Applied Tropical Agriculture
Volume 25, No.1, 23 - 28, 2020

REPRODUCTIVE AND GROWTH PERFORMANCE OF *Clarias gariepinus* INDUCED WITH SYNTHETIC HORMONE AND PITUITARY GLAND EXTRACTS

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Abstract
Bridging the gap between fish demand and supply globally, aquaculture becomes paramount. This study investigates the effects of two hormonal materials on reproductive, growth and survival of Clarias gariepinus using six females and three male broodstock. The experimental fish were induced with male pituitary gland extracts and ovaprim administered at 0.85ml and 0.5 ml respectively based on the body weight. From each female, three samples of 300mg of stripped eggs were weighed and fertilized. After yolk absorption, hatchlings were nursed on pelleted artificial diet {(56.0% crude protein)}. The result showed that artificial breeding of C. gariepinus was successfully carried out through the use of synthetic and male pituitary gland extracts. Fertilization and hatching percentage rates of C. gariepinus induced with synthetic hormone were significantly ($p<0.05$) higher than those induced with male pituitary gland extracts. Percentage weight gain, specific growth rate, feed intake, protein intake and food conversion ratio differed significantly ($p<0.05$) within the treatment (induced with ovaprim and male pituitary extract). This study has shown that C. gariepinus induced with synthetic hormone (ovaprim) produce offspring with better qualities than those induced with pituitary.

Key words: *Ovaprim, pituitary extract, reproductive performance, Clarias gariepinus*

Introduction

Production from capture fisheries has levelled off and most of the main fishing areas (industrial and artisanal) have reached their maximum potential therefore, in order to meet the growing global demand for aquatic food, aquaculture appears to have the potential to make a significant contribution to this increasing demand (Food and Agricultural Organization, (FAO) 2012). Aquaculture which could either be land based or water based but in Nigeria, is predominantly an extensive land-based system and practiced at subsistence levels in fresh waters (Anyawu-Akeredolu, 2005). Nigeria is blessed with over 14 million hectares of inland water surface out of which 1.75 million hectares are suitable for aquaculture (FAO, 2006). According to Federal Department of Fisheries (2013), aquaculture production in Nigeria is currently about 253,898 metric tonnes contributing only 26.22% of domestic fish production.

Nigerians are high fish consumers and offer the largest market for fisheries production in Africa. Thus, Nigeria has become one of the largest fish importers in the world, importing about over 750,000 metric tonnes annually (Ashley-Dejo*et al.,* 2013). Fish production from captured fisheries in spite of its being expensive and risk due to the militancy activities in the coastal line regions of Nigeria has been erratic and on the decline in recent years. To solve the high demand for fish, aquaculture production remains the only alternative to bridge the wide gap between fish demand and domestic production (Ashley-Dejo, 2012).

Stagnation and decline in capture fisheries have put pressure in fish farming as an alternative to meet increase in fish demand. Intensification of aquaculture practice is associated with progress in technologies. However, in order to achieve this target, the sector faces significant challenges (FAO, 2006). Among these, the quantity and quality of fish seeds with high rate of fertilization, survival rates, high food conversion ratios and high growth rate are a major constraint. In order to ensure the continued growth of the industry, there is an urgent need to increase fish seed which can be achieved by artificial propagation through induced breeding (Naeem *et al.,* 2005; Iskandar *et al.,* 2017).

Artificial reproduction in catfish species especially *Clarias gariepinus* has been studied by several authors and different propagation techniques have been used (Haniffa and Sridhar, 2002; Nwokoye*et al.,* 2007; Akinwande *et al.*, 2009; Ataguba*et al.*, 2009; Owodeinde and Ndimele, 2011). Induced propagation could be achieved without hormone treatment by simulating the events of flooding during rainy season could trigger the mating and spawning processes or hormone could be administered on female broodstock to fast track the mating and spawning processes. These induced females are kept in ponds

containing males for spawning and fertilisation. However, these methods could not be used for commercial purposes because very low success has been reported (Hogendoorn and Vismans 1980). The most successful propagation method recorded so far is induced breeding through hormone treatment. This method involves artificial fertilization and incubation of fertilized eggs and the subsequent rearing to fingerlings. Different hormones have been attempted which include deoxy corticosteroid acetate (DOCA), human chorionic gonadotropin (HCG) or the pituitary glands of fish and other animals as frog (Nwokoye*et al*., 2007; Owodeinde *et al*., 2011).

In addition to fish seed propagation, feeding of the hatchlings is also recognized as a major challenge (Omitoyin, 2010). After the first three days of yolk absorption of fry, the swim up larvae require exogenous source of food to live and grow. Therefore, suitable food must be provided in sufficient quantities if undesirable mortality rate is to be averted. As a result of this, it is imperative to investigate the reproductive and growth performance and nutrient utilization of *C. gariepinus* induced with synthetic hormone and pituitary gland.

Materials and Methods

Broodstock Selection

Hatchery raised gravid broodstocks were sourced from a private fish farm from Badagry, Lagos State and Ajanla farms which were located at 20km off Ibadan-Lagos Express Way, Ibadan, Oyo State. According to Ayinla *et al.* (1994), brood fish for breeding purpose should be selected by considering some external morphological features. These features include distended abdomen which must ooze out eggs when gently pressed (female) and should have reddish tip on genital papillae (male). Six female and three male *C. gariepinus* were used i.e the experiment was replicated three times. The female broodstocks weighed 800g, 850g, 880g, 900g, 960g and 1000g respectively, while the male fish weight was 1350g, 1400g and 1500g respectively. They were acclimated in concrete tank of dimensions 3m x 3m x 1m for 24 hours without feeding prior to commencement of the experiment.

Induced Breeding and Larval Rearing of *Clariasgariepinus*

Two hormonal materials {Ovaprim (Aqualife Syndel International Inc., Vancouver, BC, Canada) and pituitary gland extracts of male *C. gariepinus* (homoplastic hormones)} were used. Two artificial spawning trials were carried out.

Removal of Pituitary Gland and Injection of Hormones

Pituitary glands were extracted from mature male *C. gariepinus* using the methods of Viveen *et al.* (1985). The head and the lower jaw of the fish were cut off using a sharp knife, after which the upper jaw was washed with clean water and then turn upside down on a clean table with the brain case facing upward. The brain case was carefully cut open to locate the pituitary gland which is a creamy globule-like organ situated on the ventral side of the brain. The pituitary gland was removed using a spatula. The gland was then transferred into a sealed test tube containing acetone. Hormonal materials were administered between 8:00pm and 9:00pm same day so that stripping could commence in the early hours of the next day. Prior to homoplastic hypophysation, the weighed and stored acetone dried pituitary gland (donor fish has similar weight with recipient fish) was macerated in a porcelain mortar with a known volume of 0.6% saline solution. The clear upper solution (supernatant) i.e. the pituitary gland extracts were then drawn with 5ml hypodermic syringe and 0.6mm gauge needle. The weighed fish was then injected intramuscularly above the lateral line towards the dorsal region and pointed towards the ventral side. After withdrawal of the needle, the fish was finger-rubbed to avoid back flow of the injected extract.

The dosages of pituitary gland extracts (male) and ovaprim administered were 0.85ml and 0.5 ml respectively based on the weight of the fish (1 ml per kg body weight of fish for pituitary gland extracts and 0.5ml for ovaprim) (Viveen *et al*., 1985). The injected fish were returned separately into their tanks containing clean water at 27°C for ovulation and maturation of gonad. Stripping of matured eggs took place 10-11 hours after injection of the hormones at a mean temperature of 27.3°C, which was about 7:00am the following day.

The male broodstock was sacrificed and testes removed. The milt obtained was diluted to 3-3.5ml with physiological saline to prepare milt suspension. A drop of milt suspension was checked under microscope for sperm motility. From each female, three samples of 300 mg of stripped eggs were weighed to its nearest mg and were later fertilized with milt after sperm activation was triggered by the addition of 5 ml distilled water. The stripped yellowish-green eggs of *C. gariepinus* collected were gently mixed with 4-5 drops of spermatozoa suspension of *C. gariepinus*. The two sex products were then mixed with dry plastic spoon to avoid contamination of eggs by using 0.6% saline solution.

The arrangements were as:

Treatment I:*C. gariepinus* ♀ x *C. gariepinus* ♂ (injected synthetic hormone (Ovaprim)

Treatment II:*C. gariepinus* ♀ x *C. gariepinus* ♂ (injected with pituitary gland of male *Clarias gariepinus*)

The fertilized eggs were washed thoroughly with distilled water and released into the transparent plastic incubation tank (65 cm x 30 cm x 25 cm) provided with flow through of water (0.2 l/min) as described by (Sahoo *et al.,* 2007). Water was supplied from overhead tank filled with borehole water. Nylon mesh (1 mm) was suspended at the floor for spreading of the fertilized eggs. The eggs were spread in single layers on the suspended nylon meshed net for incubation. After complete hatchings (24 – 26 hours), the nylon meshed nets were removed with the egg shells while the hatched larvae clustered at dark corners of the incubation tank. Reproductive performance parameters were estimated in terms of fertilization percentage, hatching

percentage, percentage of survival. Hatchery water temperature, pH and dissolved oxygen were 27-28.5°C, 6.7-7.5 and 4.5-5.5 mg/L respectively.

Reproductive Performance Parameters
The number of eggs released was determined by subtracting the weight of the broodstock after spawning from the weight before spawning in grams and multiplying the difference by 700 (1 gm=700 eggs) (Viveen*et al*., 1985).

Fertilization Percentage
For calculating fertilization percentage, a sample of eggs from each replicate of each treatment was carefully collected into a petri dish which contained water. The numbers of fertilized and unfertilized eggs were counted using hand lens (Adebayo, 2006). The fertilization rate was then estimated by the equation (Adebayo, 2006).

Fertilization Percentage =
No of fertilized eggs x 100% …….. (1)
Total no eggs incubated

Hatching Percentage
The number of hatchlings in each treatment was carefully counted and the hatching percentage was determined using the equation (Adebayo, 2006).

Hatching percentage

= **No of hatchlings x 100% .....……. (2)**
Total no eggs incubated

Survival Percentage
Survival percentage was also calculated using the equation (Adebayo, 2006).
Survival percentage =
No of hatchlings alive to larvae stage × 100% …… (3)
Total No of hatchling

Feeding Trial
Fifty (50) frys were assigned to each of the three replicates of the two treatments (Induced with ovaprim and male cat fish pituitary extract). The treatments were randomly allocated into 6 different aquaria of thirty (30) litre capacities, each was filled up to two-third of its capacity. Water quality was maintained by replacing 80.0% of the volume on daily basis and siphoning of uneaten food was done regularly before feeding.

Fry in each treatment (Induced with ovaprim and male catfish pituitary extract) were gradually weaned over a five-day period unto pelleted artificial diet {(56.0% crude protein)(Table 1)}. Frys were fed *ad-libitum* for period of eight weeks. Feeding was done three times daily (7:00am, 12:00pm, and 17:00pm). Feed was dispensed evenly on the water surface of each tank to allow equal feeding opportunity. The fish were batch weighed weekly with a sensitive electronic balance model AJ 5303 (Capacity 6000g; readability 0.2g). Mortality was monitored on a daily basis.

Water Quality Parameters
Water quality parameters were monitored twice weekly according to Omitoyin (2010). Water temperature, dissolved oxygen and pH were determined using a 4 in 1 testing kit (Guacheng JPB-607) portable water quality parameter analyser.

Table 1. Nutrient composition of pelleted artificial diet (Coppens) fed to experimental fish.

Nutrient	% Composition
Crude protein	56.0
Crude fibre	10.9
Crude fat	15.0
Ash	10.9
Phosphorus	8.0
Energy	3400Kcal/kg

Each kg of the diet contained: 300mg Vit C, 200mg Vit E, 22,500 IU Vit A, 2,500 IU vit D3, 5mg Cu, E280 Preservatives and E324 Anti-oxidants.

Growth and Nutrient Utilization Parameters
The following growth and nutrient utilization parameters were determined according to Adebayo (2006).

Weight Gain
Weight Gain (WTG) = **Wi – Wo** …….. (4)
where Wi = final mean weight (g),
Wo = Initial mean weight (g)

Percentage Weight Gain
Percentage Weight Gain = $\frac{Wi - Wo}{Wo}$ x 100 ……..(5)
where Wi = final mean body weight (g),
Wo= Initial mean body weight (g)

Specific Growth Rate
Specific Growth Rate = $\frac{Loge\ Wi - Loge\ Wo}{T-t}$ x 100 …… (6)

where Wi = final weight, Wo = Initial weight,
T = Final Time (days), t = initial time (days),
Loge = Natural logarithm.

Average Daily Grow th
Average Daily Growth (ADG) = $\frac{Wi - W0}{T}$ ……(7)
where W_i= mean final weight, W_0 = mean initial weight
T = rearing period (days).

Feed Conversion Ratio

$$\text{Feed Conversion Ratio} = \frac{\text{weight of feed (g)}}{\text{Weight gained (g)}} \quad \ldots\ldots\ldots (8)$$

Statistical Analysis

The data were analysed for significant differences by Analysis of variance (ANOVA) and the differences among means were tested for significant ($P \leq 0.05$) using Duncan multiple range test (Duncan, 1955).

Results

Reproductive performance of *Clarias gariepinus* induced with ovaprim and male pituitary gland extracts of male *Clarias gariepinus*

Artificial breeding of *C. gariepinus* was successful with the use of ovaprim and pituitary extracts of male catfish to induce spawning. Percentage fertilization, hatching rate and survival percentage differed significantly ($p<0.05$), but no significant difference ($p>0.05$) was observed between survival rate of catfish induced with ovaprim and male pituitary gland extract (Table 2). The highest percentage fertilization (91.13±1.62%) and percentage hatching (87.58±2.23%) were recorded in *C. gariepinus* induced with ovaprim.

Table 2. Percentage fertilization, hatching rate and survival rate of *Clarias gariepinus* induced with ovaprim and male pituitary gland extracts

Treatments	Percentage Fertilization rate	Hatchling percentage	Survival percentage
Ovaprim	91.13±1.62[a]	87.58±2.23[a]	73.15±1.29[a]
Male Pituitary Gland extract	79.92±1.54[b]	77.32±0.51[b]	73.15±1.29[a]

Mean values with same superscripts along the columns were not significantly ($p > 0.05$) different.

Physical and chemical parameters of the culture medium of Clarias gariepinus induced with ovaprim and male pituitary gland extracts

Table 3 shows the mean values of physical and chemical parameters of the culture medium of *Clarias gariepinus* induced with ovaprim and pituitary gland extracts of male catfish. The results showed that there was no significant difference ($p>0.05$) in all treatments. The parameters include temperature, pH and dissolved oxygen. Throughout the experiment, temperature ranged between 26^0C and 27^0C, the pH range was 7.44 – 7.56, while dissolved oxygen ranged between 4.4 and 4.6.0mg/1.

Table 3. Water quality parameters of the culture medium of *Clariasgariepinus* induced with ovaprim and male pituitary gland extracts

Treatments	Temp (^{o}C)	pH	DO (mg/l)
Ovaprim	26.7±1.51[a]	7.44±0.08[a]	4.44+0.06[a]
Male Pituitary Gland extract	27.0±1.62[a]	7.56±0.08[a]	4.63±0.07[a]

Mean values with same superscripts along the columns were not significantly ($p > 0.05$) different.

Growth performance and nutrient utilization of *C.gariepinus* induced with synthetic hormone (Ovaprim) and pituitary glands of male *C. gariepinus*

Tables 4 presents the growth performance and nutrient utilization of *C. gariepinus* induced with ovaprim and male pituitary gland extract fed with experimental diets for eight weeks. Percentage weight gain, specific growth rate, feed intake, protein intake and food conversion ratio differed significantly ($p<0.05$) within the treatment (induced with ovaprim and male pituitary extract). The mean weight gain and average daily growth shows no significant difference ($p<0.05$) within the treatment (induced with ovaprim and male pituitary extract).

Table 4. Growth Performance and Nutrient Utilization Parameters of *C. gariepinus* induced with Synthetic Hormone (Ovaprim) and Pituitary Glands of Male *C.gariepinus*

Treatment	Ovaprim (C.g ♀ x C.g ♂)	Male pituitary of *C.gariepinus* (C.g ♀ x C.g ♂)
MWG	6.18±1.27[a]	5.32±1.71[a]
PWG	99.37±8.98[a]	86.23±6.13[b]
ADG	0.13±0.18[a]	0.11±0.11[a]
SRG	2.48±0.42[a]	1.48±0.22[b]
FI	21.96±0.05[a]	17.18±0.05[b]
PI	13.46±5.05[a]	10.13±4.31[b]
FCR	1.85±0.38[a]	0.96±0.05[b]

*WG = Mean weight gain; PWG = percentage weight gain; ADG = Average daily grow th; SGR = Specific growth rate, FI = Feed intake; PI = Protein intake; FCR = Food conversion ratio.
*Values in the same column and with the same superscript are not significantly different (P>0.05).

Discussion

Artificial breeding of *C. gariepinus* was successfully carried out through the use of ovaprim and pituitary gland extracts. Latency period and water quality parameters observed in this study were found to follow the study of Adebayo and Olanrewaju (2000) who reported a latency period of 12 – 14 hours at temperature ranging from 22.5 – 32.0^{0}C. It was also in agreement with the findings of Adebayo (2006) who reported that broodstock of *C. gariepinus* injected with ovaprim spawned within 11 – 18 hours at a temperature range of 23.50 – 23.77^{0}C. The dissolved oxygen and pH range were found to be within the range as reported by Boyd (1979) sited by Ndimele and Owodeinde (2012). The study showed that fertilization and hatching rates of *C. gariepinus* induced with synthetic hormone were significantly high. This finding was in line with the earlier study of Nwokoye *et al.* (2007). They reported that female *Heterobranchus bidosalis* injected with ovaprim had significant higher number of fertilized eggs and higher hatch ability rate than their counterparts injected with pituitary extract from *Heterobranchus bidosalis*. Although, fertilization and hatchability rates of *C. gariepinus* induced with male pituitary gland extracts difference significantly ($p<0.05$) compared with synthetic hormone. Male pituitary gland extract gives better result apart from its dual purpose (milt extraction and pituitary gland) and relatively safe cost. This effect could be due to the presence of domperi done in ovaprim as earlier reported by Popesku *et al.* (2008).

The growth performance of *C. gariepinus* were highest in the treatment fed combination of zooplankton and artemia also those fed combination of zooplankton, artemia and commercial feed (Coppens). The result was in line with the study of Fermin and Boliver (1996) who stated that co-feeding of larvae with live feed improved growth of *C. macrocephalus* larvae. It also agree with the findings of Okoye*et al,* (1990) that mixed diets provide the best specific growth and survival rates relative to artificial diet alone. Also, Hogendoorn and Vismans (1980)observed and reported that survival and growth of *C. gariepinus* fry was much better when fed on natural food either alone or in combination with formulated feed.

Conclusion

The need for propagation of increased number of quality fingerlings for culture is most paramount for fish hatchery farmers. This study has shown that *Clarias gariepinus* can be successfully bred using both synthetic hormone and male pituitary gland of *Clarias gariepinus.* Fingerlings obtained from *Clarias gariepinus* induced with synthetic hormone (ovaprim) had the best performances in terms of reproductive parameters, growth parameters and nutrient utilization. The synthetic hormone (ovaprim) can be recommended for induced spawning since it produced better results.

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