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Spermatozoa Revival with Addition of Diluted Dextrose Saline in African Catfish (*Clarias gariepinus* Burchell 1822)

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Abstract: *African catfish (Clarias gariepinus) production in captivity is still at the subsistent level in Nigeria largely due to its inability to reproduce naturally in captivity. In artificial breeding, rigorous operations which require high technical-know-how involving sacrifice of the male brood stock often result in colossal wastages. Thus, this study was aimed at using diluted dextrose saline to resuscitate African catfish spermatozoa in order to reduce the number of male brood stock to be sacrificed at a time as well as minimize milt wastages.*

The harvested milt was evaluated microscopically for alive or dead spermatozoa cells, active or sluggish spermatozoa cells as well as spermatozoa cells concentration every quarter hour, after adding the diluted dextrose saline. Results indicated that alive sperm cells value was as high as 79.0% in control (T_1) at 0 minute compared to 4.00% recorded at 15 minutes later and there were absolutely no more alive sperm cells at 30 minutes and beyond. Whereas, addition of diluted dextrose saline resuscitated the sperm cells in T_2 by up to 50.0% motility at 1 hour and thereafter, all the sperm cells were no longer motile. Out of these resuscitated sperm cells, 52.5% were actively motile and 47.5% were sluggish.

Consequently, diluted dextrose saline could be used as a cryoprotectant in the preservation and storage of fish milt for short term duration in artificial breeding. This technique may be practicable in hatchery operations in order to boost African catfish production in Nigeria.

Keywords: *Brood stock, milt collection, preservation, spawning*

1. INTRODUCTION

Catfish (*Clarias gariepinus*) belongs to the family of *Claridae* which is naturally carnivorous, bottom dweller, nocturnal and it is widely distributed across the African Aquatic regions. It has been described as hardy with ability to breathe and survive outside water for some time using accessory organs and it adapts to prevailing environmental conditions even with poor water quality [1]. *Clarias gariepinus* could also be described as an omnivore which basically feeds on aquatic and terrestrial insects, fish, mollusk, higher plant debris and fruits [2, 3]. According to [4], diets compositions intended for *Clarias* species should change with age such that diptera of small sizes and zooplankton predominates the ration. Little wonders *C. gariepinus* is believed to be the most suitable species for aquaculture in Africa because of its high growth rate attaining 1kg at about 6 months, resistant to handling stress, easy to propagate in captivity and it commands very high commercial value both at homes and in eateries in Nigeria. More importantly, it is typically non aggressive but could defend itself from predators by stalking non-visual primary sense organs especially barbels and other tactile organs [5, 6]. It is called "Ehęnbevbarię" among the Benin tribe of The Great Benin Kingdom in Nigeria, who highly relish it especially at ceremonies and cultural events. Therefore, *C. gariepinus* could be produced in Nigeria to augment animal protein needs of the citizenry.

At the moment, fish production in Nigeria is still at the subsistent level thus, cannot meet the demands of the populace. This was believed to be due to scarcity of good brood stock hence, the suggestion of

cryopreservation of genetic resources of available brood stock for all-year-round supply of fish seed [7]. On the other hand, it could be due to the refusal of *C. gariepinus* to breed in captive, even when a gravid female is kept with a mature male, no matter how long, with all the required management principles strictly observed, there will be no spawning in captive.

In some experimental conditions however, natural scenario was simulated and mimicked yet, there were no appreciable natural spawning. While appropriate hormones were administered in females to enhance oocytes and eggs stripping in captive catfish, the males were reported to be sacrificed in order to obtain the milt for artificial spawning. Meanwhile, in *C. gariepinus* reared in captivity at 25.0^{o}C and 12 hours photoperiod, gametogenesis was reported to be continuous in sexually mature males [1, 8, 9, 10]. The act of sacrificing the male catfish, may lead to scarcity of brood stock because there would be wastages in each round of breeding operations [11]. This probably prompted the use of syringe and needle to obtain milt from life catfish but reported seldom possibility [12]. Since cryopreservation technique requires appropriate extender, diluents, very low refrigeration temperature, follow by careful thawing and stringent expertise, it may not be feasible in most farmstead operations in Nigeria. Consequently, this study was aimed at investigating the possibility of using diluted dextrose saline to preserve and store catfish milt in order to reduce wastages of male brood stock during artificial breeding.

2. MATERIALS AND METHODS

Experimental design and fish welfare

A total of four male *C. gariepinus* brood stock, weighing about 0.60kg were bought from a sales outlet in Bukan Sidi, Lafia. They were transported in plastic container filled with clean water to Animal Science Laboratory, Faculty of Agriculture, Nasarawa State University Keffi, Shabu-Lafia Campus. The fish were allotted to two treatments in a completely randomised design based on body weight such that each treatment had two replicates. The treatments were designated as T_1 (control: without diluted dextrose saline) and T_2 (with diluted dextrose saline). The male fish were only used as a source of milt collection that were either diluted with or without dextrose saline used in this study.

Dextrose saline dilution

A sachet of dextrose saline (Glaxo®) and a litter of distilled water were procured from a reputable pharmaceutical store in Lafia. A syringe was used in taken 1ml of dextrose saline and 2ml of distilled water into a test tube and was properly mixed by shaking to prepare 50.0% dextrose saline used in the experiment. The dilution became necessary in order to avoid spermatozoa death observed by [13], who reported that 100% dextrose saline was lethal to catfish spermatozoa.

Milt quality evaluation

Two drops of the diluted dextrose saline was taken with a micropipette into a test tube containing a drop of milt and mixed carefully by turning the test tube. A drop of the diluted dextrose saline and milt mixture was then taken with a microscope slide, covered with a slip and viewed in a microscope (Olympus Microscope® Tokyo, Japan) at 10x and 40x magnifications, to determine alive or dead spermatozoa cells, active or sluggish spermatozoa cells as well as spermatozoa cells concentration as described [14, 15]. This procedure was repeated differently at every quarter hour (15 minutes) to collect data except, the milt volume and sperm cells concentration that were determined only at 0 minute.

Data collection and analysis

On arrival, the fish were transferred into an open bowl containing clean water where each fish was picked and weighed using a table scale (Five Goats®) to obtain body weight. The standard body length was taken from the mouth to the caudal fin base, while the total body length was taken from the mouth to the end of the caudal fin using measuring tape (Butterfly®). Papilla length, left and right fin length were measured using measuring tape (Butterfly®). Thereafter, all the fish were sacrificed, the abdomen was carefully dissected, thermometer probe was placed on the testes immediately to obtain testes temperature and another thermometer was hung on the wall to obtain room temperature. The left and right testes were collected carefully and the length was taken using measuring tape (Butterfly®). The testes were incised carefully using surgical blade and the milt was gently squeezed out, pooled and a known volume (0.50ml) was measured each for treatments 1 and 2 respectively. The

milt pH was determined with a pH strip differently whenever it was time to evaluate the milt quality. Data collected were subjected to analysis of variance based on statistical procedure of [16] and the mean values were separated according to Duncan Multiple Range Test of the same software package.

3. RESULTS

Table 1 shows the body linear measurements of African catfish used in the study. The mean values of all the parameters measured were not significantly different ($P>0.05$), except the right fin length value which was statistically longer ($P<0.05$) in the fish in T_2 (12.0cm) compared to 11.0cm recorded in T_1. Meanwhile, the body weight value ranged from 0.63 to 0.64kg, standard body length (42.1 to 42.2cm), total body length (48.0 to 48.1cm) and the testes length varied between 4.71 and 4.77cm.

Table 1. *Body linear measurements of African catfish (C. gariepinus)*

Parameters	Treatments (±SEM)	
	T_1	T_2
Body weight (kg)	0.64±0.005	0.63±0.02
Standard body length (cm)	42.2±0.30	42.1±0.10
Total body length (cm)	48.1±0.10	48.0±0.25
Papilla length (cm)	1.82±0.015	1.82±0.005
Left fin length (cm)	12.5±0.50	12.5±0.50
Right fin length (cm)	11.0±0.00^b	12.0±0.00^a
Room Temperature (^{0}C)	36.3±0.05	36.4±0.025
Testis temperature (^{0}C)	26.0±0.00	26.0±0.00
Left testis length (cm)	4.73±0.025	4.77±0.03
Right testis length (cm)	4.75±0.045	4.71±0.005

a, b: Means with different letters on the same row are significantly different at P<0.05; ±SEM: Plus or minus standard error of means; T_1 (control: without diluted dextrose saline); T_2 (with diluted dextrose saline).

The effect of time of addition of diluted dextrose saline on *C. gariepinus* milt is presented in Table 2. There were statistical differences ($P<0.05$) in the mean values of all the parameters measured across the treatments. However, there were no significant differences ($P>0.05$) in alive sperm cells (75.0 – 79.0%), dead sperm cells (21.0 – 25.0%), active motile sperm cells (70.0 – 85.0%) and sluggish motile sperm cells (15.0 – 30.0%) at 0minutes treatment. Similarly, values of all the parameters at 1:15hour treatment did not differ statistically ($P>0.05$) except, the sperm cells concentration which was higher (566 x10^6) in T_1 compared to 567 x10^6 recorded in T_2. Milt pH value varied from 7.60 at 30minutes treatment to as high as 8.90 at 0 and 45minutes treatments. It was observed that alive sperm cells value was very high (79%) in control at 0minute which was drastically reduced to 4% at 15minutes treatment and without any alive sperm cell activity (absolutely 0.00%) thereafter. At 30minutes and beyond, all the sperm cells (absolutely 100%) were observed dead in the control treatment (T_1) whereas in T_2, addition of diluted dextrose saline resuscitated the sperm cells up to 50.0% in 1hour. Out of these resuscitated sperm cells in T_2, 52.5% were actively motile and 47.5% were sluggish.

Table 2. *Effect of time of addition of diluted dextrose saline on African catfish (C. gariepinus) milt quality*

Parameters	Treatments (±SEM)											
	0 minute*		15 minutes*		30 minutes*		45 minutes*		1 hour*		1:15 hour*	
	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
ϕMV (ml)	0.5±0.00	0.5±0.00	0.5±0.00	0.5±0.00	0.5±0.00	0.5±0.00	0.5±0.00	0.5±0.00	0.5±0.00	0.5±0.00	0.5±0.00	0.5±0.00
Milt pH	7.8±0.2^b	8.9±0.0^a	7.9±0.1^b	8.8±0.0^a	7.6±0.4	8.8±0.0	8.9±0.0^a	8.8±0.0^b	8.8±0.0	8.8±0.0	8.8±0.0	8.8±0.0
ASC (%)	79±1.0	75±5.0	4.0±1.0^b	71.5±6.5^a	0.0±0.0^b	69.0±9.0^a	0.0±0.0^b	58.5±1.55^a	0.0±0.0^b	50±0.0^a	0.0±0.0	0.0±0.0
DSC (%)	21±1.0	25±5.0	96±1.0^a	28.5±6.5^b	100±0.0^a	31.0±9.0^b	100±0.0^a	41.5±1.5^b	100±0.0^a	50±0.0^b	100±0.0	100±0.0
AMSC (%)	70±5.0	85±0.0	6.0±4.0^b	80.0±0.0^a	0.0±0.0^b	75±5.0^a	0.0±0.0^b	62.5±7.5^a	0.0±0.0^b	52.5±2.5^a	0.0±0.0	0.0±0.0
SMSC (%)	30±5.0	15±0.0	94±4.0^a	20.0±0.0^b	0.0±0.0	25±5.0	0.0±0.0^b	37.5±7.5^a	0.0±0.0^b	47.5±2.5^a	0.0±0.0	0.0±0.0
ϕSCC (x10^6)	566±5.0^a	567±0.0^b	566±5.0^a	567±0.0^b	566±5.0^a	567±0.0^b	566±5.0^a	567±0.0^b	566±5.0^a	567±0.0^b	566±5.0^a	567±0.0^b

*ϕMilt volume and sperm cells concentration were determined only at 0 minute; *Mean values comparison was per time treatment only and not across treatments; a, b: Means with different letters on the same row at the same time per treatment are significantly different at P<0.05; ±SEM: Plus or minus standard error of means; T_1 (control: without diluted dextrose saline); T_2 (with diluted dextrose saline); MV: Milt volume; ASC: Alive sperm cells; DSC: Dead sperm cells; AMSC: Active motile sperm cells; SMSC: Sluggish motile sperm cell; SCC: Sperm cells concentration.*

4. DISCUSSION

The standard body length values of 48.5 – 50.1cm, total body length of 55.4 – 56.3cm and body weight value of about 0.6 kg were similar to the criterion for selecting mature catfish of about 4 to 12

months old [8]. The papilla and fin length values were similar to 1.31 – 1.55cm and 6.26 – 7.07cm respectively as reported [12] in sexually matured African catfish. Thus, the results probably indicated that the experimental fish were sexually matured. The milt pH value was higher than 6.2 reported [17] in mature and healthy *C. gariepinus*. The disparity could be possibly due to strain differences and experimental conditions. The sperm cells motility value was similar to a range of 70.0 – 90.0% observed by [18] and much more than 15% given by [17] in African catfish. The observed variation could be probably due to the kind of treatments involved and probably the time interval of evaluation. The sperm cells concentration value was higher than 5.16 $x10^8$ reported in healthy and mature *C. gariepinus* [17] thus, the experimental fish were probably physiologically sound.

It was observed that the milt quality was not influenced by the addition of diluted dextrose saline at 0minute but at 15minutes, the milt quality was tremendously improved. This trend was observed up to 1hr, when the sperm cells were observed to be resuscitated by the inclusion of the diluted dextrose saline. This observation contradicted the report [19] that carp sperm cell dilution and/or washing in various media (seminal fluid, saline solution) did not improve preservation. However, the findings was similar to the report of [20] that physiological saline, Ringer or saline as cryoprotectant retained sperm cell motility. Thus, dextrose saline could be used as a cryoprotectant to preserve fish milt for a short term probably without refrigeration or long term with refrigeration, during artificial breeding in other to boost catfish production in captivity.

This technique may be relevant in instances where harvested milt were not used up immediately as described by [21, 22] and needed to be preserved either in a short term or long term with or without refrigeration. Regrettably, up to this moment, catfish cannot reproduce naturally in captive according to [23] hence, the need to adopt any practicable and safe artificial measures to spawn, hatch and brood the young ones in order to boost productivity.

5. CONCLUSION

The body linear values of the experimental fish apparently indicated their sexual maturity and the milt pH, alive sperm cells motility as well as the sperm cells concentration values, seemingly showed that the fish were physiologically healthy. The overall milt quality evaluated was similar to the standards given in mature and healthy fish. It was observed that the addition of diluted dextrose saline evidently resuscitated the sperm cells for up to an hour.

Therefore, it might be a practicable technique to preserve and store catfish milt for a short term during artificial breeding operation. This may minimize the number of males that will be sacrificed in captive catfish production. Meanwhile, further in depth investigation is required to evaluate the fertilizing ability of such preserved and stored catfish milt for elucidation.

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