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IMPROVING THE QUALITY OF FERMENTED SEAWEED (EUCHEUMA COTTONII) POWDER AS A RAW MATERIAL FOR FISH FEED USING DIFFERENT TYPES OF FERMENTERS

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ABSTRACT
Various raw materials from plant-based sources, one of which is seaweed, are currently being developed for fish feed. However, the total fiber content of seaweed is relatively higher when compared to land plant-derived feedstuff. Therefore, utilizing seaweed as fish feed requires technological engineering to reduce the fiber content, including fermentation. This research aimed to analyze the effect of various types of fermenters on the chemical composition of fermented Eucheuma cottonii seaweed powder. This research used an experimental method with 4 (four) fermenter treatments: (1) the control or non-fermented group (Treatment A), (2) yeast 1.5% (Treatment B), (3) EM-4 1:50 (v/b) (Treatment C), and (4) yeast 1.5% + EM-4 0.5:50 (v/b) (Treatment D). A total of 50 g samples of E. cottonii seaweed were provided with these treatments with an additional 10 mL molasses. The fermentation process took place for 72 hours. The fermented seaweed powder was then formulated in fish feed with a concentration of 8%. The proximate test results showed that all the treatments could reduce fiber content and increase protein and Non-Nitrogen Free Extract (NFE) contents of seaweed powder compared to the control treatment (Treatment A). E. cottonii seaweed powder fermented with the EM-4 1:50 (v/b) fermenter (Treatment C) provided better results as it had the highest protein and NFE contents of 9.05% and 65.3%, respectively, and the lowest fiber content of 3.38%. Thus, when applied to feed formulation, it produced better-quality fish feed with protein and fiber contents of 49.52% and 2.02%, respectively.

KEY WORDS
EM-4, proximate, seaweed, yeast.

Fish feed is currently being developed by utilizing alternative raw materials from plant-based sources, including marine microalgae (Endraswari *et al.,* 2021; Putri *et al.,*2021; Irmadiati *et al.,* 2021). One of marine microalgae species potentially utilized as an alternative raw material for fish feed is Eucheumacottonii or *Kappaphycusalvarezii*. *E. cottonii* belongs to the group of red algae (*Rhodophyta*), which has almost 50% amino acids, is rich in aromatic amino acids, namely threonine, and has limited sulfur amino acids, namely lysine. Red algae (*Rhodophyta*) can be a source of complementary protein for human and animal needs (Xiren and Aminah, 2017; Lumbessy *et al.,* 2019).

However, when compared to land plant-derived feedstuff (tubers, fruits, cereals, and nuts), the total fiber content of seaweed is relatively higher, inseparable from its carbohydrate component, reaching 33-50% *bk* (carbohydrate content) (Dwiyitno, 2011). Furthermore, each group of seaweed has different fiber types and environmental conditions for growing. For example, Lumbessy *et al.* (2020) show that red algae of *E. cottonii* contain 5.77% fat, 15.22% fiber, 47.36% carbohydrate, and 42.88 mg/g of phycoerythrin pigment.

Hence, utilizing seaweed as fish feed requires technological engineering to reduce the fiber content, one of which is through the fermentation process. According to Aslamyah *et al.* (2017), crude fiber decreases along with increasing fermentation time. Fermentation aims to hydrolyze seaweed cells into the shortest nitrogen chains. Several previous studies have suggested that the use of *Gracilaria sp.* seaweed waste fermented with *Bacillus subtilis* bacteria as organic fertilizers leads to significant results for the growth of the planktonic *Chlorophyceae*. *Gracilaria sp.* seaweed waste contains several good nutrients for the growth of *Chlorophyceae*. Furthermore, according to Alamsjah et al. (2011), fermented seaweed experienced an increase in protein and Non-Nitrogen Free Extract (NFE) contents but, when compared to the control treatment, experienced a decrease in ash, crude fiber, and fat contents.

There has not been much information about the quality and quantity of seaweed powder after the fermentation process and its potential use in improving the quality of fish feed. Therefore, we were interested in analyzing the use of various fermenters in improving the quality of *E. cottonii* seaweed powder as a raw material for fish feed.

MATERIALS AND METHODS OF RESEARCH

The primary material (ingredient) used in this research was *E. cottonii* seaweed from seaweed farmers in Lombok, West Nusa Tenggara (NTB) waters. *E. cottonii* seaweed was washed thoroughly, cut into small pieces, and sundried. The dried seaweed was ground into powder and then sifted using the 80-meshed sieve. The fermenters used were Effective Microorganism-4 (EM-4) and yeast for cassava fermentation. During the fermentation process, molasses were added. Meanwhile, the materials used to manufacture fish feed consisted of fishmeal, soybean flour, corn flour, wheat flour, fish oil, corn oil, and premix. The equipment included a digital scale, blender, sieve, jar, steamer pan, and pellet printing machine.

This research used an experimental method with 2 (two) stages. The first stage was to ferment *E. cottonii* seaweed powder using four different fermenter treatments(1) the control or non-fermented group (Treatment A), (2) yeast 1.5% (Treatment B), (3) EM-4 1:50 (v/b) (Treatment C), and (4) yeast 1.5% + EM-4 0.5:50 (v/b) (Treatment D).

Fermentation was carried out by weighing 50 g of *E. cottonii* seaweed powder and putting it in a plastic jar. Meanwhile, the fermenters were prepared according to the existing treatments, dissolved in 10 mL of molasses, and sprayed evenly using a sprayer on the seaweed prepared earlier. Furthermore, the seaweed powder was incubated for 72 hours, allowing the fermenters to break down the substrate. After 72 hours, the seaweed powder was steamed for 1-2 minutes to inactivate the fermenter (Aslamyah *et al*., 2017). Furthermore, an analysis of the proximate content of fermented seaweed powder was carried out in each treatment.

The second stage was to formulate fish feed by adding fermented *E. cottonii* seaweed powder from various fermenters in the first stage. The fermented seaweed powder was added with a concentration of 8%. The used feed formulation is presented in Table 1 below.

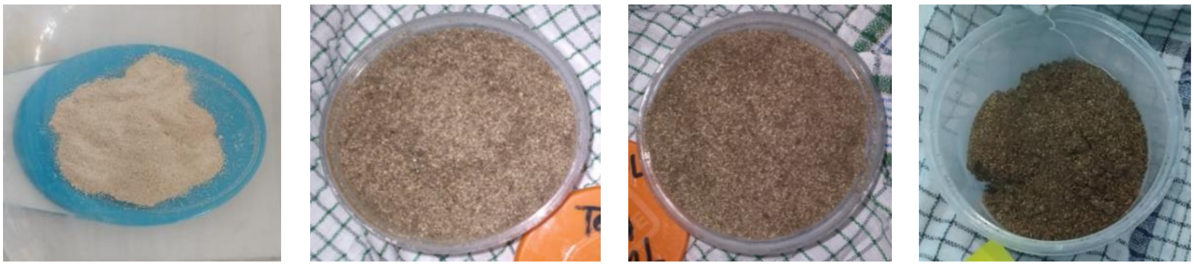
Table 1 – Feed formulation in a dose of 1 kg

Raw Materials	Weight (g)
Fish meal	390
Fermented seaweed powder (8%)	80
Soybean flour	260
Corn flour	130
Wheat flour	65
Fish oil	35
Corn oil	25
Premix	15
Total	1,000

The data obtained were processed using Microsoft Office Excel and then analyzed descriptively to be presented as narration and tables.

RESULTS AND DISCUSSION

The physical characteristics of *E. cottonii* seaweed powder fermented with different fermenters can be seen in Figure 1.



Control (A) *Yeast (B)* *EM-4 (C)* *Yeast + EM-4 (D)*

Figure 1 – Physical characteristics of *E. cottonii* seaweed powder fermented with different fermenters

Figure 1 suggests that the physical characteristics of *E. cottonii* seaweed powder in all treatments were not much different. It indicates that the fermentation process carried out in this research could maintain the physical quality of *E. cottonii* seaweed powder, including the white-to-brownish color and distinctive smell of seaweed. The distinct smell of seaweed was quite strong and stinging, expected to be able to stimulate fish appetite. Likewise, Gunawan and Khalil (2015) have found that the raw materials in the manufacture of feed pellets must have a distinctive and strong smell that can stimulate the appetite of fishes, predominantly carnivorous.

Another physical characteristic of the fermented *E. cottonii* seaweed powder in all treatments was their smooth and uniform texture despite being slightly moist due to the addition of water-containing molasses. Ninsix (2012) reveals that nutritional contents, such as moisture content, can affect the texture of feed materials (ingredients). Thus, from the physical quality, all fermenter treatments could still produce good-quality *E. cottonii* seaweed powdersuitably used as a mixed raw material for feed pellets. According to Aslamyah and Karim (2012), the quality artificial feed has a distinctive aroma liked by fish because it uses good-quality raw materials, such as having fine and uniform particle size, high homogeneity, and potential to be processed into a powder.

The nutritional contents of *E.cottonii* seaweed powder fermented with different fermenters are presented in Table 2.

Table 2 – Proximate analysis of *E. cottonii* seaweed powder fermented with different fermenters

Chemical Composition (%)						
Treatment	**Protein**	**Lipid**	**Crude Fiber**	**Ash**	**Moisture**	**NFE**
Control (A)	3.48	6.83	34	16.35	7.35	39.34
Yeast (B)	8.65	0.32	5.09	22.52	15.84	63.42
EM4 (C)	9.04	0.16	3.38	22.13	17.75	65.29
Yeast + EM4 (D)	8.96	0.56	4.16	22.18	16.24	64.14

Table 2 shows that the fermentation process decreases fat and fiber contents but increases protein, ash, and Non-Nitrogen Free Extract (NFE) contents compared to the non-fermented seaweed powder treatment (control). Thus, the fermentation process was alleged to improve the nutritional quality of seaweed powder, especially protein, NFE, and fiber contents. Supriyatna (2017) explains that fermentation of solid substrates in lingo-cellulosic feedstuff using microorganisms can increase protein, ash, and moisture contents and reduce fiber (cellulose, hemicellulose, and lignin). This result is strongly supported by the chemical structure of *E. cottonii* seaweed containing high fiber content, as shown in Treatment A of this research. Therefore, it can be concluded that the fiber content decreased from 34% in the Control Treatment (A) to around 3.38% to 5.09% after the fermentation process using various fermenters. Using the EM-4 fermenter in Treatment C resulted in the lowest fiber content.

Dwiyitno (2011) has found that the crude fiber of seaweed can be further divided into cellulose, hemicellulose, and lignin. As part of carbohydrates, fiber supports the formation of sugars in the fermentation process to be utilized by microorganisms for increasing microbial biomass containing much protein. This mechanism increases protein content at the end of each fermentation process. The highest increase in protein content was found in *E. cottonii* seaweed powder fermented with the EM-4 fermenter (Treatment C), reaching 9.04%. It might be because the EM-4 fermenter contains more beneficial microorganisms than the yeast fermenter. Qo'idah (2015) adds that Effective Microorganism 4 (EM-4) has selected species of microorganisms, particularly fermenting ones, namely lactic acid bacteria (*Lactobacillus sp.*), fermented fungi (*Saccharomyces sp.*), photosynthetic bacteria (*Rhodopseudomonas sp.*), and *Actinomycete*.

Meanwhile, yeast is formed by several fungi, such as *Rhizopus oryzae*, *Rhizopus oligosporus*, *Rhizopus orhizae*, and *Rhizopus stolonifer.* However, the most widely used fungus is *Rhizopusoryzae*, as it does not produce toxins but lactic acid. In addition, Aslamyah *et al.* (2017) assert that protein content in fermentation can get higher due to the increased fermenter cells used.

Conversely, the decrease in crude fiber content was allegedly due to microorganism activitiesthat decomposed complex substances on the substrates into simpler compounds. Astuti *et al.*(2017) state that feedstuff containing hard-to-digest nutritional bonds, such as crude fiber, can be simplified by fermentation. Indrayanti (2013) has found that the broken-down fiber will become simple carbohydrates during fermentation to increase energy and make it easy to digest. Thus, a decrease in crude fiber due to fermentation can lead to increased NFE content. This is consistent with the results of this research showing that the use of EM-4 fermenter (Treatment C), providing the lowest fiber content of 3.38%, resulted in the highest NFE content of 65.29%.Gunawan and Kholil (2015) add that carbohydrates in feed fish are found in the form of crude fiber and NFE. Therefore, a decrease in crude fiber content of a feedstuff can increase NFE content.

All treatments in this research indicated a better reduction in the fiber content of *E. cottonii* seaweed powder when compared to Aslamsyah*et al.* (2017),in which a mixture of *Bacillus sp*., *Rhizopus sp*., and fermented fungi (*Saccharomyces sp.*) was used with a composition of 1 mL + 1 g + 1 g/100 g of seaweed powder. However, this composition resulted in seaweed powder fiber content ranging from only 11.06 – 11.70%. The different results might be due to the use of different types of microorganisms as a source of fermenters.

The increase in the nutritional quality of *E. cottonii* seaweed powder fermented with the EM-4 and yeast fermenters in this research was also allegedly due to the effect of enzymes produced by microorganisms in the form of bacteria and fungi in the fermenters used. Similarly, Aslamyah*et al.* (2017)declare that enzymatic processes cause the increase in the protein and NFE contents of fermented seaweed powder by the fermenters.

Thus, it can be said that the use of EM-4 fermenters (Treatment C) in the fermentation process of *E. cottonii* seaweed powder provided the best results, with the highest protein and NFE contents of 9.04% and 65.29%, respectively, but with the lowest fiber content of 3.38%. Meanwhile, based on the number of microorganisms, the use of the mixed EM-4 and yeast fermenter (Treatment D), which was supposed to be able to provide better nutritional quality of *E. cottonii* seaweed powder, resulted in a lower increase in nutritional content when compared to the use of the yeast fermenter (Treatment B). It could happen because, although the mixed fermenters (EM-4 and yeast in Treatment D) contained more microorganisms than other treatments, the concentration of Treatment D was lower than Treatment B (the yeast fermenter) and Treatment C(EM-4 fermenter).

Table 3 – Proximate analysis of fish feed with fortification of *E. cottonii* powder fermented with different fermenters

Treatment	Chemical Composition (%)(dry weight)					
	Protein	Lipid	Crude Fiber	Ash	Moisture	NFE
Control (A)	46	16.32	2.01	7.93	8.38	27.74
Yeast Fermenter 8% (B)	41.53	14.45	2.10	9.38	10.22	32.54
EM-4 Fermenter 8% (C)	49.52	16.25	2.02	7.40	7.96	24.81
Yeast + EM-4 8% (D)	46.73	13.84	2.39	5.52	12.27	31.52

The nutritional composition of fish feed with fortified *E. cottonii* seaweed powder fermented with different fermenters was varied (Table 3). It indicates that all treatments, both the three fermented *E. cottonii* seaweed powders and the control, show a proximate content of fish feed following the Indonesian National Standard (*SNI*) 01-7242, except for the moisture content in Treatment D (EM-4 + Yeast 8%),which is slightly above the optimum limit. According to the *SNI* 01-7242, the fish feed quality standard for rearing has a maximum moisture and ash content of 12%, a minimum protein content of 25%, a minimum fat content of 5%, and a maximum fiber content of 8%.

The increase in protein content was quite good in the *E. cottonii* seaweed powder fermented with EM-4 because the seaweed powder used in this treatment had the highest protein content, as previously described (Table 1). Pinandoyo *et al.* (2015) further state that fermentation was able to increase or improve nutritional values, such as protein content. Therefore, fermentation using microorganisms can provide N as protein for other microorganisms that are mutually beneficial. Fish feed needs a large amount of protein. Karimah *et al.* (2018), explain that the most significant component of the fish body after moisture (water) is protein, ranging from 60 – 70%. Therefore, the primary energy for fish growth derives from protein.

The fat content in all fermented E. cottonii seaweed powder treatments decreased compared to the control treatment (Treatment A); it was thought to be correlated to the fermentation process. Rizky *et al.* (2017), declare that fermentation can degrade fats into simpler molecules to reduce fat content. It can happen due to the ability of some lipolytic bacteria to secrete extracellular lipase enzymes for the breakdown of fats into fatty acids and glycerol to be used by these bacteria as a carbon source. Fat is the primary energy source, containing more calories than protein and carbohydrates. Fats can provide 9 kcal/gram, while carbohydrates and protein can only result in 4 kcal/gram. However, the role of fats occupies the second function in fish because fish have a better ability to consume protein (Munisa*et al.*, 2015). Thus, although the resulting feed experienced a decrease in fat compared to the control treatment, the fat content in all treatments of fermented *E. cottoni* seaweed powder could still meet the energy needs of fish.

The fiber content in all treatments was higher than the control treatment (Treatment A) due to the addition of fermented seaweed powder. According to Dwiyitno (2011), the total fiber content of seaweed is relatively higher when compared to land plant-derived feedstuff, which is generally only rich in insoluble fiber. However, despite having higher fiber content than the control treatment, the feed fiber content value in all treatments of fermented *E. cottonii* seaweed powder still followed the digestibility of fish.

The moisture and ash contents in all fermented *E. cottonii* seaweed powder treatments provided varying results. The moisture content in feed plays a vital role as it can unite all materials (ingredients) in the feed, while ash content is a residue produced by burning organic matter in the form of inorganic compounds such as oxides, salts, and minerals (GunawanandKholil, 2015). All feed treatments showed optimum ash content, indicating that the fish feed had sufficient mineral or organic matter content and was good for the growth of fish body tissues.

All feed treatments showed varying results of NFE content because NFE content usually depends on other feed nutrient contents, such as moisture, ash, crude protein, and crude fat. Aslamyah*et al.* (2018) state that decreasing NFE content is less profitable if seen from the nutritional aspect. The lower NFE content leads to fewer components of organic matter that can be digested, ultimately producing less energy. The addition of *E. cottonii* seaweed powder fermented with yeast (Treatment B) in fish feed showed a higher NFE content of 32.54%. The small number of microorganisms used in this treatment caused a lower microbial activity than in Treatment C (EM-4 fermenter) and Treatment D (mixed EM-4 + yeast fermenter). A low microbial activity also caused low energy consumption, eventually increasing the NFE content. Hastuti *et al.* (2011) state that an increase in microbial activity in degrading substrates will also increase energy consumption (NFE). In other words, higher microbial activity can reduce the NFE content. However, the overall feed NFE content in all treatments was still optimal for fish needs.

CONCLUSION

Based on the physical characteristics, including having smooth and uniform textures, a distinctive smell of seaweed, and a white-to-brownish color, the *E. cottonii* seaweed powder fermented with various fermenters wasstill suitably used as a mixed raw material for fish feed. The quality of *E. cottonii* seaweed powder fermented with the EM-4 1:50 (v/b) fermenter (Treatment C) provided better results because it had the highest protein and NFE contents of 9.05% and 65.3%, respectively, and the lowest fiber content of 3.38%. Thus, when applied to feed formulation, it resulted in better-quality fish feed with protein and fiber contents of 49.52% and 2.02% respectively.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest. The funders had no role in the design of the study, collection, analysis or interpretation of data, writing of the manuscript or decision to publish the results.

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