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ASSESSMENT OF SELECTED PLANT EXTRACT AS EXTENDER ON SPERM VIABILITY AND FUNCTIONAL INTEGRITY OF RED SOKOTO BUCK DURING COLD STORAGE

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ABSTRACT

The use of plant-based extenders has gained interest due to their protective and antioxidant capacity. This study evaluates the effects of scent leaf (*Ocimum gratissimum*), onions (*Allium cepa*), lemongrass (*Cymbopogon nardus*), and celery (*Apium graveolens*) extracts on the viability and functional integrity of Red Sokoto buck semen during cold storage. Semen samples were collected from ten mature Red Sokoto buck, pooled, and diluted with tris egg yolk extender. In a completely randomized design, the pooled semen samples were divided into 5. The different plant extracts were added to each sample at 1.5ml/ 1 ml of semen sample with the exception of the control which only contain tris egg yolk extender. Samples were then stored at 4°C in the refrigerator for 7 days. After which it was evaluated for sperm viability (motility, livability and abnormalities) and sperm functional integrity (membrane integrity, acrosome integrity and semen concentration). The result showed no significant difference (P>0.05) in motility, livability, or functional integrity among the treatments. However, scent leaf and lemongrass exhibited a higher sperm motility and livability, while celery showed the lowest. Lemongrass extract had higher acrosome and membrane integrity, whereas onions showed the lowest. Sperm abnormalities were reduced across treatments, supporting the protective role of plant extracts in semen storage. This study suggests that plant-based extenders are effective in semen preservation and therefore may serve as cost-effective and bio secured alternatives to synthetic extenders, sustaining the quality of semen during cold storage and enhancing reproductive efficiency in Red Sokoto goats.

Keywords: Cold storage, Plant extract, Red Sokoto buck, Semen extender, Sperm viability

INTRODUCTION

Red Sokoto bucks are valuable breed in Nigeria, widely raised in the northern regions for their meat quality, adaptability and economic significance. However, successful artificial insemination and semen cryopreservation for optimum reproductive efficiency of this breed is frequently compromised due to environmental stress, oxidative damage and sperm deterioration during storage, which impact sperm quality and viability. For optimum outcome during cold storage, maintaining sperm functional integrity and viability is crucial (Yahya and Midau, 2023).

Extenders, which provide essential nutrients and protect sperm cells against cryopreservation damage, play a critical role in improving sperm preservation (Walke et al., 2023). Due to the high cost and possible negative effects of synthetic compounds on semen preservation, plant-based bioactive compounds have recently drawn attention for their potentials to improve reproductive activities (Kuralkar and Kuralkar, 2021). Several plant extracts are known to have antibacterial, anti-inflammatory, and antioxidant properties that may enhance sperm functionality and viability (Shokoohi, et al., 2018). While numerous studies have looked into using synthetic antioxidants and supplements to improve semen quality, Natural alternatives are becoming more popular because of their safety, affordability, availability and potential effectiveness (Chavda et al., 2024).

Scent leaf (*Ocimum gratissimum*), onions (*Allium cepa*), lemongrass (*Cymbopogon nardus*), and celery (*Apium graveolens*) are among the plant extracts that are being studied for their potential as natural extenders to maintain sperm viability and functional integrity of Red Sokoto buck semen while it is being stored in cold conditions. Flavonoids, phenolics, vitamins, and essential oils are among the bioactive compounds found in these plants, which have long been known for their beneficial properties that may help preserve sperm cells (Boroujeni et al., 2022)

Typically, plant extracts are added to the semen extender and utilized for cryopreservation and sperm refrigeration (Mphaphathi et al., 2024). These substances can be added to the diluents at any stage of the sperm cryopreservation procedure, in general, these natural compounds can improve sperm parameters and fertilization ability (Adewole and Attah, 2020).

The effects of various plant extracts on the functional integrity and sperm viability of Red Sokoto goats are examined in this study over a given storage period. The study aims to determine whether these natural extenders can serve as viable alternatives to conventional extenders, thereby offering a sustainable, cost-effective, and bio secured option for sperm preservation in Red Sokoto bucks

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the small ruminant unit of Prof. Lawal Abdu Saulawa Teaching and Research Farm, Federal University Dutsinma, Katsina State.

Experimental animals and management

Ten mature Red Sokoto Bucks were used in this experiment averagely weighing about 15 kg each on average. These Bucks were kept in an intensive environment and fed a concentrate diet that was supplemented with grasses. During the study, they were provided access to clean water.

Preparation of plant extracts.

The freshly obtained celery, onions, lemongrass, and scent leaf were washed to remove sand or dirt. Each ingredient was

chopped into smaller pieces before being blended. We then used a filter cloth to extract the liquid.

Semen collection and dilution

Semen samples were collected from 10 intact Red Sokoto bucks with the aid of artificial vagina, the collected semen was pooled to minimize individual differences (Bucak and Tekin, 2007). The pooled semen sample was uniformly divided into 5 parts and mixed with a pre-prepared tris egg yolk extender with the exception of the control that only contained tris egg yolk extender. The different plant extracts were added to each sample at 1.5ml/ 1 ml of semen sample. It was stored in a thermos flask at 37°C for transportation to the laboratory, where it was evaluated for sperm quality after storage in a refrigerator for 7 days.

Microscopic semen evaluation

Sperm progressive motility

Motility was determined using the method described by Kowalczyk (2022). The cryopreserved semen sample was briefly thawed in a water bath at approximately 37°C. 5 µl of the semen was then placed directly on a pre-warmed microscope slide and covered with a 22 x 22 mm cover slip. Using a Celestron Penta View digital microscope (LCD-44348 by RoHS, China) at 400x magnification, various microscopic fields were examined to assess the percentage of progressively motile spermatozoa, ensuring an accurate evaluation of sperm motility.

Sperm plasma membrane integrity

The hypo-osmotic swelling test (HOST), as reported by Hufana-Duran *et al.* (2015), was used to assess the integrity of the sperm membrane. For 30 minutes, 10 µl of semen was incubated at 37°C in a hypo-osmotic solution (9 grams of fructose and 4.9 grams of sodium citrate per 100 millilitres of distilled water). A 0.1 ml of the mixture was spread over a warm slide and covered with a cover slip. The sample was then observed under an LCD digital microscope at 400x magnification. The percentage of spermatozoa positive to the Hypo-Osmotic Swelling Test (HOST), characterized by swelling with curled tails, was determined. Spermatozoa that did not exhibit swelling and had uncurled tails were classified as having abnormal membrane integrity, indicating compromised cell function.

Sperm livability and abnormality

According to Cecere (2014), sperm abnormalities were evaluated using eosin-nigrosine. A thin layer of the eosin nigrosine solution and semen mixture was spread on the slide and allowed to dry. Under a 400x magnification LCD microscope, the proportion of spermatozoa with morphologically aberrant defects in the head, midpiece, and tail was measured. Spermatozoa that emerged white were considered live, whereas those that absorbed the stain were considered dead.

Acrosome integrity

The procedure of Ahmad *et al*. (2014) was followed in order to determine the percentage of sperm cells with undamaged acrosomes. 50 µl of semen sample was added to 500 µl of formalin citrate solution. After placing a drop of this mixture on a microscope slide, 200 spermatozoa were recorded using a 400x magnification microscope. The intactness of the acrosome was determined by the presence of a normal apical ridge on the spermatozoa, indicating healthy and functional sperm cells.

Sperm concentration

Sperm concentration was determined through microscopic analysis using the procedure of Tanga et al. (2021). The process involves diluting the semen and counting the number of sperm cells within a given volume, using a hemocytometer. This count is then used to calculate the concentration, which is expressed as the number of sperm per milliliter of semen

Number of sperm counted x dilution factor/volume x 1000 = sperm/ml.

Data analysis

Data from the experiment was subjected to analysis of variance (ANOVA). In a completely randomized design, using SPSS 2000. The means were separated by Duncan Multiple Range Test (Duncan, 1995). The model is given below.

$Yijk = \mu + Gi + \Sigma_{ijk}$

Where; Y_{ij} = the observed value of the dependent variable, µ = population mean, G_i = j^{th} effect of plant extract (onions, celery, scent leaf and celery), Σ_{ik} = Random experimenter error

RESULTS AND DISCUSSION

The effects of extract from various ingredients (scent leaf, onions, lemongrass, and celery) on motility, livability, and abnormality rate in the Red Sokoto dwarf goat are shown in Table 1. The result shows no significant difference (P>0.05) among the different ingredients, although there is a noticeable trend. Motility was slightly higher in scent leaf and lowest in celery, similar in onions and lemongrass. The highest livability was shown by the control, then lemongrass extract, followed by scent leaf extract, while the livability rates of onions and celery were relatively lower. The lowest abnormality was observed in the control, lemongrass extract, scent leaf and celery showed a moderate abnormality rate, while onions showed the highest, suggesting a greater negative impact.

The effects of extracts from scent leaf, onions, lemongrass, and celery plants on semen concentration, acrosome and membrane integrity of Red Sokoto goat sperm are shown in Table 2. All data were not statistically significant (P>0.05). For acrosome integrity, the highest was observed in the control, while lemongrass extract was found to have a higher level of acrosome integrity, followed by scent leaf and celery extract, while onions had the lowest,. Lemongrass extract also showed a higher sperm membrane integrity after the control, followed by scent leaf and celery extract, while onions showed the lowest. Celery and onion had lower concentration values than lemongrass compared to the control.

Table 1: Effect of Selected Plant Extracts on Sperm Viability of Red Sokoto Buck during cold storage

Parameters	Control	Scent leaf	Onions	Lemongrass	Celery	P value
Motility	65.23 ± 9.83	59.00 ± 10.58	43.00 ± 10.94	42.57 ± 9.55	35.86 ± 11.56	0.858
Livability	82.00 ± 5.10	75.71 ± 5.82	66.43 ± 4.10	77.86 ± 5.22	65.00 ± 5.23	0.244
Abnormality	10.20 ± 2.51	17.86 ± 5.18	24.29 ± 4.93	14.29 ± 3.52	16.43 ± 5.07	0.490

Table 2: Effect of Selected Plant Extracts on Sperm Functional Integrity of Red Sokoto Buck during cold storage

Parameters	Control	Scent leaf	Onions	Lemongrass	Celery	P value
Acrosome	80.62 ± 5.63	67.86 ± 6.71	54.29 ± 10.26	75.00 ± 6.17	47.14 ± 8.08	0.082
Membrane	92.00 ± 4.57	82.14 ± 5.18	75.71 ± 4.93	86.86 ± 3.60	81.43 ± 5.07	0.442
Con. ($x10^6$)	190.77 ± 30.52	179.43 ± 49.67	137.43 ± 36.04	183.43 ± 49.23	143.43 ± 90.86	0.819

Discussion

Reproductive efficiency is a key factor in livestock production, particularly in breeding programs aimed at genetic improvement and sustainability. The ability to preserve semen has significantly enhanced reproductive management in the livestock sector allowing a better genetic and improved fertility outcome. A promising approach in semen preservation is the use of Plant extracts as extenders which have showed the capacity of extending the shelf life of sperm cells by providing antioxidants and protective effects. Imosemi, (2020). According to the results of this investigation, extracts from scent leaf (*Ocimum gratissimum*), onions (*Allium cepa*), celery (*Apium graveolens*), and lemongrass (*Cymbopogon citratus*) sustained sperm motility and livability over a seven-day period of cold storage. These findings are consistent with those of Guerrero-Guzmán et al. (2021), who found that adding scent leaf extract to boar semen extender sustained sperm motility and preserved boar spermatozoa for up to 48 hours. The abundance of bioactive substances in scent leaf, such as flavonoids and polyphenols, which have potent antioxidant qualities, is responsible for the positive effects on sperm viability and functional characteristics.

Likewise, Ali et al. (2022), who examined the impact of adding aqueous lemongrass leaf extract to male broiler breeders' drinking water, reported that physiological parameters, were sustained, thereby corroborating the findings of this study underscoring the potential derived from plants to improve reproduction. The results of this study also support the potentials of onion extract as a natural extender, as demonstrated by Bassuony et al. (2023), who found that it sustained sperm fertility parameters after storage. Prior research on celery extract has demonstrated its beneficial effects on spermatogenesis and sperm parameters related to fertility. Vitamins E and C found in celery have antioxidant qualities that improve sperm count, acrosome integrity, membrane stability, and motility, according to Madkour (2014), while Kooti et al. (2018) found that celery leaf extract increases sperm count and motility.

Sperm abnormalities in stored and post-thawed semen have been found to be kept low by using plant extracts as extenders. This implies that bioactive substances derived from plants might be protective in preserving sperm shape during cryopreservation and storage. The potential of Nigella sativa extract to maintain sperm integrity was highlighted by Alrubaie et al. (2022), who reported a significant decrease in sperm abnormalities after incorporating the extract. These results support the theory that plant-based extenders are beneficial substitutes for traditional extenders because they can reduce structural damage and improve the overall quality of preserved semen.

CONCLUSION

Lemongrass, scent leaf, onions and celery have shown to be beneficial ingredients although perform lower than the control, it exhibited a favorable results by sustaining the sperm fertility parameters after 7 days storage. Additionally, it maintained abnormality at a lower rate, suggesting its strong potential in promoting sperm fertility and health.

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