The effect of giving fermented rice bran suspension on fecundity and production of *moina macrocopa* offspring per parent

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Abstract. The objectives of this research were: 1) determine the effect of fermented rice bran suspension concentration on the fecundity and production of *M. macrocopa* offspring per parent, 2) determine the optimal concentration of fermented rice bran suspension on the fecundity and production of *M. macrocopa* offspring per parent. This research used an experimental method with a completely randomized design consisting of six treatments of suspension of rice bran without fermentation (P0), 19.54 mg / L fermented rice bran suspension (P1), 21.98 mg / L fermented rice bran suspension (P2), 24.42 mg / L fermented rice bran suspension (P3), 26.68 mg / L fermented rice bran suspension (P4) and 29.30 mg / L fermented rice bran suspension (P5) in four replications for each. The results of the culture with a different concentration of rice bran suspension showed a significant difference (P <0.05) on the fecundity and production of *M. macrocopa* offspring per parent. The highest fecundity and production of *M. macrocopa* offspring per parent occurred in *M. macrocopa* culture with a concentration of fermented rice bran suspension of 29.30 mg / L (P5) with the fecundity 21- 25 grains/parent and production of offspring 20 - 24 ind/parent.

1. Introduction
The availability of feed, especially of natural feed is an important factor to the success of fish hatchery effort. Especially on larvae rearing phase, because natural feed certain nutrients that can not be replaced by other feed [1]. One of the natural feeds that can be used as feed for fish larvae *M. macrocopa*.

*M. macrocopa* is a natural feed for freshwater fish seed because it has a high nutritional content, easily digestible and has a high reproducibility of rapidly proliferating and has a size corresponding to the larvae fish mouth opening [2]. *M. macrocopa* average of the produce of offspring about 32 ind/day [3]. *M. macrocopa* cultivated with feed *Chlorella* sp. can result in the production of offspring per parent as much as 12-14 ind with the highest fecundity as much as 37 grains/parent [4;5].

Production *M. macrocopa* offspring per parent can be cultivated using a suspension feed rice bran. Rice bran nutritional value namely vitamin B, unsaturated fatty acids, crude fiber 12.59%, BETN 37.32%, 0.09% calcium, phosphorus 1.07%, 9.96% crude protein and crude lipid 5.96% [6;7;8]. The content of low protein and fat could be improved with a probiotic fermentation process using Effective...
Microorganisms 4 (EM4).

Effective Microorganisms 4 (EM4) contains Lactobacillus casei, Saccharomyces cerevisiae dan Rhadopseudomonas palustris. Microorganisms in Effective Microorganisms 4 (EM4) utilize complex compounds in rice bran as a nutrient for metabolic processes, so that simpler compounds are formed to be used directly by microalgae [9].

Based on the results of research conducted by [6] stated that rice bran fermented with Effective Microorganisms 4 (EM4) at different lengths of time during fermentation affects the crude protein content of 9.96% to 10.36% and crude lipid from 5.96% to 6.82 %. The concentration of protein and fat in the feed can influence the increase in fecundity, embryonic development and production speed M. macrocopa offspring per parent [7]. Based on the description above, it aims to determine the effect of fermented rice bran suspension concentration on the fecundity and production of M. macrocopa offspring per parent.

2. Materials and methods

2.1 Materials

Materials used in this study are M. macrocopa, water, rice bran, probiotics Lactobacillus casei, Saccharomyces cerevisiae and Rhadopseudomonas palustris, molasses and distilled water.

2.2 Place and time of research

This research was conducted at the Anatomy and Cultivation Laboratory of the Faculty of Fisheries and Marine Airlangga University, Surabaya. This research was conducted in March-May 2019.

2.3 Research design

This study using the experimental method with a completely randomized design (CRD). This study uses six treatments and each treatment was replicated four times. The treatment used is a suspension of rice bran without fermentation (Control), fermented rice bran suspension of 19.54 mg/L (P1), fermented rice bran suspension of 21.98 mg/L (P2), fermented rice bran suspension of 24.42 mg/L (P3), fermented rice bran suspension of 26.68 mg/L (P4) and the suspension was fermented rice bran 29.30 mg/L (P5).

2.4 Method

2.4.1 Culture media

The media for the M. macrocopa culture in this study was water taken from the water tanks owned by the Faculty of Fisheries and Marine, University Airlangga. Water from the water tanks was used to fill a 1000 L fiber tank that was aerated at least three days before using. Water from the fiber tank was filtered with 40 μm nylon before it was put into an experimental tank to eliminate other competing zooplankton.

2.4.2 Procedure making fermented rice bran

Probiotics before use activation is done in advance, done in a way, that probiotics 1 ml, molasses 1 ml and 100 ml of water allowed to stand for 12 hours in anaerobic [10]. This process aims to enable the microorganisms from an inactive condition so that when mixed with rice bran can work optimally. Bacteria active condition characterized by an increase in pH was greater than 4, aromatic like the smell of glucose [16]. After that, the already active probiotics are mixed with rice bran as much as 100 grams and then fermented for 7 days [6].

2.4.3 Suspension manufacture of fermented rice bran

Fermented rice bran suspension was made by suspending as much as 100 g of fermented rice bran into 500 ml of water, using a blender at a constant speed of 2000 rpm for 5 minutes two times. The second suspension is performed after 30 minutes of the first suspension. The water suspension was filtered
using 2 mm, 0.1 mm and 40 μm sieves. The suspensions that passed through the filtration then had more water added to reach a volume of 500 ml [12]. The results of the proximate analysis of the fermented rice bran suspension contained dissolved organic matter as follows: 32.4 mg/mL, protein 22.22%, and fat 0.61%.

2.4.4 Provision of inoculants and M. macrocopa culture

*M. macrocopa* used in this study has been adapted to feed fermented rice bran suspension in the culture at a density of 20 ind/L with a water volume of 10 L. *M. macrocopa* offspring aged less than 24 hours of culture would then become an inoculant in this study. *M. macrocopa* culture in this study using a glass jar with a volume of 300 ml. Culture was conducted over 7 days in a laboratory with daytime lighting, ranging from 700-900 lux and with the range in the evenings being 50-100 lux, as well as the use of aeration at the rate of 28 mL/min. On the second, third, fourth and fifth days, they were harvested and the *M. macrocopa* offspring were separated from their mothers by filtering. The feeding is done as much as once a day at 10:00 am. During maintenance performed a daily change of water and water quality measurements that include temperature, DO and pH [13].

2.4.5 Observations fecundity and production *M. macrocopa* offspring per parent

Observations fecundity *M. macrocopa* performed every day at 10:00 am. Fecundity observations were done by taking a sampling of individuals *M. macrocopa* using a pipette and observed with a 100x magnification microscope. After 24 hours later was observed production of *M. macrocopa* offspring per parent manner is filtered to separate between parent and offspring. The results of the sieve is *M. macrocopa* offsprings then placed on the Petri dish and calculated with the aid of a pipette. The calculation is performed from day 2 through day 7. Observations of *M. macrocopa* offspring per parent calculated using the following formula:

\[
\text{Production of offspring parent} = \frac{\text{Total M. macrocopa offspring}}{\text{Total M. macrocopa parent}}
\]

2.5 Data analysis

The observation data were analyzed using Analysis of Variance (ANOVA). If the results from the analysis of variance showed that the treatment had significantly different results, then it was followed by Duncan's Multiple Range Test with a 5% error rate to determine the best treatment.

3. Results and discussion

3.1 Fecundity *M. macrocopa*

The results showed that Fecundity *M. macrocopa* culture at a density of 20 ind/L shows the results were significantly different (Table 1).

| Treatment | Fecundity |
|-----------|-----------|
|           | 1         | 2         | 3         | 4         | 5         |
| P0        | 9.75±0.50 | 17.00±2.71| 15.75±0.50| 19.25±0.50| 16.75±0.96|
| P1        | 17.25±0.50| 20.00±0.82| 21.25±1.71| 21.25±1.26| 16.75±0.96|
| P2        | 17.50±0.58| 21.50±2.38| 22.50±0.58| 19.00±1.15| 16.25±0.50|
| P3        | 17.50±0.58| 22.00±0.82| 23.50±0.58| 19.00±1.15| 16.75±0.50|
| P4        | 18.00±0.82| 23.75±1.26| 24.50±0.58| 21.75±1.89| 18.25±1.26|
| P5        | 20.50±0.58| 24.50±1.29| 25.00±0.87| 22.50±1.29| 20.75±0.96|

Description: Different superscript letters in the same column show a significant difference (p<0.05).

Treatment P0 : Rice bran suspension not fermented 24.42 mg/L (control)
Treatment P1 : Fermented rice bran suspension 19.54 mg/L
Treatment P2 : Fermented rice bran suspension 21.98 mg/L
Treatment P3 : Fermented rice bran suspension 24.42 mg/L
Treatment P4: Fermented rice bran suspension 26.86 mg/L
Treatment P5: Fermented rice bran suspension 29.30 mg/L

**Figure 1.** Average production fecundity of *M. macrocopa* cultured with suspension feed rice bran fermented with different concentrations. Description, superscript differ on chart same show significant differences (p < 0.05).

*M. macrocopa* culture with fermented rice bran suspension produces higher fecundity than rice bran suspension without fermentation (control). The highest fecundity *M. macrocopa* occurred in *M. macrocopa* culture with a concentration of fermented rice bran suspension of 29.30 mg/L (P5) with the fecundity 25 grains/parent. Fecundity *M. macrocopa* influenced by factors different environmental conditions, especially relating to the availability of food [14].

The concentration of feed directly affects the body's metabolic rate and survival rate of *Moina* [15;16]. The quality of feed that includes protein and fat concentrations were higher in the feed can improve the ability of parthenogenetic reproduction cladocera, through increased fecundity, embryonic development and production speed offspring per parent [7, 17].

The feed is an important factor in the reproduction cladocera where the greatest energy as much as 68% is used for reproduction[18]. Fecundity and growth of *M. macrocopa* decreased when the quality and quantity of feed decreases [19]. According to [17] fecundity in the female parent of each individual depends on the age, size and quality of feed. Protein and fat content can increase the speed of embryonic development in cladocera [7]. The success of embryo development *M. macrocopa* will determine the success of the production of offspring.

### 3.2 Production of *M. macrocopa* Offspring Per Parent
The results showed that the production of *M. macrocopa* offspring per parent culture at a density of 20 ind/L shows the results were significantly different (Table 2).
Table 2. Production of *M. macrocopa* offspring per parent culture at a density of 20 ind/L.

| Treatment | Offspring 1 | Offspring 2 | Offspring 3 | Offspring 4 | Offspring 5 |
|-----------|-------------|-------------|-------------|-------------|-------------|
| P0        | 9.50±0.58   | 15.75±0.50  | 18.75±1.29  | 16.50±0.58  | 16.25±0.96  |
| P1        | 16.75±0.50  | 19.25±0.50  | 20.50±1.70  | 18.00±0.82  | 17.00±0.82  |
| P2        | 16.25±0.50  | 21.25±0.50  | 22.00±2.16  | 18.50±0.58  | 17.75±0.96  |
| P3        | 17.25±0.50  | 21.25±0.96  | 22.50±1.26  | 20.00±0.82  | 18.00±1.41  |
| P4        | 16.75±0.50  | 22.25±0.50  | 23.25±0.82  | 20.50±0.58  | 19.25±1.26  |
| P5        | 19.75±0.50  | 22.75±0.50  | 23.75±1.50  | 22.25±0.50  | 19.50±1.73  |

Description: Different superscript letters in the same column show a significant difference (p<0.05).

Treatment P0: Rice bran suspension not fermented 24.42 mg/L (control)
Treatment P1: Fermented rice bran suspension 19.54 mg/L
Treatment P2: Fermented rice bran suspension 21.98 mg/L
Treatment P3: Fermented rice bran suspension 24.42 mg/L
Treatment P4: Fermented rice bran suspension 26.86 mg/L
Treatment P5: Fermented rice bran suspension 29.30 mg/L

Figure 2. The mean production of *M. macrocopa* offspring per parent cultured with fermented rice bran suspension with different concentrations. Description, different superscript on the same graph indicate significant differences (p<0.05).

The highest *M. macrocopa* offspring per parent occurred in *M. macrocopa* culture with a concentration of fermented rice bran suspension of 29.30 mg/L (P5) with the production of offspring per parent 24 ind/parent. Increased production offspring per parent along with an increasing number of fecundity *M. macrocopa*. Production *M. macrocopa* offspring per parent after the birth of the first to
the fifth experience, the difference caused by the size of the brood is getting bigger. Brood sizes are getting bigger cause pouch size bigger, so the more the developing embryo in the pouch [17]. Speed embryonic development is affected by the quality and quantity of feed that includes protein and fat content [7]. The concentration of protein and low fat in the diet causes a decrease in the reproductive capacity of parthenogenesis cladocera [12]. In addition, the content of other nutrients in the diet such as vitamin B (thiamin (B1), pyridoxine) and some minerals are also needed increase the production of *Moina* offspring [20; 13].

The content of the rice bran covering vitamin B, unsaturated fatty acids, crude fiber 12.59%, BETN 37.32%, 0.09% calcium, phosphorus 1.07%, 9.96% crude protein and crude lipid 5.96%, [6;21; 8]. The concentration of protein and fat can be improved with the process fermentation. Fermentation starter used containing *Lactobacillus casei*, *Saccharomyces cerevisiae*, and *Rhodopseudomonas palustris* with an old best time of fermentation for 7 days [6]. The concentration of the fermented rice bran suspension in the research include protein content as much as 22.22% and 0.61% total fat in dry weight. Increasing the protein content of 20.66% to 22.22%, but the fat content decreased from 9.64% to 0.61%.

Fermented rice bran nutritional value better than the usual rice bran. The content of nutrients includes protein and fat content increased due to bacterial activity which is catabolic or breaks down the components of the complex to be simpler and easier to digest [22]. Factors affecting the increase in protein and fat in fermentation are bacteria multiply, where more and more bacteria to grow the proteins and fats in the feed increased [23].

The increase in protein content in the fermented rice bran suspension occurs because of bacteria in the fermentation solving complex proteins into polypeptides, polypeptide will be broken down into amino acids to produce enzyme protease. Amino acids will be utilized by microbes to proliferate during the fermentation process may indirectly increase the crude protein content because the microbes are single protein source. Single-cell protein is a pure crude protein derived from a single-celled microorganism or a lot more simple. The single-cell protein can be increased during the fermentation process [24]. A decrease in the fat content of fermented rice bran suspension allegedly used bacteria as nutrients in metabolic activity, resulting in lower fat content [25].

Cladosera growth is strongly influenced by the feed provided. The more the concentration of the feed contained in the media production rate cladocera offspring per parent will go faster [20].

4. Conclusion

Based on the research that has been done fermented rice bran suspension feed concentration on the culture of *M. macrocopa* effects the fecundity and production of *M. macrocopa* offspring per parent. The highest fecundity and production of *M. macrocopa* offspring per parent occurred in *M. macrocopa* culture with a concentration of fermented rice bran suspension of 29.30 mg / L (P5) with the fecundity 21-25 grains/parent and production of offspring 20-24 ind/parent.

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