Original Research Article

EFFECIENCY OF PROCEDURAL USE OF HORMONAL AGENT IN PROPAGATION OF *Clarias gariepinus*

Abstract

Administration procedures of some hormones including single, double and multiple induction protocols were investigated to determine the implications of different induction process in propagation of *Clarias gariepinus*. The hormones studied were ovulin, Carp pituitary extract (CPE) and gonopore. A total of 36 *Clarias gariepinus* of the same age comprising 27 females and 9 males were used for the study. Eggs produced among treatments were fertilized with pooled sperm from a total of 3 males per hormonal treatment. Propagation was assessed by fecundity, fertilization, hatchability and larval survival. Highest egg production was recorded at double inductions with ovulin and CPE while highest egg production was observed in single induction with gonopore. There was significant disparity ($P<0.05$) in fertilization and hatchability percentages. Best fertilization and hatching rate was recorded with ovulin at single induction (80.2%) and ovulin at multiple inductions (81.9%) respectively. There was significant difference ($P<0.05$) in number of larvae realized among the treatments. Highest larval survival was recorded with gonopore at single induction (68.1%). Findings from the study showed that hormone type and mode of administration are important in propagation of *C. gariepinus*. The study recommended double inductions in usage of Ovulin and CPE and single induction with gonopore. Adopting best procedural technology in usage of specific hormone is necessary for mass production of *Clarias gariepinus* seeds.

Key words: *Clarias gariepinus*, Fish propagation, Hormone induction, Hormone type, viable seed.

Introduction

Hormone plays overriding role in the physiology of fish reproduction. It is responsible for certain observable morphological changes in body shape and physiological changes leading to mobilization of stored energy in the liver and peculiar courtship behaviour in fishes (Skold *et. al.,* 2008). Under the influence of a releasing factor from the hypothalamus on attainment of age of sexual maturity and in response to certain environmental factors, the anterior pituitary gland produces gonadotrophic hormones which control the activity of gonads to produce gametes through the processes of oogenesis and spermatogenesis (Okubo and Nagahama, 2008). The mechanism of action and retroaction of the hormones guiding reproduction are very complex and can vary between species (Oyola and Handa, 2017). This is why some hormones will work in some species and not in others. Several synthetic hormones with trade names like ovatide, ovaprim, WOVA-FH, Ovopel, HCG, CPE, LHRH and gonopore are used for induced fish breeding. Choosing right synthetic hormone involves the selection of effective hormone formulation, proper duration of hormonal treatment, administering appropriate dosage and timing of the hormone administration (Nagaraj, Butts and Dunham, 2018). Failure of synthetic hormone can be linked to hormone type, manufacturing process, administrative procedures and biological condition of the fish (Hafeez-ur-Rehman, *et. al*., 2015). In this study, administrative procedures including single, double and

multiple induction protocols were investigated for *en-masse* propagation of *C. gariepinus*. It was viewed that best procedural technology in use of specific hormone is crucially needed to enhance fish seed propagation. The study elucidated the interaction between procedure in use of hormone type and production of quality seeds of *Clarias gariepinus*.

Materials and methods

A total of 36 *Clarias gariepinus* of the same age comprising 27 females and 9 males were used for the study. Eighteen females divided into two equal groups of 9 fish per group and kept in separate compartments were used for single and double hormonal treatments. The remaining 9 females were kept separately in triplicates for multiple hormonal treatments. Multiple hormonal treatments were carried out (with 25% recommended dosage of the respective hormone) at 7 days intervals for a total of 28 days. Double induction was carried out at 6 hours intervals in 25: 75% ratio. The final doses for multiple inductions were executed the same day the decisive doses for double and single hormonal treatments were administered. Eggs produced among treatments were fertilized with pooled sperm extracted from a total of 9 males. Each treatment group was fertilized with pooled sperm from 3 males. The hormones studied were ovulin, Carp pituitary extract (CPE) and gonopore. These hormones were administered at 0.5ml/kg, 4mg/kg and 0.5ml/kg respectively. To capture reproductive indices of fertilization, hatchability and larval survival; 100 fertilized eggs were incubated in triplicates and observed to hatch and develop in order to estimate the parameters. During the study, water quality parameters were measured every day at pre-fry stage and every other day on attainment of fry stage till the termination of the experiment. Propagation parameters analyzed included the following:

i. **Latency period** defined as the time lag between hormonal induction and egg spawning
ii. **Relative fecundity** defined as weight of eggs in gramme produced by individual fish of known body weight in a single production. It was expressed in percentage as estimated from mathematical formula: $\frac{weight\ of\ spawned\ eggs}{weight\ of\ the\ female\ spawner} \text{x} \frac{100}{1}$ (Farid *et. al.*, 2008)
iii. **Fertilization** defined as successful union of egg and sperm and evidenced in opaque green or brown colouration of the fertilized eggs as against translucent whitish colouration of the unfertilized eggs. It was estimated mathematically from the formula: $\frac{Number\ of\ fertilized\ eggs}{Total\ number\ of\ incubated\ eggs} \text{x} \frac{100}{1}$ (Farid *et. al.*, 2008).
iv. **Hatchability** was defined as emergence of larvae consequent upon cracking of egg shell that have undergone stages of swelling, cleavages and embryonic development. It was physically noticed from shaking and tail wagging against non-movement of un-hatched eggs as reported by Uka and Sikoki (2012). It was mathematically estimated from the formula: $\frac{Number\ of\ hatched\ eggs}{Total\ number\ of\ fertilized\ eggs} x \frac{100}{1}$
v. **Larval survival** was defined as hatchlings that remained alive until complete yolk reabsorption and was mathematically expressed as $\frac{Number\ of\ yolksac\ \ that\ developed\ to\ fry}{Total\ number\ of\ yolk\ sac\ larvae\ examined} \text{x} \frac{100}{1}$

Data analysis

All percentage data obtained were transformed using square root transformation prior to carrying out analysis of variance.

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Comment [CM2]: Why not 18 and 18? (same number males and females)?

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Results and Discussion

Results: Significant difference ($P<0.05$) in latency period was observed among fish exposed to the three hormones and within fish treated with ovulin under different induction protocols (single, double and multiple inductions). Average time lapse to spawning was 13:15, 12:15 and 12:45 hours for CPE, ovulin and gonopore respectively at 27^{o}C. Duration to spawning with ovulin under single, double and multiple inductions was 12 hours, 12 hours and 12:50 hours respectively (Table 1). Highest egg production was recorded with ovulin and CPE at double inductions while highest egg production was observed at single induction with gonopore (Table 1).

Table 1: Impact of procedural use of different hormones on production of *Clarias gariepinus* egg

Treatments	Latency Period (hours)	♀-Mean weight (g)	Fecundity (g)	Relative fecundity (%)
Ovulin^{s}	12.00c	1600	6.94±10.10e	0.4g
Ovulin^{d}	12.00c	1600	14.41±8.91c	0.9d
Ovulin^{m}	12.50c	1700	14.06±5.14c	0.8e
CPE^{s}	13:15a	1800	27.27±0.81b	1.5c
CPE^{d}	13:15a	1700	33.13±13.91a	1.9a
CPE^{m}	13:15a	1700	25.93±11.91	1.5c
Gonopore^{s}	12.45b	1700	27.0±5.62b	1.6b
Gonopore^{d}	12.45b	1800	8.42±3.19d	0.5f
Gonopore^{m}	12.45b	1600	8.26±0.56d	0.5f
Test	*		*	*

Superscript letter s, d and m=single, double and multiple injections. Values followed by different letter on the same row are significantly different at 5% probability level. *=$P<0.05$.

These findings have shown that reactivity of different brands of hormone administered to induce spawning in fish differ with resultant disparity in time lapse to spawning among individuals of the same species exposed to different hormones. Hafeez-ur-Rehman *et. al*. (2015) reported latency period of 43.20 - 44.45 hours in treatment of *Channa marulius* with HCG+HMG after booster induction with ovaprim and latency period of 40.25 - 42.45 hours with 41.25±0.88 mean latency hours with ovaprim+ HCG primer also boosted with ovaprim in double hormonal treatments. Ukwe, Oyekutor and Abu (2016) reported that latency period were consistently higher in ovulin treated fish when compared to ovaprim and further reported that the higher the hormone concentration, the lower the latency period in both ovulin and ovaprim. The result further revealed that reactivity of some hormones like ovulin could be adjusted to achieve shorter or extended time to spawning depending on mode of administration. Here, latency period of 12 hours under single or double induction with ovulin was extended to 12hours 50 minutes under multiple inductions with the same hormone. Cortney and Broach (2018) acknowledged that some hormones need to be administered more than once, requiring a smaller priming dose and further stated that final resolving dose should be administered after 6 to 24 hours in double induction protocol. In the present work, the final dose was given after 6 hours of priming.

There was significant difference (P<0.05) in fecundity under different protocols of usage of different hormones. Average fecundity recorded with CPE, Ovulin and gonopore was 28.78g/kg, 11.80g/kg and 14.59g/kg respectively. Highest fecundity (33.13g/kg) was achieved under double hormonal induction with CPE); while the lowest fecundity was observed under single hormonal treatment with ovulin (6.94g/kg). The highest fecundity achieved with Ovulin-14.41g/kg was under double induction, while the highest fecundity achieved with Gonopore-27.09g/kg was under single induction. The lowest fecundity obtained with CPE-25.93g/kg and Ovulin-6.94g/kg was recorded under single and multiple inductions respectively. There was significant disparity (P<0.05) in fertilization under different protocols. Best fertilization rate was recorded with ovulin at single induction (80.2%). This was closely followed by the success achieved at double induction with ovulin (75.7%) and gonopore (75.1%). Poor fertilization was recorded at single and multiple inductions with CPE (7.1%) and gonopore (30.4%) respectively. There was no significant difference (P>0.05) in hatching rate of the eggs induced to spawn under different protocols with ovulin as against significant hatching disparities (P<0.05) observed under different protocols with CPE and gonopore hormones. The best hatching success was recorded with ovulin at multiple induction protocol (81.9%). This was not statistically different (P>0.05) from the hatchability achieved with gonopore at single induction (78.4%). The average hatching percentage with CPE and gonopore was 45.7% and 57.3% respectively as against 80.3% recorded with ovulin (Table 2).

Similarities and differences in larval survival traceable to hormones and the procedures of their usage were observed among treatments (table 2). Highest larval survival was recorded with gonopore used at single induction (68.1%). This was followed by the survival recorded with ovulin also at single induction (64.3%), then ovulin at double induction (60.9%) and gonopore equally at double induction (59.8%). There was no significant difference in the survival achieved between multiple inductions with ovulin (44.4 %) and multiple inductions with CPE (43.3%). Marginal survival was observed from multiple inductions with gonopore (11.7%). Extremely poor survival was recorded under single induction with CPE (0.0 %) (Table 2).

Table 2: Impact of procedural use of hormones on fertilization, hatching and larval survival

Treatments	Fertilization (%)	Hatchability (%)	Mean H%	Survival (%)
Ovulin^{s}	80.2±5.04a	79.43±11.40a		64.30±12.70b
Ovulin^{d}	75.7±4.04b	79.50±0.75a		60.83±2.66b
Ovulin^{m}	66.7±6.96c	81.90±12.45a	80.3	44.40±29.25d
CPE^{s}	7.1±5.66f	23.33±40.41e		0.00g
CPE^{d}	59.39±7.78d	64.56±10.52c		35.50±5.45e
CPE^{m}	60.81±7.78d	49.28±1.01d	45.7	43.30±13.64d
Gonopore^{s}	75.06±2.20b	78.36±4.30a		68.06±19.85a
Gonopore^{d}	70.66±4.93bc	73.43±20.20b		59.76±3.66bc
Gonopore^{m}	30.43±8.44e	19.99±17.63f	57.3	11.76±7.13f
Test	*	*		*

Superscript letter s, d and m=single, double and multiple injections. H=Hatchability. Values followed by different letter on the same row are significantly different at 5% probability level. *=P<0.05.

The above findings have proved that egg production was significantly influenced by hormone type and the protocol of usage. Dhas *et. al.* (2017) reported disparity in fecundity among *Etroplus surarensis* induced to spawn with different hormones and posit that higher fecundity was recorded among stocks induced with HCG-LHRH as against stocks induced with ovaprim. On the other hand, Nwokoye *et a*l (2007) stated that ovaprim treatment gave significantly higher number of fertilized eggs than the homoplastic hormones in *Heteropneustes bidorsalis*. Das *et. al.* (2016) working with *Osteobrama belangeri* reported that the efficacy of synthetic hormones (Ovaprim, Ovatide and Wova-FH) was significantly ($P < 0.05$) higher than what was obtained with CPE. Also Yeasmin et. al. (2013) opined a strong influence of synthetic hormones on values of fecundity, fertilization and hatchability in *Clarias gariepinus*.

Extremely poor survival recorded with CPE under single induction could result from quality deterioration that was improved under double and multiple induction protocols. Ukwe, Oyekutor and Abu (2016) reported that ovulin performed significantly ($P<0.05$) better than ovaprim in all the parameters measured except in survival rate. The findings of Nwokoye *et al.* (2007) revealed disparity in percentage of deformed larvae attributable to differences in hormone treatments applied on their parents to induce spawning. This undoubtedly gives credence to possibility of larval deformation from hormone induction that could hamper survival in the long run as observed in the present report. This underscores the need to recognize the role of inducing agent in survival of fish seed.

There was no significant difference in water quality parameters evaluated among the treatments. The mean values of temperature, Dissolved oxygen and pH among the treatments were within limits that supports *Clarias gariepinus* propagation.

Table 3: Water quality condition in experimental units during the study

Treatments	Temperature (^oC)	Dissolved Oxygen (mg/l)	pH
$Ovulin^s$	27.20±0.02	5.56±0.06	6.45±0.26
$Ovulin^d$	27.23±0.04	5.58±0.04	6.30±0.50
$Ovulin^m$	27.24±0.04	5.58±0.05	6.40±0.19
CPE^s	27.13±0.18	5.55±0.35	6.42±0.90
CPE^d	27.28±0.11	5.62±0.18	6.38±0.72
CPE^m	27.34 ±0.06	5.54±0.11	6.41±0.45
$Gonopore^s$	27.26±0.37	5.61±0.05	6.35±0.81
$Gonopore^d$	27.37±0.11	5.49±0.10	6.40±0.90
$Gonopore^m$	27.30±0.14	5.57±0.05	6.30±0.12
Test	ns	ns	ns

ns=Not significantly different

Conclusion

Role of hormone in fish reproduction was studied. The role seems complex and was earlier reported to vary between species. It was also reported that some hormones need to be administered more than once. In this study, administrative procedures including single, double and multiple induction protocols in the use three commercially important hormones (Ovulin, Carp Pituitary Extract and Gonopore) were investigated to compare effectiveness for *en-masse* propagation of *C. gariepinus*. The findings reported here provided evidence that

reactivity of different brands of hormone and mode of usage to induce spawning in fish differs with resultant disparity in time lapse to spawning among individuals of the same species. Egg production was significantly influenced by hormone type and the protocol of usage. The study recommended double inductions in usage of Ovulin and CPE and single induction with gonopore for mass production of eggs. Reduced effectiveness as reflected in poor survival recorded with CPE under single induction could result from quality deterioration that was improved under double and multiple induction protocols.

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