Evaluation of the rice bran and cassava suspension use in the production of male moina offsprings and ephippia

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Abstract. Initial information about the reproductive characteristics of *Moina macrocopa* such as the population density and the quality and quantity feed which induces the production of sexual male and female offspring are much needed in the development of ephippia production technology. This research aimed to study the effect of feed quality and quantity, consisting of rice bran and cassava suspension, on moina population growth, male offspring production, and ephippia production. In this study, moina were raised for twelve days using four concentrations of rice bran suspension and three concentrations of cassava suspension. Moina were cultured using rice bran suspension resulted in a peak population five times the peak population produced using the cassava suspension. Feed quality also affected the number of male offspring and ephippia produced. Only moina fed the rice bran suspension feed produced male offspring and ephippia, while the moina fed the suspension cassava feed did not produce any male offspring or ephippia. The four concentrations of rice bran suspension administered in this study produced ephippia with a similar hatching rate; therefore, it could be concluded that the concentration of rice bran suspension in all treatments were adequate in producing ephippia.

Key Words: concentration, feed, culture, density, population.

Introduction. The increased price of artemia cysts has made moina a natural feed choice. However, the use of moina as a natural feed has been facing the issue of seed availability. One of the efforts that have been taken to increase the interest in using moina as a natural feed is the production of ephippia which can be stored for long periods of time and could be hatched any time (Hiruta & Tochinai 2014), either as larva feed or to provide an inoculum in the culture of an unlimited number of moina.

Moina reproduces by apomictic parthenogenesis, where the offspring produced have genetic identities similar to the parents (Simon et al 2003: Hiruta et al 2010). Moina parthenogenetic eggs can develop into sexual male or female offspring which produce ephippia (Dodson et al 2010). The combination between density, feed quality and quantity could induce the production of males and ephippia in the cladocerans. Azuraidi et al (2013) revealed that *Moina micrura* were cultured with a population density of 1,600 ind. L^{-1} fed on *Nannochloropsis oculata* (4×10^2 cells mL^{-1}) produced 186.7 ind. L^{-1} male offspring and 160 ephippia L^{-1}.

Rice bran and cassava tubers are agricultural waste and products, respectively, that abundantly available. Since moina is a nonselective filter feeder organism, rice bran and cassava suspension can be used directly to feed moina (Smirnov 2014). Rice bran and cassava tubers have different nutritional qualities. The results of a laboratory analysis revealed that rice bran suspension contains more protein (0.83%,) and fat (0.79%) than cassava suspension (0.4% protein and 0.02% fat). The low protein content of cassava suspension thereby resulting into low amino acid content; while in fact, the concentration of protein, amino acids, and fat in feed affects the offspring production (fecundity) and the speed of egg development onto hatchings (Fink et al 2011; Koch et al

2011). Rice bran also contains vitamin B (thiamine and pyridoxine) which could increase the production of moina offspring (Mehdipour et al 2011). On the other hand, the high concentration of protein and amino acids (arginine and histidine) in feed could increase the population growth, but could also decrease the production of ephippia in *Daphnia pulex* (Koch et al 2009; Koch et al 2011).

D. magna ephippia (ephippium) need a higher eicosapentaenoic acid (EPA) concentration (2.4 μg/mg dry weight) than subcutaneous (parthenogenetic) eggs (0.01 μg/mg dry weight) (Abrusan et al 2007). The production of Cladoceran ephippia is limited by the availability of poly unsaturated fatty acids, especially EPA, in feed, which also influences the hatching rate (Choi et al 2016). The fat and fatty acid content of rice bran, especially linoleic acid (6.35–6.85%) and ɑ-linolenic acid (0.20–0.27%) (Faria et al 2012), are higher than those of the cassava (0.19% linoleic acid and 0.04% ɑ linolenic acid) (Salvador et al 2014); therefore, the rice bran suspension feed is estimated to be more supportive to ephippia production.

Based on the different nutrient content data between rice bran and cassava tubers, this study was conducted with the purpose of assessing the effect of rice bran and cassava suspension, on moina population growth, male and total offspring production, and the quality and quantity of ephippia produced.

Material and Method

Research design. This study employed the Randomized Block Design based on the type of feed, namely rice bran and cassava suspension. The rice bran suspension feed block consisted of four treatments (A, B, C, D) and the suspension cassava feed block consisted of three treatments (E, F, G), each with four replications.

1. Treatment A: The initial rice bran suspension concentration was 0.3 mL L^{-1} and was increased starting the second day and the end concentration on the twelfth day was 0.6 ml L^{-1}.
2. Treatment B: The initial rice bran suspension concentration was 0.3 mL L^{-1} and was increased starting the second day and the end concentration on the twelfth day was 0.8 ml L^{-1}.
3. Treatment C: The initial rice bran suspension concentration was 0.3 mL L^{-1} and was increased starting the second day and the end concentration on the twelfth day was 1.0 ml L^{-1}.
4. Treatment D: The initial rice bran suspension concentration was 0.3 mL L^{-1} and was increased starting the second day and the end concentration on the twelfth day was 1.2 ml L^{-1}.
5. Treatment E: The initial cassava suspension concentration was 0.3 mL L^{-1} and was increased starting the second day and the end concentration on the twelfth day was 0.8 ml L^{-1}.
6. Treatment F: The initial cassava suspension concentration was 0.3 mL L^{-1} and was increased starting the second day and the end concentration on the twelfth day was 1.0 ml L^{-1}.
7. Treatment G: The initial cassava suspension concentration was 0.3 mL L^{-1} and was increased starting the second day and the end concentration on the twelfth day was 1.2 mL L^{-1}.

The production of rice bran and cassava flour suspension. An amount of 100 grams of each the rice bran and the cassava flour were suspended in 500 mL of water using a blender at a speed of 2,000 rpm for 5 minutes twice. The second suspension was done 30 minutes after the first suspension. Then the suspensions were filtered using 2 mm, 0.1 mm and 40 μm filters. The suspensions that passed the filtration then had more water added to reach a volume of 500 mL. The results of a proximate analysis of the cassava suspension revealed that it contained 72 mg mL^{-1} organic materials, 0.4% protein and 0.02% fat, whereas the rice bran suspension contained 74 mg mL^{-1} organic materials, 0.83% protein, and 0.79% fat. The rice bran suspension had a higher content of the amino acids arginine (0.050%) and histidine (0.020%) than the cassava suspension (arginine 0.005% and histidine 0.003%) (Table 1).

Table 1
The protein, fat, and amino acid (% w/w) concentrations of the rice bran suspension and cassava suspension

Parameter	*Rice bran suspension* (% w/w)	*Cassava suspension* (% w/w)
Water content	92.56	92.80
Protein	0.83	0.4
Fat	0.79	0.02
Essential amino acids		
Leucine	0.060	0.009
Arginine	0.050	0.005
Lysine	0.030	0.003
Histidine	0.020	0.003
Valine	0.040	0.006
Phenylalanine	0.040	0.005
Threonine	0.030	0.005
Methionine	0.020	0.002
Isoleucine	0.030	0.06
Non-essential amino acids		
Glutamate	0.120	0.018
Aspartate	0.070	0.012
Glycine	0.040	0.006
Serine	0.040	0.008
Alanine	0.060	0.009
Tyrosine	0.050	0.008

Media cultivation. The media for moina culture in this study used water from the processed river water collected in the water tank belonging to the Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University. The water from the tank was transfered into a 1,000 L fiberglass tank and aerated for at least three days before being used without any disinfectants. The water from the fiberglass tank was then filtered using a 40 μm filter before being transfered into the experimental containers to eliminate any competitor zooplanktons.

The moina inoculum and culture. The moina used in this study were adapted to the two types of feed for two months and were reared at a density of 20 ind. L^{-1} in containers that were filled with 10 L of water. Every three days, the offspring produced were harvested to be raised in a new container continuously so that the moina were fully adapted to the two types of feed and the offspring produced were all parthenogenetic females. These parthenogenetic female offspring were used as the inoculum in this study with similar initial densities and water volumes (Delbare & Dhert 1996). The moina were cultured began on 6 August 2015 in a closed room in the Natural Feed Laboratory, Department of Aquaculture. During the rearing period, the light intensity above the culturing containers during the day was 900-1,250 lux and at night 50-00 lux. The moina were cultured for 12 days. During the first two days of the rearing, the amount of feed given in all the treatment was the same, 0.3 mL L^{-1}, then starting on the seconds day, the amount of feed given differed between treatments as presented in Table 2.

Table 2

The total daily rice bran and cassava suspension feed concentration during the *Moina macrocopa* rearing for a volume of 10 L

Day	*Treatment*						
	A	*B*	*C*	*D*	*E*	*F*	*G*
	mL / 10 L						
0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
1	3.0	3.0	3.0	3.0	3.0	3.0	3.0
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	4.0	4.6	5.0	5.4	4.6	5.0	5.4
5	4.5	5.3	6.0	6.8	5.3	6.0	6.8
6	4.9	6.0	7.2	8.4	6.0	7.2	8.4
7	5.4	7.0	8.6	10.2	7.0	8.6	10.2
8	6.0	8.0	10.0	12.0	8.0	10.0	12.0
9	6.0	8.0	10.0	12.0	8.0	10.0	12.0
10	6.0	8.0	10.0	12.0	8.0	10.0	12.0
11	6.0	8.0	10.0	12.0	8.0	10.0	12.0
12	6.0	8.0	10.0	12.0	8.0	10.0	12.0

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

The feeds were given twice daily between 08.00-09.00 West Indonesia Time and 19.00–20.00 West Indonesia Time, each 50% of the daily concentration. During the rearing period, the containers were replaced every two days, whereas the water was replaced from the fifth day to the eleventh day. An amount of 33% of the culture media water in the containers were removed, then both the remaining water and the moina were put in a new container, and the same amount of water that was removed was added to make a volume of 10 L. During the rearing period, each container was aerated at a rate of 319 mL minute^{-1}.

The assessment parameters

Population growth. Aeration was disabled for 15 minutes so that the moina dispersed evenly, then moina samples were collected by taking 100 mL of the water from five different sites. Sampling was conducted to count the total moina numbers. Sampling was conducted from day two to day twelve. At the same time with the sample collections, adult moina that were ready to reproduce were collected (20-40 individuals) and then reared in a different container at a density of 66 ind. L^{-1} until they gave birth. The moina offspring were separated from their parents and reared for 24 hours then the number of offspring was counted and the sexes of the offspring determined using a binocular microscope (100x magnification). The results were used to calculate the offspring production per parent and the percentage of male offspring using the equations below:

$$\text{Offspring production per parent} = \frac{\text{Number of moina offspring}}{\text{Number of moina parents}}$$

$$\text{The male offspring per total offspring (\%)} = \frac{\text{Number of male moina offspring}}{\text{Total number of moina offspring}} \times 100$$

$$\text{Potential for males per liter} = \text{percentage of male offspring x total offspring per liter}$$

Collection of ephippia was done from day five to day twelve at the same time as the container change. Ephippia collection began with swirling the container so that all the feed debris and ephippia are collected in the center of the container. The feed debris and ephippia were siphoned with a pipette then moved in a petri dish. Then these were rinsed with water to expose the ephippia. The ephippia were placed in a new petri dish and rinsed three times in the same way before being identified. The ephippia were identified using a binocular microscope (100x magnification). The percentage of ephippia from the total population and the percentage of ephippia containing two, one, and no eggs were calculated using the following equation:

$$\text{\% of ephippia from the total population} = \frac{\text{Number of ephippia per liter}}{\text{Population of moina per liter}} \times 100$$

$$\text{\% of ephippia containing (X) eggs} = \frac{\text{Number of ephippia containing (x) eggs}}{\text{Population of moina per liter}} \times 100$$

Where: X is the number of eggs in the ephippia.

Hatching rate assessment. Ephippia (containing two eggs) were stored wet by placing the ephippia in an Eppendorf filled with distilled water at a density of 200 eggs/mL. The Eppendorf was then placed in a light-proof container and stored at 5±1ºC in a refrigerator (Haghparast et al 2012). After two months of storage, fifty moina ephippia from each treatment were hatched in a glass container containing 300 mL of water with a light intensity of 1800 lux (Haghparast et al 2012). The hatched moina (36-48 hours) were moved and counted. The moina ephippia hatching rate was calculated based on the equation in Haghparast et al (2012) where Ii is the hatching index and Ni is the number of larvae that hatched.

$$Hatching\ rate = \sum_{i=3}^{15} \frac{N_i}{N_e} \times I_i$$

The potential hatched moina was calculated based on the following equation:

Potential hatched moina (L) = hatching rate x (number of ephippia containing egg/50)

During the culturing period, the water quality was assessed for its dissolved oxygen, pH, temperature and total ammonia and hardness.

Chemical analysis. The analyses of the amino acids in the rice bran suspension and cassava suspension were conducted using the HPLC from the Hewlett Packard Series 1100 and the proximate analysis (protein and fat) was conducted based on the AOAC standard method (1995).

Data analysis. Data from the observation were analyzed using ANOVA. If the analysis of variance of a treatment revealed a significantly different result, it is continued with Duncan's Multiple Range Test to identify treatment with the best response at a confidence level of 95%.

Results. Moina were cultured using rice bran suspension feed resulted in a higher population than using cassava suspension feed. The peak population using the rice bran suspension treatment was five times higher than that of the cassava suspension treatment (Figure1).

Moina were cultured using rice bran suspension feed significantly produced more offspring per parent than using cassava suspension. The production of offspring using the rice bran suspension treatment was twice higher than using the cassava suspension treatment. However, the amount of rice bran suspension had the same effect on the number of offspring produced. This was similar the cassava suspension treatment; the number of offspring produced was not significantly different (Figure 2).

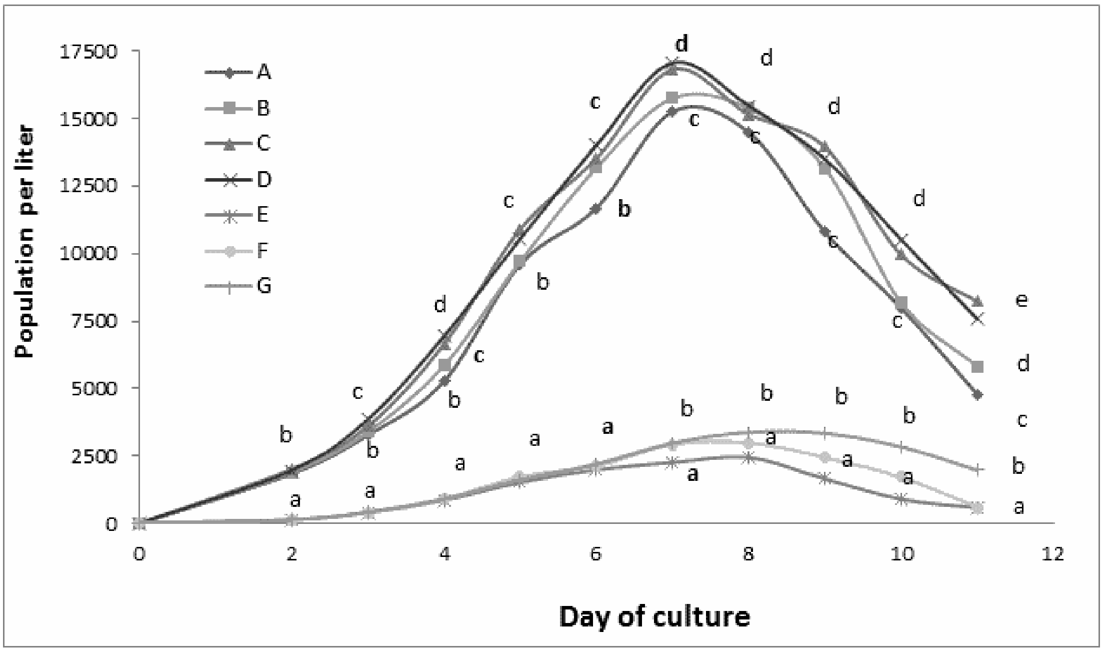

Figure 1. The population of *Moina macrocopa* (ind./L) was cultured using rice bran suspension feed (A, B, C, D) and cassava suspension feed (E, F, G). Note: Different lower case letters on the same day of sampling show significant difference (P<0.05).

As the rearing time increased, until day 7 and 8, the moina population increased (Figure 1), but as the population density increased, the production of offspring per parent decreased (Figure 2).

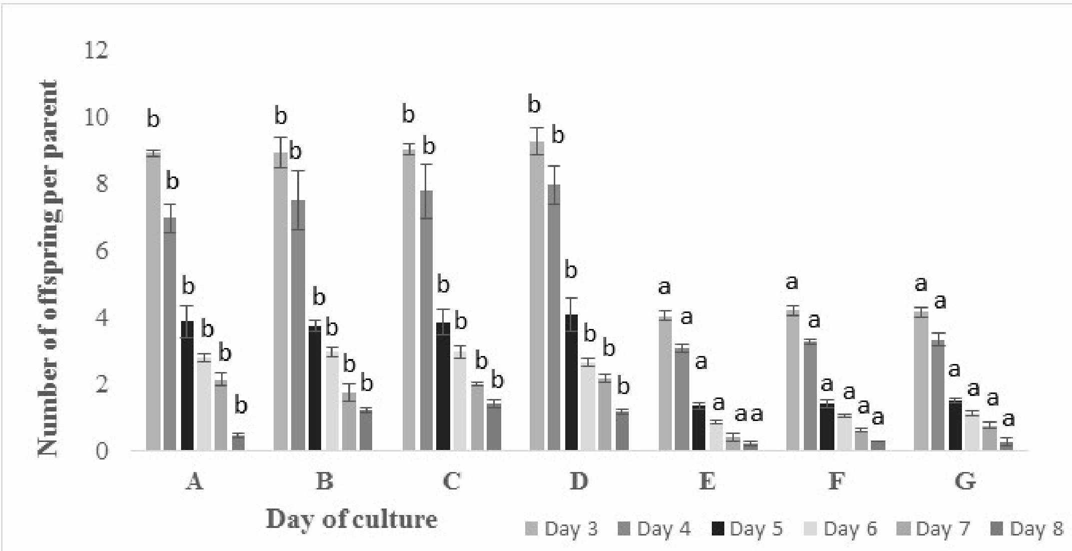

Figure 2. Production of offspring per parent in *Moina macrocopa* was curtured using rice bran suspension (A, B, C, D) and cassava suspension (E, F, G). Note: Different lower case letters on the same day of sampling show significant difference (P<0.05).

The offspring produced by the moina (Figure 2) were divided into female and male individuals. Male offspring are only produced by the moina in the rice bran suspension treatment, whereas in the cassava suspension treatment there were no male offspring found (Figure 3).

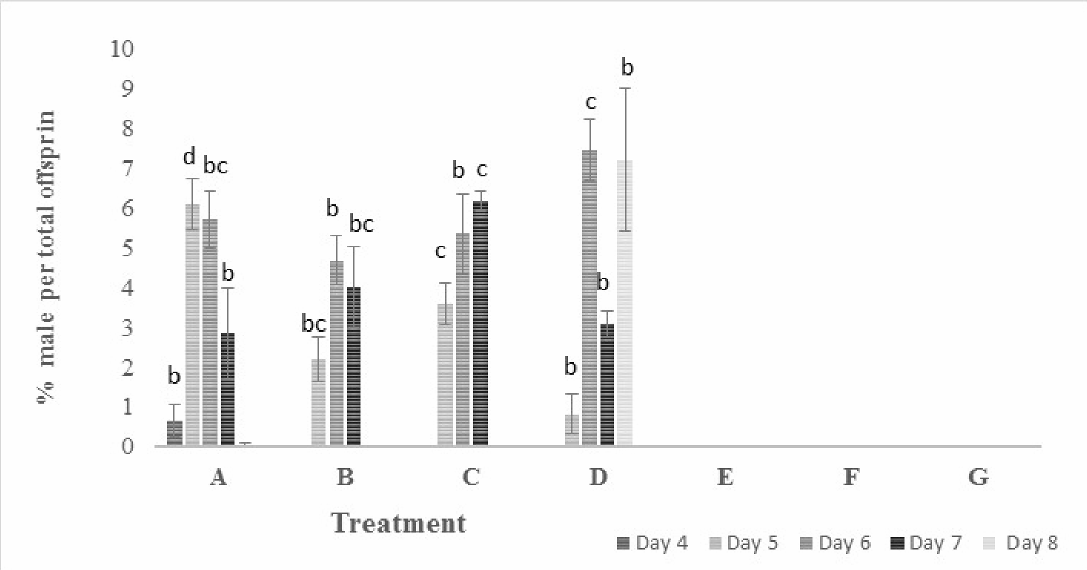

Figure 3. Production of male offspring per total offspring in *Moina macrocopa* was cultured using rice bran suspension (A, B, C, D) and cassava suspension (E, F, G). Note: Different lower case letters on the same day of sampling show significant difference (P<0.05).

The population of male offspring produced each day varied. The production of male offspring on day four in treatment A (rice bran suspension) was only 0.66%. The production of male moina offspring was the highest on day five at 6.1% in treatment A and on day six at 7.46% in treatment D (Figure 3). The total number of males in the population was the highest in treatment A at 504 ind. L^{-1} (Table 2).

Male moina can be identified by the elongated first antenna as can be seen in Figure 4A, whereas the sexual females can only be recognized after synthesizing large, dark eggs in the ovaries or after the eggs are released in the brooding room (Figure 4B).

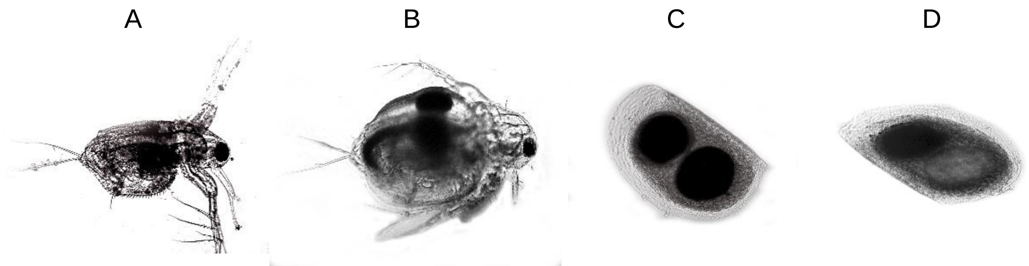

Figure 4. The morphology of male *Moina macrocopa* (A), sexual females (B), an ephippium containing two eggs (C) and an ephippium containing a single egg (D) (original).

Moina were cultured using rice bran suspension feed, produced ephippia starting from day seven with the highest daily production on day nine. The total moina ephippia production for treatments A, B, C, and D were 262±45 eggs L^{-1}, 304±82 eggs L^{-1}, 360±81 eggs L^{-1}, and 326±47 eggs L^{-1}, respectively. Moina were cultured using cassava suspension feed did not produce any ephippia (Table 3 and Figure 5).

As it is explained previously, the peak moina populations in the rice bran suspension treatments were higher than in the cassava suspension treatment. The peak moina population in treatment D was higher than that of treatment A but was not significantly different from treatments B and C (Table 3).

The ephippia production in treatment C was higher than that of treatments A and B, but was not significantly different from treatment D.

Table 3

The production, quality and hatching rate ephippia of *Moina macrocopa* was cultured using different rice bran suspension feed concentrations

Parameter	Treatment						
	Bran suspension				*Cassava suspension*		
	A	*B*	*C*	*D*	*E*	*F*	*G*
Population on day 7 (ind. L^{-1})	1,5240±1,112[b]	15,735±889[bc]	16,795±885[bc]	17,045±1048[c]	2,270±121[a]	2,937±108[a]	3,092±87[a]
Ephippia production (egg L^{-1})	262±45[b]	304±82[b]	360±81[c]	326±47[bc]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Ephippia productivity in the population (%)	1.73±0.38[b]	1.93±0.51[b]	2.15±0.53[b]	1.90±0.16[b]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Male in the population (ind. L^{-1})	504±42[c]	347±58[b]	437±111[b]	400±116[b]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
2 egg ephippia	87.50±2.89[b]	90.00±2.83[b]	88.50±1.73[b]	86.00±2.94[b]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
1 egg ephippia	9.75±1.89[b]	8.00±2.16[b]	8.00±1.41[b]	10.25±2.50[b]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Empty egg ephippia	3.00±1.41[b]	2.00±0.82[b]	3.50±0.58[b]	3.75±1.26[b]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Hatching rate (%)	16.25±4.50[b]	22.50±4.95[b]	19.63±5.23[b]	22.00±1.29[b]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Hatched moina offspring potential	81±25[b]	136±57[b]	123±36[b]	123±36[b]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]

ind. – individuals.

The ephippia productivity in the population was relatively low, only 1.72-2.14%. The ephippia were classified into three categories, ephippia containing 1, 2, and no eggs, but ephippia containing two eggs were very numerous and dominated the population in all the rice bran suspension treatments, ranging between 86 and 90% (Table 3).

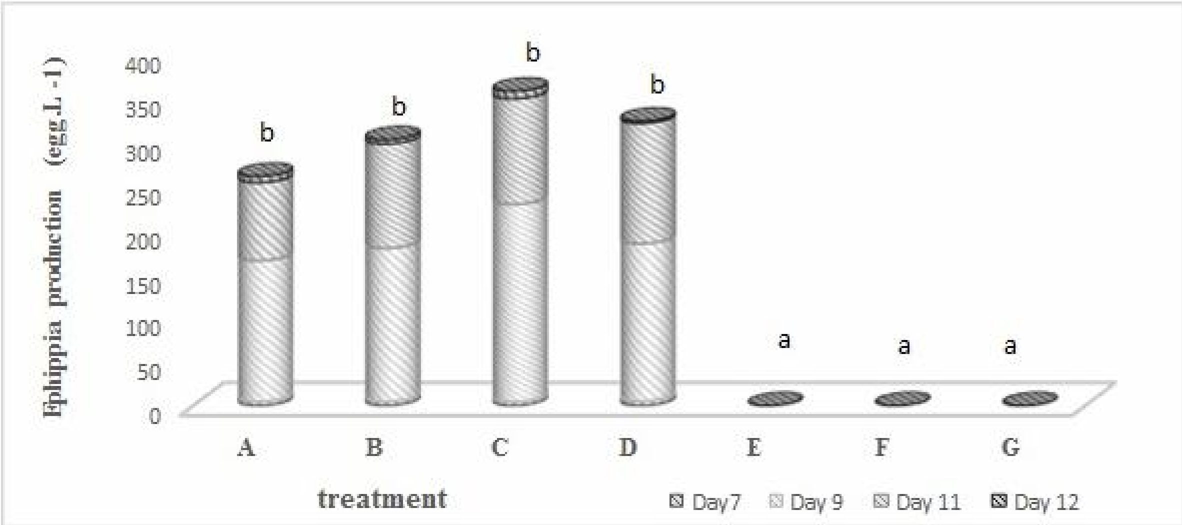

Figure 5. Production of ephippia of *Moina macrocopa* was cultured using rice bran suspension (A, B, C, D) and cassava suspension (E, F, G). Note: Different lower case letters on the same day of sampling show significant difference (P<0.05).

The moina ephippia containing two eggs produced using rice bran suspension feed treatments had similar hatching rates, between 15.63% and 22.13%. The number of potential hatched moina from the ephippia moina in treatments A, B, C, and D were not significantly different, between 76 to 131 eggs per liter cultivation media (Table 2).

Culture of moina was conducted in the pH rage of 7.7–7.4, and the water temperature during the study was varying between 27ºC and 31ºC. The dissolved oxygen at the beginning of the study ranged between 5.5 and 5.7 mg L^{-1}, but nearing the end of the study, the dissolved oxygen decreased, and the rice bran suspension treatment had a lower amount than the cassava suspension treatment. The total ammonia at the end of the study in all the treatments was less than 0.5 mg L^{-1} (Table 4).

Table 4

The quality of the water in the culture of *Moina macrocopa* using rice bran suspension feed (A, B, C, D) and cassava suspension feed (E, F, G)

Parameter	*Treatment*						
	Bran suspension				*Cassava suspension*		
	A	*B*	*C*	*D*	*E*	*F*	*G*
pH	7.7-7.5	7.7-7.5	7.7-7.4	7.7-7.4	7.7-7.4	7.7-7.4	7.7-7.4
Temperature (°C)	27-31						
Dissolved oxygen (mg L^{-1})	5.5-3.25	5.5-3.17	5.5-2.93	5.5-2.85	5.7-5.0	5.6-5.0	5.6-4.9
Total ammonia (NH3/NH4+)							
Initial (mg L^{-1})	0.00						
Day eleven (mg L^{-1})	<0.25	<0.25	0.25	0.25	<0.25	<0.25	<0.25
Water hardness (mg L^{-1})	48.73						

Discussion. The quality and quantity of feed are an important factor in the culture of moina which directly affects population growth (Hakima et al 2013). Moina were cultured using rice bran suspension resulted in a peak population five times higher than that of the cassava suspension treatment (Figure 1 and Table 3). The difference in population was because the production of offspring per moina parent in rice bran suspension feed treatments was higher than that of the cassava suspension feed treatments (Figure 2). Increasing the rice bran concentration had an effect on the increased population of moina, so the highest rice bran concentration (treatment D) resulted in the highest population. However, the population difference between treatments did not result in any difference in ephippia productivity, which led to hatching rates and numbers of offspring that were not significantly different between rice bran treatments also. Therefore, the rice bran concentrations between treatments were relatively similar in adequacy.

Rice bran suspension contains more protein (0.83%), fat (0.79%), and the amino acids arginine (0.050%) and histidine (0.020%) than cassava suspension (arginine 0.005% and histidine 0.003%). Arginine influences the endocrine and reproduction regulation (Jobgen et al 2006), while histidine influences DNA and protein synthesis (Li et al 2008). Moina were cultured using rice bran suspension feed with a higher concentration of protein, amino acids (arginine and histidine) results in a higher moina population and offspring per parent compared to moina were cultured using cassava suspension feed (Figure 2).

The protein, amino acid and fat concentration in feed influences fecundity and the production of offspring per parent and the speed of embryo development in Cladocera (Fink et al 2011; Koch et al 2011). Vitamin B (thiamine and pyridoxine) in rice bran could also increase moina offspring production (Mehdipour et al 2011). The high concentration of protein, amino acids, fat and vitamin B in rice bran suspension is the factor that caused the cultivation of moina using rice bran suspension to reach a peak population that was five times higher than that using cassava suspension.

The increased moina population density using rice bran suspension feed on day five (9,500 ind. L^{-1} – 10,500 ind. L^{-1}) was followed by a decrease in the offspring production per parent by 40% of the highest number of offspring (on day three). Population density is a factor that could induce the production of sexual male and female offspring in moina (*M. macrocopa americana*) when followed by a sudden decrease in the amount of feed available (D'Abramo 1980; Dodson et al 2010; Azuraidi et al 2013). A high population density with adequate feed could induce the production of sexual females (Azuraidi et al 2013) because cultivation in high densities and adequate feed results in smaller sexual female offspring (Frost et al 2010).

The quality and quantity of feed, especially amino acids, could affect moina's somatic growth rate and the female offspring's ability to reproduce by parthenogenesis (Koch et al 2009, 2011). Low feed quantity and poor quality (concentrations of protein and amino acid) could reduce the moina's parthenogenetic reproduction abilities and could induce the gametogenic process to produce ephippia if the other nutritional requirements, especially polyunsaturated fatty acid (PUF_{AS})/ EPA are fulfilled (Abrusan et al 2007; Zadareev & Lapotima 2007). Moina ephippia are produced by newly born female offspring that do not reproduce parthenogenetically in adequate feed conditions (Alekseev et al 2007; Azuraidi et al 2013).

Feed is an important factor in the shift from parthenogenetic reproduction to sexual reproduction in Cladocera because most of the energy from metabolism (68%) is used for reproduction (Richman 1958). Moina were cultured using rice bran suspension feed in treatment A resulted in the highest population of male offspring, 504 ind.L^{-1}. On the other hand, an increased concentration of the rice bran suspension reduced the production of male offspring. The reduced number of male offspring per parent due to an increase in the concentration of rice bran suspension was postulated to be brought about by an increased input of arginine and histidine which triggered the production of parthenogenetic female offspring and thus reduced the production of male offspring (Koch et al 2009; Koch et al 2011), even though rice bran contains a high concentration of PUFA which will induce the number of eggs in the ephippia (Abrusan et al 2007). Moina were cultured using the cassava suspension did not result in any male offspring or ephippia/sexual females. This was due to the low content of amino acids and PUFA which are important in the production of male offspring, ephippia and resting eggs (ephippium). Feed quality (PUFA concentration) could increase sexual activity, including the copulation duration, leading to an increased possibility of fertilization (Choi et al 2016). A low male to sexual female ratio increases the number of eggless or damaged ephippia, while a high male ratio would increase the number of sexual females which are mated and two-egg ephippia (Winsor & Innes 2002). The findings of the aforementioned studies are in line with this study's findings that moina given cassava suspension could not produce male offspring and ephippia/sexual females.

Moina ephippia that resulted from culture with fed a rice bran suspension had a hatching rate of 15.63-22.12%. The low hatching rate was thought to be due to the low content of the fatty acid ɑ-linolenic acid in rice bran. Rice bran has a high content of linolenic acid (6.35-6.85%) and low content of ɑ-linolenic acid (0.2-0.27%). Choi et al (2016) discovered a low hatching rate in *D. magna* ephippia fed chlorella (*Chlorella vulgaris*) at 28% compared to *Stephanodiscus hantzschii* (80%) which was caused by the low PUFA concentration in *C. vulgaris* (Faria et al 2012; Persson & Vrede 2006). Some Cladocera species are able to convert ɑ-linolenic acid to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) with a varied ability among species (Masclaux et al 2012). However, an increased concentration of EPA in *D. magna* feed increases the number of ephippia produced (Abrusan et al 2007) and increases the ephippia hatching rate (90%) (Sperfeld & Wacker 2012).

The physiological functions of the embryo during the dormant period decreases, for example, the oxygen consumption and metabolism decline (Alekseev et al 2006). The decline in physiological functions is useful in safeguarding the nutrient reserves and in preventing cellular damage due to the production of reactive oxygen species (ROS) from mitochondrial activity during storage (Murphy 2009). On the other hand, reactive oxygen (H_2O_2) plays an important role in ending the dormant period (Clegg et al 1996).

The ephippia hatching rate is also influenced by a number of factors such as illumination, temperature, pH, and storage duration (Stross 1966). Light intensity and pigment concentration on the ephippia shell could cause a variation in the hatching rate. Pigments on the ephippia shell protect the embryo from UV radiation, so a decrease in pigment concentration during storage would increase the embryos' sensitivity to light at hatching (Pinceel et al 2013).

In this study, moina ephippia were stored at a temperature of 5±1ºC fortwo months and the sensitivity at hatching was incresaed by exposure to light (1,800 lux) in accordance with the method of Haghparast et al (2012).

Conclusions. From this study, it could be concluded that differences in feed quality have an effect on the moina population, production of offspring per parent, production of male offspring, and the number of ephippia produced. The population of moina were cultured using rice bran suspension feed was higher than the population of moina were cultured using cassava suspension. The four rice bran suspension concentrations used in this study resulted in similar ephippia production with the same hatching rate.

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