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THE EFFECT OF DIFFERENT DOSES OF MULTIFLORA HONEY ON MALE BETTA FISH (*Betta splendens*) SEED PRODUCTION

Pengaruh Dosis Madu Multiflora Yang Berbeda Terhadap Produksi Benih Ikan Cupang (*Betta Splendens*) Jantan

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ABSTRACK

Multiflora honey contains potassium and flavonoids that play a role in changing fish sexuality from female to male. One factor that influences the success of sex direction is the dosage. This study aims to obtain the right dosage of multiflora honey in the formation of male sex (masculinization). This study used a Completely Randomized Design (CRD) with 4 treatments and 3 replications. Observation of male sex was carried out after 60 days of maintenance by observing the urogenital organs and 70 days of maintenance with gonad histology using HE staining. The results showed that the highest percentage of males was obtained at a dose of 4 mg/L.

Keywords: flavonoids, masculinization, potassium

ABSTRAK

Madu multiflora memiliki kandungan kalium dan flavonoid yang memiliki peran untuk merubah seksualitas ikan dari betina ke jantan. Satu faktor yang berpengaruhi dalam keberhasilan pengarahan jenis kelamin adalah jumlah dosisnya. Penelitian ini bertujuan untuk mendapatkan dosis madu multiflora yang tepat dalam pembentukan kelamin jantan (maskulinisasi). Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) dengan 4 perlakuan dan 3 ulangan. Pengamatan kelamin jantan dilakukan setelah 60 hari pemeliharaan dengan mengamati organ urogenital dan 70 hari pemeliharaan dengan histologi gonad menggunakan pewarnaan HE. Hasil penelitian menunjukan bahwa persentase jantan tertinggi diperoleh pada dosis 4 mg/L.

Kata kunci: flavanoids, maskulinisasi, kalium

INTRODUCTION

The production of male seeds plays an important role in betta fish cultivation efforts. In an effort to increase the production of male betta fish seeds, various treatments need to be considered. Masculinization is one way to increase the number of male fish through sex

differentiation. The success of directing sex differentiation of betta fish is influenced by the type of material, dosage, duration of treatment, application method and fish development phase.

In the first stage of the study, namely soaking betta fish eggs with three types of honey, the best results were in the treatment using multiflora honey. Male betta fish seeds produced by soaking in 5 ml/L Multiflora honey gave a male betta fish seed yield of 59.59%. Based on these results, research is still needed on different doses of Multiflora honey to produce the most optimal male betta fish seed production.

This second phase of research aims to determine the effect of different doses of multiflora honey on the production of male betta seeds. The results of this study are expected to provide input on the potential of multiflora honey as a natural ingredient that is useful in increasing the production of male betta fish seeds. This research is expected to be the basis for developing a more optimal and sustainable betta fish maintenance strategy.

This research is expected to not only have important scientific value, but also have practical impacts that can provide direct benefits for betta fish cultivators and business actors. The aim of this study was to determine the optimal dose of multiflora honey to increase the production of male betta fish seeds.

RESEARCH METHODS

Time and Location of Research

The research was conducted from March to May 2024 at Dwibetta Farm Serang Banten. Histology testing was carried out at the Aquatic Organism Health Laboratory, Faculty of Fisheries and Marine Sciences, IPB, Bogor.

Research Tools and Materials

The tools used in this study were 25x25x25 cm aquarium, LP100 Aerator, hose and aeration stone, bucket, small basin, sponge and small hose, petri dish, 500 mL volume sample bottle, 500 mL volume measuring cup, pH meter, DO meter, thermometer. The materials used in this study were multiflora honey, betta fish eggs, natural food, namely water fleas *(Moina* sp*)*, mosquito larvae and silk worms *(Tubifex)*.

Research Design

The experimental design used in this phase II study was a Completely Randomized Design (CRD) with 4 treatments and 3 replications. The treatments given were: A. without honey immersion (control), B. Soaking eggs with Multiflora honey at a dose of 4 ml/L of water for 10 hours, C. Soaking eggs with Multiflora honey at a dose of 5 ml/L of water for 10 hours, D. Soaking eggs with Multiflora honey at a dose of 6 ml/L of water for 10 hours (Herjayanto *et al*., 2023).

Container Preparation

The cultivation container is an aquarium measuring 25x25x25 cm as many as 12 pieces. The aquarium is cleaned, dried and filled with water as high as 20 cm (water volume 12.5 liters). The aquarium installation is aerated to keep the dissolved oxygen content in the water stable. The aquarium is arranged on a rack with a mark according to each treatment. Ketapang leaves are put into the aquarium which functions to maintain the quality of the maintenance water.

Test Fish

Ikan The test fish used were betta fish eggs that were 15 hours old after fertilization (Cindelaras et *al*., 2015). Betta fish eggs were placed in each aquarium as many as 30 fish (Herjayanto *et al*., 2023).

Betta Fish Egg Maintenance

Soaking is done by dissolving Multiflora honey according to the treatment. Then the betta fish eggs are soaked for 10 hours. The aquarium is fitted with aeration and the larvae are maintained until the betta fish fry can be distinguished by male and female morphology, namely at the age of 60 days (Ferdian *et al*., 2017). Feeding of fish larvae is done ad libitum where according to Perkasa & Hendry (2002) that ad libitum feeding aims to provide excess feed so that there is no shortage of feed so that death can be prevented. Larvae are fed after more than four days, in the form of water fleas *(Moina sp)*, silk worms *(Tubifex sp)* and mosquito larvae, given in the morning and evening (To'bungan, 2016). Feces siphoning is done every day. Water changes are carried out every five days as much as 30%.

Test Parameter Observation

Egg Hatch Percentage

The calculation to determine the hatching rate of betta fish eggs uses the following formula (Effendie, 2002):

$$\text{HR}\ (\%) = \frac{\text{Number of Fish Eggs That Hatch}}{\text{Number of Fertilized Fish Eggs}} x\ 100$$

Survival Percentage

The calculation to determine the level of fish survival uses the following formula (Effendie, 2002):

$$\text{SR}\ (\%) = \frac{\text{Number of Live Fish at End of Maintenance}}{\text{Number of Live Fish at End of Maintenance}} x\ 100$$

Gender Observation

Observation of gender is done morphologically including observation of body size and shape, tail fin shape and color. Atmadjaja & Sitanggang (2010) stated that identification of betta fish gender based on morphology is an easy and economical way because it does not require killing the animal. Male fish have dorsal fins and tail fins with longer sizes than females, the body size of males is smaller but more elongated than females.

In terms of color, male fish are more attractive and beautiful. In female fish, the stomach is generally fatter, and often the shadow of the eggs can be seen. The color of this type of fish is greatly influenced by several factors, namely gender, gonad maturity, genetics and geographic factors (Kottelat et al., 1993). Morphological observations were carried out after 60 days of maintenance. In addition to morphological observations, histological observations were also carried out using the hematoxylin-eosin (HE) staining method with a random sample of 4 fish/treatment. Histological observations of HE staining were carried out after 65 days of maintenance. This was done to see the consistency of sex morphologically after being tested with gonad histology using HE staining.

Male Gender Percentage

The main parameter that is an indicator of the success of male betta fish seed production is the percentage of male sex. The calculation of the percentage of male sex uses the following formula (Effendie, 2002):

$$\text{Male Gender Percentage (\%)} = \frac{\text{Number of Male Fish}}{\text{Total Number of Fish}} \text{ X } 100$$

Water Quality Monitoring

Water quality parameters are measured using a pH meter to measure pH, a thermometer to measure temperature, and a DO meter to measure dissolved oxygen content in water. Water quality measurements are carried out every day during maintenance. Water quality measurements are in the morning and evening.

Data analysis

Data on the percentage of egg hatching, percentage of male sex and percentage of survival obtained were analyzed using software; Microsoft Excel 2019 and SPSS Version 29. Meanwhile, water quality data was analyzed descriptively.

RESULTS

Egg Hatch Percentage

The results of calculating the percentage of egg hatching at different honey immersion doses are presented in Figure 1.

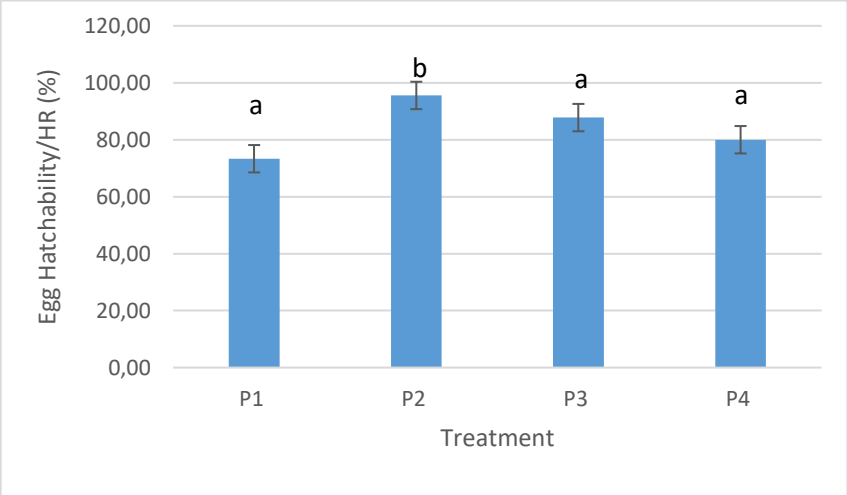

Figure 1. Percentage of Betta Fish Egg Hatching with Different Honey Dose Treatments.

Survival Percentage

The calculation of survival percentage with different honey doses is presented in Figure 2.

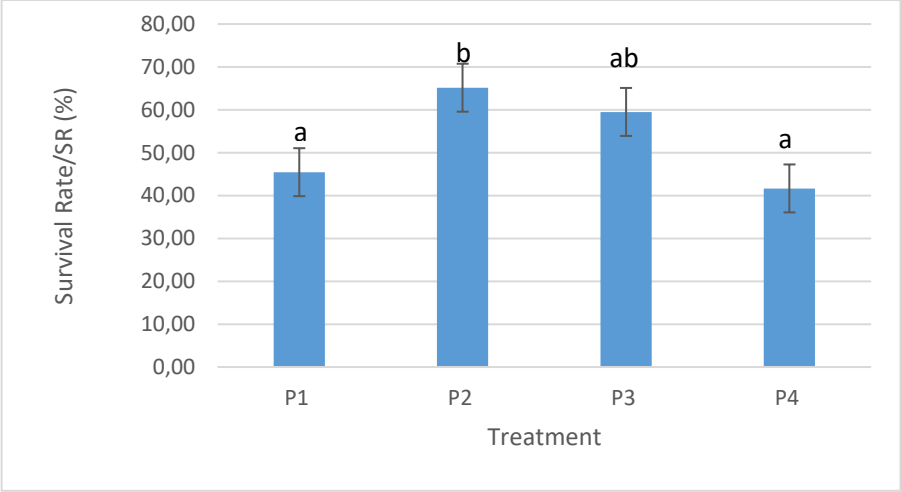

Figure 2. Percentage of Survival Rate of Betta Fish Seeds with Different Honey Dose Treatments.

Gender Observation

Observations of the morphological sex of betta fish are presented in Figure 3.

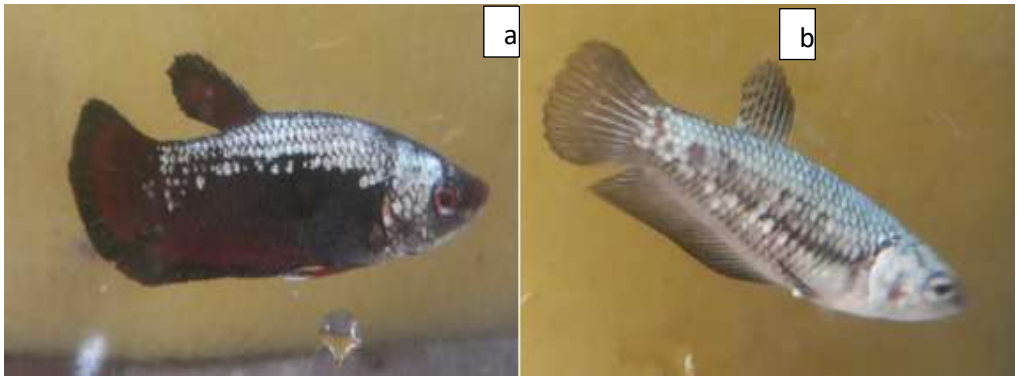

Figure 3. Morphology of betta fish
60 days old [a] Male [b] Female

Histological observations of the sex of betta fish are presented in Figure 4.

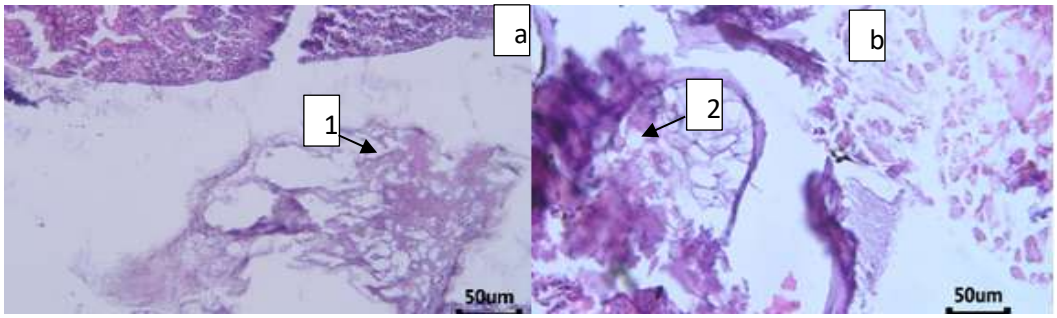

Figure 4. Types of betta fish seen from the histology of HE staining at 65 days old.
[a]Male [b]Female [1]Sperm Cell [2]Egg Cell [2]

Male Gender Percentage

The results of calculating the percentage of male sex with different honey doses are presented in Figure 5.

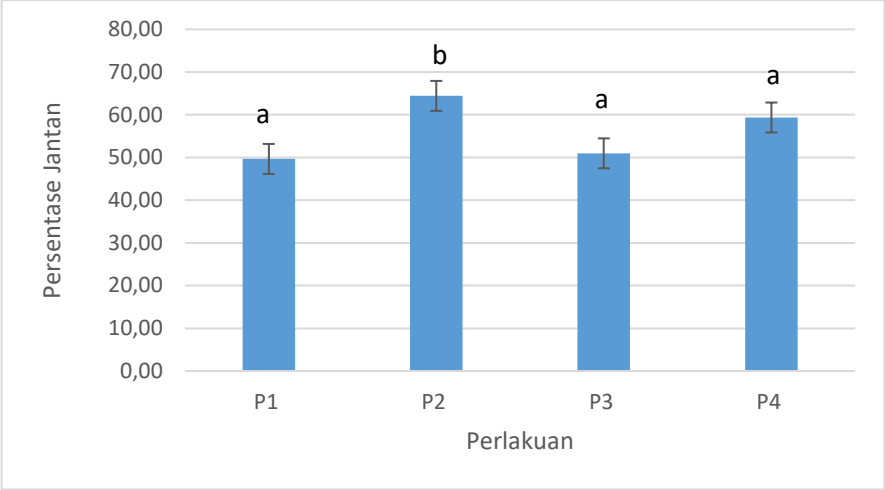

Figure 5. Percentage of Male Betta Fish Gender with Different Dosage Treatments.

Water Quality Monitoring

Hasil The results of water quality observations consisting of temperature, pH and dissolved oxygen measurements are presented in Table 1.

Results of Water Quality Measurements with Different Doses of Multiflora Honey.

Parameter	Mark	Tolerance Range
Temperature (ºC)	26 - 30	22-30 (Hasyim dkk., 2018)
pH	7,0 - 8,1	6-8 (Panjaitan dkk., 2016)
DO (mg/L)	3,1 - 6,1	3– 6,8 (Malik dkk., 2019)

DISCUSSION

Egg Hatch Percentage

Based on ANOVA calculations, the results showed that the percentage of egg hatching in treatment 2, namely immersion in a 4 ml Multiflora honey solution (95.56 ± 1.92b); was significantly different from treatment 1, namely without honey immersion (73.33 ± 3.33a); significantly different from treatment 3, namely immersion in a 5 ml Multiflora honey solution (87.78 ± 1.92a) and significantly different from treatment 4, namely immersion in a 6 ml Multiflora honey solution (80.00 ± 3.33a).

Research on the use of multiflora honey as a soaking treatment for betta fish eggs (Betta splendens) showed significant results in increasing the percentage of egg hatching. In this study, betta fish eggs soaked in a multiflora honey solution with a dose of 4 mg/L of water showed a higher hatching rate compared to the control treatment, 5 mg/L and 6 mg/L doses. This shows that multiflora honey can act as an agent to increase the hatching power of betta fish eggs. Multiflora honey comes from many types of wild flowers that bloom in the wild.

The increase in hatching percentage is thought to come from the natural antibacterial content in honey which is able to inhibit the growth of pathogenic bacteria which are often the cause of hatching failure (Almasaudi, 2021). In addition, multiflora honey contains various

enzymes and amino acids that can increase the metabolism of betta fish embryos, accelerating the development and hatching process (Belay *et al*., 2017).

In addition to its antibacterial benefits, multiflora honey also has an osmotic effect that can help in the process of water absorption by eggs, which is essential for the development of fish embryos. In several studies, eggs soaked in honey solution showed more elastic and less easily damaged eggshells, thus increasing the chances of hatching. This osmotic effect also helps reduce the risk of dehydration in eggs which is often a problem in the hatching process in suboptimal environmental conditions (Almasaudi, 2021).

Survival Percentage

Based on ANOVA calculations, the results obtained were that the percentage of survival of treatment 2, namely immersion in a 4 ml dose of Multiflora honey solution (65.15 ± 2.91b); was significantly different from treatment 1, namely without honey immersion (45.45 ± 3.96a); significantly different from treatment 4, namely immersion in a 5 ml dose of Multiflora honey solution (41.65 ± 3.64a); but not significantly different from treatment 3, namely immersion in a 6 ml dose of Multiflora honey solution (59.50 ± 1.94ab).

In this study, betta fish eggs soaked in multiflora honey solution with a dose of 4 mg/L showed higher survival compared to the control treatment, 5 mg/L dose treatment and 6 mg/L dose. This shows that multiflora honey can function as an agent to increase the survival of betta fish, especially in the early stages of their life.

The content of compounds such as flavonoids, phenolic acids, and enzymes in honey can inhibit the growth of pathogenic bacteria and other harmful microorganisms that often cause disease in betta fish. By reducing the risk of bacterial infection, multiflora honey helps create a healthier environment for betta fish, thereby increasing their chances of survival and growth (Almasaudi, 2021).

Multiflora honey is also rich in nutrients that can strengthen the immune system of betta fish. The content of vitamins, minerals, and enzymes in honey plays an important role in increasing the metabolism and endurance of fish. This study shows that betta fish that are given multiflora honey soaking treatment have higher energy levels and better growth. Additional nutrients from honey help speed up the wound healing process, increase cell growth, and repair damaged tissue, so that betta fish are more resistant to environmental stress and disease (Belay *et al*., 2017).

Gender Observation

Based on Figure 3.3, morphologically, there is a difference between male betta fish which have more attractive colors compared to female betta fish. Identification of the sex of betta fish based on morphology is an easy and economical way because it does not require killing animals (Finanta et al., 2020). Observation of the sex of betta fish morphologically is ideal because it has clear sexual dimorphism between males and females (Malik *et al*., 2019; Zairin, 2002).

Histologically using HE staining, it can be seen that the male gonad is purple while the female gonad is pink. Male phenotype betta fish have gonad tissue in the form of sperm primordia while female phenotype betta fish have gonad tissue in the form of egg primordia. The ovaries contain egg granules, in the form of a transparent gel like soft gonad tissue, occupying the posterior part of the body cavity. While the contents of the testes, sperm cells, are very smooth, and attached to the body cavity (Zairin, 2002). Identification of sex morphologically in the study obtained results that were consistent with the histological results of the HE staining carried out.

Male Gender Percentage

Based on ANOVA calculations, the results obtained were that the percentage of male sex in treatment 2, namely immersion in a 4 ml Multiflora honey solution (64.42 ± 7.24b); was not significantly different from treatment 1, namely without honey immersion (49.67 ± 5.06a); was not significantly different from treatment 3, namely immersion in a 5 ml Multiflora honey solution (50.97 ± 4.86a) and was not significantly different from treatment 4, namely immersion in a 6 ml Multiflora solution (59.31 ± 3.31a).

The nutritional content in multiflora honey, such as potassium and flavonoids, can interact with the endocrine system of betta fish. These nutrients may play a role in increasing the production of androgen hormones, which are the main hormones in the development of male characteristics. This can lead to an increase in the number of male fish compared to females.

Water Quality Monitoring

Based on water quality data, the survival of betta fish (Betta splendens) from the egg maintenance stage to adulthood can be analyzed through three main parameters: temperature, pH, and dissolved oxygen (DO) content.

Water temperature is a crucial factor in the development of eggs and the growth of betta fish. The ideal temperature range for betta fish is between 26-30°C, which is in accordance with the tolerance range of 22-30°C according to Hasyim *et al*., (2018). Low temperatures can slow down metabolism and egg development, while temperatures that are too high can cause stress and increase the risk of disease. In the temperature range of 26-30°C, betta fish can develop optimally because this temperature supports efficient physiological processes and minimizes the risk of thermal stress.

pH Water pH also affects the survival of betta fish. The ideal pH range for betta fish is between 7.0-8.1, which is within the tolerance limit of 6-8 according to Panjaitan *et al*., (2016). A pH that is too low (acidic) or too high (alkaline) can damage the gills and skin of the fish, as well as disrupt the metabolic process. At pH 7.0-8.1, the water is neutral enough to support the biological activities of betta fish, including efficient absorption of nutrients and oxygen. Therefore, maintaining the pH within this range is essential to ensure healthy growth from the egg stage to adult fish.

Dissolved oxygen (DO) content is another important parameter. The observed DO range was 3.1-6.1 mg/L, which is in accordance with the tolerance of 3-6.8 mg/L according to Malik *et al*., (2019). Sufficient DO is necessary for fish respiration, especially at the egg and larval stages which are very sensitive to oxygen deficiency. DO below 3 mg/L can cause hypoxia, which can kill eggs and inhibit larval growth. In the range of 3.1-6.1 mg/L, betta fish get enough oxygen to support their essential physiological functions and daily activities, thus ensuring good survival from the early stages to adulthood.

Thus, based on the temperature, pH, and DO parameters in accordance with the referenced research, the observed water quality supports the survival of betta fish from egg maintenance to adulthood.

CONCLUSION

The treatment of soaking betta fish eggs with different doses for 10 hours in Multiflora honey solution, gave significantly different results compared to other treatments. Multiflora honey with a dose of 4 mg/L had the greatest effect on increasing the production of male betta fish seeds, which was 64.42%. Further testing is needed using other honey dose variations for soaking betta fish eggs that have the greatest effect on increasing the production of male betta fish seeds.

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