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Reproduction

Efficacy of soursop juice extender on rooster semen quality, oxidative activity and spermatozoa kinematics

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ABSTRACT

African medicinal plant like soursop (*Annona muricate* L.) within annonaceae are known for their biological, therapeutic, and pharmacological properties with little or no toxicity. The use of such plant requires good knowledge of the toxicity dosage, purity, suitable extraction solvent and adverse effects. The leaves, seeds, fruits, barks, and roots of African medicinal plants have been used for various nutraceuticals and functional effects according to African folk medicine. The aim of this study is to evaluate the semen quality, oxidative activity and spermatozoa kinematics of rooster semen in soursop juice extender. About 30 roosters were used for the in vitro analysis. Semen was collected twice a week for 2 weeks through dorsal-abdominal massage technique. The evaluation was done hourly until semen quality declined at the 5th-hour. The pooled semen was allotted to seven treatments of semen extenders as undiluted semen, dextrose saline, 10% soursop juice extender, 20% soursop juice extender, 30% soursop juice extender, 40% soursop juice extender, and 50% soursop juice extender for the study. The percentage motility, progressive motility, nonprogressive motility, curvilinear velocity, average path velocity, straight line velocity, linearity, straightness, amplitude of lateral head, beat cross frequency and wobble were analyzed using computer aided sperm analysis. Oxidative status (antioxidant activity and lipid peroxidation) was determined by assay. Result of rooster semen at room temperature and after 1-hour dilution showed that percentage motility, nonprogressive motility, and average path velocity were significantly ($P < 0.05$) reduced by different soursop juice extenders compared to undiluted semen. After 2-hour dilution of rooster semen, nonprogressive motility, average path velocity, curvilinear velocity, straight line velocity, wobble, liveability and amplitude of lateral head parameters were significantly ($P < 0.05$) increased by different soursop juice extenders compared to undiluted semen. Antioxidant activity and lipid peroxidation in both room temperature and after 5-hour dilution were affected by different soursop juice extenders in rooster semen. In conclusion, supplementation of soursop juices as an extender to rooster undiluted semen played an improvement role on spermatozoa fertility and oxidative status during processing or preserving ejaculates for insemination.

Key words: fruit juice, organics, oxidative activity, poultry semen, semen quality

INTRODUCTION

Assisted reproductive technologies in farm animals has been regularly researched on and vastly utilized from time immemorial on Animal species that at the verge of extinction. Breeding superior quality male germplasm requires special technique to extend or preserve ejaculates for insemination. In artificial insemination, semen extender (diluents) is a chemical medium used for preservation, extension and protection of sperm cells against various shocks during processing, storage and transportation. Interestingly, there are factors that affect spermatozoa fertility during storage. These factors are pH fluctuations (buffer capacity), cold shock protection, control microbial contamination, osmotic changes, cryo-damages, and energy depletion during metabolism during freezing–thawing procedures (De Ambrogi et al., 2006; Raheja et al., 2018). Furthermore, maintaining maximum spermatozoa fertility during storage require special different types of semen diluents. These diluents (Tris, TES, MES, HEPES, PIPES, MOPS, BES, citrate, bicarbonates, sodium citrate, and dextrose saline) have been developed and can be added to spermatozoa to improve its fertility. Addition of these different types of semen diluents into ejaculated spermatozoa is to maintain its motility, increase fertilizing capacity and preserve sperm membrane integrity during insemination (Maxwell and Johnson, 1999). Each semen diluent has its function as glucose enhances preservation capacity through energy provision, compounds like bovine serum albumin protects spermatozoa from cold shock, buffer salts like sodium bicarbonate, Tris (hydroxymethyl) aminomethane (TRIS), *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulphonic acid (HEPES) prevents negative effects of pH fluctuations, basic salts of NaCl and KCl helps to maintain proper osmotic balance and bacterial growth are inhibited with antibiotics (De Ambrogi et al., 2006).

Farm animal's spermatozoa like cattle, pigs are sensitive to cooling, freezing and thawing due its plasma membrane composition. Spermatozoa are prone to plasma membrane damage due to the formation of lipid peroxidation that might lead to decrease in sperm motility (Aitken, 1995). Interestingly, using antioxidant enriched fruit juices as semen extender constituent can mitigate the cause. Also, due to low temperature, sperm's storage capacity may be affected by cell metabolism because of the microbiological conditions that cannot be slowed (Rodriguez et al., 2017; Yeste, 2018).

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Using different types of semen extenders for cryopreservation a lot of improvement has been reported in post thaw sperm quality like increases percentage motility, viability, PMAI, MMP, reduces DNA fragmentation, capacitation, reduces lipid peroxidation and reactive oxygen species (Banday et al., 2017; Askarianzadeh et al., 2018; Balamurugan et al., 2018). Depending on the type of semen diluents, they have their various functions that they perform. Therefore, evaluating whether soursop extender with regard to their capacity to delay changes is very crucial. In choice of medium for semen extension, many limitation factors are to be considered.

Poultry production is important in livestock production with a vital role of providing economic growth and development. In poultry breeding and reproduction, sperm fertility is very crucial as in or low fertility is an economic loss in poultry production (Khan, 2011). In the process of biochemical and functional changes (fertilization), lipids are part of sperm membrane that incorporate stages of maturation, capacitation, and acrosome reactions (Nolan and Hammerstedt, 1997). Oxidative stress occurs when there is decrease in the levels of antioxidants and increase in production of reactive oxygen species (ROS). These affect semen quality and its fertilizing ability resulting to imbalance of pro-oxidants and antioxidants concentrations that increase lipid peroxidation, decrease sperm motility and viability (Sheweita et al., 2005; Rehman et al., 2018). The biological (enzymatic) antioxidants are superoxide dismutase (SOD), Catalase (CAT) and glutathione peroxidase (GSH-Ox) and natural (nonenzymatic) antioxidants are vitamin E, A, and C (ascorbic acid) that are capable of protecting semen from ROS and toxic products of metabolism (Khan, 2011; El-Bahr, 2013).

The use of natural juices from fruits like pineapple, watermelon, coconut and citrus (tangerine and sweet orange) as constituents of semen extenders has been worked on different animal species of chickens and rabbits (Jimoh and Ayedun, 2020; Jimoh et al., 2020a, 2020b, 2021a). The phytochemicals/nutrients of plant extracts can be reliable, safe and cheap remedy to ameliorate reproductive anomalies in animal fertility. African medicinal plant like soursop (*Annona muricate* L.) within annonaceae is known for its biological, therapeutic, and pharmacological properties with little or no toxicity (Agu et al., 2017; Orak et al., 2019). The leaves, fruits, barks, and roots of the African medicinal plant is known and used for various functions according to African folk medicine. The phytochemicals present in Annona muricate are alkaloids, flavonoids, carbohydrates, cardiac glycosides, saponins, tannins, phytosterols, terpenoids, and proteins. Again, study has reported the presence of alkaloids, flavonoids, and phenols in high quantities especially in the fruit pulp and leaf (Agu et al., 2017). For instance, the enthopharmacological administration of soursop helps to promote digestion, manage diabetes and antimutagenic agents. The presence of acetogenins in *A. muricate* L. reveals the antioxidant, anti-inflammatory and antibiotic properties that effectively scavenge free radicals (Al-Brakati et al., 2019). Collectively, this suggests that there are numerous bioactive compounds and phytochemical properties present in Annona muricate (Soursop). Some of these chemical compounds have been linked to the ethnomedicinal properties of Soursop and its antioxidant properties. Thus, this formed the basis on which this research was designed and carried out to establish the link between Annona muricate juice as semen extender and the semen quality, oxidative activity and spermatozoa kinematics of rooster semen. However, there is very limited information on the role of Annona muricate (Soursop) in semen quality, oxidative activity and spermatozoa kinematics of rooster semen as an extender. This study, thus hypothesised that Annona muricate (Soursop) in rooster semen will promote semen quality, oxidative activity and spermatozoa kinematics as an extender. Consequently, the objective of this study is to evaluate the semen quality, oxidative activity and spermatozoa kinematics of rooster semen in soursop juices as an extender.

MATERIALS AND METHODS

Ethical Approval, Experimental Design, and Animal Study

The institutional research and ethics committee (Federal Polytechnic Ado Ekiti, Ekiti State, Nigeria) approved the animal study and its experimental protocols, which was performed in accordance with standard guidelines of University Animal Scientific procedures. This research was undertaken with IACUC approval number—FPA/EC/19/0043. The animals were handled in accordance with the National Institutes of Health (NIH Publication No. 85-23; revised 1996) guidelines for care and use of laboratory animals were followed, and appropriate measures were taken to minimize pain or discomfort on the animals.

This study used 30 ISA Brown breeder roosters and 168 ISA Brown breeder hens that were 35–40 weeks old, respectively; they were obtained from a reputable breeder farm (CHI farms, Ajanla, Oyo State). Ripe Soursop fruits were purchased from a local market (within Ado Ekiti metropolis) washed and juice from the fruits were extracted. The extracted juices were clarified by centrifuging at 4000 × *g* for 15 min. The obtained supernatant was designated as soursop juice (pH: 3.65, c titrable acidity: 174.56 mg/100 mL, and: 12.34 mg/mL, flavonoid 1.94 mg/100 g, phenolic acid 1.68 mg/100 g, alkaloids 434 mg/100 g, ascorbic acid 0.56 mg/100 g) were kept frozen 4°C in sterile 5 mL eppendorf tubes until ready for use. Dextrose saline (5% dextrose in 0.9% normal saline; Unique Pharmaceuticals, Nigeria) was used for the study.

Extension of Semen with Extenders and Evaluation

Prior to collection of the semen, the 30 roosters were trained using the dorsal-abdominal massage for 2 weeks. Semen was harvested twice a week at 3 days interval and ejaculate taken to the laboratory for in vitro analysis. All roosters were assessed for fertility and only highly fertile roosters (high motility, sperm viability and kinetics traits of high spermatozoa concentration) were used for the study. Importantly, care was taken to avoid any contamination of semen with cloacal products from roosters such as faeces.

The pooled semen was allotted as described below in a completely randomized design in a 7 × 3 factorial arrangement (treatment; T1, T2, T3, T4, T5, T6 and T7, time (0, 1 and 2 h) and diluted with assigned treatment. Diluted samples were mixed gently to allow equilibration in line with standard protocol for handling semen, semen was kept at room temperature in a closed water bath, thereafter semen assessment took place. Dilution rate is 1:2 (semen:diluent), a fixed concentration (4.30×10^7 spermatozoa) was performed to remove the concentration effect on the samples in T1 group and diluted groups T2, T3, T4, T5, T6, and T7. Semen qualitative

and oxidative status assay were assessed for each treatment. Seven treatments of different extenders formulated were used for the study as:

Treatment 1: undiluted semen (positive control)
Treatment 2: dextrose saline + 0% soursop juice extender
Treatment 3: dextrose saline + 10% soursop juice extender
Treatment 4: dextrose saline + 20% soursop juice extender
Treatment 5: dextrose saline + 30% soursop juice extender
Treatment 6: dextrose saline + 40% soursop juice extender
Treatment 7: dextrose saline + 50% soursop juice extender

The semen quality assessments were done hourly until quality declined. Extended semen, according to treatments were evaluated for sperm cell kinetics using computer assisted sperm analyzer (CASA) (SpermAnalyzeWin7 Xuzhou city, China, setting of CASA in 5th WHO manual, 51 sperm tracks, evaluated magnification × 10, image acquisition rate: number frames/s 60), the temperature of pipette tips, petri dish, media, counting chambered slides (10–20 µm deep), and the microscope stage was maintained at 37°C. The CASA setting used for the study is suitable for the rooster sperm analysis, the setting is similar to a review by Van der Horst and Du Plessis (2017); percentage motility, progressive motility, nonprogressive motility, curvilinear velocity (µm/s), average path velocity (µm/s), straight line velocity (µm/s), linearity, straightness, amplitude of lateral head (µm), beat cross frequency (Hz), wobble. Sperm concentration and liveability were determined using conventional procedures. Sperm concentration (duplicates per sample) were determined using Neubauer haemocytometer (TH-100; Hecht Assistant, Sondheim, Germany) and expressed as spermatozoa × 10^8/mL. Liveability was done by placing a drop of semen on a glass slide, one drop of eosin–nigrosin stain added and mixed gently, then smeared on a slide, air-dried and viewed under the microscope at magnification of ×400.

Oxidative Status Assay

Oxidative status assay for each treatment was conducted at zero hour and 5 h (when spermatozoa quality had declined to zero). Another batch of the various treatment samples were constituted and centrifuged at 4000 rpm for 15 min to separate seminal plasma. The treatments diluted semen samples were centrifuged and seminal plasma obtained assayed for lipid peroxidation and total antioxidant activity using standard procedures as outlined by Jimoh and Ewuola (2018). The assay for seminal lipid peroxidation involves the reaction mixture in a total volume of 3.0 mL contained 1.0 mL seminal plasma and 1.0 mL of TCA (0.67%). All the test tubes were placed in a boiling water bath for a period of 45 min. The tubes were shifted to the ice bath and then centrifuged at 2500 rpm for 10 min. The amount of malondialdehyde (MDA) formed in each of the samples was assessed by measuring the optical density of the supernatant at 532 Nm.

Seminal total antioxidant capacity activities involve a reaction mixture containing 0.5 mL of a (10 mmol/L) Na-Benzoate, 0.2 mL of H_2O_2 (10 mmol/L), 0.49 mL of phosphate buffer (100 mmol/L, pH = 7.4) (prepared by mixing 19.5 mL of KH_2PO_4 (100 mmol/L) with 80.5 mL of Na_2HPO_4 (100 mmol/L), then adjusted the pH to 7.4 and 0.2 mL of Fe-EDTA complex (2 mmol/L) (prepared freshly by mixing equal volumes of EDTA (2 mmol/L), and ferrous ammonium sulfate (2 mmol/L), then left at 25°C for 60 min. Ten microliters of the seminal plasma were added to the latter reactive mixture and were incubated at 37°C for 60 min. Finally, 1 mL glacial acetic acid (20 mmol/L) and 1 mL thiobarbituric acid (0.8% w/v in 100 mL of 50 mmol/L NaOH) were added, and the absorbance at 532 nm was measured spectrophotometrically after incubation at 100°C for 10 min. Total antioxidant capacity was calculated according to the following formula: TA capacity (mmol/L) = (CUA) (K—A)/(K—UA); where CUA (mmol/L); concentration of uric acid; K: absorbance of the control (K1 – K0); A: absorbance of the sample (A1 – A0); UA: absorbance of uric acid solution (UA1 – UA0).

Statistical Analysis

Data obtained were subjected to descriptive statistics and analysis of variance $\alpha = 0.05$, means differences separated using new Duncan's multiple range test of the general linear model procedure of statistical analysis software (SAS). The statistical model is as follows:

$Y_{ij} = \mu + B_i + e_{ij}$; where Y_{ij} represents the value of spermatozoa kinetics and oxidative stability measured in the ith diluted semen; μ is the overall mean for each character; B_i is the fixed effect of ith rooster semen diluted soursop juice extenders (i = T1 is undiluted semen (positive control), soursop juice extender was incorporated with dextrose saline at 0%, 10%, 20%, 30%, 40%, 50% as T2, T3, T4, T5, T6, and T7, respectively); and e_{ij} is the random residual effect.

RESULT

Semen Quality of Soursop Extended Rooster Semen at Room Temperature

Semen quality of rooster in soursop juice extender at room temperature is presented in Table 1. Percentage motility, nonprogressive motility, and average path velocity (VAP) parameters were significantly ($P < 0.05$) influenced by the different semen extenders of soursop compare to undiluted semen. Percentage motility of undiluted semen was significantly ($P < 0.05$) higher to treatments 2–6 but lower in treatment 7 of diluted semen treated with different semen extenders of soursop. Nonprogressive motility was significantly higher in undiluted semen and treatment 4 compare to treatments 2, 3, and 7 but lower in treatment 5 and 6 of diluted semen treated with different semen extenders of soursop. VAP of undiluted semen was significantly ($P < 0.05$) higher compared to treatments 2–4, 6, 7 but lower in treatment 5 of diluted semen treated with different semen extenders of soursop. Compared to undiluted semen, progressive motility, curvilinear velocity (VCL), VAP, straight line velocity (VSL), linearity, wobble, liveability, straightness, amplitude of lateral head (ALH), beat cross frequency (BCF) and wobble were unaffected ($P > 0.05$) by the different soursop juice extenders on rooster semen among other treatments at room temperature.

Semen Quality of Soursop Extended Rooster Semen after 1 h

The semen quality of rooster in different soursop juice extenders after 1 h is shown in Table 2. Percentage motility, nonprogressive motility, VAP, VCL, VSL, linearity, wobble, liveability, straightness and ALH parameters were significantly ($P < 0.05$) influenced by different semen soursop juice extenders except progressive motility, BCF and wobble

Table 1. Semen quality of rooster in soursop juice extender at room temperature

Parameters/ Treatments	1	2	3	4	5	6	7	SEM
Percentage motility, %	91.50^{a}	66.29^{ab}	67.12^{ab}	74.48^{ab}	69.92^{ab}	80.61^{ab}	58.73^{b}	3.64
Progressive motility, %	61.76	54.95	54.65	53.46	69.92	72.39	48.41	3.22
Non- progressive motility, %	29.74^{a}	11.33^{ab}	12.47^{ab}	20.99^{a}	2.00^{b}	5.22^{b}	10.32^{ab}	3.04
Curvilinear velocity (VCL), µm/s	14.09	5.85	5.05	13.49	5.09	5.61	6.72	1.35
Average path velocity (VAP), µm/s	11.39^{a}	5.49^{ab}	4.69^{ab}	9.75^{ab}	4.23^{b}	5.38^{ab}	5.55^{ab}	0.87
Straight line velocity (VSL), µm/s	5.88	3.63	3.42	4.79	3.07	4.33	2.92	0.38
Linearity, %	45.96	72.51	68.94	43.44	63.55	76.23	54.73	4.94
Straightness, %	55.81	75.35	74.51	50.61	72.85	79.26	60.32	4.18
Amplitude of lateral head (ALH), µm	0.51	0.22	0.20	0.45	0.19	0.23	0.27	0.04
Beat cross frequency (BCF), Hz	2.19	0.85	0.54	1.99	0.70	0.82	0.94	0.23
Wobble, %	81.88	95.46	92.63	83.97	85.32	95.45	87.32	2.32

abc: means in the same row with different superscripts are significantly ($P < 0.05$).
Treatment 1: undiluted (raw) semen.
Treatment 2: dextrose saline + 0% soursop juice extender.
Treatment 3: dextrose saline + 10% soursop juice extender.
Treatment 4: dextrose saline + 20% soursop juice extender.
Treatment 5: dextrose saline + 30% soursop juice extender.
Treatment 6: dextrose saline + 40% soursop juice extender.
Treatment 7: dextrose saline + 50% soursop juice extender.

Table 2. Semen quality of rooster in soursop juice extender after 1 h

Parameters/ Treatments	1	2	3	4	5	6	7	SEM
Percentage motility, %	94.19^{a}	70.00^{ab}	67.68^{ab}	83.52^{ab}	67.10^{ab}	77.27^{ab}	49.84^{b}	4.51
Progressive motility, %	54.71	63.33	57.98	78.32	63.84	63.16	43.18	4.55
Nonprogressive motility, %	39.48^{a}	6.67^{b}	9.70^{b}	5.20^{b}	3.26^{b}	14.11^{b}	6.67^{b}	3.00
Curvilinear velocity (VCL), µm/s	18.31^{a}	4.14^{b}	5.21^{b}	8.32^{ab}	8.22^{ab}	13.68^{ab}	3.21^{b}	1.56
Average path velocity (VAP), µm/s	14.24^{a}	4.16^{b}	4.79^{b}	5.56^{b}	5.35^{b}	8.46^{b}	3.03^{b}	0.95
Straight line velocity (VSL), µm/s	18.31^{a}	4.14^{b}	5.21^{b}	8.32^{b}	8.22^{b}	13.68^{ab}	3.21^{b}	1.56
Linearity, %	42.88^{b}	98.82^{a}	68.71^{ab}	70.33^{ab}	65.10^{ab}	40.28^{b}	92.50^{a}	6.39
Straightness, %	54.85^{b}	98.62^{a}	75.36^{ab}	75.65^{ab}	73.58^{ab}	52.65^{b}	97.48^{a}	4.86
Amplitude of lateral head (ALH), µm	0.65^{a}	0.17^{b}	0.21^{b}	0.26^{b}	0.26^{b}	0.40^{ab}	0.12^{b}	0.05
Beat cross frequency (BCF), Hz	2.79	0.56	0.62	1.94	1.85	2.98	0.36	0.36
Wobble, %	78.09	100.19	91.47	87.69	82.56	71.67	94.68	3.83

abc: means in the same row with different superscripts are significantly ($P < 0.05$).
Treatment 1: undiluted (raw) semen.
Treatment 2: dextrose saline + 0% soursop juice extender.
Treatment 3: dextrose saline + 10% soursop juice extender.
Treatment 4: dextrose saline + 20% soursop juice extender.
Treatment 5: dextrose saline + 30% soursop juice extender.
Treatment 6: dextrose saline + 40% soursop juice extender.
Treatment 7: dextrose saline + 50% soursop juice extender.

($P > 0.05$) after 1 hour of dilution. Percentage motility of undiluted semen was significantly ($P < 0.05$) higher to treatments 2–6 but lower in treatment 7 of diluted semen treated with different semen extenders of soursop. Nonprogressive motility, VAP, VSL and ALH of undiluted semen were significantly ($P < 0.05$) higher to treatments 2–7 of diluted semen treated with different semen extenders of soursop. Linearity and straightness of treatments 2 and 7 were significantly higher compare to treatments 3–5 but lower in undiluted semen and 6. Curvilinear velocity of undiluted semen was significantly ($P < 0.05$) higher to treatments 4–6 but lower in treatment 2, 3, and 7 of diluted semen treated with different semen extenders of soursop.

Semen Quality of Soursop Extended Rooster Semen after 2 h

The semen quality of rooster in soursop juice extender after 2 h is presented in Table 3. Percentage motility, progressive motility, nonprogressive motility, VAP, VCL, VSL, linearity, wobble, liveability, straightness and ALH parameters were significantly ($P < 0.05$) influenced by the different semen soursop juice extenders after 2 h of dilution. Percentage motility of undiluted semen was significantly ($P < 0.05$) higher to treatment 2–6 but lower in treatment 7 of diluted semen treated with different semen extenders of soursop. Progressive motility of treatment 3 was significantly ($P < 0.05$) higher to undiluted semen, treatments 2, 4, 5, and 7 but lower

Table 3. Semen quality of rooster in soursop juice extender after 2 h

Parameters/Treatments	1	2	3	4	5	6	7	SEM
Percentage motility, %	91.86^{a}	90.47^{ab}	90.00^{ab}	70.60^{ab}	80.34^{ab}	83.21^{ab}	61.24^{b}	3.64
Progressive motility, %	70.71^{ab}	72.72^{ab}	74.97^{a}	62.55^{ab}	65.69^{ab}	49.48^{b}	56.47^{ab}	2.86
Nonprogressive motility, %	20.84^{ab}	17.79^{ab}	15.00^{ab}	8.06^{b}	14.16^{ab}	33.67^{a}	4.44^{b}	2.76
Curvilinear velocity (VCL), µm/s	13.95^{ab}	14.45^{ab}	16.14^{ab}	13.27^{ab}	7.30^{ab}	20.89^{a}	4.93^{b}	1.71
Average path velocity (VAP), µm/s	9.85^{ab}	10.11^{ab}	9.94^{ab}	7.76^{ab}	6.41^{ab}	13.98^{a}	4.46^{b}	0.95
Straight line velocity (VSL), µm/s	5.44^{ab}	5.27^{ab}	4.85^{ab}	3.53^{b}	4.24^{ab}	6.46^{a}	3.37^{b}	0.35
Linearity, %	42.84^{ab}	42.17^{ab}	33.59^{b}	37.54^{ab}	67.49^{ab}	43.31^{ab}	78.91^{a}	5.40
Straightness, %	55.58^{ab}	54.69^{ab}	49.17^{b}	50.16^{b}	78.18^{ab}	53.56^{b}	83.23^{a}	4.01
Amplitude of lateral head (ALH), µm	0.46^{ab}	0.49^{ab}	0.48^{ab}	0.37^{ab}	0.29^{ab}	0.66^{a}	0.19^{b}	0.05
Beat cross frequency (BCF), Hz	2.61	2.87	3.64	3.11	0.95	3.42	0.60	0.39
Wobble, %	74.66	74.39	65.43	70.72	90.45	75.75	93.60	3.74

abc: means in the same row with different superscripts are significantly ($P < 0.05$).
Treatment 1: undiluted (raw) semen.
Treatment 2: dextrose saline + 0% soursop juice extender.
Treatment 3: dextrose saline + 10% soursop juice extender.
Treatment 4: dextrose saline + 20% soursop juice extender.
Treatment 5: dextrose saline + 30% soursop juice extender.
Treatment 6: dextrose saline + 40% soursop juice extender.
Treatment 7: dextrose saline + 50% soursop juice extender.

in treatment 6 of rooster semen soursop juice extenders. Nonprogressive motility and VSL of treatment 6 were significantly higher compare to undiluted semen, treatments 2, 3, and 5 but lower in treatments 4 and 7 of soursop juice extenders in rooster semen. VCL, VAP, and ALH of treatment 6 were significantly ($P < 0.05$) higher compare to undiluted semen through to treatment 5 but lower in treatment 7 of rooster semen soursop juice extenders. Straightness of treatment 7 was significantly ($P < 0.05$) higher compare to undiluted semen through to treatments 2 and 5 but lower in treatments 3, 4, and 6 of diluted semen treated with different semen extenders of soursop. Linearity of treatment 7 was significantly ($P < 0.05$) higher compare to undiluted semen—treatments 2 and 4–6 but lower in treatment 3 of rooster semen in soursop juice extenders. Compared to undiluted semen, BCF and wobble were unaffected ($P > 0.05$) by the different soursop juice extenders on rooster semen after 2 h of dilution.

Oxidative Status Assessment of Soursop Extended Rooster Semen

Antioxidant activity of rooster semen in soursop juice extenders is presented in Fig. 1. The antioxidant activity of rooster semen was influenced by the different soursop juices extenders. A significant difference between the antioxidant activity of rooster semen at 0 and 5 h was observed. The values of antioxidant activity of rooster semen at 0 hour were higher compared to the antioxidant activity of rooster semen at 5 h dilution. Furthermore, there was a gradual progression of antioxidant activity in treatments 1–5 at both 0 and 5 h dilution. However, at 5 h dilution of soursop juice extenders in rooster semen resulted in the antioxidant activity decline within treatments 5 and 6 compare to the room temperature and not after 5 h dilution.

Lipid peroxidation of rooster semen in soursop juices extender is presented in Fig. 2. The lipid peroxidation of rooster semen was affected by the different soursop juice extenders at 0 and 5 h dilution. There is a massive difference between the lipid peroxidation of rooster semen at 0 and 5 h dilution. The result revealed a sharp decline and a gradual increase of lipid peroxidation in treatments 2–7 of diluted semen treated with different semen extenders of soursop at 0 and 5 h dilution, respectively.

DISCUSSION

Semen Quality of Soursop Extended Rooster Semen

Poultry farming remains an important part of animal protein to man. The profitability and productivity of poultry production depend on factors like hatchability, fertility and reproductive efficiency to be substantiable. Furthermore, male fertility relies on semen quality for efficient reproduction for improvement and expansion. Qualitative and quantitative evaluation of semen are enhanced by the assessment of the reproductive status of male animals through artificial insemination, fertility and reproductive efficiency. Interestingly, semen analysis is the examination of physical characteristics of semen (color, odor, pH, viscosity, and liquefaction), volume, concentration, morphology, sperm viability, sperm motility and progression (Baker, 2007). In animal production, poor semen quality could be a major factors of low fertility rate, fertility capacity and high embryo mortality rate (Saacke et al., 1994). Addition of semen extenders can preserve and maintain sperm by stabilizing its properties such as sperm morphology, motility, and viability and membrane, acrosomal, and DNA integrity towards fertilization. Semen extenders provide control on semen pH, adenosine triphosphate, anti-cooling and anti-freeze shock, and antioxidant activity, thereby improving semen quality for fertilization (Bustani and Baiee, 2021).

The assessment of percentage motility, non-progressive motility, average path velocity (VAP), curvilinear velocity (VCL), straight line velocity (VSL), linearity, wobble, liveability, straightness, and amplitude of lateral head (ALH) is one of the most used parameters for semen evaluation in male farm animals. Results in Tables 1 and 2 of this study indicate that

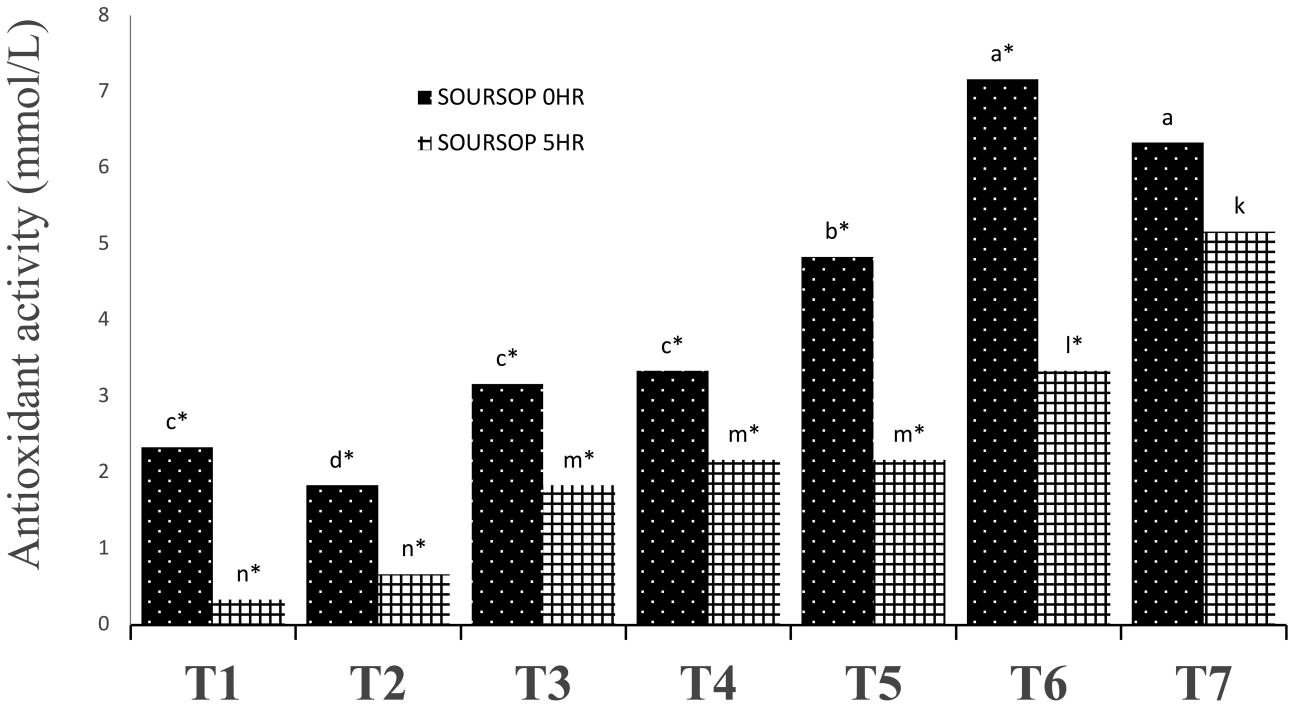

Figure 1. Antioxidant activity of rooster semen in soursop juices extender.Extenders formulated includes; Treatment 1: undiluted (raw) semen; Treatment 2: dextrose saline + 0% soursop juice extender; Treatment 3: dextrose saline + 10% soursop juice extender; Treatment 4: dextrose saline + 20% soursop juice extender; Treatment 5: dextrose saline + 30% soursop juice extender; Treatment 6: dextrose saline + 40% soursop juice extender; Treatment 7: dextrose saline + 50% soursop juice extender. The results on the Figure indicate column with superscripts; abcd: different superscripts at 0 h are significantly ($P < 0.05$) different. mnik: different superscripts at 5 h are significantly ($P < 0.05$) different. *: indicate a significant difference between 0 and 5 h within a treatment.

most parameters measured in undiluted semen were not similar to other treatments as they are higher in values compared to the diluted semen treated with different semen extenders of soursop. In Table 1, percentage motility, non-progressive motility, and average path velocity were significantly reduced by the different semen extenders of soursop at room temperature compared to undiluted semen. Furthermore, in Table 2, more parameters like percentage motility, non-progressive motility, curvilinear velocity, average path velocity, straight line velocity, wobble, liveability and amplitude of lateral head were increased in undiluted semen compared to different semen extenders of soursop after 1 hour of dilution while linearity and straightness were reduced in undiluted semen compared to different semen extenders of soursop after 1 h of dilution. Interestingly, results of Tables 1 and 2 followed similar pattern and trend. This implies that the undiluted semen was superior and has higher fertilization quality compared to the diluted semen treated with different soursop semen extenders under room temperature and after an hour dilution. The results might reveal greater incidences of total sperm abnormalities and dead spermatozoa in different soursop semen extenders at both room temperature and after an hour dilution. The superiority in fertility of undiluted semen compared to diluted treated semen is based on the evaluation of their seminal characteristics. Tables 1 and 2 results of seminal characteristics might suggest the activation in an increased output of the gonadotropic hormones in the anterior pituitary that leads to an increase in spermatogenic activities in the undiluted semen compared to the diluted treated semen (Egbuniwe et al., 2020). Again, the stability of the seminal characteristics in the undiluted semen might be due to avian spermatozoa spend time in the epidermis before ejaculation when compared to mammalian spermatozoa and also, do not undergo massive cell surface changes (Sullivan et al., 2005). In Table 3, progressive motility, non-progressive motility, average path velocity, curvilinear velocity, straight line velocity, linearity, wobble, liveability, straightness and amplitude of lateral head were increased by the different semen extenders of soursop after 2 h of dilution compared to the undiluted semen. Measured parameters of diluted treated semen were not similar to the undiluted semen as they are higher in values. Previously, reports have said that CASA parameters of sperm movement (VCL, VSL) are positively correlated with sperm mobility and egg fertility in turkeys as well as quail (Farooq et al., 2018). Again, straightness and better ALH has been linked with the ability of sperm to penetrate the cervical mucus and fuse with the oocyte in humans (Barlow et al., 1991). The results revealed that sperm motility indices (percentage, progressive and non-progressive motility) of diluted semen within 10%–50% soursop juice were suitable for artificial insemination with no negative impact on spermatozoa movement. The results indicated that the potential of soursop-dextrose saline maintains the viability and kinetics of spermatozoa at the different semen extenders of soursop after 2 h of dilution compared to the undiluted semen. The addition of soursop juice at 10%–50% range produced good spermatozoa kinetics. The result revealed that there was reduction in spermatozoa friction related by the dilution that increases surface area for sperm cell movement (Gao et al., 1995). More so, the result could be due to the antioxidants present in soursop that reduce free radical and reactive oxygen species accumulation, thereby inhibiting lipid peroxidation on sperm progressive motility. Importantly, Soursop fruits are good sources of these natural antioxidants

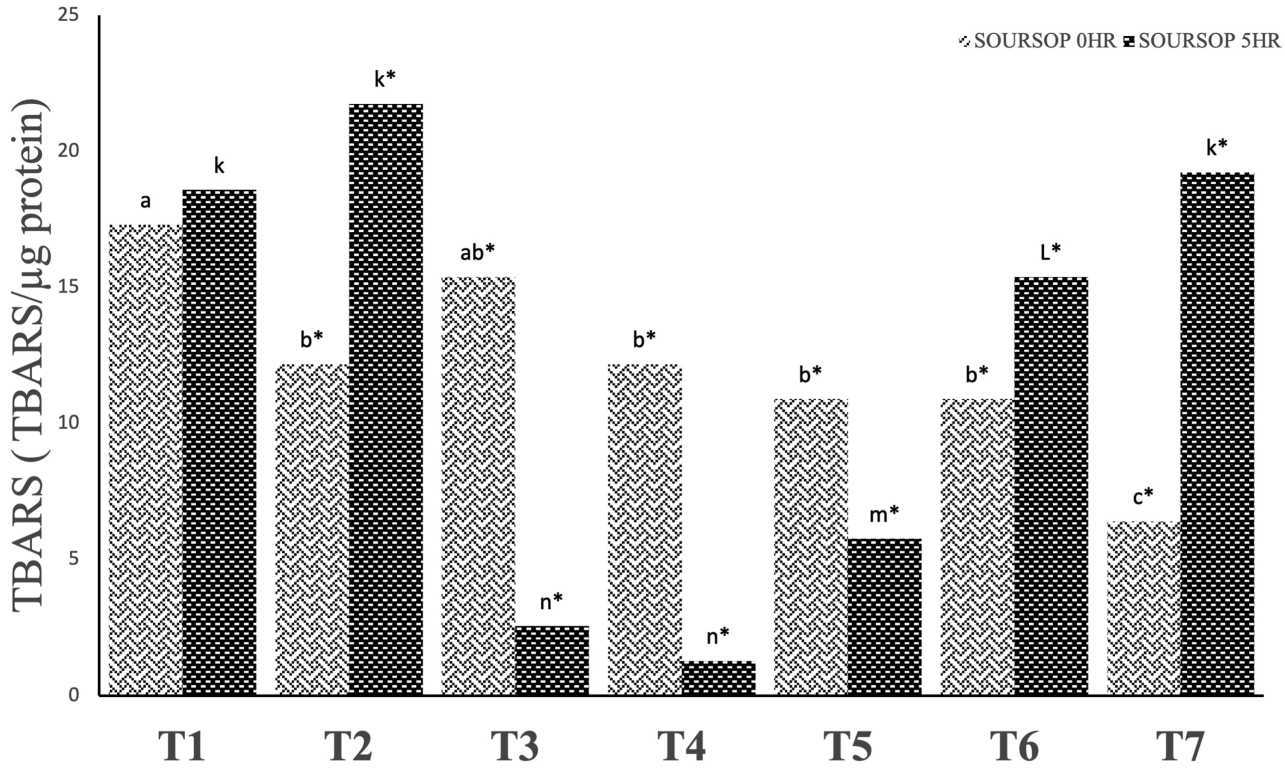

Figure 2. Lipid peroxidation of rooster semen in soursop juices extender.Extenders formulated includes; Treatment 1: undiluted (raw) semen; Treatment 2: dextrose saline + 0% soursop juice extender; Treatment 3: dextrose saline + 10% soursop juice extender; Treatment 4: dextrose saline + 20% soursop juice extender; Treatment 5: dextrose saline + 30% soursop juice extender; Treatment 6: dextrose saline + 40% soursop juice extender; Treatment 7: dextrose saline + 50% soursop juice extender. The results on the Figure indicate column with superscripts; abcd: different superscripts at 0 h are significantly ($P < 0.05$) different. mnik: different superscripts at 5 h are significantly ($P < 0.05$) different. *: indicate a significant difference between 0 and 5 h within a treatment.

comprising many different antioxidant components include steroid, alkaloid, flavonoid, saponin, tannin, phenolic acid and phytate (Agu and Okolie, 2017). Sperm progressive motility might suggest enzymatic activity in diluted semen of different soursop extenders that improve sperm survival. Possibly, the sperm survival was enhanced by the buffering capacity with the combination of the biochemical properties of the seminal plasma of different soursop juice extenders in diluted semen. Spermatozoa liveability and kinetics properties of diluted semen compared favorably with the control in a study of pineapple, watermelon and citrus (tangerine and sweet orange) diluents by (Jimoh et al., 2020a, 2020b, 2021a). The study stated that the result of motility indices of semen diluted up to 30%–50% pineapple, watermelon and citrus (tangerine and sweet orange) diluent inclusions were better than undiluted semen which our results of soursop diluents agree after 2 h observation.

Oxidative Status Assessment of Soursop Extended Rooster Semen

Rooster sperm is associated with different stresses during cryopreservation because of its biological and physiological conditions (Rezaie et al., 2021). Sperm plasma membrane of rooster is rich in polyunsaturated fatty acids that exposes to quick lipid peroxidation that causes decrease in sperm motility and viability during handling, processing and preservation. One major factor contributing to poor quality semen is seminal oxidative stress caused by lipid peroxidation (Shiva et al., 2011). The trend of result obtained showed that the exposure of rooster during semen collection and ejaculation might affect semen quality. Figure 1 showed that there was a gradual progression of antioxidant activity from treatments 1–6 at both 0 and 5 hours dilution. Our study agrees that using soursop fruit as semen extender can ameliorated cryodamages occurring during cryopreservation which plays vital role in eliminating reactive oxygen species during ejaculation stress and prevent injures on sperm cells. Our results showed that natural antioxidant like soursop fruit is rich in alkaloids, flavonoids, saponins, tannins, phytosterols, and terpenoids that preserves the sperm cells during semen preservation (Agu and Okolie, 2017). Furthermore, the antioxidant properties of soursop fruit have been shown to exhibit protective effect against a wide range of toxicants as they are better than synthetic antioxidants with lower cytotoxicity and residue (Okolie et al., 2013). Figure 2 showed a sharp decline and a gradual increase of lipid peroxidation in treatments 2–7 of diluted semen treated with different semen extenders of soursop at 0 and 5 hours dilution, respectively. This result supports the findings that during semen collection and processing, semen ejaculates are exposed to atmospheric oxygen thereby increases the susceptibility of spermatozoa to lipid peroxidation (Menegat et al., 2017). Our findings corroborate that consumption of soursop juice lowered lipid peroxidation and increase antioxidant production in the seminal plasma of heat-stressed bucks administered oral soursop compared to bucks on control group which does not receive soursop juice (Jimoh et al., 2021b). The pineapple, watermelon and citrus (tangerine and sweet orange) diluents studies conducted by

Jimoh and other researchers revealed higher antioxidant activity and reduced lipid peroxidation up to 3 h dilution compared to other semen groups which our findings corroborate up to 5 h dilution with soursop diluents. The studies showed that pineapple, watermelon and citrus (tangerine and sweet orange) juice-dextrose is a potent rooster semen diluents that sustain sperm cell mobility by reducing lipid peroxidation due to its antioxidant capacity (Jimoh et al., 2020a, 2020b, 2021a). There was a higher lipid peroxidation in undiluted semen as high antioxidant activity may be due to lower activity of specific scavengers leading to the accumulation of its oxidants and leading to lipid peroxidation but the inclusion of pineapple, watermelon and citrus (tangerine and sweet orange) juice-dextrose in groups 3–7 provided defence against the oxidation surge during handling and maintain the spermatozoa integrity and the wide array of phytoconstituents in these fruit juices played a significant role in lowering lipid peroxidation and enhancing spermatozoa progressive motility. Furthermore, soursop juice extenders enhance antioxidant defence in the reproductive system that led to the improvement of semen quality in line with this study.

Conclusion

In conclusion, added Annona muricate (Soursop) juices had minimal effects on semen quality, oxidative activity and spermatozoa kinematics, but rooster undiluted semen was important in the development of semen quality, oxidative activity and spermatozoa kinematics of the semen extender. This implies that addition of Annona muricate (Soursop) juices as an semen extender to rooster undiluted semen improves semen quality, oxidative activity and spermatozoa kinematics. The supplementation of soursop juice extender in rooster semen revealed its potential to enhance spermatozoa motility, viability and sperm kinetics after 2 h of observation. The supplementation of soursop juice extender in rooster semen elevates the total antioxidant potential and ameliorate lipid peroxidation during cryopreservation after 5 h dilution. The supplementation of soursop juice extender not above 50% in rooster semen can be used to improve spermatozoa production, enhance fertility, improve fertilizing capacity and reduces oxidative stress.

Author Contribution

Olatunji Abubakar Jimoh conducted and supervised the work. Chinwe Uchechi Nwachukwu wrote the manuscript. Both authors contributed and approved the manuscript for publication.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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