Growth and nutrient value of tilapia (*Oreochromis niloticus*) fed with *Lemna minor* meal based on different fermentation time

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Abstract. *Lemna minor* is abundant in nature especially in swamp waters, having high nutrient contents, such as 23.47% of crude protein, 3.99% of crude fat, 29.92% of crude fiber, and 19.02% of nitrogen-free extract material. However, the crude fiber of *L. minor* is difficult to digest by fishes, therefore the fermentation process was done. The study aimed to examine the growth, biomass, and nutrient content of Nile tilapia (*Oreochromis niloticus*) fed by *L. minor* leaf meal with different fermentation time. The *L. minor* meal was treated at 0, 7, 14, and 21 days. Parameters that analyzed were Total Feed Consumption (TFC), Feed Utilization Efficiency (FUE), Protein Efficiency Ratio (PER), Relative Growth Rate (RGR), Survival Rate (SR), water quality, amino acid, and fatty acid profiles of tilapia. The results showed that *L. minor* meal fermentation had a significant effect ($p < 0.05$) to TFC, FUE, PER, and RGR, while it did not have a significant effect ($p > 0.05$) to SR. The utilization of *L. minor* meal with 7 days of fermentation gave the best result of 104.43 g for TFC, 64.68% of FUE, 1.91% of maximum PER, and produced 1.90% day^{-1} of maximum RGR. The best quality of tilapia nutrient content was at tilapia fed by *L. minor* meal with 7 days fermentation time.
Key Words: fermentation time, *Lemna minor* meal, natural feed, nutrient content, production.

Introduction. Based on government target in 2018, the total production from aquaculture has set for 11.8 million tons (Rimmer et al 2013), it makes a significant increase of nile tilapia production. The biggest problem in tilapia production is a high cost of feed, while the feed is an important factor for increasing tilapia's growth and its production. Therefore, it is necessary to find a cheap and environmentally friendly feed alternative.

Several studies had developed a natural feed alternative to increase Nile tilapia (*Oreochromis niloticus*) production (Herawati et al 2015; Novelli et al 2017). One of the natural feed alternatives is *Lemna minor* (Asimi et al 2018). *L. minor* is one of the duckweeds which has 14-43% protein and sufficient amino acids for the growth of Nile tilapia. The protein content of *L. minor* is higher than *Azolla piñata* and *Salvinia molesta* (Solomon & Okomoda 2012). Newly harvested *L. minor* contain up to 43% protein by dry weight, while *Azolla piñata* has 25-35% of protein content, and *Salvinia molesta* has 12.4% of protein content (Asimi et al 2018; Kathirvelan et al 2015). Unfortunately *L. minor* has high crude fiber content which is difficult to digest by fish, therefore a method is needed to reduce the crude fiber content of *L. minor*. One of the methods to reduce the crude fiber content in *L. minor* is through fermentation. Virnanto et al (2016) stated that the fermentation process could reduce crude fiber content. Besides, fermentation could increase the nutrient value of a feed ingredient. The objective of this study was to analyze the growth and nutrient value of Nile tilapia fed by *L. minor* meal at different fermentation time. *L. minor* has been chosen because it has higher protein content than

Azolla piñata and *Salvinia molesta* so that it will improve the growth performance and nutrient value of nile tilapia.

Material and Method. The study was conducted at Aquaculture Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Science, Diponegoro University from April 2018 to July 2018. Nile tilapia were obtained from Siwarak Fish Seed Center, Semarang with 5.40±0.06 g fish^{-1} of average weight. Nile tilapia were selected then put into the containers with 15 fish container^{-1} of stocking density. Acclimatization was done for one day without giving feed to the fish. The water quality during the study was maintained in ideal condition at 28-30^{o}C of temperature, dissolved oxygen (DO) at 3.0 mg L^{-1}, and pH at 8.1–8.2. The optimum water temperature for Nile tilapia aquaculture is between 25-32^{o}C, furthermore, the optimum condition of DO and pH value for tilapia rearing are ≥ 3 mg L^{-1} and 6.0-8.5, respectively (El-Sayed & Kawanna 2008).

The *L. minor* was taken from waters then was dried and then made into a meal/flour, then *L. minor* meal was added with probiotics (*Lactobacillus* and *Saccharomyces cerevisae,* 1 mL each), it was added by 1:1 of molasses as an activator into 100 mL of water, and stored for ±3 hours in room temperature (Zahidah et al 2012; Yuniwati et al 2012; Warasto et al 2013). The *L. minor* meal then was added with an activator and fermented for 0 (A), 7 (B), 14 (C), and 21 (D) days. All treatments were conducted by three replications. The ratio of activator and *L. minor* meal were 3:10 (Warasto et al 2013). The fermented *L. minor* meal of each treatment was added by other feed ingredients to meet 100 grams of feed formulation (fish meal, soybean meal, bran flour, wheat flour, corn flour, fish oil, corn oil, vitamins, mineral, and carboxy methyl cellulose (CMC)) and mixed with 35-40% of water from total ingredients. *L. minor* meal had 2.74 g/100 g of feed composition so that the feed formulation has 30% protein content based on its proximate analysis. The composition of feeds is presented in Table 1.

Table 1

Composition of feed for Nile tilapia aquaculture

Feed ingredients	*Feed (g)*
Fish meal	32.78
Soybean meal	31.99
Lemna minor meal	2.74
Wheat flour	7.79
Bran flour	9.92
Corn flour	4.78
Fish oil	2.00
Corn oil	2.00
Vitamin mix	5.00
CMC	1.00
Total	100

After the feed of each treatment were mixed, it were formed and dried using oven at < 50°C until the moisture content of feeds was constant. The products were then analyzed for their proximate composition, essential amino acid, and fatty acid profiles. The proximate chemical composition of the samples was determined using a standard procedure (AOAC 2000; Herawati et al 2017). The crude protein content was calculated by multiplying the total nitrogen factor. The carbohydrate content was estimated by the difference. The essential amino acid composition of the samples was determined using High Performance Liquid Chromatography (HPLC) (Shimadzu LC-6A) (AOAC 2000; Herawati et al 2018). The fatty acid composition of the samples was determined using a gas chromatograph (Shimadzu) (AOAC 2000; Herawati et al 2018). Feeding methods were based on SNI (1999), with satiation feeding method of three times a day at 08.00 a.m., 12.00 p.m. and 04.00 p.m. WIB (Western Indonesian Time) for 45 days.

Nile tilapia samples about 100 grams of dry weight of each treatment were analyzed for its Total Feed Consumption (TFC) based on Pereira et al (2007), Feed Utilization Efficiency (FUE) and Protein Efficiency Ratio (PER) based on Tacon (1987), Relative Growth Rate (RGR) based on Takeuchi (1988), and Survival Rate (SR). Water quality parameters such as temperature, pH, DO, NH_3 were analyzed based on SNI (2009). After the data were obtained, then they were analyzed using analysis of variance (ANOVA) with confidence intervals of 95% to see the effect of the treatments. Before analyzing the variance, the normality test, homogeneity test, and additivity test were first carried out. Normality test, homogeneity test, and additivity test were carried out to ensure the data is spread normally, homogeneous, and additive. Data is analyzed (F test) with confidence intervals of 95%. If there are significant differences ($p < 0.05$) in the analysis of variance, then Duncan's multiple range test will be conducted to find out the differences between treatments.

$$TFC = F1 - F2$$

where: TFC = total feed consumption;
F1 = the amount of initial feed (g);
F2 = the amount of leftover feed (g).

$$FUE = \frac{W_t - W_0}{F} \times 100$$

where: FUE = feed utilization efficiency (%);
W_t = total weight of fish at the end of study (g);
W_0 = total weight of fish at the beginning of study (g);
F = total feed consumed during the study (g).

$$PER = \frac{W_t - W_0}{P_i} \times 100$$

where: PER = protein efficiency ratio (%);
W_t = total weight of fish at the end of study (g);
W_0 = total weight of fish at the beginning of study (g);
P_i = total feed consumed multiplied by fish protein content.

$$RGR = \frac{W_t - W_0}{W_0 \times t} \times 100$$

where: RGR = relative growth rate (% day^{-1});
W_t = total weight of fish at the end of study (g);
W_o = total weight of fish at the beginning of study (g);
t = time of fish aquaculture (days).

$$SR = \frac{N_t}{N_0} \times 100$$

where: SR = survival rate (%);
N_t = total of fish at the end of study (fish);
N_0 = total of fish at the beginning of study (fish).

Results and Discussion. The composition after *L. minor* was fermented for 0 (A), 7 (B), 14 (C), and 21 (D) days were presented in Table 1, and proximate profile of feeds was presented in Table 2.

Table 2

Proximate profile of feeds

Parameters	*A*	*B*	*C*	*D*
Protein (%)	33.84	34.11	33.97	35.54
Nitrogen-free extract (%)	14.65	11.87	15.45	6.79
Lipid (%)	8.94	8.43	7.73	8.65
Energy (kcal)*	262.23	259.38	255.75	251.98
Ratio of E/P**	8.74	8.65	8.52	8.40

Sources: Laboratory of Feed and Nutrition, Faculty of Animal and Agriculture Diponegoro University (2017).
Note: *Based on calculated of DE (digestible energy) with protein assumption = 3.5 kcal/g, lipid = 8.1 kcal g^{-1}, Nitrogen-free extract = 2.5 kcal g^{-1} (Wilson 1977); **E/P for optimal growth of fish range between 8-12 kcal g^{-1} (Akand et al 1991).

The highest protein and lipid content of feed were at D and A treatments, respectively. The feeding of *L. minor* meal with different fermentation time to Tilapia (*O. niloticus*) gave a significant effect ($p < 0.05$) on TFC, FUE, PER and RGR, while it did not give a significant effect ($p > 0.05$) to SR. The results of the study on the growth of tilapia were presented in Table 3.

Table 3

Growth of tilapia fed by *L. minor* meal with different fermentation time

Parameters	*A*	*B*	*C*	*D*
IBW (g)	5.40 ± 0.03^{a}	5.55 ± 0.05^{a}	5.50 ± 0.04^{a}	5.55 ± 0.08^{a}
FBW (g)	14.85 ± 0.05^{a}	25.90 ± 0.03^{ab}	22.75 ± 0.02^{b}	19.77 ± 0.01^{b}
WG (g)	10.45 ± 0.09^{a}	20.35 ± 0.10^{ab}	17.25 ± 0.08^{a}	14.22 ± 0.07^{a}
RGR (%)	1.06 ± 0.02^{a}	1.90 ± 0.03^{b}	1.54 ± 0.03^{b}	1.34 ± 0.03^{b}
TFC (g)	80.57 ± 2.11^{b}	104.43 ± 3.10^{ab}	93.41 ± 3.19^{b}	85.88 ± 5.74^{b}
PER (%)	1.30 ± 0.08^{a}	1.91 ± 0.05^{a}	1.49 ± 0.06^{a}	1.32 ± 0.04^{a}
FUE (%)	44.14 ± 2.47^{a}	64.68 ± 0.06^{ab}	55.82 ± 0.05^{b}	52.96 ± 0.07^{b}
SR (%)	80.00 ± 6.67^{a}	90.20 ± 7.70^{b}	84.44 ± 5.12^{a}	80.00 ± 7.70^{a}

Note: Value ± standard deviation from three replications, values were followed with the same superscript letter shown that they are not significantly different.

The results of the study showed that the best biomass weight, TFC, FUE, PER, RGR, and SR values were at *L. minor* meal with 7 days fermentation treatment (B), with the value of 25.90±0.03 g; 104.43±3.10 g; 64.68±0.06%; 1.90±0.03%; 1.91±0.05%; 90.20±7.70%. The lowest value was at *L. minor* meal with 0 day fermentation treatment (A) that was 10.45±0.09 g; 80.57±2.11 g; 44.14±2.47%. Tilapia fed by *L. minor* meal with 7 days fermentation treatment (B) gave higher results than the other treatments, because *L. minor* with 7 days fermentation time had higher nutrient content especially protein compared to other treatments. In addition, 7 days of fermentation time causes the feed would have a texture which favored by tilapia. The preferred feed texture increased the value of feed consumption level.

Some fishes such as trout and tilapia, rely on their sight to find prey while other species use their sense of smell (Somerville et al 2014). Fish eyes were very similar to land animals, like birds and mammals, except for their more rounded lenses. The low feed intake value of treatment A was due to *L. minor* meal didn't fermented perfectly so that the feed was difficult to digest by the fish. Therefore, the fish had lower nutrient content than the one fed by perfectly fermented *L. minor* meal. The fermentation process is a biochemical process by microorganisms that occur enzymatically. Enzymes that work on the fermentation process can cause changes in feed. Changes that occur can be the taste, color, shape, calories, and other properties. Nwachi (2013) reported the function of probiotics in cultivation, which increases fish immunity to pathogens and greatly contributes to digestive enzymes of fish.

The longer fermentation time in *L. minor* meal will decrease the feed consumption level. The results of the study found that the value of feed utilization efficiency of tilapia fish fed by *L. minor* meal with 7 days fermentation (B) was 64.68±0.08 (Table 3). It was because the feed produced by (B) treatment was consumed more than other treatments, the feed is preferred by tilapia because of its better palatability. Fermentation using probiotic bacteria provides the benefits of increasing the nutrient content of the feed, this is because fermentation is an anaerobic dissimilation process of organic compounds by the activity of microorganisms or extracts from the cells of these microorganisms. The use of bacteria in aquaculture as an activator of nutrient regeneration to promote growth and enrich nutrients in larvae feed (Nwachi 2013). Tilapia feed provision using *L. minor* meal with 7 days fermentation (B) provides better feed utilization than tilapia which fed only with soybean meal because it has higher nutrient content. This is in accordance with study conducted by Olaniyi & Oladunjoye (2012) which stated that the provision of fermented *L. minor* meal contains better nutrient content so it preferred consumed by fishes. The addition of *L. minor* meal to tilapia (*O. niloticus*) gave a high value of feed utilization. Khasani (2013), high quality artificial feed has a high level of palatability. Fish quickly respond to feeds that have compounds which stimulate their sense of smell due to a chemoreceptor mechanism.

The low value of feed utilization at (A) treatment is assumed because the fermentation process of *L. minor* meal didn’t occur. Previous study conducted by Haetami (2012) stated that fish also have limitations to digest low-quality feed with high fiber content, so that it needs high protein feed for its growth. The ability of fish to digest the feed consumed depends on the presence or absence of an appropriate enzyme and conditions required by the enzyme to react with the substrate in the digestive tract of the fish. An alternative way to improve feed efficiency in order to be easily digested and enzymes can work more effective is by adding enzyme-producing probiotics in artificial feed.

The highest PER was obtained on tilapia fed by using *L. minor* meal with 7 days fermentation (B). The effectiveness of giving fish by (B) feed treatment gave a significant effect on PER because the energy content in feed is sufficient for its energy needs. Protein content of *L. minor* in this study before fermentation is 23.47%. After fermentation process, *L. minor* protein content has increased up to 32.12%, it is a factor of tilapia (*O. niloticus*) growth enhancement. The process of fermentation is to improve the nutrient value, especially reducing the content of crude fiber as an alternative feed ingredient of fish (Putri et al 2012). In addition, in the manufacture of feed, protein content is made almost the same that is 30%, it is suspected that there is an amino acid content suitable for tilapia in the treatment using *L. minor* meal with 7 days fermentation (B). Lysine content in *Lemna* has a value that almost equal to the needs of lysine amino acids for tilapia. The need for lysine amino acids for tilapia (*O. niloticus*) is 5.12% (Santiago & Lovell 1988).

PER value is influenced by the ability of fish to digest feed. This ability is influenced by several factors, one of them is feed composition. The results showed that the value of PER in this study was higher than previous studies conducted by the authors. Solomon & Okomoda (2012) stated that on the addition of *L. minor* for tilapia (*O. niloticus*), the highest PER value in the treatment of tilapia fed by *L. minor* meal with 7 days fermentation (B) is 1.91 g and the lowest value is at tilapia fed by *L. minor* meal with 0 day fermentation (A) is 1.30 g. Olaniyi & Oladunjoye (2012) stated that on the addition of *L. minor* for tilapia (*O. niloticus*), the highest PER value in the treatment of *Lemna* with 0% and 25% dose is 0.88 g. While the lowest value is at *Lemna* 100% dose treatment. Differences in protein values in this study with previous studies is because of differences in the value of *L. minor* meal protein content. The difference in protein content itself is due to differences in *Lemna* type and its region origin. The differences in study results are due to differences in duckweed species as well as the availability of local nutrients in which they are cultivated (Solomon & Okomoda 2012). Duckweed meal has similar protein content with soybean meal which used in feed formulations, and its utilization can reduce costs (Olaniyi & Oladunjoye 2012). The lowest PER value was 1.30 obtained at tilapia fed by *L. minor* meal with 0 day fermentation (A). This is because the fermentation process in *L. minor* meal did not occur, so there is no improvement in feed nutrient value. The fermentation process in addition to reducing the height of crude fiber, it also can increase the nutrient value of a material (Virnanto et al 2016). Utilization of protein decreases with the length of fermentation process in the feed. This is reinforced by Olaniyi & Oladunjoye (2012), the use of protein decreases progressively with the increasing process of fermentation, as the probiotic bacteria work progressively decreases. This can be due to low quality of *Lemna* protein compared to soybean meal.

The results shown the best RGR value was 1.90±0.03% day^{-1} at (B) treatment. The RGR value is directly proportional to the FUE value, so a high FUE value will be followed by a high RGR value as well. The fermentation process in *L. minor* meal is thought to increase the RGR value in tilapia. This is due to the high protein content in *L. minor* cause a good nutritional balance between vegetable and animal protein in the feed. *Lemna* sp. protein is generally rich in leucine and lysine (Dewanji 1993). When compared to soybean protein, *Lemna* sp. protein has higher amino acid contents. This is supported by Ovie & Eze (2013), when the feed contains the exact amount of essential amino acids required by the fish species, the ideal protein for the species is met so there is no shortage or excess amino acid. Fish that are growing and given such feed will use only a few amino acids for energy. Amino acids will be used efficiently for maintenance, health and synthesis of new structural proteins that will result in maximum feed efficiency and growth. The low growth of fish in (A) treatment is due to the low utilization of feed. The value of fish growth is better in the treatment of *Lemna* sp. compared to only fed by soybean meal composition (Solomon & Okomoda 2012). It is also suspected that soybean meal has tannin anti-nutrient substance.

The results of the growth rate of the fish decreased with increasing of *L. minor*. This is due to an inequality of amino acid profiles between the two feed ingredients. Reduced soybean meal will reduce the presence of important amino acids that are not contained in *Lemna* sp. This is supported by Hepher (1988), excess protein has an impact that can increase energy demand for protein catabolism. The result of protein catabolism is one of which nitrogen is released through ammonia. The process can improve its function to remodel unused proteins so that the growth energy will decrease, indicating that the length of the fermentation process in the *L. minor* meal provided results in lower growth. Previous study conducted by Olaniyi & Oladunjoye (2012) reported that tilapia (*O. niloticus*) fed by *L. minor* meal with 0 day fermentation (A) showed the lowest growth result than other treatments. It is also in line with Nwachi (2013), which showed that the absence of fermentation process in *L. perpusilla* for tilapia fish feed (*O. niloticus*) also showed the lowest result compared to other treatments.

The results of ANOVA have shown that tilapia fish fed by *L. minor* meal which is fermented on artificial feed has no significant effect ($p > 0.05$) on tilapia fish (*O. niloticus*) survival rate, it is assumed that feed with addition of *L. minor* on artificial feeds have an effect only on growth, but has no effect to its survival rate. Level of survival rate of tilapia fish in this study was 73.33-93.33%. Fish survival rate is not directly affected by feed. The death of tilapia seeds that occurred during the study was suspected due to stress because of the treatments during the study (Setiawati et al 2014). It can also be because of the water quality especially fluctuating temperatures. Important factors that affect the growth and survival rate of fish beside feed is the quality of water, especially temperature (Kelabora 2010), because the temperature can affect the growth and appetite of fish. Temperature can affect important fish activities such as breathing, growth and reproduction. The high temperatures can reduce DO and affect the appetite of fish.

The value of SR in tilapia (*O. niloticus*) fed by *Lemna* sp. showed higher results compared to control treatment. Previous study conducted by Sulawesty et al (2014) stated that the treatment of carp (*Cyprinus carpio* L.) which was not fed by *Lemna* meal had 60% of survival rate, however, the carp fed by *Lemna* produced 70% of survival rate. It is also supported by Uddin et al (2007) which stated that tilapia (*O. niloticus*) that was not fed by duckweed feed was 85.34% while the fish fed duckweed yielded survival rate value of 83.68%. Fish fed with pellets and *Lemna* have higher survival rates than fish fed only with pellets (Sulawesty et al 2014). The highest SR value was tilapia which fed by *L. minor* meal with 7 days fermentation (B). The survival rate of fish is influenced by internal and external factors. Internal factors are resistance to disease, feed and age. While external factors are stock density, disease, and water quality. Externally, stocking density is one of the important factors because it is related to space for the fish. When fishes experienced stress, fish not only has lack of response to the feed given and affect on growth but also the fishes are more susceptible to pathogens, even fishes can die.

Utilization of fermented *L. minor* meal gave a significant effect to nutrient content ($p < 0.05$) enhancement. This is in line with the study that was conducted by Zahidah et al (2012) who stated that the protein content enhancement is because there is an increasing number of nitrogen and water-soluble solids during the fermentation process. The enhancement in ocean-water nitrogen is due to the activity of protease enzymes that break down proteins into water-soluble fragments. This is due to an increase in the amount of amino acids during fermentation. Amino acid profile and fatty acid profile of tilapia fed by *L. minor* meal with different fermentation time are presented in Table 4 and Table 5.

Table 4

Amino acid profile of tilapia fed by *L. minor* meal with different fermentation time

Amino acid profiles	*A (%)*	*B (%)*	*C (%)*	*D (%)*
L-Histidine	1.9 ± 0.09^a	8.0 ± 0.05^b	4.6 ± 0.08^c	4.6 ± 0.06^c
L-Serine	3.8 ± 0.04^a	12.1 ± 0.08^b	7.9 ± 0.09^c	6.9 ± 0.01^d
L-Arginine	2.7 ± 0.06^a	10.6 ± 0.06^b	7.3 ± 0.06^c	8.4 ± 0.02^d
Glycine	7.6 ± 0.03^a	17.60 ± 0.09^b	12.2 ± 0.04^c	12.0 ± 0.08^d
L-Aspartic acid	6.1 ± 0.08^a	16.5 ± 0.04^b	14.5 ± 0.09^c	16.9 ± 0.04^b
L-Glutamic acid	15.4 ± 0.09^a	35.6 ± 0.02^b	25.4 ± 0.05^c	27.9 ± 0.01^d
L-Threonine	5.4 ± 0.03^a	15.2 ± 0.08^b	8.9 ± 0.07^c	8.7 ± 0.02^c
L-Alanine	14.6 ± 0.06^a	23.1 ± 0.03^b	19.4 ± 0.06^c	17.1 ± 0.06^d
L-Proline	5.7 ± 0.04^a	6.8 ± 0.09^b	9.3 ± 0.09^c	8.7 ± 0.04^d
L-Cysteine	0.3 ± 0.01^a	0.8 ± 0.07^b	0.4 ± 0.05^a	0.3 ± 0.05^a
L-Lysine HCL	10.1 ± 0.09^a	22.8 ± 0.03^b	16.8 ± 0.07^c	15.5 ± 0.03^d
L-Tyrosine	3.5 ± 0.08^a	10.9 ± 0.05^b	7.1 ± 0.04^c	7.0 ± 0.08^c
L-Methionine	1.8 ± 0.05^a	15.4 ± 0.06^b	13.0 ± 0.08^c	8.6 ± 0.01^d
L-Valine	8.7 ± 0.08^a	16.5 ± 0.04^b	13.1 ± 0.05^c	11.7 ± 0.05^d
L-Isoleucine	6.1 ± 0.05^a	13.33 ± 0.02^b	9.9 ± 0.02^c	8.9 ± 0.03^d
L-Leucine	6.9 ± 0.09^a	19.2 ± 0.08^b	15.8 ± 0.08^c	13.8 ± 0.06^d
L-Phenylalanine	6.6 ± 0.05^a	17.2 ± 0.09^b	12.8 ± 0.09^c	13.6 ± 0.09^d
Tryptophan	2.17 ± 0.09^a	2.9 ± 0.05^b	2.5 ± 0.08^c	1.5 ± 0.01^d

Note: Value ± standard deviation from three replications, values were followed with the same superscript letter shown that they are not significantly different.

Table 5

Fatty acid profile of tilapia fed by *L. minor* meal with different fermentation time

Fatty acid profiles	*A (%)*	*B (%)*	*C (%)*	*D (%)*
Myristic	0.28 ± 0.04^a	2.41 ± 0.09^b	1.49 ± 0.04^c	0.482 ± 0.02^d
Pentadecanoic	0.10 ± 0.08^a	1.15 ± 0.08^b	1.18 ± 0.06^c	0.17 ± 0.04^d
Palmitic	2.01 ± 0.06^a	4.59 ± 0.04^b	2.29 ± 0.08^a	1.97 ± 0.08^c
Stearic	0.41 ± 0.07^a	4.91 ± 0.09^b	1.65 ± 0.02^c	0.52 ± 0.03^d
Oleic/ω9	1.62 ± 0.05^a	5.61 ± 0.01^b	0.95 ± 0.03^c	0.89 ± 0.08^d
Linoleic/ω6	0.54 ± 0.02^a	7.37 ± 0.02^b	5.46 ± 0.07^c	4.49 ± 0.07^d
Linolenic/ω3	0.19 ± 0.06^a	6.32 ± 0.01^b	2.38 ± 0.09^c	3.39 ± 0.03^d
Arachidic	0.02 ± 0.07^a	3.05 ± 0.03^b	2.83 ± 0.02^c	1.02 ± 0.04^d
Arachidonic	0.07 ± 0.01^a	1.13 ± 0.08^b	0.15 ± 0.05^c	0.15 ± 0.02^c
Eicosapentaenoic	0.27 ± 0.05^a	3.52 ± 0.06^b	2.53 ± 0.09^c	0.50 ± 0.04^d
Omega 3	0.03 ± 0.02^a	5.91 ± 0.04^b	4.01 ± 0.04^c	3.99 ± 0.08^d
Omega 6	0.63 ± 0.08^a	7.53 ± 0.08^b	5.64 ± 0.07^c	5.68 ± 0.02^c
Omega 9	1.62 ± 0.05^a	0.61 ± 0.01^b	0.95 ± 0.01^c	0.89 ± 0.08
Unsaturated fatty acid	0.88 ± 0.09^a	3.56 ± 0.07^b	4.46 ± 0.06^c	4.24 ± 0.02^c
Saturated fatty acid	0.97 ± 0.01^a	2.80 ± 0.08^b	1.09 ± 0.09^c	1.49 ± 0.07^d
Mono unsaturated fatty acid (MUFA)	1.63 ± 0.05^a	4.97 ± 0.06^b	2.64 ± 0.02^c	2.40 ± 0.04^c
Polyunsaturated fatty acid (PUFA)	1.24 ± 0.08^a	4.58 ± 0.04^b	3.82 ± 0.08^c	2.83 ± 0.02^d
AA	0.08 ± 0.09^a	6.13 ± 0.07^b	4.15 ± 0.04^c	2.15 ± 0.09^d
DHA	1.06 ± 0.02^a	6.07 ± 0.03^b	4.08 ± 0.04^c	2.07 ± 0.01^d
EPA	0.27 ± 0.03^a	5.52 ± 0.06^b	3.53 ± 0.02^c	2.50 ± 0.07^d

Note: Value ± standard deviation from three replications, values were followed with the same superscript letter shown that they are not significantly different.

The results showed that the treatment (B) had the highest amino acid and fatty acid contents compared to other treatments (Tables 4 and 5), for example in 22.8±0.03% of lysine essential amino acid and 35.6±0.02% of glutamic acid. The function of lysine amino acid as stated in Ovie & Eze (2013); Valverde et al (2013); and Herawati et al (2015) were as the building blocks of vitamin B1 and anti-viral, helps the fish to absorbs calcium, stimulates fish's appetite, and assists the fish to produce carnitine to convert fatty acids into energy. The result of fatty acid profile analysis indicated that the highest linoleic fatty acid was 7.37% at (B) treatment. Linoleic fatty acids serve as a base substrate to form long chain PUFAs. The results of this study are strengthened through the previous study conducted by Pratiwi et al (2009), and Herawati et al (2015) which stated that linoleic fatty acids act as a base substrate to form long chains of Omega 6 and Omega 3. The best treatment to increase growth and nutrient value of tilapia was at tilapia fed by *L. minor* meal with 7 days fermentation (B).

Conclusions. Based on the results, tilapia fed by *L. minor* meal with 7 days fermentation (B) was the best treatment to increase growth and nutrient value of Nile tilapia. *L. minor* meal fermentation gave a significant effect ($p < 0.05$) to TFC, FUE, PER, and RGR, while it did not give a significant effect ($p > 0.05$) to SR. The utilization of *L. minor* meal with 7 days of fermentation produced the best result of 104.43 g for TFC, 64.68% of FUE, 1.91% of maximum PER, and produced 1.90% day^{-1} of maximum RGR.

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References

Akand A. M., Hasan M. R., Habib M. A. B., 1991 Utilization of carbohydrate and lipid as dietary energy sources by stinging catfish, *Heteropneustes fossilis* (Bloch). In: Fish nutrition research in Asia. Proceedings of the fourth Asian fish nutrition workshop. Manila: Asian Fisheries Society, pp. 93-100.

AOAC, 2000 Official methods of analysis. 17th edition, Horwitz W. (ed), Academic Press, Washington DC, USA method number: 925.09.

Asimi O. A., Khan I. A., Bhat T. A., Husain N., 2018 Duckweed (*Lemna minor*) as a plant protein source in the diet of common carp (*Cyprinus carpio*) fingerlings. Journal of Pharmacognosy and Phytochemistry 7(3):42-45.

Dewanji A., 1993 Amino acid composition of leaf protein extracted from some aquatic weeds. Journal of Agricultural Food and Chemistry 41:1232-1236.

El-Sayed A. F. M., Kawanna M., 2008 Optimum water temperature boosts the growth performance of Nile tilapia (*Oreochromis niloticus*) fry reared in a recycling system. Aquaculture Research 39(6):670-672.

Haetami K., 2012 [Consumption and feed efficiency from "Jambal Siam" fish (*Pangasianodon hypophythalamus*) fed with different energy protein levels]. Jurnal Akuatika 3(2):146-158. [in Indonesian]

Hepher B., 1988 Nutrition of pond fish. Cambridge University Press, Cambridge, USA, 388 pp.

Herawati V. E., Hutabarat J., Pinandoyo, Radjasa O. K., 2015 Growth and survival rate of tilapia (*O. niloticus*) larvae fed by *Daphnia magna* cultured with organic fertilizer resulted from probiotic bacteria fermentation. HAYATI Journal of Biosciences 22:169-173.

Herawati V. E., Nugroho R. A., Pinandoyo, Hutabarat J., 2017 Nutritional value content, biomass production and growth performance of *Daphnia magna* cultured with different animal wastes resulted from probiotic bacteria fermentation. IOP Conference Series: Earth and Environmental Science 55(1):012004.
Herawati V. E., Nugroho R. A., Pinandoyo, Darmanto Y. S., Hutabarat J., 2018 The effect of fermentation time with probiotic bacteria on organic fertilizer as *Daphnia magna* cultured medium towards nutrient quality, biomass production and growth performance enhancement. IOP Conference Series: Earth and Environmental Science 116:012089.
Kathirvelan C., Banupriya S., Purushothaman M. R., 2015 *Azolla* - an alternate and sustainable feed for livestock. International Journal of Science, Environment and Technology 4(4):1153-1157.
Kelabora D. M., 2010 [Temperature effect on survival rate and growth of *Cyprinus carpio* larvae]. Jurnal Berkala Perikanan Terubuk 38(1):71-81. [in Indonesian]
Khasani I., 2013 [Fish attractant: types, functional and fish response]. Media Akuakultur 8(2):127-133. [in Indonesian]
Novelli P. K., Baroz M. M., Pezzato L. E., de Araujo E. P., Botelho R. M., Fleuri L. F., 2017 Enzymes produced by agro-industrial co-products enhance digestible value for Nile tilapia (*Oreochromis niloticus*): a significant animal feeding alternative. Aquaculture 481:1-7.
Nwachi O. F., 2013 An overview of the importance of probiotics in aquaculture. Journal of Fisheries and Aquatic Science 8(1):30-32.
Olaniyi C. O., Oladunjoye I. O., 2012 Replacement value of duckweed (*Lemna minor*) in Nile tilapia (*Oreoochromis niloticus*) diet. Transnational Journal of Science and Technology 2(9):54-62.
Ovie S. O., Eze S. S., 2013 Lysine requirement and its effect on body composition of *Oreochromis niloticus* fingerlings. Journal of Fisheries and Aquatic Science 8(1):94-100.
Pereira L., Riquelme T., Hosokawa H., 2007 Effect of three photoperiod regimes on the growth and mortality of the Japanese abalone (*Haliotis discus hanai* Iino). Journal of Shellfish Research 26(3):763-767.
Pratiwi A. R., Syah D., Hardjito L., Panggabean L. M. G., Suhartono M. T., 2009 Fatty acid synthesis by Indonesian marine diatom, *Chaetoceros gracilis*. HAYATI Journal of Biosciences 16(4):151-166.
Putri D. R., Agustono, Subekti S., 2012 [Dry content, crude fiber, and crude protein of Lamtoro leaf (*Leucene glauca*) fermented using probiotics as fish feed]. Jurnal Ilmu Perikanan dan Kelautan 4(2):161-167. [in Indonesian]
Rimmer M. A., Sugama K., Rakhmawati D., Rofiq R., Habgood R. H., 2013 A review and SWOT analysis of aquaculture development in Indonesia. Reviews in Aquaculture 5(4):1-25.
Santiago C. B., Lovell R. T., 1988 Amino acid requirements for growth of Nile tilapia. The Journal of Nutrition 118(12):1540-1546.
Setiawati J. E., Tarsim Y. T., Adipura, Hudaidah S., 2014 [The effect of probiotic with different dose on growth, survival rate, feed efficiency, and protein retention of *Pangasius hypophthalmus*]. Jurnal Rekayasa dan Teknologi Budidaya Perairan 1(2):151-162. [in Indonesian]
Somerville C., Cohen M., Pantanella E., Stankus A., Lovatelli A., 2014 Small-scale aquaponic food production. Integrated fish and plant farming. FAO Fisheries and Aquaculture Technical Paper No. 589, Rome, FAO, 262 pp.
Solomon S. G., Okomoda V. T., 2012 Growth performance of *Oreochromis niloticus* fed duckweed (*Lemna minor*) based diets in outdoor hapas. International Journal of Research in Fisheries and Aquaculture 2(4):61-65.
Standar Nasional Indonesia, 1999 [Black tilapia (*Oreochromis niloticus* Bleeker) seeds production on hatchery]. Badan Standarisasi Nasional, Jakarta, 10 pp. [in Indonesian]
Standar Nasional Indonesia, 2009 [The parent fish of black tilapia (*Oreochromis niloticus* Bleeker) main class]. Badan Standarisasi Nasional, Jakarta, 16 pp. [in Indonesian]

Sulawesty F., T. Chrismadha, Mulyana E., 2014 [Growth rate of *Cyprinus carpio* L. feed with fresh *Lemna perpusilla* Torr. on closed pond]. LIMNOTEK - Perairan Darat Tropis di Indonesia 21(2):177-184. [in Indonesian]
Tacon A. E. J., 1987 The nutrition and feeding farmed fish and shrimp. A training manual. 1. The essential nutrients. Food and Agriculture Organization of the United Nations, Brazil, 117 pp.
Takeuchi T., 1988 Laboratory work - chemical evaluation of dietary nutrients. In: Fish nutrition and mariculture. Watanabe T. (ed), JICA Textbook the General Aquaculture Course, Tokyo, pp. 179-233.
Uddin M. N., Rahman M. S., Shahjahan M., 2007 Effects of duckweed (*Lemna minor*) as supplementary feed on monoculture of GIFT strain of tilapia (*Oreochromis niloticus*). Progressive Agriculture 18(2):183-188.
Valverde J. C., Martinez-Llorens S., Vidal A. T., Jover M., Rodriguez C., Estefanell J., Gairin J. I., Domingues P. M., Rodriguez C. J., Garcia B. G., 2013 Amino acids composition and protein quality evaluation of marine species and meals for feed formulations in cephalopods. Aquaculture International 21(2):413-433.
Virnanto L. A., Rachmawati D., Samidjan I., 2016 [Utilization of azolla (*Azolla microphylla*) fermented flour as a feed combine to improve the growth and survival rate of gouramy (*Osphronemus gouramy*)]. Journal of Aquaculture Management and Technology 5(1):1-7. [in Indonesian]
Warasto, Yulisman, Fitriani M., 2013 [Fermented giant salvinia (*Salvinia molesta*) meal as feed ingredient for tilapia (*Oreochromis niloticus*)]. Jurnal Akuakultur Rawa Indonesia 1(2):173-183. [in Indonesian]
Wilson R. P., 1977 Energy relationships in catfish diets. In: Nutrition and feeding of channel catfish. Sticney R. R., Lovell R. T. (eds), Southern Cooperative Series Bulletin 218:21-29.
Yuniwati M., Iskarima F., Padulemba A., 2012 [Optimization of the condition of compost making process from organic waste by fermentation using EM4]. Jurnal Teknologi 5(2):172-181. [in Indonesian]
Zahidah W., Gunawan, Subhan U., 2012 [Analysis of population and growth of *Daphnia* sp. in floating cage culture at Cirata Reservoirs with waste fertilizer fermented by EM4]. Jurnal Akuatika 3(1):84-94. [in Indonesian]

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