

## QUALITY AND QUANTITY OF EPHIPIA MOINA MACROCOPA RESULTING FROM MATING USING TILAPIA FISH FECES SUSPENSION FEED

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### ABSTRACT

The needs of *Artemia* cysts in Indonesia currently reach  $\pm$  150 tons / year and all of them are from the imports, so it can become an obstacle in process of hatching fish and shrimp in the future. Increased Culture activities have an impact on increasing fisheries waste production, including fish feces. Fish feces have been proven to be used for the Culture of *Moina macrocopa*, but have not been tested for the production for ephippia *M. macrocopa*. This study aims to examine the use of fish aquaculture/feces as feed for the quality and quantity of ephippia *M. macrocopa*. The treatment in this study was the concentration of suspension of tilapia feces 55 mg / L, 64 mg / L, and 73 mg / L and using rice bran suspension feed control 64 mg / L. The results of this study indicate that, the concentration of suspension of tilapia feces does not affect the production and hatching rate of ephippia *M. macrocopa*. *M. macrocopa* which was cultured with suspension of tilapia feces produced ephippia production and with a lower hatching rate compared to *M. macrocopa* with rice bran suspension feed.

Key words: hatching fish, cultural activity, fisheries waste.

مبارك وآخرون

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جودة وكمية ماكروفيوفا الناتجة عن التزاوج باستعمال علف تعليق أسماك البلطي  
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### المستخلص

تصل احتياجات خراجات *Artemia* في إندونيسيا حالياً إلى 150 طنًا / سنويًا وجميعها من الواردات ، لذلك يمكن أن تصبح عقبة أمام عملية تفريخ الأسماك والروبيان في المستقبل. تؤثر أنشطة الاستزراع المتزايد على زيادة إنتاج نفايات مصايد الأسماك ، بما في ذلك براز الأسماك. لقد ثبت أن البراز السمكي يستخدم لثقافة *Moina macrocopa* ، لكن لم يتم اختباره من أجل إنتاج مادة *ephipia M. macrocopa*. تهدف هذه الدراسة إلى دراسة استخدام الاستزراع المائي للأسماك / البراز كعلف لجودة ونوعية مرض الأيبيبيا *macrocopa*. كان العلاج في هذه الدراسة هو تركيز تعليق براز البلطي 55 مجم / لتر ، 64 مجم / لتر ، و 73 مجم / لتر واستخدام التحكم في تغذية تعليق نخالة الأرز 64 ملغم / لتر. تشير نتائج هذه الدراسة إلى أن التركيز من تعليق براز البلطي لا يؤثر على معدل إنتاج وتفقيس من *ephipia M. macrocopa*. أنتجت *M. macrocopa* التي تم تربيتها مع تعليق براز البلطي إنتاج أبيتافيا مع انخفاض معدل الفقس .

كلمات مفتاحية : الاستزراع ، الروبيان ، نفايات ، مصايد الاسماك

## INTRODUCTION

The price of *Artemia* cysts is increasing and making *M. macrocopa* as one of the choice for natural food for fish and shrimp larvae (6). *M. macrocopa* as a natural food has an advantage as they meets size requirements ( $\pm 400$  um) and nutritional value comparable to *Artemia* (20), *Moina* can breed and grow rapidly, and also can be cultured using agricultural waste, livestock and food industries feed (28, 6). The utilization of *M. macrocopa* as a natural food for fish and shrimp larvae has not been maximized because the Culture of *M. macrocopa* is done like fish farming. Therefore it is necessary to find a new breakthrough so that *M. macrocopa* is no longer cultured, but using ephippia which is hatched as practically uses *Artemia* as a natural food. *M. macrocopa* Ephippia can be stored for a long time and hatched at any time (12), both as fish and shrimp larvae feed and providing inoculants in the Culture of *M. macrocopa*. *Moina* can multiply asexually (parthenogenesis) and sexually (32: 11). *Moina* sexual reproduction begins with female parthenogenesis producing male and female sexual offspring (6). *Moina*'s sexual female is a female offspring who does not reproduce parthenogenically, the eggs of a sexual female will be fertilized by males and subsequently undergo modification of carapace thickening to form ephippia (2). Our results have previously been successful in producing ephippia *M. macrocopa* with high hatchability (55%) in Culture using a combination of rice bran feed (70%) and fish meal (30%) (26). Sustainable Aquaculture development is currently a global issue. One of them is in the usage of aquaculture waste which still contains high amounts of nutrients from fish feces and excess feed for fish that has not been eaten. Aquaculture waste contains nitrogen and phosphor of 10-20% of total nitrogen in feed. In natural environments, these compounds are nutrients that are important for the growth of phytoplankton and zooplankton (16). The development of technology for the utilization of aquaculture waste can be a good asset for strengthening the potential of Indonesian fisheries in the supply of natural food, especially zooplankton, which is currently being fulfilled from imports (*Artemia* cysts).

Loh *et al.* (20,21) reported that fish feces/waste in *M. macrocopa* Culture produced a population with high density. *M. macrocopa* cultured with fish feces feed / waste gives birth at the age of 3 days and produces a high production offspring per parent (17 ind/parent). But with a lower intrinsic growth in the offspring, low intrinsic growth can inhibit reproduction of parthenogenesis (9), so that it encourages the process of gametogenesis of female offspring to produce ephippia eggs (36). The quantity and quality of ephippia *Moina* is related to the quality of nutrients received by the parent (31). Production of Ephippia and the degree of hatching of cladocera is limited by *poly unsaturated fatty acids* (PUFAs) (4) , as opposed to lower protein and amino acids that can increase the production of ephippia of *Daphnia magna* (17,18) From the analysis before it can be concluded that the problem is whether aquaculture waste/fish feces can be used for the production of good quality ephippia *M. macrocopa*. The aim of the study was to study the utilization of fish aquaculture/feces as feed for the quality and quantity of ephippia *M. macrocopa*.

## MATERIALS AND METHODS

This study is using a completely randomized design (CRD) with four treatments and each treatment was repeated three times. The treatment is the concentration of tilapia feces in *M. macrocopa* mating, which is: 1) control with rice bran suspension feed 64 mg/ L, 2). Treatment A., fish feces concentration 55 mg/ L, 3). B. Treatment of feces concentration 64 mg L, 4). Treatment C. feces concentration 73 mg L.

### Fish feces collection

Fish feces are obtained from tilapia (*Oreochromis niloticus*) Culture with dissolved oxygen system level of  $5.00 \pm 1.00$  mg / L, pH 7-8 which is maintained at room temperature (29-32 ° C). Fish is fed with commercial pellet feed which is 3-5% of the weight of the fish. Fish feces is taken by chiffling (*penyiponan*) and dredging (*penyerokan*) method. Feces are collected and dried in an oven at 50 ° C for 2 days. Feces solids are stored before usage (Looh et al 2009).

**Rice bran and fish feces production**

Each fish's rice bran and feces are 100 g in a blender glass, then 500 mL of water added and suspended using a blender at 2000 rpm for 5 minutes twice. The second suspension is done after 30 minutes of the first suspension. Furthermore, the water suspension was filtered using a 2 mm, 0.1 mm and 40  $\mu$ m filter. The suspension that passes through the filter is added with water and the volume becomes 500 ml (25).

**Analysis of protein, amino acids, fatty rice bran amino acids and fish feces**

The rice bran suspension and fish feces suspension were tested using proximate based on the AOAC standard method (1995). Amino acids and fatty acids are measured using *high-performance liquid chromatography (HPLC)* from Hewlett Packard Series 1,100, based on the Fardiaz method (1989)

**Provision of sexual females, males and mating of *M. macrocopa***

*M. macrocopa* which was used in this study came from ephippia hatching. *M. macrocopa* was adapted to rice bran suspension feed for two months in Culture with a density of 20 ind/L in a container filled with 10 L of water. Sexual female *M. macrocopa* was produced from culture with a parent density of 660 ind/L using aeration (28 ml/minute), rice bran suspension of 56-80 $\mu$ g/parent, tilapia fish *kairomone* 3.86 mg/L. *M. macrocopa* male offspring were produced from Culture without aeration with a parent density of 660 ind/L and using 45-54  $\mu$ g/mother rice bran suspension (26). Male and female offspring

were separated from the mother by screening and re-cultured with a density of 1000 ind/L with rice bran suspension feed which supporting ephippia production (36). During the preservation, concentrated rice bran suspension was given as shown in Table 1. Culture of *M. macrocopa* was carried out in a closed room with daylight illumination 700-900 lux and nighttime light 50 -100 lux. On the second day (24 hours old) the identification and separation of male offspring is carried out, especially in Culture for the supply of sexual females. Males are kept until they are 3 days old (70 hours) before being used in mating. The mating of *M. macrocopa* uses 70-hours-old female offspring, each of 30 female individuals and 9 male individuals is included in containers with a volume of one *M. macrocopa* every 2 ml water (;36) during preservation, every day 100% of the containers and water are replaced. Along with the replacement of water, the addition of feed is carried out according to each treatment

**Quality and quantity of ephippia *M. macrocopa***

Collection and calculation of the amount of ephippia produced began at the age of four days *M. macrocopa* (96 hours) to six days. The quality of ephippia was identified based on the number of eggs in ephippia using a binocular microscope with 100x magnification. The percentage of ephippia in total females in mating and the percentage of ephippia containing n eggs (n is the number of eggs in ephippia) is calculated using the equation below:

$$\text{Ephippia with (n) egg (\%)} = \frac{\text{Amount of ephippia with (n)}}{\text{Total amount of ephippia}} \times 100\%$$

$$\text{Ephippia per total female (\%)} = \frac{\text{Amount of ephippia } Moina}{\text{Amount of female } Moina} \times 100\%$$

During the Culture period measurements of water quality including dissolved oxygen, pH, temperature, and total ammonia.

**Hatching ability test of *M. macrocopa***

Ephippia containing two eggs was stored wet, with ephippia inserted in a micro tube (Eppendorf) containing 1 mL of distilled water. Then the micro tubes are inserted in a light-tight container and stored in a refrigerator

at a temperature of  $6 \pm 1^\circ \text{C}$  (10). Ephippia from each treatment after being stored for two months, was hatched in a glass container containing 300 ml of water with an intensity of exposure light with 1800 lux (10).. *M. macrocopa* which hatched is removed and counted on the second and third days. The degree of ephippia hatching is calculated based on the equation Haghparast et al. (10). It is the

hatching index and  $N_i$  is the number of *M. macrocopa* that successfully hatched.

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

### Fatty acid analysis of ephippia *M. macrocopa*

Fatty acid analysis was carried out on ephippia produced from Culture with rice bran suspension feed (control), fish feces (A), and feces with fish oil emulsion (B). Ephippia produced from Culture with an initial density of 20 ind/L with a volume of 10 ml of water. On sixth days *Moina* is fed with rice bran suspension. And on the sixth to twelfth day were given feed according to the treatment. On the eighth day ephippia was harvested. Ephippia is harvested by turning/flipping water so that ephippia and dirt accumulate in the middle of the tub. Ephippia is separated from feces and washed three times before storage (24). Analysis of fatty acid ephippia *M. macrocopa* carried out by using 10 grams of each (wet weight) based on the Fardiaz (1989) method using Shimadzu GC-2010 Gas Chromatography

### Data Analysis

Data from the observations were analyzed using ANOVA. If the results of variance analysis are known that the treatment shows results that are significantly different or very really different, then Duncan's Multiple Range Test is continued to find out the treatment with the best response at 95% confidence level.

## RESULTS AND DISCUSSION

Male *M. macrocopa* offspring can be easily identified after 24 hours of age based on their smaller size and the presence of the first antenna which is longer than the female *M. macrocopa* (Figure 1A). Sexual females have the same morphology as parthenogenetic females, sexual females can be identified after synthesizing eggs in ovaries with a large size and darker color at the age of 70 hours (Figure 1B). Mating of tilapia using the ratio of 9: 30 female male sex.

### Survival rate of *M. macrocopa*

The suspension of tilapia feces was carried out during the mating of *M. macrocopa* with a concentration of 55 mg / L dissolved organic matter, 64 mg / L, and 73 mg / L. The survival rate of *M. macrocopa* during mating is not

affected by feed treatment after first and second ephippia production (Figure 2.). The survival rate of *Moina* after the third highest day of Culture on mating was a rice bran suspension of  $80.8 \pm 3.2\%$ .

### Ephippia production

Usage of tilapia feces suspension caused a decrease in ephippia production and ephippia percentage of total females in mating (figure 3A). The concentration of tilapia feces suspension used did not cause a difference in the percentage of ephippia in females in mating. The percentage of ephippia in total female *M. macrocopa* in mating using tilapia feces suspension was 69-75%, while those using rice bran suspension feed were  $88.84 \pm 2.8\%$ . The substitution of rice bran suspension feed with tilapia feces suspension did not cause a significant difference in the percentage of ephippia containing two eggs. Reduction of the suspension feed concentration of tilapia feces (55 mg/L) caused a decrease in ephippia containing two eggs to  $89.58 \pm 4.7\%$ . The mating of *M. macrocopa* with a 9:30 female male sexual ratio produces ephippia containing two eggs which is 89.58- 100% high (Figure 3B).

### Hatching rates

*M. macrocopa* mating using feed suspension of tilapia feces produces ephippia with lower hatching rates compared to mating using rice bran suspension feed. Ephippia mating results with rice bran suspension feed have a hatching rate of  $32.50 \pm 1.18\%$  while ephippia of marital results with a suspension of tilapia feces with the same concentration of  $20.33 \pm 2.36\%$ . The increase and decrease in feces concentration of tilapia did not affect the degree of ephippia *M. macrocopa* hatching (Picture 4.). Tilapia feces contain relatively high protein of 16.58% but with a low fat content of 1.84%. Rice bran suspension contains fat (10.54%) which is higher than the suspension of tilapia feces (1.84%) (Table 2). Rice bran having high rice bran content was high in n-6 (linoleic, arachidonic, hecocentric) fatty acids (28.48%), but with low omega 3 fatty acids (0.97). Tilapia feces detected contain low omega-3 but contain EPA (0.14%) and DHA (0.2%) and total omega-6 concentrations of 12.22%. The concentration of protein, amino acids and fat in feed affects the fecundity or

production of offspring per parent and the speed of embryo development in cladocera (18). The fat concentration in feed (*D.magna*) affects the allocation of energy from metabolism for reproduction and growth. Cholesterol is fat which is the precursor to the formation of micro-crustacean hormones (27), increasing somatic growth, while PUFAs play a role in reproduction (34), cholesterol needs must be met from feed because cladocera cannot be synthesized alone. Loh *et al.* (20, 21) reported that fish feces / waste in *M. macrocopa* Culture produced a population with high density with long duration of culture because of the lower intrinsic growth of moina offspring. Low intrinsic growth can inhibit reproduction of parthenogenesis (9) and encourage the process of gametogenesis of female offspring to produce ephippia eggs (36). Our results show that Moina's mating using suspension food of tilapia feces produces low ephippia. This low ephippia production is thought to be due to the low fat content in fish feces (1.84%), because the quality and quantity of ephippia production in cladocera is influenced by the concentration of fat and fatty acids in feed (31). Production of ephippia cladocera is limited by the availability of *poly unsaturated fatty acid* (PUFAS) (4), including DHA and linolenic acid concentrations in feed (14), tilapia feces contain omega-3, EPA and DHA (0.94%) which is lower and omega-6 is 12.22%, which is lower than the content of n-6 fatty acids (linoleic, arachidonic, hecosentrionate) rice bran suspension of 28.48% in the control. Our results also show that fatty acid concentrations in feed which correlate with fatty acids in ephippia *M. macrocopa*. Married ephippia *M. macrocopa* from fish suspension feed has omega-3 of 0.55% and omega-6 of 20.48%, which is lower than the concentration of omega-6 in cultured ephippia with rice bran suspension feed (Table 2). Mated Ephippia *M. macrocopa* using rice bran suspension feed has a percentage of omega-6 acid of 26.90% and omega-3 acid concentration of 0.50%. Mated Ephippia *M. macrocopa* using feces of tilapia feces suspension had omega-6 acid percentage of 20.48% and 0.54% omega-3 acid concentration (Table 3.). Omega-3 fatty acids play an important role in the development and

function of the central nervous system and neurogenesis in the brain, as well as cell division and embryo development (3; 35), where omega-3 fatty acid diets, omega-6 affects neurogenesis in the brain (*Homarus americanus*) and growth speed related to the number of cells entering the S phase (19). In addition, the presence of omega 3 and omega 6 has been shown to increase egg hatchability and survival as in *Artemia*, fish and crustaceans (30, 15). Low percentage of omega-6 in ephippia *M. macrocopa* with fish feces is suspected to be the cause of the decrease in ephippia hatching rate even though Ephippia with suspension food of tilapia feces contains EPA fatty acids (0.55%). Linoleic acid and  $\alpha$ -linolenic acid, and both of these fatty acids are essential fatty acids for cladocera (22; 29). Some cladocera species are reported to be able to synthesize EPA and DHA from  $\alpha$ -linolenic acid, but with different synthesis capabilities (23). Tilapia feces has the potential as a feed for the production of ephippia *M. macrocopa* with increased availability of PUFAS through the addition of fish oil emulsions that are rich in EPA and DHA. Supplementation of PUFA in algae has been shown to increase ephippia production (1) and ephippia *D magna* hatching rate (33). During the male mating Moina only pursues and populates sexual females in a synthesizing eggs condition (over 70 hours old). The duration of mating lasts for 8-10 minutes (8). Mating of *M. rice branchiata* consists of three phases which begin with the male capturing the sexual female, then followed by the male movement to position themselves until perpendicular to the sexual female, then copulation which lasts for 16-25 seconds(7). The male and female sex ratio of *M. macrocopa* affected more the number of ephippia containing two eggs compared to the concentration of suspension of fish feces. Conde *et al.* (5) stated that, Moina's sexual female if not fertilized by a male will produce ephippia without eggs or damaged. This mating using the sexual ratio of male and female sexual *M. macrocopa* which is 9:30, that in order will producing ephippia containing two eggs at 100%, and lower than the sex ratio of female male *M. australiensis* at 4: 5 (13). Male *M. macrocopa* can marry more sexual females.

Water quality during mating of *M. macrocopa* includes water temperatures 29-32° C, water pH 7.5-8, hardness of 60 mg / L CaCO<sub>3</sub>.

Dissolved oxygen at the beginning of the study was 4 mg / L and decreased at the end of the study to 2.8 mg / L (Table 4.).

**Table 1. Concentration of rice bran suspension for the induction of production of male, female sexual and maintenance of *M. Macrocopa* offspring. (26).**

Day	Rice bran Suspension (mg/L)		
	Male Induction	Ehipia Induction	Offspring Preservation
1	29,60	37,00	37,00
2	32,64	44,88	44,88
3	36,86	53,85	53,85
4	40,39	53,85	64,32

**Table 2. The concentration of protein, fat, amino acids (% w / w) of tilapia rice bran and feces**

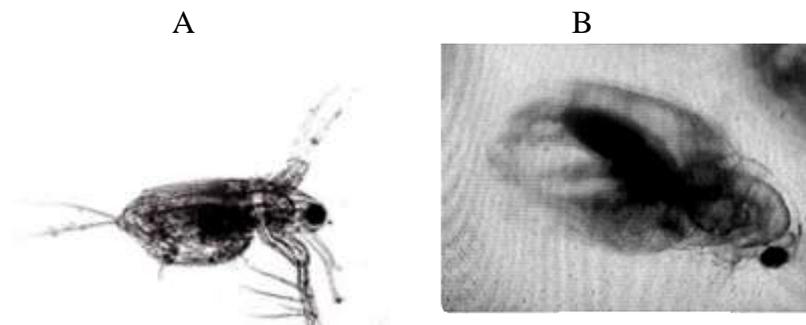
Parameter	Rice bran	Fish feses
	(% w/w)	
Protein	12,16	16,27
Fat	10.52	1,84
Carbohidrate	68,82	18,27
Essential amino acid (%)		
Leusin	4.29	6,377
Arginin	3.82	4,288
Lisin	2.11	3,408
Histidin	1.61	1,429
Valina	3.03	4,508
Fenilalanin	2.89	3,628
Treonin	2.33	3,299
Metionin	1.27	1,154
IsoJeusin	2.13	3,793
Non-esensial amino acid		
Glutamat	8.61	9,731
Aspartat	4.80	6,927
Glisin	3.17	5,223
Serin	2.69	3,738
Alanin	4.31	5,278
Tirosin	3.42	2,199

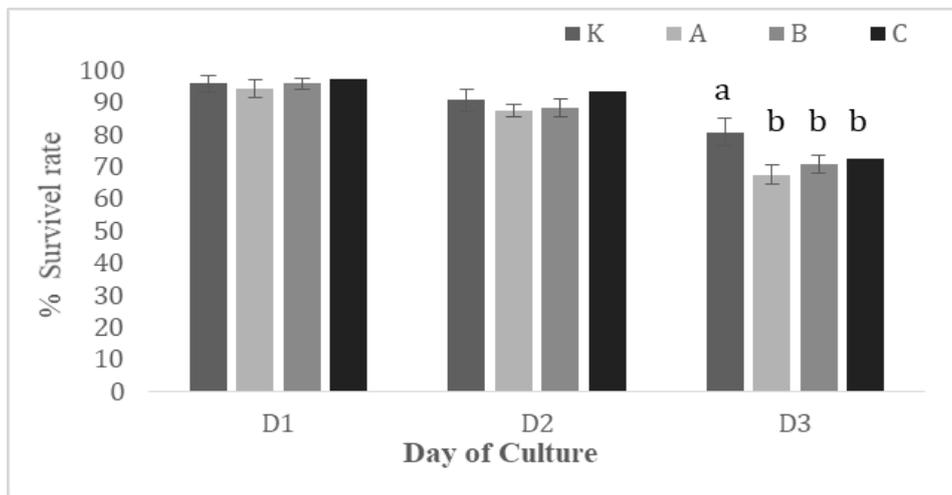
**Table 3. Concentration of fatty acid ehipia *M. macrocopa* (% w / w fat) from Culture using tilapia feed suspension and rice bran suspension**

NO	Fatty acid	<i>Ehipippia M. macrocopa</i>		Rice bran	Fish Feces
		rice bran	Fish feces		
1	12:0	0,17±0,01	0,16±0,02		0,14
2	13:0	0,04±0,01	0,04±0,01		
3	14:0	1,25±0,02	1,41±0,04	0,3	0,88
4	15:0	0,30±0,05	0,31±0,02	0,02	0,16
5	16:0	18,78±0,38	16,9±0,41	14,60	15,53
6	16:1n-7	5,89±0,11	4,52±0,35	0,01	
7	17:0	0,12±0,01	0,15±0,01	0,03	0,22
8	18:0	2,37±0,20	2,60±0,31	1,21	3,00
9	18:1n-7	0,05±0,01	0,04±0,01		
10	18: 1n-9	28,37±1,10	21,22±2,34	31,83	22,98
11	18:2n-6	23,65±1,04	17,30±1,30	28,48	12,19
12	20:0	0,11±0,01	0,13±0,04	0,04	0,26
13	18:3n-6	0,2±0,01	0,15±0,01		0,03
14	20:1	0,25±0,06	0,20±0,02		0,41
15	18:3n-3	0,5±0,08	0,5±0,04	0,97	0,61
16	20:2	0,06±0,01	0,07±0,02	0,04	0,19
17	22-0	0,22±0,01	0,21±0,02	0,26	0,27
18	20:3n-6	0,05±0,02	0,02±0,01		
19	20:5n-3		0,04±0,01		0,13
20	22:6n-3				0,20
21	Total n-3	0,5	0,54	0,97	0,94
22	Total n-6	26,90	20,48	28,48	12,22

**Table 4. Water quality of mating culture media for *M. macrocopa* using rice bran suspension feed and substitutes for suspension of fish meal with different percentages**

No	Parameter	Value
1	Temperature (° C)	29-31
2	pH	7,5-8
3	Hardness (mg/L CaCO <sub>3</sub> )	60
4	Dissolved oxygen (mg/L)	4,0 -2,7
5	Ammoniac total (mg/L)	<0,250

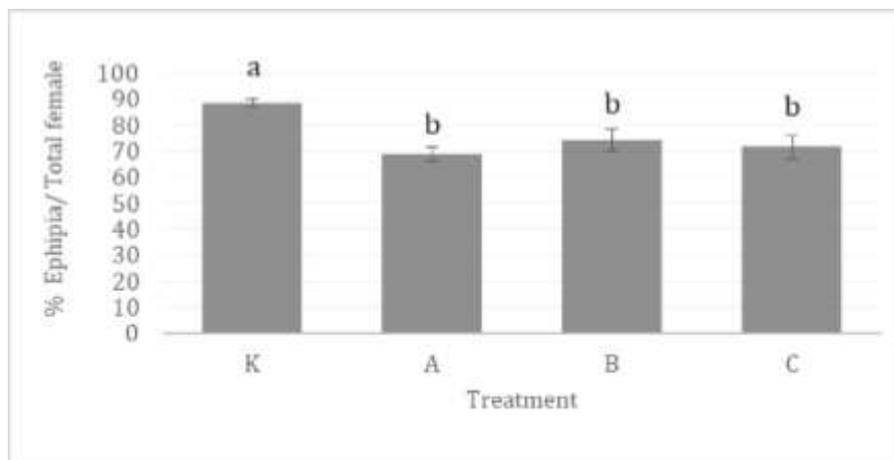
**Picture 1. Morphology of male *M. macrocopa* (A) dan female sexual with egg synthesizing (B).**



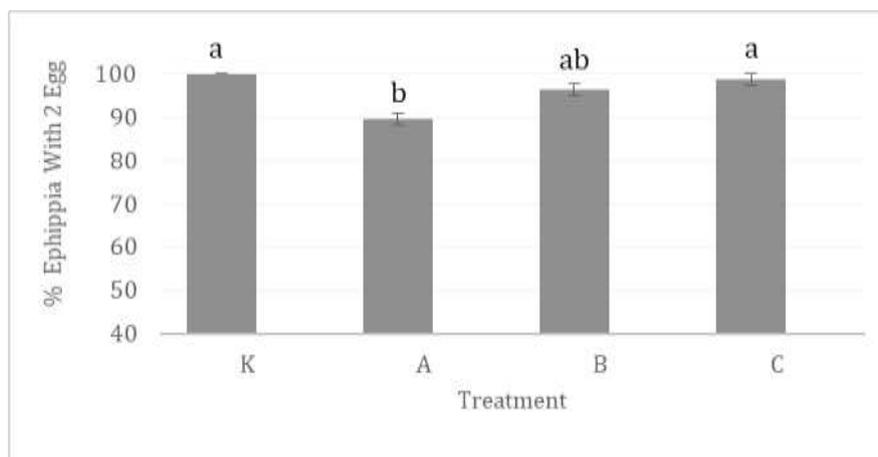
Picture 2. The survival rate of *M. macrocopa* in mating uses 70-hour-old offspring using suspension feed of tilapia feces

Notes: K; rice bran suspension feed 64 mg/L A; feces suspension feed 55 mg/L, B feces suspension feed 64 mg/L, dan C feces suspension feed 73 mg/L

A

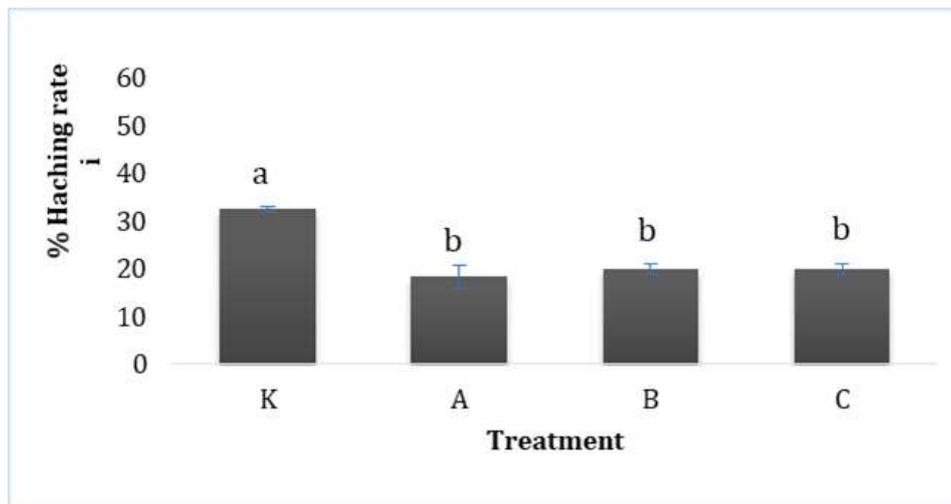


B



Picture 3. Percentage of ephippia production per female total (A) and the percentage of ephippia containing two eggs (B) marital results of *M. macrocopa*

Notes K; rice bran suspension feed 64 mg/L A; feces suspension feed 55 mg/L, B feces suspension feed 64 mg/L, dan C feces suspension feed 73 mg/L



**Picture 4. The degree of marital for ephippia *M. macrocopa* from hatching using suspension feed of tilapia feces**

Notes K; rice bran suspension feed 64 mg/L A; feces suspension feed 55 mg/L, B feces suspension feed 64 mg/L, dan C feces suspension feed 73 mg/L

Suspension concentration of tilapia feces did not affect the production and hatching rate of ephippia *M. macrocopa*. *M. macrocopa* cultured using suspension of tilapia feces producing ephippia with a lower hatching rate compared to *M. macrocopa* with rice bran suspension feed.

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