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The Effect of Fermented Tofu Dregs Using EM_4 Probiotics on Population Growth of Daphnia Magna

Pengaruh Pemberian Ampas Tahu Yang Difermentasi Menggunakan Probiotik Em_4 Terhadap Pertambahan Populasi Pakan Alami *Daphnia Magna*

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ABSTRACT

Daphnia magna is a small crustacean or often called a water flea that lives in fresh waters. According to Putri et al., (2015), the nutritional content of Daphnia magna is 39.24% protein, 4.98% fat, 4.32% carbohydrates, ash content 14.63%. One of the Daphnia magna foods that can be given is tofu pulp which has a protein content of 27.55% to support its growth. The aim of this research was to obtain the most optimal growth of Daphnia magna after being given fermented tofu dregs at different doses. The method used was an experiment with a completely randomized design consisting of 4 treatments and 3 replications. The treatment carried out consisted of giving fermented tofu dregs in Daphnia magna maintenance media with different doses, namely P0 (0 gr/control), P1 (20 gr), P2 (30 gr), P3 (40 gr). Based on the results of research that has been carried out, it was found that P2 treatment with a dose of 30 grams gave the highest growth of Daphnia magna at 1,460 individuals. The high number of Daphnia magna populations given a dose of 30 grams can be utilized optimally for the growth and survival of Daphnia magna. The research results obtained concluded that giving fermented tofu dregs at a dose of 30 gr (P2) was able to have a significant effect ($P < 0.05$) on the increase in the Daphnia magna population.

ABSTRAK

Daphnia magna merupakan krustasea berukuran kecil atau sering disebut kutu air yang hidup di perairan tawar. Menurut Putri et al., (2015), kandungan gizi Daphnia magna sebesar 39,24% protein, 4,98% lemak, 4,32% karbohidrat, kadar abu 14,63%. Salah satu makanan daphnia magna yang bisa diberikan berupa ampas tahu yang memiliki kandungan protein sebesar 27,55% untuk mendukung pertumbuhannya. Tujuan penelitian ini untuk

mendapatkan pertumbuhan daphnia magna yang paling optimal setelah diberi ampas tahu yang difermentasi dengan dosis yang berbeda. Metode yang digunakan berupa eksperimen dengan Rancangan Acak Lengkap yang terdiri dari 4 perlakuan dan 3 ulangan. Perlakuan yang dilakukan berupa pemberian ampas tahu yang difermentasi pada media pemeliharaan Daphnia magna dengan dosis yang berbeda yaitu P0 (0 gr/ kontrol), P1 (20 gr), P2 (30 gr), P3 (40 gr). Berdasarkan hasil penelitian yang telah dilakukan diperoleh Perlakuan P2 dengan dosis 30 gr memberikan pertumbuhan daphnia magna tertinggi sebesar 1.460 ekor. Tingginya jumlah populasi daphnia magna yang diberi dosis 30 gr dapat dimanfaatkan secara optimal bagi pertumbuhan dan kelangsungan hidup Daphnia magna. Hasil penelitian yang diperoleh disimpulkan bahwa pemberian ampas tahu yang difermentasi dengan dosis 30 gr (P2) mampu memberi pengaruh yang nyata (P < 0,05) terhadap pertambahan populasi *Daphnia magna*.

Kata Kunci	*Dapnia magna, ampas tahu, probiotic EM4, pertumbuhan*
Keywords	*Dapnia magna, tofu waste, EM4 probiotic, growth*
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INTRODUCTION

Natural food is fish food that grows in nature without human intervention. According to Igo et al., (2020), natural food has complete nutritional content, is easily digested by the digestive tract, does not reduce water quality and can increase the resistance of fish seeds to disease and changes in water quality. One natural food that has the potential to be developed for hatchery activities is *Daphnia magna*.

Daphnia magna is a small crustacean or often referred to as a water flea that lives in fresh waters. *Daphnia magna* is a natural food used in fish hatchery activities because it has the advantages of being easily digested by fish seeds, does not reduce water quality, is easy to obtain and has quite high nutritional content. According to Putri et al., (2015), the nutritional content of *Daphnia magna* is 39.24% protein, 4.98% fat, 4.32% carbohydrates, ash content 14.63%. Daphnia magna is usually used to meet the food needs of freshwater fish, both food fish and ornamental fish.

One of the foods that can be given to *Daphnia magna* is tofu dregs which contain the protein needed for the growth of *Daphnia magna*. According to Gaol et al., (2015), tofu dregs contain protein between 21.33% - 28.36%, nitrogen 3.41%, phosphate 1.72%, potassium 1.33. However, tofu dregs have disadvantages, namely the crude fiber content of 23.58% and the water content which is quite high, namely 84.5% of the weight, which can cause a decrease in feed digestibility and feed storage time (Nahak, 2016). Thus, it is necessary to process it first through a fermentation process before being used as a feed mixture.

Fermentation is a process of changing the physical, chemical and biological structure of a material from a complex structure to a simple one so that digestibility is more efficient. According to Herawati et al., (2016), the aim of fermentation is to enrich the nutritional value

of the fermented material by breaking down nutrients into simpler forms as well as increasing the number of microorganisms and activating metabolism. Feed ingredients that are fermented by microorganisms cause beneficial changes such as increasing the shelf life of feed, improving quality both in terms of nutritional content and digestibility (Setiawan, 2017).

Effective microorganism-4 (EM4) is an addition that functions to optimize the utilization of substances in a material because the bacteria contained in it can digest cellulose, starch, sugar, protein and fat (Megawati, 2014). EM4 can speed up the fermentation process of organic material so that the nutrients contained therein can be easily absorbed. In EM4 there are several microorganisms that are fermentative (fermenting) which consist of 4 groups of microorganisms, photosynthetic bacteria, fermenting fungi, lactic acid bacteria and Actynomycetes. These microorganisms can utilize complex compounds contained in tofu dregs waste as nutritional ingredients in their own metabolic processes so that simpler compounds are formed that can be utilized by microalgae (Sutrisno et al., 2015).

Based on the description above, it is necessary to conduct a study regarding the administration of fermented tofu dregs using EM4 probiotics to increase the population of Daphnia magna. With the aim of getting the most optimal growth of Daphnia magna after being given fermented tofu dregs at different doses.

RESEARCH METHODS

The method used was an experimental method using a Completely Randomized Design (CRD) consisting of 4 treatments and 3 replications. The treatment carried out was in the form of fermented tofu dregs in Daphnia magna maintenance media at different doses. Where P0 (Without giving fermented tofu dregs), P1 (Giving fermented tofu dregs 20 gr), P2 (Giving fermented tofu dregs 30 gr), P3 (Giving fermented tofu dregs 40 gr). Some of the procedures carried out include;

1. Preparation of containers and water
   The containers used are 12 plastic containers with a size of 10 L. The containers are washed thoroughly to avoid disease. The water used came from a drilled well and was settled for 24 hours, then filled into each test container as much as 5 L and given aeration to supply oxygen.
2. Preparation of Tofu Dregs and EM4
   The fermentation process is carried out by; 270 grams of tofu dregs that have been drained of water then mixed with 1 ml of EM4 probiotic, which has previously been activated using 1 ml of molasses (ratio 1:1) then diluted using 100 ml of water (Zakiyah et al., 2019). All ingredients are stirred until smooth, then the container is closed tightly with plastic to prevent air from entering for 1 week of fermentation until it has a fragrant and alcoholic aroma, and the texture becomes softer. In the final stage, add the fermented tofu dregs into the cloth according to the specified dosage, then tie the ends to put them in each test container.
3. Preparation and Sowing of *Daphnia magna*
   The test animal used was Daphnia magna obtained from cultivators in the Medan area, North Sumatra. Then *Daphnia magna* is cultured again in a maintenance container for reproduction. The number of seeds obtained is adjusted to all treatments and repetitions.

Next, Daphnia magna was spread to in each container treat with density 20 fishes/L (Noerdjito, 2003).

4. Care of *Daphnia magna*
   Daphnia magna maintenance is carried out for 28 days. The increase in the *Daphnia magna* population was observed every 4 days during the maintenance period. Water quality management for culture media is carried out by providing aeration as an oxygen supply. Water quality observations including temperature, pH and DO were carried out every 4 days in the afternoon for each treatment.

Several parameters tested in this research include:

1. Population Density
   Daphnia magna population density was calculated every 4 days. Observation of population density was carried out by taking 100 ml of maintenance water from each container homogeneously. When taking samples, the media was strongly aerated so that *Daphnia magna* could spread evenly. Calculations were carried out in plastic containers with a volume of 500 ml, then manual calculations were carried out using a tool in the form of a spoon, 3 repetitions in each container, then converted and averaged using Microsoft Excel.
2. Water Quality
   Water quality parameters were measured every 4 days during the research period. The water quality parameters observed were temperature, pH and DO.

The research data was analyzed using the SPSS application. Population density of *Daphnia magna* in Analysis of Variance (ANOVA), to determine whether tofu dregs fermentation has a significant effect on population density of *Daphnia magna*. If the statistical test results show a significant difference ($P<0.05$), then a further Student Neuman-Keuls test is carried out. Water quality data is displayed in tabular form and analyzed descriptively.

RESULT AND DISCUSSION

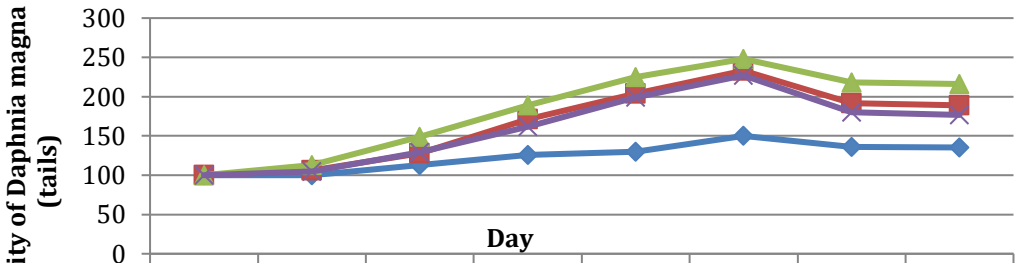
Result

From the results of the research carried out, the average number of *Daphnia magna* populations for each treatment and replication is shown in Table 1 below:

Table 1. Number of *Daphnia magna* populations during the study

Repetitions	Number Population of *Daphnia magna* (tails/l)			
	P0	**P1**	**P2**	**P3**
1	1.019	1.348	1.486	1.307
2	993	1.326	1.459	1.279
3	957	1.293	1.434	1.256
Number	2.969	3.967	4.379	3.842
Average	990 ± 6,55[a]	1.322 ± 6,55[b]	1.460 ± 5,5[c]	1.281 ± 5,5[b]

The results of observations of the average density of *Daphnia magna* individuals during the study in each treatment are presented in Figure 1 below:



	0	4	8	12	16	20	24	28
P0	100	100	113	126	130	150	136	135
P1	100	106	128	171	204	233	192	189
P2	100	113	149	189	225	248	218	216
P3	100	105	129	162	199	227	180	177

Figure 1. Individual density graph of *Daphnia magna*

Based on the results of water quality observations obtained during the research, it can be shown in Table 2 below:

Table 2. Data on Water Quality Results

Parameter	P0	P1	P2	P3	Tolerance Range
Temperature	26-28	26-29	26-29	27-29	26-31°C[a]
pH	6.7-7.3	6.8-7.2	6.8-7.4	6.7-7.4	6.7-7.4[b]
DO	4.9-5.5	4,9-5.6	4.9-5.6	5.2-5.7	3 mg/l[c]

Note: (a) Wibisono et al., (2017), (b) Firnandus (2014), (c) Agustin et al., (2017).

Discussion

The highest density of *Daphnia magna* (Table 1) was found when 30 grams of fermented tofu dregs (P2) were given, namely 1,460 individuals, this was due to the availability of feed and nutrients in quantities that could meet the needs of *Daphnia magna*. This is in accordance with the opinion of Chilmawati et al., (2015), stating that population growth is faster because it is supported by feed that contains optimal nutrition for growth. The increase in population density is because the dose given can be utilized optimally for the growth and survival of *Daphnia magna*.

The lowest density was found in the control treatment (P0), namely 990 individuals, this is thought to be due to the placement of the test container in an open room so that it is exposed to sunlight, which is one of the factors that can cause the growth of microorganisms. However, the number of microorganisms that grow due to sunlight is not sufficient for *Daphnia magna*'s food needs, resulting in competition for food between individuals. Microorganisms need a nitrogen source in the form of protein to grow and then become a food source for *Daphnia magna*. The source of protein is obtained from tofu dregs. Therefore, the difference in population numbers in the treatment given fermented tofu dregs and without treatment (control) looks very significant. This is because the number of microorganisms that grow due to fermentation of tofu dregs is different from microorganisms that grow only by relying on sunlight.

The increase in the population of Daphnia magna (figure 1) in each treatment increased whether fermented tofu dregs were given, or without fermented tofu dregs (control). This is

because the *Daphnia magna* maintenance media still contains microorganisms even though they are not treated. Apart from that, placing the treatment in an open space causes the container to be exposed to sunlight, which can trigger the growth of microorganisms which become food for Daphnia magna. The increase in population numbers in treatment 2 began to appear on the 4th day and continued to increase until the 20th day. Followed by treatments 1 and 3 which began to show an increase in population numbers on the 8th day and continued to increase on the 20th day. In the control treatment there was also an increase in population, but it was not very significant and began to appear on the 8th day and continued to increase until the 20th day. However, on days 24 and 28, each treatment experienced a decrease, this was caused by unfavorable weather or it was raining, so that the microorganisms in the containers could not reproduce because they were not exposed to sunlight. This is in accordance with the opinion of Lantang and Pakidi (2015), that the abundance of phytoplankton in the rainy season is lower than in the dry season, because the intensity of sunlight in the dry season is higher. Phytoplankton are autotrophic microorganisms which in the process of reproduction and survival require sunlight to carry out the process of photosynthesis. Apart from that, the reduced amount of nutrients contained in the culture container can also cause Daphnia magna to lack food intake. According to Astika (2015), the increase and decrease in *Daphnia magna* populations during the maintenance period is influenced by the availability of phytoplankton in the culture media.

Daphnia magna has a growth phase consisting of an adaptation phase, exponential phase, stationary phase and death phase. At the beginning of the distribution of *Daphnia magna* parents into the culture media, the parent adaptation stage to the culture media occurs. The adaptation phase that occurred during the research took place on day 0 to day 4 in each treatment. This is because there are factors that influence the adaptation process in the form of adjustments to the environment and adjustments to the organic material content in the culture media.

The exponential phase is a phase in which individuals multiply over a certain period of time due to the reproductive cycle. In each treatment, the exponential phase was seen on days 8 to 20. However, in the control treatment there was not a significant increase, this is thought to be because Daphnia magna only uses the food contained in the culture media, so there is a visible difference from the other treatments.

Next is the stationary phase, which is the peak phase of the population which is characterized by no increase in the number of *Daphnia magna* growth or a decrease in the number of growth. In this phase, optimum nutrient utilization occurs, causing a reduction in nutrients in the culture media. The stationary phase occurs on day 20.

The final phase is the death phase, which is marked by a decline in the growth graph of Daphnia magna. The death phase was seen on the 28th day where many *Daphnia magna* parents died but there were still young offspring. According to Izzah and Herawati (2014), the death phase is caused by several factors including high temperatures, reduced nutrients in the waters, changes in pH, contamination, and reduced photosynthesis processes. Apart from that, according to Putra et al., (2014), in the death phase there is a lack of nutrition in the culture media resulting in a decrease in cell density.

The results of the ANOVA test showed that fermenting tofu dregs using EM4 probiotics had a significant effect on increasing the *Daphnia magna* population ($P < 0.05$). The results of the Student Newman Keuls Advanced Test showed that there were significant differences

between treatments. Where the provision of fermented tofu dregs of 30 grams (P2) is different from other treatments, this indicates that this treatment is the best.

Another factor that can influence the growth and development of *Daphnia magna* is water quality. The water quality parameters measured during the research included temperature, pH and DO. The results of water quality measurements during the research can be seen in table 2 below:

The range of temperature, pH and dissolved oxygen in the *Daphnia magna* culture media for each treatment during the study was relatively the same and good for growth and reproduction. Temperature is an abiotic factor that can influence the increase or decrease in an organism's reproductive activity, growth and death. According to Wibisono et al., (2016), the temperature range of 22 – 31℃ is included in the range that supports the growth of Daphnia magna.

Dissolved oxygen in the cultivation media during the research period ranged from 4.9 – 5.7 mg/L. This value is still considered good for the growth and survival of *Daphnia magna* so that it can reproduce. This is in accordance with the statement of Agustin et al., (2017), who stated that the optimal dissolved oxygen concentration for *Daphnia magna* culture is >3 mg/l. One way that can be done to increase dissolved oxygen in the cultivation container is by using aeration and also placing the cultivation container outdoors so that oxygen circulation runs well.

The pH value in all treatments during the research ranged from 6.7 – 7.4, which is still in the optimal range. According to Firnandus (2014), Daphnia magna can grow well in waters with a pH of 6.5 – 8.5.

CONCLUSSION AND SUGGESTION

From the results of the research that has been carried out, it can be concluded that giving fermented tofu dregs at a dose of 30 gr (P2) had a significant effect ($P < 0.05$) on increasing the Daphnia magna population by 1460 individuals.

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