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Impact of Natural Honey on Betta sp. strain Plakat Candy Masculinization

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ABSTRACT

Betta sp. is an ornamental fish that is favoured by all ornamental fish hobbyists in Indonesia. Various strains of this fish have been produced by many farmers. However, almost all of the most popular strains are male. This study aims to determine the effect of different lengths of natural honey soaking on the masculinisation of hickey fish. The method used in this study was a completely randomised design with 4 treatments and 3 replicates. The treatment used in this study is the length of immersion (22 - 26 hours) of each treatment compared to the control (without immersion) which was then maintained for 60 days. The results of this study showed that immersion using natural honey on larvae in a 5 ml/L honey solution had no significant effect on masculinisation of hippopotamus. However, the best masculinisation was found with 22 hours of immersion at 63.6% and the lowest without immersion at 49.6%.
Keywords: Natural honey, Betta sp, Immersion, Masculinisation

INTRODUCTION

Betta splendens is a popular type of freshwater ornamental fish and is widely favored by the public. However, generally, these ornamental fish fans prefer male fish to females because they are more attractive (Rachmawati, 2016). Male hickey fish have striking colors, long fins, a smaller body size than female hickey fish, and a relatively higher price. The demand for male hickeyfish has increased in recent years, so it is necessary to find a method that can mass-produce male offspring (Purwati et al., 2004). One technique to produce male monosex fish fry is through sex reversal, either masculinization or feminization (Muslim, 2010; Mariana, 2009). However, in this fish, the attraction is the male, so the right effort is masculinization, either with steroid hormones or other materials (Utomo, 2008).

Although they tend to be more expensive and difficult to obtain, synthetic steroid hormones are often used in sex reversal technology, such as 17α-methyltestosterone, estradiol17β, and aromatase inhibitors (Ukhroy, 2008). The use of synthetic hormones such as 17α-methyltestosterone can cause pollution, liver damage in test animals, and death (Djihad, 2015). So, to overcome this, there is a need for other natural ingredients that contain steroid hormones, are safe to use, easily available, have an affordable price, and are effective for use in sex reversal techniques. An alternative material that has the potential to replace synthetic hormones is honey. Honey is a safe and economical alternative that contains chrysin, which acts as an aromatase inhibitor (Haq, 2013). Some masculinization using honey has been carried out on guppy fish, namely a dose of 50 ml/L and a duration of immersion for 15 hours, producing 56.6% male guppies (Haq, 2013). Furthermore, masculinisation with a dose of 60 ml/L honey and immersion

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for 10 hours produced 56.68% male guppies (Utomo, 2008). The highest percentage of male guppies using the same honey and for 12 hours produced 76.66% male guppies (Priyono et al., 2013).

This indicates that natural honey immersion has a significant effect on guppy masculinisation. Immersion of niloticus fish embryos with a 24-hour immersion period produced the highest value of 72.3% (Indreswari, 2017). The percentage of male niloticus fish increases with the length of natural honey immersion (Zairin, 2002). The purpose of this study was to try to reduce and increase the immersion time from previous studies to find an effective dose in masculinising the larvae.

MATERIAL AND METHODS

Study site

This research was conducted from February to April 202, at Andalas Fish Seed Centre (BBI), Gorontalo City.

Research Design

This study used a Completely Randomised Design (CRD) with 5 treatments and 3 replications. The treatments used were no soaking (control) and natural honey soaking at a dose of 5 ml.L^{-1} for 22 hours (treatment B), 24 and 26 hours for treatments C and D, respectively.

Preparation

Aquariums used for spawning and immersion were 30 x 20 x 20 cm and 20 x 15 x 15 cm. All strerilised and labelled aquariums were filled with 3 litres of water for spawning and 4 litres for larval immersion.

Broodstock spawning

The betta fish broodstock used were selected based on the level of gonadal maturity in a 1:1 ratio. After mating, the female is removed so that she does not eat the eggs while the male is left to guard the eggs until they hatch. After the betta fry are three days old, characterised by the depletion of egg yolks, the male parent is carefully removed. At the age of four days the betta fry are able to swim on their own and it is at the age of 4 days that the betta are given the treatment.

Larva Immersion

Fry at the age of 4 days was used as masculinization sample on natural honey immersion with density of 10 fish/litre. (Irmasari, 2012; Priyono et al., 2013).

Larva Rearing

Larva that has been soaked were raised for 60 days with natural food in the form of *Artemia sp, Daphnia sp*. dan *Tubifex sp*. by *ad libitum*, in the morning and evening (Nurlina et al., 2016; Sugandy, 2001). Larvae aged 4-35 days were fed with *Artemia sp*, 36 days with *Tubifex sp*, 40-60 days with *Daphnia sp.* and *Tubifex sp.* (Siregar et al., 2018).

Identification of Fish Sex

Morphological sex identification to observe reproductive organs (Siregar et al., 2018). Male seeds have characteristics such as larger body size, longer fins, brighter colours and more agile, aggressive, no white spots in the anal area. (Zairin, 2002; Djihad, 2015).

Observed Parameters

Percentage of fish fry survival by comparing the number of live fish at the end of soaking with the initial number of fish refers to: (Efendi, 2002).

$$SR = \frac{Nt}{No} \; x \; 100\%$$

Keterangan:

SR : Survival Rate (%)

N_t : Number of live fish at the end of immersion/maintenance

N_o : Number of fish at the start of immersion

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Percentage of Male Betta Fish

Percentage of male betta fish by comparing the number of male fish with the number of live fish at the end of rearing (Zairin, 2002).

$$Male = \frac{number\ of\ male\ fish}{total\ live\ fish\ at\ the\ end\ of\ rearing}\ x\ 100\%$$

Data Analysis

All data obtained during the research were analyzed using variance analysis with a 95% confidence interval and continued with a homogeneity test using the f test using SPSS version 26 (Satriani et al., 2017).

RESULTS AND DISCUSSION

Survival Rate

The difference in soaking time of the 5 ml.L^{-1} honey dose did not affect the survival of the betta larvae. Honey contains many minerals such as K, Ca, Mg, Na, Fe, Mn, Cu, Zn, and Se, depending on location and geography (Lasic et al., 2018; Dutty et al., 2020). However, only a few minerals are able to enter the bloodstream so that they do not interfere with fish metabolism (Lubis, 2016). Immersion of betta larvae in wild honey resulted in higher survival rate and percentage of male fish (Waisapy et al., 2021). The survival rate of betta larvae during immersion was highest in treatment D at 78.33%, followed by treatment B at 75%, treatment C at 70% and the lowest in the control treatment at 63.33% (Picture 1). Instead, honey has been shown to have a positive impact on the immune system of fish. Honey-fed fish showed higher survival rates and less stress when exposed to pathogens. (Csernus et al., 2020).

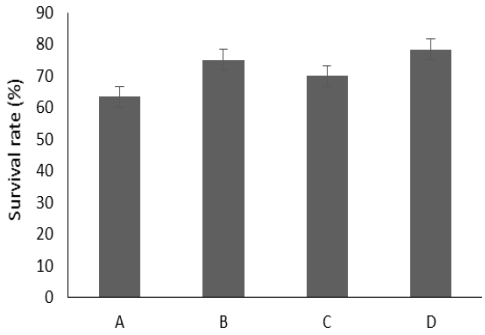

Figure 1. Survival rate of all treatments with a honey dose of 5ml.L^{-1} at different soaking times.

Masculinisation

The percentage of female betta fish in each treatment from highest to lowest was treatment A at 50.4%, B at 36.4%, C at 39.9% and D at 48.6%. Generally, hippopotamus broodstock in one spawning produces 40% males and 60% females. (Rachmawati *et al*., 2016). Sex is determined by the sex chromosomes and gonosomes at fertilisation (Rosmaidar *et al*., 2014). However, environmental and hormonal factors also influence larval sex. (Wu et al., 2021). Environmental factors are instrumental in directing phenotypes without altering genotypes, especially in the early days before sex differentials. (Rosmaidar *et al*., 2014). This study showed that immersion using natural honey on larvae had no significant effect ($p>0.05$) on the masculinisation of betta fish. However, the highest percentage of male betta fry was obtained in 22 hours of immersion at 63.6%. This is due to the lack of chrysin content entering the fish body due to the less than optimal soaking time. (Yuzrizal *et al*., 2014).

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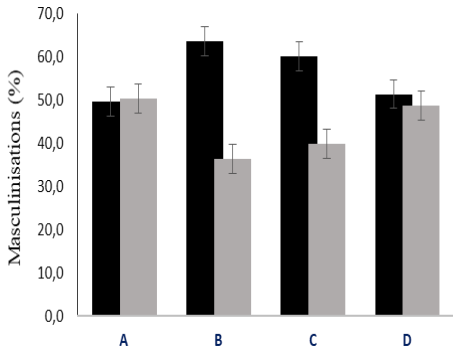

Figure 1. Masculinization of all treatments with a honey dose of 5ml.L^{-1} at different soaking times.

The hormones dissolved in the soaking medium enter the body along with the liquid, are passed on to the central nervous system and command the pituitary gland to secrete hormones. (Yuwanny, 2000). The hormone is then carried by the blood and transported to the gonads as an indication to start the formation of male or female gonads. (Arfah *et al*., 2013). One of the disadvantages of the immersion method is that the hormones are too far away to reach the hypothalamus (Zairin, 2002). In addition, the success of sex reassignment is influenced by the length of time of exposure to the hormone (Hidayani, 2016). Hormone soaking that is too short causes fish to become sterile, abnormalities. Meanwhile, giving the hormone for too long can cause fish death. (Zairin, 2002). Larvae experiencing low aromatase activity lead to the formation of testes and high aromatase activity forms ovaries (Mils et al., 2014).

Water Quality

The water temperature of this study ranged from 26.1 – 29.3 ^{0}C, pH of 6.7 – 8.1 and dissolved oxygen of 2.6 – 5.2 mg.L^{-1}, these conditions are still suitable for the survival and growth of *Betta sp. Betta sp.*, tends to like relatively warm water temperatures, which range from 24 - 30 ^{0}C (Biokani *et al*., 2014). However, the optimal temperature for fish farming depends on the species. For example, Betta rubra, which is native to Aceh and northern Sumatra, lives optimally at a temperature of 28 ^{0}C (Nur et al., 2020). Meanwhile, the optimum water temperature for Betta splenden embryo development is around 26 - 29 °C (Alsiawan dan Hasby, 2022). Furthermore, the optimal water pH for *Betta sp*. cultivation is 6 – 8 (Nugroho et al., 2016: Febriyanti et al., 2020). Fluctuations in dissolved oxygen are related to water temperature, if the water temperature increases the dissolved oxygen content decreases. This is because the use of dissolved oxygen in water increases with increasing water temperature because the metabolic rate of fish increases and vice versa. (Baxa et al., 2021).

CONCLUSIONS

The effect of honey dosage of 5 mg.L^{-1} with different immersion times did not significantly affect the masculinisation of hippopotamus. In addition, honey immersion did not affect the survival rate.

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