Culture of *Daphnia* sp. (crustacean – cladocera): the effect of manure variation on the growth, natality, and mortality

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Abstract. The objective of this research was to analyze the growth rate, reproduction rate, and mortality rate of *Daphnia* sp. which cultured in variant organic manure. This research used experimental method Randomized Completely Block Design (RCBD) with four treatment and three replications. The treatments in this research were the variant manures from chicken, quail, goat, and cow with same of growth (2.4 g/l). *Daphnia* cultured with using 100 breeders since from neonets (0 day) until growth up and died in one life cycle. At the 3-days, culture of *Daphnia* sp was give peak population with maximum age of culture using quail manure is 7 days, and other treatments are 8 days. The growth rate and the reproduction rate of using quail manure was higher than using chicken manure, goat manure, and cow manure (mean GR = 3.68 : 2.32 : 2.74 : 2.97; mean RR = 3.87 : 2.59 : 3.00 : 3.31; p < 0.05). Although all the breeders of *Daphnia* sp. died at 8th day of culture, quail manure give the lowest of mortality rate than using chicken manure, goat manure, and cow manure (mean MR = 0.19 : 0.28 : 0.26 : 0.34).

1. Introduction

*Daphnia* sp. is the best natural feed for tropical fry fish and it has become an alternative for natural fish feed [1]. The advantages of using *Daphnia* for aquaculture are its low cost and convenient to culture [2]. *Daphnia* is one of micro crustaceans from *Brachiopoda* family and it lives in fresh water [3]. All individual of *Daphnia* are females, but when the environmental quality is poor, *Daphnia* will produce male for sexual reproduction [4].

Growth and development of *Daphnia* sp. s affected by nutrient, age, temperature, respiration, and filtered particle shape [5]. *Daphnia* sp. has two reproduction methods, parthenogenesis and sexual. Parthenogenesis occurs when the environment is suitable for *Daphnia*’s life [1]. *Daphnia* is non-selective filter feeders and it is able to eat bacteria, phytoplankton, ciliate, and detritus [6]. The food is collected by filtering apparatus, while the legs make some water to flow from anterior to posterior and move the particle to the gut by setae [4].

Natural feed brings risks for of diseases and heavy metal accumulation [7]. To minimize the risk, culturing the natural feed is the safest way. Other advantages of using natural feed culture are its high quality, parasite-free or pollution-free characteristic, and sustainability. More importantly, *Daphnia* sp. lives in aquaculture media without affecting water quality [6]. There are many food sources for cultured *Daphnia*, such as organic manure, particularly chicken manure [8], cow manure, and goat manure. Nutrient from poultry waste contains nitrogen (N) 1.48 % and phosphorus (P) 0.4 %, cow manure contains nitrogen (N) 2.03 %, and phosphorus (P) 0.21 %, and goat manure contains nitrogen...
(N) 2.10 %, and phosphorus (P) 0.11 % [9]. Manure in the water will produce bacteria, detritus, and other materials, and become food for Daphnia sp. [4]. The abundance of food source in culture media will increase the growth of Daphnia. In good water condition with lack of food, Daphnia sp. use parthenogenesis as the main reproduction pathway. Parthenogenesis produces many of neonates and short of performance [10]. Each individual of Daphnia can produce 17 – 20 neonates [5]

Ammonia is a key for Daphnia culture, which is essentially the main component in animal waste. Ammonia comes in the form of uric acid in poultry manure and urea in mammals’ manure [11]. Ammonia is very toxic for all organisms, but in acidic condition, the toxicity is decreased, therefore pH monitoring is required for Daphnia culture [12].

The best method to culture a high population of Daphnia carinata is within 9 days with 500 ppt of chicken manure [8]. Another research from Sulasingkin in Izza et al. [13] reported the culture of Indonesian Daphnia using chicken manure with the dose of 12 g/5 L or 2.4 g/L [13]. With those theories, the hypothesis of this study was:

H0: The best manure for culture Daphnia sp. is chicken manure
H1: The best manure for culture Daphnia sp. is not chicken manure

Using organic manure for Daphnia culture gives good performance in terms of growth rate, natality rate, and mortality rate [14]. The biological factor of organic manure to culture Daphnia sp. is now known comprehensively yet. The objective of this research was to analyze the growth rate of Daphnia sp. cultured in different nutrient from variety of organic manure.

2. Methodology

2.1. Research design and formula.

This research used Randomized Complete Block Design (RCBD) with four treatments and three replications. The treatments were the types of manure, which were from chicken, quail, goat, and cow with same growth (2.4 g/l). If the result was significant, data would be analyzed with Honestly Significant Difference test. Growth rate of Daphnia sp. was calculated using a formula taken from Birch [15].

$$\sum e^{r-x}l_xm_x = 1097$$

(1)

\(e\) = natural logarithmic value
\(r\) = growth rate
\(x\) = maintenance time
\(l_x\) = life individual in the time (x)
\(m_x\) = neonates F1 in the time (x)

Natality rate of Daphnia sp. was calculated using a formula:

$$b = \frac{r\beta}{e^r - 1}$$

(2)

$$1/\beta = \sum L_xe^{-r(x+1)}$$

(3)

\(B\) = natality rate
\(L_x\) = \((l_x + l_x+1)/2\): average of individual in KU-x and KU-x+1
\(e\) = natural logarithm
\(r\) = growth rate
Natality rate of *Daphnia* sp. was calculated using the following formula:

\[ d = b - r \] (4)

- \( d \) = mortality rate
- \( b \) = natality rate
- \( r \) = growth rate

2.2. *Research procedure.*

The research consisted of preparation, cohort formation, and main research. We used *Daphnia* with similar age for this research. There were two methods of sample preparations. First, manure was dried and weighed 2.4 gram. Next, the manure was wrapped and tied. The second preparation was media preparation. One L of water was put in a container and installed on the aeration set. After the manure was added, the media was aerated for 3 days.

To make cohort formation, mature *Daphnia* sp. (F0) was cultured in media for 1 day. The new neonates (F1) were taken to another culture media. The neonates (F1) were cultured to adulthood (F2). The new generation (F2) was considered of the same age and used for the main research.

The second generation (F2) of *Daphnia* sp. was added in every treatment at a dose of 100 individual/L. *Daphnia* (F2) was counted everyday and moved into new media. If neonates (F3) appeared, they were (F3) separated from the previous media and counted in different place to avoid the neonates (F3) from growing up and getting difficult to be distinguished. The main research was carried out when all of the second generation of *Daphnia* (F2) died.

2.3. *Water quality.*

Water quality is an important key of *Daphnia* sp. culture. Water quality was checked every day (Table 1); involving dissolved oxygen (DO), pH, and temperature. Ammonia was checked three times: the first sample was taken from the early culture, the second sample was taken at the first spawning (4th day), and the third one was taken from the last day.

**Table 1. Water Quality Control Method.**

| Parameter | Unit   | Method         | Tools                        |
|-----------|--------|----------------|------------------------------|
| Temperature | °C     | Reading of scale | Thermometer (+ 0.01 carefulness) |
| DO        | mg/l   | Reading of scale | DO-meter (+ 0.01 carefulness) |
| pH        | -      | Reading of scale | pH-meter (+ 0.1 carefulness) |
| NH₃       | mg/l   | Nessler method  | Spectofotometer (+ 0.001 carefulness) |

3. Results and Discussion

3.1. The number of *Daphnia* sp. (F2)

Parent of *Daphnia* sp. (F2) decreased since 1st day of culture. The first day was the adaptation stage for *Daphnia* sp. to grow. Adaptation phase in culture media started from Day 0 to 3 [16]. Declamation of population occurred linearly until all of the population (F2) died. All *Daphnia* sp. from quail treatment died on 8th day, while those of the other treatment died on the 10th or 11th day. This condition was in accordance with Deckaestacker *et al.* [10] who reported 9 – 11 days as the generation time of *Daphnia* sp.. Life span of *Daphnia* sp. from being in brood chamber to adulthood and death depends on the species and environmental condition [12].
Figure 1. Parent of Daphnia sp. per-day.

The difference in culture time caused Daphnia sp (F2) from quail manure to produce more neonates than other treatment. Continuous reproduction can reduce the capacity of brood chamber or carapax to recuperate and consequently Daphnia parent dies faster. Reproduction of Daphnia sp. from other manure was lower based on the number of neonates (F3). The birth of F3 in every treatment created competition for food. Deficiency of food in medium affects the life of Daphnia [17]. The high density of Daphnia sp. in media creates a competition for survival [6].

Using quail manure shortened the culture time (x = 8 days) compared to using other manures (figure 2). Quail manure gave more food for Daphnia, thus they bred more than normal situation, resulting in large number of neonates in every reproduction and high frequency of breeding. The high reproduction rate and total neonates in every reproduction decrease the energy needed for Daphnia sp. to maintain their life, metabolism and also to recover the brood chamber; thus, the situation shortened the age of Daphnia sp.

3.2. Neonate (F3) of Daphnia
The neonates (F3) of Daphnia were related with reproduction of F2. The first and the second day, parent (F2) did not produce neonates yet because F2 was too young. The first reproduction of Daphnia sp. (F2) occurred on the third day. Daphnia sp. produce eggs every 3-4 day until they die [4]. The highest neonates in the culture occurred on the third day with 104 individuals for chicken manure, 651 individuals for quail manure, 262 individuals for cow manure, and 250 individuals for goat manure. The high population indicated that the condition of the culture media was well accepted by Daphnia sp. number of neonates declined every day after population peak. Reproduction stopped on the 9th until 11th day. This is in accordance with Deckaestacker et al. who claimed the generation time of Daphnia sp. is between 9-11 days in 20°C [10].
The difference of manure affecting the quantity of neonates. Quail manure gives the highest of neonates between other treatments. Quail media give many of food sources for *Daphnia* sp, so that the growth of *Daphnia* and the reproduction will be faster and higher than a media with poor of food source. Quail manure give chance for *Daphnia* to make more reproduction frequency and neonate’s quantity than organic manure. The abundant of food source give a good chance for *Daphnia* to make a reproduction with parthenogenesis, but if the food source is low, *Daphnia* sp. will produce male *Daphnia* for sexual reproduction. Parthenogenesis gives more of neonates then sexual reproduction in *Daphnia* [10]. Neonate’s density from parthenogenesis is different according to the food.

### 3.3. Life table of *Daphnia*

Growth rate counted by Birch [15], formula with counting the entire individual of *Daphnia* sp. in culture media. Growth rate of *Daphnia* sp. in the treatment give significantly (p < 0.05) with correlation coefficient amount 0.02. Tukey test method (p < 0.05) gives significant level for B treatment (using quail manure) for other treatment. Natality rate and mortality rate were calculated also with insignificant value (p > 0.05). The result showed in table 2.

| Treatment | Growth Rate | Natality Rate | Mortality Rate | Culture Time (x) |
|-----------|-------------|---------------|----------------|------------------|
| A         | 2.32 ± 0.14 | 2.59 ± 0.08   | 0.28 ± 0.08    | 10.0 ± 0.57      |
| B         | 3.68 ± 0.16 | 3.87 ± 0.08   | 0.19 ± 0.24    | 8.0 ± 0.57       |
| C         | 2.74 ± 0.79 | 3.00 ± 0.78   | 0.26 ± 0.10    | 9.0 ± 1.15       |
| D         | 2.97 ± 0.27 | 3.31 ± 0.15   | 0.34 ± 0.13    | 10.0 ± 1.00      |

The highest growth rate was shown by treatment with quail manure, with mean value of 2.23 ± 0.07, followed by goat manure 1.8 ± 0.06, cow manure 1.76 ± 0.08, and chicken manure 1.48 ± 0.05. Those numbers mean how much *Daphnia* could survive in the environmental condition. For example, quail manure from B2 gave growth rate of 2.31, which means that the population would increase by amount 2.31 times. Treatment B also gave significant results compared to other treatments. The difference was impacted by some factor, including food availability, reproduction frequency, and water quality of culture media.
Growth rate and natality rate gave similar results. Treatment with Quail manure showed the highest natality rate at $2.37 \pm 0.07$, followed by goat $1.99 \pm 0.44$, cow $1.98 \pm 0.28$, and chicken $1.10 \pm 0.5$. Natality rate gives probability of recruitment for the next generation of the parent. Quail treatment gave more food for *Daphnia* sp, so that it affected the growth of *Daphnia* and provided impulse to breed. According to nitrogen test, quail manure contained N-total of 2.86 %, while the N-total from chicken manure was 2.8 %. Meanwhile, cow manure and goat manure had similar N-total of 1.97%. Nitrogen functions as one of nutrients needed for the growth of microbes. Decomposition of organic material from manure helps the growth of bacteria that later becomes food for *Daphnia* sp. [13].

Food also affects mortality rate. The lowest mortality rate was from quail manure with $8.0 \pm 0.57$. Although the age of the parent was shorter than in other treatments, quail manure gave more neonates and affected mortality rate. The high amount of food can extend the life of *Daphnia* [17]. If the environment is not hospitable, growth, development, and metabolism of *Daphnia* sp. decrease [18].

Chicken, cow, and goat manures gave longer culture time than quail manure, but it had lower growth and natality rate and higher mortality rate. Decomposing Microbes in other manures were lower than in quail manure, so that the growth of *Daphnia* was not optimum. Declined filtration rate at 54% after 70 hours was due to low concentration of feed (0.025 mgC/L) [17]. Declamation of filtration rate decreases the population growth and reproduction rate. Nevertheless, *Daphnia* sp. can still survive and adapt, but more death occurs [18].

With these results, we ignored the hypothesis (H₀) that claims the best of organic manure to culture *Daphnia* was chicken manure. We found a new and better manure to culture *Daphnia* that was quail manure, with high growth rate and natality rate, and low mortality rate.

### 3.4. Water quality

Growth rate of *Daphnia* sp. was affected by some factors, such as food source, species, and environment. Environment control provides opportunity to make the media anoxic. Temperature, DO, and pH in every treatments were controlled with temperature ranged from 24.1 to 25.7 °C, DO ranged from 3.6 to 4.2 mg/L, and pH ranged from 7.5 to 8.2.

**Table 3. Water quality of media culture.**

| n  | Temperature (°C) | DO (mg/L) | pH          | Ammonia (mg/L) |
|----|-----------------|-----------|-------------|----------------|
| A  | 24.1 – 25.6     | 3.7 – 4.2 | 7.7 – 8.1   | 0.08 – 0.15    |
| B  | 24.3 – 25.6     | 3.6 – 4.1 | 7.6 – 8.3   | 0.10 – 0.38    |
| C  | 24.1 – 25.7     | 3.7 – 4.2 | 7.5 – 8.0   | 0.01 – 0.13    |
| D  | 24.2 – 25.7     | 3.7 – 4.2 | 7.6 – 8.2   | 0.01 – 0.13    |

Temperature of media = affects metabolism including for *Daphnia* as an aquatic organism. Cultured *Daphnia* sp. has good performance at temperature of 20 °C [19]. Khan and M Khan [20] stated that *Daphnia* has optimum life span at 25 °C. Ocampo *et al.* [1] suggested 20- 25 °C as the temperature for *Daphnia* culture to have normal life span. Oxygen in culture media supports respiration of *Daphnia* sp. without creating competition between *Daphnia* and decomposing microbes. DO in the culture was controlled by aeration to be within the tolerance range for *Daphnia*. *Daphnia* sp. can survive in DO > 3 mg/L, but they grow better at 6 mg/L [1]. Oxygen also gives chance for microbes to perform their metabolism and decompose organic matter in media, so that the water quality is good.

The power of hydrogen (pH) affects eggs life span zooplankton *microcrustacean* [6]. According to Ocampo *et al.* [1], optimum pH for *Daphnia* culture is between 7- 8.6. Darmawan [6], stated that neutral or base (pH 7.0- 8.2) is good for *Daphnia magna* growth. pH determines the toxicity of medium from ammonia.
Ammonia is the first substance from organic matter decomposition. Ammonia is divided into un-ionized ammonia (NH₃) and ionized ammonia (NH₄⁺) [21]. Ammonia in water gets more toxic with increased pH [11]. According to Delzer and McKenzie [22], nitrogen decomposition from ammonia: nitrite, and nitrate, were formed by microbes starting from the 6th day at temperature of 20 °C. Normally, decomposition is divided into two stages: carbonaceous (carbon decomposition that starts from day 0) and nitrogenous (nitrogen decomposition). Existence of Ammonia in media is good for Daphnia culture (table 3). Although there was fluctuation, but Daphnia still survived in every treatment. in this research, Ammonia was not the prime factor that affected mortality rate. Daphnia can maintain life by adapting even at the worst condition [18]. The main factor that affects the mortality of Daphnia is frequency of reproduction, even though the water condition has contribution too.

This current study used manure that was soaked for three days. the soaking time was not sufficient for ammonia decomposition, because decomposition of ammonia starts from so that the concentration of ammonia in the treatments was high, particularly in quail manure. The lowest concentration for un-ionized ammonia that gives effect for Daphnia sp. is 1.3 mg/L [23]. Concentration of ammonia in treatment with quail manure was between 0.10-0.38 mg/L. The high ammonia provided opportunity for decomposing microbes to perform their function and then they became food for Daphnia. Cow manure, goat manure, and chicken manure gave lower concentration of ammonia (table 3) than quail manure, so that lower bacteria was available as food for Daphnia.

4. Conclusion
Growth rate and mortality rate of Daphnia sp. cultured using quail manure was higher than chicken, cow, and goat manures. Although all the breeders of Daphnia sp. died on the 8th day of culture, quail manure gave the lowest of mortality rate. thus, null hypothesis (H₀) was ignored (Hₐ) was accepted.

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