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Utilization of Sargassum Flour Fermented with *Bacillus aerius* Bacteria in *Siganus guttatus* Rabbitfish Enlargement Feed

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Abstract— **This Sargassum is a staple food of rabbitfish in nature; although its nutritional quality is relatively low if it is used as one of the feed ingredients first carried out in the fermentation process, the results of this fermentation are used as one of the raw feed materials. The study was conducted to look at the use of fermented sargassum flour in feed at the lowest dose of 7.5% to the highest dose of 30%. This study was designed using a completely randomized design (RAL). Using 15 units of floating net cage containers. The study duration was 120 days. During the study, the test fish were fed thrice daily, namely 08.00 am, 1:00 pm, and 03:00 pm. Feeding is carried out. To determine the severity of the treatment of the biological response of test fish, sampling is carried out every 30 days. From several parameters observed, such as weight gain, specific growth rate, and daily feed consumption rate, although statistically not different (P>0.05), the tendency to increase with the increase in sargassum dose by 30%. Similarly, the total digestibility of feed, digestibility of feed protein, and digestibility of feed energy statistically do not show a difference (P>0.05) but also the tendency to increase. Based on the results of this activity, it was concluded that sargassum flour that has been fermented with *bacillus aerius* bacteria can be used well by rabbitfish, with as much as 30% in the feed.**

Keywords—***Bacillus aerius*; fermentation; sargassum; feed; rabbitfish; growth out.**

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I. INTRODUCTION

Rabbitfish, scientifically called *Siganidae* [1], encompasses a group of marine fish often recognized as spinefoot fish or *siganids*. These organisms are frequently encountered in the tropical and subtropical regions of the Indian and Pacific Oceans and the Red Sea [2]. The fish in question are commonly referred to as "*rabbitfish*" because of the distinctive feature of their lips, which resemble rabbits' oral structure. Rabbitfish are often characterized by their diminutive to moderate dimensions, featuring elongated anatomical structures and a compacted bodily form. The species under consideration possesses a discernible spinal structure on the anterior portion of their dorsal fin, thereby earning them the colloquial designation of "*spine foot*." The color of this species exhibits considerable variation [3], [4], encompassing a diverse spectrum of hues such as yellow, orange, brown, and green, frequently accompanied by elaborate patterns. Rabbitfish predominantly exhibit herbivorous eating behavior, consuming algae, seagrasses, and other plant materials. These organisms employ their distinct dental structures to engage in grazing activities on marine vegetation. These fish are recognized for their diurnal eating patterns during the daytime. The behavior of foraging for food in coral reefs and rocky areas is frequently observed in this species. Rabbitfish typically exhibit a placid demeanor and are not aggressive towards other fish [3], [4]. However, it is noteworthy that they may exhibit territorial behavior, particularly during the breeding season. Rabbitfish have acute, poisonous spines on their dorsal, anal, and pelvic fins [5]. The aforementioned spines serve as a means of protection and can potentially induce discomforting stings when touched or inadvertently encountered. It is imperative to exercise caution when handling them.

Certain kinds of rabbitfish are highly sought after in the aquarium industry owing to their distinct physical characteristics and tranquil temperament. Nonetheless, the presence of venomous spines and their particular feeding requirements necessitate the provision of a meticulously kept aquarium and suitable care. Rabbitfish hold economic significance in certain geographical areas because they are a primary food source for indigenous populations [6]–[8]. Additionally, these organisms have a crucial role as herbivores within coral reef ecosystems, contributing to regulating algal proliferation and preserving reef vitality. Rabbitfish exhibit oviparity, a reproductive strategy characterized by the laying of eggs [9]. In general, it is observed that these organisms tend to reproduce in pairs or small clusters, wherein the male and female individuals release their respective gametes (eggs and sperm) into the surrounding water. The eggs exhibit buoyancy and passively traverse the currents until they undergo hatching, transitioning into larvae. Rabbitfish exhibit a notable level of diversity and intrigue as a collective of marine fish, showcasing a range of species that have successfully adapted to distinct reef settings. Herbivorous feeding habits make them essential contributors to preserving marine ecosystems, rendering them a focal point for researchers investigating coral reefs and marine biodiversity.

Rabbitfish (*Siganus guttatus*) is one type of *Siganus* group with several advantages, such as its size reaching 500 g/head [10]. Rabbitfish can live in crowded areas. The growth rate is quite high compared to other types [9]. So, it has great potential to develop its cultivation activities. This fish is herbivorous (low food chain), so it can utilize components of vegetable materials that are high enough in feed to grow optimally [11], [12]. In nature, rabbitfish consume many aquatic plants, such as *Enhalus sp*., *Padina sp*., *Gelidium*, *Sargassum sp*., *Chaetomorpha sp*., *Enteromorpha sp*., *Cladophoropsis sp*., *Helimeda sp*., *Caulerpa sp*., *Eucheuma sp., Gracilaria sp*., Ulva sp., and others [13]–[16]. Rabbitfish are very responsive to artificial feed because it provides better growth than seaweed feed. Rabbitfish can also be cultivated in various containers such as ponds, Floating Net Cages (KJA), and concrete tubs [10], [17], [18].

In the intensive development of fish farming activities, one of the main problems often faced is the high feed price. Feed contributes to total production costs in intensive aquaculture activities, including fish farming activities in floating net cages. In addition, there is no specific commercial feed for rabbitfish as herbivorous fish. Therefore, it is necessary to develop feed for fish enlargement, which is expected to be relatively cheap and high-quality to ensure optimal fish growth. One way to get cheap feed is to utilize potential local raw materials that are abundant and have not been optimally utilized.

Sargassum is an aquatic plant (seaweed) classified as red algae often consumed by rabbitfish in nature. Its availability is quite abundant and has not been utilized optimally because its quality is quite low, as reported [12], [19], [20] that sargassum has a crude protein content of 5-8.3%, fat 1.5%, ash 19.30-40.93%, crude fiber 20-28.07%. These results show that sargassum contains crude fiber that is high enough to reduce the digestibility of feed. Even though it contains other active ingredients such as anti-oxidant, anti-bacterial, antiviral, vitamins A, B, C, D, E, and others [21], it is necessary to make efforts to improve the quality of nutrition (increase protein and reduce crude fiber) through the use of bacteria.

Several researchers have improved the nutritional content of feed raw materials through bioprocessing. [22] reported that one of the efforts to improve the quality of copra meal feeds raw materials is through bioprocessing (fermentation) using microbial types such as *Rysophus, Aspergillus niger, and Trichoderma*. Bacteria from bovine rumen fluid can decrease the crude fiber of rice straw flour from 45.15% to 25%, with an initial protein increase from 3.5% to 10.15% [23]. While [24] reported that the use of commercial bacteria in straw fermentation showed a significant decrease in crude fiber (34.13% before fermentation to 22.52% after fermentation) as well as an increase in protein (3.12% to 14.35%) and extract materials without nitrogen (12.68% to 28.54%). While some research results [25], [26] also reported that one of the efforts to optimize the potential contained in sargassum is to utilize a type of microbe/fungus, namely *Aspergillus niger,* as a microbe in the fermentation process by producing enzymes to degrade complex molecules that can be converted into simpler molecules so that they are easier to be utilized by organisms [12]. We have found a type of B*acillus aerius* bacteria that is used as a probiotic in the fermentation process of sargassum flour by successfully increasing protein, increasing BETN, and significantly reducing crude fiber.

Based on the results of this study, sargassum flour from fermentation will be tested for the level of utilization in rabbitfish enlargement feed to support the development of independent feed based on local raw materials so that lower feed prices are obtained.

II. Materials and Method

A. Materials

This research was carried out at the Brackish Water Aquaculture Research Center and Maros Fisheries Extension's floating net cage installation in Awerange Bay, Barru Regency, South Sulawesi. The test animals to be used in this study are juvenile rabbitfish with an average initial weight of 25g/head obtained from the results of fish hatcheries Windu Shrimp Hatchery Installation (IPU Barru), Maros Aquaculture Research Institute for Brackish Water Aquaculture and Fisheries Extension. The study used a Complete Randomized Design (RAL) design with five treatments, the treatment: feed without the addition of 0% fermented sargassum flour A; B with 7.5% addition; C with the addition of 15.5%; D with 22.5% addition and D with 30% addition. Each treatment is repeated silently three times, so a container of 15 pieces is needed. The containers used during the study were floating net cages measuring 1 x 1 x 1.5 m3, as many as 15 cages, each cage containing 20 test animals, so the total number of test animals was 300 heads.

This activity lasted for 120 days; during the study, fish were given treated feed in the form of dry pellets formulated by themselves (Tabe 1). The test feed to be used is pellets that have a protein content of about 30%, fat of about 8%, and total energy of about 19.32 Megajoules/kg of feed [12]. One of the main ingredients to be used is sargassum flour, which has been fermented (bioprocessed) with *Bacillus Aerius bacteria in* different doses in enlargement feed.

During the study, feeding to fish was carried out three times a day, namely at 08.00 am, 1:00 pm, and 3:00 pm. To see the treatment response to the biological performance of test fish, growth sampling was carried out every 30 days. The net change was carried out every time sampling to maintain the research container's cleanliness. This study was carried out in Awerange Bay, Barru Regency.

TABLE I
TREATMENT FEED FORMULATION USING FERMENTED SARGASSUM FLOUR USING *BACILLUS AERIUS* BACTERIA AT DIFFERENT DOSES (% DRY MATTER)

Raw Materials	**Dosage of sargassum flour in feed**				
	0	**7.5**	**15**	**22.5**	**30**
Fishmeal	22	22	22	22	22
Fermented copra meal	10	10	10	10	10
Soybean flour	20	20	20	20	20
Cornstarch	36	28.5	21	13.5	6
Fine bran	8	8	8	8	8
Fermented sargassum flour	0	7.5	15	22.5	30
Fish oil	1	1	1	1	1
Vitamin mix	2	2	2	2	2
Mineral mix	1	1	1	1	1
	100	**100**	**100**	**100**	**100**
Crude protein (%)	30.1	30.1	30.2	30.2	30.2
Crude lipid (%)	9.53	9.27	9.01	8.74	8.48
NFE	41.99	39.73	37.46	35.20	32.93
Abu (%)	9.91	11.82	13.72	15.63	17.53
Crude fiber (%)	6.47	7.06	7.66	8.25	8.84

Notes:

- Vitamin premix (in 1 kg of diet): vit. A 90.000 IU; vit. D3 30.000 IU; vit. K3 36 mg; vit. E 225 mg; vit. B1 90 mg; vit. B2 135 mg; vit. B6 90 mg; vit. B12 90 mg; vit. C 240 mg; calcium D-pantothenate 120 mg; folic acid 45 mg, biotin 300 mg, inositol 375 mg, nicotinamide 600 mg, choline chloride 450 mg;
- Mineral premix (in 1 kg of diet)): FeCl3 .4H2O 1660 mg; ZnSO4 100 mg; MnSO4 67,5 mg; CuSO4 20 mg; KI 1,5 mg, CoSO4 .7H2O 1,0 mg; Ca(H2PO4) 11.000 mg; MgSO4 .7H2O 13.000 mg; K2HPO4 .8.000 mg;
- BETN (NFE (Nitrogen free extract).

Feed digestibility was observed using an experimental container of 15 conical fiberglass tanks with a volume of 200 L and filled with 25-30 ppt salinity water, as much as 150 L, with a running water system and equipped with aeration. Using test animals, rabbitfish measuring 25 -30 g / head are stocked with a density of 20 heads/tank. The test feed added chromium oxide (Cr_2O_3) as an indicator of 0.70% [27]. Fish are satiated two times a day at 08.00 and 12.00. About 30 minutes after feeding, water is removed to remove the remaining feed by opening the tap. Test feed adaptation is carried out for seven days to obtain feces from the treated feed, not other feed types. The next day, one hour after feeding, the disposal of feed residues is carried out by opening the bottom of the container, and 2 hours after disposal of feed residues, feces collection is carried out for 1 hour to prevent the leaching of nutrients in the feces. The collected feces are immediately stored in the freezer and then dried to enough for dry proximate analysis (25 g).

Proximate analysis of feed and fish was carried out based on AOAC (1999); the dry matter content (DM) of the sample was obtained by drying it in the oven at 105°C for 16 hours, crude protein was analyzed with micro-Kjeldahl, and fat was determined gravimetrically by chloroform-methanol extraction. Coarse fibers were analyzed by heating with alternating leaching of acids and bases and ash by burning in a kiln at 550°C for 24 hours.

B. Method

1) Observed Modifiers of Growth Response and Utilization of Test Feed: The growth response of rabbitfish with the use of fermented sargassum flour using bacillus aerius bacteria in the test feed will be evaluated based on several parameters such as:

- Specific growth rate (SGR) of fish with the following formula [28]:

$$SGR(\%/day) = 100x\frac{(LnWe - LnWs)}{D} \quad (1)$$

where ln is the natural logarithm, We and Ws are the fish weights at the end and beginning of the study, and d is the number of maintenance days.

- The daily feed consumption rate (FMU) is calculated based on the formula [29]:

$$\text{FMU (\%)} = \frac{Total\ feed\ consumption\ (g)\ x\ 100}{\text{(final weight+starting weight)/2x maintenance duration (days)}} \quad (2)$$

- Feed efficiency = increase in biomass weight (wet weight)/feed consumption weight (dry weight) (Taheu).
- Protein efficiency ratio, PER = Fish weight gain (g)/Amount of protein consumed (g) [29]:
- Fish survival, SR (%) = (Number of final fish of the study/Number of initial fish of the study) ×100%.
- The digestibility coefficients (AD) of dry weight (DM), crude protein (CP), fat (L), and energy (E) are calculated based on the formula [27] follows:

$$\text{AD (\%)} = 100 * \left\{1 - \left(\frac{MD * \text{AF}}{MF * \text{AD}}\right)\right\} \quad (3)$$

wherein MD and MF, respectively, the concentration of the indicator Cr_2O_3 (% dry weight) in feed and feces. AD and AF are the concentration of nutrients (% dry weight) in feed and feces.

- Proximate analysis of fish at the beginning and end of the study

2) Data Analysis: Variables in the form of the specific growth rate of fish, daily feed consumption rate, feed efficiency, protein efficiency ratio, body chemical composition, and fish survival will be analyzed variously. Growth performance data and feed utilization were tested by variety analysis and the Smallest Real Difference test [30].

III. RESULTS AND DISCUSSION

A. Results

The results obtained in the study tested the utilization rate of sargassum flour fermented with Bacillus aerius bacteria in rabbitfish enlargement feed, as seen in Table II. Based on the parameters observed in Table 2, it can be seen that the highest final weight obtained at 30% treatment is 129.8±3.7g / head, with a weight increase of about 105 g or an increase of 423.3%, although based on variety analysis is no different (P>0.05) with all test feed doers, there tends to be an increase in growth along with increasing doses of sargassum flour that has been fermented in the feed to 30%. The highest specific growth rate (LPS) was obtained in the same two treatments, namely 22.5% and 30% with an indigo of 1.6% / day (Table 2), although based on variety analysis showed no difference (P>0.05) with all treatments.

TABLE II
FISH GROWTH PERFORMANCE AND UTILIZATION RATE OF TEST FEED WITH THE ADDITION OF DIFFERENT SARGASSUM FLOUR (% DRY MATTER)

Observed parameters	Dosage of sargassum flour in feed				
	0%	7,5%	15%	22,5%	30%
Starting weight (g)	26.4±0.9	25.6±0.1	25.6±1.1	25.3±0.3	24.8±0.7
Final weight (g)	117.5±10.1a	116.6±15.5a	114.7±8.8a	128.3±12.0a	129.8±3.7a
Specific Growth Rate (%/day)	1.5±0.28a	1.5±0.01a	1.5±0.01a	1.7±0.04a	1.8±0.03a
Daily feed consumption rate (%)	250.3±7.41b	242.9±3.08b	267.5±6.07ab	269.0±9.85a	284.1±3.30a
Feed Efficiency (%)	59.8±0.93a	59.8±0.80a	59.76±0.14a	61.4±0.41a	61.91±1.33a
Feed Conference Ratio (PCR)	2.6±0.13a	2.8±0.41a	2.8±0.08a	2.7±0.19a	2.6±0.09a
Protein efficiency ratio	2.2±0.04a	2.2±0.03a	2.2±0.9a	2.4±0.33a	2.5±0.33a
Survival (%)	98±0.01a	97±0.02a	98±0.02a	98±0.01a	97±0.01a

The highest feed consumption rate was obtained from the 30% dose treatment of sargassum flour, which was around 284.1%, although statistically, it showed no difference (P>0.05) with dose treatment of 15% and 22.5%. However, it showed a difference with 0% and 7.5% treatment. It showed that the role of *bacillus aerius* bacteria in improving the quality of sargassum flour was successful because it could increase the level of feed consumption higher than ever with increasing doses in the feed. While the protein efficiency ratio also showed no different results with all treatments (P>0.05), the feed efficiency parameters and protein efficiency ratio tended to increase with increasing doses of fermented sargassum flour (Table 2). However, the feed conference ratio (FCR) parameter showed inverse results with the previous parameter where the lowest was obtained from 2 treatments, namely 0% (without the addition of sargassum flour) and 30% with the addition of sargassum flour, with the same value of 2.6, although based on variety analysis did not show any difference (P>0.05) with all treatments. While the survival parameter was also not found to be 100%, the results of the statistical analysis of all treatments were the same (P>0.05). This showed that fish deaths during research activities were not caused by treatment feed but by the influence of sudden environmental changes due to seasonal changes and super-intensive pond waste discharges around the Bay, which caused sudden turbidity.

The highest treatment response to feed efficiency was obtained from 30% treatment, which was 61.91%, although statistically, it was not different (P>0.05) with all treatments, but the tendency to increase with the increase in fermented sargassum flour in the feed. The results of this study are much higher when compared to the results of research by [12]. In the test of the utilization of rabbitfish feed containing fermented sargassum flour with probiotics combined RICA-4 + 5 has the highest feed efficiency rate of 46.1%.

B. Discussion

The results of this study show that *sargassum*, which has been fermented with *Bacillus aerius* bacteria, can be utilized as much as 30% in the feed to provide a stronger biological performance of rabbitfish. This is because naturally, *bacillus aerius* microbes are also present in the intestines of rabbitfish used in this study, and also, sargassum is fermented using microbes isolated from the intestines of rabbitfish So directly that the sargassum used has changed chemical elements such as protein, crude fiber (essential amino acids) and extract materials without nitrogen so that it will be more easily used by rabbitfish.

As reported by and [26], the use of microbes in the fermentation process can produce enzymes that can remodel complex cell wall structures to be simpler so that organisms can utilize them more easily. The study's results are relatively the same as the results of [22] research that adding Sargassum flour as much as 29% in rohu fish enlargement feed (*Labeo rohita*) can increase SGR by 1.61% / day. However, it is better compared to previous studies' results in the same fish [12], [23]. The daily feed consumption rate increases, feed efficiency increases, feed protein efficiency ratio increases, and feed conversion ratio (FCR) is the same as control feed. This shows that the role of *Bacillus aerius* bacteria has succeeded in improving the quality of sargassum flour, making it easier for fish to use as a raw feed material.

As reported by [12], using unfermented sargassum flour in feed is only 20%. If more than 20%, it will affect fish growth and feed digestibility. This is due to the low quality of nutrients (protein and high crude fiber). *Sargassum* that has been differentiated has an increase in protein and a decrease in crude fiber due to remodeling by *bacillus aerius* bacteria containing protease enzymes, amylase, and cellulase. Based on the results of amino acid analysis, there is an increase of 10%; an increase in these amino acids affects better feed quality. Several researchers [23]–[25] explained that essential amino acids, fatty acids, and other compounds necessary for growth include anti-oxidants, minerals, and vitamins.

Based on the research results by [12], sargassum flour fermented using commercial microbial B uses a maximum of 25% in feed. Furthermore, [31] that using sargassum flour that has been fermented using a combination of Rica 4 and Rica 5 bacteria as much as 30% provided a relative weight gain response of 224.1% and a specific growth rate of 0.98%. The results of this study obtained a relative weight gain of 423.3% with a specific growth rate of 1.8%, although these two studies used the same test animals. This shows that *Bacillus aerius bacteria is appropriately used as a probiotic in the fermentation process of sargassum flour as one type of raw feed* material for rabbitfish.

The total digestibility of feed and digestibility of nutrients (protein and feed energy) in each treatment can be seen in

Table 3. Based on the table, the highest total digestibility of the feed is found in 30% of treatment feed. The statistical analysis results do not show a difference (P>0.05) with all treatments. However, there tends to be an increase in the total digestibility of feed and the addition of sargassum flour. It shows that the role of bacteria during the fermentation process can increase protein and break down carbohydrate bonds into simpler ones, making it easier to do. Please take advantage of it by fish, so fish have a more intensive lipogenesis process given the feed. As reported by [12], the utilization of unfermented sargassum flour in feed is only 20% more than it affects the total digestibility of feed. While the digestibility of feed protein obtained the same relative result (P>0.05) for all treatments, the composition of relative raw materials was different, especially sargassum flour as a treatment raw material. This shows that the nutrients of fermented sargassum flour can be utilized by rabbitfish well, especially protein. The digestibility of feed energy also showed relatively similar results (P>0.05) for all treatments, although the tendency to increase with increasing doses of sargassum flour in the feed. This shows that the digestive system in the body of rabbitfish contains many types of carbohydrate-decomposing microbes.

TABLE III
TOTAL DIGESTIBILITY OF FEED, DIGESTIBILITY OF FEED PROTEIN, AND DIGESTIBILITY OF RESEARCH FEED ENERGY USED DURING THE STUDY.

Dead parameters	**Dosage of sargassum flour in feed**				
	0%	7,5%	15%	22,5%	30%
Digestibility of feed (%)	62.10±2.02a	62.40±1.72a	62.91±0.40a	63.15±5.45a	63.98±0.83a
Protein digestibility (%)	56,23±0,67a	56,59±0,86a	56,37±0,13a	56,76±6,11a	56,79±0,54a
Energy digestibility (%)	76,34±6,42a	76,34±4,10a	77,41±6,82a	78,00±0,78a	78,95±1,85a

The results of the proximate analysis of the study's initial and late fish bodies can be seen in Table 4. The table shows the chemical composition of the fish body, especially protein. There was an increase from the initial protein content and the addition of sargassum flour dose to 30%, although statistically, it showed no difference (P>0.05) with all treatments. The highest fat content obtained from the control feed treatment (0%) statistical test results showed no difference (P>0.05) with all treatments, although the tendency decreased with the addition of sargassum flour dose in the feed to 30%, but still higher when compared to the initial fat content of fish. The crude fiber content obtained the lowest from the 30% treatment, although the statistical test results showed a difference (P>0.05) with all treatments, but still higher when compared to the coarse husky content of the initial fish.

TABLE IV
RESULTS OF PROXIMATE ANALYSIS OF FISH BODY AT THE BEGINNING AND END OF THE STUDY

Observed parameters	**Dosage of sargassum flour in feed**					
	Beginning	0%	7,5%	15%	22,5%	30%
Protein (%)	53.98	62.51±1.42a	62.51±3.51a	62.68±1.76a	62.27±1.9a	62.98±0.59a
Fat (%)	15.09	21.9±3.27a	20.5±1.76a	19.87±1309a	19.79±2.09a	19.83±7.55a
crude fiber (%)	0.87	2.0±0.39a	1.21±0.31a	1.15±0.56a	1.08±0.34a	1.01±0.14a

Changes in the chemical composition of the fish body at the end of the study can be seen in Figures 1, 2, and 3.

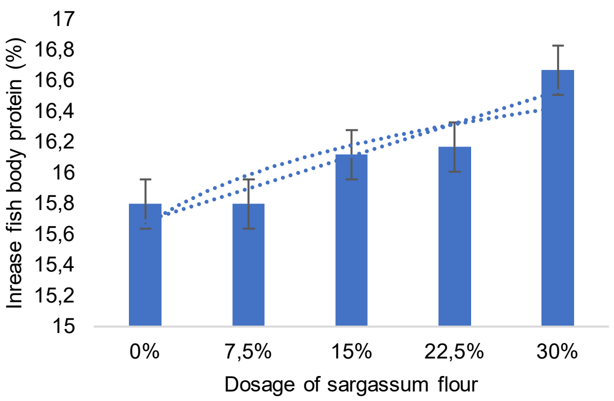

Fig. 1 Increased protein in the body of fish at the end of the study

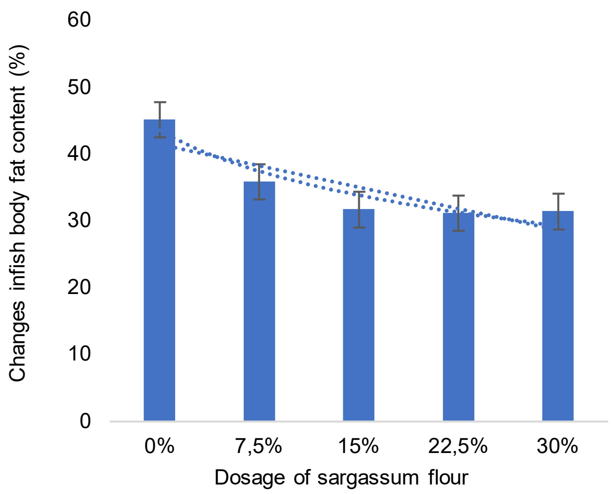

Fig. 2 Changes in fish body fat at the end of the study

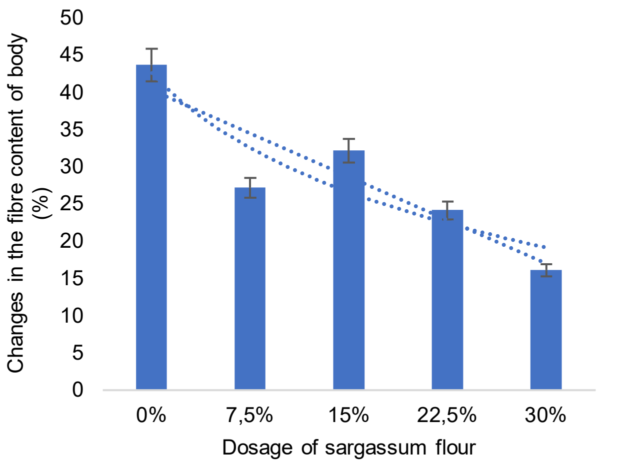

Fig. 3 Changes in fish fiber content at the end of the study

Figure 1 shows that the protein content of the fish body at the end of the study increased along with the addition of sargassum flour doses. This shows that protein from sargassum flour in feed can be utilized by enzymes in the intestines of fish and absorbed by cells in the body for growth. Changes in body fat levels, as reported by [32] in the digestive tract of *Baronang* fish, some microbes have great potential as probiotic candidates that have protease, amylase, and cellulase enzyme activity. The role of these microbes is to directly utilize feed nutrients as a substrate so that they are easily absorbed by cells in the body of fish, which has an impact on improving the chemical composition of the fish body.

IV. Conclusion

Bacillus aerius bacteria as probiotics during the fermentation process have succeeded in improving the quality of sargassum flour so that its use as one of the raw materials for rabbitfish feed as much as 30% in rabbitfish enlargement feed can still provide biological performance, total digestibility of feed, digestibility of feed nutrients and better body chemical composition of rabbitfish when compared to control feed. It is to be tested on other types of fish, such as tilapia and milkfish, at a dose of 30% to see the performance of growth and feed utilization during maintenance.

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