



The effect of nutrient content and production of *Daphnia magna* mass cultured using various wastes processed with different fermentation time

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Abstract. The objective of this study was to determine the enhancement of nutrient quality and production of *Daphnia magna* using various organic wastes as culture medium at the best fermentation time using *Lactobacillus* sp. and *Saccharomyces cerevisiae* probiotic bacteria. This study was conducted using completely randomized experimental design with four treatments and three replications. Organic wastes used were: chicken manure, quail manure, goat manure, rejected bread and tofu waste fermented by probiotic bacteria then *D. magna* was cultured for 0, 7, 14, 21, and 28 days. The results showed that the medium which used 50 g/L chicken manure, 100 g/L rejected bread, and 50 g/L tofu waste cultured for 28 days created the highest biomass production, population and nutrient content of *D. magna* those were 283,500 ind/L population density, 683.06 grams biomass production, 75.26% protein content and 7.84% fat. The highest fatty acid profile was 8.20% linoleic and 6.96% linolenic acid. The highest essential amino acid was 37.83 ppm of lysine.

Key Words: *Saccharomyces cerevisiae*, probiotic bacteria, biomass production, nutrient value, organic wastes.

Introduction. Zooplankton mass culture has been the subject of many studies during recent years because of the importance of zooplankton in fish larvae rearing (Paray & Al-Sadoon 2016). *Daphnia magna* is one of the zooplanktons which were found as the best natural feed for fish larvae rearing (Gogoi et al 2016). The advantages of using *D. magna* for aquaculture are its high nutrient content and its size which is suitable with mouth opening and nutrient needs of Nile tilapia (*Oreochromis niloticus*) larvae. Nutrient and production quality of *D. magna* highly depend on its culture medium (Nwachi 2013; Herawati et al 2017). Nutrient content of *D. magna* highly depends on its culture medium for the growth place of phytoplankton as *D. magna*'s feed (Damle & Chari 2011; Herawati et al 2017). The most commonly used *D. magna* culture medium is chicken manure (Zahidah et al 2012), and the combination of chicken, goat and cow manure (Damle & Chari 2011). Another less commonly used of culture medium is a combination of chicken manure, bran, and copra waste (Herawati & Agus 2014), and different animal waste such as chicken, goat, and quail manure (Herawati et al 2017). Bran is used as culture medium in previous study by Herawati et al (2015) because it has high nutrient content for the growth of *D. magna*.

A study using various animal wastes with fermentation time for 14 days had been done in 2017 by Herawati et al (2017); the study result stated that chicken manure is the best culture medium for *D. magna*'s nutrient quality and growth performance. The usage of organic wastes in culture medium including chicken, and quail manure mixed with the rejected bread and tofu waste based on different fermentation time with the probiotic bacteria has not been conducted as the usage of organic waste could influence the growth performance and nutrient content of *D. magna*. The highest nutrients, particularly for the content of nitrogen (N), phosphor (P), and calcium (Ca) in organic waste are the feed sources of *D. magna*. Herawati et al (2016), in their study explained that chicken

manure containing N (4.75%), P (3.57%), and Ca (4.80%), since quail manure containing N (4.06%), P (2.96%), and Ca (2.57%). Furthermore, the analysis on dried materials of tofu waste based on a previous study of Liswahyuningsih et al (2011) and Herawati et al (2017) stated that tofu waste contained crude 27.09% protein, 22.85% crude fiber, 7.37% fat, 35.02% ash, and 6.87% extract material without nitrogen/BETN. Purbowati et al (2007) and Herawati et al (2016) explained that the rejected bread contained 12.63% crude protein, 0.13 %crude fibre, 4.63% crude fat, 4.19% ash and 58.42% extract material without nitrogen.

The fermentation of the fertilizer has been proven to be effective for increasing the nutrient of culture medium. Herawati et al (2016) explained that the objective of the fermentation is to produce a product (feed materials) which has a longer storage time, better organoleptic characteristics and nutritional components. Probiotic bacteria are supportive for the health of organisms (Nwachi 2013). It also serves to decompose and ferment organic waste (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 (Zahidah et al 2012; Asadi et al 2012).

The objective of this study was to determine the best fermentation time by using probiotic bacteria (*Lactobacillus casei* and *Saccharomyces cerevisiae*) on organic wastes from various organic waste (chicken manure, quail manure, goat manure, rejected bread, tofu waste) as *D. magna*'s mass culture medium to improve its nutrient quality, biomass production, and growth performance.

Material and Method

Fermentation stage. The fermentation stage is the preparation of molasses ratio, water and probiotic bacteria. The ratio used was 1:1, i.e. 1 mL of molasses, 1 mL of probiotic bacteria and 100 mL of solvent. Chicken manure, quail manure, goat manure, rejected bread, and tofu wastes which was used as organic waste were dried. The treatments used in this study were:

- a. 100 g/L rejected bread + 100 g/L tofu waste
- b. 50 g/L chicken manure + 100 g/L rejected bread + 50 g/L tofu waste
- c. 50 g/L goat manure + 100 g/L rejected bread + 50 g/L tofu waste
- d. 50 g/L quail manure + 100 g/L rejected bread + 50 g/L tofu waste

Each of organic waste combination got fermentation treatment for 0, 7, 14, 21, and 28 days. Probiotic bacteria (*L. casei* and *S. cerevisiae*) that were already activated for 3 hours were given to fertilizers which have 200 g/L weight combination (Yuniwati et al 2012; Abu et al 2013; Herawati et al 2017). They were left for fungi growth and acidic smell to develop. Table 1 and Table 2 show the result of nutrient analysis for organic fertilizer before and after fermentation by probiotic bacteria for 0, 7, 14, 21, and 28 days for *D. magna* mass cultured.

Once the fertilizer was ready, the samples were placed into the culture medium and aerated for 14 days. And when it was ready, 1,000 ind/L of *D. magna* was inoculated (Damle & Chari 2011; Herawati et al 2017).

Water quality. The water quality during the study was maintained in ideal condition at 28-30°C temperature, DO at 0.3 ppm, and pH at 8.1-8.2. This is in line with previous studies which stated that the proper temperature for *D. magna* culture is 25-30°C, DO of 0.3-0.6 ppm, and pH of 6.5-9 (Nina et al 2012; Herawati et al 2017). Ideal condition of water quality helps the growth of phytoplankton and algae to stimulate *D. magna*'s growth.

Culture of *D. magna*. The 1,000 ind/L *D. magna* was spread for each pond containing 200 g/L fermented organic waste. Observation for the abundance of *D. magna* was conducted every two days. The water (20-25%) of this culture was replaced, and its pH level was monitored every morning at around 7:00 AM to maintain the quality. The pH was maintained at its optimum range with the addition of 1 L of dolomite/1,000 L of water.

Table 1

Nitrate (N), phosphate (P), and potassium (K) nutrient content of *Daphnia magna* mass culture medium using various organic wastes with different fermentation period

Time (days)		Nutrient (%)		
		N	P	K
0	A	1.15±0.05	0.12±0.06	0.02±0.04
	B	2.03±0.07	0.75±0.03	0.36±0.05
	C	1.85±0.05	0.32±0.09	0.22±0.03
	D	1.93±0.09	0.56±0.01	0.37±0.02
7	A	1.53±0.09	0.57±0.01	0.27±0.02
	B	2.42±0.07	1.05±0.03	0.43±0.06
	C	2.19±0.03	0.87±0.02	0.52±0.01
	D	2.53±0.09	1.07±0.01	0.47±0.02
14	A	1.80±0.09	1.07±0.06	0.52±0.03
	B	3.75±0.05	1.49±0.18	0.79±0.02
	C	2.36±0.08	1.15±0.03	0.86±0.08
	D	2.80±0.09	1.27±0.01	0.57±0.02
21	A	2.08±0.04	1.26±0.03	0.66±0.02
	B	3.93±0.03	1.74±0.08	1.09±0.03
	C	2.96±0.09	1.45±0.05	0.76±0.05
	D	3.18±0.07	1.56±0.03	0.86±0.08
28	A	2.23±0.06	1.38±0.02	0.74±0.03
	B	4.88±0.03	2.23±0.08	1.89±0.05
	C	2.96±0.09	1.45±0.05	0.76±0.05
	D	3.18±0.07	1.56±0.03	0.86±0.08

Table 2

Daphnia magna population growth phase mass cultured using various organic waste medium with different fermentation period

Time (days)		Growth phase (ind/L)			
		Lag	Exponential	Stationary	Death
0	A	18,940±3.25	32,420±3.09	155,450±3.75	22,680±0.19
	B	19,680±2.36	35,525±3.78	155,450±3.09	33,650±3.08
	C	19,157±3.18	33,750±2.68	155,450±2.98	25,325±3.45
	D	19,550±2.08	33,960±3.04	155,450±3.96	29,880±2.98
7	A	20,090±4.08	40,900±4.19	167,670±2.92	37,236±2.53
	B	24,690±3.26	45,855±3.54	167,670±3.85	40,107±3.35
	C	2,274±3.17	41,725±2.68	167,670±2.96	38,760±4.09
	D	22,850±2.95	43,643±3.06	167,670±3.08	39,875±3.05
14	A	22,890±0.08	49,840±2.68	182,720±2.95	45,210±3.27
	B	27,750±0.08	54,720±2.35	182,720±3.13	49,910±3.98
	C	24,679±0.08	51,860±2.55	182,720±3.23	47,625±2.05
	D	25,859±0.08	52,975±3.08	182,720±3.03	44,890±3.04
21	A	27,725±3.35	56,310±3.85	188,890±2.25	62,850±3.85
	B	29,760±2.75	62,325±3.06	199,120±3.17	68,220±3.9
	C	28,560±3.09	60,750±2.98	191,050±3.1	64,752±4.02
	D	28,920±2.99	59,975±2.78	192,920±2.26	65,950±3.98
28	A	34,840±3.58	65,160±2.97	203,200±2.1	97,810±3.95
	B	49,650±2.23	95,875±2.65	283,500±2.17	13,6460±2.56
	C	35,170±3.17	70,255±3.08	209,800±2.08	10,8980±2.35
	D	36,420±2.26	72,890±2.86	217,150±3.07	11,1600±2.19

Statistical analysis. This study used completely randomized design with four treatments and three replications. The weight of biomass was analyzed using variant

analysis to emphasize differences among treatments. The parameters which were analyzed were: growth, biomass production, and nutrient content of *D. magna*. The proximate chemical composition of the samples was determined using a standard procedure (AOAC 2005; Herawati et al 2017). The crude protein content was calculated by multiplying the total nitrogen factor. The carbohydrate content was estimated by the difference. The amino acid composition of the samples was determined using High Performance Liquid Chromatography (HPLC) method (Shimadzu LC-6A) (AOAC 2005; Herawati et al 2017). The fatty acid composition of the samples was determined using gas chromatography method (Shimadzu) (AOAC 2005; Herawati et al 2017).

Results and Discussion. The present study is the development of previous studies that were done in 2015, and 2017 by Herawati et al (2015), and Herawati et al (2017). In 2015 and 2016, the studies were about *D. magna* mass culture using chicken manure, rice bran and coconut oilcake fermented by probiotic bacteria. Furthermore in 2017, the study was about the usage of various animal manure for *D. magna* mass culture medium with 14 days fermentation time.

The results showed that there was an enhancement of nutrient quality in the culture medium after different fermentation time. The highest increase of quality was 4.86% in treatment with 50 g/L chicken manure + 100 g/L rejected bread + 50 g/L tofu waste that was fermented for 28 days. The average enhancement of culture medium quality for N was about 3.73%. The lowest culture medium quality was 1.15% in *D. magna* that was mass cultured using 100 g/L rejected bread + 100 g/L tofu waste fermented for 0 day. The highest K and P content was 2.23% and 1.89% in the same treatment. Nitrate (N), phosphate (P), and potassium (K) nutrient content of *D. magna* mass culture medium using various organic wastes fermented for different time is presented in Table 1. The enhancement of nutrient quality on fermented medium is the result of anaerobic dissimilation process of organic compounds by the activity of microorganisms.

Differences of nutrient content before and after fermentation contained in the *D. magna* culture medium is caused by *Lactobacillus* sp. bacteria as fermenter during fermentation process. *Lactobacillus* sp. increase the protein content of the ingredients proved by the enhancement of culture medium nutrient content. The wastes fermentation process with different time variation affects upon the amount of developed bacteria. The results of the present study in line with a previous study conducted by Hersoelistyorini et al (2010) who stated that fermentation process can increase energy, protein and crude fiber content. Microbes which are used in the fermentation process can synthesize proteins and produce enzymes that will degrade complex compounds to be simpler. Microbial proteases which are obtained through fermentative processes are capable of producing protease enzymes that will breakdown proteins. Protein which has been breakdown is converted into polypeptide, furthermore becomes a simple peptide. This simple peptide will be breakdown into amino acids. These amino acids are utilized by microbes to multiply themselves. The number of microbial colonies as the source of single cell proteins increases during the fermentation process of organic wastes in culture medium.

Nutrient enhancement in medium, especially nitrate, serves to determine the amount of phytoplankton in the culture medium as a source of *D. magna* feed other than bacteria and detritus. Based on the results of the present study, the abundance of plankton that grows and dominates the culture medium comes from the phylum *Chlorophyta*, *Euglenozoa*, *Nematoda*, *Ciliophora*, and *Rotifera*. *D. magna* is a non-selective filter feeder that feeds on unicellular algae and a variety of organic detritus including protists and bacteria, even its adult size is able to eat small crustaceans and rotifers, thus the more phytoplankton exist the faster the growth of *D. magna*. The present study results are in line with a previous study conducted by Darmawan (2014) who stated that the more abundance of phytoplankton and organic materials in the culture medium, the growth rate of *Daphnia* sp. will occur faster than in one without organic materials and less phytoplankton presence. The present study results are also strengthened by previous studies which stated that phytoplankton population

enhancement and growth in water is related with nutrient availability especially nitrate and sunlight, nitrate will increase water fertility characterized by high number of phytoplankton that exists (Sumich 1992; Tomascik et al 1997).

During cultivation, there are four phase of *D. magna* population growth phase, which are adaptation phase (lag phase), exponential phase, stationary phase, and death phase. The highest population density of the four phases occurred at *D. magna* cultured by using 50 g/L chicken manure + 100 g/L rejected bread + 50 g/L tofu waste fermented for 28 days. The present study result showed that lag phase occurred on 4th day with the highest population of 49,650 ind/L; exponential phase occurred on 12th day with the highest population of 95,875 ind/L; stationary phase occurred on 16th day with the highest population of 283,500 ind/L; death phase occurred on 20th day with the highest population of 136,460 ind/L. *D. magna* population growth phase mass cultured using various organic waste medium fermented with different time is presented in Table 2.

Biomass weight of *D. magna* mass cultured using 50 g/L chicken manure + 100 g/L rejected bread + 50 g/L tofu waste which fermented for 28 days gave the highest result of 683.06 g and *D. magna* mass cultured using 100g/L rejected bread + 100 g/L tofu waste which fermented for 0 day gave the lowest result of 112.14 g. Therefore the highest and lowest biomass weight difference is 570.92 g. Biomass weight of *D. magna* mass cultured using various animal manure based on different fermentation time is presented in Table 3. Nutrient quality of culture medium had a significant effect for the provision of plankton and bacteria to increase *D. magna* population and biomass. Damle & Chari (2011) stated that a high organic material can affect the density and biomass of *D. magna*.

Table 3
Biomass weight of *Daphnia magna* mass cultured using various animal wastes based on different fermentation period

Time (days)		Initial weight (g)	Final weight (g)
0	A	3.05±0.03	112.14±0.05
	B	3.05±0.03	212.14±0.01
	C	3.03±0.03	189.17±0.01
	D	3.07±0.07	196.20±0.01
7	A	3.03±0.02	146.19±0.09
	B	3.03±0.02	271.10±0.07
	C	3.02±0.05	208.17±0.09
	D	3.06±0.07	220.03±0.1
14	A	3.02±0.08	178.66±0.15
	B	3.02±0.08	326.23±0.20
	C	3.05±0.07	225.17±0.06
	D	3.03±0.03	296.06±0.16
21	A	3.05±0.02	252.26±0.07
	B	3.05±0.02	376.20±0.25
	C	3.02±0.04	305.23±0.26
	D	3.04±0.02	323.10±0.03
28	A	3.06±0.06	376.52±0.10
	B	3.06±0.06	683.06±0.13
	C	3.07±0.08	419.03±0.17
	D	3.04±0.05	512.26±0.19

Growth of *D. magna* mass culture using various animal waste with different fermentation time did not give significant effect between treatments at lag phase ($P>0.01$). This is because *D. magna* begins to adapt to the new environment at lag phase if cultured medium concentration is the same with natural medium, it will make *D. magna* grows faster. However, if there are differences between culture medium concentration and its

nature habitat, *D. magna* needs longer time to grow. Harrison et al (2008) and Herawati et al (2017) stated that the difference in concentration of culture medium and liquid cells in plankton will have an effect on restitution of enzyme and the concentrate substrate to a further extent for growth and presence of nutrients in cells through the diffusion process as a result of the difference in concentration between the culture medium and its body liquid.

Exponential phase is the phase where the nutrient content in *D. magna* is at the highest level while growth is not maximized and the amount of *D. magna* began to increase. The exponential phase of the study took place on the 14th day, this result is in contrast with the study of Darmawan (2014) who reported that exponential phase took place on the 9th and 10th day. The exponential and stationary phase in this study gave a significant effect between treatments ($P < 0.01$). The length of the stationary phase is correlated with the duration of *D. magna* adaptation with the new culture medium. This is because the length of the stationary phase affects the absorption of nutrients in the culture medium by *D. magna*. The results were in line with study of Fogg (1965) and Herawati et al (2017) which showed that exponential phase stopped because of nutrient lack in cell density enhancement.

The highest result of quality analysis of protein and fat are 75.26% and 7.84% in *D. magna* mass cultured using 50 g/L chicken manure + 100 g/L rejected bread + 50 g/L tofu waste which fermented for 28 days. The lowest protein and fat are 40.86% and 3.23% in *D. magna* mass cultured using 100 g/L rejected beard + 100 g/L tofu waste which fermented for 0 day. Result of proximate analysis of *D. magna* mass cultured using various animal manure based on different fermentation time is presented in Table 4.

Table 4

Proximate analysis of *Daphnia magna* mass cultured using various animal wastes based on different fermentation time

Time (days)	Proximate analysis (%)					
	Ash	Fat	Crude fiber	Protein	Carbohydrate	
0	A	21.55±0.09	3.23±0.15	4.67±0.03	40.86±0.06	29.69±0.11
	B	20.24±0.02	6.36±0.09	3.91±0.02	53.19±0.13	16.30±0.19
	C	20.14±0.03	6.25±0.11	3.18±0.02	50.23±0.25	20.20±0.12
	D	19.07±0.07	6.45±0.07	3.01±0.04	51.17±0.18	20.30±0.13
7	A	23.56±0.05	3.75±0.03	5.67±0.05	45.86±0.06	21.16±0.05
	B	20.00±0.08	6.26±0.05	3.75±0.07	56.19±0.26	13.80±0.17
	C	18.14±0.01	6.15±0.06	3.58±0.01	52.23±0.17	19.90±0.09
	D	20.15±0.08	6.37±0.07	3.21±0.02	53.17±0.18	17.10±0.12
14	A	23.78±0.05	4.02±0.12	5.63±0.01	46.58±0.05	19.99±0.05
	B	15.25±0.08	6.53±0.11	3.89±0.05	60.03±0.06	14.30±0.09
	C	19.70±0.02	6.15±0.09	3.86±0.06	55.10±0.17	15.19±0.12
	D	19.90±0.08	6.67±0.08	3.57±0.01	56.65±0.28	13.21±0.11
21	A	22.02±0.05	4.43±0.12	4.63±0.05	46.98±0.03	21.94±0.15
	B	10.15±0.08	7.23±0.09	3.69±0.03	64.43±0.06	14.50±0.11
	C	18.00±0.03	6.85±0.07	3.46±0.06	58.60±0.17	13.09±0.09
	D	20.20±0.08	7.04±0.11	3.77±0.07	61.45±0.08	17.54±0.16
28	A	23.03±0.05	5.14±0.22	4.78±0.04	47.48±0.08	19.57±0.05
	B	2.80±0.06	7.84±0.11	3.98±0.01	75.26±0.03	10.12±0.09
	C	8.15±0.07	7.40±0.07	3.56±0.03	68.19±0.25	12.70±0.18
	D	8.00±0.08	7.55±0.01	3.87±0.02	70.05±0.08	10.53±0.19

The highest result of fatty acid profiles analysis of linoleic and linolenic fatty acids are 8.20% and 6.96% in *D. magna* mass cultured using 50 g/L chicken manure + 100 g/L rejected bread + 50 g/L tofu waste which fermented for 28 days. The lowest linoleic and linolenic fatty acids are 1.88% and 1.29% in *D. magna* mass cultured using 100 g/L rejected beard + 100 g/L tofu waste which fermented for 0 day. Total fatty acid profiles

of *D. magna* mass cultured using various animal wastes based on different fermentation time is presented in Table 5.

Based on the results of the present study, the highest fat of 7.84% is lower than in a previous study conducted by Herawati et al (2016) which was 7.89%, and Herawati et al (2017) who reported 8.84%. The high level protein content and low level fat content of *D. magna* is because of the high nutrient content of its culture medium, where there are more nitrate and phosphate. Widianingsih et al (2011) stated that higher is the N and P content, higher is the protein in the cultivar. Fat content is inversely proportional to protein content. In this regard, the present study supported by the study of Lim et al (2011) who stated that higher protein content is always the exact opposite of fat because the fat in the body works two times harder than protein.

Based on the result of the study that had been done by Damle & Chari (2011), and Herawati et al (2016), factors that affecting biomass and nutrient content of *D. magna* are nutrient quality of medium, availability of phytoplankton, bacteria, and detritus as feed, and the environment. The amount of bacteria and organic particles of decomposition results can increase because of organic matter contained in fermented medium. Therefore, it can increase the availability of nutrients in the culture medium which affects *D. magna*'s population growth and biomass. This is strengthened by a study conducted by Nwachi (2013) who stated that fermentation aims to multiply the number of microorganisms as well as intensify the metabolism in *D. magna* mass cultured using 50 g/L chicken manure + 100 g/L rejected bread + 50 g/L tofu waste fermented for 28 days, resulting in new feed products using microorganisms.

The highest amino acid profile of essential lysine is 37.83 ppm in *D. magna* mass cultured using 50 g/L chicken manure + 100 g/L rejected bread + 50 g/L tofu waste which fermented for 28 days. The lowest amino acid essential lysine is 4.23 ppm in *D. magna* mass cultured using 100 g/L rejected beard + 100 g/L tofu waste which fermented for 0 day. Total amino acid profile of *D. magna* mass cultured using various animal manure based on difference fermentation time is presented in Table 6. Lysine amino acid has several functions, which are: as frame of vitamin B1 and anti-virus, helps the calcium absorption, stimulates appetite, and helps in the production of carnitine to convert fatty acids into energy.

Total fatty acid profile in Table 6 showed that the highest linoleic and linolenic fatty acid profile of *D. magna* are at mass culture medium using 50 g/L chicken manure + 100g/L rejected bread + 50 g/L tofu waste fermented for 28 days which is 8.20% and 6.96%. The lowest linoleic and linolenic fatty acid profile of *D. magna* are at mass culture medium using 100g/L rejected bread + 100g/L tofu waste fermented for 0 day which is 1.54% and 0.19%. The results of the present study showed higher values than previous studies of Herawati et al (2016) with 0.2%, and Herawati et al (2017) with 4.83%. Linoleic fatty acids serve as a base substrate in the formation of polyunsaturated fatty acids (PUFA) long chains. The results of the present study were strengthened by previous study done by Pratiwi et al (2009), Zengin et al (2013), and Herawati et al (2017) who stated that linoleic fatty acids act as a base substrate to form the long chains of Omega 6 and Omega 3.

Table 5

Total fatty acid profiles of *Daphnia magna* mass cultured using various animal manure based on difference fermentation time

Fatty acid	Day 0				Day 7				Day 14				Day 21				Day 28			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Miristic	0.11± 0.08	0.18± 0.06	0.05± 0.08	0.10± 0.04	0.19± 0.06	0.88± 0.09	0.33± 0.06	0.66± 0.02	0.25± 0.05	0.92± 0.03	0.46± 0.02	0.68± 0.01	0.29± 0.05	0.99± 0.07	0.55± 0.07	0.75± 0.07	0.35± 0.15	1.93± 0.09	1.13± 0.06	1.26± 0.08
Pentadecanoic	0.84± 0.01	1.29± 0.08	1.59± 0.04	1.47± 0.08	1.04± 0.08	1.89± 0.03	1.63± 0.02	1.52± 0.07	1.19± 0.10	1.97± 0.05	1.75± 0.03	1.72± 0.06	1.25± 0.11	2.01± 0.05	1.88± 0.01	1.93± 0.05	1.37± 0.10	2.59± 0.04	2.03± 0.05	2.14± 0.09
Palmitic	0.91± 0.02	2.91± 0.02	1.65± 0.09	1.52± 0.03	2.21± 0.02	3.09± 0.02	2.25± 0.09	2.47± 0.03	2.26± 0.12	3.47± 0.04	2.46± 0.07	2.78± 0.01	2.31± 0.09	3.63± 0.05	2.77± 0.09	2.85± 0.06	2.38± 0.07	5.98± 0.05	3.27± 0.08	3.99± 0.07
Stearic	0.85± 0.23	1.95± 0.03	1.61± 0.01	0.89± 0.08	1.25± 0.06	1.83± 0.02	1.70± 0.08	1.82± 0.04	1.31± 0.07	1.95± 0.01	1.81± 0.08	1.96± 0.03	1.43± 0.17	1.98± 0.04	1.94± 0.04	1.93± 0.05	1.56± 0.05	2.79± 0.06	2.20± 0.04	2.47± 0.03
Oleic/ω9	1.29± 0.19	3.46± 0.07	2.17± 0.02	2.49± 0.07	1.38± 0.09	3.82± 0.06	2.65± 0.08	2.78± 0.03	1.42± 0.19	3.97± 0.06	2.84± 0.02	2.77± 0.05	1.51± 0.03	4.02± 0.05	2.93± 0.07	2.89± 0.04	1.58± 0.13	5.78± 0.08	3.90± 0.09	4.06± 0.03
Linoleic/ω6	1.88± 0.19	3.32± 0.09	2.32± 0.01	2.69± 0.03	1.27± 0.19	3.86± 0.08	2.75± 0.09	2.99± 0.01	2.07± 0.09	3.94± 0.01	2.83± 0.09	3.05± 0.04	2.18± 0.11	4.32± 0.06	3.19± 0.04	3.26± 0.06	2.34± 0.09	8.20± 0.08	5.78± 0.08	6.99± 0.09
Linolenic/ω3	1.29± 0.19	3.05± 0.02	2.32± 0.03	2.45± 0.04	2.43± 0.19	3.13± 0.02	2.84± 0.04	2.92± 0.05	2.47± 0.10	3.33± 0.02	3.01± 0.05	3.05± 0.09	2.47± 0.10	4.68± 0.04	3.52± 0.07	3.59± 0.08	2.53± 0.11	6.96± 0.04	4.87± 0.07	5.78± 0.07
Arachidic	0.09± 0.19	0.18± 0.05	0.10± ±0.08	0.15± 0.02	0.29± 0.19	1.28± 0.07	0.80± 0.09	1.15± 0.05	0.69± 0.11	2.43± 0.07	1.08± 0.01	2.25± 0.05	0.72± 0.15	3.79± 0.08	1.34± 0.07	2.52± 0.07	0.89± 0.05	4.83± 0.04	3.75± 0.09	3.99± 0.06
Arachidonic	1.17± 0.19	3.52± 0.09	2.23± 0.06	2.50± 0.04	1.03± 0.19	3.37± 0.07	2.45± 0.03	2.65± 0.02	1.33± 0.07	3.40± 0.07	2.70± 0.05	2.77± 0.02	1.45± 0.13	3.59± 0.09	2.98± 0.08	2.83± 0.03	1.60± 0.03	4.19± 0.07	3.07± 0.06	3.17± 0.09
Eicosapentaenoic	1.09± 0.19	5.91± 0.04	3.17± 0.05	3.99± 0.08	1.25± 0.19	6.07± 0.01	4.66± 0.04	4.78± 0.03	2.05± 0.03	6.15± 0.07	4.87± 0.07	4.96± 0.01	2.18± 0.09	6.38± 0.07	4.93± 0.06	4.92± 0.05	2.29± 0.10	7.59± 0.08	5.23± 0.07	6.03± 0.08
Omega 3	2.08± 0.19	7.56± 0.07	4.33± 0.08	5.78± 0.02	2.85± 0.02	7.85± 0.02	5.93± 0.06	6.09± 0.03	3.15± 0.12	7.92± 0.01	5.99± 0.08	6.17± 0.04	3.24± 0.08	7.99± 0.01	5.92± 0.03	6.49± 0.09	3.38± 0.06	8.15± 0.09	6.59± 0.09	6.99± 0.05
Omega 6	0.25± 0.19	0.95± 0.01	0.61± 0.01	0.56± 0.08	0.35± 0.01	1.15± 0.01	0.83± 0.07	0.82± 0.03	0.75± 0.11	1.18± 0.01	1.03± 0.08	1.02± 0.09	1.08± 0.15	2.05± 0.05	1.42± 0.09	1.59± 0.08	1.58± 0.05	2.99± 0.09	2.03± 0.06	2.39± 0.09
Omega 9	1.17± 0.19	4.46± 0.06	3.56± 0.07	4.24± 0.02	1.03± 0.19	4.64± 0.03	3.72± 0.08	4.36± 0.01	1.13± 0.10	4.83± 0.05	3.80± 0.08	4.45± 0.05	1.27± 0.11	4.97± 0.07	3.77± 0.06	4.38± 0.09	1.33± 0.01	4.97± 0.07	3.77± 0.06	4.38± 0.09
Unsaturated fatty acid	0.09± 0.01	1.09± 0.09	0.80± 0.08	1.49± 0.07	0.15± 0.01	1.37± 0.05	1.20± 0.02	1.29± 0.06	0.35± 0.11	1.44± 0.02	1.27± 0.05	1.42± 0.07	0.67± 0.17	1.57± 0.07	1.33± 0.06	1.48± 0.06	1.40± 0.10	1.97± 0.09	1.59± 0.08	1.74± 0.05
Saturated fatty acid	1.09± 0.09	3.97± 0.02	2.49± 0.06	2.60± 0.04	1.88± 0.19	4.06± 0.02	2.97± 0.06	2.81± 0.04	2.19± 0.09	4.82± 0.02	3.19± 0.04	3.23± 0.08	2.30± 0.18	4.99± 0.06	3.34± 0.05	3.57± 0.08	2.39± 0.08	6.59± 0.06	5.15± 0.08	4.99± 0.09
Mono unsat. fatty acid	2.08± 0.10	3.58± 0.08	2.61± 0.04	2.83± 0.02	2.15± 0.14	3.74± 0.05	2.74± 0.09	2.76± 0.06	2.55± 0.04	4.66± 0.05	2.9± 0.08	3.21± 0.02	2.64± 0.14	4.78± 0.08	3.04± 0.07	3.49± 0.09	2.85± 0.04	5.55± 0.09	4.04± 0.09	4.45± 0.08
Polysaturated fatty acid	0.34± 0.21	1.15± 0.04	0.13± 0.07	0.75± 0.09	0.44± 0.07	1.63± 0.02	0.62± 0.05	1.05± 0.01	1.07± 0.17	2.26± 0.05	1.42± 0.03	1.65± 0.08	1.18± 0.19	2.43± 0.08	1.65± 0.09	1.88± 0.07	1.38± 0.09	3.23± 0.09	2.76± 0.08	2.08± 0.09
AA	0.03± 0.05	1.08± 0.04	0.07± 0.03	0.67± 0.01	0.09± 0.08	1.08± 0.04	0.07± 0.03	0.67± 0.01	0.19± 0.18	1.19± 0.08	0.33± 0.02	0.78± 0.03	0.27± 0.10	1.27± 0.09	0.46± 0.08	0.86± 0.07	0.35± 0.17	1.57± 0.08	0.79± 0.06	1.03± 0.09
DHA	0.21± 0.08	1.63± 0.02	0.52± 0.06	1.50± 0.07	0.34± 0.08	1.72± 0.02	1.15± 0.06	1.59± 0.07	0.75± 0.05	1.67± 0.02	1.23± 0.05	1.53± 0.06	0.90± 0.15	1.75± 0.02	1.32± 0.07	1.59± 0.07	0.95± 0.05	1.98± 0.07	1.47± 0.08	1.76± 0.09

Fatty acid	Day 0				Day 7				Day 14				Day 21				Day 28			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
EPA	0.21± 0.04	0.79± 0.04	0.48± 0.09	0.41± 0.02	0.28± 0.14	0.81± 0.05	0.66± 0.02	0.64± 0.01	0.39± 0.10	1.02± 0.04	0.69± 0.04	0.77± 0.01	0.43± 0.19	1.36± 0.05	0.83± 0.07	0.94± 0.09	0.48± 0.01	1.89± 0.05	0.97± 0.06	1.13± 0.07

Table 6

Total amino acid profile of *Daphnia magna* mass cultured using various animal manure based on difference fermentation time

Amino acid	Day 0				Day 7				Day 14				Day 21				Day 28			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
L-Histidine	3.19± 0.18	8.14± 0.03	6.52± 0.03	7.85± 0.05	3.56± 0.05	9.92± 0.08	6.94± 0.01	8.14± 0.03	3.78± 0.03	10.14± 0.03	8.52± 0.03	9.85± ±0.05	3.85± 0.03	9.92± ±0.08	6.94± 0.01	38.14± ±0.03	4.18± ±0.09	15.19± ±0.09	13.06± ±0.06	13.98± ±0.04
L-Serine	2.84± 0.01	5.40± 0.07	4.63± 0.07	5.76± 0.02	4.95± 0.06	6.61± 0.03	5.62± 0.01	5.99± 0.07	8.08± 0.09	11.40± 0.07	7.63± 0.07	9.56± ±0.02	10.29± 0.06	13.61± ±0.03	9.62± 0.01	10.31± ±0.07	11.46± ±0.09	19.03± ±0.03	17.10± ±0.01	17.23± ±0.07
L-Arginine	3.17± 0.02	7.39± 0.02	6.35± 0.05	8.36± 0.07	3.48± 0.05	8.61± 0.04	7.37± 0.07	8.39± 0.02	3.77± 0.03	13.30± 0.02	9.35± 0.05	10.36± ±0.07	3.85± 0.09	15.61± ±0.04	11.78± ±0.07	12.83± ±0.02	4.16± ±0.08	18.19± ±0.03	12.26± ±0.05	14.20± ±0.01
Glycine	5.43± 0.23	8.48± 0.05	9.78± 0.09	8.33± 0.02	5.53± 0.03	9.36± 0.04	8.19± 0.01	8.48± 0.05	5.66± 0.04	12.85± 0.05	10.83± ±0.09	11.23± ±0.02	5.72± 0.02	15.66± ±0.04	12.56± ±0.01	13.27± ±0.05	12.98± ±0.05	19.03± ±0.06	16.26± ±0.07	17.90± ±0.09
L-Aspartic Acid	3.26± 0.19	8.25± 0.09	9.70± 0.07	8.65± 0.03	3.47± 0.08	9.78± 0.03	10.07± 0.01	8.25± 0.09	3.59± 0.09	10.56± 0.08	9.55± 0.07	9.53± ±0.03	3.68± 0.10	13.18± ±0.03	11.37± ±0.01	12.75± ±0.09	13.79± ±0.11	18.90± ±0.09	13.80± ±0.05	14.75± ±0.08
L-Glutamic Acid	5.23± 0.19	9.76± 0.05	8.89± 0.04	8.85± 0.07	5.83± 0.07	11.51± 0.04	8.28± 0.01	9.76± 0.05	5.97± 0.09	19.76± 0.05	17.59± ±0.04	18.85± ±0.07	6.08± 0.05	20.43± ±0.04	18.28± ±0.01	19.76± ±0.05	16.27± ±0.02	24.36± ±0.08	21.67± ±0.07	22.56± ±0.02
L-Threonine	3.10± 0.19	8.37± 0.09	7.56± 0.03	7.47± 0.07	3.85± 0.03	9.02± 0.09	8.37± 0.01	7.37± 0.09	3.96± 0.02	15.85± 0.09	11.43± ±0.03	12.53± ±0.07	4.08± 0.05	19.02± ±0.09	16.37± ±0.01	17.78± ±0.09	14.43± ±0.04	21.78± ±0.06	17.89± ±0.09	18.96± ±0.08
L-Alanine	1.17± 0.06	5.21± 0.02	4.98± 0.05	4.51± 0.01	1.86± 0.05	6.65± 0.05	4.51± 0.09	5.21± 0.02	1.97± 0.02	16.34± 0.02	14.69± ±0.05	15.70± ±0.01	2.18± 0.04	20.79± ±0.05	15.51± ±0.09	17.67± ±0.02	12.17± ±0.03	23.20± ±0.09	18.95± ±0.08	20.23± ±0.09
L-Cystine	2.26± 0.09	8.74± 0.04	6.13± 0.02	5.72± 0.03	2.57± 0.09	10.24± 0.05	7.87± 0.04	7.74± 0.04	2.66± 0.08	24.57± 0.04	21.89± ±0.02	21.72± ±0.03	2.84± 0.07	25.24± ±0.05	22.93± ±0.04	23.87± ±0.04	12.84± ±0.03	25.87± ±0.07	23.75± ±0.09	24.92± ±0.01
L-Lysine HCL	4.23± 0.05	11.45± ±0.09	9.58± 0.07	10.83± 0.03	4.78± 0.05	15.54± 0.03	12.52± 0.04	14.23± 0.07	4.83± 0.05	18.32± ±0.09	15.31± ±0.05	16.62± ±0.09	5.09± 0.19	19.94± ±0.15	16.82± ±0.07	17.83± ±0.03	15.73± ±0.08	37.83± ±0.03	19.90± ±0.04	25.99± ±0.08
L-Tyrosine	2.08± 0.07	9.86± 0.06	7.99± 0.03	7.59± 0.06	2.20± 0.03	11.49± 0.06	9.89± 0.07	9.59± 0.08	2.32± 0.05	14.20± 0.06	10.26± ±0.07	12.89± ±0.09	2.57± 0.09	15.67± ±0.09	11.99± ±0.09	13.19± ±0.09	12.88± ±0.03	17.10± ±0.05	13.09± ±0.03	14.98± ±0.03
L-Methionine	2.69± 0.10	10.95± ±0.09	11.20± 0.07	10.87± ±0.02	2.76± 0.08	12.87± 0.09	10.50± 0.09	10.98± 0.09	3.23± 0.06	16.20± 0.02	12.35± ±0.06	13.86± ±0.05	3.68± 0.07	16.90± ±0.06	13.98± ±0.05	14.25± ±0.02	14.09± ±0.03	18.98± ±0.03	15.20± ±0.04	14.98± ±0.05
L-Valine	3.03± 0.05	8.67± 0.04	6.46± 0.07	7.47± 0.05	3.19± 0.03	10.20± 0.08	8.51± 0.09	8.96± 0.04	3.40± 0.05	15.23± 0.09	10.11± ±0.07	11.92± ±0.07	3.75± 0.04	16.09± ±0.02	12.23± ±0.06	12.98± ±0.05	11.16± ±0.08	15.23± ±0.02	13.09± ±0.02	13.99± ±0.03
L-Isoleucine	1.17± 0.01	5.62± 0.03	4.97± 0.05	6.41± 0.07	1.26± 0.10	8.10± 0.05	8.98± 0.08	8.09± 0.09	2.02± 0.08	10.80± 0.06	7.78± 0.09	8.90± ±0.07	2.56± 0.09	11.75± ±0.03	8.97± 0.02	10.13± ±0.06	9.23± ±0.02	13.25± ±0.03	11.38± ±0.01	12.57± ±0.01
L-Leucine	2.23± 0.06	6.82± 0.01	7.40± 0.05	6.85± 0.05	2.47± 0.05	6.63± 0.09	6.19± 0.09	6.09± 0.01	3.33± 0.07	11.13± 0.09	10.89± ±0.07	9.19± ±0.08	3.48± 0.06	12.23± ±0.04	11.09± ±0.04	11.95± ±0.07	10.06± ±0.07	15.98± ±0.01	12.98± ±0.09	14.96± ±0.06
L-Phenylalanine	3.09± 0.10	5.40± 0.05	5.63± 0.07	5.76± 0.02	3.18± 0.08	7.19± 0.01	5.98± 0.06	5.98± 0.09	3.69± 0.05	9.23± 0.05	6.88± 0.06	7.99± ±0.05	3.77± 0.04	11.03± ±0.07	8.19± 0.02	9.93± ±0.02	7.03± ±0.03	13.73± ±0.03	10.28± ±0.01	12.37± ±0.01
Tryptophan	1.26± 0.05	5.39± 0.03	4.35± 0.01	4.36± 0.05	1.46± 0.03	8.10± 0.05	8.98± 0.08	8.09± 0.09	2.57± 0.04	10.19± 0.09	6.90± 0.06	7.89± 0.05	2.60± 0.03	12.67± ±0.07	10.98± ±0.08	10.95± ±0.09	8.77± 0.05	14.97± ±0.09	12.93± ±0.07	11.17± ±0.09

Conclusions. Based on the present study results, *D. magna* mass culture using 50 g/L chicken manure + 100 g/L rejected bread + 50 g/L tofu waste fermented for 28 days gave an enhancement towards its growth, and biomass production within treatments. The highest nutrient quality based on proximate analysis, amino fatty acid profile, and amino acid was obtained in *D. magna* mass culture using the same medium.

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Received: 30 May 2018. Accepted: 20 August 2018. Published online: 30 August 2018.

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How to cite this article:

Herawati V. E., Pinandoyo, Hutabarat J., Karnaradjasa O., 2018 The effect of nutrient content and production of *Daphnia magna* mass cultured using various wastes processed with different fermentation time. *AAFL Bioflux* 11(4):1289-1299.