, antioxidant, and immunostimulant **(Abo-Raya *et al.*, 2021; Pradhan *et al.*, 2022)**.

In this study, a dose of *U. reticulata* extract of 200mg kg^{-1} in feed significantly increased growth through the induction of growth genes such as GH and IGF-1 in the red tilapia fry. **Abo-Raya *et al.* (2021)** also reported that feeding *Ulva fasciata* extract at 100mg kg^{-1} can increase the growth, growth gene expression, and health status of tilapia.

This is because *U. reticulata* extract is reported to contain active compounds such as alkaloids, flavonoids, saponins, tannins, phenols, and steroids/terpenoids. In addition, *U. reticulata* extract also has high antioxidant activity. The bioactive compounds contained in *U. reticulata* extract can be utilized as a functional feed additive that has a role as an antioxidant, anti-inflammatory, and immunomodulator, so that it can affect growth performance and health status in fish **(Tarigan *et al*., 2023)**. The bioactive compounds in *U. reticulata* extract are thought to work synergistically to support androgenic processes and stimulate digestion, appetite, and the immune system in fish. According to **Beltrán and Esteban (2022)**, bioactive compounds in plants can improve growth performance and stimulate the immune system in fish.

The highest daily growth rate of red tilapia seeds in this study was 5,94 ± 0.23% in the treatment dose of *U. reticulata* extract 200mg kg^{-1}. **Sheikhzadeh *et al*. (2022)** reported that administration of *Padina australis* extract as much as 200mg kg^{-1} was able to improve the growth performance of goldfish. Furthermore, *U. reticulata* extract was able to produce a smaller FCR value than the control. The smaller the feed conversion ratio, the more effective the feed consumed by fish is to support fish growth. Conversely, a greater feed conversion ratio value indicates that the feed provided is not effective in supporting fish growth. The decrease in FCR value is due to the presence of various bioactive compounds such as amino acids and fatty acids that can increase palability and feed intake, thereby increasing feed utilization. Bioactive substances in seaweeds can stimulate the secretion of several enzymes (amylase, lipase, and protease) that can improve the digestion of essential nutrients and their assimilation into fish tissues **(Siddik *et al*., 2023)**.

In addition, molecular fish growth data were evaluated by observing the expression of growth genes. Genes play a role in regulating the development and growth characteristics of fish, including growth hormone (GH) and insulin-like growth factor-1 (IGF-1), which form the hypothalamus-pituitary-somatotropic axis **(Abbas *et al*., 2023)**. GH can increase the synthesis of IGF-1, which acts on target cells to stimulate growth in fish **(Ahmadifar *et al*., 2021)**. Therefore, GH and IGF-1 gene expression can be used as indicators of growth performance in fish. In this study, the expression of GH and IGF-1 genes was increased in red tilapia fry following treatment with *U. reticulata* extract, especially at a dose of 200mg kg^{-1}. **Rouhani *et al*. (2022)** also reported that the administration of *Ulva intestinalis* increased the expression of the growth gene GH-IGF-1 in *Danio rerio* fish. The seaweed extract can increase the expression of growth genes so that it can directly increase the growth rate **(Abbas *et al*., 2023)**. This is thought to be because *U. reticulata* extract contains various bioactive compounds such as alkaloids, flavonoids, and phenols as antioxidant compounds that work synergistically to stimulate an increase in the GH-IGF-1 growth gene and affect the growth rate of fish. In addition, tannin compounds in *U. reticulata* extract are thought to increase the growth rate of fish. In addition, tannin compounds in *U. reticulata* extract are thought to promote fish growth by regulating the GH and IGF-1 gene axis.

The results of research by **Tarigan *et al.* (2023)** reported that *U. reticulata* extract contained alkaloids with moderate presence (++), flavonoids of 82.16mg QE 100 g^{-1} and phenols of 144.00mg QE 100 g^{-1}. Furthermore, **Chakraborty *et al.* (2014)** reported that phytochemical compounds contained in plant extracts, such as flavonoids, can stimulate growth hormones through the increased expression of GH and IGF-1 genes in animals. **Chantzi *et al.* (2024)** also reported that alkaoid compounds in feed have the ability to modulate GH and IGF-1 gene expression in rainbow trout tissues and muscles. In addition, **Ahmadifar *et al.* (2021)** reported that polyphenolic compounds can stimulate the activity of digestive enzymes and increase the synthesis of DNA, RNA, and proteins, as well as the production and function of GH and IGF-1 to increase fish growth. The addition of 0.1% tannin can improve growth performance and GH and IGF-1 gene expression in *Beluga sturgeon* **(Safari *et al.*, 2020)**.

In addition to growth, the blood picture of the fish was evaluated in this study. A blood picture is one of the parameters that can be used to assess health status and stressors in the environment. Erythrocytes are a group of cells that dominate the blood and function as carriers of nutrients and oxygen throughout the fish body **(Linda *et al.*, 2022)**. Erythrocytes carry O_2 to tissues and release CO_2 during metabolic processes **(Nursyam *et al.*, 2023)**. Dosing *U. reticulata* extract in feed increased the number of erythrocytes compared to the control treatment. This is supported by the increase in hemoglobin and hematocrit levels of fish in the *U. reticulata* extract treatment, which will play a role in carrying oxygen throughout the fish body. The resulting hemoglobin level is directly proportional to the number of erythrocytes. The results showed that the resulting fish hemoglobin levels were still in the normal range of 8,28-10,62%. According to **Kusuma *et al.* (2022)**, the range of normal hemoglobin values in tilapia ranges from 6,0-11,0%. The increase in hemoglobin and hematocrit is thought to be due to fish not experiencing stress where there is an increase in oxygen transport capacity into the fish body. According to **Gazali *et al.* (2024)**, the increase in hemoglobin levels is related to an increase in oxygen transport capacity and the fish body's mechanism against stress. This is supported by the results of research by **Zeraatpisheh *et al.* (2018)** that supplementation of *Sargassum* sp. extract as much as 400 mg kg^{-1} was able to increase hemoglobin and hematocrit in the rainbrow trout.

In addition, phagocytic activity plays a role in the immune system's defense against stress, inflammation, and infection **(Afifah *et al.*, 2023)**. The results of this study showed that administration of *U. reticulata* extract as much as 200mg kg^{-1} was able to increase phagocyte activity compared to the control treatment. An increase in phagocyte activity indicates an increase in the immune ability of fish. This is supported by the results of research by **Diab *et al.* (2023)** that the increase in fish immune ability is characterized by an increase in the number of leukocyte cells, phagocytosis activity, and phagocytosis index. The increase in phagocytic activity in this study was due to bioactive compounds in the form of flavonoids and phenols contained in the *U. reticulata* extract. Flavonoids and phenols act as immunostimulants so that they more quickly activate phagocytic activity as a defense of the immune system in fish. This is supported by the results of **Putranto *et al*. (2019)**, who showed that the flavonoid content in plant extracts can increase phagocytic activity in tilapia. Furthermore, differential observations of leukocytes in the red tilapia fry, including lymphocytes, neutrophils, and monocytes, were also evaluated (Fig. 1). Lymphocytes are the most abundant leukocyte cells found in leukocytes, followed by neutrophils and monocytes, which play a role in the immune system in fish. Lymphocytes act as providers of immune substances for body defense by building the immune system in fish **(Tian *et al*., 2022)**.

Furthermore, neutrophils also function to protect the host against bacterial infection by increasing the secretion of proteolytic enzymes and lysozyme to destroy bacteria, while the number of monocytes increases, indicating the interaction of bacteria that interfere with fish conditions **(Han *et al*., 2021)**. In this study, the lymphocyte count increased with the dose of *U. reticulata* extract compared to that of the control ($P< 0.05$). The highest lymphocyte value was observed in the treatment dose of *U. reticulata* extract 200mg kg^{-1} at 73%. However, the value of lymphocytes produced in this study is still in the normal range of 70-73%. The percentage of normal lymphocytes in tilapia fish ranges from to 68-86% **(Ariyanti *et al*., 2022)**. The number of neutrophils and monocytes was not significantly different among all treatments. This is thought to be because lymphocytes have successfully eliminated antigens from the host body. The increase in lymphocyte values is thought to be because *U. reticulata* extract contains several bioactive compounds that act as immunomodulators and can stimulate the development of lymphocytes through immune response mechanisms in fish.

Ulva extract can act as an immunomodulator and increase macrophage activity, thereby affecting the immune system in fish **(Ponce *et al*., 2020; Pradhan *et al*., 2023)**. Several studies have reported that the use of Ulva extract gave positive results on the health status of fish. Giving *Ulva* sp. seaweed extract as much as 50g kg^{-1} can improve the growth and health of *Argyrosomus japonicus* **(Madibana *et al*., 2017)**. Utilization of *U. fasciata* extract at 100mg kg^{-1} can increase growth gene expression and improve health status and antioxidants in tilapia **(Abo-Raya *et al*., 2021)**. Polyphenol bioactive compounds contained in *U. reticulata* extract in the form of phenols and flavonoids have immunomodulatory and antioxidant properties in the regulation of the immune system to improve fish health. Polyphenols regulate the immune system through immune cell regulation, cytokine synthesis, proinflammation, and immune cell gene expression to improve health status **(Yahfoufi *et al*., 2018)**. It was reported that polyphenolic compounds in seaweed extracts play a role in the regulation of the innate immune system in fish **(Salomon *et al*., 2020; Marmelo *et al*., 2024)**.

CONCLUSION

A dose of *U. reticulata* extract of 200mg kg^{-1} feed was able to increase fish growth 1.5-fold through the mechanism of increasing the expression of growth genes GH 4-fold and IGF-1 2.5-fold and to improve health through the improved haematological profiles in the red tilapia fry during rearing.

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REFERENCES

Abaho, I.; Masembe, C.; Akoll, P. and Jones, C.L.W. (2022). The use of plant extracts to control tilapia reproduction: Current status and future perspectives. J. World Aquac. Soc., 53(3):593–619.doi:10.1111/jwas.12863.

Abbas, E.M.; Al-Souti, A.S.; Sharawy, Z.Z.; El-Haroun, E. and Ashour, M. (2023). Impact of Dietary Administration of Seaweed Polysaccharide on Growth, Microbial Abundance, and Growth and Immune-Related Genes Expression of The Pacific Whiteleg Shrimp (*Litopenaeus vannamei*). Life.,13(2).doi:10.3390/life13020344.

Abo-Raya, M.H.; Alshehri, K.M.; Abdelhameed, R.F.A.; Elbialy, Z.I.; Elhady, S.S.; and Mohamed, R.A. (2021). Assessment of growth-related parameters and immune-biochemical profile of nile tilapia (*Oreochromis niloticus*) fed dietary *Ulva fasciata* extract. Aquac. Res., 52(7):3233–3246.doi:10.1111/are.15169.

Afifah, N.F.; Windarti, W. and Sarumaha, H. (2023). Haematology of saline tilapia (*Oreochromis niloticus*) fed with moringa leaf powder enriched pellets. J. Aquat. Sci., 6(3):379–388.doi:10.31258/ajoas.6.3.379-388.

Ahmadifar, E.; Fallah, H.P.; Yousefi, M.; Mahmoud, A.O.D; Seyed.;Hoseinifar, H.A.; Yilmaz, S.; Paolucci, M. and Doan, Van. H. (2021). Immune System , and Reproduction of Fish. Animals., 11(2167):1–24.

Ahmadifar, E.; Pourmohammadi, F.; Yousefi, M.; Dawood, M. A.; Hoseinifar, S. H.; Adineh, H. and Doan, H.V. (2021). The gene regulatory roles of herbal extracts on the growth, immune system, and reproduction of fish. Animals., 11(8): 2167.

Alagawany, M.; Farag, M.R.; Salah, A.S. and Mahmoud, M.A. (2020). The role of oregano herb and its derivatives as immunomodulators in fish. Rev.Aquac., 12 (4): 2481-2492. DOI: 10.1111/raq.12453.

Anderson, D.P. and Siwicki, A.K. (1995). Basic hematology and serology for fish health programs. In: Paper presented at: Second Symposium on Diseases in Asia Aquaculture, Phuket, Thailand.

Ariyanti, I.; Marnani, S.; Setiawan, A. C.; Syakuri, H. and Dadiono, M. S. (2022).

Blood profile of Tilapia (*Oreochromis niloticus*) Fed with the Addition of White Fire Mangrove *Leaf Extract* (*Avicennia marina*). J. Pantura Fish., *5*(2): 215-226.

Ashour M, Mabrouk MM, Ayoub HF, El-Feky MMMM, Zaki SZ, Hoseinifar SH, Rossi W, Van Doan H, El-Haroun E. and Goda AMAS. (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. J. Appl. Phycol., 32(5):3467–3479.doi:10.1007/s10811-020-02178-1.

Ashour, M.; Al-Souti, A. S.; Mabrouk, M. M.; Naiel, M.A.; Younis, E.M.; Abdelwarith, A. A. and Abdelhamid, A. F. (2024). A commercial seaweed extract increases growth performance, immune responses, and related gene expressions in whiteleg shrimp (*Litopenaeus vannamei*). Aquac. Rep., *36*, 102154.

Ashour, M.; Mabrouk, M.M.; Abo-Taleb, H.A.; Sharawy, Z.Z.; Ayoub, H.F.; Van Doan, H.; Davies, S.J.; El-Haroun, E. and Goda, A.M.S. (2021). A liquid seaweed extract (TAM®) improves aqueous rearing environment, diversity of zooplankton community, whilst enhancing growth and immune response of Nile tilapia, *Oreochromis niloticus*, challenged by Aeromonas hydrophila. Aquaculture., 543:736915.doi:https://doi.org/10.1016/j.aquaculture.2021.736915.

Beltrán, J.M.G and Esteban, M.Á. (2022). Nature-identical compounds as feed additives in aquaculture. Fish Shellfish Immunol., 123:409–416.doi:10.1016/j.fsi.2022.03.010.

Butt, U.D.; Lin, N.; Akhter, N.; Siddiqui, T.; Li, S. and Wu, B. (2021). Overview of the latest developments in the role of probiotics, prebiotics and synbiotics in shrimp aquaculture. Fish Shellfish Immunol., 114:263–281.doi:10.1016/j.fsi.2021.05.003.

Chakraborty, S.B.; Horn, P. and Hancz, C. (2014). Application of phytochemicals as growth-promoters and endocrine modulators in fish culture. Rev. Aquac., 6(1):1–19.doi:10.1111/raq.12021.

Chantzi, P.; Vasilopoulou, K.; Panitsidis, I.; Bitchava, K.; Efstathiou, A.; Dokou, S.; Stylianaki, I.; Tsoumani, M.; Gouva, E. and Michailidis, G. (2024). Effect of isoquinoline alkaloids (IQs) on rainbow trout performance, serum parameters, antioxidant status, fillet fatty acid profile, intestinal and skin histomorphometry and expression of growth hormones genes. Aquac. Rep., 36:102173.doi:https://doi.org/10.1016/j.aqrep.2024.102173.

Cunha, L. and Grenha, A. (2016). Sulfated seaweed polysaccharides as multifunctional materials in drug delivery applications. Mar. Drugs., 14(3).doi:10.3390/md14030042.

Diab, A. M.; Eldeghaidy, E. E.; Abo-Raya, M. H.; Shukry, M.; Abdeen, A.; Ibrahim, S. F. and Khalafalla, M. M. (2023). Assessment of growth-related parameters, immune-biochemical profile, and expression of selected genes of Red Tilapia fed with Roselle calyces (*Hibiscus sabdariffa*) Extract. Fishes., *8*(4):172.

FAO (2018). The state of world fisheries and aquaculture 2018 – Meeting the sustainable development goals. Rome: Fisheries and Aquaculture Department, Food and Agriculture Organization of the United Nations

Gazali, M.; Effendi, I.; Husni, A.; Nurjanah, N.; Wahyuni, S. and Kurniawan, R. (2024). *Sargassum* sp. extract improve hematological profile of tilapia fish (*Oreochromis niloticus*). F1000Res., 12(2): 1-16. doi:10.12688/f1000research.128819.4.

Han, G.; Yao, H.; Qiang, L.; Chen, X. and Gao, Y. (2021). Comparative study of peripheral blood cells in two marine fishes (*Synechogobius hasta* and *Sebastes schlegelii*): Morphological and cytochemical characterization. Tissue Cell.,12 (2):1-10.doi:10.1016/j.tice.2021.101633.

Henriksson, P.J.G.; Tran, N.; Mohan, C.V.; Chan, C.Y.; Rodriguez, U.P.; Suri, S.; Mateos, L.D.; Utomo, N.B.P.; Hall, S. and Phillips, M. J. (2017). Indonesian aquaculture futures – Evaluating environmental and socioeconomic potentials and limitations. J. Clean. Prod.,162:1482-1490.

Hoseini, S.M.; Gharavi, B.; Mirghaed, A,T.; Hoseinifar, S.H. and Van Doan, H. (2021). Effects of dietary phytol supplementation on growth performance, immunological parameters, antioxidant and stress responses to ammonia exposure in common carp, *Cyprinus carpio* (Linnaeus, 1758). Aquaculture., 545 (2): 737151. DOI: 10.1016/j.aquaculture.2021.737151.

Jayant, M.; Hassan, M.A.; Srivastava, P.P.; Meena, D.K.; Kumar, P.; Kumar, A. Wagde, M.S. (2018). Brewer's spent grains (BSGs) as feedstuff for striped catfish, Pangasianodon hypophthalmus fingerlings: An approach to transform waste into wealth. J. Clean. Prod., 199:716–722.doi:10.1016/j.jclepro.2018.07.213.

Jimenez-Lopez, C.; Pereira, A.G.; Lourenço-Lopes, C.; Garcia-Oliveira, P.; Cassani, L.; Fraga-Corral, M.; Prieto, M.A. and Simal-Gandara, J. (2021). Main bioactive phenolic compounds in marine algae and their mechanisms of action supporting potential health benefits. Food Chem., 341:128262.doi:10.1016/j.foodchem.2020.128262.

Klongklaew, N.; Praiboon, J.; Tamtin, M. and Srisapoome P. (2021). Chemical composition of a hot water crude extract (HWCE) from *Ulva intestinalis* and its potential effects on growth performance, immune responses, and resistance to white spot syndrome virus and yellowhead virus in Pacific white shrimp (*Litopenaeus vannam*) Fish Shellfish Immunol., 112(2):8–22.doi:10.1016/j.fsi.2021.02.004.

Kusuma, R. O.; Dadiono, M. S.; Kasprijo, K. and Nurhafid, M. (2022). Blood Profile of Tilapia (*Oreochromis niloticus*) Strains Sultana, Nirwana and Larasati against Aeromonas hydrophyla infection. J. Agroqua., 20(1):15-23.

Linda.; Nur, D.; Setyowati, A. and Putri, L. D. (2022). Blood profile of Tilapia (*Oreochromis niloticus*) on feeding with fortified *Gracilaria* sp. seaweed meal. Ijota., 5(1):11–23.

Madibana, M.J.; Mlambo, V.; Lewis, B. and Fouché, C. (2017). Effect of graded levels of dietary seaweed (*Ulva* sp.) on growth, hematological and serum biochemical parameters in dusky kob, *Argyrosomus japonicus*, sciaenidae. Egypt. J. Aquat. Res., 43(3):249–254.doi:10.1016/j.ejar.2017.09.003.

Marmelo, I.; Dias, M.; Grade, A.; Pousão-Ferreira, P.; Diniz, M. S.; Marques, A. and Maulvault, A. L. (2024). Immunomodulatory and antioxidant effects of functional aquafeeds biofortified with whole *Laminaria digitata* in juvenile gilthead seabream (*Sparus aurata*). Front. Mar. Sci., *11*, 1325244.

Nursyam, H.; Kilawati, Y. and Eka, K. R. (2023). Potential Use of Tembelekan Leaf Plant Extract (*Lantana camara* linn) on Hematological and Histopathological Profiles of Goldfish (Cyprinus carpio) infected with *Edwardsiella tarda Bacteria*. J. Surv. Fish. Sci., 10(1):1995–2010.

Ponce, M.; Zuasti, E.; Anguís, V. and Fernández-Díaz, C. (2020). Effects of the sulfated polysaccharide ulvan from *Ulva ohnoi* on the modulation of the immune response in Senegalese sole (*Solea senegalensis*). Fish Shellfish. Immunol., 100(2):27-40.

Pradhan, B.; Bhuyan, P. P. and Ki, J. S. (2023). Immunomodulatory, antioxidant, anticancer, and pharmacokinetic activity of Ulvan, a seaweed-derived sulfated polysaccharide: An updated comprehensive review. Marine drugs., 21(5):735291.

Pradhan, B.; Bhuyan, P.P.; Patra, S.; Nayak, R.; Behera, P,K.; Behera, C; Behera, A.K.; Ki, J.S. and Jena, M. (2022). Beneficial effects of seaweeds and seaweed-derived bioactive compounds: Current evidence and future prospective. Biocatal. Agric. Biotechnol., 39:102242.doi:https://doi.org/10.1016/j.bcab.2021.102242.

Pratiwi, N.; Jusadi, D. and Nuryati, S. (2016). Utilization of clove oil *Syzigium aromaticum* to improve feed efficiency on striped catfish *Pangasianodon hypophthalmus*. J. Iktiologi Indones., 16(3):233–242.

Putranto, W. D.; Syaputra, D. and Prasetiyono, E. (2019). Blood Preview of tilapia (*Oreochromis niloticus*) given fortified feed of Salam leaf (*Syzygium polyanthum*) liquid extract. Journal of Aquatropica Asia., *4*(2):22-28.

Rouhani, E.; Safari, R.; Imanpour, M.R.; Hoseinifar, S.H.; Yazici, M. and El-Haroun, E. (2022). Effect of dietary administration of green macroalgae (ulva intestinalis) on mucosal and systemic immune parameters, antioxidant defence, and related gene expression in Zebrafish (*Danio rerio*). Aquac. Nutr., 2022.doi:10.1155/2022/7693468.

Safari, R.; Hoseinifar, S. H.; Imanpour, M.R.; Mazandarani, M.; Sanchouli, H. and Paolucci, M. (2020). Effects of dietary polyphenols on mucosal and humoral immune responses, antioxidant defense and growth gene expression in beluga sturgeon (*Huso huso*). Aquaculture., *528*, 735494.

Salomón, R.; Firmino, J. P.; Reyes-López, F. E.; Andree, K. B.; González-Silvera, D.; Esteban, M. A. and Gisbert, E. (2020). The growth promoting and immunomodulatory effects of a medicinal plant leaf extract obtained from Salvia officinalis and Lippia citriodora in gilthead seabream (*Sparus aurata*). Aquaculture., 524(3):735291.

Sarangi, N. V.; Prakasan, M. V. S.; Rajkumar, R. and Srinivasan, S. (2023). Assessment of phytochemicals from marine algae *Ulva fasciata* and *Dictyota dichotoma* with antioxidant and antimicrobial potential. Chem. Eng., 6(3):1-10.

Seyedalhosseini, S. H.; Salati, A. P.; Torfi Mozanzadeh, M.; Parrish, C. C.; Shahriari, A. and Ahangarzadeh, M. (2024). Effect of dietary seaweed (*Gracilaria pulvinata and Sargassum ilicifolium*) on serum and mucosal immunity, some growth and immune-related genes expression, antioxidant status, and fatty acid profile in Asian Seabass (*Lates calcarifer*). Aquac. Nutr., 2024(1):3683163.

Sheikhzadeh, N.; Ahmadifar, E.; Soltani, M.; Tayefi-Nasrabadi, H.; Mousavi, S. and Naiel, M. (2022). Brown seaweed (*Padina australis*) extract can promote performance, innate immune responses, digestive enzyme activities, intestinal gene expression and resistance against *Aeromonas hydrophila* in Common carp (*Cyprinus carpio*). Animals., 12(23).doi:10.3390/ani12233389.

Siddik, M.A.B.; Francis, P.; Rohani, M.F.; Azam, M.S.; Mock, T.S. and Francis, D.S. (2023). Seaweed and seaweed-based functional metabolites as potential modulators of growth, immune and antioxidant responses, and gut microbiota in fish. Antioxidants., 12(12).doi:10.3390/antiox12122066.

Tarigan, N.; Sudrajat, A.O.; Arfah, H.; Alimuddin, A.; and Wahjuningrum, D. (2023). Potential use of phytochemical from ethanolic extract of green seaweed *Ulva reticulata* in aquaculture. Biodiversitas., 24(12):6868–6879.doi:10.13057/biodiv/d241248.

Thépot, V.; Campbell, A. H.; Rimmer. M. A.; Jelocnik, M.; Johnston, C.; Evans, B. and Paul, N. A. 2022. Dietary inclusion of the red seaweed Asparagopsis taxiformis boosts production, stimulates immune response and modulates gut microbiota in Atlantic salmon. Aquaculture., 546.doi:10.1016/j.aquaculture.2021.737286.

Tian, H .; Xing, J.; Tang, X.Q.; Chi, H.; Sheng, X.Z. and Zhan, W. B. (2022). Cluster of differentiation antigens: essential roles in the identification of teleost fish T lymphocytes. Mar. Life Sci. Technol., 4(3):303–316.doi:10.1007/s42995-022-00136z.

Vazirzadeh, A.; Marhamati, A. and Chisti, Y. (2022). Seaweed-based diets lead to normal growth, improved fillet color but a down-regulated expression of somatotropic axis genes in rainbow trout (*Oncorhynchus mykiss*). Aquaculture., 554(2): 738183

Wedemeyer, G.A. and Yasutake, W.T. (1977). Clinical methods for the assessment of the effects of environmental stress on fish health. Seattle: US Fish and Wildlife Service.

Yahfoufi, N.; Alsadi, N.; Jambi, M. and Matar, C. (2018). The immunomodulatory and anti-inflammatory role of polyphenols. Nutrients., 10(11):1618.

Zeraatpisheh, F., Firouzbakhsh, F. and Khalili, K.J. (2018). Effects of the macroalga Sargassum angustifolium hot water extract on hematological parameters and immune responses in rainbow trout (*Oncohrynchus mykiss*) infected with Yersinia rukeri. J. Appl. Phycol., 30(3):2029–2037.doi:10.1007/s10811-018-1395-4.