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Physiological Responses and Reproductive Potential of Yankassa Rams Supplemented Milne-Rech Seed Essential Oil-Based Diet

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ABSTRACT

This study evaluated the effects of *Piliostigma thonningii* seed essential oil (Milne-Rech seed essential oil, MSEO), commonly referred to as camel's foot essential oil, on thermoregulation, immune and oxidative stress responses, serum biochemical indices, and reproductive traits of growing sheep. Twenty-one healthy Yankassa ram lambs (6–7 months old; average body weight: 10.55 ± 0.60 kg) were randomly allocated to three treatment groups in a completely randomized design. All animals received the same basal diet, supplemented with MSEO at 0 ml/kg (T1 – control), 5 ml/kg (T2), or 10 ml/kg (T3) for 16 weeks. Rectal temperatures ranged from 38.93 to 39.95°C, with T1 exhibiting significantly higher values ($P < 0.05$) than T2 and T3, which remained statistically similar ($P > 0.05$). Earlobe temperature, heart rate (83.43–83.54 bpm), and respiratory rate (23.29–23.91 cycles/min) were not significantly affected ($P > 0.05$) by treatment. Serum total protein, globulin, and glucose levels were significantly higher ($P < 0.05$) in T2 and T3 compared to the control. Albumin levels increased significantly with higher MSEO inclusion ($P < 0.05$), peaking in T3. Serum cholesterol and liver enzymes (ALP, AST, ALT) were significantly lower ($P < 0.05$) in T2 and T3 than in T1. Blood urea nitrogen, creatinine, albumin:globulin ratio, and bilirubin showed no significant differences across treatments ($P > 0.05$). Supplementation with MSEO significantly elevated ($P < 0.05$) levels of immunoglobulins G, A, and M, triiodothyronine (T3), and superoxide dismutase, with highest values observed in T3. Antioxidant enzymes—catalase, glutathione peroxidase, glutathione reductase—and total antioxidant capacity were significantly enhanced in T2 and T3 ($P < 0.05$). Conversely, malondialdehyde (MDA) and cortisol levels were significantly reduced in these groups compared to the control ($P < 0.05$). All groups produced creamy semen, and pH values did not differ significantly ($P > 0.05$). However, ejaculate volume, progressive motility, sperm viability, and semen concentration increased significantly with MSEO inclusion ($P < 0.05$), following the order: T3 > T2 > T1. Live sperm counts and testosterone levels were significantly higher ($P < 0.05$) in supplemented groups. Libido, measured as reaction time to female exposure, was shortest ($P < 0.05$) in T3 and longest in T1. Semen abnormalities

were highest in the control group ($P < 0.05$). In conclusion, dietary inclusion of MSEO at 10 ml/kg diet did not compromise thermoregulation or health status. Instead, it enhanced antioxidant defense, immune function, and reproductive performance. These findings support MSEO as a safe, effective, and sustainable alternative to synthetic feed additives in sheep production systems.

Keywords: Sheep, immune and oxidative stress, semen, rectal temperature, pulse and respiratory rate, essential oil

1. INTRODUCTION

Globally, there has been a consistent and growing demand for animal-based protein, driven primarily by rapid population growth, increased household incomes, and evolving dietary preferences and material choices (FAO, 2009; Anaso et al., 2025a). In Nigeria, however, the supply of meat has been severely constrained by multiple factors—including persistent farmer-herder conflicts, the adverse impacts of the global COVID-19 pandemic, and suboptimal economic policies. These challenges have prompted small-scale ruminant producers to focus on enhancing feed efficiency, animal health, and meat yield from indigenous livestock breeds (Anaso et al., 2021a; 2021b; 2024b; 2024c).

Addressing this protein shortage necessitates strategies that improve the nutritional quality, physiological resilience, and reproductive efficiency of local breeds to boost overall productivity (Anaso et al., 2023; Anaso & Olafadehan, 2025; Anaso et al., 2025b). In recent times, the African livestock industry has been increasingly plagued by rising incidences of animal diseases, largely linked to the growing inefficacy of and resistance to conventional antibiotics (Anaso, 2023a; 2023b; Anaso & Alhassan, 2025; Anaso et al., 2025c). This concern has contributed to the widespread restriction—and in some regions, outright ban—of antibiotics in animal agriculture across developed countries. As a result, there is growing interest in exploring safe, natural alternatives such as phytogenic feed additives (Anaso & Alagbe, 2025), which not only support animal health and productivity but also help build environmentally sustainable livestock systems with minimal ecological impact (Toledo et al., 2017; Olafadehan et al., 2023a; Anaso et al., 2024b).

Small ruminant production in Africa, especially in Nigeria, remains hampered by a host of challenges that have significantly undermined the availability of meat required to meet the nutritional needs of the growing population (Olafadehan et al., 2023b; Anaso et al., 2024c). Among these challenges, heat stress—a transient yet critical climatic stressor—stands out as a major limiting factor affecting sheep performance in tropical regions, influencing both soil and animal physiology.

Anaso (2023a; 2023b) described essential oils as bioactive aromatic compounds derived from plants, which can be extracted and isolated for various uses. One such plant, *Piliostigma thonningii* (family Caesalpiniaceae), commonly found across the savannah regions of Nigeria, has been traditionally employed in the management of malaria and skin disorders. This plant's seed-derived essential oil is known for its aromatic, antioxidant, antimicrobial, and insecticidal properties (Anaso et al., 2023). While previous studies have reported positive outcomes when the essential oil was used in rabbit diets, little is known about its effects on the physiological and reproductive responses of rams.

Therefore, the present study was designed to investigate the following objectives:

1. Assess the thermoregulatory responses of rams fed diets supplemented with *Piliostigma thonningii* seed essential oil (MSEO);
2. Evaluate the serum biochemical profiles of these animals;
3. Examine the influence of MSEO on immune function and oxidative stress markers; and
4. Determine the impact of MSEO supplementation on the reproductive performance of rams.

2. MATERIALS AND METHODS

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

Seeds of *Milne-Rech* (*Piliostigma thonningii*) were sourced from the Federal Capital Territory of Nigeria, located within the southern Guinea savannah agro-ecological belt. Upon collection, the seeds were air-dried under shaded conditions to preserve their phytochemical integrity. Once fully dried, they were finely milled into powder and stored at room temperature in preparation for oil extraction.

To extract the essential oil, exactly 100 grams of the pulverized seed material were subjected to steam distillation. The powdered sample was immersed in 700 milliliters of distilled water and heated to 100 °C for a duration of three hours using a stainless-steel

distillation unit. This apparatus was equipped with a condenser system designed to vaporize the oil through steam, after which the vapor was cooled and converted into liquid form. The resulting essential oil droplets were collected using a Clevenger-type apparatus, in accordance with the extraction procedures described by Anaso (2023b) and Anaso and Alagbe (2025).

Experimental site

The experimental trial was carried out at the Co-farms Green Aid Revolution and Morugo Farms experimental station, geographically positioned between latitudes 08°51' and 09°37' North and longitudes 07°20' and 07°51' East. This location experiences an annual rainfall range between 1,145 mm and 1,631 mm. Seasonal temperature patterns show that during the wet season, ambient temperatures fluctuate between 25.8°C and 30.2°C, while in the dry season, temperatures soar to between 36°C and 42°C. Relative humidity, as reported by Anaso (2025a,b and c), averages approximately 60% during the rainy season and drops to about 30% in the dry season.

Experimental animals, management and diets

A total of twenty-one clinically healthy Yankassa ram lambs, aged between 6 and 7 months with an average initial body weight of 10.55 ± 0.60 kg, were procured from a reputable livestock market located in northern Nigeria for this experiment. Upon arrival, the animals were housed individually in standardized pens measuring 1.3 m² each, arranged within a larger corral structure measuring 7 × 9 × 5 meters. To ensure optimal biosecurity and hygiene, the housing facility was rigorously sanitized two weeks in advance using Morigad®—a broad-spectrum antibiotic disinfectant—and Hypo®, a sodium hypochlorite solution containing caustic soda and demineralized water.

During the mandatory two-week quarantine and acclimatization period, the ram lambs underwent a comprehensive prophylactic regimen. This included a subcutaneous injection of Avomec® at a dosage of 0.5 mL per 25 kg body weight to control endo- and ectoparasites, and an intramuscular administration of long-acting oxytetracycline (1 mL per 10 kg body weight) for bacterial disease prevention. To mitigate stress associated with handling and environmental transition, each animal received an oral dose of Vitalyte®. Additionally, all rams were immunized against peste des petits ruminants (PPR) via subcutaneous injection of 1 mL of a live attenuated vaccine containing 10^2.5 $TCID_{50}$ of the virus.

The feeding trial extended over a 90-day period and was conducted in accordance with the nutrient specifications for growing sheep outlined by the National Research Council (NRC, 2007). Details of the diet composition are presented in Table 1. Throughout the 12-week experimental phase (February to April), feed was offered at 5% of each animal's body weight on a dry matter basis, with weekly adjustments based on observed weight changes. To ensure accurate feed intake measurement and reduce wastage, rationing was carefully managed. Water was made available continuously, and feed was administered twice daily—once at 8:00 a.m. and again at 4:00 p.m.

The animals were randomly allotted to three dietary treatment groups. Group T1 served as the control and received only the basal diet. Groups T2 and T3 received the basal diet supplemented with *Piliostigma thonningii* seed essential oil (MSEO) at 5 mL/kg and 10 mL/kg of feed, respectively.

Table 1. Ingredient and chemical composition of the experimental diets (% DM)

Ingredient	Quantity
Maize	25.00
Corn bran	15.00
Biodegraded sugarcane scrapings	15.00
Groundnut cake	17.00
Cowpea husk	25.00
Salt	0.50
Limestone	2.00
Premix	0.50
Total	100.00

Body thermoregulation

Rectal temperature (RT), earlobe temperature (ET), respiratory rate (RR), and heart rate (HR) of each ram were monitored biweekly at 11:00 a.m. throughout the experimental period to assess thermophysiological responses. RT was measured using a digital clinical thermometer. Prior to insertion, the thermometer's sensor tip was disinfected with an antiseptic solution and lubricated with petroleum jelly (Vaseline) to minimize discomfort. The probe was gently inserted approximately 1.5 cm into the rectum of the animal. Once a stable temperature was reached, as indicated by the "C L0" signal on the digital display, and confirmed by an audible beep, the thermometer was removed and the recorded temperature was noted (Anaso and Alagbe, 2025).

Similarly, ET was measured by positioning the digital thermometer against the inner surface of the earlobe. The earlobe was gently folded over the thermometer to ensure consistent skin contact and to eliminate the influence of ambient temperature fluctuations. Once the device emitted a beep indicating temperature stabilization, the thermometer was withdrawn, and the reading was recorded (Olafadehan et al., 2023a).

For RR assessment, the number of visible abdominal contractions (inhalation and exhalation cycles) was counted over a one-minute interval using the seconds hand of an analog wristwatch. The observed frequency of abdominal movements was recorded as the respiratory rate, expressed in breaths per minute.

HR was determined using a standard stethoscope placed over the left thoracic region, specifically at the anatomical site corresponding to the heart. The characteristic "lub-dub" sound of a complete cardiac cycle was monitored and counted over one full minute using the analog watch as a timer. The number of heartbeats was documented as beats per minute.

Blood collection and analyses

On the final day of the experimental trial, blood samples were collected from all rams across the treatment groups. Sampling was conducted in the early morning hours prior to feeding or watering, utilizing the jugular vein as the collection site. Approximately 5 mL of blood per animal was drawn into sterile vacuum tubes, which were immediately placed in ice-packed containers and promptly transported to the laboratory for analysis.

To obtain plasma for biochemical evaluations, the whole blood samples were centrifuged at 3000 revolutions per minute for 15 minutes using a refrigerated centrifuge (NOP-350R, NOP Medical Instruments, Punjabi, India) maintained at 4°C. Plasma samples were processed and analyzed within four hours of collection to ensure data integrity.

Biochemical parameters such as total protein and cholesterol were quantified alongside the enzymatic activities of alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) using colorimetric techniques with Bio Maxima reagent kits (Lublin, Poland), read on a Metrolab 5.0 autoanalyzer (Oslo, Norway). Albumin levels were determined using the Bromocresol Green (BCG) method as described by Peter et al. (1982) and Toledo et al (2017), while blood glucose concentration was measured spectrophotometrically, following the protocol outlined by Allain et al. (1974). Globulin values were calculated by subtracting albumin concentration from the total protein content.

Additional blood chemistry analyses included assessments of creatinine, triglycerides, uric acid, and bilirubin levels. These were measured using commercially available diagnostic kits (Stanbio Laboratory, Boerne, TX, USA), in accordance with the manufacturer's protocols as detailed by Elghalid et al. (2020).

Immune function was assessed by quantifying immunoglobulin classes (IgA, IgG, and IgM), also using Stanbio-specific kits per Elghalid et al. (2020).

Oxidative stress markers evaluated included triiodothyronine (T3), total antioxidant capacity (TAC), and the enzymatic activities of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and cortisol. These were likewise analyzed using the aforementioned commercial kits and standardized methodologies.

Semen parameters

Semen was obtained from all rams in the various treatment groups using an automatic electro-ejaculator (Autojact, Neovet), operating at 12 volts and 5 amperes, in accordance with the procedure outlined by Zemjanis (1970). The rams were restrained in a standing position within a chute, and semen collection was induced through a series of 1 to 35 electrical stimuli, lasting between 30 seconds and 5 minutes. Ejaculate volume was immediately measured using a milliliter-calibrated collection vial. Following collection, semen

samples were maintained in a water bath at 37°C and evaluated sequentially based on the guidelines provided by the CBRA manual (1998).

The ejaculate volume was directly determined from the vial's graduated scale and reported in milliliters (mL). Semen pH was assessed by immersing a litmus strip into the ejaculate and interpreting the resulting color change (Anaso et al., 2023; Anaso et al., 2024d). Semen consistency was visually examined and categorized into the following classes: creamy marble, creamy, thick milky, milky, and watery (Anaso et al., 2023; Anaso et al., 2024b). Smears were prepared for each sample, air-dried, appropriately labeled, and reserved for morphological assessment.

Progressive sperm motility was evaluated by diluting 10 µL of semen in 1 mL of Tris buffer containing hydroxymethylaminomethane (3.0 g), sodium citrate (2.0 g), and fructose (1.0 g). A 10 µL aliquot of this dilution was placed between a pre-warmed microscope slide and cover slip (maintained at 37°C), and the motility of sperm cells was assessed under a light microscope at 100x magnification. Results were recorded as the percentage of sperm showing forward progressive movement.

Sperm concentration was determined using a hemocytometer. The counting was carried out using a Neubauer chamber composed of 25 large squares, each containing 16 smaller squares, amounting to a total of 400. The counting process followed a diagonal pattern across five large squares (80 small squares) as described by Rekwot et al. (1997). Prior to counting, semen was diluted at a ratio of 1:100 using formaldehyde. The diluted sample was applied to the chamber with an absorbable pipette, and care was taken to avoid the formation of air bubbles. Sperm density was then calculated based on the counted cells.

To assess sperm morphology, smears were stained using 7.5% Giemsa solution (Doles Laboratory), diluted in distilled water. The slides were immersed in the stain for two hours, air-dried in a vertical position, and examined under a microscope. Morphological evaluations included the classification of both normal and abnormal spermatozoa, based on the ruminant-specific guidelines provided by Barth and Oko (1989), and results were expressed as percentages.

The ratio of live to dead spermatozoa was determined through eosin-nigrosine staining. A drop of fresh semen was placed on a clean slide, followed by a drop of the stain. The contents were gently mixed by rotating the slide in a circular motion. Another slide was used to spread the mixture into a thin smear, which was allowed to air dry and labeled. Under microscopic observation, live sperm cells remained unstained (excluding the dye), while dead cells took up the stain and appeared colored, according to the method described by Hancock (1951).

Serum testosterone levels were quantified using a radioimmunoassay technique with commercial assay kits supplied by Siemens (Mexico, D.F). Sexual behavior (libido) was assessed by measuring the reaction time to an estrogen-treated female (doe). The time taken for the ram to attempt mounting (without penetration or ejaculation) was recorded using a stopwatch, following the procedure outlined by Angel-Garcia et al. (2015).

Statistical analyses

Data on body temperature regulation, serum biochemical markers, immune function, oxidative stress parameters, and semen characteristics were analyzed using analysis of variance (ANOVA) under a completely randomized design, employing SPSS version 23.0. To determine statistically significant differences among treatment means, Duncan's multiple range test was applied, with significance set at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

Body thermoregulation of rams fed Milne-Rech Seed essential oil supplemented diet

Table 2 presents data on the thermoregulatory responses of rams fed diets supplemented with *Milne-Rech* seed essential oil (MSEO). Rectal temperature ranged from 38.93°C to 39.95°C and was significantly elevated ($P < 0.05$) in the control group (T1) compared to the MSEO-supplemented groups (T2 and T3), which did not differ significantly from each other ($P > 0.05$). Earlobe temperature values spanned 38.74°C to 39.23°C, heart rate ranged between 83.43 and 83.54 beats per minute, and respiratory rate varied from 23.29 to 23.91 breaths per minute. These latter parameters remained statistically unaffected by dietary treatment ($P > 0.05$).

Physiological indicators such as rectal temperature (RT), earlobe temperature (ET), heart rate (HR), and respiratory rate (RR) are key markers of an animal's thermoregulatory and adaptive capacity under different environmental and dietary conditions. The recorded values for these parameters were all within the normal physiological range for healthy sheep, as reported by Hassan and

Hassan (2003). This suggests that the animals were in good health and did not experience clinical signs of thermal or physiological distress during the study period (Olafadehan et al., 2023a; Anaso and Alagbe, 2025).

The observed decrease in rectal temperature among rams receiving 5 mL and 10 mL MSEO suggests that the essential oil exerted antioxidative effects, likely mitigating oxidative stress by neutralizing reactive oxygen species (ROS), thus contributing to thermal stability at the cellular level. Rectal temperature serves as a reliable proxy for internal body temperature, though fluctuations can occur depending on body region and physiological status (Okoikhian et al., 2009). Body heat balance is influenced by both internal factors—such as metabolic heat production from feed digestion—and external environmental conditions. The findings here indicate that MSEO supplementation played a significant role in moderating core temperature, while keeping values within the physiological norm. In agreement with Okoikhian et al. (2009), a higher rectal temperature often signals increased heat stress, emphasizing the importance of this metric in evaluating animal well-being. The lower rectal temperatures observed in the MSEO groups support previous results by Ahmed et al. (2018), who demonstrated that dietary inclusion of feed additives such as extra virgin olive oil (EVOO), betaine (BET), lemongrass essential oil (LGEO), gallic acid (GA), vitamin C (VC), and vitamin E (VE) effectively reduced body temperatures in livestock, with EVOO showing the most prominent effect.

Respiratory rates across all treatment groups remained statistically consistent and within the expected physiological limits for healthy ruminants. According to Al-Haidary et al. (2012), RR serves as a sensitive physiological indicator of thermal discomfort or heat stress, reflecting the impact of ambient temperature on animal respiration. Similarly, Okoruwa et al. (2013) noted that elevated RR is a practical marker of thermal load in livestock. Therefore, the absence of significant changes in RR across treatments suggests that all rams, regardless of diet, were not subjected to thermal stress and maintained homeostatic respiratory responses.

The heart rate values, likewise unaffected by treatment, were consistent with reference values for healthy rams. This implies that cardiovascular function remained stable and adequate for ensuring effective blood circulation and thermoregulation throughout the experimental period. The stable HR further reinforces the conclusion that the rams were physiologically well-adapted and not experiencing environmental or metabolic stress.

Table 2. Body thermoregulation of rams fed Milne-Rech Seed essential oil supplemented diet

Parameters	**Treatments**				
	T1	T2	T3	SEM	RV
Rectal temperature (^{0}C)	39.95^a	39.14^b	38.93^b	0.55	38-40.0
Earlobe temperature (^{0}C)	39.23	38.75	38.74	0.25	38.6=39.7
Heart rate (bpm)	83.43	83.44	83.54	0.37	70-90
Respiratory rate (cpm)	23.91	23.67	23.29	0.36	20-30

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

T1, T2 and T3 connoting treatments one, two and three. T1, 0 ml Milne-Rech Seed essential oil; T2, 5 ml Milne-Rech Seed essential oil/kg diet; T3, 10 ml Milne-Rech Seed essential oil.

RV: Reference values as stated by Hassan and Hassan (2003)

Serum biochemical parameters of rams fed Milne-Rech Seed essential oil supplemented diet

Table 3 displays the effects of *Milne-Rech* seed essential oil (MSEO) supplementation on the serum biochemical parameters of rams. A distinct pattern was observed in the serum levels of total protein, globulin, and glucose, where the control group (T1) exhibited significantly lower values ($P < 0.05$) compared to both MSEO-supplemented groups (T2 and T3), which showed no significant difference between them ($P > 0.05$). Serum albumin increased progressively across the treatments, with T3 recording the highest concentration and T1 the lowest ($P < 0.05$). Conversely, cholesterol levels were significantly reduced in T2 and T3 compared to T1 ($P < 0.05$). Similarly, the hepatic enzymes—alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT)—were markedly lower in MSEO-treated groups, particularly T3, relative to the control ($P < 0.05$). However, no significant differences ($P > 0.05$) were observed among treatments for blood urea nitrogen, creatinine, albumin:globulin ratio, or bilirubin concentrations. Serum biochemical profiling provides vital insights into the internal physiological status of animals, revealing underlying metabolic or pathological conditions (Olafadehan et al., 2023a; Anaso et al., 2025a). Albumin and globulin, two key serum

proteins, play critical roles in maintaining oncotic pressure, supporting immune function, and transporting bioactive compounds (Jain, 1986). The concentrations recorded in this study remained within the established reference ranges for healthy ruminants, as reported in *Merck's Veterinary Manual* (2006), suggesting overall health and functional integrity of the animals.

Anaso et al (2025a) explains that serum albumin and globulin not only maintain vascular integrity and prevent fluid leakage but also support immune defense and facilitate the transport of hormones, vitamins, and other essential metabolites. Deviations from the normal range may signal disease conditions: hypoalbuminemia can suggest hepatic dysfunction, nephropathy, or malnutrition, whereas hyperalbuminemia may indicate dehydration. Similarly, abnormal globulin levels may reflect liver dysfunction, immune disorders, or hematologic malignancies (Anaso, 2025; Anaso et al., 2025a).

The elevated serum albumin and globulin concentrations in MSEO-treated rams (T2 and T3) align with the findings of Oloruntola et al. (2018), who reported improved serum protein profiles in animals receiving phytogenic supplements. These enhancements are likely attributable to the antioxidant-rich constituents of essential oils, which promote metabolic balance and immune resilience (Anaso et al., 2025a). Similar trends were also reported by Alzawqari et al. (2016) and Alagawany et al. (2021) with lemongrass-based phytogenics. These results suggest that MSEO supplementation enhances protein synthesis and immune function in rams.

Parameters such as the albumin:globulin ratio, urea nitrogen, creatinine, and bilirubin remained unaffected by treatment and were within physiologically acceptable ranges. Stable urea nitrogen levels across groups indicate consistent dietary protein quality and nitrogen metabolism. Uniform creatinine values suggest comparable muscle turnover and renal excretory efficiency, as creatinine originates from muscle metabolism and is a reliable indicator of kidney function (Hendrix, 2009; Olafadehan et al., 2023a; Anaso et al., 2025a). The absence of variation in bilirubin levels further supports the notion that no hemolytic or hepatic dysfunction occurred, as bilirubin is a product of heme degradation and its elevation typically indicates liver pathology or excessive red blood cell breakdown (Worman, 2009; Anaso et al., 2025a).

Serum glucose concentrations were within the normal physiological range for all groups. However, rams in the MSEO-supplemented groups (T2 and T3) exhibited higher glucose levels, suggesting enhanced carbohydrate utilization and dietary energy efficiency. This supports the conclusion that the animals did not suffer from hypoglycemia or glycosuria, a claim substantiated by the increased organic matter digestibility, which is a strong indicator of dietary energy availability (Weissman & Karrer, 2009; Anaso et al., 2025a).

Notably, dietary MSEO supplementation led to a significant reduction in serum cholesterol levels in T2 and T3, corroborating earlier findings by Elghalid et al. (2020) and Anaso et al. (2025a). These studies showed that phytogenics can decrease circulating cholesterol, triglycerides, and LDL concentrations by modulating lipid metabolism and protecting tissues from lipid peroxidation. This lipid-lowering effect is likely due to the inhibitory action of polyphenolic compounds and flavonoids on hepatic HMG-CoA reductase, a key enzyme in cholesterol biosynthesis (Lee et al., 2004). Additionally, enhanced LDL receptor activity, as reported by Elshater et al. (2009), facilitates greater clearance of LDL from circulation. Phytochemicals such as β-caryophyllene and oleic acid—abundant in essential oils—may also exert lipid-regulating and anti-inflammatory effects via interaction with CB2 immune receptors (Mandal, 2019; Anaso et al., 2025a). Thus, the reduced cholesterol values in T2 and T3 suggest that meat derived from these animals may be healthier for human consumption, with lower risk of contributing to hypercholesterolemia.

Furthermore, the reduced activity of liver enzymes (ALT, AST, and ALP) in MSEO-fed rams indicates improved hepatic function and cellular integrity. These enzymes typically elevate in response to hepatic or myocardial injury; hence, their reduction implies a protective or stabilizing effect of MSEO on liver tissues. Abdel-Wareth and Metwally (2020) similarly reported reduced liver enzyme activity following thyme essential oil supplementation in male pseudo-ruminants. In this study, the enzyme values remained within the normal reference ranges, indicating that none of the treatment groups experienced hepatic toxicity or stress. The lowest values observed in T3 highlight the potential of MSEO to enhance liver efficiency and maintain systemic health.

Table 3. Serum biochemical parameters of rams fed Milne-Rech Seed essential oil supplemented diet

Parameter	T1	T2	T3	SEM	RV
Total protein (g/L)	62.04^{b}	70.73^{a}	72.97^{a}	2.84	59-78
Albumin (g/L)	26.59^{c}	33.62^{b}	37.00^{a}	1.47	26-37
Globulin (g/L)	36.46^{b}	46.07^{a}	47.63^{a}	0.37	32-50

Albumim globulin ratio (g/L)	0.71	0.73	0.79	0.05	0.6-1.3
Glucose (mmol/L)	2.88[b]	3.79[a]	4.25[a]	0.78	2.4-4.5
Cholesterol (mmol/L)	1.75[a]	1.39[b]	1.18[b]	0.05	1.1-2.3
Urea Nitrogen (mmol/L)	4.96	5.02	5.03	0.05	3.7-9.3
Creatinine (mmol/L)	85.61	84.53	83.54	1.36	76-174
Bilirubin (mmol/L)	4.32	4.30	4.26	0.07	0.7-8.6
Aspartate transaminase (u/L)	124.02[a]	80.06[b]	65.84[c]	4.45	27-156
Alanine transaminase (u/L)	34.72[a]	21.63[b]	19.97[c]	0.64	15-44
Alkaline phosphatase (u/L)	108.07[a]	72.61[b]	59.66[c]	3.54	49-123

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

T1, T2 and T3 connoting treatments one, two and three. T1, 0 ml Milne-Rech Seed essential oil; T2, 5 ml Milne-Rech Seed essential oil/kg diet; T3, 10 ml Milne-Rech Seed essential oil.

RV: reference values as stated Beers (2006).

Immune and oxidative stress responses of rams fed Milne-Rech Seed essential oil supplemented diet

Table 4 outlines the immunological and oxidative stress responses of rams fed diets supplemented with *Milne-Rech* Seed Essential Oil (MSEO). Significant enhancements ($P < 0.05$) were observed in the concentrations of immunoglobulins G, A, and M, triiodothyronine (T3 hormone), and superoxide dismutase (SOD), which all followed a similar trend: lowest levels in the control group (T1) and highest in the group receiving the highest dose of MSEO (T3). Similarly, catalase, glutathione peroxidase (GPx), glutathione reductase (GR), and total antioxidant capacity (TAC) were markedly elevated in T2 and T3 compared to T1 ($P < 0.05$). In contrast, the concentrations of malondialdehyde (MDA) and cortisol, indicators of oxidative stress and physiological stress, respectively, were significantly higher in T1 and reduced in T2 and T3, which did not differ significantly from each other ($P > 0.05$).

Immunoglobulins, particularly IgG, IgM, and IgA, serve as key components of the adaptive immune system, functioning as the body's initial defense mechanism by binding to invading pathogens, neutralizing toxins, and inhibiting microbial adhesion to mucosal surfaces (Woof & Kerr, 2005; Anaso et al., 2025a; Anaso, 2025). The increased levels of these immunoglobulins in MSEO-supplemented groups (T2 and T3) suggest an enhanced immune response, likely facilitated by the antioxidative bioactive compounds present in the essential oil. These findings are consistent with those reported by Alagawany et al. (2021), who noted improved immunoglobulin levels following essential oil supplementation.

Triiodothyronine (T3), a thyroid hormone integral to metabolic regulation, growth, development, and thermoregulation—including temperature control, heart rate, and respiration—is also elevated in MSEO-supplemented rams, further suggesting improved systemic metabolic function (Bowen, 2010).

Malondialdehyde (MDA), a biomarker for lipid peroxidation and cellular membrane damage, was significantly reduced in T2 and T3, indicating diminished oxidative stress in these groups. The reduction in MDA likely results from the free radical scavenging capacity of the phenolic compounds present in MSEO. These phenolics—including ketone derivatives, catechins, and volatile oils—have been shown to inhibit oxidative damage by neutralizing superoxide and hydroxyl radicals (El-Gogary et al., 2018; Elkirdasy et al., 2015; Anaso et al., 2025a).

Supporting this antioxidative effect, bioactive constituents of MSEO such as oleic acid, heptadecanoic acid, hexadecane, limonene, alpha-pinene, and beta-caryophyllene (components of monoterpenes and sesquiterpenes) have demonstrated anti-inflammatory, antimicrobial, and antioxidant properties. These compounds have also been associated with a reduction in cortisol levels—a hormone secreted in response to stress and hypoglycemia—further reflecting improved physiological stability in the MSEO-fed rams (Howhn & Marieb, 2010; Anaso, 2023a, 2023b).

Superoxide dismutase (SOD), a key enzymatic antioxidant responsible for converting harmful superoxide radicals into hydrogen peroxide and molecular oxygen, was significantly elevated in T2 and T3. This increase suggests an upregulated antioxidant defense system, a result that aligns with earlier studies by Lin et al. (2003) and Anaso et al. (2025a, b), which documented enhanced antioxidant enzyme activity following dietary inclusion of herbal and essential oil additives. The proper functioning of SOD, however, is dependent on adequate micronutrient availability in the diet (Ashour et al., 2014).

Glutathione levels—measured via the activities of GPx and GR—were also higher in the treatment groups, implying improved intracellular defense against reactive oxygen species (ROS). Glutathione is essential for maintaining cellular integrity, as it prevents oxidative degradation of lipids, proteins, and nucleic acids, and plays a protective role in apolipoprotein B stabilization (Alagawany et al., 2015). The increased glutathione activities in T2 and T3 indicate that MSEO supplementation supports redox homeostasis and reinforces cellular antioxidant mechanisms.

Moreover, MSEO's bioactive phytochemicals may also function as natural acetylcholinesterase inhibitors, thereby preserving acetylcholine levels necessary for smooth muscle contractions, including those required for gastrointestinal motility. This contributes to more efficient nutrient digestion and absorption (Anaso et al., 2025a).

Enzymes such as catalase, glutathione peroxidase, and glutathione reductase—central to the neutralization of hydrogen peroxide and other ROS—were significantly enhanced in the MSEO-supplemented groups. These enzymes play vital roles in cellular protection by catalyzing the decomposition of hydrogen peroxide into water and oxygen, thus preventing oxidative injury (Chelikani et al., 2004; Deponte, 2013).

Table 4. Immune and oxidative stress responses of rams fed Milne-Rech Seed essential oil supplemented diet

Parameter	T1	T2	T3	SEM
Immunoglobulin G (mg/100ml)	3.998.22^{c}	5740.82^{b}	6357.27^{a}	103.50
Immunoglobulin A (mg/ml)	0.30^{c}	0.38^{b}	0.54^{a}	0.08
Immunoglobulin M (mg/ml)	3.72^{c}	5.09^{b}	5.63^{a}	0.59
Triiodothyroxine (nmol/L)	1.99^{c}	5.09^{b}	5.63^{a}	0.59
Melanodialdehyde (nmol/L)	0.92^{a}	0.48^{b}	0.46^{b}	0.03
Superoxide dismutase (nmol/mL)	887.55^{c}	1079.51^{b}	1163.91^{a}	12.07
Catalase (U/gHb)	1660.47^{b}	1990.82^{a}	2006.66^{a}	17.85
Glutathione peroxidase (U/gHb)	183.60^{b}	201.01^{a}	205.01^{a}	2.59
Cortisol (ng/ml)	14.71^{a}	6.56^{b}	6.08^{b}	0.30
Total antioxidant capacity (nmol)	1.08^{b}	1.62^{a}	1.70^{a}	0.03
Glutathione reductase (IU/10^{11}RBC)	1.38^{b}	2.08^{a}	2.27^{a}	0.30

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

T1, T2 and T3 connoting treatments one, two and three. T1, 0 ml Milne-Rech Seed essential oil; T2, 5 ml Milne-Rech Seed essential oil/kg diet; T3, 10 ml Milne-Rech Seed essential oil.

Semen quality of rams fed Milne-Rech Seed essential oil supplemented diet

All treatment groups exhibited semen of a creamy coloration, with no observable differences. Semen pH also remained statistically unaffected by dietary treatments ($P > 0.05$), as presented in Table 5. However, key semen quality indicators such as ejaculate volume, progressive motility, sperm viability, and sperm concentration showed significant variations across treatments ($P < 0.05$), following the order: T1 > T2 > T3. Notably, the proportion of live spermatozoa and serum testosterone levels were significantly higher in rams receiving MSEO supplementation ($P < 0.05$). Conversely, the libido score—measured by reaction time to an estrous female—was longest in T3 and shortest in T1 ($P < 0.05$), indicating heightened sexual responsiveness in MSEO-treated animals. Furthermore, semen abnormalities were significantly more prevalent in the control group (T1) compared to MSEO-supplemented groups ($P < 0.05$).

Semen quality assessment is crucial in livestock reproduction, as both sperm attributes and sexual behavior are principal determinants of male fertility. The improvements in semen traits observed in T2 and T3—including increased semen volume, concentration, viability, motility, and live sperm count—highlight the positive influence of MSEO on reproductive performance. These enhancements suggest a stimulatory effect on spermatogenesis, likely driven by improved nutritional status resulting from enhanced dietary intake and nutrient absorption, particularly protein. Though not directly assessed in this study, it is plausible that MSEO supplementation contributed to better nutrient digestibility and rumen microbial efficiency, thereby increasing microbial protein synthesis (Gado et al., 2009; Anaso et al., 2023; Anaso et al., 2024a; Anaso et al., 2024d; Anaso et al., 2025b).

Improved digestibility is known to support the metabolic activity of Sertoli cells and the composition of seminal plasma, which are essential for the development and nourishment of germ cells (Gado et al., 2015; Anaso, 2024). Thus, the elevated sperm concentration and ejaculate volume in the MSEO-supplemented groups signal enhanced fertility potential, which is advantageous during natural mating or artificial insemination (Oyeyemi & Okediran, 2007; Anaso et al., 2023). The modest improvements in sperm viability further imply that MSEO positively influenced the survivability and functional competence of spermatozoa (Sumalatha, 2010; Anaso et al., 2023; Anaso et al., 2024b).

Uniformity in semen coloration across all groups, characterized as creamy, aligns with previous findings in small ruminants (Oyeyemi et al., 2011; Ososanya et al., 2013; Anaso et al., 2024b). The pH values recorded in this study were slightly below the 6.9 benchmark reported by Osinowo (2016), though still within an acceptable physiological range for rams.

Sperm abnormality rates were notably lower in the MSEO-supplemented groups compared to the control, further reinforcing the positive effect of the essential oil on semen morphology. These findings surpass those reported for rams fed pineapple-waste diets, indicating a superior semen profile in this study (Ososanya et al., 2013). Since a high percentage of morphological abnormalities generally correlates with reduced fertility, the reduced abnormalities in T2 and T3 strongly suggest enhanced semen quality due to MSEO.

Testosterone, a steroid hormone synthesized by Leydig cells, is pivotal for initiating and maintaining spermatogenesis and the expression of secondary sexual characteristics in males (Sekoni et al., 2010). Its distribution throughout the body plays a critical role in modulating libido (Sajjad et al., 2007). The elevated testosterone levels in the MSEO-fed groups, though within the physiological range reported for small ruminants in Türkiye (Delgadillo et al., 1999; Polat et al., 2011), contributed to the observed improvements in sexual behavior and semen quality. The strong relationship between testosterone and reproductive parameters is well established, with low levels often linked to suboptimal semen production (Cornwall, 2009). Hence, the increased libido observed in T2 and T3—indicated by shorter reaction times to females—can be attributed to elevated testosterone levels, as supported by Gado et al. (2015) and Anaso et al. (2023, 2024b).

Table 5. Semen quality of rams fed Milne-Rech Seed essential oil supplemented diet

Parameters	Treatments			
	T1	T2	T3	SEM
Semen color	Creamy	Creamy	Creamy	
pH	6.60	6.53	6.51	0.04
EV (ml)	0.97^{c}	1.09^{b}	1.22^{a}	0.03
PM (%)	73.67^{c}	83.00^{b}	85.33^{a}	0.76
Sperm viability (%)	76.00^{c}	81.00^{b}	84.33^{a}	0.98
Sperm conc (x10^{6})	349.86^{c}	400.52^{b}	418.94^{a}	2.43
% live sperm	77.67^{b}	80.00^{b}	84.00^{a}	1.36
Abnormalities (%)	14.67^{a}	12.00^{b}	10.00^{c}	0.72
Testosterone	2.86^{b}	3.08^{a}	3.09^{a}	0.03
Libido (seconds)	14.63^{a}	13.10^{b}	11.83^{c}	0.29

$^{a, b, c}$: Means with the different superscripts along the row are significantly ($P < 0.05$) different

T1, T2 and T3 connoting treatments one, two and three. T1, 0 ml Milne-Rech Seed essential oil; T2, 5 ml Milne-Rech Seed essential oil/kg diet; T3, 10 ml Milne-Rech Seed essential oil.

Sperm conc, Sperm concentration; EV, Ejaculatory volume; PM, progressive motility

4. CONCLUSION

In conclusion, the dietary inclusion of (MSEO) at 10 ml/kg diet proved to be both safe and beneficial for growing Yankassa rams. The supplementation did not negatively affect vital physiological parameters such as body temperature regulation, heart rate, and respiratory rate, confirming that MSEO does not impose thermal or metabolic stress on the animals under typical environmental conditions. More importantly, MSEO significantly improved key indicators of health and productivity. The observed enhancement in

antioxidant enzyme activities—including catalase, superoxide dismutase, and glutathione-related enzymes—demonstrates MSEO's capacity to boost the animals' endogenous antioxidant defense system. This likely contributed to the reduction in oxidative stress markers such as malondialdehyde (MDA) and cortisol, which are often elevated under stress or poor nutritional conditions. Furthermore, the increase in serum immunoglobulins and thyroid hormones highlights MSEO's positive influence on immune competence and metabolic regulation. Notably, reproductive traits were also positively affected. Rams fed MSEO-supplemented diets showed improved semen quality, including higher sperm viability, motility, and testosterone levels, as well as reduced abnormalities. These reproductive benefits, combined with enhanced libido, point to MSEO's potential in supporting optimal male fertility, which is critical in breeding and genetic improvement programs.

Overall, the findings from this study underscore the potential of MSEO as a safe, natural, and sustainable alternative to synthetic feed additives and antibiotics in sheep production. Its multifunctional properties—ranging from health maintenance and immune modulation to reproductive enhancement—make it a promising phytogenic feed additive for improving animal welfare and productivity in a cost-effective and environmentally friendly manner.

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Author contributions

EUA; field work, writing, analysis, submission. OAO; Major editing. ICC; editing PAA; Minor editing/review, HZ; minor review JOJ; minor review

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Conflict of interest

The authors declare that there are no conflicts of interests.

Ethical approval

Ethical approval was granted (registration number 499001/1/01/SJ/002) by the Animal Ethics and Conduct Board of the Co-farms Green aid Revolution and conforms with international standards for conducting research on small ruminants, acknowledging the current international recommendations blood collection in livestock research. The Animal ethical guidelines are followed in the study for observation, identification & experimentation.

Informed consent

Not applicable.

Data availability

All data associated with this study are present in the paper.

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