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The Effect of Diluted Doses of GnRHa (Ovulin) Supplemented with Buserelin Acetate- GnRHa (Suprecur) on Final Ova Maturation and Breeding of the African Catfish (*Clarias gariepinus*)

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ABSTRACT

Studies have shown that Ovulin® is a cost-effective hormone for inducing final oocyte maturation (FOM) in *Clarias gariepinus,* and further studies have shown that dilution of the hormone can also be effective. This study was designed to investigate the spawning response of *C. gariepinus*, with an average weight of 1110.0±91.2g, by the induction method utilising serially diluted Ovulin® combined with buserelin acetate (Suprecur®), a gonadotropin hormone-releasing hormone analogue (GnRHa). Treatments administered include 0.5ml/kg Ovulin® (T1), 0.4ml/kg Ovulin® + 32µg/kg Suprecur® (T2), 0.3ml/kg Ovulin + 32µg/kg Suprecur® (T3), 0.2ml/kg Ovulin® + 32µg/kg Suprecur® (T4) and 32µg/kg Suprecur® (T5) + 5mg/kg Domperidone®. We determined the fecundity (absolute egg numbers), latency period, fertilisation rate, hatching rates, and survival (post-yolk absorption stage) in eachtreatment. The results demonstrated that diluted Ovulin® + Suprecur® together successfully induced ovulation in the experimental *C. gariepinus* broodfish. There was no significant difference ($p>0.05$) in egg weights stripped from each treated group. The application of 0.5ml/kg Ovulin®, 0.3ml/kg Ovulin® + 32 µg/kg of Suprecur® and 0.2ml/kg Ovulin® + 32 µg/kg of Suprecur® resulted in a Latency period of 12 hours compared to 11 hours for the other treatments. Results of the fertilisation percentage indicated that hormone administration did not significantly affect the fertilisation rate ($p>0.05$). Similarly, the hatching rate was not dependent onhormone administration ($p>0.05$). Also, hormonal administration to female broodstock did not affect fry survival rate to first feeding ($p>0.05$).

1. INTRODUCTION

The world population is expected to reach nine billion by 2050 from the current value of over seven billion people, with an implication for sustained food production to meet their demand **(Vicente, 2022).** Fish is consumed worldwide as part of our daily diets; hence, supply ought to keep pace with demand as our population continues to increase. Unfortunately, capture production from the wild has remained static for more than 40 years **(Oliveira *et al.,* 2021)**. An alternative means of production is therefore required to meet the demand. The alternative must sustain the production levels required to satisfy the population. Aquaculture is the alternative fish production method that can meet this criterion **(OECD and FAO, 2021)**. Interestingly, there is a sustained increase in global fish and aquatic species production from aquaculture. This increase has led to the designation of aquaculture as a rapidly growing food production sector **(Herath and Satoh, 2022)**. Between 2018 and 2020, the annual average

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growth in production from aquaculture was 6.06%, a level that almost eclipsed the 6.42% decline in the capture fisheries sector **(FAO, 2022)**. Currently, aquaculture's contribution to the total fish production in the world is 49%, which is construed as 88 million tonnes within the total global production volume of 178 million tonnes **(FAO, 2022)**. Global aquatic animal crude protein supply from aquaculture is expected to grow by 57.2% between 2018 and 2050 to meet the increasing animal crude protein intake demand of the world population **(Boyd *et al.,* 2022)**.

The availability of fish seed of sound quality is a determinant of success in a fish culture business since the production cycle will constantly replenish young fish as marketable or table size fish are dispatched from the farm **(Atanda, 2012)**. Seed quantity and quality are therefore important in this regard, with research showing that the size of broodstock, especially female broodstock, significantly affects seed quantity, quality, and survival **(Ferosekhan *et al.,* 2021)**. Selection of fast-growing offspring within cohorts of fingerlings is further enhanced if larger broodstocks are used **(Uedeme-Naa and Nwafili, 2017)**. Moreover, seed multiplication has received greater attention to best management practices for increased productivity **(Fregene *et al.,* 2021)**.

The demand for *Clarias gariepinus* in Nigeria is very high. The demand comes from farmers and consumers alike, creating a high commercial value for the species in Nigerian markets **(Dauda *et al.,* 2018)**. Previously, fish seed was sourced from the wild but with issues of biosecurity, seasonality, availability, and quality uncertainty becoming more critical, alternatives were sought **(Dauda *et al.,* 2013)**. According to **Akinrotimi *et al*. (2015)**, the level of poverty within developing countries is rising considerably due to population explosion and the overexploitation of natural resources with deleterious consequences for the environment. An increase in population translates to increased demand for fish as food, which has necessitated research into sustainable methods of increasing productivity to meet the demand. Seed production must be cost effective if the target of food security is to be met since it determines the final price consumers pay for the fish as well. Widespread use of Gonadotropin Releasing Hormone analogues (GnRHa) in inducing final oocyte maturation (FOM) and stripping is hampered by the rising cost of the hormone. **Kenoye and Godwin (2016)** have shown that ovulin is cost-effective compared to Ovaprim in inducing FOM and ovulation in *C. gariepinus*. Moreover, the dilution of ovulin up to 75% has been shown to produce a decline in fertilisation rate although it increased spawning fecundity **(Maradun *et al.,* 2019)**. Buserelin acetate is a GnRHa that is used in very small doses, and it has elicited 100% ovulation in population of females induced with >80% fertilisation and hatching rates at a dose of 40µg/kg **(El-Hawarry *et al.,* 2016)**. The potency of buserelin acetate as well as its low dose can offer a hedge for diluted GnRHa if co-administered. Therefore, this research takes novelty in co-administration of reduced dose of buserelin acetate and diluted ovulin as it affects the latency period and ovulation, fertilization, and hatching rates of *Clarias gariepinus*.

2. MATERIALS AND METHODS

2.1 Source of Broodstock

The brood stocks were obtained from Obedience fish farm No 28 Lucy Aluor Stree*t al*ong New Otupko Road Makurdi, Benue State. A total of Fifteen (15) fish comprising ten (10) females and five (5) males with an average weight of 1110.0±91.2g were purchased. All broodstock selected met the recommended morphological characteristics (Fig. 1) outlined by **(Ayinla *et al.,* 1994)**. The broodstocks were acclimatised for two (2) days.

Fig. 1. Morphological characteristics of genital papilla of male and female *C. gariepinus* selected

2.2 Source of Hormone

Suprecur® a brand of Buserelin acetate meant for women, was obtained from www.drugstore.ng #29 Ayangbure road, Ikorodu, Lagos State, Nigeria.

Preparation of Hormone

Suprecur® obtained was manufactured with a concentration of 1 mg/mL of solution. A stock solution containing 0.4 mL (400µg) of the original Suprecur® made up to 10 mL using normal saline

(9.6 mL) was made to obtain a concentration of 50 µg/mL of solution. A dosage range of 10 to 30 µg of buserelin acetate per kilogram body weight of fish was recommended by **Meenakarn and Funge-Smith (1998)**. The current trial utilised four different dosages of Ovulin®: 0.50 mL/kg, 0.40 mL/kg, 0.30 mL/kg, and 0.20 mL/kg. A tablet (10 mg) of domperidone was removed from the pack and pounded using a porcelain mortar and pestle. The powdered product was dissolved in normal saline to make 1.0 mL of solution. The dose of Suprecur® was fixed at 32 µg/kg of body weight. From the preceding, the following volumes were used in all cases:

Table 1. Doses of Hormones (Ovulin® and Suprecur®) administered to female *C. gariepinus*

Treatment	Doses of Hormones		
	Ovulin® (mL/kg)	Suprecur® (mL/kg)	Domperidone (mL/kg)
T1	0.50	–	–
T2	0.40	0.80	–
T3	0.30	0.80	–
T4	0.20	0.80	–
T5	–	0.80	0.5

2.3 Hormone Administration

The female brood stock was collected from the holding tanks using a scoop net, after which the weight of the fish was taken using a Salter® weighing scale (Model 300). Upon determining weight, the fish was covered with a clean towel and injected intramuscularly. The injected females were placed in separate plastic bowls with sufficient water and aeration.

2.4 Stripping and Fertilisation

Ripe female broodstock with signs of dripping eggs was removed from each plastic bowl after 10-12 hours (Fig. 2). The eggs were stripped into a dry bowl with slight thumb pressure on the abdomen. A sample of 10g of eggs was collected from each treatment into a petri-dish to count the total number of eggs produced from each female broodstock.

The male broodstocks were dissected, and the testes were collected before laceration to obtain the milt. The milt was squeezed onto the eggs under each treatment to fertilise them with gentle mixing of both eggs collected and sperm using a plastic spoon before adding distilled water. The bowl was vigorously shaken for a few seconds to improve fertilisation.

Fig. 2. Female *C. gariepinus* with dripping eggs after final ova maturation

2.5 Incubation

The eggs were incubated in 60 L plastic bowls that were aerated and contained about 45 L of clean water. The incubation substrate was a nylon mesh net (size 1 mm) suspended above the floor in the plastic bowls. The fertilised eggs were spread in a single layer on the net for incubation. Upon hatching (about 24 hours after incubation), the nylon meshed net was removed with the eggshells while the hatched larvae clustered at the bottom of the incubation tank.

2.6 Determination of Fertilisation Rate

The fertilisation rate was determined using 750 eggs from each cross. The eggs were covered in the dry, labelled Petri dish and were kept with labels. Use of the gravimetric approach (eggs per gram) was employed to approximate the total number of eggs. Embryonic eggs with a polar cap (Fig. 3) formed 10–20 minutes after fertilisation were counted as fertilised if they were transparent and contained embryonic eyes **(de Graaf and Janssen, 1996)**.

2.7 Hatchability

Eggs were incubated in plastic aquaria with a water volume of 40 L and a mosquito mesh net as substrate. About 24 hours after hatching was completed, the percentage hatchability was estimated.

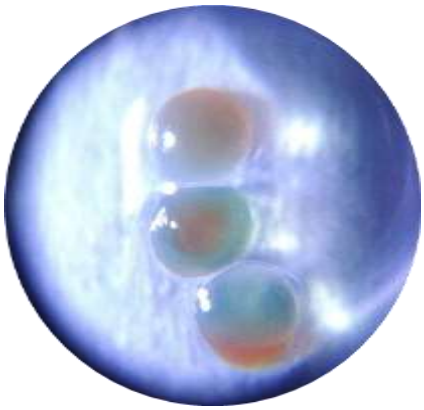


Fig. 3. Fertilised eggs with polar caps

The hatchability was estimated using the volumetric method. The incubation bowl was stirred gently to disperse the larvae evenly in the water. A beaker (100 mL) 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 count was repeated three times and the average number was taken. The value was estimated to cover 40 L water volume using a mathematical equation. The hatching rate was determined using a modified version of the formula provided by, as:

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

2.8 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 the survival rate. Here water in the holding tanks was stirred to ensure even dispersion of fry using a glass rod. A representative sample of the water (100 mL) was taken in a beaker and fry within the water volume was counted. This process was repeated three times, and the average was taken (A_{100}). The population was estimated to cover the entire water volume (40,000 mL). Therefore, the following equations were used:

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

A_{100} = Average number of fry in three counts of 100ml water

$$Survial\ rate = \frac{A_{100} \times 40000ml\ /_{100ml}}{No.\ hatched} \times 100$$

2.9 Water Quality Parameters

Water parameters such as pH, EC, TDS, and DO were measured with a Hanna multiparameter water quality probe (HI-98129). A mercury-in-glass thermometer was used to gauge the temperature of the water.

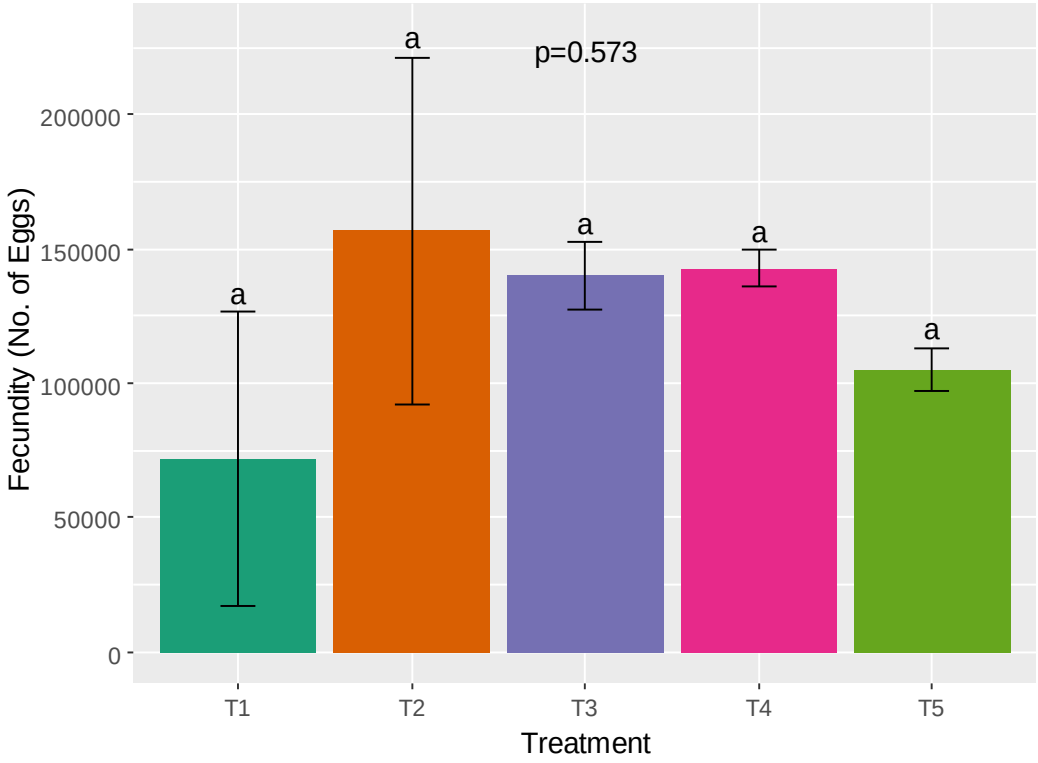
2.10 Data Analysis

Data were analyzed using R version 4.0.0 **(R Core Team, 2020)**. Descriptive statistics for hatching success were obtained using Rmisc package in R **(Hope, 2013)** and reshape2 **(Wickham, 2007)**. Differences in the hatching rates across the treatments were determined using one-way ANOVA in R via agricolae and emmeans packages **(de-Mendiburu, 2020; Lenth, 2020)**. Mean separation was done using the Tukey HSD method implemented in multcomp package **(Hothorn *et al.*, 2008)** and viewed using multcomp View **(Graves *et al.*, 2019)**. Graphs were drawn using the ggplot2 package in R **(Wickham, 2016)**.

3. RESULTS

3.1 Fecundity

Fecundity of female broodstock induced with a combination of Ovulin® and Suprecur® (Fig. 4) shows that broodstock used for the 0.4 mL/kg Ovulin® + 32 µg/kg dose of Suprecur® (T2) had the highest fecundity (157013 eggs). Closely following, were broodstock allotted to the 0.2 mL/kg dose of Ovulin® + 32 µg/kg dose of Suprecur® (T4), while broodstock administered only 0.5 mL/kg dose of Ovulin® had the least fecundity (71995 eggs). The ascending order of fecundity among the treatments was: T1<T5<T3<T4<T2. This order notwithstanding, there was no significant difference in fecundity across the treatments ($p>0.05$).



Ovulin:Suprecur:Domperidone (mL/kg): T1 = 0.5:0:0, T2 = 0.4:0.8:0, T3 = 0.3:0.8:0, T4 = 0.2:0.8:0, T5 = 0:0.8:0.5

Fig. 4. Fecundity of female broodstock of *C. gariepinus* stripped under each treatment

3.2 Spawning Performance

From the determination of breeding performance indicators (Table 2), there was no significant effect (p>0.05) of hormone administration on the egg weight obtained under each treatment. Batch egg weight ranged from 106.65 g under T1 (0.5 mL/kg Ovulin) to 220.85 g under T4 (0.2 mL/kg Ovulin + 32 µg/kg Suprecur). Fertilisation rates ranged from 79.58% (T5) to 89.37% (T3). There was also no significant effect (p>0.05) of hormone administration on the fertilisation rates recorded. Hormone administration, however, significantly affected the latency period (p<0.05).

Table 2. Egg and breeding parameters of *C. gariepinus* induced using serially diluted Ovulin® supplemented with Suprecur®

Treatment	Egg Weight (g)	Latency (Hours)	Fertilisation rate (%)	Hatchability (%)	Survival rate (%)
T1	106.65 ± 86.80	12 ± 0.0^{b}	82.32 ± 4.46	64.76 ± 4.72^{b}	33.48 ± 16.20
T2	208.35 ± 70.40	11 ± 0.0^{a}	83.72 ± 3.84	23.50 ± 1.95^{a}	42.30 ± 8.34
T3	215.70 ± 30.70	12 ± 0.0	89.37 ± 8.21	57.32 ± 1.24^{b}	44.99 ± 6.43
T4	220.85 ± 7.85	11 ± 0.0^{a}	82.78 ± 6.31	21.46 ± 1.23^{a}	57.55 ± 7.55
T5	141.30 ± 46.00	12 ± 0.0^{b}	79.58 ± 1.90	16.01 ± 0.55^{a}	13.40 ± 0.17
p-value	0.550	$<2.0\times10^{-16}$	0.774	8.28×10^{-5}	0.121

Description: Ovulin:Suprecur:Domperidone (mL/kg): T1 = 0.5:0:0, T2 = 0.4:0.8:0, T3 = 0.3:0.8:0, T4 = 0.2:0.8:0, T5 = 0:0.8:0.5. Means in the same column followed by different superscripts differ significantly (p<0.05)

The latency period was at least 11 hours, but T3 and T5 took about 12 hours to reach the stripping state. The survival rate also did not differ significantly across the treatments (p>0.05). The hatching rate significantly differed among the treatments (p<0.05), with T1 having the highest hatching rate of 64.76% and T5 having the least hatching rate of 16.01%.

3.3 Water Quality

Water quality in the incubation tanks (Table 3) reveals that the pH, temperature, and electrical conductivity (EC) were not significantly different among the treatments ($p>0.05$). Total Dissolved Solids (TDS) and Dissolved Oxygen (DO) showed a significant difference ($p<0.05$). The highest value of Total Dissolved Solids (TDS; 75.0mg/l) occurred in incubation tanks used for T3 (0.3 mL/kg Ovulin® + 32 µg/kg Suprecur®), and the least value occurred in the tanks used to incubate eggs for T4 (48 mg/L). Dissolved oxygen was least in T5 (2.05 mg/L) and highest in T3 (4.50 mg/L).

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

Treatment	pH	EC	TDS	Temp	DO
T1	7.42 ± 0.01	114 ± 5	70.0 ± 3.0^{bc}	26.5 ± 0.3	4.00 ± 0.70^{ab}
T2	7.59 ± 0.02	139 ± 3	57.0 ± 1.0^{ab}	26.15 ± 0.15	4.25 ± 0.45^{ab}
T3	7.53 ± 0.01	137 ± 15	75.0 ± 1.0^{c}	26 ± 0.3	4.50 ± 0.30^{b}
T4	7.53 ± 0.06	127 ± 17	48.0 ± 2.0^{a}	26.7 ± 0.2	4.00 ± 0.10^{ab}
T5	7.55 ± 0.03	130 ± 11	58.0 ± 4.0^{ab}	26.05 ± 0.25	2.05 ± 0.05^{a}
p-value	0.080	0.606	0.003	0.322	0.038

Description: Ovulin:Suprecur:Domperidone (mL/kg): T1 = 0.5:0:0, T2 = 0.4:0.8:0, T3 = 0.3:0.8:0, T4 = 0.2:0.8:0, T5 = 0:0.8:0.5. The means in the same column that are superscripted differently differ significantly ($p<0.05$)

3.4 Water Quality and Spawning Outcome

Correlations between water quality parameters and breeding parameters (Fig. 5) shows that there was a significantly strong and positive correlation ($p<0.05$) between TDS and latency period ($r = 0.740$) as well as between TDS and hatchability ($r = 0.830$). There was also a significantly strong and positive correlation ($p<0.05$) between fry survival and dissolved oxygen level in the water ($r = 0.850$).

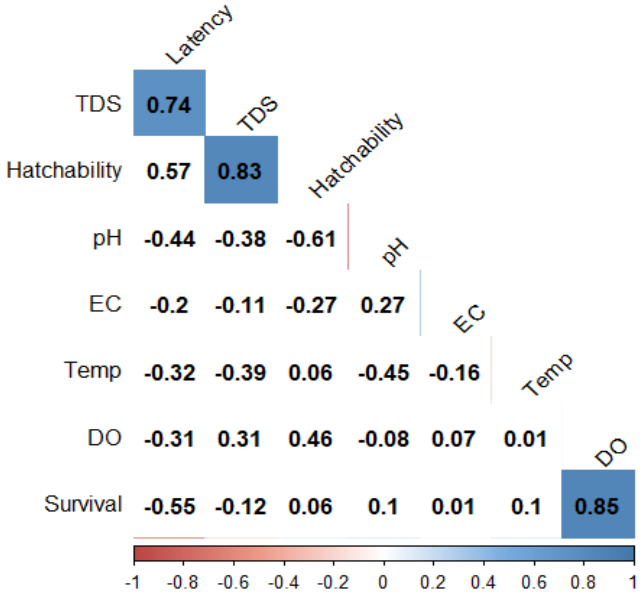


Fig. 5. Correlation plot for water quality parameters and hatchability/survival of *C. gariepinus* fry (Increasing colour intensity signifies increasing p-values and correlations without colour are not significant ($p>0.05$); Blue colour = positive correlation and Red colour = Negative correlation)

4. DISCUSSION

4.1 Fecundity

The fecundity of broodstock induced with the combination of hormones or standalone hormones was relatively high. In a similar trial by **(Kutwal *et al.*, 2017)** to determine the effect of co-administration of Ovaprim® and Ovulin® on the inducement of FOM and spawning of *C. gariepinus*, doses of both hormones at the standalone level and combined levels were used. The combination of Ovaprim® and Ovulin®, as used by these authors, obtained very similar fecundities that ranged from 111300 eggs for standalone Ovulin® to 122500 eggs for 75% Ovaprim® + 25% Ovulin® combination. This result proves that hormonal cocktails can promote higher egg numbers by ensuring better FOM than standalone administrations.

Interestingly, **Maradun *et al*. (2019)** reported better fecundity in 75% diluted Ovulin® administered to either *C. gariepinus* or *C. anguillaris* than the standalone hormone. The current result also showed a similar trend. However, the effect of broodstock weight may also be an additional factor that ensured that the fecundity in their study was far below that in the current study.

4.2 Spawning Performance

The current study's latency period between 11 and 12 hours is comparable to 11.03 hours and 12.53 hours for doses of 0.5ml/kg and 0.3ml/kg of Ovulin® administered to *C. gariepinus* by **Maradun *et al*. (2018)**. However, **Ayoola *et al*. (2012)** reported a latency period 2 - 3 hours earlier than the current report with the use of Ovulin at about 29°C water temperature. At a water temperature of 27°C, **Mamndeyati *et al*. (2018)** observed a latency period of 10 hours in diluted doses and prescribed doses of Ovulin® administered to *C. gariepinus*. The latency period depends on temperature. **Ferosekhan *et al.,* (2021)** reported a longer latency period for the Asian catfish *Clarias magur* with a margin of 5 to 6 hours more following induction with Ovatide. This dependency explains the differences observed in the latency period for the current study and the previous research considering that the water temperature in the current study was about 26°C while that of the authors cited was more. According to **Abolagba (1999)**, the latency period of *C. gariepinus* declines by 1.3 hours for every degree increase in water temperature; hence the 1°C difference between the current latency period and that reported by **Mamndeyati *et al*. (2018)**, resulted in about 1-hour difference in latency period. The temperature of the water influences the process of oocyte nuclear dislocation, and actin filaments may also play a role in the release of polar bodies during the last stages of oocyte maturation **(Yasui *et al.,* 2022)**.

Fertilisation rates in the current study were high and comparable to values obtained for various percentages of diluted Ovulin® used to induce *Clarias gariepinus* and *C. anguillaris* by **Maradun *et al*. (2019)**. However, fertilisation rate in the current trial seems to have been boosted by the co-administration of Suprecur at a diluted Ovulin dose of 0.3mg/l. This spike is like the boost given to HCG administered Glass Catfish, *Kryptopterus vitreolus,* by 10 µg/kg buserelin acetate **(Khunjaroenrak *et al.,* 2019)**. Furthermore, the results of fertilisation rates for the various treatments reported here are quite higher than the 70% rate reported for the same species by **Ayoola *et al*. (2012)** with the use of Ovulin® alone. The difference in fertilisation rates can result from improved egg release orchestrated by the co-administration of Ovulin® with Suprecur®.

Hatching rates in the current study were highly variable, hence the lack of a significant difference in the values despite the huge gaps. Hatching rates have been reported to decline with an increasing percentage of dilution of Ovulin® **(Maradun *et al.,* 2018)**. The current study did not show any significant effect of dilution on hatchability. This effect seems to be masked by the co-administration of diluted Ovulin® with Suprecur®. **El-Hawarry *et al*. (2016)**, reported hatching rates of 75.2% and 81.9% for buserelin alone and Buserelin + dopamine antagonist co-administration in *C. gariepinus*. The presence of a dopamine antagonist in Ovulin® should be the reason behind the ability of Suprecur® to elicit hatching rates of 23.5% and 32.32% at 0.4mg/kg Ovulin® + 32ug/kg buserelin acetate and 0.3mg/kg + 32ug/kg buserelin acetate respectively. Similarly, **El-Hawarry *et al*. (2012)** reported a slight increase in hatchability when Buserelin was used together with a dopamine antagonist than when used alone on the silver carp (*Hypophthalmichthys molitrix*).

The rate of survival of fry recorded in the current study was influenced by biomass at hatching as against the administration of hormones since there was no significant difference in the survival rates. On the contrary, **El-Hawarry *et al*. (2016)** reported the dependence of fry survival on the hormone administered to female broodstock before fry hatching. The co-administration of Buserelin acetate with HCG in female glass catfish *Kryptopterus vitreolus* may have also boosted fry survival with survival rates greater than 90% **(Khunjaroenrak *et al.,* 2019)**. Survival rates reported in the current study are less than those reported by **Ayoola *et al*. (2012)** with the use of Ovulin® alone.

5. CONCLUSION

Co-administration of diluted Ovulin® and Suprecur® does not reduce latency period but gives similar fertilisation, hatching and survival rates as administration of the recommended dose of Ovulin® alone. Hatchability was high with the use of Ovulin® alone, but certain factors outside the control of this study may be responsible for the non-differentiation in rates between the treatments. One plausible explanation could be the individual differences in egg volume within the ovary. Ovulin can be diluted to a dose of 0.3 mg/kg and co-administered with Suprecur at 32 µg/kg with similar results as the application of Ovulin® alone.

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