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The effect of enzymatic *Sargassum* extract on feed consumption, feed utlitization efficiency, and growth rate of Nile tilapia (*Oreochromis niloticus*)

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Abstract
Tilapia have high resistance to various diseases, tolerance to low and high temperatures, efficiency in feed and fast growth, recently growing in saline water with salinity up to 20 ppt. The addition of *Sargassum* extract into the feed is an alternative to maintain water quality and digestibility of feed in the fish body. *Sargassum* sp, has a fairly complete nutritional content, it can certainly have a growth effect when added to fish feed, according to previous research. The nutritional content of *Sargassum* sp. including crude protein content 5.19%, ash content 36.93%, fat 1.63%. *Sargassum* sp. has a protein content of 7.94%, fat 0.72%, ash content 35.84%, crude fiber 4.93%, and carbohydrates 50.57%. Based on the background, it is necessary to do research on the addition of the enzymatic extract of *Sargassum* sp. such as neutrase and viscozyme into feed on feed conversion and growth performance of tilapia (*Oreochromis niloticus*).
Sargassum collected from Pantai Karapyak, Pangandaran, West Java, Indonesia. Material and methods that used in research ranging from preparation of *Sargassum* extract, preparation of experimental animal, and experimental design. The utilization of *Sargassum* extract were effective to increase Feed Consumption (FC), Feed Utilization Efficiency (FE), and Absolute Growth Performance (AG) with the result of significant difference ($p < 0.05$) in FE and AG results. The highest result in all parameters were occurred in treatment fed by 2 g/Kg enzymatic extract of *Sargassum* (viscozyme).

Keywords: Enzymatic extract, feed consumption, growth performance, nutrition, *Sargassum*, tilapia

Introduction
The development of freshwater aquaculture according to the FAO Fisheries Commission has experienced competition, both in terms of area and water use. Therefore, there is a need for an idea to be able to use marginal land, especially in areas around the coast and apply diversification of business patterns in brackish aquaculture [1].
Tilapia have high resistance to various diseases, tolerance to low and high temperatures, efficiency in feed and fast growth, recently growing in saline water with salinity up to 20 ppt [1]. Based on, the total production of fisheries in 2018 reached 179 million tonnes [2]. The value is estimated at 401 billion USD. From that value, aquaculture sector supplies 82 million tonnes production with 250 billion USD [3]. In 2050, the production of fish as food for human is being expected double.
One of the obstacles in the aquaculture business that many farmers complain about the high price of commercial feed. Feed take an important role as an energy source for growth is the largest component of production costs, namely 40-89% [4]. In general, commercial feed can spend about 60-70% of the total production cost [5]. Therefore it is needed for additional materials to increase growth and utilization efficiency for fish production.
Based on this, it is necessary to do research to improve the value of feed nutrition. The addition of *Sargassum* extract into the feed is an alternative to maintain water quality and digestibility of feed in the fish body. *Sargassum* sp. has a fairly complete nutritional content, it can certainly have a growth effect when added to fish feed, according to previous research [6]. According to [7], the nutritional content of *Sargassum* sp. including crude protein content of 5.19%, ash content of 36.93%, fat 1.63%. *Sargassum* sp. has a protein content of 7.94%, fat

0.72%, ash content 35.84%, crude fiber 4.93%, and carbohydrates 50.57% [8].

The addition of *Sargassum* sp. in Salmon feed provides increased fish weight, better feed conversion ratio, and reduced mortality. In addition, supplementation of *Sargassum* sp. in feed can increase growth and efficiency of feed utilization in catfish fry (*Clarias* sp.)[9].

Several studies have shown that the addition of *Sargassum* sp. into the feed is able to provide additional fish weight (growth) and the efficiency of the use of feed in fish. Added 48% *Sargassum* sp. seaweed flour in the feed of tiger shrimp (*Penaeus monodon*) has a significant effect on growth and survival [10]. Supplementation of *Sargassum* meal replacing rice bran can be added up to 8% substitution for tilapia culture [11]. In addition, Enzymatic extracts from seaweed showed the potential activity in controlling *Pseudomonas aeruginosa* and *E. coli* in biofilm formation, respectively[12]. The application of enzymatic extract in degrading the cell wall of algae has been considered for the past decades and it has been proven to be effective in improving the yield of active compounds and enhancing the bioactivity [13]. Based on the background, it is necessary to do research on the addition of the enzymatic extract of *Sargassum* sp. into feed on feed conversion and growth performance of tilapia (*Oreochromis niloticus*).

Material and Methods

Preparation of *Sargassum* extract

Sargassum collected from Pantai Karapyak, Pangandaran, West Java, Indonesia. The seaweed samples were washed with distilled water and then dried under sunlight. They were ground by using a hammer mill and extracted at the central laboratory of Universitas Padjadjaran, Indonesia. In this study, *Sargassum* was hydrolysed to extract the enzyme materials, water-soluble extracts of carbohydrates (Viscozyme, Celluclast, and Termamyl) and proteases (Protamex, Kojizyme, and Neutrase) were used. Sample solutions were incubated for 50°C at 3 h. All samples were denatured for 15 min in 90 °C for filtration process. Insoluble enzymatic extracts were stored in −20 °C for total phenolic analysis to determine the insoluble phenolic compounds that may not be digested by the enzymes. Viscozyme and Neutrase *Sargassum* enzymatic extracts were prepared in triplicates for experimental diets.

Preparation of Experimental Animal

The fish used was Nile tilapia (*Oreochromis niloticus*) with a size of 10-12 grams obtained from the local fish farming ponds near Bandung, West Java. The feed used was commercial type feed with the brand Hi-Provit for tilapia with protein content of 28-30%, fat 3-5%, fiber 4-6%, ash content 10-13%, water content 11-13%. The fish used were10-12 grams in initial body weight. The number of fish used per treatment tank was 10 fish. Fish were reared for 35 days. Feed is given 4 times a day with a size of 5% biomass. The methods for maintaining fish include: 1) feeding methods: Feed is given in the morning (06.00), noon (12.00), and afternoon (16.00) at libitium.

Experimental Design

This research design used a completely randomized design with 3 treatments and 5 replications. The treatment factor is the difference in the type of main feed in the cultivation of tilapia (*Oreochromis niloticus*) fed by commercial feed coated with and without *Sargassum* enzymatic extract, namely SV1: 0 g/Kg Enzymatic *Sargassum*; SV2: 1 g/Kg of Neutrase *Sargassum* enzymatic extract; SV3: 2 g/Kg of Viscozyme *Sargassum* enzymatic extract.

The commercial feed was supplemented with *Sargassum* extract using the coating method. The enzymatic extract of *Sargassum* (by Neutrase and Viscozyme) was weighed at a concentration of 1 and 2 g/kg feed. While feed as a control without the addition of enzymatic *Sargassum* extract. Each of these ingredients was dissolved separately using 10 ml of distilled water, then homogenized using a magnetic stirrer at 500 rpm. After the three ingredients are homogeneous then put into a 30 ml spray bottle. Sprayed on the feed sequentially, namely *Sargassum* extract first, then vitamin mix and tapioca coating. Each stage of spraying the material on the feed is done drying at room temperature 25 °C until dry. Furthermore, the dry feed is packed in jars and labelled according to the treatment.

Data analysis

Data observed were feed consumption, feed utilization efficiency, and growth rate. The water quality were analyses as a supporting data. As the statistical analyses, all data were subjected to the Duncan test to determine if each parameter was significantly different.

Results and Discussion

Based on the results of the research on the effect of supplementation with the enzymatic extract of *Sargassum polycytum* seaweed on the level of feed consumption (FC), feed utilization efficiency (FE) and absolute growth (G) of tilapia (*Oreochromis niloticus*).

Feed Consumption (FC)

Based on the results of the study, the feed consumption value of tilapia (*Oreochromis niloticus*) reared for 35 days can be presented in Figure 1. On the level of feed consumption.

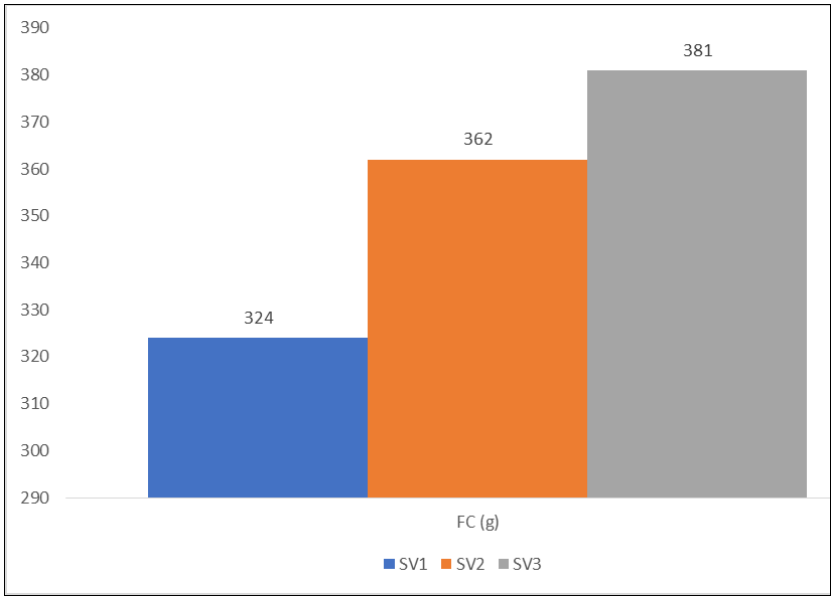

Fig 1: Feed Consumption of Tilapia reared under different feeding of enzymatic *Sargassum* types during 35 days of trial. The SV1 is trial with commercial feed without *Sargassum* enzymatic extract coating; SV2 is trial with commercial feed coated with 1 g/Kg of Neutrase *Sargassum* enzymatic extract; SV3 is trial with commercial feed coated with 2 g/Kg of viscozyme *Sargassum* enzymatic extract.

Based on the results of statistical tests according to the data obtained, the SV1 treatment showed the lowest level of feed consumption, namely 324 g, because the treatment was not given any extract (control treatment). SV2 treatment (1 g/kg feed) showed a feed consumption rate of 362 g. And SV3 (2 g/kg feed) showed the highest feed consumption level of 381 g. SV3 treatment which was supplemented into artificial feed had a significant effect on the consumption level of tilapia (*Oreochromis niloticus*), commercial feed was supplemented with an enzymatic extract of *Sargassum* (viscozyme) 2 g/kg feed.

The results from this study happened because tilapia prefer to consume feed containing *Sargassum* more than other feeds[14] stated that tilapia is an omnivorous type of fish or eats everything, but tends to be herbivores or consumes more plant-based feeds such as the Cholorophyceace, Myxophyceace, and Desmid groups. Stated that feed with 8% *Sargassum* could increase the feed consumption and growth performance [11].

Feed Utilization Efficiency (FE)

Based on the results of analysis of variance ANOVA, data on the efficiency of feed utilization in tilapia (*Oreochromis niloticus*) showed that the treatment of different concentrations of the enzymatic extract of *Sargassum* seaweed in feed on feed utilization efficiency gave a significant value ($P < 0.05$). Based on the FE data, it can be presented in Figure 2.

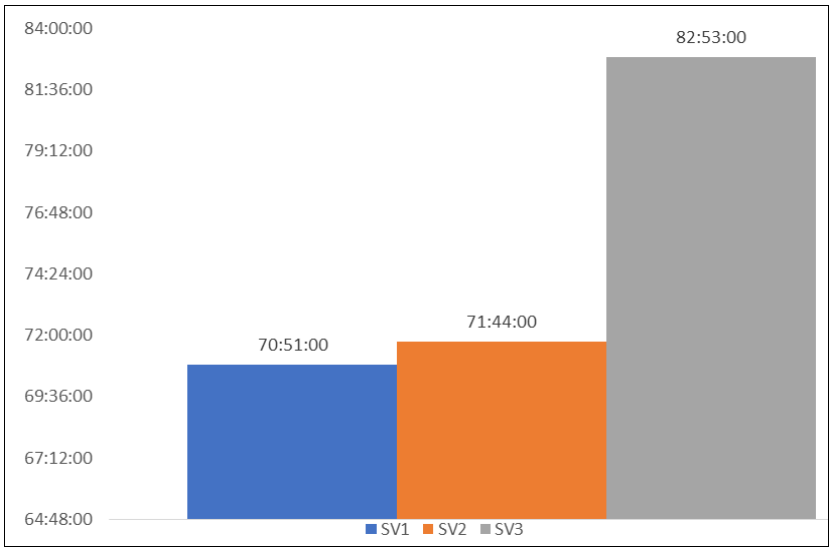

Fig 2: Feed utilization Efficiency (FE) of Tilapia reared under different feeding of enzymatic *Sargassum* types during 35 days of trial. The SV1 is trial with commercial feed without *Sargassum* enzymatic extract coating; SV2 is trial with commercial feed coated with 1 g/Kg of Neutrase *Sargassum* enzymatic extract; SV3 is trial with commercial feed coated with 2 g/Kg of viscozyme *Sargassum* enzymatic extract.

The results of statistical tests according to the data obtained from the research results, treatment SV1 showed the lowest FE value of 70.51%, because the treatment was not given the enzymatic extract of *Sargassum* seaweed (control). SV2 (1 g/kg feed) showed an FE value of 71.44%. SV3 (2 g/kg feed) showed an FE value of 82.53%. During 35 days of maintenance, there were significant difference ($p < 0.05$) with the SV3 treatment showed the highest value, because the SV3

treatment was given the highest extract at 2 g/kg of feed.
The results showed that the SV3 (2 g/kg feed) had a high feed utilization efficiency value of 82.53%, compared to the control SV1 treatment (0 g/kg feed and SV2 (1 g/kg feed). It is suspected that the feed in the SV3 treatment has good quality so that it can be utilized efficiently by tilapia (*Oreochromis niloticus*).This is because the fish not only obtain the nutrients contained in the feed, but also nutrients from *Sargassum* extract so that it helps meet the nutritional needs of the fish especially the role of viscozyme enzymatic extract.
The role of *Sargassum* polycystum immunostimulant from *Sargassum* sp. proven to be able to improve the immune system of tilapia (*Oreochromis niloticus*) and its resistance level to pathogenic bacteria, so that it can produce high survival rates and feed consumption in fish. In addition, it also has a high effect on the utilization of feed that can be utilized optimally by fish for growth. According to previous research [15], the increasing value of feed utilization efficiency indicates that the feed consumed by shrimp has good quality so that it can be utilized efficiently. Another study [16] revealed that *Sargassum* sp. contains growth promoter compounds for fish growth by increasing the efficiency of nutrient absorption from feed.

Absolute Growth Performance (AG)
The results of the study on the absolute growth of tilapia (*Oreochromis niloticus*) during the rearing process after administration of the *Sargassum* seaweed enzymatic extract at different doses for 35 days are presented in Figure 3.

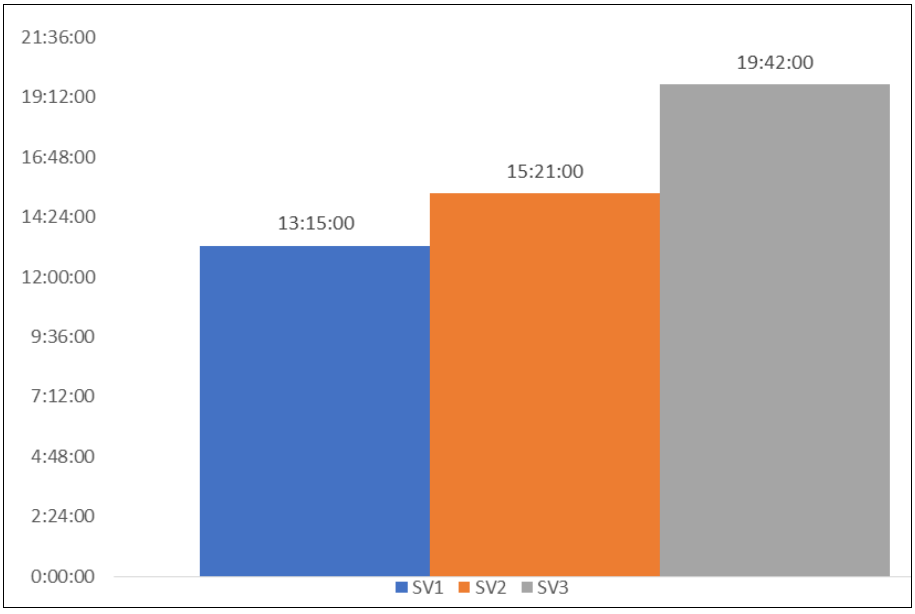

Fig 3: Absolute Growth (G) of tilapia reared under different feeding of enzymatic *Sargassum* types during 35 days of trial. The SV1 is trial with commercial feed without *Sargassum* enzymatic extract coating; SV2 is trial with commercial feed coated with 1 g/Kg of Neutrase *Sargassum* enzymatic extract; SV3 is trial with commercial feed coated with 2 g/Kg of viscozyme *Sargassum* enzymatic extract.

Based on the growth histograms of all treatments for 35 days, different concentrations of the treatment used an enzymatic extract of *Sargassum* seaweed which showed significant growth, namely the SV3 treatment with 2 g/kg extract added. In the histogram, the comparison between treatments of 0.1, 1.2 g/kg of feed was very significant, namely the concentration of the enzymatic extract of *Sargassum* with viscozyme extract of 2 g/kg of feed. The lowest growth was at the extract concentration of 0 g/kg feed (SV1). The regression analysis was observed as shown in table 1.

Table 1: Regression analysis of absolute growth performance of tilapia under different feeding of enzymatic *Sargassum* types during 35 days of trial.

SV1	*Y = 0.49x + 11.95*	*R^2 = 0.98*
SV2	Y = 0.38x + 12.01	R^2 = 0.97
SV3	Y = 0.31x + 9.95	R^2 = 0.98

*The SV1 is trial with commercial feed without *Sargassum* enzymatic extract coating; SV2 is trial with commercial feed coated with 1 g/Kg of Neutrase *Sargassum* enzymatic extract; SV3 is trial with commercial feed coated with 2 g/Kg of viscozyme *Sargassum* enzymatic extract.

The content of feed supplements in feed serves as a complement and fulfilment of micronutrient needs that are important in stimulating fish growth [9]. The micro nutrients contained in *Sargassum* sp. including vitamins A, C and mineral substances such as ash, calcium, iron, and phosphorus [7].
Besides that, the different result of FC, FE, and AG might be caused by the different type of hydrolysis extraction while SV2 were used neutrase extract and SV3 were used viscozyme extract. The choice of the time or temperature of hydrolysis can be directly gave influence to the effectiveness of enzymes. In the previous studies [17, 18, 19, 20], generally, the enzymatic hydrolytic reactions were worked for 12 h to achieve an optimum degree of the hydrolysis. Another study [20] showed the optimum conditions for some commonly used enzymes, for example, the enzymes used between 40 and 60 °C and pH 3.8 and 8 for brown or red seaweeds.

Conclusion
In conclusion, based on the present study, the utilization of *Sargassum* extract were effective to increase Feed Consumption (FC), Feed Utilization Efficiency (FE), and Absolute Growth Performance (AG) with the result of significant difference ($p < 0.05$) in FE and AG results. The highest result in all parameters were occurred in treatment fed by 2 g/Kg enzymatic extract of *Sargassum* (viscozyme). There must be further reseach to follow up which bioactive

compound and which level of enzymatic extract of *Sargassum* will be give the best result on feed and growth performance of tilapia.

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