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Potential use of Coconut Water and Saline Solutions as Diluents for Guinea Cock Semen during Short-term Storage at Ambient Temperature

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ABSTRACT

The present study was conducted to investigate the potential use of coconut water and saline solutions as diluents for Guinea cock semen during short-term storage at ambient temperatures. Thirty adult male Guinea cocks were recruited from the same hatch batch and trained for semen collection by the dorso-abdominal massage for six weeks. At the end of the training period, ten Guinea cocks were selected as semen donors. Semen was collected once weekly from the semen donors during the morning hours between 0700-0800 AM. The collected semen was pooled and evaluated for spermatozoa motility and viability at the time of collection. The evaluated semen was divided into five equal portions. One portion was not diluted and served as a control. The remaining four portions were diluted with normal saline, dextrose saline, modified Ringer's lactate solutions, and coconut water respectively at a ratio of 1:3(v/v). Aliquots were taken from the diluted semen and assessed for spermatozoa motility and viability at 0,1,2 and 3 hours post-collection and dilution. This procedure was repeated for ten weeks from July to September 2020. The results indicate that Guinea cock semen diluted in coconut water and Ringer's Lactate Solution and stored at ambient temperatures for three hours, maintained a spermatozoon motility and viability rate of 20% and 10%, respectively. It was concluded that coconut water was more effective as a diluent than Ringer's lactate, dextrose saline, and normal saline in preserving the motility and viability of Guinea cock spermatozoa stored at ambient temperatures.

Keywords: saline solutions, coconut water, semen, diluent, Guinea cock

INTRODUCTION

The helmeted guinea fowl (Numida meleagris) is a popular avian species in Sub-Saharan Africa (Houndonougbo et al., 2017). It is widely distributed in other parts of Africa, Asia, Europe, North America, and Australia (Moreki and Radikara 2013). Guinea fowls serve as a source of animal protein, and income and are used for the performance of cultural and religious ceremonies in West Africa (Kone et al., 2018; Soara et al.,2020). In Ghana, they are produced predominantly under an extensive or semi-intensive system in the Northern Regions (Agbolosu et al., 2015) and account for approximately 5.6% of the total poultry production (Ghana Statistical Services Department, 2020).

According to Annor et al (2012), the roadmap to increasing Guinea fowl production in Ghana should be based on intensive production systems and improvement in their low reproductive performance. Artificial insemination (AI) as an assisted reproductive technique, has been used to improve reproductive performance in turkeys (Iaffaldano et al., 2016), broiler breeder parents (Habibullah et al., 2015), and Guinea fowls (Seigneurin et al., 2013; Varadi et al., 2013). The sustainable implementation of AI in avian species will depend on the availability of appropriate, affordable, and effective semen extenders. Semen diluents or extenders are buffered salt solutions used to increase the volume of the ejaculate and maintain the viability of spermatozoa during in-vitro storage (Vasicek et al., 2015).

Commercially available poultry semen diluents include Beltsville poultry semen extender (Siudzińska and Łukaszewicz, 2008), CARI semen diluent (Shinde et al., 2013), and Lake semen diluent (Mohan et al.,2015; Schneider et al., 2017). These diluents are not easily available or affordable in developing countries. This has led to the search for alternative locally available materials to be used as semen extenders in avian species. Some studies have reported the use of saline solutions (Adebisi and Ewuola, 2019), coconut water (CW) based formulations (Rochmi and Sofyan, 2019) and other natural ingredients like tomato juice and pineapple juice (Al-Daraji, 2012) as semen extenders for chicken semen. According to Rochmi and Sofyan (2019), rooster semen stored in coconut water diluent and a mixture of coconut water and fructose diluent maintained a motility and viability rate of 47-51 % and 40-47% respectively, after 7 days of storage at 5°C. Similarly, Adebisi and Ewuola (2019) reported that the motility of turkey semen diluted with modified Ringer's solution, dextrose saline, and normal saline solutions, was higher than raw undiluted semen during storage at ambient temperatures for four hours. The fertility and hatchability of turkey eggs from

hens artificially inseminated with semen diluted with saline solutions were higher comparatively those inseminated with raw undiluted semen (Adebisi and Ewuola, 2019). Vasicek et al (2015) demonstrated that saline solutions were more effective than a commercially available extender (Avian Diluent, IMV Technologies, France) in preserving the motility and viability of chicken semen during short-term storage. Semen diluents increase the volume of the ejaculate and enable it to be used for the artificial insemination of more hens thereby reducing the cost of insemination. The purpose of this study was to investigate the potential use of coconut water and saline solutions as diluents for Guinea cock semen during short-term storage at ambient temperatures.

MATERIAL AND METHODS

Ethical Approval

This study protocol was approved by the Animal Ethics Committee of the College of Agriculture Education, Akenten Appiah-Menka University of Skills Development and Entrepreneurship (AAMUSTED), Ashanti Mampong Municipality, Ghana.

Management of Experimental Birds

The study was conducted at the poultry unit of the university farm. A total of thirty (30) adult Pearl Guinea cocks (GC) at 50 weeks old were housed together in groups of 6 per cage. The Guinea cocks were fed with a breeder ration containing 16.5 % crude protein and 2,800 kcal ME/kg (Yildirim, 2012) and provided with water *ad libitum* and natural lighting only.

Preparation of Semen Diluents

The materials used as diluents included 0.9% normal saline solution, 5% dextrose saline solution, Ringers' lactate solution, and coconut milk. The first three diluents were purchased from Laud K. Pharmacy shop at Boadi, Kumasi. The diluents were purchased on the day of use and were stored at room temperature (24-28°C). The normal saline diluent consisted of 0.9% w/v sodium chloride diluted to 100 ml with distilled water, while dextrose saline solution was made of 0.9% w/v sodium chloride and 0.9% w/v glucose anhydrous diluted to 100 ml with distilled water. Ringer's lactate solution was composed of 0.600g sodium chloride, 0.040 g potassium chloride, 0.320 g sodium lactate, and 0.027 g calcium chloride dihydrate diluted to 100 ml with distilled water. The diluents were manufactured by Axa Parenterals Ltd, Roorke, India.

Freshly harvested coconut fruits (*Cocos nucifera*) were purchased from the open market in the Mampong Ashanti Municipality. The husks were cut open and the coconut water was collected aseptically into a container. The coconut water was first filtered and allowed to cool for about five (5) minutes.

Table 1: Motility of Spermatozoa in Guinea Cock Semen diluted in Coconut Water and Saline Solutions

Time/Hour	Undiluted Semen	Normal Saline	Dextrose Saline	Ringers Lactate	Coconut Water	P-Value
0	74 ± 1.5	74 ± 1.5	75 ± 1.6	75 ± 1.5	74 ± 1.6	0.9671
1	34^{d}± 1.6	37^{d} ± 1.5	43^{c} ± 2.1	46^{b} ± 1.6	56^{a} ± 1.6	<0.0001
2	8^{d} ± 2.8	22^{b} ± 2.5	26^{b} ± 3.7	29^{b} ± 3.5	34^{a} ± 4.0	<0.0001
3	0	5.0^{c} ± 1.5	8^{c} ± 1.3	10^{b}± 1.5	18^{a} ± 1.5	<0.0001

** Means with different superscripts across rows are significantly different at P ≤ 0.05*

Semen Collection and Dilution

The Guinea cocks were trained for manual semen collection by dorso-abdominal massage (Rakha et al., 2015) for six weeks. At the end of the training, ten (10) Guinea cocks were randomly selected based on their response to semen collection. Semen was collected from the semen donors, once weekly for ten weeks from July to September 2020. Semen was collected between 0700-0800 GMT on Tuesdays. The collected semen samples were evaluated, pooled together, and then divided into five (5) samples (T_1, T_2, T_3, T_4, and T_5), respectively. T_1 was raw undiluted semen to serve as a control, while T_2, T_3, T_4, and T_5 were diluted with normal saline, dextrose saline, lactated Ringer's solution, and coconut water at the ratio of 1:3(v/v). The diluted samples were stored at room temperature (24-28°C). Aliquots were taken from each treatment group and evaluated for sperm motility and viability at 0,1, 2, and 3-hours post-collection and dilution in four replicates.

Parameters Measured

To ensure uniformity, the measurement of all parameters was conducted by one trained Laboratory Technician.

Spermatozoa Motility

Individual spermatozoan motility was assessed as described by Mohan et al. (2013). The number of spermatozoa with a progressive forward movement was assessed in ten (10) fields of view and the average was expressed in percentage.

Viability and Morphology of Spermatozoa

Spermatozoan morphology and viability were determined by preparing smears and staining them with Nigrosin/eosin stain and observing them under oil immersion on a light microscope at x1000 magnification (Mohan et al., 2013). Spermatozoa heads that absorbed the stain and turned pink color were evaluated as non-viable (dead), while those that remained

white were evaluated as viable (live). The viable spermatozoa were then classified either as normal (N) or abnormal (A), based on the structural integrity of the Spermatozoa. Spermatozoa with identifiable defects in their structure were referred to as abnormal. In each slide, a total of 200 spermatozoa were counted and the number of normal, abnormal, and dead spermatozoa were expressed as a percentage (%) of the total cells counted. This procedure was repeated for ten pooled semen collections from July to September 2020.

Statistical Analysis

The data obtained were analyzed using a two-way analysis of variance (ANOVA) with the help of the statistical package GraphPad Prism version and expressed as Mean ± SEM, where SEM is the standard error of the mean. The means were differentiated using Tukeys' multiple comparison tests at a significance level of 5%.

RESULTS

The effect of coconut water and saline solutions as semen diluents on the motility of Guinea cock spermatozoa during storage at room temperature is presented in Table 1. The motility of spermatozoa was not significantly different (P≥ 0.05) in all the diluents at T= 0 hours. However, with increasing time of storage

Table 2: Percent Dead Spermatozoa in Guinea Cock Semen diluted in Coconut Water and Saline Solutions

Time/Hour	Undiluted Semen	Normal Saline	Dextrose Saline	Ringers Lactate	Coconut Water	P-Value
0	11 ± 1.2	13 ± 0.7	13 ± 1.1	14 ± 1.3	13 ± 1.3	0.5976
1	56^{a} ± 1.5	49^{a} ±1.5	43^{b} ± 1.5	42^{b} ± 1.8	33^{c} ± 1.7	< 0.0001
2	68^{a} ± 0.83	63^{a}± 1.7	63^{a} ± 1.7	57^{b} ± 1.7	41^{c} ± 1.4	< 0.0001
3	91^{a} ± 3.7	83^{a} ±2.9	81^{a} ± 3.2	78^{b} ± 1.1	67^{c} ± 1.5	< 0.0001

** Means with different superscripts across rows are significantly different at P ≤ 0.05*

Table 3: Percent Normal Spermatozoa in Guinea Cock Semen diluted in Coconut Water and Saline Solutions

Time/Hour	Undiluted Semen	Normal Saline	Dextrose Saline	Ringers Lactate	Coconut Water	P-Value
0	74 ± 1.5	75 ± 1.6	75 ± 1.5	75 ± 1.5	74 ± 1.6	0.9737
1	33^{c} ± 1.5	37^{c} ± 1.5	42^{b} ± 1.5	47^{b} ± 1.8	55^{a} ± 1.7	< 0.0001
2	20^{b} ± 2.1	24^{b}± 2.8	26^{b} ± 3.2	30^{b} ± 1.7	45^{a} ± 1.7	< 0.0001
3	3.5^{c} ± 1.5	5.5^{c} ± 1.0	6^{c} ± 1.6	12^{b} ± 1.1	21^{a} ± 1.8	< 0.0001

**Means with different superscripts across rows are significantly different at P ≤ 0.05*

Table 4: Percent Abnormal Spermatozoa in Guinea Cock Semen Diluted in Coconut Water and Saline Solutions

Time/Hour	Undiluted Semen	Normal Saline	Dextrose Saline	Ringers Lactate	Coconut Water	P-Value
0	14 ± 1.2	13 ± 0.68	13 ± 0.45	14 ± 0.5	13 ± 1.1	0.6065
1	12± 0.37	13 ± 0.37	13 ± 0.40	13 ± 0.38	13 ± 0.45	0.3480
2	13 ± 0.31	13± 0.37	14 ± 0.46	13 ± 0.38	13± 0.45	0.5181
3	14 ± 0.26	14 ± 0.28	14± 0.32	14 ± 0.45	14 ± 0.48	0.7985

from T=0 to T=3, the motility (%) significantly decreased and was highest in semen diluted with coconut water (CW), followed by Ringer's lactate (RL), dextrose saline (DS), normal saline (NS), and raw undiluted semen in descending order.

The effect of saline solutions and coconut water as semen diluents on the viability and morphology of Guinea Fowl spermatozoa during storage at ambient temperatures are presented in Tables 2,3 and 4. The results show that the percentage of dead spermatozoa (D%) was similar in all the diluents at T=0 hours after collection (Table 2). At time T=3 hours post semen collection and dilution, D% was lowest in semen diluted with coconut water, but similar in the other treatment groups. Similarly, at T=3 post semen collection and dilution, the percentage of morphologically normal spermatozoa (N%) was highest in semen diluted with CW, followed by RL, but similar in the other treatment groups. The results in Table 4 indicate that irrespective of storage time and type of diluent, the percentage of abnormal spermatozoa (A%) was not significantly different (P≥ 0.05).

DISCUSSION

The short-term storage of avian semen includes the storage of chilled semen in the refrigerator at temperatures of 4-5° C. However, in developing countries where electricity is either unavailable or unreliable, avian semen can be stored at ambient temperatures. This method of semen will require the use of effective, affordable, and readily available semen diluents. Semen extenders are evaluated based on the motility and viability of spermatozoa (Fischer et al., 2014).

The present study reported that the motility of spermatozoa in all the diluents was similar at the time of collection but declined in all the diluents after three hours of storage at ambient temperatures. This finding is in agreement with reports of spermatozoa motility decline in chicken semen stored at 41°C in

different diluents (Ogbu et al., 2014; Adebisi and Ewuola, 2019; Wahyuningsih and Mirwandhono, 2021). The decline in the motility of spermatozoa in stored semen is due to the decline in nutrients such as potassium, sodium, glucose, and plasma proteins, required for the maintenance of spermatozoan metabolism during in-vitro storage (Sakar, 2020)

The present study demonstrated the comparative effectiveness of coconut water as a semen diluent over some saline solutions. This finding is in tandem with previous reports on avian species (Ogbu et al., 2014; Khaeruddin and Srimaharani,2019). The higher effectiveness of coconut water as a diluent is due to its chemical composition of essential amino acids, sugars, minerals (Rukmini et al.,2017), and anti-oxidants such as phenol, ascorbic acid, minerals, and sugars (Santos et al., 2013). These compounds serve as buffer solutions, and sources of energy, and act as antioxidants to protect the spermatozoa membrane's structural integrity. Avian spermatozoa have a larger concentration of polyunsaturated fatty acids in their cell membrane phospholipids, making them more vulnerable to lipid peroxidation (Khan, 2011). Peroxidative damage reduces sperm motility and affects sperm morphology (Long and Kramer, 2003; Zaniboni and Cerolini, 2009). The antioxidants in coconut water reduced oxidative stress during semen dilution and storage.

The comparative effectiveness of Ringer's lactate solution over dextrose saline and normal saline as diluents for avian semen has previously been reported (Adebisi and Ewuola, 2019; Abdel-Khalek et al., 2021). This difference is attributed to the fact that Ringer's lactate solution contains more electrolytes than in both dextrose saline and normal saline solution, while dextrose saline solution contains glucose as a source of energy.

The viability of spermatozoa during in-vitro storage is an important parameter when assessing semen quality. According to Malik et al. (2013), it is only viable spermatozoa that are capable of fertilizing the ovum. The observation in this study of an increase in the percentage of dead spermatozoa with increasing time of storage in the diluents is consistent with other reports in birds (Hudson et al.,2016; Prabakar et al., 2018). Coconut water was more effective in maintaining the viability of spermatozoa during short-term storage at ambient temperatures. Similar reports have been made about the effectiveness of coconut water only or in combination with skimmed milk, egg yolk, and fructose as semen diluents (Khaeruddin and Srimaharani, 2019; Rochmi and Sofyan, 2019). The minerals in coconut water help to maintain the pH and osmotic pressure of the suspension, while the antioxidants reduce the harmful effects of oxidative stress on spermatozoa membranes.

The percentage of abnormal spermatozoa was similar in diluted or undiluted semen, irrespective of the duration of storage. This finding agrees with reports on the use of Ringer's lactate solution, lactose solution, and a combination of Ringer's lactate and lactose solution as diluents for the semen of Kampong chicken stored at 40°C for eight hours (Wahyuningsih and Mirwandhono,2021). Spermatozoa are formed during spermatogenesis in seminiferous tubules and stored in the epididymides for maturation (Aire, 2014). Consequently, pathological conditions leading to the malformation of spermatozoa are encountered during the process of spermatogenesis or maturation in the epididymides. Additionally, improper handling of semen after collection or ejaculation can cause acrosomal or tail damage, resulting in abnormal spermatozoa (Partyka and Nizanski, 2021).

CONCLUSION

Coconut water is a more effective diluent than Ringer's lactate, dextrose saline, and normal saline in preserving the motility and viability of Guinea spermatozoa stored at ambient temperatures.

CONFLICT OF INTEREST

The Authors declare that there is no conflict of interest.

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