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Concentration of fermented tilapia feces suspension by decomposer bacteria as a feed to induce *moina macrocopa* sexual reproduction

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Abstract. Sexual induction of *M. macrocopa* can be induced by setting a density of feed concentration. To optimize the production of ephippia, sexual females must be given sufficient quality feed. Fermentation of tilapia fish from the results of preliminary studies showed a significant increase in protein concentration but fat concentration decreased. This research to determine the best concentration of fermented tilapia feces feed to produce ephippia *M. macrocopa*. This research is experimental by using a completely randomized design (CRD). This research consisted of 4 treatments of feed concentration is fermented feces suspension concentration of 33,30 mg/L (P1), 37,00 mg/L (P2) and 40,70 mg/L (P3) and control using rice bran suspension feed concentration of 37,00 mg/L(P0), with each using 5 replications. Induction sexual offspring is maintained at a density of 1000 ind/L for 6 days. During cultivation is calculated survival rate, *M. macrocopa* ephippia production, and some water quality parameters as support. Data were analyzed using ANOVA and Duncan test. This research showed that *M. macrocopa* cultivation using several concentrations of suspension feed of fermented tilapia feces at concentrations of 40.70 mg/L can induce sexual females and produce ephippia and showed the highest survival rate ($1186 \pm 26,45$ grains/L) and 88,13%.

1. Introduction

M. macrocopa is a natural food for fish and shrimp larvae that are spread in freshwaters [1]. Increasing the price of cyst *Artemia* sp. making *Moina* sp. as an alternative natural feed for fish and shrimp larvae [2]. *Moina* sp. reproduces in two ways sexual and asexual (parthenogenesis) [3]. Sexual female *Moina* sp. does not reproduce by parthenogenesis so that if the egg is fertilized by a male. It will experience carapace thickening and ephippia are formed [4]. Maintenance of the population of *Moina* sp. with high density and adequate feeding can induce the production of male and female sexual offspring [5].



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Fish farming activities produce waste originating from feces and fish feed residues [6]. Preliminary research results show that tilapia feces contain protein (18.19%), fat (1.46%), EPA (0.13%), DHA (0.20%), and amino acid histidine (0.26%) and arginine (0.78%).

Previous research has successfully cultivated *Moina* sp. with fecal fish feces which produce the highest fecundity [7] and populations with high densities of 1000 ind/L [8]. Tilapia feces can be used as direct feed if made in the form of suspension. To increase the solubility of tilapia fish, fermentation using decomposer bacteria can be done. This research tries to determine the effect of suspension concentration of fermented tilapia with decomposer bacteria on the induction of ephippia production from the sexual female *M. macrocopa*.

2. Materials and methods

This study was conducted in the Laboratory of the Faculty of Fisheries and Marine, Universitas Airlangga and Balai Riset dan Standardisasi Industri Surabaya, Surabaya, East Java, Indonesia.

2.1 Materials

The materials used in the research are: *M. macrocopa*, freshwater, dolomite, tilapia feces suspension, molasses, EM-4 agriculture, rice bran suspension, and detergent.

2.2 Research methods

This research uses an experimental method. The design used is a Completely Randomized Design (CRD) with 4 treatments and 5 replications, are:

P0: Feeding of *M. macrocopa* using suspension rice bran (control) concentration of 37.00mg/ L

P1: Feeding of *M. macrocopa* using suspension of fermented tilapia feces concentration of 33.30 mg/ L

P2: Feeding of *M. macrocopa* using suspension of fermented tilapia feces concentration of 37.00 mg/ L

P3: Feeding of *M. macrocopa* using suspension of fermented tilapia feces concentration of 40.70 mg/ L

2.3 Provision of inoculants and culture of *M. macrocopa*

M. macrocopa used in this study was obtained from waters in the Surabaya region, then cultivated individually (one *Moina*/20 mL) in several generations to obtain species that have the best growth and production performance of offspring. Furthermore, *Moina* is cultured with bran suspension feed for 2 months at a density of 20 / L volume of 10 L water. The cultivated *Moina* offspring now become inoculants in this study with the same initial density [3].

2.4 Research parameters

The main parameters in this research are the survival rate of the broodstock, the amount of ephippia production. The ephippia calculation is performed on the fifth to the seventh day of each treatment. Ephippia was taken based on maintenance jars for each treatment. The percentage of survival of the broodstock is calculated using the formula [9] as follows:

$$\text{Survival Rate} = \frac{N_t}{N_0} \times 100\%$$

Supporting parameters in this research are measurements of water quality in *M. macrocopa* maintenance media, which include pH, DO, temperature, and alkalinity. Observation of water quality is carried out every day in the morning and evening.

2.5 Data Analysis

Data were analyzed using Analysis of Variance (ANOVA) to determine the effect of treatment and continued with the DUNCAN test to determine and determine the treatment with the best results.

3. Results and discussion

3.1 Results

ANOVA test results showed that the cultivation *M. macrocopa* using several concentrations of fermented tilapia suspension feces showed an influence on total ephippia production and ephippia per brood ($P < 0.05$) (Table 1). The highest total production of ephippia *M. macrocopa* (3015 grains/L) and the highest production of ephippia per brood was (3.36 grains/brood) produced from aquaculture using rice bran suspension (control) feed.

Table 1. Ephippia Production of *M. macrocopa*

| Treatment | Ephippia Production (Ind/L) | |
|-----------------|-------------------------------|------------------------------|
| | Total Ephippia \pm SD | Ephippia per Brood \pm SD |
| Control | 3015 ^a \pm 57.88 | 3.36 ^a \pm 0.09 |
| P1 (33.30 mg/L) | 438 ^d \pm 35.60 | 0.52 ^d \pm 0.05 |
| P2 (37.00 mg/L) | 817 ^c \pm 53.36 | 0.95 ^c \pm 0.07 |
| P3 (40.70 mg/L) | 1186 ^b \pm 26.45 | 1.35 ^b \pm 0.05 |

Note: Different superscripts in the same column show significant differences ($P < 0.05$).

The graph of the ANOVA test results showed that the cultivation of *M. macrocopa* using several concentrations of fermented tilapia suspension feces showed an influence on the daily ephippia production ($P < 0.05$) (Figure 1).

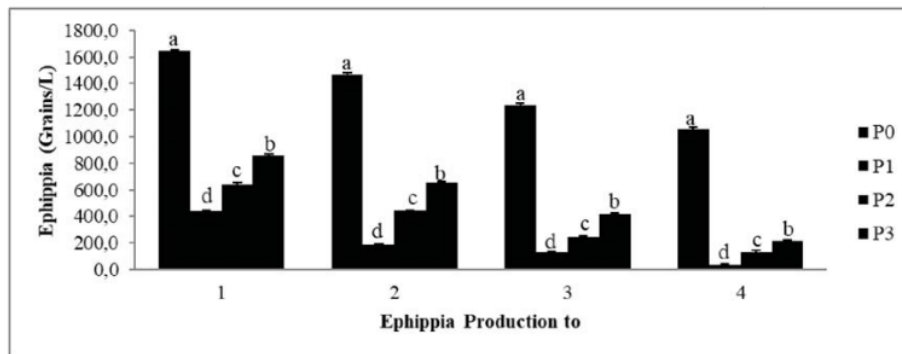


Figure 1. Daily Ephippia Production of *M. macrocopa*

ANOVA test results showed that *M. macrocopa* cultivation using several concentrations of fermented tilapia suspension did not affect the survival rate ($P > 0.05$) (Table 2). The survival rate of *M. macrocopa* on the density of 1000 ind/L with fermented tilapia suspension feces was 83.93-89.73%. Water quality during the maintenance of *M. macrocopa* that still supports life sustainability (Table 3).

Table 2. *M. macrocopa* Survival rate

| Treatment | Survival Rate (%) \pm SD |
|-----------------|-------------------------------|
| Control | 89.73 ^a \pm 2.01 |
| P1 (33.30 mg/L) | 83.93 ^c \pm 1.84 |

| | |
|-----------------|----------------------------|
| P2 (37.00 mg/L) | 85.67 ^{bc} ± 1.70 |
| P3 (40.70 mg/L) | 88.13 ^{ab} ± 2.08 |

Note: Different superscripts in the same column show significant differences (P<0.05).

Table 3. *M. macrocopa* Maintenance Parameter Water Quality

| Parameter | Value Range | Optimal Conditions |
|-------------------|---------------|--|
| pH | 8.19 – 8.25 | 7.0 – 8.0 (Miah <i>et al.</i> , 2013) |
| DO (ppm) | 5.13 – 5.49 | > 3.50 (Miah <i>et al.</i> , 2013) |
| Temperature (°C) | 28.28 – 28.64 | 25 – 31 (Tan and Wang, 2010) |
| Alkalinity (mg/L) | 24.00 – 31.00 | > 50 (Tan and Wang, 2010) |

3.2 Discussion

Induction of *M. macrocopa* sexual females in Cladosera has been developed using induction factors which include water quality, population density [10] as well as quantity and quality of feed [11]. The induction of *M. macrocopa* sexual female production in this research was carried out by setting the density of 660 ind/L by giving 37.00 mg/L concentration of rice bran suspension feed, as in previous studies that the cultivation of *M. macrocopa* with rice bran concentration of 37.00-64.40 mg/L produces females with the highest ephippia production (3052 ± 199 grains/L) [12].

The induced child is then reared at a density of 1000 ind/L and fed with fermented tilapia suspension. [10] stated that the saplings of *Moina* sp. maintained at a density of 1000 ind/L can induce ephippia production, as in research *M. branchiata* which are cultivated with densities of 750-1000 ind/L produce 70% sexual females and cultivation with child densities of 1000 ind *Moina*/L, using rice bran suspension feed concentration of 37.00-64.4 mg/L produces the highest ephippia production as many as 2102±120 grains/L.

Cultivation using fermented tilapia suspension fermented tilapia concentration of 40.70 mg/L produced the highest ephippia production (1186 ± 26.45 grains/L) compared to 33.30 mg/L concentration (438 ± 35.60 grains/L), but the ephippia production is still lower than the cultivation using bran suspension feed (3015 ± 57.88 grains / L), this is presumably due to the higher protein content in fermented tilapia suspension at 72.82%.

High protein concentration is a limiting factor in ephippia production. High protein content limits ephippia production [13], but high concentrations of fatty acids can produce ephippia [14]. The success of *M. macrocopa* in producing ephippia requires the role of omega-3 fatty acids in the form of EPA and DHA, where these needs begin in the period of previtellogenesis to the process of ovulation [14]. The availability of EPA and DHA has the potential to increase the process of gametogenesis because omega-3 fatty acids have an impact on egg production during reproduction [15]. High protein in fermented tilapia suspension (72.82%) resulted in lower ephippia production (438-1186 grains/L) compared to rice bran suspension feed (20.66%) which resulted in ephippia of (3015 grains/L).

The production of ephippia *M. macrocopa* can also be influenced by amino acids in the form of histidine and arginine. The amino acid histidine in tilapia feces was (0.26%) and arginine was (0.87%), while the amino acid histidine in rice bran suspension was (1.61%) and arginine (3.82%). The low amino acid in tilapia suspension stool feed could potentially support the production of ephippia *M. macrocopa* compared to rice bran suspension feed, but the high protein content in

fermented tilapia suspension was (72.82%) compared to rice bran suspension feed (20.66%) thought to be able to limit the production of *M. macrocopa* ephippia.

The amino acid histidine and arginine in feed can reduce the production of ephippia in Cladocera [13]. The amino acid histidine influences protein synthesis [16], while the amino acid arginine influences the reproduction of *Moina* sp. [17].

Feed concentration also affects the availability of nutrients (protein, fat and amino acids) *Moina* sp. [18]. Different treatment of fermented tilapia feces feed concentration, is thought to cause the availability of nutrients (protein and fat) for the production of ephippia *M. macrocopa*, this is consistent with the statement [8] that different nutritional values will have different effects on population development and ephippia production. High concentrations of feed can increase the availability of nutrients (protein, fat and amino acids) that can affect the body's metabolism thereby increasing the fecundity or production of ephippia [18], this is according to research [19], that Cladocera uses 68% of the energy produced by its metabolism to reproduce. According to [20] that, low concentrations of feed can reduce nutrients in feed, thus affecting the regulation of stress in Cladocera and can affect the reproductive model.

M. macrocopa began producing ephippia on the fourth day of maintenance. Production of ephippia on the fifth day and subsequently decreased, this was thought to decrease the availability of nutrients (protein and fat) in feed for the reproduction of *M. macrocopa*. The production of ephippia in aquaculture uses fermented tilapia suspension feed fermented on the first day (1200-2363 grains/L), the second day ephippia production (527-1807 grains/L), ephippia production on the third day (353-1150 grains/L), and the production of ephippia on the fourth day is (110-610 grains/L), while the production of ephippia in cultivation uses rice bran suspension feed on the first day (4590 grains/L), the second day ephippia production is (4083 grains/L), ephippia production on the third day (3450 grains/L), and ephippia production on the fourth day (2953 grains/L). According to [21] that a large nutritional deficiency in feed can cause decreased egg production produced by the ovaries.

M. macrocopa cultivation in research with fermented tilapia suspension feed with a concentration of 40.70 mg/L has a higher survival rate, but lower than rice bran suspension feed, this is because the rice bran suspension contains lower protein from tilapia suspension fermented which is thought to support the survival of *M. macrocopa*, due to the growth of *Moina* sp. supported by appropriate feed nutrition to accelerate growth [22].

Cultivation of *M. macrocopa* density of 1000 ind/L fed fermented tilapia suspension fermented fish with different concentrations had water quality values, temperatures ranged from 28.28 to 28.64°C, alkalinity ranged from 24-30 mg/L, DO ranged from 5.13-5.49 ppm, and the pH of water ranges from 8.19 to 8.25 which still supports the survival of *M. macrocopa* [23; 24]. The increase in pH value during the study was caused by the media added with dolomite CaMg(CO₃)₂. The presence of calcium (Ca) in the media reacts with H⁺ which causes the pH to increase [25].

4. Conclusion

The conclusion from the research of suspension concentration of fermented tilapia that has been fermented with decomposer bacteria, namely the daily production of ephippia, ephippia per brood and the highest total ephippia occurred in the cultivation of *M. macrocopa* concentration of 40.70 mg/L of 215.3-857.3 grains/L, 1.35±0.05 ind/L and 1186±26.45 ind/L

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