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Effect of Using Fermented *Lemna* sp. in Fish Feed on Growth Rate of Nilem Carp (*Osteochilus hasselti*)

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ABSTRACT

This research aims to determine the highest level of fermented *Lemna* sp. used in artficial feed to produce optimum growth rate of Nilem Carp. This research was conducted from March to July 2016, in the Ciparanje Laboratory and 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 five treatments and three repetitions, providing one commercial diet (as control) and four fermented *Lemna* sp. levels (10%, 20%, 30%, and 40%). Collected data are analyzed with analysis of variance F-test at 95% confidence level. The parameters observed in this research are divided into four sub-parameters: nutrition changes of fermented *Lemna* sp., daily growth rate, feed conversion ratio and survival rate. The crude fiber content of *Lemna* sp. was reduced from 18.37% to 13.57%, and protein content increased from 19.37% to 23.47%, respectively, after fermentation. Based on the results of this research, fermented *Lemna* sp. used in fish feed at 40% level produced the best results: a daily growth rate at 0.75%, a feed conversion ratio at 3.61 and a survival rate of up to 100%.

***Keywords*:** Daily growth rate, Feed conversion ratio, Fermentation, *Lemna* sp., Nilem carp, Survival rate, *Osteochilus hasselti*

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1. INTRODUCTION

Nilem fish is one of the freshwater fish cultivation commodities which are concentrated in Java, especially in West Java. Based on economic and environmental sustainability, nilem fish farming can be profitable. In the third quarter of 2015, the production of nilem fish increased by 7.19% compared to the second quarter of 2014.

In aquaculture activities, feeding is one of the most important things. Fish feed is the largest contributor to production costs, namely 50-70% of operational costs. Meanwhile, the main sources of protein that is often used in making fish feed are fish meal and soybeans, which compete with food and animal feed.

Therefore, there is a need for alternative feed ingredients that are cheap, have a good quality, and locally available. Sources of non-conventional feed ingredients as an alternative such as macrophytes are known to have been used as additional feed since the beginning of freshwater aquaculture.

Lemna is known to have considerable potential as an alternative source of raw material for fish feed because it has high productivity and locally available. Duckweed species are often difficult to identify because of their small size and elusive flowers [9]. Duckweeds often grow in thick blanket-like mats on still, nutrient-rich, fresh and slightly brackish waters. Lemna tend to have high protein content ranging from 22 - 38% in dry weight. Lemna grown in water that is rich in nutrients and have a high concentration of minerals, potassium, phosphorus, and pigments (carotene and xanthophyll), which makes Lemna could be used as an important supplementary food for fish.

Lemna also have high productivity and able to double their biomass just within two days at optimal conditions.

The downside of using Lemna as a fish feed ingredient is caused by its high crude fiber content that up to 20% which could inhibit growth in fish seeds. One of which efforts to overcome this weakness is to undertake fermentation using commercial probiotics on Lemna. Fermentation by utilizing organisms found in probiotics is considerably easier and cheaper. Fermentation results could improve the nutritional value of the product and the properties of basic ingredients such as increasing digestibility, eliminating toxic compounds, enrich the taste and smell that are favored by fish.

Based on the description above, it is necessary to ferment *Lemna* sp. using probiotics to determine the increase of nutritional value occurs after fermentation and also the effect of fermented Lemna used as an alternative raw material in the fish feed mixture on the growth rate of nilem fish. This research has never been done before.

2. MATERIALS AND METHODS

2. 1. Materials

The materials used on this research are as follows: a) The seeds of nilem fish sized 7-10 cm obtained from BBIAT Ciparay; b) *Lemna* sp. which comes from the cultivation process with 2.5% bio-slurry dose of the water volume; c) Aquasimba-D commercial probiotics containing Saccharomyces sp. cultivated from cassava starch; d) PF-800 commercial feed in the form of floating pellets.

2. 2. Research Methods

The second phase of the research design was carried out by the experimental method. This research used a completely randomized design (CRD) with five levels of treatment and three repetitions. Each treatment container was filled with ten nilem fish. The treatment on this research was in the form of giving different doses of fermented Lemna used in fish feed as follows:

Treatment A = Feed without a mixture of fermented Lemna products
Treatment B = Feed with a mixture of 10% fermented Lemna products
Treatment C = Feed with a mixture of 20% fermented Lemna products
Treatment D = Feed with a mixture of 30% fermented Lemna products
Treatment E = Feed with a mixture of 40% fermented Lemna products

2. 3. Research Procedure

The research was conducted through several stages including: a) Fermentation of Lemna sp. using probiotics; b) Test feed preparation (Table 1); c) Preparation of fish sample included seven days acclimatization by spreading 1 tail per 2 liters of water volume and fish sample fasted for one day before being observed in a container;

Table 1. Fish Feed Composition.

Treatment	Ingredients		
	Commercial Feed	Fermented Lemna	Tapioca
A (0%)	100%	0%	0%
B (10%)	89%	10%	6%
C (20%)	78%	20%	4%
D (30%)	66%	30%	2%
E (40%)	54%	40%	1%

The observation phase was carried out for 42 days where the fish sample were given fish feed as much as 5% of the weight of fish biomass. Squeezing is done to clean the remaining feed and dirt every morning before the treatment is given. Sampling is carried out every seven days including weight measurements and measurements of water quality including temperature, pH and dissolved oxygen.

2. 4. Observation Parameters

2. 4. 1. Nutritional Value of Lemna Fermentation Results

Lemna fermentation results were analyzed descriptively based on proximate data obtained including changes in nutrient content that occurred before and after the fermentation process.

2. 4. 2. Daily Growth Rate

$$G = \frac{lnWt - lnWo}{t} \times 100\%$$

Information:

G = Daily growth rate
Wt = Biomass at the end of observation (g)
Wo = Biomass 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:

FCR = Feed conversion ratio
F = Number of fish feed given during the observation (g)
Wt = Biomass at the end of observation (g)
Wo = Biomass at the beginning of observation (g)
D = Dead fish biomass during the observation (g)

2. 4. 4. Survival Rate [22]

$$SR = \frac{Nt}{No} \times 100\%$$

Information:

SR = Survival Rate
Nt = Number of fish at the end of maintenance (tail)
No = Number of fish at the beginning of maintenance (tail)

3. RESULT AND DISCUSSION

3. 1. Fermented *Lemna* sp.

Based on the proximate test results that have been carried out, it can be seen that the fermentation process causes changes in the nutrient content of the lemna in the form of increased ash and protein levels and a decrease in water content, crude fiber, crude fat, and carbohydrate (Table 2).

Changes in nutrient content are thought to be caused by a change in the substrate during fermentation where organic matter in the lemna is decomposed by microorganisms found in probiotics. That enzymes produced by microbes in the fermentation process could improve the nutritional value of fish feed.

Table 2. Nutritional Value of *Lemna* sp.

Nutrient Content	Before Fermentation (%)	After Fermentation (%)	Changes (%)
Water	94.12	7.45	-92.1
Ash	15.92	20.76	30.4
Protein	19.17	23.47	22.5
Crude Fiber	18.37	13.57	-26.2
Fats	2.70	2.29	-15
Carbohydrates	43.84	39.91	-9

Source : * Proximate Test Results of Nutrition Laboratory of Animal Husbandry Faculty Unpad

The decrease in water content by 92.1% is thought to occur after lemna through the drying process and broached. It also has an impact on increasing ash levels because ash content based on dry matter will tend to be higher than ash content based on wet material. Besides the increase in ash content indicates the high mineral content of the substrate. In accordance with the statement that ash content indicates the amount of mineral content contained in feed. Crude fiber content which has decreased by 26.2% is thought to be caused by a compound reshuffle that occurs on the substrate.

The activity of cellulase enzymes produced by yeast in probiotics can hydrolyze cellulose to glucose. The increase in protein content of 22.5% after fermentation was thought to be due to the growth of yeast Saccharomyces sp in the media. Increased protein content can be caused by two possibilities in accordance with namely an increase due to the presence of mycelium which contains a lot of protein and a result of changes in other substances such as a decrease in crude fiber.

In addition, it is known that the length of the fermentation process can also affect the increase in protein levels because the longer the time used in fermentation, the faster the increase of biomass from microorganisms. Carbohydrates that have decreased due to the activity of the amylase enzyme during fermentation which remodel carbohydrates to fulfill microbial metabolism during the fermentation process. Referring which states that microbial growth requires energy derived from carbohydrates, fats, minerals and other nutrients that causes a decrease in nutrient content

3. 2. Grow Rate

Growth rate is the result of metabolic processes in the body that cause weight gain and length in a certain period of time. The results of the observation showed that the nilem fish increased the average weight in each treatment with the highest average weight values found in treatment E (40%) which reached 18.76 g (Figure 1).

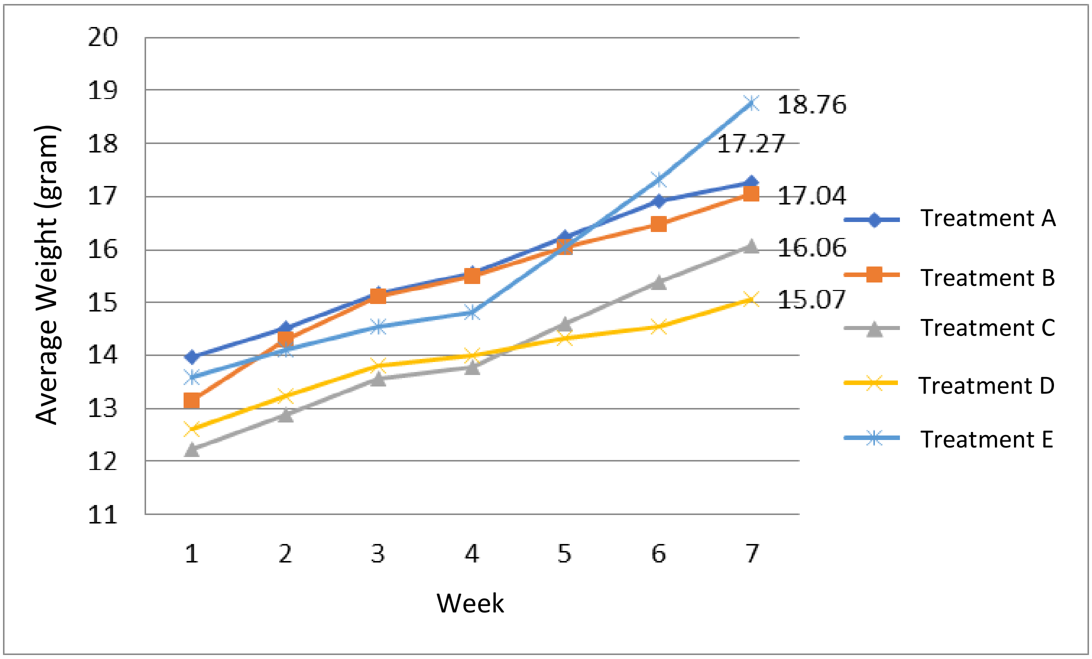

Figure 1. Graph of average increase in weight of Nilem fish

The results of analysis of variance at the 5% test level showed that the daily growth rate in the five treatments did not have a significant difference (Table 3). Treatment A (control) has a daily growth rate of 0.49% which shows that weight gain averaged 0.49 g / day, whereas in the treatment given a mixture of lemna fermented results showed a greater value of daily growth rate large as in treatment B (10%) with a daily growth rate of 0.60%, treatment C (20%) of 0.64%, and the highest value found in treatment E (40%) of 0.75%, while the lowest value was found in treatment D (30%) of 0.42%.

Table 3. Average Value of Daily Growth Rate of Nilem Fish.

Treatment	Average of DGR values (%)
A (0%)	0.49 ± 0.1921[a]
B (10%)	0.60 ± 0.2048[a]
C (20%)	0.64 ± 0.3632[a]
D (30%)	0.42 ± 0.0774[a]
E (40%)	0.75 ± 0.0914[a]

Description: Values followed by the same lowercase letter are not significantly different at 95% confidence level.

This shows that the value of the daily growth rate of nilem fish with a fermented lemna feed mixture ranging from 0.42% -0.75% is still better when compared to the administration of fresh lemna which ranges from 0.11% -0.29%. During maintenance, nilem fish shows a poor response. Even though the feed is eaten immediately when given, there is still food that is not consumed. This can be caused by the fact that fish are easier to feel full when fed with high fiber content. Feeds that contain high-fiber vegetable sources, cause fish energy to be used a lot for cellulose digestion. Daily growth rates that tend to be slow can also be caused by insufficient feed energy because not all feed eaten by fish can be used for growth. Most of the energy from food is used for metabolism, the rest is used for activity, growth, and reproduction.

3. 3. Feed Convertion Ratio

The results of the feed conversion ratio values from observations made for 42 days showed that, some feed treatments had a lower conversion value when compared to the control treatment using commercial feed. Based on observations made it can be seen that the feed conversion ratio value for the treatment with a mixture of *Lemna* sp. fermentation results ranged from 3.61-7.66 while the feed conversion ratio in the treatment of commercial feed without the mixture of lemna (control) had a value of 6.74.

Table 4. FCR Values of Each Treatment During Research.

Treatment	FCR values
A (0%)	6.74 ± 3.6317^{a}
B (10%)	5.65 ± 1.9499^{a}
C (20%)	6.39 ± 5.2824^{a}
D (30%)	7.66 ± 1.8224^{a}
E (40%)	3.61 ± 0.4504^{a}

Description: Values followed by the same lowercase letter are not significantly different at 95% confidence level.

Based on the results of the variance analysis test it is known that the feed conversion value of each treatment did not have a significant difference (Table 4). The lowest feed conversion ratio is found in treatment E (40%) with a value of 3.61. The value of feed conversion ratio in the administration of *Lemna* sp. fermentation results in treatment B (10%), C (20%), and E (40%) have a lower conversion value when compared to the treatment of commercial feed (control), while the highest feed conversion ratio is shown in treatment D (30 %) with a value of 7.66. Variable feed conversion values for each treatment showed that there were differences in the quality and quantity of nutrients contained in each feed along with different responses in each treatment given to nilem fish seeds. The high value of feed conversion is thought to be caused because there is still residual feed that is wasted and not utilized by nilem fish seeds.

The value of the high feed conversion ratio is an indication that the feed used is not good for fish production, while the smaller the value of the feed conversion ratio, the better the quality and efficiency of the feed given. In addition to the nutrient content in feed, digestive capacity and the ability to digest low protein in herbivorous fish can also be a factor that influences the value of feed conversion.

3. 4. Survival Rate

The survival rate of nilem fish in each treatment showed values ranging between 99.05% -100%. The high survival rate of nilem fish is also supported by stocking density, nutrition, water quality and space availability.

Table 5. Survival Rate (SR) of Nilem Fish.

Treatment	Average of SR values (%)
A (0%)	100 ± 0.000^{a}
B (10%)	99.05 ± 1.6512^{a}
C (20%)	100 ± 0.000^{a}
D (30%)	99.05 ± 1.6512^{a}
E (40%)	100 ± 0.000^{a}

Description: Values followed by the same lowercase letter are not significantly different at 95% confidence level.

Based on the results of analysis of variance (Table 5) shows that the treatment given does not provide a significant difference in the survival rate of nilem fish. This shows that the addition of *Lemna* sp. fermented products in feed up to the level of 40% do not have a negative influence on the survival rate of nilem fish. Referring to factors that influence the high and low survival include competitor, density, population, age and the ability of an organism to adapt to its environment. Deaths that occur are thought to be due to the limited space for fish to experience increased weight and higher metabolism while the living space of fish remains the same [38,39]. Environmental is a key factor for the survival of aquatic organisms, and any changes insalinity may affect various physiological processes in organisms.

4. CONCLUSIONS

The conclusions obtained from this research shows that fermentation using probiotics succeed in reducing crude fiber content by 26.2% and increasing protein content by 22.5% in fermented Lemna. The given of fermented Lemna products by 40% in the fish feed mixture provide the best feed conversion ratio of 3.61, the highest daily growth rate of 0.75%, and survival rate up to 100%.

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References

[1] Dossou S, Koshio S, Ishikawa M, Yokoyama S , Dawood M.A.O, El Basuini M.F, El-Hais A.M, and Olivier A. 2018. Effect of partial replacement of fish meal by fermented rapeseed meal on growth, immune response and oxidative condition of red sea bream juvenile, Pagrus major. *Aquaculture.* 490: 228-235.

[2] Kim H.S. and Cho, S.H. 2019. Dietary inclusion effect of feed ingredients showing high feeding attractiveness to rockfish (Sebastes schlegeli Hilgendorf 1880) on the growth performance, feed utilization, condition factor and whole body composition of fish (II). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. 231: 66-73.

[3] Bardach, J. E., Ryther, J. H., McLarney, W. D. 1972. Aquaculture: the farming and husbandry of freshwater and marine organisms. Wiley-Interscience, New York.

[4] Andriani Y, Irawan B, Iskandar, Zidni I, and Partasasmita R. 2019. Short Communication: Diversity of duckweed (Araceae-Lemnoideae), morphological characteristics and its potentials as food sources for herbivorous fishes in West Java, Indonesia. *Biodiversitas* Volume 20, Number 6, 1617-1623

[5] Landesman, L, N. C. Parker, C. B. Fedler, and M. Konikof. 2005. Modeling duckweed growth in wastewater treatment systems. *Livestock Research for Rural Development,* 17 (6)

[6] Ansal M.D., Dhawan A, Kaur VI. 2010. Duckweed based bio-remediation of village ponds: An ecologically and economically viable integrated approach for rural development through aquaculture. *Livestock Research for Rural Development.* 22: 7.

[7] Azer SA. 2013. Taxonomic revision of genus Lemna (Lemnaceae Gray) in Egypt. *Annals Agri Sci* 58(2): 257-263.

[8] Ceschin S, Abati S, Leacche I, Zuccarello V. 2018. Ecological comparison between duckweeds in Central Italy: the invasive Lemna minutavs. the native L. minor. *Plant Biosyst* 152(4): 674-683.

[9] Ceschin S, Della Bella V, Piccari F, Abati S, 2016. Colonization dynamics of the alien macrophyte Lemna minutaKunth: a case study froma semi-natural pond in Appia Antica Regional Park(Rome, Italy). *Fund Appl Limnol* 188(2):93-101

[10] Aslam S, and Zuberi A. 2017.Effect of duckweed by replacing soybean in fish feed on growth performance of Grass carp (Ctenopharyngodon idella) and Silver carp (Hypophthalmichthys molitrix). *Int J Fish Aquat Stud* 5: 278-282.

[11] Nopriani, U., Karti P.D.M.H, Prihantoro, I. 2014. Productivity of duckweed (Lemna minor) as alternative forage feed for livestock in different light intensities. *JITV* 19(4): 272-286.

[12] Mohapatra SB. 2015. Utilization of water hyacinth (Eichhornia crassipes) meal as partial fish protein replacement in the diet of Cyprinus carpiofry. *Eur J Exper Biol* 5: 31-36

[13] Watanabe, T. 1988. Fish Nutrition and Marine Culture. JICA Texbook. The General of Aquaculture Course. Departemen of Aquatic. *Biosciense.* Pp. 238.

[14] Ogunji, J., Toor, R.U.A.S., Schulz, C. and Kloas, W. 2008. Growth Performance, Nutrient Utilization of Nile Tilapia Oreochromis niloticus Fed Housefly Maggot Meal (Magmeal) Diets. *Turkish Journal of Fisheries and Aquatic Sciences.* 8 (1) : 141-147.

[15] Estrada G.T, Mijangos L.M.R, Romero L.A.M, González D.N.T, Salem A.Z, Mlambo V, Cuellar S.E.M, Reyes M.G, and Pleigo A.B. Potential impacts of dietary Lemna gibba supplements in a simulated ruminal fermentation system and environmental biogas production. *Journal of Cleaner Production.* 181: 555-561.

[16] Poedjiadi, A. 2006. Dasar – Dasar Biokimia. Edisi Revisi. Jakarta: UI - Press.

[17] Ali S, and Kaviraz A. 2018. Aquatic weed Ipomoea aquatica as feed ingredient for rearing Rohu, Labeo rohita (Hamilton). *The Egyptian Journal of Aquatic Research.* 44 (4): 321-325.

[18] Pagarra, H. 2010. Pengaruh Lama Fermentasi dengan Ragi Tape Terhadap Kadar Glukosa pada Umbi Gadung (Disocorea hispida DENNST). *Bionature* Vol. 11 (1): p. 7-13

[19] Limbu S.M. 2019. The effects of on-farm produced feeds on growth, survival, yield and feed cost of juvenile African sharptooth catfish (Clarias gariepinus). *Aquaculture and Fisheries.* 1-7. https://doi.org/10.1016/j.aaf.2019.07.002

[20] Omasaki S.K, Janssen K, Besson M, Komen H. Economic values of growth rate, feed intake, feed conversion ratio, mortalityand uniformity for Nile tilapia. *Aquaculture.* 481: 124-132.

[21] Katopodis C, Cai Lu, and Johnson D. 2019.Sturgeon survival: The role of swimming performance and fish passage research. *Fisheries Research.* 212: 162-171.

[22] Fu C, Cui Y.L, Xu J.J, Wang Y, and Fu S.J. 2019. A study on the physiological and behavioral mechanisms underlying the differences in survival capacity between two cyprinid fish species. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology.* 231: 74-81.

[23] Cuesta,A., Carrion, R.L., Martin del Rio, M.P., Meseguer, J., Mancera, J.M., Esteban,M.A., 2005. Salinity influences the humoral immune parameters of gilthead seabream(Sparus aurataL.). *Fish Shellfish Immunol.* 18, 255–261.

[24] Saravanan M, Ramesh M, Petkam M, and Poopal R.K. 2019. Influence of environmental salinity and cortisol pretreatment on gill Na+/K+−ATPase activity and survival and growth rates in Cyprinus carpio. *Aquaculture Reports.* 11: 1-7.