Boosting the growth of tilapia fish using marine macroalgae (*Sargassum* sp.) extracts

[1,5]Mohamad Gazali, [2]Irwan Effendi, [1]Sri Wahyuni, [2]Ronal Kurniawan, [3,5]Abdul Matin, [4]Rina Syafitri

[1] Department of Marine Science, Faculty of Fisheries and Marine Science, Teuku Umar University, 23615 West Aceh, Indonesia; [2] Department of Marine Science, Faculty of Fisheries and Marine, University of Riau, Kampus Bina Widya KM. 12,5, Simpang Baru, Kec. Tampan, Kota Pekanbaru, Riau 28293, Indonesia; [3] Department of Food Processing and Engineering, Faculty of Food Science and Technology, Chattogram Veterinary and Animal Sciences University, Khususi, Chattogram 4225, Bangladesh; [4] Department of Agribusiness, Faculty of Agriculture, Teuku Umar University, 23615 West Aceh, Indonesia; [5] Institute of Marine Biotechnology, Universiti Malaysia Terengganu, Kuala Terengganu, 21030, Malaysia. Corresponding author: M. Gazali, mohamadgazali@utu.ac.id

Abstract. *Sargassum* sp. can be an alternative addition of protein in tilapia fish feed. This study aims to determine the most effective dose of *Sargassum* sp. added to enhance the growth response in Nile tilapia (*Oreochromis niloticus*). A completely randomized design (CRD) with four treatments and three replications was implemented in this experimental research. The treatments were T0 (control), T1 (feed containing *Sargassum* sp. extract dose 2.0 g kg^{-1} feed), T2 (dose 2.5 g kg^{-1} feed), and T3 (dose 3.0 g kg^{-1} feed). The results showed that the extract of *Sargassum* sp. influenced the growth of this fish. The absolute weight growth of tilapia ranged from 4.84 to 6.18 g, the specific growth rate ranged from 2.19 to 2.60% day^{-1}, and the absolute length ranged from 3.34 to 3.79 cm. The lowest feed conversion ratio was 1.56 at T3, and the highest was 1.88 at T0, while the highest feed efficiency was 64.20% at T3, and the lowest was 53.29% at T0. Additionally, the tissue structure was still in a normal state during the rearing. This demonstrates that the dose in T3 treatment is the optimal dose in this study.
Key Words: FCR, growth rate, *Oreochromis niloticus*, *Sargassum* sp.

Introduction. Nile tilapia fish (*O. niloticus*) is one of the fish that is still very popular for consumption in Indonesia. Its farming is currently increasing, especially due to its tendency to grow rapidly (Naiel et al 2019; Abdel-Latif et al 2020), its tolerance towards environment fluctuations (El Basuini et al 2022), and its resistance to diseases (Abdelghany et al 2020). Numerous Indonesian fish farmers have opted for tilapia pisciculture due to its high demand from the market. The feeding strategy is one of the most crucial aspects of tilapia farming. Feed is one of the most significant strategic components in determining the success of pond aquaculture (Munchdar et al 2021). Because feed is essential to fish growth and survival, it has a significant impact on fish farming. If nutrition is balanced, growth will be optimal. Feeding with high protein content feed can be very expensive. Consequently, there needs to be an alternative using natural ingredients in the hope of a cheaper option.

In aquaculture, feed expenses account for about 80% of all operational expenses (Hasan & New 2013; Van Doan et al 2021). Because of this, it is essential to choose natural alternatives that are safe for the environment and can boost feed protein. Such alternatives include *Sargassum* sp. Secondary metabolites found in *Sargassum* species include terpenoids, sterols, polyphenols, sargaquinoic acid, sargachromenol, pheophytin (Rushdi et al 2020), saponins, flavonoids, alkaloids, steroids (Gazali et al 2018; Sönmez et al 2021; Gazali et al 2023), vitamins, high protein, and have balanced amino acids (El-

Shenody et al 2019; Wan et al 2019; Bowyer et al 2020; Ferreira et al 2020). Fish growth will increase if the protein content is fulfilled.

Recently, the use of *Sargassum* sp. as additional feed contributed to enhancing growth, immunity response, hematology, and tissue structure in various species of fish such as the rainbow trout *Oncorhynchus mykiss* (Sönmez et al 2021), snapper *Lates calcarifer* (Zeynali et al 2020), and Nile tilapia *Oreochromis niloticus* (Negm et al 2021; Abdelrhman et al 2022). According to Goda et al (2018), the use of seaweed in aquaculture can boost growth, nutrient utilization, the response of the immunity system, and the prevention of diseases without negative effects on fish.

This study used extracts of *Sargassum* sp. to determine how tilapia grow and whether the structure of fish tissue indicates that this extract is harmful to the body. This study aimed to determine the most effective dose for increasing the growth response of tilapia when *Sargassum* sp. was added.

Material and Method

Samples collection. The samples of *Sargassum* sp. for this research were collected from the coast of Lhok Bubon, West Aceh, Indonesia. This species was identified and determined in the South China Sea Repository and References Center, Institute of Oceanography and Environment Universiti Malaysia Terengganu (UMT) with voucher number: UMTP 3354. Subsequently, the samples were tested in the Biotechnology Laboratory, Faculty of Fisheries and Marine Science, University of Riau. This research was conducted for 30 days of maintenance, in May-June 2022.

Research method. This study employed an experimental design with one-factor, completely randomized design (CRD), and four treatment levels. Three repetitions were used to lower the error rate, requiring 12 experimental units. This study's treatment dose was consistent with the preliminary test, as follows:
T0: control;
T1: feed containing *Sargassum* sp. extract at a dose of 2.0 g kg^{-1} feed;
T2: feed containing *Sargassum* sp. extract at a dose of 2.5 g kg^{-1} feed;
T3: feed containing *Sargassum* sp. extract at a dose of 3.0 g kg^{-1} feed.

Preparation of Sargassum sp. extract feed. *Sargassum* sp. extracts were weighed for each treatment (0, 2, 2.5, and 3 g) and then were mixed into 1 kg of mashed commercial feed. The mixture was then stirred until homogeneous and a small amount of warm water was added to allow for shaping. Then, the mixture was air-dried and the feed could then be used and its proximate contents could be examined.

Preparation of fish trial and maintenance. The test fish used was tilapia weighing 5.20±0.03 g obtained from farmers in Pekanbaru, Indonesia. The fish was adapted to a 2 x 1 x 0.4 m^3 fiber pond for 2 weeks. The fish rearing was carried out in an aquarium measuring 60 x 30 x 30 cm^3 with a volume of 60 L of water and a stocking density of 1 fish/3 L. The rearing was carried out for 30 days, and the frequency of feeding three times a day was as much as 5% of body weight. Every 10 days, sampling was carried out to measure fish weight and length.

A sampling of tissue structure. The measurement and sampling of tilapia were undertaken in Laboratory twice at the start of the research and on the 30th day. Gill and kidney were used as samples, and each organ was kept in a film container with 10% formalin.

Measured parameters

Absolute length growth. The absolute length measurement was calculated using the following formula by Effendie (2002):

$$AL = Lt - Lo$$

where: AL = absolute length (cm);

Lt = the average length at the end of the study (cm);

Lo = the average length at the start of the study (cm).

Absolute weight growth (growth rate). Absolute weight growth is calculated using the formula proposed by Effendie (2002):

$$GR = Wt - Wo$$

where: GR = growth rate (g);

Wt = the average weight of fish at the end of the study (g);

Wo = the average weight of fish at the start of the study (g).

Specific growth rate (SGR). The specific growth rate was calculated using the formula proposed by Zonneveld et al (1991):

$$SGR = ((LnWt - LnWo) / t) \times 100$$

where: SGR = specific growth rate (% day^{-1});

Wt = larval weight at the end of the study (g);

Wo = larval weight at the start of the study (g);

t = research duration (day).

Feed conversion ratio. Feed conversion ratio can be calculated using the formula by Zonneveld et al (1991) as follows:

$$FCR = \Sigma F / ((Bt + Bm) - Bo)$$

where: FCR = feed conversion ratio;

ΣF = the amount of feed given during rearing (g);

Bt = fish biomass at the end of rearing (g);

Bo = fish biomass at the start of rearing (g);

Bm = dead fish biomass during rearing (g).

Feed efficiency. Feed efficiency was calculated using Syawal et al's formula (2021):

$$FE = (((Bt+Bd)-Bo)) / F) \times 100$$

where: FE = feed efficiency;

ΣF = the amount of feed given during rearing (g);

Bt = fish biomass at the end of rearing (g);

Bd = dead fish biomass during the research(g);

Bo = fish biomass at the start of rearing (g).

Data analysis. Data from growth rate, FCR, and feed efficiency measurements were collected and summarized in tables. They were then analyzed statistically using SPSS version 23. Data analysis used one-way ANOVA and its homogeneity were observed. If the results of the analysis show an effect, this will be tested further using Student Newman Keuls (SNK).

Results and Discussion

Proximate analysis. *Sargassum* sp. extract addition in fish feed could enhance the nutrient content including protein (30.46-31.33%), fatty acid (3.50-5.14%), and crude fiber (8.10-9.70%). The results of the feed proximate analysis are shown in Table 1.

Table 1

Feed proximate analysis

Extract dosage (g kg^{-1})	*Nutrition content (%)*		
	Protein	*Fatty acid*	*Crude fiber*
0 (T_0)	30.00	3.21	8.05
2.0 (T_1)	30.46	3.50	8.10
2.5 (T_2)	30.97	3.88	8.45
3.0 (T_3)	31.33	5.14	9.70

Weight and length growth of tilapia. The results showed that *Sargassum* sp. extract usage could boost the growth of tilapia fish. The administration of *Sargassum* sp. on rearing affected the growth of absolute weight and SGR of tilapia reared for 30 days and 14 days after the challenge test. The 30-day rearing showed that the absolute weight growth of tilapia ranged from 4.84 to 6.18 g, the SGR ranged from 2.19 to 2.60% day^{-1}, and the absolute length ranged from 3.12 to 3.53 cm. More details can be seen in Table 2.

Table 2

Results of measurements of absolute weight growth (g), SGR, and absolute length of tilapia during the study

Parameter	*Treatment (Sargassum sp. extract dose) (g kg^{-1})*			
	T_0 (0)	*T_1 (2)*	*T_2 (2.5)*	*T_3(3)*
Weight gain (g)	4.84±0.10^{a}	5.77±0.06^{b}	5.85±0.05^{b}	6.18±0.01^{c}
SGR (% day^{-1})	2.19±0.05^{a}	2.48±0.03^{b}	2.51±0.02^{b}	2.60±0.01^{c}
Absolute length (cm)	3.12±0.05^{a}	3.41±0.08^{b}	3.44±0.04^{bc}	3.53±0.02^{c}

Note: The average values with different superscripts on the same row indicate that there is a significant difference between treatments ($p < 0.05$).

Feed conversion ratio (FCR) and feed efficiency. The administration of *Sargassum* sp. extract affected the level of feed efficiency and FCR value ($p < 0.05$). The level of feed efficiency by tilapia given feed containing *Sargassum* sp. extract ranged from 61.18 to 64.20%, higher than fish fed without *Sargassum* sp. extract. The dose of 3 g kg^{-1} (T3) resulted in the highest feed efficiency with a value of 64.20%. While the lowest was at T0 of 53.29%. The results were directly proportional to the FCR value, where T3 produced an FCR of 1.56 (Table 3) whereas T0 yielded an FCR value of 1.88. The tilapia can use the feed optimally and adapt for their body growth as a result.

Table 3

The measurement of feed efficiency level and FCR value

Treatment (dose of Sargassum sp. extract) (g kg^{-1})	*Parameter*	
	EP (%)	*FCR*
T_0 (0)	53.29±1.16^{a}	1.88±0.04^{c}
T_1 (2.0)	61.18±0.54^{b}	1.63±0.01^{b}
T_2 (2.5)	61.36±0.67^{b}	1.63±0.02^{b}
T_3 (3.0)	64.20±0.65^{c}	1.56±0.02^{a}

Note: The average values with different superscripts on the same column indicate that there is a significant difference between treatments ($p < 0.05$).

Tissue structure of tilapia's gill. Structure of gill tissue of tilapia fed *Sargassum* sp. extract for 30 days under normal circumstances characterized by the presence of distances between secondary lamellae and visible mucus, epithelial, and lacunae cells (Figure 1).

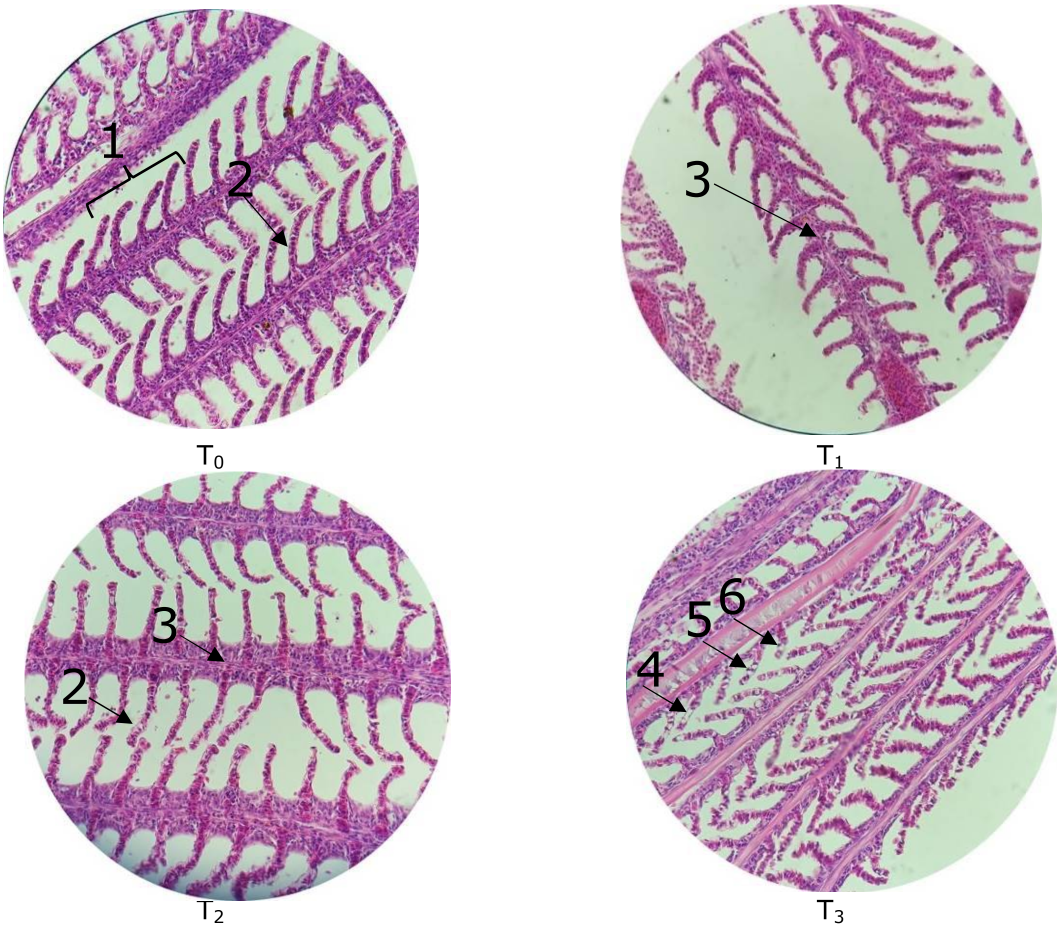

Figure 1. Structure of the gill tissue of tilapia-fed feed containing *Sargassum* sp. (1. secondary lamellae; 2. epithelial; 3. blood vessel; 4. lacuna; 5. buffer cells; 6. mucus cell.

Discussion. The administration of *Sargassum* sp. in the rearing medium affected the growth of absolute weight and SGR ($p < 0.05$). A dose of g kg^{-1} (T3) at 30 days of rearing resulted in the highest absolute weight growth, SGR, and absolute length (6.18 g, 2.60% day^{-1}, and 3.53 cm, respectively). While the lowest absolute weight growth, daily growth rate, and absolute length were at T0 (without extract administration) (4.84 g, 2.19% day^{-1}, and 3.12 cm, respectively). According to Nawir et al (2015), fish growth is restricted by protein content and the energy ratio of protein feed. The energy content of the feed consumed by fish exceeds the energy requirements for body maintenance and the excess is used for growth. Protein is the largest source of energy for the fish body, therefore the more protein is absorbed, the more energy is stored for the growth process (Pradisty et al 2017). Putri et al (2021) stated that marine macroalgae are added to feed containing carbohydrates that are used by fish as a source of energy for metabolism and movement. Thus, protein in fish feed could be optimally used for fish growth or known as the "protein sparing effect" associated with fatty acid and carbohydrate functions. The weight and length of tilapia given *Sargassum* sp. were shown to show faster growth compared to those who did not receive the treatment. According to Zulkhasyni et al (2017), the protein requirement for tilapia for optimal growth ranges from 28 to 35 g. Based on the results of the proximate test that the protein content of fish feed given *Sargassum* sp. ranged from 30 to 31% higher than regular feed so that the protein that enters the body makes the fish grow faster. During the rearing, fish continued to increase in both weight and length, which can be seen more clearly in Figures 2 and 3.

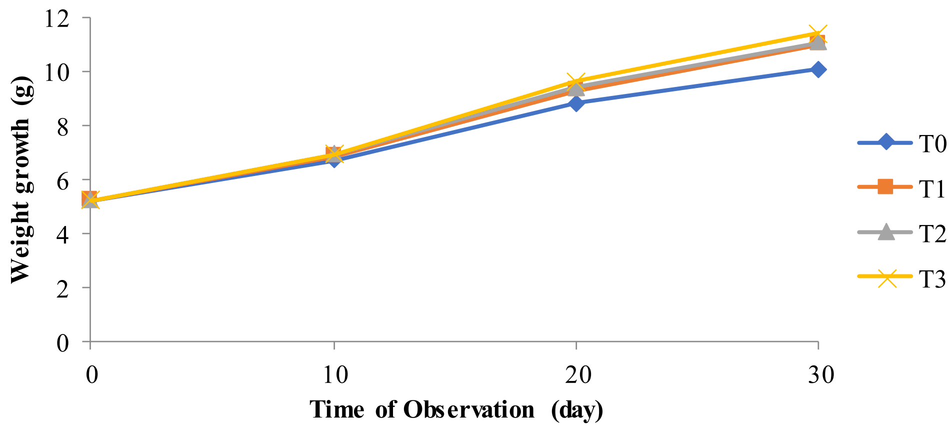

Figure 2. Weight growth of tilapia during rearing.

Weight growth continued to rise from the previous day, as shown in Figure 1. The dose of 3 g kg^{-1} of feed (T3) was the most effective one comparing to the other doses. According to Nugraha et al (2018), *Sargassum* sp. extract produced the most remarcable growth because the feed met the fish's nutritional requirements and could be utilized effectively to produce good growth. Growth in length is related to the growth in weight of the fish, whereas the length increases, the growth in the weight of the fry also increases during the rearing period. Growth in the length of tilapia-fed feed containing *Sargassum* sp. increased during the study. Fish at T3 produced the highest absolute length of 3.53 cm, while the lowest was at T0 (without adding *Sargassum* sp. extract), which was 3.12 cm. Figure 3 depicts this more clearly.

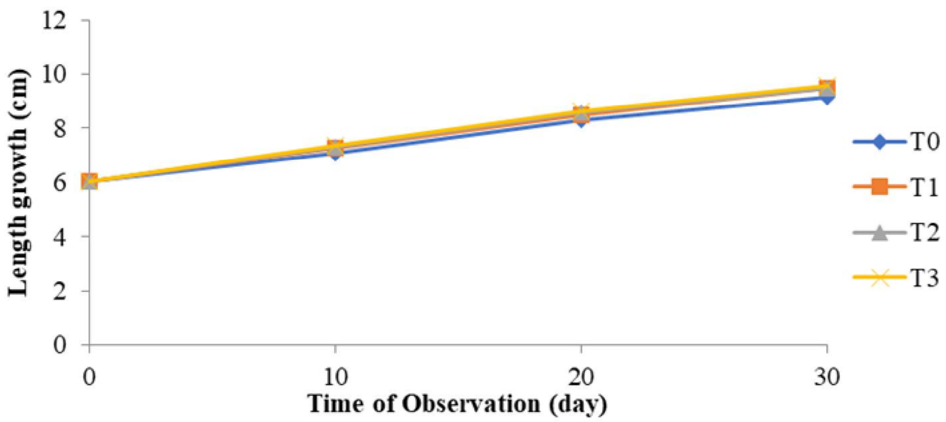

Figure 3. Length growth of tilapia during rearing.

The addition of *Sargassum* sp. extract was able to enhance feed efficiency during the rearing period. Thus, tilapia fish could grow and develop properly. The efficiency level of the feed containing *Sargassum* sp. extract ranged between 61.18 and 64.20. These values are in a good category. According to Giri et al (2016), a good feed efficiency value is ≥ 25%. The higher the feed efficiency value, the more efficient use of feed by fish. Optimal feed utilization will provide a good FCR and will produce energy for growth

The main factors affecting growth are the content of protein, fatty acids, and carbohydrates in the feed. The amount of protein in the feed needs to be at its highest possible level for fish to grow. Fish need protein from various body tissues to maintain more essential body tissues, so if the feed contains insufficient protein, it can decrease body weight. In the meantime, the protein will be catabolized if it is too much or more than needed. A significant amount of energy is required for the catabolism process; if the energy from fat and carbohydrates is insufficient, the energy from protein will be utilized, decreasing the amount of protein available for building the body and, consequently, fish growth (Adelina et al 2014).

Sargassum sp.'s vitamin C content can be used to help fish grow because it helps the body's metabolism. When feed can be properly utilized and digested by the body, vitamin C improves the efficiency of feed utilization and protein ratio (Komalasari et al 2016). Gunawan et al (2014) argued that feed containing vitamin C results in greater protein retention. It is due to an improved metabolic process that results in increased fat retention, including the retention of essential fatty acids and unsaturated fatty acids.

FCR is used to determine the level of feed efficiency in each feed treatment. The addition of *Sargassum* sp. extracts in the feed affects the FCR results in each treatment. The FCR value obtained shows how much feed is consumed to turn into biomass in the fish's body. The lower FCR value indicated that the feed usage was more efficient. FCR showed efficiency in feeding (Sulawesty et al 2014). The lower value means that the body can utilize the feed optimally. Generally, fish with the administration of *Sargassum* sp. resulted in lower FCR (1.56-1.63) compared to T0 (1.88). Therefore, the conversion value is considered good if the amount of feed that can be optimally utilized by fish has a higher digestibility level and the FCR is lower. Hariani & Purnomo (2017) asserted that the effectiveness of feed use demonstrates the worth of feed that fish can utilize by increasing their body weight. Therefore, the higher the value of feed efficiency, the higher the effectiveness of the feed used by fish.

Secondary lamellae that are neatly arranged and normal mucus cells that are visible, epithelium, and lacunae characterize the structure of the gill tissue of tilapia fed with *Sargassum* sp. for 30 days under normal circumstances. According to Wahyuni et al (2020), the epithelium, mucus cells, lacunae, supporting cells, and chloride cells make up a healthy fish's gill structure.

Marine macroalgae *Tetraselmis suecica* mixed into the feed can help increase the immune response and growth of Pacific white shrimp *Litopenaeus vannamei* (Sharawy et al 2020). Feeding red tilapia with 20 g kg^{-1} of *S. dentifolium* can improve growth performance (Abdelrhman et al 2022). It is indicated that *Sargassum* sp. contained minerals, vitamins, high protein, fatty acids, and bioactive compounds such as carotenoid (El-Shenody et al 2019; Bowyer et al 2020; Davies et al 2020; Ferreira et al 2020). The polysaccharides contained in extracts of marine macroalgae have a prebiotic activity that stimulates the growth of bacteria that are beneficial for fish growth, increases feed utilization efficiency, and digestion becomes smooth (Sharawy et al 2020). Hassaan et al (2019) stated that phenolate and polysaccharides in marine macroalgae possess positive effects in triggering fish growth. In addition, polysaccharide also helps nutrition absorption in the digestive system to enhance feed efficiency and reduce FCR value (Sotoudeh & Mardani 2018). Vitamin C content in *Sargassum* sp. can be utilized and digested by the body properly (Komalasari et al 2016).

Conclusions. The observation results reveal that the *Sargassum* sp. extract influenced fish growth. The absolute weight growth of tilapia ranged from 4.84 to 6.18 g, the SGR ranged from 2.19 to 2.60% day^{-1}, and the absolute length ranged from 3.12 to 3.53 cm. The lowest FCR was at a T3 dose of 1.56, and the highest at T0 was 1.88, while the highest feed efficiency was 64.20%, at T3 and the lowest at T0 was 53.29%. Additionally, the tissue structure was still in a normal state during rearing. This shows that the T3 treatment (3.0 g kg^{-1} of feed) was the best in this study. Further research is needed regarding the utilization of *Sargassum* extract as a phytoimmunostimulant for fish cultivators.

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Conflict of interest. The authors declare that there is no conflict of interest.

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Authors:
Mohamad Gazali, Department of Marine Science, Faculty of Fisheries and Marine Science, Teuku Umar University, Kampus Alue Peuyareng Street, 23615 West Aceh, Indonesia, e-mail: mohamadgazali@utu.ac.id
Irwan Effendi, Department of Marine Science, Faculty of Fisheries and Marine, Universitas Riau, Kampus Bina Widya KM. 12.5, Simpang Baru, Pekanbaru, Riau Province 28293, Indonesia, e-mail: helpingirwan@gmail.com
Sri Wahyuni, Department of Marine Science, Faculty of Fisheries and Marine Science, Teuku Umar University, Kampus Alue Peuyareng Street, 23615 West Aceh, Indonesia, e-mail: sri.wahyuni@utu.ac.id
Ronal Kurniawan, Department of Marine Science, Faculty of Fisheries and Marine, Universitas Riau, Kampus Bina Widya KM. 12.5, Pekanbaru City, Riau Province 28293, Indonesia, e-mail: kurniawanronal09@gmail.com
Abdul Matin, Department of Food Processing and Engineering, Faculty of Food Science and Technology Chattogram Veterinary and Animal Sciences University, Khususi, Chattogram 4225, Bangladesh, e-mail: abmatin@cvasu.ac.bd
Rina Syafitri, Department of Agribusiness, Faculty of Agriculture, Teuku Umar University, Kampus Alue Peuyareng Street, 23615 West Aceh, Indonesia, e-mail: rinasyafitri@utu.ac.id

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