Effect of cucumber juice fortification of dextrose saline extender on rooster sperm quality, oxidative stability, and kinetics

O.A. Jimoh[1,*], C.U. Nwachukwu[2], A. Patrick[1], O.O. Joseph[3] and I.U.G. Daureen[4]

[1] Agricultural Technology Department, Federal Polytechnic Ado Ekiti, Ado Ekiti, Ekiti State 360241, Nigeria

[2] Department of Agricultural Science Education, School of Vocational and Technical Education, Alvan Ikoku University of Education, Owerri, Imo State 475113, Nigeria

[3] Department of Animal Science, Adekunle Ajasin University, Akungba Akoko, Ondo State 342111, Nigeria

[4] Animal Physiology and Bioclimatology, Department of Animal and Environmental Biology, Kingsley Ozumba Mbadiwe University, Ideato, Imo State 475113, Nigeria

* Corresponding author: abubakarjimoh2011@gmail.com
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ABSTRACT

Background and Objective: Oxidative stress affects the quality of sperm cells and the success of fertilization. The use of natural antioxidants such as cucumber juice protects sperm cells from oxidative stress and increases their viability and motility. This study evaluates the efficacy of cucumber juice dextrose saline extender on sperm quality, oxidative stability, and spermatozoa kinematics in roosters.

Methodology: Thirty ISA Brown breeding roosters, aged 35–40 weeks, were used in the study. Various extenders were prepared with differing concentrations of cucumber juice (0%, 10%, 20%, 30%, 40%, and 50%) and dextrose saline. Mature cucumber fruits were processed to extract juice, which was then stored at 4°C. The roosters' sperm was collected and diluted with these extenders in a completely randomized design. The analysis focused on sperm kinetics (motility traits: progressive motility, percentage motility, and non-progressive motility; speed traits: average path velocity, curvilinear velocity, and straight-line velocity; and trajectory traits: straightness, linearity, beat cross frequency, and amplitude of lateral head) using a computer-assisted sperm analyser (CASA) and examined oxidative stress markers (n=10) through standard assays.

Main Results: The study revealed percentage motility, curvilinear velocity, and amplitude of lateral head were significantly higher in 10%, 20%, 30%, and 40% cucumber juice extenders compared to 0% and 50%. Additionally, the average path velocity and non-progressive motility were highest in the 30% extender. The antioxidant activity of rooster sperm decreased over time, with significant differences observed between 0-hour and 5-hour measurements. The antioxidant activity was highest initially and diminished progressively in all cucumber juice concentrations. The lipid peroxidation rate of raw semen was highest across the extenders at 5 hours, followed by 40% and 50%, which were higher than those on 0%, the least was observed in those in 30%.

Conclusions: The 10%, 20%, and 40% cucumber juice in dextrose saline extenders are more effective for enhancing progressive motility, while the 30% extender showed the highest kinematic performance. This indicates that cucumber juice in a dextrose saline extender is suitable for rooster semen, as it promotes motility and optimally enhances kinematics properties at 30%.

Keywords: Antioxidants, artificial insemination, oxidative stress, cucumber juice, spermatozoa

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INTRODUCTION

In the field of poultry breeding, crossbreeding plays a crucial role in increasing genetic diversity and improving flocks (Fouad *et al*., 2020). The key to this process is the development of good sperm diluents to preserve sperm cell integrity and viability during storage and transportation. Rooster semen is a valuable ingredient in poultry breeding programs. However, the quality of chicken sperm can be affected by many factors, and this includes the diluent used to store it (Balogun *et al*., 2017) as well as treatment to improve sperm quality and fertility in farm animals, which has gained popularity in recent times.

Cucumber (*Cucumis sativus* L.) is a widely used vegetable known for its high moisture content and a multitude of health benefits. Cucumber has been reported to have numerous health benefits due to its high content of vitamins, minerals, and other essential nutrients that contribute to its therapeutic properties (Sharma *et al*., 2020). They are low in calories and contain very little fat. In recent years, research on the reproductive biology of farm animals has attracted attention with particular, focus on improving sperm quality for efficient reproduction. Previous studies have shown that adding cucumber juice to sperm thinners can improve sperm count, motility, and morphology, thereby improving overall sperm quality (Daramola and Adekunle, 2015).

Importantly, oxidative stress causes damage to sperm cells by inducing DNA fragmentation and lipid peroxidation, making it an important factor in fertility and sperm quality (Jimoh, 2022). Cucumber is rich in a variety of antioxidants and other phytonutrients including ascorbic acid, carotenoids, and flavonoids, which can eliminate reactive oxygen species (ROS) and prevent oxidative damage in sperm cells. Cucumber supplementation will reduce oxidative stress thereby improving sperm quality which will lead to successful insemination (Sabeti *et al*., 2016). Additionally, the antioxidant properties of cucumber may improve fertility by helping to reduce oxidative stress and protect sperm integrity (Appiah *et al*., 2020; Leão *et al*., 2021).

Sperm movement patterns, known as kinematics, are essential for successful fertilization (Jimoh *et al*., 2021b). Cucumber contains bioactive substances that can affect sperm motility and increase sperm percentage through its hydration properties. These compounds increase the ability of sperm to travel through the female reproductive system and reach the point of fertilization. Also, added cucumber juice extender has been shown to increase the chances of successful fertilization by increasing the speed and linearity of sperm movement (Daramola and Adekunle, 2015). Additionally, the anti-inflammatory properties of cucumber can reduce pain in the male reproductive system and improve sperm quality and function. The high moisture content of cucumber can improve sperm quality by providing a higher surface area for sperm motility and nutrients to sperm cells. Using cucumber juice in sperm storage is effective in improving sperm quality, reducing oxidative stress, and improving sperm motility (Daramola *et al*., 2016). However, studies are showing the effectiveness of soursop and citrus juices on sperm quality, oxidative activity, and sperm motility in roosters (Jimoh and Nwachukwu, 2022). These studies showed that the addition of soursop and citrus juices to the uncontaminated sperm of roosters helped to improve sperm fertility, oxidative activity, and sperm kinetics outcomes. While lipid peroxidation decreased in fruit juice, lipid peroxidation increased in dehydrated sperm and 0% fruit juice extender. Sweet orange and tangerine juices inhibit lipid peroxidation in rooster sperm, increase sperm motility, and control rooster sperm kinetics (Jimoh *et al*., 2020). In particular, average path velocity, non-progressive motility, straight-line velocity, curvilinear velocity, liveability, wobble, and amplitude of lateral head parameters were no different from soursop juice diluents compared to undiluted semen collected from roosters two hours after dilution as amplitudes increased significantly.

In addition, another study investigated the effect of sperm cryopreservation using cucumber fruit juice enriched extender on the quality, motility, and oxidase activity of fish sperm and the study confirmed that the enriched extender had a positive

effect on the quality and viability of catfish sperm (Chidobem *et al*., 2022). A study on rabbit sperm diluted with watermelon juice found that it did not affect sperm quality or fertility (Jimoh and Ayedun, 2020). Another study investigated the antimicrobial effect of pineapple and cucumber juices on sperm motility and sperm storage of West African Dwarf bucks and the study showed that pineapple and cucumber juices extenders have inhibitory effects on sperm motility in sperm frozen at 5°C, leading to higher sperm count compared to control (Daramola *et al*., 2016). The above findings suggest that cucumber juice supplementation in rooster sperm may have beneficial effects on sperm motility. However, the effectiveness of cucumber juice in improving sperm quality provides a good way of improving fertility in animals. The effect of cucumber juice dextrose saline extender on sperm quality, oxidative activity, and sperm motility in rooster sperm has not been studied. This study aims to investigate the effectiveness of cucumber juice as a semen supplement on sperm quality, oxidative activity, and sperm motility.

MATERIALS AND METHODS

Experimental Design and Animal Management

This study was conducted with Institutional Ethics Committee approval, and approval number is IACUC-FPA/EC/19/0043. The guidelines of the National Institutes of Health for the care and use of laboratory animals were followed, and appropriate measures were taken to minimize pain or discomfort in the animals.

The study was conducted between June 2022 and August 2022. In this study, 30 ISA Brown breeding roosters, respectively 35-40 weeks old, from a reputable breeder farm in Ibadan, Nigeria were used. Breeder roosters were housed in clean, well-ventilated pens with controlled temperature and humidity to minimize stress. They were fed a balanced diet specifically formulated for breeders, ensuring adequate protein, vitamins, and minerals, as well as constant access to fresh water. Regular health checks and biosecurity measures were implemented to maintain optimal health and prevent disease outbreaks.

Cucumber Juice Preparation

Matured cucumber fruits were purchased, washed, and extracted for juices as described earlier by Jimoh and Ayedun (2020) using a juice extractor (Kenwood, UK) by centrifuging at 4,000 g for 15 minutes. The resulting supernatant obtained as cucumber juice was kept frozen at 4°C within 24 hours in disposable 5 mL sterile vials. Dextrose saline contains 5% dextrose in 0.9 normal saline (Unique Pharmaceuticals, Nigeria) was purchased for this study.

Extension of Semen with Extenders and Evaluation

Before sperm collection, 30 roosters were trained using the dorsal-abdominal massage technique for 2 weeks, and then sperm were collected twice a week at intervals of 3 days and taken to the laboratory for *in vitro* analysis. All roosters were evaluated for fertility and only 26 roosters with good fertility [high motility (> 90%), viability (> 90%), and kinetic traits with high sperm concentration (5×10^8 sperm cells)] were used, taking care to avoid contamination of sperm from cloacal material (such as feces). Seven treatments were created with different diluents as previously outlined in Jimoh (2020) and Jimoh *et al*. (2020; 2021a; 2021b) as follows:

Treatment 1: Raw semen (undiluted semen)
Treatment 2: Dextrose saline + 0% Cucumber juice extender
Treatment 3: Dextrose saline + 10% Cucumber juice extender
Treatment 4: Dextrose saline + 20% Cucumber juice extender
Treatment 5: Dextrose saline + 30% Cucumber juice extender
Treatment 6: Dextrose saline + 40% Cucumber juice extender
Treatment 7: Dextrose saline + 50% Cucumber juice extender

The sperm mixture was dispersed in a completely randomized design and diluted with the treatment as previously described. The diluted

samples were replicated 3 times and were mixed slowly, equalized by sperm processing standards, and immediately processed for sperm analysis. The dilution ratio was 1:2 (example: diluent). Semen analysis and oxidative status assay were evaluated for each treatment. Semen analysis was conducted immediately after extension, oxidative status assay was performed at zero hour until the test results were negative as semen quality (5 hours).

According to the treatment method, extended semen was evaluated for sperm kinetics using computer-assisted sperm analyser (CASA) where n=10 (SpermAnalyzeWin7 Xuzhon city, China, setting of CASA as in 5th WHO manual, 51 sperm tracks, evaluated magnification × 10, image acquisition rate: number frames/s 60), the temperature of pipette tips, petri dish, media, counting chambered slides (10–20 μm deep), and microscope stages were kept at 37°C. The CASA used in this study is suitable for rooster sperm analysis and is similar to the analysis of van der Horst and du Plessis (2017): sperm concentration, progressive motility, percentage motility, non-progressive motility, average path velocity (μm/s), curvilinear velocity (μm/s), straight-line velocity (μm/s), straightness, wobble, linearity, beat cross frequency (Hz), the amplitude of lateral head (μm), and liveability was determined using routine methods. Sperm concentration (replicates each sample) was determined using a Neubauer hemocytometer (TH-100; Hecht Assistant, Sondheim, Germany) and showed sperm $\times 10^8$/mL. Liveability was determined by placing the semen drop on a slide, adding a drop of eosin-nigrosin dye, and mixing gently, then smeared on a slide, air drying it, and viewing it under a microscope at 400x magnification. Acrosome integrity refers to the health of the acrosome, the cap-like structure on the head of the sperm that contains enzymes crucial for penetrating the egg during fertilization. A smear of the semen sample was prepared on a microscope slide and stained with Giemsa stain. The stained slide was observed under a microscope at 1000x magnification.

The number of sperm heads with intact acrosomes and those with damaged acrosomes were observed. Acrosome integrity was measured as a percentage of sperm with intact acrosome divided by total number of sperm counted multiplied by 100.

Oxidative Stress Status

Another batch of each treatment sample was prepared and centrifuged at 4,000 rpm for 15 minutes to separate seminal plasma. The diluted semen sample was centrifuged, and the resulting seminal plasma was analyzed for total antioxidant activity and lipid peroxidation using a standard protocol. Sperm lipid peroxidation assay according to Ohkawa *et al*. (1979) contained 3.0 mL of the reaction mixture in a total volume containing 1.0 mL of seminal plasma and 1.0 mL of TCA (0.67%). Place all tubes in a boiling water bath for 45 minutes. Transfer the tube to an ice bath and centrifuge at 2,500 rpm for 10 minutes. The amount of malondialdehyde (MDA) produced in each sample was assessed by optically measuring the density of the supernatant at 532 Nm.

The total antioxidant capacity of seminal fluid was determined according to Koracevic *et al*. (2001) by the reaction mixture (permeabilized) containing 0.5 mL of sodium benzoate (10 mmol/L), 0.2 mL of H_2O_2 (10 mmol/L), 0.49 mL of phosphate buffer [(100 mmol/L, pH = 7.4; prepared by mixing 19.5 mL of KH_2PO_4 (100 mmol/L) with 80.5 mL of Na_2HPO_4 (100 mmol/L), then adjusted the pH to 7.4 and added 0.2 mL of Fe-EDTA complex (2 mmol/L) freshly prepared in equal amounts of EDTA (2 mmol/L)], and ferrous ammonium sulphate (2 mmol/L) were mixed and left at 25°C for 60 minutes. Ten microliters of the seminal plasma were added to the reactive mixture and left to incubate at 37°C for 60 minutes. At the end, 1 mL glacial acetic acid (20 mmol/L) and 1 mL thiobarbituric acid (0.8% w/v, dissolved in 100 mL of 50 mmol/L NaOH) were added, and its absorbance was measured at 532 Nm spectrophotometrically after incubation at 100°C for 10 minutes. Total antioxidant (TA) capacity was calculated according to the following formula: TA capacity (mmol/L) = (CUA)(K – A) / (K – UA); where CUA (mmol/L) is the uric acid concentration, K is the absorbance of control (K1 − K0), A is the absorbance of the sample (A1 − A0), UA is the absorbance of uric acid solution (UA1 − UA0).

Statistical Analysis

The data obtained were analyzed using descriptive statistics and analysis of variance. The results were statistically significant at $\alpha = 0.05$, with mean differences separated using Duncan's new multiple range test from the General Linear Model procedure of the IBM SPSS 20. The statistical model was as follows: $y_{ijl} = \mu + B_i + e_{ijl}$; where y_{ijl} represents sperm kinetics and oxidative stability values measured in i^{th} diluted sperm, μ is the overall mean of each variable, B_i is the fixed effect of i^{th} rooster sperm diluted in cucumber juice-based dextrose saline (i = T1 undiluted semen (positive control), cucumber juice in dextrose saline at 0%, 10%, 20%, 30%, 40% and 50% shown as T2, T3, T4, T5, T6 and T7, respectively), and e_{ijl} is a random residual effect.

RESULTS AND DISCUSSION

The quality/characteristics of rooster semen in cucumber juice-dextrose saline diluent at room temperature is shown are Table 1. Percentage motility, curvilinear velocity and amplitude of lateral head of undiluted semen (91.60 ± 1.47%, 19.46 ± 2.56 µm/s, and 0.51 ± 0.02 µm), T3 (90.25 ± 1.58%, 18.13 ± 1.36 µm/s, and 0.49 ± 0.04 µm), T4 (91.20 ± 2.58%, 16.99 ± 2.33 µm/s, and 0.50 ± 0.20 µm), T5 (92.21 ± 0.37%, 16.74 ± 2.02 µm/s, and 0.60 ± 0.01 µm), and T6 (92.11 ± 0.78%, 19.13 ± 2.11 µm/s, and 0.56 ± 0.02 µm) cucumber juice dextrose saline-based extenders were significantly higher ($P < 0.05$) than T7 (79.85 ± 2.35%, 8.09 ± 0.36 µm/s, and 0.31 ± 0.03 µm) and T2 (72.46 ± 2.04%, 7.17 ± 1.69 µm/s and 0.28 ± 0.00 µm) cucumber juice dextrose saline-based extenders. Progressive motility of undiluted semen, T3, T4, and T6 cucumber juice dextrose saline-based extenders were significantly higher ($P < 0.05$) than T2, T5, and T7. Average path velocity, non-progressive motility and straight-line velocity of T5 cucumber juice dextrose saline-based extenders were significantly higher ($P < 0.05$) than other different cucumber juice dextrose saline-based extenders compared to undiluted semen. The linearity of T2 cucumber juice dextrose saline-based extender was significantly higher ($P < 0.05$) than other different cucumber juice dextrose saline-based extenders compared to undiluted semen. Straightness of T2, T5, and T7 cucumber juice dextrose saline-based extenders were significantly higher ($P < 0.05$) than other cucumber juice dextrose saline-based extenders compared to undiluted semen. Beat cross-frequency of undiluted semen, T3, T4, and T6 cucumber juice dextrose saline-based extenders were significantly higher ($P < 0.05$) than T5, T7, and T2 cucumber juice dextrose saline-based extenders. Wobble of T2 and T7 cucumber juice dextrose saline-based extenders were significantly higher ($P < 0.05$) than other cucumber juice dextrose saline-based extenders compared to undiluted semen. The different cucumber juice-dextrose saline-based extenders did not significantly affect the liveability or acrosome integrity of rooster sperm at room temperature ($P > 0.05$), suggesting that these extenders maintain sperm viability without compromising structural integrity.

Table 1 Quality characteristics of rooster semen in cucumber juice-dextrose saline diluent at room temperature

Parameters	T1	T2	T3	T4	T5	T6	T7	SEM
Percentage motility (%)	91.60 ± 1.47^{a}	72.46 ± 2.04^{c}	90.25 ± 1.58^{a}	91.20 ± 2.58^{a}	92.21 ± 0.37^{a}	92.11 ± 0.78^{a}	79.85 ± 2.35^{b}	1.65
Progressive motility (%)	77.04 ± 3.85^{a}	53.06 ± 6.78^{b}	83.33 ± 3.37^{a}	79.06 ± 2.59^{a}	56.89 ± 5.60^{b}	72.99 ± 3.58^{a}	59.17 ± 4.94^{b}	2.82
Non-progressive motility (%)	14.56 ± 4.39^{b}	19.40 ± 2.25^{b}	6.93 ± 1.96^{b}	12.14 ± 2.75^{b}	35.32 ± 2.94^{a}	19.12 ± 2.34^{b}	20.68 ± 4.26^{b}	2.38
Curvilinear velocity (µm/s)	19.46 ± 2.56^{a}	7.17 ± 1.69^{b}	18.13 ± 1.36^{a}	16.99 ± 2.33^{a}	16.74 ± 2.02^{a}	19.13 ± 2.11^{a}	8.09 ± 0.36^{b}	1.16
Average path velocity (µm/s)	11.15 ± 0.86^{ab}	6.54 ± 0.69^{d}	9.80 ± 1.20^{bc}	10.24 ± 0.99^{bc}	13.51 ± 1.08^{a}	11.65 ± 0.99^{ab}	7.39 ± 1.05^{cd}	0.58
Straight line velocity (µm/s)	4.95 ± 0.23^{b}	4.75 ± 0.18^{b}	4.13 ± 0.12^{b}	4.53 ± 0.18^{b}	8.06 ± 0.38^{a}	5.27 ± 0.49^{b}	4.91 ± 0.39^{b}	0.30
Linearity (%)	25.20 ± 1.06^{c}	66.98 ± 1.45^{a}	22.75 ± 2.86^{c}	26.64 ± 2.46^{c}	51.75 ± 2.05^{b}	27.65 ± 1.58^{c}	62.04 ± 2.34^{ab}	4.16
Straightness (%)	44.10 ± 1.45^{b}	72.95 ± 1.56^{a}	42.08 ± 2.05^{b}	44.22 ± 1.08^{b}	61.88 ± 3.34^{a}	45.23 ± 2.90^{b}	67.52 ± 1.20^{a}	2.91
Amplitude of lateral head (µm)	0.51 ± 0.02^{a}	0.28 ± 0.00^{b}	0.49 ± 0.04^{a}	0.50 ± 0.20^{a}	0.60 ± 0.01^{a}	0.56 ± 0.02^{a}	0.31 ± 0.03^{b}	0.03
Beat cross frequency (Hz)	4.44 ± 0.24^{a}	0.91 ± 0.02^{c}	4.62 ± 0.36^{a}	3.94 ± 0.06^{a}	2.28 ± 0.12^{b}	4.22 ± 0.75^{a}	0.95 ± 0.03^{c}	0.35
Wobble (%)	56.92 ± 2.56^{cd}	91.63 ± 2.06^{a}	54.07 ± 1.46^{d}	60.23 ± 2.45^{cd}	82.80 ± 1.47^{b}	61.10 ± 3.02^{c}	91.50 ± 1.56^{a}	3.53
Liveability (%)	91.60 ± 4.67	72.46 ± 5.07	90.25 ± 7.04	91.20 ± 4.07	92.21 ± 3.89	92.11 ± 3.90	79.85 ± 5.07	1.65
Acrosome integrity (%)	44.10 ± 10.76	72.95 ± 14.95	42.08 ± 13.66	44.22 ± 14.85	61.88 ± 19.47	45.23 ± 12.87	67.52 ± 8.93	2.91

Note: ^{abc} Means in the same row with different superscripts are significantly different ($P < 0.05$). T1 = undiluted (raw) semen, T2 = dextrose saline + 0% cucumber juice extender, T3 = dextrose saline + 10% cucumber juice extender, T4 = dextrose saline + 20% cucumber juice extender, T5 = dextrose saline + 30% cucumber juice extender, T6 = dextrose saline + 40% cucumber juice extender, T7 = dextrose saline + 50% cucumber juice extender.

Routine sperm analysis provides important information about sperm production, sperm motility and motility, male reproductive system patency, accessory organ secretions, ejaculation, and sperm output. In normal sperm analysis, sperm movement must be at least 50% A and B grade. Permanent infertility is a good indicator of reproductive failure (Moretti *et al*., 2022). Manual sperm analysis cannot measure the kinematics of sperm motility. Computer-assisted sperm analysis (CASA) is important because it allows the analysis of sperm motility (sperm head and flagella dynamics). CASA and sperm motility analysis system (SMAS) provide analysis of various sperm parameters such as curvilinear velocity, straight-line velocity, amplitude of lateral head, linearity, and beat-cross frequency. CASA and SMAS can help predict the outcome of assisted reproductive technologies such as *in vitro* fertilization or intrauterine insemination (Finelli *et al*., 2021).

The results of this study demonstrate the potential of cucumber juice to dextrose saline as a sperm supplement for roosters. The results showed significant changes in the motility parameters due to the impact of different cucumber juice-dextrose saline extenders on the motility of undiluted semen studied. Percentage motility, amplitude of lateral head, and curvilinear velocity were significantly higher with 10%, 20%, 30%, and 40% cucumber juice-dextrose saline extenders compared to 50% and 0% cucumber juice-dextrose saline extenders. This finding suggests that adding cucumber juice to dextrose saline at different concentrations has a positive effect on sperm motility.

The inclusion of cucumber juice in a tris-egg yolk extender for *in vitro* cold storage of semen obtained from WAD bucks was reported by Daramola and Adekunle (2015) indicating that these fruit juices have the ability to sustain progressive motility. This could be attributed to the high levels of vitamin C, E, and other antioxidants present in these fruits. Furthermore, the study also examined the average path velocity, non-progressive motility, and straight-line velocity to assess the quality of motility. It was observed that the 30% cucumber juice-dextrose saline extender was more effective on these parameters when compared to other cucumber juice-dextrose saline extenders and undiluted semen. This shows that 30% cucumber juice in a dextrose saline solution does have a positive effect on the quality of sperm motility.

Overall, these findings suggest that adding cucumber juice-dextrose saline extenders to undiluted semen can increase sperm motility, while 30% of cucumber juice-dextrose saline extenders show excellent results in terms of quantity and quality of motility. This study compared the straightness, linearity, and beat cross-frequency of different cucumber juice in dextrose saline extenders to undiluted semen. The results showed that the linearity of the 0% cucumber juice-dextrose saline extender was higher than all other cucumber juice-dextrose saline extenders evaluated. This indicates that sperm diluted with 0% cucumber juice in dextrose saline diluent has more linear and straight swimming trajectory when compared to other diluents.

In terms of straightness, 0%, 30%, and 50% cucumber juice-dextrose diluents were found to be more effective than other cucumber juice in dextrose saline diluents and undiluted semen. T3, T4, T5, and T6 extenders might have optimal concentrations of cucumber juice and dextrose or additional components that better maintain sperm motility and movement. These extenders might provide better nutrients or osmotic conditions for sperm cells, enhancing their motility. This shows that cucumber juice in dextrose saline concentrations had a good effect on maintaining spermatozoa straightness during swimming. When determining the beat cross-frequency, it was observed that undiluted semen and cucumber juice-dextrose saline extenders concentrations of 10%, 20%, and 40% were more effective than 30%, 50%, and 0% cucumber juice-dextrose saline extenders. T3, T4, and T6 extenders might have suitable formulations that promote progressive motility such as better buffer systems or additives that enhance sperm movement in a forward direction. This suggests that the concentration of cucumber juice in dextrose saline affects the frequency with which sperm passes through its path to swim. Overall, the results

showed that different cucumber juice-dextrose saline extenders had different effects on sperm linearity, straightness, and beat cross-frequency. T5 extenders had higher average path velocity, and straight-line velocity that reduces the viscosity of the extender or better supports the straight-line motion of sperm. The 0% cucumber juice in dextrose saline diluent showed the highest linearity, while 30% and 50% cucumber juice-dextrose saline diluents showed improved linearity. On the other hand, undiluted semen and cucumber juice-dextrose saline extenders concentrations of 10%, 20%, and 40% were found to have higher beat cross frequencies. These findings highlight the importance of selecting the appropriate concentration of cucumber juice in a dextrose saline extender to add and maintain the required sperm characteristics during sperm dilution.

Compared to the other treatments, the wobble of sperm diluted with 0% and 50% cucumber juice-dextrose saline extenders had greater. This suggests that the cucumber juice in dextrose saline extenders was more effective in maintaining the viability, motility, and stability of sperm cells during storage. Additionally, wobble observed in 0% and 50% cucumber juice-dextrose saline extenders indicate their ability to provide the necessary support and nutrients to maintain sperm motility and stability during storage. It was determined that liveability and acrosome integrity were not affected by the different cucumber juice-dextrose saline extenders used in this study. There is no significant difference in the liveability and acrosome integrity among the different cucumber juice-dextrose saline extenders indicating that these extenders did not negatively affect the quality and functionality of the sperm cells. This suggests that cucumber juice in dextrose saline extenders added at room temperature does not harm the quality and activity of sperm cells (as measured by their ability to fertilize the egg). This supports the use of cucumber juice-dextrose saline as a good alternative to sperm extenders. However, more studies are needed to investigate the long-term effects of cucumber juice in dextrose saline solution on sperm quality and fertilization rates under different storage conditions. These findings lead to continued efforts to improve the combination of methods in chicken breeding, increase genetic diversity, and improve breeding ability.

That T2 had significantly higher linearity when compared to other extenders indicates that T2 might have a formulation that better supports the linear movement of sperm, potentially through a balance of viscosity and osmotic pressure. T2, T5, and T7 had significantly higher straightness, these extenders might have optimal conditions for maintaining straight movement of sperm cells, such as appropriate nutrient concentration. Undiluted semen, T3, T4, and T6 had significantly higher beat cross-frequency compared to T2, T5, and T7, which could be due to better sperm health or more effective extender formulations for maintaining sperm vigor. It appears that all extenders were similarly effective in preserving sperm viability and acrosome integrity under the conditions tested.

The antioxidant activity of rooster sperm was influenced by the different cucumber juice dextrose saline-based extenders (Figure 1). A significant difference between the antioxidant activity of rooster sperm at 0 and 5 hours was observed. The values of the antioxidant activity of rooster sperm at 0 hours were higher compared to the antioxidant activity of rooster sperm at 5 hours of dilution. Furthermore, there was a progression of antioxidant activity in 0%, 10%, 20%, 30%, 40%, and 50% cucumber juice dextrose saline-based extenders at both 0- and 5-hour dilutions. At 5 hours, extended semen at T7 showed significantly higher antioxidant activity compared to T4, T5, and T6. The antioxidant activity of extended semen in T1 and T3 were significantly higher than those of T2 which were the least.

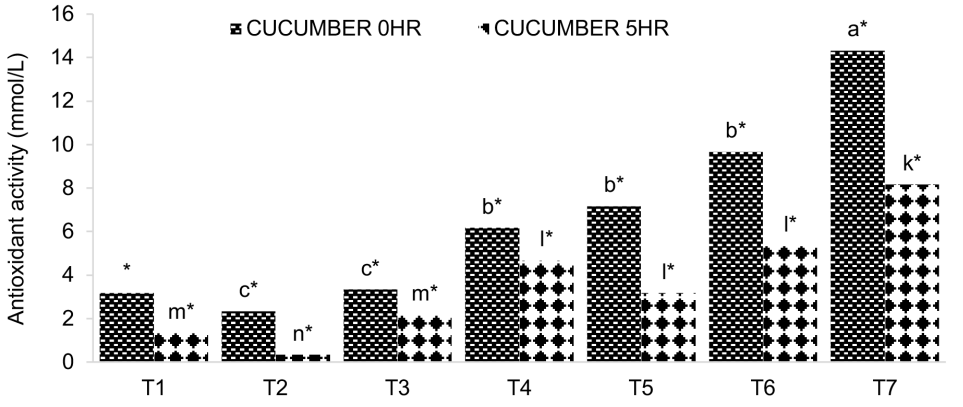

Figure 1 Antioxidant activity of rooster sperm in cucumber-dextrose-based diluent. Varying levels of extenders includes: T1 = undiluted (raw) semen, T2 = dextrose saline + 0% cucumber juice extender, T3 = dextrose saline + 10% cucumber juice extender, T4 = dextrose saline + 20% cucumber juice extender, T5 = dextrose saline + 30% cucumber juice extender, T6 = dextrose saline + 40% cucumber juice extender, T7 = dextrose saline + 50% cucumber juice extender. The results on the figure indicate column with superscripts; abc: different superscripts at 0 hours are significantly different ($P < 0.05$), klmn: different superscripts at 5 hours are significantly different ($P < 0.05$). * Indicates a significant difference between 0 and 5 hours.

The study found that the antioxidant properties of rooster sperm were affected by the addition of cucumber juice in dextrose saline extenders. The antioxidant effect of rooster sperm diluted at 0 hours is higher than that of rooster sperm diluted at 5 hours, and the antioxidant effect of rooster sperm diluted at 0%, 10%, 20%, 30%, 40%, and 50% cucumber juice-dextrose saline extenders was higher than that of rooster sperm diluted at 5 hours. The results showed that adding cucumber juice-dextrose saline extenders to the sperm diluent improved the antioxidant activity or ability of rooster sperm. The highest antioxidant activity was observed when sperm was diluted with a 50% cucumber juice-dextrose saline extender. The fact that antioxidant activity was higher at hour 0 compared to the 5-hour dilution indicates a decrease in antioxidant activity over time. In addition, the antioxidant activity of rooster sperm gradually increased with increasing concentration of cucumber juice in dextrose saline solution at 0- and 5-hour dilutions. This finding means that cucumber juice-dextrose saline can be used as a supplement to rooster sperm to help maintain sperm quality and motility. The decrease observed in the antioxidant activity of rooster semen over time can be attributed to the degradation of antioxidants in semen. These findings indicate that cucumber juice-dextrose saline can be used as a good diluent to preserve rooster sperm and maintain its antioxidant capacity. This is consistent with previous studies showing that long-term storage reduces antioxidant activity (Jimoh *et al*., 2021a). The increased antioxidant activity of more of the added concentrations of cucumber juice-dextrose saline extender suggests that cucumber juice-dextrose saline contains bioactive substances that eliminate free radicals and prevent oxidative damage. These compounds may contain various antioxidants such as vitamin C, vitamin E, and phenolic compounds. The addition of antioxidants to sperm diluents is often used to combat oxidative stress during the processing and storage of rooster sperm (Leão *et al*., 2021; Ros-Santaella and Pintus, 2021). Natural foods and food-derived antioxidants have been shown to improve sperm motility and motility in bovine sperm (Daramola and Adekunle, 2015). Other fruit juices such as orange juice and pineapple juice, have also been investigated for their antibacterial effects

on sperm (El-Sheshtawy *et al*., 2016). Antioxidant enrichment of semen extenders has several benefits, including antioxidants that combat oxidative stress during processing and conservation of semen (Leão *et al*., 2021). Enriching sperm with antioxidants has many benefits; antioxidants help prevent oxidative stress and provide a cryoprotective effect during sperm preservation and storage (Leão *et al*., 2021). Additionally, the antioxidant may also affect sperm quality by reducing the effects of reactive oxygen species (Abdel-khalek *et al*., 2022). Antioxidants have been shown to improve the fertilizing ability of rooster sperm (Leão *et al*., 2021). Plant extracts have been studied as alternative additives for sperm preservation as they contain natural antioxidants that can improve sperm motility and viability (Ros-Santaella and Pintus, 2021). Overall, antioxidant enrichment of semen extenders can improve the quality and viability of sperm leading to better reproductive outcomes. Further studies are needed to investigate the specific bioactive compounds in cucumber juice-dextrose saline responsible for the observed antioxidant activity.

Lipid peroxidation of rooster sperm in cucumber juice dextrose saline-based extenders is presented in Figure 2. The lipid peroxidation of rooster sperm was affected by the different cucumber juice dextrose saline-based extenders at 0- and 5-hour dilutions. There is a massive difference between the lipid peroxidation of rooster sperm at 0- and 5-hour dilutions. The result revealed a sharp decline and a gradual increase of lipid peroxidation in 0%, 10%, 20%, 30%, 40%, and 50% cucumber juice dextrose saline-based extenders at 0- and 5-hour dilutions respectively. The lipid peroxidation rate of T1 was highest across the extenders at 5 hours, followed by T6 and T7, which were higher than those on T2, the least was observed in those in T5.

This study investigated the effect of different cucumber juice in dextrose saline extenders on the lipid peroxidation of rooster sperm. The lipid peroxidation of rooster sperm was affected by different cucumber juice-dextrose saline extenders at 0- and 5-hour dilutions. The results showed a decrease and gradual increase in lipid peroxidation at 0- and 5-hour dilutions for 0%, 10%, 20%, 30%, 40%, and 50% cucumber juice-dextrose saline diluents, respectively. This shows that cucumber juice in a dextrose saline solution protects sperm and reduces oxidative damage. However, lipid peroxidation gradually increased in all cucumber juice with added dextrose saline after 5 hours of dilution. This shows that the protection of cucumber juice-dextrose saline supplement decreases over time and causes an increase in lipid peroxidation. It is important to note that the increase depends on the concentration of cucumber juice in the saline solution. These findings highlight the importance of using cucumber juice in a dextrose saline water solution to maintain sperm quality in roosters. The protective effect of cucumber juice-dextrose saline supplementation was observed initially but diminished over time. This shows that extenders should be used immediately to get the best results. The addition of antioxidants to sperm diluents can reduce the effects of reactive oxygen species, thereby reducing oxidative stress and improving sperm quality (Leão *et al*., 2021; Jimoh and Nwachukwu, 2022). Lipid peroxidation is a process that occurs when free radicals attack polyunsaturated fatty acids (PUFA) in cell membranes, leading to the formation of lipid peroxides (Rezaie *et al*., 2021). This process causes damage to cell membranes and causes sperm quality to decrease. Lipid peroxidation can affect rooster sperm quality in the following ways. It can lead to decreased sperm motility, an important factor in fertility (Mussa *et al*., 2020). Lipid peroxidation can cause a decrease in sperm viability, thus reducing the sperm's ability to fertilize (Safari Asl *et al*., 2018). Lipid peroxidation increases oxidative stress in sperm, causing further damage to cell membranes and reducing sperm quality (Hamilton *et al*., 2016). To combat the negative effects of lipid peroxidation, antioxidants can be added to semen extenders to reduce oxidative stress and improve sperm quality (Safari Asl *et al*., 2018; Rezaie *et al*., 2021). Antioxidants can scavenge free radicals and prevent the production of lipid peroxides, leading to better semen quality and reproductive outcomes (Ratchamak *et al*., 2023). Antioxidant enrichment of semen extenders can improve the quality and viability

of sperm, leading to better reproductive outcomes (Jakop *et al*., 2023). Pineapple and cucumber juices have been shown to have preservative effects on spermatozoa viability (Daramola *et al*., 2016; Hassanu *et al*., 2022). Other fruit juices, such as soursop, tangerine, and sweet orange, have also been studied for their effects on spermatozoa motility and oxidative status (Odeyemi *et al*., 2020; Jimoh *et al*., 2021a; Jimoh and Nwachukwu, 2022). Further studies are needed to investigate the mechanisms underlying the protection of orange juice-glucose supplementation on lipid peroxidation in chicken sperm. Understanding these processes could lead to the development of more efficient products to manage sperm quality, which will ultimately benefit the poultry industry.

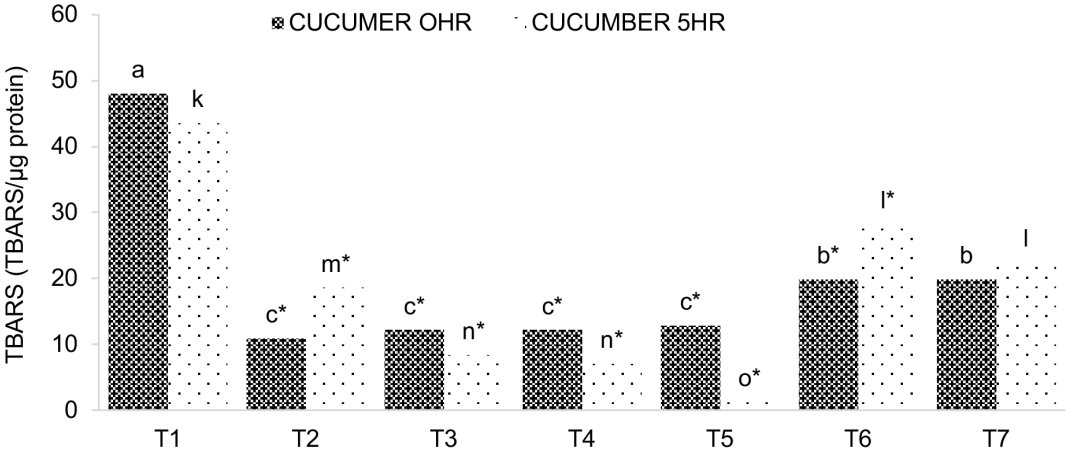

Figure 2 Lipid peroxidation of rooster semen in cucumber-dextrose-based diluent. Varying levels of extenders includes: T1 = undiluted (raw) semen, T2 = dextrose saline + 0% cucumber juice extender, T3 = dextrose saline + 10% cucumber juice extender, T4 = dextrose saline + 20% cucumber juice extender, T5 = dextrose saline + 30% cucumber juice extender, T6 = dextrose saline + 40% cucumber juice extender, T7 = dextrose saline + 50% cucumber juice extender. The results on the figure indicate column with superscripts; abc: different superscripts at 0 hours are significantly different ($P < 0.05$), klmn: different superscripts at 5 hours are significantly different ($P < 0.05$). * Indicates a significant difference between 0 and 5 hours.

The results of this study show a correlation between antioxidant activity and the levels of cucumber juice-dextrose saline-based extenders, but these results do not align with the sperm quality parameters (e.g., motility, velocity) observed. The observed increase in antioxidant activity with higher extender concentrations (T7 showing the highest activity) does not correspond to the improvement in sperm quality characteristics. For instance, while T7 had the highest antioxidant activity at 5 hours, it did not exhibit the best sperm motility or other quality parameters. Antioxidant activity might not directly translate into better sperm quality. While antioxidants neutralize harmful free radicals, the complex interactions between antioxidants and other components in the extender might be influencing sperm quality in ways not captured solely by antioxidant measurements. It is possible that while higher antioxidant activity might be beneficial, it could also be accompanied by other factors like changes in pH or ionic strength that could affect sperm quality. Further detailed investigations into the composition of the extenders might be necessary to understand these effects.

There seems to be a mismatch between the lipid peroxidation results and the antioxidant

activity, with lipid peroxidation rates not showing a straightforward relationship with the level of antioxidants in the extenders. The lipid peroxidation results indicate a decline from 0 hours to 5 hours, with T1 showing the highest peroxidation at 5 hours, which is inconsistent with the antioxidant activity results. The effectiveness of antioxidants in preventing lipid peroxidation might diminish over time due to instability or depletion of antioxidant compounds. While some extenders showed improved antioxidant activity, increasing the concentration of cucumber juice could have introduced factors that negatively impacted sperm quality. For instance, high concentrations might affect osmotic balance or introduce inhibitory compounds.

CONCLUSIONS

The addition of cucumber juice in dextrose saline to the undiluted semen of roosters improves semen quality, oxidative activity, and kinetics during sperm production, processing, or storage for insemination. Although the 30% cucumber juice extender demonstrated the highest average path velocity and straight-line velocity. Progressive motility (a crucial parameter for successful fertilization) was higher in 10%, 20%, and 40% cucumber juice extenders, and the practical application of cucumber juice-dextrose as a viable alternative to traditional semen extenders. This information can be valuable for researchers and professionals working in the field of reproductive biology and animal breeding as it provides insights into the potential use of cucumber juice in dextrose saline as an extender for semen preservation and artificial insemination techniques.

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