Jurnal Akuakultur Indonesia 22 (2), 187–199 (2023)

Original article DOI: 10.19027/jai.22.2.187-199

Male sex ratio of red tilapia *Oreochromis* sp. after soaking in different concentrations of coconut milk at larvae stadia

Nisbah kelamin jantan ikan nila merah *Oreochromis* sp. yang direndam santan kelapa pada stadia larva dengan konsentrasi berbeda

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(Received March 8, 2023; Accepted May 29, 2023)

ABSTRACT

Tilapia has sexual dimorphism, based on the size and growth. Male tilapia has a faster growth rate than female tilapia. Masculinization can be carried out to produce monosexual tilapia seeds to accelerate fish growth. As the use of the 17α-methyltestosterone synthetic hormone for masculinization activities has been limited, natural ingredients are required as a substitute, namely coconut milk. This study aimed to determine the effect of different coconut milk concentrations as a phytosteroid material for the masculinization of red tilapia by immersing the larvae to the material. Tilapia fish larvae were immersed in coconut milk for 12 hours and then reared for 60 days at 100 larvae for each rearing container. There were four different treatments, namely control treatment without coconut milk immersion, S3 (3 ml/L coconut milk), S5 (5 ml/L coconut milk), and S7 (7 ml/L coconut milk). The results showed that the 7 ml/L coconut milk treatment increased the male sex ratio and specific growth rate and reduced the feed conversion ratio without a negative impact on the survival rate of red tilapia fry. In this study, the 7 ml/L coconut milk treatment was the best treatment, which produced a male sex ratio of 67.78 ± 4.16%.

Keywords: coconut milk, masculinization, monosex, phytosteroids, tilapia

ABSTRAK

Ikan nila memiliki dimorfisme seksual yang dapat dilihat dari ukuran dan pertumbuhannya. Ikan nila jantan memiliki laju pertumbuhan yang lebih cepat ketimbang ikan nila betina. Maskulinisasi dapat dilakukan untuk menghasilkan benih ikan nila monoseks dengan tujuan mempercepat pertumbuhannya. Penggunaan hormon sintetik 17α-methyltestosteron sudah dibatasi penggunaannya sehingga diperlukan bahan alami pengganti salah satunya santan kelapa untuk kegiatan maskulinisasi. Penelitian ini bertujuan mengevaluasi pengaruh perendaman santan kelapa sebagai fitosteroid untuk maskulinisasi ikan nila merah melalui perendaman larva dengan konsentrasi berbeda. Larva ikan nila direndam santan kelapa selama 12 jam dan selanjutnya dipelihara selama 60 hari dengan kepadatan 100 ekor untuk setiap wadah pemeliharaan. Terdapat empat perlakuan berbeda, kontrol tanpa perendaman santan, S3 (Santan 3 ml/L air), S5 (Santan 5 ml/L air) dan S7 (Santan 7 ml/L air). Hasil penelitian menunjukan perlakuan perendaman santan kelapa 7 ml/L dapat meningkatkan nisbah kelamin jantan dan laju pertumbuhan spesifik, serta menurunkan rasio konversi pakan dan tidak berdampak buruk terhadap nilai tingkat kelangsungan hidup benih ikan nila merah. Dalam penelitian ini, perlakuan santan 7 ml/L merupakan perlakuan terbaik yakni dapat menghasilkan nisbah kelamin jantan sebesar 67,78 ± 4,16%.

Kata kunci: fitosteroid, ikan nila, maskulinisasi, monoseks, santan kelapa

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is an indigenous tilapia species from Africa and was firstly introduced to Indonesia in 1969 through the Center for Freshwater Culture, Bogor. This fish was then distributed to several regions in Indonesia in 1970 (Andriani, 2018). Nile tilapia is known to have high adaptability to various environmental conditions, fast growth, and easy to maintain. These advantages are the reasons for Nile tilapia as a popular and widely cultured fish.

Nile tilapia fish are highly sensitive to mating, which makes it difficult to obtain size uniformity and reach consumption size, if cultured in a mixed-sex method. This is thought to be one of the causes of minimum Nile tilapia fish production in Indonesia (Tatalede *et al*., 2019). The total Nile tilapia production in Indonesia, based on KKP (2021) in 2017 reached 1,288,735 tons, then decreased to 1,169,144 tons in 2018, and increased again in 2019 to 1,337,831 tons. However, the increased total production did not last long, as it decreased again in 2020 to 1,172,632 million tons.

Therefore, efforts are needed to increase the national Nile tilapia production to meet the national Nile tilapia production targets. One way that can be performed is through a monosex culture. The monosex culture of male Nile tilapia can increase productivity up to twice as much as mixed-sex cultivation (Dagne *et al*., 2013; Islam *et al*., 2015; Omasaki *et al*., 2016). Physiologically, male Nile tilapia fish can grow faster than female Nile tilapia fish.

This condition is caused as male tilapia spends its energy not for vitellogenesis and oocyte maturation, as found in female tilapia (Masprawidinatra *et al*., 2015; Li *et al*., 2022). Male tilapia have an average growth of 2.1 g/day, whereas female tilapia fish have an average growth of 1.8 g/day (Apriliza, 2012). Sex reversal can help fish farmers to create monosex cultures. Sex reversal is a technique to reverse the sexual development direction from female to male, or *vice versa*. One of the sex reversal methods is masculination, which leads to male sex transformation (Malik *et al*., 2019).

Naturally, Nile tilapia fish larvae hatch in a bipolar state. This condition occurs when the fish is still in its critical sensitive period. The number of male hormones and female hormones is still the same, so the sexual differentiation is absent (Fuentes-Silva *et al*., 2013). Thus, providing a treatment either with male hormones (androgens) or female hormones (estrogens), can direct the gender development according to the goals (Deswira *et al*., 2015).

Masculinization methods have been widely applied using various techniques such as the use of hormones and environmental manipulation. The effectiveness level of the masculinization process can be increased by providing androgen hormones in the gonad differentiation phase in fish. Hormonal induction in this phase can stimulate the nervous system to trigger the release of gonadotropin hormones for the formation of male gonads (Awaludin *et al*., 2019; Golshan & Alavi, 2019; Tovo-Neto *et al*., 2018). Synthetic hormones that have been widely applied for the sex reversal process include 17α-methyltestosterone (MT), 17β-estradiol (E2), and aromatase inhibitor (AI) (Mlalila *et al*., 2015). The masculinization process in Nile tilapia can also use natural ingredients that contain steroid hormones, which are easy to obtain and effective to use (Tatalede *et al*., 2019; Aziz *et al*., 2022).

Recently, the most effective hormone for the masculinization process is the synthetic hormone, namely 17α-methyltestosterone. This synthetic hormone (MT) is a duplicate or imitation of the testosterone hormone which can be produced naturally by fish (Nian *et al*., 2017). The use of the MT hormone is known to produce 91.6% male Nile tilapia fish through the soaking method at 1,800 µg/L of water. However, Permen (2019), the use of the MT hormone has been prohibited (Afpriyaningrum *et al*., 2016).

This is because MT has the potential to cause stress in fish and can affect fish health by disrupting physiological processes or causing defects in fish due to its carcinogenic properties (Tatalede *et al*., 2019). Therefore, natural ingredients are needed in the masculinization process, which can easily decompose during growth with few side effects and reduce operational costs (Mendez *et al*., 2017; Susanto *et al*., 2021). Coconut (*Cocos nucifera*) milk is a natural ingredient that has the potential for masculinizing the red Nile tilapia fish due to containing stigmasterols. Coconut milk contains stigmasterols at 10.69% of the total phytosterols in coconut milk.

Coconut milk also contains more stigmasterol than the fruit flesh, whereas reaching up to 3.85 mg/100 g of coconut milk (Ngampeerapong *et al*., 2018). In addition, coconut milk is known to have an average potassium content of 1,127 mmol/L (Khor *et al*., 2020). Stigmasterol is

an active androgenic compound that can help increase the erection levels in adult men (Putra, 2011). The inorganic ion, e.g., potassium, plays a role in changing the cholesterol level found in all fish larval body tissues into pregnenolone in the masculinization process (Malik *et al*., 2019).

The potassium content in coconut water and coconut milk is also thought to be one of the compounds that plays a role in directing the sexual differentiation in fish by modulating testosterone circulation and controlling the androgen hormones (Deswira *et al*., 2015). According to Abdullah *et al*. (2021), stigmasterol in humans is an active androgenic compound that can help increase erection levels in adult men, stimulate ovulation, and is a raw material for steroid hormones (contraceptive pills). The presence of potassium and stigmasterol compounds in coconut milk is expected to direct the sex of red tilapia larvae to become male because of the androgenic properties of these compounds.

MATERIALS AND METHODS

This study used a completely randomized design with three replications in each treatment. The coconut milk soaking treatments are described below:
S0: Soaking without coconut milk
S3: 3 ml/L coconut milk soaking
S5: 5 ml/L coconut milk soaking
S7: 7 ml/L coconut milk soaking

Materials

The fish samples used in this study were red tilapia larvae (*Oreochromis* sp.) at three days old, which were obtained from the hatchery pond, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University, Bogor. Coconut milk was obtained from the surrounding area of IPB University, Dramaga Campus.

Procedures

This study was divided into three stages, namely preparation, implementation, and analysis. The preparation stage was performed by preparing the rearing media for red tilapia seeds. For the implementation stage, red tilapia seeds were reared for 60 days and sampled to determine their developmental phase. For the analysis stage as the last stage, sex ratio was calculated to gain the best treatment during the experiment.

The containers used in this study were 12 units of a 60 cm×40 cm×50 cm aquarium. This study was performed in the Laboratory of Aquatic Organism Reproduction and Genetics (wet laboratory), Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University, Bogor. The aquaria were cleaned with water and soap, and disinfected with chlorine before rinsing and drying. The dried and sterile aquaria were ready for soaking and rearing process. The coconut milk was obtained directly from the old coconuts, that were grated and squeezed independently.

The grated old coconut was obtained from the Dramaga Market, Bogor, West Java. The coconut used was above 10 months old. Furthermore, the 250 g of grated coconut flesh was weighed and added with 250 ml of water, thus the ratio of grated coconut fleash and water was 1:1. The grated coconut was squeezed using a thin cloth, and the coconut milk produced was filtered to ensure that it was completely separated from any dirt or grated coconut. The amount of coconut milk required in this study was 225 ml.

The fish used were tilapia larvae three days after hatching. The fish were obtained from hatchery activities in the ponds, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University, Bogor. The fish larvae were previously acclimatized, when their egg yolk had run out. This study used 100 red tilapia larvae per soaking container, namely 15×15×15 cm plastic with 1 L of water and 3 L volume. The total number of red tilapia larvae in this study was 1,200 larvae for all soaking containers. Fish larvae were soaked for 12 hours in each treatment.

The water used in the soaking containers was based on the applied treatments. The coconut milk concentrations used in containers K, A, C, and D were control (without coconut milk), 3, 5, and 7 ml/L water, respectively. The acclimatized fish larvae were then stored in a soaking container. For optimum water conditions, aeration was applied. After the larvae have been soaked for 12 hours, larvae were then distributed into the rearing container and maintained for 60 days.

The larvae were fed until apparent satiation with a commercial feed containing 41% protein. Feeding was performed five times a day for one month at 08.00, 10.00, 13.00, and 17.00 WIB. After a month, the larvae were fed four times a day on 08.00, 10.00, 13.00, and 17.00 WIB. The larvae were reared at 30°C and equipped with an aquarium heater for temperature stabilization. After 60 days of rearing, the red tilapia larvae were observed their gonad with acetocarmine method.

Observations were performed by collecting 30% of the test fish samples. The acetocarmine solution was produced from 0.6 g of carmine powder dissolved in 100 ml of 45% acetic acid (45 ml of concentrated acetic acid 55 ml of aquadest). The fish were surged slowly and carefully to obtain the gonad organ with pinset. The other internal organs were removed first to easily collect the gonad. Furthermore, the gonad was placed on an object glass and minced using a scalpel until it was smooth.

Acetocarmine was dropped two or three times on a minced gonad before closing the object glass and cover glass. The gonads were observed under a microscope at 40× magnification (Putra, 2011). The water quality measurements observed in this study included temperature, pH, dissolved oxygen, and dissolved oxygen (DO) (Table 1). Temperature and pH levels were measured daily, while dissolved oxygen was measured once a week. All parameters were measured with thermometer, pH meter, and DO meter. To maintain the optimum water quality, syphonization was performed every day, and water exchange was performed at 50% every three days or when the water was turbid.

Table 1. Water quality parameters in BSN (2009).

Parameter	Unit	Optimum
Temperature	°C	25-30
pH	Scale	6.5-8.5
Dissolved oxygen	mg/L	≥ 3

Parameters

The parameters in this study were composed of specific growth rate (SGR), survival rate (SR), male sex ratio (MSR), intersex ratio (IR), feed conversion ratio (FCR), and water quality.

Survival rate

The survival rate is an organism survival level during the rearing period or experimental activity. Based on Effendie (2002), the survival rate can be calculated with the following formula:

$$SR\ (\%) = \frac{Nt}{No} \times 100$$

Note:
SR = Survival rate (%)
Nt = Total of fish on the final rearing period (fish)
No = Total of fish on the initial rearing period (fish)

Specific growth rate

The specific growth rate (SGR) is a daily weight growth of test fish during the rearing period. The specific growth rate can be calculated with the following formula (Zonneveld *et al*., 1991):

$$SGR\ (\%/day) = \frac{Ln\ Wt - LnWo}{t} \times 100$$

Note:
SGR = Specific growth rate (%/day)
t = Time (day)
Wt = Larval weight at t-day (g)
Wo = Initial larval weight (g)

Male sex ratio

The sex percentage was obtained from observations of sexual dimorphism characteristics in tilapia fish after 60 days of rearing. Male tilapia fish have a prominent part in their anus called papillae, with holes for spermatozoa and urine release. Meanwhile, female tilapia fish have three holes in their anus, whereas the urogenital hole is separated from the urine excretion hole. In addition, sex differences in tilapia fish can also be viewed from their dorsal fins. A reddish and clearly bright color is characterized by male fish, and females tend to be grayish or dark in color. Also, histological observation of the fish gonads was observed using a light microscope and acetocarmine dye (Bhagawati *et al*., 2017). The ratio of male sex can be calculated using the formula:

$$MI\ (\%) = \frac{mI}{sI} \times 100$$

Note:
MI = Male sex ratio (%)
mI = Total male fish (fish)
sI = Total observed fish (fish)

Intersex ratio

Sex percentages were obtained from observations of the gonads of 60-day-old red tilapia seeds using the acetocarmine method. The intersex ratio can be characterized by the presence of female sex cells and male sex cells in red tilapia seed gonads. The percentage of intersex gonads can be calculated using the following formula:

$$IR\ (\%) = \frac{Ij}{Is} \times 100$$

Note:
IR = Intersex ratio percentage (%)
Ij = Total intersex fish (fish)
Is = Total observed fish (fish)

Feed conversion ratio

The feed conversion ratio (FCR) describes the total feed required (kg) to produce 1 kg of fish. The feed conversion ratio can be calculated with the following formula:

$$FCR = \frac{FI}{Wt+Wm-Wo}$$

Note:
FI = Feed intake (kg)
Wt = Final biomass (kg)
Wm = Dead biomass (kg)
Wo = Initial biomass (kg)

Water quality analysis

The water quality parameters observed in this study were temperature, pH, dissolved oxygen (DO). Temperature and pH were measured every day, while dissolved oxygen levels were measured once a week. All measurements were carried out using thermometer, pH meter, and DO meter. The water quality management to maintain optimum water quality was carried out by filtering the water every day and changing the water by 50% every three days or when the water was turbid.

Data analysis

This study used a Completely Randomized Design. All data were processed using the Microsoft Excel 2013. All data from survival rate, male sex ratio, and specific growth rate were analyzed specifically using the ANOVA table with a 95% confidence interval in the SPSS 25.0 program, and continued by Duncan's test. The water quality parameters, such as temperature, pH, and DO, and cost analysis were analyzed descriptively.

RESULTS AND DISCUSSION

Results

The coconut milk soaking in red tilapia larvae at five days after hatching had a significant effect on the male sex ratio, intersex ratio, and feed conversion ratio during the rearing period. At the final study, a physical difference between male and female was yet to emerge in this size or at this age. The complete data are presented in Table 2.

Survival rate

The negative effect of materials can be found in several parameters, including survival rate (SR). The SR value describes the material eligibility as a phytohormone source for masculinization process. The survival rate of the fish during the rearing period is seen in Figure 1.

Specific growth rate

The specific growth rate (SGR) is a parameter that can determine whether a material has a negative impact on the test fish or not. The SGR values after the soaking treatment observed during 60 days of rearing can be seen in Figure 2.

Male sex ratio

An ingredient used for masculinization can be said to be effective by observing the male sex ratio parameters. A greater MSR value of the treatment than the control can illustrate the ability of a material to perform masculinization activities. The male sex ratio after the soaking treatment is shown in Figure 3.

Intersex ratio

The intersex ratio after treatment observed during the 60 days of rearing is shown in

Table 2. Survival rate (SR), initial weight (Wo), final weight (Wr), specific growth rate (SGR), male sex ratio (MSR), intersex ratio, and feed conversion ratio (FCR).

Treatment	SR (%)	Wo (g)	Wr (g)	SGR (%/day)	MSR (%)	Intersex (%)	FCR
0	94.33 ± 1.25a	0.02 ± 0.00a	5.41 ± 0.41a	9.33 ± 0.13a	48.89 ± 5.67a	0.00 ± 0.00a	1.22 ± 0.04b
3	94.33 ± 2.05a	0.02 ± 0.00a	5.64 ± 0.52a	9.40 ± 0.15a	54.44 ± 4.16ab	2.22 ± 1.92a	1.18 ± 0.06b
5	93.33 ± 2.85a	0.02 ± 0.00a	6.16 ± 0.65ab	9.54 ± 0.17a	62.22 ± 4.16bc	6.67 ± 3.34b	1.10 ± 0.01a
7	94.00 ± 2.16a	0.02 ± 0.00a	6.00 ± 0.28ab	9.50 ± 0.08a	67.78 ± 4.16c	7.78 ± 1.92b	1.11 ± 0.01a

Different superscript letters in the same line show a significant difference (Duncan's test P<0.05).

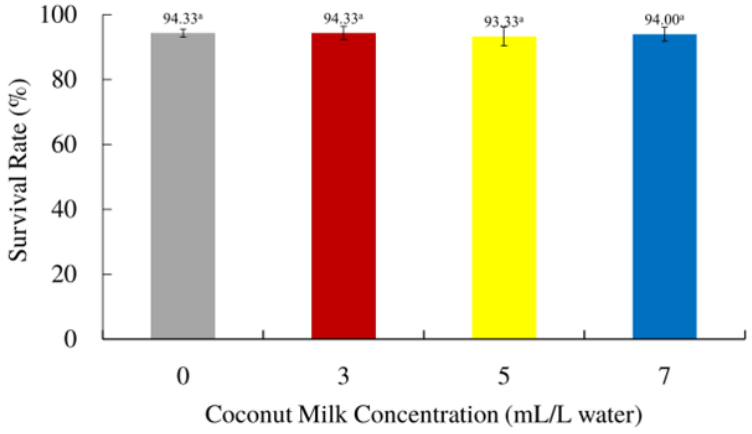

Figure 1. Survival rate of red tilapia seeds (*Oreochromis* sp.) after coconut milk soaking and 60 days of rearing.

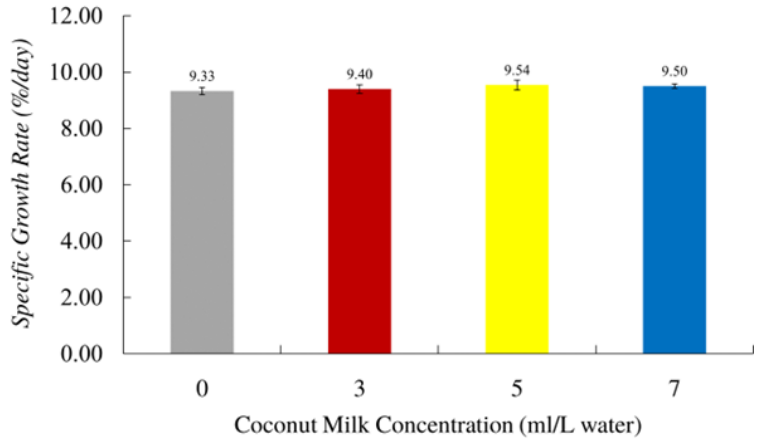

Figure 2. The specific growth rate of red tilapia after soaking treatment and rearing for 60 days.

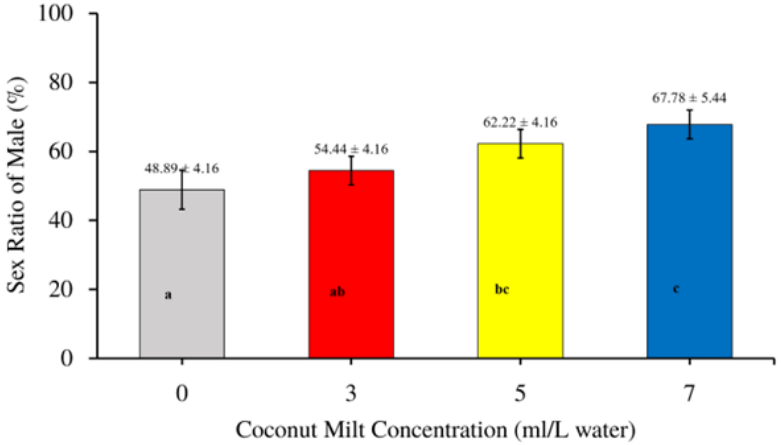

Figure 3. Male sex ratio of red tilapia seeds after coconut milk soaking treatment and 60 days of rearing. Different superscript letters in the bar show a significant difference (Duncan's test P<0.05).

Figure 4. The intersex ratio of female tilapia seeds during the rearing period is shown in Figure 4. The acetocarmine method is a histological test that can be carried out to identify tilapia gonads by staining them with acetocarmine solution. The gonad cells of male tilapia fish have a small, irregular round shape and are large in number. Meanwhile, the gonad cells of female tilapia fish are round and have larger dimensions than male gonad cells. Figure 5. The tilapia gonad

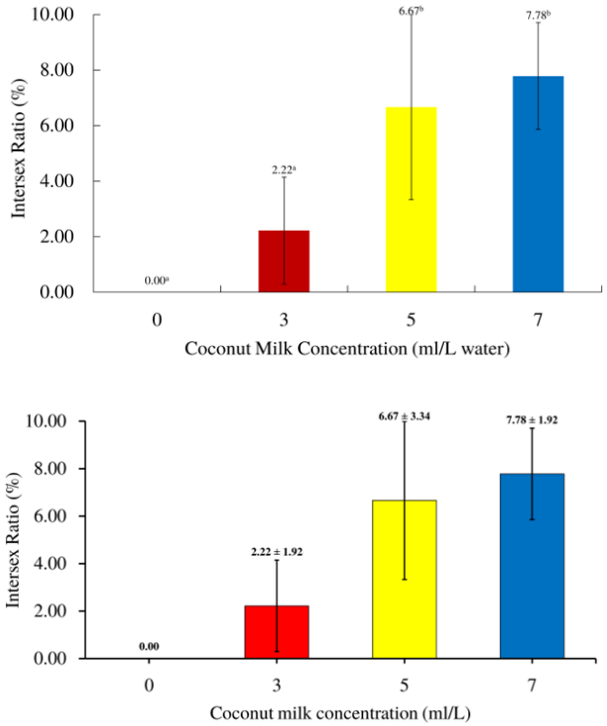

Figure 4. Intersex ratio of red tilapia after coconut milk soaking treatment and 60 days of rearing. Different superscript letters in the bar show a significant difference (Duncan's test P<0.05).

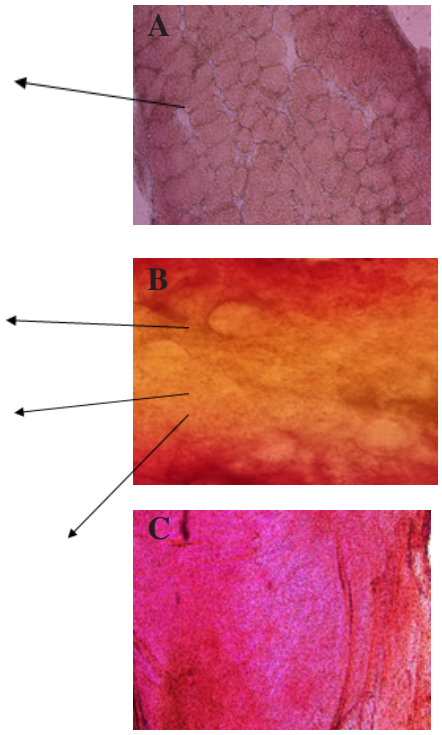

Figure 5. The gonad histology of red tilapia seeds.
Note: A. Female gonad; B. Intersex gonad; C. Male gonad.

with the acetocarmine method observed under a microscope at 40× magnification is presented.

Feed conversion ratio

The feed conversion ratio (FCR) is a parameter that can describe how much feed required to produce 1 kg of fish. The lower the FCR value (closer to 1), the better is the feed efficiency of the test fish. The feed conversion ratio after soaking is shown in Figure 6.

Water quality

Temperature is an environmental quality parameter that can affect the male sex ratio. The average temperature for each treatment is shown in Figure 7. The values of measured water quality parameters, such as temperature, pH, and dissolved oxygen (DO) levels during 60 days of maintenance showed optimum values in accordance with SNI. The range of water quality parameter values after soaking treatment and rearing for 60 days can be seen in Table 3.

Discussion

The coconut milk soaking treatment at five-day-old red tilapia larvae had a negative impact on the survival rate of red tilapia seeds. The total mortality in all treatments did not show a significant difference at 5.67-6.67%. This is illustrated by the results of observing the survival

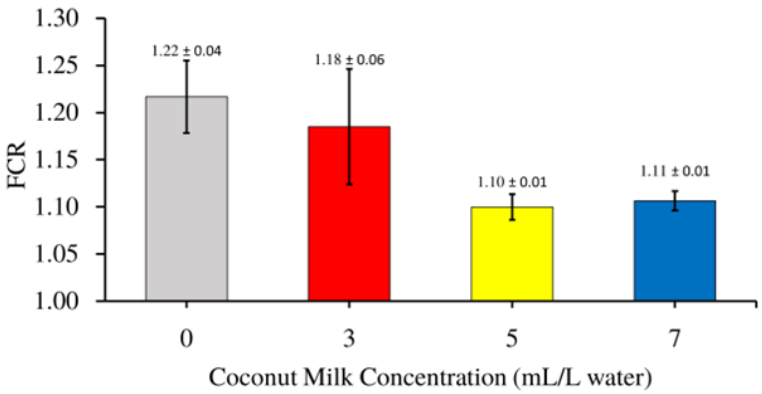

Figure 6. Feed conversion ratio after soaking treatment and rearing for 60 days. Different superscript letters in the bar show a significant difference (Duncan's test P<0.05).

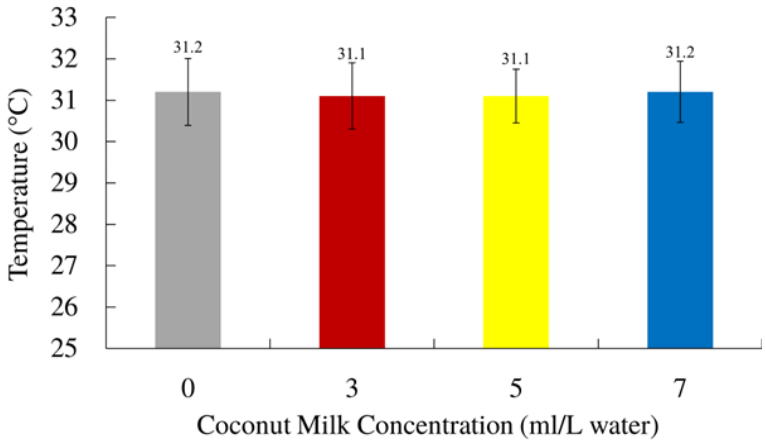

Figure 7. Average temperature in the rearing media for red tilapia seeds after soaking treatment and 60 days of rearing.

Table 3. Water quality condition after 60 days of rearing.

Parameter	Unit	Measured values	Optimal value (BSN, 2009)
Temperature	°C	29–31.5	25-30
pH	scale	6.8–8.5	6.5-8.5
DO	mg/L	3.0–7.1	>3

rate of red tilapia seeds after rearing for 60 days, as shown in Figure 1 and Table 2. The water quality of rearing media is the main factor affecting the survival rate of tilapia seeds (Azhari & Tomasoa, 2018). The water quality during the tilapia seeds rearing is shown in Table 2.

The temperature, pH, and dissolved oxygen levels are in accordance with optimal values for the survival and growth of red tilapia seeds. This was in accordance with Dahril *et al*. (2017), who stated that tilapia seeds could live and grow well at the optimal temperature, namely 25-30°C. This study was carried out indoors and used a heater so that the temperature could be maintained and stabilized in the optimal range. Prakoso (2014) stated that the best pH for the maintaining the environment for aquatic organisms is in the neutral pH range, namely 7-8.5. In this study, the pH was 6.8-8.5, as relatively neutral and safe for the survival rate of tilapia seeds.

The DO levels in this study were still in accordance with BSN (2009) regarding water quality in tilapia fish hatcheries, namely at concentrations of more than 3 mg/L. The specific growth rate (SGR) is the average of daily weight gain rate during the rearing period. The highest SGR value in this study was in the S5 treatment at 9.54%. Meanwhile, the lowest SGR value was obtained in the S0 treatment (control), but the results statistically showed no significant difference ($p<0.05$). This means that the treatments had no negative impact on the growth of red tilapia seeds but tended to have a positive impact.

Fish growth is basically influenced by two main factors, namely internal factors (genetics, age, sex) and external factors (temperature and other physical and chemical water parameters) (Hapsari *et al*., 2020). The temperature of the rearing environment can affect the growth rate of fish. The average temperature value in this study was 31.1-31.2°C, whereas this temperature range is in the optimal range that supports the growth of tilapia seeds. This was in accordance with Yanuar (2016), who reported that tilapia seeds could grow faster in the environmental temperature range of 30-32°C, compared at 25-29°C. The environmental temperatures lower than 14°C and higher than 38°C can reduce the fish growth rate.

The 30-50% of the water every day during the rearing period is also one of the factors that maintains the optimal water quality. Feed is an external factor that determines fish growth rate, besides the water quality. During maintenance, the feed used is a commercial feed with 40% protein content. The protein content of feed for tilapia in seed rearing is 35% (Bhosle *et al*., 2022) and 25-35% for the adult stage (Hossain *et al*., 2017). The tilapia growth on larval-seed stadia commonly has a similar growth rate between male and female tilapia (Putra, 2011).

When tilapia fish reach adult stadia, sexual dimorphism emerges based on their size and growth rate. Male tilapia have an average growth rate of 2.1 g/day, whereas female tilapia have an average growth rate of 1.8 g/day (Apriliza, 2012). This difference will start to emerge when fish enters the gonadal maturation phase or reaches around six months old. The higher SGR value in each coconut milk soaking treatment than in the control treatment was thought to be due to differences in MSR values between the coconut milk and the control treatments.

This indicates that there is a positive factor in the number of male fish in the population, although there was no significant difference, as the fish were still too small and the observations were carried out too early. The highest SGR value was found in the S5 treatment at 9.54% with an MSR value of 62.22%. Robisalmi *et al*. (2017) stated that rearing 100% male tilapia had a specific growth rate value of 1.76%, which was greater than rearing 25% male tilapia with SGR value of 1.50%. Githukia *et al*. (2015) also reported that tilapia reared in monosex culture method had a higher SGR value than tilapia reared in a mixed culture method, whereas the LPS value of monosex culture method was 1.83%, while the SGR value of mixed culture method was 1.47%.

Masculinization treatment by soaking tilapia larvae in coconut milk resulted in a higher male sex ratio in the S3, S5 and S7 treatments, compared to the S0 (control) treatment. Coconut milk was obtained from grated and squeezed coconuts with water in a 1:1 ratio. According to Ngampeerapong and Chavasit (2019), the Indonesian coconut flesh contains 3.07 mg of stigmasterols in each 100 g of fruit flesh. Coconut milk or coconut juice contains higher stigmasterol compounds at 3.85 mg/100 g coconut milk (Ngampeerapang *et al*., 2018). Stigmasterol is a compound derived from cholesterol that has androgenic properties (Abdullah *et al*., 2021).

These androgenic properties make coconut milk become the masculinization material, based on the male sex ratio of tilapia treated with coconut milk soaking method. The statistical test results showed a significant increase compared

to the control. The highest male sex ratio was found in the S7 treatment at 67.78%, whereas this value is still very below, when using the 17α-methyltestosterone hormone, which can reach a sex ratio of 97-100%. Temperature is an environmental parameter that can influence the functional sex of fish, especially if the temperature treatment is applied when the fish are in the phase before sexual differentiation.

The larvae at 1-14 days after hatching have similar levels of androgens and estrogens (Fuentes-Silva *et al*., 2013). The average values of temperature observed in this study for each treatment were 31.2°C (control), 31.1°C (coconut milk 3), 31.1°C (coconut milk 5), and 31.2°C (Coconut milk 7). Based on statistical analysis, the average temperature values observed in each treatment showed no significant difference ($p<0.05$). Based on the results of the temperature ranges that were not significantly different, the rearing temperature in this study did not affect the MSR value.

This condition was in accordance with Fuentas-Silva *et al*. (2013), who reported that water temperatures above 32°C could functionally change the sex of tilapia fish from female to male if the larvae exposed to this temperature were between 1-10 days after fertilization. The effect of temperature had no significant effect on the sex ratio of tilapia fish, if exposed to larvae at seven days old. The feed conversion ratio (FCR) value is a parameter that describes the amount of feed required to produce 1 kg of fish. The best FCR value in this study was found in the S 5 ml/L treatment (1.1). The highest FCR value was observed for the control treatment (1.22).

Based on the statistical tests, the results showed significantly different results between the S5 and the control treatments ($p<0.05$). Differences in FCR values were thought to be due to differences in the male sex ratio in each treatment. The S5 treatment had an MSR of 62.22%, whereas the control treatment had an MSR of 48.89%. Treatments with higher MSR values produced better feed conversion ratios. This was in accordance with Robisalmi *et al*. (2017), who reported that tilapia fish reared using the male monosexual method could produce a lower feed conversion ratio because male tilapia had the ability to utilize feed better when reared.

Apart from the male sex ratio, a good FCR value can also indicate that the feed is of good quality or in accordance with existing standards. The sex of tilapia was also observed through histological observations. The acetocarmine method is a histological test that can be used to identify tilapia gonads by staining gonads with acetocarmine solution (Widyawati *et al*., 2021; Sarker *et al*., 2022). The acetocarmine solution used in this study was created from 0.6 g of carmine powder dissolved in a 45% acetic acid solution.

The fish were first surged, and gonads were taken for further observation under a microscope. The gonad cells of male tilapia fish have a small, irregular round shape, and are large in number. Meanwhile, the gonad cells of female tilapia are round and have larger dimensions than male gonad cells (Mangaro *et al*., 2018). Intersex gonads were identified based on histological observations. Intersex status in gonads is characterized by the presence of oocytes and spermatocytes in the gonads. The intersex ratio was only found in the coconut milk soaking treatments, with the highest value of 7.78% in the 7 ml/L concentration treatment.

Intersex gonads are found in almost every masculinization activity. Aziz *et al*. (2022) reported that there were 2.3% intersex gonads in masculinization activities using a pine cone extract in tilapia. The intersex status in fish gonads occurs because of incomplete development of the gonads during the differentiation phase. Deviations in gonad development are caused by inappropriate exposure to hormones for a long time. The inoptimal use of hormone doses is also responsible for the occurrence of intersex gonadal status.

The optimal dose and length of exposure could minimize the occurrence of tilapia seeds with intersex gonad status (Depiereux *et al*., 2014). This condition was also observed in cobia fish, which did not show differences in growth during the intersex period (Dutney *et al*., 2017). The coconut milk used in this study was produced from the grated and squeezed coconuts, added with clean water in a ratio of 1:1. The coconut fruit used was the *Cocos nucifera* type that has been widely distributed throughout Indonesia. The grated coconut flesh used in this study was from the Dramaga traditional market, Bogor, West Java.

The price of grated coconut for making coconut milk in this study was the IDR. 5000/250 g. According to BPS (2021), Indonesian coconut production in 2019 reached 2,839.9 million tonnes, production in 2020 reached 2,811.9 million tonnes, and Indonesian coconut production

reached 2,853.3 million tonnes in 2021. The distribution of coconut plants in Indonesia makes it very easy to find and makes Indonesia become the second largest coconut producer and exporter country in the world (BPS, 2021). Based on these results, coconut milk soaking treatment of five-day-old tilapia larvae can affect the male sex ratio of the tilapia seeds produced.

This condition is shown by the higher percentage of male fish produced after the coconut milk soaking treatment compared to the control treatment, with significantly different results. Masculinization activities using coconut milk are a good prospect for coconut utilization in Indonesia, because the price is economical and easy to obtain. The mass masculinization process can be carried out during larval transportation, larval or seeds handling in the hatchery, either through soaking or by oral method via feed. Soaking treatment using coconut milk can be used to masculinize tilapia seeds.

The highest percentage of male tilapia seeds was found in the treatment of soaking in coconut milk in 7 ml/L of water for 12 hours, namely 67.78%. These results showed statistically significant differences compared with the control. However, these results are considered less effective, when compared to masculinization process using the 17α-methyltestosterone hormone, which can produce a male sex ratio above 90%. Afpriyaningrum *et al*. (2016) stated that masculinization with 1,800 µg/L MT could produce male tilapia of 91.6%.

Less effective results were thought because the concentration of coconut milk to the test fish did not reach the optimal point. This was shown as the MSR level did not decrease in the coconut milk treatment until the highest concentration of 7 ml/L. According to Audinah (2016), the type of target fish, treatment dose, treatment method, and environmental factors such as temperature are crucial for the success of masculinization activities in fish. Muslim (2010) also said that the length of exposure to treatment can influence the masculinization results in fish.

CONCLUSION

Soaking coconut milk during the larval stage of red tilapia fish can increase the male sex ratio of red tilapia seeds by 18.89%. The 7 ml/L coconut milk soaking treatment was the best dose with a male sex ratio of 67.78%. There were no visible differences in specific growth rate in this study, so further observation is required in a longer rearing stage.

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