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EFFECTIVENESS OF STRIPED CATFISH TESTIS FLOUR EXTRACT IN MASCULINIZATION OF GUPPY FISH (POECILIA RETICULATA, PETERS) USING THE PREGNANT FEMALE IMMERSION METHOD

Efektivitas Ekstrak Tepung Testis Ikan Patin Dalam Maskulinisasi Ikan Guppy (Poecilia Reticulata, Peters) Dengan Metode Perendaman Induk Bunting

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ABSTRACT

The guppy fish is a highly valuable ornamental fish commodity with high economic value and demand, particularly for males due to their bright colors, body shape, and more attractive tails compared to female fish. However, under normal breeding conditions, the percentage of male guppy offspring produced is often below 50%, necessitating masculinization using 17α-*methyltestosterone*, which has been banned in fish farming. This study aims to evaluate the effectiveness of striped catfish testis flour extract (ETTIP) as an alternative agent in the masculinization of guppy fish through the immersion method of pregnant females. The study used a Completely Randomized Design (CRD) with 5 treatments and 3 replicates, namely a negative control without treatment (P-), 20 mg/L 17α-*methyltestosterone* as a positive control (P+), and three doses of ETTIP: 40 mg/L (P1), 60 mg/L (P2), and 80 mg/L (P3), with the immersion of pregnant females for 24 hours. The resulting guppy offspring were maintained for 60 days, with parameters observed including the percentage of male fish, absolute length growth, survival rate, abnormalities, and water quality. The results showed that a dose of 60 mg/L ETTIP (P2) was the most effective, increasing the percentage of male fish to 84.59% with a survival rate of 100% and no harmful side effects for guppy fish. ETTIP can be used as an effective and safe alternative to maximize masculinization in guppy fish.

Keywords: Guppy, Masculinization, Striped Catfish Testis

ABSTRAK

Ikan guppy termasuk komoditas ikan hias dengan nilai ekonomis dan permintaan yang tinggi, terutama ikan jantan karena memiliki warna cerah, bentuk tubuh, dan ekor yang lebih menarik dibandingkan ikan betina. Namun dalam kondisi pemijahan normal, persentase anakan ikan guppy jantan yang dihasilkan sering kali di bawah 50%, mengharuskan pembudidaya melakukan maskulinisasi menggunakan 17α-*methyltestosterone* yang telah dilarang penggunaanya dalam budidaya ikan. Penelitian ini bertujuan untuk mengevaluasi efektivitas ekstrak tepung testis ikan patin (ETTIP) sebagai bahan alternatif dalam maskulinisasi ikan guppy melalui metode perendaman induk bunting. Penelitian menggunakan Rancangan Acak Lengkap (RAL) dengan 5 perlakuan 3 ulangan yaitu kontrol negatif tanpa perlakuan (P-), 17α-*methyltestosterone* 20 mg/L sebagai kontrol positif (P+), dan tiga dosis ETTIP 40 mg/L (P1), 60 mg/L (P2), dan 80 mg/L (P3) dengan perendaman induk bunting selama 24 jam. Anakan ikan guppy yang dihasilkan dipelihara selama 60 hari dengan parameter yang diamati berupa persentase ikan jantan, pertumbuhan panjang mutlak, kelangsungan hidup, abnormalitas, dan kualitas air. Hasil penelitian menunjukkan bahwa dosis 60 mg/L ETTIP (P2) merupakan dosis paling efektif, meningkatkan persentase ikan jantan menjadi 84,59% dengan kelangsungan hidup 100% tanpa efek samping berbahaya bagi ikan guppy. ETTIP dapat digunakan sebagai alternatif yang efektif dan aman untuk memaksimalkan maskulinisasi pada ikan guppy.

Kata Kunci: Guppy, Maskulinisasi, Testis Ikan Patin

INTRODUCTION

Indonesia has great potential to become the world's largest ornamental fish producer. Demand for ornamental fish, including guppies (Poecilia reticulata, Peters), comes not only domestically but also internationally, with high sales value. Ornamental fish production increased by an average of 7.34% per year in the 2015-2019 period, from 1.31 billion fish in 2015 to 1.67 billion fish in 2019 (DJPB, 2020). Male guppies are more popular than females due to their bright colors and attractive tail shapes, as well as their higher economic value. However, under normal spawning conditions, guppies only produce offspring with a nearly equal sex ratio between males and females (Haq *et al.,* 2013).

To increase the male guppy population, cultivation technology using the sex reversal method is required. This technique is generally carried out using androgen hormones such as 17α-methyltestosterone through immersion, injection, or oral administration through feed. The use of synthetic testosterone hormones is effective in directing sex before gonadal differentiation occurs, with a success rate of 96-100% (Rosmaidar *et al.,* 2016). However, the use of synthetic androgen hormones has been banned in fish cultivation based on the Decree of the Minister of Maritime Affairs and Fisheries No: KEP.52/MEN/2014 due to potential hazards such as carcinogenicity, environmental pollution, and undesirable side effects.

Given these issues, new, natural, safe, and effective alternative materials are needed for the sex reversal process. Safe alternative materials, such as the use of animal testicles as a source of natural hormones, are already widely used. One example is bovine testicles, which are organs that produce sperm and testosterone, and have been proven effective in masculinizing nirvana tilapia, with an increase in the number of male fish of up to 69.07% (Yustiati *et al.,* 2018) and guppy fish of 91.6% (Lutfiyah *et al.,* 2016). Waste from fisheries processing, especially catfish, also has the potential to be utilized as products with added value such as silage (Jayanti et al., 2018), fish oil (Pangestika *et al.,* 2021), and biodiesel (Azizah *et al.,* 2024). Another waste product, catfish testicles, can be developed as a safe alternative material for masculinization. Catfish testicles are a new, under-researched natural hormone source and have the potential to be used as a substitute for dangerous synthetic hormones. Catfish testis flour extract (ETTIP) is suspected to contain high levels of testosterone, seen from its content in the tail vein blood plasma of 12-month-old catfish broodstock reaching 0.256 mcg/mL (Sattang *et al.,* 2021), which is higher than the testosterone level in bovine blood plasma of 0.00613 mcg/mL (Widyaningrum *et al.,* 2015).

Treatment dosage is a critical factor influencing the success of sex reversal in guppies. This study aimed to evaluate the effectiveness of ETTIP in masculinizing guppies using the immersion method for pregnant females. The results are expected to provide a safe and effective alternative solution for increasing male guppy populations and reducing the use of harmful synthetic hormones.

RESEARCH METHODS

Time and Place

The research was conducted for 90 days, from May 12 to August 10, 2022, at the Ciparanje Wet Laboratory, Faculty of Fisheries and Marine Sciences (FPIK), Padjadjaran University. The production of catfish testis flour extract (ETTIP) was conducted at the Food Engineering Laboratory, Faculty of Agricultural Industrial Technology (FTIP), Padjadjaran University.

Tools and materials

The tools used include aquariums of various sizes, jars, aeration equipment, plastic bottles, pestles and mortars, surgical scissors, sieves, spatulas, label paper, dropper pipettes, freezers, freeze-dry machines, plastic containers, DO meters, pH meters, digital thermometers, ammonia test kits, rulers, and documentation equipment. The materials used are prospective male and female guppy fish parents, natural food (Daphnia), commercial food, 70% alcohol, synthetic hormone 17α-methyltestosterone, chlorine, methylene blue, and fresh catfish testicles.

Research Design

This research used a Completely Randomized Design (CRD) experimental method consisting of 5 treatments and 3 replications, so there were 15 experimental units. The treatment design used is as follows:

- P− : Negative control (without hormone immersion)
- P+ : Positive control, immersion in 17α-methyltestosterone 20 mg/L for 24 hours
- P1 : ETTIP 40 mg/L soaking for 24 hours
- P2 : Immersion in ETTIP 60 mg/L for 24 hours
- P3 : ETTIP 80 mg/L soaking for 24 hours

The determination of the ETTIP dosage in this study refers to Hidayani et al. (2016) who used a dose of 60 mg/L bovine testis extract with a 24-hour immersion duration on betta fish, and is reinforced by Lutfiyah et al. (2016) who used a similar immersion duration on guppies. The dose range of 40–80 mg/L was chosen to evaluate the effectiveness of the dose while remaining within the physiological safety limits of guppies.

Stages of Making Pangasius Fish Testis Flour Extract

Fresh catfish testicles obtained from the Cileunyi Healthy Market in Bandung Regency were skinned, split lengthwise, and chopped into small pieces. The testicle pieces were frozen for 24 hours, then freeze-dried for 24 hours at -50°C. The dried catfish testicles were then ground into flour using a mortar and pestle and sieved through a fine sieve. The resulting flour was stored in an airtight container in a cool, dry place until ready to use.

The catfish testis flour, according to the treatment dosage, was then simply extracted using 1 ml of 70% alcohol. The mixture was stirred and allowed to stand for 30 minutes to ensure the bioactive testosterone components could separate from the solid flour matrix and dissolve. The ETTIP was then placed in a soaking jar and vigorously aerated for 30 minutes to ensure homogenization and accelerate the evaporation of any remaining alcohol.

Implementation Stage

Three-month-old guppy broodstock were raised for one month before mating. During the rearing period, the broodstock were fed twice daily using commercial and natural feed to ensure their nutritional needs were met. After one month, the fish were mated, with each aquarium containing two males and one female for four days, and the males were separated thereafter. On the 12th day after mating, the females showing signs of pregnancy were transferred to a soaking jar and given different treatment doses for 24 hours. After the soaking period, the females were transferred back to the mating aquarium until they gave birth to their young. The resulting guppy fry were transferred to a separate rearing aquarium for 60 days. Maintenance included twice-daily feeding, regular water changes, and aquarium cleaning to maintain water quality and fish health. Daily observations were made to ensure optimal conditions throughout the study.

Research Parameters

Observed parameters included the percentage of male fish, absolute length growth, abnormalities, survival, and water quality. The percentage of male fish was calculated based on secondary morphological characteristics at 40–60 days of age using the formula:

$$\text{Ij (\%)} = \left(\frac{Ij}{Is}\right) \text{ x } 100\%$$

Information :
Ij (%) = Percentage of male fish
Ij = Number of male fish (tails)
Is = Number of fish observed (tails)

Absolute length growth is measured from the difference between the final and initial length of maintenance using the formula:

$$L = Lt - Lo$$

Information :
L = Absolute length growth (mm)
Lt = Final length (mm)
Lo = Initial length (mm)

Abnormalities were observed visually and microscopically with 40x magnification, then calculated using the formula:

$$\text{Abnormalitiy} = \frac{\text{Abnormal number of fish}}{\text{Number of fish observed}} \text{ x } 100\%$$

Survival rate was calculated after 60 days of maintenance using the formula:

$$\text{SR (\%)} = \left(\frac{N_t}{N_0}\right) \text{ x } 100\%$$

Information :
SR (%) = Life sustainability
N_t = Number of fish at the end of the experiment (tails)
N_0 = Number of fish at the start of the experiment (tails)

Water quality parameters measured included pH, DO, temperature, and ammonia. Measurements were conducted every 10 days for 60 days using a pH test kit, DO meter, digital thermometer, and ammonia test kit. The water quality of the media was based on SNI 8112:2015 and Malik *et al.* (2019).

Data analysis

The data from observations of guppy fry were analyzed statistically using Analysis of Variance (ANOVA) and if there was a significant difference, it was continued with the BNT test at a 95% confidence level.

RESULTS

Percentage of Male Fish

The analysis results showed significant differences in the proportion of male fish observed across all treatments. The untreated control group (P-) showed an average percentage of male fish of 42.90 ± 2.92%. Meanwhile, the control group treated with 20 mg/L 17α-methyltestosterone hormone (P+) experienced an increase to 74.74 ± 3.18%. Treatment with ETTIP at a dose of 40 mg/L (P1) produced a mean value of 59.44 ± 1.95%. A dose of 60 mg/L (P2) showed the highest effectiveness with a percentage of male fish reaching 84.59 ± 2.20%. However, increasing the dose to 80 mg/L (P3) actually showed a decrease in the percentage of male fish produced to 65.21 ± 4.19%. This pattern indicates that the optimal effectiveness of ETTIP in masculinization is achieved at a moderate dose (Figure 1).

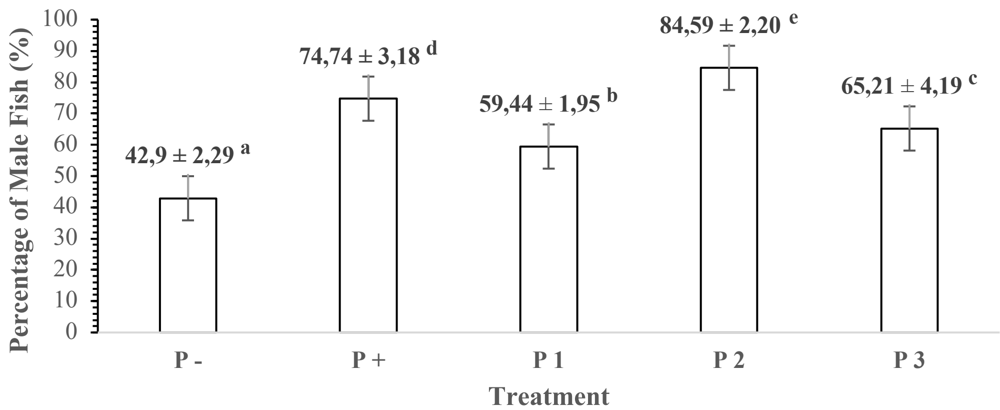


Figure 1. Percentage of Male Guppy Fish in Various Treatments

Absolute Length Growth

The average length growth in the P- and P+ groups was recorded at 22.67 ± 0.25 mm. Groups P1, P2, and P3 showed values of 22.92 ± 0.11 mm, 22.91 ± 0.16 mm, and 23.10 ± 0.29 mm, respectively. In general, all treatments did not significantly affect the absolute length growth of guppies (Figure 2).

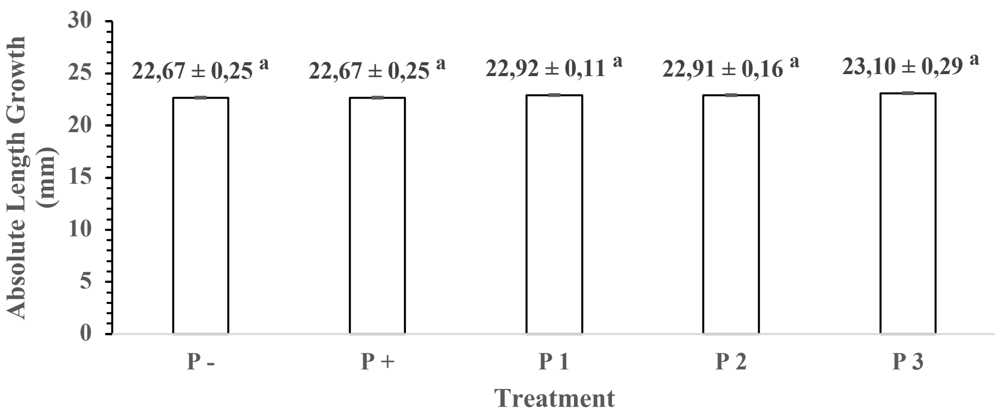


Figure 2. Absolute Length Growth of Guppy Fish in Various Treatments

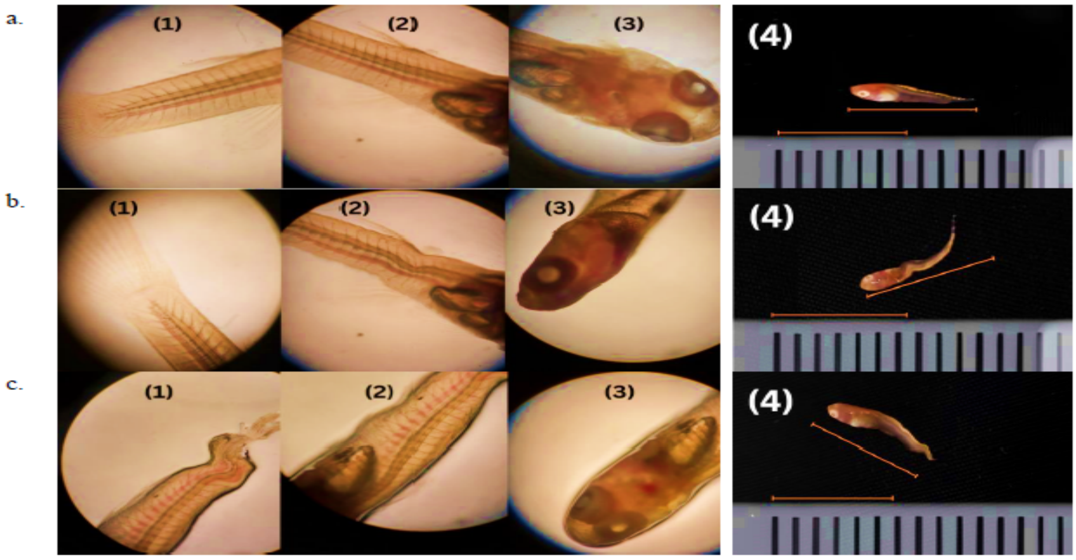
Abnormality

Morphological abnormalities in guppy fry were detected on the 10th day after treatment. Observations showed that only the P+ treatment group (treated with 20 mg/L 17α-methyltestosterone hormone) experienced abnormalities, with a percentage of 5.34%. Meanwhile, the P- control group and all ETTIP treatments (P1, P2, P3) showed no signs of abnormalities (Table 1).

Findings in the P+ group included morphological abnormalities such as spinal curvature, as shown in Figure 3. These abnormalities were likely associated with hormonal stress, as all individuals exhibiting these symptoms died before the end of the observation period. In contrast, larvae from the other groups generally exhibited symmetrical and normal body structures.

Table 1. Percentage of abnormalities in guppy fish

Treatment	Abnormality (%)
P -	0,00 [a]
P 1	0,00 [a]
P 2	0,00 [a]
P 3	0,00 [a]
P +	5,34 [b]



Source: Personal documentation (2022)
Caption: a. Normal guppy fish offspring
b. Guppy fish fry treated P+a,
c. Guppy fish fry treated with P+b,
Microscopic observation (1) tail, (2) body, (3) head, (4) Visual observation (mm)

Figure 3. Observation of Guppy Fish Abnormalities

Life sustainability

The survival of guppy fry born from pregnant broodstock treated with these treatments showed varying patterns. The negative control group (P–), as well as the P1 and P2 treatment groups, recorded the highest survival rate at 100%. The P3 treatment showed a survival rate of 96.67 ± 3.27%, and was not significantly different from the P– or other ETTIP treatments. Meanwhile, the P+ group (17α-*methyltestosterone* immersion) showed the lowest survival rate, at 85.67 ± 9.14% (Figure 4)

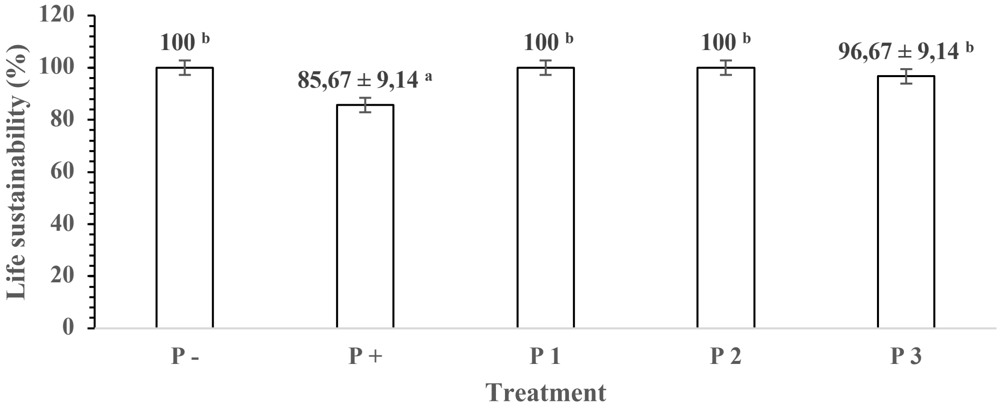


Figure 4. Survival of Guppy Fish in Various Treatments

Water Quality

The results of water quality parameter measurements are presented in Table 2.

Table 2. Water Quality of Maintenance Media

Observation Time	pH	DO (mg/L)	Suhu (°C)	Ammonia (mg/L)
Parent Maintenance	6,9 - 7,1	5,4 - 5,9	25,2 - 25,5	0,0030
Spawning	6,8 – 7,3	4,8 – 5,0	25,0 – 25,2	0,0032
Immersion				
Beginning of Immersion	7,3 – 8,5	5,8 – 6,1	26,0	0,009 - 0,08
End of Immersion	7,2 – 8,4	5,5 – 5,8	26,0 – 26,2	0,02 – 0,15
Child Care	6,2 – 6,8	5,3 – 6,9	25,5 – 26,4	0,003 – 0,006
Reference	6,0-8,0*	>3*	25,0 – 30,0*	<0,2**

Information:* SNI 8112:2015
** Malik *et al.,*(2019)

DISCUSSION

The use of catfish testis meal extract (ETTIP) in the soaking medium for pregnant broodstock at different doses significantly affected the percentage of male guppies produced. A dose of 60 mg/L ETTIP (P2) produced the highest percentage of male fish at 84.59%, compared to P+ using 17α-methyltestosterone (74.74%) and the negative control without treatment (42.90%). These results indicate that 60 mg/L ETTIP is the most effective treatment for masculinizing guppies. The effectiveness of ETTIP is closely related to the testosterone hormone content in catfish testes, which is the site of testosterone formation. Sattang et al., (2021) found that the testosterone level in blood plasma of 12-month-old catfish reached 0.256 mcg/mL, much higher than that in bovine blood plasma at 0.00613 mcg/mL (Widyaningrum *et al.,* 2015). Testosterone plays an important role in the process of gonadal differentiation by binding to androgen receptors in the primordial gonadal cell phase, then forming Leydig cells and Sertoli cells which accelerate testicular development while inhibiting ovary formation.

The sex orientation of guppy fish to become male is influenced by several factors such as the type of fish, age of the fish, type of active ingredient, duration of treatment, method used, and dosage. The success of the treatment effectiveness in the P2 group is because 60 mg/L ETTIP is the maximum dose that can be absorbed by guppy fish embryos to direct gonadal development to become male without causing negative effects. Treatment with a dose of 40 mg/L ETTIP (P1) resulted in a percentage of male fish of 59.44%. Although the results were higher than the P- group, the effectiveness of this dose was not better than in P2. This indicates that too low a hormone dose is also not enough to maximize the masculinization process. Yustiati et al. (2018) added that the use of natural steroid hormones such as ETTS with immersion treatment and oral combination in nirwana tilapia larvae showed that the use of the immersion method with a low dose alone is not effective enough to optimize masculinization results, so a combination with further oral treatment through feed is needed.

The absolute length growth of guppy fish did not show significant differences between treatments, including negative controls, positive controls, and various doses of ETTIP (Figure 2). These results indicate that the administration of synthetic and natural androgen hormones through the immersion method of pregnant broodstock does not directly affect the aspect of length growth. Length growth in guppy fish is influenced by many factors such as water quality, feed, movement space, genetics, age, immunity, and the ability to utilize feed. However, ETTIP immersion is thought to improve the quality of guppy larvae in terms of survival and early development of secondary characteristics such as color, although in a limited phase. This assumption is supported by Borg (1994) who stated that the content of androgen hormones in the testes, especially 11-Ketotestosterone, has a major role in the development of secondary sexual characteristics of male fish.

Abnormalities were observed in the positive control group (P+) treated with 20 mg/L 17α-methyltestosterone immersion, with symptoms of spinal curvature (scoliosis). These abnormalities were found in the Abdominal Vertebrae (ABV) and Urostyles (UST), as seen in Figure 3. Exposure to high doses of 17α-methyltestosterone is suspected to be the main cause that triggers a toxic response, disrupts hormonal balance, and bone tissue development. This synthetic hormone affects the spinal structure by disrupting the normal function of chondroblasts during the cartilage secretion process, which causes postural abnormalities and spinal deformities. According to Alkahemal et al. (2011), exposure to pollutants such as heavy metals or exogenous hormones can disrupt the embryonic development process and increase the risk of abnormalities.

Although Huwoyon *et al*. (2008) reported that the use of methyltestosterone in high doses did not cause negative effects, the hormone 17α-methyltestosterone is a modified form of diogenin with the addition of a methyl group. Therefore, it carries a risk of toxicity when used in excessive doses, including physiological stress and impaired bone development such as scoliosis. The accumulation of inefficiently broken-down hormones in the fish's body can inhibit normal tissue development and trigger deformities.

The P-, P1, and P2 treatments showed optimal survival rates of up to 100%. Of the three, the P2 treatment with an ETTIP dose of 60 mg/L was the most balanced in promoting masculinization without compromising fish health. Hidayani et al. (2016) also stated this dose as the optimal safe limit. Furthermore, the use of ETTIP also strengthens embryonic resistance during development because in addition to testosterone, ETTIP also contains proteins, lipids, vitamins, and minerals that are absorbed during the immersion process. Conversely, P3 (80 mg/L) experienced a decrease to 96.67%, this is suspected to be due to excessive hormone toxicity that weakens the immune system from the early stages of development. Rivero-Wendt et al., (2020) reported that high doses of hormones can trigger oxidative stress, DNA damage, and metabolic dysfunction. A similar condition also occurred in the P+ treatment with the lowest survival rate (85.67%) accompanied by the appearance of abnormalities. The negative impacts of exposure to high doses of the hormone 17α-methyltestosterone can be exacerbated if environmental parameters, especially water quality, are not within the optimal range to support the stability of the physiological functions of the fish.

Water quality plays a crucial role in maintaining physiological stability and fish survival during the masculinization process. Throughout the study, parameters such as pH, temperature, DO, and ammonia were recorded as remaining within optimal limits for guppies, according to

SNI 8112:2015 and Malik et al. (2019). A decrease in pH to 6.2 in the maintenance medium was still tolerable for guppies and did not trigger any health problems.

CONCLUSION

The use of catfish testis meal extract (ETTIP) at a dose of 60 mg/L is an effective and safe alternative for masculinization of guppies. This treatment can produce a male percentage of up to 84.59% with 100% survival without causing side effects to the fish. In contrast, the use of 17α-methyltestosterone at a dose of 20 mg/L can reduce survival and cause abnormalities in the spinal structure of guppies. Thus, ETTIP can be used as a safer and more effective substitute for synthetic hormones for masculinization of guppies.

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