

# Masculinization of Tilapia (*Oreochromis Niloticus*) by Oral Method Using Senggani Fruit Extract (*Melastoma Malabathricum* L.)

Nopita<sup>a,1,\*</sup>, Akbar Marzuki Tahya<sup>a,2</sup>, Muhammad Safir<sup>a,3</sup>

<sup>a</sup> Aquaculture Study Program, Faculty of Animal Husbandry and Fisheries, Tadulako University, Palu, Indonesia

<sup>1</sup> Email: nopita17@gmail.com\*

\* corresponding author

## ARTICLE INFO

## ABSTRACT

### Keywords

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**Introduction:** Tilapia have different growth rates in male and female fish where the growth of male fish is faster than female. Therefore, in tilapia farming activities, the production of tilapia weighing >100g/head is difficult to achieve due to uncontrolled spawning. **Method:** This study was designed using a completely randomized design (CRD) with 5 treatments and 4 replicates, namely treatment A (control), B (Senggani fruit extract 0.75 g/kg feed), C (extract 1.5 g/kg feed), D (extract 2.25 g/kg feed), E (extract 3 g/kg feed). The organisms used in the study were 7-day-old tilapia larvae. Tilapia larvae were given feed that had been added with Senggani fruit extract according to the treatment dose. Tilapia were reared for 62 days, and given artificial feed containing 41% protein, each feeding was given with a frequency of 3 times a day (morning, afternoon, evening). **Results and Discussion:** The results showed that the highest percentage of male sex was found in treatments A, B, C, D and E, which amounted to 50; 56.25; 57.5; 58.75; and 60%, respectively. Daily growth rate, survival and feed conversion ratio obtained during the study in each treatment amounted to 7.49, 7.45; 7.44; 7.46; and 7.40. for survival 73.75; 72.5; 72.5; 70; and 68.75 then the results of feed conversion ratio respectively 1.86; 1.88; 1.89; 1.86; and 1.91. The analysis showed that the highest percentage of male sex was found in the treatment of 3 g/kg feed which amounted to 60% ( $P>0.05$ ). Daily growth rate, survival rate and feed conversion ratio did not significantly affect the treatment given ( $p>0.05$ ). **Conclusion:** The results of water quality measurements obtained are still within the optimum limits for tilapia rearing. Conclusion: Feeding using Senggani fruit extract to masculinize tilapia did not have a significant effect ( $p>0.05$ ) on the percentage of male tilapia, daily growth rate and feed conversion ratio.

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## 1. Introduction

Tilapia (*Oreochromis Niloticus*) is a freshwater fish commodity that is quite popular in Indonesia and is a superior commodity. Tilapia has high nutritional and economic value. This makes the need for seeds and consumption fish tends to increase along with the expansion of aquaculture businesses [1]. Tilapia is very potential to be developed because it has several advantages including easy breeding, fast growth, high tolerance to changes in water quality and easy to cultivate [2].

Tilapia have different growth rates between male and female fish where male fish grow faster than females [3]. Therefore, in tilapia farming, the production of tilapia weighing >100g/head is difficult to achieve due to uncontrolled spawning [4,5]. This happens because the energy produced

by female tilapia is not only used for growth but also for reproduction, making the growth of female tilapia slower than male tilapia, then the seeds produced have a small size and are not in demand by consumers [6].

One way to overcome this is the cultivation of male mono sex tilapia. Mono sex male tilapia farming is widely practiced because male tilapia have a faster growth rate and larger size compared to female tilapia and are able to control wild spawning [7]. One method to produce mono sex males is through the masculinization process. The male mono sex cultivation system or masculinization in tilapia is a profitable alternative because it can avoid early gonad maturation and increase efficiency [8].

Masculinization in fish can be done with steroid hormones such as the synthetic hormone *17 $\alpha$ -Methyltestosterone* [9]. But in its development, the use of synthetic hormone *17 $\alpha$ -Methyltestosterone* for masculinization has been banned in fish farming activities because it is difficult to decompose naturally so that it has the potential to pollute the aquatic environment [10]. Therefore, its application has been limited because it can leave residues, both in fish and the aquatic environment [11].

One material that has the potential to replace synthetic materials is steroids from natural ingredients derived from Senggani plants (*Melastoma malabathricum* L.) [12]. Several studies using Senggani plant extracts have been conducted. Farizah's research [14], states that the injection of senggani leaf extract to accelerate the maturation of mangrove crab gonads at a high dose of 2 mg / g can inhibit the development of mangrove crab ovary. Giving Senggani leaf extract at 1 g/kg feed is also proven to inhibit the development of gonads in tilapia (*O. niloticus*) [14]. Senggani plant (*M. malabathricum* L.) has a distinctive fruit and is widely available in the Central Sulawesi region so that it can be produced in large quantities. The use of Senggani fruit extract has never been done to masculinize tilapia. Therefore, research on the masculinization of tilapia with the oral method using senggani fruit extract needs to be done in order to determine the effect of the addition of Senggani fruit extract added to the feed on tilapia masculinization.

## 2. Research Methodology

The test organisms used in the study were tilapia larvae aged 7 days after hatching with a weight of  $0.078 \pm 0.08$  g [14]. The 400 tilapia larvae used were obtained from the Tatanga Fish Seed Center (BBI), South Palu District, Palu City. The tools used in this study are listed in Table 1.

Table 1. Tools used in the study

No.	Tool name	Total	Usability
1.	Basin	20 units	Tilapia larvae spawning container
2.	Aerator	8 pieces	Oxygen supplier
3.	Dropper pipette	1 piece	Taking the solution
4.	Prep glass	3 pieces	Save the object
5.	Aeration hose	16 pieces	Flow oxygen into the container
6.	Wipes	1 piece	Cleaning the object
7.	Scissors	2 pieces	Dissecting fish
8.	Scales	1 piece	Weighing the fish
9.	Scalpel	1 piece	Chopping the gonads
10.	Thermometer	1 piece	Measuring water temperature
11.	pH meter	1 piece	Measuring the pH of water
12.	Ammonia kit	1 piece	Measuring ammonia
13.	DO meter	1 piece	Measuring dissolved oxygen
14.	Fish Trap	1 piece	Retrieving fish
15.	Bucket	1 piece	Water copy container
16.	Chiffon hose	1 piece	Siphoning the container
17.	Camera	1 piece	Taking documentation
18.	Stationery	1 piece	Recording data
19.	Microscope	1 piece	Observing the sex of fish
20.	Spray bottle	4 pieces	Spraying feed
21.	Blender	1 piece	Smoothing the test material

The Materials used in this study are listed in Tabel 2.

Table 2. Materials used in the study

No.	Material name	Usability
1.	Commercial feed	Test material
2.	80% and 100% Ethanol	Additional Ingredients
3.	Senggani fruit	Test material
4.	Water	Maintenance media
5.	Acetocarmin solution	Fish gonad staining
6.	Aquades	Tool sterilization

## 2.1 Research Procedures

### 2.1.1 Container Preparation

The research container used was a plastic basin with a volume of 35 L of water as many as 20 pieces. Before use, the containers were washed using detergent, then rinsed thoroughly, then dried. Each container was filled with 10 liters of fresh water [14] in each container and given aeration and treatment labels.

### 2.1.2 Preparation of Test Organisms

The organisms used were tilapia larvae aged 7 days. Larvae were obtained from natural spawning at the Tatanga Fish Seed Center (BBI), South Palu District, Palu City. Tilapia larvae were acclimatized for 1 day in two basins. During the acclimatization process, fish were fed with artificial feed (pellets) containing 41% protein [14]. After acclimatization, tilapia larvae were stocked at a density of 2 fish/liter.

### 2.1. Fruit Harvesting and Preparation of Senggani Fruit Extracts

Senggani fruit used was obtained from around Pani'i Village, Dampelas District, Donggala Regency, Central Sulawesi as much as 3 kg, then weighed to get wet weight. Then the Senggani fruit that has been obtained is dried and extracted. Furthermore, the fruit is washed first and then dried in the sun for 4 days. After drying, then weighed to obtain dry weight. Dried Senggani fruit is powdered using a blender and stored in an airtight plastic container for further testing.

A total of 100 g of fruit powder was put into a jar container, macerated with 80% ethanol solvent as much as 500 mL and closed tightly. After that, it was kept in the jar for 3 days. The results of maceration were filtered using what man brand filter paper, the macerated pulp was reused with the same procedure. The work was repeated three times. then continued at the evaporation stage using a *rotatory evaporator* with a temperature of 40 ° C so that a thick extract was obtained [13].

### 2.1.4 Preparation of Feed Treatment

Referring to Safir's research (2018), Senggani fruit extract according to the treatment dose was dissolved using 100% ethanol as much as 25 ml, then the extract was sprayed on 1 kg of feed (41% protein feed), then aerated for 1-2 hours. After drying the feed is stored into each container, then labeled and put into the refrigerator until ready for use.

### 2.1.5 Maintenance of Test Organisms

Larvae aged 7 days post-hatching were reared in a plastic basin with a volume of 35 L filled with 10 liters of water and a larval density of 2 larvae/liter of water. The frequency of feeding was done 3× a day for 62 days of rearing. Water changes were done once a week and body weight weighing was done once a week.

### 2.1.6 Observation of Sex Ratio

Sex identification of fish was done after 62 days of rearing. Sex observation was carried out using the acetocarmine staining method. Fish were taken from the rearing container using a fish trap. After that, the fish were killed using a needle and then dissected from the anus to the operculum using a surgical instrument, then the gonads were taken using tweezers and placed on a preparation glass, then chopped using a knife, then dripped with acetocarmine solution and covered with a *cover*

glass. Furthermore, it is observed using a 40 times magnification microscope so that it is known that the sperm cells only appear in the form of small dots, while the ovum cells appear in the form of large spheres [8].

## 2.2 Research Design

The experimental design in this study was a complete randomized design (CRD) using 5 treatments and 4 replications so that there were 20 units of experimental units. The treatment applied refers to research [14], which uses Senggani (*M. candidum*) leaf extract by oral method on tilapia with the best dose in the treatment of 4 g/kg feed.

This study uses senggani fruit extract with oral method

Treatment A :control (without the use of Senggani fruit extract)

Treatment B :concentration of Senggani fruit extract 0.75 g/kg feed

Treatment C :concentration of Senggani fruit extract 1.5 g/kg feed

Treatment D :concentration of Senggani fruit extract 2.25 g/kg feed

Treatment E : concentration of Senggani fruit extract 3 g/kg feed

The layout of the research container based on the randomization results is shown in Figure 1.

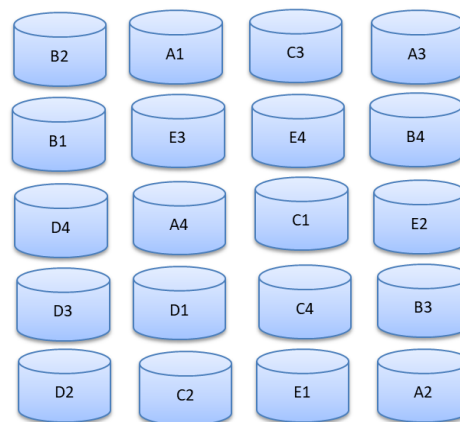


Fig 1. Layout of research containers based on randomization results.

## 3. Results and Discussion

### 3.1 Percentage of Males

Gonads in the test fish, both sperm and egg cells, can be observed using acetocarmine staining Figure 4-1.

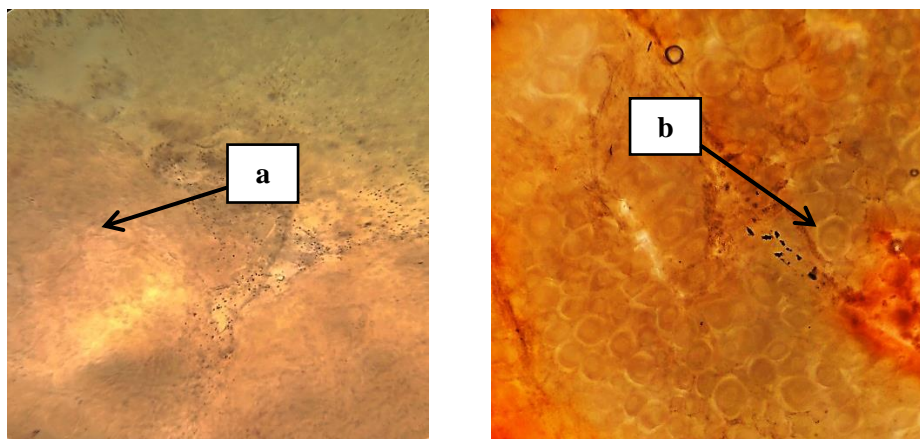


Fig.2 Observation results of tilapia gonads at 40x magnification a. Sperm cell b. Egg cell

Male tilapia were observed after 62 days of rearing. Observations showed images of sperm cells in the form of small dots spread throughout the gonad while the egg cells were seen as large spherical pieces.

The results of analysis of variance (ANOVA) showed that feeding using Senggani fruit extract to tilapia larvae did not give a significant effect ( $p < 0.05$ ) on the percentage of male tilapia (Appendix 2). The highest percentage of male tilapia was obtained in treatment E (3 g/kg) which amounted to 60%. Then followed by treatment D (2.25 g/kg) at 58.75%, treatment C (1.5 g/kg) at 57.5%, treatment B (0.75 g/kg) at 56.25% and treatment A (control) at 50% as shown in Figure 4-2.

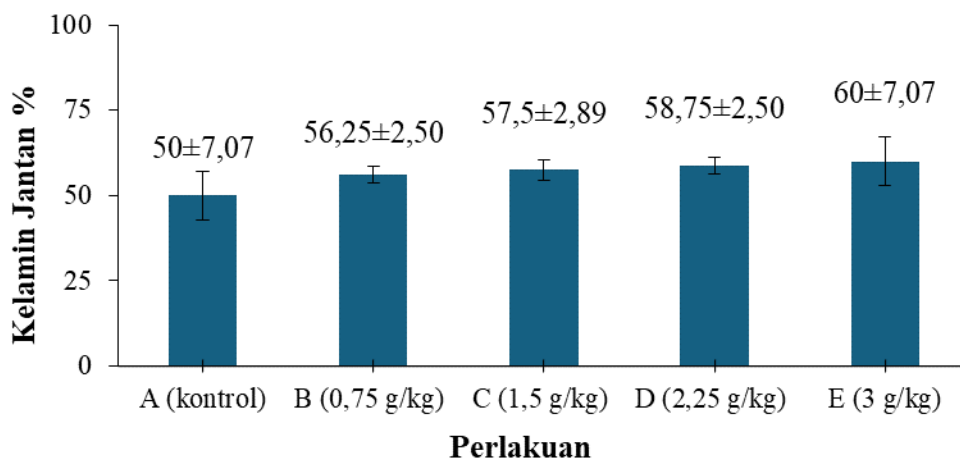


Fig. 2 Male sex percentage of tilapia (*O. niloticus*); A (control), B (0.75g/kg Senggani fruit extract), C (1.5 g/kg Senggani fruit extract), D (2.25 g/kg Senggani fruit extract), E (3 g/kg Senggani fruit extract).

### 3.2 Daily Growth Rate (LPH)

The daily growth rate obtained for 62 days is presented. The results showed that the highest value was obtained in treatment A at 7.49%, followed by treatment D at 7.46%, treatment B at 7.45%, treatment C at 7.44% and the lowest in treatment E at 7.40%. The results of the analysis of variance (ANOVA) showed that feeding using Senggani fruit extract did not have a significant effect ( $P > 0.05$ ) on the daily growth rate of tilapia fish fry during maintenance.

### 3.3 . Feed Conversion Ratio

Feed conversion ratio of tilapia (*Oreochromis niloticus*) during 62 days. The results of the feed conversion ratio of tilapia fish seeds given Senggani fruit extract during the study showed the lowest value in treatments A and D of 1.86 g, followed by treatment B of 1.88 g, then treatment C of 1.89 g and the highest in treatment E of 1.91 g.

The results of analysis of variance (ANOVA) showed that feeding using Senggani fruit extract did not have a significant effect ( $P > 0.05$ ) on the feed conversion ratio of tilapia.

### 3.4 Survival

Based on the results obtained during the study of tilapia survival from each treatment during maintenance The results showed that the survival rate of tilapia seeds from the highest to the lowest value was treatment A (73.75%), B (72.5%) C (72.5%) D (70%) and E (68.76%).

### 3.5 Water Quality

The results of measurements of water quality parameters during 62 days of rearing include temperature, pH, and dissolved oxygen respectively obtained the results of temperature 25.6-28.3 ° C, pH 7.7-8.2, dissolved oxygen 5.5-7.8 mg/L and ammonia 0.01-0.2 for all treatments (Table 4-1). Based on the results of data analysis that feeding using senggani fruit extract does not give a real effect on the percentage of male tilapia. This is due to steroid hormones contained in the extract of Senggani fruit given through the feed is lost because it is too long submerged in water, because the extract of Senggani mixed in the feed does not use the adhesive so that the feed is applied easily



washed in the cultivation medium. Therefore the content in the extract of Senggani fruit that enters the body of the fish will be reduced. States that the provision of hormones through feed has weaknesses, because the hormones contained in the feed will be washed in the cultivation medium and the possibility of degradation of hormones applied through feed can be damaged before working. [15], also added that hormones given through feed require time and sufficient amounts to affect sex differentiation. Similarly, [16] stated that steroids given through feed will be lost in water, even more than 99% of the hormone will be mixed with water in less than 24 hours and most hormones can also degrade so that their activity decreases. Several studies have examined the use of natural ingredients with masculinization methods. Furthermore, Samalei's research [14], also showed the use of *Melastoma malabacthrium* leaf extract with the oral method at a dose of 4 g/kg feed produced a male percentage of tilapia of 80.11%. Hutagalung's research [7], the use of 9% cow testicle flour / kg of feed produced a male percentage of tilapia of 77.3%.

The percentage value of daily growth rate in this study is relatively similar to that reported by Samalei [14], the use of *Melastoma* leaves with oral method to tilapia obtained the highest daily growth rate in the treatment of 1g/kg feed of 7.74%. The feed conversion ratio value in this study is still considered optimal. According to Mokoginta [17], the value of fish feed conversion ratio in general ranges from 1.5-2.5 while the optimal limit of feed conversion ratio value is around 1-3 [18]. The high and low value of feed conversion is influenced by several factors, namely the quality and quantity of feed, fish species, fish size and environmental conditions. Good environmental conditions will affect the metabolic process of fish in utilizing the energy available in order to grow optimally, the feed given must have the quality, size and quantity that is suitable for the needs of tilapia. The less the amount of feed given, the more efficient the feed is and vice versa [19].

Samalei's research [14] which used *Melastoma* leaves by oral method at doses of 0.5 and 1 g/kg feed obtained 90% survival. Furthermore, the addition of the hormone *17 $\alpha$ -Methyltestosterone* to the feed resulted in tilapia survival of 90% [5].

Ammonia measurement results obtained during the study ranged from 0.01-0.2, Ammonia levels in this study are still in accordance with the needs of tilapia. ammonia concentration in tilapia rearing activities <0.2 mg/L (SNI, 2009). However, this ammonia range can still be tolerated and the fish can still live.

#### 4. Conclusion

Giving Senggani fruit extract through feed did not have a significant effect ( $p > 0.05$ ) on the percentage of males in tilapia, daily growth rate and feed conversion ratio. The percentage of male tilapia ranged from 50-60%, the daily growth rate was 7.40-7.49% and the feed conversion ratio was 1.86-1.91.

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