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Physicochemical Characteristics and Antioxidant Content of Extenders and Their Effects on the Quality of Preserved *Clarias gariepinus* Milt

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ABSTRACT
Efficient preservation of fish sperm is essential for improving hatchery productivity and supporting selective breeding in aquaculture. This study evaluated the physicochemical properties and antioxidant composition of three semen extenders: goat milk, chicken egg yolk, and glycerol, and their effects on the milt quality of Clarias gariepinus. The extenders were assessed for pH, buffering capacity, and antioxidant content (vitamins C and E), and applied at 10%, 20%, and 30% dilution levels for storage at 4°C over 144 h. pH ranged from 6.15 ± 0.08 (egg yolk) to 7.24 ± 0.11 (glycerol), while buffering capacity varied between 0.06 ± 0.01 and 0.10 ± 0.01. Goat milk had the highest vitamin C (3.10 ± 0.18 mg/100 mL), whereas egg yolk contained the highest vitamin E (2.70 ± 0.16 mg/100 mL); glycerol lacked detectable antioxidant vitamins. Sperm quality declined with storage time across treatments. However, goat milk at 30% maintained the highest motility (66.56 ± 7.10%), viability (30.10 ± 2.39%), concentration (66.63 ± 22.26 × 10^6 cells/mL), and normal morphology (72.40 ± 0.86%). Lipid peroxidation ranged from 0.93 ± 0.18 to 1.91 ± 0.36 MDA, with glycerol showing the highest values. These results indicate that natural extenders, particularly goat milk, are more effective in preserving sperm quality during chilled storage and represent practical, eco-friendly alternatives to synthetic extenders for short-term milt preservation.

Keywords: Antioxidant content; fish sperm preservation; semen extenders; sperm quality; *Clarias gariepinus*

INTRODUCTION
African catfish (*Clarias gariepinus*) is one of the most important freshwater aquaculture species in Africa due to its rapid growth rate, high feed conversion efficiency, tolerance to adverse environmental conditions, and strong market demand. These attributes have made the species a major contributor to fish production and food security in many African countries (Musa *et al.,* 2021; Mair *et al.,* 2023). The increasing demand for catfish seed has intensified the need for efficient hatchery technologies that can ensure reliable and sustainable fry production. However, obtaining and managing high quality male gametes for artificial fertilization is often a problem for hatchery operations. In

many hatcheries, milt collection often requires sacrificing male broodstock, which reduces the availability of genetically valuable breeders and increases operational costs (Nascimento-Schulze *et al.,* 2021). In order to increase breeding efficiency and promote sustainable aquaculture production, it is now crucial to develop efficient techniques for preserving fish sperm without continuous sacrifice of broodstock.

A workable method for increasing the milt usability in hatchery operations is the short term preservation of fish sperm through chilled storage. Compared with cryopreservation, chilled storage at 4°C requires relatively simple equipment and minimal technical expertise, making it particularly suitable for routine use in aquaculture facilities, especially in developing regions (Beirão *et al.,* 2019). Despite these advantages, sperm cells are highly sensitive to environmental changes during storage, and refrigeration alone is often insufficient to maintain sperm functionality over extended periods. As a result, semen extenders are commonly used to provide a protective medium that stabilizes sperm cells during storage. These extenders help maintain osmotic balance, regulate pH, supply energy substrates, and reduce metabolic activity, thereby slowing the deterioration of spermatozoa (Bustani *et al.,* 2021) The effectiveness of chilled sperm preservation therefore depends largely on the physicochemical properties and biochemical composition of the extender used

One of the major factors contributing to the decline in sperm quality during storage is oxidative stress, because their plasma membranes contain high levels of polyunsaturated fatty acids, which are highly prone to lipid peroxidation (Wang *et al.,* 2025). During storage, the accumulation of reactive oxygen species (ROS) can damage membrane lipids, proteins, and DNA, resulting in reduced sperm motility, decreased viability, and impaired fertilization capacity (Peña and Gibb, 2022). In addition, oxidative stress can disrupt mitochondrial function and reduce the availability of adenosine triphosphate (ATP), which is essential for sperm motility and flagella movement. The inclusion of antioxidants in semen extenders has been widely investigated as a strategy for improving sperm preservation. Antioxidants neutralize reactive oxygen species and protect cellular components from oxidative damage, thereby helping to maintain the structural and functional integrity of sperm cells during storage (Kowalczyk, 2022). Various synthetic compounds have been used in semen preservation, including glycerol, dimethyl sulfoxide, and other cryoprotective agents that help stabilize cell membranes and prevent intracellular damage during freezing and thawing processes. Although these compounds are effective for cryopreservation, their suitability for short term chilled storage is sometimes limited. In addition, the cost, availability, and handling requirements associated with synthetic chemicals may restrict their application in small scale hatchery operations. (Sharafi *et al.,* 2022). These limitations have encouraged increasing interest in the use of natural biological materials as alternative semen extenders. Natural extenders derived from biological sources such as milk and egg yolk have attracted considerable attention because they are inexpensive, readily available, and environmentally friendly. These materials contain a variety of bioactive components including proteins, phospholipids, sugars, and antioxidant compounds that can contribute to sperm protection. (Bustani and Baiee, 2021)

Another important factor influencing the effectiveness of semen extenders is their dilution ratio; the proportion of extender relative to milt can influence osmotic balance,

antioxidant availability, and sperm metabolic activity. Excessive dilution may reduce the protective effects of seminal plasma and expose sperm cells to unfavorable environmental conditions, whereas insufficient dilution may limit the protective capacity of the extender. (Sharafi *et al.,* 2022) Despite the importance of this factor, the dose dependent effects of extender concentration on the preservation of Clarias gariepinus milt during chilled storage remain insufficiently understood.

Therefore, the present study investigated the physicochemical characteristics and antioxidant composition of three semen extenders goat milk, chicken egg yolk, and glycerol and evaluated their effects on the quality of *Clarias gariepinus* milt during chilled storage. The extenders were characterized for pH, buffering capacity, and antioxidant content, and were applied at dilution levels of 10%, 20%, and 30% for the preservation of milt at 4°C for up to 144 hours. Sperm quality parameters including motility, viability, morphology, concentration, and lipid peroxidation were assessed to determine the effectiveness of these extenders for short term preservation of African catfish milt.

MATERIALS AND METHODS

Study Location and Experimental Fish

The study was conducted at the National Animal Production Research Institute (NAPRI) Fertility Laboratory, Ahmadu Bello University, Zaria, Nigeria. A total of fifteen (15) mature male *Clarias gariepinus* broodstock (1.5-2.5 kg) were procured from a commercial hatchery (Enoch Farms, Zaria). Fish were transported in aerated containers and acclimatized for two weeks in holding tanks under controlled conditions. During acclimatization, fish were fed a commercial diet (42% crude protein) at 5% body weight per day, and water quality parameters were monitored regularly.

Experimental Design and Treatment Structure

The experiment was arranged in a completely randomized design (CRD) with three replicates per treatment. Three extenders (chicken egg yolk, goat milk, and glycerol) were evaluated at three inclusion levels (10%, 20%, and 30%), giving a total of nine treatments. Glycerol served as a positive control, while fresh undiluted milt served as a negative control.

Each treatment replicate consisted of 2 mL of milt-extender mixture stored under chilled conditions. Samples were evaluated at 0, 24, 48, 72, 96, 120, and 144 hours of storage.

Preparation and Composition of Extenders

Fresh chicken egg yolk (Lohmann Brown) and goat milk (West African Dwarf) were obtained from NAPRI, Zaria, while glycerol was sourced from the Chemistry Laboratory, Ahmadu Bello University.

Egg yolk was prepared under sterile conditions by disinfecting eggs with 70% ethanol and separating the yolk (Rakha *et al.,* 2018). Goat milk was centrifuged to obtain the skim fraction for improved homogeneity (Sarlak *et al.,* 2022).

A 2.9% (w/v) trisodium citrate buffer was prepared by dissolving 2.9 g of trisodium citrate in 100 mL distilled water and used as the base diluent.

Extenders were prepared as follows (per 100 mL final volume):

- 10% dilution: 10 mL extender + 90 mL buffer
- 20% dilution: 20 mL extender + 80 mL buffer
- 30% dilution: 30 mL extender + 70 mL buffer

To each 100 mL of extender solution, 1 mL penicillin (1000 IU/mL) and 0.5 mL streptomycin (1 mg/mL) were added to minimize microbial contamination.

Milt Collection, Handling, and Dilution

Mature males were identified based on genital papilla morphology. Fish were immobilized by cold shock (2-4°C water) to reduce handling stress prior to sacrifice. Euthanasia was performed by rapid decapitation, which is an accepted humane method for fish when carried out swiftly, in accordance with institutional animal care guidelines. The testes were dissected aseptically, rinsed in physiological saline (0.9% NaCl), and milt was extracted by gentle maceration and collected into sterile Petri dishes.

Fresh milt was diluted at a 1:10 (v/v) ratio (milt:extender) and gently homogenized. Aliquots (2 mL) were dispensed into labeled tubes and stored at 4°C for up to 144 hours.

Physicochemical and Antioxidant Analyses

pH Measurement

pH was measured using a digital pH meter (Metrohm, Switzerland) calibrated with standard buffers (pH 4.0, 7.0, and 10.0). Measurements were taken in triplicate at room temperature, and mean values recorded.

Buffering Capacity

Buffering capacity was determined using the method described by Kamaruddin *et al.* (2018) by titration with 0.01 M HCl until a 0.1 unit decrease in pH was achieved. It was calculated as:

$$Buffering\ capacity = \frac{VHcl \times M \times 1000}{VSample}$$

Where V_{Hcl} = volume of acid (mL)

M = molarity of Hcl

V_{sample} = sample volume (mL)

Vitamin C Determination

Vitamin C was analyzed using HPLC (Agilent Technologies) with a C18 column (150 × 4.6 mm, 5 µm). The mobile phase consisted of 0.1 M KH_2PO_4 (pH 2.5) at a flow rate of 1.0 mL/min. Detection was at 254 nm. Calibration curves were prepared using standard ascorbic acid (1-100 mg/L; $r^2 \geq 0.995$) (Kivrak *et al.,* 2019). Results were expressed as mg/100 mL.

Vitamin E Determination

Vitamin E (α-tocopherol) was quantified after saponification and hexane extraction. Analysis was performed using HPLC with fluorescence detection (excitation: 295 nm; emission: 330 nm). Quantification was based on external calibration standards (0.1-10 mg/L).

Sperm Quality Assessment

Motility

Sperm motility was assessed microscopically after activation with distilled water and expressed as percentage motile cells (Egbunike, 1994):

$$\% \, Motility = \frac{Motile \; Sperm}{Total \; Sperm} \times 100$$

Viability

Viability was determined using 0.2% eosin staining. Live sperm remained unstained, while dead sperm stained pink. At least 200 cells were counted per sample and expressed as percentage (Maulana, and Junior, 2014).

Concentration

Sperm concentration was determined using a hemocytometer and expressed as $\times 10^6$ cells/mL.

Morphology

Morphological abnormalities were assessed using Eosin-Nigrosin staining and expressed as percentage normal sperm (Blawut *et al.,* 2020).

Lipid Peroxidation (MDA)

MDA levels were determined using the thiobarbituric acid (TBA) assay. Absorbance was measured at 532 nm using a UV-Vis spectrophotometer (Jenway 6305).
MDA concentration was calculated using:

$$MDA = \frac{A532}{\in \times l}$$

Where:
$\varepsilon = 1.56 \times 10^5$ M-1 cm-1
l =1cm

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) in SPSS (version 26). Where significant differences were observed ($p < 0.05$), means were separated using Tukey's Honestly Significant Difference (HSD) post hoc test. Results were expressed as mean ± standard error.

Ethical Considerations

All procedures involving animals were conducted in accordance with the guidelines of the Animal Care and Use Committee of Ahmadu Bello University, Zaria. Fish were handled humanely, and euthanasia by rapid decapitation following cold immobilization was applied to minimize pain and distress.

RESULTS

Physicochemical Characteristics of *Clarias gariepinus* Milt Extenders

The physicochemical properties of the milt extenders differed significantly among treatments ($p < 0.05$). The pH values varied across extenders as shown in figure 1, with the glycerol-based extender recording the highest pH (7.24 ± 0.11), followed by goat milk (6.63 ± 0.58), while chicken egg yolk exhibited the lowest pH (6.15 ± 0.08). These differences were statistically significant ($p < 0.05$).

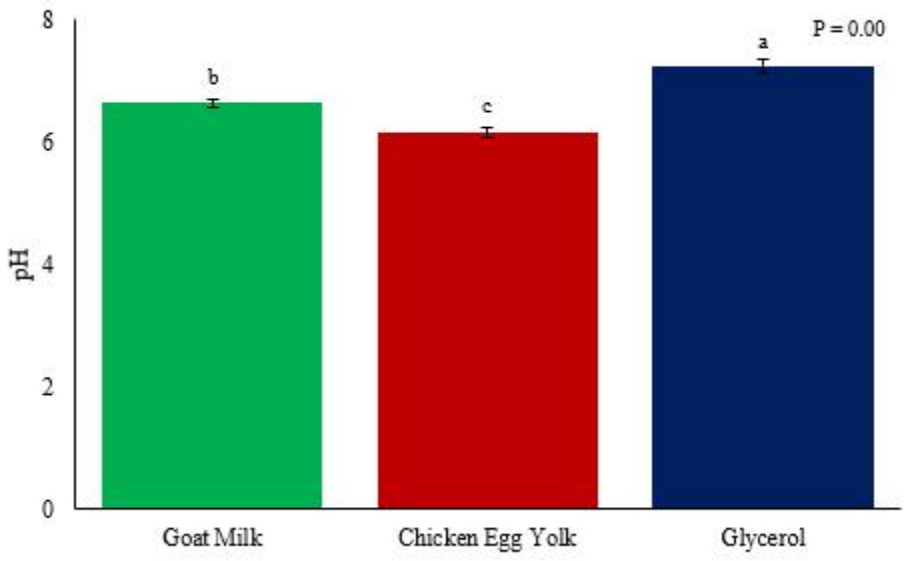

Figure 1: pH of semen extenders used for *Clarias gariepinus* milt preservation

Buffering capacity showed no significant difference among treatments ($p > 0.05$) as shown in figure 2. However, glycerol recorded the highest numerical value (0.10 ± 0.01 mM HCl/Δ0.1 pH), followed by chicken egg yolk (0.08 ± 0.02 mM HCl/Δ0.1 pH), while goat milk had the lowest buffering capacity (0.06 ± 0.01 mM HCl/Δ0.1 pH).

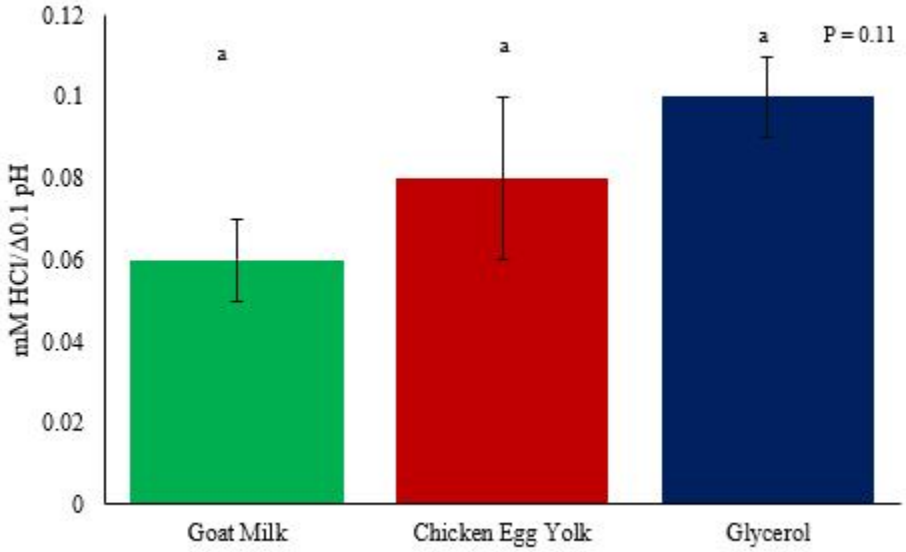

Figure 2: Buffering Capacity of semen extenders used for *Clarias gariepinus* milt preservation

The antioxidant composition of the extenders differed significantly as shown in figure 3. Goat milk recorded the highest vitamin C concentration (3.10 ± 0.18 mg/100 mL), which was significantly higher than chicken egg yolk (0.05 ± 0.00 mg/100 mL) and glycerol (0.00 ± 0.00 mg/100 mL) ($p < 0.05$). Post hoc analysis indicated that goat milk differed significantly from both egg yolk and glycerol, while the latter two were not significantly different from each other. Conversely, vitamin E concentration was highest in chicken egg yolk (2.70 ± 0.16 mg/100 mL), which was significantly greater than goat milk (0.14 ± 0.01 mg/100 mL) and glycerol (0.00 ± 0.00 mg/100 mL) ($p < 0.05$). Post hoc comparisons showed that egg yolk differed significantly from both goat milk and glycerol, while no significant difference existed between goat milk and glycerol.

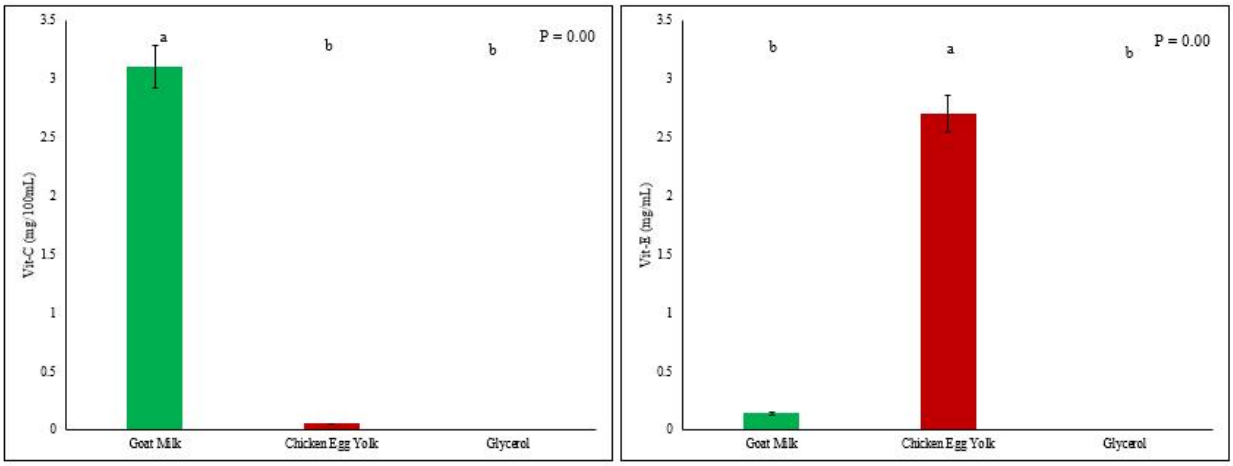

Figure 3: Antioxidant content of semen extenders used for *Clarias gariepinus* milt preservation

Quality of Preserved Clarias gariepinus

Sperm motility varied with extender type and dilution level as shown in figure 4A. At 10% dilution, no significant differences were observed among goat milk (58.61 ± 8.87%), chicken egg yolk (38.59 ± 12.03%), and glycerol (61.04 ± 8.64%) ($p > 0.05$). A similar pattern was observed at 20% dilution, where goat milk (61.23 ± 7.69%), egg yolk (51.96 ± 9.34%), and glycerol (35.07 ± 9.33%) did not differ significantly ($p > 0.05$). However, at 30% dilution, significant differences were recorded ($p = 0.05$), with goat milk showing the highest motility (66.56 ± 7.10%), followed by egg yolk (48.24 ± 10.41%), while glycerol recorded the lowest value (26.94 ± 13.09%). As shown in figure 4B, across storage duration (0-144 h), motility declined progressively in all treatments. Fresh milt recorded the highest motility at 0 h (98.44 ± 0.69%), which decreased to 0% by 72 h. Goat milk maintained higher motility throughout storage, decreasing from 87.43 ± 0.70% at 0 h to 31.10 ± 3.64% at 144 h. Egg yolk declined from 83.10 ± 0.76% to 8.23 ± 4.39%, while glycerol decreased from 85.00 ± 4.51% to 9.23 ± 5.23%. Differences among treatments were significant at all time points ($p < 0.05$).

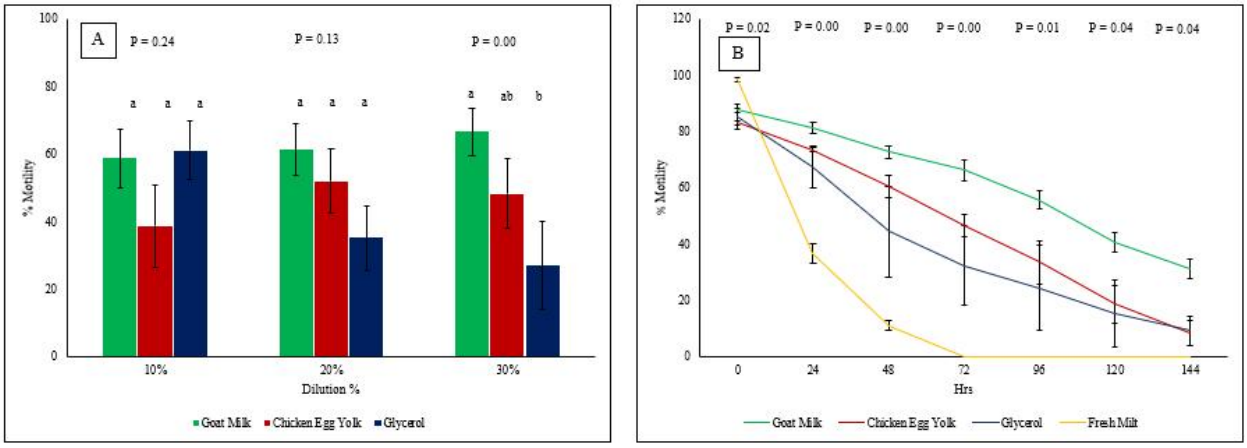

Figure 4: A - Effect of extender type and dilution level on sperm motility of *Clarias gariepinus* milt. **B** - Changes in sperm motility of *Clarias gariepinus* milt during preservation

Sperm viability followed a similar trend to motility. As shown in figure 5A, at 10% and 20% dilutions, no significant differences were observed among extenders ($p > 0.05$). At 30% dilution, goat milk recorded the highest viability (66.56 ± 7.10%) and glycerol the lowest (26.94 ± 13.09%), although these differences were not statistically significant ($p > 0.05$). Over storage time as shown in figure 5B, viability declined significantly across all treatments ($p < 0.05$). Fresh milt decreased from 98.63 ± 0.57% at 0 h to 0% by 72 h. Goat milk maintained higher viability, decreasing from 89.60 ± 0.47% to 30.10 ± 2.39% at 144 h. Egg yolk and glycerol showed sharper declines to 9.47 ± 3.27% and 9.83 ± 6.83%, respectively.

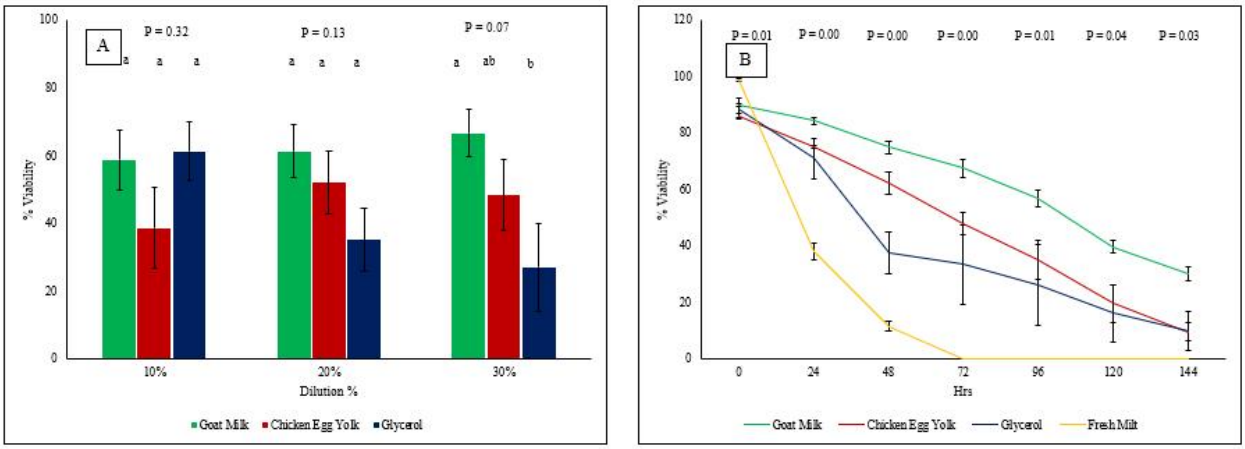

Figure 5: A - Effect of extender type and dilution level on sperm viability of *Clarias gariepinus* milt. **B** - Changes in sperm viability of *Clarias gariepinus* milt during preservation

Sperm concentration did not differ significantly among extenders at all dilution levels as shown in figure 6A ($p > 0.05$). At 10% dilution, values ranged from 126.53 ± 50.70 $\times 10^6$ cells mL^{-1} (egg yolk) to 209.57 ± 39.80 $\times 10^6$ cells mL^{-1} (glycerol), with goat milk

recording 193.06 ± 48.80 ×10⁶ cells mL⁻ ¹. Similar non-significant patterns were observed at 20% and 30% dilutions. During storage as shown in figure 6B, sperm concentration declined significantly across all treatments ($p < 0.05$). Fresh milt recorded the highest concentration at 0 h (425.62 ± 7.35 ×10⁶ cells mL⁻ ¹), dropping to zero by 72 h. Goat milk maintained higher concentrations over time (345.33 ± 1.86 to 66.63 ± 22.26 ×10⁶ cells mL⁻ ¹), compared to egg yolk (347.77 ± 1.35 to 10.23 ± 5.53 ×10⁶ cells mL⁻ ¹) and glycerol (349.87 ± 1.44 to 24.33 ± 20.33 ×10⁶ cells mL⁻ ¹). Differences were significant up to 96 h ($p < 0.05$) but not at 120 h and 144 h ($p > 0.05$).

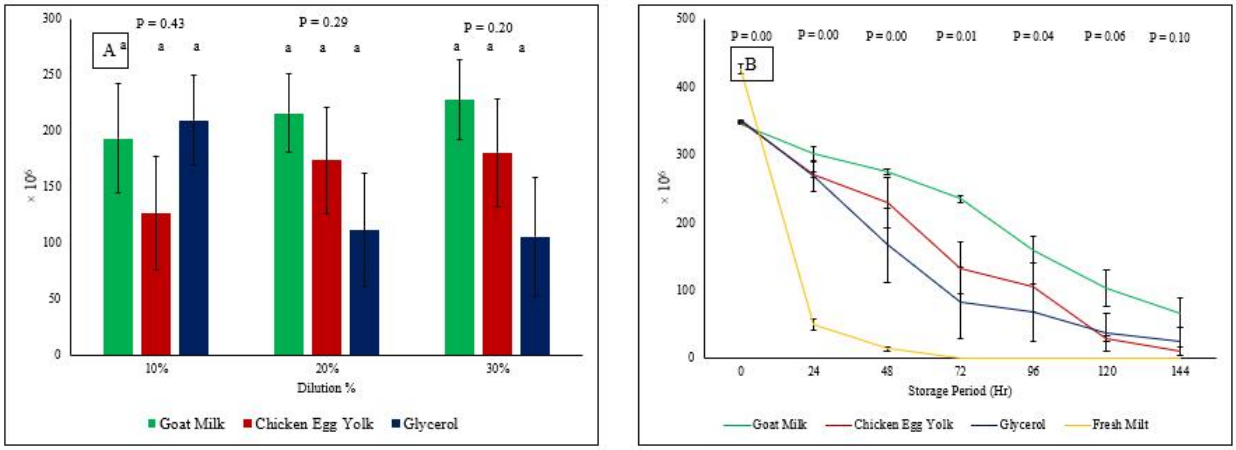

Figure 6: A - Effect of extender type and dilution level on sperm concentration of *Clarias gariepinus* milt. **B** - Changes in sperm concentration of *Clarias gariepinus* milt during preservation

As shown in figure 7A, at 10% dilution, no significant differences were observed in the percentage of normal spermatozoa among goat milk (82.97 ± 2.67%), egg yolk (75.27 ± 5.78%), and glycerol (81.89 ± 3.75%) ($p > 0.05$). At 20% and 30% dilutions, significant differences were observed ($p < 0.05$), with goat milk (84.04 ± 2.69%; 85.47 ± 2.72%) and egg yolk (80.67 ± 2.79%; 81.61 ± 2.68%) showing higher normal morphology than glycerol (56.75 ± 10.42%; 63.37 ± 4.97%). Across storage time as shown in figure 6B, morphology declined in all treatments. Fresh milt decreased from 98.20 ± 0.42% at 0 h to 0% by 72 h. Goat milk maintained higher values (92.28 ± 0.72% to 72.40 ± 0.86%), followed by egg yolk (88.99 ± 0.53% to 61.24 ± 8.67%) and glycerol (84.55 ± 4.40% to 51.26 ± 7.67%). Differences among treatments were significant from 24 h onward ($p < 0.05$).

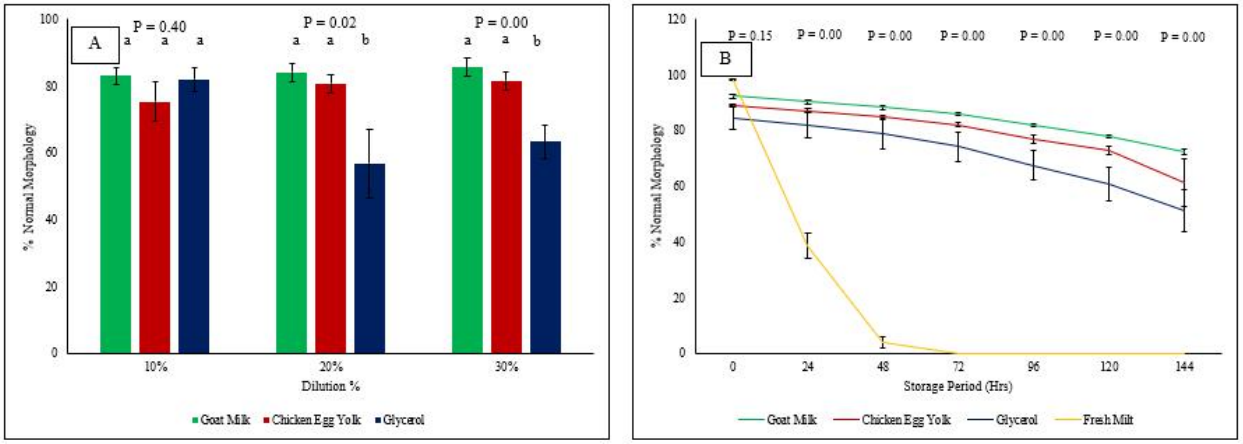

Figure 7: A - Effect of extender type and dilution level on normal sperm morphology of *Clarias gariepinus* milt. **B** - Changes in normal sperm morphology of *Clarias gariepinus* milt during preservation

MDA levels differed significantly among extenders at all dilution levels ($p < 0.05$) as shown in figure 8A. At 10% dilution, glycerol recorded the highest MDA (1.91 ± 0.36), followed by goat milk (1.49 ± 0.30), while egg yolk had the lowest (1.17 ± 0.23). Similar patterns were observed at 20% and 30% dilutions, with glycerol consistently recording higher values and egg yolk the lowest. MDA levels increased significantly with storage duration ($p < 0.05$) as shown in figure 8B. At 0 h, fresh milt recorded the highest value (6.06 ± 0.23), increasing to 9.01 ± 0.49 at 144 h. Among extenders, glycerol consistently showed higher MDA levels (1.18 ± 0.09 to 2.32 ± 0.15), followed by goat milk (0.90 ± 0.07 to 1.79 ± 0.13), while egg yolk recorded the lowest values (0.70 ± 0.05 to 1.42 ± 0.09).

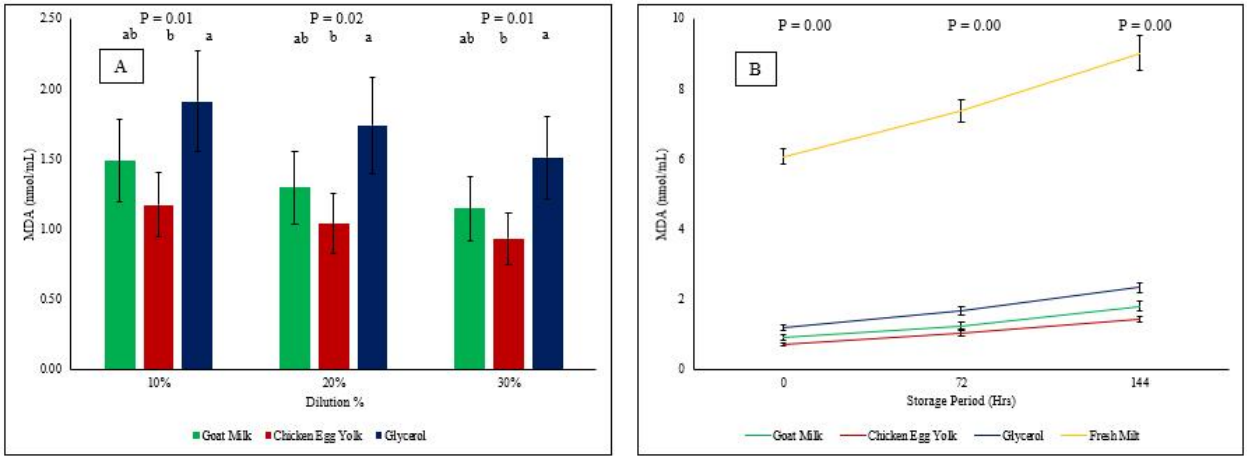

Figure 8: A - Effect of extender type and dilution level on lipid peroxidation (MDA) levels of *Clarias gariepinus* milt. **B** - Changes in sperm lipid peroxidation (MDA) levels of *Clarias gariepinus* milt during preservation

DISCUSSION

The observed differences in pH among the extenders reflect their intrinsic chemical composition and have important implications for sperm preservation. The near-neutral pH recorded for the glycerol-based extender is consistent with the chemical nature of glycerol as a relatively inert polyol that does not readily dissociate to release hydrogen ions. Such conditions are widely considered optimal for fish sperm preservation, as near-neutral pH supports membrane stability, enzyme activity, and sustained motility during storage (Bustani and Baiee, 2021; Bibi *et al.,* 2021; Kumar *et al.,* 2024). In contrast, the moderately acidic pH observed in goat milk can be attributed to the presence of lactic acid and associated milk metabolites, while the lower pH in chicken egg yolk is linked to phospholipids and protein complexes that contribute to proton release in solution (Thiangthientham *et al.,* 2024; Vodounnou *et al.,* 2025). Although mild acidity may reduce metabolic activity and prolong sperm lifespan, excessive deviation from neutrality has been associated with reduced motility and compromised membrane integrity in fish spermatozoa (Liu *et al.,* 2016).

Despite these differences in pH, buffering capacity did not differ significantly among the extenders, indicating that all treatments possessed a comparable ability to resist short-term changes in hydrogen ion concentration. This suggests that buffering alone may not be the primary determinant of extender performance under chilled storage conditions. The slightly higher buffering capacity observed in glycerol may be linked to its chemical stability, while egg yolk proteins and phospholipids likely contribute to moderate buffering through weak acid-base interactions (Bustani and Baiee, 2021; Neila-Montero *et al.,* 2024). The comparatively lower buffering capacity of goat milk aligns with its dependence on organic acids and protein systems for pH regulation. Similar findings have been reported in semen preservation studies where natural extenders exhibit sufficient buffering for short-term stability but require additional buffering systems for prolonged storage (Trentin *et al.,* 2018). This reinforces the notion that initial pH conditions and biochemical composition may exert a stronger influence on sperm quality than buffering capacity alone.

The antioxidant profiles of the extenders revealed pronounced differences that are biologically relevant to sperm preservation. Goat milk exhibited a significantly higher concentration of vitamin C, a water-soluble antioxidant known for its role in scavenging reactive oxygen species (ROS) in the extracellular environment and regenerating oxidized vitamin E (Bustani & Baiee, 2021; Ros-Santaella & Pintus, 2021). This suggests that goat milk may provide substantial protection against oxidative stress during chilled storage, thereby helping to maintain sperm viability. In contrast, chicken egg yolk was characterized by a significantly higher vitamin E content, reflecting its lipid-rich composition. Vitamin E functions as a membrane-bound antioxidant that prevents lipid peroxidation, thereby preserving the structural integrity of sperm cell membranes and enhancing fertilization potential (Asadpour *et al.,* 2011; Silvestre *et al.,* 2021). This aligns with extensive evidence supporting the effectiveness of egg yolk-based extenders in maintaining sperm quality across fish and livestock species (Michael *et al.,* 2009; Pinto *et al.,* 2020; Farid *et al.,* 2021).

Unlike the natural extenders, glycerol lacked measurable levels of both vitamin C and vitamin E, highlighting its primary role as a physical cryoprotectant rather than a

biochemical antioxidant. Glycerol functions mainly by regulating osmotic balance and reducing cold-induced cellular damage during storage, but it does not directly neutralize ROS (Silvestre *et al.,* 2021). Consequently, sperm preserved in glycerol-based systems may rely more heavily on endogenous antioxidant defenses or require supplementation with exogenous antioxidants to mitigate oxidative stress.

Sperm motility, viability, concentration, morphology, and lipid peroxidation are critical indicators of milt quality because they directly determine fertilization success in externally fertilizing fish such as *Clarias gariepinus* (Rurangwa *et al.,* 2011; Ros-Santaella and Pintus, 2021). The present study demonstrates that extender type, dilution level, and storage duration interactively influenced these parameters, with clearer differentiation among extenders emerging under higher dilution and prolonged storage conditions.

At lower dilution levels (10-20%), motility and viability did not differ significantly among extenders ($p > 0.05$), indicating that basic physicochemical protection such as osmotic balance and buffering was sufficient to sustain short-term sperm function. This is consistent with the role of residual seminal plasma, which can temporarily buffer oxidative stress and stabilize sperm membranes, thereby masking differences among extenders at early stages (Adeyemo *et al.,* 2009; Ros-Santaella & Pintus, 2021). However, at 30% dilution, where seminal plasma influence is substantially reduced, extender composition became critical. Goat milk maintained significantly higher motility (66.56 ± 7.10%) compared to glycerol (26.94 ± 13.09%) ($p = 0.05$), with egg yolk showing intermediate performance. This highlights the increasing importance of exogenous antioxidant and biochemical support at higher dilution levels.

The superior performance of goat milk in preserving motility and viability throughout storage (motility: 87.43% at 0 h to 31.10% at 144 h; viability: 89.60% to 30.10%) can be attributed to its antioxidant composition, particularly vitamin C. As a water-soluble antioxidant, vitamin C scavenges reactive oxygen species (ROS) in the extracellular environment and protects axonemal structures and ATP-generating pathways required for flagellar movement (Ros-Santaella and Pintus, 2021; Lin *et al.,* 2023). In contrast, glycerol, despite its cryoprotective properties, lacks intrinsic antioxidants and therefore showed rapid declines in both motility (to 9.23% at 144 h) and viability (to 9.83% at 144 h), likely due to cumulative oxidative and osmotic stress. Chicken egg yolk, although rich in lipid-soluble antioxidants such as vitamin E, showed intermediate performance, suggesting that membrane-level protection alone is insufficient without complementary aqueous-phase antioxidant activity.

A progressive decline in motility and viability across all treatments over 144 hours reflects the cumulative effects of metabolic exhaustion, oxidative damage, and membrane destabilization inherent to chilled storage. Notably, fresh milt, despite its high initial motility (98.44 ± 0.69%) and viability (98.63 ± 0.57%), completely lost functionality by 72 hours, reinforcing the necessity of extenders for short-term preservation (Bugau *et al.,* 2022). The parallel trends observed between motility and viability further confirm that loss of motility was primarily driven by irreversible membrane damage rather than temporary inhibition of sperm activity.

Sperm concentration was largely determined by initial dilution, with no significant differences among extenders across dilution levels ($p > 0.05$). However, during storage, goat milk maintained higher residual sperm counts (345.33 $\times 10^6$ to 66.63 $\times 10^6$ cells mL^{-1}), compared to egg yolk (10.23 $\times 10^6$ cells mL^{-1}) and glycerol (24.33 $\times 10^6$ cells mL^{-1} at 144 h). This suggests that antioxidant-mediated membrane stabilization in goat milk reduced sperm cell lysis and structural degradation over time. The rapid decline to zero concentration in fresh milt by 72 hours further emphasizes the vulnerability of unprotected sperm to chilling-induced damage.

Morphological integrity followed a similar pattern, with significant differences emerging at higher dilutions (20-30%) ($p < 0.05$). Goat milk (84-85%) and egg yolk (80-81%) maintained significantly higher proportions of normal sperm compared to glycerol (56-63%). These findings indicate that biologically derived extenders provide superior structural protection, likely through combined antioxidant activity and nutrient support that stabilize membranes and cytoskeletal components. Over time, morphology declined across all treatments, but goat milk consistently preserved structural integrity better than other extenders, highlighting its protective role against oxidative and osmotic stress.

Lipid peroxidation results, measured as malondialdehyde (MDA), provide direct evidence of oxidative stress as a key mechanism of sperm deterioration. Glycerol consistently recorded the highest MDA levels across dilution levels and storage durations (e.g., 2.32 ± 0.15 at 144 h), confirming its inability to counteract ROS. In contrast, egg yolk exhibited the lowest MDA levels (1.42 ± 0.09 at 144 h), reflecting the protective role of vitamin E in preventing membrane lipid oxidation. Goat milk showed intermediate MDA values, indicating partial protection through vitamin C and other bioactive components. The observed decrease in MDA with increasing dilution likely reflects reduced sperm density and lower total lipid substrate available for peroxidation (Larsson *et al.,* 2016; Shaliutina-Kolešová & Nian, 2022). However, MDA levels increased significantly over time in all treatments, demonstrating the cumulative nature of oxidative damage during storage.

CONCLUSION

This study demonstrates that semen extender type significantly influences the short-term preservation of *Clarias gariepinus* milt under chilled conditions. Among the extenders evaluated, goat milk consistently maintained higher sperm motility, viability, and structural integrity over the storage period, while egg yolk showed moderate effectiveness and glycerol was comparatively less effective. These differences are likely associated with variations in physicochemical properties and antioxidant composition.

The findings indicate that extenders with balanced pH and antioxidant capacity can improve the preservation of sperm quality during chilled storage. In practical terms, goat milk represents a viable and accessible alternative for hatchery operations, particularly in resource-limited settings.

Further research is recommended to optimize extender formulations and evaluate their effects on fertilization and hatchability under field conditions.

CONFLICTS OF INTREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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