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Egg Biopsy and Spawning Performance of Catfish (Burchell, 1822) using Ovaprim and Chicken Pituitary Hormone

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ABSTRACT

Study on egg biopsy and spawning performance of cat fish (*Clarias gariepinus)* induced with Ovaprim and Chicken Pituitary Hormone (CPH) was carried out to determine the effect of Ovaprim and CPH on fish egg (Oocyte) migration and the resultant effect on fertilization and hatchability percentages. Sixteen *C. gariepinus* brood stocks (sex ratio 2:1) between 1.1kg and 2.3kg in weight and four layers birds (*Gallus gallus domesticus*) between 1.2kg and 2.2kg in weight were used for the experiment. Extraction of CPH from layers birds was carried out to induce brood stock fishes. Treatment 1 was used for Ovaprim and replicated thrice whilst Treatment 2 was used for CPH and also replicated thrice. An indoor slow flow- through system vat measuring 2 ft x 6 ft x 1 ft each was used for the incubation. Water test kits and Table top scale were used to monitor essential physicochemical parameters and measurement of the fish brood stock and Chicken layers weights individually. The mean values of egg biopsy, fertilization and hatchability percentages in Ovaprim and CPH treatments was not significantly different at 5% level of probability. Also, there was no significant difference at 5% level of probability in the physiochemical parameters. This study showed that both Ovaprim and CPH induced egg ripening and migration of egg nucleus to the micropyle for effective fertilization and spawning in African catfish therefore, CPH could be recommended on the basis of spawning effectiveness and availability of the pituitary from the chicken slaughter/processing house with little or no cost.

Key words: Biopsy, Breeding, Ovaprim, Chicken Pituitary.

INTRODUCTION

Artificial fertilization and incubation of ripened fish egg is known as induced breeding. It involved environmental manipulation and the use of fish reproductive materials for the hatching. The advantage of this over natural breeding is higher rate of fertilization and hatching, better conditions for growth and survival of larvae to fingerling and better protection of larvae against unfavorable environmental condition and predators (Woynarovich and Horvath, 1980). The materials that are generally used for induced breeding comes with their different brand names and types, for example, Deoxycorticosterone Acetate (DOCA), Human Chronic Gonadotropin (HCG), Common carp *(Cyprinus carpio)* pituitary gland material, Ovaprim (Salmon Gonadotropin Releasing) and African Catfish pituitary are all non - synthetic hormones. However, this practice is usually employed on the fish that cannot breed in captivity but with high economic value and demands. One of the fish that fall into this category in this part of the continent (Nigeria) is *Clarias gariepinus*,

According to Adebayo and Popoola, (2008) *Clarias gariepinus*, is a freshwater fish of economy importance and are commonly bred in captivity through environmental condition manipulations, use of reproductive materials, synthetic or non-synthetic hormones for the purpose of mass-scale production of fish fry to take care of the pressing demand of *Clarias gariepinus* seeds for potential farmers. Artificial propagation of *C. gariepinus* under more controlled conditions and manipulation which include, stripping of eggs, collection of the milt, followed by fertilization of eggs is usually carried out in hatchery unit with hormonal inducement of either piscine or non-piscine hormone (Woynarovich and Horvath, 1980).

A non-piscine hormone from the poultry layer bird known as *Gallus gallus domesticus* for fish induced breeding/hypophyzation has been given a trial. *Gallus gallus domesticus* is one of the domesticated birds, a subspecies of the red jungle fowl. It is one of the most

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common and widespread domestic animals, with a total population of more than 19 billion as of 2011 (FAO, 2011). Male chicken is called rooster while female is been referred to as hen (Info on chicken care, 2003). The female chicken (hen) reproductive system consists of right and left ovary and oviduct which are present in embryonic stages and attached by the mesovarian ligament at the cephalic end of the left kidney respectively. The female chicken sex is called estrogens and function alongside with pituitary gland also known as hypophysis that secretes gonadotropin (Johnson, 2000).

The viability and maturity of fish eggs in induced breeding cannot be overemphasized, therefore, the fish egg biopsy need to be conducted in order to increase the fertilization and hatchability percentage of the stripped fish eggs. Hence, Fish egg biopsy is the examination of stage of development of live eggs from a live fish (Rothbard, 2010) to determine the migration percentage of oocyte for effective fertilization.

The objective of this study is to determine the effect of hormone (Ovaprim) and Chicken Pituitary Hormone (CPH) on Oocyte migration for effective fertilization and hatchability.

MATERIALS AND METHODS

A privately owned fish farm hatchery known as "Positive Farm" was used for this study due to the adequate hatchery facilities with indoor flow through system vat measuring 2 ft x 6 ft x 1 ft each. This farm is located at government assisted Fish Farm Estate in Ikorodu, Lagos State. The study was conducted in units thus, preparation of *C. gariepinus* brood stock (both males & females) for induced breeding, mounting of laboratory and hatchery facilities, female fish egg biopsy, preparation of chicken (female) pituitary hormone, brood stock priming induction (both males and females), observation of latent/ conditioning period, egg stripping and fertilization, egg treatment, egg incubation.

Experimental Design and Brood stocks Selection

The experiment was divided into two (Ovaprime treatment and Chicken pituitary treatment) and each treatment was replicated thrice making a total number of 6 experimental units. The hatchery unit runs on indoor flow through system. All accessories were assessed before the commencement of the breeding and physicochemical characteristics of the water was tested using water test kit (Ezdo Model PCT 407) to determine the suitability of water quality characteristics.

Eighteen (18) gravid brood stocks (12 females and 6 males) at ratio 2:1 of *C. gariepinus* with weight ranged between 1.1kg and 2.3kg were selected and weighed using Ohaus Scout Pro Balances Models SP-601 and subsequently acclimatized for five (5) days before the commencement of induced spawning in plastic tank of 1000 litres capacity filled with clean fresh water up to ¾ (About 700litres).

Chicken layers were selected and weighed using table top scale model CWS-3 with accuracy of 0.2gm. The weight ranged between 1.2 and 2.2kg. The chicken layers were acclimatized for five (5) days in a separate pen and fed with layer's mash ration ad- libitum.

Female Fish Brood Stock Biopsy

Fish egg biopsy is the examination of stage of development of live eggs from a live fish (Rothbard, 2010). The handling facilities for this exercise includes, bath tubs, scoop nets, bowls, syringes, hand towels, 2- phenoxy-ethylene solution (anesthesia), digital weighing scales, sera solution, Petri-dishes, droppers and compound binocular microscope. During the exercise, each female brood stock was taken out from the holding tank using a rectangular net panel scoop net. The fish was put into a bath tub of tranquilizer (10mls/l of 2-phenoxy-ethylene anesthetic solution) through a cylindrical cloth scoop net for 10 to 15 minutes thereafter the sedated brood stock fish are wiped using hand towel and weighed individually using electronic digital weighing scale and the weight recorded. After weighing, a sample of eggs was sucked from each female using a catheter.

The brood stock fish are returned to the aerated holding tanks immediately the egg samples had been siphoned from them. The eggs were gently released into a glass Petri-dish and about 10 to 15mls of sera solution added to clear the egg mass for proper observation under the microscope. The eggs-sera solution mixture was mounted onto a binocular compound microscope and observed under low power and magnification of x4. The microscope was adjusted to observe the germinal vesicle (G.V) movement and the stage of Oocyte development according to Rothbard, (2010).

Chicken Pituitary Hormone and Ovaprim Preparation and Inducement

Ovaprim does not require any special preparation. It is a commercial product that contains a salmon gonadotropin releasing analog and domperidom which helps to block the inhibitory effect of dopamine (Hill *et al*., 2005). The dosage was divided into two parts, thus, 20% for induction and 80% for resolution. Six females were injected each with 20% of 1.5ml of Ovaprim as a priming dose between 10.39hours to 10.42hours and a resolving dose of 80% at midnight (00.00hours) respectively. The males were injected with Ovaprim once, all at midnight and kept in a separate holding water tank. The conditioning/ ovulation temperature was maintained at24^{o}C in average while dissolved oxygen was improved through electric air pump and the holding tanks remained covered with netting material.

Preparation of Chicken Pituitary Hormone (CPH) was done by using four hens (layers) in ratio 1:3. The four female chickens were slaughtered and the skull compartment was opened towards the ventral's side and the pituitary gland was removed by the use of sterilized lancet. The pituitary gland was homogenized and 0.9% saline solution was added to it to make 100% hormonal solution and afterward centrifuged. The supernatant was withdrawn with calibrated syringe in preparation for induced breeding. Six females were injected each with 20% of 1.5ml of chicken pituitary Hormone as a priming dose between 10.40hours and 10.50hours, and a resolving dose of 80% at midnight between 00.00hours and 00.30hours) respectively. Each male was injected once with chicken pituitary Hormone at midnight and kept in a separate holding water tank. The conditioning/ ovulation temperature was maintained at 24^{o}C in average while

dissolved oxygen was improved through electric air pump and the holding tanks remained covered with netting material. All the induction in the experiment was administered intramuscularly, a little distance away from the head region as described by Hill *et al*., (2005).

Egg Stripping, Fertilization and Incubation

Final maturation and ovulation of the eggs are determined by inspecting the holding tank bottoms to check for the presence of eggs released or observation of the size of the belly of the females by applying gentle pressure vertically along the belly to see if eggs could freely ooze out. Female fishes that were ready are anesthetized with 2- phenoxy-ethylene solution before stripping. Healthy and developed eggs will be transparent with green-brownish coloration while the whitish eggs are those that were not healthy. The eggs were quantified by weighing them as a mass and the number obtained as the product of total eggs weight and the weight of one egg. The milt/ sperm from each male were obtained by sacrificing the male fish brood stock by removing the two lobes of the male's testes and cleaned with tissue paper and kept in a cleaned petri dish. The testes were cut open and squeeze on the ripped fish eggs and were fertilized in round bottomed sterile plastic bowls. The egg mass was homogenized using an electronic homogenizer before Fertilization. Fertilization was done by dry method thus, milt added to eggs in a dry container. Saline solution was added to the fertilized eggs to activate sperm and fresh cow milk was added to remove the stickiness of the eggs at a rate of 3% and continuously stirred in the homogenizer for 15minutes and the fertilized eggs were spread on a kakaban or spawning net of 1mm in the spawning trough and the flow through system of the hatchery unit was activated.

Hatching commenced between 20.00 hours and 48.00hours after fertilization. The hatched fries escaped through the 1mm net gauge into the vat underneath while the un-hatched eggs remained on the net. Unhatched eggs were removed from the incubator by siphoning to prevent water pollution. Feeding commences on the 4th day after their yolk sacs have been completely absorbed and were fed with small quantity of processed *Artimia salina* in *ad-libitum* at 2hours interval. During this period, water quality monitoring is an essential aspect of fish fries management (Hogendoorn *et al*., 1980). All the water quality parameters were adequately monitored

Fish Induced Breeding Parameters

Biopsy was determined by computing the percentage of developed Oocyte and migrated germinal vesicle to the micropyle for effective fertilization based on the total number of sampled eggs in the petri-dish observed under the compound microscope as described by Rothbard, (2010).

Fecundity: Initial weight – final weight of females x 66.6. This was proposed by Ayinla and Nwadude, (1988). Ezenwa, (1987) reiterated the formula for computing relative egg to body weight as (x/y) x 100/1 where: x = weight of egg for each female fish and y = weight of the female fish before injection. Also, Latency period is been calculated as Time interval between injections of the female fish and stripping of eggs.

Pseudogonadosomatic index (PGSI) can be determined using the formula;

$$\text{PGSI} = \frac{\textit{Weight of eggs collected by stripping}}{\textit{Body wt before injec} - \textit{wt of stripped eggs}} x\ 100/1$$

While, Fertilization (%) = $\frac{Numberoffertilizedeggs}{Totalnumberofeggsincubated} x\ 100$ (Ugwumba and Ugwumba, 1998).

$$\text{Hatchability \%} = \frac{Number\ of\ hatchlings}{Total\ number\ of\ fertilized\ eggs}\ x\ 100$$

$$\text{Survival Rate (After five days of hatching)} = \frac{Number\ of\ living - No\ of\ died\ hatching}{100}$$

$$\text{Relative weight gain (\%)} = \frac{\text{Initial larvae weight}}{\text{Final larvae weight}} x100$$

Statistical Analysis

The data collected were analyzed for significant differences by Analysis of variance (ANOVA) and Pearson Correlation using SPSS for windows (V.25.0).

RESULTS

The results of artificial breeding techniques and fish eggs biopsy trials are presented in Table 1 and 2 respectively for subsequent empirical discussions.

Table 1 shows the results obtained from *Clarias gariepinus* induced breeding and biopsy indices using Ovaprim and Chicken Pituitary Hormone. The initial average mean weights of female brooder fish for Ovarprim and Chicken Pituitary Hormone (CPH) for inducement were2200±100 g and 1266.67±208.17g respectively. The final mean weights after stripping were 2033±57.74 g and1000±0.00 g respectively and the weight in both treatments are significant at at 5% level of probability. The calculated percentage relative egg to body weight of fish for Ovaprim and CPH treatments were 0.66±0.015 % and 0.72±0.05 % in that order

Egg biopsy is the migration of egg nucleus to the egg micropile for effective fertilization. The mean percentage migration of egg nucleus in treatment 1 (Ovaprim) was75.4±1.15% while that of Chicken Pituitary Hormone was 77.83±1.89%.

Latency period is the duration or number of hours it takes the female broader fish to become ready for stripping. The mean hour for the latency period of female fish on Ovaprim treatment was 9.53 ± 0.54hours while in the Chicken Pituitary Hormone (CPH), the mean was 9.77 ± 0.49 hours. There is no significant difference in the hours at 5% level of probability. The mean weight of the eggs stripped from female brooder on Ovaprim inducement was 14.47±0.91g and the mean weight of eggs from Chicken Pituitary Hormone (CPH) inducement was 12.73±3.00g. There is no significant difference in the weight of fish eggs stripped from both treatments at 5% level of probability. Fecundity is the total number of eggs a fish was able to produce after the latency period. The mean of the fecundity in the Ovaprim treatment was 11100±7690while that of Chicken Pituitary Hormone (CPH) was 17760±13863.

Table 1: Spawning performances and egg biopsy of *Clarias gariepinus*

Parameters	Ovaprim Treatment and Replicate				Chicken Pituitary Hormone (CPH) Treatment and Replicate			
	T1R1	T1R2	T1R3	Mean(±SD)	T2R1	T2R2	T3R3	Mean(±SD)
Initial weight of female brooder fish (g)	2300	2100	2200	2200±100	1200	1100	1500	1266.67±208.17
Final weight of female brooder fish (g)	2000	2000	2100	2033±57.74	1000	1000	1000	1000±0.00
Weight of egg stripped (g)	15.30	13.50	14.60	14.47±0.91	9.00	8.08	10.10	12.73±3.00
No. of eggs produced (1g = 650)	9945	8775	9490	9403.33±589.80	5850	5252	6565	5889±657.37
Relative egg/body weight((x/y) x 100	0.67	0.64	0.66	0.66±0.015	0.75	0.74	0.67	0.72±0.05
Pseudogonadosomatic index (PGSI)	0.67	0.65	0.67	0.66±0.01	0.76	0.74	0.68	0.73±0.04
Fecundity	19980	6660	6660	11100±7690	13320	6660	33300	17760±13863
Biopsy (%)	75	74.5	76.7	75.4±1.15	76.5	77	80	77.83±1.89
Latency Period (hrs)	9.15	9.30	10.15	9.53±0.54	9.20	10.00	10.10	9.77±0.49
Fertilization (%)	99.45	87.75	94.90	94.03±5.90	90	80.8	101	90.6±10.11
Hatchability (%)	75.86	74.69	76.25	75.6±0.81	81.55	68.16	88.35	79.35±10.27

Table 2: Essential Physicochemical Water Parameters

Day	Temp. (^{O}C)	DO (mg/l)	pH	NH_3 (mg/l)	NO_2 (mg/l)
1	26.0	6.0	6.5	0.45	0.04
2	26.5	5.4	5.9	0.42	0.02
3	27.0	5.2	6.2	0.46	0.04
4	25.8	6.0	6.8	0.48	0.05
5	27.5	5.9	6.0	0.50	0.05
Mean	26.56	5.7	6.28	0.46	0.04
±SD	±0.70	±0.37	±0.37	±0.03	±0.01

The mean deviation of Pseudogonadosomatic index (Pgsi) in Ovarprim induced brooder fish was 0.66±0.01while the Pseudogonadosomatic index (Pgsi) of the Chicken Pituitary Hormone (CPH) mean deviation was 0.73±0.04. The Pseudogonadosomatic index (Pgsi) in Ovaprim treatment was 5.99% whereas in Chicken Pituitary Hormone (CPH) it was 9.85%. They are significant at 5% probability levels. The mean percentage fertilization recorded in Ovaprim treatment was 94.03±5.90% but in Chicken Pituitary Hormone (CPH) it was 90.6±10.11%. Therefore, it is significant at 5% level of probability. The hatchability mean percentage in Ovaprim treatment was 75.6±0.81 %and that of CPH was 79.35±10.27 %. The hatchability rate in Ovaprim and in CPH treatments is significant at 5% level of probability.

Table 2 shows all the essential physicochemical characteristics recorded during the induced breeding experiment. The water temperature during the trials ranged between 25^{o}c and 27^{o}c with average mean of 26.56±0.70^{o}c while dissolved oxygen measurement in the experiment ranged between 5mg/l and 6mg/l with average mean of 5.7±0.37. These two parameters are significant at 5% probability level and are negatively correlated. The pH of the water in Ovaprim and Chicken Pituitary Hormone treatments were recorded respectively and ranged between 5.9 and 6.8 with average mean of 6.28±0.37 and is significant at 5% level of probability. Ammonia and Nitrate concentrations in the 2 treatments were adequately monitored and recorded accordingly. This was found to be significant at 5% level of probability and the recorded value ranged between 0.42mg/l and 0.50mg/l for ammonia, 0.002mg/l and 0.05mg/l for Nitrate. There is positive correlation between Ammonia and Nitrate concentrations. All the essential physicochemical parameters observed fall within the standard range for fish hatching and breeding techniques.

DISCUSSION

In order to meet the teaming demands for the good quality fish seeds, the application of induced breeding techniques especially for those fish species that cannot breed in captivity such as *Clarias gariepinus* is essential. Artificial spawning of *C. gariepinus* for good quality fish seeds can only be achieved by the use of matured brood stock fish induced with pituitary hormone.

The importance of weight of brood stock fish for induced breeding cannot be over-emphasized as shown in the results from the experiment. Consequently, the mean weights of fish used in this study followed the suggestions of Saraswati (2018) that fish of about 2 to 4 years of age with weight between 1kg and 5 kg should be used for induced breeding. It is therefore shows from the result the significant of fish weight and relative egg/body weight of the fish in the breeding processes. An increase in the size of the brood stock increases the weights of egg and the number of the eggs produced. This corroborates the report of Bichi *et al.,* (2014) on relationship between brood stock size and egg weights.

In Table 2, Ovaprim treatment and CPH treatment met and exceeded the standard migration percentage of 70% for germinal vesicle (G.V) movement in the induced breeding techniques as proposed by Rothbard (2010). This shows that information on gamete physiology is paramount before induced breeding can be employed as reiterated by Bozkurt and Secer (2006) and supported by the publication of The Royal College of Pathologists (2019) on examination of live eggs under a microscope to determine the stage of oocyte development and the extent of germinal vesicle (G.V) migration to micropyle for effective fertilization which is often expressed in percentage (%). This could perhaps be one of the contributing factors to the success recorded on the induced breeding experiment.

The latency period in fish breeding is the time between the first hormonal injection and ovulation and female fish and stripping of eggs (Rahdari *et al.,* 2014). The latency period in treatment 1 ranged between 9.15hrs and 10.15hrs while that of treatment 2 ranged between 9.20hours and 10.10hours. None of the latency periods of these experiments exceeded the prescribed maximum latency period of 14 hours as reported by Zonneveld *et al*., (1988) and Agbebi *et al.,* (2013). This could be as result of favorable environmental condition such as water temperature which plays vital roles in determining the latency period of the brooder female fish as described by Stephen *et al*., (2005). The Temperature range and the latency period recorded in the experiment disagreed with FAO recommendation of optimum temperature of 25°C and that of latency period of about 11-13 hours (FAO, 1996). The difference might be due to geographical location (Tropical region) of the test animal and their adaptation to high temperature. All the essential physicochemical parameters range falls within the permissible threshold limit for the breeding and survival of fish larvae (Boyd and Lickotkoper, 1990; Babalola and Agbebi 2013).

The seasonal spawning potential between current and next spawning period in a female fish is being referred to as fecundity (Bichi *et al.,* 2014). In Table 2, it is obvious that the weight of the brooder fishes and their fecundity are positively correlated as explained by The Fish Site (2019), This shows that fecundity increases with the body weight of the fish as previously mentioned in the scientific report of Zamidi *et al.* (2012) on fecundity of *Eleutheronema tetradactylum* in Malaysian Coastal Water.

The fish egg size, maturity, and eggs biopsy percentage determine the fertilization success rate as showed in the fertilization percentage of fish on Ovaprim **(**94.03 %) and on CPH (90.6 %) respectively. Bozkurt and Secer (2006) emphasized that egg size and maturity are essential factor that directly affect the fertilization result. Also, Rothbard (2010) advocated for 70% and above GV migration for a successful fertilization. The results obtained on fertilization from this experiment corroborate the findings of these two researchers. The high percentage hatchability in this study for Ovaprim and CPH treatments might be due to effective fish egg fertilization and good quality milt which produces large numbers of fries. Also, healthy egg yolk could also be responsible for the high percentage hatchability as reported by Bichi *et al.* (2014).

Moreover, the results obtained from the experiment support the findings of other researchers on the use of pituitary from non- fish origin in fish hypophysation in the use of Black rat (*R. rattus*), Bull frog (*R. aspersa*), and Toad (*Bufo regularis*) (Madu *et al.,* 1999; Sule 1999, Salami *et al.,* 1992; Fagbenro *et al.,* 1991 and Nwoko 1985) as reiterated by Sunnuvu, (2004) on artificial breeding techniques for *C. gariepinus* using different types of Non-Pisces hormones.

Conclusively, it is evidence that Ovaprim and CPH can enhance spawning performance in *C. gariepinus* from the view point of insignificant difference in fertilization and hatchability percentages. However, Chicken Pituitary Hormone (CPH) could be used as a replacement for other commercial hormone products in the induced breeding of *C. gariepinus* due to its cost effectiveness and availability from the slaughter/ processing house.

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