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THE EFFECT OF DIFFERENT SOAKING DURATIONS ON LARVAE MASCULINIZATION OF GUPPY FISH (*Poecilia retriculata*) USING HONEY

Pengaruh Perbedaan Lama Perendaman Terhadap Maskulinisasi Larva Ikan Guppy (*Poecilia retriculata*) Menggunakan Madu

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ABSTRACT

Guppy fish (*Poecilia reticulata*) hold high commercial value, particularly the males, due to their aesthetic colors and morphology. This study aims to determine the effect of different soaking durations in honey on the masculinization of guppy larvae and to identify the optimal immersion time. This experimental study utilized a Completely Randomized Design (CRD) with 5 treatments and 5 replications, using a honey dosage of 5 ml/L with soaking durations of: 8 hours, 10 hours, 12 hours, 14 hours, and 16 hours. The results showed that the variation in soaking duration had a significant impact on the percentage of masculinization. The 12-hour soaking treatment yielded the best results, achieving a male percentage of 90.1%. Water quality parameters during the 45-day rearing period remained stable within the optimal range, with temperatures of 23.1–26.9°C, pH of 7.0–7.2, and dissolved oxygen (DO) of 6.1–6.7 ppm. In conclusion, the use of honey as a natural ingredient is effective in significantly inducing male sex differentiation in guppy fish larvae.

Keywords: Soaking duration, Guppy fish larvae (*Poecilia reticulata*), Masculinization, Honey.

ABSTRAK

Ikan guppy (*Poecilia reticulata*) memiliki nilai komersil tinggi, terutama pada ikan jantan karena keindahan warna dan morfologinya. Penelitian ini bertujuan untuk mengetahui pengaruh perbedaan lama perendaman menggunakan madu terhadap maskulinisasi larva ikan guppy serta menentukan durasi waktu terbaik. Penelitian eksperimental ini menggunakan Rancangan Acak Lengkap (RAL) dengan 5 perlakuan dan 5 ulangan, menggunakan dosis madu 5 ml/L dengan lama perendaman: 8 jam, 10 jam, 12 jam, 14 jam, dan 16 jam. Hasil penelitian menunjukkan bahwa perbedaan lama perendaman memberikan pengaruh signifikan terhadap persentase maskulinisasi. Perlakuan perendaman selama 12 jam merupakan hasil terbaik yang mampu menghasilkan persentase jantan sebesar 90,1%. Parameter kualitas air selama 45 hari pemeliharaan terpantau stabil pada rentang optimal, yakni suhu 23,1–26,9°C, pH 7,0–7,2, dan

oksigen terlarut 6,1–6,7 ppm. Penggunaan madu sebagai bahan alami terbukti efektif dalam memicu pembentukan kelamin jantan secara signifikan pada larva ikan guppy.

Kata Kunci: Lama perendaman, Larva ikan guppy (*Poecilia retriculata*), Maskulinisasi, Madu

INTRODUCTION

Guppies (Poecilia reticulata) are freshwater ornamental fish with high economic value due to their extraordinary adaptability to various environments (Malik et al., 2019). Sukrillah et al. (2013) stated that the popularity of these fish is driven by their ease of maintenance and the beauty of their color and pattern variations. Morphologically, male fish have a superior selling value compared to females due to their slender bodies and more brilliant fins (Matondang et al., 2018). Given the greater potential profit potential of male fish, sex reversal techniques are often applied to increase the percentage of males in a population.

Sex reassignment is a technique that manipulates the sexual differentiation of fish to artificially alter their phenotype, genetically transforming males into females, or vice versa (Himawan et al., 2018). The technique of directing the male sex to a specific species is referred to as masculinization (Malik et al., 2019). Although synthetic hormones such as 17α-methyltestosterone, estradiol-17β, and aromatase inhibitors are commonly used in this process, their use is now being banned and restricted due to their high cost and negative impacts, which can pollute the environment, damage the liver, and even cause death in test animals (Djihad, 2015; Ariyanto et al., 2016). Therefore, to support the sustainability of the masculinization process in farmed fish, innovation is needed in the use of safer and more economical natural components as alternatives to synthetic steroid hormones.

Several studies have been conducted on honey immersion for masculinization of guppies using honey, such as Priyono (2013) who conducted a 12-hour immersion in 5 ml/L of honey, which successfully increased the male sex ratio of guppies (Poecilia retriculata) by 76.66%. Habibi F. (2022) conducted a soaking treatment using 5ml/L honey for 12 hours, successfully increasing the male sex ratio of guppy fish (Poecilia retriculata) by 100%. Safitri N. (2022) conducted a soaking treatment using 10ml/L honey for 10 hours, successfully increasing the male sex ratio of guppy fish (Poecilia retriculata) by 95%. Prayadi (2024) conducted a soaking treatment using 6ml/L honey for 16 hours, successfully increasing the male sex ratio of moly fish (Poecilia latipinna) by 73.3%.

RESEARCH METHODS

Place and Time

This research on masculinization of guppy fish larvae (Poecilia retriculata) was conducted from February 23, 2026 to April 9, 2026, which took place in Kebonsari, RT 26, RW 04, Sidoarjo Regency.

Tools and Materials

The materials and tools used in this research include: a 5 liter round plastic tub, stationery, mobile phone, pH meter, DO meter, jigger kit, siphon tool, fish scoop, guppy fish larvae, honey, water, artemia feed.

Research Methods

This study used an experimental method according to Sugiono (2017), an experimental method is a research method used to find the effect of certain treatments on others under controlled conditions. This study used a Completely Randomized Design method with 5 treatments and 5 replications.

1. Treatment (A) = Treatment using the addition of 5ml/L honey with a soaking time of 8 hours.

2. Treatment (B) = Treatment using the addition of 5ml/L honey with a soaking time of 10 hours.
3. Treatment (C) = Treatment using the addition of 5ml/L honey with a soaking time of 12 hours.
4. Treatment (D) = Treatment using the addition of 5ml/L honey with a soaking time of 14 hours.
5. Treatment (E) = Treatment using the addition of 5ml/L honey with a soaking time of 16 hours.

Research Procedures

Preparation Stage

- Preparing Research Media

In preparation for the study, the first step was to wash and dry the 5-liter culture media container. This was to remove odors or chemicals from the experimental container used during the study and to prevent contamination by pathogenic microorganisms that could infect the fish being studied.

- Preparation of Guppy Fish Larvae (Poecilia retriculata)

The guppy (Poecilia reticulata) larvae used in this study were 3–5 days old and from the same strain. Each rearing tank was filled with 3 liters of water and 15 larvae, following the research procedures of Habibie (2022). Larval health criteria were determined through visual observation of their agility.

- Preparation of Soaking Solution

To prepare the honey soaking solution, 1 liter of well water that has been settled beforehand is used as a solvent with the addition of 5 ml/L of honey (milliliters per liter of water) as in previous research by Priyono. (2013) who conducted a soaking using 5 ml/L of honey for 12 hours successfully increased the male sex ratio of guppy fish (Poecilia retriculata) by 76.66%.

- Immersion Implementation Stage

Guppy larvae (Poecilia retriculata) were placed in water with a 5ml/L honey solution for 8, 10, 12, 14, and 16 hours. The tank was stocked with a density of 15 larvae and filled with 3 liters of water.

- Larva Maintenance Stage

Guppy fish larvae (Poecilia retriculata) are kept until they are 45 days old, Wardhani (2024) guppy fish (Poecilia retriculata) are kept for 45 days until males and females can be distinguished. Feeding is done ad libitum, According to Apriyani et al., (2019) ad libitum feeding aims to ensure sufficient feed so that mortality which is the main problem in fish cultivation can be avoided.

- Larva Observation Stage

After a 45-day rearing period, guppy larvae were observed morphologically. According to Matondang et al. (2018), male guppies (Poecilia retriculata) have slimmer bodies with brighter colors and fins than female guppies.

After rearing, the percentage of male guppy larvae (Poecilia retriculata) was calculated. According to Zairin (2002), the following formula can be used:

$$\text{Male Fish} = \frac{\text{Number of male fish individuals}}{\text{Number of individuals alive at the end of maintenance}} \text{x}100\%$$

- Water Quality Observation Stage

Temperature is a quantity that indicates the degree of hotness or coldness of an object. In this study, the temperature of the aquarium water was measured using a thermometer. pH is a quantity that indicates the acidity of a solution. By measuring it using a pH meter, it can

be determined whether a solution is acidic or basic. DO is the amount of dissolved oxygen in water (aquarium). A DO meter is used to measure the amount of dissolved oxygen.

Data Analysis

After the research was completed, the data were collected and then analyzed. The data observed in this study relate to the masculinization of guppy larvae (Poecilia retriculata) after being soaked in honey solution for various soaking times. To facilitate drawing conclusions, the data are presented in the form of tables and histograms. The data were analyzed using ANOVA (Analysis of Variance) with the help of the IBM SPSS statistics program 26.

RESULT

Masculinization of Guppy Fish Larvae (Poecilia retriculata)

The results of the study showed that treatment C produced the highest percentage of masculinization, as shown below:

Table 1. Masculinization for each study, Mean data for masculinization, range values, and standard deviations

Treatment	Range (%)	Average (%)	Standard Deviation
(A) 8 jam	46,6 – 60	51,7	6.06028
(B) 10 jam	83,3 – 86,6	79,3	5.94786
(C) 12 jam	86,6 – 93,3	90,1	3.67328
(D) 14 jam	60 – 73,3	68,3	6.52204
(E) 16 jam	53,3 – 66,6	59,7	6.18442

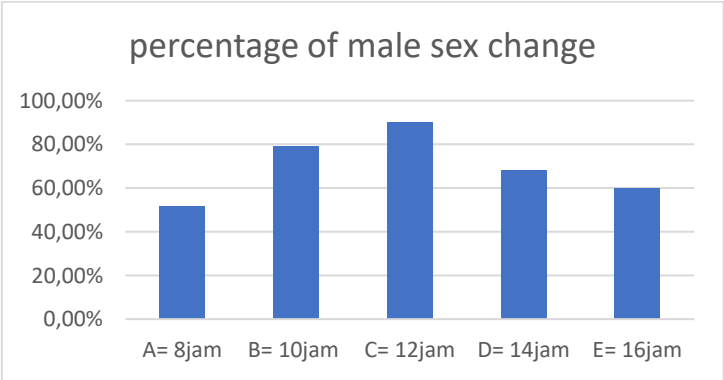

Figure 1. Percentage of male sex changes.

To determine the duration of immersion, we performed a normality test using the Kolmogorov-Smirnov test. The results of the normality test are shown in Table 2 as follows:

Table 2. Shows the results of the Normality Test of Immersion Duration on Masculinization One-Sample Kolmogorov-Smirnov Test

One-Sample Kolmogorov-Smirnov Test		
N		25
Normal Parameters	Mean	69.8800
	Std. Deviation	14.91551
Most Extreme Diffrences	Absolute	.118
	Positive	.107
	Negative	-.118
Test Statistic		.118
Asymp. Sig. (2-tailed)		.200

To determine whether the soaking time was homogeneous, a homogeneity test was performed using the Levene statistic. The results of the homogeneity test are shown in Table 3 below:

Table 3. Data from the Homogeneity of Variance Test of Soaking Time Against Masculinization

	Levene Statistic	Df1	Df2	Sig.
Stabdardized residual	,249	4	20	,907

To determine whether there was a significant difference between the treatments, a 5% one-way ANOVA test was conducted. The results of the one-way ANOVA test can be seen in Table 4 as follows:

Table 4. ANOVA test data for the percentage of masculinization of guppy larvae

ANOVA					
	Sum of Square	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	4673.816	4	1168.454	35.114	0.000
Within Groups	665.524	20	33.276		
Total	5339.340	24			

Based on the ANOVA test, the calculated F value is greater than the table F value. Therefore, another test is needed, namely a multiple comparison test using the Duncan test. The results of the Duncan test can be seen in Table 5 as follows:

Table 5. Differences in Notation of Duncan Test Results Related to Masculinization

Treatment	**N**	**Subset for alpha = 0,05**				
		1	**2**	**3**	**4**	**5**
A	5	51,7200				
E	5		59,7800			
D	5			68,3800		
B	5				79,3800	
C	5					90,1400
Sig		1,000	1,000	1,000	1,000	1,000

Water Quality Parameters

Water Temperature

To determine whether the temperature data is homogeneous, a homogeneity test was performed using the Levene statistic. The results of the homogeneity test can be seen in Table 6, as follows:

Table 6. Temperature Data Homogeneity Test

Test of Homogenity of Variance				
	Levene Statistic	**df1**	**df2**	**Sig (p)**
Based on Mean	0,636	4	20	0,643
Based on Median	0,135	4	20	0,967
Based on trimmed Mean	0,610	4	20	0,660

Furthermore, to investigate whether there are actual differences in water temperature in each process, further testing is needed, namely the ANOVA test. The results of the ANOVA test can be seen in Table 7 as follows:

Table 7. ANOVA Test for Temperature Data

ANOVA					
	Sum of Squares	**df**	**Mean squre**	**F**	**Sig (p)**
Between Groups	0,132	4	0,033	0,089	0,985
Within Groups	7,400	20	0,370		
Total	7,532	24			

pH of water

To determine whether the pH data is homogeneous, a homogeneity test was performed using the Levene statistic. The results of the homogeneity test can be seen in Table 7, as follows:

Table 8. Data for the pH Data Homogeneity Test

Test of Homogenity of Variance				
	Levene Statistic	**df1**	**df2**	**Sig (p)**
Based on Mean	0,310	4	20	0,868
Based on Median	0,300	4	20	0,874
Based on trimmed Mean	0,322	4	20	0,860

To determine whether there are significant differences between the water pH values in each treatment, a further ANOVA test is required. The results of the ANOVA test can be seen in Table 9, as follows:

Table 9. ANOVA Test Data: Water pH Data

ANOVA					
	Sum of Squares	**df**	**Mean squre**	**F**	**Sig (p)**
Between Groups	0,005	4	0,001	0,245	0,909
Within Groups	0,094	20	0,005		
Total	0,99	24			

Dissolved Oxygen

To determine whether the dissolved oxygen data is homogeneous, a homogeneity test will be performed using the Levene statistic. The results of the homogeneity test can be seen in Table 10, as follows:

Table 10. Homogeneity test data of dissolved oxygen data

Test of Homogenity of Variance				
	Levene Statistic	**df1**	**df2**	**Sig**
Based on Mean	1,015	4	20	0,424
Based on Median	0,424	4	20	0,789
Based on trimmed Mean	0,989	4	20	0,436

To determine whether there are significant differences between the water pH levels in each treatment, further testing, namely ANOVA, is necessary. The results of the ANOVA test can be seen in Table 11, as follows:

Table 11. ANOVA Test Data: Dissolved Oxygen Data

ANOVA					
	Sum of Squares	**df**	**Mean squre**	**F**	**Sig.**
Between Groups	0,049	4	0,012	0,690	0,607
Within Groups	0,352	20	0,018		
Total	0,402	24			

DISCUSSION

The results showed that the soaking time data were normally distributed (P = 0.200 > 0.05) and had a homogeneous variance (Sig = 0.907 > 0.05). Based on the results of the one-way ANOVA test, the addition of honey had a significant effect (F count > F table) sig = 0.000 < 0.05) on the masculinization of guppy larvae (Poecilia reticulata). These results are supported by previous research by Priyono (2013) and Habibi F. (2022), which showed that soaking with honey at a dose of 5 ml/L for 12 hours effectively increased the male sex ratio of guppy fish by 76.66% and 100%, respectively.

Through the Duncan test and the 5% LSD test, it was found that all treatments (A, B, C, D, and E) produced significantly different results. Treatment C (12-hour soaking) produced the highest masculinization rate, with an average of 90.1%. This highest result was followed, respectively, by treatment B (79.3%), treatment D (68.3%), treatment E (59.7%), and the lowest masculinization rate was achieved by treatment A (8-hour soaking), at 51.7%.

Honey contains chrysin, which can be used for sexual orientation (Ibrahim et al., 2016). According to Nurlina and Zulfikar (2016), chrysin is a substance that functions as an aromatase inhibitor, increasing testosterone levels and reducing estrogen levels, thus promoting male differentiation of the gonads. Honey acts as a natural masculinizing agent due to its flavonoid content, particularly chrysin, which has the biological ability to act as an aromatase inhibitor. In the hormonal cycle of guppies, the aromatase enzyme is responsible for the aromatization process, which is the conversion of androgen (testosterone) into estrogen (estradiol), which directs the development of female genitalia. By providing chrysin-rich honey during the sensitive phase of larval development, the activity of this aromatase enzyme is inhibited, resulting in a drastic decrease in estrogen production in the fish. This low estrogen level and the dominance of natural androgens then trigger gonadal differentiation toward testicular formation and the emergence of secondary male characteristics, such as more striking coloration and the modification of the anal fin into a gonopodium.

Sarida et al., (in Saputra and Iskandar, 2020), explain that masculinization of guppy larvae (Poecilia retriculata) is performed by soaking the larvae in a honey solution. The active

ingredients in the honey enter the fish's body through diffusion through the skin, gills, and lateral line, which then directs the development of the gonads into males. Other factors that can influence the success of masculinization are the length of soaking time and the accuracy of the sex determination phase. The tendency for relatively short immersion periods to result in incomplete sex reversal. The optimal time for immersion is before sex differentiation begins, during the larval stage or when the fish have just begun feeding (Erwin, 2018). In Poecilidae, sex differentiation occurs before birth and continues until shortly after larval stage. The sex differentiation phase in Poecilidae occurs during the embryonic stage until the larvae are 12 days old. The length of immersion affects the success of masculinization (Saputra et al., 2018).

Based on the homogeneity test (Levene's test), all parameters exhibited homogeneous variance, with p-values of 0.660 for temperature, 0.860 for pH, and 0.436 for dissolved oxygen ($P > 0.05$). The ANOVA test results also showed no significant mean differences between treatment tanks, either in temperature ($F = 0.089$; $P = 0.985$), pH ($F = 0.245$; $P = 0.909$), or dissolved oxygen ($F = 0.690$; $P = 0.607$). Since all p values far exceeded the 0.05 threshold, it was concluded that the environmental conditions between tanks tended to be homogeneous and the treatment did not significantly affect the stability of the three water quality parameters.

CONCLUSION

The difference in soaking time using honey with a dose of 5 ml/L has a significant effect on the success of masculinization of guppy fish larvae (Poecilia reticulata), where the 12-hour soaking treatment proved to be the best time that produced the highest percentage of male sex at 90.1% with the support of water quality in the maintenance media that was maintained homogeneously and optimally at a temperature range of 23.1–26.9°C, pH 7.0–7.2, and dissolved oxygen (DO) content of 6.1–6.7 ppm.

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