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Effect of Varying Doses of Ovatide on the Breeding Performance of African Catfish (*Clarias gariepinus* Burchell, 1822) in Sokoto, North-western Nigeria

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ABSTRACT

Effect of varying doses of Ovatide on the breeding performance of *Clarias gariepinus* in Sokoto, North-western Nigeria was investigated. The females weighed between 330 to 1078.50 g while the males weighed 317 to 542.40 g. Eight gravid brood stocks were injected intramuscularly at different dose levels of 0.10 (treatment I), 0.15 (treatment II), 0.20 (treatment III) and 0.25 (treatment IV) mL kg^{-1} b.wt. (Bw), at a water temperature of 30°C to assess their breeding performance. The experiment was replicated in a completely randomized design. The results showed that the size of eggs did not vary among the samples based on all the treatments. The female of treatment IV had the highest spawning fecundity (149,796), weight (228 g) and percentage of eggs stripped (21.14%). Treatment III had the highest percentage of fertilized eggs (95.45%) which was significantly ($p<0.05$) higher than in the other treatments. Treatment II had the highest hatchability (65.28%), number of hatchlings (3,017) and total weight of hatchlings (12.07 g). It was concluded that *C. gariepinus* of about 400 g can successfully be induced to ovulate using 0.15 mL kg^{-1} b.wt. in Sokoto, Nigeria which was lower than the manufacturer's recommended dosage of 0.20 mL kg^{-1} b.wt.

Key words: *Clarias gariepinus*, ovatide, spawning fecundity, fertilization, hatchability

INTRODUCTION

Aquaculture is the fastest growing food producing sector and according to Bartley (2005), by 2025, one out of every two fish eaten may come from aquaculture. The current demand for *Clarias gariepinus* in Nigeria stands at about 3 million metric tons per annum, with current production of only about 0.8 million metric tons, leaving a deficit of about 2.2 million metric tons that are yet to be exploited (FAO, 2008). This catfish occupies a unique and prominent position in the commercial fisheries of the country and in Africa at large, as it is tasty, hardy and can tolerate poor water quality conditions (Idodo-Umeh, 2003). It is also capable of reproducing in captivity and can grow to a size of 10 kg in large ponds (Olaosebikan and Raji, 2004).

Most of the fishes for aquaculture such as Clarias, Carps, Mullet and Milk-fish are open water breeders that breed under the influence of environmental stimuli and tend to stop spawning when subjected to confinements of ponds (Khan *et al*., 2006). Under controlled conditions, attempts are made to obtain both sperm and eggs of the highest weight and of the best quality and hence, to

produce the highest possible number of good quality seeds (Brzuska, 2003), by using various spawning agents to stimulate ovulation. These have been experimentally tested to find out those that would ensure very good effects. A large number of natural spawning agents for induced breeding of the African catfish *C. gariepinus* are available and these include Deoxycorticosterone Acetate (DOCA), Human Chorionic Gonadotropin (HCG), Carp Pituitary Extract (CPE). Other synthetic hormones such as Ovaprim, Ovopel, Dagin and Aquaspawn have also been used to induce breeding successfully (Brzuska and Adamek, 1999; Cheah and Lee, 2000; Zohar and Mylonas, 2001). These spawning agents are either difficult to quantify (CPE), ineffective (DOCA), have a short shelf life (HCG) or expensive (e.g., Ovaprim) as reported by Olubiyi *et al.* (2005) and Khan *et al.* (2006). The need for the production of quality fish seed to stock artificial ponds and natural water bodies through artificial propagation has steadily been encouraged, as it is the only practicable means of producing enough quality fish seeds (Abayomi *et al.*, 2010).

The foregoing considerations therefore prompted the need for a better and more effective substitute with fewer problems and thus, Ovatide a newly invented spawning agent as a liquid preparation containing synthetic peptide protein that is analogue to naturally occurring Gonadotropin Releasing Hormone (GnRH) and dopamine antagonist. The Ovatide was produced by Hemmo Pharma, Mumbai, India and according to its manufacturers, the recommended dosage is 0.20 mL kg^{-1} b.wt. (Bw) for catfishes. Little or no information is yet available on the use and effectiveness of Ovatide on *C. gariepinus* in Nigeria.

There is therefore the need to establish the most effective dose of the hormone in the semi arid environment of Sokoto, north-western Nigeria, so as to keep in pace with the current advances in piscine reproduction and also to contribute to the available information for mass production of *C. gariepinus* fingerlings. Hence, the objective of this study is to evaluate the breeding performance of *C. gariepinus* using varying doses of Ovatide under the semi-arid environment of Sokoto, North-western Nigeria.

MATERIALS AND METHODS

The experiment was conducted at Fish Hatchery of the Fisheries Unit, Department of Forestry and Fisheries, Usmanu Danfodiyo University, Sokoto on latitude 13°07'7"N and longitude 05°12'25"E at 275 m above the sea level (Google Earth, 2011). Eight broodstocks of *C. gariepinus* (4 males and 4 females) were collected from the Unit's brood stock pond. Identification of sex was based on external morphological characteristics (Metwally and Fouad, 2008) as shown in Fig. 1-3. The samples were then transferred to indoor holding concrete tank of dimension 1.5×1.7×1.7 m^3 filled with borehole water to acclimatize them for two days. The fish samples were each weighed with a sensitive electronic weighing balance (JT210N series) of 2,100 g maximum capacity. The samples were each placed in separate 50 L rubber bowls for induced breeding exercise.

Four dose levels of Ovatide namely, 0.10, 0.15, 0.20 and 0.25 mL kg^{-1} b.wt. were calculated for each fish sample (Table 1). The 0.20 mL kg^{-1} was to be used as the control based on manufacturer's recommendation. The females were to be given full dosage of the hormone while the males were to receive half the doses administered to the females (Viveen *et al.*, 1985). The experimental design consisted of four treatments (I-IV) based on the four varying doses of the Ovatide and these were replicated three times in a Completely Randomized Design (CRD). Twelve rubber bowls of 30 L capacity were washed, filled to fifteen litres level of water and constantly aerated with aerator pump at 30°C. A prepared net (mats) are washed and placed inside each bowl to display the eggs.

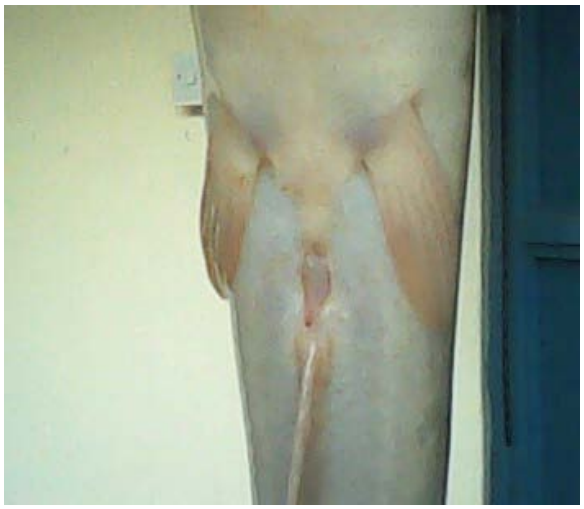

Fig. 1: Gravid male with pointed genital papilla

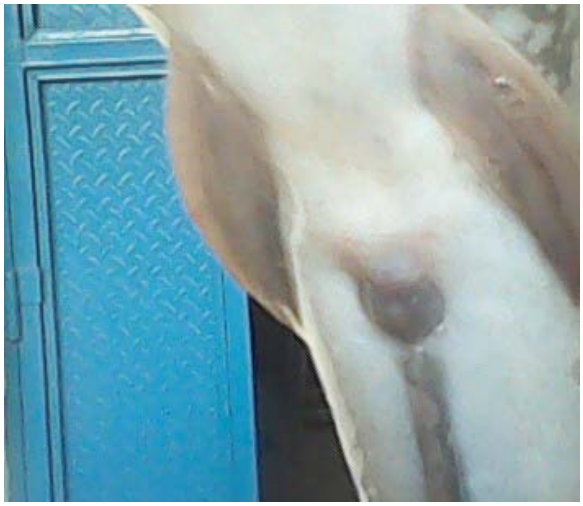

Fig. 2: Female with rounded urogenital opening

Fig. 3: Gravid female with swollen abdomen

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

Sex	Doseof Ovatide (mL)	Fish body weigth (g)	Dosage given (mL)
Female	0.10	360.00	0.04
	0.15	365.00	0.06
	0.20	330.00	0.07
	0.25	1078.60	0.27
Male	0.10	336.70	0.02
	0.15	317.60	0.02
	0.20	326.40	0.03
	0.25	542.40	0.07

The fish were injected intramuscularly above the lateral line towards the dorsal fin using a graduated hypodermic syringe of 1 mL (Haniffa and Sridhar, 2002). The needle was inserted at an angle of approximately 30° in the direction of the head as described by Viveen *et al.* (1985). The injected spawners were kept separately in containers to avoid disturbances and self injuries.

At the end of the latency period of 8 h, the milt was obtained by sacrificing the male fish. The testes were removed, mopped with clean towel from stains of blood and water and then weighed. These were incised and squeezed of the milt. The milt was diluted with 5 mL of physiological saline to prepare a sperm suspension. The milt was then used for the fertilization of eggs. Each female was stripped into a dry and pre-weighed small bowl to record the weight of the stripped eggs. The total weight of eggs from each female was recorded and used to determine the spawning fecundity of the female fish. Three sub-samples of 1 g eggs each were taken from each bowl, placed in saline solution and stirred with plastic spoon for easy counting. The mean value per bowl was used to estimate the total No. of eggs (spawning fecundity) for each female. The diameters of ten randomly selected eggs from each bowl were measured using a microscope fitted with eyepiece micrometer.

The dry method of artificial fertilization was used as described by Viveen *et al.* (1985). Three sub-samples of 10 g of eggs each were mixed with four drops of sperm solution by stirring with a plastic spoon for a minute and then washed with water to decrease the distance from the sperm to reach the micropyle of the egg. The fertilized eggs were then spread on the net in each of the 15 L bowls, prepared earlier for this purpose and continuously aerated. Three hours later, the translucent eggs containing embryonic eyes were considered fertilized (Sahoo *et al.*, 2005). After 17 h of incubation, the hatching was completed. The viable and dead eggs were determined and counted. The viable eggs were translucent while the non-viable eggs were white and opaque and these were carefully removed by siphoning. Hatching occurred at about 14 h, the percentage hatchability was estimated after two days of hatching when yolk sac has been absorbed. One hundred of the larvae were weighed, their weights multiplied by total weight of larvae in each of the bowl to estimate total hatchability per bowl.

Care of the larvae started immediately after hatching. Siphoning was carried out on a daily basis in all the bowls to prevent fouling and infection and subsequent mortality of the hatched eggs/larvae. Water was added up to 25 L and continuously aerated. Water quality parameters such as the temperature and pH were monitored with the aid of a pen pH meter that was fixed with mercury in glass thermometer.

Analytical procedure: Weight of the brood-stock, spawning fecundity, percentage fertilization and hatchability were recorded for each treatment to determine the performance (efficacy) of Ovatide at various dose levels as follows:

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

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

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

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

$$\text{Relative fecundity} = \frac{\text{No. of stripped eggs}}{\text{Body weight}}$$

Percentage fertilization: The mean fertilized eggs in all the replicated bowls was recorded and expressed as percent fertilization per female (Adebayo and Popoola, 2008) as follows:

$$\text{Fertilization} = \frac{\text{No. of fertilized eggs}}{\text{Total No. of egg counted}} \times 100$$

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

$$\text{Hatchability} = \frac{\text{No. of hatchlings (two days old)}}{\text{Total No. of fertilized egg}} \times 100$$

Survival rate: This was calculated as described by Ayinla and Akande (1988) as follows:

$$\text{SR} = \frac{\text{Ni}}{\text{N0}} \times 100$$

where, Ni is total No. of fry at the end of the experiment and No is total No. of fry at the beginning of the experiment.

The data obtained for spawning and egg quality index were subjected to one way analysis of variance (ANOVA) to determine significant differences among treatments and the treatment means were separated by Duncan's Multiple Range Tests (DMRT) at 95% (Gomez and Gomez, 1984). Computer analysis was carried out using the Statistical Package for Social Science (SPSS, 2007) 16.0 version for windows.

RESULTS

The females weighed between 330-1078.50 g while the males were 317-542.40 g. The highest weight of testes recorded was 2.60 g in fish sample with 317 g which was injected 0.02 mL of Ovatide at 0.15 mL kg^{-1} rate. The lowest weight of testes (0.40 g) was obtained in the fish weighing 542.40 g injected with 0.07 mL (0.25 mL kg^{-1}) dose level. The highest weight of stripped eggs was 228 g in the fish sample weighing 1078.60 g which was injected with 0.27 mL (0.25 mL kg^{-1}) and the least (57.20 g) was obtained in fish weighing 330 g administered 0.07 mL (0.20 mL kg^{-1}) as shown in Table 2.

Table 2: Quantity of Ovatide administered to *Clarias gariepinus* samples based on the 4 treatments

	Treatment/dosage (mL kg^{-1} b.wt.)			
Parameter	I (0.10 mL)	II (0.15 mL)	III (0.20 mL)	IV (0.25 mL)
Male				
Body weight (g)	336.70	317.00	326.40	542.40
Dosage (mL)	0.02	0.02	0.03	0.07
Weight of testes (g)	2.00	2.60	1.60	0.40
Female				
Body weight (g)	360.00	365.00	330.00	1078.60
Dosage given (mL)	0.04	0.06	0.07	0.27
Weight of stripped eggs (g)	71.50	66.30	57.20	228.00

Table 3: Characterization of stripped eggs of *C. gariepinus* samples at different dose levels of Ovatide

	Treatment/dosage (mL^{-1} kg b.wt.)			
Parameter	I (0.10 mL)	II (0.15 mL)	III (0.20 mL)	IV (0.25 mL)
Latency period (h)	8.00	8.00	8.00	8.00
Stripped eggs weight (g)	71.50	66.30	57.20	228.00
Stripped eggs (%)	19.86	18.16	17.33	21.14
Spawning fecundity	47,405	45,285	35,922	149,796
Relative fecundity (g)	131.68	124.07	108.85	138.88
No. of eggs (g)	662.67 ± 24.01^{a}	682.67 ± 12.74^{a}	627.67 ± 16.01^{b}	657.00 ± 12.29^{ab}
Egg size/diameter (mm)	1.63±0.12	1.57±0.58	1.70±0.10	1.60±0.00
Egg colour	Light green	Green	Green	Brown
Nature of egg	Matured	Matured	Matured	Matured

Means in row with same superscript are not significantly different ($p>0.05$)

The results of induced spawning of *C. gariepinus* females are presented in Table 3. The average latency period to complete ovulation was 8 h under mean temperature of 30°C in all the treatments. Weight of ovulated eggs ranged from 57.20 to 228 g. The highest was (228 g) in treatment IV and this was significantly ($p<0.05$) higher than for the other treatments. The percentage of stripped eggs, spawning fecundity, relative fecundity followed the same trend as weight of stripped eggs but this was not significantly ($p>0.05$) different from those for the other treatments. There was no significant ($p>0.05$) difference in the size of eggs for all the four treatments. The egg colours vary from light green (I), green (II and III) and brown (IV), respectively. All the eggs were mature. Female injected with treatment II had significantly more of eggs in 1 g than in for the other treatments.

Egg quality indices: Some egg quality indices for the samples administered with the four treatments are presented in Table 4. Female samples of treatment III and IV had significantly ($p<0.05$) the highest percentage fertilization of 95.43±2.45 and 91.34±1.69, respectively while the lowest fertilization (87.54±2.03) was recorded in sample of treatment II. Highest but not significant ($p>0.05$) of hatchability was obtained for sample of treatment II (65.28±12.36) while the least was found in treatments I and IV. The result of number of hatchlings and larval weight also followed same pattern as that of percentage hatchability. The incubation period had a mean of 15.50 h and yolk sac absorption was from 48.17±0.29 to 49.00±1.00 h.

Table 4: Egg quality index of *Clarias gariepinus* samples at different dose levels of Ovatide

	Treatment/dosage (mL kg^{-1} b.wt.)			
Parameter	I (0.10 mL)	II (0.15 mL)	III (0.20 mL)	IV (0.25 mL)
No. of egg (10 g)	6626.70±240.07^{a}	6826.70±127.41^{a}	6276.79±160.10^{b}	6570.00±122.88^{ab}
Fertilization (%)	90.62±3.25^{b}	87.54±2.03^{b}	95.43±2.45^{a}	91.34±1.69^{a}
Hatchability (%)	43.33±2.89^{b}	65.28±12.36^{a}	60.14±6.38^{a}	30.97±3.47^{b}
Hatchlings No.	1700.00±173.21^{b}	3016.70±741.76^{a}	2708.30±382.70^{a}	958.33±208.17^{b}
Total fry weight (g)	6.80±0.69^{b}	12.07±2.97^{a}	10.83±1.53^{a}	3.83±0.83^{b}
Initial fry length (cm)	0.51±0.06^{a}	0.50±0.06^{b}	0.52±0.06^{a}	0.50±0.00^{b}
Yolk absorption (h)	48.33±0.58	49.00±1.00	48.17±0.29	48.50±0.50
Incubation (h)	15.50	15.50	15.50	15.50

Means in row with same superscript are not significantly different (p>0.05)

DISCUSSION

The size of the brood stocks in this study was in agreement with the findings of Viveen *et al.* (1985) who reported that *C. gariepinus* becomes mature as from 200 g body weight. Spawning success in samples of this study administered with the different doses of Ovatide indicated that irrespective of weight, *C. gariepinus* responded well to Ovatide. Good breeding performance with Ovatide injection has been reported in several fish species such as carp (Thakur and Reddy, 1997), pabo catfish (*Pabda ompok pabo*) (Mukherjee and Das, 2001), stinging catfish (*Heteropneustes fossili*) and snake head murrel (*Channa punctatas*) (Marimuthus *et al.*, 2007). The highest fecundity of 149,796 obtained in female injected with treatment IV may be due to efficacy of the Ovatide or due to larger size of the fish and this is in accordance with Viveen *et al.* (1985) that larger females contain more eggs. Similar finding of higher fecundity with injection of the higher dose of Ovaprim in *C. gariepinus* was reported by Olubiyi *et al.* (2005). Percentage of stripped eggs of about 17-21% of the samples' body weight was similar to that of De-Graaf *et al.* (1995). Size of eggs of 1.65 mm in this study was larger than those reported by Ajana and Anyanwu (1995) for the same species. The number of eggs in 1 g (657) obtained in this study was similar to those reported by Viveen *et al.* (1985) and De-Graaf *et al.* (1995) in *C. gariepinus*.

The latency period of 8 hrs at 30°C recorded in this study might be due to the efficacy of the Ovatide, compared to 10-12 h of latency period for *C. gariepinus* at 28 to 30°C reported by Ajana and Aleem (1991) when pituitary gland was used. This is similar to result obtained in this study which indicated that fish respond well to the varying dose levels of Ovatide. The incubation period of 15.50 h at 30°C was similar to the 16-22 h at the same temperature reported by Ajana and Anyanwu (1995) on *C. gariepinus*. The time taken to complete any stage may be dependent on water temperature and often decreases with an increase in temperature (Legendre, 1986).

The 85% fertilization recorded for samples of this study may be an indication of the efficacy of the Ovatide used as Adebayo and Popoola (2008) reported fertilization rate of 84.50% for *C. gariepinus* while Haniffa and Sridhar (2002) reported fertilization of 70% for spotted murrel (*Channa punctatus*) by administration of Ovaprim in both cases. The high (62%) hatchability of eggs at 0.15 mL kg^{-1} b.wt. dose (treatment II) was better than those reported by Moses *et al.* (2005) for Kainji strains of *C. anguillaris* (58.58%) and *C. gariepinus* (52.44%) when Ovaprim was used.

CONCLUSION

This is the first documented scientific study to test the efficacy of Ovatide at varying the dose levels to induce spawning in *C. gariepinus* in the semi-arid environment of Sokoto. The response of *C. gariepinus* to Ovatide was found to be good considering the breeding success in this study in terms of spawning fecundity, stripped percentage, egg size and fertilization and hatchability. Therefore, the present study showed that *C. gariepinus* of mean body weight of 533.40 g can successfully be induced to ovulate from dose level 0.15-0.20 mL kg^{-1} b.wt. to obtain eggs and larvae of good quality which is below the dosage recommended by the manufacturers.

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