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Effect of fermentation time period of Ambon banana peel (*Musa acuminata*) on protein content and growth of *Daphnia magna*

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Abstract
D. magna has several advantages as a natural food, including high nutritional content with 50-70% protein, 10% carbohydrates, and low fat, body size smaller than the mouth opening of fish, and the ability to reproduce quickly. Problems in the cultivation of *D. magna* as a natural food often center on the availability and quality of nutrient sources. Dependence on natural phytoplankton, which fluctuates due to environmental changes, is a major obstacle. On the other hand, the utilization of organic waste such as banana peels as an alternative source of nutrients has great potential. This study aims to determine the effect of different fermentation times of Ambon banana peel (*Musa acuminata*) on protein content and growth of *Daphnia magna*. In this study, using experimental method with complete randomized design (RAL). The time difference in the fermentation of Ambon banana peel tested is without fermentation, 7 days, 14 days, and 21 days. Protein content of fermented Ambon banana peel and population density of *D. magna* were the two parameters observed. Analysis of variance (ANOVA) and Duncan's further test were used to analyze the data obtained. The results showed that fermentation time of Ambon banana peel can affect the growth and protein content of *D. magna*. The best fermentation time of 7 days resulted in the highest growth of *D. magna*, and 21 days of fermentation resulted in the highest protein content.

Keywords: *Daphnia magna*, growth, protein, banana peel fermentation

Introduction

Daphnia magna (*D. magna*), known as water flea, is a zooplankton organism that has great potential as a natural feed in fish farming. *D. magna* has a high nutrient content, including 50-70% protein, 10% carbohydrate, and low fat, making it an excellent feed source for zooplankton- eating fish. In addition, *D. magna* has a body size smaller than the mouth opening of fish, as well as the ability to reproduce rapidly, which makes it ideal as a continuously available natural food (Simanjuntak *et al.*, 2021) [5]. This organism is rich in essential amino acids, vitamins, and minerals necessary for fish growth and development. With a protein content of about 39.24%, *D. magna* is very useful as a protein source for fish (Rumondang *et al.*, 2023) [4].

Another advantage of *D. magna* is its ability to grow rapidly in a controlled culture system, thus allowing the provision of natural food throughout the year without depending on external environmental factors. In culture media rich in organic matter and phytoplankton, *D. magna* can grow faster than in media containing little phytoplankton and organic matter (Herawati *et al.*, 2018) [1].

On the other hand, Ambon banana peels, which are often considered as waste, have great potential as an alternative source of nutrients in *D. magna* cultivation. Banana peels contain carbohydrates, protein, fiber, vitamins, and minerals that are beneficial for the growth of microorganisms during the fermentation process (Romansyah, 2023) [6]. The fermentation process of banana peels can increase the content of energy, protein, and fiber, which serve to support the growth of *D. magna*. In addition, microbes involved in fermentation can produce enzymes that break down complex compounds into simpler ones, thus increasing the availability of nutrients (Pamungkas *et al.*, 2017) [7].

Fermented banana peels can increase protein levels in *D. magna* culture media and support its growth by providing more complex and stable nutrients. Banana peels have a fairly high protein content, which is around 10-15%, which can be increased through fermentation (Sukma and Anwar, 2022) [11]. This increase in protein content is expected to improve stocking density and nutritional quality for *D. magna*, as well as provide a sustainable solution for natural feed managemeDnt in freshwater fish farming.

The main problem in the cultivation of *D. magna* is the dependence on the fluctuating availability of natural phytoplankton, which is affected by changes in environmental conditions such as temperature, salinity, and water quality. In addition, processing banana peels through fermentation also faces challenges, such as the time required and strict quality control. Suboptimal fermentation can result in feed with inconsistent nutritional content or even harmful to the cultured organisms.

The hypothesis used in this study is to determine whether there is an effect of using banana peel fermentation on the growth of *D. magna*.

- **H0:** Banana peel fermentation time period does not affect the protein content and growth of *Daphnia magna*.
- **H1:** The time period of banana peel fermentation affects the protein content and growth of *Daphnia magna*.

Materials and Methods

To maintain *D. magna*, several tools are used, including an aerator to oxygenate the water, an aeration stone that disperses air bubbles, an aeration hose that connects the aerator to the aeration stone, a water quality meter to monitor water quality, a culture container for Daphnia growth, a fermentation container for mixing fermentation ingredients, and a suction pipette for taking samples. Tools for making fermentation are containers for fermentation. While the materials used for *D. magna* cultivation include *D. magna* as the main organism, fresh water as its habitat, EM4 (Effective Microorganisms-4) as an ingredient for fermentation, molasses as a source of energy for fermentation, and ambon banana peel as a source of natural nutrients. The treatment was conducted in an 18 L container with 14 L of water, with a reference banana peel fermentation dose of 10 g/L (Hia *et al.*, 2022) [3]. The treatments were as follows: treatment 0 with 10 gr/L banana peel without fermentation, treatment 1 with 10 gr/L banana peel fermentation for 7 days, treatment 2 with 10 gr/L banana peel fermentation for 14 days, and treatment 3 with 10 gr/L banana peel fermentation for 21 days.

- Treatment 0: 10 gr/L banana peel without fermentation
- Treatment 1: 10 g/L banana peel fermentation 7 days
- Treatment 2: 10 g/L banana peel fermentation for 14 days.
- Treatment 3: 10 gr/L banana peel fermentation 21 days

Research Procedure

The research procedure began by preparing banana peels, EM4, molasses, water, and fermentation containers. Banana peels of140 grams were mixed with 140 ml of water, 1.4 ml of molasses, and 1.4 ml of EM4, then stirred until homogeneous and allowed to stand for 7, 14, and 21 days for fermentation. An 18 L container was filled with 14 L of fresh water and aerated. 20 individuals/L of *D. magna* were prepared, and 140 ml of fermented banana peel was put into each container. Observations were made for 14 days to monitor *D. magna* growth and water quality with daily sampling.

Measurement Methods

Sampling was done twice a day for each container at the same hour. The population density of *D. magna* was calculated using the formula:

$$� = � \times \frac{�}{�}$$

Description

- a: Number of *D. magna* individuals (ind/L)
- b: Number of *D. magna* counted (ind)
- p: Volume of culture medium (L)
- q: Volume of sample taken (L)

Parameters measured include pH, temperature, and DO using measuring devices such as pH meters and DO meters. The ideal conditions for *D. magna* are temperature 25-30°C, DO 0.3-0.6 ppm, and pH 6.5-9 (Herawati *et al.*, 2018) [2]. Measurements were taken every 7 days to maintain water quality. Protein content was analyzed using the Kjeldahl method to determine the protein content in *D. magna*. *D. magna* has 42.65% protein, while banana peel contains 0.32 g protein (Romansyah, 2023) [6].

Protein Test

The protein nutrient content was analyzed from the protein results by the kjedhal method. The function of the protein test is to help determine the protein in *D. magna*. This information is very important to ensure that *D. magna* has sufficient nutrients for fish growth and development. *D. magna* has as much as 42.65% protein while banana peel has 0.32 g protein (Romansyah, 2023) [6].

The parameters measured are pH, temperature, and DO. Water quality is measured using measuring devices, such as Ph meters and DO meters. The appropriate water quality for *D. magna* is a temperature of 25-30 o C, DO 0.3-0.6 ppm, and pH 6.5-9 (Herawati *et al.*, 2018) [1]. Water quality measurements were carried out every 7 days to ensure water conditions remained ideal for *D. magna* growth.

Data Analysis

Data from the research results will be carried out using normality test and homogeneity test. The data will be checked using SPSS by presenting an analysis of variance (Anova) table. Furthermore, if the Anova test shows a significant difference in each treatment, it will be continued with the Dunnett test.

Results

After 14 days of observation, the results of *D. magna* population growth using different banana peel fermentation times are presented in the following figure:

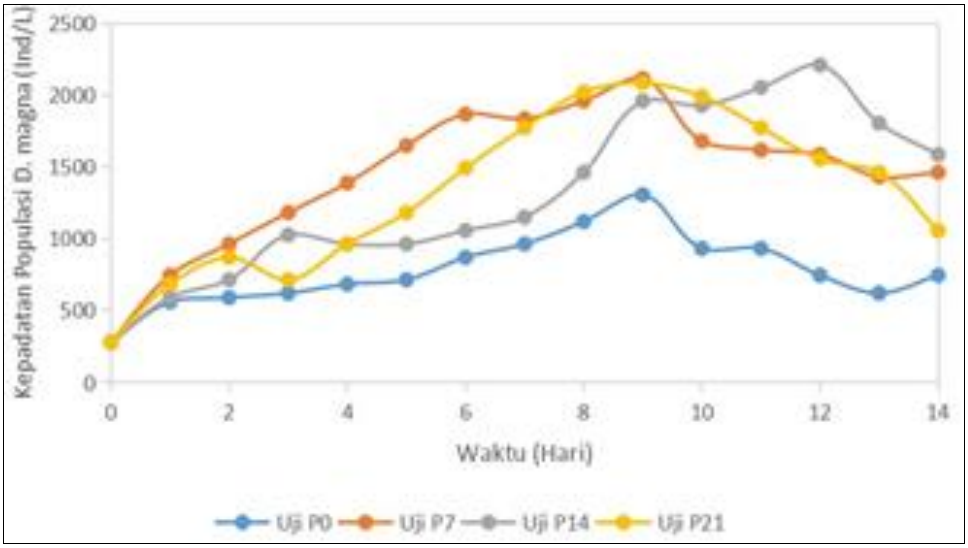

Fig 1: Graph of *D. magna* Population Growth Pattern

The calculation of *D. magna* density showed a significant growth pattern in banana peel fermentation media, with peak growth occurring on day 9 and decreasing on day 10 to day 14. Normality and homogeneity tests showed that the data were normally distributed and homogeneous, which was then continued with the ANOVA test to analyze significant differences in *D. magna* growth.

ANOVA Test Results

Table 1: Analysis of Variance of Absolute Growth Anova Data

Sk	D b	JK	KT	Fcount	F(tab le 0.05)	F(tab le 0.01)
Treatment	3	402174	13405	5,6116	2,769	4,151
		1,3	80	3**	43	94
Error	5	133780	23889			
	6	15	3,1			
Total	5	173997				
	9	56				

Description*: F count > F table = significantly affected*.

Different fermentation times have a significant effect on the growth of the *D. magna* population, namely F count> F table, then reject Ho and accept H1. Based on the Dunnett test, it is known that there are differences in influence between treatments with control, with the following results:

Table 2: Dunnett Test for Population Growth

Perlaku an	Pemband ing	Mean Differe nt	Std Error	Sig	95% Confide nce Interval Lower Bound
P7	Kontrol	0.2572	0.0794	0.00	0.0898*
		0*	3*	3*	
P14	Kontrol	0.2005	0.0794	0.01	0.0332
		2	3	9	
P21	Kontrol	0.2082	0.0794	0.01	0.0409
		4	3	5	

Description *: Most difference

Water Quality

Water quality measurements were taken every day in the morning during the study. Water quality parameters measured were DO, temperature, and pH.

Table 3: Water Quality of *D. magna*

Variables	P0	P7	P14	P21	Standard*
DO (mg/L)	4,5-	4,7-	4,9-	4,8-	2,9-5,9
	6,7	6,8	6	6	
Temperature	24,4-	23-	27-	27-	25-30
(°C)	30,7	30,5	30	30	
pH	7,9-	7,9-	7,6-	7,6-	6,5-9
	8,3	8,3	8,6	8,6	

Notes: Herawati *et al.*, 2018 [2]

Protein Content of *Daphnia magna*

D. magna is protein-rich and important as a natural fish food. Its protein content is influenced by the feed given. The results of the protein test using the Kjeldahl method are as follows:

Table 4: Protein of *D. magna* fed with fermented banana peel

Test	P0	P7	P14	P21	Standard*
Protein Content	4,48%	3,36%	2,25%	5,09%	39,24%

Notes: *Rumondang *et al.*, 2023 [4] (Fermentation of tofu pulp)

Discussion

Population Growth Pattern of *Daphnia magna*

The population growth pattern of *D. magna* in each treatment using banana peel fermentation showed a different pattern. The growth of *D. magna* in each treatment has several phases, including the adaptation phase (lag phase), growth phase (exponential), stationary phase, and death phase (Herawati *et al.*, 2018) [1]. The results of analysis of variance (ANOVA) showed that the provision of banana peel fermentation had a significant effect ($P<0.05$) on the growth pattern of *D. magna* given the fermentation time treatment of 7 days, 14 days, and 21 days. The test results were homogeneous so Dunnett's further test was conducted. The most influential treatment can be seen from the further test is the 7-day banana peel fermentation treatment, because it has the highest Mean Difference and the lowest SIG among other treatments. *D. magna* growth phases observed during the study:

Adaptation Phase: Occurs until day 3, during which *D. magna* acclimatizes to the new environment or culture medium.

Growth/Exponential Phase: Occurs from day 4 today 8, characterized by a rapid and significant increase in the number of individuals.

Stationary Phase: Occurs on day 9, when the population reaches its peak point.

Death Phase: Occurs on day 12 to day 14, where the population decreases due to a decrease in nutrients in the culture medium.

Water Quality

Water quality parameters measured during the study showed that the environmental conditions were within the optimal range for *D. magna* growth. According to Herawati *et al.* (2018) [2], *D. magna* water fleas show the best growth at temperatures between 25 to 30°C and water acidity (pH) in the range of 6.5 to 9. To keep the pH stable in the cultivation process, Alfiyanti *et al.* (2023) [10] suggested the addition of dolomite lime which plays an important role in neutralizing pH and overcoming high ammonia levels. Excessive use of organic fertilizers can produce too much carbon dioxide (CO_2) gas and reduce the amount of dissolved oxygen that is crucial for *D. magna* breathing (Djalil *et al.*, 2020) [12].

Protein Content of *Daphnia magna*

This study showed that the protein content of *D. magna* underwent significant changes as the culture time progressed. The highest protein level was recorded in the 21-day fermentation test (5.09%), while the lowest level was found in the 14-day fermentation test (2.25%). At the beginning of the culture, the protein level of *D. magna* control was 4.48%, which then decreased in the 7-day fermentation culture to 3.36%. This decrease is thought to occur because *D. magna* uses protein as the main energy source for growth and reproduction at the beginning of the culture, when nutrition is not yet optimal. A significant increase in protein levels at 21 days of fermentation indicates a change in metabolism or increased efficiency in nutrient absorption. The quality of the fermentation medium and its type greatly influence the protein content of *D. magna*. Banana peels naturally contain very small amounts of protein, only about 0.32 grams per 100 grams (Nur *et al.*, 2016) [8], which causes the potential for increasing protein from banana peels to be limited. The fermentation process on banana peels often triggers an increase in crude fiber content due to the growth of fungal mycelium containing cellulose (Has *et al.*, 2017) [9], which can actually reduce the digestibility and quality of fermented protein. At the beginning of the culture, more energy is used for growth and reproduction, so that protein levels decrease. The high population of *D. magna* is supported by the sufficient content of available nutrients because *D. magna* will eat microorganisms that grow both zooplankton and phytoplankton due to fertilization (Rumondang *et al.*, 2023) [4]. However, *D. magna* metabolism switches to energy storage in the form of body protein when nutrients from the media begin to be optimally available.

Conclusion

Based on the results of the research that has been done, it can be concluded that the fermentation time of banana peel gives a real influence on the growth of *Daphnia magna* population. Banana peel fermentation treatment for 7 days showed the best results in increasing *D. magna* population growth, with peak growth occurring on day 9. Meanwhile, the highest protein content was found in the 21-day fermentation treatment (5.09%), indicating that the longer fermentation time supported the accumulation of protein in the body of *D. magna*. Water quality parameters during the study were within the optimal range for *D. magna* growth, including temperature (23- 30.7°C), DO (4.5-6.8 mg/L), and pH (7.6-8.6). However, the use of banana peel as a fermentation medium resulted in relatively low protein levels compared to the use of other fermentation media such as tofu pulp, indicating that the type of fermentation substrate greatly affects the nutritional quality of *D. magna* as a natural feed for fish farming.

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