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COMPARATIVE STUDY ON THE EFFECTIVENESS OF HONEY, COCONUT WATER, AND METHYL TESTOSTERONE IN THE MASCULINIZATION OF NILE TILAPIA *(Oreochromis niloticus)*

Studi Perbandingan Tentang Efektivitas Madu, Air Kelapa, dan Metil Testosteron dalam Maskulinisasi Ikan Nilapia Nila (*Oreochromis niloticus*)

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ABSTRACT

This study aims to analyze the comparative effectiveness of honey, coconut water and methyl testosterone in the masculinization process of tilapia (Oreochromis niloticus). The method used was an experiment with four treatments, namely control (P1), methyl testosterone (P2), coconut water (P3), and honey (P4). The parameters observed included masculinization percentage, survival rate (SR), absolute weight, specific growth rate (SGR), and water quality. The results showed that methyl testosterone treatment produced the highest level of masculinization of 90.29% ± 3.97, followed by honey at 70.79% ± 13.34, coconut water 49.29% ± 8.61, and control 35.97% ± 8.64. The SR value ranges between 68–78% and is still considered good. Fish growth observed through absolute weight and SGR did not show significant differences between treatments, although the highest values tended to be obtained in the honey treatment. Water quality parameters during the study were within the optimal range for raising tilapia. Based on these results, methyl testosterone is the most effective treatment in masculinization, while honey has the potential to be a more environmentally friendly alternative to natural ingredients.

Keywords: Masculinization, Tilapia, Methyl Testosterone, Honey, Coconut Water.

ABSTRAK

Penelitian ini bertujuan untuk menganalisis efektivitas komparatif madu, air kelapa, dan metil testosteron dalam proses maskulinisasi ikan nila (Oreochromis niloticus). Metode yang digunakan adalah percobaan dengan empat perlakuan, yaitu kontrol (P1), metil testosteron (P2), air kelapa (P3), dan madu (P4). Parameter yang diamati meliputi persentase maskulinisasi, tingkat kelangsungan hidup (SR), berat absolut, tingkat pertumbuhan spesifik (SGR), dan kualitas air. Hasil penelitian menunjukkan bahwa perlakuan metil testosteron menghasilkan tingkat maskulinisasi tertinggi sebesar 90,29% ± 3,97, diikuti oleh madu sebesar

70,79% ± 13,34, air kelapa 49,29% ± 8,61, dan kontrol 35,97% ± 8,64. Nilai SR berkisar antara 68–78% dan masih dianggap baik. Pertumbuhan ikan yang diamati melalui berat absolut dan SGR tidak menunjukkan perbedaan signifikan antar perlakuan, meskipun nilai tertinggi cenderung diperoleh pada perlakuan madu. Parameter kualitas air selama penelitian berada dalam kisaran optimal untuk budidaya ikan nila. Berdasarkan hasil ini, metil testosteron merupakan perlakuan yang paling efektif dalam maskulinisasi, sedangkan madu berpotensi menjadi alternatif yang lebih ramah lingkungan dibandingkan bahan alami.

Kata Kunci: Maskulinisasi, Ikan Nila, Metil Testosteron, Madu, Air Kelapa.

INTRODUCTION

Tilapia (Oreochromis niloticus) is one of the aquaculture commodities that has high economic value, with market demand continuing to increase from time to time. The advantages of this fish lie in its fast growth rate, tolerance to various environmental conditions, and its ability to adapt well (Rinanto et al., 2024). According to data from the Ministry of Maritime Affairs and Fisheries (KKP), tilapia production in Indonesia in 2023 will reach 1.5 million tonnes with a value of IDR 33.62 trillion, making tilapia one of the freshwater commodities with the highest production volume (Kusumanti et al., 2023). Tilapia has very promising prospects and profit potential in the field of cultivation. However, the difference in growth rate between male and female fish is a challenge in itself, because male fish are known to grow faster than females. Therefore, cultivation with a single male population is recommended to increase production efficiency, considering that male fish are able to reach harvest size in a shorter time than female tilapia (Cahyani et al., 2021).

The sex reversal technique is one of the methods commonly used in cultivation to produce monosexual male populations of tilapia (Oreochromis niloticus). One effective way of using this technique is by using synthetic androgen hormones such as methyl testosterone (MT). MT is a sex steroid hormone that is used to direct the development of fish gonads from female to male without changing their genotype. This hormone works by providing a masculinizing effect, and is known to be more effective than natural androgen hormones because it has a longer reaction time in target cells and a slower elimination process in the body. The main content in methyl testosterone is an active androgen compound, which is able to influence fish sexual differentiation, especially during the initial phase of growth. Due to its high effectiveness, MT is one of the most widely used hormones in fish farming for masculinization purposes. The addition of the methyl group also makes 17α-MT difficult to biodegrade, so its use can cause accumulation of residues in fish bodies and the aquatic environment (Ibrahim et al., 2018). This negative impact prompted the emergence of regulations from the government, namely through Decree of the Minister of Maritime Affairs and Fisheries Number KEP.52/MEN/2014, which classifies this hormone as a hard drug because it can leave residue, both on the fish's body and in the environment, and poses a risk to food safety and ecosystem sustainability (Cahyani et al., 2021). This condition encourages the search for alternative natural ingredients that are safer, environmentally friendly, and still effective in the masculinization process.

The search for alternatives to the use of synthetic hormones in the sex reversal process of tilapia (Oreochromis niloticus) encourages the use of natural ingredients that are safer and more environmentally friendly. Honey and coconut water are two natural ingredients that cultivators are starting to pay attention to because coconut water and honey are very easy to obtain, not only that, these two natural ingredients contain bioactive compounds that have the potential to support the masculinization process. Honey contains various important components such as potassium, chrysin, enzymes, vitamins and antioxidants which play a role in supporting fish health and growth. Potassium and chrysin are the main compounds in honey which have been widely studied regarding their effect on the hormonal system (Talbiah et al., 2024). Coconut water contains high levels of essential minerals such as potassium. This content plays a role in maintaining the physiological balance of the fish's body and supports optimal growth. Apart from that, the active compounds in coconut water can also influence the process of hormonal gonad differentiation, so that the development of the sexual organs tends to lead to the formation of testes and produces male individuals (Puspitha et al., 2023).

Therefore, it is necessary to carry out research regarding the comparative effectiveness of honey, coconut water, and methyl testosterone in the *sex reversal* process of tilapia fish using the immersion method, in order to support more efficient, sustainable and environmentally friendly cultivation.

METHOD

Time and Place

This research was carried out for 60 days, in September - November 2025. The rearing of tilapia fish and the larval soaking process using honey, coconut water and methyl testosterone were carried out in the Production and Reproduction Laboratory, while observations of fish sex morphology were carried out in the Marine Hydrology Laboratory. All research activities were carried out in the Aquaculture Study Program, Faculty of Agriculture, University of Mataram.

Tools and materials

The tools used in this research include aerators, stationery, bottles, DO meters, buckets, plastic measuring cups, scissors, cameras, preparation glasses, microscopes, trays, dropper pipettes, metal racks, siphon hoses, chopsticks/stirrers, syringes, pH meters, tweezers, net scoops, thermometers, digital scales, analytical scales, and plastic jars. Meanwhile, the materials used in this research include fresh water, coconut water, distilled water, tilapia fish seeds (Oreochromis niloticus), bamboo, labels, latex, methyl testosterone, methylene blue, pure honey, commercial feed, and liquid soap.

Research methods

This research used an experimental method with the addition of natural and synthetic ingredients in the form of honey, coconut water, and methyl testosterone which were applied via the soaking method to tilapia (Oreochromis niloticus) larvae. This research was designed using a Completely Randomized Design (CRD) with 4 treatments and 3 replications so that there were 12 experimental units, consisting of one control (without treatment) and three treatments with different types of materials.

Table 1. Experimental Design

Treatment	Information
P1	Untreated fish
P2	Soaking tilapia larvae using methyl testosterone at a dose of 5 mg/L for 8 hours
P3	Soaking tilapia larvae using coconut water at a dose of 35% for 10 hours
P4	Soaking tilapia larvae using honey at a dose of 2% for 24 hours

Preparation of Containers and Maintenance Media

This research used 12 plastic jars with a capacity of 5 liters as containers for rearing tilapia fish seeds (Oreochromis niloticus) with a water volume of 4 liters per container. Before use, all jars are cleaned using soap to remove dirt and residue, then rinsed with fresh water until clean. The containers are then arranged according to the experimental design and labeled to make it easier to identify the treatment. Each jar is filled with fresh water that has been settled to maintain the quality of the media, then equipped with an aeration system to ensure oxygen availability remains optimal so as to support the survival and growth of fish during the research.

Preparation of Test Animals

The test animals used in this research were tilapia fish seeds (Oreochromis niloticus) aged 5–7 days after hatching which were obtained from the Batu Lingsar Fish Seed Center (BBI), Lingsar District, West Lombok Regency, West Nusa Tenggara. Before treatment, the seeds are acclimatized for 24 hours in settled fresh water with controlled temperature and aeration to adapt to the new environment and reduce stress levels. During the process, weak or abnormal seeds are removed. After acclimatization, the seeds were sown into rearing containers according to the experimental design. Next, the soaking process was carried out using the treatment material, while the control group was not treated. After soaking, the seeds are maintained and given commercial feed regularly until they reach a size that allows microscopic observation of sex.

Research Parameters

The research parameters were indicators observed to assess the effect of honey, coconut water and methyl testosterone treatment on sex reversal (masculinization) in tilapia (Oreochromis niloticus). The parameters observed in this research are as follows:

Survival Rate (SR)

SR calculations were carried out to determine the percentage of survival until the end of the maintenance period. By knowing the SR value, farmers can evaluate the level of success of cultivation and identify factors that influence the survival of tilapia. SR calculation formula in Kirikanang et al., (2022). as follows.

$$SR = \frac{\text{number of fish at the end of rearing}}{\text{number of fish at the start of rearing}} \times 100\%$$

Absolute Weight Growth

Absolute weight calculations were carried out to measure the increase in weight of tilapia fish during the 30 day rearing period. The following is the absolute weight formula in Usman et al., (2022).

$$W = Wt - W0$$

Specific Growth Rate (SGR)

Daily growth rate calculations were carried out to determine the growth rate of tilapia each day during the maintenance period. The SGR value provides information about fish growth efficiency over time, which is calculated based on changes in the average initial and final weight Kirikanang et al., (2022).

$$SGR\ (\%) = \frac{\ln Wt - \ln Wo}{T} \times 1000$$

Gender Percentage

The sex percentage is the percentage of the number of tilapia larvae that experience sex reversal to males of the total larvae observed. This parameter is used to determine the level of success of the masculinization process in tilapia. Determining the percentage of male sex was carried out based on the results of fish sex identification by Cahyani et al., (2021).

$$\text{Percentage of males} = \frac{\text{Number of male fish}}{\text{Total number of fish}} x\ 100\%$$

Gender Observation

Observation of the sex of tilapia (Oreochromis niloticus) is carried out in two stages, namely visual observation (macroscopic) and microscopic confirmation. Initial observations were made with the naked eye to distinguish morphological characteristics between male and female fish. Male fish have a slimmer and elongated body and pointed and prominent urogenital papillae. Meanwhile, female fish have a more rounded body with blunt and rounded urogenital papillae. This visual observation was carried out to sort the fish before further testing was carried out in the laboratory of Budin et al., (2024). After visual sorting, gender observations were confirmed through the histology method using acetocarmine solution. Fish gonads were taken from the abdominal cavity and finely chopped on a glass slide to make staining easier. The acetocarmine solution is dripped until the gonad tissue is completely submerged. Next, the object glass was covered with a cover glass and observed under a microscope with 40 times magnification. Sperm cells appear as small dots, while egg cells appear as large dots Cahyani et al., (2021).

Water Quality

The air quality parameters described in this research include temperature, pH, and DO (dissolved oxygen). Measurements were carried out every 10 days during the maintenance period, namely at the beginning, middle and end of the research, to ensure that environmental conditions continued to support the growth and development of tilapia fish.

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Survival Rate (SR)

Figure 4.1 shows that the Survival Rate (SR) value of tilapia ranges from 68.33% to 78.33%. The highest value was obtained in treatment P1 (control) at 78.33% ± 2.89, followed by P4 at 73.33% ± 2.89 and P3 at 71.67% ± 2.89, while the lowest value was at P2 at 68.33% ± 2.89. Differences in SR values between treatments indicate that the treatment given influences the ability of fish to maintain *surviva*l during the rearing period.

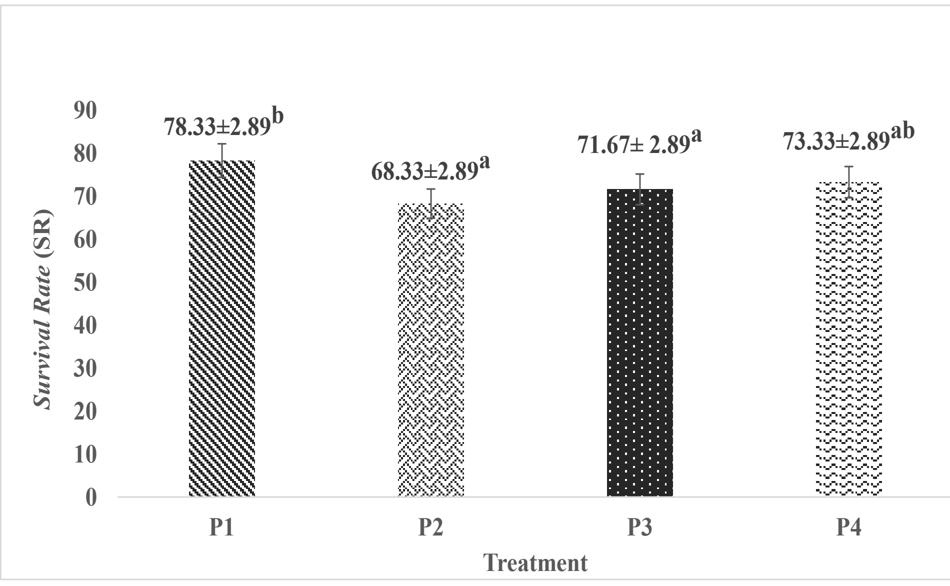


Figure 4.1 Survival Rate (SR) of Tilapia (Oreochromis niloticus)

The results of analysis using Analysis of Variance (ANOVA) show that masculinization treatment with different ingredients has a real influence on the SR value ($p < 0.05$). Based on Duncan's further test, treatment P1 was significantly different from P2 and P3, but not significantly different from P4. This indicates that the type of treatment material influences the physiological response of fish, especially in supporting survival rates during rearing.

Absolute Weight

Figure 4.2 shows that the absolute weight value of tilapia ranges from 3.95 g to 5.19 g. The highest value was obtained in treatment P4 at 5.19 ± 0.23 g, followed by P2 at 5.18 ± 0.44 g and P3 at 4.86 ± 1.21 g, while the lowest value was at P1 at 3.95 ± 0.60 g. Differences in absolute weight values between treatments indicate variations in fish growth during the rearing period.

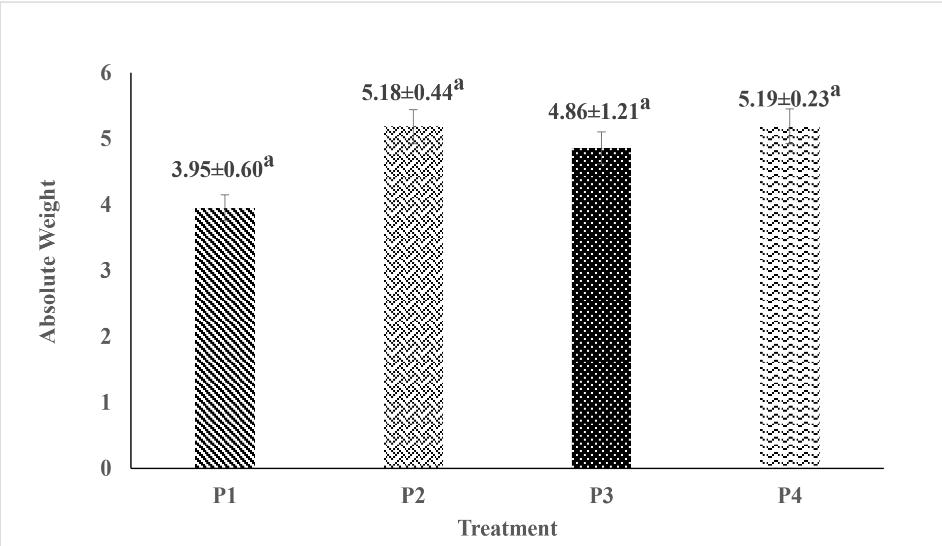


Figure 4.2 Absolute weight of Tilapia (Oreochromis niloticus)

The results of analysis using Analysis of Variance (ANOVA) showed that masculinization treatment with different ingredients did not have a significant effect on the absolute weight of tilapia ($p > 0.05$). Based on Duncan's further test, treatment P1 was not significantly different from P3, but significantly different from P2 and P4. This shows that although there are differences in descriptive values, not all treatments had a significant influence on the increase in fish weight during the study.

Specific Growth Rate (SGR)

Based on Figure 4.3, the Specific Growth Rate (SGR) value of tilapia during rearing is in the range of 2.08–2.29%/day. The highest value was recorded in treatment P4 at 2.29%/day ± 0.11, followed by P2 at 2.25%/day ± 0.12 and P3 at 2.20%/day ± 0.22, while the lowest value was obtained at P1 at 2.08%/day ± 0.04. These differences in values indicate variations in daily growth of fish in each treatment.

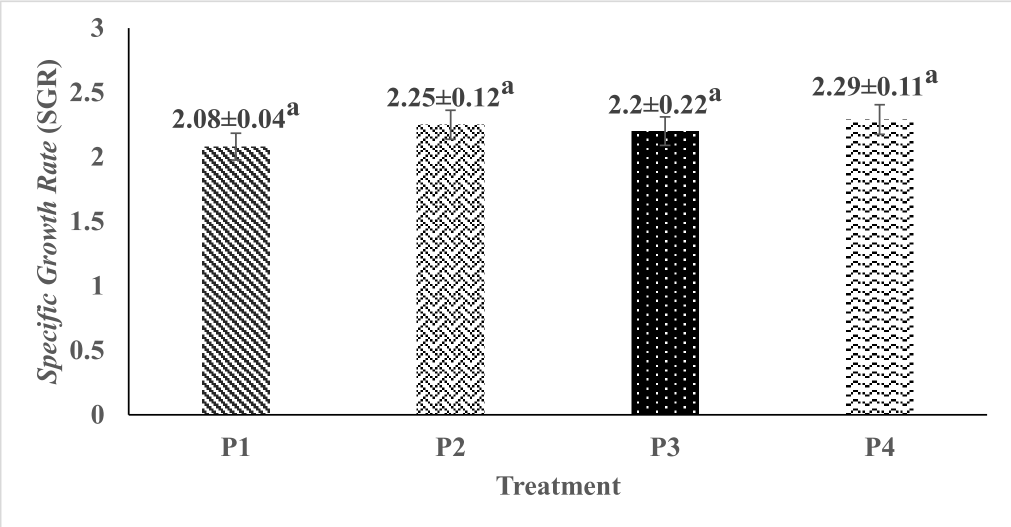


Figure 4.3 Specific Growth Rate (SGR) of Tilapia (Oreochromis niloticus)

The results of statistical tests using Analysis of Variance (ANOVA) showed that masculinization treatment with different ingredients did not have a significant effect on the SGR value ($p > 0.05$). This indicates that the growth rate of tilapia tends to be homogeneous in all treatments during the research period.

Gender Percentage

Figure 4.4 shows that the percentage of masculinization of tilapia ranges from 35.97% to 90.29%. The highest value was obtained in treatment P2 at 90.29% ± 3.97, while the lowest value was at P1 at 35.97% ± 8.64. P4 treatment resulted in a masculinization percentage of 70.79% ± 13.34, followed by P3 of 49.29% ± 8.61. These differences in values indicate the influence of treatment on the success of male sex formation in tilapia.

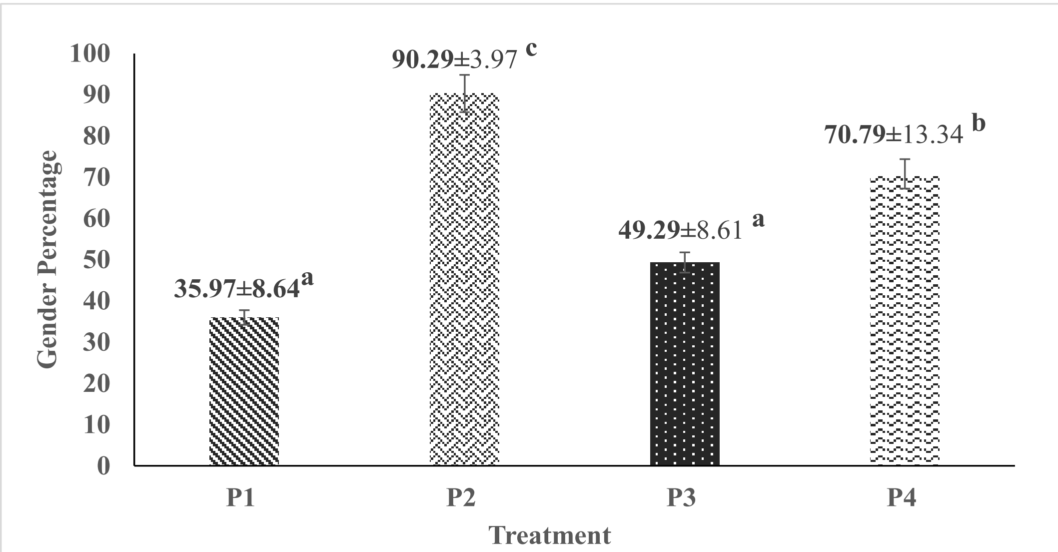


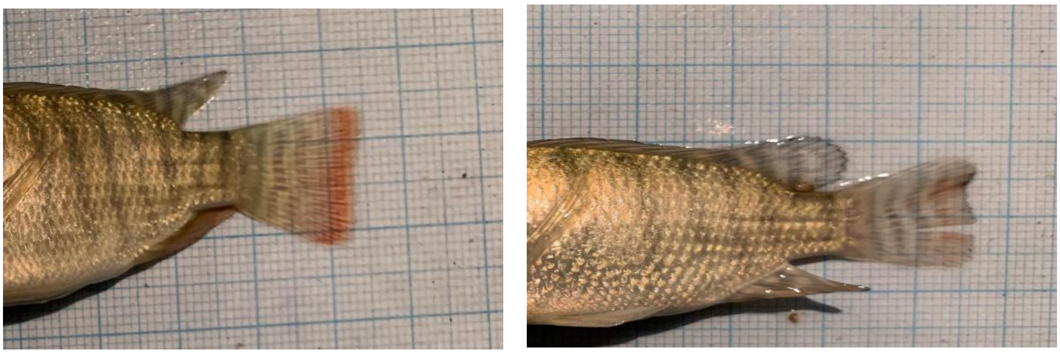
Figure 4.4 Masculinization of Tilapia (Oreochromis niloticus)

The results of analysis using Analysis of Variance (ANOVA) showed that masculinization treatment with different ingredients had a significant effect on the percentage of male genitalia ($p < 0.05$). Based on Duncan's further test, treatment P1 was significantly different from P2 and P4, but not significantly different from P3. This shows that the treatment materials used play a role in influencing the sexual differentiation process of tilapia in the early stages of development.

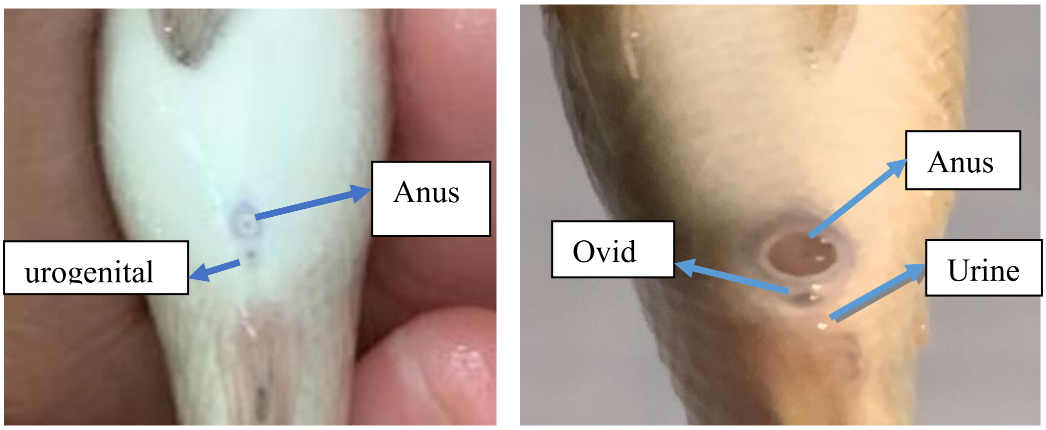
Gender Observation

a. Direct Observation (Macroscopic)

Based on Figure 4.6, morphologically male tilapia fish can be recognized by the presence of two external openings, namely a relatively larger anus and one urogenital opening which functions as a channel for urine and sperm excretion with an elongated shape. Apart from that, additional characteristics can be seen in the dorsal fin which is generally longer with a tapered tip, making the identification process easier. On the other hand, female tilapia have two clearly visible output holes, namely a round anus and a smaller oviduct located at the bottom of the anus. Females also have a urinary tract (urethra) that opens around the oviduct and often appears as a clear fluid, but does not form a separate external opening.



a. Male b. Female

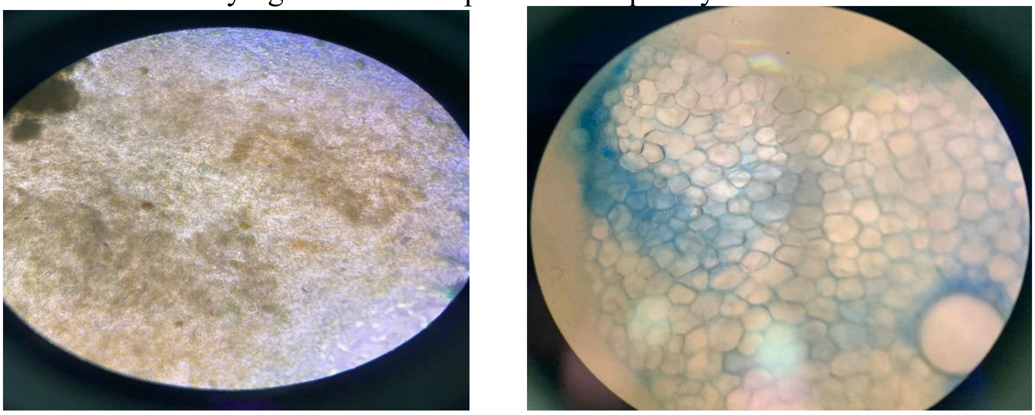


b. Male b. Female

Figure 4.6 Macroscopic Observation of the Sex of Tilapia

b. Observation Using a Microscope (Histology)

Based on the results of histological observations of tilapia gonads using a microscope with methylene blue staining, clear differences were found in the structure of gonad cells between male and female individuals. In male gonad preparations (Figure 4.5a), a densely arranged collection of spermatogenic cells with a relatively small size and a more homogeneous pattern can be seen. In contrast, in female gonad preparations (Figure 4.5b), round-shaped cells with a larger size, resembling oocytes, have clear cell boundaries and wider cytoplasm. This difference in cell morphology indicates a real separation of gonad characters, so it can be used as an indicator in identifying the sex of tilapia microscopically.



(a) (b)

Figure 4.7 Histological observations of tilapia fish (a) male genitalia, (b) female genitalia

Water Quality

The results of water quality testing during the research are presented in table 3 as follows.

Table 2. Water Quality

	Treatment				
Parameter	P1	P2	P3	P4	Literatur
Temperature (ºC)	28-28.5	27,7-28,3	28,1-28,6	27,2-28,3	25 –30 Azhari et al., 2018
DO (mg/L)	3,4-6,2	3,4-6,5	3,3-6,5	3,2-6,3	5,3-6,8 Vica et al., 2024
pH	7,7-8,2	7,7-8,2	7,7-8,1	7,8-8,2	7-8 Azhari et al., 2018

The results of water quality measurements are presented in Table 4.2, which includes temperature, pH and dissolved oxygen (DO) parameters to describe the conditions of the tilapia (Oreochromis niloticus) rearing media. In general, temperature and pH values tend to be in a stable range throughout the study period. Meanwhile, DO levels at the start of maintenance were relatively low, but showed a gradual increase until the end of the study.

DISCUSSION

Survival Rate (SR)

Survival rate (SR) is an important parameter to evaluate the ability of tilapia (Oreochromis niloticus) to maintain survival during the rearing period in aquaculture systems. The SR value is expressed as a percentage, namely the comparison between the number of live fish at the end of rearing and the number of fish at initial stocking. This concept is in line with Muhsoni et al., (2021) which states that the survival rate of fish is calculated based on the ratio of the number of individuals that survive from the start to the end of rearing.

The SR value of tilapia in the sex reversal study showed variations between treatments. Treatment P1 resulted in the highest survival rate of 78.33%, while the lowest value was in treatment P2 at 68.33%. Meanwhile, treatments P3 and P4 respectively showed SR values of 71.67% and 73.33%. Overall, the SR range was 68–78%, which indicates that the survival rate of fish during the study was still relatively good. This condition reflects the fish's ability to adapt to the treatment given and the rearing environment. These criteria are in accordance with Cahyani et al., (2021) which classifies SR values ⩾50% as good category, 30–50% medium category, and <30% low category. When compared with several previous studies, the SR value in this study was relatively higher because all treatments were above the minimum limit for the good category, thus indicating that the sex reversal treatment was still within the physiological tolerance limits of tilapia. Abdullah et al., (2023) also reported that the immersion method in sex reversal was able to increase the percentage of males, growth and survival of tilapia, although variations in treatment doses did not have a significant effect on the SR value.

During the research process, fish deaths in the soaking phase only occurred in limited numbers in treatments P3 and P4, while in treatment P2 no mortality was found at that stage. However, an increase in mortality occurred at the end of the maintenance period which was related to disruption of the aeration system, causing a decrease in dissolved oxygen levels. This condition has an impact on decreasing SR values, especially in treatment P2. This indicates that fluctuations in SR values in this study were more influenced by environmental factors and rearing management than by the sex reversal treatment itself. This statement is supported by Wicaksono et al., (2016) who stated that fish survival rates are influenced by biotic factors, such as age and adaptability, as well as abiotic factors, including water quality and food availability.

Absolute Weight

Absolute weight describes the difference in weight of tilapia (Oreochromis niloticus) between the beginning and end of rearing, which reflects the fish's ability to utilize feed and allocate energy for growth during the sex reversal process. The data in Figure 4.2 shows that there are variations in absolute weight values between treatments. The highest value was recorded in treatment P4 at 5.19 g, while the lowest value was obtained in treatment P1 at 3.95 g. These differences indicate variations in the efficiency of feed energy utilization during the rearing period.

The higher absolute weight value in treatment P4 indicates that the fish are able to allocate energy in a balanced manner between physiological needs and growth, so that the increase in body weight remains optimal. This condition is in line with Cahyani et al., (2021) which states that an optimal masculinization process allows the utilization of metabolic energy to be more efficient. In addition, Abdullah et al., (2023) reported that giving honey can increase the growth of tilapia, which is thought to be related to the prebiotic content which plays a role in increasing the efficiency of feed utilization.

However, the absolute weight growth obtained did not show significant differences between treatments. This indicates that tilapia are still able to utilize feed optimally even though they are under sex reversal treatment conditions. This finding is in line with Karim et al., (2025) who stated that fish weight growth is greatly influenced by the ability to utilize feed, so that good feed management is an important factor in supporting an increase in body weight. Apart from that, Cahyani et al., (2021) also explained that tilapia fish growth can continue well during the masculinization process, as long as environmental conditions and food availability are within the appropriate range.

Specific Growth Rate (SGR)

Specific Growth Rate (SGR) is an indicator of the daily growth rate of fish which is calculated based on changes in average weight between the start and end of rearing. This parameter is used to assess growth efficiency and the ability of fish to utilize energy for the formation of body tissue (Kirikanang et al., 2022). The data in Figure 4.3 shows that there are variations in SGR values between treatments. The highest value was obtained in treatment P4 at 2.29%/day, while the lowest value was in treatment P1 at 2.08%/day. Meanwhile, treatments P2 and P3 respectively showed values of 2.25%/day and 2.20%/day. These differences reflect variations in the growth rate of tilapia during the rearing period which is influenced by the treatment given.

The higher SGR value in treatment P4 indicates that soaking with honey not only plays a role in the *sex reversal* process, but also contributes to increasing the daily growth of tilapia. The mineral content and bioactive compounds in honey are thought to support the efficient use of feed energy for the formation of body tissue. In contrast, the lower SGR value in the control treatment (P1) indicates that without immersion treatment, the energy obtained from feed tends to be allocated more to maintaining physiological function than to growth. Abdullah et al., (2018) stated that honey not only plays a role in masculinization, but is also able to increase the growth of fish larvae through optimizing metabolism and energy utilization. This finding is also supported by Mukti, (2009) who reported that treatment with honey resulted in a higher growth rate compared to no treatment, which is thought to be related to the content of simple carbohydrates, vitamins, minerals and bioactive compounds which play a role in supporting metabolic processes. In this way, the available energy can be utilized more optimally for the growth of body tissue.

Masculinization

Masculinization is a technique for manipulating fish sexual development by directing gonad differentiation towards males (Puspitha et al., 2023). The masculinization value of tilapia in this study showed variations between treatments. Treatment P2 (methyl testosterone) produced the highest level of 90.29% ± 3.97, while control treatment (P1) showed the lowest value of 35.97% ± 8.64. Meanwhile, treatments P4 (honey) and P3 (coconut water) produced 70.79% ± 13.34 and 49.29% ± 8.61, respectively. The high success in P2 shows the effectiveness of methyl testosterone in directing sexual differentiation towards males, even higher than the report by Ngantu et al,. (2023) of 68.3%. This is in line with Ariyanto et al. (2010) who stated that synthetic androgens are able to direct gonad development towards males by regulating hormonal balance.

The effectiveness of methyl testosterone is related to its nature as an androgen hormone which can be absorbed through the gills and body surface, then influences the endocrine system in the gonad differentiation process. Steroid hormones work by diffusing into cells, binding to receptors, and regulating the expression of genes that play a role in the formation of sexual phenotypes (Rohmania et al., 2019). This process supports the dominance of androgen hormones in the early phases of development, thereby increasing the success of masculinization.

A natural ingredient treatment, honey shows higher effectiveness than coconut water. This is thought to be related to the content of bioactive compounds such as flavonoids (chrysin), vitamins, minerals and antioxidants which play a role in modulating the hormonal system. Chrysin has the potential to inhibit the activity of the aromatase enzyme so that the conversion of androgens to estrogen can be suppressed, which in turn encourages male sexual development. Apart from that, the nutritional content in honey also supports the physiological condition of the larvae so that the response to treatment becomes more optimal. This finding is in line with Abdullah et al., (2023) and Talbiah et al., (2024) who stated that honey has the potential to increase the proportion of male fish through its influence on the hormonal system. Thus, honey can be considered as a more environmentally friendly alternative natural ingredient in the masculinization process of tilapia.

Gender Observation

Identification of the sex of tilapia fish can be done by observing the morphology of the urogenital organs. Male fish generally have one urogenital opening with an elongated shape, while female fish have two separate holes, namely the anus and oviduct, with a rounded shape. These morphological characteristics indicate that sexual dimorphism has begun to develop, although visual observation still has limitations in the accuracy of identification. Bhagawati et al., (2017) reported that tilapia fry of a certain size showed sexual differences that could be identified through the shape of the dorsal fin and the structure of the exit hole. Apart from that, Ardiansyah (2016) stated that differences in external genitalia are the main indicator in visually distinguishing male and female fish.

To increase accuracy, gender identification is also carried out through histological observation of the gonads. This method allows direct observation of the cellular structure of the gonad so that the differences between male and female gonads can be determined more precisely. Preparation is done by destroying the gonad tissue on a glass object, then applying a dye such as methylene blue so that the cell structure can be seen clearly under a microscope. Damayanti et al., (2013) explained that this technique is effective for observing gonads at certain magnifications, while Cahyani et al., (2021) stated that male gonads are characterized by the presence of sperm cells, while female gonads are characterized by round cells resembling oocytes. Thus, the combination of morphological and histological observations provides more accurate sex identification results, especially in the early phases of fish development.

Water Quality

Water quality is a key factor in tilapia cultivation because it is closely related to fish growth and health (Goi et al., 2025). During maintenance, the temperature is in the range of 27.2–28.3 °C and is still in line with the optimal range of 25–30 °C (Azhari et al., 2018), thereby supporting metabolic and digestive activities (Cahyani et al., 2021). Dissolved oxygen (DO) ranges from 3.3–6.5 mg/L, still within tolerance limits and approaching the optimal range of 5.3–6.8 mg/L (Vica et al., 2024), so it is able to support fish respiration. The pH value ranges from 7.7–8.2 and is within the optimal range of 7–8 (Pratiwi et al., 2023), which supports physiological balance and efficient feed utilization. Overall, the water quality conditions during the research were still adequate and did not become a limiting factor in the growth or sex reversal process of tilapia.

CONCLUSION

Based on the research results, the effectiveness of treatment in the masculinization process of tilapia shows a clear difference, where methyl testosterone produces the highest proportion of males at 90.29% so it is considered the most effective treatment. The honey treatment also showed a fairly good ability to increase the proportion of males with a value of 70.79%, so it has the potential to be used as an alternative natural ingredient for masculinization. Meanwhile, coconut water produced a male proportion of 49.29%, lower than honey and methyl testosterone, but still higher than the control at 35.97%. These differences indicate that each treatment has a different level of success in directing the formation of male sex, so that the research objective of comparing the effectiveness and determining the most effective treatment in masculinizing tilapia can be stated to have been achieved.

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