Effect of Non-Synthetic Hormones (Dry and Wet Pituitary Gland) and Synthetic Hormone (OVULIN) on Spawning Performance of African Catfish *Clarias gariepinus*

Bawa, D. Y. and Masud Ahmed
Department of Forestry and Fisheries,
Kebbi State University of Science and Technology, Aliero
Corresponding author: bawadal505@gmail.com

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Abstract
The study is designed to find out the effect of non synthetic hormones (dry and wet pituitary gland) and synthetic hormone (ovulin) on spawning performance of catfish clarias gariepinus. The research was carried out at the hatchery unit of the Fisheries Department, Kebbi State University of Science and Technology Aliero, (KSUSTA). Ten (10) matured brood stocks measuring about 900g to 1000g was sourced from Labana Farm Aliero and transported to Fisheries Lab KSUSTA for this study. Two (2) males and three (3) females' broodfish was selected base on their quality for breeding purposes. Three (3) female brood stocks were divided in to three (3) treatments. T1, T2 and T3 respectively, each treatment was injected with the different hormones. T1 was injected with dry CgPGE hormones of 1ml/1kg, T2 was injected with wet CgPGE hormone of 1ml/1kg and T3 was injected with synthetic hormone (ovulin) at rate of 0,5ml/kg. The rate of fertilization is higher at the broodstock treated with T1 (88%) comparing it with the T2 & T3 that have (86%, 86%). The latency period of between 9.45 - 12.09hrs for all the treatments. The female injected with wet CgPGE has the lower percentage of hatchability ($88.00^{b} \pm 14.57$) and T1 has the higher percentage of the hatchability ($196.67^{a} \pm 27.28$). The Survival rates also followed the same pattern with the hatchability rate. The survival rate was higher in T3 & T1 respectively. It's concluded that the fish treated with wet CgPGE has the high percentage of survival rate (35), followed by the fish treated with dry CgPGE (33). The synthetic ovulin has the lower percentage of survival rate although it has the high percentage of hatchability rate followed by dry CgPGE hormone.

Key words: *Non-synthetic, Synthetic, Hormone, Spawning, Performance and African catfish*

INTRODUCTION

As the population increases the demand for high food resource also increases, the Population growth has a major impact on food availability, as it results in both increased in competition for available resources and demand for food resources, this make researches and famers to shift focus on production and development of aquatic resources in order to meet the global demand The role played by aquaculture in socio economic development of any society cannot be over emphasized. It is geared towards diversifying fish production to meet local consumption, generate employment and also to increase opportunities of foreign exchange earnings (Mustapha 2020)

Catfish exhibits many qualities that make it suitable as an aquaculture candidate. These include ability to withstand stress, disease resistant, fast growth rate, high yield potentials, high fecundity and good taste among others. They can also withstand low dissolved oxygen (D.O) and pH level and also grow on turbid water (Nwadukwe, 2003). *C. gariepinus* shows a seasonal gonadal maturation which is usually associated with the rainy season. The maturation processes of *C. gariepinus* are influenced by annual changes in water temperature and photoperiodicity and the final triggering of spawning is caused by a raise in water level due to rainfall (de Graaf *et al*., 1995).

Good quality and quantity of fish seed is remain the problems of many fish farmers in Nigeria. The poor quality fish seed in many states of the federation and lack of functional fish hatcheries where fish farmers can purchase fingerling and juveniles to stock their pond remain the challenges of fish production in Nigeria. (Omitoyin, 2009). Farmers have to travel long distance to source the fish seeds, or collect from the open waters bodies (wild), Even there are hatcheries in some cases, fingerlings of poor genetics quality are produced this resulted in stunted growth of fish, poor survival rate, and poor returns on an investment (Akintunde 2009). This because of the high cost of hormonal treatment used for artificial breeding, so new research is needed to carry out in finding a possible ways of artificial breeding with used of natural input that are found easily in our natural environment and maximize the cost of production for batter returns in investment. The aim of this research was to determine the effect of dry pituitary gland, wet pituitary gland of *C. gariepinus* and synthetic hormones (ovulin) on catfish (*C. gariepinus)* breeding.

MATERIALS AND METHODS

Study Area

The experiment was conducted at Kebbi State University of Science And Technology Aliero (KSUSTA) Fisheries Lab Unit of Department of Forestry and Fisheries, Faculty of Agriculture, Aliero Local Government, Kebbi State Nigeria. It is bordered in the North-West by Gwandu Local Government Area, in South-West by Jega Local Government and in the North-West by Binin Kebbi Local Government and in the East by Tambuwal Local Government of Sokoto State. The University is located between latitude 12˚16'42"N and longitude 4˚27'6"E of the equator. The annual temperature varies considerably but usually ranges between 26˚C to38˚C. There are two distinct season in the regime the rainy season which started usually around May and last till around September and dry season from October to April. The annual rainfall is frequently erratic and varies distributed from 500mm to 1300mm per annum (Smanr, 1998).

Sample Collection

Ten (10) matured brood stocks measuring about 900 to 1000g was source from Labana Farm Aliero and transported using 25 liters Jerry can around 7.00am to Fisheries Lab KSUSTA. They were acclimatized for five (5) days prior for treatment and selection. Two (2) males and three (3) females' broodfish was selected base on their quality for breeding purposes.

Feeding of Brood Stock, Selection and Injection

The brood stock was fed with commercial diet (6 mm coppens) and thereafter selected based on certain criteria. Males were examined for rigid and reddish infusion of the genital orifice particularly at the tip while females, gentle orifice, and distension of the belly and release of eggs when gentle press was applied on the abdomen. The selected samples were properly maintained separately by ensuring good water quality management and adequate feeding before being used for breeding. Tow (2) matured females brood stocks was treated with the same dose of natural (CgPGE) 1ml/kg and 0.5ml/kg of ovulin. The injection is given at intramuscular at angle of 30˚ between dorsal fins and lateral line.

Pituitary gland extracts collection

The pituitary gland was extracted from five (5) male's mature *clarias gariepinus* (CgPGE) two days before breeding. The head of the male's donor was cut off after stunning the fish, and subsequently the lower jaw was also cut off. The ventral side of the brain was opened carefully to expose the pituitary gland. Glands were collected. Three CgPGE was open dry inside the room temperature of 34ºC for two days inside conical flask and two CgPGE was place in a bottle of ethanol sprite and keeps it froze.

The dry CgPGE was grinded using motel and pistol and 0.9mlg/L normal saline solution was added, glands suspension was collected into a 2ml syringe at the range of 1ml/kg, and also wet CgPGE was diluted with saline solution and collected 1ml/kg, freshly collected pituitary gland extract (CgPGE) was immediately injected into the female *Clarias gariepinus.* And 0.5ml /kg of ovulin respectively was injected to another female broodfish and the broodstock was divided in to three treatments.

Milt and Eggs Collection and Incubation

Dry fertilization method was use for fertilizing the eggs. Eggs was stripped from females brood stocks into a clean plastic bowl by applying gentle press on both sides of the abdomen towards the genital opening after a minimum latency period of each treatment, with water temperature of about 30˚C. Male brood stock was sacrifice to collect the milt. The milt and eggs was then mixed together gently with a plastic spoon for 1-2 minutes. Small quantity of saline solution was poured onto the eggs to avoid sticking together. The fertilized eggs was then rinsed with saline solution and introduced into the incubation chamber for incubation. Spawning net placed inside the incubation chamber, clean water was used for the purpose.

Fertilized eggs were spread in a monolayer on the spawning net in the incubation chamber. Aeration was maintained in the incubation chamber. The dead eggs on the net were removed and those that fell into the container were siphoned. Water quality parameters such as temperature, Dissolved Oxygen and pH was monitored and maintained at optimum levels.
After fertilizing the eggs each treatment was kept in separate incubation chamber to determine the effect of dry , wet and ovulin hormones on *clarias gariepinus* breeding and how their support the incubation of eggs for about 72hours.

The percentage of fertilization, hatchability and survival rate was determined using the following formula.

$$\% \text{ Fertilization} = \frac{\text{Total number of fertilized eggs}}{} \times 100 \quad \text{(equation 1)}$$

$$\% \text{ Hatchability} = \frac{\text{Total number of hatched eggs}}{\text{Total number of eggs stripped}} \times 100 \quad \text{(equation 2)}$$

$$\% \text{ Survival} = \frac{\text{Total number of survived fry after 72 hours}}{\text{Total number of hatched eggs}} \times 100 \quad \text{(equation 3)}$$

Experimental Design

Three (3) female brood stocks were divided in to three (3) treatments. Treatment 1, treatment 2 and treatment 3 respectively, each treatment was injected with the different hormones.

Treatment 1; A mature female brood stock was injected with dry PGE hormones at rate of 1ml per 1kg.

Treatment 2; A female brood fish was injected with wet PGE hormone at the rate of 1ml per 1kg as treatment 2.

Treatment 3; A female brood stock was injected with synthetic hormone (ovulin) at rate of 0,5ml per kg as treatment 3.

The injected fish was kept separately in well-balanced containers containing water. The containers with the injected fish were covered to prevent the fish from leaping out before latency period.

Research Instruments and Materials

The instruments and materials to be used for this research are as follows:

Brood stock, Pituitary gland extract, Saline solution 0.9mg/L, Razor blade, Plastic bowls, Aerator, Weighing balance, Thermometer, Spawning mat, Feather, Siphoning tube, Syringe and niddle, Scoop net, plate table, Towel and Bottle respectively, motel and pistol and conical flask.

Statistical Analysis

Breeding performance of each dose obtained from the deferent treatment was subjected to statistical analysis. Data was subjected to analysis using variance (ANOVA). Multiple parameters means compression of treatment was according to Duncan multiple range tests to validate the significant (Wahua 2023).

RESULT AND DISCUSION

Results

Water quality parameters during the experiments

Temperature was measured twice daily and there was slight fluctuation during the study period. The temperature ranges between 30-31.01˚C in all dry CgPE treatments. The maximum

mean temperature was observed in 0.5ml doses of ovulin hormone treated basins during the experimental period, the temperature ranges between 31.85-33.54˚C in all treatments. In dry CgPGE hormone, the pH value of each treatment basin was in the range between 6.1 - 7.5 in all the treatment. There was a significant difference ($p<0.05$) in pH concentration among all treatments during the study periods table 1. The pH value of wet CgPGE hormone, each treatment basin was in the range between 6.1-6.7 in all treatments. There was no significant difference ($p>0.05$) in pH among all treatments during the study periods table 1. In dry CgPGE hormone, the dissolved oxygen (DO) concentration was measured in the range between 6.70-6.72 mg L-1 in all treatments. The maximum dissolved oxygen (DO) concentration was 6.85mgL-1 recorded in 0.5ml doses of ovulin hormone treated basins during the experimental period table 1. There was a significant difference ($p<0.05$) in dissolved oxygen concentration among all treatments during the study period. The range of dissolved oxygen observed in this study slightly varied among all treatments. In wet CgPGE hormone, the dissolved oxygen (DO) concentration was measured in the range between 6.70-6.72 mg L-1 in all the treatment. There was no significant difference ($p>0.05$) in dissolved oxygen concentration among all treatments during the study periods.

Table 1: Water quality parameters taken from the experimental tanks during breeding period of *C. gariepinus* induced with synthetic hormone (ovulin) and non synthetic hormones (dry and wet CgPGE).

PARAMETERS	TRT 1 (Dry CgPGE) Mean ± SE	TRT 2 (Wet CgPGE) Mean ± SE	TRT 3 (Ovulin) Mean ± SE
Temperature (˚C)	30 ± 1.7	33 ± 0.01	32 ± 1.5
pH	6.1 ± 1.5	6.1 ± 0.7	6.1 ± 1.01
Dissolve oxygen (mg/L)	6.7 ± 0.02	6.7 ± 0.11	6.7 ± 0.15

The fertilization, hatchability, and survival of C. *gariepinus* treated with synthetic hormone (Ovulin) and non synthetic (hormones dry and wet CgPE)

Broodstock injected with ovulin had a high percentage of hatchability of ($196.67^{a} \pm 27.28$), followed by the broodstock injected with dry CgPGE with total percentage of hatchability of ($173.33^{a} \pm 17.66$), while the broodstock treated with wet CgPGE had the lower hatchability rate of ($88.00^{b} \pm 14.57$). There is significant different ($P>0.05$) across all the treatments. The percentage of survival rate is also greater in the broodstock treated with ovulin ($63.33^{a} \pm 12.01$), followed the broodstock treated with dry CgPGE ($56.66^{ab} \pm 6.00$) and lower in the broodstock treated with wet CgPGE ($30.33^{b} \pm 1.76$). There is significant different of ($P>0.05$) across all the treatments, table 2. However, the latency period of T1 is shorter of (9hours and 42minutes),

followed by the T2 having 10hrs and 5minutes and lastly T3 with the latency period of 12hrs and 16minutes respectively which had the longest latency period.

Table 2: The fertilization, hatchability, and survival rate of *C. gariepinus* induced with synthetic hormone (ovulin) and non synthetic hormones (dry and wet CgPGE).

Treatments	Body weight (g)	Dosage Given (ml/kg)	Water temp (˚C)	Latency period (hrs)	Mean No of eggs fertilized	%fertilization	%hatchability	%survival
T 1 (dry)	1000	1	30	9, 42mins	500	88.02[a]	39[a]	33[ab]
T2 (Wet)	1000	1	30	10, 5mins	500	86.76[b]	20[b]	35[b]
T 3 (Ovulin)	969	0.5	30	12,16mins	500	86.97[a]	45[a]	32[a]

Discussion

During the study period, the mean temperature ranged between 30.00-33.17ºC in incubated eggs that ovulated as a result of inducing the parent stock with varying doses of dry, wet and ovulin. The maximum mean temperature was observed in 1ml/kg dose of Wet CgGPE hormone table 1. In dry CgGPE hormone, the mean temperature ranged between 30.00-31.01 ºC. The differences in the mean temperature in both hormones could be attributed to the quantity of heat absorbed by each egg in each hatchery tank, weather and environmental conditions. There is significant difference ($p>0.05$) in the mean temperature among all the varying doses of the hormones used.

The highest temperature which occurred at 1ml/kg in Wet CgGPE hormone had the lowest fertilization and hatchability rates. These results disagreed with the previous study of (Oyelese 2006) which stated that at higher temperatures, fertilization and hatchability gave the best results. However, this statement agrees with those trends in ovatide where at higher temperature, fertilization and hatchability rates were at their best. These results agrees with a study conducted by (Amaechi and Solomon 2015) which reported that optimum temperature ranges for fertilization and hatchability was between 26-27 ºC. Gomina (2011) reported that the best temperature for fertilization and hatchability was 25.9 ºC and Alemayehu (2015) reported that the best temperature for fertilization and hatchability was between 25.10-26.12

˚C.The mean water pH of the incubated eggs that were stripped as a result of using varying dosages of dry, wet CgPGE hormone to stimulate their ovulation ranged between 6.10-7.50 table 1. The differences in the mean water pH of both hormones could be attributed to the weather and environmental conditions, presence of blood stains on the eggs, fatty content of the eggs and variation in water level of the hatchery tanks used. There was no significant difference ($p>0.05$) in the mean water pH among the varying doses of the hormones used. These results agreed with the work of (Santhosth and Singh 2007) which reported that a suitable pH range for fish breeding was between 6.7-9.5. This work is also similar to the work of (Kutwal *et al*., 2015) which reported that a pH of 6.0 favored the breeding of *Clarias gariepinus* using Carp pituitary extract and ovulin-L. Isa *et al*., (2015) worked on the effect of monthly variation in water temperature on artificial breeding of common carp (*Cyprinus carpio*) on a pH range of 7.97-8.09. (Muhammed *et al*., 2014) worked on the breeding of *Sperata seenghala* using different hormones such as ovaprim, Human Chorionic Gonadotropin, Leutennizing Releasing Hormone and ovatide on pH range of 7.0-7.5.

The minimum and maximum mean incubation period across all the treatments were observed as 20-45hrs respectively in ovulin. On the other hand, a minimum and maximum mean incubation period of 20-40hours respectively was observed in dry and wet CgPGE. There was a significant difference ($p<0.05$) in the mean incubation period (hours) of the eggs that were induced with varying doses of dry and wet CgPGE and ovulin. The difference in the mean incubation period (hours) could be attributed to the effects of oxygen, temperature, pH and dissolved oxygen during incubation of the eggs and water levels in the hatchery tanks used as well as the size of the parent brood fish. These results are in agreement with the work of Ngueke (2015) which reported that the incubation period of African catfish eggs was between 20.00-21.00hours. Similarly, a research conducted by Ayoola *et al*., (2012) reported a hatching period of 21-26 hours on the eggs of *Clarias gariepinus*. Sharma *et al.,* (2010) reported an incubation period of 46.0 hours, 44.5 hours and 42.5 hours in *Clarias batrachus* injected with 0.8ml/kg, 1.0ml/kg and 0.6ml/kg (doses of ovatide) respectively. The differences in the mean incubation period (hours) obtained from this study with that of others compared could be attributed to environmental conditions and species of the fish used.

During this study the percentage of fertilization, hatchability and survival rate was observed, were pituitary extract gave a higher percentage of fertilization of (88.00) and ovulin (86.00) (fig 2), this was in agreement with the report of (Chattopadhyay, 2018) who reported a low fertilization rate in *C. gariepinus* induced with pituitary gland extract. However, there was no significant difference of ($p>0.5$) across all the treatment. The percentage of Hatchability of both *C. gariepinus* treated with both hormones are significantly different were ovulin has the higher percentage of hatchability of ($196.67^{a} \pm 27.28$) followed by dry CgPGE has the percentage of ($173.33^{a} \pm 17.66$). The wet CgPGE has the lower percentage of hatchability of ($88.00^{b} \pm 14.57$) out of all the treatments (fig 3). This agreed with (Hossain et al. 2012) who worked on *C. gariepinus* fish induced with synthetic and natural hormone. The broodstock induced with pituitary gland had higher survival (82.31%) after six weeks compared to the broodstock induced with ovaprim (79.68%), this agreed with (Chattopadhyay, 2018). The broodstock treated with ovulin has the higher survival rate after 72hrs of ($63.33^{a} \pm 12.01$) followed the broodstock treated with dry CgPGE of ($56.66^{ab} \pm 6.00$) the broodstock treated with the wet CgPGE has the lower percentage of survival rate of ($30.33^{b} \pm 1.76$).

Conclusion

The result of this study show the effect of dry, wet CgPGE and synthetic (ovulin) on the breeding of catfish (*Clarias gariepinus*). In respect of fertilization rate, hatchability rate and survival rate of *Clarias gariepinus* larvae treated with natural hormone (dry and wet CgPGE) and synthetic hormone (ovulin) and show the effectiveness of both hormone in catfish (*clarias gariepinus* breeding) after 72hrs. It's concluded that the fish treated with wet CgPGE has the high percentage of survival rate (35), followed by the fish treated with dry CgPGE (33). The synthetic ovuline has the lower percentage of survival rate although it has the high percentage of hatchability rate followed by dry CgPGE hormone.

Recommendation

i. It's recommended that the wet CgPGE is more effective than synthetic ovulin. Therefore breeder has the choice to use wet CgPGE hormone on the bvreeding of *C. gariepinus* because it bring the high survival rate then synthetic ovulin.
ii. Further studies shall be carried in breeding of catfish (*C. gariepinus*) to serve as baseline information for batter quality fish seed production.

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Antara Ghosh, 1 2Md. Anisur Rahman, 2Prianka Paul, 3Afshana Ferdous, 1Shahrear Hemal and 4Md. Aslam Ali 1Department of Aquaculture, Sher-e-Bangla Agricultural University, Dhaka 1207, Bangladesh 2Department of Fisheries and Marine Bioscience, Jashore University of Science and Technology, Jashore 7408, Bangladesh 3Department of Marine Fisheries and Oceanography, Sher-e-Bangla Agricultural University, Dhaka 1207, Bangladesh 4Fish Seed Multiplication Farm, Department of Fisheries, Gopalganj 8100, Bangladesh.

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Md. Shariful Islam1*, Nilufa Begum1 and Syed Lutfor Rahman1 *1Bangladesh Fisheries Research Institute, Brackishwater Station, Paikgacha Upazilla, Khulna District, Bangladesh.*

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