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Utilization of Fermentation Lemna as a Feed Source on the Growth of Sangkuriang Catfish (*Clarias* Sp.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This research aims to determine the nutritional content and the best treatment for the addition of *Lemna* sp. fermented for the growth of sangkuriang catfish. The research was conducted from February to August 2022, in Aquaculture Laboratory of the Fisheries and Marine Science Faculty, Universitas Padjadjaran. The method used in this research is experimental, and is of a Completely Randomized Design (CRD). It consists of four treatments and four repetitions, namely treatment A (control) treatment B (10% fermented lemna + 90% commercial feed), Treatment C (20% fermented lemna + 80% commercial feed), and Treatment D (30% fermented lemna + 70% commercial feed). Parameters observed were changes in nutrient content of lemna, daily growth rate, feed conversion ratio, survival rate, and water quality. Data were analyzed using ANOVA analysis of variance and if there was a significant difference, Duncan test was performed with a 95% confidence level, while changes in nutrients in lemna and water quality were analyzed descriptively. The results of the proximate analysis showed that the fermented lemna using BIOM-S probiotics experienced changes in the nutritional content, namely an increase in protein content

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from 25.24% to 33.66% and a decrease in crude fiber from 11.93 to 9.13%. Based on the results of this research, the treatment C (20% fermented lemna + 80% commercial feed) the best results in a daily growth rate at 1.92%/day, a feed conversion ratio at 1.41, and a survival rate of up to 97.5%.

Keywords: Sangkuriang catfish; lemna; fermentation; growth.

1. INTRODUCTION

Sangkuriang catfish is one of the most widely cultivated freshwater fishery commodities in Indonesia. The advantages of sangkuriang catfish include high production, faster harvest time, superior meat quality, more resistance to disease, and simple maintenance techniques [1]. Based on the Ministry of Maritime Affairs and Fisheries, in 2017-2018 catfish production increased by 114.82% from 841.75 thousand tons to 1.81 million tons [2].

Feed is one of the factors that greatly affect the growth of fish. Fish feed needs to be balanced with complete nutrition. Of the various types of nutrients needed by fish, protein is the main source of energy needed by fish because it plays an important role in fish growth. Fulfillment of protein in feed supplied from fish meal still relies on imports so that it has an impact on high feed prices [3]. In 2019, the percentage of feed prices to the selling price of catfish was 63% [4].

Lemna sp. or commonly called duckweed is an aquatic weed that can live anywhere (cosmopolitan) [5]. *Lemna* sp. growth is difficult to control so that within two days it can double its biomass under optimal conditions [6]. The protein content in *Lemna* sp. is quite high at 13.22% [7]. Therefore, *Lemna* sp. has great potential as a source of vegetable protein for alternative fish feed [8,9].

The main obstacle in using *Lemna* sp. is high crude fiber reaching 20.08% [7]. The high crude fiber content can inhibit the digestive and absorption ability of the fish digestive system. One way to overcome this is the fermentation process [10]. Fermentation is the processing of materials with the help of microorganisms by breaking down long polymer chains from protein to amino acids, fats to essential fatty acids, and carbohydrates to simple sugar acids [11]. Fermented feed ingredients are able to improve nutritional value and produce an aroma that is favored by fish [5].

The application of the addition of fermented lemna flour has been carried out by many studies and has a positive effect on the growth performance of several fish species. Research results showed that lemna fermentation using probiotics was able to increase protein by 22.5% and decrease crude fiber by 26.2%. The given of fermented lemna by 40% to nilem fish (*Osteochilus hasselti*) provide the best feed conversion ratio of 3.61, the highest daily growth rate of 0.75%, and survival rate up to 100% [12].

Giving the fermented lemna also gave the best growth for pomfret (*Colossoma macropomum*). The results showed that the using lemna minor flour fermented of 20% in feed formulation provided the best result were feed consumption of 0.65%, feed efficiency of 0.052%, relative growth rate of 0.090%/day, and survival rate of 100% [13]. A similar study was conducted by [14] the substitution of lemna flour in the feeding of Rainbow Trout (*Oncorhynchus mykiss*) as much as 20% gave the best results on growth performance and fillet quality. Obtained Specific growth rate of 1.25 ± 0.03%/day, Food conversion rate of 1.18 ± 0.03 grams, and Survival rate of 98 ± 1%.

The purpose of this research was to determine the effect of using probiotics in the fermentation process on the nutritional content of lemna and how big the percentage of addition of fermented lemna flour in the feed to increase the optimal growth rate of sangkuriang catfish.

2. METHODOLOGY

2.1 Research Materials

The materials used in this research are as follows: a) Sangkuriang catfish seeds measuring 8 - 10 cm with a weight of ± 6.15 g were obtained from Cileunyi, Kab. Bandung. b) *Lemna* sp. comes from nature, namely the fishing area in Lembang, Kab. West Bandung. c) BIOM-S is a commercial probiotics used for fermentation of lemna from the isolation of vegetable and fruit waste. Produced from Biomethagreen Rumah Edukasi, Tanjungsari. d) Commercial feed in the form of pellets PF 1000 with 39-41% protein content, 5% fat, 6% crude fiber, 16% ash content, and 10% moisture content produced by PT Matahari Sakti. e) CMC (Carboxymethyl cellulose) is used to glue the test feed ingredients. f) Molasses is a fermenter solution used as a medium for bacterial growth during the fermentation process.

2.2 Research Methods

The method used in this research is an experimental method using a Completely Randomized Design (CRD) consisting of 4 treatments and each 4 replications. The treatment on this research was in the form of giving different doses of fermented lemna used in fish feed as follows:

Treatment A = 100% commercial feed (control)
Treatment B = 10% fermented *Lemna* sp. + 90% commercial feed
Treatment C = 20% fermented *Lemna* sp. + 80% commercial feed
Treatment D = 30% fermented *Lemna* sp. + 70% commercial feed

2.3 Working Procedure

2.3.1 Preparation Phase

2.3.1.1 Test Feed Making

The collected lemna is washed and sorted to separate it from dirt and adhering organisms using clean water. Then drain. Lemna is dried by drying it directly under the sun. The dried lemna samples were analyzed proximately to determine the nutritional content.

Fermentation is done by mixing ± 2 kg of lemna with a fermenter solution in the form of 10 ml water and 3% molasses. BIOM-S probiotic added as much as 3 ml/liter. All ingredients are stirred until homogeneous. The resulting mixture is then put into a jar. Incubation for 9 days at room temperature under anaerobic conditions. Next, it is dried in the sun. The dried fermented lemna is ground into flour. Samples of fermented lemna flour were analyzed by proximate test to determine the nutritional content.

Commercial feed is ground using a grinding machine. The fermented lemna flour and commercial feed were mixed according to the predetermined treatment. Add 2% CMC and enough hot water. Stir the ingredients until it becomes a dough. Next, pelletizing. The molded feed was dried using an oven at a temperature of ±60oC. Then, the feed was cut into crumbles according to the fish's mouth opening.

2.3.1.2 Preparation of containers and test media

Before use, the aquarium and fiber tub were washed using detergent and rinsed thoroughly. Dry the aquarium in the sun. Next, the aquarium is filled with 40 liters of precipitated water. Heaters, hoses and aeration stones are installed in each aquarium.

2.3.1.3 Preparation of Test

Fish Test fish were acclimatized for 7 days in order to adjust the feeding during the study. The day before the study, the fish were rotated to remove the effect of the rest of the feed on the fish's body. Before the fish are put into the aquarium, the fish are weighed to determine the initial weight and determine the dose of feed given. The stocking density for each aquarium is 1 fish/2 liters of water [15].

2.3.2 Implementation Phase

Maintenance carried out for 40 days. The test fish were fed three times a day at 08.00, 12.00, and 16.00 WIB as much as 3% of the fish biomass [16]. Siphoning and water changes are carried out every 2 days to clean the rest of the feed and fish waste. Measurement of water quality pH, dissolved oxygen, and ammonia was carried out once every 10 days while the temperature was carried out every day. Growth (weight and length of fish) was carried out every 10 days. The number of fish that died was recorded during maintenance.

2.4 Observation Parameters

2.4.1 Nutritional content of lemna

Lemna before and after the fermentation process was tested proximately to determine changes in nutritional content. The proximate analysis tested included water content, ash, protein, crude fiber, crude fat, carbohydrates, and metabolic energy.

2.4.2 Specific growth rate

$$\text{SGR (\%/day)} = \frac{lnWt - lnWo}{t} \times 100\%$$

Information:

Wt = Average weight of test fish at the end of observation (g)
Wo = Average weight of fish test at the beginning of observation (g)
t = Length of observation period

2.4.3 Feed conversion ratio

$$\text{FCR} = \frac{F}{(Wt + D) - Wo}$$

Information:

F = Amount of feed given during the observation (g)
Wt = Biomass at the end of the observation (g)
Wo = Biomass at the beginning of the observation (g)

D = Weight of dead fish during the observation (g)

2.4.4 Survival rate

SR (%) = $\frac{Nt}{No}$ × 100%

Information:

Nt = Number of fish that live at the end of the observation (tail)
No = Number of fish that live at the beginning Observations (tail)

2.4.5 Water quality

Observations of water quality on the maintenance media included water temperature, pH, Dissolved Oxygen (DO), and Ammonia.

2.5 Data Analysis

Nutrient content in *Lemna* sp. and water quality were analyzed descriptively. Specific Growth Rate (SGR), Feed Conversion Ratio (FCR) and Survival Rate (SR) were processed using analysis of variance (ANOVA) to determine whether there were significant differences between treatments. If significantly different treatments were found, Duncan's test was carried out as a follow-up test with a 95% confidence level to compare between treatments, so that the best treatment could be determined.

3. RESULTS AND DISCUSSION

3.1 Nutritional Content of Lemna

Fermentation is one way of processing feed ingredients that can produce a simpler final product by changing organic matter involving microorganisms [9]. The results of fermented products can increase digestibility and improve the nutritional value of local feed raw materials so that they can be used as fish feed [10]. Based on the results of the fermentation that has been carried out, there is a change in the nutritional content of the lemna which is presented in Table 1.

Based on the proximate test analysis in Table 1, the results of fermentation using BIOM-S probiotics containing *Lactobacillus* sp., *Saccharomyces* sp., and *Bacillus* sp. [17]. There was a change in nutrition, namely an increase in protein value and a decrease in crude fiber. In line with the statement of [9], that the fermentation process is beneficial which can increase protein content and reduce crude fiber. Changes occur due to the reshuffle of the substrate so that the organic matter in the lemna is decomposed by microorganisms derived from probiotics. In accordance with [10], fermentation results are highly dependent on the substrate (basic material), inoculum (type of microbes), and environmental conditions.

The increase in protein to 33.66% came from S. *cerevisiae* which is a single cell protein (PST). *Lactobacillus* sp. in probiotics also increase the secretion of proteolytic enzymes (digestibility of feed) by breaking down protein into amino acids that can be absorbed more quickly by the intestines [18]. With the fermentation process, the energy requirements used will be less so that the excess energy will be used for growth. Protein content in lemna has met the SNI required by catfish, which is at least 30%.

Table 1. Changes in the Nutritional Value of Lemna

Nutrient Content	Before Fermentation (%)	After Fermentation (%)	Changes (%)
Water (%)	11,2	9,93	+11,34
Ash (%)	13,15	13,25	-0,76
Protein (%)	25,24	33,66	-33,36
Crude Fiber (%)	11,93	9,13	+23,47
Fats (%)	1,31	1,28	+2,29
Carbohydrates (%)	48,37	42,68	+11,76
metabolic energy (kkal/kg)	3631	3787	-4,30

Source: Results of Laboratory Analysis of Ruminant Livestock Nutrition and Food Chemistry Livestock Faculty of Animal Husbandry UNPAD 2022

There was a decrease in crude fiber to 9.13% due to the ongoing fermentation process which caused changes in the cell wall due to the hydrolysis process by microbes. The hydrolysis process is able to degrade and break lignocellulosic and lignohemicellulose bonds and dissolve silica and lignin contained in the cell walls of fibrous feed ingredients [19]. Crude fiber is needed in small amounts to help the digestive process in accordance with the statement of [20], that the classification of feed ingredients has crude fiber below 18%. The High content of crude fiber makes it difficult for fish to digest food so that it can slow down fish growth [21].

3.2 Specific Growth Rate

Growth is defined as the increase in length and weight of fish in a certain period of time [22]. Changes in the average weight of sangkuriang catfish can be seen in Fig. 1.

Based on Fig. 1, the average weight obtained from the highest to the lowest at the end of the study, namely treatment C (20% fermented *Lemna* sp. + 80% commercial feed, B (10% fermented *Lemna* sp. + 90% commercial feed), A (control), and D (30% fermented *Lemna* sp. + 70% commercial feed) with average weights, respectively namely 13.19 grams, 11.86 grams, 10.78 grams, and 9.90 grams. Fish growth in the first ten days has not increased significantly because energy is used for the adaptation process to feed and the environment. According to [23] statement, the energy contained in the feed is not all used for growth but for all body activities and maintenance through the basal metabolic process.

Based on Table 2, the specific growth rate in treatment C was significantly different from treatment A, B, and D. The highest growth rate was in treatment C (20% fermented *Lemna* sp. + 80% commercial feed) of 1.92%/day and treatment the lowest was D (30% fermented *Lemna* sp. + 70% commercial feed) of 1.20%/day. In accordance with the SNI [24], that the value of the specific growth rate is more than 1%. The greater the specific growth rate, the greater the use of feed for growth.

The increase in growth rate is due to good quality feed, which contains sufficient protein for fish needs. In line with [25] statement, that the growth rate of fish is influenced by the type and quality of feed and good environmental conditions. Fish growth occurs due to excess energy or the amount of digested feed is greater than the fish's needs for body maintenance, metabolism and activity [26]. According to [27], the availability of energy in feed that comes from non-protein (fats and carbohydrates) will increase protein reserves used for growth (protein sparing effect).

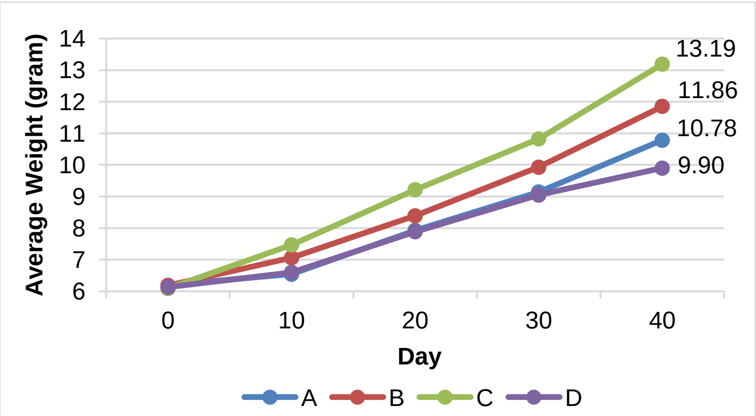

Fig. 1. Graph of Increase in Average Weight of Sangkuriang Catfish

Table 2. Average SGR of Sangkuriang Catfish during Maintenance

Treatment	SGR (%/day)
A (Control)	1,37±0,23[a]
B (10% fermented *Lemna* sp. + 90% commercial feed)	1,63±0,06[b]
C (20% fermented *Lemna* sp. + 80% commercial feed)	1,92±0,23[c]
D (30% fermented *Lemna* sp. + 70% commercial feed)	1,20±0,07[a]

Note: Values followed by the same lowercase letters show no significant difference at the 95% confidence level (F. Count > F. Table 0.05)

The high growth rate is suspected to be due to the use of probiotics in the fermentation process of feed ingredients containing microorganisms. *Bacillus subtilis* contained in probiotics is able to improve digestibility well because it is able to produce protease and lipase enzymes that can degrade amino acids and increase growth [28].

According to [29], the addition of fermented cocoa pods (*Theobroma cacao*) as raw material for sangkuriang catfish (*Clarias gariepinus*) feed was able to provide a daily growth rate of 0.99 g/day. While the research conducted by [30], the use of fermented mangrove propagule flour (*Rhizophora mucronata*) was able to provide the best growth rate for African catfish at 1.33%/day. This proves that the administration of fermented lemna in feed can be digested well by sangkuriang catfish and produces a higher growth rate.

3.3 Feed Conversion Ratio

Based on the results of the feed conversion ratio from observations made for 40 days, it can be seen in Table 3.

From the results, it was found that treatment C which was the addition of 20% fermented *Lemna* sp., was the best treatment that was able to produce the lowest feed conversion ratio. This was because Treatment C was significantly different from treatment A, B, and D. Factors that affected the low feed conversion ratio were feed quality and quantity, species, size, and water quality [31]. Feed effectiveness can be seen from the size of the feed conversion ratio.

A low FCR value indicates that the feed is of good quality so that by giving a little feed it can produce maximum weight. According to [32] stated that feed quality is influenced by nutrient composition, the ability of fish to digest and absorb feed nutrients, as well as the presence of microorganisms contained in probiotics. This is due to the fermentation process in the feed raw materials used so that there is an increase in the gastrointestinal tract to absorb and digest feed. *Lactobacillus* sp. plays a role in breaking down carbohydrates (glucose) into lactic acid which can lower the pH. In acidic conditions, it is able to increase the secretion of proteolytic enzymes (digestibility of feed) to break down protein into amino acids that can be absorbed more quickly by the intestines [18].

According to [33], the low value of the feed conversion ratio is due to the good response of fish to the feed given. The fermentation process is able to produce an aroma that is favored by fish due to the production of attractants by *Lactobacillus* sp. in probiotics through an anaerobic fermentation process so that it can affect fish appetite and support fish in responding to food [34]. Feed absorption is faster when attractants are added to the feed [33].

3.4 Survival Rate

Survival is the percentage of fish that are alive at the end of rearing. Based on data analysis regarding the survival rate of Sangkuriang Catfish the results are shown in Table 4.

Based on Table 4, it shows that there is no significant difference between treatments. The survival rate of sangkuriang catfish ranged from 93.75 to 98.75%. The highest sangkuriang catfish survival was in treatment A (control) which was 98.75% and the lowest was in treatment D (30% fermented *Lemna* sp. + 70% commercial feed) which was 93.75%. The results obtained are classified as good in accordance with the statement [35], that the survival rate is classified as good if 50%. Survival rate during rearing has met the SNI [16] that the survival rate of catfish in IV nursery is at least 80%.

Most fish deaths occurred at the beginning of the study, namely on the first day to the tenth day of the rearing period. It is suspected that the fish are experiencing stress due to the fact that they are still in the adaptation stage to the environment and the treatment given. According to [35], factors that affect fish survival are the adaptability of fish to food and the environment, stocking density, fish health status, and good water quality to support fish growth. The cause of the death of sangkuriang catfish is also due to its natural nature, namely cannibalism eating the same sex. This is marked by the loss of part of the body of the fish that died due to being gnawed by other fish. Factors that influence cannibalism are feed availability, high stocking density, water clarity, light intensity, feeding frequency and feed availability and varying sizes [36].

3.5 Water Quality

Water quality is something that needs to be considered in the maintenance of sangkuriang catfish. Based on the results of measurements of water quality in the maintenance of sangkuriang catfish for 40 days, it is obtained in Table 5.

Table 3. Average FCR of Sangkuriang Catfish during Maintenance

Treatment	FCR
A (Control)	1,37±0,23[a]
B (10% fermented *Lemna* sp. + 90% commercial feed)	1,63±0,06[b]
C (20% fermented *Lemna* sp. + 80% commercial feed)	1,92±0,23[c]
D (30% fermented *Lemna* sp. + 70% commercial feed)	1,20±0,07[a]

Note: The values followed by the same lowercase letters show no significant difference at the 95% confidence level (F. Count > F. Table 0.05).

Table 4. Average SR of Sangkuriang Catfish during maintenance

Treatment	SR (%)
A (Control)	98,75±0,50[a]
B (10% fermented *Lemna* sp. + 90% commercial feed)	96,25±0,50[a]
C (20% fermented *Lemna* sp. + 80% commercial feed)	97,5±1,00[a]
D (30% fermented *Lemna* sp. + 70% commercial feed)	93,75±0,96[a]

Note: The values followed by the same lowercase letters show no significant difference at the 95% confidence level (F. Count > F. Table 0.05).

Table 5. Water quality of Sangkuriang Catfish Rearing

Treatment	Parameter			
	Temperature (^oC)	DO (mg/L)	pH	Ammonia (mg/L)
A	27-31	4,4-6,6	6,53-7,97	0,002 - 0,012
B	26-30	3,6-6,5	6,46-8,19	0,004 - 0,015
C	26-31	3,8-6,8	6,5-8	0,004 - 0,023
D	26-31	4,1-6,7	5,83-8,1	0,008 - 0,021
SNI*	25-30	min. 3	6,5-8	Maks. 0,1

*Source: *SNI 6484.3:2014; Note: A = 100% Commercial Feed (Control); B = 10% fermented Lemna sp. + 90% commercial feed; C = 20% fermented Lemna sp. + 80% commercial feed; D = 30% fermented Lemna sp. + 70% commercial feed*

The results of temperature measurements during sangkuriang catfish rearing ranged from 26-31ºC. The temperature during maintenance fluctuates and does not meet the SNI [16], which is in the range of 25-30^{o}C. However, the increase in temperature can still be said to be feasible for the life of sangkuriang catfish because according to research [37], the average temperature obtained from the maintenance of sangkuriang catfish is between 25-31.5^{o}C. The content of dissolved oxygen (DO) during maintenance has an optimal range for the life of sangkuriang catfish, which is between 3.6-6.8 mg/L. The results obtained are in accordance with the SNI [16] of at least 3 mg/L.

The degree of acidity or pH in the maintenance medium ranged from 5.83 - 8.19. The results of pH measurements showed that the maintenance media did not meet the SNI [16], namely 6.5 – 8. Research by [38], a good pH for catfish growth ranges from 6.5 to 9. The results obtained during maintenance are still within the tolerance limit to support the life of sangkuriang catfish. The limit of ammonia based on SNI [16] is a maximum of 0.1 mg/L. The concentration of ammonia obtained during maintenance ranged from 0.002 to 0.023 mg/L. These results have met the requirements for the survival of sangkuriang catfish.

4. CONCLUSION

Based on the research that has been done, the following conclusions can be drawn:

1. Fermentation with probiotics was able to improve the nutritional value of fermented lemna, among others, an increase in protein content from 25.24% to 33.66% and a decrease in crude fiber from 11. 93% to 9.13%.
2. The treatment C (20% fermented lemna + 80% commercial feed) the best results in a daily growth rate at 1.92%/day, a feed conversion ratio at 1.41, and a survival rate of up to 97.5%.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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