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INFLUENCE OF CELLGEVITY® ON THE MILT QUALITY OF CATFISH, *CLARIAS GARIEPINUS* (BURCHELL, 1822) EXTENDED IN LACTATED RINGERS DURING CHILLED STORAGE

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ABSTRACT

The aim of the present study was to determine the influence of Cellgevity® on milt quality of Catfish, *Clarias gariepinus* extended in lactated ringers during chilled storage. Pooled milt from three bloodstocks were divided into three groups (T_1, T_2 and T_3) each in triplicate. Milt were extended in lactated ringers and supplemented with Cellgevity® at 0mg (T_1), 125mg (T_2) and 250mg (T_3). Milt were evaluated at days-0 (before storage) and days-1, 2, 3, 4 and 5 of chilled storage. Percentage mean spermatozoa motility was significantly ($P < 0.001$) lower in T_2 and T_3 than T_1 from day-0 to 4. Percentage mean spermatozoa concentrationwas significantly ($P < 0.01$) lower in T_2 than T_1 from day-0 and significantly ($P < 0.001$) lower in T_2 and T_3 than T_1 until day-3.Percentage mean live spermatozoa was significantly ($P < 0.05$) lower in T_3 than T_1 from day-0 and significantly ($P < 0.001$) lower in T_2 and T_3 than T_1 until day-3. Percentage mean total abnormal sperm cells were lower in T_2 and T_3 than T_1 from day-0 to 4 although significant ($P< 0.05$) only at day-0 and day-2.It was concluded that Cellgevity® (125 and 250 mg) resulted in a declined percentage mean spermatozoa motility, concentration, viability and total abnormalities. Hence, should not be supplemented in milt of catfish, *Clarias gariepinus* extended in lactated ringers in chilled storage.

Keywords: Cellgevity®, Chilled Storage, *Clarias gariepinus,* Milt Quality, Lactated Ringers

INTRODUCTION

African catfish, *Clarias gariepinus* is the most widely distributed and most cultured fish in Africa. One of its artificial propagation challenge is that, sexually matured bloodstocks cannot synchronize breeding by themselves. Male bloodstock has to be sacrifice each time they are needed for artificial breeding. The development of chilled storage of fish milt has the potentials to ameliorate this challenge. However, chilled storage of milt induces a significant decrease in milt quality due to oxidative stress (Figueroa *et al.,* 2018). The fish spermatozoa membrane is an ideal target for reactive oxygen species (Cabrita *et al.,* 2014). Recent studies have indicated that Cellgevity® marketed by Max International, may lower oxidative stress level in tissues (Awodele *etal.,* 2018). Itcontains the glutathione precursor molecule - riboceine (D-ribose-L-cysteine) (N'guessan *et al.,* 2018). Catfish produce a small quantity of highly viscous milt and lactated ringer's solution is the extender of choice for its dilution (Muchlisin *et al*., 2010). There is no study that has reported the use of Cellgevity® in milt of Catfish, *Clarias gariepinus* extendedin lactated ringers solution. Hence, this study aimed at accessing the influence of supplementation of Cellgevity® in milt of Catfish, *Clarias gariepinus* extended in lactated ringers in chilled storage.

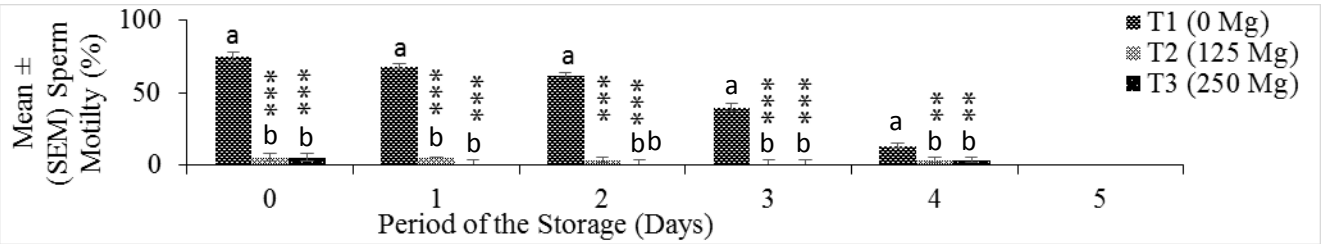
MATERIALS AND METHODS

Ethical approval for the use of the fish and milt were sorted from Animal Care and Use Committee of the Ahmadu Bello University, Zaria, Nigeria, and approval number **ABUCAUC/2021/154** was issued.Milt was collected from three sexually matured male catfish*, C. gariepinus* broodstock of average weight (2.35kg) and length (28cm)**.** Only milt with motility >70% were pooled together for for use. Lactated ringers solution

(3.0gNaCL, 0.2gKCL, 0.135g$CaCl_2$, 1.50g$C_3H_5NaO_3$, 273mOsmol/L, pH6.5) was procured from DANA® Pharmaceuticals Limited.Cellgevity® was procured from Max International Company. Milt was assigned to three (3) groups (T_1, T_2 andT_3) in triplicate and were extended in lactated ringer's solution at (1:200). T_1 served as control while T_2 andT_3 were supplemented with 125mg/ml and 250mg/ml Cellgevity®, respectively and stored at +4°c. Assessment for spermatozoa motility, concentration, viability and morphology was carried out before storage (day-0) and throughout storage until all spermatozoa stopped moving. Spermatozoa motility was determined following milt activation in bore-hole water. Spermatozoa concentration was determined using Neubauer improved haemocytometer chamber while sperm viability and morphology was evaluated using Eosin-Nigrosin Staining method (Kvist and Björndahl, 2002).Results were expressedasmean(±SEM). One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test wasusedto compare the differences in means among the groups. All tests were performed using GraphPad Prism® Version 5.03 for Windows, GraphPad Software, San Diego California USA, www.grahpad.com. Values of $P< 0.05$ were considered statistically significant.

RESULTS AND DISCUSSIONS

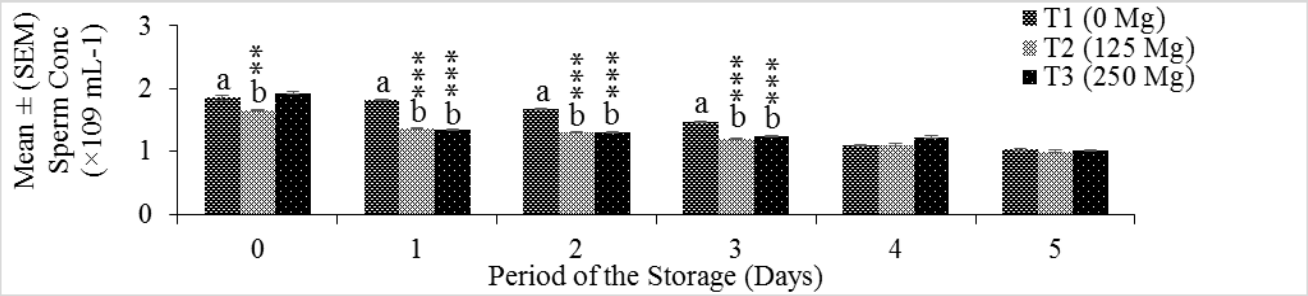
Percentage mean spermatozoa motility was significantly ($P < 0.001$) lower in T_2 and T_3 than T_1 before storage (day-0) and on subsequently days until day-3 of the storage. Similarly, it was significantly ($P < 0.01$) lower in T_2 and T_3 than T_1 on day-4 of the storage. However, by day-5, no sperm cells were detected motile (Fig. 1). The lower percentage mean spermatozoa motility in T_2 and T_3 compared to the controlis similar to the findings by Da costa *et al*., (2019) in sperm of South American silver catfish (*Rhamdia quelen*). Present findings however did not agree with the finding by; Kledmanee *et al*. (2013) in chilled carp semen; Medubi *et al.* (2019) in rats and Öğretmen et al. (2015) in sperm of common carp (*Cyprinus carpio*).



[ab]Means with different superscript letters between bars are significantly different at$P< 0.01$ (**) and $P< 0.001$ (***).

Figure 1: Percentage mean ± (SEM) spermatozoa motility in milt extended in lactated ringers and supplemented with Cellgevity® during the period of storage.

Percentage mean spermatozoa concentrationwas significantly ($P < 0.01$) lower in T_2 than T_1 before storage (day-0) and significantly ($P < 0.001$) lower in T_2 and T_3 than T_1 on subsequent days until day-3 of the storage (Fig. 2). However, by days-4 and 5, percentage mean spermatozoa concentration were not different significantly ($P> 0.05$) among the groups (Fig. 2). The lower percentage mean spermatozoa concentration in T_2 and T_3 compared to the controlin the present study is contrary to the findings by Medubi *et al.,* (2019) where sperm concentration in Cellgevity® treated groups were significantly higher.



[ab]Means with different superscript letters between bars are significantly different at$P \leq 0.01$ (**) and $P \leq 0.001$ (***).

Figure 2: Percentage mean ± (SEM) spermatozoa concentration ($\times 10^9$ mL^{-1}) in semen extended in lactated ringers and supplemented with Cellgevity® during the period of storage.

Percentage mean live spermatozoa was significantly ($P < 0.05$) lower in T_3 than T_1 justbefore storage (day-0) and significantly ($P < 0.001$) lower in T_2 and T_3 than T_1 on subsequent days until day-3 of the storage(Table 1). The lowered percentage mean viable spermatozoa in the Cellgevity® supplemented groups compared to the controlin the present study is similar to the findings that addition of cysteine alone, glutamine alone and combination of glutamine with cysteine did not improve the spermatozoa viability of the cryopreserved sperm of South American silver catfish, *Rhamdia quelen* (Da costa *et al*., 2019; Da costa *et al*., 2020). Similarly,Saroseik *et al*. (2013) reported that the supplementation of antioxidants (vitamin C and E) during the short-term storage of Perch semen *(Perca fluviatilis*) did not benefit its viability, however, same study recorded a significant increase in viability when glutathione and cysteine were supplemented in milt.

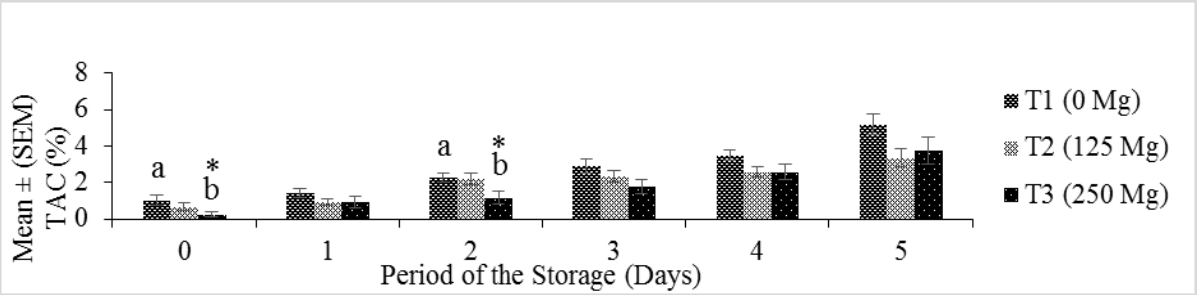
Table 1: Percentage mean ± (SEM) live and dead spermatozoa in semen extended in lactated ringers and supplemented with Cellgevity® during the period of storage.

Period	T_1 (0 Mg)		T_2 (125 Mg)		T_3 (250 Mg)	
(Days)	Live	Dead	Live	Dead	Live	Dead
0	85.00 ± 2.90[a]	15.00 ± 2.90[a]	75.00 ± 2.90[a]	25.00 ± 2.90[a]	73.33 ± 1.70[b*]	26.67 ± 1.70[b*]
1	65.00 ± 2.89[a]	35.00 ± 2.89[a]	31.67 ± 1.67[b***]	68.33 ± 1.68[b***]	33.33 ± 1.67[b***]	66.67 ± 1.68[b***]
2	63.33 ± 1.67[a]	36.67 ± 1.67[a]	8.33 ± 1.67[b***]	91.33 ± 1.67[b***]	6.67 ± 1.67[b***]	93.33 ± 1.67[b***]
3	36.67 ± 1.67[a]	63.33 ± 1.67[a]	8.33 ± 1.67[b***]	91.67 ± 1.67[b***]	5.00 ± 0.00[b***]	95.00 ± 0.00[b***]
4	8.33 ± 1.67	91.67 ± 1.67	5.00 ± 0.00	95.00 ± 0.00	3.33 ± 1.67	96.67 ± 1.67
5	5.00 ± 0.00	95.00 ± 0.01	3.33 ± 1.67	96.67 ± 1.67	3.33 ± 1.67	96.67 ± 1.68

[ab]Means with different superscript letters between columns are significantly different at$P \leq 0.05$ (*) and $P \leq 0.001$ (***).

Percentage mean total abnormal sperm cells were generally lower in T_2 and T_3 than T_1 before storage (day-0) and throughout the 5-days of the storage (Fig. 3). This indicates that Cellgevity® improved the morphology of the sperm cells in the present study. This finding is contrary to the findings thatspermatozoa abnormalities were significantly higher when cysteine was added to the cryopreserved sperm of South American silver catfish, *Rhamdia quelen* (Da costa *et al*., 2019).However, percentage mean total abnormal sperm cells progressively increased from before storage and throughout the 5 storage days in all groups. This can be

attributed to the degenerating effect on sperm cells as storage days progresses.The lower spermatozoa motility, concentration and viability in Cellgevity® supplemented groups inthis study, may be that the doses used might have impacted a toxic effect on the sperm cells. Recent studies showed that Cellgevity® could have pro-oxidant effects at higher doses (Awodele *et al*., 2018).



[ab]Means with different superscript letters between bars are significantly different at $P \leq 0.05$ (*).

Figure 3: Percentage mean ± (SEM) total abnormal sperm cells (TAC) in semen extended in lactated ringers and supplemented with Cellgevity® during the period of storage.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, extension of milt of catfish, *Clarias gariepinus* in lactated ringers and supplementation with 125 and 250 mg Cellgevity®in chilled storage resulted in a declined percentage mean spermatozoa motility, concentration, viability and total abnormalities. Hence, should not be supplemented in milt of catfish, *Clarias gariepinus.*

REFERENCES

Awodele, O., Badru, W.A., Busari, A.A., Kale, O.E., Ajayi, T.B., Udeh, R.O. and Emeka, P.M. (2018). Toxicological evaluation of therapeutic and supra-therapeutic doses of Cellgevity® on reproductive function and biochemical indices in Wistar rats. *BMC Pharmacology and Toxicology,* 19(1): 1-16. https://doi.org/10.1186/s40360-018-0253-y

Cabrita, E., Martínez-Páramo, S., Gavaia, P. J., Riesco, M. F., Valcarce, D. G., Sarasquete, C., Herraez, M.P. and Robles, V. (2014). Factors enhancing fish sperm quality and emerging tools for sperm analysis. *Aquaculture,* 432:389-401. doi:10.1016/j.aquaculture.2014.0

Da Costa, B.B., Marques, L.S., Lassen, P.G., Rodrigues, R.B., Da Rosa-Silva, H.T., Moreira, J.C.F., Oliveira, D.G. and Streit Jr, D.P. (2020). Effect of glutamine and cysteine supplementation on quality of cryopreserved sperm of South American silver catfish. *Aquaculture Research,* 52(5), 2173-2181. doi:10.1111/are.15070

Da Costa, B.B., Marques, L.S., Lassen, P.G., Rodrigues, R.B., Tais Da Rosa Silva, H., Moreira, J.C.F. and Streit, D.P. (2019). Effects of cysteine supplementation on the quality of cryopreserved sperm of South American silver catfish. *Aquaculture Research,* doi:10.1111/are.14389

Figueroa, E., Farias, J.G., Lee-Estevez, M., Valdebenito, I., Risopatron, J., Magnotti, C., Romero, J. and Oliveiro, R.P.S. (2018). Sperm cryopreservation with supplementation of ά-tocopherol and ascorbic acid in freezing media increase sperm function and fertility rate in Atlantic salmon (*Salmo salar*). *Aquaculture,* 493: 1-8.

Kledmanee, K., Somrat, T., Prawporn, T., Panida, C. and Kampon, K. (2013). Effect of L-Cysteine on chilled carp (*Cyprinus carpio*) semen qualities. *Thai Journal of Veterinary Medicine*, 43 (1): 91-97.

Kvist, U. and Björndahl, L. (2002). *Manual on basic semen analysis,* revised edition. Oxford University Press, United Kingdom. Pp1-34.

Medubi, L.J., Ama, C., Medubi, O.O., Onwosu, O.C., Lawal, O.R., and Osinubi, A.A.A. (2019). D-Ribose-L-cysteine, supplement attenuates Doxorubicin impaired spermatogenesis, testicular steroidogenesis and Redox status in Sprague-Dawley wistar rats. *African Journal of Biomedical Research,* 22: 179-185.

Muchlisin, Z.A., Nadiya, N., Nadiah, W.N., Musman, M. and Siti-Azizah, M.N. (2010). Preliminary study on the natural extenders for artificial breeding of African catfish *Clarias gariepinus* (Burchell, 1822). *AACL Bioflux,* 3 (2): 119-124.

N'guessan, B.B., Amponsah, S.K., Dugbartey, G.J., Awuah, K.D., Dotse, E., Aning, A., Kukuia, K.K.E., Asiedu-Gyekye, I.J. and Appiah-Opong, R. (2018). *In Vitro* Antioxidant Potential and Effect of a Glutathione-Enhancer Dietary Supplement on Selected Rat Liver Cytochrome P450 Enzyme Activity. *Evidence-Based Complementary and Alternative Medicine,* 1-8. doi: 10.1155/2018/7462839.

Öğretmen, F., İnanan, B.E., Kutluyer, F. and Kayim, M. (2015). Effect of semen extender supplementation with cysteine on postthaw sperm quality, DNA damage, and fertilizing ability in the common carp (*Cyprinus carpio*). *Theriogenology,* 83: 1548-1552. https://doi.org/10.1016/j.theriogenology.2015.02.001

Sarosiek, B., Dryl, K., Kucharczyk, D., Żarski, D. and Kowalski, R.K. (2013). Motility parameters of perch spermatozoa (*Perca fluviatilis* L) during short-term storage with antioxidants addition. *Aquaculture International,* 22 (1): 159-165. doi:10.1007/s10499-013-9679-9