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Addition of *Chaetomorpha* sp Extract on the Growth and Hematology of Tilapia (*Oreochromis niloticus*)

Pemberian Ekstrak *Chaetomorpha* sp terhadap Pertumbuhan dan Hematologi Ikan Nila (*Oreochromis niloticus*)

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ABSTRACT

The use of phytoimmunostimulants from *Chaetomorpha* sp which is environmentally friendly and cost-effective. Apart from that, secondary metabolite compounds found in *Chaetomorpha* sp can improve the immune system. So, it can increase the fish's resistance to disease attacks, improve the digestive system, increase the fish's appetite, and save on feed use. This can be seen from hematological and physiological parameters. Therefore, researchers are interested in researching the effectiveness of the macroalgae *Chaetomorpha* sp as a phytoimmunostimulant. This research aims to look at the growth of tilapia and the immune system, which includes hematology and physiology of fish fed with warm water extract of *Chaetomorpha* sp for 30 days of maintenance. Data analysis used One-way ANOVA, and homogeneity was seen. If the analysis results show an effect, it is tested further using Student Newman Keuls (SNK). The research results show that adding *Chaetomorpha* sp extract influences the growth rate and survival of tilapia fish that are kept for 30 days. This maintenance shows that the administration of *Chaetomorpha* sp extract with a dose of 50mL/kg feed (T2) gave the best results on growth rate, feed efficiency, hematology, and tilapia survival, namely absolute weight 18.30g, LPS 5.38%/day, absolute length 3.98 cm, FCR 1.44, feed efficiency 69.18%, total erythrocytes $1.85x10^6$ cells/mm^3, hemoglobin 6.67 g/dL, hematocrit 35.33-37.67%, total leukocytes $2.42x10^4$ cells/mm^3, leukocrit 2.67%, blood glucose 43% and survival 93.33%.

Keywords: *Chaetomorpha* sp, Growth rate, Phytoimmunostimulant

ABSTRAK

Penggunaan fitoimunostimulan dari *Chaetomorpha* sp yang ramah lingkungan dan hemat biaya. Selain itu, senyawa metabolit sekunder yang terdapat pada *Chaetomorpha* sp mampu meningkatkan sistem kekebalan tubuh. Sehingga dapat meningkatkan daya tahan ikan terhadap serangan penyakit, memperbaiki sistem pencernaan, meningkatkan nafsu makan ikan dan menghemat penggunaan pakan. Hal ini terlihat dari parameter hematologi dan fisiologis. Oleh karena itu, peneliti tertarik untuk melakukan penelitian mengenai efektivitas makroalga *Chaetomorpha* sp sebagai fitoimunostimulan. Penelitian ini bertujuan untuk melihat pertumbuhan ikan nila, sistem imun yang meliputi hematologi dan fisiologi ikan yang diberi ekstrak air hangat *Chaetomorpha* sp selama 30 hari pemeliharaan. Analisis data menggunakan One Way ANOVA dan terlihat homogenitasnya. Apabila hasil analisis menunjukkan adanya pengaruh, maka diuji lebih lanjut dengan menggunakan Student Newman Keuls (SNK). Berdasarkan hasil penelitian menunjukkan bahwa penambahan ekstrak *Chaetomorpha* sp mempunyai pengaruh terhadap laju pertumbuhan dan kelangsungan hidup ikan nila yang dipelihara selama 30 hari. Pemeliharaan ini menunjukkan pemberian ekstrak *Chaetomorpha* sp dengan dosis 50 mL/kg pakan (P2) memberikan hasil terbaik terhadap laju pertumbuhan, efisiensi pakan, hematologi dan kelangsungan hidup ikan nila, yaitu bobot mutlak 18,30 g, LPS 5,38 %/hari, panjang mutlak 3,98 cm, FCR 1,44, pakan efisiensi 69,18 %, total eritrosit $1,85x10^6$ sel/mm^3, hemoglobin 6,67 g/dL, hematokrit 35,33-37,67 %, total leukosit $2,42x10^4$ sel/mm^3, leukosit 2,67 %, glukosa darah 43% dan kelangsungan hidup 93,33%.

Kata Kunci: *Chaetomorpha* sp, Laju pertumbuhan, Fitoimmunostimulan.

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INTRODUCTION

Macroalgae contain primary metabolites such as vitamins, minerals, fiber, alginate, carrageenan, and agar, which are widely used as a cosmetic ingredient for skin maintenance. Besides that, macroalgae are also a new antibacterial source with antibacterial bioactive compounds. Recently, macroalgae have been used commercially as immunostimulants, which can increase fish's survival rate and immune system (Genovese et al., 2013). *Chaetomorpha* sp has secondary compounds, which are antibacterial, anti-fungal, anti-inflammatory, antioxidant, and free radical scavenger, effective against HIV, hypertension, and tumors (Sapna et al., 2011).

The methanol extract of *C.antennina* showed antidiabetic activity (Fernando, 2016). It affected sea cucumbers' development, food utilization, and energy levels (*Apostichopus japonicus*) (Tsuyoshi et al., 2010). *Chaetomorpha* sp extract can increase fish immunity and prevent diseases caused by *Aeromonas hydrophila* bacteria (Sattanathan et al., 2020). Antibiotics are currently used to treat *A. hydrophila* infections. Long-term and continuous use of antibiotics can have negative impacts, including bacteria becoming resistant to antibiotics, residues from these antibiotics polluting the aquatic environment, and antibiotic residues in fish flesh. Chemical residues in fish treated with antibiotics will also enter the bodies of humans who consume the fish.

One effort to prevent bacterial infections is by using natural ingredients. Using natural ingredients has been proven effective and has no negative impact on humans or the environment. One natural ingredient with active compounds that can be antibacterial and inhibit bacterial growth is *Chaetomorpha* sp (Pangemanan, 2016). So, there is a need for phytoimmunostimulants from *Chaetomorpha* sp, which is environmentally friendly and cost-effective. Apart from that, secondary metabolite compounds found in *Chaetomorpha* sp can improve the immune system. So, it can increase the fish's resistance to disease attacks, improve the digestive system, increase the fish's appetite, and save on feed use. This can be seen from hematological and physiological parameters. Therefore, researchers are interested in researching the effectiveness of the macroalgae *Chaetomorpha* sp as a phytoimmunostimulant. This research aims to look at the growth of tilapia fish and the immune system, which includes hematology and physiology of fish fed with warm water extract of *Chaetomorpha* sp for 30 days of maintenance.

MATERIALS AND METHOD

Time and place of research

This research was carried out in August - December 2023. Fish rearing was conducted at the Biotechnology Laboratory, Faculty of Fisheries and Marine, Universitas Riau. Hematological observations were conducted at the Fisheries Biology Laboratory, Faculty of Fisheries and Marine, Universitas Riau.

Methods

The method used in the research was an experimental method that applied a one-factor, Completely Randomized Design (CRD) with four treatment levels. Three repetitions were carried out to reduce the error level so that 12 experimental units were needed. The treatment dose in this study was according to the preliminary test. The treatment in question is:

TO = Commercial pellets
T1 = treatment with a dose of macroalgae extract of 25 mL/kg feed
T2 = treatment with a dose of macroalgae extract of 50 mL/kg feed
T3 = treatment with a dose of macroalgae extract of 75 mL/kg feed

Making feed containing macroalgae extract

The steps are as follows: taking macroalgae and drying the macroalgae. After drying, the macroalgae is boiled until it becomes a warm water extract and then sprayed into commercial feed at the dosage according to the treatment. A proximate test was carried out to determine the nutritional content of the pellets.

Fish maintenance

Fish rearing uses a container measuring 60x30x30 cm with a water volume of 60 L and a stocking density of 20 fish. Maintenance is carried out for 30 days. Feeding is carried out thrice daily, namely at 08.00 AM, 01.00 PM, and 05.00 PM at 5% of body weight. Every ten days, length and weight measurements were taken.

Blood collection

Blood sampling from test fish was carried out two times, namely at the beginning of rearing before treatment and after 30 days of rearing. Before taking blood, the fish was anesthetized in a clove oil solution at a dose of 0.1 mL/L, and the syringe and Eppendorf tube were rinsed first with an anticoagulant, 10% EDTA. Fish blood was taken from the linea lateralis in a caudal direction using a 1 mL syringe. The blood taken was put into an Eppendorf tube and then used for hematological observation.

Data analysis

Data obtained during research, such as growth, hematological, and physiological measurements, are collected and tabulated into tables. Next, it was analyzed statistically using the SPSS version 23 application. Data analysis used One-way ANOVA, and homogeneity was seen. If the analysis results show an effect, it is tested further using Student Newman Keuls (SNK).

RESULT AND DISCUSSION

The results showed that administering *Chaetomorpha* sp extract on rearing affects the absolute weight growth and daily growth rate of tilapia fish kept for 30 days. 30 days of maintenance showed that the absolute weight growth of tilapia ranged from 14.63g - 18.30g, the daily growth rate ranged from 4.43 - 5.38%/day, and the absolute length ranged from 3.63-3.98 cm.

Table 1. Results of measurements of tilapia growth during the research

Parameter	Treatment (mL/kg feed)			
	T0	T1	T2	T3
Absolute Weight (g)	14.63±0.48^{a}	15.67±0.35^{b}	18.30±0.19^{d}	17.09±0.33^{c}
SGR (%/day)	4.43±0.20^{a}	4.95±0.13^{b}	5.38±0.16^{c}	5.05±0.03^{b}
Absolute length (cm)	3.63±0.25	3.80±0.10	3.98±0.06	3.68±0.23
Feed Efficiency (%)	60.16±1.09^{a}	63.63±1.19^{b}	69.18±0.66^{c}	64.14±0.63^{b}
FCR	1.66±0.03^{a}	1.57±0.03^{b}	1.44±0.02^{c}	1.56±0.02^{b}
SR (%)	86.67±2.89	91.67±2.89	93.33±2.89	90.00±5.00

A dose of 50 mg/kg feed is the optimal dose that can be given to tilapia fish feed. This is thought to be due to the existing nutrient content meeting the fish's growth and metabolic activity needs. In addition, the content of secondary metabolite compounds from *Chaetomorpha* sp, such as phenols, tannins, and flavonoids, can stimulate the secretion of digestive enzymes, which can increase the amount of feed intake (Sobral-Sauza et al., 2019; Abdel-Tawwab & Hamed, 2020), fish growth and absorption of food essences (Omitoyin et al., 2019). The flavonoid and tannin content indirectly affects the work of the digestive organs so that feed nutrients can be absorbed entirely for growth (Lisnanti et al., 2019).

The survival rate of tilapia fish given feed containing *Chaetomorpha* sp extract with doses ranging from 86.67- 93.33% for 30 days of maintenance ($p>0.05$). The feed conversion value of tilapia fed with feed containing *Chaetomorpha* sp extract ranged from 1.66-1.44, and feed efficiency values ranged from 49.12-60.20%. The addition of *Chaetomorpha* sp extract in fish feed ranged from 1.57 to 1.44, lower than the control treatment, 1.66. This shows that the feed given *Chaetomorpha* sp is more efficient than control pellets. The smaller the FCR value, the higher the feed digestibility level that can be utilized optimally by the fish. The efficiency value of fish feed, given the addition of *Chaetomorpha* sp extract, ranged from 63.63 - 69.18%. The feed efficiency value is inversely proportional to the feed conversion value. The higher the feed efficiency value, the lower the feed conversion value. This shows that fish are increasingly efficient at utilizing their food for growth. According to Windarti et al. (2023), the feed efficiency value is closely related to the growth rate, where the higher growth rate will affect the weight gain and the resulting feed efficiency value. Secondary metabolite compounds, such as alkaloids and flavonoids, can increase fish feed utilization. In addition, essential oils mixed into feed can stimulate the central nervous system, resulting in increased appetite and consumption of food substances.

The red blood cells (erythrocytes) observed in this study include total erythrocytes, hemoglobin, and hematocrit. Observations of erythrocyte cells were carried out at the beginning of maintenance (D-1), day 30. At the beginning of maintenance, tilapia erythrocyte cells, such as total erythrocytes, hemoglobin, and hematocrit, ranged between 1.27-1.30 $x10^{6}$ cells/mm^{3}, 5-5.2 g/dL, and 25-26%, respectively (Table 2). Nile tilapia

erythrocyte cells were given the addition of *Chaetomorpha* sp extract on day 30. There was an increase from D-1, such as total erythrocytes ranging from 1.69-1.85x10^6 cells/mm^3, hemoglobin 6.47-6.67 g/dL, and hematocrit 33.00-35.33%. This shows that the increase also influences the increase in fish erythrocyte cells and body weight. Increasing the age and size of fish will affect oxygen requirements. Fish need oxygen for respiration, blood circulation, and metabolism, so larger fish have more erythrocytes than small ones (Syawal et al., 2021). The total erythrocytes of tilapia fish fed containing *Chaetomorpha* sp extract are higher when compared to goldfish given commercial pellets without the addition of guava leaves (control). This shows that the content of secondary metabolite compounds contained in *Chaetomorpha* sp flavonoids, tannins, and saponins can stimulate an increase in tilapia erythrocyte cells.

Table 2. Observations of hematology during the study

Parameter	Treatment (mL/kg feed)			
	T0	T1	T2	T3
Total erythrocytes (x10^6 sel/mm^3)	1.48±0.03^a	1.69±0.03^b	1.85±0.03^d	1.74±0.02^c
Hematocrit (%)	31.67±1.53^a	33.00±1.00^a	35.33±0.58^b	32.67±1.15^a
Hemoglobin (g/dL)	6.13±0.12^a	6.47±0.12^b	6.67±0.12^b	6.47±0.12^b
Total leucocyte (x10^4 sel/mm^3)	2.08±0.02^a	2.24±0.03^b	2.42±0.02^d	2.29±0.02^c
Leucocrit (%)	2.33±0.58	2.67±0.58	2.67±0.58	2.67±0.58
Blood glucose (%)	44.33±2.31	46.00±1.73	43.00±4.36	42.67±2.52

Giving *Chaetomorpha* sp. extract. with different doses had an effect between treatments ($p<0.05$) on total erythrocytes, hemoglobin, and hematocrit of tilapia that were kept for 30 days. A dose of 50 ml/kg feed (T2) gave the highest results on total erythrocytes (1.85 x10^6 cells/mm^3), hemoglobin (6.67 g/dL), and hematocrit (35.33%). This shows that a dose of 50 mL is optimal to stimulate an increase in erythrocyte cells. The total leukocytes of tilapia were fed with feed containing *Chaetomorpha* sp extract, which ranged from 2.08-2.42 x 10^4 cells/mm^3, and leukocrit 2.33-2.67%. This range is still within the normal range. The total leukocytes of tilapia fish increased based on the time they were kept. The increase in total leukocytes in fish given phytoimmunostimulants shows that there is an indicator of increased immunity.

Fish blood glucose measurements were performed thrice at the beginning of maintenance (D-1) and day 30 (D-30). Fish blood glucose at the start of maintenance (D-1) ranged from 40-43 mg/dL. The blood glucose of tilapia was fed with feed containing *Chaetomorpha* sp extract had an influence ($p<0.05$) on the blood glucose concentration of fish at 30 days of maintenance (D-30). Tilapia blood glucose ranges between 42.67% to 46% and is still within the normal range. Normal fish glucose levels range from 36-90 mg/dL (Athanasopoulus et al., 2018). In general, the blood glucose concentration of tilapia during maintenance indicates that the fish are in good condition.

CONCLUSION

The research results show that adding *Chaetomorpha* sp extract influences the growth rate and survival of tilapia fish that are kept for 30 days. This maintenance shows that the administration of *Chaetomorpha* sp extract with a dose of 50mL/kg feed (T2) gave the best results on growth rate, feed efficiency, hematology, and goldfish survival, namely absolute weight 18.30g, LPS 5.38%/day, absolute length 3.98 cm, FCR 1.44, feed efficiency 69.18%, total erythrocytes 1.85x10^6 cells/mm^3, hemoglobin 6.67g/dL, hematocrit 35.33-37.67%, total leukocytes 2.42x10^4 cells/mm^3, leukocrit 2.67%, blood glucose 43% and survival 93.33%.

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