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# Effects of a blended supplementation of *Piliostigma thonningii* and *Daniella oliveri* essential oils on reproductive performance, semen quality, and fertility indices in Bunaji bulls.

**Emmanuel U. Anaso**

*Department of Animal Science, Faculty of Agriculture, Federal University of Agriculture Mubi, Adamawa Nigeria*

## Abstract

This study investigated the effects of a blended essential oil formulation derived from *Piliostigma thonningii* (camel's foot) and *Daniella oliveri* on the reproductive performance of Bunaji bulls. The combination was selected based on the complementary phytochemical profiles of both plants, which are rich in phenolics, flavonoids, and terpenoids with documented antioxidant, antimicrobial, and endocrine-modulating activities. These bioactive compounds are associated with improved testicular function, enhanced spermatogenesis, and better semen quality. In addition, both plants are widely distributed in tropical savanna ecosystems, making their combined use a cost-effective and sustainable phytogenic strategy for reproductive enhancement in livestock. A completely randomized design was used, involving fifteen clinically healthy Bunaji bulls (22–24 months old; 156.32 ± 0.70 kg average body weight), which were allocated into three dietary treatment groups. Over a 16-week experimental period, bulls received a basal diet supplemented with 0 ml/kg (T1, control), 10 ml/kg (T2), or 20 ml/kg (T3) of the blended essential oil mixture. The results indicated that dietary treatments had no significant effect ($P > 0.05$) on semen pH or colour, which remained consistently creamy white across all groups. However, supplementation with the blended essential oil significantly improved ($P < 0.05$) sperm concentration and ejaculate volume, with the highest values observed in T3. Likewise, sperm motility, percentage of live sperm, and live-to-dead sperm ratio were significantly enhanced ($P < 0.05$) in the supplemented groups compared with the control. In contrast, bulls in the highest supplementation group exhibited the lowest proportions of dead and morphologically abnormal sperm cells ($P < 0.05$). Although most specific sperm abnormality traits, including midpiece droplet, coiled tail, detached head, free tail, and bent tail, were not significantly influenced by dietary treatment ($P > 0.05$), scrotal length and circumference increased significantly ($P < 0.05$) with supplementation, with the greatest improvement recorded in T3. In conclusion, supplementation with the blended *Piliostigma thonningii* and *Daniella oliveri* essential oil mixture at 20 ml/kg improved testicular development and semen quality without adverse effects, demonstrating its potential as a natural and sustainable phytogenic alternative to synthetic reproductive enhancers in bull production systems.

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

## Introduction

There is a persistent shortage of animal protein across Africa, driven by multiple constraints affecting ruminant production systems in small-, medium-, and large-scale operations, particularly in West Africa (Anaso and Chibuogwu, 2026; Anaso & Olafadehan, 2025; Olafadehan et al., 2023; Anaso, 2025a). The Food and Agriculture Organization reported an average per capita animal protein intake of approximately 4.5 g/day in sub-Saharan Africa, which is far below the recommended 35 g/day (FAO, 2001; Anaso and Anaso, 2025). Current projections indicate that demand for animal protein will continue to rise due to population growth, increasing income levels, and evolving dietary preferences (FAO, 2009; Anaso & Salihu, 2025; Anaso, 2026a-d). However, livestock productivity in tropical regions remains limited by poor feed quality, heat stress, and the inherently low reproductive efficiency and growth performance of indigenous breeds (Anaso, 2025b, c). These factors collectively impair feed utilization, metabolic efficiency, and reproductive performance, especially in breeding males.

Essential oils (EOs) are volatile, aromatic plant-derived compounds obtained from seeds, leaves, bark, and roots, and are increasingly recognized for their bioactive roles in animal nutrition (Anaso et al., 2025c). In this context, mixed essential oil formulations derived from *Piliostigma thonningii* (camel's foot) and *Daniella oliveri* have gained attention due to their complementary phytochemical composition and synergistic bioactivity. *Piliostigma thonningii* is a leguminous savannah tree characterized by bilobed leaves and nutrient-rich seeds, while *Daniella oliveri* is a widely distributed savannah species known for its medicinal and phytochemical richness. Both plants contain phenolics, flavonoids, terpenoids, and other bioactive constituents with antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory properties (Anaso, 2023a,b). Their combined essential oil formulation has shown potential to enhance rumen fermentation, oxidative balance, and reproductive physiology in animal systems.

Several studies have reported the benefits of phytogenic feed additives, including essential oils, in improving 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 evidence highlights their multifunctional properties, including antimicrobial activity, antioxidant effects, and improved feed palatability, positioning them as viable natural alternatives to synthetic growth promoters (Anaso, 2023a,b; Anaso and Alagbe, 2025a–c). The global restriction on antibiotic growth promoters due to antimicrobial resistance concerns has further intensified interest in plant-based feed additives capable of sustaining productivity while ensuring food safety (Soultos et al., 2009; Koné et al., 2016; Tufarelli et al., 2017; Anaso et., 2021a,b; Anaso et al., 2024d; Anaso and Dikki, 2025; Anaso, 2026a,b). Consequently, mixed essential oil formulations such as those derived from *P. thonningii* and *D. oliveri* are increasingly being explored for their antibacterial, antioxidant, and immunomodulatory potential (Miguel, 2010; Negi, 2012; Ahmed & Abdallah, 2020; Anaso et al., 2025a,b; Anaso et al., 2026).

Although individual essential oils from *Piliostigma thonningii* and *Daniella oliveri* have been 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), limited information exists on the effects of their combined essential oil supplementation on reproductive performance in large ruminants. In particular, data on semen quality, testicular development, and fertility indices in Nigerian White Fulani (Bunaji) bulls remain scarce. Given the need to enhance male reproductive efficiency under tropical production constraints, it is important to investigate the potential synergistic effects of these mixed essential oils as natural reproductive enhancers.

Therefore, the present study was designed to:

1. Evaluate the semen characteristics of White Fulani bulls fed diets supplemented with the mixed essential oil formulation.
2. Determine the semen morphology of bulls under mixed essential oil supplementation.
3. Assess the scrotal parameters of bulls receiving diets supplemented with the mixed essential oil blend.

## Materials And Methods

### *Preparation of Mixed Essential Oil Formulation*

Seeds of *Piliostigma thonningii* (Milne-Redh.) were collected from naturally occurring stands in the Federal Capital Territory, Nigeria, within the Southern Guinea Savanna agro-ecological zone. The seeds were air-dried under shade to preserve thermolabile bioactive constituents and prevent phytochemical degradation. After drying to constant weight, the seeds were milled into a fine powder and stored at ambient temperature prior to essential oil extraction.

Essential oil extraction was carried out using steam distillation. A total of 100 g of the powdered seed material was immersed in 700 mL of distilled water and heated at 100 °C for 3 hours in a stainless-steel distillation apparatus fitted with a condenser. Volatile oil fractions were collected using a Clevenger-type apparatus following the procedures described by Anaso and Alagbe (2025a) and Anaso et al. (2025c). The yield of *P. thonningii* seed essential oil was 1.42% (v/w, dry matter basis).

Gas chromatography–mass spectrometry (GC–MS) analysis (Table 1, Anaso, 2023b) revealed a terpene-rich profile dominated by β-pinene (25.02%), β-limonene (17.85%), and α-muurolene (9.63%), alongside other monoterpenes and sesquiterpenes with known antioxidant, antimicrobial, and metabolic regulatory activities.

In addition, fresh mature leaves of *Daniella oliveri* were harvested from the same agro-ecological zone. The leaves were authenticated at the Forestry Research Institute of Nigeria, thoroughly washed, air-dried to constant weight, and milled into fine powder. A 250 g portion of the powdered leaves was macerated in 1000 mL of 80% ethanol (BDH) for 48 hours in an airtight container. The extract was filtered using Whatman No. 1 filter paper and stored at 4 °C for subsequent use (Anaso, 2025a,b).

### *Preparation of Piliostigma–Daniella Mixed Essential Oil (PDEO)*

The *P. thonningii* seed essential oil was blended with the ethanolic leaf extract of *D. oliveri* to formulate a mixed essential oil system designated as PDEO (*Piliostigma–Daniella Essential Oil*). This formulation was designed to integrate the terpene-rich profile of *P. thonningii* with the phenolic- and flavonoid-rich bioactive constituents of *D. oliveri*, thereby enhancing antioxidant potential, antimicrobial activity, and reproductive functionality. The resulting PDEO was used as a composite phytogenic additive in evaluating reproductive performance in Bunaji bulls.

### *Mixed Essential Oil Formulation*

The *P. thonningii* seed essential oil (MSEO) and the ethanolic leaf extract of *D. oliveri* were subsequently combined to form a blended phytogenic formulation. This mixed essential oil/extract system was designed to harness potential synergistic interactions between the terpene-rich seed oil of *P. thonningii* and the bioactive phenolic-rich extract of *D. oliveri*, with the aim of enhancing reproductive performance, antioxidant status, and metabolic efficiency in Bunaji bulls.

**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-Isoprophylbenzene | 3.11 | 25, 48, 51, 58, 69 |
| Dodecane | 0.19 | 25, 41, 43 |
| Tridecane | 1.20 | 60, 68, 77, 103 |
| 2,3-dimethylnephthalene | 0.71 | 25, 44, 68, 92 |
| 1,8-dimethylnephthalene | 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 |
| Compounds | Percentage area | Mass peak |
| 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)

**Table 2.** Bioactive compounds of *Daniellia oliveri* essential oil detected by the gas chromatography-mass spectrometry

| *Compound* | *Relative Abundance (%)* |
|---|---|
| α-Pinene | 18.40 |
| β-Pinene | 12.70 |
| Limonene | 9.60 |
| Sabinene | 7.30 |
| Myrcene | 6.50 |
| Terpinen-4-ol | 5.80 |
| α-Terpineol | 4.90 |
| β-Caryophyllene | 8.10 |
| Germacrene D | 6.20 |
| Caryophyllene oxide | 3.40 |

Reference; Anaso (2025)

***Experimental site***

In collaboration with the Co-Farms Green Aid Revolution Ruminant Research Unit, the experimental trial was conducted at Morugo Farms. The study site is located within Nigeria's Southern Guinea Savanna agro-ecological zone, positioned between latitudes 08°51′ and 09°37′ N and longitudes 07°20′ and 07°51′ E (Anaso et al., 2025e). The area experiences a tropical climate characterized by annual rainfall ranging from 1,145 to 1,631 mm, with distinct wet and dry seasons. Relative humidity averages approximately 60% during the rainy season and 30% during the dry season, while ambient temperatures range from 25.8 to 30.2 °C in the wet season and 36 to 42 °C in the dry season.

The vegetation is predominantly derived savanna, consisting mainly of grasses interspersed with scattered shrubs, legumes, and browsable forbs that serve as feed resources for ruminants. Common browse species include *Leucaena leucocephala*, *Piliostigma thonningii*, and *Daniella oliveri*, while dominant grass species comprise *Panicum maximum*, *Andropogon gayanus*, and *Hyparrhenia rufa*. The soils are predominantly well-drained sandy loams with moderate fertility, supporting both livestock grazing and forage production.

This ecological environment provides a representative semi-intensive ruminant production system suitable for evaluating the physiological, nutritional, and reproductive responses of animals to dietary supplementation with the mixed *Piliostigma–Daniella* essential oil formulation (PDEO).

***Experimental animals, management and diets***

In this study, fifteen clinically healthy Bunaji (White Fulani) bulls with an average body weight of 156.32 ± 0.70 kg and aged 22–24 months were used. The experiment comprised a 20-day acclimation period followed by a 120-day feeding trial, giving a total duration of 140 days. The bulls were sourced from a reputable commercial farm in North-Central Nigeria (Anaso et al., 2024b) and housed in a 20 × 30 m corral equipped with 18.6 m² individual pens.

Prior to animal arrival, all pens and surrounding facilities were thoroughly cleaned and disinfected using Morigad® (a broad-spectrum antibiotic disinfectant) and Hypo® (a sodium hypochlorite-based disinfectant containing demineralized water and caustic soda) to ensure strict biosecurity conditions.

Upon arrival, the bulls underwent a two-week quarantine period during which standard prophylactic measures were implemented. Each animal received an intramuscular injection of long-acting oxytetracycline hydrochloride (10 mL/100 kg body weight) for bacterial prophylaxis, a subcutaneous administration of Avomec® (1 mL/50 kg body weight) for ecto- and endoparasite control, and an oral dose of Vitalyte® to mitigate transport and environmental stress. All treatments were administered in accordance with manufacturer guidelines under the supervision of a qualified veterinarian.

Diets were formulated in line with the nutritional requirements for cattle as recommended by the National Research Council (NRC, 2007) (Table 2). Bulls were fed at 5% of their body weight on a dry matter basis, with weekly adjustments made based on changes in body weight (Anaso et al., 2021a,b; Olafadehan et al., 2021). Clean water was provided ad libitum, and feeding was conducted twice daily at 08:00 and 16:00 h.

The experimental treatments consisted of a basal diet alone for the control group (T1), while bulls in T2 and T3 received the basal diet supplemented with the mixed essential oil formulation (PDEO) at inclusion levels of 10 mL/kg and 20 mL/kg of feed, respectively. The PDEO was thoroughly mixed with a portion of the morning ration and administered immediately to ensure uniform intake, after which the remaining feed was offered. This procedure was adopted to minimize volatilization losses, enhance palatability, and ensure consistent delivery of the mixed essential oil across treatment groups.

**Table 3**. 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) and Anaso and Chibuogwu (2026), 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:

Yij=µ+ti+eijY_{ij} = \mu + t_i + e_{ij}Yij=µ+ti+eij

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 with the mixed Piliostigma–Daniella essential oil formulation***

Table 4 presents the semen characteristics of Bunaji bulls fed diets supplemented with the mixed *Piliostigma–Daniella* essential oil formulation (PDEO). Semen colour remained consistently creamy white across all treatment groups. This uniform appearance is indicative of normal seminal plasma integrity and adequate sperm concentration, as creamy-white semen is generally associated with good fertilizing capacity, whereas altered coloration may suggest reduced sperm density or underlying reproductive dysfunction (Anaso et al., 2025d; Ososanyo et al., 2013; Bitto et al., 1988; Oyeyemi et al., 2011). The consistent semen colour across treatments indicates that PDEO supplementation did not adversely affect seminal quality or reproductive health.

Semen pH was not significantly affected by PDEO supplementation. Maintenance of physiological semen pH is essential for optimal sperm motility, metabolic activity, and fertilization potential, while deviations may reflect infection or metabolic imbalance (Anaso et al., 2025d; Zhou et al., 2015; Anaso et al., 2024a). The lack of treatment effect on semen pH suggests that PDEO did not disrupt the acid–base equilibrium of the seminal environment, consistent with reports that phytogenic additives and essential oils may not necessarily alter seminal pH (Ahmed and Abdallah, 2020; Abdel-Wareth and Metwally, 2021; Elratel et al., 2021; Anaso et al., 2023).

Ejaculate volume increased in bulls receiving PDEO supplementation compared with the control group. This parameter, which reflects the functional activity of the accessory sex glands and contributes to sperm transport efficiency, is often influenced by nutritional status and physiological condition. The observed improvement suggests a positive stimulatory effect of the mixed essential oil on reproductive secretory activity.

Sperm concentration was significantly enhanced in PDEO-supplemented bulls, indicating improved spermatogenic activity and testicular function. Higher sperm concentration is directly associated with improved fertility potential, as it increases the probability of successful fertilization. The response observed in supplemented groups suggests that PDEO may enhance nutrient utilization and antioxidant protection within the testes, thereby supporting normal sperm production and maturation.

Similarly, sperm motility and live sperm percentage were significantly improved in bulls receiving PDEO. Progressive motility is a key determinant of sperm transport through the female reproductive tract, while sperm viability reflects membrane integrity and cellular health. The elevated values recorded in supplemented bulls indicate improved functional competence of spermatozoa and enhanced reproductive efficiency.

In contrast, the proportion of dead sperm cells and morphologically abnormal spermatozoa was higher in the control group but reduced in PDEO-supplemented bulls. The reduction in sperm abnormalities suggests improved stability of spermatogenesis and enhanced protection against oxidative stress-induced cellular damage during sperm development.

Overall, the improvements observed in semen volume, sperm concentration, motility, and viability, alongside reduced sperm abnormalities in PDEO-supplemented bulls, are consistent with previous findings on phytogenic and essential oil-based feed additives (Anaso and Chibuogwu, 2026; Anaso et al., 2024b, 2023; Abdel-Wareth and Metwally, 2021). These beneficial effects are likely attributable to the synergistic bioactive constituents of the mixed essential oil formulation, which may enhance antioxidant defense mechanisms, protect spermatozoa from oxidative damage, and support overall reproductive efficiency.

**Table 4.** Semen characteristics of Bunaji bulls fed diets supplemented with the mixed *Piliostigma–Daniella* essential oil formulation

| *Parameter* | *T1* | *T2* | *T3* | *SEM* |
|---|---|---|---|---|
| Semen colour | Creamy white | Creamy white | Creamy white | |
| pH | 6.48 | 6.50 | 6.50 | 0.04 |
| Semen volume (ml) | $1.93^{c}$ | $2.26^{b}$ | $2.37^{a}$ | 0.03 |
| Motility (%) | $79.03^{b}$ | $91.04^{a}$ | $91.37^{a}$ | 0.29 |
| Semen concentration ($x10^{6}$) | $308.58^{c}$ | $358.82^{b}$ | $366.94^{a}$ | 9.12 |
| Abnormalities (%) | $12.40^{a}$ | $10.95^{b}$ | $9.81^{c}$ | 0.43 |
| Live spermatozoa (%) | $60.21^{b}$ | $80.40^{a}$ | $83.46^{a}$ | 0.72 |
| Dead spermatozoa (%) | $39.79^{a}$ | $19.59^{b}$ | $18.54^{b}$ | 0.72 |
| Live dead ratio | $1.58^{b}$ | $3.86^{a}$ | $4.12^{a}$ | 0.39 |

Means with different superscripts within the same row differ significantly ($P < 0.05$). T1, control (0 mL/kg diet of mixed *Piliostigma–Daniella* essential oil formulation, PDEO); T2, 10 mL/kg diet of PDEO; T3, 20 mL/kg diet of PDEO.

***Sperm morphology of bulls supplemented with the mixed Piliostigma–Daniella essential oil formulation***

Table 5 presents the effects of the mixed *Piliostigma–Daniella* essential oil formulation (PDEO) on sperm morphology in Bunaji bulls. Key morphological parameters including; mid-piece droplet, coiled tail, detached head, free tail, bent tail, and the proportion of normal sperm cells did not differ significantly among treatment groups. These traits are critical indices of spermatogenic efficiency, structural integrity of spermatozoa, and overall fertilizing capacity in breeding bulls, as morphological defects are known to impair motility, disrupt energy metabolism, and reduce oocyte penetration potential.

The evaluated abnormalities, which included mid-piece droplets, coiled tails, detached heads, free tails, and bent tails, alongside the proportion of normal sperm cells, are routinely used in the diagnosis of teratozoospermia, a condition associated with reduced male fertility. The absence of significant differences among treatments indicates that PDEO supplementation did not exert adverse effects on sperm morphological integrity.

The mid-piece region, which contains the mitochondrial sheath responsible for ATP production and sustained motility, showed no treatment-related disruption. This suggests that mitochondrial structure and energy-generating capacity of sperm cells were maintained in bulls receiving PDEO.

Coiled tail abnormalities, often associated with oxidative stress, microbial contamination, or membrane instability, were also unaffected by dietary treatments. This implies that the mixed essential oil formulation did not induce physiological or oxidative stress sufficient to compromise tail structure or sperm motility.

Detached head defects, commonly linked to impaired spermiogenesis or testicular dysfunction, remained unchanged across treatments, suggesting that PDEO did not interfere with testicular architecture or germ cell development. Likewise, free tail and bent tail abnormalities, which are typically associated with epididymal stress, thermal load, or nutritional imbalance, showed no treatment-induced variation. The stability of these parameters indicates that the bulls maintained normal physiological and environmental homeostasis throughout the trial.

The proportion of morphologically normal sperm cells, an overall indicator of semen quality and reproductive potential, was highest in bulls receiving the highest level of PDEO supplementation, although differences were not statistically significant. This pattern suggests a favourable trend toward improved spermatogenic efficiency and reduced cellular abnormalities with PDEO inclusion.

The maintenance of normal sperm morphology may be attributed to the synergistic bioactive constituents of the mixed essential oil formulation, including monoterpenes and sesquiterpenes such as β-pinene, β-myrcene, α-pinene, and β-caryophyllene, as well as lipid-associated compounds. These phytochemicals are known to enhance membrane stability, reduce lipid peroxidation, and protect developing germ cells from oxidative damage.

**Table 5.** Sperm morphology of bulls supplemented with mixed *Piliostigma–Daniella* essential oil formulation

| *Parameter (%)* | *T1* | *T2* | *T3* | *SEM* |
|---|---|---|---|---|
| Middle piece defect | 0.05 | 0.02 | 0.01 | 0.01 |
| Coiled tail | 13.21 | 8.40 | 6.92 | 0.51 |
| Detached head | 6.33 | 3.56 | 3.05 | 0.60 |
| Free tail | 9.18 | 6.18 | 5.95 | 1.01 |
| Bent tail | 7.04 | 5.26 | 5.00 | 1.00 |
| Normal cells | 69.69 | 76.11 | 79.77 | 3.58 |

Means with different superscripts within the same row differ significantly ($P < 0.05$).
T1, control (0 mL/kg diet of mixed *Piliostigma–Daniella* essential oil formulation, PDEO); T2, 10 mL/kg diet of PDEO; T3, 20 mL/kg diet of PDEO.

***Scrotal parameters of bulls supplemented with the mixed Piliostigma–Daniella essential oil formulation***

Table 6 presents the scrotal characteristics of Bunaji bulls fed diets supplemented with the mixed *Piliostigma–Daniella* essential oil formulation (PDEO). Scrotal measurements were assessed as non-invasive indicators of testicular development, spermatogenic capacity, and overall breeding soundness in bulls. Scrotal length increased in bulls receiving PDEO supplementation compared with the control group, with the lowest values recorded in the control and higher values observed in the supplemented groups. Bulls receiving the higher level of PDEO showed the greatest scrotal length, although values between the supplemented groups were comparable.

Similarly, scrotal circumference followed the same pattern, with the control group recording lower values, while PDEO-supplemented bulls exhibited improved measurements. The highest scrotal circumference was observed in bulls receiving the higher level of the mixed essential oil formulation.

Scrotal length is a key reproductive trait reflecting testicular elongation and parenchymal development, which are directly associated with seminiferous tubule density and daily sperm production. The lower values observed in the control group suggest comparatively reduced testicular growth potential, whereas the improved measurements in PDEO-supplemented bulls indicate enhanced testicular development and functional maturation.

Scrotal circumference is widely recognized as one of the most reliable predictors of sperm output, semen quality, and fertility potential in breeding bulls. It is positively associated with testicular weight, sperm concentration, and earlier attainment of sexual maturity. The higher scrotal circumference recorded in PDEO-supplemented bulls suggests improved spermatogenic capacity and reproductive efficiency, consistent with the superior semen quality observed in these animals.

The increase in both scrotal length and circumference in bulls receiving PDEO indicates that the mixed essential oil formulation promoted testicular growth and functional development. This response may be attributed to the synergistic bioactive constituents of *Piliostigma thonningii* and *Daniella oliveri*, which are known to enhance antioxidant status, support endocrine balance, and improve metabolic efficiency required for optimal testicular function.

Reduced scrotal dimensions are often associated with delayed sexual maturity, lower sperm production, and diminished semen quality, whereas improved scrotal traits are consistently linked to enhanced reproductive performance. Nutritional and phytogenic modulation remains a key strategy for improving male fertility in livestock production systems. The present findings therefore suggest that dietary inclusion of the mixed *Piliostigma–Daniella* essential oil formulation can effectively enhance scrotal development and reproductive potential in Bunaji bulls.

**Table 6**. Scrotal parameters of bulls supplemented with mixed *Piliostigma–Daniella* essential oil formulation

| *Parameter (%)* | *T1* | *T2* | *T3* | *SEM* |
|---|---|---|---|---|
| Scrotal length | $12.39^{b}$ | $15.57^{a}$ | $17.45^{a}$ | 0.47 |
| Scrotal circumference | $27.11^{c}$ | $31.20^{b}$ | $34.62^{a}$ | 0.40 |

Means with different superscripts within the same row differ significantly ($P < 0.05$). T1, control (0 mL/kg diet of mixed *Piliostigma–Daniella* essential oil formulation, PDEO); T2, 10 mL/kg diet of PDEO; T3, 20 mL/kg diet of PDEO.

## Conclusion

In summary, dietary supplementation with the mixed *Piliostigma–Daniella* essential oil formulation (PDEO) significantly improved testicular development, sperm viability, and overall semen quality in Bunaji bulls, particularly at the higher inclusion level, without eliciting any adverse physiological effects.

These findings indicate that PDEO has strong potential to enhance male fertility, likely driven by the synergistic action of its diverse bioactive constituents with antioxidant, antimicrobial, and endocrine-modulating properties derived from both *Piliostigma thonningii* and *Daniella oliveri*.

Overall, the mixed essential oil formulation presents a promising, safe, and sustainable phytogenic alternative to conventional synthetic reproductive enhancers, with practical applications for improving breeding soundness and fertility performance in tropical bull production systems.

## Conflict of Interest

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

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