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The Synergistic Effect of Combining Pituitary Gland and Ovaprim Hormone on Latency, Fertility, Hatchability, and Survival of African Clariid Catfish - *Clarias Gariepinus*

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Abstract
The hatchability of *Clarias gariepinus* using pituitary extract and Ovaprim hormones was carried out at the Hatchery unit of the Fisheries Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. One male to two females were used across all batch treatments (T1-T3). Batch T1 was administered 100% Ovaprim, T2 received 100% Carp Pituitary Extract-CPE, T3 a combination 50% ovaprim and 50% CPE. T4 was without any hormonal treatment. The latency periods were 12h, 16h, 10h and 0h at 27.8ºC, 26.9ºC, 27.5ºC and 26.6°C, respectively, for T1, T2, T3 and T4. T3 had the highest fertilization (78%) and hatchability (85%) rates, followed by T1 (72%, 66%), T2 (61%, 56%) and lastly T4 (26%, 28%). The hatching duration was 18h, 20h, 20h, and 23h with T3, T1, T2 and T4, respectively. Survival rates were in the order: T3 (87.77%), T1 (84.44%), T4 (81.11%) and T2 (76.66%). T3 had higher mean weight gain (0.39g) and specific growth rate (10.07%) compared to T2 (0.39g, 9.63%), T4 (0.33g, 9.54%) and T1 (0.32g, 9.44%). Economically, Ovaprim (T1) cost (₦700 or 1.71USD) while CPE (T2) cost ₦1382.4 (3.37USD). The combination (T3) had the best fertility, hatchability, survival rates, and best growth performance of African Clariid Catfish *Clarias gariepinus* fingerling production and is hereby recommended to fish breeders.
Keywords: catfish, breeding, synthetic, pituitary, combined hormone, No hormone

Introduction

The demand for fish is geometrically growing along with population growth, urbanization and increasing wealth globally to the tune of USD 416 billion in 2020 (FAO, 2022a). FAO (2022a) reported that per capita fish consumption will increase fastest in rich countries and in some parts of the world, such as China, where aquaculture thrives, leading to growing regional disparities between supply and demand. As a result, the increase in population and continued depletion of natural resources resulting from exploitation in developing countries, and the exponentially rising cost of living have made the rates of hunger and poverty surge to very high levels (Akinrotimi *et al*., 2015a; Akinrotimi *et al*., 2007; Stanley *et al*., 2003). The cost and demand for food especially the dietary fish protein has also risen considerably. In view of this, agriculture and science have been coming up with better ways of accelerating productivity to meet the population needs (Akinrotimi *et al*., 2015b; Anyanwu *et al*., 2007; Hogsetle, 1999). Most worrying, however, are the declines in projected consumption for sub-Saharan Africa, where levels of food and nutrition insecurity with poverty are alarming.

Fish make up such a high proportion of animal-source foods (32%). It is indicated that total fisheries and aquaculture production besides algae has drastically expanded in the last seven decades moving from 19 million tonnes (live weight equivalent) in 1950 to all-time high of about 170 million tonnes in 2018 with an annual growth rate of 3.3 percent (FAO 2022a). If algae production is added to that of the aquatic

animals, fisheries and aquaculture production will reach 214 million tonnes in 2020, with an overall growth of 0.3 percent (FAO 2022a). Overall, aquaculture had already overtaken capture fisheries as the primary source of aquatic production since 2013 and has reached its share of total production of 57 percent in 2020 (FAO 2022a). The stagnation experienced in the last two years is mainly associated with a slight decline in capture fisheries to 4.5 percent in 2019 in contrast to the peak of 96 million tonnes in 2018 with additional decrease by 2.1 percent in 2020 FAO 2022a). According to FAO (2022b), the long-term trend in global capture fisheries continued to be relatively stable with catches generally fluctuating between 86 million tonnes and 93 million tonnes per year since the late 1980s. Though in 2020, the global capture fisheries production (excluding algae, SOFIA 2022) was 90.6 million tonnes -a fall off 4.0 percent in comparison to the last three years. This decrease was due to the disruption in fishing operations resulting from the COVID-19 pandemic, the fluctuating catches of pelagic species and the ongoing reduction in China's catches (10 percent lower in 2020 as against average of the previous three years) (FAO 2022a, b). Global fish sales from capture fisheries in 2020 was USD 131 billion compared to USD 265 billion from aquaculture indication that capture fisheries will therefore not be able to meet the growing global demand for aquatic food (FAO 2022a).

The high demand for fish fingerlings in the high growing aquaculture industry has stimulated the need for artificial propagation of cultured warm water fisheries (FAO, 2007). African catfish (*Clarias gariepinus*) is a hardy species for aquaculture purpose. It is widely accepted in the tropics and commands good commercial values, even though, the species hardly breeds naturally in captivity. All year-round efforts are to be intensified on research for its artificial seed production (Oguntuase & Adebayo, 2014)). Sex steroids in female fish perform major roles in Oocyte maturation, ovulation, and spawning. Synthesis of Vitellogenin and increase in ovarian size during final Oocyte maturation is controlled by 17b-estradiol, which is directly related to gonad somatic index (Coccia *et al.*, 2010). Although a trend exists to apply alternative synthetic hormone substances to induce spawning in catfish and other cultured fishes, hypophysation still remains the most common technique. *Clarias* pituitary extract (CPE) and luteinizing hormone-releasing hormone analogue (LHRHa) are two well-known hormones for controlling ovulation in Channel catfish (Forbes, 2013). Among the most important advancement in the field of aquaculture to tackle this challenge in recent times, is the development of techniques to induce reproduction in fish. *Clarias gariepinus* shows a seasonal gonadal maturation which is usually associated with the rainy season. The maturation processes of *Clarias gariepinus* are influenced by annual changes in water temperature and photoperiodicity and the final triggering of spawning is caused by a rise in water level due to rainfall (de Graaf *et al*., 1995). The pituitary gland extract has advantages of better rate of fertilization and hatching, better conditions for growth and survival of larvae (Woynárovich and Horváth, 1980). In addition, it enhances free release of eggs from the genital papilla of female broodstock and stimulates the release of hormones into the blood circulatory system which induces ovulation and production of mature eggs (Oyeleye *et al*., 2016). Also, African catfish pituitary hormone is said to be readily available and cheaper than any other hormone (Adebayo and Popoola, 2008) and can be prepared in a suspension (Fagbenro *et al*., 1998). However, the extraction of the pituitary gland from other donor species is a challenge. For instance, Common carp (*Cyprinus carpio*) pituitary gland materials are not easily accessible (Olaniyi & Akinbola. 2013).

The use of synthetic hormones in African catfish particularly in female fish is now popular as a means of artificially inducing the female fish to ovulate. These include synthetic gonadotrophin releasing hormone analogues (GNRH-a) which are administered to the female brood stocks (Oyelese, 2006). Gonadotropin Releasing Hormone analogue (GnRHa) is now the best available biotechnological tool for the induced breeding of fish. These include the following which have been used to induce breeding successfully: Ovaprim, Ovatide, Ovaryprim, Ovopel, Ovupin-L, Ovulin, Dagin and Aquaspawn (Ngueku, 2015; Zhuo, *et al*., 2011; Nwokoye, et al., 2007; Cheah and Lee. 2000; Brzuska & Adamek. 1999). Among GnRHa are

Ovaprim and Ovatide which contain salmon gonadotropin releasing hormone analogue and domperidone (SGnRHa + Domperidone) often used for spawning induction in catfishes to get quality seed (Sahoo *et al*., 2014). In addition, for large scale production in hatcheries, the use of hormones may be the solution. However, some of these hormones particularly Ovaprim is usually expensive (Khan *et al*., 2006, Madu, 2006, Olubiyi *et al*., 2005). Ovaprim is reported to significantly increase ovulation in African catfish (Sharaf *et al*., 2012) and aid spawning in the matured female catfish. According to Watson *et al*. (2009), the average success rates of 50% ovulation, 54% spermiation and 1.3% mortality were recorded after injection of different species with Ovaprim. Also, it has been used successfully for hypophysation in different families of fish like the Cyprinidae (Hill *et al*., 2005), Characidae and Cobitiidae (Yanong, Martinez &Watson, 2009). The objective of the study is to determine the best hormonal approach towards improvement in breeding of African catfish fingerlings. Hence, this research work aimed at improving on the latency period, egg fertilization, hatching rate, and survival of hatchlings of African clariid (*Clarias gariepinus)* uses pituitary and Ovaprim hormone singly, in their combined form or none to breed.

Methods

The Study Area

This research work was conducted in the Department of Fisheries and Aquatic Resources Management of Michael Okpara University of Agriculture, Umudike, Nigeria which lies between latitude $5^0$29'N and $7^0$33'E. The average temperature of the area is 26^0C, with minimum of 22^0C and maximum of 32^oC. It is 122m (499ft) above sea level with an average rainfall of 21698.8mm obtainable within 148-155 days. The relative humidity ranges from 50% to 95%. The town is located within the humid rainforest zone characterized by long duration of rainfall of 7-12 months and short period of dry season.

Brood Stock Selection

A total of twelve adult (brood stocks) African catfish (four males and eight females) was used in this study. The brood stocks of the *Clarias gariepinus* were obtained from a renowned fish farm along Ussa Road, Abia State, Nigeria. The mature gravid females were selected based on well distended swollen soft abdomen, reddish vent, and easy extraction of few eggs by gently depressing the fish abdomen using the finger. Mature males were selected based on their reddish pointed genital papillae (Ajah, 2019; de-Graff & Jonsen, 1996,).

Transportation and Acclimatization of Procured Brood Stock

The brood stocks of *Clarias gariepinus* were acclimatized in concrete holding tanks of the Fisheries Department Farm 24 hours before artificially induced breeding was carried out. Both males and females were acclimatized in separate concrete ponds of 8×8×5 ft.

Sources of Hormone

Non-Synthetic Hormone: Dried Carp Pituitary Extract (CPE) was purchased from a local supplier in Calabar, Nigeria.

Synthetic Hormone: Ovaprim, produced by Syndell laboratories, Canada has been in use as an inducer for fertility since the early 1990s till today. It was purchased at Abayi Road, Aba, Abia State, Nigeria.

Hormone and Pituitary Gland Injection

Single application of ovaprim as described by (Ndimele & Falayi, 2012; Nandeesha, *et al* 1990;) was adopted. Two female brood stocks and one male were injected intramuscularly (above the lateral line, towards the tail (Figure 1) with ovaprim, at the recommended dosage of 0.5ml per kg body weight of female fish and half dosage for male (Table 1) according to Legendre (1986) and was designated as T1. Two other females and one male were injected with the recommended dosage based on their weight with 100% Carp Pituitary Extract-CPE and was tagged T2. T3 batch had a combination of 50% ovaprim and 50% CPE. T4

group which served as control had no hormonal treatment on both matured male and gravid female catfish. Upon completion of all injections at intervals of eight (8) minutes in between batches, the batches were conditioned in separate plastic tanks for a latent period of 12 hours though examined hourly from the 8th hour post injection of hormone to ascertain readiness for stripping.

Figure 1: Shows intramuscular injection of hormone on catfish

All the females were first tranquilized using anaesthetizing agent (MS222) before stripping to avert any wound and thence reduce stress/pain. Thereafter, the stomach of the two gravid and matured females were pressed gently to release the matured eggs while the males were sacrificed to extract the milts.

Table 1: Dosage of Synthetic (Ovaprim) and Non-Synthetic (CPE) Used in Female and Male Broodstock Treatments.

Treatments	ABW(g) Female	Hormones	Dose/Kg Body Weight (ml)	ABW(g) Males	Hormones	Dose/Kg Body Weight(ml)
T1	1450	100% ovaprim	0.4	750	100% ovaprim	0.3
T2	1500	100% pituitary	1.5	650	100% pituitary	0.8
T3	1350	50% ovaprim + 50% pituitary	0.19 + 0.38	550	50% ovaprim + 50% pituitary	0.25 + 0.5
T4	1350	None	-	750	None	-

ABW= Average Body Weight (g)

Milt and Egg Collection

The milts were collected by sacrificing the males. The two lobes of the male testes were removed, cleaned with tissue paper, and kept in a clean dry labelled petri dish. The abdomen of the female was well cleaned with tissue paper to avoid contact between the eggs and the water. Then the females were stripped of their eggs by a gentle application of pressure on the abdomen to release the eggs. The eggs were collected in a clean dry labelled plastic bowl.

Artificial Fertilization, Incubation and Hatching of Eggs

Dry fertilization method was used. The testes of the male were cut using a dissecting blade and the milts were squeezed out. Saline solution (0.9%) was then added to the milts to facilitate fertilization after which the semen was used in fertilizing the already stripped eggs.

Seven hundred (700) eggs were used for fertilization in each treatment. The number of eggs were estimated using gravimetric method (no of eggs/g). The translucent eggs containing embryonic eyes at the time of

polar cap formation (about 20 minutes after fertilization) were considered fertilized and counted to calculate percentage fertilization. Opaque eggs were considered unfertilized.

Incubation and hatching of eggs were carried out in four (4) hatching tanks of 600 liters capacities each filled with 500 liters of water and containing incubation nets of 1mm mesh size to serve as substrate attachment for eggs. The fertilized eggs were evenly spread on the incubation nets in the tanks and water temperatures kept between 26.7- 27.8ºC.

Experimental Design

The experiment was arranged as a complete randomized design (CRD) with four (4) experimental treatments in triplicates. In this study, 8 female brood fish and 4 male brood fish making a total of 12 brood stocks were used for the study. That is, two females to one male for each of the treatments. Brood fish were weighed before and after stripping out the eggs. The weight of the roes and eggs were taken using a sensitive weighing balance. The hatchlings of each of the treatments were allowed for a period of 3 days for endogenous feeding. Then they were fed with decapsulated artemia for 7 days after which, 30 samples were randomly selected from each of the treatments and were transferred to the experimental layout where they were raised for the period of 30 days.

Feeding Regime

The frys were fed at a fixed feeding rate of 3% body weight four times daily between 07:00 and 23:00 hours at regular intervals for a period of 30 days with Coppens Feed.

Water Quality Parameters

Water quality parameters such as pH, temperature, and dissolved oxygen were monitored daily for the first two weeks and later weekly while ammonia was monitored weekly. A smart DO meter, an accument instrument for temperature and pH were used while some water samples were taken and fixed *in situ* for ammonia determination. Each water parameter was taken thrice from each replicate and the average was recorded.

Fish Weight and Length Measurement

The initial body weight and length of each set of fish was measured using a sensitive weighing balance and meter rule, respectively, before stocking. Subsequently, bulk- weighing and length of fish in each treatment were done after every 7 days.

Determination of Reproductive Success Parameters

The following formulae (Lambert, 2008) were used:

Fertility rate:

$$\% \text{ Fertility} = \frac{\text{Number of fertilized egg} \times 100}{\text{Total number of eggs counted.}}$$

Hatchability rate:

$$\%\text{Hatchability} = \frac{\text{Number of eggs hatched (fry)} \times 100}{\text{Number of eggs incubated.}}$$

Survival rate:

$$\%\text{survival} = \frac{(\text{total no of larvae} - \text{no of dead larvae}) \times 100}{\text{total no of larvae}}$$

Analysis of Fish Growth

The growth expressed as mean weight gain, specific growth rate and survival rate were determined as follows:

Mean Weight Gain (Mwg)

The fish mean weight gain was determined as the difference between the final mean weight of the fish at the end of the experiment and the initial mean weight in grams (Duncan, 1955).

MWG = (W-1) -(W-0)/n
Where: W = initial mean weight; W_1= final mean weight; n = number of fish in the tank

Specific Growth Rate (Sgr)
This is the mean percentage increase in body weight per day over a given time interval (Abdulraheem, *et al*., 2012). In this study, the time interval was 30 days.

$$SGR = \left(\frac{InW2 - InW1)}{T}\right) \times \frac{100}{1}$$

Where, W2= final weight
W1= initial weight
T= duration of culture period
In = natural Log

Cost Analysis

i. Cost of hormone used in T1 = ₦ 1000 x 0.7ml = ₦ 700
ii. Cost of hormone used in T2 = ₦ 600 x 2.3ml + ₦ 0.6 x 4 = ₦ 1380 + ₦ 2.4 = ₦ 1382.4
iii. Cost of hormones used in T3 = ₦ 1000 x 0.44ml + ₦ 600 x 0.88ml + ₦ 0.6 x 1.5ml = ₦ 440 + ₦ 528 + ₦ 0.9 = ₦ 968.9
iv. Cost hormone used in T4= Nil

Statistical Analysis
The results obtained were subjected to one way analysis of variance (ANOVA). The statistical differences between the treatment means were separated using Tukey's test (SPSS, 2010).

Results
Water Quality Parameters Taken from the Experimental Tanks for the Hatching Period.
The temperature ranged from 26.6 – 27.8ºC, while pH was from 6.21 – 7.23, and Dissolved Oxygen between 5.2 and 5.7mgO_2/l (Table 2).

Table 2: Water Quality Parameters Taken from the Experimental Tanks during Hatching Period.

Parameters	T1	T2	T3	T4
Temperature(°C)	27.8	26.9	27.5	26.6
pH	7.10	7.23	6.52	6.21
Dissolved Oxygen (mg/l)	5.3	5.2	5.7	5.6

Induced Ovulation and Spawning of *Clarias Gariepinus* Using Synthetic (Ovaprim) and Non-Synthetic (CPE) Hormones are illustrated in Table 3. Average Body Weight (ABW), mean egg weight, fertility rate, hatchability, survival, hatching/incubation periods, and latency periods of all the treatments are presented in Table 3. T3 recorded the highest survival rate while T2 was lowest. The highest hatchability was recorded with T3 followed by T1, and T2 while the lowest hatchability was recorded in T4. The highest fertility rate was also recorded in T3 and the lowest was in T4. Latency period which is the period between the time of injection and striping was also put into consideration. The latency periods of the fish in T1, T2, T3 and T4 were 12, 16, 10hours and O hours (the control) respectively. Those in the combined effects T3 was shortened because of the synergy of the two hormones while T1 remained normal. Thus, T2 had the longest latency period of 16:00 hours while the shortest was recorded in T3. Also, the hatching periods of the treatments varied ranging from 18.00hrs-23.00hrs. The average body weight of gravid female ranged from 1350-1500g.

Table 3: Induced ovulation and spawning of *Clarias gariepinus* using synthetic (ovaprim) and non-synthetic (CPE) hormones.

Parameter	**T1**	**T2**	**T3**	**T4**
ABW(g)	1450	1500	1350	1350
Fertility Rate (%)	72.00	61.00	78.00	26.00
Hatchability (%)	66.00	56.00	85.00	28.00
Hatching/Incubation Period(hrs)	20.00	23.00	18.00	20.00
Latency(hrs)	12.00	16.00	10.00	-
Survival (%)	84.44	76.66	87.77	81.11

Percentage Survival Rate

The percentage weekly survivors per replicate per month are shown in table 4.

Table 4: Weekly survival of fries

Treatments	T1			T2			T3			T4		
Replicates	R1(%)	R2(%)	R3(%)	R1(%)	R2(%)	R3(%)	R1(%)	R2(%)	R3(%)	R1(%)	R2(%)	R3(%)
Week1 Sr	93.3	100	100	100	93.3	93.3	100	100	100	100	96.7	100
Week2 Sr	96.7	100	96.7	96.7	93.3	93.3	97	96.7	100	100	96.7	96.7
Week3 Sr	96.3	97.3	96.7	90	93.3	96.3	96.7	93.7	86.7	96.7	96.7	90
Week4 Sr	96.3	90	90	90	93.3	96.7	933	90	96.7	93.3	96.7	90
1 Month	83.3	86.6	83.8	76.6	73.3	80	86.6	83.3	93.3	80	83.3	80

Key: SR- Survival Rate

The Rate of Growth of Fry at Seven Days Intervals for 30 Days.

Table 5 shows the growth of fry taken after every seven days for 30 days, the growth parameter taken for the fry included the initial and final weights, the mean weight gain, and the specific growth rate. The initial weight was the same (0.020g) for the hatched larva. The final weight gained recorded were 0.34g recorded in T1, 0.36g in T2, 0.41g in T3, and 0.35g in T4. The fry in T3 had the highest final weight of 0.41g while T1 was the lowest with 0.32g at day 30. The mean gain weight of T3 was obviously higher with 0.39g and lowest with T1 with 0.32g induced with 50% ovaprim and 50%CPE and 100% ovaprim respectively. The specific growth rate was also highest (10.07) in T3 and least (9.44) in T1.

Table 5: Growth parameters of the fry.

Parameter	**T1**	**T2**	**T3**	**T4**
Initial weight(g)	0.020	0.020	0.020	0.020
Final weight(g)	0.34	0.36	0.41	0.35
Mean weight gain	0.32	0.34	0.39	0.33
Specific growth rate	9.44	9.63	10.07	9.54

Cost Analysis for Hatchability of *Clarias gariepinus* Using Ovaprim and CPE

Table 6: Reveals the average cost of the hormones used per treatment in this study. Treatment 4 recorded zero cost of production followed by T1 while Treatment 2 recorded the highest cost of production.

Table 6: Average cost analysis of hormones used per treatment.

Parameters	T1(₦)	T2(₦)	T3(₦)	T4(₦)
Ovaprim	700	0	440	0
Pituitary	-	1380	528	0
Normal Saline	-	2.4	0.9	0
Total	700	1382.4	968.9	0

Fish Length-Weight Relationship

T1, T2, T3, T4 had lengths of 1.41±0.78, 1.28±0.76, 1.74±1.05, and 1.37±0.87 cm, and weights of 0.14±0.16, 0.13±0.15, 0.19±0.23, and 0.13±0.15 g, respectively (table 7). Consequently, a negative allometry which indicates that the increase in the length at this stage of growth was not proportional to weight gain as observed. This is attributed to their stage of growth and feed utilization by the fries. Conversely, the coefficient of determination(r) which helps to determine the rate of growth, was positive indicating that the fry growth rate is normal.

Table 7: Length weight relationship of the experimental fish.

Treatments	Length(cm)	Weight(g)	α	B	Allometry	R	Inference
T1	1.41±0.78	0.14±0.16	0.9946	0.7881	0.7881	1	+
T2	1.28±0.76	0.13±0.15	0.996	0.8258	0.8258	1	+
T3	1.74±1.05	0.19±0.23	0.9976	0.7885	0.7885	1	+
T4	1.37±0.87	0.13±0.15	0.9986	0.8266	0.8266	1	+

Discussion

The impact of both synthetic (ovaprim) and non-synthetic (Carp pituitary extract, (CPE) hormones singly or in combination as well as non-hormonal treatment on the latency period, fertility, hatchability, growth and survival of the hatchlings and cost-effectiveness on *Clarias gariepinus* was established. The results showed the importance of inducement on breeding efficiency using synthetic and natural hormones on the targeted species to aquaculture sustainability. Average Body Weight of gravid females and males used in each treatment respectively ranged from 1350g-1500g and 550g-750g. Spawning occurred within 12h, 16h and 10h after injection of the hormones at temperatures of 27.8ºC, 26.9ºC and 26.6°C, respectively for ovaprim, pituitary and their combinations which compare favourably with Gomina (2011) who had a latency period of 10h using varying levels (0.1, 0.3 & 0.5ml/kg) of ovaprim. While the latency period, fertility rate, hatchability, incubation period, percentage survival and SGR, between Ovaprim and pituitary varied considerably, Onyia *et al*. (2021) opined that there is no difference in the use of ovaprim and pituitary extract, and that each can supplement for the other reason being that both contain Gonadotropin releasing hormone analogue (GnRTHa) hormone and Domperidone (SGnRHa _+Dompeidone) (Sahoo, *et al*., 2014) though not in the same proportion..

All treatments were significantly ($p<0.05$) different from each hormonal application. The highest temperatures of 27.8°C which occurred in T1(Ovaprim) at 0.4 ml/kg in ovaprim hormone did not yield the highest fertilization and hatchability rates rather the T3 (50% ovaprim + 50% CPE hormone) had the highest fertilization and hatchability rates of 78%, 85% respectively at temperature of 27.5ºC. These results agree with the previous studies of Oyelese (2006) who observed that at higher temperatures, fertilization and hatchability gave the best results. The findings are equally in agreement with Amaechi and Solomon (2015) who opined that optimum temperature ranges for fertilization and hatchability was between 26-27 °C. Gomina (2011) also reported that the best temperature for fertilization and hatchability was 25.9°C while Alemayehu (2015) had 25.10-26.12^{0}C. Akombo *et al.* (2018) had temperature ranges of 20.66ºC to 23.0ºC and 20.83ºC to 22.83 ºC, respectively, using Ovaprim and Ovatide for hatching using same species. The lower fertilization and hatching rates observed in T4 (without hormonal injection) was not unconnected

with the difficulty inherent in stripping out enough eggs and the readiness of the eggs fertilization hence the need for hormonal injection. culture temperature (26.6oC) was within the optimum temperature ranges for breeding (Akombo, *et al* 2018; Alemayehu 2015; Gomina 201l)

This study further revealed that ovaprim had a higher hatchability rate (66%) compared with pituitary extract (56%), although the highest hatchability rate (85%) and survival rate (87.77%) was recorded in the combined therapy while the lowest hatchability rate (28.0%) was recorded where no inducement (T4) was carried out. By implication, hormonal application is not just needed but in right proportion and better still, in a combined form to arrive at the best breeding output. Similarly, the survival rate was higher in ovaprim (84.44%) compared with pituitary (76.66%) but lowest for the non-hormonal treated batch. This agreed with Nayak *et al.,* (2001) who reported higher hatching rate of 96% using Ovaprim. It also agreed with the study reported by Belal Hossain *et al.,* (2012) that hatching rate of 76.9% in eggs spawned from Ovaprim induced individual fish compared to 72.7% in ACPE induced fish. This study differs from the finding of Olaniyi and Akinbola (2013) who reported higher survival rate from pituitary gland extract. The variation could be because of seasonal temperature variation between where this research was conducted (Abia State, Nigeria) and where these other research works were conducted or the state of the Ovaprim hormone used.

The result also indicated that there was difference in the cost of using ovaprim compared to pituitary extract which cost ₦700 (1.7005USD) and ₦ 1382.4 (3.587USD) respectively making it more economical to use ovaprim than using pituitary extract. Similarly, Olaniyi and Akinbola (2013) arrived at almost the same value of cost of production with ACPE when compared to ovaprim.

Conclusion

The synergistic effect of half the recommended doses of Ovaprim and pituitary gland have proven to be better than single acute doses of either the synthetic or natural hormone application in all parameters considered ranging from shortest latency and incubation periods, best fertility, hatchability, and survivability, as well as best mean daily growth rate and highest specific growth rate. Besides, the experiment has equally proven that breeding can be successful without the application of hormone provided the female gonad is fully matured and MS222 anesthesia applied during stripping to lessen pain on the fish species.

Recommendation

Though it has been known world-wide that hatchability of *Clarias gariepinus* could be carried out either by using Ovaprim or pituitary hormone, the current research gave credence to the combination therapy of both hormones at one half of the normal dosage of each, proven to be cost effective and gave the best results expected in the fisheries sub-sector to enable the promotion of policies to stop/reduce over-dependence on fish importation.

Alternatively, where hormones are beyond the reach of breeders, eggs could be stripped from matured broodstock that have been properly fed with balanced nutrition and fertilized by mature roe/milt with a caution to stop further stripping upon the slightest taint of blood oozing out.

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