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Masculinization of Cupang Fish (*Betta splendens*) Using Rujak Polo Fruit (*Tribulus terrestris*) Through Immersion Method

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ABSTRACT

Male betta fish have high commercial value, making it more effective and profitable if only males are produced and reared. The aim of this study was to determine the effect of Tribulus terrestris fruit on the masculinization of betta fish (Betta splendens) using an immersion method. This research is quantitative. The population in this study consisted of the entire larval population of betta fish. A sample of 300 seven-day-old betta fish larvae was taken from this population and divided into 5 containers, each containing 20 larvae. The sampling technique used was Probability Sampling. Data collection was conducted with 5 treatments, each with 3 repetitions. The data obtained were tested using a One Way ANOVA or analysis of variance with a 95% confidence level. The results showed that the average data obtained from each treatment group differed significantly. The best dosage concentration in this study was treatment P2 with a concentration of 2 mg/L, resulting in the highest percentage of male betta fish at 84.49%.

Keyword: Betta Fish (*Betta splendens*), Masculinization, Immersion, Sex Reversal, Rujak Polo Plant (*Tribulus terrestris*).

ABSTRAK

Ikan cupang jantan memiliki nilai komersial tinggi, sehingga akan lebih efektif dan menguntungkan bila hanya diproduksi dan dipelihara jenis jantan. Tujuan penelitian ini untuk mengetahui pengaruh buah tumbuhan rujak polo (*Tribulus terrestris*) terhadap maskulinisasi ikan cupang (*Betta splendens*) dengan metode perendaman. Jenis Penelitian ini adalah

penelitian kuantitatif. Populasi dalam penelitian ini adalah keseluruhan larva ikan cupang. Dari populasi tersebut diambil sampel 300 ekor larva ikan cupang berumur 7 hari, dan dibagi dalam 5 wadah setiap wadah berisi 20 ekor larva ikan cupang. Tehnik pengambilan sampel yang digunakan yaitu *probability sampling.* Pengambilan data sampel penelitian dilakukan sebanyak 5 perlakuan dengan 3 kali pengulangan. Data dianalisis mengunakan Uji One Way Anova dengan uji BNT dengan tingkat kepercayaan95%. Hasil penelitian bahwa maskulinisasi ikan cupang (*Betta splendens*) menggunakan buah rujak polo (*Tribulus terrestris*) melalui metode perendaman dengan dosis yang berbeda memberikan pengaruh yang nyata terhadap persentase ikan cupang. Konsentrasi dosis terbaik yang dapat digunakan dalam maskulinisasi ikan cupang adalah pada perlakuan P2 yaitu sebanyak 2mg/L yang menghasilkan persentase ikan cupang jantan tertinggi yaitu sebesar 84,49%.

Kata Kunci: Ikan Cupang (*Betta splendens*), Maskulinisasi, Perendaman, Sex Reversal, Tumbuhan Rujak Polo (*Tribullus terrestris*).

INTRODUCTION

Betta fish are highly favored by the public due to their attractive colors and tail fins. The high demand for betta fish, both domestically and internationally, has driven the growth of the betta fish breeding industry. Despite the promising potential of betta fish as a commercial ornamental species, (Mustaqim et al., 2019). Breeding male betta fish is more challenging, as the number of male fry obtained from each spawning is lower than that of female fry, and their quality often does not meet expectations. A single spawning cycle typically results in about 60% survival for female fry and 40% for male fry (Benediktus Rianwara Ilham Gemilang, Fajar Basuki, 2016).

In addition, male betta fish have a high selling price because of their morphology or color which becomes their aesthetic value, therefore it is necessary to know how to get a high percentage of males. An effort to increase the male fish population is to masculinize to direct the fish to be male, so that the profit value becomes higher. Sex direction is one of the monosex production techniques that applies hormonal engineering to direct sexual characteristics from females to males. The effectiveness of a treatment in sex direction can be influenced by several factors such as dose, hormone, method of administration, treatment time, environmental conditions and fish resistance to hormones (Lestari et al., 2018).

So far, sex reversal techniques to obtain monosex males (masculinization) have commonly been done using the hormone 17α-methyltestosterone. However,

recently, the use of methyltestosterone has started to be restricted due to concerns that it may pollute the environment and potentially cause cancer in humans. Consequently, the use and distribution of this hormone have been banned and it is no longer available for general sale (Asrul Ferdian, Muslim, 2017). Therefore, it is necessary to find alternative materials that can replace synthetic substances, and one natural option is derived from the plant Tribulus terrestris.

Masculinization in bette fish is conducted during the sex differentiation stage when the larvae are 7 day old. At this age, betta larvae can already swim and feed independently without parental assistance, and they have developed a strong immune system. In Anabantid fish, the sex differentiation period occurs from day 3 to day 40 post-hatching (Rachmawati et al., 2016).

This plant is native to Southern Europe, Asia, Africa, and Northern Australia. It is also known as Gokshura, Puncture Vine, Caltrop, Yellow Vine, and Goathead. In Indonesia, Tribulus terrestris is fairly easy to find and is commonly referred to as "bulu mata setan," "rujak polo," "kepala kucing," or "duri setan." The primary chemical components of this fruit include steroidal saponins, protodioscin, tribulosaponins A and B, tribulosin, and terrestrosin A–K. Other compounds include alkaloids, tribulusamides A and B, small amounts of harman and norharman, as well as flavonols like kaempferol, quercetin, and rutin. One of the compounds in this plant, known as Di-p-coumaroylquinic acid, possesses antioxidant activity and is also effective in treating idiopathic male infertility.

The dominant component in the fruit of this plant is protodioscin. Research in the fields of behavior, hematology, biochemistry, functionality, and morphology on acute, sub-chronic, and chronic toxicity of protodioscin indicates that this plant does not exhibit toxic effects. The extract of *Tribulus terrestris*, which contains protodioscin, can increase testosterone and dihydrotestosterone levels. This compound stimulates luteinizing hormone (LH) and converts testosterone into dihydrotestosterone, which enhances libido, promotes muscle development, and boosts red blood cell production (Gaziansyah et al., 2019).

Several studies report the use of *Tribulus terrestris* fruit as an aphrodisiac. Other research indicates that *Tribulus terrestris*fruit can improve sexual performance and muscle mass in men. The use of *Tribulus terrestris* fruit also impacts spermatogenesis. Studies on rats have shown an increase in primary spermatocytes, sperm concentration, and sperm morphology in the group of rats given *Tribulus terrestris* extract compared to the control group (Pelealu et al., 2015). The *Tribulus terrestris* fruit used in this study was obtained from a herbal medicine store in dried form. Therefore, the researchers are interested in conducting further research on the effects of the fruit of *Tribulus terrestris* (known as "rujak polo" in Indonesia) on the masculinization of betta fish (*Betta splendens*) through the immersion method. In his research, M. Prido Gaziansyah explained that *Tribulus terrestris*, or rujak polo, contains protodioscin, which improves reproductive function by increasing testosterone and

dihydrotestosterone levels (Gaziansyah et al., 2019). In this study, the immersion method is applied to 7-day-old betta fish larvae, an age at which the sex of the fish has not yet formed, allowing for sex reversal.

The aim of this research was to determine the effect of rujak polo (Tribulus terrestris) on the masculinization of betta fish (Betta splendens) using the soaking method.

METHODS

This research was carried out in May 2021 - July 2021. The location of this research was carried out at the Biology Laboratory, Faculty of Tarbiyah and Teacher Training, Raden Intan State Islamic University, Lampung,

This type of research is quantitative research and the methods used in this research are experimental and documentation methods.

The population in this study was all betta fish larvae. From this population, samples were taken of 300 7 day old betta fish larvae because at this age the fish larvae are stronger and can adapt to all changes, and also at this age the genitals of the fish larvae have not yet formed so it is easy to carry out gender direction or sex reversal. And divided into 5 containers where each container contains 20 betta fish larvae. The sampling technique used is Probability Sampling. This technique is a method of determining research samples which is carried out randomly. If in the experiment there are fish that die, then the dead fish remain in the data as consideration for determining the treatment dose used in the experiment.

Data Collection

Data collected in this research using experimental methods were survival after immersion, survival after rearing and the percentage of male betta fish. Research data collection is carried out by direct research on test samples in order to obtain test results that are in accordance with the treatment given. Research sample data was collected in 5 treatments with 3 repetitions. The treatment used was soaking 7 day old betta fish larvae with Tribulus terrestris fruit for a soaking time of 8 hours because in research conducted by Afpriyaningrum MD, soaking 10 and 14 day old tilapia fish larvae with 1,800 µg/L MT for 8 hours produced males. as many as 98.3% of fish are male (MD et al., 2016). The doses used are as follows:

1. P0 = Control (without administration of rujak polo extract)
2. P0+= Positive control (using the hormone 17a methyltestosterone) 2mg/L (MD et al., 2016)
3. P1 = Concentration of rujak polo fruit soaking 1 mg/L
4. P2 = Concentration of rujak polo fruit soaking 2 mg/L
5. P3 = Concentration of rujak polo fruit soaking 3 mg/L.

Tools and Materials

The tools used are as follows: a plastic container with a capacity of 2 L for spawning betta fish and soaking young betta fish, a Styrofoam box with a capacity of 5 L used for enlarging betta fish, a fish filter, a digital scale, a camera, a stopwatch for calculating the soaking time, a beaker and a spatula. to make soaking water.

The ingredients used are the following 7 day old betta fish larvae,

dried and finely ground fruit of the rujak polo plant (Tribulus terrestris), the hormone 17a-methyltestosterone, 70% alcohol, natural food, and water.

Preparation Stage

Prepare maintenance containers by cleaning 2 L plastic containers. Next, fill each container with 1 L of immersion solution and label according to treatment group. This research used 300 7-day-old betta fish larvae obtained through natural breeding.

Experiment Stage

Soak the larvae in a 2L capacity plastic container containing rujak polo fruit soaking water that is adapted to the treatment and has been left for 24 hours first. The larvae used were 7 days old. Each container was filled with 20 betta fish larvae. The soaking time was 8 hours and survival was observed during soaking. After 8 hours, the fish larvae were transferred to the rearing container. The larvae, which had been soaked in the water from the rujak polo fruit, were then kept in a styrofoam box with a water volume of 3 liters for 60 days. Larvae rearing are given natural food in the form of Artemia sp., and water fleas Moina sp. Artemia sp. given to larvae aged 7-20 days. After the larvae are 20 days old, feed Artemia sp. stopped and given only natural food. After 60 days, the sex of the Betta fish larvae was identified using morphological observations.

Results Statement Stage

1. **Post-Soaking Survival Percentage**

The survival percentage of fish seeds is done by comparing the number of live fish at the end of soaking with the number of fish at the start of soaking. The formula used to calculate survival is as follows:

$$KH=\frac{Nt}{No}\times 100\%$$

Where:
- KH = Survival rate (%)
- Nt = Number of fish alive at the end of immersion (individuals)
- No = Number of fish at the beginning of immersion (individuals)

2. **Post-Maintenance Survival Rate Percentage**

The survival rate of the fish was calculated by comparing the number of live fish at the end of the maintenance period with the number at the start. The formula used to calculate the survival rate is as follows:

$$KH = \frac{Nt}{No}\times 100\%$$

where:
- KH = Survival rate (%)
- Nt = Number of fish alive at the end of maintenance (individuals)
- No = Number of fish at the start of maintenance (individuals)

3. **Percentage of Male Betta Fish**

The percentage of male betta fish was determined by comparing the number of male fish to the total number of surviving fish at the end of the maintenance period. The formula is as follows:

$$\frac{\boldsymbol{Nm}}{\boldsymbol{Nt}}\ \boldsymbol{x}\ \mathbf{100\%}$$

where:
- Nm = Number of male fish (individuals)
- Nt = Total number of live fish at the end of maintenance (individuals)

Data Analysis Technique

The data obtained were survival after immersion, percentage of male betta fish, and survival after rearing.

Data on the percentage of male Betta fish were analyzed statistically using analysis of variance (ANOVA) with a confidence level of 95%. If the data shows a real effect, Duncan's further test is carried out.

RESULTS AND DISCUSSION

1. Post-Immersion Survival

Based on the research results, data were obtained on the number of live betta fish larvae at the end of immersion across various treatment groups and repetitions, as shown in Appendix 1. The post-immersion survival rate of betta fish larvae ranged from 75% to 100%. The highest survival percentage was observed in the K- treatment (control without *Tribulus terrestris* extract), while the lowest percentage was found in the P3 treatment group with an immersion concentration of *Tribulus terrestris* extract at 3 mg/L. The average post-immersion survival rate of betta fish larvae can be seen in Table 1.

Table 1. Post-Immersion Survival Data

Treatment	Preplication			Percentage			Average
	1	2	3	1	2	3	
K-	20	20	20	100%	100%	100%	100%
K+	19	20	18	95%	100%	90%	95%
P1	20	20	19	100%	100%	95%	98%
P2	17	18	18	85%	90%	90%	88%
P3	15	15	16	75%	75%	80%	77%

2. Post-Maintenance Survival

Data on the percentage of survival of Betta fish larvae after rearing is 87% to 100%. The highest survival percentage was in the K- treatment, while the lowest post-rearing survival percentage was in the P1 and P3 treatments.

Detailed data on the post-maintenance survival of betta fish larvae can be seen in Table 2.

Table 2. Post-Maintenance Survival Data of Betta Fish Larvae

Treatment	Preplication			Percentage			Average
	1	2	3	1	2	3	
K-	20	20	20	100%	100%	100%	100%
K+	19	19	18	100%	95%	100%	98%
P1	18	19	18	90%	95%	95%	93%
P2	16	18	18	94%	100%	100%	98%
P3	15	13	15	100%	87%	94%	93%

3. Percentage of Male Betta Fish

Based on the results of this study, it can be seen that the effect of soaking betta fish larvae in rujak polo fruit soaking water with a dose of 0 or no mixture (as a negative control), 2mg of the hormone 17α-methyltestosterone (as a positive control), 1, 2, and 3mg on The average percentage of male Betta fish obtained was 41.67%, 80.41%, 59.84%, 84.49% and 83.76%. The highest average percentage of male betta fish was obtained in treatment P2 at 2 mg/L, with 84.49%, while the lowest percentage was in the K- treatment (negative control) at 41.67%.

Table 3 shows the percentage of male betta fish following immersion in *Tribulus terrestris* extract at varying doses. The lowest number of male fish was observed in the 1 mg/L dose group (P1), with an average of 59.84%, while the highest percentage was in the 2 mg/L dose group (P2), with an average of 84.49%. The negative control group yielded an average male percentage of 41.67%, whereas the positive control (K+) showed an average male percentage of 80.41%.

Table 3. Percentage of Male Betta Fish

Treatment	Preplication			Percentage			Average
	1	2	3	1	2	3	
K-	8	10	7	40%	50%	35%	41,67%
K+	14	16	15	74%	84%	83%	80,41%
P1	11	13	9	61%	68%	50%	59,84%
P2	13	16	15	81%	89%	83%	84,49%
P3	12	11	13	80%	85%	87%	83,76%

One Way ANOVA Test Percentage of Male Betta Fish (Betta splendens)

The results of the One Way ANOVA test above show a significance result of 0.001, where in the test results 0.001 ≤ 0.05, it can be concluded that H0 is not accepted and H1 is accepted because the average data obtained from each treatment group is significantly different at the level significance α5%. So there is an influence of polo salad fruit (*Tribulus terrestris*) on the results of masculinization in betta fish larvae (Betta splendens).

Duncan's test

Because the results of the One Way ANOVA test showed significantly different results in each treatment group, it was continued with the Duncan test to further investigate which groups were significant. The results of Duncan's follow-up test were carried out using the IBM SPSS Statistics 22 application with a significance level of α 5% can be seen in table 5 below.

The average survival rate of betta fish larvae after immersion indicates that the dose used in the *Tribulus terrestris* immersion solution significantly affected the survival of the larvae in each treatment. Higher doses of *Tribulus terrestris* immersion solution led to lower survival rates in betta fish larvae. This reduced post-immersion survival rate is likely due to the larvae's vulnerability to environmental changes, transitioning from a neutral medium to an immersion medium containing *Tribulus terrestris* extract, which caused stress and led to mortality. And also the low survival value of betta fish larvae can also be caused by the saponin content contained in the fruit of the rujak polo plant, in some animals the saponin is toxic, especially in cold-blooded animals.

In some cases, saponins are traditionally used by fishermen to stun fish. In aquaculture, saponins are commonly employed to eliminate predator organisms in ponds without harming shrimp, due to shrimp's resistance to saponins, which is about 50 times stronger than that of fish. Saponins' effects on fish range from stunning to death, depending on the concentration used.(Puspito, 2018).

The low survival rate of betta fish larvae during maintenance is suspected to be due to the initial mortality of larvae at the start of the rearing period. This may be attributed to the larvae still being affected by saponin from the immersion phase. Additionally, the larvae are vulnerable to the media transition, moving from an immersion medium with *Tribulus terrestris* extract to one without it. This shift may have caused stress in

the larvae, leading to mortality. Most of this mortality occurred during the first three days of maintenance (when the larvae were between 7 and 9 days old), with no further deaths observed after the fourth day.

According to Sumantadinata and Carman (1995), cited in Iskandar (2014), the administration of hormones aims to disrupt hormonal balance in the blood, which, during the period of sex differentiation, determines whether an individual will develop as male or female (Iskandar et al., 2014). A high dose and prolonged immersion time may have a paradoxical effect, resulting not in an increase in male betta fish but rather an increase in the number of females.

The average percentage data of male betta fish showed an increasing trend in male percentage with *Tribulus terrestris* doses up to 2 mg/L, as observed in treatments P1 and P2. However, a decrease was seen with the 3 mg/L dose in treatment P3. This aligns with Zairin's (2002) statement that hormone administration and dosage have a tendency: too low a dose may lead to sterility and abnormalities, while too high a dose can cause fish mortality (M, 2002).

The high percentage of male betta fish is also likely due to the presence of the compound protodioscin in *Tribulus terrestris* (rujak polo). This compound stimulates luteinizing hormone (LH) and converts testosterone into dihydrotestosterone, which enhances libido, muscle development, and red blood cell production. Research in behavioral, hematological, biochemical, functional, and morphological fields on the acute, sub-chronic, and chronic toxicity of protodioscin suggests that this plant does not exhibit toxic effects. The effects of *Tribulus terrestris* extract, which contains protodioscin, can increase testosterone and dihydrotestosterone levels.

Studies on the hormonal effects of *Tribulus terrestris* on erectile dysfunction in primates, rabbits, and sterilized rats have shown that protodioscin can increase testosterone, dihydrotestosterone, and dehydroepiandrosterone sulfate (DHEA-S) levels. Additionally, hydroalcoholic extract from *Tribulus terrestris* can protect testicular tissue and sperm in cisplatin-induced rats, likely due to compounds like protodioscin, flavonoids, lignin, and alkaloids (Gaziansyah et al., 2019).

The use of *Tribulus terrestris* (rujak polo) fruit immersion as a masculinization agent for betta fish can be considered economical and environmentally friendly compared to using the hormone 17α-methyltestosterone. The use of a 2 mg/L *Tribulus terrestris* immersion solution resulted in a male betta fish percentage similar to that achieved with 17α-methyltestosterone at the same dosage. Therefore, immersion in *Tribulus terrestris* fruit at a dose of 2 mg/L for an 8-hour duration can be a viable alternative or substitute for 17α-methyltestosterone in the masculinization process of betta fish.

CONCLUSIONS AND RECOMMENDATIONS

Based on the results of Duncan's further tests, it can be concluded that the masculinization of Betta fish (*Betta splendens*) using rujak polo fruit (*Tribulus terrestris*) through the method of soaking Betta fish larvae at different doses has a significant effect on the percentage of male Betta fish, but an insignificant effect on the percentage of Betta fish (Betta

splendens). survival after immersion, and had no significant effect on the percentage of survival after rearing. The best dose concentration that can be used to masculinize betta fish is the P2 treatment, namely 2mg/L which produces the highest percentage of male betta fish, namely 84.49%.

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