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Potentials of Coconut Water and Skimmed Milk as Semen Extenders in Chilled Cobb 500 Cock Semen

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Abstract

This study evaluated the efficacy of citrated egg yolk, skimmed milk, and coconut water on the preservation of Cobb 500 cock semen under chilled storage. Semen was collected from six 60 –week- old roosters and extended at a 1:10 dilution ratio. Semen quality parameters including sperm motility, viability, concentration, morphology, mass activity, and pH were assessed at 0, 18, 24, 48, 72, and 96 hours of storage at 4oC. Phytochemical analysis of coconut water revealed the presence of phenols, tannins, and alkaloids, which are known to possess antioxidant properties.

Results showed that Coconut Water extender exhibited the highest sperm motility (75.00± 4.33%) and viability at 24 hours but declined sharply to zero by the 48th hour, likely due to its unstable pH and lack of buffering capacity. Citrated egg Yolk and Skimmed Milk demonstrated more stable sperm motility and viability profiles, with significant preservation up to 72 hours, however, by 96 hours, all extenders recorded zero sperm motility and viability, likely due to oxidant degradation. Although Coconut Water initially supported superior sperm quality, its acidification over time limited its long- term effectiveness, Citrated Egg Yolk, with its strong buffering capacity and clear optical clarity, proved to be the most reliable extender for longer-term storage, followed by Skimmed Milk. It was concluded that Coconut Water may serve as a natural, short-term semen extender, and recommends the inclusion of buffering agents such as sodium citrate to improve its pH stability and extend its efficacy in poultry artificial insemination programs.

Keywords: Cobb 500 Cock, Semen Extenders, Coconut Water, Citrated Egg Yolk, Skimmed Milk, Artificial Insemination

Introduction

The use of artificial insemination (AI) in poultry production has gained increasing attention due to its role in enhancing genetic improvement, optimizing fertility, and promoting disease control in breeding flock. A critical component of AI is the use of effective semen extenders that maintain sperm viability and functionality during storage. Semen extenders are solutions that dilute semen to preserve spermatozoa and protect them from damage caused by environmental factors, particularly during storage and transport.

In poultry, semen is typically stored at low temperatures to slow down metabolic processes and prolong sperm viability. However, chilling can induce cold shock, resulting in reduced motility, increased abnormalities, and compromised fertilizing potential. To counteract these effects, extenders are formulated with protective agents such as energy substrates, pH buffers, and cryoprotectants. Traditionally, citrated egg yolk has been the standard extender for avian semen preservation due to its rich content of phospholipids and low-density lipoproteins, which protect sperm membranes from cold shock. However, the risk of microbial contamination, zoonotic disease transmission, and variability in composition has prompted the search for alternative extenders from safer, more stable, and readily available sources. Coconut water, the clear liquid inside young coconuts, has been explored in various species for its nutrient richness, isotonic nature, and antioxidant properties. It contains sugars, amino acids, minerals, and vitamins that may support sperm metabolism and viability. Its potential as a semen extender has been reported in rams and bulls, but its efficacy in poultry, particularly under chilled

conditions, remains underexplored.

Skimmed milk, another potential alternative, is composed mainly of water, lactose, casein, and minerals. It's buffering capacity and membrane-stabilizing proteins, such as casein, may help preserve sperm integrity during storage. While extensively used in mammalian semen preservation, application of skimmed milk in avian needs further evaluation to determine its effectiveness relative to established extenders like citrated egg yolk.

The aim of this study was to investigate and evaluate the effects of different semen extenders on the quality and viability on cobb 500 cock semen and to identify the most effective and suitable extenders to enhance the semen quality and fertilization potential in artificial insemination procedures [1-8].

Materials and Methods

Experimental Animals and Management

Six (6) Cobb 500 broiler breeder cock, aged 60 weeks and weighing between 4 - 4.5 kg, were used for this study. The selection of birds of this age corresponds to the peak of reproductive performance in the breed. The birds were conditioned to the experiment environment for two weeks before commencement of study. The cocks were fed with commercial broiler breeder feed. The feed was administered ad libitum, and access to clean, fresh water was provided at all times. The roosters were housed in a well-ventilated cage. Housing conditions were designed to mimic commercial poultry practices, providing adequate ventilation and lighting.

Experimental Design

Semen Collection and Extension

Three different groups of extenders were used for this study. These groups included;

Group 1 (Control): Egg Yolk-Citrate Extender
Group 2: Skimmed Milk Extender
Group 3: Coconut Water Extender

Semen collection was carried out through the dorso-abdominal massage technique. This non-invasive method involves gentle abdominal massage to stimulate the roosters' reproductive organs, leading to the collection of ejaculates in sanitized containers.

A pooled semen samples were obtained from the roosters, which was used for this study. Baseline data were obtained, before semen extension at the dilution ratio of 1:10.

Phytochemical Screening of Coconut Water

Coconut water was evaluated for presence of secondary metabolites such as flavonoids, alkanoids, triperpenoids, resine, tannins, saponins and steroids, according to established protocols [9,10].

Semen Evaluation Parameters

The parameters measured include:

- **Sperm Motility (%):** Sperm motility was evaluated under a light microscope (×400 magnification) using a drop of diluted semen placed on a warm slide. The percentage of progressively motile sperm was recorded.
- **Mass Activity:** Mass activity was evaluated on a 0–5 scale under low power (×100) magnification.
- **3 Semen pH:** Semen pH was obtained by using a pH strip via making a drop of semen/extended semen on the strip and observing the reading on the reference scale.
- **Percentage Live Sperm:** Live-dead staining was done using eosin-nigrosin stain. A smear was prepared and at least 200 sperm cells were examined under oil immersion.
- **Sperm Concentration:** Sperm concentration ($\times 10^9$ cells/mL) was determined using a Neubauer hemocytometer after appropriate dilution in a sodium bicarbonate-formalin solution.
- **Sperm Abnormalities (%):** A stained smear was prepared and observed under oil immersion to identify morphological abnormalities. At least 200 sperm cells were evaluated per slide.
- **Optical Clarity:** The clarity of each extender was observed under a microscope to assess turbidity and ease of sperm visualization and photograph was taken.

Extension Procedure

4.5 ml of each of the extenders from Groups 1, 2 and 3 were separately measured into 3 different plain sample bottles. 0.5 ml of the semen sample was added into Groups 1, 2 and 3 extenders respectively, using a dilution ratio of 1:10. 0.5 ml of the now extended semen samples were dispensed into 6 sample bottles for each extender, making a total of 18 sample bottles, and properly labelled for each day of analysis. The experiment was for a duration of 5 days. The extended semen samples were assessed on day 1 of the experiment, 18 hours later and subsequent samples of the extended semen were analysed at 24, 48, 72 and 96 hours, respectively.

Semen Evaluation

Sperm Motility

A subsample of semen was placed on a pre-warmed microscope slide to assess sperm motility, and motility parameters were recorded.

Sperm Morphology

A portion of the semen was smeared onto glass slides and stained using the eosin-nigrosin stain. Sperm morphology was evaluated under high-power microscopy, and abnormalities were recorded.

Statistical Analysis

Data were analysed using One-Way ANOVA followed by Duncan's Multiple Range Test for means separation. Significant differences were considered at $p<0.05$. Results were presented as mean ± standard error of mean (SEM). Statistical analysis was performed using SPSS P C version 25. SPSS Inc. 444 N. Michigan Avenue, Chicago, U S A

Results

Qualitative test for the coconut water revealed the presence of tannin, alkaloid and phenol while saponin, flavonoid, tarpenoid, steroid and balsam were absent. Baseline values for the pooled semen sample before dilution were volume = 0.9 mL, motility = 98%, Mass activity = 3.00, Semen pH = 6, Percentage live = 98%, Sperm concentration = 9,3x109/ml, Sperm abnormalities = 7 %

Sperm Motility

The sperm motility of group 2 (Skimmed milk extender) at 18 hour of storage was significantly lower than group 1 (Citrated egg yolk) and significantly higher than group 3 (Coconut water). ($p<0.05$) (Table 1). At 24th hour group 2 was significantly lower than both groups 1 and 3 respectively ($p<0.05$). At 48th hour the motility of sperm cells in group 3 was zero, which was significantly lower than groups 1 and 2, respectively ($p<0.05$) group 2 was also significantly lower than group 1. A similar trend continued at 72nd hour and by 96th hour of storage the sperm motility of groups 1, 2 and 3 were zero and there were no significant differences ($p>0.05$).

Duration (Hours)	Group 1 Citrated Egg Yolk (Control)	Group 2 Skimmed Milk	Group 3 Coconut Water
0	96.67±1.53	96.67±2.07	97.33±1.00
18	92.33±2.52a	90.33±1.37ab	87.33±2.65b
24	71.00±3.61a	65.00±4.47b	75.00±4.33a
48	57.67±2.52a	44.33±3.61b	0.00±0.00c
72	10.33±1.53a	6.33±1.37b	0.00±0.00c
96	0.00±0.00	0.00±0.00	0.00±0.00
a, b, c Means in the same row with different superscripts are significantly different (P<0.05)			

Table 1: Mean (±Sem) Sperm Motility of Semen of Cobb 500 Cocks Stored in Different Extenders

Mass Activity

Mass activity did not show any significant differences among groups 1, 2 and 3, respectively, (Table 2) ($p>0.05$).

Duration (Hours)	Group 1 Citrated Egg Yolk (Control)	Group 2 Skimmed Milk	Group 3 Coconut Water
0	3.00±0.00	3.00±0.00	3.00±0.00
18	3.00±0.00	3.00±0.00	3.00±0.00
24	2.00±0.00	2.00±0.00	2.00±0.00
48	2.00±0.00	2.00±0.00	0.00±0.00
72	1.00±0.00	1.00±0.00	0.00±0.00
96	0.00±0.00	0.00±0.00	0.00±0.00

Table 2: Mean (±Sem) of Mass Activity of Semen of Cobb 500 Cocks Stored in Different Extenders

Mean pH

The base line pH of group 3 was lower than those of groups 1 and 2, the difference was statistically significantly lower in group 3 compared to 2 ($p<0.05$) (Table 3).

The pH of group 3 at the 18th hour was significantly lower than those of groups 1 and 2 ($p<0.05$). At 24th and 48th hour this parameter was significantly lower in group 3 compared to groups 1 and 2 ($p<0.05$). However at 72nd and 96th hour of storage group 2 had a significantly lower pH than groups 1 and 3 ($p<0.05$).

Duration (Hours)	Group 1 Citrated Egg Yolk (Control)	Group 2 Skimmed Milk	Group 3 Coconut Water
0	6.00±0.00b	6.50±0.00a	5.83±0.08b
18	6.00±0.00a	6.00±0.00a	5.67±0.08b
24	6.00±0.00 a	6.00±0.00 a	5.50±0.00 b
48	6.00±0.00 a	6.00±0.00 a	5.50±0.00 b
72	5.50±0.00 a	4.00±0.00 b	5.50±0.00 a
96	5.50±0.00 a	4.00±0.00 b	5.00±0.00 a
a, b, Means in the same row with different superscripts are significantly different (P<0.05)			

Table 3: Mean (±Sem) Ph of Semen of Cobb 500 Cocks Stored in Different Extenders

Percentage Live

At 18th hour percentage live was significantly higher in group 3 compared to groups 1 and 2 ($p<0.05$) (Table 4). At 24th hour this parameter was significantly lower in group 2 compared to groups 1 and 3 ($p<0.05$). At 48th and 72nd hour percentage live was 0 in group 3 and significantly lower in group 2 compared to group 1 ($p<0.05$) (Table 4). By 96th hour all cells were dead in all the three groups and there were no significant differences in all groups ($p>0.05$).

Duration (Hours)	Group 1 Citrated Egg Yolk (Control)	Group 2 Skimmed Milk	Group 3 Coconut Water
0	96.67±0.88	96.00±0.73	96.00±0.76
18	81.00±2.08b	81.00±2.03b	89.33±0.33a
24	60.33±1.45b	54.00±1.32c	70.00±1.44a
48	54.00±1.45a	40.00±0.73b	0.00±0.00c
72	10.00±1.16a	5.00±0.73b	0.00±0.00c
96	0.00±0.00	0.00±0.00	0.00±0.00
a, b, c Means in the same row with different superscripts are significantly different (P<0.05)			

Table 4: Mean (±Sem) Percentage Live (%) Semen of Cobb 500 Cocks Stored in Different Extenders

Sperm Concentration

Sperm concentration at 0 and 18th hours were significantly higher in group 3 compared to groups 1 and 2 ($p<0.05$) (Table 5). At the 24th, 48th and 72nd hours group 3 was significantly higher than groups 1 and 2, however group 2 was significantly lower than group 1. At 96th hour, the sperm concentration was zero in all groups, there were no significant differences among groups 1, 2 and 3, respectively ($p>0.05$).

Duration (Hours)	Group 1 Citrated Egg Yolk (control)	Group 2 Skimmed Milk	Group 3 Coconut Water
0	6.70±0.12b	6.43±0.09b	8.60 ±0.10a
18	6.50±0.17b	6.00±0.07c	8.50±0.12a
24	5.00±0.06b	4.00±0.07c	7.67±0.10a
48	4.30±0.17b	3.00±0.00c	7.00±0.09a
72	2.00±0.12c	1.50±0.04a	5.50±0.09b
96	0.00±0.00	0.00±0.00	0.00±0.00
a, b, c Means in the same row with different superscripts are significantly different (P<0.05)			

Table 5: Mean (±SEM) Sperm Concentration (X109/Ml) of Semen of Cobb 500 Cocks Stored in Different Extenders

Sperm Abnormalities

Sperm abnormalities were significantly higher in group 1 compared to groups 2 and 3 at 0 hour ($p<0.05$) (Table 6). However, percentage abnormalities increased in groups 2 and 3 compared to group 1 at the 18th, 24th, and 48th hours of storage but there was no significant difference among the three groups at 72nd and 96th ($p>0.05$) (Table 6).

Duration (Hours)	Group 1 Citrated Egg Yolk (Control)	Group 2 Skimmed Milk	Group 3 Coconut Water
0	8.00±1.15	7.33±0.76	7.67±0.44
18	10.00±1.15b	15.00±0.73a	14.67±1.01a
24	15.00±0.58c	20.00±0.73b	25.00±0.58 a
48	25.00±1.73 c	35.00±0.73 a	30.00±0.58 c
72	60.00±0.58 a	70.00±0.37c	50.00±0.87 b
96	0.00±0.00	0.00±0.00	0.00±0.00
a, b, c Means in the same row with different superscripts are significantly different (P<0.05)			

Table 6: Mean (±SEM) Sperm Abnormalities (%) Semen of Cobb 500 Cocks Stored in Different Extenders

Optical Clarity

Optical clarity of the extenders under the microscope showed that group 3 had lowest optical presence of fat globules (Fig.1)

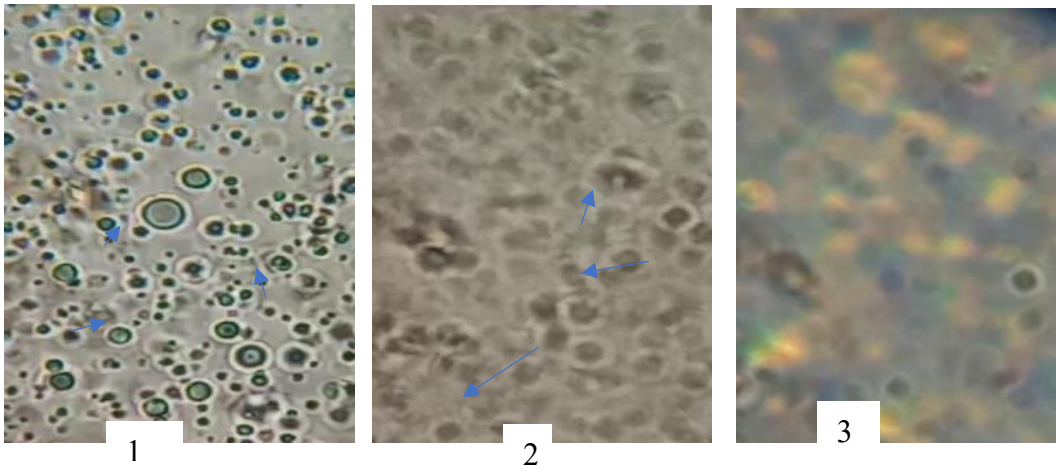

Figure 1: Photomicrograph of Gross Morphological Appearance of Extended Cock Semen in Different Extenders (1 = Citrated Egg Yolk Extender with Sperm Cells Hiding Under Fat Globules (Arrow Heads), 2 = Skimmed Milk Extender with Sperm Cells Hiding Under Fat Globules (Arrows), 3 = Coconut Water Extender with No Clear Fat Globules) Wet Mount, X40 Objective

Discussion

The sperm motility of Skimmed milk extender at 18th, 24th, 48th hour of storage was significantly lower than Citrated egg yolk ($p<0.05$), and a similar trend continued at 72nd hour and by 96th hour of storage the sperm motility of all extenders was zero. This observation is similar to the report of in canine extension where skimmed milk diluent performed significantly lower than Tris diluent which maintained motility till day 3 (72 hours) while skimmed milk diluent lost motility within 2 days (48 hours). This finding aligns with the findings of who reported that under standard operations semen diluted in milk is difficult to evaluate which could be as a result of fat droplets present in milk extenders rendering the optical clarity poor, also this could be due to reflection and refraction of light in the sample when light is passing, this makes observation of individual sperm difficult. This attribute of milk has also been confirmed by who reported that milk has an adequate viscosity for the maintenance of spermatozoa in a liquid medium [11-14].

By the 24th hour of storage, Coconut water showed the highest motility among the three extenders, which was 75.00 ±4.33 %. This was observed before the sudden drop to zero at 48th hour which could be as a result of the decreasing pH of the extender. High percentage motility have been observed in coconut water, according to a previous report by who stated that sperm cell extended with Coconut water has a high percentage motility. It also agreed with the report of that a Coconut water-based extender is effective for cryopreservation of semen [15,16].

The findings may be due to the sugar, vitamins, and amino acids constituents of Coconut water. This report also corroborates the finding of who reported high motility and fertilization rate in African catfish milt extended in Coconut water, the observation suggested that the integrity of the sperm cells in the extender was very intact as at day 2 of cold milt storage Phytochemical screening of Coconut water reported that it contains phenols devoid of steroids and phenols have been reported to have potential antioxidant properties and protect cells from free-radical oxidation reactions. It also has anti-inflammatory property. These combined properties ranging from antioxidative effect, presence of vitamins and minerals, may have played synergistic roles in giving Coconut water extender an advantage as at 24th hour of storage. Coconut water extenders can efficiently harness its potassium content for the survival of the spermatozoa as

Coconut water is rich in potassium.

Unstable pH was a serious shortfall of Coconut water extender in the present study. The pH of Coconut water at 0 and 18th hour was significantly lower than that of Skimmed milk and Citrated egg yolk extenders ($p<0.05$). The unstable pH may have contributed to an acidic medium leading to sudden drop of motility to zero at 48th hour of storage. In the present study there was no inclusion of a buffering agent in the Coconut water extender. Lack of a buffering agent may have been responsible for the decreasing pH observed over time [17-20].

Addition of sodium citrate as a buffer may have mitigated this effect and championed Coconut water as a perfect medium for cock semen extension. In another perspective, Cock semen in Coconut water may slightly lower pH of the medium due to its compositions. pH value of Coconut water were 5.83± 0.08 and 5.67±0.08 at 0 and 24 hours respectively compared to other extenders (citrated egg yolk 6.00±0.00 and 6.00±0.00 while skimmed milk was 6.50±0.00 and 6.00±0.00 at 0 and 24 hours respectively. This observation is in contrast to what was reported in catfish by Columbus et al., (unpublished data) where pH in Coconut water extender was 6.00±0.57.

It is possible that cock semen has chemical constituents that react with Coconut water which alters its pH. The pH continued decreasing throughout the 96 hours of the chilled storage and consequently semen motility became zero in Coconut water extender as at 48 hour of storage. Milk diluents are effective buffers. The buffering action may be due to its protein composition which includes casein and whey. These two proteins can serve as protein buffers. This observation is consistent with the report of which stated that milk has a buffering capacity, bactericidal action and an adequate viscosity for the maintenance of spermatozoa in a liquid medium. Hence in the present study, both citrated egg yolk and skimmed milk showed superiority as regards shelf life over coconut water likely because of their buffering capacities. Mass activity did not show any significant differences among the semen extenders within the first 24 hours. It subsequently followed the same trend with the sperm motility, which was expected [21].

At 18th hour, percentage live was significantly higher in coconut water compared to Skimmed milk and Citrated egg yolk ($p<0.05$). At 24th hour this parameter was significantly higher in Coconut water followed by Citrated egg yolk which was significantly higher than Skimmed milk ($p<0.05$). At 48th hour percentage live was significantly lower in Coconut water than Skimmed milk which was significantly lower than Citrated egg yolk extender. By 48 hours of storage semen pH in Coconut water extender had become more acidic, motility had stopped completely and percentage live was significantly lower in the Coconut water extender compared to skimmed milk and citrated egg yolk. This observation may be a reflection of the decreasing pH in that extender. The trend continued at 72nd hour of storage, by 96th hour all cells were dead in all the three extenders, Oxidative damage may be responsible for cell death with longer period of storage.

Sperm concentration at zero. 18th, 24th, and 48th hour of storage was significantly higher in Coconut water compared to Skimmed milk and Citrated egg yolk extenders ($p<0.05$). By 72nd hour sperm concentration was significantly higher in Skimmed milk than Coconut water which was significantly higher than Citrated egg yolk extender ($p<0.05$). Sperm concentration was zero in all extenders by 96th hour of storage. The observation here may be attributed to the poor optical clarity of the skimmed milk and citrated egg yolk extenders. But with the decreasing pH in coconut water, massive cell death at 72nd hour might have changed the sperm concentration significantly that skimmed milk count became higher than coconut water.

In conclusion, the study demonstrated that Coconut water, Citrated egg yolk, and Skimmed milk extenders have varying capacities in preserving cock semen during cold storage, The lack of buffering capacity in Coconut water, led to a sharp decline in pH, resulting in a complete loss of motility and viability by the 48th hour. Citrated egg yolk and Skimmed milk exhibited more stable buffering effects, prolonging sperm motility and viability up to 72 hours, though with declining efficiency over time. By the 96th hour, no viable sperm was observed in any of the extenders, likely due to cumulative oxidative damage. The initial superior performance of Coconut water was likely due to its rich nutrient composition and antioxidant properties, but its effectiveness was significantly limited by acidification over time in the absence of a buffering agent. Citrated egg yolk and skimmed milk extenders also had the clear optical presence of fat globules.

It was recommended that inclusion of a buffering agent in Coconut water should be considered to improve the storage potential of Coconut water extender, it is strongly recommended to supplement it with sodium citrate or similar buffers. This would stabilize its pH and potentially extend sperm viability beyond 48 hours. Coconut water can be considered a suitable extender for short-term semen preservation (≤24 hours), particularly where natural, nutrient-rich, and antioxidant media are preferred. For storage periods extending beyond 24 hours, Citrated egg yolk remains the most effective extender, followed by Skimmed milk due to their superior buffering capacities and sustained sperm viability. Further studies should explore, the effect of adding buffers to Coconut water and the interaction of cock semen components with Coconut water that may be contributing to pH drop.

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