**COMPARATIVE EFFECTS OF INDUCING OVULATION IN *Clarias gariepinus* ON THE GROWTH AND SURVIVAL OF THE OFFSPRING USING ARTIFICIAL AND PITUITARY EXTRACT.**

**COMPARATIVE STUDY ON INDUCE BREEDING OF *Clarias gariepinus* ON GROWTH AND SURVIVAL USING SYNTHETIC HORMONE AND PITUITARY EXTRACT**

**Abstracts**

**Aim**: To investigate the effects of inducing broodstock with Artificial and Natural hormones on the growth and survival of *Clarias garipeinus*.

**Study Design**: Treatments were assigned using complete randomized design.

**Place and Duration of the Study:** Fish farm complex of Akwa Ibom State University (AKSU), Nigeria.

**Methodology:** Five (5) broodstock of *Clarias gariepinus* (3male and 2 females) with mean weight of 2 kg each were selected from Aksu farm for breeding, using Ovaprim and Pituitary extract separately. The fertilized eggs were incubated accordingly. Fourteen-day old fry from each of the treatments were randomly stocked at 50 fry/m2 in a 2x 2 x1 m3 culture media in four replicates. Feeding was twice daily at 10 % body weight using Coppens commercial feed for twelve weeks. Water parameters and weekly growth data were observed and recorded.

**Result:** The result showed no significant difference in all the growth parameters observed in this study. Final mean length and weight 18.8 ± 0.04 cm and 37.89 ± 0.06g were higher with pituitary extract than 14.8 ±0.02 cm and 29.86 ±0.02g respectively from the offspring treated with ovaprim. Offspring from pituitary extract treatment recorded the highest value of weight gain and specific growth rate 3.16 ±1.14(g) and 8.72 ± 1.20 more than the offspring from the broodstock induced with ovaprim with 2.49 ±3.92(g) and 8.44 ±1.06 but shows no significant (p<0.05) difference. Offspring from ovaprim treatment had the least % survival of 68.93 ± 4.46%. While offspring from pituitary extract treatment had higher survival value of 79.60±3.28%. Offspring from pituitary extract recorded 0.25 ± 0.02 value of food conversion ratio (F.G.R) while ovaprim treatment had 0.26 ±0.03. Offspring from ovaprim hormones had PER value of 12.09 ± 6.36 while pituitary treatment had PER value of 12.43 ± 4.12. The Condition factor (CF) from the ovaprim treatment was 1.46±0.15 higher than 1.44±0.13 obtained from the sample treated with pituitary extract. Statistically, analysis revealed that there was no significant (P<0.05) difference in the specific growth rate (SGR), Food conversion ratio (FCR), Protein Efficiency Ratio (PER) and Condition factor of all the treatments. However, the offspring from broodstock induced with P.E had the best food conversion ratio value of 0.25 ± 0.02.

**Conclusion:** Since pituitary extract can equally compete and perform effectively with ovaprim hormone, due to cost, scarcity, preservative problems and Government policy on import duties, pituitary extract which is readily available is recommended for artificial propagation of African catfish (*Clarias gariepinus*).

**Keywords: Pituitary Extract, Ovaprim, *Clarias gariepinus*, Growth, Survival, Ovulation**

1. **Introduction**

The global increase in the population, alongside huge demand of protein for human sustainability, re-enforced overfishing of wild caught fishes (Otoh, *et al* 2023 a, b), which eventually lead to decline in capture fisheries (Otoh, *et. al* 2024a, b, c, d) and projected aquaculture as the only alternative to meet the protein need of the masses from aquatic sources. Global production of protenous foods through aquaculture fills the deficit in global fish supply (Otoh, *et al*., 2023 a). Aquaculture being interesting venture, lucrative and economical, require hard work and passion. It is economical and enhances maintenance of human health (Udoh and Otoh, 2023 b).

In Nigeria, African catfish belonging to the family Clariidae (*Heterobranchus* and *Clarias* species) are the most cultivable species of significance (Otoh and Udoh, 2018 a, b; Oyeleye *et al*., 2016). This is due to the unique characteristics of the species such as fast growth rate, good taste, high stocking density, high market price and high resistance to disease and ability to reproduce in captivity, which makes it economical to culture (Otoh, *et al*., 2023 a; Udoh and Otoh, 2017; Udoh and Otoh 2016; Udoh and Otoh, 2023 a; Otoh, *et al*., 2020; Nya, *et al*., 2017 and otoh, *et al*., 2024 e).

However, the growth of *Heterobranchus longifilis* is remarkable in the history of aquaculture, but depends on availability of good feed of which a single feed stuff component cannot achieve (Otoh and Udoh; 2018 c; Otoh, *et al*.,2023 a; Otoh; *et al*., 2024 c; Ekanem, *et al*.,2000). *Heterobranchus* and *Clarias*, readily accept supplementary feed and grow faster within a short period of culture compared to other species (Nlewadim, *et al*., 2011; Otoh and Udoh, 2018 b; Asangusung, *et al*., 2020). These species dominate fresh water environments such as lakes, rivers and streams (Adewunmi and Olaleye, 2011).

Fish growth rate and feed conversions are significantly affected by feeding level and fish cultured environment (Nlewadim, *et al*., 2011; Udoh and Otoh, 2017; Otoh, *et al*., 2024 b; Otoh, *et al*.,2023 a, b; Otoh and Udoh, 2019). The species are also significant because they easily spawn in captivity and can be manipulated to breed all year round through administration of hormones to induce ovulation. Collection of wild fish seed is limited in quality and quantity, (Udoh and Otoh, 2017), prone to disease infection and high level of cannibalism (Udoh and Otoh, 2023). Administration of hormones to induce ovulation and spawning in fish is achievable through administration of natural or synthetic hormones (Asanausung, *et. al*., 2020, Otoh, *et.al*., 2023a).

However, irrespective of feed types available, genetical status and environmental factors, time plays vital role in the growth of any living organism of which is not exceptional (Otoh and Udoh 2018c; Otoh and Udoh 2020, Otoh *et al*., 2023a). It has been observed that fish growth rate and feed conversion are influenced significantly by feeding levels (Nlewadim, *et al*., 2011, Udoh and Otoh 2017). This study seeks to determine the effects of inducing ovulation in *Clarias gariepinus* with natural and artificial hormones on the growth and survival rate of their offspring,

**2. Materials and Methods**

**2.1 Study Area**

The research was conducted at the Fish Farm Complex of Akwa Ibom State University Obio Akpa campus which is located between latitude 5017’N and 7027’N, Longitude 7027’E and 7058’E with an annual rainfall ranging from 3500mm -5000mm and average monthly temperature of 250C. Akwa Ibom State is a coastal state lying between latitude 4028’N and 503’N and between longitude7027’E and 8020’E with a relative humidity `between 60 -70%. It is in the tropical rainforest zone of Nigeria (Otoh, *et al*., 2022, Otoh and Nlewadim, 2019).



**Fig. 1: Map of the Study Area Showing the Location of the Fish Farm Complex**

**2.2 Broodstock**s Acquisition **and care**

Five farmed raised broodstocks: 3 males and 2 females, averagely 2.3 kg each, were sourced from Akwa Ibom State University fish farm. Selection was based on their fitness and morphological condition (Otoh, *et al*., 2020). The broodstocks were maintained in an indoor concrete breeding tanks at temperature of 26 0C, and water depth of 5cm3 in the hatchery all through the study.

**2.3 Hormonal Inducement, Collection of Gametes and Fertilization**

One male spawner was sacrificed for hypophysation. Following its extraction, the pituitary was macerated in saline solution and the *Clarias gariepinus* pituitary extract suspension administered to induce ovulation in one female broodstock of equal weight. The second female was induced to breed by administration of a single dosage of 0.5 ml ovaprim kg-1 female body weight. The two induced female were allowed a 10-hour latency period at 260 C, before stripping manually to obtained eggs (Otoh and Udoh, 2018c, Otoh, *et .al*, 2020; 2022). The eggs from each hormonal treatment were arranged in batches of 3g eggs (containing approximately 2000 oocytes) in three replicates in separate bowls.

**2.4 Milt Extraction**

Milt from the remaining two males were collected and pooled together into one volume, to remove any variation, after sacrificing the males and extracting the testes. The pooled milt was diluted in saline solution and then divided into equal portions of approximately 2ml each and preserved at below 70C temperatures in a refrigerator, until utilized. The three replicates (3g of eggs containing 2000 oocytes) each from ovaprim and pituitary extract treatments, were inseminated and fertilized with pooled milt (2ml of pooled semen per replicate). The fertilization, hatchability and reproductive performances were determined as described in Otoh, *et. al*., (2022).

 **2.5 Growth Studies**

After three days post-hatching, the hatchings spawned from parental inducement with natural and artificial hormones, were fed to satiation with 0. 2mm size Coppens starter feed at 3 hourly intervals for 10 days. Fourteen –day –old hatchery –raised fry from each treatment were randomly stocked in four replicates (n= 200 each per replicate), separately, into 8 different outdoor nursery concrete pond tanks of 2x2x1m3 at 50 fry/ m-2 for cumulative of 12 weeks. After stocking, they were fed twice daily in split-rations at 10% body weight while adjusting the pellet size from 0.2mm initially and gradually increasing to 0.5, 0.8, 1.2, 1.5, 2.0, 3.0 and finally 4.5 mm size till end of experiment in week 12.

Fry were handled under similar water quality conditions. The experimental tanks were screened with mosquito net to exclude predators. Water temperature, pH and dissolved oxygen were monitored in each compartment using thermometer, digital pH meter and Microprocessor, Oximeter, respectively. Survival was measured every week in each experimental unit. The fish samples were measured to the nearest 0.1cm total length (TL) and 0.1g total weight (TW), using meter rule and electronic weighing balance, respectively. The amount of feed administered was recalculated based on the new body weight at each sampling. Fish feed and growth indices were calculated as described by Udoh and Otoh (2017):

Survival % = (No. of fish stocked–Mortality/No. of fish stocked) x 100

Mortality % = (initial number−final number/final number ×100

Weight Gain (g) = Final mean weight (W2)–Initial mean weight (W1)

Average weight gain (%) = [(Final mean weight–Initial mean weight)/Initial mean weight] ×100

Specific growth rate (% g day-1) = [Ln (final mean weight) – Ln (initial mean weight) x 100/days of feeding

 SGR = LnW2– LnW1 x 100

 T2 – T1 1

Protein-energy ratio (PER) =wet weight gain (g)/amount of protein fed (g)

PER = weight gain (g wet fish)

 Wet of protein fed (g crude protein fed)

Feed conversion ratio, FCR = Amount of feed provided (g) / Weight gain (g)

FCR = Diet feed (g)

 Weight gain (g)

Nitrogen metabolism (Nm) = (0.549) (a + b) h (Zeitoun*, et.al*., 1973);

 where, a = initial weight of fish, b = final weight of fish, h = culture period in days.

Gross feed conversion efficiency, GFCE (%) = FCR-1.100 (Lovell, 1989).

**2.6 Monitoring of Water Quality**

Dissolved oxygen and pH of the water were monitored daily using pH meter (VIVOSUN pH meter) and dissolved oxygen meter (Extech, 407510 Dissolved oxygen meter) while mercury in glass thermometer was used to take temperature readings.

2.7 Statistical Analysis

The Data was analyzed using one-way ANOVA at 0.05 significant levels to check the significant difference in fertilization, hatchability and survival rates in the different hormonal treatment.

**3.0 Results**

**3.1 Mean Water Quality of the Incubating Tanks**

The physiochemical parameters observed in each of the treatment showed no significant (P>005) difference. Dissolved oxygen, temperature and pH measurement ranged between 5.25± 0.01- 5.26 ± 0.01, (MgL-1), 27.05 ± 0.01- 27.06 ± 0.01 0C and 6.95 ± 0.01 – 6.96 ± 0.1 respectively (Table 1).

**Table 1. Mean Water Quality Parameter of the Rearing Tanks**

|  |  |  |  |
| --- | --- | --- | --- |
| **Timing/Mean** | **Dissolved Oxygen mg/L** | **Temperature 0C** | **pH** |
| Morning | 5.25-5.26 | 27.05- 27.06 | 6.95- 6.96 |
| Evening | 5.25- 5.26 | 27.05-27.06 | 6.95-6.96 |
| Mean ± SE | 5.23±0.01 | 27.06± 0.01 | 6.96± 0.01 |

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**3.2** **Comparative Effects of Inducing Ovulation in *Clarias gariepinus* on the Growth of the Offspring Using Artificial and Pituitary Extract.**

The results of the Comparative effects of inducing Broodstock with Artificial and Natural Hormone on the growth and survival of the offspring of *Clarias gariepinus* is presented in Table 2. Initial mean length and weight of the fry in all the treatments which were 1.2 ±0.04 – 1.2 ±0.05 and 0.03 ± 0.06 – 0.3 ±0.03g were not significantly different (P> 0.05) which the final mean length and weight at the end of the study showed no significant (P>0.05) difference.

Treatment ‘A’ the offspring from the broodstock induced with artificial hormones (Ovaprim) had the final mean length and weight of 14.8 ± 0.02 and 29.86±0.02 respectively while the offspring from the broodstocks induced with natural hormones pituitary extract had the final mean length and weight of 18.8 ±0.04 and 37.89 ± 0.06. Although a gradual increase was observed in the length and weight of the treatment, yet no significant (P>0.05) difference was observed. Offspring from Natural hormones treatment recorded the highest value of weight gain and specific growth rate 3.16 ±1.14(g) and 8.72±1.20 more than the offspring from the broodstock induced with artificial hormones with 2.49±3.92(g) and 8.44 ±1.06 but show no significant difference. Offspring from artificial hormones had % survival of 68.93 ±4.46 while offspring from natural hormones had 79.60 ± 3.28. Offspring from artificial hormone recorded FCR of 0.26 ± 0.03 while natural hormone showed FCR of 0.25 ±0.02. Offspring from artificial hormones had PEM value of 12.09 ±6.36 while the other one had PEM value of 12. 43 ±4.12 C.F of 1.46 ± 0.15 was observed in the offspring from artificial hormone while C F value of 1.44 ± 0.13. was observed in the offspring from natural hormone.

 Statistically, analysis revealed that there was no significant difference in the specific growth rate (SGR), Food conversion ratio (FCR), Protein Efficiency Ratio (PER) and condition factor of all the treatments. However, the offspring from broodstock induced with P.E had the best food conversion ratio value of 0.25 ± 0.02.

**Table 2: Growth Performance of offspring of *Clarias gariepinus Broodstock* induced with Artificial and Natural Hormones and Reared in Concrete Aquaculture Ponds for 12weeks (*Mean ± SE*)**

|  |  |  |
| --- | --- | --- |
| **Growth indices** | **Artificial hormones** | **Natural hormones** |
| Initial length (cm) | 1.2±0.04a | 1.2±0.05a |
| Final length (cm) | 14.8±0.02a | 18.8±0.04a |
| Initial weight (g) | 0.03±0.06a | 0.03±0.03a |
| Final weight (g) | 29.86±0.02a | 37.89±0.06a |
| Weight gained (g) | 2.49±3.92a | 3.16±1.14a |
| Growth rate, *SGR*(%/day) | 8.44±1.06a | 8.72±1.20a |
| Survival (%) | 68.93±4.46a | 79.60±3.28a |
| *FCR* | 0.27±0.03a | 0.26±0.02a |
| *ADWG* (g) | 0.36±0.18a | 0.45±0.16a |
| Weight gain (%) | 42.99±3.92a | 43.70±4.21a |
| *PER* | 12.09±6.36a | 12.43±4.12a |
| *Nm* | 0.03±0.01a | 0.06±0.02a |
| *GFCE*% | 430.67±39.04a | 436.95±42.09a |
| Condition Factor | 1.46±0.15a | 1.44±0.13a |

a, b values with same superscript in each row are not significantly different (p>0.05)

Where*: SGR*= Specific Growth rate, ADWG = Average daily weight gain *FCR*= Feed conversion ratio

*PER* = Protein-energy ratio, *Nm* = Nitrogen metabolism, GFCE % = Gross feed conversion efficiency

**4. Discussion**

The condition factor (Wellbeing) of the fish revealed higher value with the offspring from broodstock induced with artificial hormones (Ovaprim) while the least value of condition factor was observed in the treatment with pituitary extract but analysis revealed that there was not significant (P>0.05) difference among the two treatments. This is in agreement with the report of Otoh and Udo (2018); Otoh, *et.al* 2023b. There was no significant (P>0.05) difference in the specific growth rate (S.G.R), Food Conversion Ratio (F.C.R) and Protein Efficiency Ratio (P.E.R) in both treatments.

This result revealed that, both artificial (ovaprim) and natural Hormones (pituitary extract) performed equally in terms of growth performances of the offspring. The result agrees favorably with the findings of Otoh, *et.al* (2023 a) that both ovaprim and pituitary extract are good to be used for artificial propagation of African catfish. However, initial length and weight of the offspring from both treatments were observed to increase with culture period. Similar growth conditions were observed by Otoh and Udoh 2018c, Nlewadim, *et.al* 2011). This could be attributed to similar culture environment as observed by management and maintenance of water quality of the culture species. The result of water quality of this study revealed there was no significant (P>0.05) difference, it could also be attributed to food source that was not different and management system of the hatchery level. Similar result was also observed by Nlewadim, *et.al* (2011) during their studies on the effect of *Moringa Oleifera* on the growth performances and survival of *Oreochromis niloticus*.

The offspring obtained from the broodstock induced with pituitary extract revealed higher percentage survival value of 79.60 ± 3.28 while the offspring from the artificial hormones had the least percentage survival value of 68.93 ± 4.46 but were not significantly (P>0.05) different. This result could be attributed to the management system at the hatchery level. The results align favorably with the assertion of Ajah (2007); Otoh, *et.al*., 2022, Otoh, *et.al*., 2023a and otoh, *et.al*., (2024 a). Based on the results of findings, it is observed that both hormones are active for artificial propagation of catfish.

**5.0 Conclusion**

It has been observed that both artificial and pituitary extract are very active for artificial propagation of *Clarias gariepinus* and the growth parameters and survival rate of the offspring from the two treatments were not significantly different. It is therefore recommended that, due to high cost of artificial hormone, unavailability, Problem of preservation, high cost of importation and Government policy, Pituitary extract which is readily available and affordable should be used for artificial propagation of Clarias *gariepinus.*

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