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Comparative Studies of Some Biochemical Characteristics of Seminal Plasma in Wild and Cultured Male Catfish (*Clarias gariepinus*), Burchell, 1822 Broodstock

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ABSTRACT

Purpose: *In aquaculture, the most important aspect to consider is ensuring the development of valuable offspring by using high-quality gametes from fish broodstock. This research aims to compare the biochemical constituents of seminal plasma in both wild and cultured Clarias gariepinus broodstock.*

Research Method: *Apparently healthy adult male Clarias gariepinus (n=9, 900±300g mean weight per fish type (wild or cultured), 49±8.5cm mean total length) were collected from fishermen on the Oshin River, Nigeria for the wild samples and from a reputable fish farm in Ilorin and examined for some seminal plasma's biochemical parameters following standard procedures. T-test statistics were used to compare the differences between classes.*

Findings: *Higher values of most of the semen biochemical compositions were recorded among the cultured Clarias gariepinus with significant variations ($p<0.05$) recorded in the mean values of total cholesterol, high-density lipoprotein-cholesterol (HDL-Cholesterol), low-density lipoprotein-cholesterol (LDL-Cholesterol), uric acid, Aspartate Aminotransferase (AST), total bilirubin, albumin, potassium, chloride and urea. Creatinine, glucose, sodium, and triglycerides, on the other hand, did not vary significantly ($p>0.05$) between cultured and wild Clarias gariepinus semen.*

Originality/ Value: *The current study's findings on biochemical compositions could lead to more effective gamete management, higher yields, and improved semen suitability for short-term storage.*

Keywords: *Semen, African catfish, Total cholesterol, Potassium, Uric acid*

INTRODUCTION

Clarias gariepinus is one of Africa's, Asia's, and Europe's most important tropical fish (Viveiros *et al.*, 2000). *Clarias gariepinus* is commonly cultivated in Nigeria due to its high marketability, rapid growth, and ability to withstand harsh pond conditions (Adeleke *et al.*, 2021). However, to ensure consistent fish production, gametes must be available throughout the year. In aquaculture, the most important consideration is to ensure the development of valuable offspring by using high-quality gametes from fish broodstock The aquaculture industry has put a greater emphasis on the quality of eggs and larvae than on the quality of sperm, even though sperm quality affects the supply of healthy larvae (Migaud *et al.*, 2013; Ochokwu *et al.*, 2015). However, milt is often inadequate in quantity and quality in commercial hatcheries, and it does not always result in effective fertilization in the artificial insemination procedures widely used for aquaculture organisms (Idahor *et al.*, 2018; Mylonas *et al.*,

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2010). This is especially true for species like the African catfish (*Clarias gariepinus*), where manual stripping by abdominal massage failed to retrieve sperm, necessitating intra testicular sperm extraction from the testes (Beirão *et al.*, 2019). Understanding variations in sperm quality between male fish can help determine the relative success of each broodstock, which can help solve some of the challenges listed above (Rurangwa *et al.*, 2004). The use of high-quality gametes enhances larvae generation survival, and a simple assessment of sperm quality will aid in selecting the best broodstock with high-quality milt (Rurangwa *et al.*, 2004). The chemical and biochemical constituents of seminal plasma, which serves as an indicator in determining the content of sperm in many fish species, have effect on the sperm quality (Alavi *et al.*, 2012). In addition to having the proper pH and viscosity for sperm viability and motility, seminal plasma also has the proper concentration of fructose and other ions to support the growth of spermatozoa (Zadmajid *et al.*, 2019). Seminal plasma offers the majority of physiologic antioxidant defense against oxidative damage since spermatozoa are compact cells with little antioxidant capability. Spermatozoa cultured in a media devoid of seminal plasma exhibit a substantial reduction in motility and an increase in oxidative stress indicators after just 2 hours (Cheng and Ko, 2019; Tvrdá *et al.*, 2016).

Seminal plasma contains unique organic and inorganic components that aid spermatozoa viability (Ciereszko *et al.*, 2000; Lahnsteiner *et al.*, 1993; Lahnsteiner *et al.*, 1994; Piironen and Hyvärinen, 1983). Organic constituents such as triglycerides, glycerol, fatty acids, glucose, and lactate provide energy for metabolism. The inorganic constituents such as K^+, Na^+, Ca^{2+}, and Mg^{2+} aid in sperm motility and many enzymes such as acid phosphatase, alkaline phosphatase, malate dehydrogenase, lactate dehydrogenase, adenosine triphosphatase and aspartate aminotransferase are regulating spermatozoan metabolic process (Rurangwa *et al.*, 2004). Seminal plasma contains a variety of biochemical constituents that are essential for sperm function and metabolism, such as glucose, cholesterol, proteins, metabolites, intracellular and antioxidant enzymes, and mineral elements (Asadpour, 2012; Juyena and Stelletta, 2012). By designing a triggering media for reproduction, determining the consistency of semen based on these criteria aids in the improvement of artificial fertilization methods (Rodina *et al.*, 2004). Awareness of the effects of these parameters on semen quality aids in the development of appropriate media for artificial fertilization or the use of a cryopreservation technique (Golpour *et al.*, 2013). Despite the fact that the spermatozoa's microenvironment can significantly affect sperm motility and viability, this subject matter has attracted little study attention (Gallego and Asturiano, 2018; Mann and Lutwak-Mann, 1981). This study thus examines some semen biochemical characteristics of wild and cultured male catfish (*Clarias gariepinus*, Burchell, 1822) broodstock

METHODOLOGY

Description of the Study Area

The experiments were carried out at the Department of Aquaculture and Fisheries, Faculty of Agriculture, University of Ilorin, Ilorin, Kwara State, Nigeria. Oshin River in Ilorin East and a reputable private fish farm in Ilorin, Kwara State, Nigeria were used as test sites for this experiment. Ilorin is situated at 8°30'N and 4°32'E latitude and longitude.

Sample Collection

During April-May 2019, Apparently healthy adult male *Clarias gariepinus* (n=9 per fish type (wild or cultured), 900±300g mean weight, 49±8.5cm mean total length) were collected from fishermen landing on the Oshin River, Nigeria for the wild samples and from broodstock market section of a reputable fish farm in Ilorin for the cultured samples. *Clarias gariepinus* was identified using fish guide by (Olaosebikan and Raji, 2013) and

matured male samples were identified with well vascularized genital papilla.

Broodstock Care and Transportation

The samples were collected in plastic bowls half-filled with water, which were then covered with sacks to prevent the fish from jumping out, mitigating tension, and transported to the University of Ilorin's Department of Aquaculture and Fisheries. The wild and cultured broodstocks were conditioned in separate fish tanks and acclimatized for two days without food to enable the alimentary tract to empty before testes were collected. The broodstock was well-cared for, and the water was changed regularly to avoid contamination.

Collection of Semen

Clarias gariepinus broodstocks were dried with a clean damp towel along the abdomen and sacrificed because the milt could not be stripped out. Each sacrificed broodstock's abdomen was dissected, and the two testes were carefully removed from the body cavity without harming them. The gonads were immediately extracted and measured on a sensitive scale to ascertain their actual weight. The testes were put in a buffer solution before it was time to release the sperm (Adeyemo *et al.*, 2007). The milt was obtained by carefully macerating the milt sac with a new surgical blade to release the seminal fluid into 5mL sterilized test tubes that were labelled based on the sample identification (Adewumi, 2004; Lamai and Eyo, 1999) To dilute the seminal fluid, a 0.9 per cent NaCl solution was applied to each test tube. Each sample was kept in the refrigerator until it was time to move on to the next sample. The test tubes containing semen, were transported in a cooler containing ice cubes to keep the temperature of the collected semen at 4^0C. All analyses were conducted at the University of Ilorin Central Laboratory

Evaluation of the seminal plasma Composition

To separate the spermatozoa from the seminal plasma, the milt samples were centrifuged at 3000rpm for 10 minutes at room temperature, and the supernatant (seminal plasma) of each sample was carefully removed into different test tubes depending on the sample identity, with care taken to avoid contact with spermatozoa. Every tube's seminal plasma was held in the refrigerator at -20^0C until the biochemical analysis began. Sample tubes were gently placed in racks that were held in cool water with ice packs at the start of the experiment. Biochemical parameters; glucose (mg/dl), total protein (mg/dl) , Total cholesterol (mg/dl), HDL-cholesterol (mg/dl), LDL-cholesterol (mg/dl), urea (mg/dl), uric acid (mg/dl), total bilirubin (mg/dl), ALT (U/l), AST (U/l), sodium (mEq/L), chloride (mEq/L), potassium (mEq/L), triglycerides (mg/dl), creatinine (mg/dl), albumin (mg/dl), were estimated from the samples using quantitative biochemical kits of serum or plasma parameters (RANDOX^{TM} United Kingdom), and all the parameters were measured using UV-2601 double beam UV-VIS spectrophotometer (Model:UV-2601 UK).

Procedures of Some Biochemical Parameters

Total Protein

10 µL of seminal plasma were collected into separate tubes, along with 1 mL of total protein reagent, which was combined in each tube as explained by Lowry method (Lowry *et al.*, 1951). All tubes were left at 20-25^0C for 30 minutes before being measured in a spectrophotometer (550 wavelength) for readings. The reagent blank was used to compare the absorbance of the sample and the normal.

Calculation;

$$Total\ protein\ concentration = \left(\frac{A_{Sample}}{A_{Standard}}\right) \text{x}\ Standard\ concentration$$

Uric Acid

10 µL of seminal plasma were collected into separate tubes, and 500 µL of reagent were applied to the tubes, which were combined and incubated at 20-25^{0}C for 15 minutes. The absorbance of the sample and of the norm was measured against the reagent blank within 30 minutes after which is measured in the spectrophotometer (520 wavelengths) for readings.

Calculation;

$$Urea\ concentration = \left(\frac{A_{Sample}}{A_{Standard}}\right) \text{x}\ Standard\ concentration$$

Creatinine

50 µL of seminal plasma were collected into separate tubes, and 500 µL of reagent were applied to the tubes, which were combined and then placed in the spectrophotometer (492 wavelengths), where the sample was read after 30 seconds and then again after exactly 2 minutes.

Calculation;

$$Creatinine\ concentration = \left(\frac{A_{Sample}}{A_{Standard}}\right) \text{x}\ Standard\ concentration$$

Sodium

50 µL of seminal plasma were collected into separate tubes, along with 1 mL of filtrate reagent, which was combined and centrifuged for 10 minutes. Following that, 50 microliters of supernatant, 1 ml of acid reagent, and 50 µL of colour reagent were applied to each tube and mixed thoroughly. For readings, each sample was placed in a spectrophotometer (550 wavelengths).

Calculation;

$$Sodium\ concentration = \left(\frac{A_{of\ blank} - A_{of\ sample}}{A_{of\ blank} - A_{of\ standard}}\right) \text{x}\ Standard\ concentration$$

Potassium

10 µL of seminal plasma were collected into separate tubes, along with 1 mL of potassium reagent, which was mixed and allowed to stand for 3 minutes. For readings, each sample was placed in a spectrophotometer (500 wavelengths).

Calculation;

$$Potassium\ concentration = \left(\frac{A_{of\ unknown}}{A_{of\ standard}}\right) \text{x}\ Standard\ concentration$$

Chloride

10 µL of seminal plasma were collected into separate tubes, along with 1.5 mL of chloride reagent, which was combined and allowed to stand for 5 minutes. For readings, each sample was placed in a spectrophotometer (480 wavelengths).

Calculation;

$$Chloride\ concentration = \left(\frac{A_{of\ unknown}}{A_{of\ calibrator}}\right) \text{x}\ Standard\ concentration$$

All other parameters were calculated using the RANDOX biological kits' manufacturer's procedures.

Ethical Statement

Experimental animals were handled and employed in accordance with accepted standards for animal welfare during transit, housing, and the conclusion of the study. The Animal Research: Reporting of In-Vivo Experiments (ARRIVE) rules were adhered to while reporting this study.

Statistical Analysis

Data were expressed as mean and standard deviation using SPSS statistical software (IBM SPPS 2011, version 20). T-test statistics were

used to compare the differences between the mean values of the biochemical parameters.

RESULTS AND DISCUSSION

The biochemical characteristics of both wild and cultured *Clarias gariepinus* seminal plasma were evaluated in this study. The fish seminal plasmas are needed to determine effective artificial fertilization and semen handling techniques (Cejko *et al.*, 2022). Knowledge of fish biology, especially reproductive characteristics, is critical for their management and conservation in the wild. In this regard, endemic fish species must be given special consideration, since they are the most significant component of local water bodies (Lind *et al.*, 2012; Romero *et al.*, 2021). Table 1 shows the semen biochemical parameters of cultured and wild *Clarias gariepinus* in the Ilorin metropolis. Higher values of most of the semen biochemical compositions were recorded in the cultured *Clarias gariepinus* with significant variations ($p<0.05$) recorded in the mean values of total cholesterol, HDL-cholesterol, LDL-cholesterol, uric acid, AST, total bilirubin, albumin, potassium, chloride and urea. In fish that fertilize externally, the ions in the seminal plasma influence the motility of the sperm (Ercin *et al.*, 2009).

According to a study of the literature, Na^+ and Cl^- are most likely the key electrolytes involved in sustaining the osmolality of seminal plasma (Morisawa *et al.*, 1979) and spermatozoa viability.

Potassium (K^+) is involved in the static storage of spermatozoa (Baynes *et al.*, 1981). Morisawa and Suzuki (1980) have discovered that a potassium ion is an effective inhibitor of sperm movement in seminal plasma. The mean values of potassium ion for cultured fish groups were higher than those of the wild fish. This potentiates the tendency of lower sperm movement in cultured catfish showing a better sperm motility in wild catfish.

Table 01: Seminal plasma biochemical parameters of cultured and wild *Clarias gariepinus* in Ilorin metropolis

Parameters	Cultured	Wild	T-value
Total cholesterol (mg/dl)	571.67 ± 190.15	206.33 ± 75.55	5.36***
HDL-C (mg/dl)	128.00 ± 9.82	89.67 ± 5.92	10.03***
LDL-C(mg/dl)	376.89 ± 168.69	91.89 ± 75.27	4.63***
Uric acid (mg/dl)	5.11 ± 0.33	2.22 ± 0.67	11.63***
AST (U/I)	307.33 ± 73.71	121.89 ± 8.67	7.50 ***
ALT (U/I)	146.78 ±14.30	108.22 ± 23.88	4.16 ***
Total bilirubin (mg/dl)	3.56 ± 1.01	15.11 ± 4.99	-6.81 ***
Albumin (mg/dl)	5.56 ± 0.88	1.78 ± 1.30	7.21***
Potassium (mEq/L)	4.67 ± 0.71	1.44 ± 0.53	v10.96 ***
Chloride (mEq/L)	10.11 ± 1.36	54.67 ± 5.20	-24.88 ***
Total protein (mg/dl)	12.22 ± 0.67	13.44 ± 1.33	-2.46 *
Urea (mg/dl)	3.67 ± 0.50	33.11 ± 2.85	-30.55 ***

*SD= standard deviation; T-values having *** are highly significant ($p<0.001$), ** values indicate moderately significant ($p<0.01$), * indicate significant values ($p < 0.05$) and NS shows non-significant values ($p > 0.05$); HDL- cholesterol: high density lipoprotein cholesterol; LDL- cholesterol: low density lipoprotein cholesterol; AST-Aspartate Aminotransferase; ALT - Alanine Aminotransferase*

Total protein, according to White and Macleod (1963), plays a defensive function. This study found that total protein concentrations in cultured and wild fish (12.22±0.67 mg/dl, 13.44±1.33 mg/dl) are higher than those recorded for rainbow trout: 1.74±0.79 mg/ml (Loir et al., 1990), 1.34±0.67 mg/ml (Ciereszko and Dabrowski, 1993), and 1.47±0.84 mg/ml (Lahnsteiner *et al.*, 1998). However, research by Bozkurt *et al.* (2006) revealed that high protein content (9.42±3 g/dl) is needed for fish semen. The study's high protein concentration may be due to the high demand for protein in *Clarias gariepinus*' diet. Notable urea concentrations were observed in cultured samples (3.67±0.50 mg/dl), which had lower content than wild samples (33.11±2.85 mg/dl), although this is in contrast to *Cyprinus carpio* (54.72±3.49 mg/dl) (Ercin *et al.*, 2009) and within the range established in *Oncorhynchus mykiss* (31.65±0.78 mg/dl); (Akcay *et al.*, 2004) Urea contamination of sperm can result in decreased sperm motility and fertilization capacity (Dreanno *et al.*, 1998), affecting the variability of other semen parameters (Glogowski *et al.*, 2000).

However, there were no significant differences ($p>0.05$) between the groups in this experiment for creatinine (Figure 1), glucose (Figure 2), sodium (Figure 3), or triglycerides (Figure 4).

Extracellular carbohydrates, such as glucose, can be used by fish spermatozoa. Monosaccharides including glucose are commonly used as energy sources for sperm motility in fish (Lahnsteiner *et al.*, 1993; Stoss, 1983). The presence of this carbohydrate in seminal plasma, on the other hand, has been linked to the high energy demand for the testes during spermatogenesis or spermatozoa lipid synthesis (Soengas *et al.*, 1993). In this analysis, glucose was found in seminal plasma at concentrations of cultured fish (100.11±39.23 mg/dl) and wild fish (114.44 ±11.23 mg/dl), respectively, which were higher than those found in other fish such as Rainbow trout (1.33±0.76 mg/dl)(Akcay *et al.*, 2004) and *Barbus grypus* (9.41±1.29 mg/dl) (Khodadadi *et al.*, 2016)

The mean Na^+ concentration for cultured fish and wild fish in our study was lower than that for perch (124 mmol/l) (Lahnsteiner *et al.*, 1995), Asian catfish (164 mmol/l)(Tan-Fermin *et al.*, 1999); and muskellunge (129 mmol/l)(Lin *et al.*, 1996); it can be concluded that the low levels of Na^+ are due to a defect in the formation of seminal plasma, resulting in lower sperm motility in the semen. These variations are most likely due to species-specific characteristics (Ciereszko *et al.*, 2000).

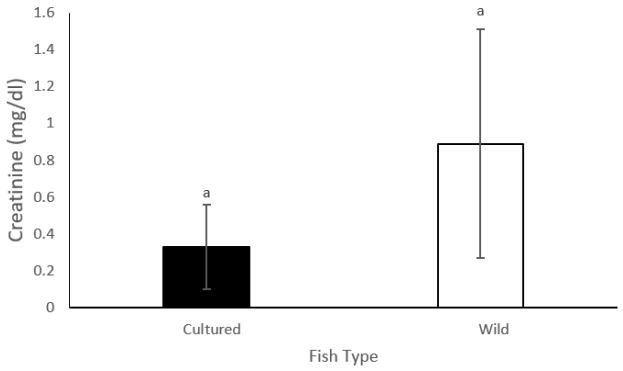

Figure 01: **Semen creatinine concentration (n=9, 900±300g mean weight, 49±8.5cm mean total length) of cultured and wild *Clarias gariepinus* in Ilorin metropolis. Bars represented by mean ± SD. Bars with the same alphabets are not significantly different (p>0.05) from each other.**

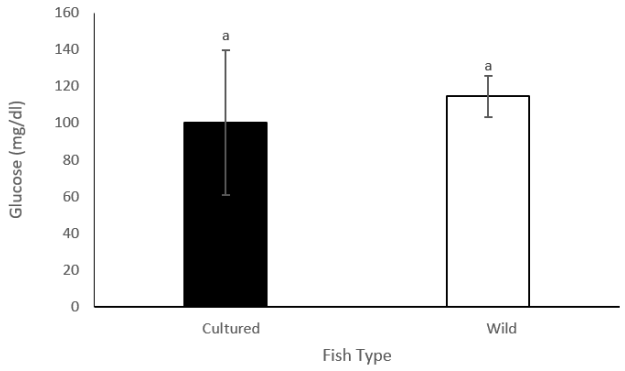

Figure 02: **Semen glucose concentration (n=9, 900±300g mean weight, 49±8.5cm mean total length) of cultured and wild *Clarias gariepinus* in Ilorin metropolis. Bars represented by mean ± SD. Bars with the same alphabets are not significantly different (p>0.05) from each other.**

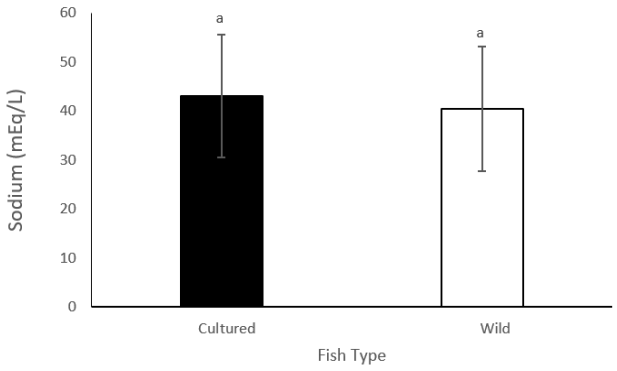

Figure 03: **Semen sodium concentration (n=9, 900±300g mean weight, 49±8.5cm mean total length) of cultured and wild *Clarias gariepinus* in Ilorin metropolis. Bars represented by mean ± SD. Bars with the same alphabets are not significantly different (p>0.05) from each other.**

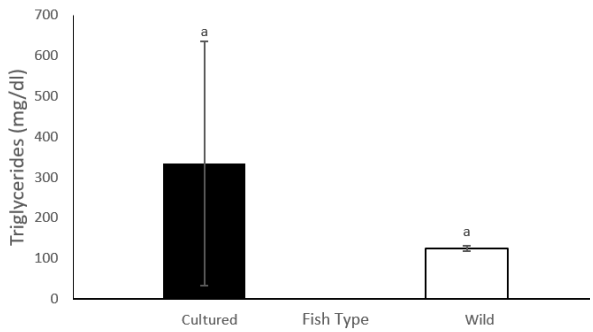

Figure 04: **Semen triglycerides concentration (n=9, 900±300g mean weight, 49±8.5cm mean total length) of cultured and wild *Clarias gariepinus* in Ilorin metropolis. Bars represented by mean ± SD. Bars with the same alphabets are not significantly different (p>0.05) from each other.**

Uric acid is a significant antioxidant found in sperm (Banihani, 2018; Lewis *et al.*, 1997) and it helps to protect fish spermatozoa from oxidative damage while also their viability. The mean uric acid in the wild group (2.22±0.67) was lower than in the cultured group (5.11±0.33), which may be a toxicity marker from exogenous oxidants in the water body, according to the findings (Narayana, 2007; Narayana *et al.*, 2006).

The mean triglycerides of both cultured and wild samples in our study were (333.89±301.50 mg/dl; 124.11±6.15 mg/dl), which were higher than those recorded in *Salmo trutta macrostigma* (6.24±0.08 mg/dl) (Bozkurt *et al.*, 2011) and *Cyprinus carpio* ((10.31±.01 mg/dl) (Ercin *et al.*, 2009). Triglycerides are an energy source for spermatozoa during immotile storage and after motility regeneration ((Lahnsteiner *et al.*, 1993). A low triglyceride level may indicate a lack of energy, a reduced motility rate, and a reduced fertilization capability

Freshwater fish have cholesterol in their seminal plasma (Billard *et al.*, 1995). Cholesterol can protect against environmental changes (particularly temperature changes) that occur when fish semen is released (Bozkurt *et al.*, 2008). . The cholesterol levels in cultured catfish groups were (571.67±190.15 mg/dl) than wild catfish group (206.33±75.5 mg/dl) in this study. Seminal plasma transaminases are assessed as an index of measurement of injury to spermatozoa sustained during various conditions (Hinton *et al.*, 1998; López Rodríguez *et al.*, 2013). AST and ALT play an important role in the defense of spermatozoa from oxidative stress (Hinton *et al.*, 1998; López Rodríguez *et al.*, 2013; Sirat *et al.*, 1996). The AST concentration for the cultured group (307.33±73.71U/l) was higher than that of the wild group (121.89±8.67U/l), which also had a higher content than males of jundia (*Rhamdia quelen*) (247.8±23.4). However, AST value for the cultured and wild African catfish fell within the range established range for jundia (*Rhamdia quelen*) (Borges *et al.*, 2005).

CONCLUSION AND RECOMMENDATION

The capacity to evaluate the reproductive ability of various fish species requires knowledge of the biochemical constituents of seminal plasma. This may also contribute to a deeper understanding of the processes of fertilization. Our findings show that cultured male *Clarias gariepinus* have high-quality sperm as a result of their well-balanced

diet, the composition of certain nutrients in their feed, which helps to improve sperm viability, and the feeding regimen. While wild male *Clarias gariepinus* may be employed in artificial fertilization in commercial aquaculture to reduce inbreeding depression and stunted growth of fingerlings, which are typical issues for catfish producers, and so boost the farm's profitability. The results of this study's chemical composition analysis could lead to more effective gamete management, increased yields, and improved semen suitability for short-term storage. Further research is needed to establish the relationship between the physical and biochemical characteristics of *Clarias gariepinus* semen, both wild and cultured.

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