SECTION OF PREFERENCE: ANIMAL PHYSIOLOGY AND REPRODUCTION THE POTENTIAL OF DEXTROSE SALINE FORTIFIED WITH CARROT JUICE AS SEMEN EXTENDER IN LAYER BREEDER CHICKENS

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ABSTRACT
Artificial insemination is one of the best management technique that can be utilized to improve the reproductive efficiency of layer breeder chickens. Carrot juice was extracted from carrot fruits by using standard procedure. The pH of the supernatant fluid was examined with the aid of pH meter and was buffered with drop of potassium hydroxide (1g of the potassium hydroxide into 5ml of water) to pH of 7.0. The prepared carrot juice ((CJ) was used to fortified 1% dextrose saline at different concentrations 25% (T25), 50% (T50), 75% (T75) and 100% (T100) to form the extenders. Semen collected from three Isa Brown breeder cocks were pooled and divided into five portions. Each portion was extended with the formulated extenders at ratio 1:1, while dextrose saline (DS) served as the control. The extended semen was evaluated in vitro for quality characteristics (sperm cells motility and livability). In the second hour DST (60.50±0.50%) was significantly (p<005) higher than CJT25% (56.75±2.50%), CJT50% (55.25±8.54%), CJT75% (51.50±2.89) and CJT100% (50.50±0.50%). The same trend was obtained in the third hour. DST (50.00±0.00%) was significantly (p<005) higher than CJT25% (41.25±1.25%), CJT50% (43.75±2.39%), CJT75% (46.25±1.25%) and CJT100% (45.25±1.25%), while the semen fortified with carrot juice extract sustained spermatozoa livability up to third hour. CJT25% (60.00±3.66%), CJT50% (60.00±3.65%), CJT75% (60.00±3.70%) and CJT100% (63.75±3.75%), was significant (p< 0.05) higher than the sperm cell livability of the DST (40.00±0.00%). Dextrose saline fortified with 25% carrot juice extract did not enhance sperm cell motility beyond 2 hours and livability beyond 4 hours in vitro.
Keywords: ISA Brown breeder, Semen extender, Dextrose saline, Carrot-juice extender

INTRODUCTION

Artificial insemination is one of the best management technique that can be utilized for the improvement of the reproductive efficiency of layer breeder chicken (Penfold *et al.,* 2000). Artificial insemination has been proven to be a useful tool in the improvement of reproductivity in the poultry industry (Penfold *et al.,* 2000) as it has the advantage of efficient utilization of poultry semen compare to natural mating (Penfold *et al.,* 2000, Brillard, 2003). During natural mating a cock will release an average of 0.06ml of semen per ejaculate (Penfold *et al.,* 2000, Olujimi and Ewuola, 2023) which is relatively small compare to volume of semen require to inseminate the laying capacity of the female strain of the bird. However, the efficient utilization of the poultry semen required the dilution of the poultry semen with suitable diluent and extender that are easily available, affordable and which are capable of mitigating the adverse effect of reactive oxidative species (ROS) which imposes stress on the sperm cell qualities. The oxidative stress in the sperm cells has been attributed to the consistence increase in the level of oxidation of the cellular components in combination with the high production of reactive oxygen species (ROS).

Despite the potential of the female strain of the bird to lay up to 300 eggs in her first laying year (Haftu, 2016). Artificial insemination protocol has not been developed to enhance the reproductive efficiency of the male strain of the bird, in term of the development of best semen extender for the mitigation of the oxidative stress which compromises the fertility potential of the sperm cells.

Evidence has shown that carrots are excellent source of vitamins which have much antioxidant (anthocyanins) which contains more adequate non-provitamin carotinoid hydrocarbon compound called lycopene (Ting *et al.* 2009). It is capable of protecting the body cells against oxidative stress and environmental toxin (Singh *et al.,* 2018). In addition, it has recently been proved that the consumption of carrots improved the level of fertility in male (Lizette, 2013), as it has been documented to have best results on sperm motility and livability (Lizette, 2013, Ewuola, *et al.,* 2015)

Despite the potential of this additive, there is death of documentation on how it can be utilized to improve the fertility potential of Isa Brown Layer Breeder Chickens as semen extender.
Therefore, this study was conducted to assess the effect of fortifying dextrose saline, a good energy sources (Adebisi and Ewuola, 2019, Bolarinwa and Ewuola, 2021) with carrot juice extract on the sperm cell qualities.

MATERIALS AND METHODS

Extraction of the Carrot Juice-Extender

The carrot juice was extracted from twenty pieces of carrots with the aid of juice extractor. The carrot was washed with distilled water, cut into pieces and macerated. The carrot juice was collected and sieved with the aid of sieve gauze and centrifuged at 4600rpm for 10minuets after which the supernatant was decanted into a storage tube. The pH of the supernatant fluid was examined with the aid of pH meter and was buffered with drop of potassium hydroxide (1gram of the potassium hydroxide into 5ml of water) to pH of 7.0.

The Reconstitution of the Dextrose Saline and Formulation of the Extenders

4ml of normal saline was diluted with 1ml of 0.9% dextrose saline in a separate bottle to form 1% dextrose saline and the pH of the diluted dextrose saline solution was measured to be 7.00.
The prepared carrot juice extract was used to fortify 1% dextrose saline at different concentrations 25%, 50%, 75% and 100% as indicated
100% of dextrose saline + 0% of carrot juice extract DST
75% of dextrose saline + 25% of carrot juice extract CJT_{25}
50%of dextrose saline + 50% of carrot juice extract CJT_{50}
25% of dextrose saline +75% of carrot juice extract CJT_{75}
0% of dextrose saline + 100% of carrot juice extract CJT_{100}

Semen Extension using Formulated Extenders

Semen collected from three breeder cocks were pooled and divided into five portions. Each portion was extended with the formulated extenders at ratio 1:1. The extended semen was evaluated *in vitro* for quality characteristics.

The *In vitro* Evaluation of Semen extended with Dextrose Saline Fortified with Carrot Juice Extender

Sperm Cells Motility

A little drop of the extended semen was gently placed on a warmed glass slide. The mixture was then covered with a coverslip and then assessed under a microscope at (magnification ×400) progressive motility of the sperm cells was scored on a scale of 0-100%

Sperm Cells Livability

The sperm cells livability was determined by rubbing a drop of extended semen and *Eosin Negrosin* stain on a microscope glass slide. The glass slide was air dried for some minutes then examined for some minutes under the microscope and scored in percentage (0-100%).The sperm cells that absorbed the eosin nigrosin solution to any extent was scored as dead sperm cells and those that did not absorb the eosin nigrosin were scored as live sperm cells and rated in percentage (Ewuola and Egbunike, 2010).

Statistical Analysis

Data collected were subjected to log transformation before analysed using descriptive statistics, analysis of variance (ANOVA) using (SAS, 2023), while Duncan multiple ranged tests was used to separate the means at $\alpha_{0.05}$

RESULTS

The *in vitro* Sperm Cells Motility of the Semen Extended with Graded Levels of the Carrot Juice -Extender

The *in vitro* sperm cell motility of the semen extender fortified with graded levels of the carrot juice extract is presented in Table 1. There was no significant different in the sperm cell motility of the $CJT_{25\%}$, $CJT_{50\%}$, $CJT_{75\%}$ and $CJT_{100\%}$ in the zero and first hour. The values obtained ranged from 90.00±0.00% - 92.25±1.31% and 79.00±3.53% - 83.75±1.25%, respectively. In the second hour DST (60.50±0.50%) was significantly ($p<005$) higher than $CJT_{25\%}$ (56.75±2.50%), $CJT_{50\%}$ (55.25±8.54%), $CJT_{75\%}$ (51.50±2.89%) and $CJT_{100\%}$ (50.50±0.50%). The same trend was obtained in the third hour.

DST (50.00±0.00%) was significantly (p<005) higher than CJT25% (41.25±1.25%), $CJT_{50\%}$ (43.75±2.39%), $CJT_{75\%}$ (46.25±1.25%) and $CJT_{100\%}$ (45.25±1.25%).

Sperm Cells Livability of the Semen Extended with Graded Levels of the Carrot Juice - Extender

The sperm cell livability of the carrot-juice extender fortified with dextrose saline at graded level is presented in Table 2. There was no significantly (p>0.05) difference in the sperm cell livability at the first, second, and third hour of storage. While at the fourth hour interval the sperm cell livability $CJT_{25\%}$ (60.00±3.66%), $CJT_{50\%}$ (60.00±3.65%), $CJT_{75\%}$ (60.00±3.70%) and $CJT_{100\%}$ (63.75±3.75%), was significant (p< 0.05) higher than the sperm cell livability of the DST (40.00±0.00%).

Table 1: Sperm Cell Motility (%) of the Semen fortified with Graded Levels of Carrot Juice - Extender

Treatment	CJT_{25} (%)	CJT_{50} (%)	CJT_{75} (%)	CJT_{100} (%)	DST (%)
0 hour	92.25±1.31	91.25±2.39	90.75±2.17	90.75±1.25	90.00±0.00
1 hour	80.00±2.04	83.75±1.25	80.25±1.25	79.00±3.53	80.00±3.54
2 hour	56.75±2.50^{b}	55.25±8.54^{b}	51.50±2.89^{c}	50.50±2.50^{c}	60.50±0.50^{a}
3 hour	41.25±1.25^{c}	43.75±2.39^{ab}	46.25±1.25^{b}	45.25±1.25^{b}	50.00±0.00^{a}

^{abc}=means on the same row with different superscripts are significantly (P < 0.05) different, DST= dextrose saline treatment, CJT= carrot juice treatment, % = percentage

Table 2: Sperm Cells Livability (%) of the Semen Fortified with Graded Levels of the Carrot Juice -Extender

Treatment	CJT_{25} (%)	CJT_{50} (%)	CJT_{75} (%)	CJT_{100} (%)	DST (%)
0 hour	94.25±2.17	90.50±2.50	92.25±1.49	93.50±1.89	93.50±1.50
1 hour	80.25±2.17	80.50±2.50	80.25±1.49	80.25±1.49	80.25±4.34
2 hour	70.50±5.00	70.25±2.99	70.25±1.49	70.75±2.39	72.75±2.66
3 hour	65.00±2.04	63.25±2.39	62.75±1.25	62.50±1.44	66.50±1.44
4 hour	60.00±3.66^{a}	60.00±3.65^{a}	60.00±3.70^{a}	63.75±3.75^{a}	40.00±0.00^{b}

^{abc}=means on the same row with different superscripts are significantly (P < 0.05)
different, DST= dextrose saline treatment, CJT= carrot juice treatment, % =percentage

Discussion

The harmful effects of the reactive oxygen species (ROS), which do affect the qualities of the sperm cells as a result of the gaseous exchange (aerobic) of the cell, is expected to be mitigated by the antioxidant (lycopene) that is present in the carrot juice (extract). The sperm cell motility was significantly higher in the TCJ_{25} (treatment group fortified with the 25% of the carrot juice extract) at the 2rd hour of the *in vitro* evaluation compared to the other treatment groups TCJ_{50} (the treatment group fortified with the 50% of the carrot juice extract), TCJ_{75} (treatment group fortified with the 75% of the carrot juice extract) and TCJ_{100} (treatment group fortified with the 100% of the carrot juice extract). Although the sperm cells livability result revealed that there was decrease in the livability of the sperm cells across the various treatment groups from the 0 hour till the 4th hour, however, the livability of the sperm cells did not drop below 60% across the various treatment groups. It could be suggested the carrot juice extract did not have the capacity to improve the sperm cell motility beyond the 2nd hour which corroborates the report of (Ewuola *et al.,* 2015) who supplemented the carrot juice extract with milk based extender. Carrot juice milk based extender did not enhance the sperm cells motility beyond the 2nd hour but improved the quality of the sperm cells livability from 0 hour till the 4th hour. Evidence has shown that the purple-orange carrot used in this study did not have much antioxidant (anthocyanins) property compared to the purple- yellow carrot which contains more adequate non-provitamincarotinoid hydrocarbon compound called lycopene (Ting *et al.* 2009), which is capable of protecting the body cells against oxidative stress and environmental toxin (Singh *et al.,* 2018).

CONCLUSION

Dextrose saline-carrot juice extender sustained spermatozoa livability up to the third hour *in vitro* at 27.6ºC in Isa Brown breeder chicken**,** while the dextrose saline fortified with 25% carrot juice extract did not enhance sperm cell motility beyond 2 hours in ISA Brown breeder chicken**.**

Therefore, dextrose saline fortified with 25% carrot can be used in Nigeria layer breeder industry for the storage of ISA Brown layer breeder semen.

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