

EFFECTS OF THE CARBONATED DRINK AS AN EXTENDER ON SEMEN CHARACTERISTICS, FERTILITY AND HATCHABILITY IN NIGERIAN INDIGENOUS CHICKEN

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Abstract: Semen extenders are liquid diluents that buffer sperm cells and preserve their fertilizing potentials. The commercial carbonated drink (CD) as an extender was evaluated on semen characteristics, fertility and hatchability in Yoruba ecotype chickens (YECs). The fructose of the CD was 1.52 ± 0.05 mg/ml. Under the conditions of 37°C, 5% and 10% of CD were added to the egg yolk citrate solution to make 100%. Semen was obtained from ten matured Yoruba ecotype chicken cocks with an average weight of 1.8 ± 0.2 kg. The semen was pooled in a test tube and added to the extenders for preservation at 0, 30 and 60 minutes, respectively, in a factorial design layout. Percentage motility of sperm cells was significantly ($p < 0.05$) higher in 5% CD inclusion compared with 10% CD inclusion and control. Motility decreased with an increase in preservation time across the treatments. The percentage of dead sperm cells decreased ($p < 0.05$) in 5% CD inclusion when compared with 10% CD inclusion and control. The sluggish sperm percentage increased significantly ($p < 0.05$) with semen preservation time. Fertility and hatchability of eggs were significantly ($p < 0.05$) higher in 5% CD inclusion. It was concluded that carbonated drinks at 5% inclusion in an extender could preserve cock sperm cells for 60 minutes with improved fertility and hatchability of eggs.

Key words: semen, extender, carbonated drink, motility, Yoruba ecotype chickens.

Introduction

Poultry is important livestock in the agricultural economy of Nigeria, both as sources of good quality protein and income generation. Huge resources are committed to importing birds while the Nigerian indigenous chicken (NIC) remained uncharacterized and unimproved (Ige et al., 2012). Nigerian indigenous chickens (NICs) are relatively small-sized birds that produce semen of low volume and high concentration (Akanbi, 2018). Improving the YEC would require

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committed mating, which could be facilitated by artificial insemination (AI). The increasing use of AI in poultry emphasizes the need for qualitative semen. The efficacy of AI could be ascertained by an efficient extender with an improved diluent (Mian et al., 1990). The advantages of the semen extender include the maximum use of semen in short supply and a reduction in the male to female mating ratio. NIC produces about 0.2ml of semen per ejaculate (Akanbi, 2018), which may be difficult to handle in AI. Diluents enhance the spread of semen over many hens (Mian et al., 1990), and the application of an extender is essential to sustain semen quality (Bootwalla and Miles, 1992), which could be preserved without impairing the viability and fertilizing ability of the semen (Lukaszewicz et al., 2008). An appropriate semen extender has to maintain sperm cell motility, fertility capacity and membrane integrity (Rihast et al., 2006).

Generally, an extender will facilitate semen handling, maintain sperm viability, and inhibit pathways detrimental to sperm cell survival. Several sources such as coconut, tomato and carrot (Banerjee, 2011) and *Borassus aetiopium* (Adeyina et al., 2017) have been reported for their semen extending potential, and there are a number of buffered salt solutions available as extenders for chicken semen. These extenders have a different composition, sometimes with special additives such as skimmed milk, egg albumin, glutamine sulphate and carbonic acid (Iaffaldano et al., 2007). The carbonated drink is one of such solutions with the composition that could support semen extending potentials. The carbonated drink contains sugar, mild acid, sodium citrate and other minerals that could serve as minimum essential medium (MEM) for semen survival. The availability and affordability of the carbonated drink (CD) could make it a ready-made additive of importance in semen extenders for reproductive improvement. The need to evaluate the potential of CD in enhancing the viability and fertility of semen for the successful application of AI has necessitated this research.

Material and Methods

The experiment was conducted at the poultry unit of the Teaching and Research Farm, Faculty of Agriculture, University of Ilorin, Ilorin, Nigeria.

The carbonated drink (CD) was obtained from a commercial outlet within the University campus. The drink was contained in a green bottle produced by a globally renowned carbonated drink manufacturer. The solution was colourless and drizzling when opened. The solution was opened and allowed to settle for about five minutes, after which 2 ml of the CD was taken and analyzed for the mineral composition, according to Volpe et al. (2015).

Sodium citrate solution was prepared by weighing and dissolving 2.90g of sodium citrate dehydrate into 100ml of distilled water. The solution was sterilized and kept in the refrigerator, according to Singh (2005). Egg yolk solution was prepared by breaking an egg at the narrow end with a forcep and draining out the

albumin. The egg yolk membrane was broken, and the clear yolk was poured into a measuring cylinder. The egg yolk citrate solution was prepared by adding 40% of the egg yolk into a 60% sodium citrate solution to serve as a control. Treatment one had 40% egg yolk added to a 55% sodium citrate solution and 5% CD, while treatment two had 40% egg yolk added to a 50% sodium citrate solution and 10% CD. The solutions were then placed in a water bath maintained at 37°C.

Ten matured YEC cocks (average weight 1.8 ± 0.2 kg) aged 12 months were obtained from the stock of YEC being raised on the Teaching and Research Farm, University of Ilorin. The birds were trained to be used to the milking procedures and then milked to obtain semen using the massaging method as described by Adeyina et al. (2016). The undiluted semen was pooled into a test tube and analyzed for semen quality and characteristics using the method of Wishart (1995). From the pooled semen, 0.5 ml was taken into 2.5 ml of the extender in test tubes already placed in the water bath in the ratio of 1:5, according to Adeyina et al. (2017). All the semen solutions were maintained at the same temperature in the water bath. Samples, in triplicate from the semen solutions, were taken on a slide and observed under a microscope (Olympus model x40) at 0, 30 and 60 minutes according to the method described by Wishart (1995). Sixty actively laying hens of YEC (weighing 1.3 ± 0.2 kg) were selected and inseminated using semen with 0%, 5% and 10% inclusion of CD. The birds were divided into three treatments and four replicates (five hens per replicate). The insemination was done using 0.1 ml of the extended semen at 0, 30 and 60 minutes. The hens were kept individually in a battery cage system and fed with a commercial layer mash of 17.5% CP and 2700 kcal/kg M.E, water was offered *ad libitum*. Eggs from the birds were collected after 24 hours following insemination for four days before the insemination was repeated. A total of 150 eggs, 50 eggs /treatment, were set in the incubator.

Table 1. The percentage composition of experimental extenders.

Egg yolk	40	40	40
Sodium citrate (buffer)	60	55	50
Carbonated drink (CD)	0	5	10
Total	100	100	100

The experiment was conducted in line with the university's guideline for the ethical treatment of experimental animals in accordance with the best practices within Institutional Animal Care and Use Committee (IACUC) guidelines.

Statistical analysis

All data obtained were subjected to statistical analysis using the analysis of variance (ANOVA) procedure following a factorial (2x3) model (SAS 1999), and the level of interaction was determined using the same procedure.

Results and Discussion

Table 2 shows the semen characteristics and biochemical properties of undiluted cock semen. The value of the average volume of semen from the cock was within 0.2 and 0.3 ml, corroborating the findings of Akanbi (2018), who reported the semen volume of YEC as 0.3ml. The percentage motility of 98% recorded in this study indicates that YEC sperm is of very good quality in accordance with the value presented for NIC by Ajayi et al. (2014). The sperm concentration of $256 \times 10^6/\text{ml}$ recorded in this study was higher compared with $248 \times 10^6/\text{ml}$ reported by Akanbi (2018), supporting the fact that semen of low volume is usually of high concentration. The value of the fructose is higher than 2.0mg/ml reported for cocks by Singh (2005). Fructose is an energy source for semen metabolism, and the high fructose concentration is a reflection of energy required to support the metabolism of a sperm cell with high concentration.

Table 2. Characteristics of undiluted semen.

Parameters	Values $\pm$ SD
Volume (ml)	0.2 ± 0.08
Concentration ($\times 10^6/\text{ml}$)	256 ± 18.57
Motility (%)	98 ± 5.64
Sluggish (%)	0 ± 0.0
Dead (%)	2 ± 0.61
Total protein (mg/ml)	2.95 ± 0.51
Fructose (mg/ml)	2.95 ± 0.06
Potassium (mg/ml)	18.83 ± 2.32
Sodium (mg/ml)	34.95 ± 1.07
Magnesium (mg/ml)	2.98 ± 0.33
Calcium (mg/ml)	0.95 ± 0.11
Osmolarity (Osm/L)	388.94 ± 21.55
pH	7.1 ± 0.46

Table 3 shows the chemical composition and the properties of carbonated drink. The presence of minerals is necessary for the maintenance of the certain physiochemical process essential to life. It is believed that this edible CD was formulated to provide the needed energy for the mass activities of sperm cells.

Table 4 shows the effect of the CD and the preservation period on semen quality. There was a significant ($p < 0.05$) reduction in sperm cell motility while the percentage of sluggish and dead cells significantly ($p < 0.05$) increased with an increase in preservation time. However, the value of motility of 75% at 60 minutes was still within the range of good quality semen. This suggests that carbonated drinks in diluents supported a minimum essential medium (MEM) for survivability. According to Singh (2005), MEM for sperm cell survival includes soluble sugars

and minerals (Fukuhara and Nishikawa, 1993), which were adequately available for up to 60 minutes.

Table 3. Properties of the carbonated drink.

Parameters	Value $\pm$ SD
Fructose (mg/ml)	1.52 $\pm$ 0.05
Potassium (mg/ml)	7.35 $\pm$ 2.13
Sodium (mg/ml)	274.07 $\pm$ 10.24
Magnesium (mg/ml)	0.94 $\pm$ 0.03
Calcium (mg/ml)	0.94 $\pm$ 0.08
Osmolarity (Osm/L)	615.83 $\pm$ 20.68
pH	5.6 $\pm$ 0.07

Table 4. The effects of carbonated drinks and preservation periods on semen characteristics.

Parameters	Carbonated drinks				Preservation periods (minutes)				
	control	5%CD	10%CD	SEM	0	30	60	SEM	CD*PP
Motility (%)	85.17 ^b	88.00 ^a	85.00 ^b	1.02	97.17 ^a	86.00 ^b	75.00 ^c	3.06	*
Sluggishness (%)	1.83	1.50	1.66	0.67	0.33 ^c	2.04 ^b	3.75 ^b	0.74	*
Dead cell (%)	13.50 ^a	10.50 ^b	13.33 ^a	1.24	2.50 ^c	11.88 ^b	21.25 ^a	2.25	*

a, b, c means with different superscripts within the same row are significantly different ($p < 0.05$). S=significant and SEM=standard error of the means.

Table 5 shows the effect of CD and time of preservation on sperm cell motility. Sperm motility was significantly ($p < 0.05$) reduced with an increase in preservation time. However, the motility (%) in the 5% inclusion of CD was higher than that of control. At 60 minutes, the motility was still above 70%, signifying that the inclusion of CD supported the good fertilizing ability of the semen (Adeyina *et al.*, 2017). The inclusion of CD at 5% and 10% did not affect the motility at 0 minute compared with the control because the CD is edible and contains minerals supportive of life processes, including semen metabolism due to the presence of MEM. More importantly, the sperm cell motility in 5% and 10% inclusions of CD is of importance to the overall usefulness of CD as an extender.

Table 5. The effect of the CD and preservation time on sperm cell motility (%).

Treatment	Control	5% CD	10% CD
Periods			
0	96.33 ^a	98.00 ^a	98.00 ^a
30	85.17 ^b	88.00 ^b	85.00 ^b
60	74.00 ^c	78.00 ^c	72.00 ^c
SEM	10.27	8.29	9.13

a, b, c means with different superscripts in the same column are significantly different ($p < 0.05$). SEM=standard error of the means.

Table 6 shows the effect of the CD and time of preservation on sperm cell abnormality. Sperm cells abnormality significantly ($p<0.05$) increased with the period of preservation, but it was still within the range of 9.9–12.8% for successful AI (Tselusi et al., 1999).

Table 6. The effect of the CD and preservation time on sperm cell abnormality (%).

Treatment	Control	5% CD	10%CD
	Periods		
0	0.67 ^c	0.00 ^c	0.00 ^c
30	1.84 ^b	1.50 ^b	1.67 ^b
60	3.00 ^a	3.00 ^a	3.33 ^a
SEM	0.23	0.40	0.62

a, b, c means with different superscripts in the same column are significantly different ($p<0.05$). SEM = standard error of the means.

Table 7 shows the effect of the CD and time of preservation on semen dead cells. The percentage of dead cells increased with time across the treatments. According to Tselusi et al., (1999), the survivability of semen reduces with time. The inclusion of 5% CD reduced dead cells compared with 10% inclusion and the control at 60 minutes.

Table 7. The effect of the CD and preservation time on dead sperm cells (%).

Treatment	Control	5% CD	10% CD
	Periods		
0	3.00 ^c	2.00 ^c	2.00 ^c
30	13.0 ^b	10.50 ^b	13.34 ^b
60	23.00 ^a	19.00 ^a	24.67 ^a
SEM	4.01	3.82	2.93

a, b, c means with different superscripts in the same column are significantly different ($p<0.05$). SEM = standard error of the means.

Table 8 shows the effect of CD on the fertility and hatchability of eggs. The inclusion of CD at 5% was significantly ($p<0.05$) higher in fertility and egg hatchability compared with that of 10% and the control. This could be due to the maintenance of sperm cell integrity in 5% CD as a result of moderate metabolic activity and energy availability. The CD provides additional energy for sperm cell function, having been constituted with sugar (fructose). The availability of carbonate in the CD could have caused the production of CO₂, which could reversibly immobilize sperm cell. According to Long (2006), CO₂ narcosis is an effective means to maintain the viability and fertilizing ability of spermatozoa. Carbonated water is the water in which bubbles were added (Anon, 2004).

However, 10% CD seems to have resulted in toxicity of the sperm cells, hence, the poor fertility and hatchability in this treatment.

Table 8. The effect of CD on the fertility and hatchability of eggs.

Treatment	Fertility (%)	Hatchability (%)
Control	77.00 ^b	67.00 ^a
5% CD	83.00 ^a	70.00 ^a
10% CD	54.00 ^c	46.00 ^b
SEM	4.83	3.72

a, b, c means with different superscripts in the same column are significantly different ($p < 0.05$). SEM = standard error of the means.

Conclusion

Carbonated drinks up to 10% have the capacity of a potential extender for cock semen, and they are supportive of semen storage on a short-term basis. They could preserve cock's sperm cells for up to 60 minutes. It can, therefore, be concluded that CD at 5% inclusion in the egg yolk citrate diluent provides considerably high fertility and hatchability of eggs and could be used in the storage of YEC cock semen for AI over a short distance before usage.

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References

- Adeyina, A.O., Ojetunji, C.T., Adeyina, O.A., Annongu, A.A., & Akanbi, A.S. (2016). Effect of cold aqueous extract of *Moringa oleifera* on reproductive parameters and relative organ weight in cockerels. *Wayamba journal of Animal Science*, 9, 1539-1548.
- Adeyina, A.O., Akanbi, A.S., Okupke, K.M., Ogbah, I., Alli, O.I., Adegboye, A.A., & Bolaji, M. (2017). Effect of *Borassus aethiopum* extract as a biological extender on semen characteristics, fertility and hatchability in chickens. *Journal of Agricultural Sciences*, 62 (4), 385-393.
- Ajayi, F.O., Agaviezor, B.O., & Ebogomo, D. (2014). Comparative studies of semen and haematology quality of Nigerian indigenous and exotic chicken breeds in the humid tropical zone of Nigeria. *Global Journal of Bio-science and Biotechnology*, 3 (2), 164-168.
- Akanbi, A.S. (2018). Physiological and reproductive responses of Yoruba ecotype chickens to dietary and hormonal manipulations. *Ph.D. Thesis Faculty of Agriculture, University of Ilorin, Ilorin, Nigeria*.
- Anon (2004). Detection of syrup addition to apple juice by capillary gas chromatography recommendation No 4, in *IFU Compendium of Analytical Methods for Fruit Juices and Nectars*, Swiss Fruit Juice Union, Zug, Switzerland.

- Banerjee, C.C. (2011). *A Textbook of Animal Husbandry*. Eight edition. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi.
- Bootwalla, S.M., & Miles, R.D. (1992). Developments of diluents for domestic fowl semen. *Poultry Science Journal*, 48, 121-126.
- Fukuhara, R., & Nishikawa, Y. (1993). Effects of various substrates on respiration, glycolysis and motility of goat spermatozoa. *Japanese Journal of Zoo Science*, 44, 271-274.
- Iaffaldano, N., Manchisi, A., & Rosato, M.P. (2007). The preservability of turkey semen quality during liquid storage in relation to strain and age of males. *Journal Animal Reproduction Science*, 11, 024. DOI: 101016
- Ige, A.O., Salako, A.E., Yakubu, A., & Adeyemi, A.S. (2012). Qualitative Traits Characterization of Yoruba and Fulani Ecotype Indigenous Chickens in Derived Savannah Zone of Nigeria. *International Journal of Poultry Science*, 11 (10), 616-620.
- Long, J.A. (2006). Avian semen cryopreservation: what are the biological challenges? *Poultry Science*, 85, 232-236
- Lukaszewicz, E., Jersey, A., & Partyka, A. (2008). Efficacy of evaluation of rooster sperm morphology using different staining methods. *Research Veterinary Science*, 85, 583-588.
- Mian, Z., Nasim, A., Shakoar, A., & Qureshi, M.S. (1990). Effect of dilution of fowl semen with normal saline on the fertility of RIR hens through Artificial Insemination. *Pakistan Journal of Agriculture*, 11 (3), 201-204.
- Rihast, L., Apolen, D., Pivko, J., Grafenau, P., & Kubovicova, E. (2006). Influence of implementing sheep fertility out of season. *Slovak Journal of Animal Science*, 4, 180-182.
- SAS (1999). Statistical Analysis Institutes. User's guide SAS institute Inc. Cary N.C.
- Singh, B.K. (2005). *Textbook of Andrology and Artificial Insemination in Farm Animals*, (pp. 440-514) New Delhi: Jaypee Brothers Medical Publisher (P) LTD.
- Tselusi, K., Seigneurin, F., & Blesbois, E. (1999). Comparison of cryoprotectants and methods of cryopreservation on fowl spermatozoa. *Poultry Science*, 78, 586-590
- Volpe, M.G., Michele Di Stasio, M.N., Siano, F., Raffaele Coppola, R., & De Marco, A. (2015). Content of micronutrients, mineral and trace elements in some Mediterranean spontaneous edible herbs *Chemistry Central Journal*, 9, 57.
- Wishart, G.J. (1995). New approaches to evaluating male and female fertility. In: Bakst, M.R., & Wishart, G.J. (Ed.), *Proceedings of first International Symposium on the Artificial Insemination of Poultry*. Poultry Science Association (pp. 207-223). Savoy, IL.

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UTICAJI GAZIRANOG PIĆA KAO RAZREĐIVAČA NA KARAKTERISTIKE SPERME, PLODNOST I IZVODLJIVOST KOD NIGERIJSKIH DOMAĆIH PILIĆA

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R e z i m e

Razređivači sperme su tečni razblaživači koji puferiraju ćelije sperme i čuvaju njihov potencijal oplodjenja. Komercijalno gazirano piće (GP) kao razređivač procenjeno je u odnosu na karakteristike sperme, plodnost i izvodljivost kod pilića ekotipa Joruba. Sadržaj fruktoze u gaziranom piću iznosio je $1,52 \pm 0,05$ mg/ml. U uslovima temperature od 37°C, 5% i 10% gaziranog pića dodati su citratnom rastvoru žumanceta kako bi se dobilo 100%. Sperma je uzeta od deset zrelih petlova pilića ekotipa Joruba prosečne težine $1,8 \pm 0,2$ kg. Sperma je sakupljena u epruvetu i dodata je razređivačima radi čuvanja u vremenskom periodu od 0, 30 odnosno 60 minuta u faktorskom dizajn rasporedu. Procenat pokretljivosti spermatozoida bio je značajno ($p < 0,05$) veći kod 5% GP u poređenju sa 10% GP u rastvoru i kontrolom. Pokretljivost se smanjivala sa povećanjem vremena čuvanja tokom tretmana. Procenat mrtvih ćelija sperme se smanjio ($p < 0,05$) kod 5% GP u poređenju sa 10% GP i kontrolom. Procenat slabo pokretnih ćelija sperme se značajno povećao ($p < 0,05$) sa vremenom čuvanja sperme. Plodnost i izvodljivost takođe su bili značajno ($p < 0,05$) veći kod 5% GP. Zaključeno je da gazirana pića u količini od 5% u razređivaču mogu da sačuvaju spermatozoide petla tokom 60 minuta uz poboljšanu plodnost i izvodljivost.

Ključne reči: sperma, razređivač, gazirano piće, pokretljivost, pilići ekološkog tipa Joruba.

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