The population growth and the nutritional status of *Moina macrocopa* feed
with rice bran and cassava bran suspensions

Perkembangan populasi dan status gizi *Moina macrocopa* yang diberi pakan
suspensi dedak dan tepung ketela pohon

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ABSTRACT

*Moina macrocopa* culture density can be improved by optimizing the fecundity, and somatic growth through the
regulation of quality and quantity of feed. The purpose of this study were to determined how to use effectively the
rice bran and cassava bran *Manihot utilisima* suspension on Moina, based on population, neonates production,
adult percentage, biomass, metabolisme and nutritional state. In this study, Moina were cultured for eighth days
using four concentrations of rice bran suspension and three concentrations of cassava suspension. This research
found that *M. macrocopa* culture with rice bran suspension has higher population, neonates production, adult
percentage and biomass than its culture with cassava bran suspension (P<0.05). This study also found that Moina
culture with rice bran suspension has higher total value of RNA, total value of DNA, the ratio RNA/ DNA, FCR,
and concentration of protein and amino acid than Moina culture with cassava bran suspension. Treatment D with
the initial rice bran suspension concentration was 0.3 mL/L and was increased starting the second day and the end
concentration on the eighth day was 1.2 mL/L has highest peak population of Moina 17,975 ind/L in seventh day,
weight wet biomass 439 mg/L in eighth day and lower FCR 0.94.

Keywords: suspension, rice bran, cassava, population, ratio RNA/DNA

ABSTRAK

*Kepadatan populasi dalam budidaya Moina macrocopa* dapat ditingkatkan dengan mengoptimalkan fekunditas dan
pertumbuhan somatik melalui pengaturan kualitas dan kuantitas pakan. Penelitian ini bertujuan untuk menganalisis
efektifitas penggunaan pakan suspensi dedak dan tepung ketela pohon *Manihot utilisima* dalam budidaya *M.
macrocopa* terhadap populasi, produksi anak per induk, persentase dewasa, biomasa, FCR, dan metabolismenya
(asam amino, DNA, RNA, dan RNA/DNA). Di dalam penelitian ini, *M. macrocopa* dibudidayakan selama delapan
hari menggunakan empat konsentrasi dedak dan tiga konsentrasi tepung ketela pohon. Hasil penelitian ini
menunjukkan bahwa, budidaya *M. macrocopa* dengan pakan suspensi dedak menghasilkan populasi, produksi
anak/induk, persentase dewasa dan biomasa yang lebih tinggi dibandingkan budidaya Moina dengan pakan
suspensi ketela pohon (P<0.05). Budidaya *M. macrocopa* dengan pakan suspensi dedak juga menghasilkan total
RNA, total DNA dan nisbah RNA/DNA, konsentrasi protein, dan asam amino yang lebih tinggi dibandingkan
Moina dengan pakan suspensi ketela pohon. Perlakuan D dengan pakan suspensi dedak awal 0,3 mL/L dan
meningkat mulai hari kedua dengan konsentrasi hari ke delapan 1,2 mL/L menghasilkan puncak populasi tertinggi
pada hari ketujuh sebanyak 17,975 ind/L, berat basah biomasa hari kedelapan kultur 439 mg/L, dan FCR yang
rendah yaitu 0,94.

Kata kunci: suspensi, dedak, ketela pohon, populasi, nisbah RNA/DNA
INTRODUCTION

Moina has become an important candidate as natural feed for both fish and shrimp larvae as a result of artemia cysts price increment (Dodson et al., 2010). It has a higher nutritional value compared to naupli and can breed and growth fast. It can tolerate low dissolved oxygen (DO) level and a high ammonia concentration (Loh et al., 2013). Moina can be cultured using agricultural, animal, and food industry wastes as feed (Patil et al., 2010). However, the use of Moina macrocopa is limited due to its low commercial availability.

M. macrocopa culture with an initial inoculant of 40–50 individual/L using Chlorella spp. (1.0×10⁶ cell/mL) as feed resulted in producing about 15,000–20,000 individual/L, which was higher compared to Moina production using animal wastes (fowl and cow manures) i.e. 1,301 individual/L (45 individual/L) (Ventura et al., 2012; Siddque et al., 2004; Malla & Banik, 2015). Moina culture using Chlorella spp, at a density of 1.0×10⁶ cell/mL, generated a production of 12–14 larvae/broodstock (Malla & Banik, 2015). According to Rietzler et al. (2014), M. macrocopa can reach a maximal fecundity of 37 eggs/broodstock and both fecundity and optimum population growth were obtained at water hardness of 50 mg/L, a temperature of 25–31 °C, pH of 7–8 and DO higher than 4 mg/L (Tan & Wang, 2010).

M. macrocopa population density could be enlarged by mean of increasing fecundity and decreasing the reproductive period by manipulating both quality and quantity of the feed (Hakima et al., 2013), including protein concentration, amino acids concentration (Koch et al., 2011), lipid concentration (Wacker & Creuzburg, 2007), and vitamin B (Mehdipour et al., 2011). M. macrocopa fecundity and growth decreased when the population density increased and both feed quality and quantity decreased (Loh et al., 2016; Zadereev & Lopotina, 2012).

Rice bran is a potential feed for Moina since it contains various nutrients such as protein (12–13%), lipid (16–20%), linoleic acid (6.35–6.85%), acids α linolenate (0.2–0.27%), vitamin B, and minerals (6–9%), which are dominated by calcium and iron (Faria et al., 2012; Murtaza et al., 2011). Another agricultural product candidate that is abundantly available is cassava bran (Manihot utilisima) that also contains nutrients such as carbohydrates (56–94%), Vitamin B1 (thiamin) (2.16–48 μg/g), high vitamin C (50–510 μg/g), low protein content (1.5–4.7%), lipid (0.3–3.2%), and lower mineral compared to rice bran (Salvador et al., 2014; Faria et al., 2012).

Rice bran was used as feed in both Daphnia and Artemia cultures (Sorgeloos et al., 1980; Depauw et al., 1981). Rice bran and cassava bran could directly be used as M. macrocopa feed, although they have to be processed into small particles suspension in order to fit the the mouth of M. macrocopa. Protein and amino acids concentrations of the feed directly affect fecundity and population growth of M. macrocopa. Indeed, the amino acids, arginine, affects both endocrine system and reproduction (Jobgen et al., 2006), while histidine affects DNA and protein synthesis. Glycine, tirocsin, phenilalanine, and lysine affect the speed of embryo development in the incubating cavity (Li et al., 2008).

Moina fecundity and population growth are also affected by fat and fatty acids concentrations. Cholesterol is a proccurssor for hormone formation that could not be synthesized by microcrustacean (Nagaraju, 2011). In Cladocera, it plays a role in increasing somatic growth, while PUFAs (polyunsaturated fatty acids) play important roles in reproduction, growth performance, and survival (Fereidouni et al., 2013). High PUFAs concentration hampers somatic growth and induces sexual reproduction in Daphnia magna that results in ephippia (Choi et al., 2016).

Base on the above information, it is believed that both rice bran and cassava bran suspensions with specific concentrations can enhance both M. macrocopa fecundity and population growth. The present research was aimed at determining the effectiveness of using rice bran and cassava bran suspensions in M. macrocopa culture on population growth, metabolism, nutritional status, protein and amino acids contents.

MATERIALS AND METHODS

Research design

A completely randomised design with two major parameters i.e. rice bran suspension concentration and cassava bran suspension concentration, were used in the present research. Rice bran suspension consisted of 4 treatments (A, B, C, and D), while the cassava bran suspension had 3 treatments (E, F, and G). Each treatment was replicated 4 times. Both rice bran and cassava bran specific concentrations were...
determined in a preliminary research, where the concentration of 0.3 mL/L was discerned as the optimum concentration that sustains both survival and reproduction of *M. macrocopa* (at a maximal level) at the beginning of the rearing period. Rice bran and cassava bran concentrations of 1.2 mL/L was the concentration that generated a hardness level that did not affect *M. macrocopa* survival level. *M. macrocopa* population was observed to decrease with a cassava bran concentration of 0.6 mL/L, thus, was not used in the present study.

Both rice bran and cassava bran suspension concentrations were 0.3 mL/L on the first day of the research and increased on the second day (according to each treatment) up to final concentrations on day 8 of the research. The treatment were as follows: final rice bran suspension concentration of 0.6 mL/L (A), final rice bran suspension concentration of 0.8 mL/L (B), final rice bran suspension concentration of 1.0 mL/L (C), final rice bran suspension concentration of 1.2 mL/L (D), final cassava bran suspension concentration of 0.8 mL/L (E), final cassava bran suspension concentration of 1.0 mL/L (F), and final cassava bran suspension concentration of 1.2 mL/L (G).

### Preparation of rice bran and cassava bran suspensions

Hundred grams of both rice bran and cassava bran were separately suspended in 500 mL water (from a water tank) using a blender at a speed of 2000 rpm for 5 minutes (twice) in order to increase the concentration and decrease organic matter size in the suspension. A second suspension process was performed 30 minutes after the first, then filtered using a net (2 mm, 0.1 mm) and nilon (40 µm). Finally, water was added to the obtained suspension (up to 500 mL).

Proximate analysis results of cassava bran suspension revealed that it contained organic matters (72 mg/mL), protein (0.4%), and fat (0.02%), while rice bran suspension contained organic matters (74 mg/mL), protein (0.83%), and fat (0.79%).

### Culture medium

Water from a water tank in the faculty of fisheries and marine science (Bogor Agricultural University) was used as medium for *M. macrocopa* culture in the present study. Water from the tank was accommodated in a 1000 L fiber tank and used as water supply during *M. macrocopa* culture. Water was aerated for 3 days and filtered using a 40 µm nylon filter prior to stocking it in experimental tanks in order to dispose of other zooplankton competitors.

### Innoculant availability and *M. macrocopa* culture

*M. macrocopa* used in the present study was brought from Surabaya and individually cultured (1 individual/20 mL) during several generations to obtain a high *M. macrocopa* seed quality (in terms of growth and reproduction). Afterwards, *M. macrocopa* was cultured at a density of 20 individual/L with water volumes ranging between 300 mL and 10 L, and aclimatised to both feed (rice bran and cassava bran) for two months. Finally, seeds from the 10 L water treatment was used as inoculant in the present study.

Twenty individuals per liter water was used as inoculant in the present study and cultured in 10 L containers. *M. macrocopa* culture lasted for 8 days in an indoor room (closed) with photoperiods of 900–1250 lux in the afternoon and 50–100 lux at night. During the first two days, all of the treatments received the same amount of feed i.e. 0.3 mL/L. On the second day, various feeding rate were applied according to each treatment as presented in Table 1, and feeding (50% from the daily concentration) was done twice daily at 8.00–9.00 am and 7.00–8.00 pm. During the rearing period, both water and container exchange were performed every two days, starting on day 3 until day 7. Thirty three percent (33%) of the water in previous container was disposed and *M. macrocopa* placed into a new container and filled with the percentage of disposed water (until it reached 10 L). Container exchange was performed in order to prevent the formation of filamentous layer that could trap *M. macrocopa*, leading to death.

### Tested parameters

#### Population growth

Sampling was carried out by randomly collecting 100 mL of water from 5 collecting points (both center and corners) after turning off the aeration system for 15 minutes. Data on population, total broodstock, and offspring were collected from day 2 until day 8 of the culturing period. In addition to the sampling, *M. macrocopa* broodstock, ready for spawning, were selected (20–40 individual) and stocked at a density of 66 individual/L. offspring production per broodstock and *M. macrocopa* broodstock percentage were determined using the following formula:
Offspring production (individual/broodstock) =
\[
\frac{\text{Number of } M. \text{ macrocopa offspring}}{\text{Number of } M. \text{ macrocopa broodstock}}
\]
Broodstock percentage (%) =
\[
\frac{\text{Number of } M. \text{ macrocopa offspring} \times 100}{\text{Number of } M. \text{ macrocopa broodstock}}
\]

Harvesting and M. macrocopa final weight measurement (wet weight) were carried out on day 8 of the research. M. macrocopa was dried using a paper tissue on a nylon filter before weighing and the mentioned data was used to determine FCR as follows:

FCR = \( \frac{F}{(Wt - Wo)} \)

FCR was determined based on the total feed weight (F), initial weight (Wo), and final weight (Wt) of M. macrocopa.

Water quality parameters such as dissolved oxygen, pH, temperature, total ammonia, and hardness were also measured throughout the research.

M. macrocopa DNA and RNA analysis

RNA and DNA concentrations were determined based on Ramalho et al. (2004) method. RNA and DNA were isolated from 20 mg M. macrocopa (wet weight), that were collected on day 5 and day 7 of the culture (7 h after morning feeding). DNA and RNA concentrations were measured using Gene Quant from Biotech Pharmacy with absorbance (\( \lambda = 260 \text{ nm and } 280 \text{ nm} \)). RNA concentration results was used to determine RNA/DNA ratio as indicator for nutritional status of M. macrocopa from culture systems using rice bran and cassava bran suspensions.

Protein, feed amino acids and M. macrocopa analysis

Rice bran, cassava bran, and M. macrocopa were collected from each group of treatment on day 8 and used to analyse amino acids content by mean of a high-performance liquid chromatography (HPLC) (Hewlett Packard Series 1.100) and proximate test based on AOAC (1995) method.

Data analysis

Data on population, fecundity, broodstock percentage, biomass, DNA, RNA, RNA/DNA ratio, and FCR were analysed using ANOVA, which was followed by a Duncan’s post-hoc comparison test if significant differences were found. Data on amino acids concentration and water quality were descriptively analysed.

RESULTS AND DISCUSSION

Results

M. macrocopa culture using rice bran suspension resulted in higher population peaks compared to cassava bran suspension. Treatment D had the highest population among rice bran and cassava bran treatments, starting on day 3 until the population peak (17,975 individual/L) on day 7. M. macrocopa culture using cassava bran with the same concentration (treatment G) resulted in
a population peak of 1,970 individual/L (Figure 1).

The differences in \( M. \) macrocopa population were consequences of differences in broodstock fecundity and \( M. \) macrocopa fed on rice bran suspension produced about 13.25 individual per broodstock on the 2\(^{nd} \) day, which decreased to 10.50–12.75 individual on the 3\(^{rd} \) day. The mentioned performances were higher than those of \( M. \) macrocopa fed on cassava bran suspension i.e. about 3.39–3.92 individual on the 2\(^{nd} \) day and 3.42–3.5 individual on the 3\(^{rd} \) day. Increasing the population density resulted in a decrease in offspring production per broodstock, which was about 2.0–2.25 individual in rice bran suspension treatment, but higher compared to cassava bran suspension, being about 0.25–0.75 individual (Figure 2).

Fecundity of \( M. \) macrocopa broodstock with low cassava bran suspension resulted in a higher percentage of broodstock compared to that of rice bran suspension. \( M. \) macrocopa cultured with cassava bean suspension had higher \( M. \) macrocopa broodstock on the 3\(^{rd} \) day (30–32\% of the total population) than in the rice bran suspension (3.2–3.3\% of the total population). \( M. \) macrocopa broodstock population increased after the 3\(^{rd} \) day with the highest percentage on day 7 i.e. 50\% of the total population resulting from \( M. \) macrocopa culture using cassava bran suspension (treatment E). The highest \( M. \) macrocopa broodstock percentage in the culturing system using rice bran suspension was 42.5\% of the total population (treatment D) on the day 8 (Figure 3).

The final biomass of \( M. \) macrocopa cultured in rice bran suspension (280–439 mg/L) was higher compared to that of cassava bran suspension (31–57 mg/L) (Table 2). \( M. \) macrocopa cultured with rice bran suspension as feed (treatment D) had the highest biomass i.e. 439 mg/L with a FCR of 0.94, while \( M. \) macrocopa cultured using cassava powder suspension (treatment E) had the lowest biomass, being 31 mg/L, with a FCR of 14.13 (Table 2).

Protein and amino acids concentrations of \( M. \) macrocopa cultured with rice bran suspension were higher compared to those of \( M. \) macrocopa cultured with cassava bran suspension. \( M. \) macrocopa cultured with rice bran suspension had a protein content of 3.78\% (wet weight), while \( M. \) macrocopa cultured with cassava bran suspension had a protein content of 2.57\% (wet weight) (Table 3). Both protein and amino acids concentrations in rice bran suspension were higher compared to cassava bran suspension. For instance, rice bran suspension had an arginine concentration of 3.82\%, while arginine was only 0.89\% of the total protein in cassava bran suspension. \( M. \) macrocopa cultured in cassava bran suspension had higher glutamate (11.47\%) and phenylalanine (3.98\%).

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**Figure 1.** Population of \( M. \) macrocopa using rice bran suspension (A, B, C, D) and cassava tree suspension (E, F, G). Different letters on the same day indicate significant differences (P<0.05).
concentrations. However, the concentrations of the other amino acids were lower in cassava bran suspension compared to *M. macrocopa* cultured in rice bran suspension (Table 3).

*M. macrocopa* fed on rice bran suspension had a total DNA of 0.272–0.292 µg/µg on day 5 and the highest total DNA, 0.292 µg/µg, was observed in treatment A. Total DNA experienced a decrease on day 7 with 0.119 µg/µg as the highest *M. macrocopa* DNA value (treatment E) (Table 4). *M. macrocopa* fed on rice bran suspension had a RNA of 0.055–0.069 µg/µg on day 5 with the highest total RNA of 0.069 µg/µg in treatment B. Total RNA experienced a decrease on day 7 except in treatment B with a total RNA of 0.083 µg/µg.

The RNA/DNA ratio of *M. macrocopa* on day 5 ranged between 0.20–0.24 and experienced an increase on day 7 i.e. 0.36–0.82 in *M. macrocopa* fed on rice bran suspension and 0.32–0.45 in *M. macrocopa* fed on cassava bran suspension. The highest *M. macrocopa* RNA/DNA ratio (on day 7) was observed in treatment B, being 0.82 and the lowest in treatment E, being 0.32 (Table 4).

![Figure 2. *M. macrocopa* offspring production per broodstock in rice bran suspension (A, B, C, D) and cassava tree suspension (E, F, G). Different letters on the same day indicate significant differences (P<0.05).](image)

![Figure 3. *M. macrocopa* broodstock percentage during the culturing period using rice bran suspension (A, B, C, D) and cassava powder suspension (E, F, G). Different letters on the same day indicate significant differences (P<0.05).](image)
Water quality parameters were observed to support *M. macrocopa* growth (Table 5). *M. macrocopa* cultured with rice bran suspension had a dissolved oxygen of 3.9 mg/L, while that of the cassava bran suspension was 4.0–5.0 mg/L. In addition, total ammonia and pH were 0.51 mg/L and 7.3–7.5, respectively.

**Discussion**

*M. macrocopa* population density could be enlarged by mean of increasing fecundity and decreasing the reproductive period by manipulating both quality and quantity of the feed (Hakima et al., 2013), including protein concentration, amino acids concentration (Koch et al., 2011), lipid concentration (Wacker & Creuzburg, 2007), and vitamin B (Mehdipour et al., 2011). Feed quantity and quality directly affect population growth and survival rate (Zadereev & Lopotina, 2012; Hakima et al., 2013). *M. macrocopa* population peak in rice bran suspension occurred on day 7, about 13.25 individual, which was higher than that of cassava bran on day 8 (1,975 individual/L). This was due to a high reproductive capacity of rice bran suspension (13.25 individual) compared to cassava bran suspension (4.00 individual). Observation results also showed that *M. macrocopa* culture using rice bran suspension can accelerate the reproductive cycle, leading to first reproduction within 55 h and consecutively every 18–21 h.

Cladocera fecundity is affected by factors such protein concentration, fat, and amino acids (especially arginine and histidine) (Koch et al., 2011). Feed protein will be digested into amino acids using various networks to synthesize new protein during growth and reproduction, or even change the existing protein (Li et al., 2008). Increments in arginine and histidine concentrations in feed can increase not only fecundity but also offspring development (Koch et al., 2011). Indeed, arginine affects endocrine regulation, and reproduction (Jobgen et al., 2006; Chen et al., 2013), while histidine affects both DNA and protein synthesis (Li et al., 2008). Rice bran suspension used in the present study contained protein (0.83%) and fat (0.79%) that were higher compared to those of cassava bran suspension (0.4% and 0.02%, respectively). In addition, arginine (3.82%) and histidine (1.61%) concentrations were also higher in rice bran suspension compared to cassava bran (0.89% and 0.56%, respectively).

Table 2. *M. macrocopa* biomass and FCR fed on rice bran and cassava bran suspensions

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
<th>Rice bran suspension</th>
<th>Cassava bran suspension</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Population on day 8 (×10³ ind/L)</td>
<td>14.2±0.36b</td>
<td>14.9±0.71bc</td>
<td>15.6±1.01cd</td>
</tr>
<tr>
<td>Feed weight (g)</td>
<td>7.52</td>
<td>8.82</td>
<td>10.06</td>
</tr>
<tr>
<td>Feed weight in suspension (g)</td>
<td>2.71</td>
<td>3.16</td>
<td>3.62</td>
</tr>
<tr>
<td>Moina initial weight (mg/L)</td>
<td>2.8</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>Moina final weight (mg/L)</td>
<td>280±14b</td>
<td>322±30c</td>
<td>364±44d</td>
</tr>
<tr>
<td>FCR</td>
<td>0.98±0.1a</td>
<td>1.00±0.1a</td>
<td>1.01±0.1a</td>
</tr>
</tbody>
</table>

Note: A, B, C, and D are rice bran suspension; E, F, and G are cassava bran suspension. Different letter in the same row showed the significant different between the treatment (P<0.05).
protein and amino acids (arginine and histidine) concentrations in rice bran (61.82% higher than cassava bran suspension). Lysine concentration in *M. macrocopa* fed on cassava bran suspension (3.33 g/100 g protein) was lower compared to that of *M. macrocopa* fed on rice bran suspension (6.39 g/100g protein), indicating a lysine deficiency that caused a decrease in embryo growth in the embryonic developmental cavity (Li *et al.*, 2008).

Fat concentration in *Cladosera D. magna* feed affects the allocated energy from metabolism. Indeed, cholesterol is a precursor in hormone formation that cannot be synthesized in micro-crustacean (Nagaraju, 2011) and plays a role

### Table 3. *M. macrocopa* amino acids concentrations (% amino acid weight per protein weight) fed on rice bran and cassava bran suspensions.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>M. macrocopa fed on rice bran</th>
<th>M. macrocopa fed on cassava bran</th>
<th>Rice bran suspension</th>
<th>Cassava bran suspension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein % (<em>w/w)</em></td>
<td>3.78</td>
<td>2.57</td>
<td>1.38</td>
<td>0.51</td>
</tr>
<tr>
<td>Amino acid % (<em>w/w)</em></td>
<td>2.98</td>
<td>1.86</td>
<td>0.70</td>
<td>0.10</td>
</tr>
<tr>
<td>Essential amino acids (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>6.80</td>
<td>6.48</td>
<td>4.29</td>
<td>1.81</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.72</td>
<td>4.85</td>
<td>3.82</td>
<td>0.89</td>
</tr>
<tr>
<td>Lysine</td>
<td>6.39</td>
<td>3.33</td>
<td>2.11</td>
<td>0.59</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.32</td>
<td>2.13</td>
<td>1.61</td>
<td>0.56</td>
</tr>
<tr>
<td>Valine</td>
<td>5.13</td>
<td>5.06</td>
<td>3.03</td>
<td>1.22</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.36</td>
<td>3.98</td>
<td>2.89</td>
<td>1.03</td>
</tr>
<tr>
<td>Threonine</td>
<td>4.11</td>
<td>3.97</td>
<td>2.33</td>
<td>1.02</td>
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<tr>
<td>Methionine</td>
<td>1.95</td>
<td>1.88</td>
<td>1.27</td>
<td>0.35</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>4.21</td>
<td>4.10</td>
<td>2.13</td>
<td>1.20</td>
</tr>
<tr>
<td>Non-essential amino acids (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Glutamine</td>
<td>11.37</td>
<td>11.47</td>
<td>8.61</td>
<td>3.60</td>
</tr>
<tr>
<td>Asparagine</td>
<td>8.06</td>
<td>7.73</td>
<td>4.80</td>
<td>2.39</td>
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<tr>
<td>Glycine</td>
<td>4.58</td>
<td>4.08</td>
<td>3.17</td>
<td>1.21</td>
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<td>Serine</td>
<td>4.26</td>
<td>4.09</td>
<td>2.69</td>
<td>1.47</td>
</tr>
<tr>
<td>Alanine</td>
<td>5.95</td>
<td>5.72</td>
<td>4.31</td>
<td>1.67</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>4.58</td>
<td>3.31</td>
<td>3.42</td>
<td>1.51</td>
</tr>
</tbody>
</table>

### Table 4. Total DNA, RNA, and RNA/DNA ratio concentrations of *M. macrocopa* fed on rice bran and cassava bran suspension.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Day 5</th>
<th>Day 7</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(RNA)µg /µg</td>
<td>(DNA)µg/µg</td>
<td>RNA/DNA</td>
</tr>
<tr>
<td>Rice bran</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.066±0.001a</td>
<td>0.292±0.008a</td>
<td>0.23±0.01a</td>
</tr>
<tr>
<td>B</td>
<td>0.069±0.001a</td>
<td>0.284±0.010a</td>
<td>0.24±0.01a</td>
</tr>
<tr>
<td>C</td>
<td>0.055±0.001b</td>
<td>0.272±0.006ab</td>
<td>0.20±0.01ab</td>
</tr>
<tr>
<td>D</td>
<td>0.056±0.005b</td>
<td>0.246±0.002b</td>
<td>0.23±0.02a</td>
</tr>
<tr>
<td>Cassava bran</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>0.043±0.002c</td>
<td>0.213±0.018bc</td>
<td>0.20±0.03ab</td>
</tr>
<tr>
<td>F</td>
<td>0.043±0.001c</td>
<td>0.179±0.014c</td>
<td>0.24±0.02a</td>
</tr>
<tr>
<td>G</td>
<td>0.048±0.001b</td>
<td>0.255±0.005b</td>
<td>0.19±0.01b</td>
</tr>
</tbody>
</table>

Note: A, B, C, and D are rice bran suspension; E, F, and G are cassava bran suspension. Different letter in the same column showed the significant different between the treatment (P<0.05).
in increasing somatic growth in Cladosera species. Meanwhile PUFAs play important roles in reproduction (Wacker & Creuzburg, 2007) by increasing both growth and survival rate (Fereidouni et al., 2013). Rice bran contains linoleic acid (6.35–6.85%), and acid α linoleic (0.2–0.27%) i.e. fatty acids that are essential for cladosera (Faria et al., 2012; Persson & Vrede, 2006). Some Cladosera species have the ability to convert acid α linoleic into eicosapentaenoic acid (EPA) and decosahexanoic acid (DHA) with varying abilities (Masclaux et al., 2012). EPA availability in copepod species feed affects eggs production (Jónasdóttir et al., 2009).

*Macrocopa macrocopa* population growth and fecundity (on day 5 and day 7) that were high in rice bran suspension (treatment A, B, C, and D) were supported by high RNA/DNA ratio on day 5 (0.20–0.24) and day 7 (0.36–0.82) compared to those of cassava bran suspension (0.19–0.24 and 0.32–0.45, respectively). RNA/DNA ratio also becomes an indicator of both nutritional condition and growth of marine organisms (Chícharo & Chícharo, 2008). Feed availability (chlorophyll-a concentration) controls copepod *Calanus sinicus* growth and increase RNA/DNA ratio during plankton blooming (Ning et al., 2013). Protein concentration increment (from 40% to 50%) in rainbow trout (*Oncorhynchus mykiss*) larvae significantly increased RNA/DNA ratio (Labh et al., 2014). A high RNA/DNA ratio indicated high protein synthesis capacity per cell and a better nutritional status (Fathallah et al., 2010).

Variations in total Cladosera DNA also reflect changes in reproductive activities. In an organism that reproduces sexually, DNA synthesis is active after fertilization (Kermi et al., 2017). In Cladosera (*Moina*) that reproduces asexually (parthenogenesis), the cell of the egg will develop into embryo without fertilization i.e. after being placed in the embryonic developmental cavity (Hiruta et al., 2010). Cladosera DNA concentration increases at the beginning of gonadal development (Gorokhova & Kyle, 2002). Transcription program and active differentiation occur at the end of embryogenesis (Kermi et al., 2017), so that RNA concentration will increase in late developmental period of embrionic growth (Gorokhova & Kyle, 2002).

A decrease in fecundity of broodstock on day 7 was a consequence of a drop in eggs production in gonads that was followed by a cut in *Macrocopa macrocopa* DNA concentration. The rise of *Macrocopa macrocopa* DNA/RNA ratio cultured in rice bran suspension on day 7 was due to a decrease in the total *Macrocopa macrocopa* DNA value (0.078–0.100 µg/µg) and an increase in total RNA (0.023–0.083 µg/µg). The total RNA of *Macrocopa macrocopa* cultured in cassava bran suspension (0.016–0.039 µg/µg) also faced a decrease on day 7. The highest total *Macrocopa macrocopa* RNA concentration on day 7 (0.083 µg/µg) was observed in treatment B.

*Macrocopa macrocopa* fed on rice bran suspension had lower feed conversion ratio (±1.00) compared to those fed on cassava bran suspension (7.8–14.1).

*Macrocopa macrocopa* cultured in rice bran had a protein content of 53.69% (dry weight), which was higher than that of cassava bran suspension (39.5%, dry weight). Protein content of *Macrocopa macrocopa* fed on rice bran suspension was still within the normal range for *Macrocopa macrocopa* i.e. 50% (Gogoi et al., 2016), while *Macrocopa macrocopa* fed on cassava bran suspension had lower protein content.

*Macrocopa macrocopa* culture using rice bran as feed (treatment D) resulted in the highest population peak, being 17,975 individual/L, with a biomass of 439 mg/L (wet weight) and a FCR of 0.94±0.09, which was lower than that of *D. magna* fed on cassava bran suspension (1.00–2.00). A decrease in feed concentration (rice bran suspension) that was lower than that of treatment D was caused by a decline in both fecundity and growth. Zadereev and Lopotina (2012), reported a decrease in broodstock fecundity (from 14 to 10) due to a decline in *Chlorella vulgaris* density from 800 cell/mL to 100 cell/mL.

### Table 5. Water quality parameters of 10 L *M. macrocopa* using rice bran and cassava bran suspensions

<table>
<thead>
<tr>
<th>Water quality</th>
<th>Research results</th>
<th>Optimal conditions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolved oxygen (mg/L)</td>
<td>5.30–3.92</td>
<td>&gt; 3.50</td>
<td>Miah et al. (2013)</td>
</tr>
<tr>
<td>pH</td>
<td>7.8–7.4</td>
<td>7.0–8.0</td>
<td>Miah et al. (2013)</td>
</tr>
<tr>
<td>Ammonia (mg/L NH₃)</td>
<td>0.42–0.51</td>
<td>&lt; 2</td>
<td>Miah et al. (2013)</td>
</tr>
<tr>
<td>Hardness (mg/L) CaCO₃</td>
<td>59.34</td>
<td>&gt; 50</td>
<td>Tan and Wang (2010)</td>
</tr>
</tbody>
</table>
CONCLUSION

Rice bran suspension is better than cassava bran suspension as feed in *M. macrocopa* culture due to high population, fecundity, broodstock percentage, and biomass. *M. macrocopa* culture using rice bran suspension resulted in high RNA/DNA ratio, FCR, protein concentration and amino acids concentration compared to cassava bran suspension.

REFERENCES


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