Effect of testic brahman (*Bos indicus*) mixed with commercial feed and different time for genital of male and female in Tilapia (*Oreochromis niloticus*)

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**Abstract.** The Tilapia is hermaphrodite fish, and from larva they can changing of genital for examples male or female. Male tilapia has faster growth rate than female tilapia. Many technologies were produces genital of fish male dominant population. But to produces that genital were need to prepare for the diet, ones of that were give of the feeding. These results were used of the brahman testicles and mixed with commercial feed, the data were using by immersion method. The results were found value of male seen the larvae compared for the fry tilapia. To give that feed were used at the different time of immersion treatments. The time of immersion was separated to P1 (16 hours), P2 (32 hours), P3 (48 hours) and P0 as (control). The results were observed of male tilapia, body weight, length and water quality parameters. The results of immersion time shown of tilapia larvae very significant effect compared the female. The results were found from the age of 60 days, immersion time of P0 was observed with value of 52.83%, P1 of 85.71%, P2 of 74.49% and P3 was observed and value of 62.41%.

1. Introduction

The tilapia fish is higher economic value and that is a commodity in Indonesia easy to growth and very cheap. The market demand for tilapia has increased every year, because of that the fishermen have to continued maintain especially in the hatchery process [1].

To increase production of tilapia have to loke some various even they bringing new strains from outside, improving seeding and cultivation technology, as well as genetic improvement. Improvement of seeding and aquaculture is done by using advanced technology, such as to make of single-sex culture (monosex), appropriate to applied and tilapia was obtained fast growth with male and faster grow compared to female fish [2]

Cow testicles are wastes and difficult to fine because not often utilized in the abattoir industry. The bovine testes function as a producer of spermatozoa for male sex cells and can secrete hormones (testosterone) [3]

The success of changing sex is not only determined by the type and dosage of hormones used, but is also influenced by the duration of hormone administration. Therefore, information of duration of larval in the optimal testicular flour solution is needed to change the sex of females to be male sex or genitals type.
2. Materials and methods

2.1. Tools and materials
These experiment were used of 12 units bucket, 60 litres of water capacity and the larval rearing, 9 containers and 5 litre water capacity for soaking larvae, aeration equipment, thermometer, pH meter, DO meter, rough fishnet, dipper, cleaning sponge, small hose, gloves, analytical scales, millimetre blocks, test tubes, measuring cups, ovens, blenders, porcelain bowls, fine sifter, plastic bags, digital cameras, surgical instruments, drop pipettes, object glass, cover glass, drip pipettes, and microscope.

The materials used in this study were tilapia larvae (*Oreochromis niloticus*), water as a living medium, Brahman testicles (*Bos indicus*), acetocarmine solutions, artemia and tubifex as larval natural feed (after hatching 3-10 days) and conventional feed, methylene blue, and 70% alcohol solution.

2.2. Making testicular flour
The Cow testicles were used of 250-500 g, take it from Brahman cattle (*Bos indicus*) in Mabar Slaughterhouse abattoir place. Fresh cow testicles from cutting skinned, cut and cut then chopped until smooth, pieces of cow testicles put in the oven at 30°C for 7 days, then dry testicles removed from the oven and grounded at 3 porcelain bowls until shaped like flour, then blended testicles and put in the airtight jar and tightly closed and ready for use, before used the flour keep in a freezer.

2.3. Container preparation
The containers were used in these study consisted for two types containers namely for immersion form a jar with a capacity of 5 litres and 9 units, and for maintenance we need 12 unit a bucket with capacity of 60 litres of water. To maintenance the growth of fish were used in the government lab Tuntungan BBI area Medan. Before we used that water needed aclimitasation at ± 24 hours. Then the water is transferred to the maintenance media with aerated for 2 days to increase the oxygen content in the water.

2.4. Hormone concentration and soaking time
This research was used of immersion method with concentration of hormones at 5ppm. Beef testicle flour were used of 0.5 grams with concentration of 4.5% alcohol or 4.5 ml. The Immersion carried out after 16 hours (P1), 32 hours (P3) and 48 hours (P3) and the P0 (control) without immersion testicular flour solution.

2.5. Immersion of larvae
Needed a jar filled the water with 2 litres/container. Testicle flour was dissolved in water as 10 ml. Each soaking container is equipped with an aerator to increase the level of dissolved oxygen in the water and homogenize mixture of beef testicles. Before the process of soaking the larvae, the immersion container be quiet during 60 minutes. The larvae of tilapia fish (*Oreochromis niloticus*) were used from the age of 3 days after hatching. Stocking density were used of 15 heads/litre of water.

2.6. Larval rearing
After the immersion was complete, the larvae of fish moved to maintenance container with density of 1 fish/litre. The fed of larvae fish must be mashed like powder. During 60 days maintenance of fish the growth increase and type of feed will be differences not powder but the size around 3mm pellets. Feed were give in a manner of libitum (as needed) with giving of 3 to 4 times/day (SNI 6141-2009). The maintenances of fish during 60 days.

2.7. Water quality measurement
The water quality for these experiment needed analysis of water temperature, dissolved oxygen (DO) and degree of acidity (pH). The water temperature DO and pH were measured twice a day in the
morning and evening during 60 days of observation with using digital pH, thermometer and digital DO meter.

2.8. Examination of gonad fish
The experimental immersion solution of gonad in fish during 60 days old. After 60 days maintenance of fish, the fish was take out from container entirely. After that the fish was dissected with a scalpel. The gonads are taken with tweezers and placed in the glass object to be chopped with a scalpel blade until smooth. The gonad chopped given 2 drops of aceticarmine solution. To facilitate observation, then the sample put it in the glass cover to observed under microscope.

2.9. Percentage of sex of male Tilapia fish
The similar result was follow [4] and calculation of sex percentage of male fish following the formula below this.

\[
\% \text{ male} = \frac{\text{number of male}}{\text{total number of fish}} \times 100\%
\]

2.10. Fish survival rate
According [1] to determine of survival rate during 60 days experiment were calculation number of fish were using the formula.

\[
\text{SR} \, (\%) = \frac{N_t}{N_0} \times 100\%
\]

Where:
SR= Survival of fish during the experiment (%)
Nt= Number of fish at the end of the experiment (tail)
No= Number of fish at the beginning of the experiment (tail)

2.11. Fish growth
According to [1] growth of fish observed by calculating of weight gain from each treatment that was weighed during 60 days following the formula.

\[
W = W_t - W_0
\]

Where:
W = Fish growth (g)
Wt = Average individual weight at the end of maintenance (g)
Wo = Average individual weight at the start of maintenance (g)

Meanwhile, to determine the growth of absolute weights was calculated using to [1] follows.

\[
\Delta P = P_t - P_0
\]

Where:
\(\Delta P\) = Growth in absolute length (cm)
Pt = Average individual length on the t-day (cm)
Po = Average individual length on the 0th day (cm)

2.12. Data analysis
The experimental design was used completely randomized design (CRD) referring to [5] with 4 treatments and 3 replications, namely:
1. P0 treatment, soaking without beef testicular flour solution.
2. Treatment of P1, cow testicular flour concentration of 5 ml/litre with 16 hours soaking time.
3. P2 treatment, beef testicular flour concentration of 5 ml/litre with 32 hours soaking time.
4. Treatment of P3, cow testicular flour concentration of 5 ml/litre with 48 hours soaking time.

The research design model used follows:

\[ Y_{ij} = \mu + \tau_i + \epsilon_{ij} \]  

Where:
\( Y_{ij} \) = Observation value of i-th treatment, j-th test
\( \mu \) = General average
\( \tau_i \) = Effect of i-th treatment
\( \epsilon_{ij} \) = Random effects that spread normally

To determine effect of treatment were used analysis of variance (ANOVA) and Duncan's multiple range test at the level of 5%.

3. Results and discussion

3.1. Percentage of male tilapia

Based on the results of research carried out of tilapia fish for 60 days. The treatment was changing sex by immersing solution of testicles brahman with different time was shown in table 1. The results was found higher in immersion solution of 16 hours with value of 85.71% (P1) of male sex, compared the other immersion solution. The results of male sex of control (P0) was lowers with value of 52.83% with 0 time immersion solution and followed of P2 with value of 74.49% and 32 hours immersion solution, and P3 was found with value of 62.4% with 48 immersion solution.

| Treatment | Immersion solution | Percentage of Male Tilapia |
|-----------|--------------------|---------------------------|
| P0        | 0 hours            | 52.83 ± 13.89031          |
| P1        | 16 hours           | 85.71 ± 6.19100           |
| P2        | 32 hours           | 74.49 ± 6.13271           |
| P3        | 48 hours           | 62.41 ± 5.66394           |

Notation: \(^a\), \(^ab\), \(^bc\), and \(^c\) state that there are significant differences between treatment interactions.

3.2. Body weight increase

The different results was shown in sex, growth of fish was different between the P0 was higher weight of 9.63g compared P1, P2 and P3 was lowest weight of 8.23 g, 7.83 g and 7.99 g during 60 days. The treatment of P0 without immersion solution may be the growth of body weight was higher without immersion solution, the body weight increase was shown in figure 1.
Figure 1. The body weight of tilapia during 60 day maintenance.

3.3. Survival of fish
From the results of the average survival value of the test of tilapia in each treatment showed difference in the immersion period. The significant effect of each treatment was shown higher live with value in treatment P2 was found the value of 91.11% compare to P0, P1, and P3. The lower value of 62.22% was shown in P0, but the same results was found in P01 and P3 which value is 85.56% seen from the different letter notation. Where the treatment of P0 (5 ppm, 0 hours) the survival value of 62.22% where this value is significantly different from the survival value of the treatment P1 (5 ppm; 16 hours), P2 (5 ppm; 32 hours), P3 (5 ppm; 48 hours). The average survival value of tilapia can be seen in table 2.

Table 2. The average and standard error of survival.

| Treatment | Information | Average (%) |
|-----------|-------------|------------|
| P0        | 0 hours     | 62.22 ± 2.082 |
| P1        | 16 hours    | 85.56 ± 1.528 |
| P2        | 32 hours    | 91.11 ± 2.517 |
| P3        | 48 hours    | 85.56 ± 2.517 |

Notations: a and b were different percentage of tilapia with 60 days maintenance time.

3.4. Water quality parameters
Measurement of water quality parameters during 60 days the study was analyse seen immersion solution prepare and carried out for a maximum of 48 hours. The data was obtained is relatively stable because maintenance is carried out in a controlled manner. The study was conducted indoors so that the environmental conditions were relatively homogeneous. The parameters of water quality was measured of temperature, pH and DO. The results were shown in table 3.

Table 3. Mean of water quality measurement in container.

| Parameter  | Unit   | P0    | P1    | P2    | P3    |
|------------|--------|-------|-------|-------|-------|
| DO         | mg/L   | 5.14  | 5.29  | 5.13  | 5.24  |
| pH         | the unit | 6.67  | 6.73  | 6.66  | 6.7   |
| Temperature | °C     | 25-28 | 25-28 | 25-28 | 25-28 |
The water quality parameters for maintenance of container was observation during 60 days. The data was obtained relatively stable because maintenance was controlled manner. The study was conducted indoors so that the environmental conditions were relatively homogeneous. The parameters of water quality measured are temperature, pH, and DO. Water quality parameter the results was shown in table 4.

| Parameter | Unit | P0     | P1     | P2     | P3     |
|-----------|------|--------|--------|--------|--------|
| DO        | mg/L | 5.17   | 5.19   | 5.22   | 5.21   |
| pH        | the unit | 6.3 – 7.8 | 6.3 - 8 | 6.5 – 8.2 | 6.3 - 7.8 |
| Temperature | °C   | 26-28  | 25-28  | 26-28  | 27-28  |

The water quality immersion for 60 days of maintenance was observation not much different. Water quality parameters measured during research on maintenance media was collected of temperature, pH, and DO. The results were found of 5.17 mg/L for control (P0), P1 was found with value of 5.19 mg/L, P2 of 5.22 mg/L and P3 of 5.21 mg/L. Then the measurement for, pH value obtained at treatment P0 ranged from 6.3 to 7.8. P1 ranges from 6.3-8. P2 ranges from 6.5 to 8.2 and P3 ranges from 6.3 to 7.8. While the results of water temperature measurements obtained in the treatment P0 range from 26-28 oC, P1 ranges from 25-28 °C, P2 26-28 oC and P3 ranges from 27-28 °C.

4. Conclusions
The conclusions of this study are as follows: 1) The administration of brahman testicular flour solution significantly affected the formation of male sex in the larvae of tilapia during 3 days old with a success percentage value of 85.71%. 2) The optimum immersion time to change the sex of tilapia into male with immersion method for brahman testicle flour solution was found at 16 hours with a concentration of 5 ppm beef testicular flour solution. 3) For body weight between treatment was different, but without immersion solution and was shown in control (P0) was higher wight compared other experimental namely, P1, P2 and P3.

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