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Full Length Research

Comparative study on African catfish (Burchell, 1822) hypophysation using ovaprim and chicken pituitary gland extract

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ABSTRACT: Comparative study on African catfish hypophysation indices using ovaprim and chicken pituitary gland extract (CPGE) was carried out. Sixteen (16) African catfish brood stocks between 1100 and 2300 g in ratio 2:1 and four (4) numbers of layer birds (*Gallus gallus domesticus*) between 1200 and 2200 g in ratio 1:3 were used respectively. The experiment was batched into "A" and "B". Batch 'A' was placed on ovaprim while batch 'B' was placed on CPGE. An indoor hatchery vat, measuring 0.6096 m x 1.8288 m x 0.3048 m each was used for the incubation. Digital measuring kits were used to monitor water quality parameters and measurement of the brood stocks weights independently. Brood stocks on ovaprim and CPGE treatments had the following mean hypophysation indices in these order: fecundity (11100±7690 and 17760±13863), latency period (9.53±0.54 and 9.77±0.49), fertilization percentage (94.03±5.90% and 90.6±10.11%), hatchability percentage (75.6±0.81% and79.35±10.27%), number of eggs produced (5889±657.37 and 9403.33±589.80), total number of larvae hatched (4717.33±1111.0 and 7111.33±506.64), SGR (0.67±0.31 and 1.1±0.31), and survival rate (64.81±0.71 and66.24±2.19) respectively. There was no significant difference ($p = 0.05$) in the mean values of fecundity, latency period, fertilization and hatchability percentages in both treatments but there was significant difference ($p \leq 0.05$) in number of eggs produced and total number of larvae hatched in both treatments. The water quality parameters were within the range for induced breeding techniques and there was no significant difference ($p= 0.05$) in both experiments. Both ovaprim and CPGE had excellent results from the induced breeding of *Clarias gariepinus* and yielded good results. Therefore, CPGE could be recommended from the view point of excellent results and its availability from chicken slaughter houses as against ovaprim which are imported.

Keywords: Chicken pituitary gland extract, *Gallus gallus domesticus* hypophysation, ovaprim, *Clarias gariepinus*.

INTRODUCTION

All over the world, aquaculture practices depend largely on the supply of inputs especially quality fish seeds which must be readily available whenever it is needed for the smooth running of the farm in order to meet ever growing market demands for fish because the output of natural propagation in fish is very low and cannot meet the protein requirement of its consumers. This however, led to researches into the artificial propagation techniques under simulated environmental conditions to produce large quantities and good quality fish fries and fingerlings (FAO, 1996). These researchers had used various branded piscine hormones such as: pituitary extracts from common carp and salmon fish and non-piscine hormone such as: Deoxycorticosterone Acetate (DOCA), Human Chronic Gonadotropin (HCG), Black rat, Bull frog, and Toad (Nwoko, 1985; Fagbenro et al., 1991; Salami et al., 1992; Sunnuvu, 2004) in the practice of induced breeding which in turn had given some encouraging results.

Chicken pituitary gland extract was found to be physiologically similar to fish pituitary glands and is readily available in the commercial slaughter houses. A chicken pituitary gland extract was also found to be compatible with

teleost fish as a spawning induction agent in terms of potentiality, compatibility and effectiveness (Muchlisin et al., 2014).

C. *gariepinus* is a freshwater fish that belong to the family of Clariidae (De Graaf et al., 1995). Information on the reproduction of C. *gariepinus* has been documented in Africa (Clay, 1981; Nawar and Yoakim, 1984; Quick and Bruton, 1984). Despite the popularity of *C*. *gariepinus* as a species for culture and its great market potentials, production is still basically at subsistence level due largely to inadequate availability of seed for stocking, *C*. *gariepinus* seldom reproduce in captivity.

The aim of this research work is to use the chicken pituitary gland extract (*Gallus gallus domesticus*), a non-piscine hormone as a replacement for ovaprim, a piscine hormone and compare the result for plausible recommendations.

MATERIALS AND METHOD

Study design

A wet laboratory attached to government assisted Fish Farm Estate in Ikorodu, Lagos State of Nigeria was used to conduct the experiment. The facility has indoor hatchery tub measuring 0.6096 m x 1.8288 m x 0.3048 m each which was used for the experiment. The experiment was conducted in two batches. Batch 'A' (ovaprim inducement) and batch 'B' (Chicken pituitary extract inducement), each batch was replicated three times.

Broodstock fish

Eighteen (18) gravid brood stocks were selected (12 females and 6 males) at ratio 2:1 of *C*. *gariepinus* weighing between 1.1 and 2.3 kg using Ohaus Scout Pro Balances Models SP-601for the weight and afterwards acclimatized in plastic tank of 1 cm^3 of water for five (5) days before the commencement of induced spawning. Layer birds were also selected and weighed using table top scale model CWS-3 with accuracy of 0.2 gm. The weight ranged between 1.2 and 2.2 kg. The egg layers birds were acclimatized for five (5) days in a separate pen and fed with layer's mash ration *ad-libitum*. The layer birds are selected on ratio 1:3 based on the weight range between the fish and the layer birds.

Hormones source, preparation and injection

Ovaprim (salmon gonadotropin) used was sourced commercially (Syndel brand). Ovaprim was administered at the dosage of 0.5 ml per kg of each test animals as recommended by the manufacturer. The dosage was divided into two parts, 20% for decisive dosage and 80% for resolution dosage. Six females were injected each with 20% of 1.5 ml of ovaprim as a priming dose between 10.39 and 10.42 hours and a resolving dose of 80% at midnight (00.00 hours) respectively. The males were injected with ovaprim once at midnight in a separate holding tank. The hormonal induction in the experiment was administered intramuscularly, a little distance away from the head region as described by Adebayo and Popoola (2008).

Four hens (layers) in ratio 1:3 were used. Four female chickens were sacrificed and the skull compartment was opened towards the ventral's side and the pituitary gland was removed by the use of sterilized lancet. The pituitary gland was homogenized and 0.9% saline solution was added to it to make 100% hormonal solution and afterward centrifuged at 1000 rpm for 5 minutes and the supernatant was removed with calibrated syringe. Six females were injected each with 20% of 1.5 ml of chicken pituitary gland extract as a preparing dose between 10.40 and 10.50 hours, and a resolving dose of 80% at midnight between 00.00 and 00.30 hours, respectively. These procedures follow the order of Muchlisin et al. (2014) with slight modifications.

Physicochemical parameters

All the essential water quality parameters {Temp.(°C), DO (mg/l), pH, NH_3 (mg/l), NO_2 (mg/l)} for fish breeding were constantly monitored throughout the induced breeding period in both batch "A" and batch "B" by using water test kits (Ezdo Model PCT 407).

Sperm and eggs collection

The presence of eggs at the bottom of the holding tank and application of gentle pressure vertically along the belly of female brood stocks to see if eggs could freely ooze out are the test for final maturation of the eggs (Fagbenro et al., 1991). Female fishes that were ready are anesthetized with 2- phenoxy-ethylene solution before stripping. The eggs were quantified by weighing them as a mass and the number obtained as the product of total eggs weight and the weight of one egg. Healthy and developed eggs will be transparent with green-brownish coloration while the whitish eggs are those that were not healthy. The egg mass was homogenized using an electronic homogenizer before Fertilization (Rottmann et al., 1991).

The milt from each male was gotten by slaughtering the male fish brood stock and removing the two lobes of the male's testes. The testes were cut open and squeezed on the ripened fish eggs and were fertilized in round bottomed sterile plastic bowls. Fertilization was done by dry method thus, milt added to eggs in a dry container. Saline solution was added to the fertilized eggs to activate sperm and fresh cow milk was added to remove the stickiness of the eggs at a rate of 3% and continuously stirred in the homogenizer for 15 minutes and the fertilized eggs were spread on a kakaban or spawning net of 1 mm in the

spawning trough and the flow through system of the hatchery unit was activated according to Jhingran and Pullin (1985) with slight amendment.

After dry fertilization and activation of flow through system of hatchery, hatching of fertilized eggs commenced from 20.00 to 48.00 hours. The hatched fries were separated from un-hatched eggs by 1 mm net gauge and escaped into the vat underneath. Un-hatched eggs were removed from the incubator by siphoning to prevent water pollution. Feeding commences on the 4th day after their yolk sacs have been completely absorbed and were fed with small quantity of processed *Artimiasalina* in *ad-libitum* at 2 hours interval. During this period, water quality was monitored being an essential aspect of fish fries management as reiterated by Hogendoorn et al. (1980).

Parameters

The following performance parameters were used for the comparative studies: Specific Growth Rate (SGR): SGR=(In (final weight in grams) – In (initial weight in grams) x 100)/ t (in days).

Specific growth rate for fish larvae was obtained by: (Inw2-Inw1) x 100/ (t2 -t1), where w1 and t1 were total wet weight and time at the beginning of experiment and w2 and t2 were final wet weight and time.

Fecundity assessment = Initial weight – final weight of females x 66.6

Relative egg to body weight is calculated as (x/y) x 100/1 where: x = weight of egg for each female fish and y = weight of the female fish before injection.

Latency period is been calculated as Time interval between injections of the female fish and stripping of eggs.

$$\text{PGSI} = \frac{Weight\ of\ eggs\ collected\ by\ stripping}{Body\ wt\ before\ injec - wt\ of\ stripped\ eggs} x\ 100$$

$$\text{Fertilization}\ (\%) = \frac{Numberoffertilizedeggs}{Totalnumberofeggsincubated} x\ 100$$

$$\text{Hatchability}\ \% = \frac{Number\ of\ hatchlings}{Total\ number\ of\ fertilized\ eggs}\ x\ 100$$

$$\text{Survival Rate}\ = \frac{Number\ of\ living - No\ of\ died\ hatching}{100}$$

$$\text{Relative weight gain}\ (\%) = \frac{\text{Initial larvae weight}}{\text{Final larvae weight}} x100$$

These follow the procedures of Tsadu et al. (2012) and Olaniyi et al. (2013) on fish induced breeding. Note that the Survival Rate is after five days of hatching.

Statistical analysis

One-way T-test was used for the experimental design using R 3.6. 2, version in order to determine the two sample mean to ascertain whether the two means are from the same treatment or different treatments.

RESULTS AND DISCUSSION

Tables 1 and 2 shows the results obtained from *C. gariepinus* induced breeding indices using ovaprim and chicken pituitary gland extract (CPGE) respectively. The initial average mean weights of female brooder fish for ovarprim and CPGE for inducement were 2200±100 and 1266.67±208.17 g, respectively and the final mean weights after stripping were 2033±57.74 and 1000±0.00 g, respectively and the weight in both treatments were significant at p=0.05.

The mean hours for the latency period of female fish on ovaprim treatment was 9.53±0.54 hours while in the CPGE was 9.77±0.49 hours. There is no significant difference in the hours at 5% level of probability. The mean weight of the eggs stripped from female brooder on ovaprim inducement was 14.47±0.91 g and the mean weight of eggs from CPGE inducement was 12.73±3.00 g. The number of eggs produced (5889±657.37 and 9403.33±589.80). There is no significant difference in the weight of fish eggs stripped from both treatments at 5% level of probability. The mean fecundity in the ovaprim treatment was 11100±7690 while that of CPGE was 17760±13863. The weight of eggs produced in ovaprim treatment ranged between 13.50 g and 15.30 g with average and the percentage relative to fish weight ranged between 0.64 and 0.67%. Conversely, in CPGE treatment, the fish egg weight ranged between 8.08 and 10.10 g and the relative egg/body weight percentage ranged between 0.67 and 0.75%.

The mean percentage fertilization recorded in ovaprim treatment was 94.03±5.90% but in CPGE it was 90.6±10.11%. The hatchability mean percentage in ovaprim treatment was 75.6±0.81% and that of CPGE was 79.35±10.27%. The hatchability percentage and percentage fertilization in ovaprim and in CPGE treatments were significant at p<0.05 level of probability. The mean total numbers of hatched larvae from fish broodstock on ovaprim and CPGE treatments were 7111.33±506.64 and 4717.33±1111.0 respectively. Initial and final total mean weights of hatched larvae from ovaprim (48100±16930 and 144265 ±50803.98 g) and CPGE (75250±28600 and 225750±85799 g) were recorded. The initial and final mean weight of larvae from ovaprim (0.163± 0.06 and 0.217±0.07 g) with average daily weight gain of 0.01±0.00 g and fish larvae from CPGE (0.07±0.02 and 0.1±0.01 g) with average daily weight gain of 0.004667±0.00 g were also reported. The mean percentage relative weight gain and specific growth rate of

Table 1. *Clarias gariepinus* induced breeding using ovaprim (Batch 'A')

Parameters	Treatment and replicate			
	T1R1	T1R2	T1R3	Mean (±SD)
Initial weight of female brooder(g)	1200	1100	1500	1266.67±208.17
Final weight of female brooder(g)	1000	1000	1000	1000±0.00
Weight of egg stripped (g)	9.00	8.08	10.10	12.73±3.00
No. of eggs produced (1g = 650)	5850	5252	6565	5889±657.37
Relative egg/body weight((x/y) x 100	0.75	0.74	0.67	0.72±0.05
Fecundity	13320	6660	33300	17760±13863
Latency Period (hours)	9.20	10.00	10.10	9.77±0.49
Fertilization (%)	90	80.8	101	90.6±10.11
Hatchability (%)	81.55	68.16	88.35	79.35±10.27
Total number of larvae hatched	4772	3580	5800	4717.33±1111.0
Initial larvae total weight (g)	106700	50800	68250	75250±28600
Initial average larvae weight (g)	0.05	0.07	0.08	0.07±0.02
Final larvae total weight(g)	320100	152400	204750	225750±85799
Final average larvae weight (g)	0.10	0.09	0.11	0.1±0.01
Average daily weight gain (g)	0.003	0.005	0.006	0.004667±0.00
Relative weight gain (%)	50	77.78	72.73	66.83667±14.80
Specific Growth Rate (g/day)	1.0	0.4	0.6	0.67±0.31
Survival Rate after 5 days	65.33	64.01	65.1	64.81±0.71

Legend: T1R1 = treatment 1 replicate 1, T2R2 = treatment 2 replicate 2 and T3R3 = treatment 3 replicate 3.

Table 2. *Clarias gariepinus* induced breeding using chicken pituitary gland extract (Batch 'B')

Parameters	Treatment and replicate			
	T1R1	T1R2	T1R3	Mean (±SD)
Initial weight of female brooder (g)	2300	2100	2200	2200±100
Final weight of female brooder(g)	2000	2000	2100	2033±57.74
Weight of egg stripped (g)	15.30	13.50	14.60	14.47±0.91
No. of eggs produced (1g = 650)	9945	8775	9490	9403.33±589.80
Relative egg/body weight((x/y) x 100	0.67	0.64	0.66	0.66±0.015
Fecundity	19980	6660	6660	11100±7690
Latency Period (hours)	9.15	9.30	10.15	9.53±0.54
Fertilization (%)	99.45	87.75	94.90	94.03±5.90
Hatchability (%)	75.86	74.69	76.25	75.6±0.81
Total number of larvae hatched	7544	6554	7236	7111.33±506.64
Initial larvae total weight (g)	34880	42205	67180	48100±16930
Initial average larvae weight (g)	0.22	0.16	0.11	0.163± 0.06
Final larvae total weight(g)	104640	126615	201540	144265 ±50803.98
Final average larvae weight (g)	0.29	0.21	0.15	0.217±0.07
Average daily weight gain (g)	0.01	0.01	0.01	0.01±0.00
Relative weight gain (%)	75.86	76.19	73.33	75.127±1.56
Specific Growth Rate (g/day)	1.4	1.0	0.8	1.1±0.31
Survival Rate after 5 days	64.67	65.30	68.74	66.24±2.19

larvae from ovaprim inducement were 75.127±1.56% and1.1±0.31 g/day respectively. The larvae from CPGE mean percentage relative weight gain and specific growth rate were 66.83667±14.80% and 0.67±0.31 d/day.

The fish larvae mean survival rate after five days of hatching from ovaprim treatment was 66.24±2.19 while that of CPGE was 64.81±0.71. There was no significant difference in the survival rates in ovaprim and in CPGE at 5% level of probability.

Tables 3 and 4 shows all the essential physicochemical

Table 3. Essential physicochemical water parameters for ovaprim treatment (Batch 'A').

Day	Temp. (°C)	DO (mg/l)	pH	NH_3 (mg/l)	NO_2 (mg/l)
1	26.0	6.0	6.5	0.45	0.04
2	26.5	5.4	5.9	0.42	0.02
3	27.0	5.2	6.2	0.46	0.04
4	25.8	6.0	6.8	0.48	0.05
5	27.5	5.9	6.0	0.50	0.05
Mean±SD	26.56±0.70	5.7±0.37	6.28±0.37	0.46±0.03	0.04±0.01

Table 4. Essential physicochemical water parameters for CPGE treatment (Batch 'B').

Day	Temp. (°C)	DO (mg/l)	pH	NH_3 (mg/l)	NO_2 (mg/l)
1	26.1	5.9	6.4	0.44	0.04
2	26.4	5.5	6.0	0.43	0.02
3	26.0	5.3	6.2	0.45	0.04
4	26.8	6.1	6.9	0.49	0.05
5	27.4	5.8	6.1	0.50	0.05
Mean±SD	26.55±0.69	5.6±0.36	6.27±0.36	0.46±0.03	0.04±0.01

characteristics recorded during the induced breeding experiment. The water temperature during the trials ranged between 25.8 and 27.5°C while dissolved oxygen measurements in the experiment ranged between 5.2 and 6.1 mg/l. These two parameters are significant at 5% probability level and are negatively correlated. The pH of the water in ovaprim and CPGE treatments were recorded respectively and ranged between 5.9 and 6.9 and are significant at $p<0.05$. Ammonia and Nitrate concentrations in the 2 treatments were adequately monitored and recorded accordingly. This was found to be significant at $p=0.05$ and the recorded value ranged between 0.42 and 0.50 mg/l for ammonia, 0.002 and 0.05 mg/l for Nitrate. There is positive correlation between Ammonia and Nitrate concentrations. All the essential physicochemical parameters observed fall within the standard range for fish hatching and breeding techniques.

In order to overcome the challenges of breeding fish that cannot spawn in captivity, hypophyzation is the most commonly used method in inducing breeding in such species of fish where the administered synthetic or non-synthetic hormone stimulates the growth, development, maturity and ovulation of eggs. In the present study, the brood stock weights from the results and the corresponding number of eggs produced and the fecundity explains the significant roles age, size and weight of the fish plays in the fish induced breeding. Similar findings were presented by Quintero and Davis (2015) who reported that brood fish maturity affected reproductive performance in terms of spawning success and egg production of channel catfish.

There are significant differences in the number of eggs produced and the fecundity between ovaprim and CPGE treatments. This agreed with the publication of FAO (1996) and Muchlisin et al. (2014) who reported that the potentiality, compatibility and effectiveness of CPGE in induced breeding of fish could be as a result of similarity in their physiology. The latency period, fertilization and hatchability in both ovaprim and CPGE treatments are encouraging. This could be attributed to good environmental condition and the nutritional status of the brood stock fish used in the experiment. This also in the agreement with Agbebi et al. (2013) and also, good management practices during the experiment contributed significantly to the production of both good quality and quantity of gamete, and fish fries. The outcome of this corroborates the findings of Haylor (1993) on induced spawning of a tropical ornamental fish.

Three variance factors that exist between fertilization, hatchability and total number of larvae hatched are the environmental condition, brood stock management and the quality of the hormone used. It shows from the results that these three factors played good roles and met the standard for good fish fry yield in batch 'A' and 'B' of the experiments (Agbebi et al., 2013). The reported specific growth rates *vis-à-vis* good quality fish eggs in both batches agreed with the findings of other researchers such as Bobe and Labbé (2010) and Rønnestad et al. (2013) which shows that the egg yolk is of high quality as indicated in the specific growth rate of the fry after 5 days of hatching before the introduction of exogenous feeding.

The insignificant differences observed in the survival rate of larvae in ovaprim and CPGE treatments though high shows that there is no inherent negative effect of CPGE on larvae in the induced breeding that could result into neonatal mortality provided all the necessary hatchery management standards are adequately observed (Quintero and Davis, 2015). All the reported essential

physicochemical parameters are within the threshold limits for the fish induced breeding (Ukwe et al., 2016). This could probably be one of the factors responsible for the success recorded in the induced breeding trials using ovaprim and CPGE.

In conclusion, the use of ovaprim and CPGE had excellent results in the induced breeding of *Clarias gariepinus* but CPGE could be recommended for use in artificial breeding from the view point of its cheapness and availability from chicken slaughter houses over ovaprim which is imported.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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