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Regular Research

Enhancing the Nutritional Value of Coconut Meal for Aquaculture Feed through Fermentation with *Rhizopus* sp. and EM4

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Abstract: Coconut meals are an underutilized agricultural byproduct that still contains valuable nutrients. This study aimed to evaluate the effect of fermentation on the nutritional composition specifically protein, fat, and carbohydrate content of coconut meal. Three treatments were compared: unfermented coconut meal, coconut meal fermented with Rhizopus sp., and coconut meal fermented with EM4 (a microbial consortium). The unfermented coconut meal contained 34.77% protein, 8.84% fat, and 27.73% carbohydrates. Fermentation with Rhizopus sp. increased protein content to 37.37%, reduced fat content to 6.11%, and slightly reduced carbohydrates to 23.04%. In contrast, EM4 fermentation raised protein to 35.87%, decreased fat to 6.55%, and increased carbohydrates to 26.86%. These findings suggest that Rhizopus sp. fermentation is more effective in enhancing protein content and reducing fat, while EM4 is more effective in increasing carbohydrate levels. In conclusion, fermentation can significantly improve the nutritional quality of coconut meals, making it a more valuable and nutritious alternative for aquaculture feed.

Keywords: Aquaculture feed, coconut meal; EM4; fermentation; Rhizopus sp.

1. Introduction

Coconut is a fruit with many uses, consisting of husk, shell, meat, and coconut water. The inner coconut meat and coconut water are usually used as food and beverage ingredients. Coconut meat is widely used as coconut milk or in the form of coconut oil [1]-[4]. The result of squeezing oil from coconut meat is coconut meal [5].

Coconut meal is a by-product of the coconut oil extraction process that is widely used as animal feed due to its high nutritional content. However, the utilization of coconut meals in animal feed is often limited by several factors, such as high crude fiber content and nutritional values that can vary. Therefore, efforts need to be made to improve the nutritional quality of coconut meals to provide more optimal benefits as animal feed. One method that can be used to improve the nutritional quality of coconut meals is through a fermentation process. Fermentation is a biochemical process that involves microorganisms, such as bacteria, fungi, or molds, to break down complex organic matter into simpler and easily digestible compounds. The fermentation process can not only increase the content of certain nutrients, such as protein, but can also reduce unwanted components, such as fat and crude fiber [6].

One of the potential microorganisms to be used in coconut meal fermentation is Rhizopus sp. Rhizopus sp is a type of mould that has long

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been known in the fermentation industry. This microorganism can increase protein content through the fermentation process, where enzymes produced by Rhizopus sp can break down complex components into amino acids and other nitrogen compounds that are more easily absorbed by the livestock body [7]. This study focuses on the effect of fermentation using two types of microorganisms, namely Rhizopus sp and Effective Microorganisms 4 (EM4), on the protein, fat and carbohydrate content of coconut meal. Rhizopus sp is known as a mould that is effective in increasing protein content through the fermentation process, while EM4 is a consortium of microorganisms often used in agriculture and animal husbandry to improve the quality of organic materials [8], [9].

An alternative to obtain cheaper fish feed is to utilize food waste. Food waste does not mean rotten rubbish that cannot be utilized. Food waste in question is a by-product when processing food products and this by-product has very potential nutritional content when utilized as an alternative fish feed. Like food waste from the community that is useless and just waste that can be processed as a source of nutrition for fish, for example coconut meal, tofu pulp. This can answer the global problem of supplying fishmeal and fish oil, several studies have shown that alternative food waste can be a good nutritional substitute for fish development [10].

Due to the high cost of artificial feed, which can reach up to 70% of production costs, fish farmers often experience difficulties with the relatively high price of fish feed on the market. Therefore, it is necessary to make independent feed from local raw materials that have nutritional value for fish, are easily available and have a low price. Using food waste can reduce costs but is rich in nutrients, this has been applied in European Union fish farming [11].

Fermentation technology is one of the efforts that can be made to increase the utilization of protein as an energy source in fish. Fermentation can be done anaerobically or aerobically where the way fermentation works is to break down organic compounds into simpler compounds with the help of microbes. The fermentation process can increase the protein contained in the feed with the help of microbes that convert starch compounds into protein. Fermentation occurs through enzyme reactions produced by microorganisms to convert complex organic materials such as proteins, carbohydrates and fats into simpler molecules, due to the activity of microorganisms, the fermented material (substrate) will undergo both physical and chemical changes. The principle of applying fermentation is to maximize the work of microorganisms that can change the components of feed ingredients such as reducing crude fiber levels [12], [13] and reducing anti-nutritional substances in feed ingredients [14].

Some microorganisms that can improve the nutritional quality of an ingredient are Rhizopus sp. and EM4 probiotics which contain several microorganisms such as lactobacillus casie and saccharomyces. Based on the above background, this study was conducted to determine the proximate content of fermented feed. Proximate characteristics include protein, fat and carbohydrate content.

The purpose of this study was to compare the protein, fat, and carbohydrate contents of unfermented coconut meal, coconut meal fermented with Rhizopus sp, and coconut meal fermented with EM4. The results of this study are expected to provide useful information regarding the most effective fermentation method in increasing the nutritional value of coconut meal, to provide better quality and efficient alternative animal feed. Through this research, it is hoped that a better understanding of how the fermentation process can affect the nutritional composition of coconut meals and how its application can provide real benefits in the livestock industry can be obtained. In addition, the results of this study can also serve as a basis for the development of more effective and sustainable fermentation technologies in utilizing coconut meal as a source of high-value animal feed.

2. Materials and Methods

This study used an experimental method with a complete randomized design (CRD) with 3 treatments and 3 replicates, as for the treatments in this study, namely P1: feed without fermentation, P2: EM4 fermentation, P3: fermentation of Rhizopus sp.

1. Research Procedure

a. Fermentation of coconut meal with EM4

The fermentation process referred to research [15] with modifications. Fermentation began with the preparation of probiotic solution, namely by adding 15 ml EM4 to 500 ml water and 15ml brown sugar. The addition of sugar is aimed at a food source for decomposing bacteria. Next, the probiotic solution was mixed with 1kg coconut meal, then put in a container and tightly closed. The fermentation process was carried out anaerobically for 4 days. After fermentation, drying was carried out and after drying, it was crushed using a blender and sieving process.

b. Fermentation of coconut meal with Rhizopus sp.

Coconut meal was first steamed for about 30 minutes and then cooled, 5 grams/kg of Rhizopus sp. was mixed until homogeneous in the coconut meal, then wrapped in plastic and punctured and fermented for 4 days at room temperature. After the coconut meal fermentation process was complete, it was dried in the sun, broken up if there were clumps and mashed.

c. Ground fish meal

Rucha fish was first cleaned from dirt, then steamed for 20 minutes, then dried in the sun. Then the fish was ground using a blender and sieved.

d. Feed making

The processing process began with mixing the mashed ingredients such as unfermented/unfermented coconut meal, fish meal, bran, tofu pulp and tapioca flour. Next, 550 ml of warm water is added and moulded using a moulding machine, then dried in the sun for 2-3 days.

Table 1. Composition of feed ingredients

No.	Ingredients	Formulations		
		P1	P2	P3
1.	Unfermented coconut meal	30	0	0
2.	EM4 fermented coconut meal	0	30	0
3.	Tempe yeast fermented coconut meal	0	0	30
4.	Fish meal	30	30	30
5.	Tofu dregs	20	20	20
6.	Bran	10	10	10
7.	Cassava flour	10	10	10
	Total	100	100	100

3. Results

Coconut meals were a by-product of coconut processing that was often used as animal feed due to its good nutritional content. The fermentation process could influence the nutritional composition of coconut meals, including its protein, fat, and carbohydrate content. The following were the results of a study on the nutritional content of coconut meal that had undergone fermentation treatments with *Rhizopus* sp. and EM4, as presented in Table 2.

Table 2. Proximate test results of coconut meal

Treatments	Parameters		
	Protein	Fat	Carbohydrates
P1	34,77	8,84	27,73
P2	37,37	6,11	23,04
P3	35,87	6,55	26,86

Description, P1 is without fermentation, P2 is Tempe yeast fermentation dan P3 is EM4 fermentation.

4. Discussion

4.1 Unfermented Coconut Meal

Unfermented coconut meal had a protein content of 34,77%, which was quite high and made it a good source of protein. The fat content was 8,84%, which was quite significant, and the carbohydrate content was 27,73%.

4.2 Coconut Meal Fermented with Rhizopus sp.

Fermentation of coconut meals with *Rhizopus* sp. increased the protein content to 37.37%, which was higher than that of the unfermented sample. This indicates that *Rhizopus* sp. fermentation can enhance the protein value of coconut meal. Additionally, the fat content decreased to 6.11%, reflecting a reduction in lipid levels due to the fermentation process. The carbohydrate content showed a slight increase to 23.04%. These results demonstrate the potential of microbial fermentation, particularly with *Rhizopus* sp., to improve the nutritional quality of coconut meals, making it a more suitable and valuable ingredient for aquaculture feed.

4.3 Coconut Meal Fermented with EM4

Fermentation with EM4 also increased the protein content to 35.87%, which, although lower than the increase observed with *Rhizopus* sp., was still higher than that of the unfermented coconut meal. The fat content decreased to 6.55%, though the reduction was not as pronounced as that achieved with *Rhizopus* sp. fermentation. Notably, the carbohydrate content increased significantly to 26.86%. These findings suggest that EM4 fermentation is effective in enhancing the carbohydrate and protein content of coconut meals, with moderate effects on fat reduction, indicating its potential for improving the nutritional value of coconut meal for use in aquaculture feed.

5. Comparative Analysis

5.1 Protein

Fermentation with *Rhizopus* sp. resulted in the highest increase in protein content (37.37%), followed by fermentation with EM4 (35.87%), while the unfermented coconut meal had the lowest protein content (34.77%). In terms of fat content, *Rhizopus* sp. fermentation led to the greatest reduction (6.11%), followed by EM4 fermentation (6.55%), whereas the unfermented coconut meal had the highest fat content (8.84%). Regarding carbohydrate content, EM4 fermentation yielded to the highest level (26.86%), followed by *Rhizopus* sp. fermentation (23.04%), while the unfermented coconut meal had a carbohydrate content of 27.73%.

The increase in protein content following fermentation with *Rhizopus* sp. and EM4 is attributed to microbial activity that breaks down complex compounds into simpler, more bioavailable forms. The highest protein level was observed in the *Rhizopus* sp. treatment (37.37%), followed by the EM4 treatment (35.87%), while the lowest protein content was found in the unfermented sample (34.77%). These findings demonstrate the potential of microbial fermentation to enhance the nutritional quality of coconut meals, particularly in terms of protein enrichment.

5.2 Fat

5.2.1 Effect of Fermentation on Protein Content

The results of this study show that fermentation significantly affects the protein content of coconut meal. As presented in Table 2, the highest protein content was observed in the *Rhizopus* sp. fermentation treatment (P2), reaching 37.37%. This was followed by the EM4 fermentation treatment (P3) with 35.87%, while the unfermented coconut meal (P1) showed the lowest protein content at 34.77%.

The increase in protein content following fermentation is attributed to microbial activity that breaks down complex compounds into simpler forms. During fermentation, *Rhizopus* sp. produces protease enzymes that hydrolyze complex proteins into peptides and free amino acids, increasing the protein bioavailability in the substrate. Additionally, the accumulation of microbial biomass (single-cell proteins) during fermentation contributes to the overall increase in crude protein levels. The presence of *Rhizopus* sp. also helps reduce anti-nutritional factors and enhances amino acid content, making the feed more digestible and nutritionally valuable for aquaculture use.

Although EM4 fermentation also increased the protein content, its effectiveness was lower compared to *Rhizopus* sp., with only a 1.07% increase over the unfermented control. This lower efficiency may be due to the slower growth rate of *Lactobacillus* spp. in EM4, which limits its ability to hydrolyze proteins within a short fermentation period. However, the increase is still significant and aligns with previous findings, such as the 4.79% protein increase observed in cassava peel fermented with EM4 [20].

5.2.2 Effect of Fermentation on Fat Content

Fat content also decreased following fermentation. The unfermented coconut meal (P1) had the highest fat content at 8.84%. After fermentation, fat content decreased to 6.11% in the *Rhizopus* sp. treatment (P2) and 6.55% in the EM4 treatment (P3). The reduction in fat content is primarily due to the utilization of fat as an energy source by fermenting microorganisms. *Rhizopus* sp. is known to produce lipase enzymes that break down fat into fatty acids and glycerol, which are then used for microbial growth. The fat content in coconut meals fermented with *Rhizopus* sp. decreased by approximately 4.4% compared to the unfermented sample. This reduction is beneficial for feed quality, as lower fat levels reduce the risk of rancidity and improve feed stability during storage. Although EM4 also led to a decrease in fat content, the reduction was not as pronounced as in the *Rhizopus* sp. treatment. This may be due to the lower lipase activity of the microorganisms present in EM4, resulting in less efficient fat hydrolysis. Nonetheless, the decline in fat content is consistent with previous research reporting a 4.42% decrease in fat content after fermentation [21].

5.2.3 Effect of Fermentation on Carbohydrate Content

Carbohydrate content also changed following fermentation. The unfermented coconut meal contained 27.73% carbohydrates. EM4 fermentation (P3) resulted in the highest carbohydrate content at 26.86%, followed by *Rhizopus* sp. fermentation (P2) at 23.04%. The reduction in carbohydrate content, particularly in the *Rhizopus* sp. treatment, is due to enzymatic degradation of starch by microbial enzymes. Carbohydrates are metabolized by microorganisms during fermentation as a carbon source to support growth. Interestingly, EM4 treatment retained more carbohydrates compared to *Rhizopus* sp., possibly due to differences in microbial composition and enzymatic activity. This suggests that EM4 fermentation may be more suitable when higher carbohydrate retention is desired in the final feed product.

5.2.4 Nutritional Implications for Aquaculture Feed

Overall, fermentation improves the nutritional quality of coconut meals, particularly in increasing protein content and reducing fat. These improvements are beneficial for aquaculture feed formulation, where high protein content and stable fat levels are essential for fish growth and feed shelf life. All treatments met the protein content requirement set by the Indonesian National Standard (SNI 9043-4:2022), which mandates a minimum crude protein content of 30% for aquaculture feed. Fermentation, especially with *Rhizopus* sp., also contributes to the reduction of anti-nutritional factors and increases amino acid availability, further enhancing the functional value of the feed. Although EM4 was less effective in increasing protein, it showed better retention of carbohydrates, which could be advantageous depending on the energy requirements of the aquaculture species being cultivated.

5.3 Carbohydrate

5.3.1 Effect of Fermentation on Carbohydrate Content

Based on the data presented in Table 2, carbohydrate content was found to be highest in the unfermented coconut meal (P1) at 27.73%. In contrast, the lowest carbohydrate content was observed in the *Rhizopus* sp. fermentation treatment (P2) at 23.04%, while the EM4 treatment (P3) yielded a carbohydrate content of 26.86%. The reduction in carbohydrate content following fermentation, particularly with Rhizopus sp. reduced carbohydrate content by 8.35%. This decrease is likely due to the activity of lignocellulolytic enzymes produced by Rhizopus sp., which can hydrolyze the bonds between lignin and cellulose, hemicellulose, or protein. The breakdown of these complex structures leads to a reduction in crude fiber and available carbohydrate content as the carbohydrates are metabolized for microbial growth and energy.

Similarly, fermentation with EM4 also resulted in a reduction in carbohydrate content, although the decrease was less pronounced compared to *Rhizopus* sp. This may be attributed to the microbial composition of EM4, which includes *Lactobacillus* spp. and *Saccharomyces cerevisiae*. These microbes can utilize carbohydrates as energy sources. According to [6], *Saccharomyces cerevisiae* in fermented tofu pulp was shown to reduce carbohydrate content by 6.45% through the production of enzymes that degrade complex carbohydrates. This enzymatic activity is largely due to its high amylolytic enzyme production, which facilitates the conversion of starches and other polysaccharides into simpler sugars that are then used for microbial metabolism.

Probiotic microorganisms such as *Lactobacillus* and *Saccharomyces* are also known to secrete several hydrolytic enzymes, including amylase, protease, lipase, and cellulase. These enzymes aid in the degradation of complex nutrients like carbohydrates, proteins, and fats into simpler compounds monosaccharides, amino acids, and fatty acids which are more bioavailable and metabolically useful for fish nutrition.

Despite the reduction in carbohydrate content due to fermentation, the final values remained within the optimal range recommended for aquaculture feed. According to standard nutritional requirements for fish, the carbohydrate content of feed should be in the range of 20–30% (SNI 9043-4:2022). Thus, all treatments in this study, including fermented and unfermented samples, met this requirement. The reduction in carbohydrates, while expected due to microbial metabolism, indicates active fermentation and nutrient conversion. Moreover, maintaining carbohydrates within the optimal range is essential, as balanced carbohydrate levels in aquaculture feed contribute to efficient nutrient metabolism and support optimal growth performance in fish.

6. Conclusions

The fermentation process using *Rhizopus* sp. and EM4 significantly improves the nutritional quality of coconut meals, making it more suitable as an alternative ingredient for aquaculture feed. Fermentation with *Rhizopus* sp. was the most effective in enhancing protein content (37.37%) and reducing fat content (6.11%). This improvement is attributed to the enzymatic activity of *Rhizopus* sp., particularly protease and lipase enzymes, which break down complex macromolecules into simpler, more digestible forms. Additionally, the carbohydrate content decreased to 23.04% due to lignocellulolytic enzyme activity that degraded fiber components.

Fermentation with EM4 also increased protein content (35.87%) and reduced fat (6.55%), although less effectively than *Rhizopus* sp. However, it retained a higher carbohydrate level (26.86%), suggesting that EM4 fermentation may be advantageous when a higher energy supply is desired for aquaculture species. All treatments met the minimum protein content requirement for aquaculture feed as outlined in the Indonesian National Standard (SNI 9043-4:2022), which is ≥30%. Therefore, it can be concluded that fermentation, particularly with *Rhizopus* sp. is a promising method to enhance the nutritional value of coconut meal. This process adds value to agricultural by-products and supports the development of cost-effective, high-quality feed alternatives for the aquaculture industry.

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