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Research Article

Effect of different milt extenders on sperm parameters, fertilization rate, embryo quality, and fry production traits in the African catfish (*Clarias gariepinus*)

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Abstract

The fertility indices of the African catfish (*Clarias gariepinus*) milt diluted in different extenders were investigated using four adult males and two adult females. There were four groups with three replicates each, comprising an undiluted fresh milt group (FM; Control) and three groups of different milt extenders: soya beans milk (SM), citrated egg yolk (CEY), and citrated water (CW). Each extended milt group was diluted at a 1:10 ratio and stored at 4 °C for 48 h prior to spawning. After milt extension, the milt pH, sperm motility and viability, and sperm concentration were determined daily for 7 days. The egg fertilization and hatchability rates, fry survival rate, embryo viability rate (at 8 h and 24 h), and fry gross biometry (13 days post-spawning) were evaluated. The results showed no significant difference ($p > 0.05$) in the diluted milt pH of the different groups. The sperm viability, motility, and concentration significantly decreased ($p<0.05$) in all groups, although the CEY group had the highest values ($p<0.05$) compared to SM and CW treatments. No fertilization was observed in SM treatment. It was concluded that as a milt extender for the African catfish, chilled CW was similar to CEY with respect to fertilization, hatchability, and fry survival rates, while a higher fry body weight was obtained in CW compared to CEY. On the other hand, the chilled SM extender stored for 48 h was not suitable for producing fry due to unsuccessful fertilization.

Keywords: African sharptooth catfish, Fertilization, Hatchability, Fry Characteristics, Milt Extenders

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Introduction

The African Catfish (*Clarias gariepinus*) is one of the most appreciated fish species cultured in the tropical and subtropical regions (Al-Khalaifah *et al*., 2020). The inadequate supply of good-quality fish eggs is an important factor limiting aquaculture development in many developing countries, including Nigeria. The stocking of good quality fingerling fish and the provision of optimum conditions that enhance rapid growth and early harvest are crucial to the success of aquaculture (Ogunremi *et al.,* 2022). Consequently, there is a need to improve the quality and quantity of fish egg production. Attempts are being made to achieve this through the use of extenders to dilute the usually-viscous milt, with the ultimate goal of increasing seminal fluid volume and thereby reducing the cost of production.

In the artificial propagation of the African catfish, milt quality is among the principal factors that determine the production of viable larvae (Kowalski and Cejko, 2019). Milt quality is a measure of the ability of sperm to successfully fertilize an egg, and this is influenced by the qualitative parameters of the milt such as the composition of seminal fluid, milt volume, sperm density, and sperm motility (Ogidan *et al*., 2018). Artificial propagation involves a complex reproductive process and is typically not 100% successful. Hence, it is important to estimate the percentage of fertilized eggs during the process of artificial spawning (Okomoda *et al.,* 2018). The most widely used method to estimate fertilization rate involves monitoring a small portion of the unfertilized eggs from the time of stripping until the eggs become completely opaque (dead), and by evaluating the percentage of good (fertilized) eggs and bad (opaque/unfertilized) eggs (Ataguba *et al.,* 2013). The synchronous reproductive process involves the hatching of fertilized eggs into hatchlings. The time it takes for catfish eggs to hatch depends on the water temperature. African catfish typically spawn when the water temperature is between 21–29°C. The hatchability rate is determined after 24 h by counting the larvae hatched from the incubated eggs.

In modern practice, saline water and other chemical solutions are commonly used as extenders in the artificial breeding and cryopreservation programs of fish milt. The use of conventional extenders presents some challenges (Muchlisin, 2009). According to Bustani and Baiee (2021), egg yolk can be an effective agent in milt extenders for the protection of spermatozoa against cold shock and lipid-phase transition effect. However, the use of chilled-stored milt diluted in egg yolk-based milt extenders is limited by its relatively short-time fertilization capacity (Aurich *et al*., 1997). The use of a soybean milk extender reduced the fertility of *Clarobranchus* (African giant catfish) milt after 6 h of extension (Onyia *et al.,* 2017). Hence, to maintain the milt for a longer period, it is important to find the best milt extenders to increase fry productivity in aquaculture sectors. The

aim of this study was to compare the characteristic performance of different catfish milt diluents with respect to milt quality, fertilization rate, hatchability rate, embryo quality, fry survival rate, and fry quality post-hatch.

Materials and methods

Study area

The study was carried out in a standard fish hatchery (Maudy Fish Farms, Arab Road, Kubwa, Abuja, Nigeria). Geographically, it is located on latitude 9°10′9.234″ N and longitude 7°19′2.772″ E within the North Central part of Nigeria (NPC, 2006).

Experimental design

The study involved four groups (with three replicates each) comprising an undiluted fresh milt group (FM; Control) and three groups of different milt extenders: soya beans milk (SM), citrated egg yolk (CEY), and citrated water (CW). The extended groups were all diluted at a ratio of 1:10, placed into 10 sterile containers (1 ml each), and stored at 5 °C. The extended milt samples were used for spawning after 48 h of storage and were evaluated daily for milt pH, percentage sperm motility and viability, and sperm concentration for 7 days. The FM group was a fresh milt sample used for fertilization on spawning day, as a positive control. After spawning, the groups were evaluated for fertilization rate, embryo quality (at 8 h and 24 h of bench incubation), hatchability rate (after 24 h of incubation), fry survival rate (at 13 days of age under hatchery pond condition) and fry biometry (at 13 days of age). Baseline and post-hatch water quality parameters were also evaluated. The different milt extenders were prepared according to well-established methods (Chanda and Ramachandra, 2019).

Experimental animals

The animal experiments were performed in accordance with the principles of laboratory animal care and welfare (CACC, 1993). The study was approved by the University of Abuja Ethical Committee on Animal Use (No.: UAECAU 2022/0010). Four male catfish (eighteen months old), weighing 2.01±0.14 (kg) and measuring 57.2 ±0.8 (cm) in length were used for milt collection. The milt from three males was used for dilution with the extenders while the fourth male catfish provided fresh milt was used as the control group (D group). Two female catfish (eighteen months old), weighing 1.82±0.03 (kg) and measuring 27.5±0.4 (cm) in length were used for spawning and egg stripping, to collect the eggs used for fertilization. The brood stocks were housed in a concrete pond and acclimatized for two weeks during which feed (Blue crown® 9mm; OLAMS, Nigeria) was provided once a day.

Milt collection, preparation, and dilution

Milt (semen) was collected by sacrificing the brood stocks. The milt sacs were exteriorized and washed with normal saline. The sacs were dried with

filter paper. Milt was extracted into three vials of 5 ml sample bottles and was maintained at room temperature. The color of the pooled milt was noted and the volume was measured and recorded. For microscopic examination, a drop of freshly-pooled milt was placed on a pre-warmed glass slide and mixed with a drop of water to activate motility. The sample was viewed under the microscope at ×100 magnification to evaluate individual sperm motility. Sperm concentration was determined using a hemocytometer at ×400 magnification. A drop of freshly-pooled milt was placed on a glass slide and mixed with a drop of eosin-nigrosin stain. A thin smear was made on the glass slide for the evaluation of sperm viability. The milt pH was determined using a benchtop digital pH meter (Apera PH700; Apera Instruments LLC, Columbus, Ohio, USA).

The fresh milt (1 ml) was diluted by mixing with 9 ml of the extenders (SM, CEY, or CW), respectively. Diluted milt (1 ml each) was distributed into nine sterile 5 ml sample bottles and stored at 5°C. This was carried out for the three replicates. For post-dilution examination, extended milts in the SM, CEY, and CW groups were evaluated starting from day 0 to day 7, post-dilution. The parameters evaluated were milt pH, percentage sperm motility and viability, and sperm concentration, according to the methods already described (Akanmu *et al*., 2019). Fresh milt used in the control (FM group) was also subjected to macroscopic and microscopic examination before being used in spawning.

Spawning, fertilization, and incubation of eggs

Spawning was carried out at 48 h post-dilution with the chilled extended milt or fresh milt (control), as indicated. A female catfish was injected with 0.5 ml/kg gonadotropin analogue (Ovuline; Ningbo Farmland Training Co. Ltd., China) for ovulation induction 12 h prior to spawning. The eggs were stripped after 12 h when they were ready for spawning.

Egg fertilization and incubation were carried out by placing 7 g of stripped eggs (estimated to be 3300 eggs) in a bowl, for each replicate in each group. The eggs were mixed with 0.5 ml of normal saline. The extended milt (1 ml) was added and mixed thoroughly with the eggs. A drop of groundwater was added to the mixture for activation. A total of 200 eggs (in 2 ml of the activated egg mixture) were taken and dispensed into a petri dish for the determination of fertilization rate. The observations were made between 60 and 120 min of activation, and the hatchability rate was determined after 24 h of incubation at a temperature of 35°C. Eggs were placed on a substrate with an opening of 1 mm^2, and incubated in hatching tanks of 300 l water capacity for total fry recovery. One hundred (100) fertilized eggs were also incubated in petri dishes in a mini-incubation trough of 500 ml water capacity, for the estimation of hatchability rate. The incubation water was first sterilized with 0.1% formalin to

prevent fungal and microbial growth, and incubation was done in a dark environment (Fig. 1).

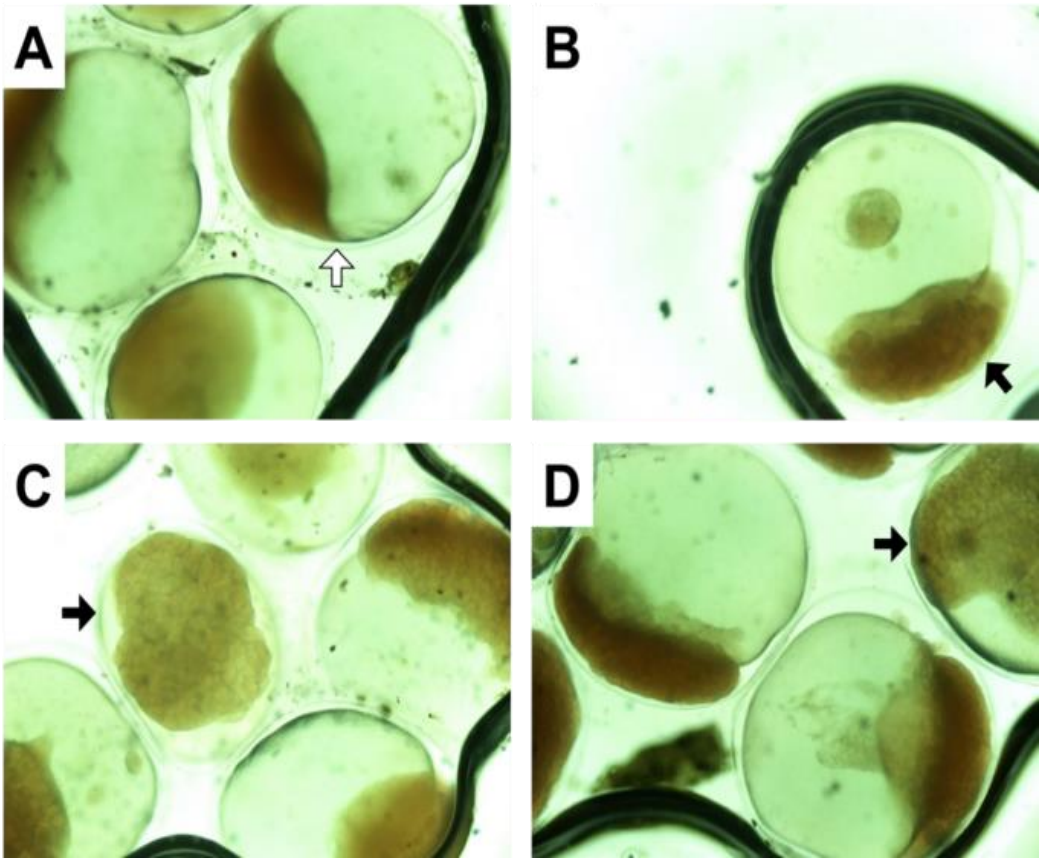

Figure 1: Micrographs of African catfish (*Clarias gariepinus*) eggs after 60 min of incubation in different milt extenders. A: white arrow shows no evidence of fertilization and absence of embryonic cells in the soya milk (SM) group. B, C and D: black arrows show evidence of fertilization and presence of embryonic cells in the citrated egg yolk (CEY), citrated water (CW) and fresh milt (FM) groups, respectively. (×40 magnification).

Estimation of fertilization rate, hatchability rate, fry survival rate, and embryo quality

The fertilization rate was estimated by mounting a representative sample of the activated mixture of the eggs and semen (roes and milt) on a glass slide, evaluating microscopically at × 4 magnification. A total of 100 eggs were counted for each slide. The number of the dividing embryos was determined with the aid of a computer screen using the modified method of Okomoda *et al.* (2018). Micrographs were obtained using a Moticam 2.0 camera (Motic, Carlsbad, CA, USA). The number of fertilized eggs observed was expressed as a percentage of the total number of eggs counted on the slide. The hatchability rate (Fig. 2) was estimated according to the modified method of Arun and Shigeharu (2004). The hatchability rate was expressed as a percentage of the total number of fertilized eggs in the petri dish. The fry survival rate was estimated by counting

the total number of live fry in the main incubation tanks after 13 days, and the value was then divided by the total number of hatched larvae and multiplied by 100. Estimation of embryo quality was carried out with the remaining activated gametes in the petri dish (following the determination of the fertilization rate). These were incubated on a bench at 28°C and observations were made grossly with the naked eyes to subjectively assess the quality of the developing embryos (identification of those that would turn white). Evaluations were made at 8 and 24 h of incubation, and photographs were taken using a digital camera.

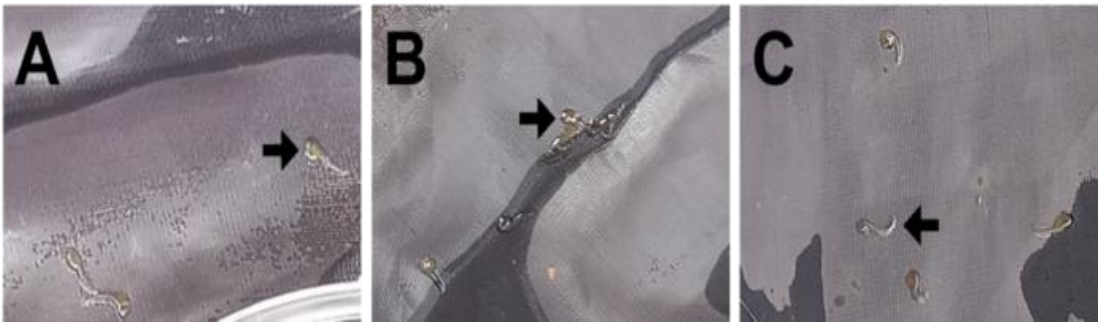

Figure 2: Photographs of African catfish (*Clarias gariepinus*) hatched fry after 24 h of incubation in different milt extenders. A, B and C: black arrows show hatched fry in the citrated egg yolk (CEY), citrated water (CW) and fresh milt (FM) groups, respectively.

Post-hatch gross biometry of fry on day 13

Fry body weight, body length, and head width were measured by randomly selecting 20 fry from each replicate. Fry body weight was measured with an Ohaus digital scale (Pine Brook, NJ, USA), while body length and head width were measured with a tape rule, respectively.

Statistical analysis

Data were analyzed using SPSS, version 26 (IBM Corp, Armonk, NY, USA). Post-dilution milt and sperm parameters, and post-hatch and larva rearing water parameters were analysed using mixed ANOVA. Fry body measurement data were analyzed by One-way ANOVA. Means with significant differences were confirmed using Tukey's HSD posthoc multiple comparison. Values were expressed as mean ± standard deviation (mean±SD). Fertilization, hatchability, survival rate, and embryo quality parameters were analyzed using Kruskal-Wallis test and Dunn's multiple comparisons test, with *P* values adjusted for multiple comparisons. Values were expressed as median (interquartile range) i.e. median (Q1, Q3). The results were considered significant when $p<0.05$.

Results

Baseline data of the pre-dilution parameters of the African catfish fresh pooled milt

The pre-dilution milt parameters recorded were as follows: color

(creamy), volume (13 ml), pH (6.02), sperm motility (95%), sperm viability (98%), and sperm concentration ($2{,}740\times10^6$/mL).

Post-dilution milt parameters

The results showed significant differences in sperm viability for the different milt extenders within each day post-dilution (F [2, 6]=55.883, p<0.001, partial eta-squared [$\eta p2$] = 0.949). Pairwise comparison showed that from Days 3-5, sperm viability in both CEY and CW were not different, but were higher than in SM. From days 6-7, sperm viability was highest in CEY followed by CW, but was lowest in SM (Table 1). Sperm viability also differed significantly (p<0.05) within each of the milt extenders as the days increased, post-dilution. Sperm viability was high in all groups from days 0-2, but decreased significantly (p<0.05) in all the groups by day 7, with SM showing the most decrease followed by CW and then CEY.

Table 1: Effect of different milt extenders on the post-dilution daily sperm viability (%) in the African catfish (*Clarias gariepinus*).

Days post dilution	Milt Extender		
	Soya Milk (SM)	Citrated Egg Yolk (CEY)	Citrated Water (CW)
0	98.3 ± 0.6^{a}	98.3 ± 0.6^{a}	98.0 ± 1.0^{a}
1	98.0 ± 1.0^{a}	98.0 ± 1.0^{a}	97.7 ± 0.6^{a}
2	$94.7 \pm 2.1^{b\,(x)}$	$97.7 \pm 0.6^{a\,(y)}$	$96.7 \pm 1.2^{a\,(xy)}$
3	$85.7 \pm 1.5^{c\,(x)}$	$90.3 \pm 1.5^{b\,(y)}$	$89.3 \pm 1.5^{b\,(y)}$
4	$69.3 \pm 2.1^{d\,(x)}$	$81.2 \pm 1.9^{c\,(y)}$	$79.3 \pm 2.0^{c\,(y)}$
5	$56.0 \pm 1.0^{e\,(x)}$	$63.1 \pm 2.0^{d\,(y)}$	$61.0 \pm 1.0^{d\,(y)}$
6	$48.0 \pm 1.0^{f\,(x)}$	$55.0 \pm 1.0^{e\,(y)}$	$51.0 \pm 1.0^{e\,(z)}$
7	$40.0 \pm 1.0^{g\,(x)}$	$48.0 \pm 1.0^{f\,(y)}$	$43.0 \pm 2.0^{f\,(z)}$

Values represent mean±SD (n=3). $^{x\text{-}z}$Rows with different superscripts represent significant differences (p<0.05) in groups within each day post dilution. $^{a\text{-}g}$Columns with different superscripts represent significant differences (p<0.05) in the days post dilution within each group.

There were significant differences in sperm motility for the different milt extenders within each day, post-dilution (F [2, 6] = 226.51, p<0.001, $\eta p2$=0.987). Sperm motility was not different in CEY and CW at days 0 and 1, but both groups had higher values than SM. On day 4, the sperm motility was higher in CEY compared to SM and CW. From days 5-7, sperm motility was highest in CEY followed by CW, but was lowest in SM. Sperm motility was high in all the groups from days 0-1, but decreased significantly (p<0.05) in all the groups, with SM showing the most decrease followed by CW and then CEY (Table 2).

Table 2: Effect of different milt extenders on the post-dilution daily sperm motility (%) in the African catfish (*Clarias gariepinus*).

Days post dilution	Milt Extender		
	Soya Milk (SM)	Citrated Egg Yolk (CEY)	Citrated Water (CW)
0	$85.0 \pm 1.0^{a\,(x)}$	$90.0 \pm 0.9^{a\,(y)}$	$90.0 \pm 1.0^{a\,(y)}$
1	$85.0 \pm 1.1^{a\,(x)}$	$88.0 \pm 1.0^{a\,(y)}$	$87.7 \pm 1.0^{b\,(y)}$
2	$80.0 \pm 1.0^{b\,(x)}$	$82.7 \pm 1.2^{b\,(y)}$	$81.3 \pm 1.5^{c\,(xy)}$
3	$73.0 \pm 2.0^{c\,(x)}$	$78.7 \pm 1.5^{c\,(y)}$	$76.7 \pm 1.2^{d\,(y)}$
4	$60.0 \pm 1.1^{d\,(x)}$	$69.0 \pm 1.9^{d\,(y)}$	$63.0 \pm 2.0^{e\,(x)}$
5	$43.7 \pm 2.5^{e\,(x)}$	$56.3 \pm 1.5^{e\,(y)}$	$49.7 \pm 1.5^{f\,(z)}$
6	$29.3 \pm 2.1^{f\,(x)}$	$49.3 \pm 1.5^{f\,(y)}$	$35.3 \pm 1.5^{g\,(z)}$
7	$18.7 \pm 2.1^{g\,(x)}$	$31.0 \pm 2.0^{g\,(y)}$	$23.0 \pm 1.7^{h\,(z)}$

Values represent mean±SD (n=3). $^{x\text{-}z}$Rows with different superscripts represent significant differences (p<0.05) in groups within each day post dilution. $^{a\text{-}h}$Columns with different superscripts represent significant differences (p<0.05) in the days post dilution within each group.

The results revealed significant differences in sperm concentration for the milt extenders within each day post-dilution (F [2, 6]=12.447, p<0.01, $\eta p2$=0.806). Pairwise comparison showed that from Days 0-3, the sperm count in all groups was not different. From days 5-7, sperm count was highest in CEY followed by CW, but was lowest in SM. As the days increased post-dilution, sperm concentration also differed significantly (p<0.05) within each of the milt extenders. From days 0-2, sperm count was high in all groups but decreased significantly (p<0.05) in all the groups by day 7, with SM showing the most decrease followed by CW and then CEY (Table 3).

Table 3: Effect of different milt extenders on the post-dilution daily sperm concentration (×10^6/mL) in the African catfish (*Clarias gariepinus*).

Days post dilution	Milt Extender		
	Soya Milk (SM)	Citrated Egg Yolk (CEY)	Citrated Water (CW)
0	174.8 ± 6.2^{a}	170.7 ± 4.9^{a}	172.5 ± 10.3^{a}
1	161.5 ± 5.3^{b}	167.3 ± 3.9^{a}	164.1 ± 8.9^{b}
2	145.0 ± 9.5^{c}	161.0 ± 5.8^{b}	156.6 ± 11.6^{c}
3	141.0 ± 9.2^{d}	157.2 ± 4.9^{c}	153.0 ± 10.9^{d}
4	$126.8 \pm 5.9^{e\,(x)}$	$152.0 \pm 6.6^{c\,(y)}$	$143.1 \pm 4.8^{e\,(y)}$
5	$116.0 \pm 6.0^{f\,(x)}$	$143.0 \pm 4.7^{d\,(y)}$	$129.3 \pm 3.2^{f\,(z)}$
6	$79.7 \pm 8.3^{g\,(x)}$	$126.7 \pm 4.4^{e\,(y)}$	$112.8 \pm 3.8^{g\,(z)}$
7	$55.4 \pm 8.0^{h\,(x)}$	$114.2 \pm 5.8^{f\,(y)}$	$97.6 \pm 4.0^{h\,(z)}$

Values represent mean±SD (n=3). $^{x\text{-}z}$Rows with different superscripts represent significant differences (p<0.05) in groups within each day post dilution. $^{a\text{-}h}$Columns with different superscripts represent significant differences (p<0.05) in the days post dilution within each group.

Fertilization, hatchability, survival, and embryo viability rates

The fertilization rate was not significantly different (p>0.05) in the CEY, CW, and FM groups. There was zero fertilization rate in the SM group, which was significantly different (p<0.05) from the CW, FM, and CEY groups (Table 4). Hatchability rates in the CEY, CW, and FM groups were not

significantly different (p>0.05). The survival rate was high in all former groups (CEY, CW, and FM), but was only significantly higher (p<0.05) in FM compared to CW. There was no significant difference (p>0.05) in embryo viability rate in the three groups at 8 h post-fertilization. However, at 24 h post-fertilization, the CW group recorded a zero-viability rate which was significantly different (p<0.05) compared to the 100% viability rate recorded in the CEY and FM groups, respectively.

Water quality parameters

The evaluation of the baseline and post-hatch water quality parameters revealed slight variations which were not significantly different among the treatment groups. The values were also within the acceptable ranges for each water quality parameter.

Table 4: Effect of different milt extenders on the fertilization, hatchability, survival, and embryo quality rates (%) in the African catfish (*Clarias gariepinus*).

	Soya Milk (SM)	Citrated Egg Yolk (CEY)	Citrated Water (CW)	Fresh Milt (FM)
Fertilization rate (%)	0.0 (0.0, 0.0)^{a}	95.0 (94.0, 96.0)^{b}	100.0 (100.0, 100.0)^{b}	100.0 (100.0, 100.0)^{b}
Hatchability rate (%)	-	76.0 (75.0, 77.0)	74.0 (73.0, 75.0)	73.0 (72.0, 74.0)
Survival rate (%)	-	94.7 (93.8, 95.6)^{ab}	92.4 (91.0, 94.8)^{a}	96.6 (95.4, 97.8)^{b}
Embryo viability at 8 h Post-fertilization (%)	-	100.0 (100.0, 100.0)	100.0 (100.0, 100.0)	100.0 (100.0, 100.0)
Embryo viability at 24 h Post-fertilization (%)	-	100.0 (100.0, 100.0)^{a}	0.0 (0.0, 0.0)^{b}	100.0 (100.0, 100.0)^{a}

Values represent median (Q1, Q3); n=3. Rows with different superscripts represent significant differences (p<0.05) in groups. Fertilization was zero in the SM group therefore other parameters were not determined for the group.

Post-hatch gross biometry of fry on day 13

The results for the average body measurements showed that fry length was highest (p<0.05) in CEY, followed by CW, but was lowest in FM. The fry head width was significantly high (p<0.05) in CW, but it was not significantly different (p>0.05) between CEY and FM groups. The fry weight was high and also not significantly different (p>0.05) in both CW and FM but it was significantly low (p<0.05) in CEY treatment (Table 5).

Table 5: Effect of different milt extenders on the biometrical parameters of the African catfish (*Clarias gariepinus*) fry at 13 days post-hatch.

	Milt Extender		
	Citrated Egg Yolk (CEY)	Citrated Water (CW)	Fresh Milt (FM)
Fry length (cm)	1.03 ± 0.01^{a}	0.91 ± 0.02^{b}	0.87 ± 0.02^{c}
Fry head width (cm)	0.13 ± 0.01^{a}	0.18 ± 0.02^{b}	0.11 ± 0.02^{a}
Fry weight (mg)	51.2 ± 3.0^{a}	101.1 ± 2.6^{b}	100.3 ± 2.1^{b}

Values represent mean±SD (n=20). Rows with different superscripts represent significant differences (p<0.05) in groups.

Discussion

In this study, a total egg weight of 7 g (estimated to be 3300 eggs) was stripped during the spawning season. This observation revealed an average of 471 eggs per g of egg, which differed from previous authors (Bichi *et al.*, 2014) who estimated 1 g to yield approximately 700 eggs. This difference may be due to the different sizes of female broodstocks utilized for the studies (Ataguba *et al*., 2013).

A relatively high percentage of sperm motility and viability were still observed in the diluted milt samples as on day 3 post-collection. A previous study reported that collected sperm sacs stored in the fridge could still be used for spawning, with good results after 48 h (Rahman *et al.*, 2020). However, on day 7, sperm viability, motility, and concentration had decreased markedly in all groups, with SM showing the most decrease followed by CW and then CEY treatments. This could be attributed to a deterioration of the sperm cells during the prolonged storage, an observation that may hamper milt fertility. During hypothermic storage of milt, oxidative damage to spermatozoa has been reported as the potential cause of the decline in sperm motility and fertility (Agarwal *et al*., 2019).

A very interesting finding of this study was observed with the SM extender. By day 3 post dilution, sperm viability, and motility were not different between CEY and CW but had significantly decreased in SM. Taken together, these findings provide evidence of significant sperm deterioration under chilled storage in SM extender. Regardless of the promising semen quality of the SM group at 48 h of storage, its fertilization rate was zero, and hence hatchability was also zero. This observation may not be strange in the catfish. There was a report of lowered fertility of the African giant catfish (*Clarobranchus*) milt extended in soya milk, and stored up to 6 h (Onyia *et al.*, 2017). This report is consistent with our present finding. This observation implies that motility may not correlate with fertilization, and therefore differs from a previous study that reported sperm motility as the most evaluated criteria for sperm quality assessment in fish due to its correlation with fertility (Ogidan *et al*., 2018). The observed fertilization failure in SM extender may be due to other factors. It is also possible that some components of SM extender may have some adverse effects on the egg-sperm interaction or on the biochemical processes of fertilization. It will be of interest to investigate the effects of soya fat globules on egg micropyle or surface coating as it affects sperm penetration and fertilization. Soya milk also contains a toxic substance- saponin which can be harmful, whether cooked or not, but can be broken down by the enzymes used in fermentation. The soya extract used in this study contained saponin in addition to flavonoid, steroid, and glycoside as revealed by the phytochemical analysis. Soya milk also contains anti-trypsin (Palliyeguru *et al.*, 2011). Trypsin was detectable in membranes of spermatozoa, and trypsin and or trypsin-like protease is an essential and

multifunctional factor in spermatogenesis (Shachong *et al.*, 2008). Anti-trypsin substance in soya beans necessitates heating, to be suitable for consumption. However, it is also possible that the level of heating during SM preparation may not have been enough to inhibit the anti-trypsin or the other component factors. Moreover, the toxic substance- saponin, is heat-stable even after ultra-high temperature heating. Shachong *et al.* (2008) reported that trypsin is associated with fertilization in fish. Therefore, the presence of anti-trypsin in the SM extender may have impacted negatively on the fish gametes and the fertilization process.

The observation on SM extender was a sharp contrast to CEY and CW with the fertilization rates of 95% and 100%, respectively, compared to 100% recorded for the fresh milt. Thus, CEY and CW both showed relatively high rates of fertilization, hatchability, and survival, compared with the control (fresh milt). This observation is not in agreement with the findings in other animal species. Aurich *et al*. (1997) reported that the use of chilled-stored stallion semen diluted in egg yolk-based extenders was limited by its relatively short-time fertilization capacity. The use of quail egg yolk in this study instead of chicken egg yolk, as well as the animal species studied, may be responsible for the different observations. Embryo viability at 8 h post-fertilization was very high in both CEY and CW (100%), as also recorded for the FM control. However, by 24 h post-fertilization, viability had plummeted to zero for CW whereas it remained very high (100%) in both CEY and FM. This implied that under bench laboratory incubation, CEY yielded better embryo quality, possibly due to the other components of the extender including antibiotics. However, it should be noted that the embryo quality in a petri dish was a test of stability under stress. Therefore, under hatchery rearing conditions, fry quality is related to body measurements and weight as discussed later.

The baseline water quality parameters were within the normal range acceptable for fish production, as reported previously (Warish *et al.*, 2017). It is noteworthy that all the observed changes in water parameters did not adversely affect the fry survival rate in all the groups. This is consistent, as the recorded values were within the acceptable range suitable for fry production in accordance with Warish *et al.* (2017).

Evaluation of the body measurements of fry at day 13 post-hatch revealed group differences in body parameters. Whereas CEY recorded the highest fry length, CW recorded the highest fry head width. Fry body weight was also lower in CEY compared to CW and FM. These differences may be attributed to variations in the daily larvae rearing water parameters in the different groups studied. It will be interesting to carry out further investigations on the body parameters in adult catfish reared under these different groups.

The growth and welfare of cultured fish are closely dependent on the physical,

biological, and chemical parameters of water. The growth rate and internal body temperature of fish can be regulated by water. This influence of water can also be reflected through dissolved material and waste product exchange within the system. The most crucial parameter to monitor continuously was dissolved oxygen. In establishing an efficient artificial fish production system, the source and quality of water, assessed by pH, ammonia, nitrite, alkalinity, temperature, suspended substances, heavy metal, and dissolved oxygen levels, play important roles in healthy fish growth. From the findings of this study, it was concluded that as a milt extender for the African Catfish, chilled CW was comparable to CEY with respect to fertilization, hatchability, and fry survival rates, but was superior to CEY with respect to fry quality (body weight). On the other hand, the chilled SM extender stored for 48 h was not suitable for producing fry due to failed fertilization. It was recommended to apply medicated citrated water in the extension and chilled storage of African catfish milt. Soya bean milk should also undergo further investigations as a milt extender to determine the mechanisms causing fertilization failure in catfish production.

References

Agarwal, A., Parekh, N., Selvam, M.K.P., Henkel, R., Shah, R., Homa, S.T., Ramasamy, R., Ko, E., Tremellen, K., Esteves, S. and Majzoub, A., 2019. Male oxidative stress infertility (MOSI): proposed terminology and clinical practice guidelines for management of idiopathic male infertility. *The World Journal of Men's Health*, 37(**3**), 296-312. DOI:10.5534/wjmh.190055

Akanmu, O.A., Ipinmoroti, M.O., Iyiola, A.O. and Abifarin, P., 2019. The use of Cocos nucifera L, water as milt extender in artificial breeding of *Clarias gariepinus* (Burchell, 1822). *Nigerian Journal of Fisheries*, 16(**2**), 1762-1767.

Al-Khalaifah, H.S., Khalil, A.A., Amer, S.A., Shalaby, S.I., Badr, H.A., Farag, M.F.M., Altohamy, D.E. and Abdel Rahman, A.N., 2020. Effects of Dietary Doum Palm Fruit Powder on Growth, Antioxidant Capacity, Immune Response, and Disease Resistance of African Catfish, *Clarias gariepinus* (B.). *Animals (Basel)*, 10(**8**), 1407. DOI:10.3390/ani10081407

Arun, P.B. and Shigeharu, S., 2004. Decline in Fertilization and Hatching Rate of African Catfish *Clarias gariepinus* after Ovulation. *Suisanzoshoku*, 52(**1**), 37-44.

Ataguba, G.A., Okomoda, V.T. and Onwuka, M.C., 2013. Relationship between brood stock weight combination and spawning success in African catfish (*Clarias gariepinus*). *Croatian Journal of Fisheries*, 71, 176-181. DOI:10.14798/71.4.694

Aurich, J. E., Schonherr, U., Hoppe, H. and Aurich, C.. 1997. Effects of antioxidants on motility and membrane intergrity of chilled stored stallion semen. *Theriogenology*, 48,

185-192. DOI:10.1016/s0093-691x(97)84066-6

Bichi, A.H., Isyaku, S., Danba, E.P., Kurawa, I.A. and Nayawo, A.A.. 2014. Effect of brood stock size on egg fertilization, hatchability and fry survival rate of African catfish (*Clarias gariepinus*). *Bayero Journal of Pure and Applied Sciences*, 7(**2**), 150-154. DOI:10.4314/bajopas.v7i2.26

Bustani, G.S. and Baiee, F.H., 2021. Semen extenders: An evaluative overview of preservative mechanisms of semen and semen extenders. *Veterinary World*, 14(**5**), 1220-1233.

Canadian Council on Animal Care Guide (CACC), 1993. Guidelines on Laboratory Animal Facilities: Characteristics, Design and Development. http://www.ccac.ca/Documents/Standards/Guidelines/Experimental_Animals_Voll.pdf

Chanda, S. and Ramachandra, T.V., 2019. Phytochemical and Pharmacological Importance of Tumeric (*Curcuma longa*): A Review. *Scientific Technical and Medical Journals*, 9(**1**), 16-23.

Kowalski, R.K. and Cejko, B.I., 2019. Sperm quality in fish: Determinants and affecting factors. *Theriogenology*, 135, 94-108. DOI:10.1016/j.theriogenology.2019.06.009.

Muchlisin, Z.A., 2009. A General Overview on Some Aspects of Fish Reproduction. Syiah Kuala University, Banda Aceh, Indonesia. *Aceh International Journal of Science and Technology*, 3(**1**), 43-52. DOI:10.13170/AIJST.0301.05

National Population Commission Nigeria (NPC), 2006. Population census of the Federal Repubic of Nigeria. Abuja, Nigeria.

Ogidan, I.M., Chukwuka, A.V., Omiwole, R.A., Ibor, O.R. and Adeogun, A.O., 2018. Gamete quality and reproductive performance in *Clarias gariepinus* chronically exposed to industrial effluent mixtures. *African Journal of Biomedical Research*, 21(**2**), 201-210.

Ogunremi, J.B., Igbani, F., Onimisi, M.M. and Shetur, C.Y., 2022. Assessment of Fish Farming Practices, Development and Constraints among Fish Farmers in Ibi Local Government Area, Taraba State, Nigeria. *Nigerian Agricultural Journal*, 53(**1**), 260-266.

Okomoda, V.T., Koh, I.C.C. and Shahreza, S.M., 2018. A Simple technique for accurate estimation of fertilization rate with specific application to *Clarias gariepinus* (BURCHELL 1822) *Aquaculture Research*, 49(**2**), 1116-1121. DOI:10.1111/are.13528

Onyia, L.U., Ochokwu, I.J. and Mohammed, U., 2017. Effects of different sperm extenders on the fertility, hatchability and survival of *Clarobranchus*. *CAED International Journal of Medical Science and Applied Biosciences*, 2(**3**), 19-29.

Palliyeguru, M.W., Rose, S.P. and Mackenzie, A.M., 2011. Effect of trypsin inhibitor activity in soya bean

on growth performance, protein digestibility and incidence of sub-clinical necrotic enteritis in broiler chicken flocks. *British Poultry Science*, 52(**3**), 359-367. DOI:10.1080/00071668.2011.577054. PMID: 21732882

Rahman, S.M., Alsaquft, A.S., Alkhanis, Y.A., Rahman, M.M., Ahsan, M.N., Mathew, R.T. and Hossain, Q. Z., 2020. Short term storage of Asian walking catfish *Clarias batrachus* Linnaeus, 1758) Gametes. *Advances in Animal and Veterinary Sciences*, 8(**12**), 1394-1401. http://dx.doi.org/10.17582/journal.aavs/2020/8.12.1394.1401.

Shachong, Y., Sam, K.C., Zhisheng, L. and Baojun, X., 2008. Elimination of trypsin inhibitor Activity and Beany Flavor in Soy milk by Consecutive blanching and ultr high-temperature (UHT) processing. *Journal of Agricultural and Food Chemistry*, 56(17), 7957-7963. DOI: 10.1021/j1801029h.

Warish, K., Abdul, V., Adil, M. and Najib, H., 2017. Water quality requirements and management strategies for fish farming (A case study of Ponds around Gurgaon Canal Nuh Palwal). *International Journal of Trend in Scientific Research and Development*, 2(**1**), 388-393. DOI:10.31142/ijtsrd5914