




Evaluation of induced breeding of catfish (*Clarias gariepinus*), using different doses of normal saline diluted ovaprim

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ABSTRACT

With the aim of reducing the cost of ovaprim in induced breeding, the present study was conducted to evaluate the use of diluted ovaprim with normal saline solution at levels of 0% (control), 25%, 50%, 75% and 100% in the induced breeding of *Clarias gariepinus*. Ten gravid female and ten matured male CG were obtained from the Pilot Aquaculture Centre (weight range of 500 g–1000 g). In all, ten (10) spawning tests were conducted. Females under each treatment were injected around the dorsal fin and after close to 24 hours, each female was stripped of her eggs, total number of eggs A-12982 (40.2 g), B-12659 (39.2 g), C-12336 (38.2 g), D-0 (0 g) and E-0 (0 g). After twenty-four hours of the incubation period, total number of hatched eggs were determined and the unfertilized eggs that turned whitish were determined using the volumetric counting method. The percentage of hatchability of stripped eggs was A-65% (6299), B-62% (6008) and C-69% (6686), with no noteworthy ($P > .05$) among the three treatments, whereas percentage survival of the fry was 3124 (49.6%), 2644 (44%) and 3610 (54%) for A, B, and C treatments respectively. From the findings of our research, the study recommends the use of treatment C in induced breeding of catfish.


KEYWORDS

Clarias gariepinus;
fertilization; hatchability;
ovaprim; survival rate

Introduction

Fish farming project's victory occurs based on seed readiness of excellent eminence fish. This stands because, as the marketable ones are being vented out of the farmhouse, undeveloped ones are being supplied to replace the inventory for industry sustenance.

The African catfish could be a huge, eel-like angle, usually of dull gray or dark coloration on the back, blurring to a white stomach. It is a nighttime fish

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like numerous catfishes. *C. gariepinus* are omnivorous bottom feeders that sometimes nourish at the surface. Feed on a wide range of prey at night, such as insects, plankton, invertebrates and fish, but also take young birds, rotting flesh and plants. It also nourishes on living, as well as dead animal matter. The quickly developing intrigued among fish farming operators to culture the freshwater catfish of the sort *Clarias* is due to the exceptionally empowering data on the science of the fish such as its disease resistance, hardness, capacity to outlive hypoxic condition, capacity to acknowledge pelleted bolster, quick development in imprisonment, fabulous adjustment to surrounding climate and tall showcase esteem (Adewolu and Adoti 2010). As a result, the request for an adequate supply of fingerlings of the species has as of late picked up among fishpond operators.

The African catfish is West African and other parts of the African continent's favorite aquaculture fish. The species dominates in freshwater settings such as streams, lakes, and dams (Adewumi and Olaleye 2011). The African catfish, *C. gariepinus* was preferred in aquaculture owing to its ease of cultivation, quick growth rate, high disease resistance, broad temperature tolerance, low dissolved oxygen as well as elevated salinity and, most significantly, high commercial value (Oyeleye, Ola, and Omitogun 2016; Shourbela, Tohamy, and Waleed 2019). *C. gariepinus* is a remarkable species for aquaculture because it is omnivorous, develops quick, and endures moderately destitute water quality (Rad, Kurt, and Bozaoğlu 2004). *C. gariepinus* is widely distributed in tropical Africa and Asia (Sudarto 2007). *C. gariepinus* relocate to waterways and transitory streams to produce; bringing forth, for the most part, takes put at night within the shallow, overwhelmed regions of the streams, lakes, and waterways. Highly aggressive interactions between males precede courtship. Romance and mating take place in shallow waters between separated sets of females and males. The male lies in a curved U-shape around the female's head, held for a few seconds. A collection of eggs and milts is released followed by a vigorous female tail swish to disperse the eggs across a wide region. There's no parental care for guaranteeing the survival of the offspring but by careful choice of the correct location. The advancement of eggs and hatchlings is quick, and the hatchlings can swim inside 48–72 hours after fertilization (De Graaf and Janssen 1996). Because they usually do not breed in ponds, *C. gariepinus* culture is obliged by seed accessibility. Induced spawning have been created, but generation frameworks and incubation center that make the seed of great quality promptly accessible to all ranchers are however to be built up in most African nations (Brummett 2007). Also, it has been identified that the largest mature catfish, *C. gariepinus* would usually give the best spawn weight in induced breeding, but there's no literature available as to whether the fish with the leading produce would similarly grant the leading survival and bet development execution (Odedeyi 2007).

Due to problems associated with wild fish seed which include seasonal availability, the uncertainty of species of fish seed collected, disease infestation and limited quality of harvestable fish seed, it is questionable concerning the sustenance of commercial fish farming and subsequently the require for seeds generation utilizing hormones (Olumuji and Mustapha 2012). Although a few normal water bodies are known to create the fingerlings, such preparations are not sufficient to meet the showcase request. The inherent insufficiency catfish fingerlings may be produced regarding the low survival rate of hatchlings due to factors such as pests and parasites, predation and poor water quality in the natural environment. Because of this, an artificial propagation technique (induced spawning) under more controlled conditions has been discovered to produce reliable sources of fish fingerling dissemination (Akande and Diei-Ouadi 2010).

The most effective and efficient way to ensure the supply of good quality fish seeds throughout the year and the sustainability of the catfish aquaculture industry has been described as artificial fish propagation. This requires the use of natural or synthetic hormones (hypophysation) to induce ovulation and spawning in farmed fish (Odedeyi 2007; Olumuji and Mustapha 2012). Agreeing to Madu and Offor (Madu and Offor 2005), hormone input accounts for around 50–60% of the repetitive consumption of a catfish fingerling generation venture in Nigeria. A significant number of natural spawning agents for African Catfish *C. gariepinus* is available, including Deoxycorticosterone Acetate (DOCA), Human Chorionic Gonadotropin (HCG) and Pituitary Gland Extract (PGE) (Kutwal et al. 2017). These hormones are known to be utilized effectively, be that as it may, they are insufficient in different ways. For occasion, Deoxycorticosteroid Acetic acid derivation (DOCA) causes serious ulcer on the infused female, Human Chorionic Gonadotropin (HCG) is costly, and Pituitary Gland Extract (PGE) is troublesome to evaluate (Ngueku 2015; Nwokoye, Nwuba, and Eyo 2007). Other ranchers too utilize non-piscine pituitary hormones such as Bullfrog (*Rana catesbeiana*) and the toad (*Bufo regularis*) to urge a cheaper but similarly compelling hormone.

Ovaprim is one of the foremost broadly satisfactory and promptly available synthetic hormones since it is exceptionally successful. Ovaprim is a liquid peptide preparation of a salmon gonadotropin releasing hormone analog (sGnRH_a, D-Arg6-Pro9-Net, 20 µg/mL) and a dopamine antagonist (Domperidone, 10 mg/mL). It is delivered as either an intramuscular or intracoelomic injection and acts directly on the pituitary stimulating the release of gonadotropic hormones while concomitantly preventing dopaminergic inhibition of gonadotropin secretions (DiMaggio, Broach, and Ohs 2013; Khan et al. 2006; Sahoo, Giri, and Chandra 2008).

Due to the cost of hormone production, artificial fish breeding continues to be one of the main problems for fish breeders. Up till date, ovaprim is used

undiluted which is still more expensive for the average fish breeders, thereby increasing the cost of fish production among fish farmers and consumers. To reduce the cost of ovaprim on fish breeders in the induced breeding of *C. gariepinus* and overall cost of the fish production while at the same time achieving a high spawning, hatchability and survival success of the fish, this work was therefore done to assess the effectiveness of different doses of ovaprim with usual saline, additionally, diminish the sacrificing of males for their pituitary organ by farmers within the induced breeding of *C. gariepinus*.

Materials and methods

Study area

The study was carried out at the Pilot Aquaculture Center (P.A.C), Ghana, which is a governmental organization under the Ministry of Fisheries and Aquaculture Development, to produce fingerling of catfish and tilapia and to promote fish farming in the Ashanti region and Ghana as a whole. The facility is located 30 km from Kumasi along Asante – Mampong road in the Ashanti region of Ghana. It is situated within the catchment of river Adousu, between the Kona community and Tano – Odumasi. It lies between GPS $6^{\circ} 54' 15.17''$ N and $1^{\circ} 29' 50.47''$, in the Sekyere South District of the Ashanti region.

Selection of *Clarias gariepinus* broodstock

According to (Haimovici and Canziani 2000; Øvredal and Totland 2002), weight and length and relationships are of great importance in fisheries examination because they provide information on populace parameters. Body measurements of a hundred and fifty (150) (Fagbenro 2002) selected *C. gariepinus* broodstocks (gravid females and matured males) thus, seventy five (75) each were taken. The weight of the broodstock ranged between 500 g to 1000 g. All the broodstocks were obtained from P.A.C. When the abdomen is well distended, the females were considered ready and the eggs oozed freely after the abdomen was gently squeezed anteroposteriorly, whereas the males were considered ready when the top of the genital papilla is reddish.

Materials used for the experiment

The materials used were the weighing scale for taking their weights, the rule for measuring the standard and total lengths; scissors were used for dissection of fish. The syringe was used for the injection of the females, knife for cutting off the head of the males, the poultry feather was used for the stirring of the eggs during fertilization, and the bowl was used to collect the eggs. The plastic netting of 2 mm mesh size is what the eggs were placed on in the tank.

Experimental design

The experiment was conducted using five (5) therapies with three (3) replicates each. The five (5) treatments were based on consideration levels of undiluted ovaprim 0%, ordinary saline diluted ovaprim at 25%, 50%, 75%, and 100%, respectively, identified as A, B, C, D, and E.

Extracting the testis

In a separate concrete tank, both males and females were weighed and acclimatized for two days, during which they were fed a standardized diet of 40% crude protein twice daily at 8–9 am and 4–5 pm at 5% of total fish biomass. When the samples were collected, all seventy-five (75) males were sacrificed by cutting off their heads with a sharp knife and the testes were removed by using a scissor to cut their belly. Each male had a pair of testes, with its color being yellowish pink. The testicles of the individual males were removed and placed in different containers containing a saline solution to avoid contamination. The milt was collected in a Petri dish using a clean razor blade to lacerate the testis into 25 ml of normal saline.

Injection of hormone

Females were injected using a 2 ml graduated syringe intramuscularly at an angle of 30–45° at the dorsal fin with 0%, 25%, 50%, 75% and 100% consideration levels of ordinary saline. Each treatment-injected brooder was secured in different holding troughs to avoid them from injury and for easy identification.

Stripping of eggs

Injected gravid females were then left overnight in their respective tanks for 12–16 hours at 24°C. The following day depending on temperature, each female was stripped of her eggs into sterile name bowls by squeezing the belly delicately. The weights of individual brood stock's eggs were taken using an electronic scale and the means of replicated treatment each was recorded. Treatment (A) was higher followed by treatment (B) and treatment (C). Therefore, this was used to calculate the total number of eggs per treatment by using the volumetric counting method to first measure one (1) gram of the eggs.

Fertilization of eggs

Fertilization was accomplished by mixing the eggs and milt for a couple of minutes with a chicken quill to obtain a uniform mixture. No other equipment, such as a brush was used for the stirring of the eggs and milt to prevent

the tips of the brush penetrating the eggs. Ten milliliters of clean water were added to the mixture and again stirred for a minute to allow the water to activate the sperms for maximum contacts with the eggs.

Incubation of eggs

After fertilization, the eggs obtained from each set of treatments were kept in a fiberglass tank containing 20 litres of clean water on a plastic net substrate of 2 mm mesh size. The substrates were kept interior the fiberglass tank to prevent the eggs from sticking together. To achieve a well-oxygenated source, the water flowed in and out of the trough (flow-through system) reducing ammonia accumulation, improve the rate of hatching and prevent spoilage of the eggs.

Estimation of percentage hatchability and survival of the larvae

After twenty-four hours of the incubation period, the eggs that hatched swam and passed through the substrate net and hid under the stone in the tanks because they are nocturnal fishes. Each fiberglass tank for each treatment was siphoned to separate the unhatched eggs from the hatched ones. The total number of hatched eggs was determined 24–30 hours after hatching and the unfertilized eggs that turned whitish were collected in a bowl and determined using the volumetric counting method (Olumuji and Mustapha 2012). Subtracting the number of unfertilized eggs from the total number of stripped eggs estimated the total number of hatched eggs. The survival rate was recorded from the day after hatching to the 8th week. Percentage survival, relative fecundity, and hatchability were examined using the method below (Adebayo and Popoola 2008).

Water quality

DO and pH of the water were monitored daily using pH meter (VIVOSUN pH Meter) and dissolve oxygen meter (Extech 407510 Dissolved Oxygen Meter) while mercury in glass thermometer was used to take temperature readings.

Statistical methods

Data were processed using Microsoft Excel 2010 for their mean values and presented in tables and graphs. Information was analyzed using one-way ANOVA at 0.05 significant levels to check the significant difference in hatchability and survival.

Results

The results of the experiment of induced breeding of *C. gariepinus* utilizing undiluted ovaprim as control and four diverse dosages of typical saline diluted with ovaprim at 25%, 50%, 75%, and 100% is described in this section. Treatment A, the control, showed the highest average egg weight (40.2 g) followed by B (39.2 g) and C (38.2 g). This has also been used to calculate the total number of eggs per treatment by first weighing one (1) gram of the eggs followed by tallying and it was 323, meaning 1 g of eggs equals 323 single eggs. The total number of eggs was determined by the weight of the total eggs multiplying the 323. Treatment A was 12,982, treatment B 12,659 and treatment C 12,336. Both treatments D and E didn't produce any eggs. For uniformity in the research work, 30 g (9690) of eggs were collected from each treatment and used as a sample size for fertilization. Be that as it may, there was no critical contrast ($P > .05$) within the weight of eggs discharged within the three (3) treatments. In treatments D and E, spawning or release of eggs did not occur. Those that hatched and survived from treatments A, B and C are shown in Figure 1.

Hatchability

Each treatment was divided into three different tanks. Treatment C-69% (6686) confirmed the highest percentage of hatchability after hatching as compared to treatments A-65% (6299) and B-62% (6008), but there was no significant difference ($P > .05$) in the hatchability percentage of the three

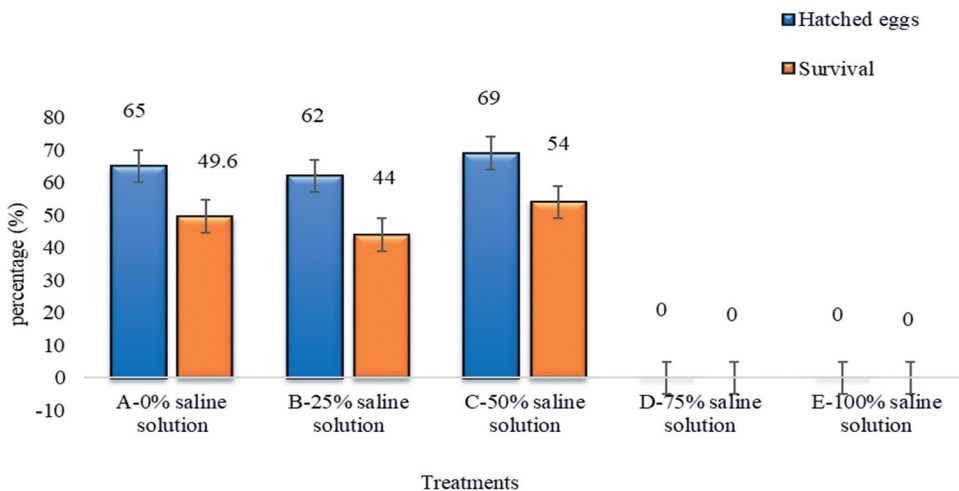


Figure 1. Percentage hatchability and survival of *C. gariepinus* in the use of diluted ovaprim with normal saline solution in rates of 0%, 25%, 50%, 75% and 100% after 24 hours of the incubation period and 8 weeks of survivor test respectively with no significant difference ($P > .05$).

treatments, as shown in [Figure 1](#). Unhatched eggs from these three treatments were; Treatment A- 3391, B- 3682 and C- 3004.

Survival

The survivorship of the treatments was determined from the first day after the eggs hatched to the eighth week. After eight weeks, the number of fries of the treatments were determined by counting each manually and after counting, treatment C- **3610** had the highest number of fries followed by A- **3124**, B- **2644**. Treatments D and E were zero as represented in [Table 1](#). The rate of treatment survival was determined and treatment C was the highest, 54%, taken after by A- 49.6%, B- 44%, D. 0.00 and E. 0.00 but there was no significant difference ($P > .05$) between the three (3) treatments ([figure 1](#)). [Table 2](#) gives a breakdown from the number of eggs stripped to the quantity of fries that survived from the first day after the eggs hatched to the eighth week.

Discussion

The average weight of the brooders' collected eggs shows that standard saline diluted ovaprim at **25%** and **50%** is successful in induced breeding of *C. gariepinus*. It should also be remembered that the latency time varies significantly with usual saline dilution rates and this may be the explanation why hatching in treatments D and E does not occur at all.

Concerning the time of latency, both *C. gariepinus* brood fish spawn after hormone injection for 11–13 hours. Treatment C latency period was very higher than the rest of the treatments and also higher than results reported by

Table 1. Weight of gravid catfish before and after egg stripping and environmental parameters.

Treatments	Mean Weight before stripping (g)	Mean Weight after stripping (g)	Mean Weight loss (g)	Mean Temp. at Hatching (°C)	Mean pH	Mean DO	The latency period (hours)
A	502.5	485.43	17.07	24.5	6.30	6.50	12.30
B	504.5	488.10	16.40	24.5	6.70	6.30	12.00
C	550.5	534.24	16.26	24.5	6.50	6.80	15.00
D	535.5	535.5	0.00	0.00	0.00	0.00	0.00
E	484	484	0.00	0.00	0.00	0.00	0.00

Table 2. A quantitative breakdown of eggs stripped from each treatment, the quantity fertilized, quantity of hatched and unhatched eggs and the quantity of survivals.

Treatments	A	B	C	D	E
Total eggs	12982 (40.2 g)	12659 (39.2 g)	12336 (38.2 g)	0	0
30 g of eggs each for fertilization	9690	9690	9690	0	0
Hatched eggs	6299 (65%)	6008 (62%)	6686 (69%)	0	0
Unhatched eggs	3391 (35%)	3582 (38%)	3004 (31%)	0	0
Survived fries	3124 (49.6%)	2644 (44%)	3610 (54%)	0	0

(Shinkafi and Ilesanmi 2014), and this could be the reason why hatchability and survival rate is high in treatment C than the rest. Temperature ($^{\circ}\text{C}$) values ranged between 24.5 and 24.6 $^{\circ}\text{C}$ were recorded within the test and typically in line with (Ayinla and Akande 1988), which documented changes in the interval between the start of embryonic development (fertilization) and hatching (incubation period) with temperature increase, (Adeniji and Ovie 1982; Madu 1989) suggested the best temperature range for optimum output of *Clarias* species is 25–31 $^{\circ}\text{C}$. Afzal et al. (2008), recommended for good fish results a temperature range of between 25 to 32 $^{\circ}\text{C}$ for good performance of fishes. Due to the high effects of physio-chemical parameters such as the high absorption of dissolved oxygen, the high hatchability and survival observed in treatment C. The water's pH ranged from 6.3 to 6.7 and this is accepted with the organization of the world health. The universal freshwater standard is 7.0–8.pH. It also compares with the work of Huet, Timmermans, and Kahn (1986), which suggests that neutral or slightly alkaline with a pH range of 7 to 8 is the best water for production.

The value for the dissolved water oxygen content ranged from 6.3 to 6.8 mg/l and agreed with that of (Ufodike and Garba 1992), which states that the minimum constant value of 5.0 mg/l dissolve oxygen DO is sufficient for most organisms and stages of aquatic life and (Brain et al. 2006) that recorded increased levels of DO are necessary to support the increase in metabolic rates and reproduction.

Also, low dissolved oxygen provided a lower percentage of fertilization and hatching. Hypoxia diminishes in general regenerative victory by aggravating endocrine capacities, which in turn influence gametogenesis, gamete quality, fecundity, fertilization success, and viability. And this could be the reason why hatchability and survival rate in treatment A and B are low. And this might be the reason why hatchability and survival rate in treatment A and B are moo. A little sum of stress includes a positive impact and more extreme stressors hurt regenerative execution. For example, physical conditioning with low-stress levels, but sufficient to activate the hypothalamic-hypophyseal-interrenal (HPI), can improve a stress-resistance condition in fish (Schreck 2000). Also, it ought to be said that African catfish offspring can be significantly influenced when their mothers have stress amid the actuated producing, uncovered by brought down survival rates, expanded recurrence of morphological peculiarities. These discoveries may be of intrigued both in conceptual and connected conditions as they interface the fish environment to the consequent reasonability of the offspring. During induced breeding (injection and stripping of eggs) brood fish are often disturbed in catfish hatcheries, and such activities can affect egg quality.

The hatchability rate observed in the experiment was identical to (Olubiyi, Ayinia, and Adeyemo 2005), where he inspected the impacts of different measurements of ovaprim on the regenerative execution of *C. gariepinus*.

Nevertheless, this work showed that diluting generic ovaprim with normal saline at 75% and 50% could also result in egg production and hatchability as well as fry survival, which has performed successfully in comparison with undiluted ovaprim. This shows, therefore, that normal saline may improve the growth, hatchability of eggs and fry survival. Several techniques, such as multiple ovaprim dilution techniques, have been developed. This strategy includes employing a diluted ovaprim. Several studies were conducted and the findings were expected to achieve high spawning, hatchability and survival success at the same time (Olubiyi, Ayinia, and Adeyemo 2005).

Egg production and hatchability as well as fry survival were compared to undiluted ovaprim. The normal saline could, therefore, improve production, egg hatchability and fry survival. Bromage (1998) reported similar findings. Nonetheless, (Olumuji and Mustapha 2012) found that the survival rate was relatively lower compared to (Nwokoye, Nwuba, and Eyo 2007) tests. This could be due to the size of the receptacle (tank) in which the experiment was done, which was relatively smaller and also fertilized more eggs, and the tank was not aerated as stated by (Olumuji and Mustapha 2012).

Often, because of import duties, the price of ovaprim raises indiscriminately. Therefore, to reduce production costs resulting from the use of ovaprim, an alternative must be found.

Comparing the costs within the 3 treatments, it is obvious that treatment C with 50% consideration of ordinary saline diluted ovaprim is exceedingly cost-effective, diminishing the taken fee of the hormone utilized by 50%.

Conclusion

Standard saline diluted ovaprim at an inclusion point of 50% will induce breeding in *C. gariepinus* from the study which will be equivalent to generic ovaprim with equal effectiveness, performance, and efficacy. With this, it is possible to save, 50% percent of the costs incurred on the hormone without jeopardizing its output during the induction of C breeding. This will reduce farmers' costs and ensure high output at an affordable price.

It is therefore recommended that farmers should dilute generic ovaprim at 50% during induced spawning to help them get cheaper and quality seed for their production. To effectively test the survival rate, further research should be performed because the tank in which the experiment was conducted was relatively smaller, resulting in high mortality. Therefore, further studies are needed to enhance the viability, survival, and growth of larvae used in farming conditions. Therefore, *C.gariepinus* fries should be grown in concrete tanks and hapas built-in ponds to compare the early life growth and survival rates of the fish species from the three stocks to the fry pond culture undertaken in this research.

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Authors' contribution

Kwabena Anane, Daniel Assan and Akwasi Ampofo-Yeboah (main supervisor) were in charge of the formation and design of the study. Emmanuel D. Abarike helped in the acquisition of data, analysis and interpretation of data. Emmanuel D. Abarike and Elliot H. Alhassan helped in drafting the article and revising it censoriously for significant intellectual content. All authors have approved the final article for publication.

Compliance with ethical standards

This study's protocols were reviewed and approved by the ethics board of the Animal Care Unit of the University for Development Studies.

Disclosure statement

No potential conflict of interest was reported by the authors.

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