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Effect of Different Doses of Saline-diluted Ovatide on the Breeding Performance of *Clarias anguillaris* and *Clarias gariepinus*

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Abstract

A study on the effect of different doses of Ovatide hormone suspended in saline on the breeding performance of *Clarias anguillaris* and *Clarias gariepinus* was carried out. The experiment was conducted in a 2x5 factorial experiment in a Completely Randomized Design (CRD) at the Hatchery Unit of the Department of Fisheries and Aquaculture, Usmanu Danfodiyo University, Sokoto. Species and hormone dilutions constituted the factors; specie having 2 levels (*C. anguillaris* and *C. gariepinus*) and ovatide hormone suspended in saline at 5 levels (0%, 25%, 50%, 75% and 100%). The result showed that species levels did not significantly ($p>0.05$) affect the breeding performance in all the parameters observed. However, fertilization rate, hatching rate and survival rate were significantly affected ($p<0.05$) by different levels of ovatide suspended in saline, but did not have significant influence ($p>0.05$) on egg weight, spawning fecundity and relative fecundity. It could be concluded from this study that the synthetic hormone ovatide can effectively be suspended in saline at different dilution levels of 25%, 50% and 75% to induce breeding in *C. anguillaris* and *C. gariepinus* and successfully record good fecundity, fertilization, hatching and high survival of larvae which in turn reduces the quantity of the synthetic hormone to be used.

Keywords

African Catfish, *Clarias gariepinus*, Induced Breeding, Synthetic Hormone, Ovatide

1. Introduction

The African catfish is widely considered as the leading cultured fish in Nigeria. Some of the credentials of African catfish are: high growth rate reaching market size of 1 kg in 5–6 months under intensive management conditions: highly adaptable and resistant to handling and stress; can be artificially propagated by induced spawning techniques for reliable mass supply of fingerlings; commands a very high commercial value where it is highly cherished as food in Nigerian homes and hotels [1-2].

Clariid catfish is the most sought after fish species among fish farmers and consumers because it commands a very good commercial value in Nigerian markets [3] According to Benedict *et al.* [4] *Clarias anguillaris* and *Clarias gariepinus* are the two species most readily acceptable in Nigeria, because they grow to large sizes. African catfish hardly

reproduces in captivity but with the popular induced breeding technique (artificial method of spawning, incubation and hatching of eggs under controlled environmental conditions), it has been made possible to produce fish seed all year round [5].

Hypophysation has been used to achieve mass production of African Catfish. Hormone substances used in hypophysation include acetone dried carp pituitary at 4mg/kg for *C. gariepinus* [6-7] or fresh pituitary gland by matching weight to weight in a recipient/donor fish. Other substances used are mammalian hormones like Human Chorionic gonadotropin (HCG) [8]. Production has also been achieved by using other substances like; Luteinizing Hormone (LH) or Follicle stimulating hormone (FSH), and deoxycorticosterone acetate (DOCA) which only induces pre-ovulation [9]. Some of these spawning agents are either difficult to quantity, ineffective or of short shelf life, and for that, many breeders are reluctant to use them in field conditions. However, the commercially available synthetic inducing hormones in ready-made form containing GnRHa and dopamine (Ovaprim, Ovopel, Ovulin, Ovatide, Dagin and Aquaspawn) are becoming very popular and found to be efficient in successful spawning of fishes [10-12].

This study was carried out using Ovatide hormone suspended saline for induced breeding of *C. anguillaris* and *C. gariepinus* in order to test the effect of various doses of the normal saline diluted-hormone with undiluted ones on the fertilization, hatching and larval survival of both *Clarias anguillaris* and *Clarias gariepinus*.

2. Methodology

2.1. Study Area and Broodstock Procurement

The experiment was conducted at the Hatchery unit of the Department of Fisheries and Aquaculture located at the main campus of Usmanu Danfodiyo University, Sokoto (N13º07'45.12" E5º12'18"). Eighty (80) broodstock, forty (40) each of *C. anguillaris* and *C. gariepinus* (30 females, 10 males each) were collected from the Departmental Fish Farm of the university. The fish were conditioned at the hatchery complex of the farm and were fed commercially produced industrial feed (Coppens) at 3% body weight twice daily for two weeks before the commencement of the experiment.

2.2. Experimental Setup and Design

The experiment was set up in a 2 factor (2 × 5) factorial experiment in a Completely Randomized Design (CRD) with two levels of *Clarias* species (*C. anguillaris* and *C. gariepinus*) and five inclusion levels of Ovatide hormone suspended in saline (at 0%, 25%, 50%, 75% and 100%). All the treatments were replicated three times to give a total of 30 spawning trials (i.e. 2 × 5 = 10, replicated 3 times = 30). Induced breeding was carried out and data collected was subjected to statistical analysis.

The treatment combinations were therefore; A_0B_0, A_0B_1, A_0B_2, A_0B_3, A_0B_4, A_1B_0, A_1B_1, A_1B_2, A_1B_3, A_1B_4. Where A_0 = *Clarias anguillaris*, A_1 = *Clarias gariepinus*, B_0 = 0%, B_1 = 25%, B_2 = 50%, B_3 = 75% and B_4 = 100%. These were randomly distributed in triplicate tanks in a CRD.

2.3. Injection, Egg Collection and Fertilization

Ovatide was used to induce ovulation at a recommended dosage of 0.5ml/kg body weight of female broodstock, while half dosage was administered to male broodstock [13]. Hormone administration was carried out via intramuscular injection with 0%, 25%, 50%, 75% and 100% inclusion levels of normal saline and the injected fish were kept separated in well-labelled closed containers containing water. After a latency period of about 8 hours, eggs were collected from each female through stripping by gently pressing the abdomen of the fish and milt was obtained by sacrificing the males. Each male was dissected carefully and their milt sac obtained.

The milt obtained from the male fishes was squeezed gently onto the stripped eggs obtained from the females accordingly and stirred gently and thoroughly using plastic spoon for about 2 minutes to allow contact and adequate fertilization.

2.4. Incubation and Hatching

Checking the mixture of the eggs and milt were distributed in a single layer on the spawning nets in the well aerated incubation bowls. Three gram of egg was collected from each sample and incubated in 60-litre plastic containers for the experiment, for easy assessment of fertilization and hatching rates [14].

2.5. Data Collection

Data on induced breeding performance (ovulation response, fecundity, fertilization rate, hatching rate and larval survival rate) were recorded.

2.6. Data Analysis

The data collected on the induced breeding parameters was subjected to statistical analysis using SPSS (Version 20). All data with discrete counts and percentages was transformed before analysis was carried out. The data were analyzed using analysis of variance (ANOVA) to test for significant differences ($P<0.05$) in fertilization rate, hatching rate and larval survival, and means were separated using Duncan's Multiple Range Test (DMRT) where significant difference exist [15].

3. Results

3.1. Mean Weight, Dosage, Incubation Period and Latency Period

The mean initial weight of the broodstocks used for the experiment ranged from 326.67g to 500.00g (Table 1). Dosage administered for injection of the broodstocks ranged from 0.06 ml to 0.1ml at the recommended dosage of 0.2ml/kg body weight and the quantity of egg used for incubation was 3 grams for each treatment. The number of eggs obtained in 1 g

of egg was between the range of 623 to 660. Latency period was between 8hrs to 8hrs and 10mins and incubation period ranged between 21hrs 10mins to 23hrs and 4mins for all the treatments.

Table 1. *Weight, dosage, latency period and incubation period during induced breeding with ovatide.*

Treatments	Parameters				
	Initial Weight (g)	Dosage (ml)	No of Egg (1g)	Latency Period	Incubation Period
A_0B_0	500.00	0.10	623	8h 9m	23h 4m
A_0B_1	440.00	0.08	634	8h 2m	22h 57m
A_0B_2	340.00	0.06	645	8h	22h 10m
A_0B_3	373.33	0.07	629	8h 1m	22h
A_0B_4	340.00	0.06	-	-	-
A_1B_0	373.33	0.07	664	8h 1m	22h 20m
A_1B_1	408.67	0.08	695	8h 10m	21h 30m
A_1B_2	403.33	0.08	661	8h	22h 55m
A_1B_3	396.67	0.08	660	8h	21h 10m
A_1B_4	326.67	0.07	-	-	-

3.2. Breeding Performance of *C. Anguillaris* and *C. Gariepinus* Induced with Ovatide Suspended in Saline

The result of breeding performance of *C. anguillaris* and *C. gariepinus* induced with different levels of ovatide hormone suspended in saline is shown in Table 2. The result indicates that species levels did not have significant effect on the breeding performance in this experiment ($p>0.05$) with both *C. anguillaris* and *C. gariepinus* producing statistically similar mean values in terms of egg weight, spawning fecundity and relative fecundity, as well as the breeding performance parameters (fertilization rate, hatching rate and larval survival rate). The result further showed that different doses of ovatide suspended in saline significantly affected the breeding performance in this experiment. In terms of egg weight, spawning fecundity and relative fecundity, no significant difference was observed between the dilution levels (positive control, 25%, 50% and 75%). However, negative control (100% saline with 0% ovatide) did not produce any value since spawning did not occur. 0% saline dilution (positive control) produced relatively higher mean values than all the other dilution levels in terms of fertilization rate, hatching rate and survival rate. However, it was statistically similar ($p>0.05$) with 50% dilution level but significantly different ($p<0.05$) from 25% and 75% with respect to fertilization rate and hatching rate. It was also statistically similar ($p>0.05$) to 25% dilution level but significantly different ($p<0.05$) from 50% and 75% with respect to survival rate.

Table 2. *Main effects specie and ovatide suspended in saline effects on induced breeding performance of C. anguillaris and C. gariepinus.*

Factors	Parameters					
	EW (g)	SF	RF (g)	FR (%)	HR (%)	SR (%)
Specie						
C. anguillaris	38.72	26,039	64.55	67.11	57.27	74.81
C. gariepinus	30.69	20,724	53.63	65.33	55.67	74.05
SEM	2.45	1688.85	5.17	1.74	2.04	0.44
Hormone dilution						
0%	46.37^{a}	$30,777^{a}$	69.30^{a}	93.33^{a}	82.21^{a}	94.67^{a}
25%	42.02^{a}	$28,829^{a}$	69.37^{a}	84.44^{b}	67.04^{bc}	93.47^{ab}
50%	39.33^{a}	$26,732^{a}$	75.29^{a}	88.33^{ab}	74.20^{ab}	92.36^{b}
75%	45.80^{a}	$30,569^{a}$	81.51^{a}	65.00^{c}	58.90^{c}	91.65^{b}
100%	00.00	0.00	0.00	0.00	0.00	0.00
SEM	3.88	2670.31	8.18	2.74	3.22	0.70
Interaction	NS	NS	NS	NS	NS	NS

NS = Not significant.

Means with the same superscripts on the same column are not significantly different (P>0.05).

EW = Egg weight, SF = Spawning Fecundity, RF = Relative Fecundity, FR = Fertilization Rate, HR = Hatching Rate, SR = Survival Rate.

4. Discussion

4.1. Fish Weight, Latency Period and Incubation Period

The size range of the broodstocks used in the experiment was in agreement with Viveen *et al.* [16] who opined that African catfish clarias can become mature and breed as from 200g body weight. And it agrees also with de Graaf and Janssen [17] who reported that the ideal broodfish weight should be between 300-800 grams, as larger fish are difficult to handle and often results in substantial egg losses prior to stripping. The time taken to achieve ovulation (latency period) is dependent upon water temperature as reported by [18], as such the higher the temperature the quicker the eggs ovulate. In other words, the higher the temperature the shorter the latency period. The mean latency period observed in this study fall within 8hrs at mean temperature of between 27 – 28°C and was similar to what was reported by [17] for *Clarias*

gariepinus. The result also showed no significant variation of latency time between the treatments except for treatment induced with 100% normal saline which could be the reason why ovulation did not occur for that particular treatment in all the phases which is due to the lack of hormone effect that foster ovulation in fish. This was similar to what was observed by [19] on induced breeding of *Clarias gariepinus* using different doses of normal saline-diluted ovaprim.

De Graaf and Janssen [17] reported that the development process of fish from fertilized egg to hatching is like all other biological processes, that is, it is dependent upon water temperature, as such the higher the water temperature the faster the eggs hatch. The incubation period observed in this experiment was in the range of 21 to 23hrs at a temperature range of about 25.9 to 28.6°C which was similar to observations of Viveen, *et al* and was also in comparison with the findings of Shinkafi and Ilesanmi for *Clarias gariepinus* that achieved incubation period of 15hrs at a temperature of 30°C [16, 20].

4.2. Breeding Performance

The spawning fecundity observed in the study showed that different doses of ovulin suspended in saline at 25%, 50% and 75% inclusion levels can be effective in the induced breeding of *C. anguillaris* and *C. gariepinus*. The highest mean fecundity value (30,777) was observed in 0% dilution level (100% hormone) which is the positive control. This was in agreement with Viveen *et al.* (1985), that larger female fishes contain more eggs than smaller fishes and therefore have higher fecundity values and this could also be due to the efficacy of the hormone used which indicates that even a small quantity of hormone can be diluted with saline and be effective in the induced breeding of African catfish.

4.3. Relative Fecundity

The relative fecundity observed in the study showed that the highest mean values of 81.51 was observed in 75% dilution level but there was no significant difference ($p>0.05$) between the different dilution levels except the negative control (100% normal saline) that did not spawn completely. This value was relatively lower than what was obtained by Shinkafi and Ilesanmi for *C. gariepinus* induced with varying doses of Ovatide [20]. This could be due to larger weight of fish they used compared to what was used in this experiment as was reported by Viveen, *et al* that larger fishes produce more eggs than smaller fishes [16].

4.4. Fertilization Rate

The highest mean fertilization rate in the experiment was observed in positive control treatment (0% normal saline dilution) with mean value of 93.33 respectively and this was significantly different ($p<0.05$) from the other dilution levels. This was similar to what was obtained by Olumuji and Mustapha who examined the effect of varying doses of normal saline-diluted ovaprim on the induced breeding of *C. gariepinus* [19]. This work however, showed that suspending generic hormone in saline at 25%, 50% and 75% dilution levels can be effective in the induced breeding of African catfish, which agrees with the findings of [20] that even small quantity of hormone below the manufacturer's recommended dose can successfully induce ovulation in African catfish.

4.5. Hatching Rate

The highest mean hatching rate of 82.21% was observed in positive control treatment (0% dilution) and this was significantly different from the other dilution levels. This was relatively higher than what was obtained by [19] on the induced breeding of *C. gariepinus* using different doses of normal saline-diluted ovaprim and this could be attributed to the efficacy of the hormones used in this study. The mean hatching rate obtained was also higher than what was obtained by [21] for Kainji strains of *C. anguillaris* (58.58%) and *C. gariepinus* (52.44%) using ovaprim and [22] using ovatide and ovaprim on *C. gariepinus* with mean values of 59.70% and 66.37% respectively.

4.6. Larval Survival Rate

The highest larval survival rate recorded in this experiment was 94.67 in 0% dilution level, which was comparatively higher than what was obtained by several authors working on *Clarias;* [23] who worked on induced breeding of *C. gariepinus* under varying broodstock ratios; [24] on the effect on breeding performance and egg quality of *C. batrachus* at various doses of ovatide during spawning induction. Likewise, the result obtained was higher than what was obtained by [19] on the induced breeding of *C. gariepinus* using different doses of normal saline-diluted ovaprim, and this can be related to the spawning medium (tank) used to run the experiment which was larger in this experiment with more space and constant aeration using aerators that provide dissolved oxygen into the medium which agrees with [25, 26] that physico-chemical parameters of water such as high concentration of dissolved oxygen affects the hatchability and larval survival of fish.

It has been observed that there is no statistically significant difference between *Clarias anguillaris* and *Clarias gariepinus* induced using synthetic hormone (ovatide) in terms of egg, fecundity and all the breeding performance parameters such as fertilization rate, hatching rate and larval survival rate.

5. Conclusion

As observed from the result of the experiment, different levels of hormones suspended in saline at 25%, 50%, and 75% produced significantly different results on induced breeding performance of African catfish with respect to fertilization rate, hatching rate and larval survival rate. It has also been observed from the experiment that positive control (0% dilution level) stand out as the best performing treatment in relation to breeding performance with respect to *C. anguillaris* and *C. gariepinus*. And the study further showed that 100% saline dilution level did not produce any result and cannot be used to induce breeding in African catfish.

Therefore, the present study demonstrates that *Clarias anguillaris* and *Clarias gariepinus* can both be successfully induced to spawn with ovatide hormone at the recommended dosage and produce good results. This study also showed that the synthetic hormone ovatide can effectively be suspended in saline at different dilution levels of 25%, 50% and 75% to induce breeding in *C. anguillaris* and *C. gariepinus* and successfully record good fecundity, fertilization, hatching and high survival of larvae which in turn reduces the quantity of the synthetic hormone to be used.

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