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Homoplastic Hypophysation of African Catfish *(Clarias gariepinus*, Burchell 1822) Using Catfish Pituitary Gland with Coconut Water as Extender Agent

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study aimed to evaluate the effects of coconut water as an extender agent on the reproductive variables of *Clarias gariepinus*. The study used 18 healthy broodstocks with body weight ranging from 300 g to 750 g. The four treatments used were: 100% diluted pituitary gland for T1, 75% coconut water and 25% diluted pituitary gland for T2, 50% coconut water and 50% diluted pituitary gland for T3, and 25% coconut water and 75% diluted pituitary gland for T4. T1 had the highest mean fecundity value with 33,366.67 eggs, followed by T3 with 33,416.67 eggs, while T2 had the lowest with 27,466.67 eggs. The GSI was highest in T1 at 12.96%, while T2 had the lowest at

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10.69%. The fertilization rate was significantly different among treatments, with T1 having the highest rate of 95.94%, followed by T4 at 92.43%. T3 and 2 had rates of 88.26% and 87.14%, respectively. The hatchability rate was also significantly different among treatments, with T1 having the highest rate of 91.5%, followed by T4 at 84.1%. T3 had a hatchability rate of 76.2%, and T2 had the lowest hatchability rate of 73.1%. The water temperature was within the optimum range, but the pH fell short of the optimum range. Overall, the study suggests that using coconut water as an extender agent can improve fertilization and hatchability rates in *C. gariepinus* injected with pituitary gland extract.

Keywords: Clarias gariepinus; fecundity; hatchability; GSI; fertility; coconut water.

1. INTRODUCTION

Aquaculture is a rapidly growing sector with the potential to provide livelihoods and affordable animal protein, particularly in developing countries [1]. Catfish is an important freshwater food fish in Southeast Asia due to its ability to tolerate poor water quality and high stocking density [2]. Its reproduction is seasonal and influenced by water temperature, photoperiod, and water level [3-5]. Extenders are important for inducing reproduction, and the pituitary gland is the main source of hormones responsible for reproduction in animals [6-7].

Chemical solutions are commonly used as extenders but may be toxic to fish sperm, and the use of extenders can prolong the life of spermatozoa in storage [8,9]. The young coconut water is more effective than old coconut water and sugarcane water for this purpose. Research on induced breeding practices using pituitary extracts has been conducted for cyprinids, catfish, and sturgeon [10,11].

Breeding fish using pituitary extract is cheap as it uses natural inducing hormones, but it may not always be available in developing countries [12,13]. Developing fish seed production has been identified to augment the dwindling fish supply from the capture fisheries [14]. Despite its potential effectiveness, coconut water has not been well explored as an extender for catfish breeding [15]. Thus, this study aimed to evaluate the effectiveness of coconut water as an extender agent for *C. gariepinus.* The goal is to introduce an innovative and cost-effective method of farming to local farmers by using pituitary glands extended with coconut water, thereby increasing and accelerating their production.

2. MATERIALS AND METHODS

2.1 Experimental Design and Set-Up

The study was conducted at RG Aqua Hatchery in Monkayo, Compostella Valley, which is a supplier of catfish and tilapia fingerlings in the area. The experiment utilized a Complete Randomized Design (CRD) with four treatments and three replicates per treatment. The treatments consisted of different ratios of pituitary gland extract and coconut water, namely: 100% diluted PG (T1), 75% coconut water and 25% diluted PG (T2), 50% coconut water and 50% diluted PG (T3), and 25% coconut water and 75% diluted PG (T4).

2.2 Broodstock Source and Management

The study used 18 healthy broodstocks of *C. gariepinus*, consisting of 12 females and 6 males. The broodstocks were conditioned in an earthen pond measuring 12 meters in width, 15 meters in length, and 20 inches in depth for a week. They were fed with commercial feeds twice daily at 7:00 A.M. and 5:00 P.M., with a feed amount equivalent to 5% of the total fish biomass.

2.3 Collection and Preservation of Pituitary Gland

The pituitary glands were extracted from six male *C. gariepinus* specimens by removing the top part of the head and skull, located under the brain mass, using a sharp knife. The procedure was done with utmost care to avoid damage and preserve the potency of the PG, following the method described by De Graaf and Janssen [16]. After being collected, the pituitary glands were placed in a bowl of distilled water to prevent degradation of glycol- or macro-proteins through enzymatic action. The minced pituitary glands were then mixed with distilled water in a bowl.

2.4 Collection of Coconut Water

The coconut water collected from coconuts with a green shell and soft flesh was used to dilute the pituitary gland (PG). The young coconut water was chosen due to its composition, which includes 5.20% sucrose and fructose, 81.80 mg

L-1 magnesium, and 730.40 mg L-1 potassium, as reported by Barlina et al. [17]. As the coconut matures, the composition changes to 3.00% sucrose and fructose, 70.60 mg L-1 magnesium, and 772.40 mg L-1 potassium.

2.5 Pituitary Gland Injection

The female *C. gariepinus* breeders were injected intramuscularly at an angle of 30°- 45° at the dorsal fin with different dose of hormone in every treatment. Each injected breeder was secured in different holding basin to prevent them from inflicting injury on one another.

2.6 Stripping, Fertilization, Incubation, and Water Monitoring

After a 12-hour post-injection, the female *C. gariepinus* breeders were taken out from their individual basins and their eggs were stripped into a clean, dry bowl. Meanwhile, the testes of the male breeders were extracted through the abdomen and kept in a refrigerator until used. The extracted testes were then squeezed onto the eggs to fertilize them. These fertilized eggs were spread onto an improvised hatching basin with a flow-through of clean freshwater until hatching. The water quality parameters, such as pH and temperature, were monitored hourly from the incubation of eggs until hatching.

2.7 Reproductive Variables

The reproductive variables such as fecundity, fertility, hatchability, and GSI were computed according to the corresponding formula for each variable:

fertilization rate (%) = no.of fertilized eggs / total no.of eggs x 100

hatching rate = no.of egg hatched / total no.of fertilized eggs x 100

Gonadosomatic Index = weight of gonad / body weight x 100

To determine the fecundity, after removing excess water using filter paper, the ovaries were carefully weighed. The number of eggs per gram was then counted and used to calculate the total number of eggs, following the method described by Dada and Ebhodaghe [18].

2.8 Statistical Analysis

The SPSS was used to analyze the data via One-way Analysis of Variance (ANOVA) at $p < 0.05$ probability levels.

3. RESULTS

The variation in spawning fecundity of *C. gariepinus* injected with *C. gariepinus* pituitary gland (PG) extract is shown in Fig. 1. In T1, the range of spawning fecundity was from 31,800-35,700 eggs, with a mean value of 33,366.67 ± 975 eggs. For Treatment 2, the range was from 26,450-28,100 eggs, with a mean value of 27,466.67 ± 412.5 eggs. In Treatment 3, the range was from 29,200-39,750 eggs, with a mean value of 33,416.67 ± 2,637.5 eggs. Finally, in Treatment 4, the range was from 25,200-32,150 eggs, with a mean value of 28,850 ± 1,737.5 eggs.

The Gonadosomatic Index (GSI), fertility rate, and hatchability rate of *C. gariepinus* is shown in Fig. 2. The GSI is a commonly used metric in fish biology that measures the ratio of gonad weight to body weight and is used to evaluate the reproductive status of fish. In this study, the highest value was observed in T1 at 12.96%, while T2 had the lowest value at 10.69%.

Fig. 1. The experimental set-up showing the catfish eggs on the hatching basins waiting to hatch (Left) and the administration of the diluted PG (Right)

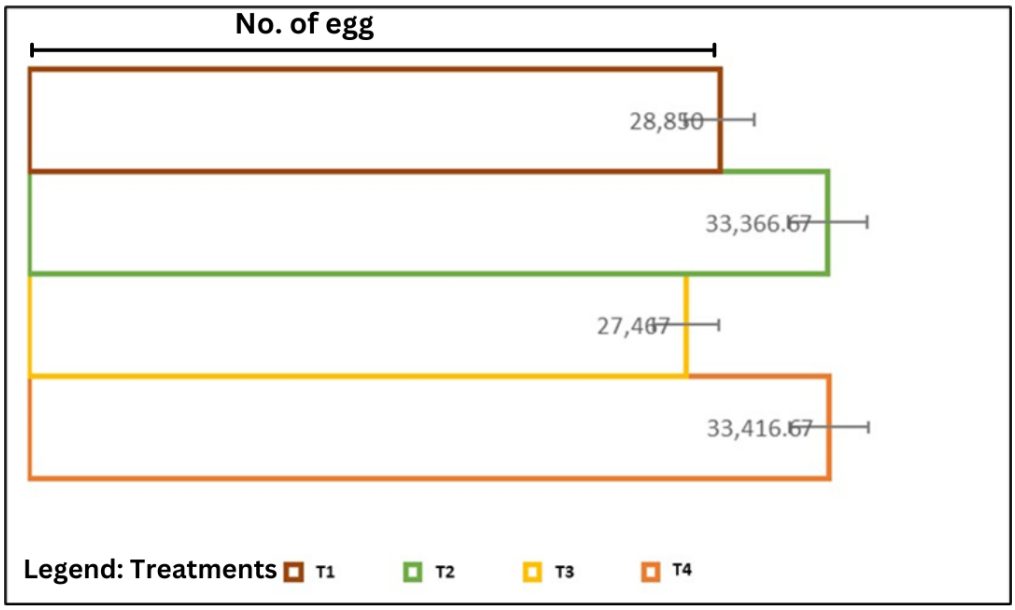

Fig. 2. The fecundity of *C. gariepinus* using different treatments

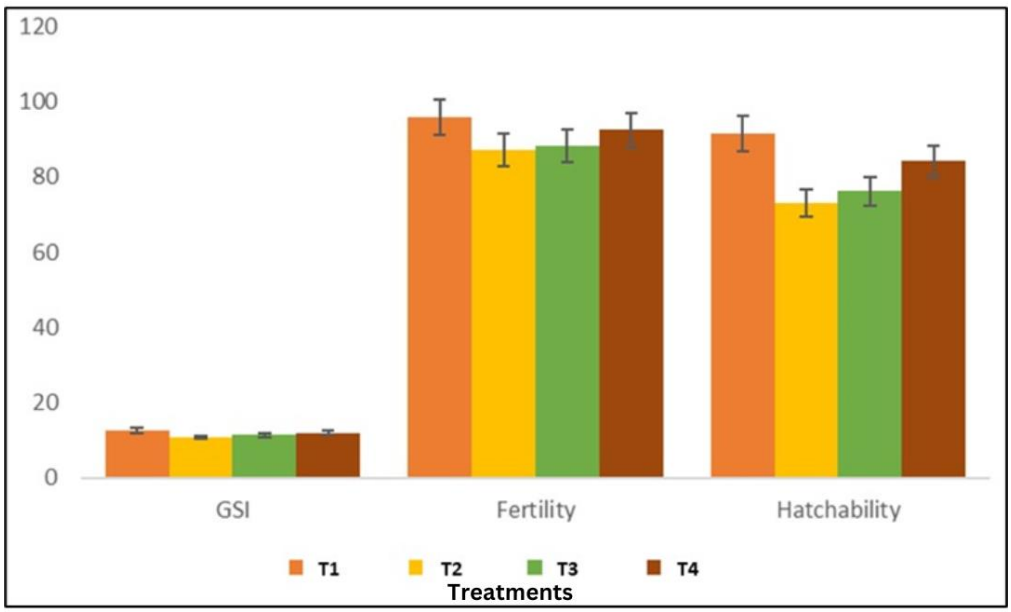

Fig. 3. The GSI, fertility rate, and hatchability rate of *C. gariepinus* using different treatments

Table 1. Physico-chemical parameters of the water

Treatment	Temperature		pH	
	Actual	Optimum range	Actual	Optimum range
1	26.27°C	26-28°C	6.92	7.0-8.5
2	26.24°C		6.85	
3	26.30°C		6.83	
4	26.26°C		6.83	

Treatment 3 and 4 obtained values of 11.36% and 11.96%, respectively. Furthermore, the fertilization rate was notably highest in Treatment 1 with 95.94%, followed by Treatment 4 at 92.43%, while Treatment 3 and 2 had rates of 88.26% and 87.14%, respectively. Finally, after

36 hours of incubation, the hatchability rate of *C. gariepinus* was measured. It was observed that T1 had the highest hatchability rate of 91.5%, while Treatment 2 had the lowest hatchability rate of 73.1%. Treatment 3 and 4 had hatchability rates of 76.2% and 84.1%, respectively.

The statistical analysis revealed that the fecundity and GSI of *C. gariepinus* were not significantly different among all treatments. However, there was a significant difference ($p<0.05$) in the fertilization rate among treatments, with T1 and 4 being the most effective. Similarly, there was a significant difference ($p<0.05$) in the hatchability rate among treatments, with T1 producing the highest hatching rate.

Table 1 presents the physico-chemical parameters of the water used in the study. T1 exhibited the highest pH value of 6.90, while T3 and 4 had a pH of 6.83. T2 had a pH value of 6.85. The highest water temperature was recorded in T3 at 26.30°C, whereas T2 had the lowest temperature at 26.24°C. T4 and 1 had water temperatures of 26.26°C and 26.27°C, respectively.

4. DISCUSSION

Fecundity variations are common in fishes, and are influenced by factors such as size, age, and condition of the fish, as well as space and food availability. Fecundity increases with size and weight, as indicated by Bagenal [19], who used length and weight as reliable indicators of egg production capacity. Evaluating fish fecundity is essential to assess their reproductive potential, as noted by Duarte and Araujo [20] Bagenal [19] defined fecundity as the number of vitellogenic oocytes in mature females before the next spawning season, specifically referring to ripe, spawnable eggs larger than 1.0 mm in the fish's ovary. However, other authors, such as Clay, Eyo and Mgbenka, and Ezenwaji [21-23], have included all available eggs in the brood stock's ovary when defining fecundity. GSI indicates gonadal development and maturity of fish which increases with the maturation of the fish and declines abruptly thereafter [24,25] also reported that GSI was widely used especially for the bony fishes in order to examine the spawning period because its value was directly related to the development of the gonad.

In a study conducted by Muchlisin [26] to investigate the effectiveness of natural extenders for fish sperm, it was found that coconut solution at a dilution ratio of 1:20 had the highest fertility rates among the three natural extenders tested. Soybean milk at the same dilution ratio also showed similar results. Additionally, the coconut water at 1:20 dilution ratio resulted in higher hatching rates compared to other natural extenders. This outcome is likely due to the pH and ion composition of the diluents.

The effects of acidic water on the viability and development of fish eggs have been reported to vary. Some studies have shown that eggs in acidic water are susceptible to predation for a longer duration than those in neutral water. However, contradictory findings have also been reported for salmon species. For instance, Daye and Garside [27] observed no effect of acid stress on the development rate of Atlantic salmon within the pH range of 6.8-3.7. Similarly, Menendez [28] found no impact on *S. fontinalis*. In contrast, Trojnar [29] recorded faster development of *S. fontinalis* at pH levels below 5.

5. CONCLUSION

This study demonstrated that coconut water was an effective extender agent for homoplastic hypophysation of African catfish, with reproductive variables such as fecundity, fertility, hatchability, and gonadosomatic index being influenced by the ratio of coconut water to diluted pituitary gland.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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