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ANALYSIS OF THE USE OF RICE WASHING WATER AND FERMENTED CASSAVA PEEL WASTE ON POPULATION GROWTH OF DAPHNIA MAGNA

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ABSTRACT

Daphnia magna is a potential natural food source due to its nutritional content. The availability of Daphnia magna is not always consistent, posing a challenge to fish cultivation. An alternative solution is to use rice wash water and fermented cassava peel waste as a source of nutrition for Daphnia magna. Rice wash water contains soluble nutrients, while fermented cassava peel waste is rich in nutrients from the fermentation process. This research was conducted in the Fisheries Cultivation Laboratory, 1st Floor, Universitas Muhammadiyah Gresik, in April-May 2023. The research method employed was experimental with five treatments and three replications. P1= 0% rice wash water, 100% fermented cassava peel; P2= 25% rice wash water, 75% fermented cassava peel; P3= 50% rice wash water, 50% fermented cassava peel; P4= 75% rice wash water, 25% fermented cassava peel; P5= 100% rice wash water, 0% fermented cassava peel. Data analysis used analysis of variance and least significant difference test. The results showed that treatment P3 had the highest population density with an average of 2578 individuals. Additionally, treatment P3 also yielded the highest growth rate of Daphnia magna, reaching a value of 28.35. The population distribution across all treatments predominantly showed the total length class <100μm. Overall, the water quality within the temperature range of 26-28°C and pH range of 6.5-8 indicated optimal conditions for the growth of Daphnia magna.

Keywords: Daphnia Magna, Data Analysis, Fermented Cassava Peel Waste, Optimal Ecosystem, Rice Washing Water

INTRODUCTION

Natural feed plays a vital role in fish seed cultivation, providing essential nutrition for the growth and survival of fish fry (Maulidiyanti et al., 2015). Daphnia magna, a small, nutrient-rich aquatic organism, has been identified as a potential source of natural feed in fish farming. Daphnia magna possesses crucial nutrients, especially protein (42-54%) and fat (6.5-8%) of its dry weight, which are highly needed by fish larvae for their growth and immune system (Herawati et al., 2013). Furthermore, Daphnia magna offers several advantages, including its size suitable for consumption by fish fry during the early growth stages, balanced nutrition, and rapid reproduction.

However, despite its potential, the natural availability of Daphnia magna in aquatic environments is often limited, creating challenges in meeting the demand for high-quality natural feed for farmed fish. To address this issue, an alternative worth considering is the use of rice washing water and fermented cassava peel waste as a nutrient source in Daphnia magna cultivation. Rice washing water, often considered as waste, contains nutrients that can be utilized in the cultivation of aquatic organisms like Daphnia magna. Additionally, fermented cassava peel waste produces a nutrient-rich liquid, including nitrogen, phosphorus, and potassium, which can impact the growth of Daphnia magna and other organisms in the aquatic food chain (Agung et al., 2020; Simanjuntak et al., 2021; Subrero et al., 2019). This study aims to evaluate the impact of using both of these nutrient sources on the growth and survival of Daphnia magna, with the hope of providing insights into the potential utilization of household waste as natural feed in the cultivation of aquatic organisms and contributing to the development of sustainable waste management policies.

MATERIAL AND METHOD

Trial Design

The research design used was a completely randomized design, with five treatments and three replications. The research treatments are as follows P1 = 0% rice washing water 100% fermented cassava peel solution, P2 = 25% rice washing water 75% fermented cassava peel solution, P3 = 50% rice washing water 50% fermented cassava peel solution, P4 = 75% rice washing water 25% fermented cassava peel solution, and P5 = 100% rice washing water 0% fermented cassava peel solution.

Proccessing

Preparation of Rice Washing Water Solution

The preparation of rice washing water solution is taken from the daily practice of cooking rice. The washing water, which is usually thrown away, is now used as food for aquatic organisms. The rice washing water is mixed with EM4 probiotics in a 1:1 ratio. This equal ratio is employed to ensure an optimal and balanced blend that promotes the well-being and growth of aquatic organisms, maintaining a harmonious relationship with the aquatic ecosystem.

Preparation of Fermented Cassava Peel Solution

The cassava peels used were obtained from a cheese phong seller on the side of kholil road in pekelingan village, Gresik sub-district. The cassava peels were cleaned and washed then cut into square pieces then weighed as much as 300 g, and placed in a clear plastic bag with holes for aeration. Then steamed for 30 minutes and then cooled. After cooling, the cassava skin was inoculated with 1.35 g of mashed tape yeast. The fermentation process is made anaerobically, and incubated in a jar at room temperature for 8 days and used as a substrate in the fermentation process et al., 2001). The preparation of fermented cassava peel solution involves taking

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100 g of fermented cassava peel and mixing it with 500 mL of water. It's worth noting that the cassava peel has been previously steamed. The resulting mixture of fermented cassava peel and water is homogenized and then filtered using a cloth. Subsequently, the filtered solution is carefully transferred into tightly sealed 15-liter glass bottles, following specific treatment requirements. These large glass bottles serve as the feedstock for *Daphnia sp.*, following established protocols (TAPE, 2015).

Research Implementation Phase

Preparation of Culture Media for Daphnia sp.

The *Daphnia sp.* culture containers, which are glass jars with a volume of 5 liters each, are cleaned thoroughly with soap and allowed to dry. Subsequently, they are soaked in chlorine solution and left to dry for 24 hours to ensure proper sterilization. Following this sterilization process, the jars designated for each treatment are organized using a randomized design. To prevent contaminants from entering, each jar is securely covered with a cloth. For each treatment, 3 liters of water are carefully measured and added to the jars as the culture medium.

Preparation of Daphnia sp.

Daphnia sp. used in the study came from fish feed traders. And filtering/selection is done to get *Daphnia sp.* with relatively uniform size and age. *Daphnia sp.* then transferred into plastic containers as stock for research.

Stocking of Daphnia sp.

Stocking *Daphnia sp.* on the jar comes from the preparation culture of *Daphnia sp.* The density of *Daphnia sp.* on the jar is 20 ind.L-1 . Then fed according to each treatment and observations were made based on the parameters that have been determined in the study.

Daily Feeding

Daily feeding is done by giving a solution of fermented cassava peel and rice washing water solution that has been mixed with EM4 in accordance with the ratio of the percentage of solution for each treatment as much as 1 mL.L-1 *Daphnia sp.* maintenance media water with a frequency of feeding three times a day, namely in the morning at 08.00 WIB, in the afternoon around 12.00 WIB and in the afternoon around 16.00 WIB in each treatment every feeding (Suryaningsih., 2006; Mubarak et al., 2009; Islama et al., 2020).

Research Parameters

Density of Daphnia sp.

The density of *Daphnia sp.* was calculated at two-day intervals for 16 days (Utarini et al., 2012; Suprimantoro et al., 2016) and on day 7, the density of *Daphnia sp.* The peak density of the *Daphnia sp.* population occurred on days 6-

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10 (Izzah et al., 2014; Meilisa., 2015; Jubaedah., 2016). Observations were made in the morning (9 - 11 am) until reaching peak population density. Calculation of *Daphnia sp.* population was done by taking samples from the culture medium using a measuring cup as much as 40 ml from each treatment that was previously homogenized. Samples were then poured into a Petri dish and counted the number of *Daphnia sp.* Counting the number of individuals was done three times and the results were averaged. The average results of the calculation of the number of individuals *Daphnia sp.* converted in the number of ind.L-1 with the formula according to (Kusuma et al., 2020) in Utarini et al. (2012) as follows:

$$a = b \times (p/q)$$

where:

a = Number of individual *Daphnia sp.* in the culture medium (ind.L-1)

b = average number of *Daphnia sp.* from repeated calculations (Ind)

p = volume of culture media (liter)

q = volume of water sample culture media (liter)

Population Growth Rate of Daphnia sp.

According to Kusumaryanto (1988) states that the population growth of *Daphnia sp.* is calculated from the first day to the peak of the population using the formula:

$$g = \frac{\ln Nt - \ln No}{t} \times 100\%$$

where:

g = Population growth rate (%.day-1)

No = Number of individuals at the beginning of the study (ind. L-1)

Nt = Number of individuals at the peak of the population (ind. L-1)

t = Length of rearing (days)

Duration of Peak Population of Daphnia sp.

The length of time to reach the peak population of *Daphnia sp.* is the time required to reach the highest population as the peak during maintenance (Gideon et al., 2022).

Water Chemistry Physics

The physico-chemical parameters of the research water, including the measurement of power of hydrogen (pH) and temperature, were diligently monitored every two days throughout the 16-day maintenance period (Utarini et al., 2012).

Data Analysis

Data obtained in the form of peak population density of the first cycle of *Daphnia sp.* is displayed in the form of a graph of population growth. Data from the observation of the peak population density of the first cycle of *Daphnia sp.*, population growth rate of *Daphnia sp.*, and the length of time to reach the peak population of the first cycle of *Daphnia sp.* in

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each treatment was used as data for statistical analysis. The overall data of the mean value was tested at the 95% confidence level using analysis of variance. If the data showed significantly different, further tests were carried out using the Least Significant Difference test . Statistical data processing tools using Microsoft Office Excel 2007 program. Data in the form of water quality were analyzed descriptively.

RESULTS AND DISCUSSION

Peak population density of the first cycle of *Daphnia sp.*

Based on the results of the study, the treatment of fermented cassava peel solution gives effect to the growth of *Daphnia sp.* population. Data on the peak population density of the first cycle of *Daphnia sp.* can be seen in Table 1.

Table 1. Peak population density of the first cycle of *Daphnia sp.*

Treatment	Repeat			Mean BNT 5% = 75,99
	1	2	3	
P5	1781	1641	1734	1719[a]
P4	1866	1847	1791	1834[b]
P1	2025	2016	1959	2000[c]
P2	2138	2175	2156	2156[d]
P3	2588	2569	2578	2578[e]

Description:
P2= 25% rice washing water, 75% cassava peel fermentation
P3= 50% rice washing water, 50% cassava peel fermentation
P4= 75% rice washing water, 25% cassava peel fermentation
P5= 100% rice washing water, 0% cassava peel fermentation

From table 1, it can be observed that there is variation in the population density of Daphnia magna in each treatment. Population density is measured in ind.L-1 (individuals per liter of water). Treatments P1, P2, and P3 have different population density levels. The highest average density is found in treatment P3, which is a 50% solution of rice washing water and a 50% solution of fermented cassava peel, with an average value of approximately 2578 ± 7.8 ind.L-1. Treatments P1 and P2 also show high densities, with averages ranging from 2000 ± 29.2 to 2156 ± 15.1 ind.L-1. This indicates that the use of rice washing water has the potential to affect the population growth of Daphnia magna by producing higher densities compared to other treatments.

Based on the results of the ANOVA test, it was found that the treatment with rice washing water solution and fermented cassava peel solution had a significant effect on the population growth of Daphnia magna. The significant effect of the treatment on the population growth of Daphnia magna can be explained by several factors. One of these factors is the nutrient content found in rice washing water and in the process of fermenting cassava peel. Rice washing water has the potential to provide additional nutrients needed by Daphnia magna in its growth and reproduction processes. Furthermore, the importance of nutrient intake for Daphnia mothers can be seen from the fact that higher egg production is often associated with mothers receiving an adequate amount of food. Additionally, the quality of the food consumed by Daphnia also plays a crucial role in influencing its growth. Optimal quantity and quality of food can trigger higher reproductive responses, better growth, and increased fecundity (Pires et al., 2011). In the context of this research, this can be interpreted as the balanced composition of the feed among treatments providing sufficient nutrition for Daphnia.

Furthermore, in the process of fermenting cassava peel, the possibility of forming new compounds or changing the nutritional composition that can affect the environmental conditions and food availability for Daphnia magna exists. Herawati et al.'s (2013) study also showed an improvement in nutritional quality after fermentation in Daphnia magna feed. These findings align with Suprimantoro's research (2016), which found the influence of fermented cassava peel on the population growth of Daphnia magna.

Based on the results of post hoc tests using the Least Significant Difference (LSD) test on treatment P3 (50% rice washing water, 50% fermented cassava peel), it is evident that the population density is significantly higher compared to the other treatments. The combination of 50% rice washing water and 50% fermented cassava peel has a significant positive impact on population growth, possibly due to a synergistic interaction between the nutrients present in rice washing water and compounds produced during cassava peel fermentation. This combination likely provides a more optimal environmental condition for the growth and reproduction of Daphnia magna (BNT, $p<0.05$). In this study, significant differences were found between treatments P1, P2, P4, and P5. These differences could be attributed to factors such as suboptimal nutrient concentrations or interactions between rice washing water and fermented cassava peel at extreme percentages (P1 and P5), which reduced the positive effect on the population growth of Daphnia magna. However, among all treatments, treatment P3 yielded the best results with the highest population density of Daphnia magna (BNT, $p<0.05$). These results confirm that a balanced diet and nutrition greatly contribute to the increase in population density of Daphnia magna (Chilmawati & Sumianto, 2010). Daphnia magna is a non-selective filter feeder capable of filtering various types of food, such as phytoplankton, suspended organic particles, and bacteria. Its food is directed to its mouth through 5-6 antennae. However, it is important to note that the presence of excessive dissolved particles in the water can reduce Daphnia's nutrient uptake capacity (FAO, 1996). Treatment P3 showed the best results because there were not many dissolved particles in the water, optimizing nutrient absorption by Daphnia. High growth in P3 is associated with efficient filtering area, while low growth indicates difficulties in obtaining sufficient food, both in quantity and quality. A larger screen helps Daphnia to intake more food, but at high food availability, a large screen is not needed as the food is already abundant. However, at high food concentrations, a large filtering area can reduce Daphnia's energy efficiency and lead to excessive food rejection, resulting in decreased population growth.

Population growth rate of *Daphnia sp.*

The population growth rate of *Daphnia sp.* fed with fermented cassava peel solution during the study can be seen in Table 2.

Table 2. Population growth rate values of *Daphnia sp.* (%.day-1)

Treatment	Repeat			Mean BNT 5% = 75,99
	1	2	3	
P5	23,96	23,06	24,13	23,72[a]
P4	23,62	23,43	24,29	23,78[ab]
P1	25,19	26,33	25,32	25,61[c]
P2	26,21	26,88	26,55	26,55[cd]
P3	28,54	28,38	28,12	28,35[e]

Description:
P1= 0% rice washing water, 100% cassava peel fermentation
P2= 25% rice washing water, 75% cassava peel fermentation
P3= 50% rice washing water, 50% cassava peel fermentation
P4= 75% rice washing water, 25% cassava peel fermentation
P5= 100% rice washing water, 0% cassava peel fermentation

In this study, the population growth of Daphnia magna was measured from the beginning until reaching the population peak. Data on population growth rates during the study are recorded in Table 7. The table indicates that treatment P3 (50% rice washing water, 50% fermented cassava peel) exhibited the highest growth rate with an average of 30.37% per day, indicating the fastest population growth among all treatments. Treatment P2 also displayed a high growth rate with an average of 29.25% per day. Meanwhile, treatments P1, P4, and P5 showed similar growth rates, ranging from 27.83% to 28.78% per day on average. These results suggest that P3 provides optimal conditions for the growth of Daphnia magna.

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Analysis using ANOVA (Table 8) revealed that the treatment involving a combination of rice washing water and fermented cassava peel significantly influenced population growth. This is confirmed by the calculated F-value exceeding the critical value at a significance level of 0.05, indicating a significant difference among treatments. Furthermore, the Least Significant Difference (LSD) post hoc test in Table 9 confirms significant differences among the five treatment groups regarding the growth rate of Daphnia magna. Treatment P3 had the highest growth rate, followed by P2, while P1, P4, and P5 had lower growth rates.

Rice washing water contains additional nutrients such as carbohydrates and proteins, which are essential as a source of energy and building materials (Agung et al., 2020). These nutrients enhance food availability and support the growth and reproduction of Daphnia magna. On the other hand, the fermentation of cassava peel produces new compounds that affect the environment and nutrient availability for Daphnia magna (Suprimantoro et al., 2016). The growth rate test results indicate that the combination of 50% rice washing water and 50% fermented cassava peel in treatment P3 provides the most optimal conditions for population growth. This combination may synergistically contribute positively to the growth rate of Daphnia magna. Therefore, the use of this combination of rice washing water and cassava peel fermentation is crucial in understanding the environmental impact on population growth.

Population Distribution of Daphnia magna

The study analyzed the population distribution of Daphnia magna based on their total length (µm) at harvest on day 16. The data is presented in Table 3.

Table 3. Population distribution among total length classes (ind.L-1)

Treatment	<100	100-200	200-300	>300	Total
P5	113,33^{a}	88,67^{a}	72,67^{a}	59,33^{a}	334
P4	134,67^{a}	113^{b}	85^{ab}	81^{b}	414
P1	220,33^{b}	127,33^{bc}	99^{ab}	81,33^{bc}	528
P2	241^{bc}	180^{c}	105,33^{ab}	90,67^{bc}	617
P3	264,67^{bc}	184,33^{cd}	119^{ab}	93,33^{bc}	661

Description:
P1= 0% rice washing water, 100% cassava peel fermentation
P2= 25% rice washing water, 75% cassava peel fermentation
P3= 50% rice washing water, 50% cassava peel fermentation
P4= 75% rice washing water, 25% cassava peel fermentation
P5= 100% rice washing water, 0% cassava peel fermentation

Table 3 summarizes the population distribution among different size classes for each treatment (P1, P2, P3, P4, and P5), with each treatment repeated three times. Size classes include <100 μm (class I), 100-200 μm (class II), 201-300 μm (class III), and >300 μm (class IV).

The results reveal that treatment P3 (50% rice washing water, 50% fermented cassava peel) had the highest population distribution of Daphnia magna across all size classes. This suggests that the balanced food ratio in P3 supported optimal growth and reproduction. Treatment P1 (0% rice washing water, 100% fermented cassava peel) also showed even population distribution across size classes, but P3 consistently had the highest number of individuals at various lengths.

Additionally, the observations shed light on food preferences and the zooplankton's ability to use resources in different treatments. Differences in harvest rates among size classes may indicate Daphnia magna's preference for specific length sizes.

These findings underscore the importance of the right food proportion for efficiently managing Daphnia populations and potentially enhancing their growth and abundance. Moreover, the growth of individual Daphnia magna within specific length classes influences population distribution in each treatment (Sarida, 2007). For example, in treatment P3, which exhibited the highest growth rate, more individuals tended to reach larger lengths (classes III and IV). This could be attributed to the rapid and optimal growth of Daphnia magna in treatment P3.

In summary, the study provides valuable insights into Daphnia magna's population distribution and growth under various conditions. It emphasizes the significance of food proportions in managing populations efficiently.

Population Distribution of Daphnia magna

The results of the measurement of the physico-chemical parameters of the *Daphnia sp.* rearing media water obtained during the study can be seen in Table 4.

Table 4. Water Quality Parameters

Treatment	Water Quality Parameters	
	Temperature (C°)	pH
P1	26-28	6,5-8
P2	26-28	6,5-8
P3	26-28	6,5-8
P4	26-28	6,7-8
P5	26-28	6,7-8

Changes in water pH can have a significant impact on Daphnia magna. Firstly, alterations in pH can affect the availability of nutrients in the water, which is crucial for the growth and reproduction of Daphnia magna (Peng et al., 2022). An increase in pH can reduce the quality of food resources available to these organisms. Secondly, variations in pH can influence the solubility of chemical compounds, such as heavy metals, which may become more soluble at higher pH levels. Therefore, variations like P4 and P5 with higher pH levels potentially increase the concentration of hazardous compounds in the water, posing a threat to the health and survival of Daphnia magna (Na et al., 2021). Finally, changes in pH can also affect the chemical stability of the aquatic ecosystem as a whole, including the availability of dissolved oxygen and ecological interactions between Daphnia magna and other organisms (Tkaczyk et al., 2021). Hence, variations in pH across different treatments, such as P1-P5, should be taken into account when analyzing the environmental impact on the growth and survival of Daphnia magna in each variation.

CONCLUSION

Based on this study, it can be concluded that the population density of *Daphnia sp.* in the first cycle reached its peak on day 16. Environmental factors such as water temperature, food availability, and predation pressure played an important role in determining the peak population density. The population density graph showed a significant increase over time, reflecting the typical *Daphnia sp.* population growth pattern. In addition, the P3 variation had the highest *Daphnia sp.* population growth rate, indicating that the environmental conditions in this variation were very favorable for *Daphnia sp.* The difference in pH between the P1-P5 variations may also affect water quality and environmental conditions for *Daphnia sp.* In conclusion, environmental factors, such as temperature, food availability, and pH, play an important role in influencing *Daphnia sp.* population growth, and need to be considered in aquatic ecosystem management.

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