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Manipulation of Synthetic Hormones in Induced Breeding of Catfish *Clarias gariepinus* (Burchell, 1822)

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Abstract
The present study was conducted to determine the efficacy of two synthetic hormones (Ovaprim and Ovulin) to producing high number of fingerlings at reduced cost. 20 matured broodstocks of *Clarias gariepinus*; ten males, ten females, Ovaprim and Ovulin hormones were sourced. Broodstock of the ratio one: one were used. The females weighed 1.5 kg each were hypophysized respectively. A latency period of 14hrs was observed and the males were later dissected to obtain the milt used for fertilization. The number of eggs spawned was estimated using standard formula. The result revealed that, all the females hypophysized spawned including the control. Treatment (3) gave the best fecundity, fertilization, hatchability and survival rate of 122,500 eggs, 87.41%, 93.9% and 58.9% respectively which were significantly different ($p<0.05$) from other treatments. The research indicated that 75% Ovaprim combine with 25% Ovulin is recommended for induce breeding in *Clarias gariepinus.*

Keywords: ovaprim, ovulin, intramuscular, hypophysation, clarias gariepinus

1. Introduction
The world population is increasing daily and this might bring about shortage of food in a decade to come. It is important for us to profer solution to food production as a proactive measure and solving respectively. Proteins are important part of food for any population to be healthy and productive [15]. Among the different sources of animal protein, freshwater fish is considered as one of the most promising commodities that can contribute in increasing food production [15]. African Catfish is an important source of animal protein, constituting 23% of human daily animal protein intake [18] as cited by other workers [3]. They maintained that, it is an important food for over 400 million Africans, contributing essential proteins, minerals and micronutrients to their diets. Despite the high dependence on fish as a source of animal protein, fish consumption in sub-Saharan, Africa is the world's lowest [3]. This could be because of low production of fish fingerlings and can determine the low production of table size fish. However, the rate of fish consumption can be high, giving priority to massive production to fish seeds which can increase the availability of fish for consumption. More attention needs to be directed towards increasing fish production through aquaculture in Nigeria, because of the economic and nutritional importance of fish to the populace [21]. Researchers [24] postulated that, by 2025, one out of every two fish eaten should come from aquaculture. To achieve the statement made [24], we need a holistic approach from government, private and individuals in fish production.
One of the important areas in the field of aquaculture today, is the use of various hormones to induce spawning in fish. 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) and Carp Pituitary Extract (CPE). While synthetic hormones such as Ovaprim, Ovatide, Ovaryprim, Ovopel, Ovupin-L, Ovulin, Dagin and Aquaspawn among others have also been used to induce breeding successfully [9, 10, 27]. These spawning agents are either difficult to quantify (e.g. CPE) or to determine the efficacy (e.g. DOCA) or for shelf life (e.g. HCG) are usually expensive (e.g. Ovaprim) as established [22, 16]. The synthetic hormones now cost #6.500. Among these hormones, Ovaprim and Ovulin were featured as the best when treated with others.
The present study was aimed to determine the effectiveness of two synthetic hormones (Ovaprim and Ovulin) manipulation that best enhances fecundity, fertilization, hatchability and fry survival rate of *Clarias gariepinus*.

2. Materials and Methods
The present study was carried out from University of Jos, Plateau State, Nigeria. It is located between latitude 9° 93'N and longitude 8° 89' E at an elevation of 1186 meters above sea level. Brood stocks of *Clarias gariepinus* were sourced from Tetu Fish Farm, behind ECWA Staff Secondary School Jos Plateau State. Ovaprim and Ovulin hormones were also obtained from Green Water Fish Store, adjacent to Dilimi central pharmacy, opposite Plateau State Library, Jos.
Water samples from acclimatized broodstocks and hatchlings were collected fortnightly to determine the water quality parameters using the standard methods [4]. The parameters were pH; Temperature; Conductivity; TDS (total dissolved solid); Total alkalinity and Corbon (iv) oxide. Dissecting kit with surgical blade, a dissecting table, Weighing balances, Bouin's fluid (preservation) or 0.9% NaCl, Kakaban made

with nylon net, 6 bowls 20L, power supply, water supply, 1'' inch polyvinyl chloride (PVC) pipes, stainless(plastic) plates, bird's feather, gloves, plastic tray were purchased for surgical operation of the male.

There was a continuous flow of freshwater from the borehole empowered by Power Holding Company (PHC), NESCO and Generator alternatively when the need arises. The pumping of the water was monitored which helped in maintaining the flow-through system.

Ten graduated bowls of ten liters capacities were bought. The bowls were perforated at the bottom center and join with 3/4'' inch PVC to serve as incubator outlet for draining the water as more was coming in through 1/2'' inch PVC pointing downward on top of the bowls. The inlet was controlled through open and close cork fixed on the ½ " inch PVC while the outlet was determined by the inlet current. Kakaban made in circular form with nylon net was used. Method of hypophyzation was used intramuscularly above the lateral line just below the dorsal fin and above the lateral line. The manipulated concentrations were 100% Ovaprim (treatment 1), 100% Ovulin (treatment 2), 75% Ovaprim + 25% Ovulin (treatment 3), 50% Ovaprim + 50% Ovulin (treatment 4) and 25% Ovaprim + 75% Ovulin (treatment 5). The injected areas were massaged with a finger in order to make sure that the administered hormones concentrations doses were evenly distributed throughout the muscles and was also to prevent back flow of the hormones. The injected fish were kept in separate bowls of 10 litre capacity for a latency period of 14hrs at 24ºC overnight.

The milt from male brooders was extracted using methods of some researchers [25, 12] where the males were not sacrificed or killed as used by others [2, 20, 6, 5] where the male was sacrificed in order to remove the gonad (testes).

The male fish was placed dorso-ventrally on a wet disinfected white cloth spread on clean table with the head covered with a piece of wet towel. The surface of the abdomen was cleaned with part of a clean towel before incision was made on the ventral side of the abdomen. The incision portion was extended towards the head with the new blade 3-5cm long to expose the internal organs including the testes. One testis each was removed before suturing back using uninterrupted method with sterilized sewing needle. The operation was assisted by another person.

The female being ready for stripping, was removed using clean wet towel covering the head with one hand after reducing the water to the barest minimum to prevent struggling and subsequent wasting of egg. While the other hand was used to gently pressed the abdomen towards the posterior part with the assistance of another person holding the caudal part firmly using part of the clean towel[14]. Gentle pressure applied on the abdomen of the female brooder enabled the ovulated eggs to oozed out freely from the genital opening in to a clean, dried, stainless steel bowl without any contaminants. The removed testis was cut into small pieces using the new blade, then placed in a net mesh size 0.5mm and milt was finally squeezed out on the eggs in between a thumb and two fingers(dry method) after which the fertilized eggs were then incubated. Data collected were subjected to statistical analysis using one-way analysis of variance (ANOVA) and LSD

3. Results and Discussion

The mean values of water quality parameters of treatment tank are given in Table 1 and 2. Results indicated that there were no significant differences ($p>0.05$) between each of the water quality parameters of the different treatments in these investigations. It was observed that the required values for the Dissolved Oxygen (DO), pH, Conductivity, Temperature, CO_2, Total Dissolved Solids (TDS) and Alkalinity of 8.3mg/l, 6.4, 0.63ppm, 25.5ºC, 20.1ppm, 78.37mg/l and 85.14mg/l respectively recorded during the period of the study were considered to be within the limit of aquatic life survival [8, 11, 7] but was contrary to the works done by other researchers [1, 6] as there could probably have been variations in temperature and other environmental factors.

Table 3 showed that broodstock selected had the same weight of 1.5kg each, were induced with the manipulated synthetic hormones and results observed that the mean weight of their eggs were significantly different ($p<0.05$) from each other. This result is contrary to the other peoples work [25, 6, 19] where they stated that, the weight of eggs stripped corresponded to the weight of broodstocks injected. This could be because of different concentrations of the synthetic hormones manipulations. The highest weight of eggs (175g) was from a combination of 75% Ovaprim and 25% Ovulin treatment 3, followed by treatments 4 (169g), treatment 5 (165g) treatment 1 (161g) and treatment 2 (159g) in that order.

The results of the effect of Hormones Manipulation on Fecundity is shown in Table 3 where it was highest in treatment 3. This work corroborated with the work done by other researchers [1, 6, 19, 25] because they observed that fecundity is depended on the weight of eggs produced in respective of hormones concentration.

The result of Fertilization in Table 4 also indicated that treatment 3 had the highest number of fertilized eggs. This is in line with some published works [6, 19, 7, 23] but contrary to the work recorded by other people [28, 26] where they reported that fertilization does not follow fecundity. This could be because of the quantity and viability of the sperm used.

The result of Hatchability in Table 4 showed that treatment 3 had the highest hatchlings. This follows the pattern of fertilization [5, 17, 6, 23]. The result of Survival rate in Table 5, recorded the highest in treatment 3. This did not follow the train of hatchability [28, 26] where they reported that survival rate is independent of the hatchlings produced. This could be because of factors like the state of the broodstocks used, time of stripping and the management skills. In this study, the effect of hormone manipulation on fecundity indicated that the higher the weight of eggs produced, the higher the fecundity. Similarly, fertilization, hatchability and survival rate were observed to follow the same pattern and were significantly different ($P<0.05$) than other treatments. The results showed that treatment 3 had the highest fecundity (122,500 eggs), fertilization (87.41%), hatchability (93.9%) and fry survival rate (58.9%) which were significantly different ($p<0.05$) from other treatments.

Table 1: Mean Values of Water quality parameters of Broodstocks (*Clarias gariepinus*).

Parameter	Values
Dissolved Oxygen(DO) mg/L	8.30± 0.95
pH	6.40 ±0.44
Conductivity(µs/cm)	0.63 ±0.12
Temperature(˚C)	24.50 ±1.24
Carbon Dioxide (mg/L)	21.10 ± 0.35
Total dissolved solids(TDS) (mg/L)	78.37 ±1.67
Alkalinity(mg/L)	85.14 ±21.13

Table 2: Mean Values of Water quality parameters of Hatchlings (*Clarias gariepinus*).

Parameter	Values
Dissolved Oxygen(DO) mg/L	8.30± 0.95
pH	6.40 ±0.44
Conductivity(µs/cm)	0.63 ±0.12
Temperature(ºC)	25.50 ±0.35
Carbon Dioxide (mg/L)	20.10 ± 0.35
Total Dissolved Solids(TDS)(mg/L)	78.37 ±1.67
Alkalinity(mg/L)	85.14 ±21.13

Table 3: Mean Induced Ovulation and Spawning of *Clarias gariepinus* using two synthetic hormones (Ovaprim and Ovulin) and effect of the hormones on weight of Eggs and Fecundity.

TRT	Wt of fish(g)	Wt of eggs(g)	T. No of eggs
TRT1	1500	161	112700
TRT2	1500	159	111300
TRT3	1500	175	122500
TRT4	1500	169	118300
TRT5	1500	165	115150
Mean	1500	166±2	116130±2240

Where TRT=Treatment; N.S= Normal Saline; Ovp=Ovaprim; Ovl=Ovulin;
T=total; ml=mil; g= gram; no=Number

Table 4: Mean Effect of Different Doses of Two Hormones (Ovaprim and Ovulin) Concentration on Fertilizaton and Hatchlings Rate of Clarias gariepinus.

Trt	T. Fert. Eggs	% Fert.	T. Hatchlings	% Hatch.
TRT1	81703	72.50	62284	76.22
TRT2	83912	75.40	68168	81.25
TRT3	107072	87.41	100511	93.90
TRT4	88958	75.20	75392	84.75
TRT5	88320	76.70	69058	83.35
	89993±5073.8	77.46±2.98	75083±7645.4	83.89±3.54

Table 5: Mean Effect of Hormones Manipulation on Hatching and Survival Rate of *Clarias gariepinus.*

TRT	T. HATCH.	% HATCH.	T. SURVIVAL	% SURVIVAL
TRT1	62284	76.22	23742	38.12
TRT2	68168	81.25	24968	36.63
TRT3	100511	93.90	59202	58.90
TRT4	75392	84.75	36809	48.82
TRT5	69058	83.35	25500	36.93
MEAN	75083(7645)	83.89 (3.54)	34044(7092)	44(4.44)

4. Conclusion

The cost of synthetic hormones is very expensive at not less than #6500.00 per bottle (10ml). Getting carp pituitary extract is tedious, laborious and time consuming. The cost of carp fish is about #1000.00, while the use of urine from pregnant women is also tedious, laborious and has negative thinking from the donors(not easy to be convinced), farmers and consumers alike. But 0.4ml and 0.1ml of Ovaprim and Ovulin respectively at the cost of 500.00 per ml and combining the percentage fraction will go a long way in producing a large number of fingerlings at a lower cost. This work has given an insight to fish breeders to buy synthetic hormones in fractions and combine which is cheaper and effective.

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