

Effects of *Piliostigma Thonningii* (Schumach.) milne-rech essential oil supplementation on reproductive performance, semen quality, and fertility indices in bunaji bulls

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Abstract

This study examined the impact of camel's foot essential oil, also known as *Piliostigma thonningii* seed essential oil (Milne–Rech seed essential oil, MSEO), on the reproductive performance of Bunaji bulls. *Piliostigma thonningii* was selected because its seeds are rich in bioactive phytochemicals, including phenolics, flavonoids, and terpenoids, which have demonstrated antioxidant, antimicrobial, and endocrine-modulating properties capable of enhancing testicular function, spermatogenesis, and semen quality. In addition, the plant is widely available in tropical savanna ecosystems, making it a cost-effective and sustainable phytogenic alternative to synthetic reproductive enhancers. A completely randomized design was used to allocate fifteen clinically healthy bulls (aged 22–24 months; average body weight: 160.55 ± 0.60 kg) to three dietary treatments. For 16 weeks, bulls were fed a common basal diet supplemented with 0 ml/kg MSEO (T1, control), 10 ml/kg MSEO (T2), or 20 ml/kg MSEO (T3). Dietary treatments did not affect semen pH or its creamy white coloration ($P > 0.05$). However, MSEO supplementation significantly increased ($P < 0.05$) sperm concentration and ejaculate volume, with T3 recording the highest values. Sperm motility, percentage of live sperm, and live-to-dead sperm ratio were significantly improved ($P < 0.05$) in T2 and T3 compared with the control. Conversely, bulls in T3 exhibited the lowest proportions of dead and morphologically abnormal sperm cells ($P < 0.05$). Percentages of normal sperm cells and certain sperm abnormalities, including midpiece droplet, coiled tail, detached head, free tail, and bent tail, were not significantly affected by dietary treatment ($P > 0.05$). Scrotal length and circumference increased significantly ($P < 0.05$) with MSEO inclusion, with the highest values observed in T3. In conclusion, dietary supplementation with 20 ml/kg MSEO enhanced testicular development and semen quality without adverse effects, supporting its potential use as a natural, sustainable phytogenic alternative to synthetic reproductive enhancers in bull production systems.

Keywords: *Piliostigma thonningii* essential oil, Bunaji bulls, Reproductive performance, Semen quality, Scrotal traits, Natural feed additive

INTRODUCTION

There is a severe lack of animal protein to meet the continent's rising nutritional needs due to numerous challenges confronting ruminant production systems across Africa, particularly within small-, medium-, and large-scale operations in West Africa (Anaso & Olafadehan, 2025; Olafadehan et al., 2023; Anaso, 2025a). The Food and Agriculture Organization reported that average daily animal protein intake in sub-Saharan Africa is approximately 4.5 g per capita, far below the recommended 35 g per day (FAO, 2001; Anaso and Anaso, 2025). Recent projections indicate that demand for animal protein continues to rise, driven largely by rapid population growth, increasing incomes, and shifts in dietary preferences (FAO, 2009; Anaso & Salihu, 2025). However, livestock productivity in tropical regions remains constrained by several physiological and environmental factors, including poor-quality nutrition, heat stress, and the inherently slow growth and reproductive rates of indigenous breeds (Anaso, 2025b, c). These stressors negatively affect feed intake, metabolic efficiency, and reproductive performance, particularly in breeding males.

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Essential oils (EOs) are naturally occurring, volatile, aromatic compounds extracted from various plant parts such as seeds, leaves, bark, and roots, and are increasingly recognized for their bioactive roles in animal nutrition (Anaso et al., 2025c). Among these, *Piliostigma thonningii* which is commonly known as camel's foot or monkey bread is a leguminous tree widely distributed across the savannah zones of West Africa. Morphologically, the plant is characterized by its distinctive bilobed leaves resembling a camel's hoof, flattened woody pods, and nutrient-rich seeds. Biologically, *P. thonningii* seeds are rich in secondary metabolites, including phenolics, flavonoids, terpenoids, and essential oils, which confer antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory properties (Anaso, 2023a,b). The seed essential oil (Milne-Rech seed essential oil; MSEO) has attracted particular interest due to its stability, bioavailability, and demonstrated ability to modulate rumen fermentation, oxidative balance, and reproductive physiology in animal models.

Several studies have documented the benefits of phytogetic feed additives, including EOs, in enhancing nutrient utilization, health status, and productive performance in ruminants (Kholif et al., 2017, 2020; Morsy et al., 2018; Anaso et al., 2025d-g). More recent research highlights the multifunctional properties of Eos such as antimicrobial activity, antioxidant capacity, and palatability enhancement, which make them attractive natural alternatives to synthetic feed additives (Anaso, 2023a, b; Anaso and Alagbe, 2025a-c). The global ban on antibiotic growth promoters, prompted by increasing concerns over antimicrobial resistance, has further accelerated the search for safe, plant-based dietary supplements capable of sustaining livestock productivity while ensuring food safety (Soultos et al., 2009; Koné et al., 2016; Tufarelli et al., 2017; Anaso et al., 2024d; Anaso and Dikki, 2025; Anaso, 2026a,b). Consequently, interest in phytogetic additives like MSEO has resurged due to their proven antibacterial, antioxidant, and immunomodulatory effects (Miguel, 2010; Negi, 2012; Ahmed & Abdallah, 2020; Anaso et al., 2025a, b; Anaso et al., 2026).

Although *Piliostigma thonningii* seed essential oil has been extensively evaluated in rabbits and small ruminants (Anaso, 2023a,b; Anaso et al., 2023; Anaso et al., 2024a-d; Anaso, 2025a-e; Anaso et al., 2025a-g), information regarding its influence on reproductive performance in large ruminants remains scarce. In particular, data on its effects on semen quality, testicular development, and fertility indices in Nigerian White Fulani (Bunaji) bulls are limited. Given the urgent need to improve male reproductive efficiency under tropical production constraints, further investigation is warranted to elucidate the potential of MSEO as a natural reproductive enhancer and its possible synergistic interactions with conventional feeding systems.

Therefore, the present study was designed to:

1. Evaluate the semen characteristics of White Fulani bulls fed diets supplemented with MSEO.
2. Determine the semen morphology of bulls under MSEO supplementation.
3. Assess the scrotal parameters of bulls receiving MSEO-supplemented diets.

MATERIALS AND METHODS

Collection of Milne-Rech seeds and extraction of the essential oil

Piliostigma thonningii *Piliostigma thonningii* (Milne-Rech) seeds were collected from the Federal Capital Territory, Nigeria, located within the Southern Guinea Savanna agro-ecological zone. The seeds were air-dried under shade to preserve their bioactive constituents and prevent degradation of thermolabile phytochemicals. After complete drying, the seeds were milled into a fine powder and stored at ambient temperature prior to extraction.

Essential oil extraction was performed by steam distillation using 100 g of powdered seed material immersed in 700 mL of distilled water. The mixture was heated at 100 °C for 3 h in a stainless-steel distillation apparatus equipped with a condenser to facilitate vaporization and condensation. Volatile oil fractions were collected using a Clevenger-type apparatus. The extraction protocol followed the procedures described by Anaso and Alagbe (2025a) and Anaso et al. (2025c). The essential oil yield obtained from the seeds was 1.42% (v/w, dry matter basis), providing an estimate of extraction efficiency and potential economic implications for practical application.

The chemical composition of the extracted *P. thonningii* seed essential oil (MSEO) was determined using gas chromatography–mass spectrometry (GC–MS) in Table 1 referenced from Anaso (2023b). The oil was characterized by a terpene-rich profile, with β -pinene (25.02%) identified as the predominant constituent, followed by β -limonene (17.85%) and α -muurolene (9.63%). Other notable components included β -myrcene (6.05%), γ -terpineol (5.11%), α -terpineol (3.70%), 1,3-dimethyl-4-isopropylbenzene (3.11%), hexenyl acetate (2.83%), terpinen-4-ol (2.60%), and α -cubebene (2.09%), alongside several minor mono- and sesquiterpenes. These major and minor constituents are widely reported to possess antioxidant, antimicrobial, and physiological modulatory activities. In particular, the dominance of monoterpenes and oxygenated terpenoids in the oil may explain the observed improvements in semen quality, sperm viability, and testicular development in Bunaji bulls supplemented with MSEO in the present study.

Experimental site

In cooperation with the Co-Farms Green Aid Revolution Ruminant Research Unit, the experimental trial was carried out at Morugo Farms. The study site is situated in Nigeria's Southern Guinea Savanna agro-ecological zone, between latitudes

08°51' and 09°37' N and longitudes 07°20' and 07°51' E (Anaso et al., 2025e). The region has a tropical climate with annual rainfall ranging from 1,145 to 1,631 mm with distinct wet and dry seasons. Relative humidity averages 60% and 30% during the rainy and dry seasons, respectively, while ambient temperatures range from 25.8 to 30.2 °C and 36 to 42 °C.

Savanna forest makes up the majority of the vegetation, which is primarily composed of grasses and sporadic shrubs with leguminous forbs and browsing species that ruminants frequently use in between. While browsing plants like *Leucaena leucocephala*, *Piliostigma thonningii*, and *Daniellia oliveri* are common, dominant grass species include *Panicum maximum*, *Andropogon gayanus*, and *Hyparrhenia rufa*. The region's soils are sandy loams that drain well, are somewhat fertile, and can be used for raising livestock as well as fodder. In semi-intensive ruminant production systems, this ecological setting offers a representative habitat for assessing the physiological and nutritional impacts of feed additives.

Table 1. Bioactive compounds of *Piliostigma thonningii* essential oil detected by the gas chromatography-mass spectrometry

Compounds	Percentage area	Mass peak
Azulene	0.42	25, 40, 42, 57
Beta myrcene	6.05	25, 72, 102, 106, 110
Beta limonene	17.85	25, 41, 43, 57
Cis- linaloxide	0.21	25, 39, 47, 49, 55
Alpha cymene	0.15	25, 44, 48
Beta ocimene	0.06	25, 88, 106
Trans beta ocimene	0.30	25, 39, 66, 70
4-methylpropiohenone	0.12	25, 88, 106, 109
1,3 dimethyl-4-Isopropylbenzene	3.11	25, 48, 51, 58, 69
Dodecane	0.19	25, 41, 43
Tridecane	1.20	60, 68, 77, 103
2,3-dimethylnepthalene	0.71	25, 44, 68, 92
1,8-dimethylnepthalene	0.22	25, 81, 87, 102, 117
Alpha thujene	0.19	25, 29, 38, 44
Beta pinene	25.02	25, 62, 68, 100
Terpinen-4-ol	2.60	25, 42, 47, 58
Alpha terpineol	3.70	25, 88, 93, 106
Gamma terpineol	5.11	25, 71, 79, 100
Alpha cubebene	2.09	25, 63, 69
Alpha yiangene	1.02	25, 41, 55, 108, 127
Alpha selinene	1.10	25, 40, 48, 51
Chrysathenine	0.08	25, 47, 57, 63
Alpha allyltoluene	0.29	25, 39, 47, 58
Borneol	1.44	25, 33, 39
Hexenyl acetate	2.83	25, 28, 37, 108
Camhene	1.50	25, 63, 68, 101
n-Nonanol	0.04	25, 69, 104
Trans-pinocarveol	0.62	25, 27, 29, 41, 55, 67
Cis pinocarveol	1.77	25, 41, 55, 67, 81, 104
Fenchol	0.96	25, 41, 58, 138
Alpha murolene	9.63	25, 39, 51, 105, 109

Reference; Anaso (2023b) and Anaso et al. (2024d)

Experimental animals, management and diets

In this investigation, 15 clinically healthy Bunaji (White Fulani) bulls weighing an average of 160.55 ± 0.60 kg and between the ages of 22 and 24 months were employed. A 20-day acclimation period and a 120-day feeding phase made up the 140-day feeding trial. A 20 x 30 m corral with 18.6 m² cages was utilized to house the bulls, who were purchased from a respectable commercial farm in North-Central Nigeria (Anaso et al., 2024b).

Morigad®, a broad-spectrum antibiotic sanitizer, and Hypo®, a sodium hypochlorite-based disinfectant that contains demineralized water and caustic soda, were used to thoroughly clean and disinfect all of the pens and adjacent facilities before the animals arrived.

The bulls were quarantined for two weeks after arrival, at which time conventional preventative measures were put in place. Each animal was given an intramuscular injection of long-acting oxytetracycline hydrochloride (10 mL/100 kg body weight) for bacterial prophylaxis, a subcutaneous injection of Avomec® (1 mL/50 kg body weight) for ectoparasite and endoparasite control, and an oral dose of Vitalyte® to reduce environmental and transport stress. Every therapy was carried out in compliance with the guidelines provided by the manufacturer and overseen by a licensed veterinarian.

Following the National Research Council's (NRC, 2007) recommendations, diets were developed to satisfy the nutritional needs of cattle (Table 2). Bulls were fed 5% of their body weight (dry matter basis), with weekly feed adjustments made in response to body weight fluctuations (Anaso et al., 2021a,b; Olafadehan et al., 2021). Clean water was accessible at all times, and feeding took place twice a day at 8:00 and 16:00.

While animals in T2 and T3 got the identical diet supplemented with 10 mL/kg and 20 mL/kg of MSEO, respectively, animals in the control group (T1) simply received the basal diet. The essential oil was first thoroughly combined with a portion of the morning ration, given right away, and after the feed was fully taken, the remaining feed was given to ensure efficient intake and reduce volatilization. This process promoted consistency between treatments by guaranteeing consistent oil intake and preventing feed refusal.

Table 2. Ingredient composition of the experimental diets (% DM)

Ingredient (DM basis)	Quantity (kg)
Panicum Maximum (hay)	30
Maize	28
Biodegraded sugarcane scrapings (BDSS)	15
Groundnut Cake	12
Cowpea husk	7
Cassava peels	3
Molasses	2
Salt	0.5
Limestone	2
Vitamin–mineral premix	0.5
Total	100

Testicular and semen parameters

Semen analyses and scrotal measurements were performed in accordance with accepted standard practices. In accordance with Akpa et al. (2012), scrotal length (SL), which is the distance along the caudal surface of the scrotum from the place of attachment at the perineum to the distal tip, was measured once a week using a flexible measuring tape. According to Akpa et al. (2012), scrotal circumference (SC) was calculated as the biggest circumference around the pendulous scrotum following a gentle push of both testes to the bottom of the scrotal sac.

Following the protocols described by Zemjanis (1970), semen was extracted from all experimental bulls by electro-ejaculation using an automatic electro-ejaculator (Autojact, Neovet) running at 12 V and 5 A. A series of 1–35 electrical shocks were given to the bulls over a period of 30 seconds to 5 minutes while they were held in a standing position inside a chute in order to induce ejaculation (Anaso et al., 2024b). The volume of ejaculate (measured in milliliters) was promptly recorded from the calibrated collection vial. Following that, samples were kept in a water bath at 37 °C, and further tests were carried out in compliance with the recommendations of the CBRA (1998).

Semen Evaluation Procedures

While pH was instantly evaluated by dipping a narrow-range litmus paper into the semen and watching for a color shift, ejaculate volume was measured straight from the graduated collection tube (Anaso et al., 2023). Semen appearance (color and consistency) was evaluated visually and classified as watery, thick milky, creamy marble, or creamy (Anaso et al., 2023). As soon as possible after collection, the initial fructose content was measured using Mann's technique (1948). For additional microscopic evaluation, thin smears of every sample were made, allowed to air dry, appropriately labeled, and kept (Anaso et al., 2024b).

Sperm Motility and Concentration

By diluting 10 µL of semen in 1 mL of Tris buffer (which contains 3.0 g hydroxymethylaminomethane, 2.0 g sodium citrate, and 1.0 g fructose), progressive motility was assessed. A 100× magnification light microscope was used to examine a 10 µL aliquot of this mixture that had been sandwiched between a coverslip and a glass slide that had been preheated to 37 °C. The percentage of progressively motile spermatozoa was used to express the results.

A Neubauer hemocytometer, which has 25 large squares that are further divided into 16 smaller squares (for a total of 400), was used to measure the concentration of sperm. In accordance with Rekwot et al. (1997), sperm cells were counted diagonally over five large squares (equivalent to 80 small squares). Semen samples were diluted 1:100 in formaldehyde solution to immobilize the sperm cells before counting. To make sure there were no air bubbles, a drop of the diluted sample was put into the counting chamber. The sperm concentration per milliliter of semen was then calculated by extrapolating the quantity of sperm cells (Anaso et al., 2024b).

Sperm Morphology and Viability

Smears stained with 7.5% Giemsa solution (Doles Laboratory) diluted in distilled water were used for morphological evaluation. To distinguish between normal and aberrant spermatozoa, slides were submerged for two hours, allowed to air dry upright, and then inspected under a microscope. Morphological anomalies were categorized using Barth and Oko's (1989) criteria. Percentages of total spermatozoa were used to express the results.

The eosin–nigrosine staining method was used to calculate the live-to-dead sperm ratio (Hancock, 1951; Anaso et al., 2024b). On a sterile glass slide, a drop of semen and an equal drop of eosin-nigrosine stain were carefully combined. Another slide was used to create a thin smear at a 45° angle, which was then labeled and allowed to air dry. Dead spermatozoa absorbed the dye and turned pink or crimson under the microscope, whereas healthy spermatozoa appeared unstained.

Statistical analyses

A completely randomized design (CRD) was used to analyze data on reproductive and scrotal parameters using a one-way analysis of variance (ANOVA), with nutrition therapy acting as the fixed effect. The SPSS program (version 22.0; IBM Corp., Armonk, NY, USA) was used for all statistical analyses. Duncan's Multiple Range Test (DMRT) was used to compare treatment means, and differences were deemed statistically significant when $P < 0.05$. The statistical model used for the analysis is expressed as:

$$Y_{ij} = \mu + t_i + e_{ij} \quad Y_{ij} = \mu + t_i + e_{ij}$$

where:

- $Y_{(ij)}$ = observed value of the dependent variable,
- μ = overall mean common to all observations,
- t_i = fixed effect of the i^{th} dietary treatment ($i = 3$), and
- e_{ij} = random error term associated with each observation.

A strong statistical foundation for evaluating treatment differences was provided by this analytical method, which made sure that observed changes in scrotal features and reproductive success were due to treatment effects rather than chance experimental error.

RESULTS AND DISCUSSIONS

Semen characteristics of bulls supplemented *Piliostigma thonningii* essential oil-based diet

Table 3 presents the semen characteristics of Bunaji bulls fed diets supplemented with MSEO. Semen colour, an important qualitative indicator of sperm density and seminal plasma integrity, remained uniformly creamy white across all treatments (T1, T2, and T3). Creamy-white semen is widely associated with high sperm concentration and good fertilizing capacity in domestic animals, whereas translucent or discoloured semen often indicates low sperm density, contamination, or underlying reproductive disorders (Anaso et al., 2025d; Ososanyo et al., 2013; Bitto et al., 1988; Oyeyemi et al., 2011). Therefore, the uniform semen colour observed across treatments suggests that dietary MSEO supplementation up to 20 mL/kg did not adversely affect seminal quality or reproductive health.

Semen pH, a critical determinant of sperm motility, metabolic activity, and fertilization ability, was not significantly influenced by MSEO inclusion. Maintaining semen pH within the physiological range is essential, as deviations may impair sperm function or indicate infection and metabolic disturbances (Anaso et al., 2025d; Zhou et al., 2015; Anaso et al., 2024a). The absence of treatment effects on pH aligns with previous reports showing that phytogetic feed additives and essential oils do not necessarily alter seminal acid–base balance (Ahmed and Abdallah, 2020; Abdel-Wareth and Metwally, 2021; Elratel et al., 2021; Anaso et al., 2023).

Ejaculate volume, which reflects the functional capacity of the accessory sex glands and contributes to sperm transport within the female reproductive tract, ranged from 1.95 to 2.23 mL. Bulls in T3 recorded significantly ($P < 0.05$) higher ejaculate volume compared to the control. Although ejaculate volume can be influenced by non-nutritional factors such as collection frequency, handling, and environmental conditions (Soderquist et al., 1992; Anaso et al., 2025d), the observed increase in MSEO-supplemented groups suggests a positive physiological response to dietary inclusion.

Sperm concentration, a direct measure of spermatogenic efficiency and testicular function, increased significantly ($P < 0.05$) in T3 compared with T1. Higher sperm concentration is closely linked to improved fertility potential, as it enhances the likelihood of successful fertilization. The elevated sperm counts observed in T2 and T3 may indicate improved antioxidant defense and nutrient utilization, supporting normal spermatogenesis and epididymal sperm maturation.

Sperm motility and live sperm percentage, both critical predictors of fertilizing ability, were significantly ($P < 0.05$) higher in bulls receiving MSEO supplementation. Progressive motility is essential for sperm transport through the female reproductive tract, while a high proportion of live spermatozoa reflects membrane integrity and cellular viability. According

to Osinowo (2016), sperm motility above 65% is indicative of good reproductive potential in breeding bulls; values recorded in T2 and T3 exceeded this threshold, confirming the beneficial effect of MSEO on semen quality.

Conversely, dead sperm cells and abnormal spermatozoa, which negatively affect fertility and embryo development, were significantly ($P < 0.05$) higher in the control group and lowest in T3. Reduced sperm abnormalities suggest improved spermatogenic precision and protection against oxidative damage during sperm development.

The overall improvements in semen volume, sperm concentration, motility, and viability, coupled with reduced abnormalities in MSEO-supplemented groups, are consistent with previous findings on essential oils and phyto-genic additives (Anaso et al., 2024b, 2023; Abdel-Wareth and Metwally, 2021). These effects are likely mediated by the antioxidant and antimicrobial properties of MSEO's dominant bioactive constituents, particularly β -pinene, which may enhance testicular function, protect sperm cells from oxidative stress, and improve overall reproductive efficiency.

Table 3. Semen characteristics of bulls supplemented *Piliostigma thonningii* essential oil-based diet

Parameter	T1	T2	T3	SEM
Semen colour	Creamy white	Creamy white	Creamy white	
pH	6.48	6.51	6.50	0.04
Semen volume (ml)	1.95 ^c	2.06 ^b	2.25 ^a	0.04
Motility (%)	80.12 ^b	90.04 ^a	90.37 ^a	0.39
Semen concentration ($\times 10^6$)	308.58 ^c	328.22 ^b	346.54 ^a	8.94
Abnormalities (%)	12.40 ^a	10.95 ^b	9.81 ^c	0.43
Live spermatozoa (%)	61.22 ^b	79.41 ^a	80.47 ^a	0.72
Dead spermatozoa (%)	38.78 ^a	20.58 ^b	19.53 ^b	0.72
Live dead ratio	1.58 ^b	3.86 ^a	4.12 ^a	0.39

Means with the different superscripts along the row are significantly ($P < 0.05$) different

T1, 0 ml *P. thonningii* essential oil; T2, 10 ml *P. thonningii* essential oil; T3, 20 ml *P. thonningii* essential oil/kg diet.

Sperm morphology of bulls supplemented *Piliostigma thonningii* essential oil-based diet

Table 4 presents the effects of MSEO supplementation on sperm morphology of Bunaji bulls. Parameters such as mid-piece droplet, coiled tail (8.92–10.21%), detached head (3.75–5.33%), free tail (6.95–8.18%), bent tail (5.47–6.04%), and normal sperm cells (70.37–76.47%) showed no significant ($P > 0.05$) differences among treatments, which are critical indicators of spermatogenic efficiency, structural integrity, and fertilization capacity in breeding bulls. Morphological defects compromise sperm motility, energy metabolism, and the ability to penetrate the oocyte, thereby reducing reproductive success.

Parameters assessed included mid-piece droplets, coiled tails, detached heads, free tails, bent tails, and the proportion of normal sperm cells, all of which are routinely used to diagnose teratozoospermia which is described as a condition associated with impaired male fertility. In the present study, none of these parameters differed significantly ($P > 0.05$) among treatments, indicating that dietary MSEO supplementation did not adversely affect sperm morphology.

The mid-piece, which houses the mitochondria and serves as the primary energy center of the spermatozoon, is essential for sustained motility and progressive movement toward the site of fertilization (Anaso et al., 2024b). The absence of treatment effects on mid-piece droplets suggests that mitochondrial integrity and ATP-generating capacity were preserved in bulls fed MSEO-supplemented diets.

Coiled tail abnormalities, often associated with oxidative stress, bacterial contamination, or membrane damage, reduce sperm motility and fertilizing ability (LLU, 2023). The lack of significant differences in coiled tail incidence across treatments indicates that MSEO inclusion up to 20 mL/kg diet did not induce oxidative or pathological stress capable of disrupting tail structure.

Detached sperm heads are typically linked to testicular degeneration, impaired spermiogenesis, or prolonged sexual inactivity, all of which negatively affect fertilization potential (Anaso, 2024). The consistency of this parameter across treatments implies that the bioactive compounds present in MSEO did not disrupt testicular architecture or spermatogenic processes.

Similarly, free and bent tails, which are commonly attributed to epididymal dysfunction, thermal stress, or nutritional imbalance, showed no treatment-related variation. Tail abnormalities are widely recognized as stress-responsive indicators of compromised sperm maturation (Barth and Oko, 1989). Their stability in this study suggests that bulls were not subjected to environmental, nutritional, or oxidative stress during the experimental period.

The percentage of normal sperm cells, an integrative measure of overall semen quality and reproductive competence, was numerically highest in bulls receiving 20 mL/kg MSEO (T3), although differences were not statistically significant. A higher proportion of morphologically normal sperm cells is indicative of efficient spermatogenesis and a reduced risk of fertility

disorders such as oligozoospermia or azoospermia. This trend suggests a favorable, albeit subtle, influence of MSEO on spermatogenic performance.

The preservation of normal sperm morphology observed in this study may be attributed to the antioxidant and cytoprotective properties of MSEO, particularly its dominant monoterpenes and sesquiterpenes, including β -pinene, β -myrcene, α -pinene, and β -caryophyllene, as well as fatty acid derivatives such as oleic and heptadecanoic acids (Anaso et al., 2024a; 2024c; 2024d; 2025a–d). These compounds are known to stabilize sperm membranes, mitigate lipid peroxidation, and protect developing germ cells against oxidative injury.

Overall, the absence of deleterious effects on sperm morphology confirms that MSEO is physiologically safe at the tested inclusion levels and supports its suitability as a phyto-genic feed additive for improving reproductive performance in Bunaji bulls.

Table 4. Sperm morphology of bulls supplemented *Piliostigma thonningii* essential oil-based diet

Parameter (%)	T1	T2	T3	SEM
Middle piece defect	0.03	0.01	0.01	0.00
Coiled tail	10.21	9.40	8.92	0.71
Detached head	5.33	3.86	3.75	0.70
Free tail	8.18	7.18	6.95	1.02
Bent tail	6.04	5.96	5.47	1.00
Normal cells	70.37	75.19	76.47	3.58

Means with the different superscripts along the row are significantly ($P < 0.05$) different

T1, 0 ml *P. thonningii* essential oil; T2, 10 ml *P. thonningii* essential oil; T3, 20 ml *P. thonningii* essential oil/kg diet.

Scrotal parameters of bulls supplemented *Piliostigma thonningii* essential oil-based diet

Table 5 presents the scrotal characteristics of Bunaji bulls fed diets supplemented with MSEO. Scrotal measurements were selected because they are widely accepted non-invasive indicators of testicular development, spermatogenic capacity, and breeding soundness in bulls. Scrotal length values were 12.79, 14.57, and 15.45 cm for T1, T2, and T3, respectively. Bulls in T1 recorded significantly ($P < 0.05$) shorter scrotal lengths than those in T2 and T3, which did not differ ($P > 0.05$). Similarly, scrotal circumference values were 27.81, 30.20, and 31.62 cm for T1, T2, and T3, respectively, with T1 being significantly ($P < 0.05$) lower and T3 exhibiting the highest mean value.

Scrotal length is an important reproductive trait because it reflects testicular elongation and parenchymal development, which are directly associated with the number of seminiferous tubules and the efficiency of spermatogenesis. Bulls with greater scrotal length generally possess higher testicular mass and increased daily sperm production (Akpa et al., 2012). The significantly lower scrotal length observed in control bulls (T1) suggests comparatively reduced testicular growth potential, while the improved values in T2 and T3 indicate enhanced testicular development following MSEO supplementation.

Scrotal circumference is considered one of the most reliable predictors of sperm output, semen quality, and fertility potential in breeding bulls. Larger scrotal circumference is positively correlated with testicular weight, sperm concentration, and earlier attainment of puberty (Swanepoel & Heyns, 1990; Anaso et al., 2024b). The significantly higher scrotal circumference recorded in T3 bulls suggests improved spermatogenic capacity and reproductive efficiency, supporting the superior semen characteristics observed in MSEO-supplemented groups.

The observed increase in both scrotal length and circumference in bulls receiving MSEO, particularly at 20 mL/kg (T3), indicates that the essential oil stimulated testicular growth and functional development. This response is likely linked to the bioactive constituents of MSEO, which may enhance metabolic efficiency, endocrine regulation, and antioxidant defense mechanisms necessary for optimal testicular function.

Ruminants with reduced scrotal dimensions often exhibit lower sperm production, delayed sexual maturity, and suboptimal semen quality, whereas increases in scrotal parameters are consistently associated with improved reproductive performance and breeding soundness. Nutritional modulation remains a key strategy for improving male fertility in livestock (Smith & Akinbamijo, 2000; Anaso et al., 2024b), and the present findings demonstrate that dietary inclusion of *Piliostigma thonningii* seed essential oil up to 20 mL/kg diet can be effectively used to enhance scrotal development and reproductive potential in Bunaji bulls.

Table 5. Scrotal parameters of bulls supplemented *Piliostigma thonningii* essential oil-based diet

Parameter (%)	T1	T2	T3	SEM
Scrotal length	12.79 ^b	14.57 ^a	15.45 ^a	0.47
Scrotal circumference	27.81 ^c	30.20 ^b	31.62 ^a	0.30

Means with the different superscripts along the row are significantly ($P < 0.05$) different

T1, 0 ml *P. thonningii* essential oil; T2, 10 ml *P. thonningii* essential oil; T3, 20 ml *P. thonningii* essential oil/kg diet.

CONCLUSION

In summary, Bunaji bulls' testicular growth, sperm viability, and semen quality were all markedly enhanced by dietary supplementation of *Piliostigma thonningii* (Milne-Rech) seed essential oil (MSEO) at a rate of 20 mL/kg diet without causing any negative physiological consequences. These results show that MSEO has the ability to increase fertility, which is probably due to its diverse range of bioactive substances that have antibacterial, endocrine-modulating, and antioxidant qualities. As a result, *P. thonningii* essential oil presents a viable approach to enhancing breeding soundness and fertility performance in tropical bull management systems while also being a safe, sustainable, and environmentally friendly substitute for pharmaceutical reproductive enhancers.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the research, authorship, or publication of this manuscript.

Author Contribution

All authors contributed equally to the study design, data collection, analysis, and manuscript preparation.

Ethics committee approval

This study was approved by the Cofarms Green aid ethics committee (approval no: 19/001/05; date: 2/12/2025).

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