*Original Research Article*

**EFFECT OF ADMINISTERING SERIALLY DILUTED SUPRECUR AND MOTILIUM (Dopamine Blocker) ON THE BREEDING PERFORMANCE OF *Clarias gariepinus***

**ABSTRACT**

This study was designed to determine the effects of using serially diluted Buserelin acetate (Suprecur®), i.e a luteinizing hormone-releasing hormone analogue (LHRHa) with dopamine antagonist (Motilium®). Treatments administered include 50ug/l, 40ug/l, 20ug/l and 10ug/l of Suprecur® in tandem with 5mg/kg of Motilium®. Metrics obtained include egg numbers, latency period, fertilization rate, hatching rates and survival to first feeding. The results obtained demonstrated that the use of Suprecur® (LHRHa) together with dopamine antagonist (Motilium®) successfully induced ovulation in the experimental *Clarias gariepinus* broodfish. There was no significant difference in egg weights stripped from each treated group. The application of 50 µg/kg of Suprecur® with 5mg/kg of Motilium® resulted in earlier synchronization of ovulation (Latency period; 12 hours). Results of the fertilization percentage indicated that increase in dose of LHRHa did not significantly affect fertilization rate in treated groups of broodfish. Overall superiority of 50 µg/kg of Suprecur® plus 5mg/kg of Motilium® in spawning induction was proved by significant high hatchability, 83.56%.

**Keywords: *Hormone, Ovulation, Fertilization, Hatchability, Hypophysation, Synthesis***

**INTRODUCTION**

Wild fry collection has become outdated for *Clarias gariepinus* species since artificial hatchery technology exists, and availability of seed is the bedrock of commercial aquaculture [1]. Accordingly, induced spawning of captive African catfish becomes popular with merits such as improved fertilization and hatching rates, higher survival rates, as well as all year production of fry. However, one major challenge of commercial farming of the African catfish is the availability of seed (fingerlings) since *C*. *gariepinus* does not freely breed in captivity [2], and the need to constantly sustain improved seed quality for commercial availability all year round. Increasing population and market demand for fish in Nigeria has led to increased demand for seed of the species for grow-out production [1]. The insufficiency of quality seeds can be attributed to the absence of environmental cues, necessary for gonadal maturation and spawning [3] as well as stress induces ovarian atresia [4]. Some farmers obtain their seeds from the wild but this mode of obtaining fingerlings is unreliable and does not guarantee quality (uniform size, and parasite or disease free) seeds, and the process requires long period of waiting, time consuming and unprofitable for commercial production of the fish [5].

Successful attempts have been made to mimic or manipulate the environmental cues (temperature and water depth) [6] critical to stimulate gonadal maturation and spontaneous spawning in the African catfish. Studies have indicated that *C*. *gariepinus* can be induced to spawn under controlled conditions (water depth and temperature at appropriate stocking densities) but did not show that this technique can be used in the commercial production of fingerlings, moreover, this technique is seasonal [7].

Over time, the most effective approach to overcome the challenges of breeding catfish under captivity is through hypophysation to induce the final oocyte maturation, ovulation and spawning of fish through hormonal injection, used to breed fish species that do not posses the ability to spawn under confinement.

In the case of the African catfish, hypophysation techniques have been employed in semi artificial or semi natural propagation methods (where the female African catfish is injected with natural or synthetic hormone and placed together with the males in ponds or tanks to spawn) and artificial propagation method (where the female African catfish is injected with natural or synthetic hormone and eggs stripped into a receptacle to be fertilized with milt collected from the gonads of sacrificed males) [8].

Inducement is done through injection of one of several hormones including; fish pituitary extracts, HCG hormone, gonadotropin hormone (GTH), luteinizing hormone-releasing hormone (LHRH) and LHRH agonists (LHRHa) (i.e. gonadotropin-releasing hormone (GnRH) and GnRHa) in commercial synthetic forms such as: Ovatide®, Ovaprim®, Ovopel®, Ovupin-L®, Ovulin®, Aquaspawn® and many others [9] [10] [11] [12].

Hormone-induced spawning of fish has been used for almost 60 years in fish hatcheries for production of fry or fingerlings which contributes significantly to the overall aquaculture production [13]. It has opened the door of a new era throughout the world for high quality and high quantity of fish production [14]. In Africa induced breeding started after the Second World War, with the first successful production of fingerlings being that of *Clarias garepinus* in Egypt [15]. Surprisingly, the same procedures, with only minor modifications, have been used to spawn an entire range of fishes from the ancient sturgeon and paddlefish to carp, catfish, salmon, sea bass, sea bream and mullet. In addition to breeding other desirable fish species, Inducement of spawning using hormones provides a direct control over the final stages of the reproduction cycle in teleosts [16].

Induced spawning of *C. gariepinus using* GnRHhas remained a method of producing the species for research [17] [18] [19] [20] [21]; recently in Egypt [22] [23] [24]. On the other hand, the use of HCG is the popular protocol to induce spawning in many fish species such as Sea bream, *Sparus aurata* [25]; the Japanese eel, *Anguilla japonica* [26]; Benni, *Barbuss sharpeyi* [27]; Pigfish, *Orthopristis chrysoptera* [28]. The stimulation of final oocyte maturation, ovulation and spawning of African catfish by using of human chorionic gonadotropin (HCG) was presented by many authors [22] [29] [23] [30].

 Buserelin acetate (Suprefact®) is a Gonadotropin releasing hormone analog (GnRHa) that is co-administered with domperidone (DOM), an antagonist of dopamine which is produced when fish are stressed. This cocktail has been frequently used (Peter *et al.,* 1993). Buserelin is an analogue of GnRHa, which regulates gonadotropin hormone (GtH). GtH comprises luteinizing hormone (LH) and follicle stimulating hormone (FSH) which can affect the development of ovary and testis. The essential hormones for ovulation, GnRH together with the GnRH receptor, which are located on the gonadotrope membrane in the pituitary gland, can stimulate gonadotropin production. Gonadotropin then will be released into the blood by G protein-coupled receptor systems [31] [32].

Induced breeding using hormones also contributes to cost of fingerling production. This is true considering Suprefact (Buserelin acetate, an LHRHa) used in aquaculture. Efforts at increasing the number of fish induced using one vial of hormone will greatly optimize production and reduce cost. Therefore this study will attempt to induce fish using diluted suprecur, a brand of Buserelin acetate.

1. **please write in the sentences**To determine the latency period and fecundity of *C. gariepinus* induced with serially diluted suprecur with a dopamine- antagonist.
2. Determine the fertilization and hatching rates of *Cl. gariepinus* hatched from broodstock induced with serially diluted suprecur..

**MATERIALS AND METHOD**

The study was carried out at the fishery Hatchery of the Department of fisheries and Aquaculture, Joseph Sarwuan Tarka University Makurdi (University of Agriculture Makurdi).

The brood stocks were from Obedience Fish Farm Makurdi, Benue State. A total number of Twelve [12] fish, eight [8] females and four [4] males ware purchased. All brood stocks were selected by external morphological characteristics using the method of [33]. The brood stocks were acclimatized for Two [2] days

Suprecure® a brand of Buserelin acetate meant for females was obtained from [www.drugstore.ng](http://www.drugstore.ng) #29 Ayangbure road, Ikorodu, Lagos State and motilum was acquired from Wino pharmacy Makurdi, Benue State.

**Experimental Design**

**Preparation of Hormone**

Suprecur® obtained was manufactured with a concentration of 1mg/ml of solution. A stock solution containing 2ml (2000µg) of the original Suprecur® made up to 20ml using normal saline (18ml) was made to obtain a concentration of 100µg/ml of solution. All ten motilium tablets (100mg) were also removed from the pack and pounded using a porcelain mortar and pestle. The volume of the powdered product was determined to be 4ml. This was made up to 10ml by adding 6ml of normal saline. The dosage of Suprecur® for walking catfish as recommended by [34] is 10-30µg/kg body weight in combination with 5-10mg/kg body weight for Motilium®. The current trial utilized four different dosages of Suprecur®: 50 µg/kg, 40µg/kg, 20µg/kg, and 10µg/kg. The dose of Motilium® was fixed at 5mg/kg of bodyweight. From the foregoing, the following volumes were used in all cases:

Table 1: Dose of Hormones (Suprecur® and Motilium®) administered to female *C. gariepinus*

|  |  |
| --- | --- |
| **TREATMENT**  |  **DOSES OF HORMONES** |
| SUPRECUR® (µg/kg) | MOTILIUM® (mg/kg) |
| T50 | 50 (0.5ml/kg) | 5.0 (0.5ml/kg) |
| T40 | 40 (0.4ml/kg) | 5.0 (0.5ml/kg) |
| T20 | 20 (0.2ml/kg) | 5.0 (0.5ml/kg) |
| T10 | 10 (0.1ml/kg) | 5.0 (0.5ml/kg) |

 **Hormone Administration**

The female brood stock was collected from the holding tanks by using a scoop net after which the weight of the fish was taken using a Salter® weighing scale. The weighed fish was then covered with clean towel and injected intramuscularly above the lateral line towards the dorsal section and pointed towards the ventral side. After withdrawal of the needle the fish was finger rubbed to avoid flow of the injected fluid. The injected females were returned separately into their respective plastic bowls.

 **Stripping and Fertilisation**

Injected female brood stock were removed from plastic bowl after 12-13 hours and stripped in dry bowl by holding the fish at the head and tail by an assistant. The ovulated eggs oozed out on slight pressure by thumb into the dry plastic bowl and 10g of eggs were collected from each sample into a petri-dish for counting so as to know the total number of eggs produced from each of the female brood stock. The male brood stock were removed after dissecting them and the milt was collected by laceration of the testes with a clean razor blade. The sperm was then used to fertilize each treatment by mixing both eggs collected and sperm with a plastic spoon before adding distilled water. The bowl was vigorously shaken for a few seconds to improve fertilization.

**Incubation**

Incubation of the fertilized eggs was carried out in 60 liters plastic bowl containing about 45 liters of clean water which was equipped with water aerators. Nylon mesh size (1mm) was suspended above the floor in the plastic bowl for spreading of fertilized eggs. The fertilized eggs were spread in a single layer on the suspended nylon meshed net for incubation. Upon hatching (about 24 hours after incubation), the nylon meshed net was removed with the egg shells while the hatched larvae clustered at the bottom of the incubation tank.

**Determination of Fertilization Rate**

Fertilization rate was determined using 750 eggs from each cross. The eggs were covered in the dry, labeled Petri dish and were kept with labels. The number of eggs were estimated using the gravimetric method (number of eggs/g). The translucent eggs containing embryonic eyes at the time of polar cap formation 10 - 20 minutes after fertilization were considered fertilized and counted to estimate fertilization rate [35].

 **Hatchability**

Eggs were incubated in plastic aquaria with a water volume of 40L and mosquito mesh as substrate. Percentage hatchability was estimated 24 hours after hatching was completed. This was estimated using the volumetric method. To do this, the incubation bowl was stirred gently to disperse the larvae evenly in the water. A beaker (100ml) was used to collect water from the bowl with the dispersed larvae swimming freely inside. The number of larvae in the volume of water was counted. This was repeated three times and the average number was taken. The value was then estimated to cover 40 litres water volume using mathematical relationship. The hatching rate was determined using a modified version of formula provided by [36] as:

$$Hatching Rate= \frac{Total Number of Hatched Eggs}{Total Number of Incubated Eggs}×100$$

**Survival**

The survival rate of larvae was estimated four days after hatching i.e. post yolk sac absorption. The volumetric method was employed in determining survival rate. Here water in the holding tanks was stirred to ensure even dispersion of fry using a glass rod. After this, a representative sample of the water (100ml) was taken in a beaker and fry within the water volume were counted. This was repeated three times and the average was taken. The population was then estimated to cover the entire water volume (40,000ml). Therefore the following equations were used:

$$A\_{100}=\frac{\sum\_{}^{}No. of fry in three samples}{3}$$

$$Survial rate=\frac{^{A\_{100}×40000ml}/\_{100ml}}{No. hatched}×100$$

**Water Quality Parameters**

Water quality parameters such as pH, Electrical Conductivity, Total Dissolved Solids (TDS) and Dissolved Oxygen of the water were monitored using Hanna Multiparameter Water Quality Probe Model HI-98129. A mercury in glass thermometer was used to take temperature readings.

**Statistical Analysis**

Data was analysed using R version 4.0.0 [37] Descriptive statistics for hatching success were obtained using Rmisc package in R [38] and reshape2 [39]. Differences in the hatching rates across the treatments were determined using one-way ANOVA in R [37] via agricolae and emmeans packages [40]; [41]. Mean separation was done using the Tukey HSD method implemented in multcomp package [42] and viewed using multcompView [43]. Graphs were drawn using the ggplot2 package in R [44].

 **RESULTS**

 **Fecundity**

Fecundity of female broodstock to be induced with a combination of Suprecur® and Motilium® (Figure 1) shows that broodstock used for the 40µg/kg dose had the highest fecundity followed closely by broodstock allotted to the 20µg/kg dose while broodstock selected for the 50µg/kg dose had the least fecundity. The fecundity of the species was independent of treatments to be administered and is therefore a random effect in the current experiment.



*Figure 1: Fecundity of Female Broodstock of C. gariepinus stripped under each treatment*

**Breeding Performance**

The effect of each dose administered on respective breeding parameters (Table 2) shows that the weight of eggs stripped from each female for each treatment was not significantly different (p>0.05) and also reflective of the fecundity (Figure 1).Fertilization rates did not differ across the treatments (p>0.05). Latency period differed significantly (p<0.05) among the treatments with the least period of 12 hours being recorded for fish treated with 50µg/kg of Suprecur® and 5mg/kg of Motilium®. Hatchability differed significantly across the treatments (p>0.05) with the highest hatchability (83.56%) beingobserved for fish administered 50µg/kg of Suprecur® and 5mg/kg of Motilium®. Survival at yolk sac absorption was not significantly different among the treatments (p>0.05).

Table 2: Egg and breeding parameters of *C. gariepinus* induced using serially diluted suprefact and motilium

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatment | Egg\_Wt | Latency | Fertilization | Hatchability | Survival |
| T10 | 199.85 ± 23.70 | 13.00 ± 0.00b | 72.10 ± 8.80 | 11.44 ± 0.76a | 45.93 ± 14.90 |
| T20 | 178.70 ± 6.40 | 13.00 ± 0.00b | 88.98 ± 0.56 | 56.32 ± 1.36b | 77.92 ± 14.00 |
| T40 | 205.75 ± 42.30 | 13.00 ± 0.00b | 85.03 ± 1.59 | 64.12 ± 2.89b | 63.52 ± 20.30 |
| T50 | 165.95 ± 29.60 | 12.00 ± 0.00a | 85.66 ± 2.94 | 83.56 ± 2.91c | 56.16 ± 25.60 |
| p-value | 0.749 | <2.0×10-16 | 0.199 | 8.79×10-5 | 0.709 |

Means in the same column followed by different superscripts differ significantly (p<0.05)

 **WATER QUALITY**

Water quality in the incubation tanks (Table 3) reveals that the pH, temperature, Electrical conductivity (EC) and Dissolved Oxygen (DO) were not significantly different among the treatments (p>0.05). Total Dissolved Solids (TDS) was highest in incubation tanks used for 10µg/kg dose of Suprecur® and least in the tanks used to incubate eggs derived from 50µg/kg dose of Suprecur®.

Table 3: Water quality parameters in aquaria used for incubation of *C. gariepinus* eggs

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatment | pH | EC µS/cm | TDS (mg.l-1) | Temp | DO (mg.l-1) |
| T10 | 7.55 ± 0.03 | 138.00 ± 4.00 | 75.0 ± 1.0b | 26.00 ± 0.30 | 4.2 ± 0.1 |
| T20 | 7.49 ± 0.06 | 132.00 ± 4.00 | 57.0 ± 1.0a | 26.15 ± 0.15 | 4.6 ± 0.2 |
| T40 | 7.58 ± 0.07 | 126.50 ± 5.50 | 70.0 ± 3.0b | 26.50 ± 0.30 | 4.8 ± 0.1 |
| T50 | 7.52 ± 0.04 | 139.00 ± 3.00 | 48.0 ± 2.0a | 26.70 ± 0.20 | 4.3 ± 0.2 |
| p-value | 0.723 | 0.272 | 0.002 | 0.306 | 0.156 |

Means in the same column followed by different superscripts differ significantly (p<0.05)

**RELATIONSHIP BETWEEN WATER QUALITY AND BREEDING**

Correlations between water quality parameters and breeding parameters: hatchability and survival (Figure 2) shows that there was no significant correlation (p>0.05) between the water quality parameters themselves and the breeding parameters as well.



*Figure 2: Correlation plot for water quality parameters and hatchability/survival of C. gariepinus fry (Increasing colour intensity signifies increasing p-values and correlations without colour are not significant (p>0.05); Blue colour = positive correlation and Red colour = Negative correlation).*

 **DISCUSSION**

 **Fecundity**

Fecundity recorded in the current study are quite higher compared to a range of 9918.83 to 11544.13 eggs obtained from *Heterobranchus bidorsalis* induced to spawn with homoplastic hormone ovaprim by [45]. The egg number recorded in the current study was higher in fish treated with 40 µg/kg of Suprecur® followed by those treated with 20 µg/kg. This result is in line with a previous report by [26], where number of ovulated eggs using 50 µg/kg of Buserelin acetate (LHRHa) with 10mg/kg of dopamine antagonist was 33856 compared to 2541 eggs with LHRHa alone and even more than all other hormone cocktails used.

**Latency Period**

Latency period observed in the current study was least in the highest dose of Suprecur® (50 µg/kg) with a value of 12 hours. According to Sharaf [47], latency period of *C. gariepinus* induced using GnRHa and the dopamine blocker pimozide ranged from 9.5-12 hours. In an experiment using LHRHa and pimozide, a dopamine antagonist, [48] reported a latency period of 12.3 hours for all the females of African catfish (C. gariepinus). However, in another trial using the same combination of hormones, [49] reported a latency period of 16 hours. The time between injection and stripping of eggs from female broodstock in the hatchery is actually indicative of the physiological response of the females to inducement by artificial hormones [50]. Results from previous breeding trials using various hormonal treatments did show that the effect of a hormonal treatment on spawning performance and larval quality can be very inconsistent with a tilt of performance being observed in a particular spawning agent over the others considering the spawning success and survival of larvae produced [23]. This notwithstanding, of the plethora of factors that can be used to determine an appropriate inducing hormone, its ability to synchronize ovulation is critical. Synchronization of spawning is an important factor in hatchery management with benefit of maximizing time spent on breeding with equal disposition on the output.

**Fertilisation Rate**

Percentage fertilization in each treatment group of this study shows that co-administration of Suprecur® and Motilium® (Dopamine antagonist) successfully increased fertilization rate across all treatments regardless of the decreasing levels of Suprecur®. Studies that utilized a hormone in tandem with a dopamine blocker in clariid fish species including: *C. gariepinus* [18] and Vundu catfish (*H. bidorsalis*) [44] had concluded that combination of a hormone with a dopamine antagonist showed much more effectiveness compared to the LHRHa or GnRHa hormone alone. A confirmation of the current lies in the report by [51] where simultaneous injection of pimozide (10 mg kg−1) and a higher dose of LHRHa (100 μg kg−1) elicited >75% fertilization rate.

**Hatchability**

The hatchability rates recorded in this study differed significantly (p<0.05) among the different treated broodstock. The range of hatchability observed in this study is wider than the range observed by [23] Similarly, [52] reported a significantly lower hatching rate in GnRHa treated females than those injected with ovatide or GnRHa and a dopamine antagonist (Domperidone) with their results being attributed to the poor quality of eggs.

 **Survival Rate**

The survival rate of *C. gariepinus* larvae was generally above 50% except in the group obtained from 10 µg/kg of Suprecur® which had about 45% survival to first feeding. Again, despite the large number of eggs produced in the treatment with 10 µg/kg of Suprecur®, the lower survival rate recorded in this treatment might be attributed to the poor quality of eggs stripped. The survival rates recorded in this study are similar to those obtained by [53] who recorded a 50–60% survival rate of hatchlings of African catfish (HCG injected) and (10–30%) for those injected with “Ovaprim®”.

**CONCLUSION**

The use of Suprecur (LHRHa) together with dopamine antagonist (Motilium®) successfully induced ovulation in *C. gariepinus* broodfish. The addition of dopamine antagonist successfully increased fertilization rates. However, the obtained results clearly indicated an overall superiority of using 50 µg/kg of Suprecur® together with 5mg/kg of Motilium® to induce spawning with regard to the recorded high hatchability percentage. With effective management, survival rate can be increased.

Suprecur® can be used to induce *C. gariepinus* at a dose between 40 µg/kg and 50 µg/kg with co-administration of a dopamine antagonist: Motilium® at 5mg/kg body weight. Reduction of dosage below 40 µg/kg is not advisable in order to optimize fry production.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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