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The Addition of Bali Sardinella Fish Oil in Rice Bran Suspension to Successful Induction of *Moina macrocopa* Ephippia Production

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Abstract

The production of *Moina macrocopa* ephippia is limited by the availability of polyunsaturated fatty acids (PUFAs) in the feed. The lack amount of ω-3 fatty acids in rice bran suspension limits ephippia production. Bali sardinella fish oil containing eicosapentaenoic acid (EPA) has the potential to be added in rice bran suspension to increase ephippia production. The purpose of this study was to determine the effect and optimal concentration of Bali sardinella fish oil addition in rice bran suspension, which results in the highest ephippia production. This study used the Completely Randomized Design (CRD) method with five treatments adding fish oil in rice bran suspension of 0%, 1.5%, 3%, 4.5%, and 6%), with four replications. Sexual female offspring are produced from cultures with a density of 660 ind/l with regulated feed concentration. Furthermore, the offspring are cultured with feed according to the treatment. During the culture, daily production, total production, and production ephippia per parent. These results indicate that the addition of Bali sardinella fish oil to rice bran suspension as a feed of *M. macrocopa* affects the production ephippia. The addition of Bali sardinella fish oil with a concentration of 6ml/100g in rice bran produces the highest total *M. macrocopa* ephippia production (3452 ± 43.0 ephippia/l).

INTRODUCTION

The sexual female of M. macrocopa produces ephippia. Sexual reproduction begins with female parthenogenesis providing male offspring and sexual females (Mubarak *et al*., 2017). The nutrient quality received by *M. macrocopa* sexual female can affect the quality and quantity of *M. macrocopa* ephippia (Bouchnak and Steinberg, 2014). The production of cladoceran ephippia, including *M. macrocopa*, is limited by the availability of polyunsaturated fatty acids (PUFAs) (Choi *et al*., 2016). Ephippia requires higher EPA concentrations (2.4 µg/mg dry weight) than subcutaneous eggs (parthenogenesis) (0.01 µg/mg dry weight) (Abrusán *et al*., 2007).

Culture of *M. macrocopa* with the aim of producing ephippia using rice bran suspension feed resulted in a total production of ephippia (3052 ± 199 eggs/L) with ephippia containing two eggs (14.22% ± 2.08%) and ephippia containing one egg (8.73% ± 3.14%) (Mubarak *et al*., 2017). The low quality and quantity of ephippia are caused by the

low ω-3 fatty acids content in rice bran suspension. The availability of ω-3 fatty acids is a limiting factor in the production of copepod eggs (Mubarak *et al.*, 2017). ω -3 fatty acids contained in eggs affect the initial stage of embryogenesis. They will determine the development of embryos in ephippia (Mokoginta, 1992). Mokoginta *et al.*, (2003) states that the excess or deficiency of ω-3 fatty acids in eggs causes the embryogenesis process is inhibited. ω -3 fatty acids play an essential role in the process of embryogenesis, especially in the formation of cell membranes (Borlongan and Benitez, 1992). So, it is necessary to add fatty acids from other sources such as Bali sardinella fish oil.

Bali sardinella fish oil contains EPA and DHA of 8.97% and 6.56% (Maulana *et al.*, 2014). Enrichment of bran with fish oil has not been done in *M. macrocopa* culture. The high ω-3 fatty acids in Bali sardinella fish oil are expected to increase the amount of *M. macrocopa* ephippia production. The purpose of this study was to determine the effect of fatty acids addition from Bali sardinella fish on bran suspension to the creation of *M. macrocopa* ephippia. Also, to determine the optimal concentration of fatty acids from Bali sardinella fish on bran suspension, which produced the highest production of *M. macrocopa* ephippia.

METHODOLOGY

Time and Place

This research was conducted in April - May 2019 in the Anatomy and Aquaculture Laboratory of the Faculty of Fisheries and Marine Airlangga University.

Research Material

The tool used in this study consisted of a 1-liter basin, 30 ml small round jar, petri dish, dropping pipette, filter, small plastic cup, aerator, binocular microscope, and blender.

The material used in this research is *Moina macrocopa* obtained from hatching ephippia, rice bran derived from rice milling in the Sidoarjo Gedangan area, Bali sardinella fish oil obtained from fish canning industry waste in Banyuwangi, and water collected from water reservoirs at the Faculty of Fisheries and Marine Airlangga University.

Research Design

This research is experimental using the Randomized Completely Design Method (CRD) which consists of 5 treatments including P0 (100% bran suspension), P1 (100% bran suspension + 1.5% bali sardinella fish oil), P2 (100% bran suspension + Bali sardinella fish oil 3%), P3 (100% bran suspension + Bali sardinella fish oil 4.5%), P4 (100% bran suspension + 6% bali sardinella fish oil) with 4 replications.

Work Procedures

M. macrocopa culture media is prepared in a 1 m^3 tub with a size of 10 liters for female sexual induction. A glass jar with a capacity of 300 ml is used to induce *M. macrocopa* ephippia. Preparation of culture media is done by cleaning the tubs and jars that will be used for culture with running water then dried. The water used in the study was obtained from a reservoir in the Faculty of Fisheries and Marine, Airlangga University, treated with CaCO3 to reach a water alkalinity of 40mg/L.

The rice bran suspension is made by preparing 100 g bran and 500 ml water. It is then homogenized twice using a blender at 2000 rpm until they are evenly mixed for ± 5 minutes. The second suspension is done after 30 minutes of the first suspension. Furthermore, the bran suspension is grade filtered using a filter size of 2 mm, 0.1 mm, and 40 µm. The suspension passed through the filter was added with water so that the volume became 500 ml (Mubarak *et al*., 2017). The addition of Bali sardinella fish oil was given according to treatment, namely 1-6%. If added afterward, it will not be mixed evenly.

Induction of *M. macrocopa* sexual female is cultivated with a density of 660 ind/l. The culture is carried out for five days with a daytime light intensity of 700-900 lux and 50-100 lux at night. Light measurements are carried out using a lux meter. Aeration treatment with a discharge of 28 ml/min. Replacement of containers and cultivation media is done every day. Bran suspension is given according to the cultivation age (Mubarak *et al*., 2017), as shown in Table 1.

Table 1. Concentrations of bran suspension in the induction of sexual females.

Day	Concentration of feed given (mg/l)
1	37.00
2	44.88
3	54.85
4	64.34

On the third day, *M. macrocopa* offspring were screened. Then, the induction of *M. macrocopa* ephippia production was carried out with a 70-hour-old sexual female culture resulting from the maintenance of 660 ind/l. Each culture density is up to 1000 ind/l sexual females. During maintenance, 100% of containers and water are replaced every day. Along with water replacements, feeding is done according to each treatment.

The parameters observed in this study were daily production of *M. macrocopa* ephippia, the total output of *M. macrocopa* ephippia, ephippia production per *M. macrocopa* parent, and *M. macrocopa* survival rate. Ephippia production per parent is estimated using the following formula (Mubarak *et al*., 2017):

$$\text{Ephippia production} = \frac{\text{Total ephipia}}{\text{Total broodstock}}$$

Survival rate is calculated according to Effendie (1997):

$$SR = \frac{Nt}{No} x\ 100\%$$

SR is the value of survival rate (%), Nt is the number of *M. macrocopa* that lived at the end of the experiment and No is the number of *M. macrocopa* that lived at the beginning of the experiment.

Sampling was carried out starting on the fourth day by using a dropping pipette and petri dish.

Data Analysis

Statistical data analysis of the effect of fish oil administration on bran on *M. macrocopa* ephippia production was performed using Analysis of Variance (ANOVA). Duncan Multiple Range Test was used if the study showed significant differences between treatments.

RESULTS AND DISCUSSION

Suspension of rice bran without the addition of Bali sardinella fish oil (P0) has a fat content of 0.70%. The addition of Bali sardinella fish oil by 6%/100g bran produces 2.01% fat in the bran suspension. This shows that the addition of Bali sardinella fish oil in the bran suspension can add fat content to the bran suspension. The results of the analysis of fat content can be seen in Figure 1.

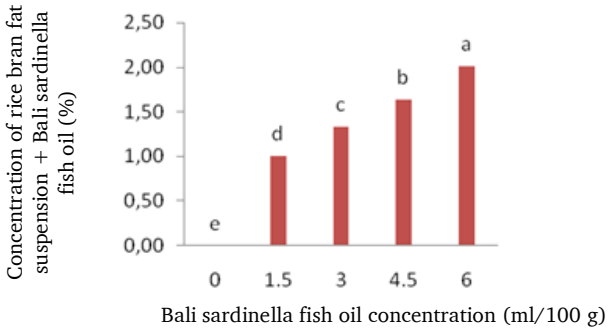

Figure 1. Concentration of fat in bran suspension after adding Bali sardinella fish oil.

The average survival rate result of *M. macrocopa* cultured by suspension of rice bran enriched with bali sardinella oil with different concentrations ranged from 84.24% - 92.33% (Table 2).

Table 2. Survival rates of *M. macrocopa*.

Bali sardinella fish oil concentration (%)	Survival rate (%)
0	84.24 ± 2.31[c]
1.5	87.83± 1.23[b]
3	90.83 ±0.64[a]
4.5	90.58 ± 1.13[a]
6	92.33 ± 1.4[a]

ANOVA test results showed that the addition of Bali sardinella fish oil in rice bran suspension with different concentrations as *M. macrocopa* feed affected the survival rate ($P < 0.05$). The increasing Bali sardinella in rice bran suspension increases the survival rate of *M. macrocopa*. The addition of Bali sardinella fish oil to rice bran suspension with a concentration of 3-6%/100 g bran produced a survival rate that was not significantly different from 90.83-92.33%, but substantially different from the survival rate of *M. macrocopa* culture without Bali sardinella fish oil and suspension with fish oil concentration of 1.5%/100 g bran with survival rates of 84.24% and 87.83%, respectively.

Daily production of *M. macrocopa* ephippia cultured by using rice bran suspension feed added with Bali sardinella fish oil with different concentrations can be seen in Table 3.

Table 3. Daily ephippia production of *M. macrocopa* (ephippia/l) fed with bran suspension with the addition of Bali sardinella fish oil with different concentrations.

Day	Ephippia Production	Bali sardinella fish oil concentration				
		0 ml/100g	1.5ml/100g	3ml/100g	4.5ml/100g	6 ml/100g
0	-	-	-	-	-	-
1	-	-	-	-	-	-
2	-	-	-	-	-	-
3	Ephippia production – 1	483 ±9.35[d]	504 ±7.82[d]	574 ±5.12[c]	603 ±5.95[b]	656 ±8.44[a]
4	Ephippia	512 ±8.83[d]	515 ±6.18[d]	584 ±5.59[c]	613 ±4.72[b]	669 ±8.88[a]

	production – 2					
5	Ephippia production – 3	406 ±8.13^{d}	426 ±7.72^{d}	463 ±8.50^{c}	490 ±7.89^{b}	514 ±8.54^{a}
6	Ephippia production – 4	263 ±12.69^{d}	390 ±6.99$_{c}$	418 ±8.30^{b}	452 ±6.29^{a}	468 ±9.19^{a}
7	Ephippia production– 5	128 ±10.70^{d}	307 ±7.68^{c}	400 ±7.05^{b}	432 ±4.03^{a}	456 ±16.12^{a}
8	Ephippia production – 6	75 ±5.13^{d}	258 ±6.76^{c}	279 ±8.09^{c}	310 ±2.86^{b}	388 ±19.38^{a}
9	Ephippia production – 7	27 ±3.71^{d}	232 ±4.73^{c}	245 ±5.89^{c}	273 ±3.80^{a}	302 ±5.74^{a}
	Total	1893	2632	2964	3173	3452

ANOVA test results showed that the addition of Bali sardinella fish oil on rice bran suspension with different concentrations as *M. macrocopa* feed affected the daily production of *M. macrocopa* ephippia (P <0.05). The first, second, third-highest daily ephippia production was produced with rice bran suspension added with Bali sardinella fish oil concentration of 6 ml/100 g bran, each of 656 ± 8.43 ephippia/l (first production), 669 ± 17, 76 ephippia/l (second production), and 514 ± 17.07 ephippia/l (third production).

The first, second, third, third-lowest daily ephippia production was produced from cultivation using suspension of rice bran without added Bali sardinella fish oil respectively 483 ± 9.35 ephippia/l, 512 ± 8.83 ephippia/l, and 406 ± 8.13 ephippia/l which is not significantly different from the addition of Bali sardinella fish oil on bran suspension with a concentration of 1.5%/100 g bran. The fourth and fifth highest ephippia production resulted in the addition of Bali sardinella fish oil to rice bran suspension with a concentration of 6%/100 grams of bran at 468 ± 9.19 ephippia/l and 456 ± 16.12 ephippia/l, which were not significantly different from the addition of Bali sardinella fish oil on bran suspension with a concentration of 4.5%/100 grams of bran.

After the fifth ephippia production, there was a decrease in ephippia production in all treatments, with the highest ephippia production in the sixth ephippia in addition of bran suspension with Bali sardinella fish oil concentration of 6%/100 grams of bran 388 ± 19.38 ephippia/l was significantly different from all treatments. The seventh highest production of ephippia resulted from the addition of Bali sardinella fish oil in a suspension of bran concentration of 6%/100 grams of rice bran by 302 ± 5.74 ephippia/l, which was not significantly different from the addition of Bali sardinella fish oil to rice bran suspension with a concentration of 4.5%/100 grams of 273 ± 3.80 ephippia/l, the lowest ephippia production occurred in rice bran suspension feed without added Bali sardinella fish oil by 27 ± 3.71 ephippia/l.

Total ephippia production, ANOVA test results showed that *M. macrocopa* fed with rice bran suspension enriched with Bali sardinella oil with different concentrations affected the total ephippia production of *M. macrocopa* (P <0.05). Total ephippia production in *M. macrocopa* cultured with Bali sardinella fish oil feed on rice bran suspension with different concentrations during the maintenance period is shown in Figure 2.

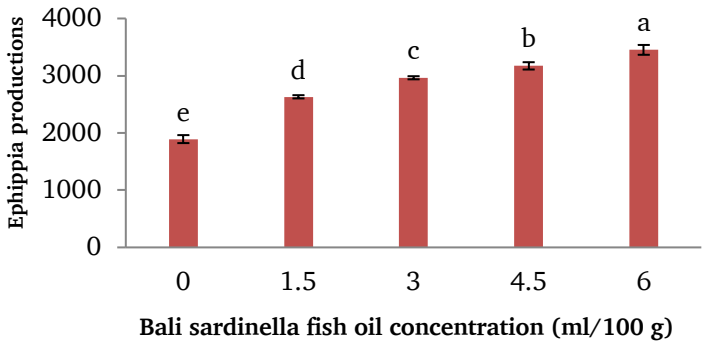

Figure 2. Production of ephippia *M. macrocopa* fed with bran suspension feed added to Bali sardinella fish oil with different concentrations.

The addition of Bali sardinella fish oil with a higher concentration in the suspension of rice bran can increase ephippia production. The highest ephippia production was produced from culture using a rice bran suspension enriched with Bali sardinella fish oil by 6%/100 g bran producing ephippia at (3452 ± 43.0 ephippia/l) (Figure 2), while the lowest ephippia production in the bran suspension feed without added with Bali sardinella fish oil is (1893 ± 34.8 ephippia/l).

In ephippia per parent production, ANOVA test results showed that the addition of Bali sardinella fish oil with different concentrations in rice bran suspension feed significantly affected the production of *M. macrocopa* per parent ($P < 0.05$). The graph of *M. macrocopa* ephippia production per parent can be seen in Figure 3.

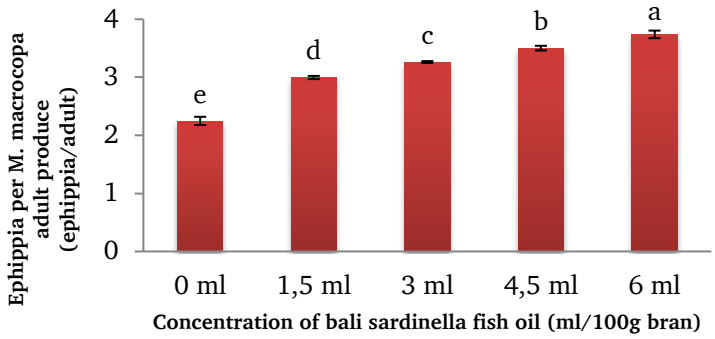

Figure 3. Production of ephippia per *M. macrocopa* parent fed with rice bran suspension enriched with Bali sardinella fish oil with different concentrations.

The increased concentration of Bali sardinella fish oil led to an increase in the production of ephippia per parent. The highest production of ephippia per parent occurred in the suspension of rice bran enriched with Bali sardinella oil with a concentration of 6%/100 g rice bran by 4 ± 0.065 ephippia/parent (Figure 4). The lowest ephippia per parent were produced by bran suspension feed without the addition of Bali sardinella fish oil by 2 ± 0.070 ephippia/parent.

The addition of Bali sardinella fish oil in rice bran suspension as *M. macrocopa* feed affected the survival rate, average daily ephippia production, total ephippia production, and ephippia production per *M. macrocopa* parent. It is increasing the concentration of Bali sardinella fish oil given to rice bran suspension as *M. macrocopa* feed increases the value of survival rates, the average daily ephippia production, total ephippia production and ephippia production per *M. macrocopa* parent. According to

Hakima *et al*. (2013), the quality and quantity of feed in the culture of *M. macrocopa* is an important factor that can affect the reproductive mode and production of *M. macrocopa* ephippia.

Rice bran suspension as *M. macrocopa* feed contains nutrients needed for growth, including the content of Vitamin B (Thiamin (B1), pyridoxine) (Mehdipour *et al*., 2011), protein by 11.01%, fat 8.57% (Hadipernata *et al*., 2012). The most unsaturated fatty acids contained in rice bran are dominated by ω- 6 fatty acids (Jumari *et al*., 2015), while rice bran has a low ω-3 fatty acid content, not more than 1% (Hadipernata *et al*., 2012). The addition of Bali sardinella fish oil in rice bran suspension is expected to increase the content of ω-3 fatty acids. The ω-3 fatty acids found in Bali sardinella fish oil include EPA of 7.1% and DHA of 5.67%. The addition of Bali sardinella fish oil to bran proved to be able to increase the value of total fat in the bran suspension (Table 1).

The survival rate of *M. macrocopa* is influenced by the feed given (Dewi *et al*., 2019), biotic and abiotic factors including density, competitors, age, population and the ability of organisms to adapt to their environment (Pujihastuti *et al*., 2009). Ω-3 fatty acids have an important role in supporting the survival rate for *M. macrocopa* (Izquierdo *et al*., 2001). EPA is a good substrate of the lipolytic digestive enzyme (Izqueirdo *et al*., 2001). In addition, EPA is a good substrate for forming eicosa and oil (Bell *et al*., 1994; Copeman *et al*., 2002).

The addition of 3-6% bali sardinella fish oil produced *M. macrocopa* survival rates that were not significantly different in the range of 90% -92%, which was higher than *M. macrocopa* which was fed with bran suspension and bran suspension with the addition of bali sardinella fish oil below 3 % with a survival value of 84% - 87%.

Ephippia is produced by the sexual female *M. macrocopa*. Induction of ephippia production in female parthenogenesis of *M. macrocopa* does not directly produce ephippia, but it produces a female sexual offspring that will produce ephippia. Induction of female sexual production can be done with *M. macrocopa* parthenogenesis parent culture with a density of 660 ind/l using 22.2-24.42 mg/l bran suspension feed (Mubarak *et al*., 2019), then the female child is re-cultured with density of 1000 ind/l to induce ephippia production with bran suspension feed concentration of 37 mg/l on the first day, 49 mg/l on the second day, 58.5 mg/l on the third day, and 70 mg/l on the fourth and fifth days (Lopatina and Zadereev, 2012; Mubarak *et al*., 2019).

Lopatina and Zadereev (2012) stated that the culture of *M. branchiata* parents and offspring parthenogenesis with a density of 750-1000 ind/l produced sexual females with ephippia production of 70%. Besides being affected by density, the production of *M. macrocopa* ephippia is also influenced by the quality of feed, including the availability of ω-3 fatty acids. EPA and DHA limit the production of ephippia in *M. macrocopa* (Abrusán *et al*., 2007). The ω-3 fatty acid is needed in reproduction for embryonic development (Pangkey, 2011). EPA and DHA are needed in the period of previtellogenesis to the process of ovulation. The availability of ω-3 fatty acids has a significant impact on ovarian development, as well as the number of eggs during reproduction (Mazorra *et al*., 2003) so that if the availability of EPA and DHA are met, it can potentially increase the process of gametogenesis in *M. macrocopa*.

ω-3 fatty acids have an important role in the success of ephippia production in *M. macrocopa* (Abrusán *et al*., 2007). The concentration of EPA needed in the formation of ephippia in cladocerans is 2.4 μg/dry weight, which is higher than the EPA needed in the formation of subcutaneous eggs (0.01 μg/dry weight) (Abrusán *et al*., 2007). In line with this, an increase in the concentration of ω-3 fatty acids affects the average daily ephippia

production, total ephippia production, and ephippia production per parent.

The addition of Bali sardinella fish oil with a concentration of 6% produced the highest average daily ephippia production of 669 ± 8.88 ephippia/l, as well as the total ephippia production of 3,452 ± 43.0 ephippia/l. This shows that the addition of Bali sardinella fish oil in bran suspension contributes to the increased production of ephippia produced. This study is in line with research by Utiah *et al*. (2007) which states that increasing EPA and DHA in feed can increase fecundity in yellow catfish (*Hemibagrus nemurus* Blkr) and Marzuqi *et al*. (2015) which states that increasing fatty acids in feed affect increased gonad development in prospective milkfish broodstock.

Production of *M. macrocopa* ephippia per parent in culture with rice bran suspension enriched with Bali sardinella oil with a concentration of 6% has a value of 4 grains/parent higher than *M. macrocopa* culture without adding 2 grains of Bali sardinella fish/parent. The addition of Bali sardinella fish oil in this study had an influence on the increased production of ephippia produced. The addition of Bali sardinella fish oil to rice bran suspension increased the fatty acid content in the feed, so that fat reserves in the female sexual parent increased, which would have an effect on increasing the frequency of gametogenesis in *M. macrocopa* broodstock (Pangkey, 2011). The results of this study are in line with Pan *et al*. (2014) that the availability of fatty acids influences fecundity, egg development, egg hatching, and copepod embryo development.

CONCLUSION

The results showed that there was a very significant difference between the production of ephippia fed rice bran suspension with ephippia production fed rice bran suspension with the addition of Bali sardinella fish oil with different concentrations. The optimal concentration of Bali sardinella fish oil, which can increase the production of *M. macrocopa* ephippia by adding 6% Bali sardinella fish oil to rice bran suspension with a total production of ephippia (3,452 ± 43.0 ephippia/l).

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