Evaluation of the Use of Various Microbes in *Lemna* sp Fermentation: A Review

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ABSTRACT

The use of Lemna sp as a fish feed ingredient has a very good opportunity to add nutritional value to commercial feed. However, the high content of crude fiber will make it difficult for fish to digest Lemna sp in fresh conditions. Fermentation is one way to reduce the levels of crude fiber contained in lemna, improve digestibility, and have an aroma favored by fish. Some fermentation processes are carried out with the addition of probiotics. Probiotics are a consortium of microbes such as Lactobacillus sp., Bacillus sp., Pseudomonas sp., Azotobacter sp., Saccharomyces sp., Aspergillus sp., Rhizopus oryzae, Rhodopseudomonas palustris. These probiotics can be an alternative to improve the nutritional quality of Lemna sp through the fermentation process.

Keyword: *Fermentation, Lemna sp., Protein, Nutrition, Microbes, Nutrition*

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1. Introduction

Lemna sp or duckweed is an aquatic plant that belongs to the monocotyledons of the Lemnaceae family and is classified as a higher plant or macrophyte. Lemna is easily propagated because it reproduces vegetatively and is easy to obtain to serve as fish feed. *Lemna* sp includes aquatic plants that live by floating on the surface of the water. According to Andriani, lemna can live and thrive in rice fields and ponds [1]. Under optimal conditions such as nutrient availability, sunlight, and ideal temperature, lemna can double its biomass in just two days [2]. Ansal reported that the productivity of lemna planted with an effective cropping system reached 12−38 tonnes dry weight/ha/year [3]. The use of *Lemna* sp as feed does not compete with human needs so it has the potential as an alternative feed source that can be used as a sustainable feed supply. These aquatic plants have fulfilled the requirements for determining feed raw materials including It has high nutritional value, is easy to obtain, does not contain toxins, is relatively inexpensive, and does not compete with human needs [4].

According to Hillman and Culley, *Lemna* sp as a natural protein source has a better amino acid composition than most other vegetable proteins and is more like animal protein [5]. The use of lemna as fish feed can be given directly in fresh, dry, or pre-processed form. This plant contains up to 18-43% protein dry weight and can be used without further processing as a complete feed for fish [6]. According to Iskandar, the nutritional content of *Lemna* sp is 15.92% protein, 18.37% crude fiber, 2.70% fat and 43.4% carbohydrates [7]. In addition, lemna also contains N as much as 0.75 mg/L, P as much as 0.33-3.0 mg/L, K as much as 100 mg/L, Ca as much as 360 mg/L, Mg as much as 72 mg/L, Na as much as 250 mg/L, and Fe as much as 100 mg/L [8]. *Lemna* sp has the potential as an alternative forage for several types of fish because it contains high crude protein reaching 10-43% dry weight [9]. However, *Lemna* sp has a barrier, namely the high crude content. According to Sutriana, the high crude fiber content in the feed will make it difficult for fish to digest the feed [10]. Meanwhile, the need for crude fiber for maximum fish growth is 10% [11].

The high content of crude fiber in feed can inhibit the ability of digestion and absorption in the digestive system of fish. According to Virnanto, the fermentation process can reduce the high crude fiber [12]. In addition, fermentation can also increase the nutritional value of a material. Astuti reported that fermentation increased the content of folic acid, niacin, riboflavin, nicotinamide, and pyridoxine [13].

2. Fermentation

According to Khairudin, fermentation is a process to increase the digestibility of the material because the fermented material changes from being difficult to digest to single-cell protein which is more easily digested [14]. Fermentation is a way to reduce crude fiber by overhauling long polymer chains from protein to amino acids, fats to essential fatty acids, and carbohydrates to simple sugar acids, so that the feed is easily absorbed and digested by the fish's body [15]. The fermentation process increases the content of vitamin B12, B complex vitamins and C, lysine, and tryptophan as well as various minerals [16] [17].

The fermentation process using probiotics has a more distinctive smell (attractant) that stimulates fish to approach and consume the feed given [18]. Abun stated that fermented feed ingredients would produce an aroma that fish liked [19]. Fermented feed has several advantages, including the shelf life of the feed being longer because during the fermentation process there is a decrease in pH thereby inhibiting spoilage bacteria. According to Surono, that antibacterial activity is produced during fermentation especially lactic acid accompanied by a decrease in pH [20]. A decrease in pH will inhibit the growth of *E. Coli* bacteria which grow optimally at pH 6 -7. The length of the fermentation process can also affect the increase in protein content because the longer the time used in fermentation, the faster the increase in the biomass of microorganisms [7].

3. Probiotic Microbes

3.1 Bacillus

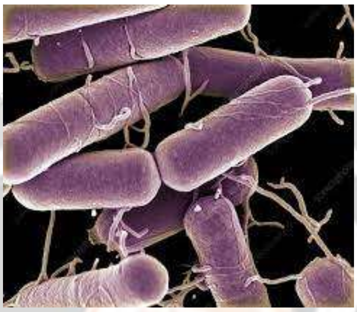

Figure 1: *Bacillus* sp.

Bacillus subtilis can improve digestibility well. This bacterial activity forms colonies and attaches to the intestines of fish. This will urge pathogenic bacteria to grow so that it will not inhibit the digestive process in fish [21]. *Bacillus subtilis* is also capable of producing protease and lipase enzymes which can degrade amino acids and increase growth [22].

Bacillus sp. bacteria play a role in breaking down proteins into amino acids. These amino acids are then used by bacteria to reproduce themselves, so as to increase feed protein and reduce crude fiber, so as to increase protein and carbohydrates in feed [23]. According to Wizna research, the fermentation process using *Bacillus amyloliquefaciens* as an inoculum can increase the crude protein content by up to 36% and reduce the crude fiber content by up to 32% [24]. Meanwhile, according to Santoso, fermentation can lead to improvements in the properties of raw materials, such as increasing digestibility and creating a preferred taste and aroma [25].

The increase in protein after fermentation was caused by the presence of *Bacillus* sp. which belongs to the sacrolytic bacteria, namely bacteria that can decompose disaccharides or polysaccharides into simpler molecular groups. Furthermore, *Bacillus* sp. will decompose proteins into amino acids [26]. Changes in nutrient content are thought to be caused by an overhaul of the substrate during fermentation where the organic matter in the lemna undergoes decomposition by microorganisms present in probiotics.

3.2 Saccharomyces

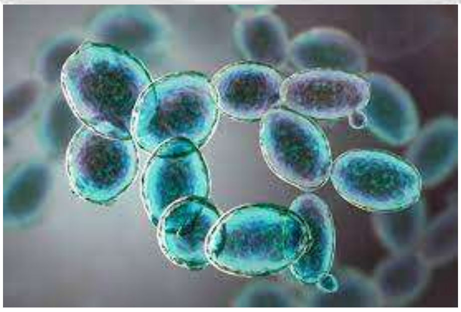

Figure 2: *Saccharomyces* sp.

Saccharomyces sp. has a chemical composition consisting of 50-52% crude protein, 30-37% carbohydrates, and 7-8% minerals, as well as vitamins, especially vitamin B, so it can be used as a probiotic and immunostimulant [27]. *Saccharomyces cerevisiae* will grow better under aerobic conditions but will ferment sugar much faster under anaerobic conditions [28].

According to Razak, the addition of *Saccharomyces cerevisiae* bacteria can increase the activity of peptidase, protease, and amylase enzymes in the digestive tract of fish so that the feed will be easily absorbed and increase fish growth [29]. In line with Rachmawati's statement, feed containing *Saccharomyces* sp. can increase protein digestibility so that the use of feed becomes more efficient [30]. The use of *Saccharomyces* sp. feed is also an immunostimulant and prevents disease caused by pathogenic bacteria or viruses because it contains essential ingredients such as β 1.3 glucan [31].

3.3 Lactobacillus

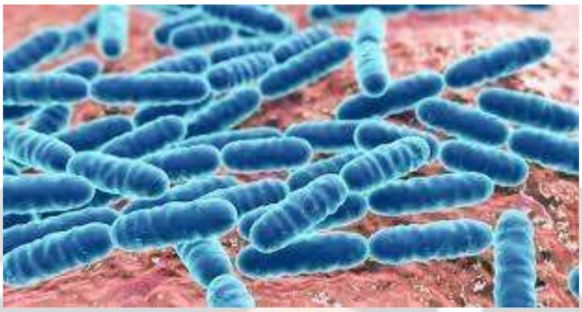

Figure 3: *Lactobacillus* sp.

Lactobacillus bulgaricus is a homofermentative bacterium that mainly produces lactic acid through the process of glycolysis or glucose breakdown [32]. This microbe is a gram-positive bacterium, in the form of non-sporing cocci or rods, generally the optimum growth temperature is 40° - 45°C. According to Syadillah, *Lactobacillus* sp. plays a role in increasing fish appetite due to the production of attractants through an anaerobic fermentation process [33].

Lactobacillus sp. is a type of bacteria that is often used as a probiotic for aquaculture activities. These bacteria can break down carbohydrates (glucose) into lactic acid which can lower the pH thereby stimulating the production of endogenous enzymes to increase nutrient absorption, feed consumption, growth, and inhibit the growth of pathogenic bacteria [34]. *Lactobacillus* sp.bacteria are useful for fermenting organic matter into lactic acid compounds, photosynthetic bacteria which function to absorb toxic gases and heat from the fermentation process, yeast which has a role in fermenting organic matter into alcohol compounds, sugars and amino acids [35].

Sandra mentioned that *Lactobacillus* sp., can produce cellulase enzymes that can break down crude fiber [36]. The activity of *Lactobacillus* sp.. bacteria, which are contained in probiotics, can produce lactic acid from sugars and carbohydrates produced by photosynthetic bacteria and yeast. According to Arief, *Lactobacillus* sp.bacteria play a role in balancing digestive tract microbes so that they can increase the digestibility of fish by converting carbohydrates into lactic acid which can lower pH, thereby stimulating the production of endogenous enzymes to increase nutrient absorption, feed consumption, growth and inhibit pathogenic organisms [37]. Ahmadi added that *Lactobacillus* sp.in probiotics will also increase the secretion of proteolytic enzymes (feed digestibility) breaking down protein into amino acids which can be absorbed more quickly by the intestine [21].

4. Improved Nutrition of Lemna Through Fermentation Process

Improvement of nutrition in Lemna *sp* through a fermentation process with a microbial consortium, dosage, incubation period, and different results are shown in Table 1.

Table 1: Various Research Results on Increasing Lemna Nutrition through the Fermentation Process

Mikrobial type	Dose and incubation period	Result	Reference
Lactobacilus sp. *Bacillus licheniformis* and *Saccharomyce*	-	Increasing protein by 22.5%, reducing carbohydrates by 9%, and reducing crude fiber by 26.2%.	[7]

cervisiae.			
Lactobacilus sp. Bacillus licheniformis and *Saccharomyce cervisiae.*	Probiotic dose of 5% and incubation period for 7 days	Increased levels of crude protein from 13.22% to 18.64% and decreased crude fiber from 20.08% to 12.45%.	[38]
Lactobacillus casei, *Saccharomyces cerevisiae*, and *Rhodopseudomonas palustris*	Probiotic dose 0.3% incubation period of 7 days	Increasing the water content by 91.15%, crude protein by 5.60% and crude lipid by 5.76% and can reduce the ash content by 3.93% and crude fiber by 15.27%.	[39]
Trichoderma harzianum	The number of inoculums was 3,107 microbes / 100 grams of substrate and the fermentation time was 24 hours	The increase in the crude protein content of the substrate from 18.19% to 19.07% and the decrease in the crude fiber content of the substrate from 15.1% to 3.6%.	[40]
Lactobacillus sp., *Bacillus sp.*, *Azotobacter sp.*, *Saccharomyces sp.*, *Aspergillus oryzae*, *Rhizopus oryzae*, and *Pseudomonas sp.*	Probiotic dose 0.3% incubation period for 10 days	Reducing crude fiber content by 9.02% and increasing protein by 29.29%	[41]

5. Conclusion

Based on the description above, it can be concluded that the use of microbes can be done as an effort to improve the nutritional quality of *Lemna* sp through a fermentation process. Given the huge potential that *Lemna* sp has as a plant that is easy to breed and easy to find. So, the utilization of *Lemna* sp by fermentation can be an alternative solution in processing lemna into fish feed

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