

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

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 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, Synthetic 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 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 [9] [10] [11].

36
37 Prior to the work reported by Olumuji and Mustapha [12], the synthetic hormone for fish
38 breeding has been used undiluted unlike natural hormone (pituitary extract). Normal saline
39 which is commonly used form of saline solution is prepared by dissolution of 9g of NaCl in 1
40 litre of water [13]. Therefore, not much, if any, has been reported on the use of Ovulin diluted
41 with normal saline on induced breeding of *C. anguillaris* and *C. gariepinus*.
42

43 This study therefore, was carried out using Ovulin hormone diluted with normal saline for
44 induced breeding of *C. anguillaris* and *C. gariepinus* in order to test the effectiveness and
45 efficiency of the hormone in induced breeding of African catfish and to compare the effect of
46 various doses of the normal saline diluted-hormone with undiluted ones on the fertilization
47 rate of the eggs, hatching rate of the eggs and larval survival of both *Clarias anguillaris* and
48 *Clarias gariepinus*.
49

50 **2. MATERIAL AND METHODS**

51 **2.1 Description of the Study Area**

52 The experiment was conducted at the Hatchery unit of the Department of Fisheries and
53 Aquaculture located at the main campus of Usmanu Danfodiyo University, Sokoto (N13° 07'
54 45.12" E5° 12' 18").

55 **2.2 Broodstock Collection and Management**

56 Eighty (80) broodstock, forty (40) each of *C. anguillaris* and *C. gariepinus* (30 females, 10
57 males each) were collected from the Departmental Fish Farm of the department of Fisheries
58 and Aquaculture, Usmanu Danfodiyo University Sokoto. The fish were conditioned at the
59 hatchery complex of the farm and were fed commercially produced industrial feed (Coppens)
60 at 3% body weight twice daily for two weeks.

61 **2.3 Experimental Design and Procedures**

62 The experiment was set up in a 2 factor (2 x 5) factorial experiment in a Completely
63 Randomized Design (CRD) with two levels of *Clarias* species (*C. anguillaris* and *C.*
64 *gariepinus*) and five inclusion levels of Ovulin hormone suspended in saline (at 0%, 25%,
65 50%, 75% and 100%). All the treatments were replicated three times to give a total of 30
66 spawning trials (i.e. 2 x 5 = 10, replicated 3 times = 30). Induced breeding was carried out
67 and data collected was subjected to statistical analysis.
68

69 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 ,
70 A_1B_3 , A_1B_4 . Where $A_0 = Clarias\ anguillaris$, $A_1 = Clarias\ gariepinus$, $B_0 = 0\%$, $B_1 = 25\%$, $B_2 =$
71 50% , $B_3 = 75\%$ and $B_4 = 100\%$. These were randomly distributed in triplicate tanks in a
72 CRD.
73

74 **2.4 Induced Spawning and Hormone Treatment**

75 Artificial hormone (Ovulin) was used for inducing ovulation at a recommended dosage of
76 0.5ml/kg body weight of female broodstock, while half dosage was administered to male
77 broodstock [14]. Hormone administration was carried out via intramuscular injection with 0%,
78 25%, 50%, 75% and 100% inclusion levels of normal saline and the injected fish were kept
79 separated in well-labelled closed containers containing water. The containers with the
80 injected fish were covered and heavy stones were put on the lid of the containers that
81 prevented the fish from jumping out.

82 **2.5 Procurement of ripe eggs and milt**

83 After a latency period of about 8 hours, at a temperature of about 28⁰C, the eggs were
84 collected from each female through stripping by gently pressing the abdomen of the fish. The
85 eggs were collected into clean bowls labelled accordingly. The weights of the eggs were
86 recorded. Milt was obtained by sacrificing the males. Each male was dissected carefully and
87 their milt sac obtained. A small incision was made on the lobes of the testes with a sharp
88 razor blade and the milt was squeezed into a dry Petri dish containing the collected eggs.

89 **2.6 Artificial fertilization**

90 Dry method of fertilization was used where the milt obtained from the male fishes was
91 squeezed gently onto the stripped eggs obtained from the females accordingly and stirred
92 gently and thoroughly using plastic spoon for about 2 minutes to allow contact and adequate
93 fertilization. Normal saline was added before spreading the eggs on the spawning nets in the
94 incubation units prepared earlier for that purpose [15].

95 **2.7 Incubation and hatching**

96 The mixture of the eggs and milt were distributed in a single layer on the spawning nets in
97 the well aerated incubation bowls. Three gram of egg was collected from each sample and
98 incubated in 60-litre plastic containers for the experiment, for easy assessment of fertilization
99 and hatching rates [16] [17] [18].

100 **2.8 Data collection**

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

103 **2.9 Data analysis**

104 The data collected on the induced breeding parameters was subjected to statistical analysis
105 using SPSS (Version 20). All data with discrete counts and percentages was transformed
106 before analysis was carried out. The data were analyzed using analysis of variance
107 (ANOVA) to test for significant differences ($P < 0.05$) in fertilization rate, hatching rate and
108 larval survival, and means were separated using Duncan's Multiple Range Test (DMRT)
109 where significant difference exist [19].
110

111 3. RESULTS AND DISCUSSION

112 3.1 Water quality parameters

113 The water quality parameters recorded during the experiment are shown in table 2. The
114 mean temperature recorded during latency period ranged from 27.47 to 28.17 for all the
115 treatments and the mean temperature recorded during incubation ranged from 25.90 to
116 28.00. pH of the water during the experiment was in the range of 7.10 to 7.73 for all the
117 treatments while the mean dissolved oxygen value recorded was between 6.40 and 7.27.

118
119 The physico-chemical parameters of water are important to the growth, productivity and
120 survival of aquatic organisms especially fish as they play a vital role in the biology and
121 physiology of the fish [20]. According to Madu [14], the best temperature range for optimum
122 production of *Clarias* species is 25 – 31°C. The water quality parameters recorded during
123 this experiment are within the recommended level for catfish breeding. The mean
124 temperature values recorded during the experiment was similar to what was observed by
125 [21] for *Clarias gariepinus* that exhibited a latency period of about 8 hours at 28°C. The
126 mean pH value recorded during the experiment also falls within the normal range of 6.5 to
127 8.0 for catfish according to [21]. And this is in agreement with many researchers that the
128 best water for fish cultivation is that which has a pH range of between 7 to 8. Dissolved
129 oxygen level during the experiment was also within the recommended level for catfish.

130
131 **Table 2: 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 ₀ B ₀	27.97	26.73	7.17	6.47
A ₀ B ₁	27.73	25.90	7.23	6.50
A ₀ B ₂	27.60	27.57	7.13	6.87
A ₀ B ₃	27.70	27.40	7.53	6.63
A ₀ B ₄	27.53	-	-	-
A ₁ B ₀	27.93	27.00	7.27	6.93
A ₁ B ₁	28.17	27.23	7.73	6.40
A ₁ B ₂	28.17	28.00	7.10	6.77
A ₁ B ₃	27.47	27.53	7.17	7.27
A ₁ B ₄	27.63	-	-	-

132

133 Mean weight, dosage, incubation period and latency period

134 The mean initial weight of the broodstocks used for the experiment ranged from 326.67g to
135 480.00g (Table 3). Dosage administered for injection of the broodstocks ranged from 0.16 ml
136 to 0.24 ml at the recommended dosage of 0.5 ml/kg body weight and the quantity of egg
137 used for incubation was 3 grams for each treatment. The number of eggs obtained in 1 g of
138 egg was between the range of 623 to 645. Latency period was between 7hrs and 58mins to
139 8hrs and 12mins and incubation period ranged between 22hrs to 23hrs and 4mins for all the
140 treatments.

141

142 The size range of the broodstocks used in the experiment was in agreement with [21] who
143 opined that African catfish *clarias* can become mature and breed as from 200g body weight.
144 And it agrees also with [22] who reported that the ideal broodfish weight should be between
145 300-800 grams, as larger fish are difficult to handle and often results in substantial egg

146 losses prior to stripping. The time taken to achieve ovulation (latency period) is dependent
 147 upon water temperature as reported by [22], as such the higher the temperature the quicker
 148 the eggs ovulate. In other words, the higher the temperature the shorter the latency period.
 149 The mean latency period observed in this study fall within 8hrs at mean temperature of
 150 between 27 – 28^o C and was similar to what was reported by [22] for *Clarias gariepinus*. The
 151 result also showed no significant variation of latency time between the treatments except for
 152 treatment induced with 100% normal saline which could be the reason why ovulation did not
 153 occur for that particular treatment in all the phases which is due to the lack of hormone effect
 154 that foster ovulation in fish. This was similar to what was observed by [12] on induced
 155 breeding of *Clarias gariepinus* using different doses of normal saline-diluted ovaprim.

156
 157 de Graaf and Janssen [22] reported that the development process of fish from fertilized egg
 158 to hatching is like all other biological processes, that is, it is dependent upon water
 159 temperature, as such the higher the water temperature the faster the eggs hatch. The
 160 incubation period observed in this experiment was in the range of 21 to 23hrs at a
 161 temperature range of about 25.9 to 28.6^o C which was similar to observations of Viveen *et al.*
 162 (1985) and was also in comparison with the findings of [23] for *Clarias gariepinus* that
 163 achieved incubation period of 15hrs at a temperature of 30^oC

164
 165 **Table 3: Weight, dosage, latency period and incubation period during induced**
 166 **breeding with ovulin**

Treatments	Parameters				
	Initial weight (g)	Dosage (ml)	No. of Egg (1g)	Latency period	Incubation period
A ₀ B ₀	453.33	0.23	623	8h 9m	23h 4m
A ₀ B ₁	433.33	0.22	634	8h 2m	22h 57m
A ₀ B ₂	426.67	0.21	645	8h	22h 10m
A ₀ B ₃	480.00	0.24	629	8h 1m	22h
A ₀ B ₄	346.67	0.17	-	-	-
A ₁ B ₀	363.33	0.18	641	8h 12m	22h 32m
A ₁ B ₁	380.00	0.19	643	7h 58m	22h 8m
A ₁ B ₂	326.67	0.16	630	8h	22h 20m
A ₁ B ₃	433.33	0.22	641	8h 3m	22h 25m
A ₁ B ₄	413.33	0.21	-	-	-

167

168 **Breeding performance of *C. anguillaris* and *C. gariepinus* induced with ovulin**
 169 **suspended in saline**

170 The result of breeding performance of *C. anguillaris* and *C. gariepinus* induced with different
 171 levels of ovulin hormone suspended in saline is shown in Table 4. The result indicates that
 172 species levels did not significantly ($p>0.05$) affect the breeding performance in this
 173 experiment with *C. gariepinus* producing relatively higher mean values compared to *C.*
 174 *anguillaris* in egg weight, spawning fecundity and relative fecundity, as well as the breeding
 175 performance parameters (fertilization rate, hatching rate and larval survival rate). However,
 176 there was no significant difference ($p>0.05$) between the means. The result further showed
 177 that different doses of ovulin suspended in saline significantly affected the breeding
 178 performance in this experiment. In terms of egg weight, spawning fecundity and relative
 179 fecundity, positive control (0% saline), 25%, 50% and 75% dilution levels produced
 180 significantly similar ($p>0.05$) mean values while the negative control (100% saline) did not
 181 produce any value since spawning did not occur. 0% normal saline dilution (positive control)

182 produced relatively higher mean values that are significantly different ($p < 0.05$) from the other
183 dilution levels (25%, 50% 75% and 100%) in terms of breeding performance parameters
184 (fertilization rate and hatching rate) except for survival rate where no significant difference
185 ($p > 0.05$) exist between the mean values. 25% and 50% dilution levels produced statistically
186 similar ($p > 0.05$) result in terms of fertilization rate while 50% and 75% dilution levels
187 produced similar result statistically ($p > 0.05$) in terms of hatching rate. There was no
188 significant interaction between the factors in this experiment.

189
190 The spawning fecundity observed in the study showed that different doses of ovulin
191 suspended in saline at 25%, 50% and 75% inclusion levels can be effective in the induced
192 breeding of *C. anguillaris* and *C. gariepinus*. The highest mean fecundity value 33,939 was
193 observed in 75% dilution level. The value obtained was in agreement with [21], that larger
194 female fishes contain more eggs than smaller fishes and therefore have higher fecundity
195 values and this could also be due to the efficacy of the hormone used which indicates that
196 even a small quantity of hormone can be diluted with saline and be effective in the induced
197 breeding of African catfish.

198
199 The highest mean fertilization rate was observed in positive control treatment (0% normal
200 saline dilution) with mean values of 92.22 and this was significantly different ($p < 0.05$) from
201 the other dilution levels. This was similar to what was obtained by [12] who examined the
202 effect of varying doses of normal saline-diluted ovaprim on the induced breeding of *C.*
203 *gariepinus*. This work however, showed that suspending generic hormone in saline at 25%,
204 50% and 75% dilution levels can be effective in the induced breeding of African catfish,
205 which agrees with the findings of [23] that even small quantity of hormone below the
206 manufacturer's recommended dose can successfully induce ovulation in African catfish.

207
208 The highest mean hatching rate of 86.62 was observed in positive control treatment (0%
209 dilution) and this was significantly different from the other dilution levels. This was relatively
210 higher than what was obtained by [23] on the induced breeding of *C. gariepinus* using
211 different doses of normal saline-diluted ovaprim and this could be attributed to the efficacy of
212 the hormones used in this study. The mean hatching rate obtained was also higher than
213 what was obtained by Moses *et al* for Kainji strains of *C. anguillaris* (58.58%) and *C.*
214 *gariepinus* (52.44%) using ovaprim and [24] using ovatide and ovaprim on *C. gariepinus* with
215 mean values of 59.70% and 66.37% respectively.

216
217 The highest larval survival rate recorded in this experiment was 93.73 in 75% dilution level.
218 The value obtained were comparatively higher than what was obtained by several authors
219 working on *Clarias*; [25] who worked on induced breeding of *C. gariepinus* under varying
220 broodstock ratios; [26] on the effect on breeding performance and egg quality of *C.*
221 *batrachus* at various doses of ovatide during spawning induction. Likewise, the result
222 obtained was higher than what was obtained by [12] on the induced breeding of *C.*
223 *gariepinus* using different doses of normal saline-diluted ovaprim, and this can be related to
224 the spawning medium (tank) used to run the experiment which was larger in this experiment
225 with more space and constant aeration using aerators that provide dissolved oxygen into the
226 medium which agrees with [27] and [20] that physico-chemical parameters of water such as
227 high concentration of dissolved oxygen affects the hatchability and larval survival of fish.

228

229 **Table 4: Main effects specie and ovulin suspended in saline effects on induced**
 230 **breeding performance of *C. anguillaris* and *C. gariepinus***

Factors	Parameters					
	EW(g)	SF	RF(g)	FR(%)	HR(%)	SR(%)
Specie						
<i>C. anguillaris</i>	34.92	22,245	49.13	60.67	56.87	73.09
<i>C. gariepinus</i>	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

231 Means with the same superscripts on the same column are not significantly different
 232 ($p>0.05$)

233 NS = Not significant

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

236

237

4. CONCLUSION

238

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

246 Therefore, the present study demonstrates that *Clarias anguillaris* and *Clarias gariepinus*
 247 can both be successfully induced to spawn with ovulin diluted with normal saline and
 248 successfully record good fecundity, fertilization, hatching and high survival of larvae which in
 249 turn reduces the quantity of the synthetic hormone to be used.

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