

**EFFECTS OF DIETARY CAULERPA LENTILLIFERA SUPPLEMENTATION ON GROWTH PERFORMANCE AND SURVIVAL RATE OF MILK FISH, *Chanos chanos* (Forsskål, 1775)**

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**Abstract:** The study aimed to investigate the dietary supplementation effect of sea grape, *Caulerpa lentillifera*, on the growth performance and survival rate of milkfish, *Chanos chanos* (Forsskål, 1775). Two hundred fish weighing in the range of 0.20-0.25 g/ind were randomly distributed into 20 of 25 L tanks. The 0 (control), 10, 20, 30, and 40 g/kg of *Caulerpa lentillifera* powder was supplemented into four formulated feeds and then fed for 49 days. The result exposed that the administration of *Caulerpa lentillifera* powder in the diet provided a significant effect on the absolute weight gain, absolute length gain, specific growth rate, and feed efficiency ( $P < 0,05$ ), but there was no significant effect on survival rate ( $P > 0,05$ ). The optimum specific growth rate, absolute weight, absolute length and feed efficiency were found at treatment C (20 g/kg diet) with the values of  $1.597 \pm 0.137$  %/days,  $4.47 \pm 0.08$  g,  $2.98 \pm 0.77$  cm and  $69.86 \pm 2.72$  %, respectively. Regarding this result, the supplementation of sea grape (*Caulerpa lentillifera*) at a 20 g/kg diet is recommended to obtain the optimum growth of milkfish (*Chanos chanos*).

**Keywords:** sea grape; powder; feed conversion ratio.

**Abstrak:** Penelitian ini bertujuan untuk mengetahui pengaruh suplementasi pakan anggur laut *Caulerpa lentillifera* terhadap performa pertumbuhan dan kelangsungan hidup ikan bandeng, *Chanos chanos* (Forsskål, 1775). Dua ratus ikan sampel berbobot berkisar 0.20-0.25 g/ind didistribusikan secara acak ke dalam 20 tangki dengan volume masing-masing 25 L. Tepung anggur laut dengan dosis 0 (kontrol), 10, 20, 30, dan 40 g/kg ditambahkan ke dalam lima formulasi pakan dan diberi makan sebanyak 5% dari biomassa ikan dengan frekuensi 2 kali sehari selama 49 hari. Hasil penelitian menunjukkan bahwa pemberian tepung *C. lentillifera* dalam ransum memberikan pengaruh nyata terhadap laju pertumbuhan spesifik, bobot mutlak, panjang mutlak dan efisiensi pakan ( $P < 0.05$ ), tetapi tidak berpengaruh nyata terhadap kelangsungan hidup ( $P > 0.05$ ). Laju pertumbuhan spesifik, bobot mutlak, panjang mutlak dan efisiensi pakan yang optimum terdapat pada perlakuan C (diet 20 g/kg) dengan nilai  $1.597 \pm 0.137$  %/hari,  $4.47 \pm 0.08$  g,  $2.98 \pm 0.77$  cm dan  $69.86 \pm 2.72$ %. Berkaitan dengan hasil tersebut, disarankan penambahan suplemen anggur laut (*C. lentillifera*) pada pakan 20 g/kg memberikan pertumbuhan yang optimum bagi ikan bandeng (*Chanos chanos*).

**Kata kunci:** anggur laut; tepung; rasio konversi pakan.

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## Introduction

Allah SWT has blessed Indonesia as one of the countries located on the equator and has the longest coastline in the world. For this blessing, Indonesia has abundant fisheries resources (Muchlisin et al., 2017; Putra et al., 2018a; Rizwan et al., 2017). Fish is a source of animal protein that is very important for humans. Besides fishing, fish consumed by humans also come from aquaculture activity. Milkfish, *Chanos chanos* (Forsskål, 1775) belongs to Southeastern waters (Evendi et al., 2017), and Indonesia is one of the largest fish producers besides China and the Philippines. Small-scale production has also been carried out in several countries such as Thailand, Malaysia, Vietnam, Sri Lanka and several Pacific countries (Vasava, 2018). According to a report, Indonesia was the first country to start milkfish cultivation 700 years ago (Ronquillo, 1975), followed by Taiwan and the Philippines since 300 years ago.

In addition, Aceh province is one of the milkfish-producing provinces in Indonesia (Fadhla, 2019). In general, the dominant districts for cultivating milkfish are found in the east coast of Aceh, such as Banda Aceh City, Sabang, Aceh Besar District, Pidie, Pidie Jaya, Bireuen, Lhokseumawe City, North Aceh, East Aceh, Langsa City and Aceh Tamiang and several districts in the west coast such as Aceh Jaya, Nagan Raya, Southwest Aceh and Simeulue (DKP, 2018). Milkfish cultivators in Aceh generally carry out extensive or traditional cultivation systems. So even though the area of the milkfish culture pond is quite large, it is not followed by optimum production.

A common problem experienced by the fish farmer, including milkfish farmers, is the high price of feed on the market (Muhammadar et al., 2019). In aquaculture, fish feed aspects contribute as much as 60-70% of production costs (Putra et al., 2019a). Fish meal, which is the main raw material for fish pellets, is still imported from fish meal producers like China and South America countries, so the price is strongly influenced by fluctuations in dollar prices. This is a challenge that all parties, including academics, must solve. Therefore, it is necessary to seek alternative feed raw materials that are easy to obtain, affordable, and well digested by fish.

There are many studies on macroalgae as an alternative source of protein. In the world's marine waters, more than 400 macroalgae have been found that contain abundant protein, amino acids and minerals (Saleh, 2020), so they have the potential to be used to increase fish growth. The use of the macroalga *Ulva*

*lactuca* as a feed additive for growth has been given to African catfish, *Clarias gariepinus* and Nile tilapia, *Oreochromis niloticus* L. (Abdel-Warith et al., 2016; Natify et al., 2015), *Ulva fasciata* and *Enteromorpha flexusa* applications on the growth of rabbitfish, *Siganus rivulatus* fry (Abdel Aziz & Ragab, 2017). The use of *Enteromorpha flexusa* for the growth of hybrid red tilapia (*Oreochromis mossambicus* × *Oreochromis niloticus*) juvenile nutrition (Saleh, 2020) and screening of several macroalgae *Ulva rigida*, *Gracilaria* sp.; *Fucus vesiculosus* and *Saccharina latissima* as functional raw material compositions (Neto et al., 2018).

One of the macroalgae found in Aceh waters is the sea grape, *C. lentillifera*. *C. lentillifera* is a simple plant species because it cannot be distinguished between true roots, stems, and leaves (de Gaillande et al., 2017). Sea grapes are edible and have a fairly high nutritional content as vegetable protein, minerals and vitamins. The nutritional content of sea grapes is 0.16-18% crude fiber, 0.08-0.80% crude lipid, 94-96% wet content and 0.43-0.55% crude protein (Nofiani et al., 2018). Recently, the use of sea grapes, *C. lentillifera* in fish feed raw materials has been very limited. Previous research has explored the potential use of the macroalgae *C. lentillifera* as raw material for tilapia and tiger prawns (Putra et al., 2019b; Putri et al., 2017). However, the application of *C. lentillifera* flour as a raw material for milkfish has never been made. Therefore, this study aimed to investigate the use of sea grape, *C. lentillifera* flour in the formulated diet on the growth and survival of milkfish, *Chanos chanos*.

## Materials and Method

This research was carried out at the Brackish Water Seed Center (BBAP) Ujung Batee Street, Aceh Besar District, for 49 days. The complete randomized design was used with five treatments and four replications. The treatment was as follows:

1. Treatment A= Control (no sea grape supplementation)
2. Treatment B = Supplementation of sea grape at 10 g/kg feed
3. Treatment C = Supplementation of sea grape at 20 g/kg feed
4. Treatment D = Supplementation of sea grape at 30 g/kg feed
5. Treatment E = Supplementation of sea grape at 40 g/kg feed

Round plastic containers were used with 25 L volume placed in sequences based on the label numbering. Before the experiment, the containers were sterilized through immersion with 30 mg/L of chlorine for 24 hours to eliminate the bacteria and fungi. The water was then filled into the containers and aerated.

## Preparation of Sea Grape (*Caulerpa lentillifera*) flour

The sea grape, *C. lentillifera*, was dried, refined, and mixed with other feed ingredients. The raw material mixed with other feed ingredients has been adjusted

evenly, then printed according to the treatment. Feeding was carried out in the morning and evening at 08.00 and 17.00 WIB with a feeding rate at 5% of body weight and two times feeding frequency per day. The protein content was adjusted at 30%.

### **Fish preparation**

A two hundred milkfish, *Chanos chanos* fingerling obtained from the Brackish Water Seed Center (BBAP) Ujung Batee, Aceh Besar district. Milkfish fry, *C. chanos* was weighted at 2-3 cm length and 0.20-0.25 g/ind weight. The random distribution of milkfish was spread as much as 10 ind/m<sup>2</sup> at 40 cm water height.

### **Water quality management**

Water quality management was applied to maintain the water conditions to prevent decreasing water quality parameters. The water quality measurements were to determine the changes that occur in the environment during fish rearing. Water quality measurements were carried out once a week. Measured parameters include temperature (°C), pH, and dissolved oxygen. Siphoning of leftover feed was carried out every day in the afternoon to prevent the poor quality of rearing water.

### **Data collection and parameters**

The growth performance measurement of milkfish, *Chanos chanos* was conducted every week from day 0 stocking until day 49. The research parameters were absolute length gain, absolute weight gain, specific growth rate, feed efficiency, and survival rate. The formula is described as follows:

#### **Absolute Length Gain**

Absolute length growth using the formula as follows (Putra et al., 2019c; Silva & Anderson, 1994):

$$L = L_t - L_o \quad \dots\dots\dots(1)$$

- L = absolute length gain (cm),
- L<sub>t</sub> = the average length of the end of the study (cm),
- L<sub>o</sub> = the average length of the beginning of the study (cm)

#### **Absolute Weight Gain**

The absolute weight gain using the formula (Putra et al., 2018a; Werner, 1989):

$$W = W_t - W_o \quad \dots\dots\dots(2)$$

- W = Absolute weight gain (g),
- W<sub>t</sub> = Total fish weight of the test fish at the end of the experiment (g),
- W<sub>o</sub> = Total fish weight of the test fish at the beginning of the experiment (g).

#### **Specific Growth Rate**

Specific growth rate using the formula (Safriani et al., 2019; Werner, 1989):

$$SGR = \frac{(\ln(W_2) - \ln(W_1))}{(t_2 - t_1)} \times 100\%$$

SGR = Specific growth rate (% per day),

W<sub>2</sub> = Biomass of fish at the end of the study (gr),

W<sub>1</sub> = Biomass of fish at the beginning of the study (gr),

t<sub>2</sub> = Final time of the study (days),

t<sub>1</sub> = initial time of the study (days)

### Feed Efficiency

The formula of feed efficiency, according to (Tacon 1987) are:

$$EP = \frac{W_t - W_o}{F} \times 100\%$$

EP: Feed efficiency (%),

W<sub>t</sub>: Total weight of fish at the end of the study (g),

W<sub>o</sub>: Total weight of fish at the beginning of the study (g),

F: Amount of feed consumed during the study (g)

### Survival rate

Survival rate formula using (Putra et al., 2021; Putra, et al., 2019d) :

$$SR = (N_o - N_t) / N_o \times 100\%.$$

SR = Survival rate (%),

N<sub>t</sub> = Number of fish that live after the study (ind),

N<sub>o</sub> = Number of test fish at the beginning of the study (ind)

### Data analysis

The data obtained in this study were analyzed using analysis of variance (one way ANOVA). Duncan's further test would be carried out from the study results if there were a significant.

### Results and Discussion

The experimental results have shown that supplementation of sea grape flour, *Caulerpa lentillifera* to artificial feed with different doses showed a significant effect on the growth performance of milkfish, *C. chanos*. Table 1 shows that the feed supplemented with sea grape flour showed a more significant feed efficiency and survival rate growth than fish not given sea grape flour. In the proportion of sea grape flour supplementation from (B) 10 g/kg to (C) 20 g/kg feed, the growth of milkfish increased significantly. Interestingly, fish growth slowed down when the dose of the sea grape diet increased. According to our previous diet test, the salt content of sea grape flour was quite high, proven that when we performed an organoleptic test on sea grape flour which showed a fairly high level of saltiness. When the salt content is high enough to exceed the fish's tolerance, the fish are not interested in eating and will even stay away from the

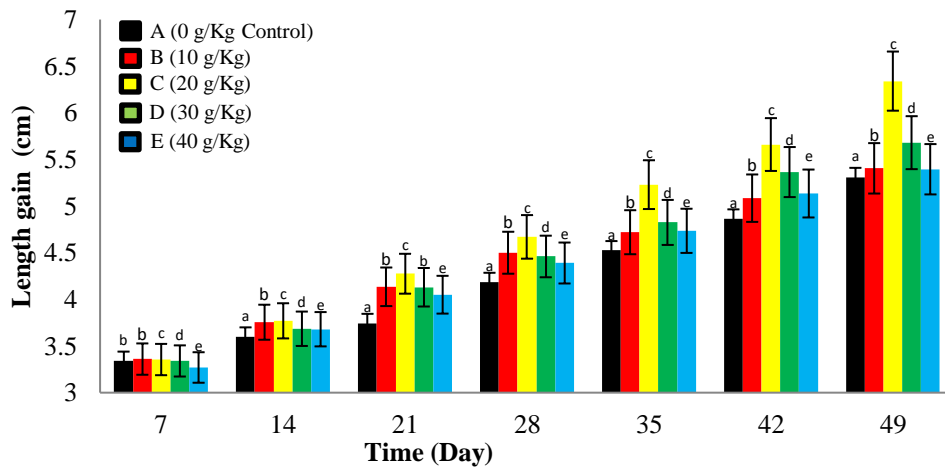
pellet. Therefore this can affect its growth performance. As stated by (Salman 2015), this is in accordance that excessive salt content in the fish feed may negatively impact fish growth and feed efficiency. Moreover, excessive salt consumption in living creatures, like humans, can also have a bad impact. When it enters the human body, excess salt can cause various diseases such as high blood pressure, heart attacks, autoimmune, etc. (Agócs et al., 2020).

**Table 1.** The growth performance and survival rate of milkfish, *Chanos-chanos*.

Treatment	Absolute length gain (cm)	Absolute weight gain (gr)	Specific Growth Rate (%/day)	Feed Efficiency (%)	Survival Rate (%)
A (0 g/Kg Control)	1,97±0,12 <sup>d</sup>	3,18±0,14 <sup>d</sup>	1,278±0,401 <sup>d</sup>	54,15±2,19 <sup>c</sup>	77,50±5,00 <sup>a</sup>
B (10 g/Kg)	2,04±0,05 <sup>cd</sup>	3,45±0,04 <sup>c</sup>	1,356±0,123 <sup>c</sup>	60,51±3,24 <sup>b</sup>	77,50±9,57 <sup>a</sup>
C (20 g/Kg)	2,98±0,77 <sup>a</sup>	4,47±0,08 <sup>a</sup>	1,597±0,137 <sup>a</sup>	69,86±2,72 <sup>a</sup>	90,00±8,16 <sup>a</sup>
D (30 g/Kg)	2,34±0,81 <sup>b</sup>	3,68±0,03 <sup>b</sup>	1,417±0,110 <sup>b</sup>	61,08±1,77 <sup>b</sup>	87,50±9,57 <sup>a</sup>
E (40 g/Kg)	2,12±0,82 <sup>c</sup>	3,39±0,12 <sup>c</sup>	1,341±0,335 <sup>c</sup>	60,81±2,39 <sup>b</sup>	82,50±5,00 <sup>a</sup>

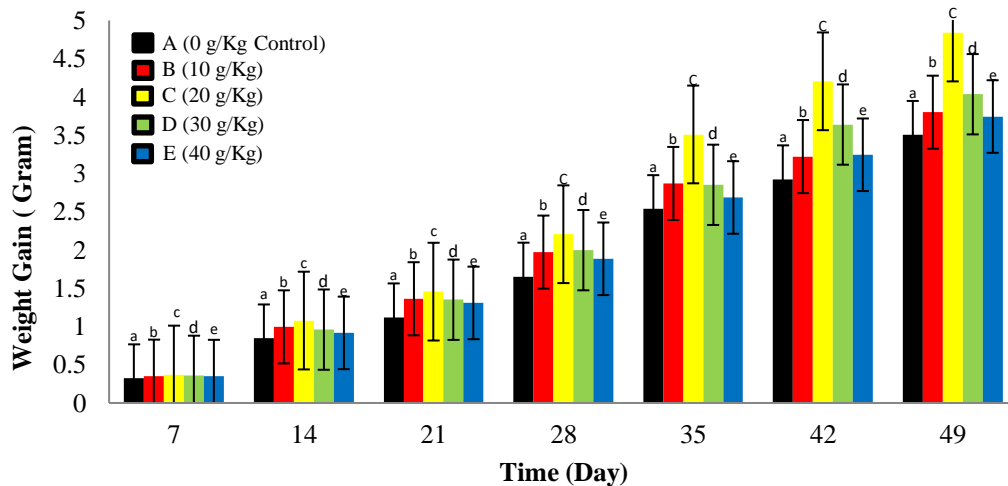
Note: Different *superscript* letters behind the average number in the same column indicate a significant effect (P<0.05 ).

Figures 1 and 2 show that supplementation of sea grape flour, *Caulerpa lentillifera* of 20 g/kg of feed showed a significant increase in length and weight of milkfish. A similar result was also obtained: adding 20% of sea grape to Nile tilapia, *Oreochromis niloticus* showed a significant increase in fish weight (Putri et al., 2017). However, different results were obtained from adding sea grape flour, *Caulerpa lentillifera* to tiger prawns, *Penaeus monodon* to gain optimum growth performance at 30 g/kg supplementation. This shows that supplementation of sea grape flour with a certain dose in the fish or shrimp diet may act as a booster of nutritional content for fish and shrimp. Moreover, a study reported that macroalgae, *Caulerpa lentillifera* has a fairly good range of nutritional content, namely protein 6.6–19.4%, ash 22.2–48.9%, lipid 0.8–7.2%, carbohydrates 11.8–64.0% and moisture 74.7–94.3% (de Gaillande et al., 2017). In addition, macroalgae also have other important nutrients such as amino acids, fatty acids, beneficial polysaccharides, antioxidants, vitamins and minerals (Ismail et al., 2017; Saleh, 2020).



**Figure 1.** The length gain of milkfish fed with different doses of *C. lentillifera* supplementation for 49 days of rearing. Different letter data significantly differ ( $p < 0.05$ ) among treatments.

In addition to the use of macroalgae *Caulerpa lentillifera* on milkfish growth, the application of other types of macroalgae as fish feed supplementation has been widely reported. Sahara et al. (2015) has researched that the addition of *Sargassum* sp. in feed can improve growth performance in catfish fry, *Clarias* sp. Supplementation of *Ulva* sp. flour to the feed of tilapia, *Oreochromis niloticus* is also known to increase the growth of these fish (Harpeni et al., 2015). In addition, the addition of *Kappaphycus alvarezii* flour in feed can improve the growth quality of tilapia, *Oreochromis niloticus* (Tasruddin & Erwin, 2015). A recent study found that *Gracilaria* sp. flour has also been shown to increase the growth performance of fish, Tilapia, *Oreochromis niloticus* (Endraswari et al., 2021). Apart from being a supporter of fish growth, macroalgae have also been shown to have the ability as an immunostimulant. Several microalgae are reported to be immune system stimulants, including *Sargassum* sp, *Gracilaria* sp, carrageenan and *spirulina* sp. (Baleta et al., 2013; Chen et al., 2014; Harpeni et al., 2015; Kitikiew et al., 2013).



**Figure 2.** The weight gain of milkfish fed with different doses of *C. lentillifera* supplementation for 49 days of rearing. Different letter data significantly differ ( $p < 0.05$ ) among treatments.

The highest feed efficiency was obtained at treatment C 20 g/kg feed. This is assumed due to the effect of sea grape which contains an immunostimulant reaction. Therefore it indirectly has a positive impact on the health of the test fish. Increasing fish health will affect the increase in appetite and feed efficiency levels. So that all nutrients, including iron, will be adequately absorbed. The iron in food is carried through the blood and circulated throughout the fish's body tissues. On the other hand, feed efficiency is the percentage of feed consumed by fish and is directly proportional to the increase in fish biomass. This can be defined by increasing feed efficiency, which indicates the quality of feed used by fish (Muhammadar et al., 2012).

Survival is the ability of fish to survive in a certain period. Treatment C (20 g/kg) showed the optimum survival rate among other treatments even statistically not significant. It is estimated that the nutritional content of sea grape *Caulerpa lentillifera* has an important role in immunostimulants. Therefore, it can help fish body reactions to physiological stress, disease prevention and fish growth processes. In addition, other nutritional content of *Caulerpa lentillifera* can have potential abilities such as reducing antioxidative stress (Balasubramaniam et al., 2020), anti-diabetic agents (Nofiani et al., 2018; Sharma & Rhyu, 2014), immunological activity (Zhang et al., 2020), and also the content of sulphated polysaccharide CLGP4 from *C. lentillifera* can function as an anti-inflammatory (Sun et al., 2020). A recent full review reported that sea grape of the genus *Caulerpa* has promising benefits such as antiviral, antimicrobial, cytotoxic, hypolipidemic, anti-obesity, cardiac protective hepatoprotective, and anti-proliferative agents (Rushdi et al., 2020).

Water quality is one of the supporting environmental factors in the milkfish, *C. chanos* rearing. The environment can be divided into biotic and abiotic such as rearing media, fish handling methods, water quality, DO, pH, weather support,



ammonia, fish diseases, fish age and the feed and nutrients content. The results of the measurement of the physico-chemical parameters of water in this study showed that the parameters were still in the normal range or those that could be tolerated by milkfish (*C. chanos*) to grow and develop. The water quality measured were temperatures in the range of 27-30°C, acidity (pH) in the range of 7.3-7.5, dissolved oxygen (DO) in the range of 5-8 mg/L and salinity in the range of 25-35 ppt. According to (Evendi et al., 2017), the water quality above was still within the tolerance range of milkfish, *C. chanos* rearing.

## Conclusion

Based on the results of the study, it can be concluded that supplementation of sea grape flour, *C. lentillifera* significantly affected absolute length gain, absolute weight gain, specific growth rate, and feed efficiency but had no significant effect on milkfish survival rate. Thus, it can be recommended that supplementation of sea grape, *C. lentillifera* flour, as much as 20 g/kg of feed can improve the growth performance of milkfish, *C. chanos*.

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