International Journal of Research in Agriculture and Food Technology

(IJORAF)

Journal Home page: www.ijoraf.com

Publishingrealtime

Effects of Semen Cryopreservation with Cucumber Fruit juice Fortified-extender on Milt Quality and Anti-oxidative Enzyme activity of *Hetreoclarias hybridfish*

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ARTICLE INFO

Article history:

Received September 27, 2022

Received in revised form October 10, 2022

Accepted October 25, 2022

2023 Available online November 13, 2022

Keywords:

Cryopreservation

Cucumber

fruit juice

Hetreoclarias hybridfish

Semen

oxidative enzyme activity

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https://doi.org/10.36265/ijoraf.2022.010102

ISSN– Online **2736-1411**

Print **2736-142X**

ABSTRACT

The present study investigated the quality and oxidative enzyme activity of catfish *Hetreoclarias hybrid* semen fortified with cucumber fruit juice. The highest milt fertility rate of 75.56% was recorded in 10% CJ on day 3 compared with the least rate of 56.21% in control on day 1. The least hatchability rate of 57.56% was recorded on control day 1 compared with the highest value of 75.07% in 10% CJ on day 2. The highest milt survival rate of 99.07% was recorded among the control group on day 3 compared with the least survival rate of 87.48% recorded on day 1 of 20% CJ. There was a significant difference ($P< 0.05$) between the CAT control and treatments of CJ. The lowest value of 0.29 µmolemm-1 mg protein-1 was recorded in control on days 1, 2 and 4 compared with the highest value of 0.34µmolemm-1 mg protein-1 in 15% CJ on day 4. There was no significant difference in SOD activity between the control and CJ. Recorded values ranged between 0,026-0.165 Umole-1mgprotein-1 in 15% on day 2 and 10% on day 1 respectively. There was no significant difference in LPO activity between the control and CJ. However, a range of 0.022-0.135 mMoleTbarsmm-1 protein-1 was recorded on day 1in 10% and day 4 in 15% respectively. 20% and 10% Cucumber fruit juice showed greater values in fertility rate than control at respective storage periods of 3 days in *Hetreoclarias hybridfish* semen, and could in combination with DMSO improve its cryopreservation at short periods.

1.0 Introduction

Increasing global demand for dietary fish protein has led to widespread overfishing in wild fisheries, resulting in a considerable decline in fish supply and, in some regions, outright depletion. Aquaculture has been the primary contributor to the rise in fisheries and aquaculture production, with an average annual growth rate of 5.3% between 2000 and 2018, hitting a record high of 82.1 million tonnes in 2018. (FAO, 2020). Animal proteins are vital for human health, yet they are severely lacking in the majority of the world, particularly in underdeveloped nations. An estimated one billion individuals, or one-third of the global population, lack access to high-quality protein (Adewolu et al., 2008). Around one in six people in the globe are undernourished, and the majority of them reside in developing nations with inadequate food production. The FAO recommends a daily minimum protein intake of 70 milligrammes, of which 35 milligrammes must come from animal protein (FAO, 2000). In Nigeria, the African catfish (Hetreoclarias hybridfish) is commonly cultivated. This species of fish is one of the most productive aquaculture species (FAO, 2000). It is well-known for its resilience to handling stress, capacity to withstand a broad variety of environmental parameters, wide dietary range, high fertility, rapid growth, disease resistance, large stocking densities under culture conditions, and high meat quality (Kryzyzosiak et al., 2000; Reza et al., 2011). Thus, it is necessary to store this breed's sperm so that it can be utilised for artificial insemination over a longer length of time. Artificial insemination using sperm that has been

kept is a promising approach for genetic improvement of this breed.

By saving energy, the extender prevents the activation of spermatozoa, preserving the vitality and viability of sperm. The extender also serves as a medium for cryoprotectants, which are substances that reduce cell damage caused by the production of intracellular ice crystals during cryopreservation during the freezing and thawing of sperm. Important stage in creating a cryopreservation technique for a species is identifying the type of cryoprotectant and its optimal concentration in extender medium (Muchlisin et al., 2009). According to reports, the use of fruit juices as cryopreservatives is suitable for the cryopreservation of key fish species. Moreover, numerous cryopreservation experiments have demonstrated that fruit juices result in a higher fertilisation and hatching rate than artificial cryopreservants.

The toxicity of a cryopreservative is dependent on its kind, concentration, temperature, and duration of exposure (Van et al, 2017). In order to preserve the viability of spermatozoa following collection in seminal plasma and long-term storage at low temperatures, sperm must be diluted with a suitable extender. The benefits of freezing catfish sperm are well-established. It is not only a useful management tool, but it also offers several benefits, such as stock protection from extinction due to sudden outbreaks of disease, natural disasters, or overexploitation; improvement in selective breeding so that stock can be maintained more economically and effectively; and experimental material for advanced studies, such as gene transfer.

Fruits are rich in natural antioxidants and include a variety of antioxidant compounds (Cao et al., 1996). Carotenoids, vitamins, phenolic compounds, and flavonoids comprise these antioxidants, which have been shown to work as singlet and triplet oxygen quenchers, free radical scavengers, and peroxide decomposers (Gardner et al., 2000). Oxidative stress is an imbalance between reactive oxygen species (ROS) and antioxidants inside cells and tissues. It has been observed that DMSO and other cyroprotectants act as chemo-preventive agents against cell injury damage (Kiwon et al., 2003; Ondei et al., 2009), but frequently produce oxidative stress. It has been shown that cucumber (Cucumis sativus) fruit juice and seed extracts exhibit significant levels of antioxidant and antibacterial properties (Sheila et al., 2018), making them potentially ideal for reducing the oxidative stress posed by DMSO in milk cryopreservation. Cryopreserved catfish sperm is more effective than natural sperm at fertilising eggs (Agarwal, 2011). Cryopreservation using extenders increases the volume of fish sperm so that it can be utilised for numerous inseminations (Agarwal, 2011). Many experimental trials revealed that cryopreserved sperm resulted in a much better percentage of fertilisation and hatchability than freshly retrieved sperm (Kovacs and Urbanyi, 2010). Determining the quality and oxidative stress of cryopreserved fish milk with cucumber juice as cryoprotectants was the objective of this investigation.

This study aims to examine the effect of semen cryopreservation with cucumber fruit juice fortified- Extender on milt quality, anti-oxidative enzyme activity, and the viability of Hetreoclarias hybridfish larvae fertilised by cryopreserved milt. The objectives of the study are to:

1) Evaluate the impact of adding various concentrations of cucumber fruit juice on the quality of cryopreserved milt from Hetreoclarias hybridfish.

2) Compare the anti-oxidative enzymes of cryopreserved fish sperm in cucumber fruit juice with DMSO as a control

2.0 Materials and Methods

2.1 Materials

In other to achieve the required objectives of this study, the following materials were used;

- Binocular microscope of various lens magnification
- Slide for sample view under the microscope
- Cover slide for placing and securing the sample so it can be viewed
- Matured male and female brood stock
- Razor blade for dissection
- Ovulin hormone for inducing ovulation in the female brood stock
- 5ml Syringe for hormone injection.
- Cryovials for semen collection
- Beakers for extender and diluents preparation
- PH meter for water pH determination
- Dissolved oxygen meter for water D.O determination.
- Scale balance for weight determination
- Oharus Coperation Digital chemical balance
- Bowls and nets (kakaban) for incubation.
- Hand gloves and nose mask, with lab coat
- Extender materials (saline solution, cucumber juice, sodium citrate and dimethyl sulphide).
- Blender for crushing the fruits into fine liquid
- Sieve for separating the chaff from the liquid
- Two hand towels for capturing broodstock
- Masking tape and maker for labeling
- Atmospheric thermometer for temperature determination
- Programmable deep freezer to achieve freezing at -30^{0}C.
- Spectrophotometer for determining the oxidative stress

2.2 Research Area

This research was carried out at Ekohtim farm Ihe in Agwu Local government area of Enugu State. The location lies at 6.2327^o N and 7.4544^o E. The average daily temperature was 26^{o}C and the annual rainfall of 1800mm to 2100mm.

2.3 Experimental Methods

2.3.1 Diluent preparation

Diluents (mixture of Extender and Cryoprotectant) was prepared in the first day of the research and stored in a refrigerator at 4^{0}C for 24hrs before use following the method of Argwal *et al*., 2009. 2% of Dimethyl Sulphide (DMSO) was prepared and used as Cryoprotectant. The 2% Dimethyl sulphide was prepared by addition of 2mls of DMSO to 98mls of saline solution.

2.3.2 Preparation of cucumber fruit juice

The fruit-juice was prepared according to the procedure by Adeyemo *et al*., (2007) with some modifications: fresh cucumber was washed thoroughly using distilled water and thereafter cut into pieces and the seeds removed and then the fruit were blended for five minutes, placed in a sieve and pressure was applied manually to squeeze out the juice from the blended fruit. The supernatant fluid obtained were decanted into a clean beaker and used immediately for the experiment.

2.3.4 Preparation of buffer

About 100ml of distilled water was heated to boiling point and allowed to cool under running tap water. The preparation of 2.9% weight/volume (w/v) of sodium citrate was then made by using 2 x 2.9g of sodium citrate. This amount was dissolved in 200ml flat bottomed flask. This solution was then shaken together and allowed to cool for 3 - 5 minutes.

2.3.5 Semen Extraction

The method of Agarwal, (2011), was used in the present study. Semen was extracted by dissecting male broodstocks from the abdomen towards the pointed papilla, using a new sharp razor blade to dissect the testis. The male brood stock was first weighed to be 1.2kg and was cleaned with a clean towel before dissection. The testis obtained was washed with saline solution to remove bloodstains and mucus in order to

avoid contamination. Thereafter, the testis was opened into a cryovial to obtain of the semen.

2.3.6 Method of Semen Dilution

Milt obtained from adult male fishs was diluted with the prepared cryoprotectant at ratio of 1:20 containing no inclusion of the cucumber fruit juice which serves as the control but fortified with 5%, 10%, 15% and 20% fruit juice from cucumber fruit juice respectively in treatments 2, 3, 4 and 5.

Five beakers were washed and rinsed with saline solution, then with a 5ml syringe, diluents was collected and poured into the five beakers and subsequently cryovials containing catfish semen was gradually emptied into beakers accordingly, to avoid osmolarity and pH shock. The beaker containing the diluted semen was shaken to obtain even dilutions before storage.

2.3.7 Method of stripping

The female broodstock was held by two persons, then and small pressure was applied on the abdomen of the fish and the eggs freely flowed out and was collected with five dry 100ml beaker. Each beaker contained about 12g of stripped eggs. After wards the female broodstocks was kept, and then fertilization commenced. After each egg collection with each beaker, the eggs were fertilized with 1.0ml of the cryopreserved semen. 1.0ml of semen was spread on the eggs, afterwards it was gently stirred to ensure even fertilization. Afterwards the three replicates of the fertilized eggs were incubated, Agarwal, (2011).

2.3.8 Method of Freezing

The stored semen in cryovial (Syringe) was laid in the Freezer, before then the freezer was allowed to freeze up to -30^{0}C before the semen was kept. The semen was stored in the freezer at -30^{0}C for 4days but at the end of each day, each tube containing the cryopreserved semen was thawed and examined its quality.

2.3.9 Semen Storage

Ampoules/vials/visotube method was used in storing semen for freezing (Mounibs, 1978). The diluted semen was stored with syringe (visotube). This was done for the five beakers containing the diluted semen, therefore five syringes were used to store the semen for freezing. The volume of semen in each syringe was 4ml. After the storage, the semen were allowed to stay for equilibration before freezing. The semen was kept in the fridge at 4^{0}C for equilibration. The period of this equation is termed "equilibrium time. This is the time allowed to facilitate the penetration of Cryoprotectant into the cells for effective protection during freezing. (Grout and Morris 1987). The semen was allowed to equilibrant for 45mins with the time of gradual addition of diliuent to semen and storage taken into account. Equilibrium time was not longer than 45 – 60mins to maximize later recovery. The most appropriate equilibrium time is 45mins for DMSO (Sanni, 2008).

2.3.10 Thawing and artificial fertilization with cryopreserved semen

The semen was thawed in a water bath at 57^{0}C for 10secs. But prior to the time of removal from the freezer, hot water was boiled to be used for the thawing (warming). The hot water was used for thawing at 57^{o}C for 10secs according to (Rana 1995). In several fresh water fish species, thawing is done at 30 – 80^{0}C in a water bath. The tube containing the cryopreserved semen was laid in the water for 10secs. before used for artificial fertilization and quality evaluation.

2.3.11 Method of Incubation

The same incubation method observed earlier was still observed. This had three replicates contained in a kakaban with 5l of water in a bowl. At the time of this incubation was 24hrs of incubation done in day4 of this research with fertilized eggs of cryopreserved semen. Then great hatchability was observed within 20hours. This was observed in the three replicates and hatchability was evaluated and data was recorded.

2.4 Experimental Design and Procedure

The experiment was arranged in a completely randomized design (CRD) with five (5) treatments and each replicated three (3) times. The experiment lasted for 1 week.

Sperm viability

Fertilized eggs incubated for 24h at appropriate temperature and pH of water. Fertilization, hatchability and survival rate of larvae was used to assess the viability of the milt.

$$\text{Fertilization rate (\%)} = \frac{\textit{Number of egg cells hatched}}{\textit{Total number of egg cells counted}} \times \frac{100}{1}$$

$$\text{Hatchability rate (\%)} = \frac{\textit{Number of egg cells hatched}}{\textit{Total number of egg in a batch}} \times \frac{100}{1}$$

$$\text{Survival rate (\%)} = \frac{\textit{Number of hatchling alive to larval stage}}{\textit{Total number of hatchling}} \times \frac{100}{1}$$

2.5 Oxidative Stress Parameters

Samples were analyzed for changes in catalase activity (CAT), superoxide dismutase (SOD) and lipid peroxidase (LPO) using AP – 120 spectrophotometer.

Determination of Catalase Activity

Catalase activity was determined according to Sinha, (1972). 5g of potassium was dissolved in 100m of distilled water, 2ml of acetic acid was added. Hydrogen perioxide was prepared using 0.9ml of phosphate buffer at pH of 7.4 and 0.2ml was added to each test tube containing 0.1ml of sample. 2ml of potassium dichromate was also added and immediately stop watch was started and absorbance at 530nm for 15, 30, 60, 90 and 120 seconds against the blank (H_2O).

$$\text{Catalase activity (CAT)} = \frac{2.303}{DT} \times \frac{Log\ A1}{A1\ A2}$$

Where

A1 = Initial absorbance

A2 = Final absorbance (Sinha, 1972)

Determination of Lipid Peroxidation (LPO)

Lipid Peroxidation (LPO) was determined according to Wallin *et al,* (1993) by estimation of thiobarbitunic acid reactive substance (TBARS), 0.5ml of sample was added, 0.5ml of 10% TCA and 0.5ml of 10% TBA was also added and boiled for 30 minutes. Then allowed to cool, after cooling, 1ml of distilled water added for the blank.

Absorbance was read at 532nm and 600nm wavelength and results taken.

$$\text{LPO} = \frac{\textit{Reading at 532} - \textit{reading at 600nm} \times 2 \times 10}{10066}$$

Determination of Superoxide Dismutase (SOD)

Superoxide Dismutase (SOD) was determined according (Eldred and Hoffert, 2001), in the preparation of blank, 0.5ml of bicarbonate was added to 0.5ml of EDTA and 1ml of water and incubated for 5minutes at room temperature, while for the 0.5ml of sample was added with 1ml of distilled water incubated at room temperature for 3 minutes and 0.3ml of

Ephinephrine was added and immediately absorbance was read at 480nm for 30 minutes and records taken (Eldred and Hoffert, 2001).

2.5 Statistical Analysis

Data was collected using Statistical Package for Social Sciences (SPSS) computer package, version 20.0 and subjected to two-way analysis of variance (ANOVA). Mean differences was separated using the Duncan Multiple Range Test.

3.0 Results

The results of milt fertility rate, hatchability rate, survival and oxidative stress is given in table 1 below.

Table 1: Milt Fertility Rate, Hatchability Rate, Survival Rate, CAT, SOD and LPO Using Varying Levels of Cucumber Juice for 4 Days

Parameters	**Cucumber Juice (%)**	**Days**			
		1	2	3	4
Fertility rate (%)	Control	56.21 ± 0.65^{Aa}	67.79 ± 0.92^{Dd}	64.43 ± 0.38^{Ac}	59.38 ± 0.96^{Ab}
	5	59.38 ± 0.96^{Ba}	59.35 ± 0.59^{Aa}	71.46 ± 0.88^{Cc}	67.31 ± 1.02^{Cb}
	10	62.34 ± 0.38^{Ca}	62.04 ± 2.54^{Ba}	75.56 ± 1.41^{EC}	72.21 ± 0.68^{Eb}
	15	65.64 ± 0.65^{Da}	66.43 ± 4.91^{Ca}	73.89 ± 0.79^{Dc}	70.91 ± 0.11^{Db}
	20	62.84 ± 0.35^{Ca}	75.27 ± 0.59^{Ed}	70.25 ± 1.86^{Bc}	65.01 ± 0.01^{Bb}
Hatchabilityrate (%)	Control	57.56 ± 0.82^{Aa}	66.38 ± 0.42^{Ad}	64.44 ± 0.89^{Ac}	59.40 ± 0.41^{Ab}
	5	60.69 ± 0.71^{Ba}	71.00 ± 0.07^{Bd}	66.65 ± 0.26^{Bc}	62.63 ± 0.46^{Bb}
	10	63.27 ± 0.65^{Ca}	75.07 ± 0.42^{Ed}	70.14 ± 0.78^{Dc}	65.11 ± 0.59^{Cb}
	15	63.52 ± 1.32^{Ca}	73.39 ± 1.20^{Cd}	70.88 ± 0.94^{Dc}	65.61 ± 0.88^{Cb}
	20	63.75 ± 1.11^{Ca}	74.32 ± 0.37^{Dd}	69.75 ± 0.55^{Cc}	65.33 ± 1.08^{Cb}
	Control	97.69 ± 0.42^{Da}	99.06 ± 0.17^{Dc}	99.06 ± 0.29^{Dc}	98.58 ± 0.30^{Db}
Survival rate (%)	5	91.84 ± 0.84^{Cb}	94.49 ± 0.43^{Cd}	93.48 ± 0.54^{Cc}	89.52 ± 1.74^{Ca}
	10	88.52 ± 0.32^{Bb}	89.35 ± 0.44^{Bc}	88.55 ± 0.46^{Ab}	87.97 ± 0.43^{Aa}
	15	87.94 ± 0.66^{Aa}	88.52 ± 1.21^{Ab}	89.38 ± 0.96^{Bc}	87.81 ± 0.62^{Aa}
	20	87.48 ± 0.67^{Aa}	88.78 ± 0.95^{Ab}	89.30 ± 0.60^{Bc}	88.05 ± 0.55^{Bb}
Catalase (umolemm-1 mg protein)	Control	0.29 ± 0.01^{Aa}	0.31 ± 0.03^{Bb}	0.29 ± 0.01^{Aa}	0.29 ± 0.01^{Aa}
	5	0.31 ± 0.01^{Bb}	0.30 ± 0.00^{Aa}	0.30 ± 0.01^{Ba}	0.30 ± 0.00^{Ba}
	10	0.32 ± 0.01^{Ca}	0.32 ± 0.01^{Ca}	0.32 ± 0.12^{Ca}	0.33 ± 0.01^{Cb}
	15	0.33 ± 0.00^{Da}	0.33 ± 0.01^{Da}	0.33 ± 0.01^{Da}	0.34 ± 0.01^{Db}
	20	0.31 ± 0.01^{Ba}	0.33 ± 0.01^{Dc}	0.32 ± 0.01^{Cb}	0.33 ± 0.00^{Cc}
SOD (Umole-1mgprotein-1)	Control	0.05 ± 0.00^{Aa}	0.05 ± 0.01^{Aa}	0.05 ± 0.00^{Aa}	0.06 ± 0.01^{Aa}
	5	0.06 ± 0.00^{Aa}	0.06 ± 0.00^{Aa}	0.56 ± 0.01^{Bb}	0.05 ± 0.01^{Aa}
	10	0.16 ± 0.03^{Bb}	0.56 ± 0.00^{Bc}	0.06 ± 0.01^{Aa}	0.06 ± 0.00^{Aa}
	15	0.07 ± 0.17^{Aa}	0.03 ± 0.00^{Aa}	0.06 ± 0.00^{Aa}	0.06 ± 0.01^{Aa}
	20	0.56 ± 0.00^{Cb}	0.05 ± 0.00^{Aa}	0.06 ± 0.00^{Aa}	0.05 ± 0.00^{Aa}
LPO (mMoleTbarsmm-1protein -1)	Control	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.01 ± 0.00^{A}	0.01 ± 0.00^{A}
	5	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.01 ± 0.00^{A}	0.01 ± 0.00^{A}
	10	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.01 ± 0.00^{A}	0.01 ± 0.00^{A}
	15	0.01 ± 0.00^{A}	0.01 ± 0.00^{A}	0.01 ± 0.00^{A}	0.01 ± 0.00^{A}
	20	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.01 ± 0.00^{A}	0.01 ± 0.00^{A}

Same superscript on a row and column did not differ but different superscripts differed significantly at $p< 0.0$

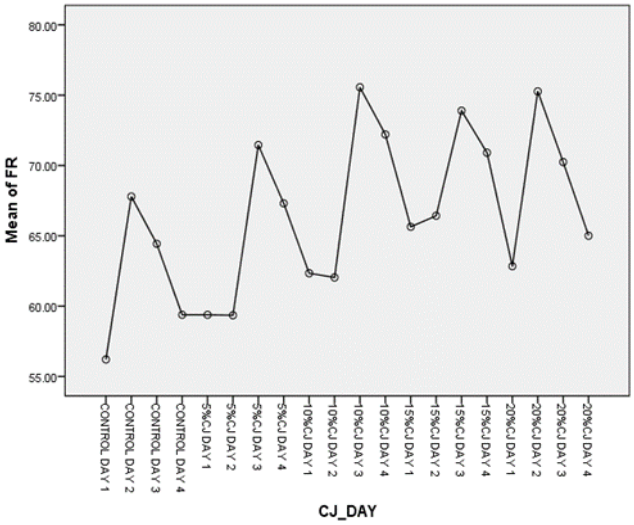

Figure 1: Fertility Rate

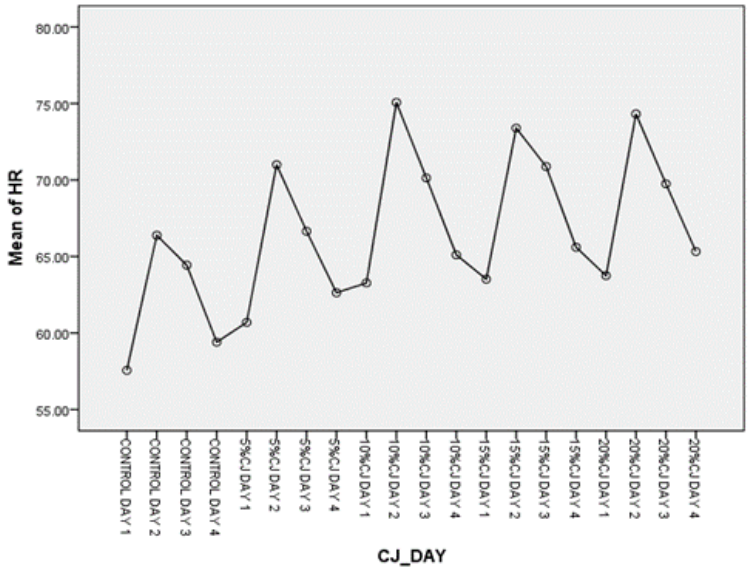

Figure 2: Hatchability Rate

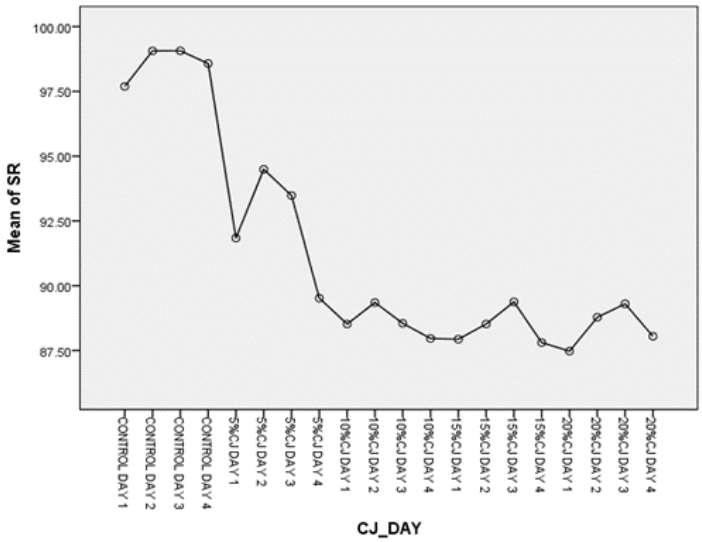

Figure 3: Milt Survival Rate

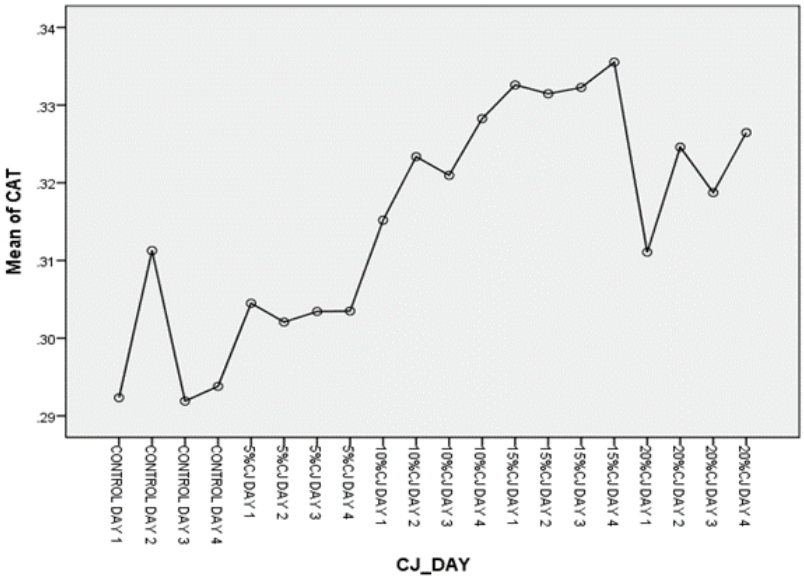

Figure 4: Catalase Activity

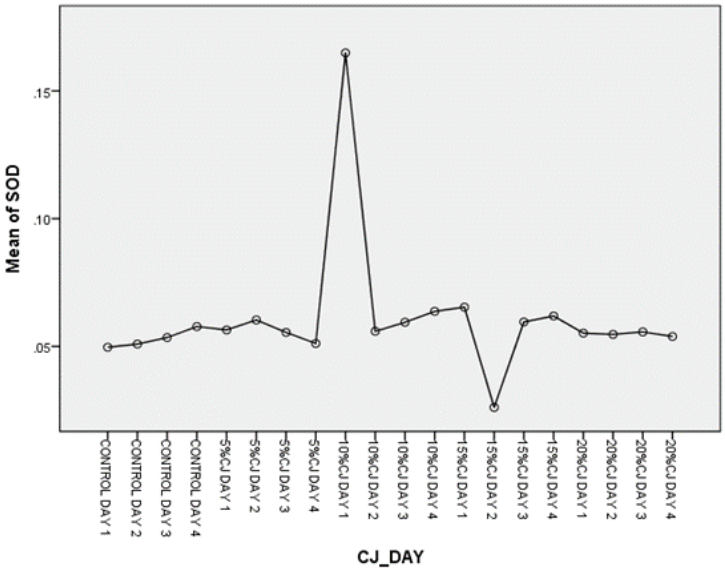

Figure 5: Superoxide Dismutase

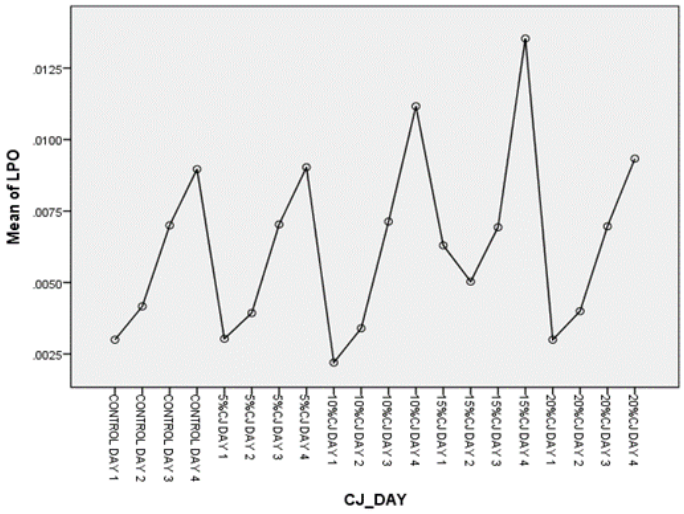

Figure 6: Lipid Peroxidase

4.0 Result and Discussion

The use of fruit juices as cryopreservatives is suitable for the cryopreservation of key fish species (Heinstra et al., 2005; Horvath; Ubanyi, 2009; Chidobem et al., 2022). Moreover, numerous cryopreservation experiments have demonstrated that fruit juices result in a higher fertilisation and hatching rate than artificial cryopreservants. From the above table and figures, the fertility and hatchability rate was significantly elevated. The highest milt fertility rate of 75.56% recorded in 10% CJ on day 3 compared with the least rate of 56.21% in control on day 1. There was a significant difference ($P<$ 0.05) between the control and treatments of CJ The least hatchability rate of 57.56% recorded in control day 1 compared with the highest value of 75.07% in 10% CJ on day 2 There was a significant difference ($P<$ 0.05) between the control and treatments of CJ. Cryopreserved catfish sperm is more effective in fertilising eggs than wild sperm (Agarwal, 2005; Agarwal, 2011). Cryopreservation using extenders increases the volume of fish sperm so that it can be used for numerous fertilisations (Agarwal, 2011). Many experimental trials revealed that cryopreserved sperm resulted in a much better percentage of fertilisation and hatchability than freshly retrieved sperm. (Kovacs and Urbanyi, 2010; Ezike et al., 2019).

Van Vuren and Steyn (2017) stated, nonetheless, that the quantity, kind, concentration, temperature, and exposure time of the fruit juice serve to evaluate the survival of the milt. The highest milt survival rate of 99.06% was recorded among the control group on day 2 which compared with the least survival rate of 87.48% recorded on day 1 of 20% CJ. As seen in the table 1 and figure 3 above, significant differences ($p < 0.05$) was shown to be higher in the control.

Cryopreservation of African catfish sperm often yields improved motility and fertilising capacity following thawing in species such as Hetreoclarias hybridfish; this was demonstrated on 10% CJ day 3 with a fertility rate of 75.56 1.41096. With a mean embryo survival of 80%, than in freshwater species such as Atlantic salmon, where embryo survival using cryopreserved sperm is approximately 65%. (Suquet et al., 2000; Gwo, 2011).

Catalase activity (CAT) in semen enriched with fruit juice increased significantly compared to control and cucumber juice- enriched semen. The trend was witnessed throughout the duration of the study as shown in figure 4. There was a significant difference ($P<$ 0.05) between the control and

treatments of CJ. The lowest value of 0.29 µmolemm-1mgprotein-1 recorded in control on day 3 compared with the highest value of 0.34 µmolemm-1mgprotein-1in 15% CJ on day 4.

Similarly, dismutase superoxide (SOD) increased significantly among semen enhanced with fruit juice compared to control throughout the experimental period. There was significant difference in SOD activity between the control and CJ. Recorded values ranged between 0.026-0.164 Umole-1mgprotein-1 in 15% on day 2 and 10% day 1 respectively.

The CAT-SOD system of enzyme in the semen may have successfully eliminated reactive oxygen species ROS by a trigger from fruit antioxidants created during storage (Krzyzosiak et al., 2000), hence limiting the induction of LPO as shown in table 1 and figure 6. Significant differences as seen in the above table and figure 6 did not exist ($p>0.05$) between control in all the treatment days. However, a range of 0.0022-0.0135 mMoleTbarsmm-1protein-1 was recorded on day 1in 10% and in 15% day 4 respectively

Carotenoids, vitamins, phenolic compounds, and flavonoids are fruit antioxidants that have been shown to work as singlet and triplet oxygen quenchers, free radical scavengers, and peroxide decomposers (Anghel et al., 2010; Daramola et al., 2016;). Due to the fact that the majority of artificial cryoprotectants used have an oxidative effect on fish milt spermatozoa, the preservation of fish spermatozoa has become a challenging endeavour. Cucumber (Cucumis sativus) and orange (Citrus sinensis) are fruit-rich natural antioxidants recognized for their high vitamin and antioxidant content (Cutis et al., 2013; Reda et al., 2016; Okiyele et al 2019).

Hence, the use of cucumber fruit juice could facilitate the cryopreservation of fish sperm, namely that of Hetreoclarias hybridfish (Adeyemo et al 2007; Boryshpolets et al., 2011; Liu et al., 2015).

5.0 Conclusion

The control indicated higher survival rates than cucumber fruit juice. But 10% cucumber fruit juice showed greater values in hatchability and fertility rate than control at respective storage periods for 2 and 3 days in *Hetreoclarias hybridfish* semen. Cucumber fruit juice could in combination with DMSO improve its cryopreservation in 4 days.

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