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The effect of fermented duckweed (*Lemna minor*) in feed on growth and nutritional quality of tilapia (*Oreochromis niloticus*)

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Abstract. Herawati VE, Pinandoyo, Darmanto YS, Rismaningsih N, Widarto S, Radjasa OK. 2020. The effect of fermented duckweed (*Lemna minor*) in feed on growth and nutritional quality of tilapia (*Oreochromis niloticus*). *Biodiversitas* 21: 3350-3358. One of the raw materials used for making tilapia fish feed is soybean meal. Soybean prices continue to rise and currently reach 4,500 rupiah/kg, so that alternative feed ingredients that are cheap and environmentally friendly need to be sought. Alternative feed ingredients that can be utilized in making feed, one of which is *Lemna minor* meal. Duckweed (*Lemna minor*) which has a high nutrient content, such as 23.47% crude protein, 3.99% crude fat, 29.92% crude fiber, 23.6% ash, and 19.02% nitrogen-free extract, will be the best candidate as an alternative feed ingredient. This study aims to find growth performance and nutritional quality of tilapia fed by different feed formulations using different amounts of fermented *L. minor* meal. The dosage treatments of feed with fermented *L. minor* meal are 0% (A), 2.5% (B), 5% (C), and 7.5% (D). Feeding of tilapia using fermented *L. minor* meal has a significant effect ($P < 0.05$) on total feed consumption, feed utilization efficiency, protein efficiency ratio, and relative growth rate, and had no significant effect ($P > 0.05$) on survival rate. The results found that the best biomass weight, total feed consumption, feed utilization efficiency, relative growth rate, protein efficiency ratio, and survival rate value was in the treatment B (usage of 2.5 % *L. minor* meal) which were 38.58g, 110.17g, 70.10%, 2.01%, 1.98%, and 94.44%, respectively. The best nutritional quality was in the treatment B (usage of 2.5 % *L. minor* meal) which was amino acid lysine, the highest value of amino acid lysine was 25.22% and the highest value of linoleic fatty acid was 4.37%.

Keywords: Aquaculture, fermentation, *Lemna minor*, natural feed, tilapia

INTRODUCTION

The Nile tilapia (*Oreochromis niloticus*) is a freshwater fish that is easy to cultivate. Production continues to increase and quality of tilapia rearing is an important commercial factor; therefore, feed is an important factor in increasing the production and quality of tilapia. In freshwater fish aquaculture, fish feed costs more than 50% of the production costs (Appenroth et al. 2018; Chakrabarti et al. 2018; Pramana et al. 2019). One of the raw materials used to produce tilapia fish feed is soybean meal. Soybean prices have continued to rise and currently, the price is 4,500 rupiah/kg (Handajani, 2011); therefore, alternative, inexpensive, and environment-friendly feed ingredients are needed. Several alternative feed ingredients may be used in the feed, one of which is duckweed (*Lemna minor*) meal. *L. minor* is a water weed that is rarely utilized but has a high-protein content.

Duckweed is a water weed that grows rapidly. The high productivity of *Lemna* sp. supports its use as an alternative feed or feed supplement. However, the use of *Lemna* sp. as a feed ingredient is constrained by the high amount of crude fiber, which reduces feed digestibility. *Lemna* spp. have great potential for use as feed because they are

abundant in water. Additionally, their growth is fast and they can multiply with weights double their initial weight within 3-4 df. Duckweed can contain 28%-43% crude protein, 5% fiber (dry weight), and high concentrations of minerals, such as phosphorus and potassium, as well as xanthophylls and carotenes (del Carmen Flores-Miranda et al. 2015). Duckweed meal is known for its high nutritive value of as much as 40% and high-crude protein, depending on the aquaculture system (Sogbesan et al. 2015). The protein content of duckweed is relatively high, namely 20-30% (dry weight). Research on *L. minor* as a substitute for soybean meal by Solomon and Okomoda (2012) demonstrated that *L. minor* with high-protein content and amino acids based on the needs of the fish could be used to replace soybean meal in feed formulations in fish farms. *L. minor* can be a substitute feed ingredient for soybean meal to reduce production costs and provide high nutrients for tilapia rearing. The rapid growth rate coupled with the high-protein content (41%-45%) is a unique property of duckweed, which may make it suitable for supplementation or substitution of other plant or animal protein in fish diets. Duckweed is an excellent alternative to the use of soybean meal and fish meal in fish feed (Sogbesan et al. 2015).

One way to reduce the crude fiber content in *Lemna* is through a fermentation process. According to Virnanto et al. (2016), the fermentation process can reduce high crude fiber content. Additionally, fermentation can also increase the nutritional value of some ingredients. The goal of the fermentation process is to increase the content and quality of protein, maintain the nutritional value during storage, and reduce the anti-nutrient substances of the feed ingredients. The fermentation process can also increase the nutritional value of ingredients in the meal (Nwachi 2013). Fermentation is the anaerobic dissimilation process of organic compounds mediated by the activity of microorganisms or extracts from the cells of these microorganisms (Abu et al. 2013). The purpose of fermentation is to shorten the long chain of amino acids and fatty acids to facilitate the absorption of feed during metabolic processes using probiotic bacteria to enhance growth and enrich nutrients in the larval feed (Nwachi 2013). The advantages of the fermentation process include the ability to improve nutrients, especially proteins and fats, produce specially scented food, and increase energy efficiency and ease of use.

Consequently, given the high nutritional quality of *L. minor* and the continued increase in the price of soybean meal, in this study, we reported the substitution of soybean meal using fermented *L. minor* meal. The objective of this study was to determine the growth performance and nutritional quality of tilapia feed different formulations using different amounts of fermented *L. minor* meal (0%, 2.5%, 5%, and 7.5%). Furthermore, the best feed formulation among the treatments based on the results was determined.

MATERIALS AND METHODS

Lemna minor meal fermentation

Lemna minor plants were obtained from Lake Rawa Pening, Semarang, Indonesia. They were cleaned of dirt and dried in the sun. The method of drying *L. minor* was according to the research conducted by Solomon and Okomoda (2012), where drying was also conducted using sunlight. The dried leaves were then crushed and sieved. *L. minor* meal fermentation was performed by mixing the probiotic microorganisms *Lactobacillus casei* and *Saccharomyces cerevisiae* evenly with *L. minor* meal. The duckweed was washed, dried, and fermented using 1 mL of commercially available bacteria containing *Lactobacillus casei* and *S. cerevisiae* probiotic bacteria and molasses as an activator with a ratio of 1: 1 in 100 mL of water (Pinandoyo et al. 2019). The mixed product was then put in a plastic bag and stored at room temperature. The results of the fermentation of *L. minor* meal were indicated by an acidic odor. The next step was to open the plastic bag containing the fermented *L. minor* meal to allow cooling.

This research was conducted experimentally, using a completely randomized design (CRD) with four treatments with three replications. The treatments of this research were as follows: Treatment A: Feeding without fermented *L. minor* meal substitution (0%), B: Feeding with fermented *L. minor* meal substitution (2.5%), C: Feeding with fermented *L. minor* meal substitution (5%), D:

Feeding with fermented *L. minor* meal substitution (7.5%). Determination of the above dose refers to previous research, conducted by Solomon and Okomoda (2012) about the growth performance of tilapia fed by duckweed meal. The results of the research which was carried out using *L. minor* meal without fermentation process and got the best results at a dose of 5%.

Diet preparation

The fermented *L. minor* meal was dried and the nutrient composition was tested. The test feed in this study was artificially pressed in the form of pellets using local ingredients, such as fish meal, soybean meal, *L. minor* meal, bran flour, wheat flour, comstarch, fish oil, corn oil, vitamins, minerals, carboxymethylcellulose, and water. First, the feed was mixed with the lowest to the highest percentage concentration based on Table 2 and stirred until homogeneous. Then, warm water was added and constituted as much as 35%-40% of the total ingredients. The water was added while the ingredients were stirred until evenly distributed and smooth such that clumps could be formed using a mill and dried in an oven at a temperature of <50°C until the water content of the feed was less than 10% of the initial water content.

The nutritional quality of feed

Proximate analysis was carried out for the dried feed. *L. minor* meal nutrient content before and after the fermentation process is presented in Table 1. Proximate analysis of feed formulations in the study is presented in Tables 1 and 2, respectively. The feed formulation using fermented *L. minor* meal as soybean meal substitution used in this study is presented in Table 2. The results of the Proximate analysis for each test feed used during the study are presented in Table 3.

Water quality parameters

Water quality parameters measured include Dissolved Oxygen (DO), pH value, temperature, and ammonia content. The data of temperature, DO, pH, and ammonia are presented in Table 4.

Essential amino acid profile analysis

The amino acid composition of the sample was determined using High-performance liquid chromatography (HPLC) (Shimadzu LC-6A) (AOAC, 2000; and Herawati et al. 2014). The essential amino acid profile of *L. minor* was determined by examining its essential amino acid content. The essential amino acid analysis was conducted using an HPLC type 1100 with a Eurospher 100-5 C18, 250 x 4.6 mm column that has P/N: 1115Y535 pre-column. The effluents were: A) 0.01 M acetate buffer pH 5.9; and B) 0.01 M MeOH acetate buffer at pH 5.9; THF > 80: 15: 5 Δ Fluorescence: Ext: 340 nm Em : 450 nm. About 2.5 g of the sample was put into a sealed glass. Then, 15 mL of HCl 6N was added. The mixture was then vortexed for homogeneity and underwent hydrolysis using an autoclave at 110 °C for 12 hours before being cooled down to room temperature and neutralized with NaOH 6N. After the addition of 2.5 mL of 40% Lead Acetate and 1 mL of 15%

3) lalate acid, around 3 mL of the mixture was filtered with 0.45 μm miller. For the injection into HPLC, 25 μL of the filtered mixture plus 475 μL of OPAA solution was vortexed and incubated for 3 minutes. Finally, 30 μL of the final mixture was put into the HPLC (Herawati et al. 2014). Amino acid profile of feed using fermented *L. minor* meal for tilapia is presented in Table 5.

Table 1. Proximate analysis results for *Lemma minor* meal before and after the fermentation process

Ingredients	Components (%)				
	Protein	NFE	Fat	Crude fiber	Ash
<i>L. minor</i> meal	23.47	19.02	3.99	29.92	23.6
Fermented <i>L. minor</i> meal	32.13	15.96	5.13	28.58	18.20

Note: Protein and ash had a significant effect; NFE, fat and crude fiber have no significant effect.

Table 2. Feed formulation using fermented *Lemma minor* meal as soybean meal substitution used in the study

Feed ingredients	Feed (g)			
	A (0%)	B (2.5%)	C (5%)	D (7.5%)
Fish meal	32.86	32.78	32.71	32.64
Soybean meal	33.89	31.99	30.07	28.18
<i>L. minor</i> meal	0.00	2.74	5.46	8.17
Wheat flour	6.85	7.79	6.89	3.83
Rice bran meal	8.46	9.92	12.86	16.37
Cornstarch	7.94	4.78	2.01	0.90
Fish oil	2.00	2.00	2.00	2.00
Corn oil	2.00	2.00	2.00	2.00
Vitamins-minerals mix*	5.00	5.00	5.00	5.00
Carboxymethylcellulose (CMC)	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00

Note: Calculation of feed formulations based on Proximate Composition and Analysis of Feed Materials used during research (% Dry Weights) (Wilson 1982). * Vitamins-mineral mix (PT. Indosco Dwijayasakti, Surabaya, Indonesia) contained vitamin A, vitamin D3, vitamin K3, vitamin E, vitamin B1, vitamin B2, vitamin B6, vitamin B12, vitamin C, folic acid, biotin, inositol, nicotinamide, choline chloride, Co, Cu, I, Mn, Se, and Zn. This is a commercially vitamins-minerals mix; the quantity applied to the feed was 5 grams.

Table 3. Proximate composition of experimental diets used in the present study

Contents	Feed (g)			
	A (0%)	B (2.5%)	C (5%)	D (7.5%)
Protein (%)	33.84	34.11	33.97	35.54
NFE (%)	14.65	11.87	15.45	6.79
Fat (%)	8.94	8.43	7.73	8.65
Energy (kcal) *	262.23	259.38	255.75	251.98
E/P ratio **	8.74	8.65	8.52	8.40
Fiber (%)	24.37	25.14	22.57	25.83
Ash (%)	18.20	20.45	20.28	23.19

Note: * Based on calculation of DE (digestible energy) with the assumption for protein = 3.5 kcal/g, fat = 8.1 kcal/g, Nitrogen-free extract (NFE) = 2.5 kcal/g (Wilson 1982). ** E/P values for the optimal growth of fish ranged from 8-12 kcal/g (De Silva, 1987)

Table 4. Water quality parameter measurement results of tilapia (*Oreochromis niloticus*) aquaculture media during the study

Water quality parameters	Range of water quality parameter	References (feasibility)
Temperature ($^{\circ}\text{C}$)	25-28	25-32
pH	7.0-7.5	6.0-8.5
DO (mg/L)	3.00-3.60	≥ 3
NH_3 (mg/L)	0.0028-0.0437	< 0.02
Salinity	0.00	0.00

Reference: Asadi et al. 2012; Solomon dan Okomoda (2012)

Table 5. Amino acid profile of feed using fermented *Lemma minor* meal for tilapia (*Oreochromis niloticus*)

Amino acid	A (0%)	B (2.5%)	C (5%)	D (7.5%)
L-Histidine	1.8 \pm 0.01	5.5 \pm 0.05	3.8 \pm 0.05	4.2 \pm 0.05
L-Serine	1.5 \pm 0.03	4.6 \pm 0.02	2.7 \pm 0.02	3.1 \pm 0.02
L-Arginine	2.6 \pm 0.05	5.8 \pm 0.06	3.4 \pm 0.06	2.9 \pm 0.06
Glycine	1.4 \pm 0.04	4.7 \pm 0.09	2.9 \pm 0.09	2.1 \pm 0.09
L-Aspartic acid	2.9 \pm 0.08	4.3 \pm 0.04	2.7 \pm 0.04	4.6 \pm 0.04
L-Glutamic acid	1.5 \pm 0.09	3.6 \pm 0.02	1.9 \pm 0.02	1.2 \pm 0.02
L-Threonine	3.5 \pm 0.03	5.25 \pm 0.08	2.8 \pm 0.08	3.4 \pm 0.08
L-Alanine	3.5 \pm 0.06	5.89 \pm 0.03	4.9 \pm 0.03	3.7 \pm 0.03
L-Proline	0.7 \pm 0.04	3.8 \pm 0.09	2.9 \pm 0.09	2.3 \pm 0.09
L-Cystine	0.3 \pm 0.01	2.4 \pm 0.07	0.6 \pm 0.07	2.1 \pm 0.07
L-Lysine	1.9 \pm 0.02	5.8 \pm 0.03	2.5 \pm 0.03	3.9 \pm 0.03
L-Tyrosine	2.4 \pm 0.03	4.9 \pm 0.05	1.8 \pm 0.05	0.7 \pm 0.05
L-Methionine	0.4 \pm 0.04	5.4 \pm 0.06	2.2 \pm 0.06	1.7 \pm 0.06
L-Valine	1.8 \pm 0.05	3.7 \pm 0.04	2.1 \pm 0.04	2.4 \pm 0.04
L-Isoleucine	2.6 \pm 0.08	6.7 \pm 0.02	2.6 \pm 0.02	3.9 \pm 0.02
L-Leucine	3.1 \pm 0.05	9.2 \pm 0.08	1.9 \pm 0.08	2.7 \pm 0.08
L-Phenylalanine	3.6 \pm 0.04	5.2 \pm 0.09	4.5 \pm 0.09	4.3 \pm 0.09
Tryptophan	0.1 \pm 0.01	2.3 \pm 0.05	0.9 \pm 0.05	5.9 \pm 0.05

Fatty acid profile analysis

The fatty acid composition of the sample was determined using a gas chromatograph (Shimadzu) (AOAC 2000; and Herawati et al. 2014). The fatty acid profile of *L. minor* can be determined by analyzing its total fatty acid content. The equipment used for this purpose was a Gas Chromatograph (GCMS) QP-2010 and the Mass Spectrophotometer with a W Cot fused Silica Counting CP-SIL-88 column of 50 m length, 0.22 mm diameter and at a column temperature of 120-200 $^{\circ}\text{C}$. The method employed was in situ transesterification. 100 mg of *L. minor* sample was homogenized using 4 mL of water. The resulting 100 μL homogenate was then transferred into a reaction tube. One hundred μL of methylene chloride was then added, along with 1 mL of NaOH 0.5 N in methanol. Once nitrogen was added and the tube was sealed, it was heated to 90 $^{\circ}\text{C}$ for 10 minutes. The reaction tube was then cooled, and 1 mL of 14% BF_3 in methanol was added. After the nitrogen addition, heating ensued at the same temperature for the next 10 minutes. Afterward, the reaction tube was cooled to ambient temperature, and 1 mL of water and 200-500 μL of hexane were added. The mixture was then vortexed for 1 min to extract the fatty acids methyl ester. After centrifugation, the upper layer of the sample was ready for GC analysis (Herawati et al., 2014). Fatty acid

profile of feed using fermented *L. minor* meal as a meal for tilapia is presented in Table 6.

Feeding of Nile tilapia

Nile tilapia (*O. niloticus*) obtained from the Siwarak Fish Seed Center, Semarang District, Indonesia. The tested fish seed, which was used, had an average weight of 5.40 ± 0.06 g/fish. The initial average weight was measured to obtain homogenous initial data. Furthermore, the initial weight is needed to measure the final weight and weight gain during the study. The rearing container used in this study is a 30 Liter capacity plastic bucket as a rearing container filled with 20 Liters of water with a stocking density of 1 fish/L. The bucket is closed with waring so that the test fish does not jump out of the container (Handajani 2011). The method of feeding was using *at satiation* method (fed as much as they could consume each day) with the frequency of feeding three times a day at the morning (08:00), noon (12:00), and afternoon (16:00) of Western Indonesia Time (Srirangam 2016; Pinandoyo et al. 2019).

Data calculations and analysis

On the 45th day of feeding, fish in each container were weighed and counted. The weighing was based on each treatment and replication. The parameters measured include the total feed consumption (TFC), efficiency of feed utilization (FUE), protein efficiency ratio (PER), relative growth rate (RGR) and survival rate (SR). The total feed consumption and relative growth rate can be calculated using the Tacon (1987).

Data in the form of percentages include feed utilization efficiency (FUE), protein efficiency ratio (PER), relative growth rate (RGR) and survival rate (SR), arcsin data transformation, normality test, homogeneity test, and additivity test are carried out to ensure that data is normal, homogeneous and additive. Data were analyzed using ANOVA to see the effect. Duncan's multiple range test was conducted to determine the differences between treatments. The proximate composition of the samples was determined using a standard procedure (AOAC, 2000; Herawati et al. 2015).

RESULTS AND DISCUSSION

Results

The study of giving feed to tilapia using fermented *L. minor* meal as a substitute for soybean meal gave results in the form of biomass weight, relative growth rate (RGR), total feed consumption value (TFC), protein efficiency ratio (PER), feed utilization efficiency (FUE), and survival rate (SR). The results showed that feeding using fermented *L. minor* meal had a significant effect ($P < 0.05$) on TFC, FUE, PER and RGR and did not have a significant effect ($P > 0.05$) on SR. The results of the study on the growth of tilapia fed by *L. minor* meal are presented in Table 7.

The results of the study found that the best value of biomass weight, TFC, FUE, RGR, PER, and SR is at the treatment of tilapia which was fed using 2.5% of fermented *L. minor* meal as a substitute for soybean meal (B), they are 38.58 ± 0.08 ; $110.17 \text{ g} \pm 3.11$; $70.10\% \pm 0.07$; $2.01\% \pm 0.05$; $1.98\% \pm 0.04$; $94.44\% \pm 0.70$ respectively, while the lowest value was in tilapia fed by using 0% of fermented *L. minor* meal as a substitute for soybean meal (A), they are $23.05 \text{ g} \pm 0.05$; $88.17 \text{ g} \pm 2.11$; $52.14\% \pm 0.47$; $1.15\% \pm 0.02$; $1.35\% \pm 0.08$; $88.00\% \pm 0.67$. The amino acid profile of tilapia fed by fermented *L. minor* meal as a substitute for soybean meal is presented in Table 8.

Based on the analysis results, the highest amino acid profile is at tilapia fed by fermented *L. minor* meal as a substitute for soybean meal with a 2.5% formulation and found at lysine, which is 25.22% in essential amino acids and arginine is 10.35% in non-essential amino acids. The fatty acid profile of tilapia fed by fermented *L. minor* meal as a substitute for soybean meal is presented in Table 9.

The analysis results of the highest fatty acid profile in tilapia fed by 2.5% of fermented *L. minor* meal as a substitute soybean meal were found in linolenic fatty acids which are 18.96% in essential fatty acids, and palmitic fatty acids which are 7.59% in non-essential fatty acids.

Table 6. Fatty acid profile of feed using fermented *Lemma minor* meal for tilapia (*Oreochromis niloticus*)

Fatty acid profile (%)	A (0%)	B (2.5%)	C (5%)	D (7.5%)
Myristic	0.49±0.04	0.48±0.09	0.41±0.02	0.50±0.05
Pentadecanoic	0.18±0.06	0.15±0.08	0.17±0.04	0.08±0.02
Palmitic	2.29±0.08	2.59±0.04	1.97±0.08	2.12±0.01
Stearic	1.65±0.02	2.91±0.09	0.52±0.03	2.08±0.05
Oleic/ω9	0.95±0.03	3.21±0.01	0.89±0.08	0.55±0.03
Linoleic/ω6	0.46±0.07	4.37±0.02	1.09±0.07	0.75±0.02
Linolenic/ω3	0.08±0.09	3.32±0.01	0.39±0.03	0.56±0.07
Arachidic	2.83±0.02	3.05±0.03	1.02±0.04	1.25±0.05
Arachidonic	0.15±0.05	0.13±0.08	0.15±0.02	0.06±0.08
Eicosapentaenoic	2.62±0.08	3.58±0.04	2.83±0.02	3.17±0.01
AA	0.15±0.04	0.13±0.07	0.15±0.09	2.18±0.03
Docosa-hexaenoic acid (DHA)	0.08±0.04	1.07±0.03	0.07±0.01	0.39±0.08
EPA	0.53±0.02	1.52±0.06	1.50±0.07	1.09±0.02

Table 7. Growth of tilapia fed by fermented *Lemna minor* meal

Parameters	Treatments			
	A (0%)	B (2.5%)	C (5%)	D (7.5%)
Initial Body Weight (IBW) (g)	5.40±0.03 ^a	5.55±0.08 ^a	5.50±0.04 ^a	5.55±0.05 ^a
Final Body Weight (FBW) (g)	23.05±0.05 ^a	38.58±0.08 ^b	33.67±0.02 ^b	30.01±0.06 ^b
Weight Gain (WG) (g)	17.65±0.12 ^a	33.03±0.11 ^b	28.17±0.15 ^b	24.46±0.17 ^b
RGR (%)	1.15±0.02 ^a	2.01±0.05 ^b	1.48±0.04 ^b	1.74±0.09 ^b
TFC (g)	88.17±2.11 ^a	110.17±3.11 ^b	98.11±3.19 ^b	95.13±3.10 ^b
PER (%)	1.35±0.08 ^a	1.98±0.04 ^a	1.66±0.06 ^a	1.47±0.05 ^a
FUE (%)	52.14±0.47 ^a	70.10±0.07 ^b	65.82±0.05 ^b	63.38±0.06 ^b
SR (%)	88.00±0.67 ^a	94.44±0.70 ^a	90.20±0.12 ^a	90.20±0.70 ^b

Note: ^a: the value was not significantly different. ^b: the value was significantly different

24

Table 8. Amino acid profile of tilapia (*Oreochromis niloticus*) fed by using fermented *Lemna minor* meal

Amino acid profile (%)	A (0%)	B (2.5%)	C (5%)	D (7.5%)
L-Histidine	3.14±0.03	3.52±0.03	3.85±0.05	4.92±0.08
L-Serine	1.40±0.07	2.63±0.07	2.76±0.02	5.61±0.03
L-Arginine	8.39±0.02	10.35±0.05	9.36±0.07	8.61±0.04
Glycine	1.48±0.05	3.78±0.09	5.33±0.02	6.36±0.04
L-Aspartic acid	8.25±0.09	4.70±0.07	5.65±0.03	6.13±0.03
L-Glutamic acid	1.76±0.05	2.89±0.04	2.85±0.07	2.51±0.04
L-Threonine	3.37±0.09	5.56±0.03	5.47±0.07	4.02±0.09
L-Alanine	3.21±0.02	2.98±0.05	3.51±0.01	4.65±0.05
L-Proline	1.98±0.07	3.44±0.04	3.08±0.09	2.25±0.05
L-Cystine	5.74±0.04	5.13±0.02	2.72±0.03	3.24±0.05
L-Lysine	10.14±0.05	25.22±0.05	10.89±0.06	13.92±0.08
L-Tyrosine	2.86±0.06	3.99±0.03	3.59±0.06	3.57±0.04
L-Methionine	5.95±0.09	2.20±0.07	7.87±0.02	5.97±0.03
L-Valine	10.10±0.04	4.46±0.07	5.45±0.05	5.44±0.05
L-Isoleucine	5.62±0.03	3.97±0.05	5.41±0.07	5.49±0.07
L-Leucine	5.82±0.01	4.40±0.05	6.85±0.05	5.10±0.08
L-Phenylalanine	10.40±0.05	6.43±0.07	5.00±0.02	5.61±0.03
Tryptophan	10.39±0.03	5.35±0.01	7.36±0.05	6.61±0.03

24

Table 9. Fatty acid profile of tilapia (*Oreochromis niloticus*) fed by using fermented *Lemna minor* meal as a substitute for soybean meal

Fatty acid profile (%)	A (0%)	B (2.5%)	C (5%)	D (7.5%)
Myristic	1.49±0.04	4.41±0.09	1.82±0.02	2.01±0.05
Pentadecanoic	2.81±0.06	3.75±0.08	2.36±0.04	2.38±0.02
Palmitic	4.29±0.08	7.59±0.04	5.97±0.08	6.12±0.01
Stearic	2.35±0.02	4.71±0.09	3.08±0.03	2.01±0.05
Oleic/ω9	1.78±0.03	8.90±0.01	5.76±0.08	4.16±0.03
Linoleic/ω6	4.65±0.07	10.05±0.02	7.83±0.07	6.30±0.02
Linolenic/ω3	3.38±0.09	18.96±0.01	12.77±0.03	13.43±0.07
Arachidic	2.83±0.02	3.05±0.03	1.02±0.04	1.25±0.05
Arachidonic	0.15±0.05	0.13±0.08	0.15±0.02	0.06±0.08
Eicosapentaenoic	2.53±0.09	5.52±0.06	3.50±0.04	3.98±0.02
AA	1.65±0.04	3.19±0.07	2.15±0.09	2.38±0.03
Docosa-hexaenoic acid (DHA)	4.15±0.04	5.07±0.03	4.17±0.01	3.35±0.08
EPA	1.53±0.02	3.87±0.06	2.53±0.07	2.19±0.02

Discussion

The present study demonstrated that 2.5% fermented *L. minor* meal as a substitution in fish feed resulted in the best growth performance of tilapia. This was because feed that was made at a dose of 2.5% had a texture and smell that were preferred by tilapia. This was shown by the results of research on weight gain (33.03 g) and the highest relative

growth rate (2.01%). The smell of the feed was caused by the amino acid glycine. The content of glycine in treatment B feed was higher than that in the other treatments. The function of the amino acid glycine is a natural attractant in *L. minor* meal that is used as a source of vegetable protein. This was reinforced by the results reported by Yilmaz (2004) and Khasani (2013), who found that glycine was a

natural attractant found in sources of animal and vegetable protein. The relative growth performance of tilapia fed with 2.5% fermented *L. minor* meal resulted in better nutritional balance compared to that of the other treatment compositions. The feed in this treatment was consumed more compared to the feed in other treatments. This was because of the increased feed palatability. The use of 2.5% *L. minor* meal resulted in better feed utilization compared to that of soybean meal. This was supported by a study conducted by Khasani (2013), which stated that high-quality artificial feed has a high level of palatability. Fish respond quickly to feed that have compounds that stimulate their sense of smell because of their chemoreceptor mechanism.

The use of different protein sources in combination can prevent a high inclusion level of any single anti-nutritional factor in the diet and can also be a means of compensating for an essential amino acid deficiency in any single protein source (Hossain and Jauncey, 1990). This is because feed containing *L. minor* meal has a high protein content, especially amino acids, and fatty acids, which are suitable for the nutritional needs of tilapia; thus, it can support the growth of tilapia. The utilization of protein in tilapia decreased with increasing levels of *L. minor* meal fermentation in the feed. This was confirmed by a study conducted by Olaniyi and Oladunjoye (2012), which stated that protein utilization decreased progressively with increasing levels of substitution of *Lemna* sp. in the feed. This research showed that increasing the composition of *L. minor* meal fermentation yielded lower values for growth and increases in biomass. The lysine content in feed was almost the same as the amino acid requirements for lysine by tilapia. According to Santiago and Lovell (1988), the need for lysine by tilapia (*O. niloticus*) is 5.12%. The results showed the content of lysine and leucine were highest in treatment B, namely 5.8% lysine and 9.8% leucine. According to Dewanji (1993), *Lemna* sp. is generally rich in leucine and lysine. When compared to soybean protein, *Lemna* sp. protein is higher in amino acid content. The results of this study were reinforced by the findings of Ovie and Eze (2013); if the feed contains the right amount of essential amino acids needed by fish species, the ideal protein for the species is fulfilled such that there is no lack or excess of amino acids. The growth rate was directly proportional to the value of efficiency of feed utilization such that the high efficiency of feed utilization resulted in relatively high growth rates. The higher the use of fermented *L. minor* meal as a substitution for soybean meal in this study, the greater the decrease in fish growth rate because of the low amino acid profile between these feed ingredients. Those responsible were the amino acids lysine and leucine in the feed, which could improve growth and nutritional quality. The results of the study were reinforced by the results reported by Ilyas (2014), which stated that the addition of *L. perpusilla* at a dose of 100% in tilapia feed showed the lowest growth rate compared to that of other treatments. Olaniyi and Oladunjoye (2012) also reported that the addition of *L. minor* meal, with as much as 100%, to the feed of tilapia, showed the lowest growth results among the treatments.

The results of this study agreed with those of Srirangam (2016), which stated that increasing levels of protein, especially amino acids, could be accomplished by the fermentation process. During the fermentation process, there is an increase in the amount of nitrogen, with the increase caused by the activity of protease enzymes that break down proteins, such that they are more soluble in water. This resulted in an increase in the amino acid content of tilapia after being fed with fermented *L. minor* meal as a substitute for soybean meal. The fermentation process also aimed to increase the growth of tilapia by increasing the nutrients in the feed and facilitating the optimal absorption of the feed nutrients. Olaniyi and Oladunjoye (2012) stated that the fermentation process was not only able to reduce the high crude fiber content, but could also increase the nutritional value of the feed ingredients. Nwachi (2013) and Abu et al. (2013) stated that the purpose of fermentation was to increase the number of microorganisms and intensify their metabolism in feed, thereby producing new feed products with microorganisms to increase growth rates and enrich nutrients in larval feed. When the *L. minor* meal was not subjected to the fermentation process, the feed was difficult to digest by tilapia and had low nutrient content. The fermentation process is a biochemical process caused by microorganisms that occur enzymatically. Enzymes that work during the fermentation process can cause changes in feed ingredients. Changes that occur can be in the form of taste, color, shape, calories, and other properties. Asadi et al. (2012) stated that the function of fermentation was to contribute to the digestive enzymes of the fish and the absorption of organic material mediated by probiotics. Nwachi (2013), in his research, explained the function of probiotics in aquaculture, as increasing fish immunity to pathogens and significantly contributing to fish digestive enzymes.

The results showed that the fermentation process could improve the protein quality of the duckweed meal, as observed in the protein efficiency ratio (PER). However, it is important to note that in addition to their role in fermentation, bacteria are an important source of up to 70% high-quality protein (Aas et al. 2006a, b). In addition to high nutritional quality, the feed of tilapia using fermented *L. minor* meal as a substitute for soybean meal has a better level of feed palatability than that exhibited by other treatments; thus, it was highly preferred by the fish for consumption. Khasani (2013), Paul (2013), and Talukdar (2012) stated that high-quality artificial feed had a high level of palatability. In addition to higher nutritional quality, tilapia feed with 2.5% fermented *L. minor* as a substitute for soybean meal also had better taste than that exhibited by other treatments. This affects the choice of feed preferred by fish and feeds consumption considerably. Accordingly, it gives a higher PER yield compared to other treatments. This statement was reinforced by the results of Khasani (2013), Paul (2013), and Talukdar (2012), which stated that high-quality artificial feed has a high level of deliciousness.

Fish respond quickly to feed that has compounds that stimulate their sense of smell because of their

chemoreceptor mechanism. These compounds are known as attractants. Olaniyi and Oladunjoye (2012) stated in their study that the addition of *L. minor* to tilapia feed resulted in high feed utilization values. The PER is influenced by the ability of fish to digest feed. Several factors influence this ability, one of which is the composition of feed, where the higher the protein used by the body, the higher the value³⁷ protein efficiency. Based on the results of this study, the highest PER value was in tilapia fed with 2.5% of fermented *L. minor* meal, apparently because of the energy and nutrient content in the feed being suitable for the needs of the tilapia. The amino acid content in the feed satisfied the needs of the tilapia. The lysine content in this study for the 2.5% *L. minor*¹¹ meal was 5.8%. This is higher than that of the lysine amino acid requirements for tilapia. According to Santiago and Lovell (1988), the need for lysine amino acids by tilapia is 5.12%. The results showed that the PER in this study was higher than that in previous studies. The PER for the 2.5% *L. minor* in addition to the feed of tilapia was 1.98% compared to the PER of 0% *L. minor*, which was 1.35% (Solomon and Okomoda, 2012). The highest PER values were for the treatment with 0% and 25% concentration of *L. minor* in tilapia feed, having a value of 0.88% (Olaniyi and Oladunjoye, 2012). The difference in PER values in this study compared to that in previous studies was caused by differences in the protein content of *L. minor* meal. The difference in protein content was in agreement with that reported by Salomon and Okomoda (2012), which stated that it was caused by differences in the type of *L. minor* and nutritional availability in the areas where they were cultivated.

The difference in protein content is thought to be caused by differences in the type of *L. minor* and their origin. This was confirmed by the results reported by Salomon and Okomoda (2012), who stated that the differences in results were caused by differences in the type of *L. minor* and nutritional availability in the areas where they were cultivated.

The nutritional quality of tilapia feed with fermented *L. minor* meal instead of soybean meal based on the analysis of amino acids and fatty acids showed that the best value was 2.5% *L. minor*, as shown in Tables 5 and 6. In this study, the best value was the 2.5% formulation (Table 8) for lysine (45.22%) and arginine (74.35%). This was in agreement with the results reported by Asadi et al. (2012), wherein the protein of *Lemna* sp. was generally rich in leucine and lysine. Compared to soybean protein, the protein in duckweed had a higher²³ amino acid content. Amino acids are widely used for body maintenance, health, and synthesis of new str⁴⁴uctures from new structural proteins that will produce maximum feed efficiency and growth. The high amino acid content in feed with 2.5% *L. minor* meal fermentation compared to that in other feeding treatments can better meet the amino acid requirements of tilapia¹⁴ which is 5.12%. Fish that continue to grow will only use a small amount of the amino acids for energy. The amino acid lysine serves to increase growth and provide an energy source because lysine aids in the production of carnitine, which decomposes the¹⁴ source of nutrients in food into fatty acids. This statement is in agreement with the

results reported by Ovie and Eze (2013), Valverde et al. (2013), and Herawati et al. (2015), which stated that the amino acid lysine serves as a framework for vitamin B1 formation, anti-viral immunity, aids in calcium absorption, stimulates appetite, and helps in the production of carnitine, which converts fatty acids into energy.

Based on the results of this study, the highest fatty acid profile was for linoleic fatty acids. Linolenic fatty acids fur³⁰n as basic substrates for the formation of long chains of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Linolenic essential fatty acids cannot be produced in the body and must be obtained from feed. After this, with the help of enzymes, they are converted into long hydrocarbon chains. The results of the analysis of fatty acid profiles in feed using fermented *L. minor* meal as a substitute for soybean meal was 4.37% (Table 6); thus, the excess of these fatty acids is stored as an energy source to support growth and aid in¹⁵ the metabolic process. The results of these studies are in agreement with the findings of Gao et al. (2011). Freshwater fish do not need lo²² chain unsaturated fatty acids (HUFA) but require C18 n-3 fatty acids, namely linolenic acid (18: 3-n-3), with concentrations ranging from 0.5% to 1.5% in feed. Another function of linolenic fatty acids described by Gao et al. (2011) is the formation of double bonds in HUFA, EPA, and DHA, which is very important for metabolic function and the components in cell membranes.

Based on the results of this study, the survival rate of tilapia fed with fermented *L. minor* meal as a substitute for soybean meal was 94.44%. This was higher than the survival rate of tilapia without the *L. minor* meal substitution, which was 88.00%. This survival rate was higher than that reported by Sulawesty et al. (20¹⁷) using carp (*Cyprinus carpio* L.) that were not fed with *L. minor*, which ha¹⁷ survival rate of 60%. However, those that were fed with *L. minor* had a survival rate of 70%. Tilapia fed with fermented *L. minor* meal as a substitute for soybean meal exhibited a higher survival rate. The high survival rate of tilapia was caused by internal and external factors. Internal factors included feed, age, and resis¹⁰ to disease, whereas external factors comprised the stocking dens¹⁰ disease, and water quality. Kelabora (2010) stated that an important factor affecting the growth and survival rate of fish other than feed is water quality, especially temperature. As tempe¹³re can affect the growth and appetite of fish, the temperature can affect important activities of fish, such as breathing, growth, and reproduction. High temperatures can reduce dissolved oxygen and affect fish appetite.

The best growth was for the treatment of tilapia fed with 2.5% fermented *L. minor* meal because it produced a weight gain of 33.03% and an RGR of 2.01%. The results showed⁴ that feeding tilapia with fermented *L. minor* meal had a signi⁴ficant effect ($P < 0.05$) on TFC, FUE, PER, and RGR, but did not have a significant effect ($P > 0.05$) on SR. Currently, only a few previous studies have focused on *L. minor* as a feed ingredient. The results of this study, which showed higher values than those reported by previous studies, will have an important impact on research

on the use of *L. minor* as a feed substitution for fish, especially tilapia.

The use of fermented *L. minor* meal as a substitute for soybean meal was possible because tilapia is an omnivorous fish that tend to be herbivorous; consequently, it is easier for them to adapt to feed with vegetable sources. Additionally, 2.5% fermented *L. minor* meal can be used by farmers, thereby reducing production costs, especially for the feed.

In conclusion, the growth performance and nutritional quality of tilapia fed by fermented *L. minor* meals had been reported. Feeding of tilapia using fermented *L. minor* meal has a significant effect ($P < 0.05$) on total feed consumption, feed utilization efficiency, protein efficiency ratio, and relative growth rate, and had no significant effect ($P > 0.05$) on survival rate. Treatment B (the usage of 2.5% fermented *L. minor* meal as a substitute for soybean meal) gave the best result as the best feed formulation which can be applied for future fish aquaculture rearing.

5

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9
