HAYATI Journal of Biosciences 22 (2015) 169–173

Contents lists available at ScienceDirect

HAYATI Journal of Biosciences

journal homepage: http://www.journals.elsevier.com/hayati-journal-of-biosciences

Growth and Survival Rate of Tilapia (*Oreochromis niloticus*) Larvae Fed by *Daphnia magna* Cultured With Organic Fertilizer Resulted From Probiotic Bacteria Fermentation

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ARTICLE INFO

Article history:
Received 2 February 2015
Accepted 19 August 2015
Available online 11 December 2015

KEYWORDS:
D. magna,
fermentation,
growth,
survival,
tilapia

ABSTRACT

Daphnia magna is a potential feed for fish. The aim of this research was to find the best treatment and effect of *D. magna* culture addition from fermented organic fertilizer, to growth and survival rate of *Oreochromis niloticus* larvae. There were five treatments, each with three repetitions used in the study. All treatments used chicken dung, and different combinations of rice bran, coconut oilcake waste and tilapia larvae. Feeding on tilapia was given by *ad libitum* method for five times a day until 14 days. Water quality during the research was maintained at temperature 28–29°C, DO 0.3 ppm and pH 8.1–8.2. Observed variables include relative growth rate, survival rate, food consumption rate and water quality. Our results showed that *D. magna* cultured by fermented organic fertilizer for tilapia larvae (*O. niloticus*) had high significant effect ($p < 0.01$) on the relative growth rate and survival rate. Treatment of *D. magna* cultured by 1.2 g/L chicken manure, 0.9 g/L rice bran and 0.3 g/L coconut oilcake showed the highest value on the relative growth rate (10.86%); survival rate (98.46%) and food consumption at first week (106.43%) and second week (152.76%).

1. Introduction

Oreochromis niloticus has an excellent faster growth and tolerance even in bad environmental water condition. Quality of its larvae is highly determined by food nutrient and suitability for mouth opening. *Daphnia magna* is one of the most potential natural feed for fish larvae due to its high nutritional content. *D. magna* nutritional content largely depends on its culture medium because the growth of phytoplankton, which is the feed for *D. magna*, depends on it (Damle and Chari 2011). Nowadays, the most commonly used culture medium is fermented chicken dung (Zahidah 2012).

The mass-cultured *D. magna* used probiotic bacteria as it improves both biomass production and nutritional content of *D. magna*. Probiotic bacteria are really supportive for the health of organisms (Nwachi 2013). It also serves to decompose and ferment organic materials (Yuniwati *et al.* 2012). Decomposition is a biological process that makes the most of bacteria's ability to produce growth substances, hormones, vitamins, and other enzymes (Asadi *et al.* 2012; Zahidah *et al.* 2012).

The goal of this research was to find the best treatment and effect of *D. magna* culture addition from fermented organic fertilizer, to growth and survival rate of *O. niloticus* larvae.

2. Materials and Method

2.1. Fermentation stage

The first stage of fermentation is the preparation of the ratio of molasses, water and probiotic. The ratio was 1:1, i.e. 1 mL of both molasses and probiotic bacteria and 100 mL of solvent material. The organic materials used were chicken dung, rice bran, and coconut oilcake, all of which were dried (Herawati and Agus 2014). The treatments are (A) *D. magna* cultured with 1.2 g/L chicken dung and 1.2 g/L rice bran; (B) *D. magna* cultured with 1.2 g/L chicken dung, 0.9 g/L rice bran and 0.3 g/L coconut oilcake; (C) *D. magna* cultured with 1.2 g/L chicken dung, 0.6 g/L rice bran and 0.6 g/L coconut oilcake; (D) *D. magna* cultured with 1.2 g/L chicken dung, 0.3 g/L rice bran and 0.9 g/L coconut oilcake; and (E) *D. magna* cultured with 1.2 g/L chicken dung and 1.2 g/L coconut oilcake.

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Peer review under responsibility of Institut Pertanian Bogor.

http://dx.doi.org/10.1016/j.hjb.2015.08.001

Table 1. Nutrient analysis for organic fertilizer fermented with probiotic bacteria for the mass culture of *Daphnia magna*

Parameters	Unit (%)	A	B	C	D	E	Test method
N	%	0.67 ± 0.02	0.92 ± 0.09	0.73 ± 0.08	0.90 ± 0.05	0.88 ± 0.07	Kjedahl
P	%	0.20 ± 0.08	0.39 ± 0.03	0.33 ± 0.06	0.55 ± 0.07	0.35 ± 0.09	AOAC
K	%	0.43 ± 0.05	0.70 ± 0.06	0.52 ± 0.02	0.64 ± 0.04	0.64 ± 0.06	AOAC

The total combination of organic fertilizer (2.4 kg) was then added with probiotic bacteria (*Lactobacillus casei* and *Saccharamyces cerevisiae*) that were already activated for 3 hours (Yuniwati *et al.* 2012; Abu Elala *et al.* 2013). The result of nutrient analysis for organic fertilizer fermented with probiotic bacteria for the mass-cultured *D. magna* is available in Table 1.

2.2. Water quality

The water quality during the research was maintained at temperature 28–29°C, DO 0.3 ppm and pH 8.1–8.2. This is in line with Mokoginta *et al.* (2003), Jusadi *et al.* (2005) and Nina *et al.* (2012), who described that the proper temperature for *Tilapia* is 25–30°C, DO 0.3–0.6 ppm, and pH 6.5–9.0.

2.3. *D. magna* culture

One hundred individuals/L of *D. magna* was spread in each pool (Damle and Chari 2011). Observation for the abundance of *D. magna* was conducted every 2 days to monitor the population of *D. magna*. The water was routinely replaced and its temperature and pH level was monitored every morning (around 7 AM) to maintain its quality. The pH was maintained at its maximum with the addition of 1 L of dolomite/1000 L of water.

2.4. Fatty acid profile

The fatty acid profile of *D. magna* can be determined by analyzing its total fatty acid content. The equipment used for this purpose was a gas chromatograph and 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 used was *in situ* transcertification. One hundred milligrams of *D. magna* sample was homogenized using 4 mL of water. The resulting 100 μL homogenate was then transferred into a reaction tube (Park and Goins 1994).

One hundred microliters 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°C for 10 minutes. The reaction tube was then cooled and 1 mL of 14% BF3 in methanol was added. After nitrogen addition, heating was 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 minute to extract the fatty acid's methyl ester. After centrifugation, the upper layer of sample was ready for GC analysis.

2.5. Essential amino acid profile

The essential amino acid profile of *D. magna* was determined by examining its essential amino acid content. Essential amino acid analysis was conducted using an HPLC type 1100 with a Eurospher 100-5 C18, 250 × 4.6 mm column that has P/N: 1115Y535 precolumn. 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 (AOAC 1999). Then, 15 mL of HCl 6 N 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 6 N. After the addition of 2.5 mL of 40% Pb acetate 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.

2.6. Tilapia larvae culture

Tilapia larvae 3 days of age was cultured for 14 days with density of 48 individuals/L. Feeding in first week larvae culture, *D. magna* size was 0.5 mm with density of 1022 *D. magna*/L. At second week, *D. magna* size was 0.6–1.0 mm with density of 1467 *D. magna*/L. *D. magna* was given five times a day *ad libitum*.

3. Result

The growth of *D. magna* in mass culture with the use of probiotic bacteria is shown in Figure 1. The growth of *D. magna* in mass culture with the use of fermented organic fertilizer was significantly influential ($p < 0.01$) among treatments, in which the lag phase occurred in the 6-day period, while the exponential phase occurred on day 8. The organic fertilizer with 1.2 g/L chicken dung, 0.9 g/L rice bran and 0.3 g/L coconut oilcake has resulted in the highest breeding at 4.937 individuals/L.

The biomass weight of *D. magna* in mass culture with organic fertilizer fermented with probiotic bacteria is presented in Figure 2. The highest biomass weight of 161.83 g was obtained from the treatment using 1.2 g/L chicken dung, 0.9 g/L rice bran and 0.3 g/L coconut oilcake.

The proximate result of *D. magna* in mass culture probiotic bacteria can be found in Table 2. Based on the result of nutrient value content from proximate analysis, the highest fatty acid and amino acid profile was found in the treatment of organic fertilizer with 1.2 g/L chicken dung, 0.9 g/L rice bran and 0.3 g/L coconut oilcake. The highest protein content was 73.90%; saturated fatty acid found was palmitic (3.78%); unsaturated fatty acid found was linoleic (0.20%) and essential amino acid found was lysine (44.16 ppm). Table 3 presents the profile of fatty acid of *D. magna* in mass culture using the probiotic fermented organic fertilizer. Table 4 presents the profile of amino acid of *D. magna* in mass culture using organic fertilizer fermented with probiotic bacteria.

D. magna cultured by fermented organic fertilizer for tilapia larvae (*O. niloticus*) had high significant effect ($p < 0.01$) on the relative growth rate (Figure 3) and survival rate in treatment of *D. magna* cultured by 1.2 g/L chicken dung, 0.9 g/L rice bran and 0.3 g/L coconut oilcake (highest value on the relative growth rate was 10.86%, and survival rate was 98.46%). Table 5 presents relative growth, survival rate, food consumption of tilapia larvae fed by *D. magna*.

4. Discussion

The organic fertilizer containing 1.2 g/L chicken dung, 0.9 g/L rice bran and 0.3 g/L coconut oilcake gives the highest growth of *D. magna*. The nutrient in the culture media, particularly the highest N and P content that probably enhanced the growth of phytoplankton and bacteria as the food source for fulfilling the needs for nutrient

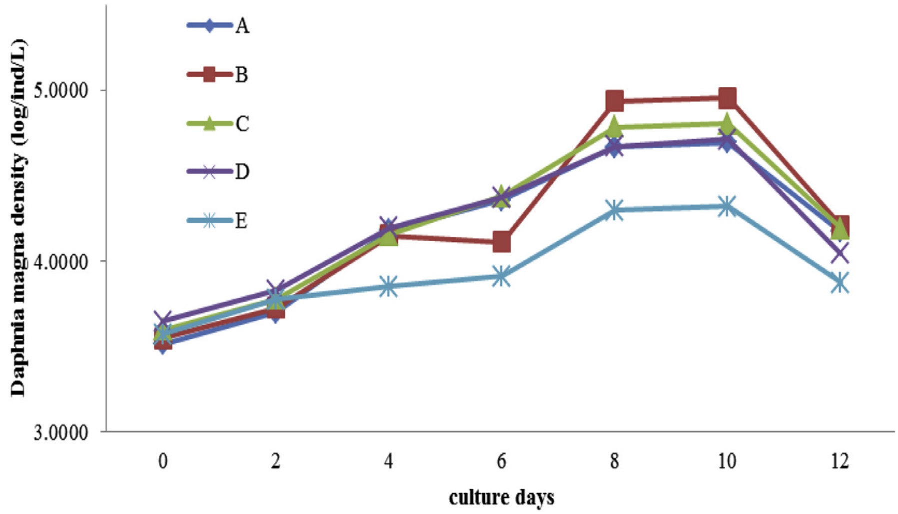

Figure 1. The growth of *Daphnia magna* in mass culture using the waste of husbandry and fermented with probiotic bacteria.

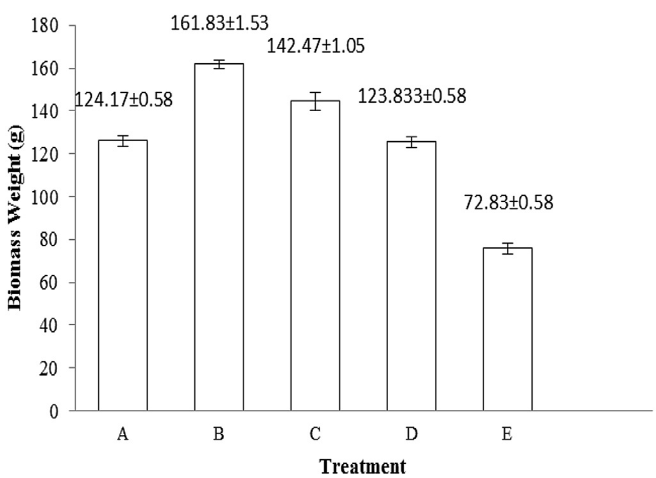

Figure 2. Biomass weight of *Daphnia magna* in mass culture with organic fertilizer fermented with probiotic bacteria.

required in the growth of *D. magna*. As revealed in the research by Pandriyani *et al.* (2012) and Rakhman *et al.* (2013), the organic substance in culture media can increase the number of bacteria and organic particles. The result of the decomposition by the bacteria can enhance the provision of nutrient in culture media. This nutrient provision of culture media was highly influential for the provision of plankton and bacteria to enhance the growth of the population and biomass of *D. magna*. Damle and Chari (2011), stated that fertilizing process in cultivation period can bring an effect on the density and biomass of *D. magna*. In this research, it has been proved that the nutrient in culture media was highly influential on the abundance of phytoplankton proliferating in culture media and used as a food source for *D. magna*. Thus, it could bring an effect on the population of *D. magna*. This statement is supported by the result of the research conducted by Yuniwati *et al.* (2012), which explained that the nutrient in culture media could determine the abundance of phytoplankton proliferating in culture media as the food source for *D. magna*.

Lag phase 3.74×10^4 individuals/L was found in the treatment of organic fertilizer with 1.2 g/L of chicken dung and combination of 0.3 g/L of rice bran and 0.9 g/L of coconut oilcake. This was in view of the density of culture media in that treatment was equal as in nature; thus, it could bring an effect on the rate of growth period of *D. magna*. Exponential phase of growth occurred on day 8. The length of the stationary phase was correlated with the duration of adapting phase of *D. magna* with the new culture media. The result of this research was in line with the research conducted by Fogg (1965), that showed the discontinuation of exponential phase for the lack of nutrient in the increase of cell density.

For the nutrient content based on the proximate analysis, it was found that the highest protein reached 73.90%, which is higher than the level obtained from the result of a research conducted by Mokoginta *et al.* (2003), reaching only 68.12%, and the research of Nina *et al.* (2012), reaching only 4% of wet weight. Furthermore, in this research, the lowest level of the fat was obtained at 4.24% in the same treatment. This was because the fat content was always inversely proportional to the protein content. The result of this research is supported by the argument of Lim *et al.* (2011), stating that higher protein content is always conversely proportional to the fat since the fat in body works double compared to the protein. In addition, the high protein content and the low fat content in this research were due to the nutrient in the culture media of *D. magna* in which the higher the nitrate and phosphate content, the higher

Table 2. Proximate analysis of *Daphnia magna* in mass culture using organic fertilizer fermented with probiotic bacteria

Treatment	Dry weight content percentage				Gross fiber (%)
	Protein (%)	Carbohydrate (%)	Gross fat (%)	Dust (%)	
A	67.45 ± 0.02	15.07 ± 0.05	7.57 ± 0.02	8.13 ± 0.03	1.78 ± 0.03
B	73.90 ± 0.04	14.22 ± 0.03	4.24 ± 0.03	5.19 ± 0.03	2.45 ± 0.08
C	69.44 ± 0.06	14.87 ± 0.05	7.89 ± 0.02	3.61 ± 0.02	4.19 ± 0.05
D	72.07 ± 0.02	12.25 ± 0.02	6.40 ± 0.02	4.68 ± 0.06	4.60 ± 0.02
E	72.26 ± 0.06	12.40 ± 0.02	6.04 ± 0.04	3.67 ± 0.07	5.63 ± 0.06

Table 3. Fatty acid profile of *Daphnia magna* in mass culture using the organic fertilizer fermented with probiotic fermentation

Fatty acid	*D. magna*				
	A	B	C	D	E
C 4:0	0.03 ± 0.01	0.03 ± 0.03	0.05 ± 0.05	0.04 ± 0.03	0.04 ± 0.03
C 6:0	0.02 ± 0.01	Nd	Nd	Nd	0.02 ± 0.01
C 8:0	0.01 ± 0.05	0.02 ± 0.03	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
C 10:0	0.02 ± 0.02	0.02 ± 0.01	0.01 ± 0.02	0.02 ± 0.01	0.01 ± 0.09
C 11:0	Nd	Nd	0.01 ± 0.01	Nd	0.01 ± 0.01
C 12:0	0.28 ± 0.06	0.15 ± 0.04	0.15 ± 0.06	0.21 ± 0.05	0.03 ± 0.17
C 13:0	0.01 ± 0.05	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
C 14:0	0.67 ± 0.04	0.39 ± 0.04	1.17 ± 0.02	0.67 ± 0.04	0.34 ± 0.26
C 14:1	0.08 ± 0.07	0.07 ± 0.04	0.40 ± 0.08	Nd	0.15 ± 0.02
C 15:0	0.06 ± 0.06	0.12 ± 0.06	0.27 ± 0.05	0.13 ± 0.05	0.24 ± 0.04
C 15:1	Nd	0.01 ± 0.01	0.02 ± 0.02	0.01 ± 0.01	0.01 ± 0.11
C 16:0	3.40 ± 0.07	3.78 ± 0.05	2.31 ± 0.04	3.72 ± 0.05	3.31 ± 0.02
C 16:1	0.04 ± 0.03	0.62 ± 0.04	0.03 ± 0.02	0.06 ± 002	0.87 ± 0.02
C 17:0	0.05 ± 0.04	0.04 ± 0.02	Nd	0.13 ± 0.02	0.08 ± 0.07
C 17:1	Nd	0.07 ± 0.04	0.08 ± 0.04	0.04 ± 0.01	0.03 ± 0.35
C 18:0	1.00 ± 0.05	0.32 ± 0.05	1.25 ± 0.08	1.17 ± 0.04	0.88 ± 0.36
C 18:1	1.33 ± 0.10	1.07 ± 0.05	0.10 ± 0.06	0.19 ± 0.05	0.11 ± 0.53
C 18:2	0.14 ± 0.17	0.20 ± 0.02	0.12 ± 0.04	0.08 ± 0.03	0.06 ± 0.04
C 20:0	0.04 ± 0.03	Nd	0.12 ± 0.06	Nd	Nd
C 18:3	Nd	0.01 ± 0.01	Nd	Nd	Nd
C 20:1	Nd	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	Nd
C 21:1	0.01 ± 0.05	0.03 ± 0.02	0.05 ± 0.02	Nd	0.01 ± 0.03
C 20:2	Nd	Nd	Nd	Nd	Nd
C 22:0	Nd	Nd	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.04
C 20:3 W6	Nd	Nd	Nd	Nd	Nd
C 20:3 W3	0.19 ± 0.04	0.39 ± 0.05	0.66 ± 0.03	0.29 ± 0.04	0.25 ± 0.03
C 20:4 W6	Nd	0.10 ± 0.09	Nd	Nd	Nd
C 22:1	0.20 ± 0.01	0.20 ± 0.18	0.03 ± 0.02	0.02 ± 0.01	0.01 ± 0.09
C 23:0	Nd	0.10 ± 0.08	Nd	Nd	Nd
C 22:2	Nd	Nd	Nd	Nd	Nd
C 20:5 W3	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
C 22:6 W3	0.02 ± 0.01	0.30 ± 0.02	Nd	0.01 ± 0.01	0.01 ± 0.07

Nd = not determined.

its protein content and the lower its lipid content. Widianingsih *et al.* (2011), stated that the higher the N and P content, the higher protein inside.

Profile of the total fatty acid showed that the highest fatty acid was palmitic (3.78%). Linoleic fatty acid reached 0.20% and was found in the treatment of organic fertilizer with 1.2 g/L chicken dung, 0.9 g/L rice bran and 0.3 g/L coconut oilcake. The palmitic content was found as the highest in this research because its function as an energy storage both in phytoplankton and zooplankton. This statement strengthened the result of Lim *et al.* (2011), which stated that the palmitic act as an energy storage that later used for the biosynthesis process of saturated fatty acid.

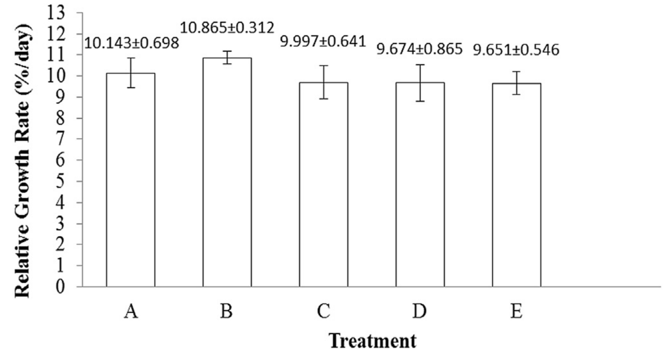

Figure 3. The growth of *Oreochromis niloticus* fed with *Daphnia magna* in mass culture using the waste of husbandry and fermented with probiotic bacteria.

The linoleic acts as a substrate to form long chain of PUFA. This result is in line with Pratiwi *et al.* (2009) and Ouli (2012) who stated that linoleic acts as the basic substrate of forming the long chain of Omega 6 and Omega 3.

The organic fertilizer with 1.2 g/L chicken dung, 0.9 g/L rice bran and 0.3 g/L coconut oilcake also provided the highest profile of essential amino acid (lysine, 44.16 ppm). The function of lysine in the research of Herawati *et al.* (2012), Ovie and Eze (2013), and Valverde *et al.* (2013), is as the building frame of vitamin B1 and anti-virus, assisting in calcium absorption, stimulating appetite, and assisting in carnitine production to alter the fatty acid to energy.

Tilapia larvae fed with *D. magna* that were cultured using fermentation fertilizer showed a significant effect ($p < 0.01$) in relative growth rate and survival rate during the research. The highest relative growth and survival rate in this research was 10.86% and 98.46%, which was the treatment of tilapia fed with *D. magna* cultured with 1.2 g/L chicken dung, 0.9 g/L rice bran and 0.3 g/L coconut oilcake. *D. magna* in this treatment has the highest nutrient content especially protein composition which can provide better growth rate than other treatments. The result of this research is in agreement with Nina *et al.* (2012) and Ovie and Eze (2013), where protein is really needed for the fish larvae especially in early stage, because protein has a function to repair and maintain its cells. Gao *et al.* (2011) explained that omnivore fish larvae like *O. niloticus* need as much as 38% protein and 4% fat.

Feeding consumption rate at first week, but not at second week, of culture showed significant difference ($p < 0.01$) in each treatment. Setyawati and Suprayudi (2003), stated that the daily

Table 4. Amino acid profile of *Daphnia magna* in mass culture using organic fertilizer with probiotic fermentation

Amino acid	*D. magna*				
	A	B	C	D	E
L-aspartic acid	48.92 ± 0.08	53.94 ± 0.01	45.52 ± 0.05	36.14 ± 0.05	37.85 ± 0.05
L-serine	15.61 ± 0.03	17.62 ± 0.01	17.63 ± 0.07	13.40 ± 0.05	14.76 ± 0.02
L-glutamic acid	76.61 ± 0.04	72.37 ± 0.07	64.35 ± 0.03	54.39 ± 0.03	57.36 ± 0.07
Glycine	19.36 ± 0.04	19.19 ± 0.01	18.78 ± 0.06	16.48 ± 0.04	17.33 ± 0.02
L-histidine	9.78 ± 0.03	9.70 ± 0.01	9.50 ± 0.05	8.48 ± 0.04	8.65 ± 0.03
L-agrinine	20.51 ± 0.04	27.28 ± 0.01	27.89 ± 0.08	18.56 ± 0.05	20.85 ± 0.07
L-threonine	19.02 ± 0.09	20.37 ± 0.01	20.56 ± 0.03	16.17 ± 0.02	18.47 ± 0.07
L-alanine	40.65 ± 0.05	32.51 ± 0.09	32.98 ± 0.05	29.21 ± 0.05	32.51 ± 0.01
L-proline	20.25 ± 0.05	19.00 ± 0.06	17.44 ± 0.04	18.98 ± 0.07	18.08 ± 0.09
L-valine	30.24 ± 0.05	28.87 ± 0.04	28.13 ± 0.02	23.74 ± 0.04	25.72 ± 0.03
L-metheonine	11.10 ± 0.08	10.40 ± 0.04	13.40 ± 0.05	7.82 ± 0.04	10.89 ± 0.06
L-lysine HCl	39.57 ± 0.04	44.16 ± 0.01	40.99 ± 0.03	28.56 ± 0.06	31.59 ± 0.06
L-isoleucine	19.97 ± 0.03	22.79 ± 0.04	21.20 ± 0.07	16.95 ± 0.05	18.87 ± 0.02
L-leucine	32.44 ± 0.05	36.88 ± 0.05	35.46 ± 0.07	30.67 ± 0.04	31.47 ± 0.05
L-phenylalanine	15.49 ± 0.07	16.98 ± 0.10	15.97 ± 0.05	14.62 ± 0.03	14.41 ± 0.07

Table 5. Relative growth, survival rate, and consumption rate of *Tilapia larvae* fed by *Daphnia magna* in mass culture using organic fertilizer fermentation

Feed	W0 (g)	Wt (g)	RGR (%)	SR (%)	FC 1 (%)	FC 2 (%)
D. magna A	0.053 ± 0.03	0.278 ± 0.06	10.14 ± 0.55	96.60 ± 0.53	106.40 ± 0.02	152.75 ± 0.01
D. magna B	0.052 ± 0.03	0.320 ± 0.15	10.86 ± 0.23	98.46 ± 0.53	106.43 ± 0.01	152.76 ± 0.04
D. magna C	0.052 ± 0.03	0.278 ± 0.23	9.99 ± 0.39	96.91 ± 0.53	106.37 ± 0.01	152.74 ± 0.01
D. magna D	0.050 ± 0.03	0.307 ± 0.06	9.67 ± 0.16	95.99 ± 0.93	106.40 ± 0.05	152.75 ± 0.02
D. magna E	0.052 ± 0.03	0.295 ± 0.15	9,65 ± 0.23	96.63 ± 0.93	106.40 ± 0.01	152.75 ± 0.01

amount of food consumed by fish is one of the factor that can affect growth rate, and the daily consumption rate has a relation with stomach capacity. Natural feed consumption rate closely related with growth rate of Tilapia larvae. Nutrient from natural feed that larvae take, will be absorbed and become an energy source for metabolism and larvae growth. Some important factors that have an effect on natural feed utilization rate are size of larvae, species, and size of natural fish food, nutrient value, and feeding dose (Melianawati *et al.* 2012).

Based on the result of this research, organic fertilizer 1.2 g/L chicken dung, 0.9 g/L rice bran and 0.3 g/L coconut oilcake contributed to the growth of highest biomass production and nutritional value content for *D. Magna*. Tilapia fed with *D. magna* cultured with organic fertilizer 1.2 g/L chicken dung, 0.9 g/L rice bran and 0.3 g/L coconut oilcake also showed the highest relative growth, survival and consumption rate.

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