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TOMATO JUICE SUPPLEMENTATION IN PHYSIOLOGICAL SOLUTIONS AND EGG YOLK EXTENDERS IMPROVES BROILER ROOSTER SEMEN EXTENSION

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ABSTRACT

The use of semen extenders is essential for maximizing the potential of artificial insemination in the poultry sector. The aim of this study was to assess whether a tomato-based semen extender is appropriate for semen extension in a Broiler parent stock. Three broiler roosters were used the experiment was replicated three times. The process of semen collection involved the stimulation of the copulatory organ through gentle massage of the abdomen and back areas surrounding the testes. The semen was evaluated at room temperature. Pre-extension Semen parameters evaluated were semen volume, pH, motility, concentration, morphological abnormalities and viability which were; 0.45 ± 0.05, 7.25 ± 0.35, 85 ± 7.07, 2.65 ± 0.35 ($\times 10^9$), 8 ± 2.43 and 94 ± 1.41 respectively. Post-extension semen parameters were evaluated at 0, 1, 2,3,4,5,6,7,8 and 9 hours. Extenders used were; Sodium citrate-Egg yolk (control), Sodium citrate and Tomato juice, Physiological solutions (Normal saline, Dextrose, and Dextrose-saline), Tomato juice and Physiological solutions and Tomato juice and Egg yolk. Data collected were subjected to descriptive statistics and means were analyze using ANOVA. Generally, sperms motility decreased significantly with time in all the extenders. However, the combination of sodium citrate, Tomato juice and Egg-yolk based extender significantly improved semen extension up to 60% at 9 hours post extension. The study recommends the use of combination of Sodium citrate, Tomato juice and Egg-yolk for the extension of broiler rooster semen.

INTRODUCTION

The use of semen extenders is essential for maximizing the efficiency of artificial insemination (AI) in the poultry industry. Chicken semen is highly concentrated, containing approximately 4 to 6 billion spermatozoa per milliliter (Santiago-Moreno and Blesbois, 2020). It exhibits a viscous consistency and low volume, rendering sperm cells particularly susceptible to dehydration as a result of water evaporation (Tomaiuolo *et al*., 2022). The motility and fertilizing capacity of raw, undiluted chicken semen decline markedly within one hour of collection (Rajput *et al*., 2024). Consequently, immediate dilution of semen post-collection is imperative to maintain sperm viability. This practice not only provides optimal physiological conditions for sperm survival but also increases the number of insemination doses obtainable per collection and ensures uniform sperm distribution within the diluent (Hobbs *et al*., 2023). Semen extenders are indispensable for effective sperm preservation and successful fertilization. Beyond their fundamental role in maintaining spermatozoa viability, extenders support sperm metabolism, regulate pH during and after thawing, inhibit bacterial proliferation, and mitigate cryogenic damage (Malik *et al*., 2018). An effective semen extender must satisfy several physicochemical and biological requirements, including maintenance of a physiological pH range of 6.8–7.2 (Liu *et al*., 2016), provision of adequate energy substrates (Mohamed *et al*., 2019), inclusion of antioxidants to counteract oxidative stress (Mousavi *et al*., 2019), incorporation of antimicrobial agents to prevent contamination (Schulze et al., 2020), and protection of spermatozoa from freezing-induced injury (Tariq *et al*., 2020). These attributes are critical for sperm preservation and transport, as well as for the successful application of assisted reproductive techniques (ARTs) such as in vitro fertilization and intracytoplasmic sperm injection. Semen extenders are commonly formulated in two main types: chilled (liquid) extenders, suitable for short-term storage of up to three days, and cryopreserved extenders, designed for long-term storage extending over several years (Hameed *et al*., 2024).

With the global demand for animal-derived protein steadily increasing, poultry, particularly chicken, remains one of the most affordable and widely consumed sources. Nevertheless, commercial poultry populations frequently experience fertility constraints, largely due to the impaired natural mating ability of heavy, muscular males (Mengesha *et al*., 2022). This limitation has necessitated increased reliance on assisted reproductive technologies, particularly AI, to enhance fertility outcomes. As a result, optimization of semen collection, processing, and preservation under both liquid (short-term) and frozen (long-term) storage conditions has become a research priority. Artificial insemination plays a pivotal role in avian reproduction through the collection, dilution, and

storage of rooster semen to maintain sperm viability and fertilizing potential. Extending sperm longevity requires deceleration or temporary cessation of cellular metabolism. Spermatozoa, specialized for the delivery of paternal genetic material and the activation of the oocyte during fertilization, are indispensable to successful reproduction (Zafar, 2021). Empirical evidence indicates that preservation of avian semen at sub-room temperatures for extended durations represents an effective reproductive management strategy. The two principal methods of sperm preservation in poultry are liquid storage and cryopreservation. Under practical AI conditions, diluted semen is typically maintained at room temperature or under chilled conditions for no longer than 24 hours (Zafar, 2021).

Given the central role of semen preservation in the success of poultry AI programs, the identification of effective, economical, and locally available semen extenders is crucial for improving the reproductive efficiency of broiler breeder males. Accordingly, this study was designed to evaluate the suitability of a tomato-based semen extender for use in broiler parent stock.

MATERIALS AND METHODS

Study Area

The research was conducted in the Department of Theriogenology and Animal Production at the Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto. The experiment was conducted between October and November 2024

Buffer Preparation

This study followed the method described by Goje (2015). Sodium citrate (SC) was prepared by dissolving 2.9 grams of sodium citrate in 100 mL of distilled water, this was followed by vigorous stirring until the solution was homogeneous.

The physiological fluids utilized were: 0.9% Normal Saline (Dele fluids®-NS), 5% Dextrose in 0.9% Saline (Unidexal®-9) and 5% Dextrose in water (Unidex®-5)

Preparation of Extension Media

Sodium Citrate (SC) (100%): Exactly, 2 mL of sodium citrate was mixed with 500 IU of penicillin-streptomycin and 500 µL aliquot was transferred into Eppendorf tubes.

Normal Saline (NS) (100%): 2.5 mL of normal saline was thoroughly mixed with 500 IU of penicillin-streptomycin and 500 µL aliquot was transferred into Eppendorf tubes.

5% Dextrose: 2.5 mL of 5% dextrose was mixed with 500 IU of penicillin-streptomycin and 500 µL aliquot was transferred into Eppendorf tubes.

Dextrose Saline (DS) (100%): 2.5 mL of dextrose saline was combined with 500 IU of penicillin-streptomycin and 500 µL aliquot was transferred into Eppendorf tubes.

Tomato Juice (TJ) (100%): Fresh tomato was disinfected with methanol, rinsed with distilled water, chopped, and blended until homogeneous. The juice was sieved, centrifuged at 1000rpm and 2.5 mL was mixed with 500 IU of penicillin-streptomycin. A 500 µL aliquot was transferred into Eppendorf tubes.

Egg Yolk- Citrate (EYC): An intact egg was disinfected with methanol, rinsed with distilled water, and cracked to separate the yolk from the albumin using filter paper. The yolk was stirred to achieve homogeneity, to this, sodium citrate was added at the ratio of 1:4 (20% Egg yolk: 80% Sodium citrate) and a 500 µL aliquot of the mix was transferred into Eppendorf tubes.

Multi- components semen diluents

Tomato juice and Sodium citrate (TJSC); Tomato juice and Normal Saline (TJNS); Tomato juice and 5% Dextrose (TJD); Tomato juice and Dextrose Saline (TJDS);Tomato Juice, Sodium Citrate and Egg Yolk (TJSCEY) a mixture of 80% sodium citrate, 10% tomato juice, and 10% egg yolk (2 mL sodium citrate, 0.25 mL tomato juice, and 0.25 mL egg yolk) with 500 IU of penicillin-streptomycin was prepared and500 µL aliquot was transferred into Eppendorf tubes.

Tomato Juice, Normal Saline, and Egg Yolk (TJNSEY); A mixture of 80% normal saline, 10% tomato juice, and 10% egg yolk (2 mL normal saline, 0.25 mL tomato juice, and 0.25 mL egg yolk) with 500 IU of penicillin-streptomycin was prepared and 500 µL aliquot was transferred into Eppendorf tubes. Tomato Juice, Dextrose Saline, and Egg Yolk (TJDSEY); A mixture of 80% dextrose saline, 10% tomato juice, and 10% egg yolk (2 mL dextrose saline, 0.25 mL tomato juice, and 0.25 mL egg yolk) with 500 IU of penicillin-streptomycin was prepared. and 500 µL aliquot was transferred into Eppendorf tubes.

Tomato Juice, 5% Dextrose, and Egg Yolk (TJDEY): A mixture of 80% dextrose, 10% tomato juice, and 10% egg yolk (2 mL 5% dextrose, 0.25 mL tomato juice, and 0.25 mL egg yolk) with 500 IU of penicillin-streptomycin was prepared and 500 µL aliquot was transferred into Eppendorf tubes.

Semen Collection, Extension and Evaluation

Semen Collection:
Semen was collected by stimulating the copulatory organ through dorso-abdominal massage around the testes. The tail was pushed forward, and semen was extracted using the thumb and index finger. The semen was transferred into sterile Eppendorf tubes and stored in a water bath at 37.5°C within a Thermos flask.

SemenExtension:
For each diluent, 0.125 mL of semen was added to 2.5 mL of the diluent, achieving a 1:20 dilution ratio (Patel *et al*., 2016).

Semen Evaluation:

Pre-extension Evaluation: Parameters assessed include sperm motility, morphology, concentration and viability as described by Zemjanis (1977).

Progressive Motility: Semen was mixed with two drops of 2.9% sodium citrate on a pre-warmed slide and observed under × 40 objective lens, as described by Singh (2005). Morphological Abnormalities: Using a × 100 objective lens and oil immersion, 200 spermatozoa were evaluated for head, mid-piece, and tail defects across four fields (Singh, 2005).

Sperm Concentration: Measured using a Neubaeur Haemocytometer and reported as spermatozoa $\times 10^9$ sperm cells /mL.

Viability: Assessed using Eosin-Nigrosin stain. Cells were stained, and live-to-dead ratios were calculated by observing 200 cells under a ×100 objective lens (Singh, 2005).

Post-extension Evaluation:
Individual motility, livability, and morphology were assessed for each diluent at various intervals (1-9 hours) at room temperature.

pH Assessment:
Post-extension, the pH of the diluents was measured using universal pH paper (Universal pH 1-14®).

Data Analysis
Descriptive statistics were conducted to calculate mean and standard error of the mean. One-way ANOVA was used to compare means, with significance set at $p<0.05$. Statistical analyses were performed using SPSS (version 20).

RESULT

Pre-Extension Semen Characteristics of Broiler rooster
Table 1 presents the pre-extension semen evaluation results for broiler rooster. The average semen volume was 0.45 ± 0.05 mL, Progressive motility was 85 ± 7.07%. Sperm concentration was 2.65 ± 0.35 ($\times 10^9$). The percentage of abnormal sperm morphology was 8 ± 2.43, while the sperm live-to-dead ratio averaged 94 ± 1.41%. The mean semen pH was 7.25 ± 0.35.

Table 1: Pre-Extension Semen Characteristics

Characteristics	Motility%	Volume(ml)	Concentration $\times 10^9$/ml	M/A %	L/D %	pH	Colour
1st Trial	90	0.4	2.9	10	93	7	Creamy
2nd Trial	80	0.5	2.4	7	95	7.7	Creamy
Mean±(SD)	85±7.07	0.45±0.05	2.65±0.35	8±2.43	94±1.41	7.27±0.35	Creamy

The pH values of the prepared extenders after reconstitution were 3.5 ± 0.70 for the tomato juice which was found to be more acidic than other extenders and TJSCEY was almost neutral 7.5 ± 0 as presented in Table 2.

Table 2: Different pH of Prepared Extenders

Trial	1ST	2ND	Mean±(SD)
TJ	3	4	3.5±0.70
NS	5	5.5	5.25±0.35
5%D	4	4.5	4.25±0.35
DS	4	4	4±0
SC	5.5	5.5	5.5±0
TJNS	4	5	4.5±0.70
TJD	5	6	5.5±0.70
TJDS	4	5	4.5±0.70
TJSC	5	5	5±0
EYSC	6.5	6.6	6.5±0
TJNSEY	6.5	6	6.25±0.35
TJDEY	5.5	6	6.5±0.70
TJDSEY	7	6	5.75±0.35
TJSCEY	7.5	7.5	7.5±0

Post-Extension Percentage of Progressive Sperm Motility

The percentage of motile sperm cells in single diluents showed a progressive decline over time, from 0 to 2 hours, in diluents such as DS, D, NS, and SC. However, in the TJ, sperm motility dropped to 0% in less than an hour after extension (Figure 1)

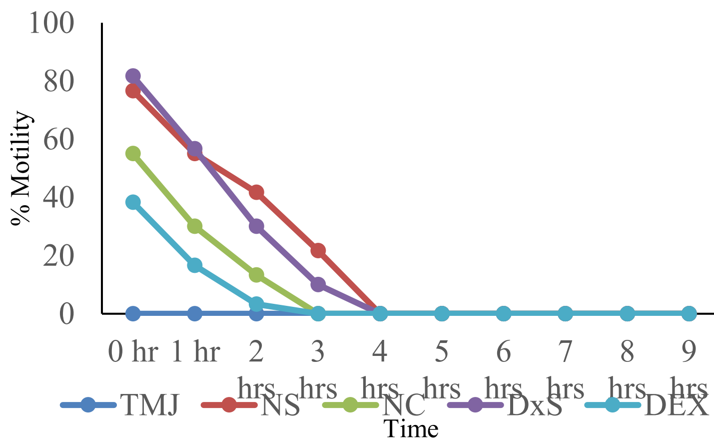

Figure 1: Percentage Motility of Sperm Cells in Single Extender

Percentage Motility of Sperm Cells in Double Extenders: The motility of sperm cells gradually declined over time, from the initial zero hour to 2.5 hours, across the double extenders (TJDS, TJD, TJNS, and TJSC). Figure 2 shows the mean (±SD) of the progressive motility of spermatozoa in these double extenders

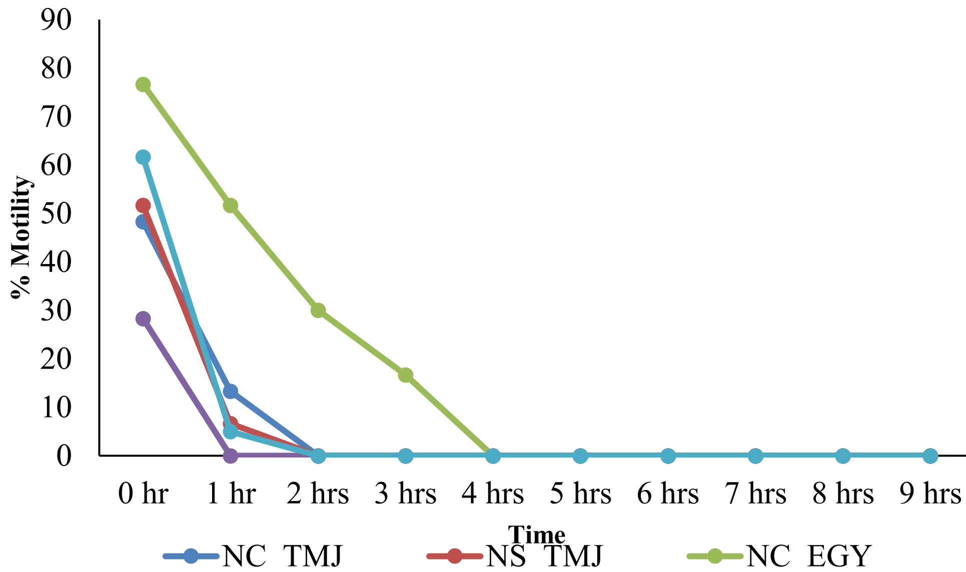

Figure 2: Percentage Motility of Sperm Cells in Combination of Two Extenders

Sperm Motility in Various Extenders: The motility of sperm cells decreased progressively from 0 to 3 hours across most extenders, including TJDSEY, TJDEY, and TJNSEY. However, the extender TJSCEY maintained sperm motility for up to 9 hours (Figure 3). Semen storage beyond 3 hours post-extension showed better results with extenders containing TJ, EY, and SC. By the 3rd hour, all extenders (TJ, DS, D, NS, SC, TJNS, TJDS, TJD, TJSC, SCEY, TJEYDS, TJEYNS, and TJEYD) recorded 0% sperm motility, except for TJEYSC, which retained over 60% motility.

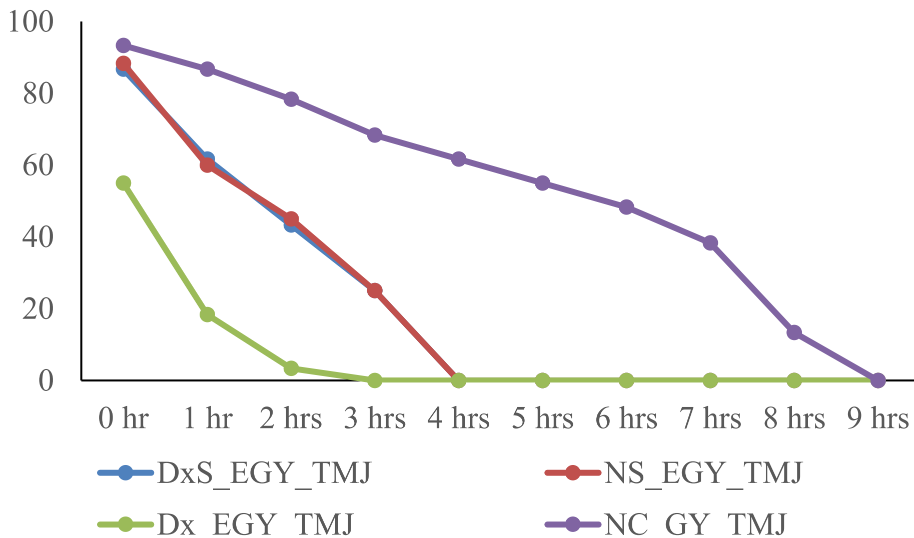

Figure 3: Percentage Motility of Sperm Cells in Multiple Extenders

Post extension Percentage Morphological Abnormalities

The morphological abnormalities in the tomato juice extender were significantly higher at 30 minutes compared to 0

minutes. However, for the other extenders, there was no significant difference in morphological abnormalities between 0 and 30 minutes, as presented in Table 3.

Extenders	Mean±SD.	
	0 mins	30 mins
TJ	8±0.03[a]	57±0.01[a]
EYSC	4±0.00b[c]	6±0.01[b]
TJNSEY	5±0.01[b]	9±0.02[c]
TJSCEY	3±0.01b[c]	6±0.02[b]

Table 3: % Morphological Abnormalities

Values along the same column with different superscript letters [(a,b and c)] are significantly different

The livability of sperm cells shows a significant difference when using tomato juice extender at both 0 and 30 minutes, whereas no significant differences observed at these time points for the other extenders, as shown in Table 4.

Table 4: % Live-Dead ratio

Extenders	Mean±SD	
	0 mins	30 mins
TJ	53±0.05[a]	0±0[a]
SCEY	96±0.01[b]	94±0.02[b]
NSEYTJ	97±0.01[b]	91±0.01[b]
TJEYSC	97±0.01[b]	93±0.01[b]

Values along the same column with different superscript letters [(a,b)] are significantly different

DISCUSSION

The average ejaculate volume of a rooster has been estimated to be 0.7 ml across various bird breeds (Tuncer et al., 2008). The volume recorded in this study was lower than 0.7 ml. Several factors, such as breed, age, body weight, over-stimulation, seasonal variations and environmental conditions could have contributed to this lower sperm count. Bah et al. (2001) and Tuncer et al. (2008) reported an average ejaculate volume of 0.28 ± 0.14 mL, which is even lower than the present study's findings. In this study, the ejaculate volumes ranged from 0.34 to 0.59 ml, consistent with the range often reported for bird sperm (Etches, 1998). The creamy white color of the ejaculates observed in this study aligns with findings by Peters et al. (2008), who also reported creamy white ejaculates. Machebe and Ezekwe (2005) suggested that variations in sperm color could be linked to contaminants or low sperm concentration, however, such issues were not encountered in this study.
Sperm motility in this study was 85±7.07, slightly higher than the 83.2±0.6 reported by Bah et al. (2001) for White Leghorns. High sperm motility is a good indicator of sperm quality and fertilizing capacity (Malejane *et al*., 2014). Peters et al. (2008) reported 82.50 ± 10.00% sperm motility in improved indigenous crossbred chickens (Alfa). In contrast, Mosenene (2009) found lower sperm motility in Rhode Island Red, New Hampshire, and White Leghorn (59.6 ± 14.5%, 61.6 ±

14.1%, and 58.8 ± 12.5%, respectively). These variations in sperm motility could be attributed to the pH of extenders and season in which semen was collected. The semen in this study had a slightly alkaline pH of 7.24 ± 0.35, which is within the range commonly reported for bird sperm (Etches, 1998). Other researchers recorded pH values of 7.02 ± 0.01, 7.4 ± 0.2, and 7.68 ± 0.01 for rooster sperm (Bah *et al*., 2001; Peters *et al*., 2008; Tuncer *et al*., 2008). The technique used for sperm collection and gonadal stimulation could influence sperm pH, as gonadal fluid is usually alkaline (Bah *et al*., 2001).

Sperm concentration typically reflects the number of spermatozoa per unit volume of seminal plasma (Malejane *et al*., 2014). Sperm concentration in cocks ranges from over 1.2 billion sperm cells per mL (Bolarinwa 2021) to less than 0.7 billion sperm per ml (Hafez, 1978). In this study, the sperm concentration of 2.65 ± 0.35x10^9/mL aligns with previous studies. Variations in sperm concentrations are likely due to differences in genetic backgrounds.

Tselutin et al. (1999) reported a live sperm count without abnormalities of 91 to 94%, consistent with this study's result of 94 ± 1.41%. Sperm motility decreased progressively from zero hours to 9 hours in all extenders, likely due to the accumulation of metabolites in the medium. However, sperm storage was more effective with diluents containing TJ, EY, and SC. At 3 hours post-extension, all diluents (TJ, DS, D, NS, SC, TJNS, TJDS, TJD, TJSC, SCEY, TJEYDS, TJEYNS and TJEYD) had 0% sperm motility except for TJEYSC, which maintained over 60% motility. This may be attributed to the phospholipids, cholesterol, low-density lipoproteins in egg yolk and the antioxidant properties of tomato juice, which synergistically protect sperm from cold shock and oxidative stress (Sunday *et al*., 2018). Lycopene is a predominant natural carotenoid, which can be found in ripe tomato fruit, watermelon or pink grapefruit. Although used as a food colorant for many years, it has also become a subject of interest with respect to its properties in alleviating numerous chronic or inflammatory diseases (Ilahy *et al*., 2015). Lycopene is a highly unsaturated straight chain hydrocarbon with 13 double bonds, 11 of which are conjugated, which makes it a very powerful antioxidant. Lycopene has been shown to quench singlet oxygen twice as efficiently as β-carotene and ten times faster in comparison to α-tocopherol (Heymann *et al*., 2019).

Several human studies have shown that lycopene administration leads to a significant improvement of semen parameters in patients diagnosed with idiopathic or antibody-mediated infertility (Castleton *et al*., 2022).

Moreover, animal *in vivo* reports revealed that lycopene may prevent testicular degeneration, improve sperm motility and morphology and stabilize the antioxidant profile of testicular tissue exposed to drugs (Antonuccio *et al*., 2020), organic pollutants (Halliwell and Aruoma, 1991) or mycotoxins (Krinsky, 1992). Another mechanism that accounts for the antioxidant activity of lycopene is its reaction with free radicals (Krinsky, 1998).

The pH of the extenders with the TJ, EY, and SC combination was slightly alkaline (7.5), whereas other extenders were more acidic. This may explain why the TJ, EY, and SC combination was more effective in maintaining sperm motility.

Tomato juice extender alone only maintained sperm motility for up to one hour, likely due to its acidic nature. The combine effect of antioxidant in tomato and properties of egg yolk and sodium citrate could have been responsible for the significance improvement recorded in the TJEYSC extender.

CONCLUSION

In conclusion, supplementing broiler semen extender with Sodium citrate, Egg yolk and Tomato juice is beneficial as it extends semen shelf life for up to 9 hours compared to other extenders.

RECOMMENDATION

This study recommends using tomato juice, egg yolk and sodium citrate for extending broiler rooster semen. Further research is needed to explore the beneficial components of tomato juice for semen preservation in broiler roosters.

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Conflicts of interest

The authors wish to declare no conflict of interest

Authors Contributions

Shehu Sidi conceived the idea, Shehu Sidi, Oloye Abimbola and Muhammad Sanusi design the work, Hashim Muhammad, Shehu Sidi and Saifullahi Umar conducted the experiment, Rahila Hassan carried out the statistics and Kabir Ibrahim help with the review of the manuscript.

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