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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

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Abstract. The nutrient quality and growth performance of *D. magna* are highly depend on the organic fertilizer which is used in its culture medium. The objective of this study was to identify the best fermentation time by using probiotic bacteria on organic fertilizer as mass culture medium to improve its nutrient quality, biomass production, and growth performance. This study was conducted using completely randomized experimental design with five treatments and three repetitions. Organic fertilizers used cultured medium with chicken manure, rejected bread and tofu waste fermented by probiotic bacteria then cultured for 0, 7, 14, 21 and 28 days. The results showed that medium which used 25% chicken manure, 25% tofu waste and 50% rejected bread cultured for 28 days created the highest biomass production, population density and nutrient content of *D. magna* those are 233,980 ind/L for population density; 134.60 grams for biomass production, 0.574% specific growth rate; 68.06% protein content and 6.91% fat. The highest fatty acid profile is 4.83% linoleic and 3.54% linolenic acid. The highest essential amino acid is 53.94 ppm lysine. In general, the content of ammonia, DO, temperature, and pH during the study were in the good range of *D. magna* life. The conclusion of this research is medium which used 25% chicken manure, 25% tofu waste and 50% rejected bread cultured for 28 days created the highest biomass production, population and nutrient content of *D. magna*.

Keywords: *D. magna*, fermentation time, nutrient value, probiotic bacteria, various wastes

1. Introduction

Daphnia sp. is a zooplankton which is the best natural food for freshwater fish larvae and ornamental fish culture because the nutrient content and size of *Daphnia* sp. suitable with mouth opening and nutrient needs of Nile tilapia larvae. The nutrient content is highly depends on the culture medium where it grows and breeds [1, 2]. Nutrient content of *D. magna* highly depends on its culture medium as the growth place of phytoplankton as *D. magna*'s feed [3, 4]. Recently, not only chicken manure is used as common culture medium for *D. magna* [5], but also the used of goat, and cow manure as culture medium [3]. Another medium used is a combination of chicken dung, bran, and copra waste [6] and different animal wastes those are chicken, goat and quail manure [4]. The organic matters in bran are highly nutritious for the growth of *D. magna*.



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The research using various animal waste with fermentation time for 14 days has been done in 2016, the study result stated that chicken manure is the best culture medium for *Daphnia magna*'s nutrient quality and growth performance. The use of organic fertilizers in culture media including the wastes/faeces of chicken, and quail mixed with the rejected bread and tofu waste based on different fermentation time with the probiotic bacteria has been conducted as the use of organic fertilizer could impact the growth performance and nutrient content of *D. magna*. The highest nutrient, particularly for the content of N, P and Ca in organic fertilizer are the food sources of *D. magna*. Chicken waste contained N (4.75%); P (3.57%) and Ca (4.80%) and quail waste contained N (4.06%); P (2.96%) and Ca (2.57%) [2]. Furthermore, the analysis on the dried materials of tofu waste has been done by previous study and the results showed that it contained crude protein (27.09%), crude fiber (22.85%), fat (7.37%), ash (35.02%), and extract material without nitrogen/BETN (6.87%) [4][7]. Rejected bread contained crude protein (12.63%), crude fiber (0.13%); crude fat (4.63%); ash (4.19%) and the extract material without nitrogen (58.42%) [10].

Fermentation of fertilizer has been proven to be effective to increase nutrient of culture medium. The aims of the fermentation are to produce a product (food materials) that contains the nutrients, to have a longer storage time, and to have a better organoleptic characteristics and nutritional components [2]. Probiotic bacteria are really supportive for the health of organisms. It also serves to decompose and ferment organic matters [9]. Decomposition is a biological process that makes bacteria produces growth substances, hormones, vitamins, and other enzymes [5][10].

The objective of this study was to identify the best fermentation time by using probiotic bacteria on organic fertilizer as mass culture medium to improve nutrient quality, biomass production, and growth performance of *Daphnia magna*. The fertilizer itself is fermented with probiotic bacteria (*Lactobacillus casei* and *Saccharomyces cerevisiae*).

2. Materials and Methods

2.1. Fermentation Stage

Fermentation stage is the preparation of molasses ratio, water and pro-biotic. The ratio used was 1 : 1, i.e. 1 mL of molasses, 1 mL of probiotic bacteria and 100 mL of solvent. The organic matter used was a combination of 25% chicken manure + 50% rejected bread + 25% tofu waste then it was dried. The treatments used in this research are as follow: A. 0 day fermentation; B. 7 days fermentation; C. 14 days fermentation; D. 21 days fermentation; E. 28 days fermentation. Pro-biotic bacteria (*L. casei* and *S. cerevisiae*) that were already activated for 3 hours were given to fertilizer with a combination weight of 200g/L [4][9][11]. They were left for fungi to grow and acidic smell to develop. Once the fertilizer was ready, the samples were put into the culture medium and aerated for 14 days. And when it was ready, 100 ind/L of *D. magna* was inoculated [3].

2.2. Water Quality

Water quality during the study was maintained at 28-30°C temperature, 0.3 ppm DO and 8.1-8.2 pH, which is ideal. This is in line with the previous study that the proper temperature for *D. magna* culture is 25-30°C, DO at 0.3-0.6 ppm, and pH at 6.5-9 [4][12]. Excellent water quality helps the growth of phytoplankton and algae for *D. magna* to stimulate its growth.

2.3. *D. magna* Culture

The 100 ind/L *D. magna* was spread for each pool containing 200 g/L fermented organic fertilizer. Observation for the abundance of *D. magna* was conducted every two days to monitor the population of *D. magna*. The water (20-25%) of this culture was replaced, and its pH level was monitored every morning at around 7 a.m. to maintain the quality. pH level was maintained at its optimum range with the addition of 1 L of dolomite /1.000 L of water.

3.4. Statistical Analysis

This study had a completely random design with five treatments and three repetitions. The biomass weight was analyzed using variant analysis to determine differences among treatments. Parameters analyzed were growth, biomass production, and nutrient content of *D. magna*.

Proximate analysis. The proximate chemical composition of the samples was determined using a standard procedure [4][13]. Crude protein content was calculated by multiplying total nitrogen factor. Carbohydrate content was estimated by the difference.

Essential amino acid profile. The amino acid composition of the samples was determined using HP 6C (Shimadzu LC-6A) [4][13].

Fatty acid profile. The fatty acid composition of the samples was determined using a gas chromatograph (Shimadzu) [4][13].

3. Results and Discussion

This study is the development of previous study about *D. magna* mass culture using chicken manure, rice bran and coconut oil cake fermented by probiotic bacteria in 2015 and 2016 with the use of various manure in *D. magna* mass culture media. The results of the study found that organic matters in the form of fermented various chicken manure mixed with rejected bread and tofu waste had improved nutrient quality. To determine the quality of *D. magna* cultured medium, nutrient analysis through N, P, and K and medium nutrient through proximate analysis were done. Cultured medium N, P and K nutrient content before fermented at different times is presented in Table 1.

Table 1. N, P, and K nutrient content of organic fertilizer before fermented at different times

Parameters	Results (%)					Methods
	A	B	C	D	E	
Nitrogen	1.05	2.31	2.75	2.86	3.07	Kjeldhal
Potassium (K)	0.18	0.22	0.09	0.22	0.44	AOAC 983.02.2000
Phosphor	0.54	0.54	0.58	0.60	0.62	AOAC 983.02.2000

Study result found that cultured medium before fermented which has highest Nitrogen content is cultured medium with 28 days fermentation (E) about 3.07 % and the lowest Nitrogen content is at cultured medium without fermentation (A) about 1.05%. Cultured medium which has the highest Potassium (K) and Phosphor contents is cultured medium with 2 days fermentation (E) those are 0.44% and 0.62%, respectively. N, P, and K nutrient content of organic fertilizer after fermented at different times is presented in Table 2.

Table 2. N, P, and K nutrient content of organic fertilizer after fermented at different times

Parameters	Results (%)					Methods
	A	B	C	D	E	
Nitrogen	1.58	2.96	3.06	3.79	4.86	Kjeldhal
Potassium (K)	0.10	1.43	1.09	1.61	2.65	AOAC 983.02.2000
Phosphor	0.47	1.50	1.49	2.12	2.97	AOAC 983.02.2000

The results showed that enhancement of cultured medium nutrient quality after fermentation at different times occurred. The highest enhancement occurred at cultured medium fermented in 28 days (E) about 4.86%. It increased about 1.79% than before and cultured medium nutrient quality average enhancement for Nitrogen was about 0.53% - 1.79%. The highest Potassium (K) and Phosphor content occurred at cultured medium fermented in 28 days (E) those are 2.65% and 2.97%. Organic fertilizer nutrient content (Proximate) before and after fermented at different times is presented in Table 3.

Table 3. Organic fertilizer Nutrient content (Proximate) before and after fermented at different times

Materials	Content in 100% Dried Matter					Methods
	Ash (%)	Crude Fat (%)	Crude Fiber (%)	Crude Protein (%)	Carbohydrate (%)	
A (Without Fermentation)	30.57	11.59	17.32	17.67	22.85	Kjeldhal
B (7 days Fermentation)	29.62	11.61	18.84	19.04	20.89	
C (14 days Fermentation)	19.26	13.75	19.28	28.79	18.92	
D (21 days Fermentation)	17.99	14.78	16.80	29.69	20.74	
E (28 days Fermentation)	17.58	15.98	13.20	33.69	19.55	

The highest proximate analysis result was at cultured medium fermented for 28 days (E) for protein and fat are 33.69% and 15.98% respectively; while the lowest result was cultured medium without fermentation (A) those are 22.85% and 11.59%. The improvement of nutrient quality on fermented medium is an anaerobic dissimilation process of organic compounds by the activity of microorganisms or extracts from the cells of microorganisms.

Differences between nutrient and nutrient content contained in the culture medium *D. magna* caused by *Lactobacillus* sp. bacteria as fermenter during fermentation process. *Lactobacillus* sp. increase the protein content of the ingredients proved by the increase of nutrient and nutrient content in the culture medium. Time difference in the fermentation process has an effect on the amount of bacteria that develops. This study results are accordance with previous study stated that fermentation process can increase energy, protein and crude fiber content [14]. Microbes used in the fermentation process can produce enzymes that will degrade complex compounds to be simpler and synthesize proteins. The statement is strengthened by previous study stated that *Lactobacillus* sp. as fermenter has unique characteristic because it can neutralize acidic or alkaline organic matters [15]. Proteolytic microbes are capable of producing protease enzymes that will breakdown proteins. Protein breakdown is converted into polypeptide, then becomes a simple peptide, then this peptide will be breakdown into amino acids. These amino acids will be utilized by microbes to multiply themselves. The number of microbial colonies that are the source of single cell proteins increases during the fermentation process.

Improvement of nutrient medium, especially nitrate, serves to determine the amount of phytoplankton present in the culture medium as a source of *D. magna* feed other than bacteria and detritus. Based on the results of the 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 in adult sizes able to eat small crustaceans and rotifers, thus the more phytoplankton exist the faster the growth of *D. magna*. This study results is strengthened by previous studies stated that the more abundance of phytoplankton and organic matter in medium, growth rate of *Daphnia* sp. will occur faster [3]. This study results strengthened by previous study stated that phytoplankton population enhancement and growth in water related with nutrient availability especially nitrate and sunlight, nitrate will increase water fertility characterized by high number of phytoplankton exists [16-17].

Population density graph of *D. magna* cultured using organic fertilizer based on fermentation time difference is presented in Figure 1.

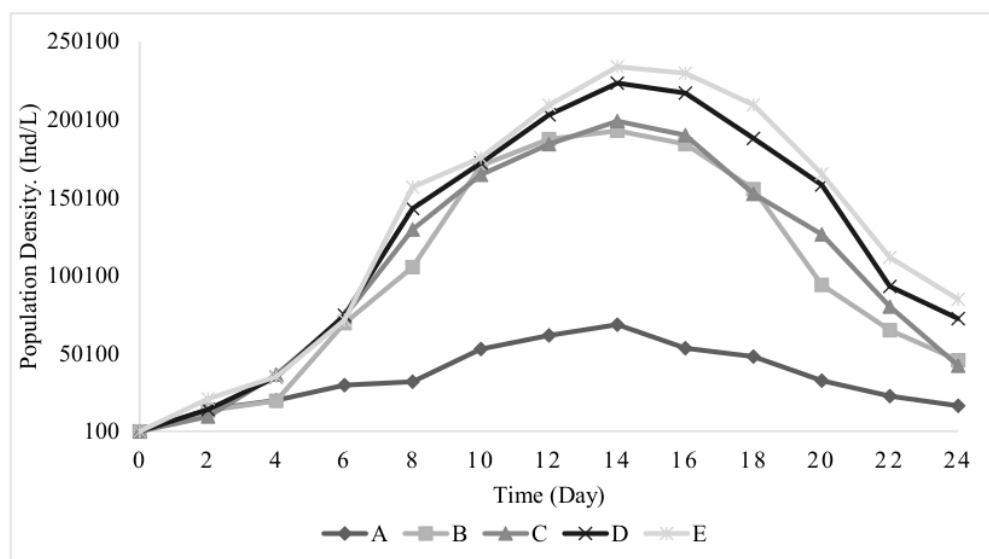


Figure 1. Population density graph of *D. magna* cultured using organic fertilizer based on fermentation time difference

D. magna growth phase cultured using organic fertilizer based on fermentation time difference is presented in Table 4.

Table 4. *D. magna* growth phase cultured using organic fertilizer based on fermentation time difference

Phase	Treatments (ind/L)				
	A	B	C	D	E
Lag	63,490.00 ^a	102,280.00 ^a	119,640.00 ^a	123,330.00 ^a	127,500.00 ^a
Exponential	61,620.67 ^a	187,590.33 ^b	184,390.33 ^b	203,200.33 ^b	209,370.33 ^b
Stationary	68,570.33 ^a	192,920.00 ^b	199,120.33 ^b	223,500.33 ^b	233,980.33 ^b
Death	23,813.33 ^a	68,123.33 ^{ab}	82,776.67 ^{ab}	107,820.00 ^b	220,426.67 ^b

Notes: ^adifferent between treatments; ^bnot significantly different between treatments; ^{ab}significantly different between treatments

D. magna population growth phase during cultivation consists of adaptation phase (*lag phase*), exponential phase, stationary phase, and death phase. Lag phase occurred on day 4 with highest population density of 127,500.00 ind/L; exponential phase occurred on day 10 with highest population density of 209,370.33 ind/L; stationary phase occurred on day 14 with highest population density of 233,980.33 ind/L; death phase occurred on day 16 with highest population density of 220,426.67 ind/L. *D. magna* growth rate cultured using organic fertilizer based on fermentation time difference is presented in Figure 2.

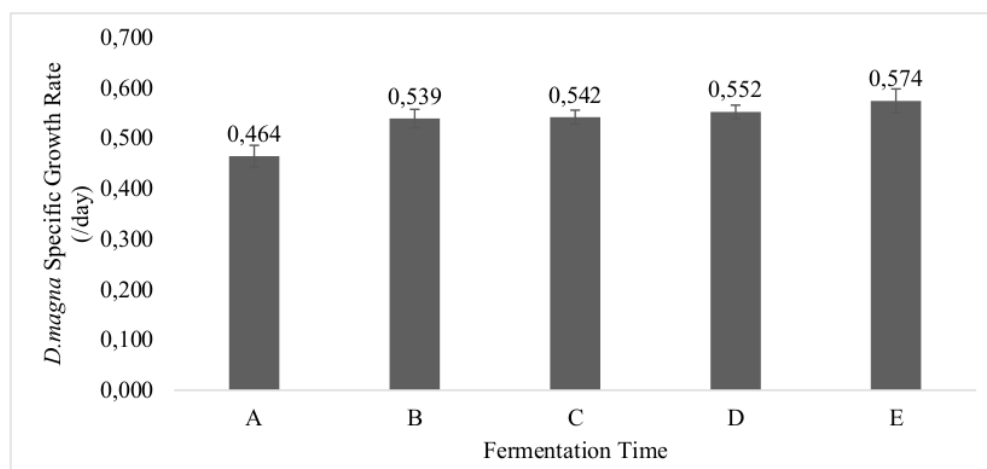


Figure 2. *D. magna* growth rate cultured using organic fertilizer based on fermentation time difference

Based on the results, the highest *D. magna* growth rate occurred in *D. magna* which cultured using organic fertilizer fermented for 28 days (D) is $0.574 \pm 0.005/\text{day}$. The lowest *D. magna* growth rate occurred in *D. magna* without fermentation (A) is $0.464 \pm 0.002/\text{day}$.

Growth of mass-cultured *D. magna* use 25% Chicken manure + 50% rejected bread + 25% tofu waste with different fermentation length gave no significant effect between treatment at lag phase ($P > 0.01$), lag phase happened on day 4 and the highest by 25% Chicken manure + 50% rejected bread + 25% tofu waste with 28 days fermentation (E), that is $127,500.00 \pm 0.01 \text{ ind/L}$. This is due to *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, *D. magna* needs longer time to grow. 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 the liquid body [2][18].

Exponential phase occurs on day 10, with the highest population is 209,370.33 ind/L stationary phase occurred on day 14, with the highest population is 233,980.33 ind/L; The death phase occurred on day 16, with the highest population is 220,426.67 ind/L. Exponential phase is the phase where the nutrient content in *D. magna* is highest while growth is not maximized and *D. magna* began to increase. The exponential phase of the study took place on the day 10, the results were in line with previous study stated that *Daphnia* sp. is in lag or exponential phase on day 9 and 10 [19]. The exponential and stationary phase in this study gives a very real effect between treatments ($P < 0.01$). The length of the stationary phase is correlated with the duration of adapting the *D. magna* phase 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 previous study which showed about exponential phase stop because of nutrient lack in cell density enhancement [2][20].

D. magna biomass weight cultured using organic fertilizer based on fermentation time difference is presented in Figure 3.

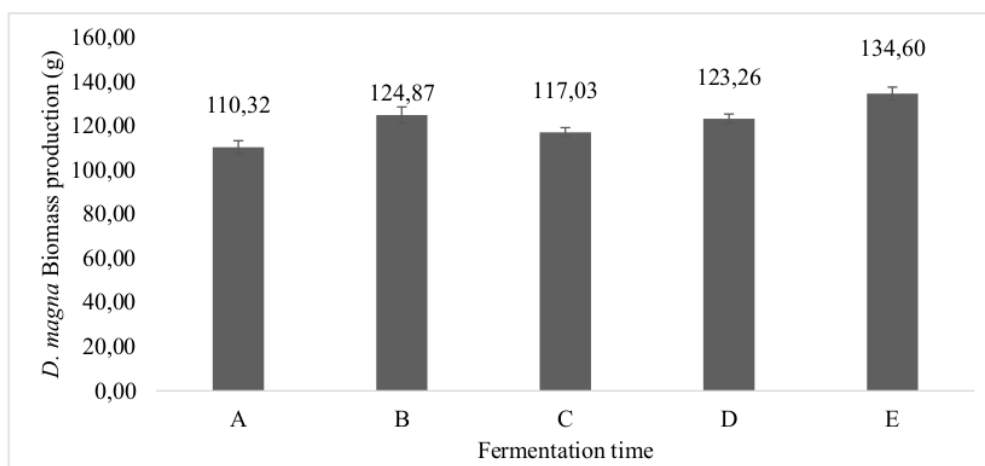


Figure 3. *D. magna* biomass weight mass cultured using organic fertilizer based on fermentation time difference

The highest *D. magna* biomass weight was *D. magna* cultured using organic fertilizer which fermented for 28 days (E) that is 134.60 ± 0.005 g and the lowest *D. magna* biomass weight was *D. magna* cultured using organic fertilizer which fermented for 0 day (A) that is 110.32 ± 0.001 g. Quality of nutrient culture medium give effect to the supply of plankton and bacteria to increase population growth and biomass of *D. magna*. High organic matters can have an effect on the density and biomass of *D. magna* [3]. Proximate analysis result of *D. magna* cultured using organic fertilizer based on fermentation time difference is presented in Table 5.

Table 5. Proximate analysis of *D. magna* cultured using organic fertilizer based on fermentation time difference

Treatments	Carbohydrate (%)	Ash (%)	Crude fat (%)	Crude fiber (%)	Crude protein (%)
A (Without Fermentation)	21.87	5.73	5.59	4.03	62.78
B (7 days Fermentation)	18.00	6.36	6.76	4.31	64.58
C (14 days Fermentation)	15.09	5.34	6.58	5.50	67.49
D (21 days Fermentation)	16.30	4.94	6.61	4.50	67.65
E (28 days Fermentation)	13.76	6.81	6.91	4.46	68.06

The highest *D. magna* protein and fat nutrient quality analysis result was *D. magna* cultured using organic fertilizer fermented for 28 days (E) those are 68.06% and 6.91%; the lowest protein and fat nutrient content was *D. magna* cultured using organic fertilizer fermented for 0 day (A) those are 62.78% and 5.59%. *D. magna* total fatty acid profile analysis result cultured using organic fertilizer based on fermentation time difference is presented in Table 6.

Table 6. *D. magna* total fatty acid profile analysis result cultured using organic fertilizer based on fermentation time difference

Fatty acids profile (%)	Seed	A	B	C	D	E
Miristic	0.28 ± 0.04	0.49 ± 0.04	0.48 ± 0.09	0.41 ± 0.02	0.50 ± 0.05	0.52 ± 0.05
Pentadecanoic	0.10 ± 0.08	0.18 ± 0.06	0.15 ± 0.08	0.17 ± 0.304	0.08 ± 0.02	0.09 ± 0.06
Palmitic	2.01 ± 0.06	2.29 ± 0.08	2.59 ± 0.04	1.97 ± 0.08	2.12 ± 0.01	3.14 ± 0.09
Stearic	0.41 ± 0.07	1.65 ± 0.02	2.91 ± 0.09	0.52 ± 0.03	2.08 ± 0.05	2.71 ± 0.07
Oleic/ω9	1.62 ± 0.05	0.95 ± 0.03	2.61 ± 0.01	0.89 ± 0.08	2.55 ± 0.03	3.07 ± 0.02
Linoleic/ω6	1.54 ± 0.02	2.46 ± 0.07	3.37 ± 0.02	2.49 ± 0.07	3.75 ± 0.02	4.83 ± 0.09
Linolenic/ω3	0.19 ± 0.06	2.38 ± 0.09	3.32 ± 0.01	2.39 ± 0.03	2.56 ± 0.07	3.54 ± 0.05
Arachidic	0.02 ± 0.07	2.83 ± 0.02	3.05 ± 0.03	1.02 ± 0.04	1.25 ± 0.05	2.30 ± 0.08
Arachidonic	0.07 ± 0.01	0.15 ± 0.05	0.13 ± 0.08	0.15 ± 0.02	0.06 ± 0.08	0.07 ± 0.02
Eiksapentaenoic	0.27 ± 0.05	2.53 ± 0.09	3.52 ± 0.06	0.50 ± 0.04	0.05 ± 0.02	0.06 ± 0.07
Omega 3	0.03 ± 0.02	4.01 ± 0.04	5.91 ± 0.04	3.99 ± 0.08	2.02 ± 0.04	4.06 ± 0.08
Omega 6	0.63 ± 0.08	5.64 ± 0.07	7.53 ± 0.08	5.68 ± 0.02	5.03 ± 0.05	6.05 ± 0.02
Omega 9	0.62 ± 0.05	0.95 ± 0.01	0.61 ± 0.01	0.56 ± 0.08	0.90 ± 0.03	1.48 ± 0.02
Unsaturated fatty acid	0.88 ± 0.09	4.46 ± 0.06	3.56 ± 0.07	4.24 ± 0.02	2.68 ± 0.04	3.65 ± 0.08
Saturated fatty acid	0.97 ± 0.01	1.09 ± 0.09	2.0 ± 0.08	1.49 ± 0.07	0.97 ± 0.02	1.24 ± 0.02
Mono unsaturated fatty acid (MUFA)	1.63 ± 0.05	2.642 ± 0.02	3.97 ± 0.06	2.40 ± 0.04	3.57 ± 0.05	3.93 ± 0.03
Polyunsaturated fatty acid (PUFA)	1.24 ± 0.08	2.62 ± 0.08	3.58 ± 0.04	2.83 ± 0.02	3.17 ± 0.01	4.31 ± 0.09
AA	0.08 ± 0.09	0.15 ± 0.04	0.13 ± 0.07	0.15 ± 0.09	2.18 ± 0.03	2.71 ± 0.03
DHA	0.06 ± 0.02	0.08 ± 0.04	1.07 ± 0.03	0.07 ± 0.01	0.39 ± 0.08	0.83 ± 0.05
EPA	0.27 ± 0.03	0.53 ± 0.02	1.52 ± 0.06	1.50 ± 0.07	1.09 ± 0.02	2.08 ± 0.08

The highest *D. magna* linoleic acid and linolenic acid profile analysis result was *D. magna* cultured at medium fermented for 28 days (E) those are 4.83% and 3.54%; the lowest linoleic acid and linolenic acid profile analysis result was *D. magna* cultured using organic fertilizer fermented for 0 day (A) those are 1.54% and 0.19%. The highest *D. magna* amino acid profile cultured using organic fertilizer fermented for 28 days (E) those are non-essential amino acid aspartic acid 10.70 ppm and essential amino acid Lysine 53.94 ppm. The lowest amino acid for this treatment (E) was leucine 12.40 ppm. The lowest fatty acid profile was non-essential amino acid aspartic acid 8.25 ppm, essential amino acid Lysine 30.14 ppm, and amino acid leucine 5.82 ppm at *D. magna* cultured using organic fertilizer fermented for 0 day (A). *D. magna* amino acid profile mass cultured using organic fertilizer based on fermentation time difference is presented in Table 7.

Table 7. Amino acid profile of *D. magna* cultured using organic fertilizer based on fermentation time difference

Amino Acid Profile	Seed	17 A (ppm)	B (ppm)	C (ppm)	D (ppm)	E (ppm)
Histidine	30.85 ± 0.07	38.14 ± 0.03	44.52 ± 0.03	39.85 ± 0.05	48.92 ± 0.08	50.94 ± 0.01
Serine	10.76 ± 0.05	15.40 ± 0.07	16.63 ± 0.07	16.76 ± 0.02	15.61 ± 0.03	18.62 ± 0.01
Arginine	50.36 ± 0.03	57.39 ± 0.02	64.35 ± 0.05	58.36 ± 0.07	76.61 ± 0.04	79.37 ± 0.07
Glycine	15.33 ± 0.09	18.48 ± 0.05	19.78 ± 0.09	17.33 ± 0.02	19.36 ± 0.04	20.19 ± 0.01
Aspartic Acid	7.65 ± 0.01	8.25 ± 0.09	9.70 ± 0.07	8.65 ± 0.03	9.78 ± 0.03	10.70 ± 0.01
Glutamic Acid	16.85 ± 0.05	19.76 ± 0.05	28.89 ± 0.04	22.85 ± 0.07	20.51 ± 0.04	27.28 ± 0.01
Threonine	15.47 ± 0.09	17.37 ± 0.09	23.56 ± 0.03	20.47 ± 0.07	19.02 ± 0.09	23.37 ± 0.01
Alanine	30.51 ± 0.03	33.21 ± 0.02	35.98 ± 0.05	30.51 ± 0.01	40.65 ± 0.05	42.51 ± 0.09
Proline	15.08 ± 0.04	18.98 ± 0.07	19.44 ± 0.04	20.08 ± 0.09	20.25 ± 0.05	23.00 ± 0.06
Cystine	23.72 ± 0.02	25.74 ± 0.04	28.13 ± 0.02	25.72 ± 0.03	30.24 ± 0.05	32.87 ± 0.04
Lysine	25.89 ± 0.05	30.14 ± 0.05	45.22 ± 0.05	30.89 ± 0.06	48.92 ± 0.08	53.94 ± 0.01
Tyrosine	27.59 ± 0.06	29.86 ± 0.06	30.99 ± 0.03	33.59 ± 0.06	39.57 ± 0.04	44.16 ± 0.01
Methionine	15.87 ± 0.02	19.95 ± 0.09	21.20 ± 0.07	20.87 ± 0.02	19.97 ± 0.03	22.79 ± 0.04
Valine	31.47 ± 0.05	33.67 ± 0.04	35.46 ± 0.07	32.47 ± 0.05	32.44 ± 0.05	36.88 ± 0.05
Isoleucine	12.41 ± 0.07	15.62 ± 0.03	18.97 ± 0.05	16.41 ± 0.07	15.49 ± 0.07	19.98 ± 0.10
Leucine	7.85 ± 0.05	5.82 ± 0.01	7.40 ± 0.05	6.85 ± 0.05	10.10 ± 0.08	12.40 ± 0.04
Phenylalanine	14.76 ± 0.02	15.40 ± 0.05	17.63 ± 0.07	15.76 ± 0.02	15.61 ± 0.03	17.62 ± 0.01
Tryptophan	42.36 ± 0.07	45.39 ± 0.03	47.35 ± 0.01	44.36 ± 0.05	46.61 ± 0.03	52.37 ± 0.04

The difference between highest result and lowest result of non-essential amino acid aspartic acid is 2.45 ppm, essential amino acid lysine is 23.8 ppm, and essential amino acid leucine is 6.58 ppm.

Nutrient content based on proximate analysis as shown in Table 5, found that the highest protein and fat of *D. magna* are on mass-cultured *D. magna* using organic fertilizer which is fermented for 28 days (D) that is 68.06% and 6.91%; the lowest protein and fat are on mass-cultured *D. magna* using organic fertilizer which is fermented for 0 day (A) that is 62.78% and 5.59%. the result is lower than previous study which reached 73.90% ± 0.04 protein [2], higher than other previous studies which reached 66.12% [21] and 4% from wet weight [12]. The highest fat content about 8.84% ± 0.01 higher than previous study which reached 7.89% ± 0.02 [2]. A high protein content and low fat from the study due to the high nutrient in the culture medium *D. magna* where the higher nitrate and phosphate levels. The higher the N and P content, the higher the protein in the culture medium [22]. The protein content is always the opposite of fat, because the fat in the body works twice as much as the protein [23].

Factors that affecting biomass and nutrient content *D. magna* are quality of nutrient medium, availability of phytoplankton, bacteria, and detritus as food, and the environment [2][3]. Organic matter contained in fermented medium can increase the number of bacteria and organic particles of decomposition results, thus increasing the availability of nutrients in the medium, this affects growth of population and biomass of *D. magna*. Fermentation aims to multiply the number of microorganisms as well as intensify the metabolism in food, resulting in new food products using microorganisms [1].

Total fatty acid profile found the highest linoleic and linolenic fatty acid profile of *D. magna* are by mass-cultured using organic fertilizer fermented for 28 days (E) that is 4.83% and 3.54% (Table 6); the lowest are by mass-cultured using organic fertilizer fermented for 0 day (A) that is 1.54% and 0.19%.

This results were higher than previous study which reached 0.2% [2]. Linoleic fatty acids serve as a base substrate in the formation of PUFA long chains. Linoleic fatty acids act as a base substrate forming long chains of Omega 6 and Omega 3 [2][24-25].

The study result the highest of amino acid profile of *D. magna* are by mass-cultured using organic fertilizer and fermented for 28 days (E), the result are non-essential amino acids aspartic acid are 10.70 ppm, lysine essential amino acids are 53.94 ppm, and the amino acid leucine that is 12.40 ppm; the lowest amino acid profile of *D. magna* are by mass-cultured using organic fertilizer and fermented for 0 day (A), the result are non-essential amino acids aspartic acid are 8.25 ppm, lysine essential amino acids are 30.14 ppm, and the amino acid leucine that is 5.82 ppm. The function of the lysine amino acid is as frame of vitamin b1 and anti-virus, helps in the absorption of calcium, stimulates appetite, and helps in the production of carnitine to convert fatty acids into energy [2][22][26].

4. Conclusion

Mass-cultured *D. magna* using fermentation medium of 25% chicken manure + 50 % rejected bread + 25% tofu waste combination was dried for 28 days fermentation (E) provide the highest improvement to growth and biomass production. The highest nutrient quality based on proximate, fatty acid profile, and amino acid profile analysis of *D. magna* was also showed in *D. magna* cultured by the same treatment (E).

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