BREEDING, BIOTECHNOLOGY AND PHYSIOLOGY (ORAL)
SURVIVABILITY OF COCK SEMEN STORED UNDER ROOM TEMPERATURE IN EXTENDERS CONTAINING SOME ANTIOXIDANTS

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ABSTRACT
The study was conducted to determine the survivability of cock sperm stored under room temperature in extenders containing some antioxidants. A volume of 2ml of pooled semen collected from four mature Heavy Ecotype cocks was stored in Honey-Ringers solution and three other extenders containing Honey-Ringers solution supplemented with the antioxidant vitamins C and E. The semen samples were observed under a microscope at 0, 6 and 12 hours to determine sperm motility by scoring. Survivability of the sperm cells was taken as the average percentage number of motile spermatozoa in each extender recorded during 0, 6 and 12 hours storage duration. The experimental layout was a completely randomized design. The generated were analysed using one way analysis of variance (ANOVA) in SPSS. The result showed that Average sperm survivability was significantly ($P<0.05$) higher for semen stored in supplemented with the antioxidant vitamins C and E compared to semen stored in Ringers solution. The proportion of spermatozoa that survived within the 6 hour storage time was highest in the Vit E supplemented extender followed by the Vit C group and lowest in the control group. From the results, it was concluded that extenders supplemented with the antioxidant preserved semen motility and viability.
Key words: survivability,semen extender, antioxidants, Vitamin C, Vitamin E.

INTRODUCTION
The modern day farm animal reproduction is hinged on better reproductive techniques, one of which is artificial insemination (AI) (Akandi *et al.,* 2015). It is an effective and globally accepted method of breeding animals including poultry. Success in AI requires quality spermatozoa which have the capacity to reach the site of fertilization, fertilize the egg and activate embryonic development (Wilson, 2016). Its application is widely based on stored semen for future use. However, the length of storage of undiluted or extended semen is restricted and presents a significant decrease in spermatozoa viability and fertilizing capacity after 24 hours of storage (Donoghue and Wishart, 2000). Lipid peroxidation of sperm membrane lipids is a major cause of decreased motility and fertilizing ability of mammalian spermatozoa (Aurich *et al*., 1997). Avian semen is characterized by high level of unsaturated fatty acid within its phospholipids (Surai *et al.*, 1998c). This makes their semen susceptible to lipid peroxidation and spermatozoa deterioration during storage (Surai *et al.*, 1997; 1998c). Bozhedomov *et al.*, (2009) reported that Oxidative stress occurs when production of potentially destructive reactive oxygen species (ROS) exceeds natural antioxidant defenses thereby resulting in cellular damage. Toxic lipid peroxides are reported to cause impairments of sperm cells and may play a major role in the etiology of infertility in males (Firozeh *et al.*, 2010).
Avian semen contains natural antioxidants including ascorbic acid, tocopherol, catalase, glutathione peroxidase and superoxide dismutase that help to balance lipid peroxidation and prevent excessive peroxide formation (Beconi *et al.*, 1993). However, the endogenous antioxidant activity of seminal plasma and spermatozoa may not be enough to prevent peroxide damage after extension and *in-vitro* storage thus supplementation with antioxidants could improve the semen shelf life (Donoghue and Donoghue, 1997).

MATERIALS AND METHODS
Location of the experiment
The experiment was carried out at the poultry Unit of the Teaching and Research Farm, Department of Animal Science, University of Nigeria, Nsukka in Enugu State, Nigeria.
Experimental diets, animals and management
Thirty (30) mature heavy ecotype cocks with an average weight of 2.4±0-5kg were used for the experiment. The birds were kept in battery cage, one cock per cell to avoid physical contact between

the cocks and to avoid fight. Routine management practices including vaccination and drug administration where necessary were observed with water and feed provided *ad libitum.*

Experimental Design

The experiment was carried out in a completely randomized design (CRD). Ringer's solution + Honey extender was prepared at room temperature, and divided into 3 parts which were supplemented with the antioxidant vitamins C and E, and control corresponding to groups DII, DIII and DI, respectively. No antioxidant was added to the extender in the control group (DI). The compositions of the different extenders per L are shown in Table 1.

Table 1 Composition of Extenders

Ingredients	DI (Ringer)	DII (Ringer + Vit. C)	DIII (Ringer + Vit. E)
Sodium Chloride (g)	9.5	9.5	9.5
Potassium Chloride (g)	0.2	0.2	0.2
Calcium Chloride (g)	0.26	0.26	0.26
Sodium bicarbonate (g)	0.2	0.2	0.2
Honey (g)	1	1	1
Vitamin C (g)	0	0.4	0
Vitamin E (g)	0	0	0.08
CMC (ml)	0	0	5
Distilled water (L)	1	1	1

Fresh honey used in this research was purchased from local suppliers in Nsukka Main Market, Enugu State. The honey was used as purchased without further processing.

Semen Collection and Evaluation

Semen was collected from the toms using the abdominal manual massage method as described by Al-Daraji, (2007). On the day of semen collection, 2 mL of pooled semen was diluted (1:4) in each of the constituted extenders. Semen samples from the four cocks were pooled to reduce any likely variation in semen quality that may be caused by individual differences in genetic blueprint of the cocks. The determination of the different physical characteristics of the semen was made as described by Peters et al., (2008). The percentage motility of spermatozoa was evaluated immediately after semen collection. For this, a drop of semen was placed on a warm glass slide and covered with a cover slip to evenly spread the semen in the slide. The slide was afterwards viewed in the microscope (400 x magnification). Observations were widely made across different areas on the slide for the various samples. Motility was expressed as percentage of sperm showing normal forward progressive movements.Motility assessment was made by the same person to minimize differences during interpretation. Motility scores taken immediately after dilution were regarded as score for 0 hour storage, while experimental motility scores were recorded for 0, 6 and 12 hours of storage. Sperm concentration was determined by the method of direct cell count using an improved Neubauer hemocytometer. The percentages of live and dead spermatozoa were determined using an eosin–nigrosin stained smear with oil emulsion and observed under a light microscope at 400x magnification. Unstained spermatozoa were regarded as live whereas stained or partially stained spermatozoa were counted as dead. This determination was made over observations of about 200 spermatozoa per slide.

Statistical Analysis

The data generated were analysed using one way analysis of variance (ANOVA) in SPSS software (IBM® version 20.0). Differences found among the treatment means were compared using Duncan's New Multiple Range Test (Duncan, 1955) and accepted at 5% or 1% level of probability.

RESULTS AND DISCUSSION

Table 2: Mean (±SE) effect of diluents and storage duration on sperm survivability of Nigerian ecotype cock

Duration of storage (hrs)	Traits (%)	Ringer solution	Ringer solution + vit C	Ringer solution + vit E	P-value
0	Motility	85.56±3.91	87.22±4.41	87. 22±2.64	0.655
	Live	83.00±6.22	81.78±7.10	81.33±6.54	0.844
	Dead	17.00±6.22	18.22±7.10	18.67±6.54	0.844
6	Motility	70.00±3.54^{c}	75.00±3.54^{b}	80.00±5.00^{a}	0.000
	Live	64.56±5.43^{c}	70.44±7.43^{b}	78.11±6.47^{a}	0.000
	Dead	35.44±5.43^{a}	29.56±7.43^{b}	21.89±6.47^{c}	0.000
12	Motility	62.78±4.41^{c}	70.00±7.07^{b}	75.56±3.91^{a}	0.000
	Live	61.78±4.02	62.56±4.98	65.56±7.40	0.407
	Dead	38.22±4.02	37.44±4.98	34.44±7.40	0.407

^{a,b,c} – Means in the same row with different superscripts are significant at 5 or 1 % (* P < 0.05; ** P<0.01), NS- Not significant, Vit- Vitamin.

The result of the study on the effect of diluents and storage duration on sperm survivability of Nigerian ecotype cock are presented in Table 2.

The result showed that within 0 hour of storage, no significant difference (P > 0.05) was observed in semen progressive motility, percentage live and dead sperm cells among the various treatment groups. All extenders maintained high motility. At 0 hour post semen dilution, spermatozoa motility were 85.56±3.91%, 87.22±4.41%, and 87.22±2.64% for DI, DII, and DIII respectively. The percentage live spermatozoa at 0 hour post dilution were 83.00±6.22%, 81.78±7.10%, and 81.33±6.54% for DI, DII, and DIII respectively while percentage dead sperm cells were 17.00±6.22%, 18.22±7.10%, and 18.67±6.54% for DI, DII, and DIII respectively.

At 6 hours post dilution, significant difference (P < 0.05) was observed in progressive semen motility. Spermatozoa motility reduced to 70.00±3.54%, 75.00±3.54 %, and 80.00±5.00% for DI, DII, and DIII respectively. The same trend was followed for percentage live sperm cells. The percentage live sperm cells for DI, DII, and DIII were 64.56±5.43%, 70.44±7.43 %, and 78.11±6.47% respectively while percentage dead sperm cells recorded 35.44±5.43%, 29.56±7.43%, and 21.89±6.47% for DI, DII, and DIII respectively. The percentage of sperm cells that were motile within the 12th hour of storage was 62.78±4.4 %, 70.00±7.07 %, and 75.56±3.91% for DI, DII, and DIII respectively. There was distinct variation (P<0.05) in the motility of spermatozoa in the extenders. The proportion of spermatozoa that were motile within the 12 hours of storage time was highest in the DIII and lowest in DI. The percentage live sperm cells for DI, DII, and DIII were 61.78±4.02%, 62.56±4.98%, and 65.56±7.40 % respectively while percentage dead sperm cells recorded 38.22±4.02%, 37.44±4.98%, and 34.44±7.40% for DI, DII, and DIII respectively. Although these figures differed numerically, they were statistically similar (P>0.05).

DISCUSSION

Motility, expressed as the percentage of spermatozoa moving through the power of their tail is used as an indicator of fertility. The mean motility value (86.33 ± 3.65) from this study was within the established normal range proposed by Sexton (1981) who reported a mean motility of 80% and above as excellent.

The motility results decreased across the groups with increase in storage time. This drop in motility was witnessed more in the control group compared with the Vit. C and Vit. E treated groups. This is because of the antioxidative properties of Vit. C and Vit. E.

The results from this study showed that antioxidant supplementation of cock semen extenders improved the viability of stored cock semen over the control. The vitamin E supplemented group (DIII) had a better viability followed by vitamin C (DII) while the least values were observed in the control (DI) over the storage period. This finding is supported by the earlier report of Donoghue and Donoghue (1997). Similar results were also reported by Tabatabaei *et al.* (2011) who observed that supplementing semen extender with vitamin E improved spermatozoa quality parameters including motility and viability of cock semen. Al-Daraji (2002) also reported an improvement in semen

quality of roosters supplemented with vitamin C and E. The better viability observed in vitamin E and C group compared to the control could be attributed to the larger distribution of Vit E within the plasma membrane of spermatozoa and its lipid solubility allows it to suppress peroxidative damage across the plasma membrane and the efficiency of ascorbic acid (Vit C) as an antioxidant which scavenges ROS thus protecting sperm membrane against lipid peroxidation.

CONCLUSION

The findings of this study have shown that antioxidant supplementation of cock semen extenders helped to preserve semen motility and viability. Cock semen supplemented with vitamin E performed better, followed by vitamin C supplemented group. These antioxidants hold great prospect in our quest to develop local extenders that are of low cost and environmentally friendly and this is of immense benefit in rural communities in many developing countries, where commercially known extenders are not readily available to farmers for AI.

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