Efficiency of Procedural Use of Hormonal Agent in Propagation of *Clarias gariepinus*

A. Uka a* and G. I. Obilo a

a Department of Fisheries and Aquatic Resources Management, College of Natural and Environmental Resources Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Authors’ contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJFAR/2022/v17i130393

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/85554

Received 26 January 2022
Accepted 05 April 2022
Published 08 April 2022

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.

Keywords: *Clarias gariepinus*; fish propagation; hormone induction; hormone type; viable seed.

*Corresponding author: Email: anyaeleuka@gmail.com;
1. 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 [1]. 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 [2]. The mechanism of action and retroaction of the hormones guiding reproduction are very complex and can vary between species [3]. 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 [4]. Failure of synthetic hormone can be linked to hormone type, manufacturing process, administrative procedures and biological condition of the fish [5]. 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.

2. MATERIALS AND METHODS

A total of 36 Clarias gariepinus of the same age (13 months) 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 within 21 days and full dose at day 28. 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:

Latency period: defined as the time lag between hormonal induction and egg spawning.

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:

\[
\text{Relative fecundity} = \frac{\text{weight of spawned eggs}}{\text{weight of the female spawner}} \times 100
\]

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:

\[
\text{Fertilization} = \frac{\text{Number of fertilized eggs}}{\text{Total number of incubated eggs}} \times 100
\]

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 unhatched eggs as reported by Uka and Sikoki [7]. It was mathematically estimated from the formula:

\[
\text{Hatchability} = \frac{\text{Number of hatched eggs}}{\text{Total number of fertilized eggs}} \times 100
\]

Larval survival: was defined as hatchlings that remained alive until complete yolk reabsorption and was mathematically expressed as
Number of yolk sac that developed to fry $\times \frac{100}{\text{Total number of yolk sac larvae examined}}$

Data Analysis

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

3. RESULTS AND DISCUSSION

3.1 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°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).

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 [5] reported latency period of 43.20 - 44.45 hours in treatment of Channa marulius with HCG+HMG after booster induction with ovarprim and latency period of 40.25 - 42.45 hours with 41.25±0.88 mean latency hours with ovaprim+ HCG primer also boosted with ovarprim in double hormonal treatments. Ukwe, Oyekutor and Abu [8] reported that latency period were consistently higher in ovarprim treated fish when compared to ovarprim and further reported that the higher the hormone concentration, the lower the latency period in both ovarprim and ovarprim. The result further revealed that reactivity of some hormones like ovarprim 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 12 hours 50 minutes under multiple inductions with the same hormone. Cortney and Broach [9] 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 28.78 g/kg, 11.80 g/kg and 14.59 g/kg respectively. Highest fecundity (33.13 g/kg) was achieved under double hormonal induction with CPE; while the lowest fecundity was observed under single hormonal treatment with ovulin (6.94 g/kg). The highest fecundity achieved with Ovulin-14.41 g/kg was under double induction, while the highest fecundity achieved with Gonopore-27.09 g/kg was under single induction. The lowest fecundity obtained with CPE-25.93 g/kg and Ovulin-6.94 g/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 1. Impact of procedural use of different hormones on production of *Clarias gariepinus* egg

| Treatments | Latency Period (hours) | ♀ Mean weight (g) | Fecundity (g) | Relativefecundity (%) |
|------------|-----------------------|------------------|---------------|-----------------------|
| 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                  |

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

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 | 60.83±2.66b  |
| Ovulin d   | 75.7±4.04b        | 79.50±0.75a      | 60.83±2.66b | 60.83±2.66b  |
| Ovulin m   | 66.7±6.96c        | 81.90±12.45a     | 60.83±2.66b | 60.83±2.66b  |
| 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 | 43.30±13.64d |
| 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  |

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 [10] reported disparity in fecundity among *Etroplus suratensis* 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 al. [11] stated that ovaprim treatment gave significantly higher number of fertilized eggs than the homoplastic hormones in *Heteropneustes bidorsalis*. Das et al [12] 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 [13] 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 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 [11] 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 (°C) | Dissolved Oxygen (mg/l) | pH        |
|------------|-----------------|-------------------------|-----------|
| Ovulin\textsuperscript{s} | 27.20±0.02       | 5.56±0.06               | 6.45±0.26 |
| Ovulin\textsuperscript{o} | 27.23±0.04       | 5.58±0.04               | 6.30±0.50 |
| Ovulin\textsuperscript{m} | 27.24±0.04       | 5.58±0.05               | 6.40±0.19 |
| CPE\textsuperscript{s}   | 27.13±0.18       | 5.55±0.35               | 6.42±0.90 |
| CPE\textsuperscript{o}   | 27.28±0.11       | 5.62±0.18               | 6.38±0.72 |
| CPE\textsuperscript{m}   | 27.34±0.06       | 5.54±0.11               | 6.41±0.45 |
| Gonopore\textsuperscript{s} | 27.26±0.37     | 5.61±0.05               | 6.35±0.81 |
| Gonopore\textsuperscript{o} | 27.37±0.11      | 5.49±0.10               | 6.40±0.90 |
| Gonopore\textsuperscript{m} | 27.30±0.14     | 5.57±0.05               | 6.30±0.12 |
| Test       | ns              | ns                      | ns        |

\( \text{ns}= \text{Not significantly different} \)

4. 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 \( \text{C. gariepinus} \). 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Skold HN, Amundsen T, Svensson PA, Mayer I, Bjelvenmark J, Forsgren E. Hormonal regulation of female nuptial coloration in a fish. Hormone and Behavior. 2008;54(4):549-556.
2. Okubo K, Nagahama Y. Structural and functional evolution of gonadotropin-releasing hormone in vertebrates. Acta Physiol (Oxf). 2008;193(1):3–15. (PubMed)
3. Oyola MG, Handa RJ. Hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes: sex differences in regulation of stress responsivity. Stress. 2017;20(5):476-494. DOI: 10.1080/10253890.2017.1369523. Epub 2017 Aug 31. PMID: 28859530; PMCID: PMC5815295.
4. Nagaraj GC, Butts IAE, Dunham RA. Hormone Preparation, Dosage Calculation, and Injection Techniques for Induced Spawning of Foodfish: Revision. SRAC Publication. 2018; 0425:6. Available:https://fisheries.tamu.edu>2019/01>SRAC 0425.
5. Hafeez-ur-Rehman M, Ashraf M, Abbas F, Iqbal KJ, Qureshi IA, Andleeb S. Effect of Different Synthetic Hormones and/or Their Analogues on Induced Spawning in Channa marulius. Pakistan Journal of Zoology. 2015;47(3).
6. Farid SM, Miah MI, Habib MAB, Rahman MM. Effect of pituitary gland (PG) doses on Artificial propagation of endangered Tarabaim, Macrognathus aculeatus (BLOCH). Progress Agric. 2018;19(2):111-118.
7. Uka A, Sikoki FD, Edun MO. Effects of salinity on larval dimension of \( \text{Tilapia guineensis} \). J. Fish. Aquat. Sci. 2012;8:166-171.
8. Ukwe I, Oyekutor K, Abu OM. Evaluation of efficacy and cost effectiveness of Ovulin and Ovaprim Hormones for spawning African catfish ( \( \text{Clarias gariepinus} \)). J. of Fisheries. 2016; 10(4): 053-062.
9. Cortney LO, Broach JS. Hormone Preparation, Dosage Calculation, and
Injection Techniques for Induced Spawning: Baitfish and Ornamental Fish. SRAC Publication No. 0428 September; 2018.

10. Dhas SA, Selvaraj T, Citarasu T, Punitha SM, Babu MM. Role of Synthetic Hormones on Reproductive performance in Etroplus suratensis. Journal of Fisheries & Livestock Production. 2017; 5(3):248.

11. Nwokoye Charles, Ononuju Nwuba, Lily Afuluenu, Eyo Joseph Effiong. Induced propagation of African catfish, *Hetrobranchus bidorsalis* (Geoffery Saint Hillarie,1809) using synthetic and homoplastic hormones. African Jol. of Biotech. 2007;6(23):2687-2693.

12. Das P, Behera BK, Meena SK, Singh Mandal, Sahoo SC, Das S, Yadav AK, Bhattachariya. Comparative efficifcy of different inducing agents on breeding performance of a near threatened cyprinid *Osteobrama belangeri* in captivity. Aquaculture Reports. 2016;4:178–182.

13. Yeasmin SM, Rahman MA, HAQ M. Effects of Hormone on ovulation, fecundity, fertilization and hatching of common Carp (*Cyprinus carpio*). Int. J. anim. Fish. Sci. 2013;1(1):7.

© 2022 Uka and Obilo; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here: https://www.sdiarticle5.com/review-history/85554