Induced Breeding of *Clarias gariepinus* (Burchell, 1822) using Ovulin and Chicken Pituitary Gland Extract as Spawning Agents

Supported by tetfund
TERTIARY EDUCATION TRUST FUND

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Received: February 14, 2025, Accepted: April 28, 2025

Abstract: The African catfish *Clarias gariepinus*is one of the most relished fish for aquaculture in West Africa, and in particular Nigeria. However, there is decline in fish production due to the scarcity of fish seed for stocking. Hence, the study aimed at addressing the shortage in fish seed through the use of hypophysation, by comparing the effectiveness of Ovulin and Chicken pituitary gland extract. Two matured and healthy female broodstock were selected and induced with intramuscular injection of Ovulin and Chicken pituitary gland extract. The first broodstock was administered with Ovulin (0.5ml/g) and the second was administered with two chicken pituitary gland extract (1.5mL/kg). The latency period for the two induced female broodstock was 13 hours, after which stripping and fertilization with milt collected from the two lobes of two matured male broodstock. The fertilized eggs were incubated at 28 hours. One hundred hatchlings were selected from each fertilized female broodstock eggs and growth performance, and survival rate were monitored for 8 weeks, to evaluate the effectiveness of the two hormones. Data were collected at each stage, analyzed using ANOVA, while the means were separated using Turkey range test. The Ovulin and Chicken pituitary gland extract treatments had no significant difference (P>0.05) in the number of fertilized eggs, however Ovulin was much higher with 82 fertilized eggs. The hatchability was not significantly different (P>0.05), however the hatchability rate was higher in Ovulin than Chicken pituitary gland extract with 1.66. The growth performance of the hatchlings from the two brooders were not significantly different (P>0.05); However, the weight was higher in Chicken pituitary gland extract treatment than Ovulin hormonal treatment. The mean frequency of shooters was also not significantly different (P>0.05). There is no major advantage in terms of fertilization, hatchability, growth and survival rate, however Ovulin and Chicken pituitary gland extract treatments were effective in inducing the female broodstock for fish production.

Key words: *Clarias gariepinus*, Chicken pituitary, Induced breeding, Latency period, Ovulin

Introduction

Aquaculture in the world is the fastest growing food production among other production system (FAO, 2016). It's expected to bridge the gap between demand and supply for fish and to contribute to food security and community settlement through improved food supply, employment and increased income. The greatest production occurs in developing communities, besides being sustainable and environmentally friendly, therefore professional aquaculture technologies should start in a base of small-scale producers who are backbone of the advance aquaculture industry (Hagar, 2015).

The African catfish *Clarias gariepinus* is the favorite fish for aquaculture in West Africa because of its high fecundity, fast growth rate, resistance to diseases and high feed conversion efficiency (Adewumi et al., 2011), and other countries of the African continent. In recent years *Clarias gariepinus* has been cultured extensively (Hagar, 2015). The African catfish *Clarias gariepinus* has been reported to be a suitable Aquaculture species in Nigeria and other countries in Africa (Eyo et al., 2015), popular with consumers in Nigeria for its high nutritive value (Udeze et al., 2012).

However due to problems associated with wild fish seed such as, seasonality in availability, uncertainty of species of fish collected, diseases infestation and limited quality of harvestable fish seed (Olumuji and Mustapha., 2012). Seed availability which is hindered by the scarcity of natural spawning in captivity, photophobic behavior, and the shortage of high-quality fingerlings are the bottleneck for successful culture of this species (Waleed *et al*., 2016). In addition, the dependence on natural resources for seed collection is seasonal reliant, restricted, unreliable, time consuming and uneconomic (Dadebo et al., 2014). As a result, spawning induction of captive fish becomes the best scenario to overcome such problems through injection of one of the possible hormones. The hormones promote reproduction in fish which is controlled by several factors such as sex steroid in regulation of reproductive process (Nwokoye et al., 2007). Administration of these hormones to induce ovulation and spawning in fish is achieved through artificial propagation with either natural or synthetic hormones (Nwokoye et al., 2007, Ngueku, 2015). The use of synthetic hormones in African catfish particularly a female fish is now popular as a means of artificially inducing the female fish in order to ovulate. A Chicken pituitary gland extract was also found to be compatible with teleost fish pituitary as a spawning induction agent in terms of potentiality, compatibility and effectiveness (Muchlisin et al., 2013). This study was aimed to investigate the effectiveness of Ovulin and Chicken pituitary on the reproductive performance of *Clarias gariepinus*

Materials and Methods

Description of Experimental Area

The experiment was conducted at Fisheries Research Unit, Federal University Wukari, Taraba State which is located in Wukari Local Government Area which lies along Latitudes $7^0$52'17.00" N and Longitude 9^0 46' 40.30"E of the equator with an elevation of 189m above level.

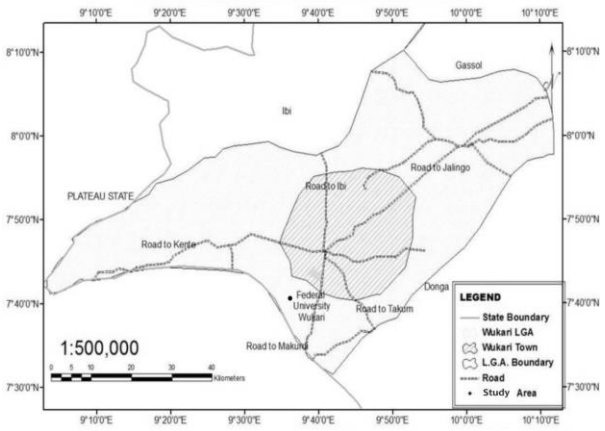

Figure 1: Map showing experimental site

Procurement of Materials

A total of six (6) *Clarias gariepinus* broodstocks (4 males and 2 females) were used in this study. The broodstock were procured from Anointed fish farm Gboko. The hormone was procured from Agro-service centre at Makurdi, Benue State. The broodstocks used were of average weight between 800g - 1000g.

Selection of Broodstocks

Each brood stock was selected by consideration of external morphological characteristics as described by Ukwe and Abu, (2016).

Transportation/Acclimation

The broodstocks of *C. gariepinus* collected were transported in fifty litres (50Ltrs) jerricans to Fisheries and Aquaculture research farm and were acclimatized in fiber reinforced holding water tanks for 72 hours before artificially induced breeding was carryout.

Preparation of spawning agents

The Ovulin was packaged in sealed bottles by the manufacturer and no special preparation is needed. The hormones were taken directly from the bottle using a syringe at appropriate dosage while the pituitary glands were taken from freshly slaughtered chicken heads at local market. The glands were washed with 45% alcohol and gently ruined in a muller. Approximately 5mL of physiological solution (0.9% saline water) were added into the muller then centrifuge for 5 minutes at 15,000 rpm, then extract was taken using a syringe.

Injection of Female Spawner

The two female broodstocks were separately induced to spawn through intramuscular injection with synthetic hormone at 0.5mL/kg and Chicken pituitary gland suspension at 1.5mL/kg and the injection was carried out towards the head region at an angle of 45º. The injected areas were finger robbed to prevent retraction of the hormone. The injected specimen were kept in different tanks and the success of ovulation was monitored at two hours interval during the latency period.

Milt collection

After the latency period, the milt was collected by sacrificing the male. The two testes lobes of the males were removed, and then cleaned with tissue papers following Omeji et al., (2013).

Stripping of the eggs

The female brooders were mopped dry by the use of towel to prevent water and mucus slime coming in contact with eggs. Pressure was applied gently on the abdomen of the female brooders. Ovulated eggs were collected in stainless bowls with labels hormone and weighed separately.

Artificial fertilization

The testes of the males were cut open using razor blade and the milt was squeezed out, and then normal saline was added to the milt to activate the milt before it was poured on the stripped eggs.

Setting of Indoor Experiment and Daily Survival of Hatchlings

Each treatment was in triplicates. One hundred (100) hatchlings of each after taken the pooled weight were collected and placed in each flow through container. The survival of fries in each container per treatment were taken daily and weekly for 8 weeks, while pooled weight, pooled length and final survival were taken on the 56th day.

Feeding of Larvae

After yolk absorption by the fry, Artemia was used to feed the fry for 21days followed by starter feed for 7weeks. During these periods, measurement such as weight and length of fish were taken on weekly basis.

Data collection

Data on number of fertilized eggs, hatchability, survival rate, growth Performance and frequency of shooters following Ukwe and Abu, (2016).

Evaluation of the growth performance

Fish weights in gram were recorded at the beginning at day 3 and the end of the feeding experiment at week 8 for all fry of each treatment were to determine the difference between the initial and final weights of fry following formulae of Olaniyi et al., (2013).

Specific Growth Rate (SGR)

$$= \frac{(\text{Ln Final Mean Weight} - \text{Ln Initial Mean Weight})}{\text{Length of Feeding Trial (days)}}$$

Determination of shooters frequency

The incubation tanks were cleaned and restocked with post hatchling with complete yolk sac absorption. During the rearing period, the fry were fed with artemia for 14 days followed by artificial feeds during the experimental feeding which last for 8 weeks. The pellet size was changed due to the growth of the frys. A flow through system was maintained throughout the rearing period and water quality of the rearing environment was monitored. At the expiration of the rearing period, the fry was sorted in to different sizes following Nwadukwe et al., 2000 and Oyebola, 2015.

Water quality parameters

Temperature, pH value, dissolved oxygen, ammonium level and other factors all have an impact on water quality. They were measured *in situ* using a thermometer to determine temperature and a pH reagent to determine pH. Other parameters were measured by immersing the measuring instrument probes in the water at a depth of about 4cm in the middle of the cultured water until equilibrium was reached.

Data analysis

Results were expressed as the mean values ± standard deviation (S.D) by measuring three independent replicates. Analysis of variance (ANOVA) was performed to test the significance difference between means obtained among the

treatments at the 5% level of significance using SPSS software (version 21, IBM SPSS). Results

Reproductive success of *Clarias gariepinus* induced with Ovulin and Chicken pituitary gland extract.

Table 1 indicated the result of the reproductive success of *Clarias gariepinus* induced with Ovulin and Chicken pituitary gland extract, with no significance difference P>0.05 for all parameters. However, the number of fertilized eggs were higher in Ovulin (1450.00±50.00) than Chicken pituitary gland extract (1368.33±57.00), the number of hatchlings were higher in Ovulin (959.33±89.21) than Chicken pituitary gland extract (882.33±33.70), the fertilization (%) were higher in Ovulin (80.55±5.00) than Chicken pituitary gland extract (76.44±8.00),the hatchability (%) were higher in Ovulin (66.13±8.00) than Chicken pituitary gland extract (64.47±9.00), and survival rate (%) was higher in Ovulin (76.53±14.00) than Chicken pituitary gland extract (74.14±16.00).

Table 1: Reproductive success of *Clarias gariepinus* induced with Ovulin and Chicken pituitary gland extract.

Spawning agents	Fertilized eggs	Hatchlings	Fertilization (%)	Hatchability (%)	Survival (%)
Ovulin	1450±50.00[a]	959.33±89.21[a]	80.55±5.00[a]	66.13±8.00[a]	76.53±14.00[a]
Chicken	1368.33±57.00[a]	882.33±33.70[a]	76.44±8.00[a]	64.47±9.00[a]	74.14±16.00[a]

Means with the same superscript along the Column are not significantly different (P>0.05).

Growth performance of *Clarias gariepinus* induced with Ovulin and Chicken pituitary gland extract.

Table 2 Shows the growth performance of *Clarias gariepinus* induced with the spawning agents which reveal that initial weight was lesser in Ovulin (1.06±0.03) than Chicken pituitary gland extract (1.45±0.23), final weight was also lesser in Ovulin (3.23±0.18) than in Chicken pituitary gland extract (4.15±0.41) while the initial length was lesser in Ovulin (0.60±0.06) than Chicken pituitary gland extract (0.68±0.06), the final length was also lesser in Ovulin (3.37±0.32) than Chicken pituitary gland extract (4.80±0.06), the mean weight gain was lower in Ovulin (2.07±0.28) than chicken pituitary gland extract (2.46±0.37), the growth rate was also lower in Ovulin (0.04±0.03) than Chicken pituitary gland extract (0.05±0.01), the mean length gain was lesser in Ovulin (2.37±0.06) than Chicken pituitary gland extract (4.12±0.09), the length gain was lesser in Ovulin (0.05±0.04) than Chicken pituitary gland extract (0.07±0.00), and also the specific growth rate was also lower in Ovulin (0.09±0.00) than Chicken pituitary gland extract (0.05±0.02), this trend is as shown in figure 2 and 3.

Table 2: Growth performance parameters of *Clarias gariepinus* induced Ovulin and Chicken pituitary gland extract

Spawning agents	Initial weight(g)	Final weight(g)	Initial length(cm)	Final length(cm)	MWG(g)	GR (g/d)	MLG (cm)	LG (cm)	SGR(g/d)
Ovulin	1.06±0.03[a]	3.23±0.18[a]	0.60±0.06[a]	3.37±0.32[a]	2.07±0.28[a]	0.04±0.03[a]	2.37±0.06[a]	0.05±0.04[a]	0.09±0.00[a]
Chicken	1.45±0.23[a]	4.15±0.41[a]	0.68±0.06[a]	4.80±0.06[a]	2.46±0.37[a]	0.05±0.01[a]	4.12±0.09[a]	0.07±0.00[a]	0.045±0.02[a]

Means with the same superscript along the Column are not significantly different (P>0.05).

Where: MWG=Mean weight gain; MLG=Mean length gain; LGR=Length gain rate and SGR=Specific growth rate.

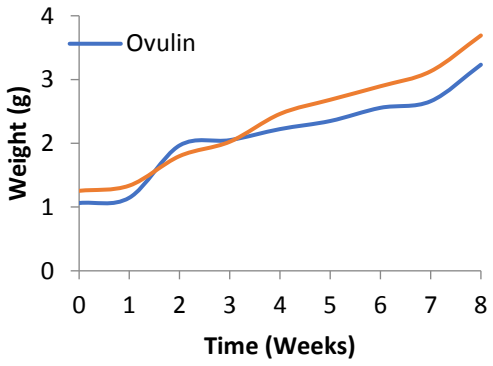

Figure 2: Showing weight gained trend of *Clarias gariepinus* induced with Ovulin and Chicken pituitary gland extract.

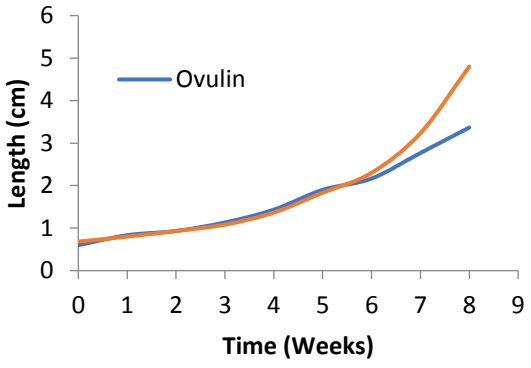

Figure 3: Showing length gained of *Clarias gariepinus* induced with Ovulin and Chicken pituitary gland extract.

Weekly Survival Rate of Clarias gariepinus induced with Ovulin and Chicken pituitary gland extract

Table 3 Showed the weekly survival rate of *Clarias gariepinus* induced with Ovulin and Chicken pituitary gland extract. With no significant difference at P>0.05 in all the parameters. Week 1 reveals higher survival in Ovulin (95.33±1.83) than Chicken pituitary gland extract (82.00±1.15) while at the end of the rearing period week 8 lower survival rate was observed in Ovulin (60.00±8.18) compared to chicken pituitary gland extract (62.66±6.35).

Table 3 Weekly Survival Rate of *Clarias gariepinus* induced with Ovulin and Chicken pituitary gland extract.

Spawning agents		Weeks							
		1	2	3	4	5	6	7	8
Ovulin	100±0[a]	95.33±1.85[a]	93.00±1.52[a]	86±1.52[a]	81.66±1.20[a]	71.66±6.00[a]	66.6±8.35[a]	62.60±8.95[a]	60.00±8.18 [a]
Chicken	100±0[a]	82.00±1.15[a]	79.00±0.57[a]	77.66±0.80	76.00±1.00[a]	74.66±0.66[a]	71.66±1.45[a]	62.66±6.35[a]	62.66±6.35[a]

Means with the same superscript along the Column are not significantly different (P>0.05).

Mortality Rate of *Clarias gariepinus* induced with Ovulin and Chicken pituitary gland extract.

Table 4 Showed significant different (P>0.05) in mortality rate at week 1, were mortality was lower in Ovulin (4.66±1.15) than Chicken pituitary gland extract (18.00±1.15). However, at the end of the rearing periods at week 8 there was a significant difference (P>0.05) between ovulin (3.00±1.00) and chicken pituitary gland extract (0.00±0.00).

Table 4: Weekly Mortality rate of *Clarias gariepinus* induced with Ovulin and Chicken pituitary gland extract

Spawning agents	Number of weeks							
	1	2	3	4	5	6	7	8
Ovulin	4.66±1.85[a]	2.33±1.20[a]	7.00±1.52[a]	4.33±0.33[a]	10.00±5.02[a]	4.33±2.84 [a]	4.66±0.33[a]	3.00±1.00[b]
Chicken	18.00±1.15[b]	3.00±1.00[a]	1.33±0.33[a]	1.66±0.66[a]	1.33±0.33[a]	3.00±1.00[a]	9.00±5.00[a]	00.0±0.00[a]

Means with the same superscript along the Column are not significantly different (P>0.05).

Mean weekly frequency of Shooters of Clarias gariepinus induced Ovulin and Chicken pituitary gland extract.

Table 5 Showed the mean weekly shooters frequency of *Clarias gariepinus* induced with Ovulin and Chicken pituitary gland extract, with no significant difference in all the parameters at P> 0.05. in week 0 shooters frequency was lesser in Ovulin (0.33±0.33) than Chicken pituitary gland extract (0.66±0.33), the same for week 1 were shooters frequency were lesser in Ovulin (0.66±0.33) than Chicken pituitary gland extract (2.00±0.00) while at week 8 shooters frequency were lower in Ovulin (1.66±0.33) than in Chicken pituitary gland extract (2.00±0.12).

Table 5: Mean weekly Frequency of shooters of *Clarias gariepinus* induced with Ovulin and chicken pituitary gland extract

Spawning agents	Weeks								
	0	1	2	3	4	5	6	7	8
Ovulin	0.33±0.33[a]	0.66±0.33[a]	1.00±0.57[a]	1.66±0.88[a]	1.33±0.33[a]	2.66±0.88[a]	1.66±0.33[a]	2.00±0.58[a]	1.66±0.33[a]
Chicken	0.66±0.33[a]	2.00±0.00[a]	2.00±0.57[a]	2.66±0.66[a]	2.33±0.66[a]	1.33±0.33[a]	2.33±0.88[a]	2.33±0.66[a]	2.00±0.12[a]

Means with the same superscript along the Column are not significantly different (P>0.05).

Water Quality of the Culture system.

Table 6 showed the result of the water quality for the cultured *Clarias gariepinus* during the 8 weeks culture period. The values observed were not significantly different P>0.05. The temperature was the same for the culture period at (27.00±0.05), pH ranged from Chicken pituitary gland extract (8.30±0.11) to Ovulin (8.10±0.17), Dissolve oxygen(mg/L) ranged from Ovulin (5.93±0.27) to Chicken pituitary gland extract (5.73±0.17), NH_3 (mg/L) ranged from Ovulin (0.06±0.01) to Chicken pituitary gland extract (0.05±0.00), and Electrical conductivity (µS/m) ranged from Chicken pituitary gland extract (29.66±0.33) to Ovulin (29.00±0.78).

Table 6: Water quality parameters of *Clarias gariepinus* in the rearing system

Spawning agents	Temperature(°C)	pH	Dissolved oxygen(mg/l)	NH_3(mg/l)	Electrical conductivity (µS/m)
Ovulin	27.00±0.05[a]	8.10±0.17[a]	5.93±0.27[a]	0.06±0.01[a]	29.00±0.78[a]
Chicken pituatry	27.00±0.05[a]	8.30±0.11[a]	5.73±0.17[a]	0.05±0.00[a]	29.66±0.33[a]

Means with the same superscript along the Column are not significantly different (P>0.05).

Discussions

Reproductive success of Clarias gariepinus induced Ovulin and Chicken pituitary gland

This study showed that Ovulin and Chicken pituitary gland extract have successfully induced spawning of African catfish. The effectiveness of Ovulin was sufficient to trigger the activity of reproductive hormones in the body of fish, by inducing gonad maturation and egg ovulation faster compared to Chicken pituitary gland extracts as reported (Rokade et al., 2006; Muchlisin et al., 2014). However, Chicken pituitary gland extract has successfully induced the ovulation of *Clarias gariepinus*, this is probably because pituitary gland contains the similar hormones as Ovulin. But the composition and concentration might be lower than Ovulin (Bowen, 2004). The pituitary gland produces gonadotropin that has an important role in gonad maturation of animals. Muchlisin et al., (2014), reported potentiality, compatibility and effectiveness of chicken pituitary gland extract in induced breeding of fish could be as a result of similarity in their physiology with other inducing hormones.

The highest hatchability rate was recorded in the recipients of synthetic hormone (Ovulin) and the lowest in the recipients of pituitary extract from chicken at 66.13% and 64.47% respectively. This record was better when compared to similar report that used the same source of pituitary for inducing female African catfish. For instance, the report of (Gadissa and Devi, 2013) showed that, the mean hatchability rate was 45.3% and 42.9% from female African catfish which were injected by African catfish and common carp pituitary extract respectively. However, the hatchability rate recorded in this present study, is lower, when compared to other studies Shourbela et al., (2014); Adebayo, (2006) that reported 89.1% and 71.7% respectively.

The higher fertilization and hatchability rate in both Ovulin and Chicken pituitary gland could be attributed to environmental condition and the nutritional status of the fish used in the experiment is in agreement with Agbebi et al., (2013), and also good management practice during the experiment contributed significantly to the production of both good quality and quantity gamete, and fish fry. The outcome of this corroborates the findings of Haylor, (1993), on induced spawning of a tropical ornamental fish.

Growth Performance of C. gariepinus induced with Ovulin and Chicken pituitary gland

Growth in fish differs between species, strains or population within the same species and even between individuals within the same population. The difference in growth performance of the fish indicates differences in adaptability between Ovulin and Chicken pituitary gland extract to local breeding conditions. Significance differences were observed in growth indices between the two hormones. In terms of weight, the highest fry value of 4.15g was observed in Chicken pituitary gland extract when compared to 3.23g in Ovulin, the highest fry length value of 4.8cm was observed in chicken pituitary gland extract when compared 3.36cm in Ovulin. This might be attributed to the fluctuation in the environment condition during the rearing period. The growth rate observed in terms of Length-Weight were higher in Chicken pituitary gland than in ovulin in the present study when compared to the values obtained by Zanial et al., (2014) in the induced spawning of Seurukan fish *Osteochilus* using Ovaprim, Oxytocin and Chicken pituitary gland extract which disagreed with the findings of Babalola et al., (2021) in the induce breeding of *C. gariepinus* using Ovaprim and Chicken pituitary gland extract, who reported lower values in chicken pituitary gland than in Ovaprim.

Survival of C. gariepinus induced with Ovulin and Chicken pituitary gland extract

After the egg yolk absorption, the highest survival was observed in Ovulin compared to Chicken pituitary gland extract, this is in agreement with the findings of (Tilahum et al., 2016), who reported that the progeny of the broodstocks, exhibited a decline trend in survival when advanced towards fry and fingerlings. Survival of fry reared for a period of eight (8) weeks declined towards their fingerlings stage, during these period, Survival rate in Ovulin was much higher for the first four 4 weeks as compared with Chicken pituitary gland extract, which could be due to the effectiveness of the hormone Ovulin, and the fluctuation in environmental conditions, which go in line with the findings (Zanial et al., 2014), who reported high survival rate of Ovaprim and Chicken. The last 4 weeks revealed an impressive rise in survival rate in Chicken pituitary gland extract than Ovulin, this might be attributed to its adaptability to the environment, this is incongruent with the findings of (Zanial et al., 2014), who stated Chicken pituitary gland has high survivorship but not as high as Ovaprim. The insignificant differences observed in the survival rate of larvae in Ovulin and Chicken pituitary gland extract shows that no inherent negative effect of chicken pituitary gland extract on larvae in the induced breeding that could result into neonatal mortality provided all the necessary hatchery management standard are adequately observed (Quintero and Davis, 2015).

Frequency of shooters of C. gariepinus induced with Ovulin and Chicken pituitary extract

Induced hormone variation significantly affected the shooters heterogeneity frequencies. The higher frequency of shooters was obtained in chicken pituitary gland extract and the less in Ovulin, this could be attributed to the higher rate of cannibalism among the fry and also environmental factors. Although, the result obtained is lower than the findings of Oyebola et al., (2015) who reported higher frequency of shooters in the induce breeding of African catfish pituitary and common carp pituitary.

Water Quality Parameters

Water quality parameter includes all physical, chemical and biological factors that influence the beneficial use of water. Careful monitoring of the water quality parameters was necessary in order to maintain condition within acceptable limit as recommended by Emmanuel et al., (2013). The physio-chemical parameters showed no significance difference among the cultured waters. The optimum growth of African Catfish requires a Temperature range of 28-30ºC, < 15mg/l dissolve oxygen, and 6.5-9.0 pH, in the rearing water. The values of water quality for these findings are within the acceptable ranges recommended for pisciculture. This could probably be one of the factors responsible for the success recorded in the induced breeding trials with Ovulin and Chicken pituitary gland extract.

Conclusion

The Ovulin and Chicken pituitary gland extract were effective in inducing female broodstocks for fish reproduction. However, the growth rate of individual fish and the numbers of shooters were higher in Chicken pituitary gland extract

than Ovulin hormonal treatment. However, fish farmers can also use Chicken pituitary gland extract as an alternative hormone in induced breeding program of *Clarias gariepinus*.

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