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Comparative Study of Carp Pituitary Extracts (CPE) and Ovupin-L on Induced Breeding in African Catfish (Clarias gariepinus)

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Abstract
A comparative study of Carp Pituitary Extract (CPE) and Ovupin-L on induced breeding of African catfish (*Clarias gariepinus*) was conducted in applied hydrobiology and fisheries unit of Zoology Department, University of Jos. 24 brood stocks (12 males and 12 females) were sourced and selected from a private fish farm at Gangare, behind Murtala house Jos between the months of July to August 2010. Four of the females were hypophysized with 3mg, 4mg, 5mg and 6mg/kg body weight (BW) respectively of carp pituitary extract; four were hypophysized with 0.3, 0.4, 0.5, and 0.6mg/kg (BW) ovupin-L while the remaining four were injected with 0.3, 0.4, 0.5, and 0.6ml/kg (BW) of normal saline which serve as a control. The females were isolated separately and kept in plastic tanks (A-L) for latency period of 14hrs after hypophysation, while the males were kept in a single plastic tank. And were later dissected and testis removed to obtain milt which was used to fertilize the stripped eggs. The number of eggs spawned was estimated using standard formula. The result revealed that, all the females hypophysized with CPE and ovupin-L spawned in all the dosages but no fish spawned in all the dosages of normal saline. Ovupin-L at 0.5 ml/kg gave the best result in all the four parameters of fecundity investigated. There was a significant difference between the CPE and ovupin-L on fecundity and fertilization of *Clarias gariepinus* with ovupin-L performing better than CPE in all the parameters ($p < 0.05$). Ovupin-L at dosage of 0.5ml/kg highly significant than other tested parameters in *Clarias gariepinus*, the dosage with the lowest output was obtained with CPE at 0.4mg/kg body weight.
Keywords: carp pituitary, extract, ovupin-L, African catfish, induced breeding, hypophysation.

Introduction

Fish breeding naturally has been an important tool for fish perpetuation and for food by man and his livestock. This natural breeding is triggered by suitable physiological and environment changes at particular seasons or periods of the year (Audu and Ofojekwu, 2010). The most important area in the field of aquaculture in recent times is the use of various hormones to induce spawning in fish to meet the demand of fish for food by the increasing population of human beings worldwide (Audu and Ofojekwu, 2010). The big problem to most inland fresh water aquaculturist today, is the lack of knowledge and skills by the farmers regarding the use of hormones to improve the commercial production of fishes. many hormones including lutenizing hormone releasing hormone (LHLRH), deoxycorticosteroids acetate (DOCA), human chorionic gonadotropin (HCG), ovaprim, ovatide, crude pituitary extract(CPE) of carp, catfish, other fishes and ovupin-L have been used in the induction of different fish species over the past two decades (Zonnevald *et al*. 1988).

Many researchers have reported on the use of CPE in combination with other hormones or separately on the induction of *Clarias gariepinus* and other related species. Audu and Ofoejekwu (2010) induced African catfish (*Clarias gariepinus*) with crude carp pituitary extract and ovaprim. They reported that all the six fishes hypophysized with CPE and ovaprim using three females respectively spawned well having the highest fecundity from females hypophysized with 5mg/kg body weight of ovaprim.

Ofojekwu *et al*, (2001) induced common carp (Cyprinus carpio) with piscine and non-piscine pituitary extracts i.e. carp and frog respectively. They also reported that all nine fish hypophysized with CPE spawned. They also reported that the number of eggs spawned increased with increasing dosage of the hormones. (Salami *et al*. 1994) had reported that a similar increase in egg output due to administration of HCG and CPE in *Clarias gariepinus*. Obi and Popoola(1994) injected different fish with different pituitary extracts and found that fish injected with CPE and Clarias pituitary extracts spawned satisfactorily. Omoregie *et al*, (1998) compared the efficacy of pituitary extracts of carps, tilapia and *Clarias* on common carps but all the six fish given the CPE spawned positively.

These studies compare the effect of CPE and ovupin-L on the fecundity and fertilization of the African catfish (*Clarias gariepinus*, teugels 1986) and determine the most effective hormone and dosage in the induction

of this catfish.

Materials and Methods

Twelve matured male and twelve gravid female catfish, *Clarias gariepinus* were collected from a private fish farm at gangare behind murtala house Jos, plateau state, Nigeria. The males and the females were identified by their external genital structure. The males have distinct papilla located just behind the anus. This sexual papilla is absent in females which have rounded vents. Gravid females (with distended abdomen which release eggs on slight pressure on the abdomen) and matured males (with reddish tip of papilla) were distinguished by sex and transported to the hatchery in another private fish farm adjacent AP filling station terminus, Jos.

Before the introduction of the brood stock in the hatchery, two concrete tanks(ponds) A and B were washed with high concentration of salt and rinsed thoroughly with municipal water and sterilized with malachite green, the water depth was maintained at 0.8m (about 300 liter each). The spawning mats and basins were treated with malachite green to prevent it from fungus and bacterial attacks. The tanks were all covered with wire netting which prevented the brood fish from jumping out.

The pollution in the tanks due to uneaten died and feacal matter were removed by changing the water every four days for two weeks and the water quality parameters like temperature, free carbon dioxide (CO2), dissolved oxygen (DO), PH and alkalinity were monitored using the APHA (1985) methods.

Extraction of CPE and Source of Ovupin-L

Both sexes of matured carp (*Cyprinus carpio*) were used for the extraction of carp pituitary extract (Vivean *et al*., 1986). All the extracted pituitary glands of each were kept immediately in a vial containing 10ml acetone which was refreshed after 10 minutes and later after 8 hours. It was drained off completely after 24 hours; air dried by evaporation and sealed in a vial for storage at 4˚C.

Ovupin-L (salmon GnRHa) a synthetic commercial hormone was obtained from green water fish store, adjacent dilimi central pharmacy close to plateau state library Jos, a day before induction and stored at room temperature.

Administration of CPE and Ovuipin-L and removal of Testis

During the administration, only the female replicates were hypophysized intramuscularly with hormones and the fish were given knock-outs (final or resolving) dose as recommended by Garcia *et al*, (2000).

CPE 3mg, 4mg, 5mg, and 6mg/kg were administered to replicate 1, 2, 3, and 4 respectively of group A, ovupin-L 0.3ml, 0.4ml, 0.5ml and 0.6ml/kg body weight were administered to replicate 1,2,3 and 4 respectively of group B, while replicates 1, 2, 3 and 4 of group C(the controls) were injected with 0.3ml, 0.4ml, 0.5ml and 0.6ml/kg body weight of o.9% normal saline. The fish were all injected with the hormone based on their body weight per kg at 6.00 pm using a wet towel to cover the head and eye before injection which serves as anesthesia. All the hypophysized females (brooders) were kept separately in plastic basins with 20 liters of water each and covered with heavy wire mesh to prevent jumping out.

The following day after a latency period of 14 hours, the induced brooders were checked for spawning before the males were dissected to remove the testis. It was to maintain high potency. When the female was found to be ready, a male was dissected using a new sterilized razor blade in the presence of 0.9% normal saline. The male head was covered with clean towel and the belly turned upward and the dorsal part downward. The belly was dissected from the anterior part to the posterior, carefully not to temper with the internal organs. The testes were located with the hand using hand gloves and the testes were cut with the blade. The dissected portion was sutured back with a sterilized needle and a string and then released into the bowl. The testis were cleaned with 0.9% normal saline and wrapped in tissue paper. The female with ripped over (eggs) was then stripped by holding slightly on its side, tail down. Gently hand pressure was applied to the abdomen moving downward the vents.

The stream of egg was directed into a clean dry bowl. This was weighted to determine the approximate number of eggs per female brood fish (fecundity). Care was taken to prevent mixture with water, feaces and body fluid on the egg because they can render the eggs unviable for fertilization.

Fertlization

Immediately after stripping, the preserved testis kept at room temperature in a clean tissue paper was incised with the same razor blade wrapped in a clean nylon net and was squeezed with the thumb to release milt on the eggs. It was then mixed properly using pre-disinfected bird feather to ensure complete fertilization of the eggs. Little amount of normal saline (0.9%) was also added for complete mixing. The fertilized eggs were spotted (counted) for approximate number of fertilized eggs per brood fish.

Estimation of Spawning Success

The estimation of the eggs spawned and percentage fertilization was carried out using the method put forward by Nikolski, (1969), dekimpe and micha (1974) and Hogendoorn (1979). The spawning was calculated as follows.

Total number of eggs spawned=differences between pre-spawning weight + 666 (Hogendoorn, 1979) and the weight of striped eggs + 666.

Percentage fertilization = N/n×100 (Dekimpe and Micha, 1974).

Where N= total number of eggs spawned

n= number of unfertilized eggs

Estimation of Fertilized Eggs

Before the estimation of fertilization, the fertilized eggs were differentiated from the unfertilized eggs once by the presence of 'eye spot' swelling. It was carried out using the formula,

a/7×N (Nikolski, 1969).

Where y=total number of fertilized eggs estimated.

a= number of eggs counted from the 7 sampled bunches on spawning mat.

N= total number of bunches on the spawning mat. The eggs in each sample were counted using a tally counter.

Estimation of Unfertilized Eggs

The unfertilized were estimated using the formula

Z=b/7×N (Nikolski, 1969).

Where:

Z=estimated number of unfertilized eggs from 7 bunches of sample on the mat.

b= number of unfertilized eggs from 7 bunches on the mat.

N= total number of bunches on the spawning mat.

Statistical Analysis

The data obtained were analyzed using one way analysis of variance (ANOVA) to determine significant difference between treatments. The differences were checked at p=0.05 level of significance.

Results

The mean physicochemical parameter of tanks A and A showed that temperature (˚C),pH, dissolved oxygen (DO) alkalinity (mg/l) and free carbon dioxide (mg/l) were 24.14, 6.0, 5.91,6.0 and 3.96 respectively for both tanks as represented in table 1.

The effect of CPE on fecundity and fertilization in Clarias gariepinus is presented in table 2. The table showed that female 3 (f3) (870g weight) which was injected with 0.5mg/kg body weight of the hormone gave the highest fecundity (65,268), followed by female 4 (f4) (960g weight) whose fecundity was 53,680. The third female whose weight was 900g/kg body weight gave a fecundity of 41,225. Female 2(f2) 880g gave the lowest fecundity of 34,699.

The effect of hormone on fertilization shows that F3 which was injected with 0.5g/kg body weight gave the highest number of fertilized eggs (51,235) which constitute 78.5% of the total number of stripped eggs (65,268. The second highest number of fertilized eggs of 40,851 was observed in F4 which made up to 76.1% of the total fecundity. The third highest number of fertilized eggs of 28,569 was also observed in F1 with 69.3% of the total number of fecundity. Female 2 (F2) showed the lowest number of fertilized eggs (22,138). This value constitutes about 63.8%of the total number of stripped eggs.

The effect of ovupin-L on fecundity and fertilization in *Clarias gariepinus* is shown in table 3. The table indicates that F3 (870g) was injected with 0.5ml/kg body weight of ovupin-L gave the best fecundity (99900). This was followed by F1 (950g) with fecundity of 83,250. This was followed by F2 (weighed 900g) with fecundity of 66,893. The lowest fecundity 73,260 was observed in F4 (1000g) after injection of 0.6ml of ovupin-L per kilogram weight of fish.

The table also indicated that F3 which was induced with 0.5ml of ovupin-L body weight gave a very high number of fertilized eggs (96403) which constituted 96.5% of the total fecundity of 99900, F1 (950g) injected with 0.3ml of ovupin-L gave the second highest number of fertilized eggs (75425) which is 90.6% of the total stripped eggs of 83,250. F2 (900g) injected with 0.4ml of ovupin-L gave the third highest number of fertilized egg (66893) with a percentage of 83.7% of the total stripped eggs of 79920. The lowest fertilized eggs (60733) was observed in F4 (1000g) injected with 0.6ml of ovupin-L gave 83.9%.

It was observed in both table 2 and 3 that the fecundity and fertilization of the eggs was directly proportional to weight of the eggs stripped and not weight of the fish (brood fish).

Table 2 shows that eggs stripped from F3 gave a weight of 98g, with fecundity 65368, fertilized egg of 51235 and a percentage fertilization of 78.5%. The second weight of eggs was from F4, the third was from F1 and the last was from F2. The fertilization and percentage fertilization followed the same pattern respectively.

Table 3 followed the same pattern with F3, 150g; F1, 125g; F2, 120g and F4 with 110g for the fecundity,

fertilized eggs and percentage fertilization from the higher weight to the lower weigh of eggs stripped respectively. The various doses of normal saline (0.3, 0.4, 0.5 and 0.6ml/kg body weight) showed no effect on the fecundity and fertilization of *Clarias gariepinus* as shown in table 4.

Table 1. Mean Physiochemical Parameters of Tanks A and B

Parameter	Tank A	Tank B
Temperature(˚C)	24.14±0.5	24.11±0.5
pH	6.0±0.5	6.0±1.8
Dissolved Oxygen(DO)	5.91±0.7	5.87±0.7
Alkalinity(mg/l)	6.0±1.8	6.0±1.8
Carbon Dioxide(CO2)	3.96±0.3	3.96±0.3.

Table 2. Effect of CPE on Fecundity and Fertilization in *Clarias gariepinus*

Female Broodstock	fish wt (g)	Dosage (ml)	Egg wt (g)	No of stripped egg	Fertilized eggs	% Fertilization
	900	0.3	61.9	41225	28569	69.3
	880	0.4	52.1	34699	22138	63.8
	800	0.5	98.0	65268	51235	78.5
	960	0.6	80.6	53680	40851	76.1
Mean(S.E)	885± 40	0.45 ±0.08	73.15 ±22.95	48718 ±30569	35068±6207.25	71.93 ±3.68

Table 3. Effect of Ovupin-L on Fecundity and Fertilization on *Clarias gariepinus*

Female Broodstock	Fish wt (g)	Dosage (ml)	Egg wt (g)	No of stripped egg	Fertilized eggs	% Fertilization
	950	0.3	125	83250	75425	90.6
	900	0.4	120	79920	66893	83.7
	870	0.5	150	9990	96403	96.5
	930	1000 0.6	110	73260	60733	83.9
Mean ± S.E)	930±32.5	0.45±0.08	126.25±10.00	84082.5 ±23143.5	74863.5 ±35670.0	88.43 ±3.85

Table 4. Effects of Normal saline on Fecundity and fertilizationin *Clarias gariepinus*

Female Broodstock	Fish wt (g)	Dosage (ml)	WT of Eggs (g)	No of stripped egg
	800	**0.3**	**0**	**0**
	1000	0.4	0	0
	910	0.5	0	0
	950	0.6	0	0
Mean ± S.E	915±50.0	0.45±0.08		

Discussion

After hypophysation of the various brood stock with different dosages of CPE, ovupin-L and normal saline, all the fish injected with CPE and ovupin-L in all the dosages spawned except those injected with the normal saline which served as the control. This result agrees with the findings of Ofojekwu *et al*, (2001) and Audu and Ofojekwu (2010). It also agrees with the findings of Obi and Popoola (1994) and Omoregie *et al*, (1998) that all fish injected with CPE spawn satisfactorily.

The result of this work show that fecundity does not increase with increase in dosage of the hormones as earlier reported by Salami *et al*, (1994), Ofojekwu *et al*, (2001) and Haniffa and Sridhar (2007). But rather fecundity and fertilization increases with increase in the weight of eggs striped (table 2 and 3). This could be because of maturity of the brood stocks.

In comparing the effects of four doses of CPE (3, 4, 5 and 6mg/kg) body weight and ovupin-L (0.3, 0.4, 0.5, and 0.6ml/kg) body weight on fecundity. It could be seen from the result that ovupin-L at a dose of 0.5ml/kg body weight perform significantly better than all the doses of CPE. This is in agreement with the report of Okoro *et al*, (2007) an Audu and Ofoejekwu (2010) who effectively induced Oocyte maturity with 0.5ml/kg of ovaprim in *Clarias gariepinus* and found it worthy of recommendation for use.

The percentage fertilization show that ovupin-L at 0.5ml/kg body weight was significantly different from the same dose (5mg/kg) or other doses (3, 4 and 6 mg/kg) body weight of CPE. The highest percentage fertilization

of 96.5% was recorded with the 0.5ml/kg body weight dose of ovupin-L. The highest percentage fertilization with CPE was 78.5 % and this was recorded with the 5mg/kg dose which is much less than that of ovupin-L. This shows the superiority of ovupin-L over CPE.

Conclussion

In this research work, the effect of Ovupin-L on fecundity and fertilization of African catfish (*Clarias gariepinus*) was investigated. Carp pituitary extract and Ovupin-L doses of 0.3, 0.4, 0.5 and 0.6 ml/kg body weight in *Clarias gariepinus* induced ovulation, but Ovupin-L was the best compared to CPE with respect to percentage fertilization especially Ovupin-L administered at 0.5ml/kg in the fish had the highest of 96.5% fertilization.

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