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Masculinization of Mutiara's Catfish (*Clarias gariepinus*) Using Cow's Testicles Flour Through Immersion Technique

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ABSTRACT

The production of male catfish is faster, resulting in a shorter harvest period, which can increase profits for farmers. To produce superior male catfish fry, masculinization is conducted. Cow's Testicles Flour (CTF) is one of the natural alternative materials that can be used for the masculinization process in fish. This study aims to determine the best concentration of cow's testicles flour to enhance the success of masculinization in mutiara's catfish (*Clarias gariepinus*) through immersion techniques. The method used is an experimental method consisting of 4 treatments with 4 replicates (0 mgL^{-1}, 45 mgL^{-1}, 60 mgL^{-1}, and 75 mgL^{-1} CTF). The parameters observed were the percentage of male sex, survival rate, growth, and water quality. The highest percentage of male catfish was obtained at a concentration of 60 mgL^{-1} CTF, at 72%. The highest absolute length and specific length growth rate were found at the 60 mgL^{-1} CTF dosage, measuring 5.43 cm and a specific length growth rate of 3.05%. The highest absolute weight in catfish was found at the 75 mgL^{-1} CTF dosage, at 4.69 g. The highest specific weight growth rate in catfish was observed in the control treatment, at 2.40%. The ideal water quality to support the survival of catfish includes a temperature range of 26.26-29.85°C; pH range of 6.5-8.3; and DO range of 4.6-6.7 mgL^{-1}.

INTRODUCTION

Catfish (*Clarias gariepinus*) is one of the freshwater fish species that has great potential to be cultivated in Indonesia. This is evident from the rapid growth of catfish production centers including hatcheries, nurseries, enlargement, and the culinary sector spread throughout Indonesia (Harianto and Budiardi, 2021). Based on production data for the fourth quarter of 2022, catfish occupies the second position in the highest aquaculture production with a total of 359 thousand tons, experiencing an increase in growth of 32.3% (KKP, 2022).

Mutiara's catfish has several advantages over other types of catfish, such as high growth and feed efficiency, same size, resistance to disease and poor environmental conditions, and thicker meat proportions, making it more profitable. Its growth rate can reach 20-70% higher than that of other catfish fry (Matasina and Tangguda, 2020).

According to Findayani and Madinawati (2022), male catfish fry show an advantage over female fry, as males grow faster and have a shorter harvest period. In contrast, female catfish grow slower as their energy is used for reproduction. In the artificial spawning of catfish, it is necessary to cut male catfish to retrieve their sperm, so the availability of male fish is very important.

One of the efforts to increase catfish production is through masculinization techniques. Masculinization is a method of directing male sex by administering androgen hormones during the differentiation phase of the fish gonads. Administration of these hormones changes the environment and stimulates the nervous system of the fish, which in turn triggers the release of gonadotropin hormones for the formation of male gonads (Qotijah et al., 2021).

The synthetic hormone 17α-methyltestosterone is a commonly used hormone in the masculinization process. However, based on the decree of the Minister of Marine Affairs and Fisheries KEP.52/MEN/2014, the use of this hormone is restricted because it can have negative impacts on fish, the environment, and humans (Rohmaniah et al., 2019). Cow's Testicles Flour (CTF) is one of the alternative natural ingredients for the fish branding process, because it contains high natural testosterone hormone. Radio Immuno Assay (RIA) test showed that CTF extract has 30% higher testosterone levels compared to mice, sheep, and goat testes (Iskandar et al., 2014).

The successful use of CTF in fish masculinization has been proven by Hidayani et al. (2016) on cupang fish (*Betta* sp.). A dose of 60 mgL^{-1} with immersion for 24 hours produced the highest percentage of male sex (88.50%). Research by Setiawan et al. (2017) also showed that soaking cow testicles flour for 24 hours at the same dose gave the best results. In addition, research by Saputra et al. (2022) noted that a 24-hour soaking time with cow testicles flour extract produced the optimal number of male fish. Research by Sholeh and Kusuma (2022) also stated that immersion of goat testis flour for 24 hours at a dose of 60 mgL^{-1} was the best treatment. The findings regarding the concentration and duration of testosterone administration can be used as a reference for further research.

Based on this background, the problem that can be identified is how the effect of giving cow testicles flour through soaking techniques with different concentrations on the masculinization of mutiara's catfish (*Clarias gariepinus*). The purpose of this study was to determine the best concentration of cow testicles flour addition in the successful masculinization of mutiara's catfish (*Clarias gariepinus*) through immersion technique.

LITERATURE REVIEW

Catfish is the people's choice as a source of animal protein because of its affordable price, ease of processing, and delicious taste. The high demand for catfish encourages farmers to increase production optimally. One of the efforts to optimize catfish production is to improve seed quality and feed efficiency, which are the main factors in the catfish cultivation process (Muntafiah, 2020).

Masculinization techniques have been widely applied to various fish species using various materials, including the administration of androgen hormones at the gonadal differentiation phase. Environmental changes caused by external hormone administration can stimulate the fish nervous system and trigger the release of gonadotropin hormones, which play a role in the formation of male gonads (Laheng and Widyastuti, 2019).

The successful use of natural compound cow testicles flour in the process of masculinizing fish has been proven by Hidayani et al. (2016) on beta fish (*Betta* sp.). Compared to other doses, the percentage of males using a dose of 60 mgL^{-1} soaked for 24 hours showed the highest results (88.50%). The existence of research on the concentration and length of time of testosterone hormone administration can be used as a research reference. This result is the same as the research of Setiawan et al. (2017) which said immersion of cow testicles flour for 24 hours at a dose of 60 mgL^{-1} was the best result (62.67% of males). Research by Saputra et al. (2022) also said that the immersion time of cow testicles flour extract for 24 hours got the best male fish results. In addition, research by Sholeh and Kusuma (2022) mentioned that the 24-hour immersion time of goat testicles flour with a dose of 60 mgL^{-1} was the best treatment (73.37%).

METHOD

Stages of Research

This research was conducted from February 2024 to May 2024 at the UPTD Fish Seed Center Ciparay Dispakan Bandung Regency which includes spawning, fish rearing, and observation of fish gonads. The making of cow testicles flour was carried out at the Ciparanje Wet Laboratory, Faculty of Fisheries and Marine Science, Padjadjaran University, which is located in Jatinangor.

Tools and Materials

The tools used are a knife, oven, grinder, coolbox, cutting board, 32 aquarium units, 16 aeration installations, 16 heaters, fish surgical tools, spoons, bowls, water quality measuring instruments, microscopes, laptops, millimeter blocks, stationery, electric scales, hoses. The materials used are 4-day-old catfish larvae (length 1.2cm and weight 0.06 g), Cow Testicles Flour (CTF) ice cubes, silica gel, physiological NaCl, acetocarmin, flour feed, and silkworms.

Research Design

This study used a completely randomized design (CRD) method consisting of 4 treatments with 4 replicates. The treatment was immersion of mutiara's catfish larvae using cow testicle flour with different concentrations. The four treatments can be explained as follows:

Treatment A: without the administration of cow testicles flour (control)
Treatment B: immersion with a concentration of 45 mgL^{-1} CTF
Treatment C: immersion with a concentration of 60 mgL^{-1} CTF
Treatment D: immersion with a concentration of 75 mgL^{-1} CTF

Research Procedure

1) Preparation of Cow Testicles Flour

Purchase fresh cow testicles from MBC Baleendah slaughterhouse. Skinning the fresh cow testicles, then splitting them lengthwise. Cut into small pieces and chopped the skinned cow testicles. Put the chopped cow testicles on a baking sheet and put them in the oven to dry at 60° Celsius for 24 hours. Grind the dried cow testicles using a grinder. Sift the cow testicles flour using a fine sieve. Put the cow testicles flour in plastic and store it in the freezer before use.

2) Aquarium preparation

Prepare 32 units of aquarium, 16 units for soaking, and 16 units for rearing. Wash both immersion and rearing aquariums thoroughly and then dry the aquariums in the sun until dry. Filled all rearing aquariums with 10 L of water. Installed aeration and heater to maintain good water quality

3) Larval Immersion

Preparing 4-day-old mutiara's catfish larvae that will be used as test fish with as many as 1600 tails (10 tails L^{-1} water x 10 L x 16 aquariums). Preparing a pre-dried immersion aquarium and then putting water and dissolved cow testicles flour (with different concentrations) into the aquarium and adding NaCl and aeration to dissolve the cow testicles flour and maintain the availability of dissolved oxygen for the larvae. Putting the mutiara's catfish larvae into the immersion aquarium after the cow testicles flour dissolves (± 30 minutes), then the larvae are immersed for 24 hours.

4) Larval rearing

Transfer the mutiara's catfish larvae that have been soaked in CTF for 24 hours into the previously prepared rearing aquarium at a density of 10 fish L^{-1} water. Feed flour to the larvae 4 times a day (07.00, 11.00, 15.00, 19.00 WIB) using the ad satiation method. Siphon feces and change water in the rearing aquarium every 2 days or when the water looks cloudy. Measure water quality (temperature, DO, pH) once a week. Maintain mutiara's catfish larvae until 60 days old.

5) Identification of Catfish Gonads

Take mutiara's catfish when the fish is 60 days old from the maintenance aquarium. Identify sex by morphological observation (primary sex characteristics) and gonad identification using a microscope by giving acetocarmin solution. Dissect mutiara's catfish samples (25% of each rearing aquarium) using a scalpel then take the gonads using tweezers and place them on an object glass. Chopping the gonads using a scalpel blade, then adding 2 drops of acetocarmin solution to the gonads to facilitate observation. Close the object glass using a cover glass, then observe the gonads under a microscope with 10x and 40x magnification.

Parameters

1) Sex Percentage of Catfish

Sex proportion aims to see the results of the sex ratio of male fish and female fish. Based on Saputra et al., (2022) the percentage of male and female individuals can be calculated using the following formula:

$$SR = A/B \times 100\%$$

Description:

SR : Sex ratio (male and female) (%)

A : Number of specific fish species (male and female)

B : Total number of individual fish present (fish)

2) Catfish Survival

Based on Saputra et al., (2022) the percentage of fish survival can be calculated using the following formula:

$$SR = Nt/N0 \times 100\%$$

Description:

SR : Survival rate (%)

N0 : Number of fish at the beginning of rearing (fish)

Nt : Number of fish at the end of rearing (fish)

3) Absolute Length

Absolute length growth was calculated using the formula of Zonneveld et al. (1991) in Indra et al. (2021):

$$PM = Lt - L0$$

Description:

PM : Absolute length growth of catfish (cm)

L0 : Fish length at the beginning of rearing (cm)

Lt : Fish length at the end of rearing (cm)

4) Specific Length Growth Rate

Calculation of the specific growth rate of catfish reared can be done using the Effendie formula (1979) in Indra et al. (2021):

$$LPPS = (\ln Lt - \ln L0)/t \times 100\%$$

Description:
LPPS : Specific Length Growth Rate of catfish (%)
L0 : Fish length at the beginning of rearing (cm)
Lt : Fish length at the end of rearing (cm)
t : Length of rearing time (days)

5) Absolute Weight

Calculation of absolute weight growth using the Effendi formula (1979) in Indra et al. (2021) as follows:

$$BM = Wt - W0$$

Description:
BM : Growth of absolute weight of catfish (g)
W0 : Fish weight at the beginning of maintenance (g)
Wt : Fish weight at the end of maintenance (g)

6) Specific Weight Growth Rate

Calculation of the specific growth rate of catfish can be done using the Effendie formula (1979) in Indra et al. (2021):

$$LPBS = (\text{Ln } Wt - \text{Ln } W0)/t \times 100\%$$

Description:
LPBS : Specific Weight Growth Rate of catfish (%)
W0 : Fish weight at the beginning of rearing (g)
Wt : Fish weight at the end of maintenance (g)
t : Length of rearing time (days)

7) Water Quality

Water quality measurements were carried out once a week on each maintenance media in various treatments, the measured water quality parameters include temperature (°C), pH, and DO (ppm).

Data Analysis

The data obtained were analyzed statistically using analysis of variance (ANOVA) with a confidence level of 95%, including data on the number of male catfish, survival, and growth including length and weight of catfish. If the treatment has a significant effect, it is continued with Duncan's multiple range test. While water quality is analyzed descriptively.

RESULT AND DISCUSSION

Catfish Sex Percentage

The highest percentage of the male sex of catfish is obtained by giving CTF with a concentration of 60 mgL^{-1}, which is 72%, followed by a concentration of 45 mgL^{-1} with a percentage of 64%, a concentration of 75 mgL^{-1} with a percentage of 57%, and the lowest percentage is obtained in the control treatment, which is without CTF immersion treatment with a percentage of 51%. Based on the results

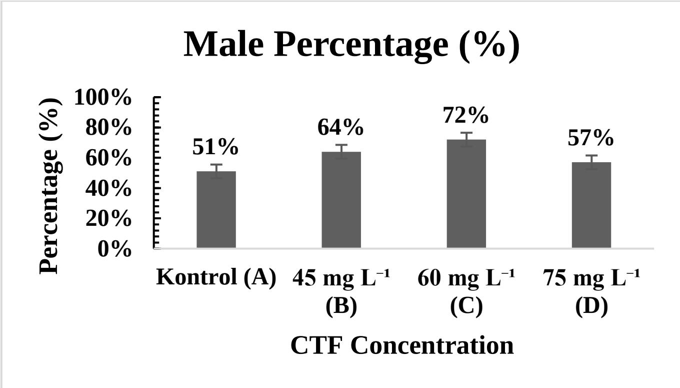

Figure 1. Male Percentage (%)

of the Duncan test, it is known that treatments A, B, and D are not significantly different, treatments B and C are not significantly different, while treatment C is significantly different from treatments A and D.

Treatment C with CTF concentration of 60 mgL-^{1} obtained the highest male sex results, so it can be said that the CTF concentration of 60 mgL-^{1} is the best concentration for the masculinization of catfish. Determination of the right dose of steroid hormone (testosterone) can inhibit the formation of ovaries while accelerating the development of male gonads so that the gonads will develop into male gonads. Administration of testosterone hormones in high concentrations and long duration can cause a paradoxical effect, where what happens is not an increase in the number of male fish, but instead an increase in the number of female fish (Setiawan et al., 2017). Conversely, administering low doses of steroid hormones will not be sufficient to optimally establish a male population (Muslim et al., 2011). In this study, CTF concentration of 60 mgL-^{1} proved to be the best concentration for catfish masculinization.

Hormones entering the larval body are thought to be through the process of osmosis, where the concentration of hormones in the rearing medium is higher than the concentration of hormones in the larval body. This causes the hormones in the media to diffuse into the larval body, and the longer the immersion process, the more hormones affect the gonads (Irmasari et al., 2012). The right dose of steroid hormones in cow testis flour can accelerate the formation of male gonads, which then develop into testes, resulting in more male-phenotyped fish compared to female-phenotyped fish (Hidayani et al., 2016).

The percentage of male fish in this study was higher than that of Setiawan et al. (2017) who used cow testicles flour on rainbow trout, which reached 62.67%; the use of cow testicles flour extract by Irmasari et al. (2012) on tilapia, at 69.07%; the use of coconut water in catfish by Laheng and Widyastuti (2019), at 65.56%; and the use of 17α-methyltestosterone in catfish by Ibrahim et al. (2018), which reached 71.11%. This suggests that the use of cow testicles flour is very effective in catfish breeding. A factor that is thought to contribute to the high percentage of male fish in this study is the use of catfish larvae that are still 4 days old. There is a tendency that the older the larvae, the lower the percentage of male fish obtained (Mulia et al., 2016).

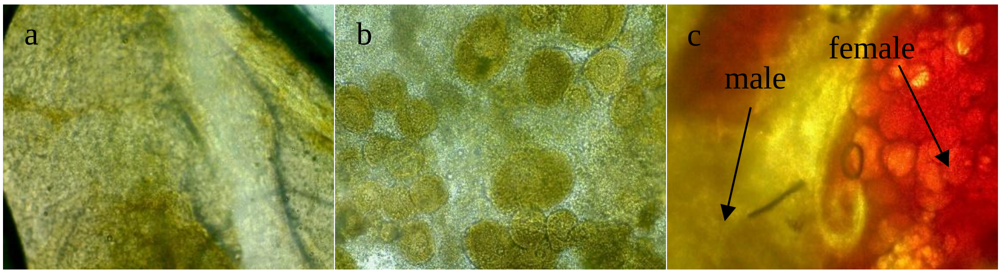

Figure 2. Catfish gonads at 10x magnification; a. male, b. female, c. intersex

In this study, intersex fish were found, namely the presence of male and female gonadal cells in one individual. In the CTF treatment with a dose of 45mgL-^{1} found 2 fish and 60 mgL-^{1} as much as 1 fish. In the control treatment (without administration of CTF) and 45 mgL-^{1} CTF was not found intersex fish.

The appearance of intersex fish may be due to the inability of testosterone to fully sex-change the fish into males, resulting in incomplete gonadal differentiation. Administration of steroid hormones in low concentrations can produce intersex individuals, which is caused by the functional inability of exogenous steroids produced by body tissues, as well as genetic factors and existing physiological activities, which can even cause pathological effects on gonadal development (Syaripudin et al., 2015).

Survival Rate

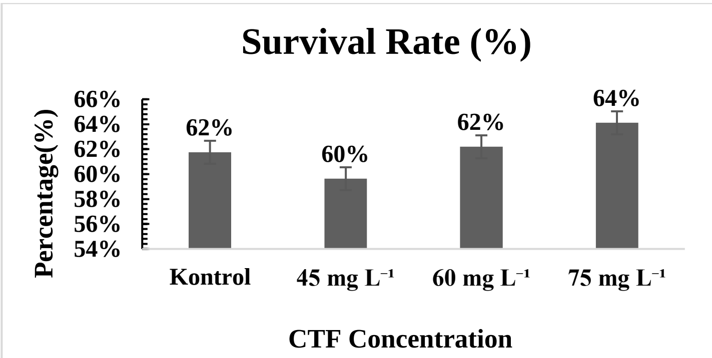

Figure 3. Survival Rate (%)

The survival rate of catfish calculated from post-immersion to the end of maintenance in each treatment is as follows: control treatment (without giving CTF) 62%, CTF dose 45 mgL-^{1} 60%, 60 mgL-^{1} 62%, and 75 mgL-^{1} 64%. Based on the results of the ANOVA test, it is known that there is no significant difference between treatments on the survival rate of catfish.

Cow testis flour has no effect on fish survival, which indicates that this flour is not toxic to fish (Hidayani et al., 2016). In treatment D, the survival rate of catfish was recorded to be the highest from post-immersion to the end of maintenance. The high survival rate after giving cow testicles flour is thought to be caused by the high protein content in the flour. As stated by Irmasari et al. (2012), the protein content in cow testicles flour reaches 76.26-77.08%. This can be achieved if cow testicles flour is made from fresh cow testicles and immediately stored in the freezer (Hidayani et al., 2016).

The survival results calculated from post-immersion to the end of catfish rearing in this study were higher than the results of research by Irmasari et al. (2012), amounting to 14.33-21.67%; Mulia et al. (2016), amounting to 17.78-31.11%.

Absolute Length

Table 1. Absolute Length (cm)

CTF Treatment	Absolute Length (cm)
Control	4.93 ± 0.25
45 mgL^{-1}	5.18 ± 0.48
60 mgL^{-1}	5.43 ± 0.48
75 mgL^{-1}	5.3 ± 0.41

The data above shows the highest absolute length of catfish was found in the dose of 60 mgL^{-1} CTF, which amounted to 5.43 cm, while the lowest absolute length was obtained in the control treatment (without giving CTF) of 4.93 cm, the absolute length of the dose of 45 mgL^{-1} CTF of 5.18 cm, and the dose of 75 mgL^{-1} CTF of 5.30 cm. Based on the results of the ANOVA test, it is known that there is no significant difference between treatments on the absolute length growth of catfish.

The high absolute length in treatment C is due to the dominance of male catfish, which have a faster growth rate than female fish (Mulia et al., 2016; Hardiana et al., 2019; Laheng and Widyastuti, 2019; Findayani and Madinawati, 2022). Larval growth is strongly influenced by the feed provided, where silk worm contains 57% protein (Saputra et al., 2022).

Specific Length Growth Rate

Table 2. Specific Length Growth Rate (%)

CTF Treatment	SLGR (%)
Control	2.91%
45 $mg\ L^{-1}$	2.98%
60 $mg\ L^{-1}$	3.05%
75 $mg\ L^{-1}$	3.01%

The data above shows that the highest Specific Length Growth Rate (SLGR) in catfish is obtained at a dose of 60 mgL-^{1} CTF, which is 3.05%, while the lowest specific length growth rate is obtained in the control treatment (without giving CTF) at 2.91%, LPPS dose of 45 mgL-^{1} CTF at 2.98%, and dose of 75 mgL-^{1} CTF at 3.01%. Based on the results of the ANOVA test, it is known that there is no significant difference between treatments on the specific length growth rate of catfish.

The specific length growth rate is the sum of the daily length gain of fish during rearing expressed in percent (%) (Ratulangi et al., 2022). Specific growth rate describes the speed of growth over time and shows how well fish can convert feed into energy in a certain period (Ririhena and Palinussa, 2021).

Absolute Weight

Table 3. Absolute Weight

CTF Treatment	Absolute Weight (g)
Control	4.32
45 mg L^{-1}	4.44
60 mg L^{-1}	4.57
75 mg L^{-1}	4.69

The data above shows that the highest absolute weight of catfish was found in the dose of 75 mgL-^{1} CTF, which amounted to 4.69 g, while the lowest absolute weight was obtained in the control treatment (without giving CTF) of 4.32 g, the absolute weight of the dose of 45 mgL-^{1} CTF of 4.44 g, and the dose of 60 mgL-^{1} CTF of 4.57g. Based on the results of the ANOVA test, it is known that there is no significant difference between treatments on the growth of absolute weight of catfish.

Fish weight increased in all treatments when compared to the initial weight. The growth rate is related to the increase in body weight resulting from the utilization of protein in feed (Huda et al., 2018). The increase in absolute weight in treatment D is thought to be caused by the high protein content in cow testicles flour.

Specific Weight Growth Rate

Table 4. Specific Weight Growth Rate (%)

CTF Treatment	SWGR (%)
Control	2.40%
45 mg L^{-1}	2.34%
60 mg L^{-1}	2.30%
75 mg L^{-1}	2.24%

The data above shows the highest Specific Weight Growth Rate (SWGR) in catfish obtained in the control treatment (without giving CTF), which amounted to 2.40%, while the lowest specific length growth rate was obtained at a dose of 75 mgL-^{1} CTF at 2.24%, LPBS dose of 45 mgL-^{1} CTF at 2.34%, and a dose of 60 mgL-^{1} CTF at 2.30%. Based on the results of the ANOVA test, it is known that there is no significant difference between treatments on the growth rate of specific weight of catfish.

The high growth rate of specific weight in the control treatment compared to treatments B, C, and D is thought to be due to the stress experienced by the fish during the soaking process, which resulted in reduced appetite. In addition, the limitations of the research media are also one of the factors that affect growth (Ibrahim et al., 2018).

Water Quality

Table 5. Water Quality

Treatment	Temperature (°C)	pH (unit)	DO (mgL^{-1})
Control	26.43-29.85	6.7-8.1	4.6-6.7
45 mgL^{-1}	26.26-29.83	6.6-8.3	4.7-6.3
60 mgL^{-1}	26.42-29.39	6.5-8.1	4.7-6.3
75 mgL^{-1}	26.38-29.77	6.6-8.1	4.7-6.3

Based on the results of the water quality measurement of the rearing media, it shows that the rearing media has an ideal quality to support the survival of catfish, namely the temperature range of 26.26-29.85°C; pH range of 6.5-8.3; and DO range of 4.6-6.7 mgL-^{1}.

The ideal water conditions to support the survival of catfish are water with a pH between 6.5-8.5; a temperature between 25-32°C; and dissolved oxygen (DO) levels of more than 3 mgL^{-1} (Anis and Hariani, 2019).

Abnormality

Figure 4. Catfish abnormalities; a. absence of both fins, b. absence of left fin, c. absence of right fin

Based on the results of the research conducted, 10 catfish that have defects (abnormal), 2 abnormal catfish in the treatment of 60 mgL-^{1} CTF dose with no left fin, and 8 abnormal catfish at a dose of 75 mgL-^{1} CTF, where 4 tails do not have left fins, 3 tails do not have right fins and 1 catfish that does not have right and left fins.

The number of abnormal fish in treatment D is caused by a higher CTF concentration compared to other treatments, resulting in the fish receiving more thyroxine. Administration of thyroxine at high doses can lead to various abnormalities in several fish species, including reduced pigmentation, abnormalities in the dorsal fin, lordosis and scoliosis in the bones, an imbalance between tail length and total length, as well as mortality (Oktaviani et al., 2017).

CONCLUSION

The best treatment for masculinization of mutiara's catfish is treatment C, which uses a CTF concentration of 60 mgL^{-1}, resulting in a male sex ratio of 72%, a survival rate of 62%, an absolute length of 5.43 cm, and a specific growth rate of 3.05%.

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