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The Efficacy of Synthetic and Non-Synthetic Hormones in the Induced Spawning of the African Catfish (*Clarias gariepinus* Burchell, 1822)

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Abstract
The efficacy and economical utilization of the pituitary extract of C*larias gariepinus* (CPE), Generic Ovaprim (GO) and Normal Saline Diluted Ovaprim (NSD) as designated hormones were evaluated. The extraction and dosage was discussed alongside the preliminary rearing of larvae in the hatchery gutters. Two female spawners were injected 0.4 ml/kg Ovaprim at 0% and 25% saline dilution in a single dose while one was injected 1.0 ml/kg *C. gariepinus* pituitary extract in a single dose. Relative fecundity ranged from 126.87-131.25. *C. gariepinus* injected with Ovaprim (GO and NSD) had the highest percentage fertilization (88.37 and 87.93%). Percentage hatchability was high and ranged from 62.50-75.00 % and showed similar patterns to percentage survival (55.20-68.66%). The latency period was 7-9hrs and the incubation period was 20 – 21hrs for all treatments. Water quality parameters were observed to be within recommended standards. The results showed that all designated hormones were effective and efficient and therefore conclude that the economical use of both available resources in the induced spawning of *C. gariepinus* is an added value to the function of profitability.

Keywords: *Clarias gariepinus*, Induced spawning, Ovaprim, Saline solution, Larvae, Water quality parameters.

1. Introduction
With our economy that is heavily dependent on importation of goods with little concern for local alternatives, the mobilization, application and utilization of available resources becomes paramount. *Clarias gariepinus* (African Catfish) one of the widely distributed fish species in Nigeria as well Nasarawa State is yet to be fully harnessed (Ngueku, 2015) [1].

In Nigeria fish was once the cheapest and most available source of animal protein, today it is not the case as it is hampered by low supply caused by low productivity of our water bodies that have been over-exploited, badly managed farms and lack of government sincere support for aquaculture. Today the result is National protein deficiency resulting in malnutrition especially in the young and rural populace and is a major factor adversely affecting the health, well-being and socio-economic development of people in Nigeria and developing countries. Inadequate supply of protein in diets is the main cause of malnutrition. Fish the best source of protein is a major concern in food protein security and can best be achieved if fish farming is given due attention.

Fish seed (fingerlings) quality has been a major problem in the attempts by many fish farmers to culture fish, and is a fundamental pre-requisite in large scale production of fish. Today, many fish farmers in Nigeria are unable to produce their own fingerlings due to financial, technical and logistical demands and have relied on the government that pays little or no attention. Induced spawning guarantees a reliable source of fish seed (Fingerlings) in quality and availability. Today prominent synthetic hormone brands like Ovaprim, Ovatide and Ovuline are in use but still not readily within the fish farmers reach, Ovaprim is one of the most widely and readily available synthetic hormones because it has been found to be very effective (Olubiyi, Ayinia and Adeyemo, 2005) [2]. Saline solution in which natural hormones in pituitary are dissolved prior to administration in recipient fish is cheap and affordable. The highly viscous Ovaprim is diluted with saline solution to cut down the cost of induced spawning.

In this research, natural and synthetic hormones are to be evaluated for their efficacy in respect

to fecundity, fertilization, hatchability, survival rate while taking into cognisance the economical use of both resources. The objective of this study is to emphasize the economical use of available fish spawning resources which will be an added value to the function of profitability.

2 Materials and Methods

2.1 Research Location

The experiment was conducted at Xtreme Idea Farms Ltd. fish seed hatchery Tudun Wamba-Ko in Wamba LGA, Nasarawa State. Induced spawning trails were conducted in May and early June, 2015.

2.2 Experimental Design

Three (3) spawning trails were conducted. Three treatments based on Clarias pituitary extract (CPE) Generic Ovaprim (GO) and 25% Normal Saline Diluted Ovaprim (NSD) was carried out on selected spawners.

2.3 Selection of Spawners

Male and Female spawners of 1.6kg-1.8kg were collected from the broodfish pond. A gentle press was made on the abdomen with thumb towards the vent or tail, thinly separated greenish and brownish eggs were seen oozed out freely from the papilla and the males papilla were observed to be reddish in color indicating that they were gravid or matured. They were disinfected with concentrated common salt solution and brought into the hatchery and allowed rest for 24hrs without food.

2.3.1 Collection of Pituitaries

A male Brood fish of 1.6kg was killed and decapitated not sooner than one hour before the planned time of injecting the female spawner. The lower jaw of the fish was cut away with a knife and the palate of the mouth opened with pincers at location of the pituitaries in the skull situated on the ventral portion of the brain. The pituitaries were collected with a pair of Tweezers and grinded in a mortar containing 2ml normal saline solution (0.9%. NaCl).

2.3.2 Collection and Preservation of Milt

Milt was collected from the same males that were sacrificed for the pituitaries by dissecting to remove the testes. Incision was made on the testes lobes to collect the milt. The milt was collected in a small bottle with 5ml saline solution (0.9% NaCl) and was preserved at 4^0C in a Refrigerator and was used after the latency period.

2.4 Hormone Injection

1.6kg female spawner was injected using a 2ml graduated syringe intramuscularly with 1.6ml pituitary suspension that was collected from a male of equal size and weight immediately after grinding in a mortar containing 2ml normal saline solution (0.9% NaCl) at an angle of 30-45^o in the dorsal muscles in the direction of the tail. Another 1.6kg female spawner was injected in same way with 0.64ml undiluted generic Ovaprim and a third spawner 1.6kg injected in same way with 0.64ml 25% normal saline diluted Ovaprim. Each injected female spawner was secured in different holding troughs to prevent fighting. The males were not administered with hormones.

2.5 Stripping and Fertilization

Injected spawners were stripped of their eggs after the latency period into clean dry bowls and weighed. The preserved chilled milt was evenly distribute over the egg mass to some portion of the treatment while the milt of a second male spawner collected after dissecting and lacerating the testes with a clean razor blade to evenly distribute the milt over the egg mass in the bowl with 5ml normal saline (0.9% NaCl) was done to another portion due to the large egg mass and a proper mixture done with the aid of a feather and afterwards some clean fresh water was added.

2.6 Incubation and Care of Larvae

Fertilized eggs were then thinly and evenly distributed in a single layer on a plastic netting substrate (Kakaban) of 2mm mesh size in the incubation gutters or troughs containing water supported by Aerators for adequate supply of Oxygen. Hatching occurred 20-25hrs. Siphoning was done after 3 days after the egg yolks were completely depleted. External feeding began on the 4th day with Shell Free Artemia to the larvae for 10 days supported by aeration and flow of water.

2.7 Estimation of Fecundity, Fertilization, Hatchability and Survival Rate

Twenty-five hours after fertilization of sample (1g = 700 eggs) dead and unviable eggs which have turned whitish were collected after removal of plastic netting by siphoning and percentage fertilization determined. Percentage hatchability and survival were estimated at thirty hours and the fifth day after hatching respectively using the method described by Adebayo and Popoola, (2008) [3]. Thus, calculations involving relative fecundity, fertilization rate, hatching rate and survival rate are calculated as shown below

$$\text{Relative Fecundity} = \frac{\text{Total No. of eggs}}{\text{Body weight}}$$

$$\text{Fertilization rate (\%)} = \frac{\text{No. of fertilized eggs in a sample}}{\text{Total No. of eggs in a sample}} \times 100$$

$$\text{Hatching rate (\%)} = \frac{\text{No. of hatched eggs in a sample}}{\text{Total No. of eggs in a sample}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{No. of larvae alive}}{\text{Total No. of hatchlings}} \times 100$$

2.8 Water Quality Parameters

2.8.1 pH and Temperature

The water pH and Temperature were determined using a portable digital display pen type device embedded with pH meter and Thermometer (ATC 009(III) model). The electrode of the device was dipped into water up to immersion level and was allowed for about 4 minutes to stabilize readings before records were made.

2.8.2 Dissolved Oxygen (DO)

The Alsterberg (Azide) method was employed to determine dissolved oxygen. The water samples were collected using 250ml stopper bottle at 250ml below the water surface. The bottle was corked inside the water to avoid any trapping of air. The water samples were then fixed by adding 2ml of Manganese Sulphate and 2ml of Alkaline-iodide (Sodium Azide). The water was restoppered and a careful shaking was done for proper mixing. The samples were allowed to settle for few minutes and 2ml of concentrated Sulphoric acid was added. Careful mixing was done by shaking until a solution is formed. About 200ml of the solution formed was transferred

into a conical flask and titrated to pale yellow using 0.025N Sodium Thiosulphate. When 1ml of 1% starch solution was added, the solution turned blue immediately. Titration was carried out until the blue color first disappears. The volume of the 0.025N Sodium Thiosulphate used in the titration was recorded as the amount of oxygen in the water sample (APHA/AWWA/WPCF, 1995) [4].

3. Results and Discussion

3.1 Results

The results of trials are summarized in table 1 while that of water quality parameters are summarized in table 2. Spawning and hatching of larvae was observed in the African cattish. (*C. gariepinus*) treated with CPE, GO and NSD. Weight of spawners used in the experiment ranged from 1.6kg – 1.8kg. All the spawners irrespective of their size and weight responded well to hormone injection and spawned at 7 and 9hrs (Latency period) and hatched within 20-21hrs at temperature range of 29.56 -30.40 ºC.

Fecundity among treatments ranged from 203,000 – 210,000 while eggs diameter ranged from 1.2 – 1.3mm.The percentage fertilization ranged from 76.19 – 88. 37%. 62.50 –75.00% for hatchability and 55.20 – 68.66% for survival. The mean water pH ranged from 7.26 – 7.40, 29.56 – 30.40 ºC for mean water temperature and 4.70 mg/L for Dissolved Oxygen.

Table 1: Summary Result of Induced Spawning of *C. gariepinus* Using Synthetic and Non-Synthetic Hormones.

Parameters	CPE	GO	NSD
Body weight of			
Females (kg)	1.60	1.60	1.60
Fecundity	210,000	206,500	203,000
Relative Fecundity	131.25	129.06	126.87
Egg weight (g)	300	295	290
Egg Diameter (mm)	1.20	1.20	1.30
Fertilization rate (%)	76.19	88.37	87.93
Hatching rate (%)	62.50	75.00	72.50
Survival rate (%)	55.20	68.66	68.27
Latency period (hrs)	7.00	7.00	9.00
Incubation period (hrs)	21.00	20.00	20.00

Table 2: Summary Result of Water Quality Parameters

Parameters	CPE	GO	NSD
Mean pH	7.40	7.35	7.26
Mean Temperature (T ºC)	29.56	30.40	30.40
Mean DO (mg/L)	4.70	4.70	4.70

3.2 Discussion

The Summary result of the induction of *C. gariepinus* as indicated in table 1 revealed that mean weight of eggs was highest in *C. gariepinus* injected with Clarias Pituitary Extract and showed a significant difference from other treatments. The fecundity was significantly different among hormone treatments as that of *C. gariepinus* injected with Normal Saline Diluted Ovaprim (NSD) was lower. There was no significant difference among egg diameters of the different hormone treatments.

Fertilization rate was highest in *C. gariepinus* injected with Generic Ovaprim (GO) (88.37%) compared to NSD (87.93%) and CPE (76.19%). The percentage hatchability showed a similar pattern to percentage fertilization and was highest in *C. gariepinus* injected with GO (75. 00%) which was significantly different among treatments. Percentage survival rate ranged from 55.20 – 68.66% with similar patterns to fertilization and hatchability. The Latency period ranged from 7- 9hrs and the incubation period from 20 – 21hrs which was not significantly different among treatments.

The percentage fertilization of *C. gariepinus* injected Clarias Pituitary Extract (CPE) and Generic Ovaprim (GO) was 76.19% and 88. 37%. This is slightly higher than that reported by Adebayo and Popoola (2008)[3]. But, similar to that reported by Olumuji and Mustapha (2012)[5] in that treated with GO. This could be attributed to species differences and culture systems.

The percentage fertilization of *C. gariepinus* injected with Normal Saline Diluted Ovaprim (NSD) at 25% was 87.93% similar to that reported by Olumuji and Mustapha (2012) [5]. The percentage hatchability and survival of *C. gariepinus* injected with CPE and GO were (62.50 – 75.00 and 55.20 – 68.66%) above that reported by Adebayo and Popoola (2008)[3] and that reported by Olumuji and Mustapha (2012)[5] in that treated with GO and NSD. This could also be attributed to species differences and culture systems.

The summary result of the water quality parameters during the period of the experiment as recorded in table 2 showed that pH, Water temperature and Dissolved Oxygen were not significantly different among treatments and were within recommended standards for fish culture.

4. Conclusion

Hormone injection and stripping revealed that single intramuscular injection of CPE, GO and NSD induced ovulation in all the female Catfish at the specified dosages. The synthetic hormone Ovaprim (GO and NSD) was more efficacious and economical compared to the pituitary extract (CPE). However, the major purpose of this study is to present the efficacy of both hormones and how they can be harnessed at a single breeding regime to maximize profit while avoiding wastage. This research is targeted on the frugal use of resources to maximize profit. As pituitary extract and milt from male broodfish is potentially utilize alongside the Ovaprim in a single production in order to cut down cost of production while maximizing profit.

Good fecundity is the function of proper feeding as exhibited by the female gravids used in this research. My experience in fish spawning and management over the years thought me that. Well-fed male gravid broodfish will not need to be injected hormone. Well-fed female gravid broodfish will give excellent results in respect to fecundity, Latency period, egg mass, egg size, egg yolk, fertilization, hatchability and survival rate. Hatchery tanks or gutters be well aerated to enhance dissolved Oxygen, hatching and eliminate or reduce the effect of H_2S gas for normal healthy development of embryos. Water temperature can be regulated to enhance Latency, incubation and hatching.

Thus, concludes that the proper utilization and economical use of available resources in fish production is an added value to the function of profitability. That is, more profit to the fish farmer at low cost of production.

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