EFFECTS OF VITAMIN E SUPPLEMENTATION IN SUGARCANE-BASED EXTENDER ON SEMEN QUALITY OF DARAG NATIVE CHICKEN (*Gallus gallus domesticus*) AT ROOM AND LOW TEMPERATURE STORAGE

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ABSTRACT

Eleven five-month-old Darag native roosters were used as semen donors to evaluate the effects of vitamin E supplementation in a sugarcane-based extender on semen quality. Semen was pooled and initially assessed for color and consistency; only samples with ≥70% initial motility were processed. Pooled semen was randomly assigned to five treatments: T1 (Ringer's solution), T2 (sugarcane-based extender, SBE), T3 (SBE with 1% vitamin E), T4 (SBE with 2% vitamin E), and T5 (SBE with 3% vitamin E), and stored at either room (20-25°C) or low (4-9°C) temperatures. Samples were evaluated for motility, viability, and morphologically normal sperm. All treatments stored at low temperatures had the longest shelf life, with motility maintained above 20%. SBE with 1% and 2% vitamin E had significantly higher motility than Ringer's solution after 8 hours (p=0.011). Treatments with vitamin E showed significantly higher viability than Ringer's solution after 8 hours (p=0.007), and only 2% and 3% vitamin E treatments were higher after 12 hours (p=0.010). No significant differences in morphology were observed among treatments. This study demonstrates the benefits of vitamin E and sugarcane-based extender in preserving avian semen quality.

Keywords: Darag native chicken, semen, semen extension, sugarcane-based extender, vitamin E

INTRODUCTION

Artificial insemination (AI) remains one of the most significant and commonly practiced assisted reproductive technologies, enabling the rapid distribution of superior male genetics (Vishwanath, 2003). AI in poultry involves two main steps: collecting semen from male birds and manually transferring it to females (Aisha and Zain, 2010). As society progressed and the animal industry developed, additional steps were incorporated into the AI procedure, such as semen extension. Semen extension or dilution maximizes the survival of spermatozoa by providing an optimal environment to maintain semen quality, protecting sperm metabolic functions, and controlling pH and microbial growth (Raheja *et al.*, 2018).

During *in vitro* storage, the fertility and motility of sperm decrease one hour after collection (Dumpala *et al.*, 2006; Oluwatoba *et al.*, 2017). Normal room temperature and conditions make it impossible to halt metabolic processes in cells, leading to the

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accumulation of free radicals, which cause oxidative stress (Sikka *et al.*, 1995). Antioxidants, substances that neutralize free radicals, are crucial in combating this issue. Vitamin E, a well-known antioxidant, prevents excessive production of reactive oxygen species (ROS) and has been shown to improve motility and viability, and decrease sperm morphology defects in extended semen of commercial chicken strains (Tabatabaei *et al.*, 2011; Farid *et al.*, 2021). Additionally, plant extracts, which serve as economical and accessible sources of antioxidants, have emerged as a novel method for semen preservation, with sugarcane extract proving particularly effective (Ros-Santaella & Pintus, 2021; Salifu *et al.*, 2023).

Despite these advancements, more research is needed to establish the positive effects of plant juices like sugarcane extract on sperm parameters and to investigate vitamin E supplementation in extended semen of native chickens. Darag, a native chicken breed from Western Visayas, is popular among backyard raisers and a significant source of income for rural farmers (Contreras *et al.*, 2014). Investigating ways to maintain semen quality and prolong its shelf life through techniques like semen extension and antioxidant supplementation is essential. This study aims to explore the effects of using sugarcane extract as an extender and vitamin E supplementation on the quality of Darag native chicken semen, potentially boosting production and performance in native chickens.

MATERIALS AND METHODS

The study was conducted at the Animal Physiology Laboratory of the Institute of Animal Science, College of Agriculture and Food Science, University of the Philippines Los Baños from April to August 2023. All experimental and laboratory procedures were approved by the Institutional Animal Care and Use Committee of the University of the Philippines Los Baños with assigned protocol review number CAFS-2022-024.

Animal Care and Management

Eleven 5-month-old Darag native roosters with weights ranging from 1.2 – 2.2 kg. were used in the study. Birds were placed individually in a battery cage with a 0.2 m^2 floor space area. The cages were manually cleaned every one to two weeks. The roosters were maintained at a normal farm temperature and exposed to a maximum of 12h lighting. The birds were fed daily with 100 grams of commercial feeds. Fresh and clean drinking water was available *ad libitum*.

Experimental Design

The experiment was carried out in a randomized complete block design following a 5 x 2 factorial experiment with five types of extenders stored at room temperature (20-25 ^{o}C) and low temperature (4-9 ^{o}C). The collection period was used as the blocking factor. The five types of extenders are T1 (Ringer's solution), T2 (sugarcane-based extender (SBE), T3 (SBE supplemented with 1% vitamin E), T4 (SBE supplemented with 2% vitamin E), and T5 (SBE supplemented with 3% vitamin E).

Semen Collection

Semen was collected every other day at 8:00 AM. A week before collecting semen, the birds were routinely trained for semen collection by massaging their abdomen and back

for 30-60 seconds. Semen was collected using the abdominal massage method (Burrows & Quinn, 1937). In this method, the back of the birds was gently massaged with firm and rapid strokes. The collected ejaculates were pooled in a glass funnel and transferred in a 1 cc syringe. Each ejaculate was initially evaluated for its color and consistency. The total volume and initial motility of the pooled semen were also recorded. Only those having initial motility higher than 70% were used in the study. Semen samples having lower than 70% motility were discarded. Ringer's solution was used as the basal extender.

Preparation of Sugarcane-based Extender

Sugarcane stalks were acquired from the Institute of Plant Breeding, University of the Philippines, Los Baños, specifically the Phil 2004-1011 variety provided by the Sugar Regulatory Administration - Department of Agriculture (SRA-DA). The sugarcane stalks were first cleaned using a damp cloth before it was peeled with a knife. Sugarcane stalks were cut horizontally into small chunks measuring approximately 2 inches. Each chunk, excluding the nodes, was cut into smaller thin strips. These thin strips were then sliced into smaller fine pieces. A blender was used to grind the fine pieces to be able to extract the juice. Sugarcane juice was extracted manually using a cheesecloth. The composition of the sugarcane-based extender is enumerated in Table 1. The extender was filtered three times using filter paper to remove solid particles that may affect the accuracy of measurement of semen quality parameters under the microscope.

Table 1. Composition of sugarcane-based extender (Salifu *et al.*, 2023).

COMPONENT	COMPOSITION, mL
Sugarcane extract	40.0
Distilled water	30.0
Egg-yolk citrate extender	30.0
TOTAL	100.0

Semen Quality Evaluation

The semen quality parameters measured in the study are motility (%), viability (%), and normal morphology (%). Sperm normal morphology and motility were evaluated using the Gallus module of CASA (Ceros II, IMV Technologies, China) which operates at a frame capture rate of 60 hertz and a camera exposure time of 4 milliseconds. In a glass slide, about 7 µl of each sample was transferred and covered with a cover slip. Five different frames were captured for each observation. The motility and normal morphology measurements are the average of these five fields. Sperm viability was determined by evaluating the acrosome integrity using the Trypan Blue-Giemsa staining method. A uniform amount of 5µl trypan-blue solution and 5 µl semen sample was thoroughly mixed in a glass slide. The slide was allowed to air dry vertically, followed by a fixation using a formaldehyde-neutral red solution for approximately 2-3 minutes. The slide was then rinsed with distilled water and air-dried. Next, the slide was covered with a Giemsa stain for 2.5 hours and incubated at 37 ^{o}C. Finally, the slide was washed with distilled water and air-dried. A total of 200 spermatozoa were

evaluated in each observation. Sperm having purple acrosome and white head are viable while sperm having pale lavender acrosome and blue head are non-viable. All semen quality parameters were observed every 4 hours until motility dropped to 20%.

Statistical Analyses

Data were statistically analyzed using SPSS version 26 (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp). Variables analyzed were percent motility, percent viability, and percent morphologically normal sperm. After satisfying the tests for assumption, a two-way ANOVA was used to compare the values per time period of the three sperm parameters. To measure the specific differences between pairs of means, the Tukey HSD post-hoc test was used. Paired t-test or Wilcoxon signed-rank test was used to compare initial (0H) and final (12H) values. All tests were run at a 0.05 significance level.

RESULTS AND DISCUSSION

The semen color and consistency of the freshly collected *Darag* native chicken ejaculates were observed (n=49). Thirty-seven (37) ejaculates are white and have a thick creamy consistency while twelve (12) ejaculates were white and had a milky consistency. Semen color indicates the density of the ejaculate. The fluid released by different reproductive organs and ducts of domestic fowl ranges in consistency from a dense, opaque suspension to a clear watery liquid (Peters *et al.*, 2008). Semen color can also vary depending on the type of breed utilized but in general, it should be creamy denoting a high sperm concentration (Cole & Cupps, 1977). The average semen volume of each pooled sample and the number of roosters were also recorded. The average semen volume of each ejaculate ranges from 0.10-0.13 ml. This is lower than the findings of Esguerra *et al.* (2020) who reported that the average semen volume of *Paraoakan* native chicken semen is 0.16 ml which ranged from 0.05-0.27 ml. The average ejaculate volume when using the abdominal massage method is about 0.25 ml (Gordon, 2005). Studies on the semen quality of roosters are typically conducted using pooled semen samples because poultry semen has low volumes. However, as a result of probable interactions involving sperm competitiveness, semen quality may be compromised (Schneider *et al.*, 2017).

Semen Shelf Life

The average hours it took for samples in each treatment combination to drop to 20% motility is summarized in Table 2. It can be observed that semen samples stored at room temperature exhibited a rapid decline, reaching the 20% threshold after at most 16.80 hours, compared to those stored at low temperatures taking up to at least 42.40 hours before reaching the 20% threshold. Figure 1 illustrates the average time for each type of extender stored at different temperatures to drop to 20% motility. There is no significant difference in the mean length of hours before sperm motility reaches the 20% threshold according to types of extender ($p=0.330$). In contrast, semen samples stored in low temperatures have a significantly longer time to reach the 20% threshold compared to those stored in room temperatures ($p<0.001$). Traditionally, semen has been stored at temperatures of 5°C or less. This is because, at this temperature, the spermatozoa's metabolism decreases. It is also

more practical as a majority of refrigeration units have a temperature between 2-5°C (Van Wambeke, 1978). Mohan *et al.* (2017) reported that very good fertility can be observed for chicken semen stored at 7-8°C.

Table 2. Mean ± SEM in hours for the Darag native chicken semen diluted with different types of extender and stored at two storage temperatures to drop below 20% motility.

TYPE OF EXTENDER	TEMPERATURE		
	Low (4-9 °C)	Room (20-25 °C)	Mean for type of extender
T1	42.40±8.45	16.00±1.26	29.20±5.96
T2	54.40±4.83	16.80±2.33	35.60±6.76
T3	52.80±4.45	13.60±1.60	33.20±6.90
T4	55.20±4.96	16.00±2.19	35.60±7.02
T5	60.00±6.07	16.80±2.94	38.40±7.87
Mean for Temperature	52.96±2.70	15.84±0.91	
p-value			
Extender x Temperature	0.414		
Extender	0.330		
Temperature	<0.001*		

Statistical test used: Two-way ANOVA; *significant at p<0.05

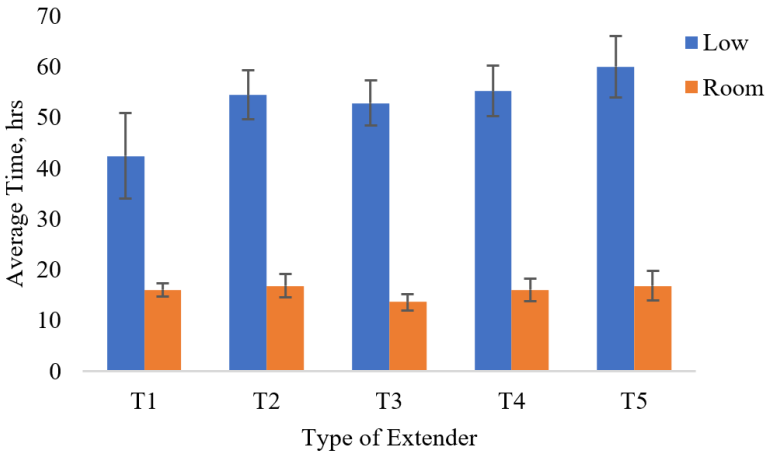

Figure 1. Average time (hours) for the Darag native chicken semen diluted with different types of extender and stored at two storage temperatures to drop below 20% motility.

Percent Motility

The effects of different treatments on sperm motility of extended *Darag* native chicken semen during different time periods are summarized in Table 3. The initial semen motility of samples that received T1 exhibits a notable difference of at least 13% compared to the starting semen motility of samples that received the other four extenders. The analysis reveals a consistent decline in sperm motility among samples subjected to room temperature over varying time intervals. In contrast, samples exposed to lower temperatures exhibit notable fluctuations in sperm motility values. Particularly noteworthy is the observed increase in motility during the eighth hour of extension. After four hours of storage, no significant difference was found in sperm motility across extenders and temperatures nor was any interaction effect observed.

After eight hours of extension, the average sperm motility of samples placed in low temperatures was significantly higher compared to those at room temperature ($p<0.001$). Furthermore, a significant difference in sperm motility was observed across different types of extenders ($p=0.011$). Post-hoc tests revealed that T2, T3, T4, and T5 did not exhibit a significant difference, but T3 and T4 were significantly higher than T1. These results imply that the samples diluted with sugarcane-based extender and supplemented with 1% and 2% vitamin E recorded significantly higher motility than samples diluted with Ringer's solution. High motility values of samples diluted with a sugarcane-based extender were due to the sucrose content of the sugarcane extract. Sucrose, a disaccharide composed of glucose and fructose, was utilized as an energy source by the spermatozoa during the storage period (Lukman *et al.*, 2023). Pure sugarcane juice is composed of 0.54% sugar and 18.08% sucrose, which are more abundant than any other nutrients in sugarcane extract (Erwinda & Susanto, 2014). Lukman *et al.* (2023) found that low percentages of sperm motility are observed in low concentrations of sugarcane (15%). Similarly, sperm motility will likewise decrease at high concentrations and this could be because spermatozoa may have too little or too much energy available to them. Sperm motility may decrease with low concentrations of sugarcane extract in the extender. Conversely, excessively high concentrations can be lethal to the sperm, also resulting in low motility (Salifu *et al.,* 2023). Vitamin E was also able to influence the high motility values of treatments 3 and 4. Vitamin E is a lipid-soluble compound that effectively stabilizes the membrane of cells by acting as an antioxidant (Bjørneboe *et al.*, 1990). Lipid peroxidation can impair the function of sperm membranes, which reduces their motility and viability. (Prihantoko *et al.*, 2022). Vitamin E scavenges lipid peroxyl radicals to prevent uncontrolled lipid peroxidation and stop the chain from propagating, regardless of the kind of free radicals that cause chain initiation (Niki, 2021). However, it is important to note that high amounts of vitamin E in the sample can make the semen sample more viscous. An increase in semen fluid viscosity can deteriorate sperm parameters, especially motility.

After 12 hours of extension, a two-way ANOVA detected an interaction effect between the type of extender and temperature on sperm motility ($p=0.042$). Subsequent post hoc tests indicated that sperm motility in T1-L, T2-L, T3-L, T4-L, and T5-L was significantly higher than in T1-R, T2-R, T3-R, T4-R, and T5-R after twelve hours of extension. Additionally, sperm motility at low temperatures remained significantly higher compared to high temperatures after 12 hours of extension.

Tests of comparison reveal that of the ten treatment combinations, all setups placed at room temperature have a significant drop in sperm motility (T1-R: $p=0.004$, T2- R:

p=0.003, T3-R: p=0.003, T4-R: p=0.003, and T5-R: p=0.002). Reduced sperm motility in mammals has been strongly linked to elevated ROS levels and oxidative stress in birds has a similar effect on poultry sperm motility (Alahmar, 2019).

Table 3. Effects of extender type and storage temperature on *Darag* semen motility (%) at different extension periods.

	0H	4H	8H	12H	p-value[2]
	Mean±SEM				
Extender x Temperature					
T1-L	67.68±6.93	74.26±6.53	61.14±7.95	64.80±2.70^{b}	0.620
T1-R	66.14±7.01	57.86±3.27	37.94±5.26	28.60±6.52^{a}	0.004*
T2-L	81.38±4.11	70.84±5.78	80.66±1.99	86.96±3.41^{b}	0.451
T2-R	79.68±7.54	74.62±9.02	45.16±7.43	22.66±4.39^{a}	0.003*
T3-L	83.54±4.49	68.32±9.29	80.02±3.65	86.12±1.53^{b}	0.537
T3-R	79.18±8.24	54.46±10.51	64.50±10.62	19.58±5.14^{a}	0.003*
T4-L	86.74±2.14	80.02±5.80	80.64±1.65	79.02±4.24^{b}	0.107
T4-R	79.60±4.75	68.84±6.53	59.16±4.86	26.84±7.37^{a}	0.003*
T5-L	87.38±3.20	62.18±12.99	81.24±8.22	77.44±7.41^{b}	0.303
T5-R	77.48±6.38	73.42±7.62	52.56±7.67	21.44±5.45^{a}	0.002*
Extender					
T1	66.91±4.65	66.06±4.40	49.54±5.93^{a}	46.70±6.89	0.019*
T2	80.53±4.06	72.73±5.09	62.91±6.94^{ab}	54.81±11.03	0.093
T3	81.36±4.48	61.39±7.00	72.26±5.89^{b}	52.85±11.37	0.047*
T4	83.17±2.73	74.43±4.52	69.90±4.32^{b}	52.93±9.58	0.006*
T5	82.43±3.75	67.80±7.34	66.90±7.14^{ab}	49.44±10.29	0.007*
Temperature					
Low(4-9 °C)	81.34±2.34	71.12±3.69	76.74±2.75	78.87±2.41	0.510
Room(20-25 °C)	76.42±3.01	65.84±3.59	51.86±3.61	23.82±2.49	<0.001*
p-values[1]					
Extender x Temperature	0.941	0.371	0.618	0.042*	-
Extender	0.053	0.518	0.011*	0.540	-
Temperature	0.187	0.313	<0.001*	<0.001*	-

Statistical test/s used: [1]Two-way ANOVA, Tukey HSD Post-hoc Test, [2]Paired t-test or Wilcoxon Signed Rank Test (0H vs 12H); values with the same superscripts are not significantly different; *significant at p-value<0.05

Percent Viability

Table 4 summarizes the changes in percent viability for different treatments at various time periods. Initially, the average sperm viability for different treatments is at least 90%, aligning with the reported viability of diluted rooster semen by Masoudi *et al.* (2019) and Rochmi and Sofyan (2019). This is higher than the 85.40% viability reported for Philippine native roosters by Baguio and Capitan (2008). No significant differences were observed in the initial sperm viability across the different treatment groups ($p>0.05$).

Table 4. Effects of extender type and storage temperature on the viability (%) of Darag native chicken semen at different extension periods.

	0H	4H	8H	12H	p-value[2]
	Mean±SEM				
Extender x Temperature					
T1-L	91.60±2.47	85.10±1.68	81.90±3.01	73.70±6.06	0.022*
T1-R	91.70±1.49	65.90±4.46	50.60±3.24	36.80±6.33	0.001*
T2-L	96.30±1.20	90.50±2.48	89.20±2.78	88.00±2.76	0.079
T2-R	93.60±2.13	73.30±6.56	61.30±5.34	40.70±6.54	0.001*
T3-L	94.60±0.94	92.00±2.17	92.00±2.79	87.90±2.66	0.085
T3-R	95.70±1.70	74.80±5.55	65.70±6.52	35.70±4.32	<0.001*
T4-L	96.80±1.08	92.90±3.46	92.00±3.05	90.80±3.86	0.115
T4-R	94.40±2.74	74.10±3.55	69.60±5.15	46.50±1.37	<0.001*
T5-L	95.60±2.21	94.60±2.50	94.50±2.85	93.10±3.10	0.264
T5-R	97.60±1.26	79.90±4.45	68.50±6.47	47.40±2.26	<0.001*
Extender					
T1	91.65±1.36	75.50±3.91	66.25±5.62^{a}	55.25±7.41^{a}	0.005*
T2	94.95±1.24	81.90±4.38	75.25±5.45^{ab}	64.35±8.56^{ab}	0.007*
T3	95.15±0.93	83.40±4.01	78.85±5.51^{b}	61.80±9.02^{ab}	0.007*
T4	95.60±1.44	83.50±3.91	80.80±4.68^{b}	68.65±7.63^{b}	0.005*
T5	96.60±1.24	87.25±3.44	81.50±5.47^{b}	70.25±7.83^{b}	0.012*
Temperature					
Low(4-9 °C)	94.98±0.79	91.02±1.23	89.92±1.48	86.70±2.11	<0.001*
Room(20-25 °C)	94.60±0.89	73.60±2.25	63.14±2.64	41.42±2.14	<0.001*
p-value[1]					
Extender x Temperature	0.623	0.982	0.894	0.511	-
Extender	0.099	0.073	0.007*	0.010*	-
Temperature	0.743	<0.001*	<0.001*	<0.001*	-

Statistical test/s used: [1]Two-way ANOVA, Tukey HSD Post-hoc Test, [2]Paired t-test or Wilcoxon Signed Rank Test (0H vs 12H); values with the same superscripts are not significantly different; *significant at p-value<0.05

A general decline in sperm viability was observed across all treatment groups over time. Sperm viability in groups stored at room temperature significantly decreased after 12 hours. After four hours of extension, sperm viability of samples stored at room temperature was significantly higher than those stored at low temperatures ($p<0.001$). The viability continued to decline significantly at both low and room temperatures after 8 hours ($p<0.001$) and 12 hours ($p<0.001$).

Table 5. Effects of extender type and storage temperature on Darag semen normal morphology (%) at different extension periods.

	0H	4H	8H	12H	p-value[2]
	Mean±SEM				
Extender x Temperature					
T1-L	98.74±0.35	98.84±0.43	97.38±0.53	97.92±0.15	0.103
T1-R	98.60±0.47	96.42±0.73	94.32±1.73	94.40±1.30	0.055
T2-L	98.28±0.42	99.08±0.77	98.14±0.94	97.84±0.59	0.500
T2-R	97.54±1.06	98.12±0.45	95.26±1.29	92.26±1.11	0.004*
T3-L	96.36±0.77	98.88±0.80	97.90±0.50	98.96±0.26	0.005*
T3-R	99.06±0.27	96.28±1.75	95.70±0.99	91.74±1.59	0.013*
T4-L	96.72±0.93	97.80±0.93	97.08±0.35	98.38±0.32	0.095
T4-R	96.70±1.45	96.38±1.26	95.48±0.78	92.10±0.81	0.023*
T5-L	95.72±2.46	96.50±1.64	97.82±0.92	97.52±0.73	0.574
T5-R	97.60±0.39	96.56±1.16	95.98±1.29	91.54±1.45	0.043*
Extender					
T1	98.67±0.28	97.63±0.57	95.85±1.00	96.16±0.85	0.028*
T2	97.91±0.55	98.60±0.45	96.70±0.89	95.05±1.10	0.022*
T3	97.71±0.59	97.58±1.00	96.80±0.64	95.35±1.42	0.333
T4	96.71±0.81	97.09±0.78	96.28±0.48	95.24±1.12	0.273
T5	96.66±1.21	96.53±0.95	96.90±0.81	94.53±1.26	0.221
Temperature					
Low(4-9 °C)	97.16±0.56	98.22±0.45	97.66±0.29	98.12±0.22	0.241
Room(20-25 °C)	97.90±0.39	96.75±0.49	95.35±0.53	92.41±0.56	<0.001*
p-values[1]					
Extender x Temperature	0.446	0.728	0.939	0.414	-
Extender	0.298	0.419	0.829	0.563	-
Temperature	0.283	0.038*	0.001*	<0.001*	-

Statistical test/s used: [1]Two-way ANOVA, Tukey HSD Post-hoc Test, [2]Paired t-test or Wilcoxon Signed Rank Test (0H vs 12H); values with the same superscripts are not significantly different; *significant at p-value<0.05

Significant differences in sperm viability across types of extenders were observed after 8 hours (p=0.007) and 12 hours (p=0.010) of storage. After 8 hours, the viability of T3, T4, and T5 was significantly higher compared to T1, and after 12 hours, the viability of T4 and T5 remained higher than T1. These differences are attributed to the sugarcane-based extender and vitamin E supplementation, which provide effective antioxidants for semen preservation. Studies have shown that sugarcane extract and vitamin E improve motility, viability, and morphology of diluted semen (Lukman *et al.*, 2023; Tabatabaei *et al.*, 2011; Arif *et al.*, 2023). However, sperm viability significantly decreased after 12 hours due to lower energy content, increased lactic acid concentration, and oxidation reactions producing free radicals, damaging the spermatozoa plasma membrane (Sartika *et al.*, 2022). Despite these challenges, the natural antioxidants in sugarcane and vitamin E play crucial roles in maintaining sperm quality during storage.

Percent Morphologically Normal Sperm

Initially, no significant differences in normal morphology were observed at the beginning of the extension period, as shown in Table 5. After four hours, however, the average normal morphology of semen samples stored at low temperatures was significantly higher compared to those stored at room temperature (p=0.038). By the eighth and twelfth hour of extension, there were still no significant differences in normal morphology based on the type of extenders used, but the disparity between semen stored at low and room temperatures increased. Interestingly, the average normal morphology of sperm stored at low temperatures was higher after 12 hours compared to the initial measurement.

Furthermore, the average normal morphology of semen stored at low temperatures was significantly higher than that stored at room temperature (8H: p=0.001, 12H: p<0.001), suggesting that low temperatures are more effective in maintaining normal morphology of rooster sperm. This finding is consistent with Esguerra *et al.* (2020), who found that low temperatures better maintained normal morphology in extended Paraoakan native chicken semen, and Suriyasomboon *et al.* (2005), who reported that high temperatures and humidity negatively affected sperm morphology in Duroc boars.

There was no significant difference in the normal morphology of sperm samples that received treatments T3 (p=0.333), T4 (p=0.273), and T5 (p=0.221). Additionally, there was no significant difference in the normal morphology of semen stored at low temperatures before and after twelve hours (p=0.241). Abnormalities in sperm can affect fertility since morphological defects may lead to unsuccessful fertilization (Zaenuri *et al.,* 2017), though Sumadiasa *et al.* (2023) argued that morphologically normal sperm are essential for fertilization. Lukman *et al.* (2022) emphasized that freshly collected semen suitable for processing and extension should have ≤ 20% abnormal sperms.

SUMMARY AND CONCLUSION

The use of antioxidants and natural extracts as components of semen extenders to improve semen quality has been gaining popularity, enhancing artificial insemination in the livestock and poultry industry. The volume, color, and consistency of freshly collected Darag chicken semen align with the typical characteristics of native chickens, and extended semen samples stored at low temperatures exhibited longer shelf life. Supplementation of

vitamin E improved sperm motility and viability, with 1% and 2% vitamin E showing higher motility values compared to semen extended with Ringer's solution after 8 hours of storage. Additionally, 1%, 2%, and 3% vitamin E significantly increased viability over Ringer's solution after 8 hours, and only 2% and 3% vitamin E were significantly higher after 12 hours. These improvements are attributed to the high sucrose content of sugarcane and the antioxidant properties of vitamin E, which provide energy and defense against oxidative stress. While no significant differences in normal morphology were observed among treatments, samples stored at low temperatures had significantly higher normal morphology percentages, as low temperatures reduce sperm metabolism. Though abnormalities in sperm morphology are not directly related to fertilizing capacity, it is unlikely that sperm with high morphology defects will achieve successful fertilization.

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