

**SHORT TERM HOLDING CAPACITY OF PLANT BASED EXTENDER ON TWO
ECONOMIC POULTRY SPECIES MICROSCOPIC SEMEN QUALITY**
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ABSTRACT

Response of different poultry species variability to dilution and holding period of their semen after ejaculation has been reported by various studies. This study is conducted to test if formulated plant based, Tris Coconut-water Orange Juice, extender (TCWOE) for dilution of poultry semen is specie specific or not. TCWOE was prepared. Semen ejaculates were collected and pooled from five toms and cocks separately. The pooled semen for each species was divided into two portions each, making four treatments and was diluted with extender at the ratio of 1:3 (semen: extender). Experimental design used was completely randomized design consisting of three trials. Microscopic semen parameters such as motility, viability membrane and acrosome integrity were examined and recorded for freshly extended semen examined immediately after dilution and 12h preserved semen at 4-8°C. Percentage motility was significantly ($p < 0.05$) higher in extended cock semen, but was not significantly different to un-extended Cock and Tom semen. Percentage live sperm and acrosome integrity revealed no significant different ($p > 0.05$) in both species (extended and un-extended). At 12h, motility and membrane integrity of both extended and un-extended poultry species semen is significantly different ($p < 0.05$) from each other. Although, extended cock semen had highest value recorded for sperm motility and membrane integrity. In conclusion, dilution of semen with Tris coconut-water orange juice extender revealed a significant positive impact on the sperm cells activities of both Cock and Tom, but had more significant impact on dilution and short term holding potentials on Cock semen than Tom semen.

Keywords: Tom, Cock, Semen, Dilution, Coconut-water, Refrigeration.

Introduction

Semen dilution is an important approach in semen processing for keeping sperm cells active within the period of ejaculation and insemination. The major purpose of extenders is to serve as multiplying agent to increase the volume of semen from one ejaculate, and allows effective distribution of sperm cells which may be package in several breeding doses for artificial insemination (AI). However, the extended semen can further be stored at room-temperature (RT), liquid storage (LS) and frozen. A reliable extender is expected to provide sperm cells with source of adequate nutrients, energy, antioxidant, and buffer condition (Balogun, 2019). Moreover, Extenders provide adequate protection for sperm cells, gives room for viability and encourage fertility over time by stabilizing plasma membrane, providing energy substrates, buffers, antibiotic activity and osmotic balance over time (Linde-Forsberg, 1995).

Formulating a natural based extender suitable for preserving poultry semen of all species is challenging because of their unique sperm structural characteristics compared to other farm animal species. Among this major component of the extenders, coconut- water seems to have exhibited promising reliable results over the years in our previous studies (Balogun, 2019; Balogun *et al.*, 2017).

It is regarded as better alternative extenders to Coconut- milk and other natural based extender components used for dilution and preservation of poultry semen. Though, 100% concentration of coconut water has been associated with drastic reduction in sperm activities of turkey and domestic fowl semen after 4h (Balogun *et al.*, 2020; Balogun, 2019). However, combining it with tris buffer and supplementing with orange juice has shown tremendous result over the years (Balogun, 2019; Balogun *et al.*, 2017), especially for immediate use and preservation of chicken semen for period of 72h stored at refrigeration (4-8°C). This study therefore aimed at comparing the semen qualities of two economic poultry species (Cock and Tom) semen extended with tris coconut-water orange juice extender stored for 12h

Materials and methods

This experiment was carried-out at Oyo State College of Agriculture and Technology teaching and research farm. Ripe coconut was purchase from the market, the coconut was pierced from the top and the water was drained. It was filtered and diluted with tris buffer at the ratio of 1:1. 10% Orange juice was supplemented to the tris coconut-water extender. It was stored in the refridgerator for further use. Five matured toms and cocks at their reproductive stage were selected and used for the experiment. They were trained for 2weeks for the semen collection exercise.

After two weeks, semen was ejaculated from tom and cock individually and pooled for both tom and cock separately. The pooled semen was divided into two portions for each species. One portion from each species was diluted with TCWOE at the ratio of 1:3 (semen: extender). Both diluted and undiluted semen for each species were examined for motility, viability, membrane and acrosome integrity immediately after dilution and 12h preservation period at 4-8°C. Three different trials were conducted and the results were recorded. Data collected were subjected to analysis of variance using SPSS® statistical software package.

Results and Discussion

Comparative effect of dilution on Tom and Cock semen with TCWO extender is presented in table 1. Percentage motility was significantly ($p<0.05$) higher in extended Cock semen, but was not significantly different to un-extended Cock and Turkey semen. Percentage live sperm and acrosome integrity revealed no significant different ($p>0.05$) in both species (extended and un-extended). Membrane integrity was significantly ($p<0.05$) higher in extended Cock semen although was not significantly different ($p>0.05$) from un-extended Cock and extended Turkey semen. Convincingly, TCWO extender revealed a favorable impact and important of diluting the poultry semen before insemination in this study. This could be attributed to the presence of substances in these fruit such as vitamins and phenolic compounds known to function as antioxidants (Gebhardt and Thomas, 2002; Kiwon *et al.*, 2003; Cutler *et al.*, 2008). In addition, it has been reported by (Zuo *et al.*, 2002) that ferulic acid acts synergistically with other antioxidants to reduce free radical damage to cell membranes.

Table 1: Comparative effects of diluting Tom and Cock semen with TCWO extender

TURKEY			COCK		
Parameters (%)	Un-extended Semen	Extended Semen	Un-extended Semen	Extended Semen	SEM
Motility	90.00 ^{ab}	88.75 ^b	93.75 ^{ab}	95.00 ^a	1.00
Live Sperm	96.00	96.00	96.50	98.25	0.51
Membrane Integrity	84.25 ^b	90.00 ^{ab}	91.00 ^a	93.00 ^a	1.19
Acrosome Integrity	100.00	100.00	100.00	100.00	0.00

Comparative effect of dilution on Tom and Cock semen with TCWO extender preserved for 12h at 4-8°C is presented in table 2. Motility and membrane integrity of both extended and un-extended Tom and Cock semen are significantly different ($p<0.05$) from each other. However, result recorded for both extended cock and tom semen are statistically similar ($p>0.05$). Lives sperm and acrosome integrity of extended and un-extended semen of both species were not significantly different ($p<0.05$). Although, extended cock semen had highest ($p<0.05$) value recorded for sperm motility and membrane integrity, but both extended cock and tom semen with TCWO extender possessed the higher value of sperm motility and membrane integrity compare to their un-extended counterparts in both species. This is evidence that TCWO extender has a positive impact on retaining the sperm activities of both economic poultry species used in this study. It implies that this extender is not species specific. The finding of this study corroborated AX *et al.* (2000) report that the survival of turkey ejaculated sperm in seminal plasma alone is limited to a few hours. But in contrary to the

observation of Leighton *et al.* (1969) Lake and Ravie, (1982) who reported that poultry semen rapidly lose viability and fertilizing capacity when stored either undiluted or diluted at physiological temperatures. The result of this work revealed that rate of loss in viability of poultry semen is evidently reduces by diluting it with TCWO extender. The improvement in this parameter could be attributed to conjugate synergy or complimentary effects of coconut water and orange juice being a plant source that contained vitamins and phenolic compounds which are known to function as antioxidants (Balogun, 2019). The result of this study also corroborated the report of Reza *et al.*, (2011) who stated that stored semen spermatozoa motility could be improved by antioxidant supplementation.

Table 2: Comparative effects of preserving Tom and Cock semen with TCWO extender for 12h at 4-8°C

	TURKEY		COCK		
Parameters (%)	Un-extended Semen	Extended Semen	Un-extended Semen	Extended Semen	SEM
Motility	40.00 ^c	75.00 ^{ab}	66.25 ^b	85.00 ^a	4.85
Live Sperm	89.50 ^b	94.75 ^a	92.00 ^{ab}	93.75 ^{ab}	0.84
Membrane Integrity	41.00 ^c	74.00 ^{ab}	64.75 ^b	87.75 ^a	5.27
Acrosome Integrity	100.00	100.00	100.00	100.00	0.00

CONCLUSION AND RECOMMENDATION

In conclusion, dilution of semen with TCOWE revealed a significant positive impact on the sperm cells activities of both Tom and Cock, but had more significant impact on dilution and short term cold storage holding potentials on cock semen than tom semen. It is therefore recommended that fertility trial should be further carried out on both species to ascertain the fertility potentials of both diluted and stored semen.

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