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Effect of Different Doses of Ovulin Hormone Suspended in Saline on the Induced Breeding Performance of African Catfishes *Clarias anguillaris* and *Clarias gariepinus* in Sokoto, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors HFM and LAA designed the study, wrote the protocol, wrote the first draft of the manuscript and performed statistical analysis. Authors MYA and FU managed the literature searches and analysis of the study. Authors LIK and AMS collected samples. All authors read and approved the final manuscript.

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ABSTRACT

A study on the effect of different doses of Ovulin 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 at the Hatchery Unit of the Department of Fisheries and Aquaculture, Usmanu Danfodiyo University, Sokoto. Species and hormone dilutions constituted the factors with specie having 2 levels (*C. anguillaris* and *C. gariepinus*) and Ovulin suspended in saline at 5 levels (0%, 25%, 50%, 75% and 100%). The result

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showed that species levels did not significantly (P>0.05) affect the breeding performance in all the breeding performance parameters observed. However, fertilization rate, hatching rate and survival rate were significantly affected (P<0.05) by different levels of Ovulin 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 Ovulin suspended in saline can have significant influence on the breeding performance of African catfish.

Keywords: African catfish; ovulin; hormone; Clarias anguillaris; Clarias gariepinus.

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].

Fertilization, hatching and early survival of larvae are vital for successful aquaculture of the African catfishes [3]. Richter and Van der Hurk [4] reported that the problem of inadequate supply of fish seed can only be solved through induced breeding by the application of various inducement materials. Various types of fishes have been induced to spawn, using various hormonal materials [5,6,7,8]. Some of these spawning agents are either difficult to quantify, 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 [9,10,11].

Prior to the work reported by Olumuji and Mustapha [12], the synthetic hormone for fish breeding has been used undiluted unlike natural hormone (pituitary extract). Normal saline which is commonly used form of saline solution is prepared by dissolution of 9g of NaCl in 1 litre of water [13]. Therefore, not much, if any, has been reported on the use of Ovulin diluted with normal saline on induced breeding of *C. anguillaris* and *C. gariepinus*.

This study therefore, was carried out using Ovulin hormone diluted with normal saline for induced breeding of *C. anguillaris* and *C. gariepinus* in order to test the effectiveness and efficiency of the hormone in induced breeding of African catfish and to compare the effect of various doses of the normal saline diluted-hormone with undiluted ones on the fertilization rate of the eggs, hatching rate of the eggs and larval survival of both *Clarias anguillaris* and *Clarias gariepinus*.

2. MATERIALS AND METHODS

2.1 Description of the Study Area

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").

2.2 Broodstock Collection and Management

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 Department of Fisheries and Aquaculture, Usmanu Danfodiyo University Sokoto. 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.

2.3 Experimental Design and Procedures

The experiment was set up in a 2 factor (2 x 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 Ovulin 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 x 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.

2.4 Induced Spawning and Hormone Treatment

Artificial hormone (Ovulin) was used for inducing ovulation at a recommended dosage of 0.5 ml/kg body weight of female broodstock, while half dosage was administered to male broodstock [14]. 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. The containers with the injected fish were covered and heavy stones were put on the lid of the containers that prevented the fish from jumping out.

2.5 Procurement of Ripe Eggs and Milt

After a latency period of about 8 hours, at a temperature of about 28°C, the eggs were collected from each female through stripping by gently pressing the abdomen of the fish. The eggs were collected into clean bowls labelled accordingly. The weights of the eggs were recorded. Milt was obtained by sacrificing the males. Each male was dissected carefully and their milt sac obtained. A small incision was made on the lobes of the testes with a sharp razor blade and the milt was squeezed into a dry Petri dish containing the collected eggs.

2.6 Artificial Fertilization

Dry method of fertilization was used where 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. Normal saline was added before spreading the eggs on the spawning nets in the incubation units prepared earlier for that purpose [15].

2.7 Incubation and Hatching

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 [16,17,18].

2.8 Data Collection

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

2.9 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 [19].

3. RESULTS AND DISCUSSION

3.1 Water Quality Parameters

The water quality parameters recorded during the experiment are shown in Table 1. The mean temperature recorded during latency period ranged from 27.47 to 28.17 for all the treatments and the mean temperature recorded during incubation ranged from 25.90 to 28.00. pH of the water during the experiment was in the range of 7.10 to 7.73 for all the treatments while the mean dissolved oxygen value recorded was between 6.40 and 7.27.

According to [20], the physico-chemical parameters of water are important to the growth, productivity and survival of aquatic organisms especially fish as they play a vital role in the biology and physiology of the fish. This statement agrees with the findings of [14] who opined that the best temperature range for optimum production of *Clarias* species is 25 – 31°C and the water quality parameters recorded during this experiment are within those recommended levels for catfish breeding. The mean temperature values recorded during the experiment was similar to what was observed by [21] for *Clarias gariepinus* that

exhibited a latency period of about 8 hours at 28°C. The mean pH value recorded during the experiment also falls within the normal range of 6.5 to 8.0 for catfish according to [21]. And this is in agreement with many researchers that the best water for fish cultivation is that which has a pH range of between 7 to 8. Dissolved oxygen level during the experiment was also within the recommended level for catfish.

3.2 Mean Weight, Dosage, Incubation Period and Latency Period

The mean initial weight of the broodstocks used for the experiment ranged from 326.67g to 480.00 g (Table 2). Dosage administered for injection of the broodstocks ranged from 0.16 ml to 0.24 ml at the recommended dosage of 0.5 ml/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 645. Latency period was between 7 hrs and 58mins to 8 hrs and 12 mins and incubation period ranged between 22 hrs to 23 hrs and 4 mins for all the treatments.

The size range of the broodstocks used in the experiment was in agreement with [21] who opined that African catfish clarias can become mature and breed as from 200 g body weight. And it agrees also with [22] 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 [22], 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 8 hrs at mean temperature of between 27 – 28°C and was similar to what was reported by [22] 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 [12] on induced breeding of *Clarias gariepinus* using different doses of normal saline-diluted ovaprim.

de Graaf and Janssen [22] 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 23 hrs at a temperature range of about 25.9 to 28.6°C which was similar to observations of Viveen et al. 1985) and was also in comparison with the findings of [23] for *Clarias gariepinus* that achieved incubation period of 15hrs at a temperature of 30°C.

3.3 Breeding Performance of *C. anguillaris* and *C. gariepinus* Induced with Ovulin Suspended in Saline

The result of breeding performance of *C. anguillaris* and *C. gariepinus* induced with different levels of ovulin hormone suspended in saline is shown in Table 3. The result indicates that species levels did not significantly ($p>0.05$) affect the breeding performance in this experiment with *C. gariepinus* producing relatively higher mean values compared to *C. anguillaris* in egg weight, spawning fecundity and relative fecundity, as well as the breeding performance parameters (fertilization rate, hatching rate and larval survival rate). However, there was no significant difference ($p>0.05$) between the means. The result further showed that different doses of ovulin suspended in saline significantly affected the breeding performance in this experiment. In terms of egg weight, spawning fecundity and relative fecundity, positive control (0% saline), 25%, 50% and 75% dilution levels produced significantly similar ($p>0.05$) mean values while the negative control (100% saline) did not produce any value since spawning did not occur. 0% normal saline dilution (positive control) produced relatively higher mean values that are significantly different ($p<0.05$) from the other dilution levels (25%, 50% 75% and 100%) in terms of breeding performance parameters (fertilization rate and hatching rate) except for survival rate where no significant difference ($p>0.05$) exist between the mean values. 25% and 50% dilution levels produced statistically similar ($p>0.05$) result in terms of fertilization rate while 50% and 75% dilution levels produced similar result statistically ($p>0.05$) in terms of hatching rate. There was no significant interaction between the factors in this experiment.

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 33,939 was observed in 75% dilution level. The value obtained was in agreement with [21], 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.

The highest mean fertilization rate was observed in positive control treatment (0% normal saline dilution) with mean values of 92.22 and this was significantly different ($p<0.05$) from the other dilution levels. This was similar to what was obtained by [12] who examined the effect of varying doses of normal saline-diluted ovaprim on the induced breeding of *C. gariepinus*. 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 [23] that even small quantity of hormone below the manufacturer's recommended dose can successfully induce ovulation in African catfish.

The highest mean hatching rate of 86.62 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 [23] 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 Moses *et al* for Kainji strains of *C. anguillaris* (58.58%) and *C. gariepinus* (52.44%) using ovaprim and [24] using ovatide and ovaprim on *C. gariepinus* with mean values of 59.70% and 66.37% respectively.

The highest larval survival rate recorded in this experiment was 93.73 in 75% dilution level. The value obtained were comparatively higher than what was obtained by several authors working on *Clarias;* [25] who worked on induced breeding of *C. gariepinus* under varying broodstock ratios; [26] 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 [12] 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 [27] and [20] that physico-chemical parameters of water such as high concentration of dissolved oxygen affects the hatchability and larval survival of fish.

Table 1. Mean water quality parameters recorded during the experiment with ovulin

Treatments	Parameters			
	Latency temp (°C)	Incubation temp (°C)	pH (mg/l)	DO (mg/l)
A_0B_0	27.97	26.73	7.17	6.47
A_0B_1	27.73	25.90	7.23	6.50
A_0B_2	27.60	27.57	7.13	6.87
A_0B_3	27.70	27.40	7.53	6.63
A_0B_4	27.53	-	-	-
A_1B_0	27.93	27.00	7.27	6.93
A_1B_1	28.17	27.23	7.73	6.40
A_1B_2	28.17	28.00	7.10	6.77
A_1B_3	27.47	27.53	7.17	7.27
A_1B_4	27.63	-	-	-

Table 2. Weight, dosage, latency period and incubation period during induced breeding with ovulin

Treatments	Parameters				
	Initial weight (g)	Dosage (ml)	No. of Egg (1 g)	Latency period	Incubation period
A_0B_0	453.33	0.23	623	8h 9m	23h 4m
A_0B_1	433.33	0.22	634	8h 2m	22h 57m
A_0B_2	426.67	0.21	645	8h	22h 10m
A_0B_3	480.00	0.24	629	8h 1m	22h
A_0B_4	346.67	0.17	-	-	-
A_1B_0	363.33	0.18	641	8h 12m	22h 32m
A_1B_1	380.00	0.19	643	7h 58m	22h 8m
A_1B_2	326.67	0.16	630	8h	22h 20m
A_1B_3	433.33	0.22	641	8h 3m	22h 25m
A_1B_4	413.33	0.21	-	-	-

Table 3. Main effects specie and ovulin 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	34.92	22,245	49.13	60.67	56.87	73.09
C. gariepinus	35.77	22,876	61.90	65.56	58.84	74.27
SEM	3.71	2463.64	6.00	1.86	1.98	1.13
Hormone dilution						
0%	39.93^a	$25,295^a$	65.74^a	92.22^a	86.63^a	92.08^a
25%	45.42^a	$29,034^a$	71.82^a	82.78^b	75.09^b	90.61^a
50%	37.97^a	$24,536^a$	62.69^a	77.22^b	65.80^c	92.00^a
75%	53.42^a	$33,939^a$	77.33^a	63.33^c	61.76^c	93.73^a
100%	00.00	0.00	0.00	0.00	0.00	0.00
SEM	5.87	3895.35	9.49	2.94	3.13	1.70
Interaction	NS	NS	NS	NS	NS	NS

Means with the same superscripts on the same column are not significantly different (p>0.05)
NS = Not significant
EW = Egg weight, SF = Spawning Fecundity, RF = Relative Fecundity, FR = Fertilization Rate, HR = Hatching Rate, SR = Survival Rate

4. CONCLUSION

It has been observed from the result of this experiment that there is no statistically significant difference between *Clarias anguillaris* and *Clarias gariepinus* induced using Ovulin synthetic hormone in terms of egg weight, fecundity and all the breeding performance parameters such as fertilization rate, hatching rate and larval survival rate. It has also been observed that positive control (100% hormone) stand out as the best performing treatment in relation to breeding performance with respect to *C. anguillaris* and *C. gariepinus* compared to other treatments where hormone was diluted with normal saline.

Therefore, the present study demonstrates that *Clarias anguillaris* and *Clarias gariepinus* can both be successfully induced to spawn with ovulin diluted with normal saline 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. And this is in agreement with the findings of [12] who worked on the breeding performance of *C. gariepinus* induced with different doses of ovaprim diluted in normal saline.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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