EFFECT OF VARYING DOSAGE OF OVULIN ON THE BREEDING PERFORMANCE OF *Clarias gariepinus* IN IMPROVISED HATCHERY TANKS IN BENUE STATE UNIVERSITY, MAKURDI, BENUE STATE, NIGERIA

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Abstract: Fish production under controlled conditions (artificial breeding) using hormones depends on high gamete quality and progeny performance. This study is aimed at determining the effect of varying doses of Ovulin on the breeding performance of *Clarias gariepinus* in Benue State, North-Central Nigeria. Five brood stocks comprising of three females which weighed between 253.73 – 296.85 g and two males which weighed 260.06 and 282.14 g were bought from a fish pond in Gboko and University of Agriculture fish pond, Makurdi. The brood stocks were injected intramuscularly at different doses of 0.10 ml (treatment I), 0.30 ml (treatment II) and 0.50 ml (treatment III) for the females. After a latency period of 10 h, the female eggs were stripped and males sacrificed to obtain milt which was then fertilized and incubated in a flow through hatchery system at water temperature of 30ºC. This was replicated in a completely randomized design and data collected were subjected to analysis of variance. The result showed that the female of treatment III had the highest fecundity (12051), weight of stripped eggs (44.42 g) and percentage of stripped eggs (14.96%). Treatment I had the highest percentage of fertilized eggs (15.27%) highest hatchability (23.77%), there was a significant difference ($P<0.05$) among the treatments while Treatment II had the highest survival rate of 35.42% with survival hatchling number of 59 larvae. It was concluded that *Clarias gariepinus* of about 253.73 and 277.13 g can successfully be induced using 0.3 and 0.1 ml/kg.b.wt. of Ovulin in Benue State, Nigeria, which was lower than the manufacturers recommended dosage of 0.5 ml/kg.b.wt. if other water quality parameters are well monitored.

Keywords: *Clarias gariepinus*, hatchery, latency, Ovulin, survival

Introduction

According to Abdulraheem *et al*. (2012) fishes are aquatic ‘‘cold-blooded’’ animals which are distinguished from other vertebrates by their possession of permanent gills and fins adapted solely for aquatic life. They belong to the super class Pisces and phylum Chordata. Fish forms an important source of human diet as they provide proteins, fats and especially vitamins A and D. Special importance of fish is that they contain vitamin B, which is not present in the plant food. Fish is the good source of calcium; polyunsaturated fatty acids belonging to linolenic acid series (18:3) are present in fish (Oguntuase and Adebayo, 2014).

Hypophysation is a process of inducing fish to spawn through the use of hormones. Different hormones have been reported to be widely used to induce fish breeding although the dosage used varies with fish breeders since no standard dose exist for all fish species (Gomina, 2011). This study is aimed at comparing the effect of varying dosage of Ovulin on the hatchability, survival and breeding performance of *Clarias gariepinus* in an improvised hatchery tanks at Benue State University, Makurdi, Nigeria.

Materials and Method

Study area

The experiment was conducted in the Zoology Laboratory of the faculty of Sciences, Benue State University, Makurdi, Nigeria located on latitude 7^0 43’ 42.6’’ (7.7285^0) North and longitude 8^0 33’ 14.2’’ (8.554^0) East (Google Earth, 2017).

Brood stock collection, selection and identification

Five (5) healthy brood stocks of the African Catfish *Clarias gariepinus* [three (3) females and two (2) males] were purchased from two different locations to avoid inbreeding. Female brood stocks were purchased from a fish pond in Gboko and the male brood stocks were purchased from Federal University of Agriculture, Makurdi fish farm, Benue State. Identification of sex of fish was based on external morphological characteristics (Akombo *et al*., 2015). The brood fish were transported to the Zoology Laboratory of the Department in black 50 L plastic cans. Weights were measured using a sensitive electronic weighing balance (ADAM AFP-4100L), the samples were conditioned in a separate rubber bowls of 35 litres to acclimatize them for three (3) days. The incubation tank (hatchery) is a flow through system with 10 L capacity plastic rubber as the improvised hatchery tank.

Experimental design

Three (3) treatments A, B and C with three (3) replicates of each of the varying doses of ovulin levels 0.10, 0.30, 0.50 ml/kg^{-1}b.wt, respectively. The 0.50 ml/kg^{-1}b.wt was used as the control based on manufacturer’s recommendation.

Injection of spawners

The fish samples were injected intramuscularly above the lateral line just below the dorsal fin, using a graduated hypodermic syringe of 1 – 2 mL (Haniffa and Sridhar, 2002). The needle was inserted parallel to the fish pointing posteriorly at an angle of approximately 30 - 45^0 in the direction of the head with different doses of ovulin (Shinkafi and Ilesanmi, 2014). After injection, the injected area was rubbed with one finger to distribute the hormone suspension evenly throughout the muscles (Akombo *et al*., 2015). The breeders were handled with care using a wet towel. Three dose levels of ovulin 0.10, 0.30 and 0.50 ml/kg^{-1}b.wt was calculated for each fish sample. The 0.50 mLkg^{-1}b.wt was used as the control based on manufacturer’s recommendation. The injected spanners were kept separately in the plastic container containing 10 L (Shinkafi and Ilesanmi, 2014). The container where the injected brood stocks were kept was covered. This helps to speed up the latency period of the fish (Akombo *et al*., 2015).

Collection of milt and stripping egg

Milt were obtained by sacrificing the male fish (weight 500 g) prior to stripping the female and dissecting the testes with a pair of scissors, the testes were carefully removed without squeezing them and dried with a piece filter paper. At the end of the latency period of 10 h, each female was strip gently until some blood appears; the mingling of the blood is

prevented. Eggs were strip into a dry and pre-weighed plastic container to record the weight of the stripped eggs. The total weight of eggs from each female was recorded (1 gram egg mass contains about 700 eggs) and was used to determine the number of eggs that can be fertilized. Three sub-samples of 1 g eggs each were taken from each bowl, placed in petri-dish and saline solution was added and stirred with plastic spoon for easy counting. The mean value per bowl was used to estimate the total number of eggs (spawning fecundity) for each female.

Fertilization and incubation

The dry method of artificial fertilization was used (shinkafi and Ilesanmi, 2014). The total stripped egg from each female was mixed with the prepared sperm solution by stirring with a plastic spoon for one minute. Three (3) sub-samples of 10 g of eggs each were taken and placed in a petri-dish and mixing was facilitated by adding few drops of physiological saline and then washed with water to decrease the distance from the sperm to reach the micropyle of the egg.

After one minute of stirring and fertilization was complete, the fertilized eggs were then spread over a nylon mosquito net mesh (kakaban) placed in a 10 litres plastic trough (the hatchery) containing about 8 L of water connected in a flow through system with inlet and outlet of water channels. The incubation tank (hatchery) was connected in a running water incubation tank (the flow through system) containing the kakaban and the fertilized eggs were spread homogeneously in a single layer on the kakaban to avoid overlapping of the eggs which can result in clogging. Three hours later, the translucent eggs containing embryonic eyes were considered fertilized (Sahoo *et al*., 2005).

Estimation of percentage of stripping, fecundity, fertilization, hatchability and survival rate of African catfish; Clarias gariepinus

Stripping percentage: This was calculated as described in Brzuska (2003) as follows:

$$\text{Stripping (\%)} = \frac{weight\ of\ stripped\ eggs}{body\ weight} \times 100$$

Spawning fecundity: The total number of eggs stripped (spawned) was estimated by counting the eggs in 1 g as described by Sahoo *et al*. (2005).

Relative fecundity: This was calculated as described by Kahkesh *et al*. (2010) as follows:

$$\text{Relative fecundity} = \frac{number\ of\ stripped\ eggs}{body\ weight}$$

Percentage Fertilization: The mean fertilized eggs in all the plastic bowls was recorded and expressed as percentage fertilization per female as described by Adebayo and Popoola (2008) as follows:

$$\text{Fertilization (\%)} = \frac{number\ of\ fertilized\ eggs}{total\ number\ of\ counted\ eggs} \times 100$$

Percentage Hatchability: Hatchability was described by direct counting of the number of hatchlings of two days old as described by Haniffa and Sridhar (2002) and estimated as follows:

$$\text{Hatchability (\%)} = \frac{number\ of\ hatchlings\ (two\ days\ old)}{total\ number\ of\ fertilized\ eggs} \times 100$$

The survival rate was estimated and calculated using the formula described by Ayinla and Akande (1988) as follows:

$$\text{Survival Rate (SR)} = \frac{Ni}{N_0} \times 100$$

Where: Ni is total number of fry at the end of the experiment

N_0 is total number of fry at the beginning of the experiment.

Water quality parameters

The pH, Temperature and Dissolved Oxygen of the water were all monitored four times daily using pH meter, Thermometer and Dissolved Oxygen meter, respectively.

Statistical analysis

The data for the number of stripped eggs in 1 and 10 g were subjected to simple mean while data obtained for spawning, number of hatchings, percentage fertilization, hatchability and survival rate were subjected to one way analysis of variance (ANOVA) to determine differences among treatments and the treatment means were separated Fisher's Least Significant Difference (F-LSD) at 95% (Ogbaji, 2003).

Results and Discussion

Table 1 presents the dosage of Ovulin hormone administered to *Clarias gariepinus* brood stock samples based on body weight. Five brood stocks with three females which weighed 277.13 g as treatment I (0.1 mL) was given 0.14 mL, 253.73 as treatment II (0.30 mL) was given 0.13 mL 296.85 g treatment III (0.5 mL) was given 0.15 mL of Ovulin and two males which weighed 260.06 g was given 0.07 mL and 282.73 g were given 0.08 mnL of the hormone. Weight of stripped eggs for treatment I was 40.01 g, treatment II was 36.56 g, treatment III was 44.42 g and weight of testes obtained for 260.06 g male brood stock was 3.77 g and weight of testes obtained for 282.73 g male brood stock was 4.17 g.

Table 1: Dosage of Ovulin administered to *Clarias gariepinus* based on body weight

Sex	Dosage of Ovulin (mL/kg b.wt.)	Fish body weight (g)	Dosage given (mL)	Weight of gonads/eggs gotten (g)
Female	0.1	277.13	0.14	40.01
	0.3	253.73	0.13	36.56
	0.5	296.85	0.15	44.42
Male	0.3	260.06	0.07	3.77
	0.5	282.73	0.08	4.17

The results of the dosage of Ovulin used on *Clarias gariepinus* females are presented in Table 2. The latency period for all treatments (I, II and III) to complete ovulation was 10 h. The percentage of stripped eggs for treatments I, II and III were 14.44, 14.41 and 14.96%, respectively. Relative fecundity recorded for treatments I, II and III were 35.645, 29.77 and 40.60 g, respectively. The number of eggs recorded in 1 g for treatment I was 188.67±2.33, treatment II was 219.67±9.67 and treatment III was 231.67±9.33. Number of estimated eggs in 10 g for treatments I, II and III were 1886.70±23.30, 2196.70±96.70 and 2316.70±93.30, respectively. The number of hatchlings of two days old counted was 201±4.67 for treatment I, 167±4.33 for treatment II and 115±3.00 for treatment III. The number of Fry obtained at the end of the study (seven days after hatching) was 51±2.00, 59±1.67 and 39±1.33 for treatments I, II and III, respectively. The colour of the stripped eggs obtained for all treatments was green.

Table 2: Characterization of stripped eggs of *Clarias gariepinus* samples at different dosage of Ovulin

Parameters	Treatment/dosage mL/kg b.wt. (mL)		
	I (0.10)	**II (0.30)**	**III (0.50)**
Latency Period (h)	10.0	10.0	10.0
Stripped egg (%)	14.44	14.41	14.96
Relative fecundity (g)	35.64	29.77	40.60
Mean no. of eggs (1g)	188.67 ±2.33	219.67 ±9.67	231.67 ±9.33
No. of eggs (10 g)	1886.70 ±23.30	2196.70 ±96.70	2316.70 ±93.30
No. of hatchlings (2 days old)	201± 4.67	167± 4.33	115±3.00
No. of fry (seven days old)	51±2.00	59±1.67	39±1.33
Colour of eggs	Green	Green	Green

For no. of eggs in 1g; F_{cal}=10.19, LSD=24.08, P<0.05. For no. of hatchlings; F_{cal}=15.86, LSD=12.64, P<0.05. For no. of Fry; F_{cal}= 4.75, P>0.05. Note that F_{tab} for all was 5.14.

The spawning fecundities of female *Clarias gariepinus* at different dose levels of Ovulin are presented in Fig. 1 where treatment I had 9876, treatment II had 7553 and treatment three had 12051 spawning fecundities. This increased with increase in weight of the brood stocks. Fig. 2 presents the percentage fertilization of female *Clarias gariepinus* stripped eggs. Treatment I had the highest fertilization percentage of 15.27%±0.39, followed by 15.07%±0.52 of treatment II while treatment III had the lowest fertilization rate of 13.04%±0.46. There was significant difference among the treatments.

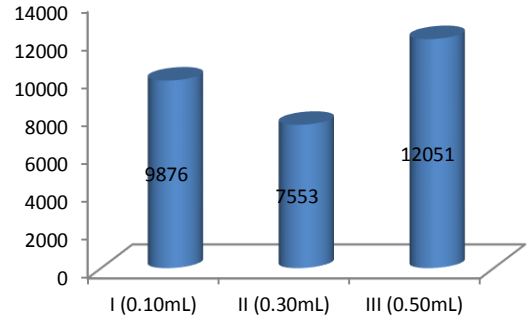

Fig 1: Fecundity of *Clarias gariepinus* females at different dosage of Ovulin

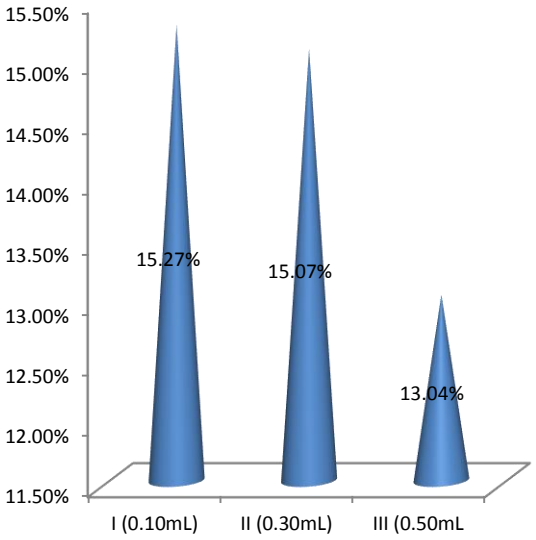

Fig 2: Fertilization percentage of stripped eggs of female *Clarias gariepinus*
F_{cal}=9.70, F_{tab}=5.14, LSD=1.37, P<0.05

The percentage hatchability of eggs of *Clarias gariepinus* at different dose levels are presented in Fig. 3 below with treatment I having the highest recorded percentage hatchability of 23.77%±2.27 followed by treatment II with percentage hatchability of 16.80%±1.05 and treatment III with the lowest percentage hatchability of 12.67%±0.64. There was significant difference among the treatments. The result showed that percentage hatchability decreased with increasing dosage of hormone level in this study.

Table 3 reveals the physico-chemical parameters of the water. The mean temperature of the water observed was 27.23^{0}C±0.45, mean dissolved oxygen (DO) was 5.38 mg/l±0.23 and mean pH level was 6.71±0.09. Survival rate of *Clarias gariepinus* fry of seven (7) days old in Fig. 4 reveals that Treatment II had the highest survival fry rate of 35.42%±2.22, followed by treatment III with survival rate of 34.22%±4.04 and treatment I had the lowest survival rate of 25.87%±4.80. There was no significant difference in the treatments.

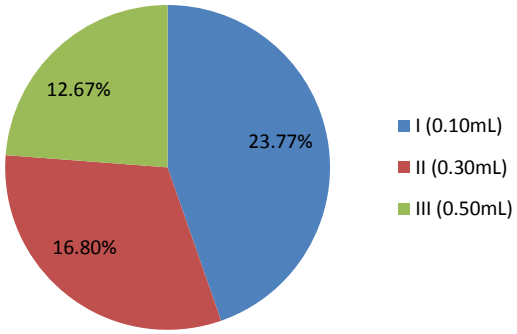

Fig 3: Percentage hatchability of incubated eggs in 10 g of eggs of *Clarias gariepinus*
F_{cal}=18.74, F_{tab}=5.14, LSD=4.49, P<0.05

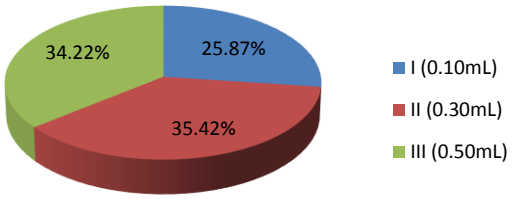

Fig 4: Survival rate (%) of *Clarias gariepinus* fry of seven (7) days old
F_{cal}=2.23, F_{tab}=5.14, P>0.05

Table 3: Physico - chemical parameters of water

Parameters	Results
Mean temperature (^{0}C)	27.23±0.45
Mean dissolved oxygen (DO) mg/l	5.38±0.23
Mean pH	6.71±0.09

The size of the brood stocks in this study was in agreement with the findings of FAO, (1996); Nwachi and Esa (2006) and Ataguba *et al*. (2012) who reported that *Clarias garipinus* becomes mature as from 200 g body weight.

In this study, fecundity generally increased with increase in hormone dosage. This may be due to efficacy of the Ovulin or larger size of the fish and this agrees with Ataguba *et al*. (2012) who reported increase in spawning fecundity with increase in weight of brood stocks and Shinkafi and Ilesanmi (2014) who used Ovatide and recorded higher fecundity for higher dosage in *Clarias gariepinus*.

The small number of eggs (1886.70±23.30, 1886.70±23.30 and 2316.70±93.30) in 1 g obtained in this study was low and this could be as a result of low spawning fecundity. This result is in contrast with the work of FAO (1996) who reported that stripped eggs should contain at least about 600 eggs in 1 g and Shinkafi and Ilesanmi (2014) who reported about 657 of eggs in 1 g.

Percentage of stripped eggs in this study disagrees low compared with FAO, (1996) who reported that percentage of stripped eggs of a mature brood fish should be at least 15-20% and Megbowon *et al*. (2013) who reported about 18.98% of the fish body weight. This result is however agrees with the work of Ipinjolu *et al*. (2013) who reported about 11.7% of stripped eggs in a similar study.

The latency period of this study might be due to the efficacy of the Ovulin. This is similar to the work of Olaniyi and Akinbola (2013) whose latency period was 9 - 12 h when Ovaprim and Catfish pituitary Extract Hormone were used. The low fertilization percentage in this study could be as a result of low quality eggs and sperm produced by the brood stocks as Ayoola *et al*. (2012) reported fertilization of 67.00% for *Clarias gariepinus* when Ovulin was used at 0.5 ml/kg b.wt.

The low hatchability rate obtained in this study might be due to low quality eggs and sperm and also low level of dissolved oxygen as Shinkafi and Ilesanmi (2014) reported highest hatchability rate of 65.28 in treatment II at 0.15 mL/kg b.wt when Ovatide hormone was used.

The low survival rate of this study might be due to stress by the larvae when passing through the Kakaban as a result of the small size diameter of the Kakaban and low level of dissolved oxygen and water hardness as Nwokoye *et al*. (2007) reported a survival rate of 99.61% when pituitary extract from *Heterobranchus bidorsalis* was used to induce *Heterobranchus bidorsalis*.

Conclusion

It was concluded that *Clarias gariepinus* of about 253.73 and 277.13 g can successfully be induced using 0.3 and 0.1ml/kg.b.wt. of Ovulin to produce fry in Benue State, Nigeria, which was lower than the manufacturers recommended dosage of 0.5ml/kg.b.wt. if other water quality parameters are well monitored.

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