The effects of seaweed, *Sargassum* sp. meal dosages in the artificial diet on growth, feed intake, feed efficiency, protein efficiency ratio, and nutritional body composition of Rabbitfish, *Siganus guttatus*

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Abstract. *Sargassum* sp. meal is a plant ingredient that has not been used properly, and its potential has not fully realized as an herbivorous fish diet. This experiment aims to determine the potential use of sargassum meal in the grow-out of the rabbitfish diet. Rabbitfish (initial weight of 51.76 ± 0.11 g ind⁻¹) those were cultivated in fifteen floating net cages sized 1×1×1.5 m³ at 20 fish cage⁻¹ stocking density lasted for 90 days. This experiment was designed as Completely Randomized Design with five treatments of sargassum meal dosages in diets, which were 0.0, 7.5, 15.0, 22.5, and 30.0%, with three replications each. The results showed that no significant effect (P>0.05) of different sargassum meal dosages on the specific growth rate and survival rate of rabbitfish. However, feed intake increased with increasing sargassum meal, where the highest was obtained at 22.5% (275.6±9.2 g fish⁻¹) and 30.0% (270.8±8.4 g fish⁻¹) dosages which were significantly higher (P<0.05) than the other dosages. At the same time, sargassum meal dosages were significantly different (P<0.05) on feed efficiency and protein efficiency ratio, where they both seem to decrease with the increase of sargassum meal. The highest feed efficiency was obtained at the dosage of 0.0% (68.6±3.6%) that had no significant effect (P>0.05) with 7.5% (63.7±2.2%) and for protein efficiency ratio was obtained at 0.0% (2.29±0.12) that had no significant effect (P>0.05) with 7.5% (2.20±0.08) and 15.0% (2.12±0.05) dosages. The use of sargassum meal as much as 30.0% caused a lower crude lipid and higher crude protein contents in the nutritional body composition of rabbitfish, and was significantly different (P<0.05) compared to 0.0% dosage. Based on the growth rate of fish, the optimum dosage of sargassum meal in rabbitfish grow-out diet was estimated at 19.46%, but only 7.5% of sargassum meal could be used in the diet based on feed efficiency.

1. Introduction

Rabbitfish (*Siganus guttatus*) sells for an adequately high price and is a favorite in seafood restaurants in South Sulawesi. Therefore, it is very feasible to develop its cultivation both in ponds and floating net cages. Rabbitfish are classified as herbivorous and can consume natural plant food as their main diet in the form of seaweed such as *Glaucaria, Gelidium, Eucheuma, Chaetomorpha, Enteromorpha, Ulva, Sargassum* and others [1, 2].

Fish that are cultured in floating net cages cannot consume natural food in nature, so the fish must be fed sufficiently in both quality and quantity to grow optimally. In intensive fish farming activities,
including the grow-out of fish in floating net cages, the proportion of costs of fish diets can reach 60-75% of the total production costs [3, 4]. This is partly because the price of fish diet continues to rise, while the price of fish from cultivation tends to stagnate. Therefore, efforts should be made to reduce the price of feed. An effort that can be done to reduce the price of feed is to use locally source draw materials that are widely available, and which have not been used optimally.

*Sargassum* is a genus of brown seaweed that grows in Indonesian coastal waters, especially during the rainy season and approaching the dry season. Until recently, the sargassum has not been utilized properly, and therefore its potential as an ingredient in the herbivorous fish diet has not been fully realized. Sargassum has growth-promoting compounds that can increase the absorption efficiency of nutrients in the diet, so that the feed can be used more efficiently [5]. Furthermore, sargassum contains essential amino acids and other growth-promoting substances, which can affect fish growth [6]. Ash and mineral contents in sargassum are very potent and also contains high levels of vitamin C and vitamin A [7]. Dry sargassum meal contains 7.3% protein, 0.7% crude lipid, 37.3% ash, 26.2% crude fibre and nitrogen-free extract (BETN) of 28.5% [8]. Sargassum has high crude fibre and ash contents, while crude protein and crude lipid contents are relatively low. Therefore, it can be used as feed ingredients for herbivorous fish in the form of pellets with the addition of other ingredients.

Based on these, this research was carried out with the aim to determine the potential use of sargassum meal in the grow-out of the rabbitfish diet. The results of this study are expected to be used as an information and reference material on fish feed-making based on local ingredients, especially in coastal areas and islands where terrestrial plant materials are very limited.

### 2. Material and methods

This research was carried out for three months in the Floating Net Cage Research Unit of Research Institute for Coastal Aquaculture and Fisheries Extension, located in Awerange Bay, Barru Regency of South Sulawesi.

#### 2.1 Test diets

Five test diets were made from local ingredients that contained approximately 29.0% crude protein and 4500 kcal/kg gross energy. The diets were prepared with different dosages of sargassum meal which were 0.0%, 7.5%, 15.0%, 22.5%, and 30.0% by reducing the content of rice bran in the test diet. Fresh sargassum was taken from the coastal waters around the cultivation site, washed with freshwater, dried, and grounded using a hammer mill. The dry sargassum meal had a proximate composition of 7.3% crude protein, 0.7% crude lipid, 26.2% crude fibre, 37.3% ash, and 28.5% nitrogen-free extract. The ingredients and proximate analysis of test diets are presented in Table 1.

| Ingredients         | Sargassum meal dosages (%) |
|---------------------|----------------------------|
|                     | 0.0 | 7.5 | 15.0 | 22.5 | 30.0 |
| Fish meal           | 22  | 22  | 22   | 22   | 22   |
| Copra cake meal     | 10  | 10  | 10   | 10   | 10   |
| Soybean meal        | 20  | 20  | 20   | 20   | 20   |
| Corn meal           | 36  | 28.5| 21   | 13.5 | 6    |
| Sargassum meal      | 0   | 7.5 | 15   | 22.5 | 30   |
| Rice bran           | 8   | 8   | 8    | 8    | 8    |
| Fish oil            | 1   | 1   | 1    | 1    | 1    |
| Vitamin premix\(^a\) | 2   | 2   | 2    | 2    | 2    |
| Mineral premix\(^b\)| 1   | 1   | 1    | 1    | 1    |

| Proximate analysis (% dry matter) |  |
|----------------------------------|---|
| Crude protein                    | 29.9 |
| Crude lipid                      | 10.3 |
| Crude fibre                      | 7.1  |

\(^a\)Inositol

\(^b\)Calcium carbonate
Ash 10.4 12.0 13.5 15.0 16.9
Nitrogen-free extract 42.3 39.7 38.6 36.4 32.4
Gross energy (kcal/kg)* 4690 4591 4479 4408 4324

*a Vitamin mix (in 1 kg of diet): Vit. A 60,000 IU; Vit.D 20,000 IU; Vit.K 24 mg; Vit. E 150 mg; Vit B1 60 mg; Vit B2 90 mg; Vit B6 60 mg; Vit B12 60 mg; Vit C 160 mg; Calcium D-Pentathenate 80 mg; Folic acid 30 mg, Biotin 50 mg, Nicotinamide 400 mg, Cholin chloride 300 mg.

*b Mineral mix (in 1 kg of diet): Calcium 325 mg; Phosphor 100 mg; Iron 60 mg; Manganese 40 mg; Zinc 73.5; Copper 3 mg; Sodium 1 mg; Cobalt 1 mg; Iodine 0.75 mg; Potassium 0.035 mg.

2.2 Feeding experiment

Test fish used were rabbitfish with an initial weight of 51.8 g fish⁻¹. The selected fish were distributed to 15 units of 1 x 1 x 1.5 m³ floating net cages at a stocking density of 20 fish cage⁻¹ and assigned randomly to five treatments in accordance with a 5 × 3 completely randomized design (CRD). The test fish were fed with test diets three times a day in the morning, noon, and afternoon to apparent satiation. Feeding was stopped as soon as the fish had been satiated to avoid wasting feed. Growth monitoring and change of nets were done every 30 days.

2.3 Sample collection and chemical analysis

At the end of the feeding experiment, three fish from each cage were euthanized, dried, and blended and stored at −20°C to be analyzed for nutritional body composition. Proximate analysis (moisture, crude protein, crude lipid, and ash content) was carried out according to AOAC methods [10]. Briefly, moisture was analyzed after drying the samples at 105°C for 16 hours using an oven (Memmert, Germany). Crude protein was determined according to the micro-Kjeldahl procedure, and crude lipid was extracted using chloroform and methanol. Ash was analyzed using a muffle furnace at 550°C (Barnstead, Thermolyne, USA).

2.4 Calculation and statistical analysis

The following equations were used in calculating each variable evaluated:

Specific growth rate (SGR, % day⁻¹) = 100 × [(In final weight – In initial weight)/days of the experiment] [11].

Feed intake (FI, g fish⁻¹) = (total amount of dry feed consumed) / (fish numbers × days) [12].

Feed efficiency (FE, %) = (wet body weight gain) / (total dry feed consumed) [12].

Protein efficiency ratio (PER) = (body weight gain)/(total feed consumed × protein content in diets) [12].

Survival rate (SR, %) = 100 × (final amount of fish / initial amount of fish).

Variables of specific growth rate, feed intake, feed efficiency, protein efficiency ratio, survival rate, and nutritional body composition of fish were analyzed using a one-way analysis of variance (ANOVA). Significant differences (P<0.05) were assessed using Tukey’s HSD (honestly significant difference) test. All the statistical analyses were done using software SPSS version 21.0. In addition,
the estimated optimum dosage of sargassum meal in the diet for maximum fish growth rate was determined by a quadratic regression using CurveExpert 1.4.

3. Results and discussion

Growth performance and test diet utilization of rabbitfish after 90 days of cultivation are presented in Table 2. The final weight and specific growth rate of rabbitfish after given test diets showed no significant effect (P>0.05) between treatments. This shows that the use of sargassum meal of up to 30.0% was still able to provide a fish growth rate that was relatively the same as the control diet that did not contain sargassum meal. However, the regression analysis of growth rate of fish and sargassum meal dosages showed that the equation of \( Y = -0.0022x^2 + 0.0870x + 0.8042 \) \( (R^2 = 0.603) \) resulted in the maximum specific growth rate of 1.65%/day at the optimum dosage of sargassum meal of 19.5% (Figure 1). The highest specific growth rate at 19.5% thought to be caused by the nutrient contents of sargassum could affect fish growth and could be well consumed by rabbitfish. This seaweed contains essential amino acids and other growth-promoting substances [6]. The use of sargassum meal gave good essential amino acid ratio, fatty acid, and other compounds that were needed for fish growth, including antioxidants, minerals, and vitamins [13-17]. Immunostimulant components in sargassum can also increase the health condition and growth of fish [18]. While the lowest specific growth rate on the use of sargassum meal was 30.0%, which may be caused by high crude fibre and ash contents and unable to be well digested by rabbitfish. Sargassum meal contains high crude fibre and ash, and in 30.0% dosage of sargassum meal contained 13.3% of crude fibre and 16.9% of ash, so that the negative effect of these two high nutrients outweighed the positive effect of several important nutrients in sargassum meal on fish growth. The results of other researches on the optimum dosage of sargassum meal in several fish species diets include 10% in Litopenaeus vannamei diet [19], 3% in Clarias sp. diet [20], 7.5% in Oncorhynchus mykiss diet [17], and 3% in Cyprinus carpio diet [21]. The difference in the optimum dosage of the use of sargassum meal in the diet was due to differences in species, fish size, and diet formulations used.

![](image1.png)

Figure 1. The relationship between Sargassum sp. meal dosages in test diet and growth rate of rabbitfish. The peak of the curve showed the maximum value of SGR (1.65% a day\(^{-1}\)) at the optimum dosage of sargassum meal (19.46%) in the test diet.

The use of sargassum meal with different dosages in the diet was significantly different (P<0.05) with feed intake, feed efficiency, and protein efficiency ratio. The increase of sargassum meal dosages up to a certain extent (22.5%) also increased feed intake, but on the other hand, it decreased feed efficiency and protein efficiency ratio. The use of sargassum meal at 0.0% to 15.0% dosages gave no significant effect (P>0.05) on feed intake of rabbitfish but were significantly different (P<0.05) with 22.5% and 30.0% dosages. While the highest feed efficiency was obtained at the dosage of 0.0% that
had no significant effect (P>0.05) with a 7.5% dosage but was significantly higher than 15.0%, 22.5%, and 30.0% dosages. Therefore, the highest protein efficiency ratio obtained at 0.0% had no significant effect (P>0.05) with 7.5% and 15.0% dosages but was significantly higher than 22.5% and 30.0% dosages.

**Table 2. Growth performance of rabbitfish and diet utilization.**

| Variables               | 0.0      | 7.5      | 15.0     | 22.5     | 30.0     |
|-------------------------|----------|----------|----------|----------|----------|
| Initial weight (g)      | 51.9±1.5 | 51.8±1.3 | 51.6±0.7 | 51.8±0.4 | 51.7±0.3 |
| Final weight (g)        | 204.4±13.9a | 206.5±7.2a | 200.5±2.7a | 210.6±10.6a | 191.8±7.8a |
| Specific growth rate (% day⁻¹) | 1.52±0.11a | 1.54±0.04a | 1.51±0.03b | 1.56±0.06a | 1.46±0.05a |
| Feed intake (g fish⁻¹) | 222.0±12.3a | 242.8±10.6a | 242.9±4.9a | 275.6±9.2b | 270.8±8.4b |
| Feed efficiency (%)     | 68.3±3.6d | 63.7±2.2ed | 61.3±1.4bc | 57.4±2.0bd | 51.7±1.5e |
| Protein efficiency ratio| 2.29±0.12e | 2.20±0.08bc | 2.12±0.05bc | 2.02±0.07bd | 1.82±0.05a |
| Surviva rate (%)        | 100      | 100      | 100      | 100      | 100      |

Value in each row with different superscripts have significant differences (P<0.05).

Fish consumes feed to meet its energy needs for living. As an organism that lives in water, the feeding process of fish is very complex because it is influenced by many internal and external factors that are interrelated [22]. Therefore, when the feed was given to fish on the satiation method in a normal environment, the feed intake of fish highly depends on chemical-nutritional factors and physical characteristics of feed, which were basically affected by the characteristics of its composition ingredients [23]. This research showed a correlation between an increase in the feed intake of rabbitfish with the increase of sargassum meal dosages. However, on 90 days, observation of rabbitfish showed that the use of sargassum meal at 22.5% dosage gave the highest feed intake, whereas it had no significant effect at 30.0% dosage, which descriptively decreased. This can be an early warning (if attention was only paid to the rate of fish growth) that the use of sargassum meal in the rabbitfish diet should be maximum dosage of 22.5% and optimized at 19.5% dosage.

The different feed intake between treatments was affected by the nutrients and digestibility value of each test diet. Trout, which were fed fishmeal diets had lower feed intake and higher feed efficiency compared with trout on plant-based diets [24]. This was due to the dietary ingredients in the fishmeal diet had higher digestibility as well as some nutrients being more bio-available to the trout. The findings of that report were in line with the results of this research, where the increased dosages of sargassum meal slightly decreased crude protein, crude lipid, NFE, and energy total of test diets and conversely increased feed crude fibre and ash (Table 1). This was due to the high crude fibre and ash contents of sargassum meal. Although rabbitfish are classified as herbivorous and can consume plant-ingredients, they also have limitations in digesting crude fibre because the activity of cellulase enzymes in fish is very limited [22, 23], as well as the report of milkfish [27]. Likewise, ash in the diet does not contain energy, though the ash component contains micro and macro minerals that are also needed by fish for their metabolic processes. High crude fibre and ash in diet ingredients will affect and reduce the digestibility of diet energy [28-30]. Therefore, fish that were given higher dosages of sargassum meal could consume more diet than those given lower dosages to fulfill their daily energy needs.

The increase of feed intake of fish that were fed with higher dosages of sargassum meal was not followed by a significant increase in body weight, causing feed efficiency and protein efficiency ratio to decrease by increasing sargassum meal dosages. It showed that consumed feed and protein were not used maximally for fish growth. Protein is a nutrient component of feed that plays a role in fish growth [31]. The cause of the decreased of feed efficiency and protein efficiency with increased sargassum meal was thought to be due to decreased digestibility of nutrient value, especially protein and amino
acids in test diets due to the crude fibre content being too high. High crude fibre reduces the nutrient digestibility value, including the digestibility of protein, lipid, and carbohydrate [32]. However, this research found relatively the same protein efficiency ratio at 0.0% - 15.0% dosages of sargassum meal in the diet. This indicated that the use of sargassum meal up to 15.0% could still be tolerated by rabbitfish in using protein diet to grow. While the use of sargassum meal at 22.5% dosage and above had significantly reduced the feed efficiency and protein efficiency in the test feed. This may be due to the amount of crude fibre content in the test diet significantly reducing the digestibility value of other nutrients, including protein. That excessive amount of crude fibre in diet can reduce the digestibility of protein, thereby reducing the protein efficiency ratio of fish [33]. Rabbitfish being herbivorous means they can consume relatively more carbohydrates as a source of energy than carnivorous and omnivorous fish, and thus needs lower crude protein in the diet to keep a normal growth. However, rabbitfish can well consume high protein diet of around 32 – 34% without reducing their growth rate [34]. While rabbitfish (Siganus guttatus) juvenile needs crude proteins in the diet as much as 35% to grow optimally [35]. However, in this research, a lower protein of 29.0% was used due to the fish size being larger.

Table 3. Nutritional body composition (% dry matter) of rabbitfish fed the test diet for 90 days.

| Variables       | Initial | 0.0     | 7.5     | 15.0    | 22.5    | 30.0    |
|-----------------|---------|---------|---------|---------|---------|---------|
| Crude protein   | 64.0±0.7| 60.0±1.6a| 60.6±0.7ab| 59.2±3.1ab| 61.5±0.4ab| 63.3±1.1ab|
| Crude lipid     | 15.0±0.1| 26.1±0.7b| 24.3±1.3b| 24.8±1.8b| 23.8±1.0b| 21.2±0.8a|
| Crude fibre     | 3.5±0.9 | 1.5±0.1a | 3.7±1.6a | 3.1±1.6a | 3.2±1.6a | 2.6±0.7a |
| Ash             | 17.1±0.7| 12.3±0.1a| 10.8±2.3a| 12.4±1.1a| 11.0±1.8a| 12.2±0.9a|

Value in each row with different superscripts have significant differences (P<0.05)

The nutritional body composition of rabbitfish after 90 days of cultivation is presented in Table 3. Crude protein of the fish body tended to decrease, while crude lipid increased at the end for all the treatments. As a result, the fish at the end of the study were found to be fatter than at the beginning of the study. A fairly high increase of lipid for all treatments indicated that all of the test diets contained enough energy content for the living and growth of rabbitfish, hence the lipid synthesis process occurring in the fish body.

The use of sargassum meal for each treatment was significantly different (P<0.05) to the nutritional body composition of rabbitfish, especially the crude protein and crude lipid variables, while there was no significant effect (P>0.05) to crude fibre and ash contents. Crude protein of the fish body tended to increase, while crude lipid decreased with the increase of sargassum meal in diet. Fish that were given a diet with 30.0% of sargassum meal had the highest crude protein and lowest crude lipid. This was due to the low amount of energy digested as a result of high crude fibre and ash contents. The availability of low digestible energy in fish causes lipid deposit in the body to be low, and conversely, the availability of high digestible energy will cause lipid deposit in the body to increase [36]. This is due to the excess digestible energy consumed becoming accumulated lipid deposit in the fish body [37]. Furthermore, it is said that high excessive energy consumption results in a higher lipid deposit in the fish body. The increase of lipid in the fish body with the decrease amount of sargassum meal could cause protein in the fish body to decrease.

4. Conclusions
The use of sargassum meal of up to 30.0% in diet gave no effect on the growth rate, but the optimum dosage of sargassum meal for rabbitfish grow-out was estimated at 19.46%. However, a higher dosage of sargassum meal tends to decrease the feed efficiency and protein efficiency ratio, so that if it was based on feed efficiency, sargassum meal that could be used in the grow-out of rabbitfish diet is 7.5%. The use of sargassum meal as much as 30.0% in diet caused a lower lipid and higher protein contents in the nutritional body composition of rabbitfish.
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