Induction of Ovulation Using Ovaprim And Its Impact on Reproductive Parameter of Clarias gariepinus

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Abstract

Sustainability of the aquaculture industry can be secure through the induce breeding technique. One way that induce breeding can do is to increase the rate of maturation and ovulation in making sure the availability of good quality fish seeds all year round. Here, we investigate how Ovaprim hormone impact the reproduction of Clarias gariepinus including spawning rate, fecundity, hatching and survival rate. For this purpose, six broodstocks were divided in three experimental treatments and injected intramuscularly with different doses of Ovaprim hormone of T1: 0.25ml/kg for female and 0.125ml/kg for male, T2: 0.5ml/kg for female and 0.25ml/kg for male and T3: 1.0ml/kg for female and 0.5ml/kg for male. According to our results, broodstock injected with Ovaprim hormone of dose of T3: 1.0ml/kg for female and 0.5ml/kg for male showed higher eggs production and hatching rate compared to other treatment. The results of this study suggest that a dosage of 1.0ml/kg Ovaprim for female and 0.5ml/kg for male have a better effect on the reproductive parameter of Clarias gariepinus.

Keywords: Induction, Induced breeding, Ovaprim, Reproductive parameter, Catfish.

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INTRODUCTION

In aquaculture industry, the success of fish farming enterprises depend on the availability of good quality fish seeds as a stock for sustainable of the fish production to meet the needs (Olumuji and Mustapha, 2012). In natural environment, fish seed usually can be produce by natural reproduction, collected from wild but all this technique contributed to the low quality and quantity of seed as well as seasonal dependence. Therefore, breeding technology such as induce breeding is crucial needed to overcome these problem (Nuraini et al., 2017).

Induced breeding through hormone treatment and artificial incubation of fertilized egg has advantages of better rate of fertilization and hatching, better conditions for growth and better protection of larvae against unfavourable environmental condition and predators (Olaniyi and Akinbola, 2013). Therefore, study on the usage of hormonal dosage could help to determine the best concentration of hormone that could increase the production of larvae.

There are various hormones that can be used in induced breeding for seed production including pituitary extract or hypophysis from similar or different fish, Deoxycorticosterone Acetate (DOCA), Ovaprim, Ovulin, Ovamide, Human Chorionic Gonadotropin (HCG), Ovopel, Dagin and Aquaspawn (Brzuska and Adamek, 1999; Cheah and Lee, 2000; Zohar and Mylonas, 2001; Hassan et al., 2018). The commercially available synthetic inducing hormones in readymade containing GnRH and dopamine blocker receptor such as ovaprim,
ovopel, dagan and aqua spawn are becoming very popular and found to be efficient in successful spawning of fishes (Hassan et al. 2018).

Ovaprim is one of the most popular and effective hormones to stimulate the maturation of male and female adult fish. Ovaprim is used as an effective spawning inducer for artificial breeding of fishes (Chaube et al., 2014). Ovaprim is made from Salmon Gonadotropin Releasing Hormone (SGnRH) and Dopamine antagonist in a stable solution, prepared in glycerine and alcohol at certain quantity (Sasmita, 2016). Ovaprim are used to quicken the ovulation process in female catfish and make sperm more fertile in male fish. Ovaprim can accelerate the final oocytes maturation and induced spawning (Zadmajid et al., 2017). Fish treated with the Ovaprim exhibited increase in gonadosomatic index, egg diameter, and wet weight relative to controls. Fish that injected with high dosage of hormones produce large number of egg production (Zadmajid et al., 2017).

Artificial breeding of fish, especially C. gariepinus, remains the most capable and reliable way to make sure obtainability of good quality fish larvae (Hassan et al., 2018). Clarias gariepinus hardly reproduces in captivity (Hassan et al., 2018). In order to make C. gariepinus to reproduce faster throughout the year, induced breeding technique was applied to the male and female fish in order to produce sperms and eggs in control condition. Thus, in the present study, the following questions were addressed: (1) How is the egg production of Clarias gariepinus affected under different Ovaprim hormones concentration? (2) How does the exposure of egg to different concentration of Ovaprim hormones affect the embryonic development of Clarias gariepinus? (3) How is the hatching rate of Clarias gariepinus affected under different Ovaprim hormones concentration?

**Research Method**

**Broodstocks Selection**

This study was conducted at Aquaculture Laboratory, Blok Pakar, Campus Sultan Abdul Jalil, University Pendidikan Sultan Idris (UPSI), Tanjung Malim Perak. Selected broodstocks of *Clarias gariepinus* (3 males and 3 females) were acquired from commercial farmer in Hulu Bernam, Selangor. The weight of broodstocks ranged from 2.3 kg - 1.025 kg for male and 2.76 kg - 1.6 kg for female. Males and females of *C. gariepinus* can be determine by looking at the genital papillae of the fish. Male fish have genital papillae that is coned shape and female fish have genital pore that is rounded shape. They were transported back to the Aquaculture Laboratory in UPSI. All of the broodstocks were separated in different aquarium tank. The fish were fed with commercial pellet food.

**Hormone Preparation and administration**

Ovaprim hormone were purchased from Syndel Laboratories Ltd, USA. For this experiment, ovaprim hormone was prepared for three different doses (Table 1) of 0.125mL/kg (BW), 0.25mL/kg (BW) and 0.5mL/kg (BW) for male and 0.25mL/kg (BW), 0.5mL/kg (BW) and 1.0mL/kg (BW) for female. The females were given full dosage of hormone while the males were received half of the doses administered to the females (Viveen et al., 1985). Fish were injected intramuscularly above the lateral line using 1mL syringe and 5mL syringe.
Table 1. Dosage of Ovaprim hormone administered to Clarias gariepinus broodstock based on body weight

| Treatment | Sex   | Dose of Ovaprim (mL/Kg) | Fish body weight (kg) | Dosage given (mL) |
|-----------|-------|-------------------------|-----------------------|-------------------|
| T1        | Male  | 0.125                   | 1.025                 | 0.1               |
|           | Female| 0.25                    | 1.60                  | 0.4               |
| T2        | Male  | 0.25                    | 1.80                  | 0.45              |
|           | Female| 0.5                     | 2.36                  | 1.2               |
| T3        | Male  | 0.5                     | 2.83                  | 1.4               |
|           | Female| 1.0                     | 2.70                  | 2.7               |

Experimental Design
Immediately after administering the hormone, males and females broodstock were released into separate aquarium tank. Approximately, after 10-11 hours, the males and females broodstock reached final oocytes maturity stage by checking the female by pressing the abdomen. The eggs from female's fish were stripped out by pressing the abdomen and the eggs was put in the bowl and weight. Males fish was sacrifice to collect sperm. Then, sperm from male was cut into pieces and mixed with the egg by gently stir with chicken feather in order to fertilized the eggs. Along this, latency period and eggs production were also measured. Eggs production was determined by taking sample of eggs (0.25g) from the total eggs released by female. Total number of eggs in 1g were counted and multiplied by total weight of eggs.

Fertilized eggs (10g) were transferred into different 2L aquaria (T1, T2 and T3) for incubation. The eggs were monitored until hatching (20 hours) and the hatching rate were determined by calculate the total number of hatched eggs and divided by the number of eggs.

Reproductive parameter
The reproductive parameter measure in this study were egg weight g.bw hatching percent (%) and larval survival (%). These parameters were measured as follow:

Hatching percent = number of viable embryos / total number of eggs x 100

Egg weight/g. bw = the number of collected eggs (g) / total body weight (g)

Statistical Analysis
Statistical software package SPSS version 24.0 was used for data analysis. Data normality was investigated by Kolmogrov-Smirnov test. Differences between mean were analyzed by One-way analysis of variance (ANOVA) followed by Tukey comparison P< 0.05.

RESULTS AND DISCUSSION
Embryonic Development
Maturation, ovulation and spawning were achieved from females injected with Ovaprim hormone at 0.125ml/kg (T1), 0.50ml/kg (T2) and 1.0ml/kg (T3). Injected females spawned at 10-11 h after injection. The embryonic development characteristic stages are described in Table 2.
Table 2. Embryonic characteristic and duration of development in Clarias gariepinus

| Stage          | After Fertilization (hours) | Description                                                                 |
|----------------|-----------------------------|----------------------------------------------------------------------------|
| one-cell stage | -                           | Protoplasmic layer bulges at the animal pole or submicropilar area         |
| Four-cell stage| 1 hour 2 hours 1 hour       | The second cleavage just at right angle to the first division producing leads to the formation of the four-cell stage |
| Eight-cell stage| 2 hours 3 hours 2 hours     | Third cleavage parallel to first cleavage plane; 8 cells formed            |
| Sixteen-cell stage| 4 hours - 3 hours         |                                                                           |
| Stage                  | Time 1 | Time 2 | Time 3 |
|-----------------------|--------|--------|--------|
| Thirty-two cell stage | 5 hours| -      | 5 hours|
|                       |        |        |        |
| Early morula stages   | 5 hours| 5 hours|        |
|                       |        |        |        |
| Gastrula: 50% epiboly | 6 hours| 9 hours| 7 hours|
|                       |        |        |        |
| Blastoderm expanded   | 6 hours| 9 hours| 7 hours|
|                       |        |        |        |
| Gastrula: gastrulation| 11 hours| 13 hours| 13 hours|
| ends early somite     |        |        |        |
| blocks formation      |        |        |        |
|                       |        |        |        |
| Paired blocks of cells|        |        |        |
| that developed along   |        |        |        |
| the back of the embryo|        |        |        |
| forming the vertebral |        |        |        |
| column                |        |        |        |

Fourth cleavage parallel to second cleavage plane; a layer of 16 cells formed.

Early morula stages whereby continuous divisions and cleavages lead to heterogeneous formation of many cells.

Blastoderm expanded; cell randomized transitional movement; formation of embryonic axis.

Paired blocks of cells that developed along the back of the embryo forming the vertebral column.
Somite formation begins with noticeable pairs of cells developing along the back of the embryo.

| Somite                | 16 hours | 19 hours | 18 hours |
|-----------------------|----------|----------|----------|
| Somite complete       |          |          |          |

All of body part was complete.

| Hatched larva         | 18 hours | 21 hours | 20 hours |
|-----------------------|----------|----------|----------|
|                       |          |          |          |

Break-off of the embryo out of the egg capsule through the tail.

**Egg Production**

All females *Clarias gariepinus* injected with ovaprim hormone at doses of 0.25ml/kg (T₁), 0.50ml/kg (T₂) and 1.0ml/kg (T₃) successfully produced an egg after 14 hours of injection (Table 3). There is significant effect ($F_{2,8} = 3817824.817; P = 0.001$) of hormone dosage on the total number of eggs produced by females. The egg produced from the male and female injected with higher dose (T₃) was higher (Mean: 304912.00 ±12.00) compared to the male and female that been injected with a standard dose (T₂; Mean: 276596.33±116.629) followed by the lower dose group (T₁) with a mean value of egg production of 153539.00±39.00.
Table 3. Effect of Ovaprim hormone dosage on the egg production of Clarias gariepinus

| Treatment | Sex | Dose of Ovaprim (mL/Kg) | Fish body weight (kg) | Dosage given (mL) | Weight of stripped egg (g) |
|-----------|-----|-------------------------|----------------------|------------------|-------------------------|
| T₁        | Male | 0.125                   | 1.025                | 0.1              | -                       |
|           | Female | 0.25                   | 1.60                 | 0.4              | 103.21                  |
| T₂        | Male | 0.25                    | 1.80                 | 0.45             | -                       |
|           | Female | 0.5                    | 2.36                 | 1.2              | 250.51                  |
| T₃        | Male | 0.5                     | 2.83                 | 1.4              | -                       |
|           | Female | 1.0                    | 2.70                 | 2.7              | 295.81                  |

Hatching Rate
There is no significant effect of hormone dosage on the hatching rate of *C. gariepinus* eggs (Table 4; $F_{2,5} = 9.616, P = 0.050$). A pairwise comparison (Turkey) was then used to determine the hatching rate between groups. There are significant differences ($F_{2,5} = -705.500, P = 0.045$) of hatching rate between lower dose of Ovaprim hormone (T₁) with the high dose of hormone (T₃). Meanwhile, the percentage of egg hatched into larvae in T₂ (standard dose) was 15.21% respectively (Figure 1).

Table 4. Effect of Ovaprim hormone dosage on the egg production of Clarias gariepinus

| Treatment | Sex | Dose of Ovaprim (mL/Kg) | Fish body weight (kg) | Dosage given (mL) | Egg weight (g) | Hatching percent (%) |
|-----------|-----|-------------------------|----------------------|------------------|---------------|----------------------|
| T₁        | Male | 0.125                   | 1.025                | 0.1              | -             | -                   |
|           | Female | 0.25                   | 1.60                 | 0.4              | 103.21        | 11.76               |
| T₂        | Male | 0.25                    | 1.80                 | 0.45             | -             | -                   |
|           | Female | 0.5                    | 2.36                 | 1.2              | 250.51        | 15.21               |
| T₃        | Male | 0.5                     | 2.83                 | 1.4              | -             | -                   |
|           | Female | 1.0                    | 2.70                 | 2.7              | 295.81        | 18.70               |
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Figure 1. Hatching rate of Clarias gariepinus egg from a female injected with Ovaprim hormone at 0.25ml/kg (T₁), 0.50ml/kg (T₂) and 1.0ml/kg (T₃). Data are means ± standard error.* Significant difference at 5% level.

CONCLUSION
The effect of Ovaprim hormone on eggs production, embryonic development and hatching rate on Clarias gariepinus was studied. In this study, the concentration of Ovaprim hormone injected on female broodstock (T₁= 0.25 ml/kg, T₂= 0.5 ml/kg , T₃= 1.0 ml/kg) has effect on the eggs production of C. gariepinus. The result show high concentration 1.0ml/kg (T₃) of Ovaprim hormone injected into female broodstock resulted in more eggs production compare to the low concentration. Different concentration of Ovaprim hormone used in this study also affect the embryonic development of C. gariepinus. In this experiment, the lower concentration (T₁) of Ovaprim hormone affect the embryonic development of C. gariepinus by trigger the eggs to hatched faster compare to control (T₂) and high concentration (T₃). The effect of Ovaprim hormone on the hatching rate was also studied. In this study, the concentration of Ovaprim hormone effect the hatching rate of C. gariepinus. The result shows that high concentration (T₃) of hormone increased the hatching rate compare to low concentration (T₁) and control (T₂).

In this study, the concentration of ovaprim hormone play an important role for eggs production in C. gariepinus. Higher hormone concentration, 1.0ml/kg (T₃), used to injected female fish has produce higher number of eggs compare to other treatment (T₁ and T₂). This result are similar with Hassan et al., (2018), Nuraini et al., (2017) and Sharma et al., (2010) where high dosage of hormone influenced the weight of eggs produced as the increase in dosage resulted in more eggs being produced by the fish.

The concentration of Ovaprim hormone also has affected the hatching rate of C. gariepinus eggs. High concentration hormone, T₃ (male=0.5ml/kg, female=1.0ml/kg) have contribute to produced more hatching rate of C. gariepinus compare to low concentration, T₁ (male=0.125ml/kg, female=0.25ml/kg). This result is similar to Nuraini et al., (2017) where their study showed that Cyclocheilichthys apogon, breeding using high concentration of Ovaprim hormone produced greater hatching rate. Ghosh et al., (2012) also reported that
A high concentration of Ovaprim hormone produced a high hatching rate of induced breeding, embryonic, and larval development of koi carp (Cyprinus carpio).

In low doses, a reproductive hormone does not support the maturation of eggs for example germinal vesicle migration and breakdown and subsequently ovulation (Rottmann et al., 1991). At high concentration doses, the combination of sGnRH and domperidone in Ovaprim solution might stimulate gonadotropin secretion in the pituitary gland of the fish and therefore, induced the ovulation and maturation of gonad (Nuraini et al., 2017) which explain the higher egg production and hatching rate at increased dosage of ovaprim. In addition, the optimum dosage of stimulating hormone results in better ovulation and improves egg quality. Prostaglandin plays an important role in inducing ovulation and significantly influences fertilization and hatching rates. This is because prostaglandin contains arachidonic acid derived from essential fatty acids that determine the egg quality (Rottman et al., 1991). In addition, the quality of eggs is influenced by several factors including the hormone levels, nutrition genetics and the environment (Rottman et al., 1991). The quality of eggs is reflected in higher fertilization and hatching rates. In this study the effect of nutrition, genetic and environmental factor on the reproduction of catfish was not investigate. Therefore, future work is needed to look at this factor as it can influence the ovulation and also the quality of egg.

The use of Ovaprim in induced breeding appear to be a promising alternative to seed production. However, results of the study showed that the Ovaprim hormone did not significantly affected the hatching rate of C. gariepinus. On the other hand, Ovaprim hormone significantly affected the egg production. In conclusion, the results in the present study showed that the Ovaprim at dose 1.0 ml/kg improves more reproductive parameter of catfish.

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