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

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ABSTRACT

Water flea (*Daphnia magna* Straus, 1820) is the best natural feed for tilapia larvae rearing, however, the quality of its nutrients and production is very dependent on the culture medium. The purpose of this study was to enhance the production and nutritional quality of tilapia fed with *D. magna* grown in medium that was fermented at different time intervals. This study was conducted using a completely randomised experimental design with five treatments and three replicates. *Daphnia magna* was mass-cultured using chicken manure as a culture medium fermented with probiotic microorganisms *Lactobacillus casei* and *Saccharomyces cerevisiae* for 7, 14, 21, and 28 days whereas the control was used on 0 days. Tilapia larvae fed four times daily with *D. magna* cultured in fermentation medium for 28 days produced the best final weight (2.58 g), relative growth rate (19.98 %), biomass weight (2.72 g), survival rate (98.55 %), net protein utilisation (1.90 %) and protein efficiency ratio of (2.95 %). The best tilapia's nutritional quality was also seen in the in the same treatment that was fermented for 28 days and gave the highest value for fatty acid profile of 9.36 % linoleic acid, and amino acid profile was in lysine of 32.19 ppm. The study showed that provision of *D. magna* feed mass-cultured using 200 $g.L^{-1}$ of chicken manure fermented for 28 days gave the best results for growth of *Oreochromis niloticus* (Linnaeus, 1758) larvae.

Keywords: amino acid, fatty acid, fermentation time, fish production, natural feed, water flea

INTRODUCTION

Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) is a freshwater fish with rapid growth and is the second most important cultured fish in the world after carps (Ogello et al., 2014). Its demand has increased consistently every year, and Indonesia is the world's second-highest producer of Nile tilapia after China (Ogello et al. 2014; Fitzsimmons, 2017). The increased production of tilapia is due to the availability of the high quality of larvae (Herawati et al. 2017). The quality of larvae is determined by feed size given that is suitable for mouth openings of the larvae and its nutritional contents. Live feed such as water flea *Daphnia magna* Straus, 1820 serves as the best natural feed for tilapia's larval rearing (Pangkey, 2009). The advantage of water flea is that it has a high nutrient content and suitable size according to the mouth opening of fish larvae. Nutritional quality and production of water fleas are highly dependent on the culture medium in which they are maintained (Nwachi, 2013; Herawati et al. 2015). Fermentation of the culture medium to grow *D. magna* can improve its nutritional quality (Damle and Carie, 2011; Herawati et al. 2017).

Nutritional quality of *Daphnia* sp., especially protein and fat are needed by fish larvae for their growth and survival (Lim et al. 2011; Herawati et al. 2015; Herawati et al. 2018). The protein content of *Daphnia* sp. is high ranging from 45-72 % and the fat ranges from 6.5-8 % of its dry weight. Furthermore, its linoleic and linolenic fatty acids contents are 7.5 % and 6.7 %, respectively (Rakhman et al. 2013; Herawati et al. 2015; Herawati et al. 2018).

The fermentation of the culture mediium has been proven to be effective for increasing the nutrient of the medium. According to Herawati et al. (2018), the fermentation of the culture medium is to produce feed materials that have a longer storage time, better organoleptic

characteristics and nutritional components. Probiotic microorganisms serve to decompose and ferment organic materials (Yuniwati et al. 2012) and are also support the health of organisms (Nwachi, 2013). Furthermore, decomposition is a biological process that increases the microorganism's ability to produce growth substances, hormones, vitamins, and other enzymes (Zahidah et al. 2012; Asadi et al. 2012).

Since the nutritional content of *D. magna* is highly dependent on the phytoplankton it feeds on, the culture medium for phytoplankton is important (Damle and Chari, 2011; Herawati et al. 2018). Research on increasing production and nutritional quality of *D. magna* cultured using organic wastes with various animal manures, expired bread and tofu waste based on different duration of fermentation has been conducted previously by Herawati et al. (2018). Therefore, this research is a continuation of *D. magna* application as a natural source of feed for improving the nutrient quality and growth of tilapia larvae.

The present study investigates the optimum time required to ferment the organic waste culture medium to produce high-quality *D. magna* for good growth performance and nutritional quality of tilapia larvae. The purpose of this study is to determine the growth and nutritional quality of tilapia fed with *D. magna* mass cultured using chicken manure as organic waste that was fermented at different time intervals. Chicken manure which has uric acid and ammonia salts cannot be used directly as food for organisms. The fermentation process is to modify the basic characteristic of the raw wastes to improve the feed value (Murugan, 1989). The chicken manure is fermented with probiotic microorganisms *Lactobacillus casei* and *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Fermentation stage

The fermentation process using *L. casei* and *S. 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. Chicken manure as the material for culture medium was dried. Probiotic microorganisms (*L. casei* and *S. cerevisiae*) that were already activated for 3 hours were added to the culture medium with a combined weight of 200 $g.L^{-1}$ (Yuniwati et al. 2012; Abu Elala et al. 2013; Herawati et al. 2018). The duration of the fermentation treatment was for 0, 7, 14, 21, and 28 days. The fermented chicken manure was used as culture medium for *D. magna*'s mass-culture. The mass-cultured *D. magna* itself was used as a natural feed for Nile tilapia larvae.

D. magna culture

In this study, 6 ponds with 600-1,000 L of water capacity were used. The sampling for further measurement and analysis was done at 6 spots on each pond with three replicates. The 1,000 $ind.L^{-1}$ of *D. magna* was spread in each pond containing 200 $g.L^{-1}$ of fermented organic fertiliser and aerated for 14 days (Damle and Chari, 2011; Herawati et al. 2018). Observation for the abundance of *D. magna* was conducted using hand counter equipment (Kenko HT-302) every 2 days to monitor the population of *D. magna*. The water (20-25 %) of this culture was replaced regularly, and its pH level 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)

Tilapia larvae culture

Larvae of Nile tilapia used were 3 days old after hatching, with an initial length of 0.9-1.0 cm and weight of 0.05 g. The newly hatched tilapia larvae were taken from a fish hatchery and place in hapa net, measuring 100 x 50 x 40 cm, for 2 days of acclimatisation. The experimental design was similar to the one conducted by Herawati et al. (2015), where 3 days old tilapia larvae were used at a stocking density of 48 fish.L^{-1}. The size of *D. magna* used for feeding in the 1st week of larval rearing was 0.5 mm with the amount of 1,022 per litre of water, and in the 2nd week was 0.6 -1.0 mm size with the amount of 1,467 per litre of water. *Daphnia magna* was given 4 times a day at 6 a.m, 12 noon, 6 p.m, and 12 midnight *ad libitum* and fed for 21 days.

To feed the tilapia larvae, *D. magna* were collected from the stock culture medium using a scoop net and then rinsed with freshwater. The *Daphnia* sp. were then separated using a 1 μm plankton net to obtain smaller than < 0.5 mm size as feed in the 1st week and 0.6-1.0 mm as feed for the 2nd week (Herawati et al. 2015). The number of *D. magna* was calculated using a measuring cup and drop pipette until the appropriate amount of feed for tilapia larvae was obtained.

Water quality

The water quality for Nile tilapia rearing was maintained at 28-30 ^{o}C that was measured with Checktemp Digital Thermometer (Model HI98501), 0.3 ppm dissolved oxygen (DO) measured by a DO meter (AMTAST DO-820) and 8.1-8.2 pH measured by using a pH meter (PH-061). These parameters were similar to the recommendation by Nina et al. (2012); Herawati et al.

(2015); and Herawati et al. (2018) that the ideal temperature for Nile tilapia larvae is 25-30 ^{o}C, DO at 0.3-0.6 ppm, and pH at 6.5-9.

Statistical Analysis

This research was conducted using a completely randomised design with five treatments and three replicates. The five treatments where Nile tilapia larvae were fed *D. magna* that was cultured in fermented medium were as follow; treatments A, B, C, D and E fermented for 0, 7, 14, 21 and 28 days respectively.

The biomass weight was analysed using analysis of variance (ANOVA) to determine the differences among the treatments. The parameters analysed were growth, biomass production, and nutritional content of tilapia larvae fed *D. magna* feed.

Biochemical analysis

Proximate analysis. The proximate chemical composition of the samples was determined using a standard procedure (AOAC, 2000; and Herawati et al. 2018). The crude protein content was calculated by multiplying the total nitrogen factor. The results of the proximate analysis of *D. magna* mass cultured using organic wastes based on different duration of fermentation is presented in Table 1.

Table 1. Proximate analysis of *Daphnia magna* mass-cultured using organic waste-based at different duration of fermentation of the culture medium.

Proximate analysis of *D. magna*	Treatments at different duration of fermentation				
	A (0 days)	B (7 days)	C (14 days)	D (21 days)	E (28 days)
Ash	20.24 ± 0.02	20.00 ± 0.08	15.25 ± 0.08	10.15 ± 0.08	2.80 ± 0.06
Fat	6.36 ± 0.09	6.26 ± 0.05	6.53 ± 0.11	7.23 ± 0.09	7.84 ± 0.11

Crude Fiber	3.91 ± 0.02	3.75 ± 0.07	3.89 ± 0.05	3.69 ± 0.03	3.98 ± 0.01
Protein	53.19 ± 0.13	56.19 ± 0.26	60.03 ± 0.06	64.43 ± 0.06	75.26 ± 0.03
Carbohydrate	16.30 ± 0.19	13.80 ± 0.17	14.30 ± 0.09	14.50 ± 0.11	10.12 ± 0.09

Notes:

Mean value ± standard error.

The best proximate results of *D. magna* mass cultured using 200 $g.L^{-1}$ of chicken manure were obtained from medium that was fermented for 28 days and gave 75.26 % protein and 7.84 % fat.

Essential amino acid profile. The amino acid composition of the sample was determined using high performance liquid chromatography (HPLC) (Shimadzu LC-6A) (AOAC, 2000; and Herawati et al. 2015). Total amino acid profile of *D. magna* mass cultured using organic waste based on different duration of fermentation is presented in Table 2.

Table 2. Total amino acid profiles of *Daphnia magna* mass-cultured using organic waste based on different duration of fermentation.

Amino Acids of *D. magna*	Treatments at different duration of fermentation				
	A (0 days)	B (7 days)	C (14 days)	D (21 days)	E (28 days)
L-Histidine	8.14 ± 0.03	9.92 ± 0.08	10.14 ± 0.03	9.92 ± 0.08	15.19 ± 0.09
L-Serine	5.40 ± 0.07	6.61 ± 0.03	11.40 ± 0.07	13.61 ± 0.03	19.03 ± 0.03
L-Arginine	7.39 ± 0.02	8.61 ± 0.04	13.30 ± 0.02	15.61 ± 0.04	18.19 ± 0.03
Glycine	8.48 ± 0.05	9.36 ± 0.04	12.85 ± 0.05	15.66 ± 0.04	19.03 ± 0.06
L-Aspartic Acid	8.25 ± 0.09	9.78 ± 0.03	10.56 ± 0.08	13.18 ± 0.03	18.90 ± 0.09
L-Glutamic Acid	9.76 ± 0.05	11.51 ± 0.04	19.76 ± 0.05	20.43 ± 0.04	24.36 ± 0.08
L-Threonine	8.37 ± 0.09	9.02 ± 0.09	15.85 ± 0.09	19.02 ± 0.09	21.78 ± 0.06
L-Alanine	5.21 ± 0.02	6.65 ± 0.05	16.34 ± 0.02	20.79 ± 0.05	23.20 ± 0.09
L-Cysteine	8.74 ± 0.04	10.24 ± 0.05	24.57 ± 0.04	25.24 ± 0.05	25.87 ± 0.07
L-Lysine HCL	11.45 ± 0.09	15.54 ± 0.03	18.32 ± 0,09	19.94 ± 0.15	37.83 ± 0.03
L-Tyrosine	9.86 ± 0.06	11.49 ± 0.06	14.20 ± 0.06	15.67 ± 0.09	17.10 ± 0.05
L-Methionine	10.95 ± 0.09	12.87 ± 0.09	16.20 ± 0.02	16.90 ± 0.06	18.98 ± 0.03
L-Valine	8.67 ± 0.04	10.20 ± 0.08	15.23 ± 0.09	16.09 ± 0.02	15.23 ± 0.02
L-Isoleucine	5.62 ± 0.03	8.10 ± 0.05	10.80 ± 0.06	11.75 ± 0.03	13.25 ± 0.03
L-Leucine	6.82 ± 0.01	6.63 ± 0.09	11.13 ± 0.09	12.23 ± 0.04	15.98 ± 0.01
L-Phenylalanine	5.40 ± 0.05	7.19 ± 0.01	9.23 ± 0.05	11.03 ± 0.07	13.73 ± 0.03
Tryptophan	5.39 ± 0.03	8.10 ± 0.05	10.19 ± 0.09	12.67 ± 0.07	14.97 ± 0.09

Notes:

Mean value ± standard error.

Fatty acid profile. The fatty acid composition of the sample was determined using gas chromatograph (Shimadzu) (AOAC, 2000; and Herawati et al. 2018). The fatty acid profiles of *D. magna* mass-cultured using organic waste as culture medium based on different duration of fermentation as feed for tilapia larvae is presented in Table 3.

Table 3. Total fatty acid profiles of *Daphnia magna* mass cultured using organic waste based on different duration of fermentation.

Fatty Acids of *D. magna*	Treatments at different duration of fermentation				
	A (0 days)	B (7 days)	C (14 days)	D (21 days)	E (28 days)
Miristic	0.18 ± 0.06	0.88 ± 0.09	0.92 ± 0.03	0.99 ± 0.07	1.93 ± 0.09
Pentadecanoic	1.29 ± 0.08	1.89 ± 0.03	1.97 ± 0.05	2.01 ± 0.05	2.59 ± 0.04
Palmitic	2.91 ± 0.02	3.09 ± 0.02	3.47 ± 0.04	3.63 ± 0.05	5.98 ± 0.05
Stearic	1.95 ± 0.03	1.83 ± 0.02	1.95 ± 0.01	1.98 ± 0.04	2.79 ± 0.06
Oleic/ω9	3.46 ± 0.07	3.82 ± 0.06	3.97 ± 0.06	4.02 ± 0.05	5.78 ± 0.08
Linoleic/ω6	3.32 ± 0.09	3.86 ± 0.08	3.94 ± 0.01	4.32 ± 0.06	8.20 ± 0.08
Linolenic/ω3	3.05 ± 0.02	3.13 ± 0.02	3.33 ± 0.02	4.68 ± 0.04	6.96 ± 0.04
Arachidic	0.18 ± 0.05	1.28 ± 0.07	2.43 ± 0.07	3.79 ± 0.08	4.83 ± 0.04
Arachidonic	3.52 ± 0.09	3.37 ± 0.07	3.40 ± 0.07	3.59 ± 0.09	4.19 ± 0.07
Eicosapentaenoic	5.91 ± 0.04	6.07 ± 0.01	6.15 ± 0.07	6.38 ± 0.07	7.59 ± 0.08
AA	1.08 ± 0.04	1.08 ± 0.04	1.19 ± 0.08	1.27 ± 0.09	1.57 ± 0.08
DHA	1.63 ± 0.02	1.72 ± 0.02	1.67 ± 0.02	1.75 ± 0.02	1.98 ± 0.07
EPA	0.79 ± 0.04	0.81 ± 0.05	1.02 ± 0.04	1.36 ± 0.05	1.89 ± 0.05

Notes:

Mean value ± standard error.

Results

The results showed that feeding mass-cultured *D. magna* using 200 $g.L^{-1}$ of chicken manure fermented for 28 days (treatment A) to tilapia aged 3 days to 17 days (D3-D17) larvae gave the best results (Table 4).

Table 4. The growth of Nile tilapia fed by *Daphnia magna* which was mass-cultured using organic waste as culture medium based on different duration of fermentation.

Parameters	Treatments at different duration of fermentation				
	A (0 days)	B (7 days)	C (14 days)	D (21 days)	E (28 days)
IBW (g)	0.06 ± 0.03[a]	0.05 ± 0.03[a]	0.05 ± 0.03[a]	0.06 ± 0.03[a]	0.06 ± 0.03[a]
FBW (g)	1.10 ± 0.15[a]	1.18 ± 0.01[a]	1.25 ± 0.06[a]	1.59 ± 0.23[a]	2.52 ± 0.06[b]
WG (g)	1.04 ± 0.03[a]	1.13 ± 0.07[b]	1.20 ± 0.07[b]	1.53 ± 0.01[b]	2.46 ± 0.02[b]
RGR (%)	7.45 ± 0.03[a]	9.41 ± 0.03[b]	11.75 ± 0.09[b]	12.95 ± 0.09[b]	19.98 ± 0.13[b]
CR1 (g)	106.93 ± 0.15[a]	108.17 ± 0.01[b]	112.05 ± 0.02[b]	116.45 ± 0.23[b]	122.19 ± 0.10[b]
CR2 (g)	164.19 ± 0.03[a]	165.77 ± 0.09[a]	166.81 ± 0.07[a]	167.17 ± 0.03[a]	173.03 ± 0.03[b]
NPU (%)	1.23 ± 0.06[a]	1.27 ± 0.03[a]	1.35 ± 0.04[b]	1.45 ± 0.07[b]	1.90 ± 0.06[b]
PER (%)	1.07 ± 0.05[b]	1.42 ± 0.01[ab]	1.72 ± 0.03[b]	1.76 ± 0.04[b]	2.95 ± 0.02[b]
SR (%)	97.46 ± 0.08[a]	97.09 ± 0.26[a]	97.15 ± 0.08[a]	98.23 ± 0.02[b]	98.55 ± 0.13[b]

Notes:
Mean value ± standard error. The values in the same row with different superscrip ere significantly different ($P < 0.05$). Initial body weight (IBW) (g); Final body weight (F V) (g); Weight Gain (WG) (g) = final body weight (FBW) - initial body weight (IBW); relative growth rate (RGR) (%/day); feed intake (FI) (G fish) = (Total feed consumption (g)/number of fish); protein efficiency ratio (PER) (%); Net Protein Utility (NPU) (%) = Weight (g) / Protein of feed x 100% Survival Rate (SR) (%).

Providing *D. magna* feed mass-cultured using the organic waste of 200 g.L^{-1} of chicken manure fermented for 28 days (treatment E) given 4 times daily to tilapia larvae. Feeding was carried out during maintenance since the fish age 3 days to 17 days (D3-D17), gave final body weight on 17 days old tilapia of 2.52 g; relative growth rate of 19.98 %; biomass weight of 2.72 g; survival rate of 98.55 %; net protein utility of 1.90 %; and protein efficiency ratio of 2.95 % (Table 4).

Table 5. Proximate analysis of Nile tilapia fed by *Daphnia magna* mass-cultured using organic waste based on different duration of fermentation.

Proximate	Treatments				
	A (0 days)	B (7 days)	C (14 days)	D (21 days)	E (28 days)
Ash	21.36 ± 0.12	20.75 ± 0.11	15.25 ± 0.05	16.50 ± 0.05	9.26 ± 0.06
Fat	15.24 ± 0.08	13.16 ± 0.07	14.89 ± 0.01	14.43 ± 0.06	15.98 ± 0.11
Crude Fiber	6.19 ± 0.07	6.10 ± 0.06	7.53 ± 0.04	4.23 ± 0.07	3.84 ± 0.01
Protein	41.91 ± 0.09	46.80 ± 0.09	46.30 ± 0.09	50.69 ± 0.05	60.82 ± 0.03
Carbohydrate	15.53 ± 0.11	13.16 ± 0.07	16.03 ± 0.06	14.15 ± 0.10	10.10 ± 0.09

Notes:

Mean value ± standard error.

Treatment E gave the highest value of protein at 60.82 % and fat of 15.98 % of proximate analysis of tilapia fed *D. magna* mass-cultured using organic waste in the form of 200 $g.L^{-1}$ of chicken manure fermented for 28 days (Table 5). The lowest protein value of 31.98 % and fat of 12.27 % was in treatment A with a fermentation time of 0 days. Treatment E gave the highest fatty acid profile of linoleic acid namely 9.36 %, while the lowest value was in treatment A, fermented at 0 days that gave 2.27 %. The total of fatty acid profiles of Nile tilapia fed *D. magna* mass-cultured using organic waste based on different duration of fermentation are presented in Table 6.

Table 6. The fatty acid profile of tilapia fed by *Daphnia magna* mass cultured using organic waste based on different duration of fermentation.

Fatty Acid Profiles	Treatments				
	A	B	C	D	E
	(0 days)	(7 days)	(14 days)	(21 days)	(28 days)
Miristic	0.45 ± 0.04	0.68 ± 0.08	0.95 ± 0.03	0.98 ± 0.07	1.98 ±0.02
Pentadecanoic	1.28 ± 0.08	1.99 ± 0.03	2.25 ± 0.05	2.08 ± 0.05	2.81 ± 0.04
Palmitic	1.91 ± 0.02	3.11 ± 0.02	3.45 ± 0.04	3.78 ± 0.05	5.98 ± 0.05
Stearic	1.95 ± 0.03	1.93 ± 0.02	1.95 ± 0.01	2.98 ± 0.04	3.18 ± 0.06
Oleic/ω9	3.45 ± 0.07	3.82 ± 0.06	3.95 ± 0.06	4.12 ± 0.05	5.95 ± 0.08
Linoleic/ω6	3.32 ± 0.09	3.96 ± 0.08	4.05 ± 0.01	5.82 ± 0.06	9.36 ± 0.08
Linolenic/ω3	3.05 ± 0.02	3.13 ± 0.02	3.75 ± 0.06	4.88 ± 0.03	7.75 ± 0.02
Arachidic	1.28 ± 0.07	3.52 ± 0.09	2.93 ± 0.07	3.79 ± 0.08	5.95 ± 0.04
Arachidonic	2.52 ± 0.09	3.37 ± 0.07	3.55 ± 0.07	3.89 ± 0.09	5.35 ± 0.07
Eicosapentaenoic	5.91 ± 0.04	6.07 ± 0.01	6.15 ± 0.07	6.38 ± 0.07	7.59 ± 0.08
AA	7.56 ± 0.07	7.85 ± 0.02	7.92 ± 0.01	7.99 ±0.01	8.15 ± 0.09
DHA	4.46 ± 0.06	4.64 ± 0.03	4.83 ± 0.05	4.97 ± 0.07	4.97 ± 0.07
EPA	7.56 ± 0.07	7.85 ± 0.02	7.92 ± 0.01	7.99 ± 0.01	8.15 ± 0.09

Notes:

Mean value ± standard error.

The highest amino acid profile of tilapia larvae fed by *D. magna* was in the treatment E which was fermented for 28 days and produced 32.19 ppm lysine essential amino acid while the lowest

value of 2.13 ppm was in treatment A. Total amino acid profiles of Nile tilapia fed by *D. magna* mass-cultured using organic waste based on different duration of fermentation are presented in Table 7.

Table 7. Amino acid profile of tilapia fed by *Daphnia magna* mass cultured using organic waste based on different duration of fermentation.

Amino Acid Profiles	Treatments				
	A (0 days)	B (7 days)	C (14 days)	D (21 days)	E (28 days)
L-Histidine	8.25 ± 0.09	10.95 ± 0.02	10.69 ± 0.03	10.15 ± 0.04	17.85 ± 0.09
L-Serine	6.23 ± 0.07	6.98 ± 0.03	11.98 ± 0.07	14.06 ± 0.03	19.99 ± 0.03
L-Arginine	7.99 ± 0.02	9.25 ± 0.04	14.15 ± 0.02	15.98 ± 0.04	20.25 ± 0.03
Glycine	8.98 ± 0.05	9.98 ± 0.04	13.10 ± 0.05	16.15 ± 0.04	19.88 ± 0.06
L -Aspartic Acid	8.95 ± 0.09	10.12 ± 0.03	11.17 ± 0.08	14.29 ± 0.03	25.85 ± 0.09
L-Glutamic Acid	10.15 ± 0.07	12.19 ± 0.03	20.26 ± 0.09	21.96 ± 0.08	26.17 ± 0.09
L-Threonine	8.85 ± 0.09	9.98 ± 0.09	17.25 ± 0.09	20.59 ± 0.09	23.26 ± 0.06
L-Alanine	6.23 ± 0.02	7.10 ± 0.05	7.75 ± 0.02	17.10 ± 0.05	23.03 ± 0.09
L-Cysteine	7.59 ± 0.07	8.88 ± 0.03	11.98 ± 0.07	14.06 ± 0.03	25.56 ± 0.03
L-Lysine	4.85 ± 0.09	14.19 ± 0.03	12.96 ± 0,09	21.76 ± 0.15	32.19 ± 0.03
L-Tyrosine	8.25 ± 0.09	15.40 ± 0.01	16.76 ± 0.02	20.98 ± 0.04	22.25 ± 0.03
L-Methionine	7.23 ± 0.07	7.24 ± 0.03	11.98 ± 0.07	14.06 ± 0.03	19.06 ± 0.03
L-Valine	9.05 ± 0.09	10.92 ± 0.03	12.17 ± 0.08	14.29 ± 0.03	23.06 ± 0.09
L-Isoleucine	6.19 ± 0.07	8.98 ± 0.03	11.98 ± 0.07	14.06 ± 0.03	20.56 ± 0.03
L-Leucine	9.60 ± 0.09	10.98 ± 0.09	18.25 ± 0.09	20.59 ± 0.09	25.06 ± 0.06
L-Phenylalanine	6.80 ± 0.07	6.98 ± 0.03	11.98 ± 0.07	14.06 ± 0.03	23.10 ± 0.03
Tryptophan	6.23 ± 0.03	9.95 ± 0.05	13.26 ± 0.09	13.68 ± 0.07	19.06 ± 0.09

Notes:

Mean value ± standard error.

Discussion

This research is a follow up study on feeding *D. magna* mass-cultured using chicken manure, rice bran, and coconut cake which were fermented by probiotic microorganisms for 14 days as reported by Herawati et al. (2015) and Herawati et al. (2016) A recent study by Herawati et al. (2018) used animal manures, tofu waste, and coconut cake with different duration of fermentation). The present study was the application of *D. magna* mass-cultured using chicken

manure as culture medium based on different duration of fermentation to increase production and nutritional quality of Nile tilapia larvae.

The quality of *D. magna*'s culture medium is an important factor because its quality determines the production and nutrients quality of *D. magna*. The fermentation process using probiotic microorganisms *Lactobacillus casei* and *S. cerevisiae* produces high quality *D. magna* that shorten the long chains of C and N in the nutrients of the culture medium making it more easily absorbed and utilised for improving the nutrient quality of *D. magna*. The statement is in line with research conducted by Nwachi (2013) who stated that the purpose of fermentation is to obtain a new feed with higher nutritional quality.

Based on the results of table 1, the ash content of 28th day fermentation treatment was much lower compared to other treatments. Low ash content is caused by two parameters. First is the effect of the fermentation time. Ash content decreases proportional to the fermentation time on culture medium. Second, the ash content in an organism depends on the type of the organism and the environment where it lives. Each organism, has a different ability to regulate and absorb inorganic metals / minerals.

The results of the analysis of variance showed that tilapia larvae fed *D. magna* mass-cultured using 200 g.L^{-1} of chicken manure fermented with *L. casei* and *S. cerevisiae* for 28 days had a significant effect on the relative growth rate value of tilapia larvae and showed the highest growth rate of 19.98%. The length of fermentation time influences the quality of culture media and the phytoplankton in it.

Daphnia magna is herbivorous zooplankton and typically primary consumer that utilise phytoplankton as their food source. *Daphnia magna* is a filter-feeder that obtains food from the water by filtration and is sometimes used as control agents against phytoplankton proliferation in a method known as biomanipulation. It relies on energy obtained from phytoplankton for population growth. The nutrient content and biomass of prey phytoplankton are important factors in *D. magna* growth. Therefore, the quality and quantity of phytoplankton consumed are crucial factors controlling the growth of *D. magna* populations (Choi et al., 2014). Thus culture medium that was fermented at different durations had an indirect effect on the nutritional quality of *D. magna*. This is because the phytoplankton and microorganisms that dominated in the culture medium is a source of natural feed for *D. magna*. Hence, the results of the study showed that with 28 days of fermentation, gave the highest protein and fat values of 75.26% and 7.84% respectively for *D. magna*.

Treatment E showed the highest growth rate value of 19.98 % in tilapia larvae fed by *D. magna* mass-cultured in medium fermented for 28 days. And the lowest growth rate of 5.95 % was in treatment A. This was because the nutrient content of *D. magna* in treatment E was optimal and suitable to meet the nutritional requirements needed by the tilapia larvae. Furthermore, the results of this study showed that the first and second-week feed intake was 122.19 g and 173.03 g respectively that gave a relative growth rate of 19.98 %. Feed intake was increased proportional to the size of fish that grew bigger.

The rate of utilisation of natural feed is closely related to the growth rate of tilapia larvae. Nutrients in the natural feed that are eaten by larvae would be absorbed by the body and used as an energy source for metabolism and growth of larvae. Hutabarat (2019), reported that several

important factors influence the rate of natural feed utilisation namely larvae size, type and size of natural feed used, nutritional value, and the amount given to the larvae. The size of natural feed given must be smaller than the larvae mouth opening. Fish need energy to grow and develop and the source of energy is from the feed and nutrients consumed by the fish. Fish could grow well if the nutrients obtained from the feed can be used effectively and efficiently.

Growth rates related to the nutritional content of feed include protein, carbohydrates, fats, vitamins, and minerals contained in the feed, which is consumed by the fish. In this study, the highest level of protein efficiency ratio is 2.95 %, and the net protein utility is 1.90 %. The results showed that the highest growth rate of tilapia was in treatment E, where tilapia was fed by *D. magna* mass-cultured in fermented medium for 28 days. The high growth was seen in treatment E because the protein and fat in *D. magna* were 75.26 % and 7.84 % respectively. The protein requirements of tilapia larvae for growth is ranged from 35-40 %. The results of this study are in line with the statement by Gao et al. (2011) that omnivorous fish larvae such as tilapia requires 38 % protein and 10 % fat. Furthermore, El-Sayed et al. (2008) explained that the optimum growth of tilapia larvae requires feed containing more than 30 % protein. The excess protein contained in the feed is used to form body tissues which are shown in the increase in weight and size of fish. Besides protein, fat is an energy source and a source of essential fatty acids which according to Mokoginta (2003) maintain cell membrane integrity, and are precursors of prostaglandin, prostacyclin, thromboxane, and leukotriene compounds. In addition to the high nutritional value, *Daphnia* sp. contains digestive enzymes such as protease or peptidase, amylase, lipase and cellulase which has a function as exoenzymes in the digestion system of fish larvae (Pangkey, 2009).

The survival rate of tilapia larvae is strongly influenced by environmental conditions (water quality) which must be maintained in optimum conditions. The other crucial factors are the quantity, size and quality of the feed must be suitable. Based on the results of the analysis of variance showed that feeding to tilapia larvae using *D. magna* mass cultured using organic waste based on different duration of fermentation showed no significant effect on survival rate. The average value of survival rate ranged from 97.09 % to 98.55 %.

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