Fermentation Using Mold to Improve the Quality of Fish Feed

Yuli Andriani[1], Rusky I. Pratama[1],

[1]Staff at Fisheries Department, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran, Indonesia

ABSTRACT

Fermentation using a mold is one method that can be used to increase the nutritional value of feed. The application of mold fermentation has been shown to increase the crude protein content, reduce the crude fiber content and increase the buoyancy of fish feed. Types of molds that can be used in fermenting feed ingredients include Aspergillus niger, Trichoderma spp. and Rhizopus sp. Fermentation methods that can be used are submerged culture fermentation and solid substrate fermentation. Substrate fermentation is mostly carried out because the volume of water in the fermentation process is lower, harvesting is easier and the remaining substrate in solid substrate fermentation still has nutritional value. Fermentation of feed ingredients using Trichoderma viride mold on fermented rice straw can increase the crude protein content from 4.65% to 6.65% and fiber content from 45.93% to 52.62%.

Keyword: *feed, fermentation, fiber, mold, protein*

1. INTRODUCTION

One important aspect of fish farming is feed. Feed is a source of materials and energy that helps fish survive and grow, but the feed is also a large part of production costs (50-70%). Every fish farmer must face the challenge of increasing the price of fish feed without increasing the selling price of cultured fish. Therefore, to reduce production costs, it is necessary to process feed ingredients that can increase growth and feed efficiency using mold fermentation. Fermentation of feed ingredients using mold can be used as a solution to reduce production costs, achieve optimal feed growth and efficiency, and reduce feed waste that pollutes the environment.

Fermentation is a method for converting substrates into desired products by utilizing microbes such as mold. Molds are microscopic fungi with masses of transparent spores and mycelia. Due to its cotton-like shape, its growth can be seen clearly on the substrate. At the beginning of its growth, it is white, however when the spores have appeared it will change color to vary, depending on the type of mold [1]. The utilization of mold for fermentation can increase the number of nutrients present in the substrate and decrease the amount of fiber. Protein content can be affected by the use of mold in fermentation. Protein content can be affected by the type of microorganism used in the fermentation process. This paper is a review based on a literature search regarding improving feed quality with the fermentation method using the mold.

2. ADDITION OF MOLD TO MOLD

2.1 Definition and Working Mechanism

Molds are multicellular fungi and have filaments. The growth of mold on the substrate is easy to observe because it looks like cotton threads. According to [1], the growth of the mold is initially can be seen as white colored, however once the spores appear, the color will change to a more specific color depending on the type of mold. Mold can be divided into two groups based on the structure of the hyphae, namely hyphae insulated or septate and hyphae not insulated or non-septate according to [2]. The nuclei of the septate cells are scattered along the hyphae. The mold septum is an insulating wall that allows the cytoplasm to move freely between the chambers because it is not tightly closed. The *Ascomycetes*, *Basidiomycetes*, and *Deuteromycetes* mushroom classes are examples of insulated molds, while the *Phycomycetes* (*Zygomycetes* and *Oomycetes*) are non-insulated molds.

The addition of microorganisms such as mold to feed is an alternative method to produce feed that has high nutritional value and indirectly improves feed quality. The employ of microorganisms as a source of enzymes has several advantages, including the production of microorganisms in producing enzymes can be practically increased. During the fermentation process, microorganisms can maintain their physiological properties and produce enzymes rapidly, and their yield products do not cause disturbance to the environment [3]. Mold is the main microorganism that produces cellulase enzymes. Types of molds that produce these enzymes include *Aspergillus* spp., *Trichoderma* spp. and *Penicillium* sp. One type of mold that is useful at producing cellulase and amyloglucosidase enzymes is *Aspergillus niger*. *Aspergillus niger* produces enzymes that are classified as extracellular enzymes. This enzyme functions to break down complex molecules into simple molecules.

In addition to cellulase enzymes, *Aspergillus flavus, A. niger*, and *Penicillium* sp. are known to produce the amylase, hemicellulase, catalase, pectinase, and xylanase enzymes [4],[5]. Amylase and cellulase enzymes can break down long-chain polysaccharides into glucose monomers or short chains by breaking alpha- and beta1,4-glycoside bonds. This causes a decrease in polysaccharide levels after fermentation. The sugar will then be used by microorganisms as a carbon source and produce biomass. Mycelial protein from this biomass is also important in increasing protein value after fermentation [5].

2.2 Types of Molds

1) *Aspergillus niger*

Aspergillus *niger* is a species of mold from the genus *Aspergillus* which is most widely used in fermented feed ingredients. Based on the results of [6] study, the *Aspergillus niger* mold is a non-hazardous species of the *Aspergillus* genus because it does not produce mycotoxins. The use of *Aspergillus niger* in fermented feed can increase the value of crude protein and reduce crude fiber. For example, the fermentation of feed made from cocoa waste using *Aspergillus niger* can increase the crude protein content from 8.80% to 12.34% and reduce the crude fiber content from 18.2% to 11.05% [7].

Figure 1. *Aspergillus niger*

2) *Trichoderma* spp.

Trichoderma spp. is known to produce commercial cellulase enzymes and can induce different enzymes according to the substrate used. *Trichoderma* spp. can also produce other enzymes, namely protease, lipase, and pectinase. According to [8], *Trichoderma* spp. is a parasitic mold that can attack other molds and steal their nutrients. Mechanisms used by *Trichoderma* spp. antagonist agents against pathogens are mycoparasites and antibiosis. In addition, the mold *Trichoderma* spp. has advantages such as easy isolation, broad adaptability, the ability to grow rapidly on substrates, extensive microparasitism, and is not pathogenic [9]. The use of *Trichoderma* spp. in fermented feed can increase the value of crude protein and reduce crude fiber. For example, fermented feed with feed ingredients, namely coffee cherries which fermented by *Trichoderma* sp. contains 13.67% protein, 26.95% crude fiber, and 1.03% crude fat. *Trichoderma* was able to provide a difference (decrease) in the crude fiber content of 2.19%, this indicated that *Trichoderma* mold was able to break down the fiber bonds of the coffee berry skin during the fermentation process [10].

Figure 2. *Trichoderma* spp.

3) *Rhizopus* sp.

Rhizopus sp. is known to the Indonesian people because it is applied for fermenting soybeans with the final product being tempeh. This mold belongs to the Mucoraceae family, genus Rhizopus, order Mucorales, and class Zygomycetes. As the *Rhizopus* sp. aged, it changes color from whitish to brownish-gray and reaches a height of about 10 mm. There are various species of the genus *Rhizopus*, including *R. arrhizus*, *R. microspores*, *R. oligosporus*, *R. oryzae,* and *R. stolonifer*. *Rhizopus* produces important enzymes such as amylase, lipase, and protease [11]. The utilization of *Rhizopus* sp. in feed fermentation can increase feed buoyancy. For example, the fermentation of a commercial sinking feed mixed substrate made from cassava pile and duckweed using *R. oryzae* initiates the buoyancy of the fermented product. Fermented floating feeds absorb water up to about 4 times their initial dry weight, whereas commercial floating feeds absorb up to about 2 times their initial dry weight. For three hours, both fermented and commercial floating feed floated 100% without sinking [12].

Figure 3**.** *Rhizopus* sp.

3. FERMENTATION OF FEED INGREDIENTS USING MOLD

3.1 Definition of Fermentation

According to [13], fermentation is a process of altering a compound into another compound using microorganisms which are carried out under aerobic or anaerobic conditions. Fermentation is also the process of breaking down organic compounds into simpler compounds by involving microorganisms [14]. Fermentation is one way to increase the biological value of the material. Through the biosynthesis of vitamins, essential amino acids, and proteins, the fermentation process can increase the nutritional value of a material and facilitate its digestion by reducing the crude fiber content [15].

3.2 Fermentation Method

Fermentation can be done by two methods based on moisture and substrate content. Fermentation varies into submerged culture fermentation with a moisture content of about 90%, and solid substrate fermentation with a moisture content of 40-75%. Solid substrate fermentation is usually applied to ferment plantation by-products (palm and coconut oil cake), food crops (rice bran and wheat polish), and the agricultural industry (cassava skin, cassava pile from tapioca factories) for feed ingredients. Compared to submerged cultures with higher water content, solid substrate fermentation is considered better because the volume of the fermentation process is lower. Harvesting in solid substrate fermentation is easier because the microorganism cells with the remaining substrate do not need to be separated, while cells with centrifugation or filtration in submerged cultures need to be separated. This separation process certainly increases the cost of feed production. The rest of the substrate in solid substrate fermentation still has nutritional value because it contains polymer substrates that are easier to digest or simpler and contain hydrolytic enzymes that play a role in fish digestion [4].

3.3 Potential and Constraints of Mold Utilization

Fermentation is biological processing, namely processing by utilizing microorganisms that will form enzymes to create transformations to complex molecules such as proteins, carbohydrates, and fats into simpler molecules. Fermentation has the potential to utilize agricultural and plantation wastes such as carrots, cocoa, and coffee skin wastes as alternative feeds. This is an effort to deal with rising feed prices by making feed alternatives that are easy to produce and relatively affordable [10]. Fermentation using mold can increase the value of crude protein and reduce the content of crude fiber. This is in accordance with the results of research employing cocoa pod shell powder feed ingredients with *Aspergillus niger* mold which can reduce crude fiber by 51.48%, increase protein content by 78.67% and reduce the content of fiber fractions. Likewise, *Lamtoro* leaf flour which was fermented experienced a decrease in crude fiber by 46.61% and an increase in protein by 18.25%.

Fermentation using mold also has problems that require to be cautious for. Mold can be a pollutant or toxic to feed or feed ingredients, thus, it has the opportunity to instigate disease in farmed fish. The disease is caused by mold (mycoses) or its toxic metabolites (mycotoxicosis). The infection process is characterized by the presence of pathogenic mold contamination in the feed, then the mold will invade fish that have low immunity. Diseases caused by mold are easier to treat than toxins that already enter the fish. The secondary metabolites of mold metabolism are mycotoxins. Mycotoxins are cytotoxic, capable of damaging cell structures, and damaging important cell formation processes such as DNA, RNA, and proteins. There are five types of mycotoxins that need to be watched out for because they are harmful to health, namely aflatoxins, fumonisins, ochratoxins, trichothecene, and zearalenone [16].

4. QUALITY IMPROVEMENT OF FEED INGREDIENTS THROUGH FERMENTATION EMPLOYING MOLD

The high cost of obtaining protein-sourced feed ingredients such as fish meals and soybean meals has led to the widespread use of alternative feed ingredients. Fermentation has been used to process high-fiber feed's material. Fermentation is a method that can be apply to improve the quality of feed ingredients. The utilization of mold in fermentation allows the reorganization of material components that are difficult to digest into more easily digestible ones, thus increasing their nutritional value. The following are the results of research on the mold fermentation application in feed.

Table 1. Quality Improvement Through Fermentation of Various Feed Ingredients

Mold Species	Result	Reference
Trichoderma sp.	The feed ingredient is coffee cherries skin fermented by *Trichoderma* sp. and has a protein content of 13.67%, crude fiber of 26.95%, and crude fat of 1.03%. *Trichoderma* can give a difference (decrease) content of crude fiber by 2.19%, this show that *Trichoderma* is capable to break bond in fiber skin coffee cherries during the fermentation process	[10]
Rhizopus sp.	Floating fish feed fermented by *Rhizopus* sp. has buoyancy ≥ 95% for 60 minutes, with stability in water without aeration of 80.3-87.1%. However, in aerated water,	[17]

	the buoyancy of fermented feed decreases to 0-2.5%	
Rhizopus oryzae	Fermentation of a mixture of commercial sinking feed, cassava pile and duck weeds using *R. oryzae* gave rise to the fermented product buoyancy. Fermented floating feeds absorb water up to about 4 times their initial dry weight, whereas commercial floating feeds absorb up to about 2 times their initial dry weight. For three hours, both fermented and commercial floating feed floated 100% without sinking.	[12]
Phanerochaete Chrysosporium and *Neurospora crassa*	Fermentation of durian fruit waste with comparison inoculum dose of *Phanerochaete chrysosporium* mold and *Neurospora crassa* (1:1) has the highest protein value of 17.79%	[7]
Aspergillus niger	Fermentation of seaweed flour (*Sargassum* sp.) using *Aspergillus niger* resulted in a crude fiber content of 15.90% and ash content of 11.76%	[18]

5. CONCLUSIONS

Based on the results of this literature review, mold fermentation can improve the quality of fish feed. The nutritional value of a feed can be increased by decreasing the crude fiber content, increasing the crude protein value, and increasing the buoyancy of the feed. This can be a solution for the supply of quality feed ingredients at a more economical price.

6. REFERENCES

[1]. Ali, A. (2005). “Basic Microbiology Volume I”. State University of Makassar-Press. Makassar.
[2]. Waluyo, L. (2004) “General Microbiology” UMM Press. Malang
[3]. Akhtar, M. (1997) “Method of enhancing bio-pulping efficacy” US patent 005. 620.564.
[4]. Purwadaria, M.B.T., Sinurat, A.P. Haryati, T., Sutikno, I. Supriyati and Dharma, J. (1998). “Correlation between the activity of mannanase and cellulase enzymes on the fiber content of fermented palm sludge with *Aspergillus niger*” *J. Livestock and Veterinary Science* 3(4), pp 230-235.
[5]. Iyayi, E.A. (2004). “Change in Cellulose, Sugar, and Crude Protein Contents of Agro-industrial by-products Fermented with *Aspergillus niger, Aspergillus Flavus,* and *Penicillium* sp.” *Afr J Biotechnol*. 3, pp 186-188.
[6]. Gray, W.D. (1970) “*The Use of Fungi as Food and in Food Processing*” Ohio, CRC Press.
[7]. Guntoro, E.J. (2017) “Comparison of Composition and Inoculum Dosage of *Phanerochaete chrysosporium* and *Neurospora crassa* in Animal Feed Fermentation Process” *Husbandry Stock* 1(1), pp 1-7.
[8]. Purwantisari, S. (2009) “Isolation and identification of *indigenous rhizosphere fungi* of potato plants from organic potato farms in Pakis Village, Magelang” *BIOMA Journal*. I11 (2), pp 45.
[9]. Arwiyanto, T. (2003). “Biological control of bacterial wilt of tobacco” *Indonesian Journal of Plant Protection* 3(1), pp 54-60.
[10]. Daning, D.R., and Karunia, A.D. (2018) “Fermentation Technology Using *Trichoderma* sp Mold to Improve the Nutritional Quality of Coffee Peels as Ruminant Animal Feed. *Agriekstensia Journal* 17(1), pp 70-76.
[11]. Bujang A, Taib, N.A. (2014). “Changes in amino acid content in soybean, garbanzo bean, and groundnut during pretreatments and tempeh making” *Malaysian Science*. 43, pp 551-557.

[12]. Zaman, A.B., Sriherwanto, C., Yunita, E., and Suja'i, I. (2018). "Physical Characteristics of Non-Extruded Floating Fish Feed Made Through *Rhizopus oryzae* Fermentation" *J Biotechnology Biosains Indonesia* – 5(1), pp 27-35.
[13]. Stephanie, and Purwadaria, T. (2013) "Fermentation of Cassava Skin Solid Substrates as a Poultry Feed Material" *Wartazoa* 23(1), pp 15-22.
[14]. Pamungkas, W. (2011). "Fermentation Technology, Alternative Solutions in Efforts to Utilize Local Feed Materials" *Aquaculture Media* 6(1), pp 43-48.
[15]. Oboh, G. (2006). "Nutrient enrichment of cassava peels using a mixed culture of *Saccharomyces cerevisiae* and *Lactobacillus* spp solid media fermentation techniques" *Process Biotechnology* 9(1), pp 550-554.
[16]. Ahmad, R.Z. (2009) "Mold contamination in feed and its control" *Journal of Agricultural R&D, 28(1),* pp 15-22.
[17]. Sriherwanto, C., Sujai, I., and Soraya, S. (2017). "Utilization of *Rhizopus* sp. as a biological agent for floating fish feed" *Journal of Indonesian Mycology* 1(2), pp 70-81.
[18]. Suryaningrum, L.H., and Samsudin, R. (2018). "Quality Improvement *of Sargassum* sp. as a fish feed ingredient using mold fermentation" *Proceedings of the 10th National Fish Seminar*, pp 649-657.