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Masculinization of Betta Fish (*Betta* sp) Using extracts of Cow Testicles Trough Immersion with Different Doses of Immersion

Maskulinisasi Ikan Cupang (*Betta* sp.) Menggunakan Ekstrak Testis Sapi Melalui Perendaman Dengan Dosis Perendaman Yang Berbeda

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ABSTRACT

Ornamental fish is a fish that is much favored by the public because it has a beautiful color. The demand for ornamental fish is increasing every year in both the domestic and international markets, based on data from the Ministry of Maritime Affairs and Fisheries, in 2018 the export value of ornamental fish reached USD 32.23 million and by 2020 Indonesia is targeting to produce 1.8 billion ornamental fish. One of the ornamental fish that is much favored by the public is betta fish (*Betta* sp). From an economic point of view, the cultivation of male betta fish is more profitable because of the good color and body morphology. So that male betta fish are more popular than female fish. Based on the results of the study, it was found that the highest male sex presentation of betta fish was in treatment P4 with a total of 91.11%, treatment P3 78.89%, treatment P5 with a total of 73.33%, treatment P2 58.89%, and P1 with a total of 35.56% male.

ABSTRAK

Ikan hias merupakan ikan yang banyak digemari oleh masyarakat karena memiliki warna yang indah. Permintaan ikan hias semakin meningkat setiap tahunnya baik di pasar domestik maupun international, berdasarkan data dari KKP pada tahun 2018 nilai ekspor ikan hias mencapai USD 32,23 juta dan pada tahun 2020 indonesia menargetkan akan memproduksi iakn hias sebanyak 1,8 milyar ekor. Salah satu ikan hias yang banyak digemari oleh masyarakat adalah ikan cupang (*Betta* sp). Dari segi ekonomi, budidaya ikan cupang jantan lebih menguntungkan karena warna dan morfologi tubuhnya yang bagus. Sehingga ikan cupang jantan lebih digemari dibandingkan ikan betina. Berdasarkan hasil penelitian di dapatkan hasil bahwa presentasi kelamin jantan ikan cupang tertinggi terdapat pada perlakuan P4 dengan jumlah sebesar 91.11%, perlakuan P3 78,89%, perlakuan P5 dengan jumlah 73,33%, perlakuan P2 58.89%, dan P1 dengan jumlah 35.56% jantan.

Kata Kunci	*Segi ekonomi, ikan cupang jantan*
Keywords	*In terms of economy, male betta fish*
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INTRODUCTION

Ornamental fish are fish that are popular with people because they have beautiful colors. The demand for ornamental fish is increasing every year in both domestic and international markets. Based on data from the Ministry of Maritime Affairs and Fisheries, in 2018 the export value of ornamental fish reached USD 32.23 million and in 2020 Indonesia targets to produce 1.8 billion ornamental fish. One of the ornamental fish that is very popular with people is the Betta fish (*Betta* sp). From an economic perspective, cultivating male Betta fish is more profitable because of their good color and body morphology. According to Arfah et al., (2013) said that male Betta fish have several advantages over female fish, including high price, more aesthetic morphology and color.

Natural spawning of Betta fish has the same percentage of male and female fish. Based on the results of research conducted by Saputra (2018), natural spawning produces 40.72% male fish. The demand for male betta fish is increasing so it is necessary to find a method that can produce a large number of male offspring or a higher percentage of males compared to females. One way that can be done to produce male genitalia is by masculinizing.

Masculinization is a method of directing the male genitalia from the female. According to Sulasy (2018), the masculinization technique is a sex reversal technology that directs sexual differentiation to become male and is carried out when the fish's gonads have not yet differentiated. In the masculinization process, synthetic steroid hormones such as 17a-methyltestosterone are generally used, but the use of this material is starting to be limited because it has the weakness of being difficult to decompose, can be carcinogenic and pollute the environment and can even cause side effects in farmed fish. Apart from that, this material is relatively expensive and difficult to obtain (Riani et al, 2010). Therefore, it is necessary to look for ingredients that have the same content at a relatively cheap price and are easy to obtain and are safer and more environmentally friendly in the sex reversal process.

Cow testicles are a natural ingredient that is often used in the process of directing fish sex. Based on various research results that have been carried out using cow testicle flour, it is stated that there is a very high natural testosterone hormone in cow testicles (Muslim, 2011). Testosterone hormone from cow testicle flour is given in the early larval phase when sexual differentiation has not yet occurred. Apart from that, factors that can influence the success of masculinization are the dose and duration of administration. According to Purwati (2004) that high doses require a shorter time, whereas low doses require a long time. Based on the results of research conducted by Hidayani et al. (2016) that the use of cow testicle flour showed the best results at a dose of 60 mg/L with a percentage of male fish of 88.55%. The results of other research on catfish using cow testicle extract showed that a dose of 120 mg/L was 73.33% (Ramadhan, 2021). Therefore, it is necessary to carry out research using testicular flour extract soaked in water directly so that it can produce high male genitalia.

METHODS

This research will be carried out for 45 days at the Reproduction Laboratory of the Aquaculture Study Program, Faculty of Agriculture, Mataram University, aquariums, stationery, sieves, cameras, knives, fresh water, betta fish, water fleas, cow testicles, ethanol. This research used an experimental method by conducting experiments on administering cow testicle extract with different immersion doses. The design used in the research was a Completely Randomized Design (CRD) which consisted of 5 treatments and 3 repetitions to produce 15 experimental units. The treatment in this research is as follows:

Treatment

P1 No cow testicle extract
P2 Soaking 0.06 mg/L water
P3 Soaking 0.08 mg/L water
P4 Soaking 0.10 mg/L water
P5 Soaking 0.12 mg/L water

The research procedures for 45 days are as follows:

Making Cow Testicle Flour and Extract

Making cow testes in accordance with research (Irmasari et al., 2012) Iskandar (2010). First, the cow testicles are cut in half, then chopped into small pieces, then dried for three days, after drying, the testicles are blended until they become powder and the powder is sifted to produce a fine powder, flour ready for use. The next stage, the cow testicle flour extract is soaked in 96% ethanol solvent ratio with cow testicle flour extract, namely (1 kg of cow testicle flour extract: 3 liters of ethanol) for 3 days

Container Preparation

The container used in this research is an aquarium measuring 30cm X 30cm X 35 cm. First wash the aquarium using detergent and then dry the aquarium. Next, the aquarium was arranged according to the experimental design that had been prepared previously and filled 10 L times with water.

Parent Spawning

There are 4 pairs of parent Betta fish ready to be spawned, mated in pairs in an aquarium. Female and male Betta fish parents were selected randomly. After mating, the female parent fish is removed so that the eggs are not eaten, while the male parent is left in the aquarium with the aim of guarding the eggs until they hatch. Betta fish larvae that have run out of egg yolk last for three days when the male parent is removed. At the age of four days, bettas can swim on their own and are ready to be used as test material (Herjayanto et al., 2016).

Soaking Test Animals

The seeds tested were betta fish larvae from spawning. The larvae used were four days old, the larvae were soaked using cow testicles according to the treatment used. The seed stocking density used was 30 individuals. Making soaking containers is done by dissolving cow testicle flour with 40 mg/L of water then putting each into a jar filled with one liter of water. The solution is given an aerator so that the testicle flour dissolves in water. The aquarium used was 12 larvae soaking in a solution of cow testicle flour with different soaking times (Ali, 2014).

Test Fish Keeping

After soaking, the Betta fish seeds are kept in their respective aquariums. During the rearing, the Betta fish seeds are fed with water fleas three times a day, namely in the morning, afternoon and evening. Feeding is done ad libitum with food that is available continuously. After that, siphoning is carried out so that the water quality remains stable,

siphoning is done every day and water changes are carried out as much as 10% of the water volume. Caring for young bettas until they are 45 days old or until they can be identified as male or female (Mualim, 2011).

Test Fish Gender Check

Checking the sex of betta fish can be done when the fry enter the juvenile period (body length 2.5 cm), by externally examining the body of the young betta fish. Observations of male fish can be characterized by brighter colors, longer fins and larger body size and when viewed from the back they look slender. Meanwhile, female fish are characterized by white spots around the anus, less bright colors and shorter fins. Apart from that, female fish are less aggressive compared to male fish. (Pebriansyah, 2016).

The parameters in this research are as follows:

Gender Proportion

Sex proportion aims to see the results of the sex comparison of male and female fish by looking at the physical characteristics of the fish. The presentation of individual males can be calculated using the formula Hidayani et al., (2016):

$$\% \text{ ikan jantan} = \frac{jumlah\ jantan}{jumlah\ total\ ikan} x\ 100\%$$

Survival Rate

Survival is the ratio of the number of fish alive at the end of rearing to the number of fish stocked. The survival rate can be calculated using the formula Hidayani et al., (2016):

$$\text{Kelangsungan Hidup} = \frac{jumlah\ ikan\ hidup}{jumlah\ total\ ikan} x\ 100\%$$

Water Quality Parameters

Water quality parameters aim to determine the effect of cow testicles on the rearing environment. The water quality observed is do, pH and temperature.

Data Analysis

The data obtained were analyzed using analysis of variance (ANOVA) and if a significant effect was found, Duncan's further test was carried out to determine the best soaking time. (Hidayani *et al*., 2016).

RESULT AND DISCUSSION

Gender Proportion

Based on the percentage of male sex in betta fish, it is expressed in percent and calculated using the equation, namely the number of male fish at the end of rearing divided by the number of live fish at the end of rearing. The percentage of maleization of Betta fish is presented in the figure 1.

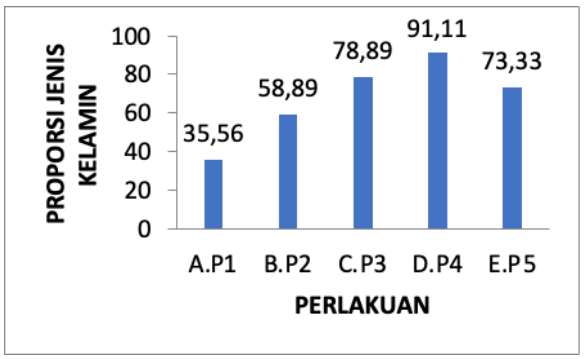


Figure 1. Betta Fish Sex Proportion Chart

Based on the research results, it was found that the highest male genital presentation of Betta fish was in treatment P4 with a total of 91.11% followed by treatment P5 with a total of 73.33%, treatment P3 78.89% to treatment P2 58.89% male and followed by male genital presentation. the lowest was in treatment P1 with a total of 35.56%. Based on the results of the analysis of variance (ANOVA) test, it showed that the length of time the betta fish larvae were soaked in cow testicle extract with different soaking times had an effect on the percentage of males produced ($P<0.05$), so a test was carried out to determine the differences between each treatment.

Based on the results of further tests, Duncan showed that the length of soaking time affected the percentage of males produced. The percentage of male yield in treatment P1 was not significantly different from treatments P2, P3 and P5 but was significantly different from treatment P4, while treatment P4 was not significantly different from treatment P3 and treatment P5. Previous research (Arrasidin et al., 2017), showed that the highest maleization results were found in the treatment using a dose of 0.10 mg/L of water with a soaking time of 24 hours resulting in a percentage of males of 91.11%. The high percentage of male betta fish in this study is thought to be influenced by the testosterone hormone contained in cow testicle extract because the testosterone hormone is an element that will maintain the continuity of the spermatogenesis process and is the main mediator of spermiation, so this study has the highest percentage of males, with the highest percentage of males. obtained at a dose of 0.10 mg/L water with a soaking time of 24 hours with a male percentage of 91.11%.

The high percentage of males in the P4 treatment was due to the correct immersion time in the test fish which was in accordance with the ability of testosterone to inhibit the action of the aromatase enzyme and the low percentage of males in the P2 treatment was due to the decreased palatability of testosterone in inhibiting the action of the aromatase enzyme in the test animals. The high or low percentage of male fish produced is influenced by the fish's ability to absorb and maintain the palatability of testosterone in the test fish's body through the suitability and carrying capacity of the environment. Tetoteron functions as an aromatase inhibitor or inhibitor of the work of the aromatase enzyme, which has the maximum time to inhibit the work of the estrogen hormone in the fish's body. After that, the presence of testosterone will decrease and run out over time. However, it is not yet known precisely how long the maximum time for tetoterone to exist to inhibit the work of the aromatase enzyme in the body of biota and what things affect the palatability of testosterone so that its effect on biota can be as maximal as possible.

The percentage of males produced is influenced by the length of soaking time, correct dosage, appropriate soaking procedure and environmental temperature (Hartami et al., 2013). A good soaking time can influence the production of a good number of males as well. If the soaking time is too long, the presence of hormones in the fish's body for genital direction will be saturated so that it will reduce the palatability of the ingredients used, whereas if the soaking time is too short, the working power of these hormones will not be at the highest point for the influence of genital direction in the test animals so that the function These hormones are not distributed properly. This statement is in accordance with the statement of Pifferre and Donaldson (1989) in Fariz (2014) in Arrasyidin et al. (2017) high doses and soaking for too long will also be paradoxical, namely the result obtained is not an increase in the number of male fish, but an increase in the number of female fish.

Apart from the correct length of soaking time, environmental temperature also influences the number of males produced. If the temperature at the time of rearing tends

to be cold, the resulting fish will have their genitals become female and vice versa, if the temperature tends to be constant at high temperatures, the genitals in the fish will tend to produce male fish. Arfah et al. (2013) in Arrasyidin et al. (2017) stated that if the temperature is relatively higher, it will affect the development of the fish's gonads into males, while relatively lower temperature conditions will affect the gonads into females.

Betta Fish Survival Rate

Survival rate is a comparison value between the number of living organisms at the end of rearing and the number of organisms at stocking, expressed as a percentage. Fish were kept for 45 days and the survival rate was calculated at the end of rearing. The survival rate of betta fish in this study is presented in the figure 2.

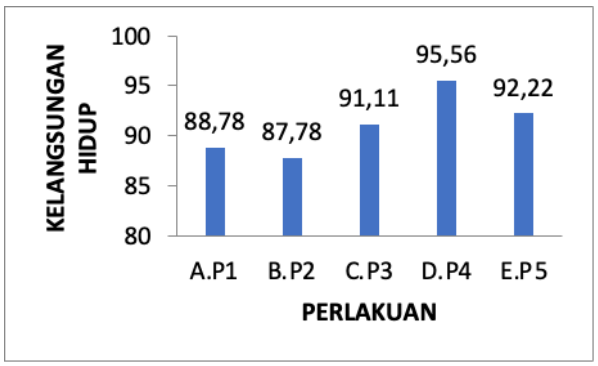


Figure 2. Betta Fish Survival Rate Graph

Survival is expressed in percent where the number of live fish is divided by the number of fish kept and multiplied by 100%, the survival rate is obtained (treatment P1 87.78%, treatment P287.78%, treatment P3 with survival 91.11% Treatment P4 95.56% and the survival rate of betta fish in the treatment P592.22%). Using a long soaking time for honey also has a maximum time to be used so that the treatment used is not wasted, so using 24 hours is a good time for the maleization treatment using treatment.

Based on the results of the analysis of variance (ANOVA) test, it showed that the length of time the betta fish larvae were soaked in cow testicle extract for different times had no effect on the survival rate of the betta fish produced ($P<0.05$).

The use of testissapi extract in the maleization process on the survival of betta fish did not show significantly different results between treatments P1, P2, P3, P4 and P5. The survival results showed that P4 treatment with a dose of 0.10 mg/L of soaked bovine testicle extract in water for 24 hours resulted in no different survival. This is because the dose and length of soaking time used are still within normal levels so it does not affect the survival of the betta fish being kept. High number of larval survival rates.

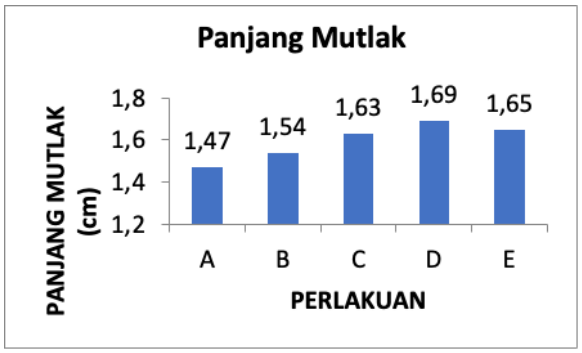
On the other hand, if the dosage and soaking time are not used correctly, the larval survival rate will decrease. Soaking the larvae for a long time will stress the larvae and make the fish sterile and the larvae will die (Zairin, 2002 in Hartami et al., 2013). Using a long soaking time will also trigger the growth of fungus in the rearing container which will cause the fish larvae to be unable to move so that within a short period of time the larvae will die.

The survival rate of fish being kept is also influenced by the type of feed and the availability of feed that is even used during rearing. The appropriate type of feed will increase the survival of the biota being kept because it is easy for the fish to swallow and process the feed for growth. A limiting factor that also influences the survival of fish is the availability of food which is periodically found in the media where the fish live so that the fish feed is sufficient and the fish can remain alive. The high number of survival rates in

this study shows that the type and availability of feed used is appropriate for keeping Betta fish.

Absolute Length

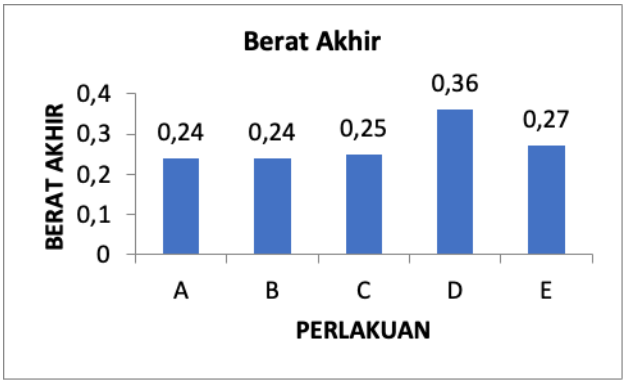
Data on the absolute length of Betta fish can be seen in the following table.



Based on the research results, the highest absolute length results in Betta fish were seen in the P4 treatment, which was 1.69, the P5 treatment was 1.65, the P3 treatment was 1.6 and the P2 treatment was 1.54, while the absolute length had the lowest value. was in treatment P1 1.47. The results of the study showed that the treatment with the dose of cow testicle extract given and the age group of the larvae had a significant effect on increasing the final weight of betta fish ($p>0.05$). Based on the results of research on Betta fish, the absolute length obtained in the P4 treatment was 1.69, the P5 treatment was 1.65, the P3 treatment was 1.63, the P2 treatment was 1.54 and the P1 treatment was 1.47. This means that the dose of cow testicle extract given did not have a significant effect on the growth of betta larvae in any age group. Larval growth is more affected by the feed given. Betta fish larvae are given pellet food until the end of rearing. According to Lithner (2009), water fleas or Daphnia sp. Contains 40-70% protein. The protein content in silk worms is 57% (Satyani, 2003. The high protein content can help the growth of betta fish during maintenance.

Final Weight

Data on the final weight of Betta fish can be seen in the following picture.



Based on the research results, the highest final weight for betta fish was seen in treatment P4, which was 0.36, treatment P5 0.27, and treatment P3 0.25, while treatment P2 0.24 and P10.24 both had the lowest values. The results of calculating data on the average absolute weight growth of Betta fish show that the provision of pellets in the maintenance of Betta fish. Protein is a source of energy for fish and protein is absolutely necessary for fish. Protein can be useful for repairing damaged cells, as a form of cell membrane, and can also

be a source of energy for fish. According to Rukmini (2013), growth speed depends on the amount of feed consumed, the amount of protein contained in the feed, water quality, and other factors such as heredity, age and endurance as well as the ability of the fish to utilize feed. According to Anggraeni and Nurlita (2013) that fish growth is closely related to the availability of protein in feed, because protein is a source of energy for fish and protein is also a nutrient that is really needed by fish for growth, the amount of protein will greatly influence fish growth.

This is also because feed is one of the factors that plays a role in fish growth, the higher the protein content, the faster the growth rate. According to Alang (2013) protein plays an important role in the formation of fish tissues and organs. In the feed given to fish, protein must be available in sufficient quantities. Low protein will result in slow fish growth.

Water Quality

Water quality has an important role in supporting the life and growth of betta fish. The results of observations of water quality parameters including temperature, pH and DO are presented in Table 1 below.

Table 1, Water Quality Parameters

No	Parameter·	Range	References
1	DO	5,2-6,3	5 mg/L (Sunari 2008)
2	Temperature	27,0-28,8 °C	24-29°C (Innes, 1996 *dalam hartami 2013)*
3	pH	6,6-6,7	6,5-7,2 (Atmadjaja 2010)

Temperature

Temperature is the condition of a water environment that can be hot or cold. Temperature measurements were carried out at the beginning, middle and end of maintenance. Water temperature conditions are expressed in degrees. From the table above it can be seen that the water temperature at the beginning of maintenance ranged from 27.0-28.8°C. In the middle of maintenance, the water temperature ranged from 27.6-28.3°C, while at the end of maintenance the water temperature ranged from 27.8-28.6°C. The high and low temperature values depend on the environmental conditions at the time the organism is maintained. Water temperature has a very important effect on respiration, appetite levels and affects the body's metabolic system. However, this temperature range is a normal range for the life of Betta fish being kept. This statement is in accordance with the statement (Innes, 1996 in Hartami et al., 2013) that the temperature tolerance range for the life of Betta fish is around 24-29°C.

Temperature is one of the factors that influences the success of the fish maleization process. If these temperature conditions are related to the maleization process in the test fish, then a temperature of 26.3-28.5°C has shown the optimum temperature range for the development of the test fish's gonads in directing the fish to become male. Arfah et al. (2013) in Arrasyidin et al. (2017) stated that if the temperature is relatively higher, it will influence the development of fish gonads to become more male, while relatively lower temperature conditions will influence the fish gonads to become female. An increase in temperature in the range of 26.3-28.5°C can increase the male fish population.

Acidity (pH)

pH or degree of acidity is a description that shows how acidic or wet the condition of the waters used in a cultivation activity is. pH measurement in this study. The results obtained were the pH range of the water every time the measurements were taken, at the beginning of maintenance the pH range was in the range of 6.6-6.7. The pH in the middle

of maintenance was around 6.7-8.2. Meanwhile, at the end of maintenance the pH range is 7.2-8.1. High and low pH values can inhibit the growth process in Betta fish and can even cause death. According to Atmadjaja (2010) the pH range for the life of betta fish is 6.6¬¬-8.2. So, the pH range above in this study shows the optimal pH range for the life of betta fish. Good water quality can support the growth, development and survival of fish (Effendi, 2002 *dalam* Rachmawati *et al.*, 2016).

Dissolved oxygen (DO)

Dissolved oxygen is the most important environmental condition in cultivation activities because dissolved oxygen plays an important role in the life of cultivated organisms. Dissolved oxygen in water is expressed as 5.2-6.3 mg/L. Dissolved oxygen value. Dissolved oxygen is an important factor in cultivation activities to support fish survival. From the research results, the results of dissolved oxygen measurements were obtained, namely, at the beginning of maintenance the dissolved oxygen content ranged between 5.2-6.3 mg/L, the dissolved oxygen range in the middle of maintenance ranged from 6.6-7.7mg/L, then measurements were carried out dissolved oxygen at the end of maintenance and the measurement results obtained were in the range. The high and low dissolved oxygen content in the rearing media reaches 7.4-7.9 mg/L because the fish become stressed and the fish's appetite decreases.

It can be concluded that the dissolved oxygen content is normal for the life of betta fish. Betta fish are one of the fish that can survive at very low levels of dissolved oxygen in water, both in godfish and larvae. In this research, the supply of dissolved oxygen to the maintenance of Betta fish is gradual, because the oxygen requirements of each age of fish are different. Small sized fish or still larvae require dissolved oxygen that is not too high because it can make the movement of the larvae unstable and cause the larvae to become stressed. Likewise, when fish have just reached the age and size of fry, the presence of dissolved oxygen will be increased because the larger the fish, the more oxygen it needs for its survival. So in this study the presence of dissolved oxygen was supplied gradually depending on the age and size of the biota so that the presence of oxygen did not affect the survival of the fish being kept. Water conditions that have dissolved oxygen of 6.2-7.6 mg/L Betta fish are still able to survive because Betta fish are labyrinth fish which are able to take oxygen directly from the air. However, it would be better if the dissolved oxygen content was high enough because if it is too low it can cause a decrease in appetite, fins not developing properly, and an unattractive body shape. It is very important to always maintain the dissolved oxygen content above 7.4-7.9 mg/L (Sunari, 2008 in Rachmawati et al., 2016). Setyowati et al., (2014) in Austraningsih (2017) stated that suitable waters for keeping ornamental fish, including betta fish, are pH 6-7, temperature 26-27oC and DO 4.06-4.89mg/L

CONSLUSSION

Based on the results of research on the effect of cow testicle extract on the masculinization of betta fish (Betta splendens), it can be concluded that it was given to the test animals through the soaking method. Effective for masculinizing Betta fish (Betta splendens). Soaking the test larvae in cow testicle extract was able to increase the presentation of male and control male fish by 60 mg/L to 91.11%. Giving cow testicle extract did not affect the survival rate of the test animals.

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