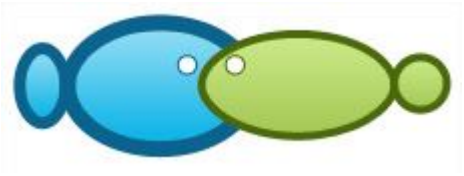

Feeding effectiveness of frozen and preserved *Daphnia magna* mass-cultured using fermented organic waste, and artificial feed on growth and nutritional quality of tilapia (*Oreochromis niloticus*)

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Abstract. The availability of *Daphnia magna* is very dependent on nature, so an alternative is needed to use frozen and preserved *D. magna* to maintain the availability of natural feed. The research objective was to find the nutritional quality and growth of tilapia (*Oreochromis niloticus*) fed by frozen and preserved *D. magna* mass-cultured using fermented organic waste, and artificial feed through feed utilization rates, survival and nutritional quality of tilapia. Experimental research was using completely randomized design (CRD) with three treatments, three replications each: treatment A (frozen *D. magna*), B (preserved *D. magna*) and C (artificial feed). Tilapia which used was D3 (3 days old) fish larvae, and feed was given four times a day by applying the fixed feeding rate method of 30% biomass weight with a rearing time of 14 days. The three different treatments had a very significant effect on relative growth rate, final body weight, efficiency of feed utilization, net protein utility, final body weight, protein efficiency ratio, and had a significant effect on survival rate. Treatment A (frozen *D. magna*) gave the best results at a relative growth rate of 2.3%, final body weight 2.42 g, feed utilization efficiency 17.03 g, net protein utility 1.90%, protein efficiency ratio 2.75% and the survival rate was 98.55%. Based on the best nutritional content in the same treatment, namely the highest protein and fat, 59.92% and 14.78% respectively, the highest essential fatty acids were linoleic and linolenic acids, 8.36% and 6.75% respectively. In contrast, the highest amino acids were lysine and methionine, 33.19 ppm and 27.06 ppm, respectively.

Key Words: *Daphnia magna*, growth, postlarvae, tilapia.

Introduction. Nile tilapia, *Oreochromis niloticus* is an omnivorous fish which lives in freshwater, having a fast growth (Herawati et al 2019). Quality of larvae is highly determined by food which is suitable for mouth openings and its nutrient needed. Natural feed in the form of *Daphnia magna* is the best type of feed for larval rearing. The advantage of *D. magna* is that it has a high nutritional content and a size that will fit the mouth of the fish larvae (Nwachi 2013; Herawati et al 2017, 2018). The need for feed-in tilapia larvae depends on the availability of feed-in each season. A method for storing feed is needed so that the feed can last a long time, and the nutritional content in the feed does not change, in this case, it can be done by the freezing and preserved method. Nutrients are a major factor required for growth and survival. In addition to nutrients in the feed, an adequate and appropriate frequency of feeding is also needed to support the growth of tilapia larvae. Nutrients in feed, especially protein, are used as a source of energy for growth and reproduction (Ovie & Eze 2013).

Fermentation is a way to improve the nutritional quality of *D. magna* culture media as a natural feed source (Nwaichi 2013; Herawati et al 2018). The use of *D. magna* in this study is an implementation of the mass culture of *D. magna* using organic

waste fermentation in the form of frozen, preserved and artificial feed. Live *D. magna* cannot be stored for a long time because its growth pattern is short and very dependent on natural conditions, so it is necessary to find other alternatives, by freezing or preserving it.

Feed that is frozen in the freezer at a temperature of 0^{o}C does not change its shape, like a living condition, so it is safe if tilapia larvae will consume it. The level of feed consumption will affect both individual growth and biomass at the end of rearing, which is related to larval growth. *D. magna* is the right choice as natural feed because it has a relatively small size and nutrient content needed by tilapia larvae, it can adjust the digestive tract of tilapia larvae which is still in a simple form (Lim et al 2011; Herawati et al 2015).

This study aims to find the nutritional quality and growth of tilapia larvae by feeding *D. magna* in frozen and preserved form from mass culture results using organic waste fermentation and artificial feed to increase tilapia production.

Material and Method. The study was conducted at Aquaculture Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Science, Diponegoro University from April to July 2020. Nile tilapia were obtained from Siwarak Fish Seed Center, Semarang with 0.05±0.02 g fish^{-1} of average weight. The treatments conducted in this study are as follows:

A. feeding of tilapia by using frozen *D. magna*;
B. feeding of tilapia by using preserved *D. magna*;
C. feeding of tilapia by using artificial feed (commercially available).

Fermentation stage. The fermentation used 1 mL of molasses, 1 mL of probiotic bacteria and 100 mL of solvent. The organic materials used (chicken manure, rejected bread, and tofu wastes) were dried. The treatment conducted during the study was feeding tilapia using *D. magna* mass cultured by 50 g L^{-1} of chicken manure + 100 g L^{-1} of rejected bread + 50 g L^{-1} of tofu waste fermented for about 28 days (Herawati et al 2018). The fermentation process using *Lactobacillus casei* and *Saccharomyces cerevisiae* in culture medium was to increase growth and enrich the nutrients of Nile tilapia larvae's feed. The first stage of culture media fermentation was the preparation of the ratio of molasses and probiotic microorganisms. The ratio used was 1:1 with 1 mL of molasses and 1 mL of probiotic microorganisms. Furthermore, 100 mL of water was added as a solvent. Then 50 g L^{-1} of chicken manure + 100 g L^{-1} of rejected bread + 50 g L^{-1} of tofu waste as the material for culture medium were dried then fermented for 28 days (Herawati et al 2018). Probiotic microorganisms (*L. casei* and *S. cerevisiae*) that were already activated for 3 h were added to the culture medium with a combined weight of 200 g L^{-1} (Abu-Elala et al 2013; Herawati et al 2018).

D. magna culture. The 1000 individuals L^{-1} *D. magna* was spread into 6 ponds with a volume of 600 liters with 3 repetitions. For each pond, the amount of fermented organic fertilizer put into the pond is 200 g L^{-1}. Observation for the abundance of *D. magna* was conducted every two days. The water quality was monitored every morning at around 7 a.m. The pH was maintained at 8.1-8.2, with the addition of 1 L of dolomite / 1.000 L of water (Herawati et al 2018, 2019).

Frozen D. magna. The freezing process was as follows: *D. magna* was harvested from a mass culture pond then cleaned and stored in a refrigerator with a temperature around the value of -20°C so that the decay process does not occur. The freezing process for *D. magna* is efficient, then its product also easy to be fed to tilapia.

Preserved D. magna. *D. magna* is added with preservatives in the form of organic acids such as citric acid, formic acid, acetic acid and sodium alginate. Based on Nugroho et al (2015), *D. magna* was preserved using formic acid 3% as much as 70 mL, 20 mL of 40% acetic acid, 1 g of 100% citric acid and 1 g of alginate. Preservation takes for about 3 days.

Artificial feed. The artificial feed given to tilapia is commercial feed in powder form. Proximate nutritional content, amino acids and fatty acids are presented in Tables 1-3.

Proximate analysis. The parameters used for proximate analysis were protein, fat, ash, crude fiber, and water. The analysis was performed once the sample is dried. An amount of 20 g of dry weight for sample was used for proximate analysis. Lipid samples were analyzed by gravimetric method using the weight of the sample to find the lipid levels. The proximate chemical composition of the samples was determined using a standard procedure (AOAC 2005; Herawati et al 2018). Protein analysis was performed using the Kjeldahl method, while carbohydrate analysis was carried out manually based on the results of the proximate analysis.

Essential amino acid profile. 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 at pH 5.9; and B) 0.01 M MeOH acetate buffer at pH 5.9; THF > 80:15:5 Λ Fluorescence: Ext: 340 mm Em: 450 nm. About 2.5 g of 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 110ºC for 12 hours, before bein g cooled down to room temperature and neutralized with NaOH 6N. After the addition of 2.5 mL of 40% Pb Acetat and 1 mL of 15% oxalate acid, around 3 mL of the mixture was filtered with 0.45 µm millex. 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 final mixture was put into the HPLC. (Shimadzu LC-6A) (AOAC 2005; Herawati et al 2018).

Fatty acid profile. The equipment used for this purpose was a Gas Chromatoghrap and the (GCMS) QP-2010 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°C. The method employed was *in situ* transcertification. An amount of 100 mg of 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 90ºC for 10 minutes. The reaction tube was then cooled and 1 mL of 14% BF3 in methanol was added. After the nitrogen addition, heating ensued at the same temperature for the next 10 minutes. Afterwards, 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 acid's methyl ester. After centrifugation, the upper layer of sample was ready for GC analysis (AOAC 2005; Herawati et al 2018).

Application of feed on tilapia larvae culture. There were three feed treatments in the study, with 3 replications each: *D. magna* from mass culture using organic waste fermentation in the form of frozen and preserved *D. magna*, and artificial feed for 17 days of maintenance. The artificial feed was given in powder form. Feeding was done using the *ad libitum* method, which was given four times a day, namely at 08:00, 12:00, 16:00 and 20:00 Western Indonesian Time (Herawati et al 2015).

Water quality. The water quality during the research was maintained at 28-30^{o}C temperature, 0.3 ppm DO and 8.1-8.2 pH. These are assumed to be the ideal conditions for *O. niloticus* rearing. Thus, according to Schlotz et al (2012), Herawati et al (2015, 2019), that the proper temperature for *O. niloticus* larvae is 25-30^{o}C, DO at 0.3-0.6 ppm, and pH at 6.5-9.

Statistical analysis. 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.

Relative growth rate (RGR), efficiency of feed utilization (EFU), net protein utility (NPU), protein efficiency ratio (PER), survival rate (SR), and final body weight (FBW) were calculated based on Tacon (1983):

- RGR = $((W_t - W_0)/(W_0 \times t)) \times 100$, where RGR is relative growth rate (% day^{-1}), W_t is total weight of fish in the end of the research (g), W_o is total weight of fish in the beginning of the research (g), and t is time of fish aquaculture (days);

- EFU = $((W_t - W_0)/F) \times 100$, where EFU is efficiency of feed utilization (%), W_t is final biomass in the end of the study (g), W_0 is initial biomass in the beginning of the study (g), F is total feed consumed during the study;

- NPU = $((P_b - P_a)/P_i) \times 100$, where NPU is net protein utilization (%), P_b is the total protein content of tested animal in the end of the study (%), P_a is the total protein content of tested animal in the beginning of the study (%), and P_i is the amount of feed protein consumed by fish (%);

- PER = $((W_t - W_0)/P_i) \times 100$, where PER is protein efficiency ratio (%), W_t is total weight of fish in the end of the research (g), W_0 is total weight of fish in the beginning of the research (g), P_i is total feed consumed multiplied by fish protein content;

- SR = $N_t/N_0 \times 100$, where SR is survival rate (%), N_t is total of fish in the end of the research (fish), and N_0 is total of fish in the beginning of the research (fish)

- FBW = W_t-W_0, where FBW is the final biomass weight of tilapia (g); W_t is final tilapia larval weight (g), W_0 is initial body weight of tilapia larval (g).

Results. The proximate analysis results for each treatment are presented in Table 1. Treatment A (frozen *D. magna*) showed the highest proximate result for protein and fat, with 56.19% of protein, 10.26% of fat, then carbohydrate was 13.80%, 6.75% of crude fibre and 10% of ash.

Table 1

Proximate analysis of frozen and preserved of *D. magna*, and artificial feed

Proximate composition (%)	*Frozen D. magna*	*Preserved D. magna*	*Artificial feed*
Ash	10.00±0.08	16.24±0.02	29.24±0.02
Fat	10.26±0.05	6.36±0.09	7.36±0.09
Crude fiber	6.75±0.07	7.91±0.02	13.91±0.02
Protein	56.19±0.26	53.19±0.13	33.19±0.13
Carbohydrate	13.80±0.17	16.30±0.19	16.30±0.19

The results of total amino acid content for each treatment are presented in Table 2. The total amino acid content of the treatments showed that treatment A (frozen *D. magna*) had the highest level of lysine (36.53 ppm) for essential amino acids and for non-essential amino acids were glutamic acid (24.36 ppm); the lowest amino acid was tryptophan in treatment C (artificial feed).

Table 2

The total amino acid profile of frozen and preserved *D. magna* mass culture results using organic waste fermentation, and artificial feed

Amino acid profile (ppm)	*Frozen D. magna*	*Preserved D. magna*	*Artificial feed*
Histidine	15.19±0.09	9.92±0.08	8.14±0.03
Serine	19.03±0.03	13.61±0.03	5.40±0.07
Arginine	18.19±0.03	16.61±0.04	7.39±0.02
Glycine	19.03±0.06	15.66±0.04	8.48±0.05
Aspartic acid	18.90±0.09	13.18±0.03	8.25±0.09
Glutamic acid	24.36±0.08	15.43±0.04	9.76±0.05
Threonine	21.78±0.06	19.02±0.09	8.37±0.09
Alanine	23.20±0.09	20.79±0.05	5.21±0.02
Cystine	25.87±0.07	18.24±0.05	15.74±0.04
Lysine	36.53±0.03	25.94±0.15	15.45±0.09
Tyrosine	17.10±0.05	15.67±0.09	9.86±0.06
Methionine	28.98±0.03	14.90±0.06	14.95±0.09
Valine	15.23±0.02	16.09±0.02	8.67±0.04
Isoleucine	13.25±0.03	11.75±0.03	5.62±0.03
Leucine	15.98±0.01	12.23±0.04	6.82±0.01
Phenylalanine	13.73±0.03	11.03±0.07	5.40±0.05
Tryptophan	14.97±0.09	12.67±0.07	5.39±0.03

The fatty acid profile of each treatment was presented in Table 3. The results of fatty acid content showed that treatment A had the highest essential fatty acid levels (linoleic, 8.20%) and non-essential fatty acids (palmitic, 5.98%), while the lowest fatty acids were in treatment C (DHA and myristic). The results of the study found that treatment A on the larvae of tilapia at stage D3-D17 gave the best results.

Table 3

Total fatty acid profile of frozen and preserved *D. magna*, the result of mass culture using organic waste fermentation and artificial feed

Fatty acid profile (%)	*Frozen D. magna*	*Preserved D. magna*	*Artificial feed*
Myristic	1.93±0.09	0.99±0.07	0.18±0.06
Pentadecanoic	2.59±0.04	2.01±0.05	1.29±0.08
Palmitic	5.98±0.05	3.63±0.05	2.91±0.02
Stearic	2.79±0.06	1.98±0.04	1.95±0.03
Oleic/ω9	5.78±0.08	2.02±0.05	3.46±0.07
Linoleic/ω6	8.20±0.08	2.32±0.06	4.32±0.09
Linolenic/ω3	6.96±0.04	2.68±0.04	3.05±0.02
Arachidic	4.83±0.04	2.79±0.08	0.18±0.05
Arachidonic	4.19±0.07	1.59±0.09	3.52±0.09
DHA	2.98±0.07	0.75±0.02	1.63±0.02
EPA	3.89±0.05	0.36±0.05	1.79±0.04

The growth performance of tilapia fed by frozen *D. magna*, preserved *D. magna*, and artificial feed presented in Table 4. Feeding tilapia larvae (in stage D3-D20) *D. magna* in the frozen form four times a day was the best treatment based on the following parameters: final weight of 2.42 g, relative growth rate of 2.3% per day, biomass weight of 2.48 g, survival rate of 98.55%, net protein utility of 1.90% and protein efficiency ratio of 2.75%.

Table 4

Growth performance of tilapia fed by frozen *D. magna*, preserved *D. magna*, and artificial feed

Parameters	*Frozen D. magna*	*Preserved D. magna*	*Artificial feed*
IBW (g)	0.06 ± 0.02^{a}	0.06 ± 0.02^{a}	0.05 ± 0.05^{a}
FBW (g)	2.42 ± 0.05^{ab}	1.39 ± 0.02^{a}	1.45 ± 0.07^{a}
WG (g)	2.48 ± 0.02^{ab}	1.45 ± 0.01^{a}	1.40 ± 0.01^{a}
RGR (% day^{-1})	2.3 ± 0.03^{ab}	1.45 ± 0.09^{b}	1.29 ± 0.09^{b}
UFE (g)	17.03 ± 0.03^{ab}	9.92 ± 0.03^{a}	10.35 ± 0.07^{a}
NPU (%)	1.90 ± 0.06^{ab}	1.45 ± 0.07^{b}	1.35 ± 0.04^{b}
PER (%)	2.75 ± 0.02^{ab}	1.66 ± 0.04^{b}	1.52 ± 0.03^{b}
SR (%)	98.55 ± 0.13^{b}	98.23 ± 0.02^{b}	97.15 ± 0.08^{b}

Note: Values with different superscripts in the same column indicate significant differences ($p < 0.05$).

The results of the proximate analysis of tilapia by feeding frozen and preserved *D. magna*, mass culture using fermented organic waste and artificial feed as feed are presented in Table 5. The proximate analysis showed that tilapia fed the frozen form of *D. magna* had the highest fat and protein levels (59.92% and 14.78% respectively), whereas the lowest levels were found in tilapia fed artificial feed (46.30% of protein and 9.89% of fat).

The total fatty acid profile of tilapia by feeding frozen *D. magna*, preserved mass culture results using organic waste fermentation and artificial feed as feed is presented in Table 6. Analysis of the fatty acid profiles revealed that tilapia fed *D. magna* in the frozen form had the highest levels of linoleic and linolenic acids (8.36% and 6.75%, respectively), whereas the lowest levels were recorded in tilapia fed artificial feed (4.05% and 3.75%, respectively).

Table 5

The results of proximate analysis of tilapia fed by frozen and preserved *D. magna*, mass culture results using organic waste fermentation and artificial feed

Proximate composition (%)	*Before treatments*	*Frozen D. magna*	*Preserved D. magna*	*Artificial feed*
Ash	19.86±0.06	9.46±0.06^{b}	16.50± 0.05^{b}	18.25± 0.05^{ab}
Fat	10.98±0.11	14.78±0.11^{ab}	10.43±0.06^{a}	9.89±0.01^{a}
Crude Fiber	13.24±0.01	5.74±0.01^{b}	8.23±0.07^{b}	10.53±0.04^{ab}
Protein	40.22±0.03	59.92±0.03^{ab}	50.69±0.05^{b}	46.30±0.09^{b}
Carbohydrate	15.70±0.09	10.10±0.09^{b}	14.15±0.10^{b}	16.03±0.06^{ab}

Note: Values with different superscripts in the same column indicate significant differences ($p < 0.05$).

Table 6

The profile of total fatty acids of tilapia by feeding frozen and preserved *D. magna*, mass culture results using organic waste fermentation and artificial feed

Fatty acids (%)	*Before treatment*	*Frozen D. magna*	*Preserved D. magna*	*Artificial feed*
Myristic	0.45±0.04	1.98±0.02^{a}	1.05±0.07^{a}	0.95±0.03^{a}
Pentadecanoic	1.28±0.08	2.81±0.04^{a}	2.08±0.05^{a}	2.25±0.05^{a}
Palmitic	1.91±0.02	5.98±0.05^{b}	3.78±0.05^{b}	3.45±0.04^{b}
Stearic	1.95±0.03	3.18±0.06^{b}	2.98±0.04^{b}	1.95±0.01^{b}
Oleic/ω9	3.45±0.07	5.95±0.08^{b}	4.12±0.05^{b}	3.95±0.06^{b}
Linoleic/ω6	3.32±0.09	8.36±0.08^{b}	4.82±0.06^{b}	4.05±0.01^{b}
Linolenic/ω3	3.05±0.02	6.75±0.02^{b}	3.88±0.03^{b}	3.75±0.06^{b}
Arachidic	1.28±0.07	5.95±0.04^{b}	3.79±0.08^{b}	2.63±0.07^{b}
Arachidonic	2.52±0.09	5.35±0.07^{b}	3.89±0.09^{b}	2.55±0.07^{b}
Eicosapentaenoic	3.91±0.04	5.59±0.08^{b}	4.38±0.07^{b}	3.15±0.07^{b}
AA	2.56±0.07	2.15±0.09^{a}	1.99±0.01^{a}	1.92±0.01^{a}
DHA	4.46±0.06	4.97±0.07^{b}	4.97±0.07^{a}	0.83±0.05^{b}
EPA	3.56±0.07	5.15±0.09^{b}	1.99±0.01^{b}	1.82±0.01^{b}

The total amino acid profile of tilapia by feeding frozen *D. magna*, preserved mass culture using organic waste fermentation and artificial feed as feed is presented in Table 7. Amino acid profile analysis showed that lysine and methionine were recorded at the highest levels (33.19 ppm and 27.06 ppm, respectively) in tilapia fed frozen *D. magna* (A).

Table 7

Total amino acid profile of tilapia by feeding frozen and preserved *D. magna*, mass culture results using organic waste fermentation and artificial feed

Amino acid (ppm)	*Before treatment*	*Frozen D. magna*	*Preserved D. magna*	*Artificial feed*
Histidine	8.25±0.09	19.85±0.09^{b}	10.69±0.03^{b}	10.15±0.04^{b}
Serine	6.23±0.07	17.99±0.03^{b}	11.98±0.07^{b}	14.06±0.03^{b}
Arginine	7.99± 0.02	20.25±0.03^{b}	14.15±0.02^{b}	15.98±0.04^{b}
Glycine	8.98±0.05	18.88±0.06^{b}	13.10±0.05^{b}	16.15±0.04^{b}
Aspartic acid	8.95±0.09	20.85±0.09^{b}	11.17±0.08^{b}	14.29±0.03^{b}
Glutamic acid	10.15±0.07	26.17±0.09^{b}	20.26±0.09^{b}	21.96±0.08^{b}
Threonine	8.85±0.09	20.26±0.06^{b}	17.25±0.09^{b}	20.59±0.09^{b}
Alanine	6.23± 0.02	23.03±0.09^{b}	7.75±0.02^{b}	17.10±0.05^{b}
Cystine	7.59±0.07	25.56±0.03^{b}	11.98±0.07^{b}	14.06±0.03^{b}
Lysine	10.85±0.09	33.19±0.03^{b}	22.96 ±0,09^{b}	21.76±0.15^{b}
Tyrosine	8.25±0.09	19.25±0.03^{b}	16.76±0.02^{b}	20.98±0.04^{b}
Methionine	12.23±0.07	27.06±0.03^{b}	19.98±0.07^{b}	14.06±0.03^{b}
Valine	10.05±0.09	23.06±0.09^{b}	12.17±0.08^{b}	14.29±0.03^{b}
Isoleucine	6.19±0.07	20.56±0.03^{b}	11.98±0.07^{b}	14.06±0.03^{b}
Leucine	9.60±0.09	25.06±0.06^{b}	18.25±0.09^{b}	20.59±0.09^{b}
Phenylalanine	11.80±0.07	22.10±0.03^{b}	11.98±0.07^{b}	14.06±0.03^{b}
Tryptophan	10.23±0.03	20.06±0.09^{b}	13.26±0.09^{b}	13.68±0.07^{b}

Discussion. This study is a development on previous research on mass culture of *D. magna* using chicken manure, rice bran, and coconut cake fermented by probiotic bacteria (Herawati et al 2015), as well as the research of Herawati et al (2018) using various types of wastes in mass culture media of *D. magna* with the fermentation of culture media for 14 days, using manure, tofu waste, and coconut cake with different fermentation times. The present study is an application of feeding *D. magna* mass cultured using fermented organic waste in frozen, preserved, and artificial feed to increase the production and nutritional quality of tilapia.

Feeding tilapia with *D. magna* from mass culture using organic waste fermentation in the frozen form (A) showed the best results at a relative growth rate of 2.3%, final body weight of 2.42 g, feed utilization efficiency of 17.03 g, net protein utility of 1.90%, protein efficiency ratio of 2.75%, and survival rate of 98.55%. Analysis of variance showed that feeding tilapia larvae using treatment A significantly affected the relative values of growth rate, final body weight, feed utilization, net protein utility, protein efficiency ratio, and survival rate. Treatment A had a higher nutrient content, that met the nutritional requirements of larvae and could be maximally utilized by tilapia larvae compared to treatments B and C. Additionally, the odor of frozen *D. magna* as a distinctive attractant was the same as that of fresh *D. magna*.

The rate of feed utilization was closely related to the growth rate of tilapia larvae. The highest protein and fat contents were recorded in treatment A (59.92% and 14.78%, respectively). The nutrients in natural feed eaten by the larvae are absorbed by the body and used as energy for metabolism and larval growth. The growth of tilapia is influenced by both internal and external factors. Internal factors, including heredity, sex, age, parasites, and disease, are difficult to control. The main external factors that affect growth include feed, temperature, dissolved oxygen, ammonia, pH, and salinity (Ovie & Eze 2013; Herawati et al 2015).

The high protein content of *D. magna* in the frozen form as feed can meet the requirement of tilapia larvae to support their growth. The excess protein in the feed is used to form body tissue, which is reflected by the increase in fish weight and size. Fish can grow well if the nutrients obtained from the feed can be used effectively and efficiently. This statement follows the results of previous studies that show that the nutritional content of feed that meets the nutritional requirements will accelerate their growth (Lim et al 2011; Nwaichi 2013). The fish body utilizes protein, fat, and carbohydrates in the feed as energy sources (Limsuwatthanathamrong et al 2012). *Daphnia* sp. contain digestive enzymes, including proteinase, peptidase, amylase, lipase, and cellulase, which function as exo-enzymes during digestion (Nguyen et al 2012).

In the present study, tilapia fed artificial feed recorded the lowest results; this is because the nutrient content of the artificial feed (protein and fat 33.19% and 7.36% respectively) cannot meet the nutritional requirement of tilapia. This statement follows the observations of Gao et al (2011) that omnivorous fish larvae such as Nile tilapia require feed with a minimum of 38% protein and 10% fat content. Furthermore, Ovie & Eze (2013) demonstrated that for optimum growth of Nile tilapia larvae feed with more than 35% protein content is required.

The fatty acid analysis results showed that the highest linoleic and linolenic acid contents were found in treatment A (8.36% and 6.75%, respectively). Linoleic and linolenic fatty acids play important roles in fat transport and metabolism, immune function, and cell membrane function and integrity. Linoleic and linolenic fatty acids function to eliminate chylomicron lipoproteins from the plasma and reduce the production of triglycerides and β (beta) apolipoproteins in the liver (Nguyen et al 2012). Linoleic and linolenic fatty acids are essential for proper functioning of the brain and retina for normal growth and function of all tissues that cannot be synthesized (Nguyen et al 2012; Tocher 2015). Derivatives of these fatty acids are arachidonic acid from linoleic acid, and eicosapentaenoic acid (EPA) and docosahexaenoic (DHA) from linolenic acid. Linoleic and linolenic acids are precursors of a group of hormone-like eicosanoid compounds such as prostaglandins, prostacyclin, thromboxane, and leukotrienes. These compounds regulate blood pressure, heart rate, immune function, nervous system stimulation, muscle contraction, and wound healing (Ovie & Eze 2013).

The highest levels of amino acids in treatment A were lysine (33.19 ppm) and methionine (27.06 ppm). Lysine functions as a building block for vitamin B, stimulates appetite, and helps in the production of carnitine to convert fatty acids into energy (Ovie & Eze 2013; Valverde et al 2013; Herawati et al 2018). Methionine improves the balance and utilization of other amino acids to increase fish growth, and also plays an important role in protein synthesis and other physiological functions. In addition, methionine and cysteine are the primary sources of amino acid sulfate in animals (Bhagavan 1992). The body requires methionine to form nucleic acids and tissues in protein synthesis and is also a building block for other amino acids (cysteine) and vitamins (choline). Methionine functions together with vitamin B12 and folic acid to assist the body in regulating excessive protein supply in a high-protein diet. Fish require methionine in their feed at a level of 2.30%. Tissue protein synthesis is predominantly determined by the completeness and levels of amino acids that are transported to the tissues. Gao et al (2011) showed that the synthetic process of ribosomes is highly dependent on the presence of the amino acids. Methionine is required by fish to initiate protein synthesis and assist muscle growth (Belghit et al 2014). It has been proven that the addition of methionine in feed increases growth and immune responses (Yuan et al 2011; Kuang et al 2012; Boonyoung et al 2013; Ma et al 2013; Gao et al 2011). Methionine deficiency can lead to decreased growth and survival in carp (*Cyprinus carpio*) (Tang et al 2009), cobia (*Rachycentron canadum*), and rainbow trout (*Oncorhynchus mykiss*) (Poston 1986) and also causes cataracts in red snapper (Takagi et al 2001).

Environmental conditions also greatly influence the survival rate of tilapia larvae. Water quality must remain optimal, and the feed must be of high quality and at appropriate amount and size. Based on the analysis of the variance of tilapia, treatment A had a significant effect on survival, exhibiting the highest survival rate (98.55%). The factors that influence the survival rate include the transition period of the larval rearing media (that can cause stress to fish larvae), changes in feed intake from egg yolk to natural food when yolk is depleted, water quality, and contamination of larval rearing media. The results of this study are consistent with those of Tocher (2015), who stated that the environmental conditions (water quality) must be optimal, and the amount and size of available feed must be based on the quality and quantity of available feed sources that are known to support the survival rate of tilapia larvae.

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References

Abu-Elala N., Marzuok, M., Moustafa, M. 2013. Use of different *Saccharomyces cerevisiae* biotic form as immune modulator and growth promoter for *Oreochromis niloticus* challenged with some fish pathogens. International Journal of Veterinary Science and Medicine 1:21–29.

AOAC, 2005 Official methods of analysis. 18th edition, Association of Official Analytical Chemists, Benjamin Franklin Station, Washington, 26 pp.

Bhagavan N. V., 1992 Medical biochemistry. Jones and Barlett Publisher, 980 pp.

Belghit I., Skiba-Cassy S., Guerden I., Dias K., Surget A., Kaushik S., Panserat S., Seiliez I., 2014 Dietary methionine availability affects the main factors involved in muscle protein turnover in rainbow trout (*Oncorhynchus mykiss*). British Journal of Nutrition 112(4):493-503.

Boonyoung S., Haga Y., Satoh S., 2013 Preliminary study on effects of methionine hydroxy analog and taurine supplementation in a soy protein concentrate-based diet on the biological performance and amino acid composition of rainbow trout [*Oncorhynchus mykiss* (Walbaum)]. Aquaculture Research 44(9):1339-1347.

Gao W., Liu Y. G., Tian L. X., Mai K., Liang G. Y., Yang H. J., Huai M. Y., Luo W. J., 2011 Protein sparing capability of dietary lipid in herbivorous and omnivorous freshwater finfish: a comparative case study on grass carp (*Ctenopharyngodon idella)* and tilapia (*Oreochromis niloticus* x *O. aureus*). Aquaculture Nutrition 17(1):2-12.

Herawati V. E., Hutabarat J., Pinandoyo, Radjasa O. K., 2015 Growth and survival rate of tilapia (*Oreochromis niloticus*) larvae fed by *Daphnia magna* cultured with organic fertilizer resulted from probiotic bacteria fermentation. HAYATI Journal of Biosciences 22(4):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 Science: Earth and Environmental Science 116(1):012089.

Herawati V. E., Hutabarat J., Pinandoyo, Rismaningsih N., Karnaradjasa O., 2019 Mass culture of *Daphnia magna* Straus, 1820 in fermented medium as feed to enhance nutrient quality and growth performance of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) larvae. Asian Fisheries Science 32:182-189.

Kuang S. Y., Xiao W. W., Feng L., et al, 2012 Effects of graded levels of dietary methionine hydroxy analogue on immune response and antioxidant status of immune organs in juvenile Jian carp (*Cyprinus carpio* var. Jian). Fish and Shellfish Immunology 32(5):629-636.

Lim C., Yldirim-Aksoy M., Klesius P., 2011 Lipid and fatty acid requirements of tilapias. North American Journal of Aquaculture 73(2):188-193.

Limsuwatthanathamrong M., Sooksai S., Chunhabundit S., et al, 2012 Fatty acid profile and lipid composition of farm-raised and wild-caught sandworms, *Perinereis nuntia*, the diet for marine shrimp Broodstock. Asian Journal of Animal Sciences 6:65-75.

Ma R., Hou H., Mai K., Bharadwaj A. S., Cao H., Ji F., Zhang W., 2013 Comparative study on the effects of L-methionine or 2-hydroxy-4-(methylthio) butanoic acid as dietary methionine source on growth performance and anti-oxidative responses of turbot (*Psetta maxima*). Aquaculture 412-413:136-143.

Nguyen B. T., Koshio S., Sayikama K., Ishikawa M., Yokoyama S., Kader M. A., 2012 Effects of polychaete extracts on reproductive performance of kuruma shrimp, *Marsupenaeus japonicus* Bate. Part II. Ovarian maturation and tissue lipid compositions. Aquaculture 334-337:65-72.

Nugroho I. I., Subandiyono, Herawati V. E., 2015 [Utilization rate of frozen *Artemia* sp., preserved *Artemia* sp. and silkworms for growth and survival of gurami (*Osphronemus gouramy*, Lac.) larvae]. Journal Aquaculture Management and Technology 4(2):117-124. [in Indonesian]

Nwachi O. F., 2013 An overview of the importance of probiotic in aquaculture. Journal of Fisheries and Aquatic Science 8(1):30-32.

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.

Poston H. A., 1986 Response of rainbow trout to source and level of supplemental dietary methionine. Comparative Biochemistry and Physiology Part A 83(4):739-744.

Schlotz N., Sorensen G. J., Martin-Creuzburg D., 2012 The potential of dietary polyunsaturated fatty acids to modulate ecosanoid synthesis and reproduction in *Daphnia magna*: a gene expression approach. Comparative Biochemistry and Physiology Part A 162(4):449-454.

Tacon A. G. J., 1993 Feed ingredient for warmwater fish, fish meal and other processed feedstuffs. FAO Fisheries Circular (FAO) no. 856, 64 pp.

Takagi S., Shimeno S., Hosokawa H., Ukawa M., 2001 Effect of lysine and methionine supplementation to a soy protein concentrate diet for red sea bream *Pagrus major*. Fisheries Science 67(6):1088-1096.
Tang L., Wang G. X., Jiang J., Feng L., Yang L., Li S. H., Kuang S. Y., Zhou X. Q., 2009 Effect of methionine on intestinal enzymes activities, microflora and humoral immune of juvenile Jian carp (*Cyprinus carpio* var. Jian). Aquaculture Nutrition 15(5):477-483.
Tocher D. R., 2015 Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective. Aquaculture 449:94-107.
Valverde J. C., Martinez-Liorens S., Vidal A. T., et al, 2013 Amino acids composition and protein quality evaluation of marine species and meals for feed formulations in cephalopods. Aquaculture International: Journal of the European Aquaculture Society 21(2):413-433.
Yuan Y. C., Gong S. Y., Yang H. J., Lin Y. C., Yu D. H., Luo Z., 2011 Effects of supplementation of crystalline or coated lysine and/or methionine on growth performance and feed utilization of the Chinese sucker, *Myxocyprinus asiaticus*. Aquaculture 316(1-4):31-36.

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