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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 pengunaan 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 suspensi dedak dan tiga konsentrasi suspensi 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 kedelapan 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 cyts 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 oxgyen (DO) level and a high amonnia 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 availibilty.

M. macrocopa culture with an initial innoculant of 40−50 individual/L using *Chlorella* spp. (1.0×10^6 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 1.0×10^6 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 proccessed 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 proccursssor for hormone formation that could not be synthesized by micro-crusteacean (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 dipose 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 acclimatised to both feed (rice bran and cassava bran) for two months. Finally, seeds from the 10 L water treatment was used as innoculant in the present study.

Twenty individuals per liter water was used as innoculant 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:

Table 1. *M. macrocopa* volume air 10 L Total rice bran and cassava bran daily suspension concentrations in *M. Macrocopa* culture in 10 L water

Culture days	A	B	C	D	E	F	G
	mL/10L						
0	3	3	3	3	3	3	3
1	3	3	3	3	3	3	3
2	3.2	3.3	3.4	3.5	3.3	3.4	3.5
3	3.6	3.8	4.1	4.3	3.8	4.1	4.3
4	4	4.6	5	5.4	4.6	5	5.4
5	4.5	5.3	6	6.8	5.3	6	6.8
6	4.9	6	7.2	8.4	6	7.2	8.4
7	5.4	7	8.6	10.2	7	8.6	10.2
8	6	8	10	12	8	10	12
Total (mL)	37.6	44.0	50.3	56.6	44.0	50.3	56.6

Note: A, B, C and D are rice bran suspension; E, F, and G are cassava bran suspension.

Offspring production (individual/broodstock) =

$$\frac{\text{Number of } \textit{M. macrocopa} \text{ offspring}}{\text{Number of } \textit{M. macrocopa} \text{ broodstock}}$$

Broodstock percentage (%) =

$$\frac{\text{Number of } \textit{M. macrocopa} \text{ offspring}}{\text{Number of } \textit{M. macrocopa} \text{ broodstock}} \times 100$$

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:

$$FCR = F/(Wt - Wo)$$

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 (λ= 260 nm and 280 nm). DNA and 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 ANNOVA, 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 2nd day, which decreased to 10.50−12.75 individual on the 3rd 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 2nd day and 3.42−3.5 individual on the 3rd 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 3rd 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 3rd 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%)

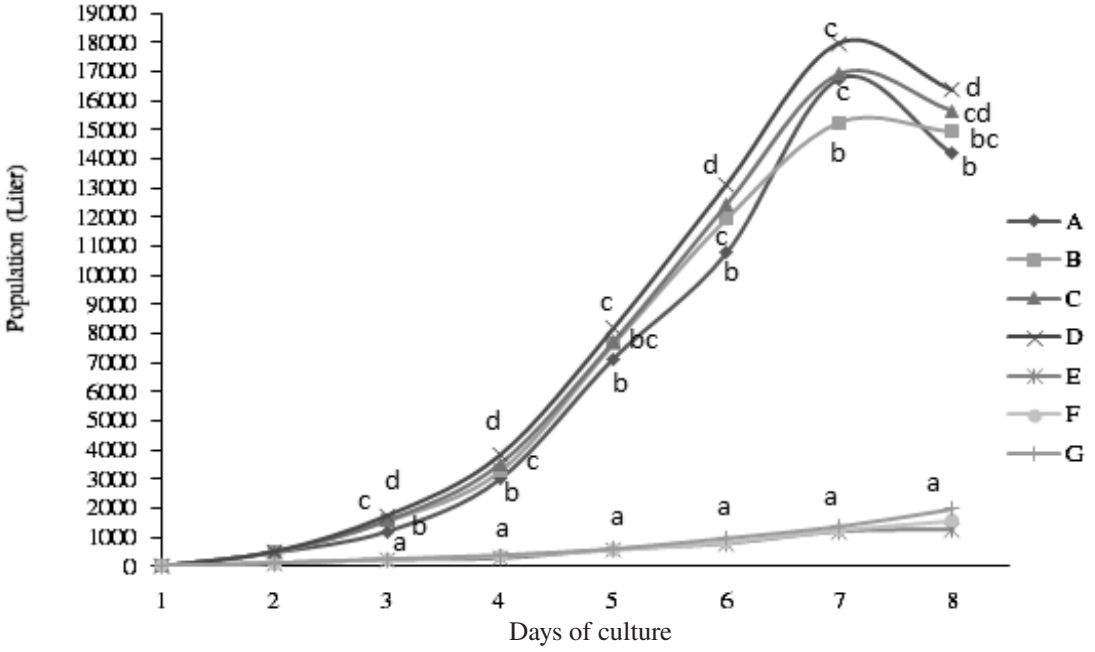

Figure 1. Population of *M. macrocopa* using rice bran suspension (A, B, C, D) and cassava tree suspension (E, F, G). Different letters on the same day indicate significant differences (P<0.05).

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).

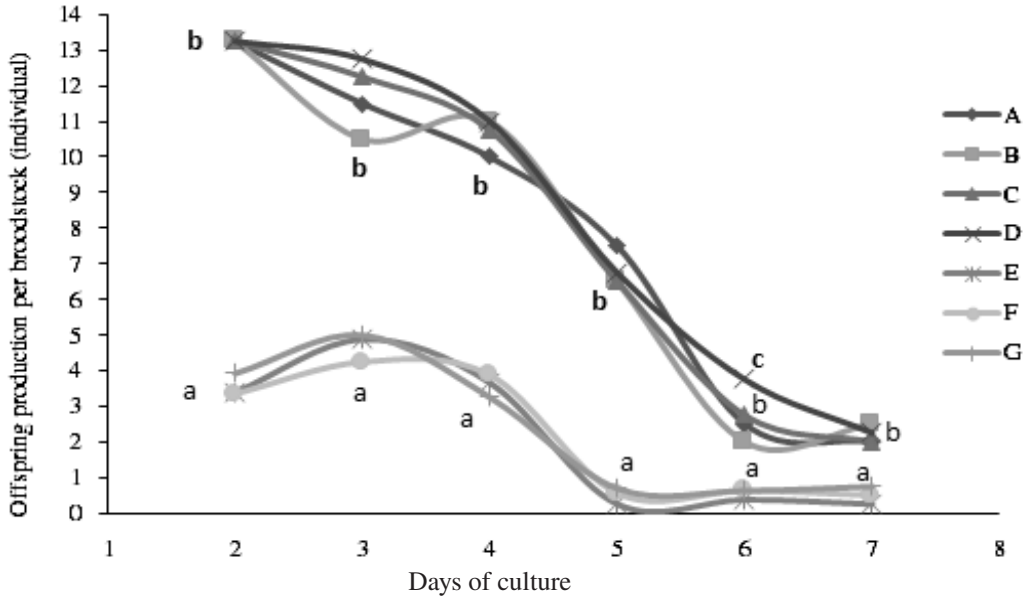

Figure 2. *M. macrocopa* offspring production per broodstock in rice bran suspension (A, B, C, D) and cassava tree suspension (E, F, G). Different letters on the same day indicate significant differences (P<0.05).

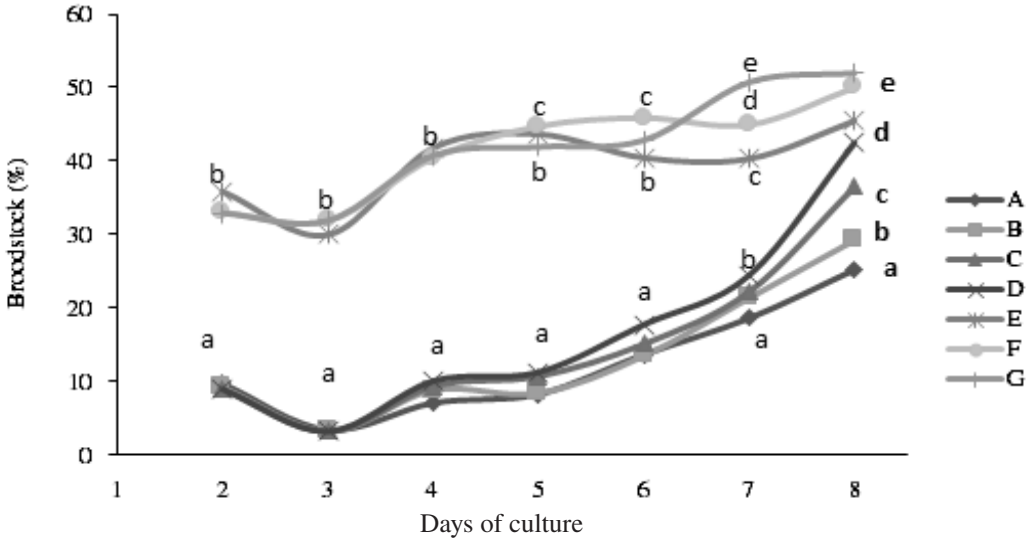

Figure 3. *M. macrocopa* broodstock percentage during the culturing period using rice bran suspension (A, B, C, D) and cassava powder suspension (E, F, G). Different letters on the same day indicate significant differences (P<0.05)

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

Parameters	Treatments						
	Rice bran suspension				Cassava bran suspension		
	A	B	C	D	E	F	G
Population on day 8 ($\times 10^3$ ind/L)	14.2±0.36b	14.9±0.71bc	15.6±1.01cd	16.3±1.35d	1.27±0.46a	1.56±0.18a	1.97±0.19a
Feed weight (g)	7.52	8.82	10.06	11.32	8.8	10.06	11.32
Feed weight in suspensions (g)	2.71	3.16	3.62	4.08	3.27	3.74	4.21
Moina initial weight (mg/L)	2.8	2.8	2.8	2.8	2.8	2.8	2.8
Moina final weight (mg/L)	280±14b	322±30c	364±44d	439±40e	31±14a	41±90a	57 ±60a
FCR	0.98±0.1a	1.00±0.1a	1.01±0.1a	0.94±0.1a	14.13±7.4b	10.30±2.0b	7.82±1.0b

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).

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).

M. macrocopa fed on rice bran suspension resulted in offspring production per broodstock (on the second day) higher (13.25 individual) than that fed on cassava bran suspension (4.00 individual), which was a consequence of high

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

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

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

Treatments		Day 5			Day 7		
		(RNA)µg /µg	(DNA)µg/µg	RNA/DNA	(RNA)µg/µg	(DNA)µg/µg	RNA/DNA
Rice bran	A	0.066±0.001a	0.292±0.008a	0.23±0.01a	0.034±0.000a	0.078±0.001b	0.43±0.01b
	B	0.069±0.001a	0.284±0.010a	0.24±0.01a	0.083±0.001a	0.100±0.001ab	0.82±0.02a
	C	0.055±0.001b	0.272±0.006ab	0.20±0.01ab	0.023±0.000c	0.065±0.007b	0.36±0.03b
	D	0.056±0.005b	0.246±0.002b	0.23±0.02a	0.031±0.001bc	0.067±0.001b	0.46±0.02ab
Cassava bran	E	0.043±0.002c	0.213±0.018bc	0.20±0.03ab	0.039±0.00ab	0.119±0.006a	0.32±0.02c
	F	0.043±0.001c	0.179±0.014c	0.24±0.02a	0.037±0.001b	0.096±0.000b	0.38±0.01b
	G	0.048±0.001b	0.255±0.005b	0.19±0.01b	0.016±0.001c	0.035±0.001c	0.45±0.03b

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).

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

Water quality	Research results	Optimal conditions	References
Dissolved oxygen (mg/L)	5.30–3.92	> 3.50	Miah *et al*. (2013)
pH	7.8–7.4	7.0–8.0	Miah *et al*. (2013)
Ammonia (mg/L NH_3)	0.42–0.51	< 2	Miah *et al*. (2013)
Temperature (°C)	27–30	25–31	Tan and Wang (2010)
Hardness (mg/L) CaCO3	59.34	> 50	Tan and Wang (2010)

in increasing somatic growth in Clodosera 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 Clodosera 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).

M. 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*., 3017). 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.,* 3017), 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 *M. macrocopa* DNA concentration. The rise of *M. macrocopa* DNA/RNA ratio cultured in rice bran suspension on day 7 was due to a decrease in the total *M. 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 *M. macrocopa* cultured in cassava bran suspension (0.016–0.039 µg/µg) also faced a decrease on day 7. The highest total *M. macrocopa* RNA concentration on day 7 (0.083 µg/µg) was observed in treatment B.

M. 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). *M. 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 *M. macrocopa* fed on rice bran suspension was still within the normal range for *M. macrocopa* i.e. 50% (Gogoi *et al*., 2016), while *M. macrocopa* fed on cassava bran suspension had lower protein content.

M. 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.

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.

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