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# Coconut Water (*Cocos nucifera*) as an Agent of Sex Reversal in Tilapia Larvae (*Oreochromis niloticus*)

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

Tilapia is one of the leading commodities because it has high nutritional and economic value. The growth of male tilapia is faster than that of female tilapia. The research dealt with sex reversal of tilapia using coconut water. the study is important in the field of aquaculture production since, sex reversal of tilapia to male can cause improvement on the growth of tilapia. This implicates more harvest and could aid in food security. The effort to incease the percentage of tilapia males can be done by using a maculinization with 17α- Methyltestosterone. However, this chemical material is a type of drug which can cause environmental problem and food poisoning. So, it is important to use as an natural agent on masculinization process of tilapia male which is environmetally friendly, cheaper and easy for application such as coconut water. The study aims to determaine the effect of coconut water (C. nucifera) and its optimal concentration on masculinization process of tilapia

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larvae. This study consider effect of coconut water on zootechnical performance of this tilapia such as survival rate, absolute weight gain and FCR. This study used compelately randomized design (CRD) with four treatments and three replications. There were four different treatments namely A (350 mL/coconut water); B (300 mL/L coconut water); C (250 mL/L coconut water and K (control treatment without coconut water immersion). Tilapia larvae were immersed in coconut water for 8 hours and then reared for 60 days at stocking density 30 fish larvae for each rearing container. The result showed that 300 mL/ L coconut water treatment increased the male sex ratio, survival rate and specific growth rate and reduced feed convertion ratio without a negative impact of survival rate of tilapia fry. In this study, the 300 mL/L cocunat water was the most optimal trreatment which produced a male sex ratio of 86.4%, survival rate of 91.1%, absolut weight gain of 61.3 grams, and feed convertion ratio of 1.17.

*Keywords: Sex reversal; masculinization; coconut water; tilapia.*

## 1. INTRODUCTION

Indonesia is one of the countries whose main commodity comes from aquaculture. This country is a maritime country with a tropical climate, making it easy to cultivate, especially in the fisheries sector. One of the most popular fisheries in Indonesia is tilapia cultivation, this is supported by the easy cultivation process. Tilapia *(Oreochromis niloticus)* has several advantages compared to other freshwater fish, including being easy to maintain, easy to breed, a high level of tolerance to environmental changes, high survival rate, and relatively fast growth. Tilapia also has unique characteristics, where the growth of male tilapia is faster than that of female tilapia (Burhani et al., 2022).

The growth characteristics between male tilapia and female tilapia are very different. Male tilapia have faster growth characteristics with the ability to utilize nutrients in feed faster than female tilapia (Zhu et al., 2022). Female tilapia convert more feed for reproduction than for growth. This is because female tilapia are easytospawn, have relatively fast gonad maturation, and can spawn repeatedly (Robbani, 2017). Then according to (Hutagalung, 2020), the growth rate of female tilapia to reach consumption size is 0.83-1.05 g per day, while male tilapia reaches 1.53-1.69 g per day. So to get a higher size and economic value, most tilapia farmers carry out size and sex selection to separate males and females, to prevent excessive mating and spawning, and to increase fish production. However, carrying out size and sex selection is not easy, due to the large number of individuals that must be separated between males and females. So that many tilapia farmers are constrained at this stage, especially if the stocking density is carried out in large quantities.

To overcome the above problems, farmers can use the sex reversal method. Sex reversal is one of the applications of aquaculture biotechnology that has been widely carried out and developed by fish farmers where before gonad maturation occurs, a treatment is carried out so that the fish will later differentiate into male tilapia. This method is also commonly called the masculinization technique. This technique has been carried out very often, one of which is by using chemicals such as *17α-Methyltestosterone* (Susenoet al., 2020).

*17α-Methyltestosterone* is a synthetic androgen hormone that is commonly used to increase the percentage of male sex in tilapia cultivation. *17α-Methyltestosterone* can affect environmental sustainability and food health, so its application is limited because it can produce residues that are harmful to fish and waters (Ningsih et al., 2018).

Coconut water contains potassium ions (K) which can play a role in the sex reversal process. Young coconut water contains 250 mg/100 grams of young coconut water (Yong et al., 2009). The results of the study concluded that the ability of potassium can change cholesterol into prenegnelone, then this prenegnelone will affect the process of progesterone hormone formation. Progesterone hormone is a sexual hormone that is widely found in humans or male animals. So with the influence of pregnenolone, the differentiation of tilapia can be directed into fish with male sex through a hormonal process (Islama & Nisa, 2017). In addition, coconut water also has various nutritional contents, such as enzymes, phytochemicals, and natural hormones such as testosterone and androgen hormones. These natural testosterone and androgen hormones

play an important role in the sex reversal process (Maulana et al., 2023).

Research on the use of coconut water for masculinization in tilapia (*O. niloticus*) has been widely conducted. Among them are research conducted by (Ernani et al., 2015) which concluded that coconut water has a real effect of up to 90% on the ratio of male tilapia, then research conducted by (Cahyani et al., 2021) concluded that coconut water has an effect on the percentage of male sex up to 86.87%. However, previous studies have had a low survival rate due to the side effects of coconut water. Previous studies also still have a wide range of concentrations, making it difficult to determine the most optimal concentration in masculinizing tilapia. The aim of this study was to evaluate optimal concentration of coconut water on masculinization process of larvae tilapia (*O. niloticus*).

## 2. METHODS

### 2.1 Time and Place of Research

The research was conducted for 64 days, on February - April 2024 at the Brackish Water Aquaculture Research Center and Fisheries Extension, Maros, South Sulawesi, Indonesia.

### 2.2 Research Variables

This research consists of two variables, namely the independent variable and the dependent variable. The independent variable in this study is the treatment of tilapia larvae using coconut water, while the dependent variable is the percentage (%) of males produced, survival rate, absolute weight gain, and food convertion ratio after 60 days after being given treatment.

### 2.3 Tools and Materials

**Tools:** The tools used in this study consisted of an aquarium (60 x 30 x 35 cm), basin, sieve, measuring kettle, digital scales, stationery, camera, machete, bucket, water hose, shipon pump, ½" PVC pipe, aeration hose, aeration stone, aerator, ½" external thread socket (SDL) and water quality meter (YSI Proquatro Multiparameter).

**Materials:** The materials used in this study consisted of tilapia larvae (4 days old), coconut water, commercial feed (MS PREO 320 32% Protein), fresh water, sponge and detergent.

### 2.4 Research Procedure

**Preparation of containers:** This study used two containers, namely a basin and an aquarium with a volume of 60 liters (60cm x 30cm x 35 cm). The basin is used as a container for soaking tilapia larvae as a form of treatment for giving coconut water concentration. The aquarium functions as a container for cultivating tilapia after soaking. Before use, the container and other equipment such as water hose, shipon pump, ½" PVC pipe, aeration hose, aeration stone, and ½" external thread socket (SDL) are washed using a sponge and detergent, then dried for 24 hours.

**Preparation of maintenance media:** The media used in research activities is fresh water. The water is first put into an aquarium that has been previously cleaned, then equipped with an aeration system. After that, the media is left to stand by giving aeration for 2 x 24 hours which is useful for increasing the dissolved oxygen levels in the waterso that fish growth is optimal.

**Preparation of tilapia larvae:** Larvae the tilapia sed in this study were obtained from the Takalar Brackish Water Aquaculture Center located in Galesong District, Takalar Regency. The tilapia seeds used were 3- day-old tilapia seeds (*O. niloticus*) with the sultana tilapia variety. After the seeds were taken from the spawning location, the fish were first acclimatized for 6 hours to adjust the temperature in the quarantine container so that the fish were not stressed (Palimbu & Mandiangan, 2019). After acclimatization, the fish were then quarantined for 1 day to find out which seeds were healthy and good to be used as experimental materials (Pane et al., 2023).

**Preparation of coconut water:** The coconuts used in this study were from local variety species mostly cultivated by the farmers. The coconuts used in this study were coconut from the same tree. Use of coconut water as an agent for sex reversal of tilapia larvae (Cahyani et al., 2021). The coconuts were split to get the water, then collected in the same container. After getting enough coconut water, it was filtered to separate the coconut water from the fiber and coconut shell.

**Treatment process for tilapia larvae:** The coconut water was given to tilapia larvae using a previously prepared basin, then filled with coconut water with different concentrations, namely 250

ml/L, 300 ml/L, and 350 ml/L, with a stocking density of 30 fish/container. Then soaked each for 8 hours. Duration of time choosen 8 hours because the previous study more the 10 hours. If soaking tilapia larvae more times will affect survival rate of larvae

**Fish maintenance:** Fish maintenance was carried out for 60 days after treatment. The feed used is a type of commercial feed with a feed size that is adjusted to the level of fish development. The frequency of feeding is 6 times a day, namely at 07.00, 10.00, 13.00, 16.00, 19.00, and 22.00 WITA.

**Water quality management:** Water quality management includes monitoring temperature, pH, and DO twice a week. Water quality measurements use a tool called the YSI Proquatro Multiparameter by submerging the sensor at the end of the tool. The purpose of this water quality measurement is to determine the condition of the maintenance media. In addition, siphoning is also carried out 3 times a week which aims to clean dirt and leftover feed that settles at the bottom of the research container. Water changes of 25% are carried out twice a week so that water quality can be maintained properly.

**Examination of the sex of test fish:** Examination of the sex of test fish is carried out during maintenance reaching 60 days after treatment (the sex of the fish can be distinguished). Before the observation of sex is carried out, the water in the maintenance container is drained first

so that catching the test fish is easy to do. Examination of the sex of the test fish is carried out one by one by looking at the primary sexual characteristics of the test fish.

## 2.5 Experimental Design

The research used Competely Randomized Design (CRD) wit four treatments and three replications The treatments were carried out by immersion of tilapia larve stage in coconut water. The treatments were as follows:

A = 350 ml/L coconut water immersion
B = 300 ml/L cocnut water immersion
C = 250 ml/L coconut water immersion
K = Control (Without coconut water immersion)

## 2.6 Observation and Analysis of Research Results

Observation of research results was carried out by observing 5 parameters consisting of survival rate, percentage of sex, absolute weight gain, FCR (Food Conversion Ratio), and water quality.

**Survival rate:** Survival is a comparison of the number of fish alive at the end of the study with the number released at the beginning of the study. Survival rate calculation can be calculated using the formula (Ernani et al., 2015).

$$Survival\ rate = \frac{\Sigma \text{ fish at the end of the research}}{\Sigma\ fish\ used\ at\ the\ beginning\ of\ the\ research} \text{ x } 100\%$$

**Percentage of sex:** The percentage of male tilapia is carried out after the maintenance period, namely 45 days after treatment. Calculation of the percentage of male sex can be using the formula (Ernani et al., 2015).

$$\text{Male fish } (\%) = \frac{\Sigma \text{ male fish}}{\Sigma\ fish\ at\ the\ end\ of\ the\ research} \text{ x } 100$$

**Absolute weight gain:** Absolute growth is the addition of fish weight that is maintained until the end of maintenance. Absolute weight gain can be calculated using the formula (Arief et al., 2009).

$$W = Wt - W0$$

**Description:**

W : Absolute weight gain
Wt : Final weight of the research
W0 : Initial weight of the research

**Food Conversion Ratio (FCR):** Food conversion ratio (FCR) or feed conversion ratio (g or kg) is the total amount used during cultivation. The smaller the FCR value the more optimal the feed conversion is said to be. According to the FCR formula can be calculated using the following formula (Siegers et al., 2023).

$$\text{FCR} = \frac{\text{Total Feed Used}}{\text{Final Weight} - \text{Initial Weight}}$$

**Table 1. Observed Water Quality Parameters**

| NO | Parameters | Optimal Range(SNI) |
|---|---|---|
| 1 | Temperature (°C) | 25 - 32 |
| 2 | DO (mg/l) | ≥ 3 |
| 3 | pH | 6,5 – 8,5 |

## 3. RESULTS

### 3.1 Survival Rate 8 Hours, 3 Days, 60 Days After Treatment

The survival rate is the number of fish that survive from the total fish used in each treatment group. Based on the results of the research that has been conducted, it was found that at the treatment stage, three days, and 60 days after treatment, there were several fish that died so that the survival rate decreased. The average survival rate obtained can be seen in Table 2.

Based on Table 2, it shows that the treatment of tilapia larvae using coconut water has a significant effect on the percentage of tilapia survival rate. The higher the concentration of coconut water given, the lower the survival rate produced. Coconut water can become acidic when used in the process of soaking tilapia larvae, so that the pH decreases which will cause stress to the fish, which has an impact on the survival rate. The lowest survival rate was found in treatment A, which was 82.2%, which was significantly different from treatments B, C and K. Treatment B obtained results that were not significantly different from treatment C with an average of 91.1% and 92.2% respectively.

### 3.2 Percentage of Male Sex 60 Days After Treatment

The percentage of male sex is the number of males obtained from the total number of fish that survived until the end of the study. Based on the results of the analysis of the percentage of male sex in each treatment, the percentage of males was obtained which can be seen in Table 3.

Based on Table 3, it shows that the higher the concentration of coconut water given, the higher the percentage of male sex obtained. Treatment A is the most optimal concentration in obtaining the percentage of male sex, which is 89.4%, which is not significantly different from treatment B and significantly different from treatments C and K. Treatment B obtained a percentage of male sex of 86.4% so that the results obtained were not significantly different from treatment A and significantly different from treatment C. In the concentration treatment group, Treatment C obtained the lowest percentage, which was 79.7% and was said to be significantly different from treatments A, B, and K. Treatment K obtained a percentage of male sex of 50.2% so that it was said to be significantly different from treatments A, B, and C.

### 3.3 Absolute Weight Gain 60 Days After Treatment

Absolute weight gain is the addition of fish weight obtained during the study which is reduced by the initial weight of the study. Based on the results of the research that has been carried out, absolute weight growth data was obtained which can be seen in Table 4.

**Table 2. Survival Rate**

| | | Survival Rate | | |
|---|---|---|---|---|
| **Group** | **Total Fish** | **Treatment Process (8 hours) (%)** | **3 days (%)** | **60 days (%)** |
| **A** | 30 | 92.2[a] | 86.6[a] | 82.2[a] |
| **B** | 30 | 97.8[b] | 94.4[b] | 91.1[b] |
| **C** | 30 | 98.9[b] | 95.5[b] | 92.2[b] |
| **K** | 30 | 100[b] | 98.9[b] | 97.8[c] |

***Description:*** *The same letter notation indicates "not significantly different", while treatments with different letter notation indicate "significantly different" based on Duncan's test with a 95% confidence level (α = 0.05).*
*A = 350 ml/L coconut water immersion*
*B = 300 ml/L cocnut water immersion*
*C = 250 ml/L coconut water immersion*
*K = Control (Without coconut water immersion)*

**Table 3. Obtaining Male Sex**

| Group | Survival Rate (60 days) | Obtaining of Male Sex | |
|---|---|---|---|
| **A** | 24.6 | 22 | 89.4[a] |
| **B** | 27.3 | 23.6 | 86.4[a] |
| **C** | 27.6 | 22 | 79.7[b] |
| **K** | 29.3 | 15 | 50.2[c] |

*Description*: *The same letter notation indicates "not significantly different", while treatments with different letter notation indicate "significantly different" based on Duncan's test with a 95% confidence level (α = 0.05).*

**Table 4. Absolute Weight Gain (g)**

| Group | $\bar{X}$Initial Weight | $\bar{X}$Final Weight | Absolute Weight Gain |
|---|---|---|---|
| **A** | 0.6 | 64.2 | 63.6[a] |
| **B** | 0.6 | 61.9 | 61.3[a] |
| **C** | 0.6 | 54.3 | 53.7[b] |
| **K** | 0.6 | 45.3 | 44.7[c] |

*Description*: *The same letter notation indicates "not significantly different", while treatments with different letter notation indicate "significantly different" based on Duncan's test with a 95% confidence level (α = 0.05).*

Table 4 showed that the treatment of tilapia larvae using different concentrations of coconut water has a significant effect on the absolute weight gain obtained. It can be seen that treatment A obtained the highest absolute weight gain of 63.6 grams, while the lowest absolute weight gain was obtained in treatment K, which was 44.7. This absolute weight gain is influenced by the number of males produced. Male tilapia have faster growth, resulting in greater weight.

## 3.4 Food Conversion Ratio (FCR) 60 Days After Treatment

Based on the results of the research that has been done, the FCR for each treatment can be seen in Table 5.

Table 5 showed that the treatment of tilapia larvae with different concentrations of coconut water has a significant effect on the level of FCR produced. The higher the concentration of coconut water given, the lower the FCR obtained. FCR shows the level of productivity in fish, the lower the FCR obtained, the higher the level of productivity of the fish (Robbani, 2017). It can be seen that treatment A gave results that were not significantly different from treatment B with each FCR being 1.14 and 1.18. Treatment B obtained an FCR that was significantly different from treatments C and K, where treatment C obtained an FCR of 1.34 and treatment K of 1.62. So that the most optimal concentration of coconut water was obtained in treatment A.

## 3.5 Range of Water Quality

During the research, physical water quality control was carried out with a minimum-optimum range as seen inTable 6.

Based on the Table 6, it can be seen that at the time the research was conducted, the water quality in the maintenance media was well controlled and in accordance with the established Indonesiannational standards (SNI).

**Table 5. Food Convertion Ratio**

| Group | Total Feed Used (g) | Absolute Weight (g) | FCR |
|---|---|---|---|
| **A** | 72 | 63.6 | 1.13[a] |
| **B** | 72 | 61.3 | 1.17[a] |
| **C** | 72 | 53.7 | 1.34[b] |
| **K** | 72 | 44.7 | 1.61[c] |

*Description*: *The same letter notation indicates "not significantly different", while treatments with different letter notation indicate "significantly different" based on Duncan's test with a 95% confidence level (α = 0.05).*

**Table 6. Range of Water Quality Obtained**

| Group | Range of Water Quality Obtained | | |
|---|---|---|---|
| | Suhu (°C) | pH | DO (mg/L) |
| **A** | 26,8-29,2 | 7,20-7,81 | 4,82-6,92 |
| **B** | 26,8-29,3 | 7,18-7,79 | 5,11-6,86 |
| **C** | 26,8-29,2 | 7,14-7,73 | 5,12-6,97 |
| **K** | 26,8-29,2 | 6,93-7,68 | 5,17-6,83 |
| **Optimum (Indonesian National Standard)** | 25-32 | 6,5-8,5 | ≥ 3 |

## 4. DISCUSSION

The results of the study that have been conducted using coconut water in several treatments, namely 350 ml/L, 300 ml/L, 250 ml/L, and Control showed different results between each treatment. There are four main parameters in this study that must be considered to determine the most optimal treatment from this study, including the survival rate, percentage of male sex, absolute weight growth, and total feed conversion (FCR). The four above parameters are important indicators that must be considered in the process of cultivating tilapia (*Oreochromis niloticus*) in order to obtain optimal results.

Based on Table 1, the results of the study show that the survival rate decreases as the concentration of coconut water given increases. The table shows that the highest survival rate is in the control, and the lowest survival rate is in treatment A which is the treatment with the highest concentration of coconut water. This is thought to be due to the level of concentration of coconut water given affecting the quality of the water in the treatment media. The higher the concentration of coconut water given, the lower the water quality in the treatment media, because coconut water easily changes color to cloudy and the aroma becomes smelly, then triggers an increase in ammonia levels in the treatment media. The treatment media using coconut water can increase ammonia levels up to 2 Mg/L. When ammonia levels are high, fish will easily become stressed and cause death (Masprawidinatra et al., 2015).

Based on Table 2, it shows that the percentage of male sex increases along with the higher concentration of coconut water. Potassium (K) content or commonly called potassium is a mineral that can increase androgen hormone secretion through the process of converting cholesterol to pregnenolone. This pregenenolone compound is a source of steroid hormone biosynthesis which will cause or trigger the formation of testosterone (Islama & Nisa, 2017). In accordance with the results of the study which showed that the group giventhe highest concentration of coconut water got the highest percentage of males, while the control treatment got the lowest percentage of males. The results of the study obtained by (Masprawidinatra et al., 2015; Cahyani et al., 2021; Laheng & Widyastuti, 2019) concluded that the potassium contained in coconut water has a significant effect on tilapia larvae. So coconut water has great potential to be used as a natural ingredient to increase the percentage of male sex in tilapialarvae.

Based on the research results in Table 3, it shows that giving coconut water with different concentrations has a significant effect on the level of absolute weight gain (grams) on tilapia larvae. The higher the concentration of coconut water, the higher the absolute weight gain. Absolute weight gain is the total weight gain obtained during the study. This is because by giving coconut water concentration, it will trigger an increase in the number of male sex in each treatment. With the increase in the number of male sex, the level of productivity in each treatment will also increase. This is in accordance with the theory of (Thongprajukaew, 2017), which states that male tilapia are more productive in converting feed so that the absolute weight gain produced is also faster. So that the absolute weight gain is closely related to the survival rate and the percentage of males obtained. In accordance with the results of the study above, it can be concluded that the most productive absolute weight gain in this study was in treatment A and treatment B.

Food conversion ratio is the number of feed effectiveness on fish weight gain. Based on Table 4, it is found that the higher the concentration of coconut water given, the lower the FCR level obtained. This figure is used as a benchmark to determine the efficiency of feed on fish growth, where the lower the FCR level obtained, the better the cultivation productivity

obtained. This is interrelated with the percentage of male sex obtained, namely the higher the percentage of males, the lower the FCR obtained. Fish with male sex have a relatively fast feed conversion rate. This is because the characteristics of male tilapia fish have a relatively high level of productivity and their ability to utilize nutrients in feed faster than female fish (Robbani, 2017). In relation to the discussion above, it can be seen that the treatments with the lowest FCR levels are in treatments A and B.

The advantage of tilapia (*Oreochromis niloticus*) is its characteristics that easily adapt to environmental changes. So that tilapia is included in the group of fish that are easy to maintain. However, to ensure the safety of tilapia, water quality must still be maintained so that the fish do not experience stress to death. In accordance with this study, water quality control is still carried out, in order to get maximum results as expected. It can be seen in Table 5 where the water quality obtained is in accordance with the national standards (SNI). that have been set. So it can be concluded that this study was carried out with good water quality control.

## 5. CONCLUSION

Immersion during the fish larvae stage on coconut water can increase the male sex ratio of tilapia seeds. The 300 mL/L coconut water immersion treatment was the optimal concentration with a male sex ratio of 86 % and survival rate, absolute weight gain and FCR, 91.1 %, 61.3 grams and 1.17 respectively.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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