EFFECTS OF VITAMIN C SUPPLEMENTATION IN SUGARCANE-BASED EXTENDER ON SPERM MORPHOLOGY, VIABILITY, AND MOTILITY OF BANABA NATIVE CHICKEN (*Gallus gallus domesticus*)

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ABSTRACT

Nine sexually mature Banaba roosters were used as semen donors to assess the effects of vitamin C supplementation in a sugarcane-based extender on semen quality. Collected semen were pooled and initially assessed for color and consistency. Only samples with at least 70% initial motility were further processed. Pooled semen samples were randomly distributed into five experimental groups: RC (Ringer's solution, control), SC (sugarcane-based extender, control), T1 (SBE with 2 mg/mL vitamin C), T2 (SBE with 4 mg/mL vitamin C), and T3 (SBE with 6 mg/mL vitamin C), and stored at either room (20-25°C) or low (4-9°C) temperatures. Samples were evaluated for motility (%), viability (%), and morphologically normal sperm (%). Results showed a significant interaction effect ($p<0.001$) between the type of extender and temperature on extension time, with T2 showing superior preservation. While all samples stored at room temperature exhibited decreased motility over time, a significant interaction effect ($p=0.006$) was noted for sperm motility at 8 hours, with T2 at low temperature maintaining the highest motility. Moreover, T2 showed the highest viability, particularly at low temperatures. All groups demonstrated comparable sperm morphology at both 4 and 8 hours of extension at room and low temperatures. These findings validate the potential use of vitamin C and a sugarcane-based extender in avian semen processing, crucial for successful artificial insemination and poultry breeding programs.

Keywords: Banaba native chicken, semen extension, sugarcane-based extender, vitamin C

INTRODUCTION

Artificial insemination (AI) is a valuable tool in the poultry industry, facilitating the rapid spread of genetically advantageous traits. AI allows for the efficient use of genetically superior cockerels with high productive performance (Getachew, 2016) and involves manually collecting semen from the male and inseminating it into the female. Key processes include semen dilution, storage, and evaluation, depending on farm or laboratory goals (Bakst and Daymond, 2013). As AI's role grows, ideal semen storage conditions have become crucial since semen degrades significantly within an hour of in vitro storage, necessitating suitable extenders to maintain viability (Dumpala, *et al.,* 2006; Siudzińska & Łukaszewicz,

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2008). Semen extension enables transporting sperm to distant farms, inseminating more females, and enhancing the use of exceptional males' sperm. However, avian spermatozoa are susceptible to oxidative stress and freezing damage, especially during storage (Bansal & Bilaspuri, 2010; Donoghue & Wishart, 2000).

Avian sperm cell membranes are rich in polyunsaturated fatty acids, making them especially vulnerable to lipid peroxidation (LPO) and oxidative stress, which are linked to increased cellular component oxidation and excess reactive oxygen species (ROS) generation (Partyka & Nizanski, 2021). Excessive ROS production can result from unfavorable environmental conditions (Khan, 2011). Antioxidants are widely used to mitigate these challenges by eliminating reactive oxygen metabolites and shielding cells from structural damage. Effective antioxidant defense systems disrupt free radical chain reactions, prevent ROS from interacting with cell components, and eliminate the by-products of ROS interactions with cellular macromolecules (Partyka & Nizanski, 2021). Examples of antioxidants include Vitamin E, Lycopene, Glutathione, Vitamin A, and Vitamin C. Vitamin C is particularly noted for its defense against free radicals and oxidative stress, accounting for 65% of the seminal plasma's antioxidant capability. Research has shown that supplementing broiler chickens with vitamin C enhances sperm quality and fertility (Uzochukwu *et al.,* 2020).

The poultry industry in the Philippines primarily involves the production of chickens, ducks, and their eggs. Native chickens, often kept in rural areas, can adapt, endure, and reproduce in harsh environments with minimal care and input (Lopez *et al.,* 2014). The Banaba native chickens, derived from Red Jungle Fowls and found mostly in Batangas, are known for their bright orange hackles, black tail and wing feathers, and yellow-red plumage, with adult males renowned for their high-flying abilities (Pangga & Collantes, 2019). These chickens are prized as fighting cockerels due to their high spirit and impressive flight capabilities (Yan, 2020). Consumers and entrepreneurs favor them for their strong flavor, high degree of pigmentation, leanness, and meat versatility (Lambio *et al.*, 2010). This study aims to improve and prolong the shelf life of extended semen for maximum AI utilization by examining the effects of vitamin C supplementation, diluted with a sugarcane extract-based extender, on the quality of semen in Banaba native chickens.

MATERIALS AND METHODS

This study was conducted at the Animal Physiology Laboratory, Institute of Animal Science, College of Agriculture and Food Sciences, University of the Philippines Los Baños, from January to June 2024. All laboratory and experimental procedures were approved by the University of the Philippines Los Baños Institutional Animal Care and Use Committee under protocol number CAFS-2022-024.

Animal Care and Management

Nine 6-month-old Banaba native roosters, each weighing 1.5 to 2.0 kilograms, were used for the experimentation. They were sourced from the University Animal Farm in Tuntungin Putho, Los Baños, Laguna. Each bird was housed in a battery cage with a floor area of 0.2 square meters. The built-up waste was manually removed from the cages, which were then cleaned and disinfected by soaking and brushing with soapy water. The birds

were exposed to a maximum of 12 hours of light and kept under prevailing environmental conditions. They were fed a daily ration of commercial feed appropriate for their age and weight, with water provided *ad libitum*. Before semen collection, all roosters were properly prepared and trained.

Experimental Design

This experiment utilized a completely randomized design (CRD) with five experimental groups based on the type of extender used with or without vitamin C: RC (Ringer's solution; control), SC (sugarcane-based extender; control), T1 (sugarcane-based extender with 2 mg/mL vitamin C), T2 (sugarcane-based extender with 4 mg/mL vitamin C), and T3 (sugarcane-based extender with 6 mg/mL vitamin C). Samples were stored at low temperature (4-9 °C) and room temperature (20-25 °C). Rooster semen was pooled and randomly assigned to each treatment. Parameters observed included normal morphology (%), motility (%), and viability (%). The experiment involved five replicates from five independent pooled semen collections.

Semen Collection

Semen collection was done every other afternoon at 4:00 PM. Before the experiment, roosters underwent routine training for collection, involving firm and moderately rapid back massages (4-6 times) until cloacal eversion occurred. Pooled semen ejaculates were initially collected in a glass funnel, then transferred to a 1 cc syringe, and extended to a 1:5 dilution using commercially available lactated Ringer's solution. Characteristics such as color, consistency, and initial motility were observed and recorded. Only semen collections ≥70% motility proceeded to further extension and evaluation.

Semen Extension

Semen samples with ≥70% motility were further extended using a sugarcane-based extender developed by Salifu *et al.* (2023). This extender consists primarily of sugarcane extract, distilled water, and egg yolk citrate extender. The sugarcane stalks used for extraction were sourced 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 basal extender was pre-mixed and pre-heated to 37°C using a water bath before collection to minimize any potential decline in semen quality due to prolonged intervals between collection and extension. Additionally, the extract underwent filtration five times to remove contaminants that could interfere with accurate evaluation by CASA. Table 1 illustrates the components and composition of the sugarcane extract-based extender.

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 Evaluation

The primary method used for data collection in this study was semen evaluation, focusing on three key parameters: motility (%), viability (%), and normal morphology (%). Computer Assisted Semen Analysis (CASA) (Ceros II, IMV Technologies, China) facilitated the measurement of sperm motility and normal morphology. Glass slides containing 5 µL of each sample were loaded into the CASA apparatus, with five frames captured per sample for analysis. The program provided quantitative results for motility (%) and normal morphology (%). Sperm viability was assessed using the Trypan blue-Giemsa staining method, which relies on acrosomal integrity to differentiate viable from non-viable sperm. This involved preparing slides with equal parts of 0.2% Trypan blue solution and semen (5 µL), air-drying vertically, fixing with formaldehyde-neutral red solution, and staining with Giemsa for 2.5 hours at 37 °C. Evaluation under a microscope involved manually counting 200 spermatozoa per slide; sperm with white heads and purple acrosomal regions were classified as viable, while those with blue heads and pale lavender acrosomal regions were deemed non-viable. These assessments were conducted at 4-hour intervals until motility observed by CASA dropped below 10%.

Statistical Analysis

All data collected from this experiment were analyzed using IBM SPSS Statistics for Windows, Version 26.0. After satisfying the tests for assumption, a Two-way ANOVA was employed to compare variable values across different time periods. Additionally, the Wilcoxon signed-rank test was utilized to compare sperm viability between 0 hours and 8 hours. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Initial Semen Evaluation

During the study, significant increases in the heat index were observed, which likely affected the semen volume collected. According to Pimprasert *et al.* (2023), seasonal variations significantly impact AI technology in roosters, with environmental conditions playing a crucial role in sperm quality. McDaniel *et al.* (1995) also noted that ambient temperature notably influences male bird fertility. The semen volume per bird ranged from 0.17 to 0.21 mL, all displaying a thick, creamy consistency and appearing white. Pamulaklakin *et al.* (2024) reported that semen samples from various genetic groups of native chickens typically exhibit either thick or thin creamy consistency and color, with the Banaba native chicken generally producing the highest semen volume among breeds like Paroakan, and Joloano.

Shelf Life of Extended Semen

Table 2 shows the mean hours (± SEM) it takes for Banaba native chicken semen to fall below a 10% motility threshold using different extenders at two storage temperatures (low and room). The extenders used were Ringer's solution (RC), sugarcane extract extender (SC), and sugarcane extract extenders with varying concentrations of vitamin C (T1, T2, T3).

The analysis shows significant effects of the extender type ($p<0.001$), storage temperature ($p<0.001$), and their interaction ($p<0.001$) on semen motility retention. There is a significant interaction between extender type and temperature, indicating that the

extender's effectiveness is influenced by the storage temperature. The mean hours vary significantly across different extenders, with T2 showing the highest motility retention at low temperatures (48.80±1.50 hours) and T3 the lowest among the treated groups (40.80±0.80 hours). Semen stored at low temperatures had significantly higher mean motility retention (39.04±1.76 hours) compared to room temperature (8.16±0.16 hours). This data underscores the critical role of both extender type and storage temperature in preserving semen motility. It particularly highlights the superior effectiveness of the sugarcane-based extender supplemented with vitamin C and stored at a low temperature, significantly enhancing extender performance.

A study by Achi and Achi (2018) found similar results when vitamin C was added to bull semen. They observed a significant improvement in the motility and viability of bull spermatozoa, concluding that vitamin C supplementation helped maintain these parameters in extended semen stored at a low temperature (5 °C). Additionally, an experiment by Amini *et al.* (2015) on post-thawed rooster semen showed that supplementation with 2 mg/mL of vitamin C resulted in higher motility compared to other extenders.

Table 2. Mean ± SEM in hours for the Banaba native chicken semen to fall below 10% motility threshold with different extenders at different storage temperatures.

TYPE OF EXTENDER	TEMPERATURE		MEAN FOR TYPE OF EXTENDER
	Low (4-9 °C)	Room (20-25 °C)	
RC	24.80±2.94[e]	8.00	16.40±4.42
SC	37.60±0.98[d]	8.00	22.80±7.01
T1	43.20±0.80[b]	8.80±0.80	26.00±8.14
T2	48.80±1.50[a]	8.00	28.40±9.67
T3	40.80±0.80[c]	8.00	24.40±7.75
Mean for temperature	39.04±1.76	8.16±0.16	
p-value			
Extender x Temperature	<0.001*		
Extender	<0.001*		
Temperature	<0.001*		

Statistical test used: Two-way ANOVA; values with the same superscripts are not significantly different; *significant at $p<0.05$

Sperm Viability

Table 3 shows the effects of different combinations of extenders and storage temperatures on the viability (%) of Banaba native chicken semen over various incubation periods (0H, 4H, 8H).

There is no significant interaction effect between extender type and temperature (p-values > 0.05). However, significant differences are noted among extenders at all time

points, indicating that the choice of extender affects semen viability (p-values < 0.05). Highly significant differences are observed between low and room temperature storage, with low temperature consistently preserving higher viability (p-values < 0.001). Across all temperatures, sugarcane-based extenders (SC, T1, T2 & T3) generally maintain higher viability than Ringer's solution (RC). Notably, T2 (Sugarcane-based extender + 4 mg/mL vitamin C) consistently shows the highest viability over time.

Table 3. Effects of extender type and temperature on Banaba semen viability (%) at different extension period.

	0H	4H	8H	p-value
	Mean±SEM			
Extender x Temperature				
RCLT	89.70±0.73	80.90±3.20	66.70±5.63	0.063
RCRT	89.70±0.73	46.10±3.90	21.30±1.93	0.063
SCLT	92.00±0.61	81.90±1.65	76.80±3.81	0.063
SCRT	92.00±0.61	51.80±2.00	27.40±2.09	0.063
T1LT	90.30±0.73	84.20±0.44	79.90±2.43	0.125
T1RT	90.30±0.73	59.30±3.53	34.30±1.53	0.063
T2LT	90.90±0.86	86.10±3.17	84.00±1.67	0.063
T2RT	90.90±0.86	66.30±6.95	34.20±1.72	0.063
T3LT	89.30±1.10	82.50±3.08	78.30±2.55	0.063
T3RT	89.30±1.10	50.60±4.83	29.40±0.75	0.063
Extender				
RC	89.70±0.49^{ab}	63.50±6.27^{b}	44.00±8.07^{b}	0.002*
SC	92.00±0.41^{a}	66.85±5.16^{ab}	52.10±8.48^{a}	0.002*
T1	90.30±0.49^{ab}	71.75±4.48^{ab}	57.10±7.72^{a}	0.004*
T2	90.90±0.57^{ab}	76.20±4.88^{a}	59.10±8.38^{a}	0.002*
T3	89.30±0.73^{b}	66.55±5.96^{ab}	53.85±8.25^{a}	0.002*
Temperature				
Low (4-9 °C)	90.44±0.39	83.12±1.11	77.14±1.85	<0.001*
Room (20-25 °C)	90.44±0.39	54.82±2.36	29.32±1.20	<0.001*
p-value[1]				
Extender x Temperature	1.000	0.283	0.873	-
Extender	0.020*	0.012*	<0.001*	-
Temperature	1.000	<0.001*	<0.001*	-

Statistical test/s used: [1]Two-way ANOVA, Tukey HSD Post-hoc Test, [2]Wilcoxon Signed Rank Test (0H vs 8H); values with the same superscripts are not significantly different; *significant at p<0.05

At low temperature, semen viability remains high across all extenders over time, with only slight declines observed by 8 hours (8H). At room temperature, a significant drop in viability is noted for all extenders by 8H, with the most dramatic declines in the Ringer's solution (RC) groups.

Initial sperm viability at 0H is high for all extenders, with no significant differences (p-value =1.00). After 4 hours (4H), low temperature storage maintains higher viability across extenders compared to room temperature. By 8 hours (8H), sperm viability drops significantly in room temperature storage, whereas it remains relatively high in low temperature storage.

The data indicate that both the type of extender and the storage temperature are crucial for maintaining semen viability. Sugarcane-based extenders with added vitamin C (particularly T2) are more effective in preserving viability, especially when stored at low temperatures. These findings align with those of Giesen and Sexton (1983), which also showed a significant decline in sperm viability when extended semen was stored at 25 °C. Additionally, the use of 40% sugarcane extract in semen extender preparations in this study is consistent with the research by Salifu *et al.* (2023), which concluded that a 40% inclusion rate of sugarcane in the extender significantly improves sperm quality after incubation. The superiority of T2 among the other extenders further highlights the benefits of including vitamin C at 4 mg/mL, as its antioxidant properties have proven to enhance sperm viability. This finding also coincides with a study conducted by Tabatabaei (2012) wherein significantly higher viability was observed in a treatment having 1% ascorbic acid supplemented to the extended semen of Ross-308 broiler breeder roosters. Overall, temperature plays a significant role, with low temperature storage markedly enhancing semen viability over time.

Sperm Motility

Table 4 provides a comprehensive analysis of how various combinations of extenders and temperatures influence Banaba semen motility (%) over time. An interaction effect (p-value=0.006) at 8 hours of extension between extender type and temperature highlights significant differences in semen motility, underscoring the impact of these factors on motility outcomes. Specifically, the use of a sugarcane-based extender stored at low temperature demonstrated superior motility compared to a sugarcane-based extender stored at room temperature and Ringer's solution (RC) stored under both conditions (low and room temperatures). Notably, T2 exhibited the highest observed motility. Significant differences in semen motility (%) were also observed among different extender types (RC, SC, T1, T2, T3) across all temperatures. Sugarcane-based extenders (SC, T1, T2, T3) consistently exhibited higher motility than RC, suggesting a substantial influence of extender type on semen motility throughout incubation periods (0H, 4H, 8H). Moreover, T2 (sugarcane-based extender with 4 mg/mL vitamin C) consistently demonstrated higher motility percentages compared to other sugarcane-based extenders (SC, T1, T3) at 4H and 8H.

The impact of temperature on semen motility was notably significant, with low temperatures consistently preserving higher motility compared to room temperature across all extenders. This finding, however, demonstrates a potential attenuation of the antioxidant effects of vitamin C, particularly evident when extenders are stored at room temperature. This observation aligns with the findings of Arif *et al.* (2023), where extended semen supplemented with vitamin C showed negligible improvements in motility under room temperature storage conditions. The lower motility observed at room temperature storage

across all extender types may be attributed to increased lipid peroxidation (LPO), which accelerates cellular component oxidation and elevates reactive oxygen species (ROS) production over time (Partyka & Nizanski, 2021).

Table 4. Effects of extender type and temperature on Banaba semen MOTILITY (%) at different extension period.

	0H	4H	8H	p-value[2]
	Mean±SEM			
Extender x Temperature				
RCLT	83.33±1.12	52.72±9.58	40.78±8.87^{b}	0.063
RCRT	83.33±1.12	37.98±5.68	7.34±1.05^{c}	0.063
SCLT	86.50	75.32±3.15	64.50±3.87^{a}	0.063
SCRT	86.50	54.78±5.23	8.22±0.73^{c}	0.063
T1LT	82.60±1.59	70.53±2.85	58.74±4.16^{ab}	0.125
T1RT	82.60±1.59	60.46±1.97	8.80±0.97^{c}	0.063
T2LT	85.08±1.15	82.06±0.62	73.64±3.43^{a}	0.063
T2RT	85.08±1.15	70.58±3.33	8.66±0.45^{c}	0.063
T3LT	80.04±1.92	67.28±6.17	57.20±5.78^{ab}	0.063
T3RT	80.04±1.92	64.06±5.66	7.68±0.49^{c}	0.063
Extender				
RC	83.33±0.75^{abc}	45.35±5.80^{b}	24.06±6.99^{b}	0.002*
SC	86.50^{a}	65.05±4.47^{a}	36.36±9.56^{a}	0.002*
T1	82.60±1.06^{bc}	64.93±2.30^{a}	33.77±8.56^{ab}	0.002*
T2	85.08±0.77^{ab}	76.32±2.49^{a}	41.15±10.95^{a}	0.002*
T3	80.04±1.28^{c}	65.67±3.98^{a}	32.44±8.69^{ab}	0.002*
Temperature				
Low(4-9 °C)	83.51±0.70	69.54±3.04	58.97±3.17	<0.001*
Room(20-25 °C)	83.51±0.70	57.57±2.94	8.14±0.34	<0.001*
p-value[1]				
Extender x Temperature	1.000	0.549	0.006*	-
Extender	<0.001*	<0.001*	0.003*	-
Temperature	1.000	<0.001*	<0.001*	-

Statistical test/s used: [1]Two-way ANOVA, Tukey HSD Post-hoc Test, [2]Paired t-Test, Wilcoxon Signed Rank Test (0H vs 8H); values with the same superscripts are not significantly different; *significant at p<0.05

Time-dependent changes in semen motility (0H vs 8H) were also influenced by extender type and storage temperature. For example, T2 consistently maintained higher

motility percentages than T1 and T3 across different time points and temperatures. Room temperature storage consistently resulted in lower semen motility compared to low temperature storage, irrespective of the extender type.

Overall, these findings highlight the critical role of both extender type and storage temperature in maintaining Banaba semen motility, with sugarcane-based extenders, particularly when supplemented with vitamin C like T2, proving advantageous in preserving semen quality over extended incubation periods and under varying environmental conditions.

Table 5. Effects of extender type and temperature on Banaba semen normal morphology (%) at different extension period.

	0H	4H	8H	p-value[2]
	Mean±SEM			
Extender x Temperature				
RCLT	99.56±0.15	99.24±0.33	97.94±1.72	0.625
RCRT	99.56±0.15	97.78±0.75	95.76±2.66	0.125
SCLT	97.64±1.20	98.80±0.32	99.54±0.19	0.188
SCRT	97.64±1.20	98.34±1.05	98.28±0.57	0.813
T1LT	99.50±0.12	98.94±0.34	98.68±0.74	0.313
T1RT	99.50±0.12	98.26±0.36	98.14±0.87	0.313
T2LT	98.88±0.32	99.04±0.44	98.30±0.46	0.438
T2RT	98.88±0.32	98.78±0.55	97.12±1.06	0.188
T3LT	99.04±0.59	99.40±0.37	98.18±0.88	0.375
T3RT	99.04±0.59	98.46±0.32	99.24±0.43	0.813
Extender				
RC	99.56±0.10[a]	98.51±0.46	96.85±1.54	0.275
SC	97.64±0.80[b]	98.57±0.52	98.91±0.35	0.322
T1	99.50±0.08[a]	98.60±0.26	98.41±0.54	0.193
T2	98.88±0.21[b]	98.91±0.34	97.71±0.58	0.131
T3	99.04±0.39[ab]	98.93±0.28	98.71±0.49	0.734
Temperature				
Low(4-9 °C)	98.78±0.37	98.62±0.28	98.04±0.66	0.259
Room(20-25 °C)	99.07±0.18	98.79±0.19	98.20±0.35	0.381
p-value[1]				
Extender x Temperature	1.000	0.823	0.723	-
Extender	0.025*	0.890	0.411	-
Temperature	1.000	0.030*	0.279	-

Statistical test/s used: [1]Two-way ANOVA, Tukey HSD Post-hoc Test, [2]Wilcoxon Signed Rank Test (0H vs 8H); values with the same superscripts are not significantly different; *significant at $p<0.05$

Sperm Morphology

Table 5 illustrates the effects of various extenders and temperatures on Banaba semen normal morphology (%) throughout different incubation periods. There is no significant interaction effect between extender type and temperature on normal morphology across all time points (p-value = 1.000), indicating that the combination of extender type like Ringer's solution (RC), sugarcane-based extender (SC), or sugarcane-based extenders with different inclusion levels of vitamin C (T1, T2, T3), with either low or room temperature, does not alter normal sperm morphology differently than expected based on individual effects. At the initial 0H time point, there were no significant differences in normal morphology across all extenders and temperatures (p-value = 1.000). However, significant differences in normal morphology were observed among different extender types (p-value < 0.025), with Ringer's solution (RC) generally showing a slightly higher percentage compared to sugarcane-based extender (SC) and sugarcane-based extender with vitamin C (T2) at 0H extension, although not consistently statistically significant at 4H and 8H extension. Temperature alone did not significantly affect normal sperm morphology at 0H (p-value = 1.000), but it did show a significant effect at later time points (4H and 8H) (p-value < 0.030). Generally, low temperature storage tended to preserve slightly higher normal morphology percentages compared to room temperature storage, although not always statistically significant. Time-dependent changes in normal morphology were evident across different extender types and temperatures, with differences becoming more pronounced as incubation time increased from 0H to 8H, though statistical significance varied. Ringer's solution (RC) consistently showed slightly lower normal morphology percentages compared to sugarcane-based extenders (SC) and other sugarcane-based extenders supplemented with vitamin C (T1, T2, T3) after longer incubation periods, notably at 8H. This finding aligns with the study conducted by Bukola *et al.* (2023), which indicated that the use of Ringer's solution may have adverse effects on sperm morphology. The differences in normal morphology between low and room temperatures were more evident at later time points (4H and 8H), highlighting the critical role of temperature control in semen preservation. Overall, the varying effects of different extenders and temperatures on Banaba semen normal morphology emphasize potential impacts on fertility outcomes.

SUMMARY AND CONCLUSION

In summary, our investigation into Banaba native chicken semen supplemented with vitamin C in sugarcane extract-based extenders demonstrated significantly enhanced sperm motility and viability during low temperature storage compared to room temperature conditions. The inclusion of antioxidants in the extenders effectively protected semen from potential lipid peroxidation, thereby maintaining optimal quality. These findings underscore the effectiveness of supplemented extenders combined with low temperature storage in preserving sperm quality, albeit with variations depending on specific environmental conditions

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