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Evaluation of Feed Intake and Hematological Profile of West African Dwarf Does Fed with *Piliostigma thonningii* Seed Essential Oil-Based Diet

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Abstract

This study evaluated the impact of *Piliostigma thonningii* (Milne-Rech.) seed essential oil (MREO) supplementation on feed intake, hematological responses, and oxidative stress biomarkers in lactating West African Dwarf does. A total of 21 clinically healthy does with comparable initial body weights were randomly allocated to three dietary treatment groups in a completely randomized design. Animals were fed a common basal diet supplemented with 0 ml/kg (T1; control), 6 ml/kg (T2), or 12 ml/kg (T3) of MREO over a 90-day feeding period. Dry matter, ether extract, organic matter, neutral detergent fiber, cellulose, and hemicellulose intakes increased significantly with rising MREO inclusion, reaching the highest levels in T3. Likewise, crude protein, non-structural carbohydrates, and acid detergent fiber intakes were significantly elevated in MREO-supplemented groups compared to the control. Hematological indices, including red blood cells, hemoglobin, mean corpuscular volume, white blood cells, eosinophils, neutrophils, and lymphocytes, followed a dose-dependent improvement (T3>T2>T1). Packed cell volume and mean corpuscular hemoglobin were significantly higher in the MREO groups, while the neutrophil-to-lymphocyte ratio was significantly reduced in T2 and T3 compared to T1. Mean corpuscular hemoglobin concentration was also significantly elevated in T3. In conclusion, dietary inclusion of MREO at 12 ml/kg enhanced feed intake and promoted favorable hematological and immunological profiles, suggesting improved health and metabolic performance in does. These findings support the potential of MREO as a viable, natural alternative to synthetic additives in sustainable small ruminant production systems.

Introduction

Small ruminant production remains a critical component of the livestock sector in West Africa, especially in Nigeria, where it serves as a major source of income, food, and livelihood for rural households [1]. However, the sector is plagued by persistent challenges that have exacerbated the region's chronic shortage of animal protein, making it difficult to meet the dietary requirements of a rapidly expanding population [2-5]. One of the major constraints limiting productivity is the poor genetic potential and low growth rate of indigenous breeds, which are further compounded by suboptimal feeding practices and the harsh tropical environment [6, 7]. High ambient temperatures prevalent in the region often lead to heat stress, depressed appetite, and compromised metabolic functions, resulting in reduced feed efficiency and lower productivity [8-10]. In response to these challenges, the last decade has witnessed a surge in the exploration of natural bioactive materials, particularly essential oils (EOs), for use in animal nutrition. Essential oils are volatile aromatic compounds extracted from various plant parts and are renowned for their antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory properties [10-13]. When strategically incorporated into ruminant diets, these compounds can modulate rumen microbial populations, improve nutrient digestibility, and enhance feed conversion efficiency, thereby contributing to improved animal health and performance [14, 15].

One such plant of growing interest is *Piliostigma thonningii* (Milne-Rech.), a member of the Caesalpiniaceae family and a dominant species in Nigeria's savannah agro-ecological zone. This plant is characterized by its irregular, crooked growth and dark fissured bark, and its seeds yield an essential oil (MREO) rich in bioactive constituents such as β-pinene, limonene, and γ-terpineol [16, 17]. These phytochemicals have demonstrated promising antioxidant and antibacterial effects, as well as potential benefits for feed palatability and gut health [18]. Studies have shown that essential oils from such plants may offer dual benefits: enhancing productivity while mitigating enteric methane emissions, thereby aligning with sustainable, climate-smart livestock practices [19, 20]. In light of increasing global restrictions on the use of antibiotic growth promoters due to the alarming rise in antimicrobial resistance (AMR), the shift toward natural alternatives such as phytogenic feed additives has gained momentum [21, 22]. Essential oils, which are generally recognized as safe (GRAS), are now widely used across both food and feed industries for their functional properties and minimal side effects [23, 24].

While considerable research has focused on the use of crop residues, agro-industrial by-products, and herbal supplements to reduce feed costs and improve animal performance [1,2,6,13,25,26], empirical data on the direct supplementation of *Piliostigma thonningii* seed essential oil in ruminant diets remain limited. To date, there is a noticeable gap in the literature regarding the influence of MREO on feed intake, hematological indices, and general physiological responses, particularly in lactating West African Dwarf (WAD) does. Therefore, this study was designed to evaluate the nutritional and hematological impacts of MREO supplementation in WAD does, aiming to provide evidence for its efficacy as a sustainable feed additive in small ruminant production systems. Therefore, the present study was undertaken with the following specific objectives: (1) to evaluate the effects of dietary supplementation with Milne-Rech seed essential oil on feed intake parameters in lactating West African dwarf (WAD) does, and (2) to investigate the influence of Milne-Rech seed essential oil-based diets on the hematological indices and overall blood health profile of WAD does.

Materials and Methods

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

Milne-Rech seeds were sourced from the Federal Capital Territory, located within Nigeria's southern Guinea savannah ecological zone. After collection, the seeds were air-dried under shade to preserve their phytochemical integrity, then milled into a fine powder and stored at room temperature until needed for oil extraction. For the extraction, 100 g of the powdered seed material was subjected to steam distillation. This involved suspending the sample in 700 ml of distilled water and heating it at 100 °C for three hours using a stainless-steel Clevenger-type distillation unit (Model E215, LabTech, Italy) equipped with a condenser. The vaporized essential oil passed through the condenser and was converted back into liquid form. The resulting oil droplets were captured using the Clevenger-type apparatus, following the methodology described by Anaso [27, 28] and Anaso and Alagbe [29].

Experimental site

This experimental study was carried out in collaboration with the Ruminant Research Unit of Co-farms Green Aid Revolution at Morugo Farms. The research site is geographically situated between latitudes 08°51′ and 09°37′N and longitudes 07°20′ and 07°51′E. The region experiences annual rainfall ranging from 1,145 mm to 1,631 mm. Seasonal temperature variations show that during the wet season, ambient temperatures range from 25.8°C to 30.2°C, while in the dry season, they rise significantly, ranging from 36°C to 42°C. As reported by Anaso [7], relative humidity averages approximately 60% in the rainy season and drops to around 30% during the dry season.

Experimental animals, management and diets

A total of twenty-one clinically healthy West African Dwarf (WAD) does, each with a comparable initial body weight (23.73 ± 0.40 kg), were assigned randomly to three dietary treatment groups using a completely randomized design. Each treatment group consisted of seven animals. The does were housed individually in standardized pens measuring 1.9 m², located within a larger well-ventilated corral facility measuring 10 × 15 × 8 meters. Before animal arrival, the housing facility underwent thorough sanitation and disinfection using Morigad® (an antimicrobial solution) and Hypo® (a sodium hypochlorite-based disinfectant composed of caustic soda and demineralized water), ensuring strict adherence to biosecurity and hygiene protocols [3]. Upon arrival, the animals were subjected to a two-week acclimatization and quarantine period, during which prophylactic health measures were administered. These included: a subcutaneous injection of Avomec® at 0.5 ml per 25 kg body weight for internal and external parasite control; a long-acting oxytetracycline intramuscularly administered at 1 ml per 10 kg body weight to prevent bacterial infections; an oral dose of Vitalyte® to manage oxidative stress; and a subcutaneous injection of a live peste des petits ruminants (PPR) vaccine (1 ml containing 10^2.5 $TCID_{50}$ virus titer) to confer immunity against PPR. The feeding trial lasted 90 days and was conducted from February to April. Diets were formulated in line with the nutrient requirements for growing sheep as outlined by the National Research Council [30], and the specific composition is detailed in Table 1. Feed was offered at a rate of 5% of each animal's body weight on a dry matter basis, with weekly adjustments based on observed changes in body weight to maintain accuracy and consistency. Feeding was carried out twice daily at 08:00 and 16:00 hours, and clean drinking water was made available at all times. The experimental groups were as follows: treatment 1 (T1: control) received the basal diet alone; treatment 2 (T2) received the basal diet supplemented with 6 ml/kg of Milne-Rech seed essential oil (MREO); and treatment 3 (T3) received the basal diet supplemented with 12 ml/kg of MREO. Feed intake was closely monitored to ensure minimal losses and to maintain dietary precision throughout the trial.

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

Feed intake and body weight

Feed intake was calculated by subtracting the weight of the feed refused from the quantity offered on the previous day. To determine nutrient intake, the amount of dry matter consumed was multiplied by the nutrient composition of the respective diets. Body weights of individual animals were measured using a standard small ruminant scale. Baseline weights were recorded at the start of the experiment, while final weights were taken at the end of the trial, before the morning feeding.

Blood collection

On the final day of the feeding trial, blood samples were collected from the jugular vein of each ram early in the morning, before feeding and watering. Approximately 5 mL of blood was drawn into vacuum tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. The samples were immediately stored in cool flasks and transported to the Hematology Laboratory for analysis within 1 to 4 hours post-collection. Hematological parameters were analyzed using an ABACUS ROSS hematology analyzer (Model 212, India). The complete blood count included red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), packed cell volume (PCV), and leukocyte differentials. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated using standard formulas based on RBC, Hb, and PCV values. Packed Cell Volume (PCV) was measured as described by [31], by filling microhematocrit tubes with anticoagulated blood, centrifuging at 10,000–12,000 rpm for 5 minutes, and reading the packed cell percentage using a hematocrit reader. Hemoglobin (Hb) was determined using the cyanmethemoglobin method [32], where blood was mixed with Drabkin's reagent and absorbance was measured at 540 nm with a spectrophotometer. Red Blood Cells (RBC) were counted using Hayem's solution as the diluent. The mixture was loaded onto a hemocytometer and examined under a microscope [33]. White Blood Cells (WBC) were counted using an acetic acid–based diluent with gentian violet, then counted under a microscope using a hemocytometer. Differential Leukocytes were counted according to [32]. A blood smear was stained with Giemsa or Wright's stain, and 100 leukocytes were counted under a microscope to determine neutrophils, lymphocytes, eosinophils, basophils, and monocytes. The following calculations were performed to determine red cell indices, as outlined by Jain [31]:

- MCH (pg) = (Hb × 10) / RBC
- MCHC (g/dL) = (Hb × 100) / PCV
- MCV (fL) = (PCV × 10) / RBC

Chemical analyses and calculations

Following the guidelines provided by AOAC [34], the crude fiber (CF), ash, CP, and ether extract (EE) of the experimental diets were calculated and estimated. The Van Soest et al. [35] methods were used to assess lignin, neutral detergent fiber (NDF), and acid detergent fiber (ADF). Calculated as the discrepancies between NDF and ADF and ADF and lignin, respectively, the concentrations of hemicellulose and cellulose were determined (NDF – ADF=Hemicellulose; ADF – Lignin=Cellulose). NSC = 100 - (CP + EE + ash + NDF) % was the formula used to compute non-structural carbohydrate (NSC).

Data analysis

Data were analyzed using a completely randomized design (CRD) with the Statistical Package for the Social Sciences (SPSS) version 23.0. Analysis of variance (ANOVA) was employed to determine significant differences among treatment means at a 5% level of significance ($P \leq 0.05$). Where significant differences were detected, means were separated using Duncan's Multiple Range Test (DMRT) within the same software.

Results and Discussion

Dry matter and nutrient intake of West African Dwarf Does

Table 2 presents the dry matter and nutrient intake patterns of West African Dwarf (WAD) does fed diets supplemented with Milne-Rech essential oil (MREO). Results revealed a significant linear increase ($P < 0.05$) in the intake of dry matter (DM), ether extract (EE), organic matter (OM), neutral detergent fiber (NDF), cellulose, and hemicellulose across the treatment groups, with the highest values recorded in treatment 3 (T3: 12 ml MREO/kg diet) and the lowest in the control group (T1: 0 ml MREO/kg). Similarly, the intake of crude protein (CP), non-structural carbohydrates (NSC), and acid detergent fiber (ADF) was markedly higher ($P < 0.05$) in the MREO-supplemented groups (T2 and T3) compared to the control. The pronounced increase in DM intake observed in T3 suggests that supplementation with 12 ml/kg MREO did not compromise feed palatability or acceptability; rather, it potentially enhanced both. As noted by Anaso et al. [1, 22], dry matter intake is influenced by multiple factors, including nutrient composition, physical characteristics, digestibility, and flavor of the feed. DMI is a critical determinant of nutrient availability and is strongly associated with animal performance metrics such as growth, milk yield, and reproductive function [4]. Low digestibility diets, on the other hand, are known to reduce intake due to delayed passage through the digestive tract and prolonged rumen retention time [13, 36].

Milne-Rech essential oil, rich in bioactive monoterpenes like β-myrcene and limonene, possesses aromatic and flavor-enhancing properties that likely contributed to the increased feed consumption, especially in T3. These compounds may stimulate sensory receptors and improve overall feed appeal, aligning with earlier findings by Anaso et al. [13], who reported similar improvements in feed intake, serum biochemistry, and immune indices in rabbits supplemented with MREO. Corresponding evidence by [37] also confirms that dietary inclusion of garlic oil, cinnamon, and other essential oils can significantly boost DMI in ruminants. The enhanced nutrient intakes observed in T3, including higher values of CP, EE, OM, NDF, ADF, and NSC, are indicative of MREO's potential to improve not only palatability but also gastrointestinal motility and nutrient assimilation. This is consistent with reports by Omer et al. [38] who demonstrated that phytogenic additives such as fennel seeds and oregano improved

Table 2 Dry matter and nutrient intake of West African dwarf does supplemented with Milne-Rech seed essential oil-based diets.

Intake (g/day)	T1	T2	T3	SEM
Dry matter	508.02[C]	639.06[b]	679.24[a]	3.12
Crude protein	62.43[b]	67.14[a]	68.49[a]	0.65
Ether extract	27.81[c]	36.44[b]	37.81[a]	0.48
Organic matter	400.72[c]	447.78[b]	455.29[a]	2.74
Non-structural carbohydrates	101.29[b]	120.10[a]	122.63[a]	1.25
Neutral detergent fibre	140.29[c]	156.06[b]	159.96[a]	0.38
Acid detergent fibre	86.16[b]	98.77[a]	103.69[a]	2.40
Cellulose	75.94[c]	87.32[b]	90.75[a]	0.59
Hemicellulose	68.48[c]	75.94[b]	79.82[a]	0.62

Means with the different superscripts along the row are significantly (P < 0.05) different.
T1, 0% MREO; T2, 6ml/kg MREO; T3, 12ml/kg MREO. SEM: Standard error of the mean

nutrient intake and digestibility in rabbits. Anaso et al. [13] similarly emphasized that nutrient utilization is a function of both the quantity of feed consumed and its nutrient density. The inclusion of 10–12 ml/kg MREO (particularly in T3) appeared to stimulate voluntary feed intake, translating to higher fiber and carbohydrate consumption, nutrients essential for optimal rumen microbial activity and fermentation processes. Although this study did not directly assess digestibility metrics, the elevated DMI in T3 implies improved ruminal fermentation efficiency, enhanced gastrointestinal turnover, and potentially better nutrient bioavailability. These observations align with previous reports by Okunade and Olafadehan [39] and Anaso and Olafadehan [13], who suggested that increased intake accelerates gut clearance and reduces digesta retention time, thus supporting improved animal productivity. Conversely, poorly digestible rations have been shown to restrict intake due to delayed transit time [13]. In conclusion, the data support the hypothesis that MREO enhances feed palatability and nutrient intake, with the 12 ml/kg diet dosage offering optimal results. This makes MREO a promising phytogenic additive for improving the feeding behavior and performance of small ruminants in tropical systems.

Hematological indices of West African dwarf does

Table 3 summarizes the hematological profiles of West African Dwarf (WAD) does fed diets supplemented with Milne-Rech Essential Oil (MREO). Significant improvements (P < 0.05) were observed in red blood cell count (RBC), hemoglobin concentration (Hb), mean corpuscular volume (MCV), white blood cell count (WBC), eosinophil, neutrophil, and lymphocyte levels, with values increasing in the order: T1 (control) < T2 (6 mL/kg) < T3 (12 mL/kg). Additionally, packed cell volume (PCV) and mean corpuscular hemoglobin (MCH) were significantly elevated in the MREO-treated groups (P<0.05). The neutrophil-to-lymphocyte (N/L) ratio was notably higher in the control group (T1), indicating relatively lower immune efficiency compared to MREO-supplemented groups. Mean corpuscular hemoglobin concentration (MCHC) was significantly increased in T3 compared to T1, while T2 remained statistically intermediate (P>0.05). Basophil and monocyte counts did not differ significantly among the treatment groups. Hematological parameters are essential biomarkers for evaluating physiological, nutritional, and immune status in livestock [22]. In this study, no signs of morbidity, clinical illness, or mortality were recorded in any treatment group, indicating the non-toxic nature of MREO at the tested inclusion levels. This observation corroborates the findings of [40], who reported the safety of thyme extract and similar essential oils in livestock diets. Likewise, Ogbiko et al. [41] found *Piliostigma thonningii* leaf extract to be non-toxic when administered orally at doses up to 2 g/kg body weight in small ruminants.

The improved hematological indices in T2 and T3 groups, particularly RBC, Hb, PCV, and WBC, are indicative of enhanced oxygen transport, improved nutritional status, and stronger immune competence. These findings align with the results of Anaso et al. [22], who reported similar enhancements in rabbits supplemented with essential oil from *P. thonningii* seeds. Bassiony et al. [42] also observed elevated blood parameters in response to dietary supplementation with cinnamaldehyde and thymol in goats. Packed cell volume (PCV), a reliable indicator of anemia and protein adequacy, remained within the normal reference range of 27–45% for healthy goats [43]. The higher PCV observed in T2 and T3 may reflect improved dietary protein utilization and enhanced erythropoiesis. The parallel increase in Hb levels, which also fell within the accepted physiological range of 9–15 g/dL [43], underscores MREO's role in enhancing hemoglobin synthesis,

Table 3 Hematological indices of West African dwarf does supplemented with Milne-Rech seed essential oil-based diets.

Parameter	T1	T2	T3	SEM	RV
Red blood cells (x10^{12}/L)	11.98^c	13.29^b	14.95^a	0.49	9-15
Hemoglobin (g/dL)	13.14^c	14.32^b	14.88^a	0.20	9-15
Packed cell volume (%)	37.95^b	42.40^a	43.53^a	0.56	27-45
Mean corpuscular value (fl)	29.95^c	34.20^b	35.52^a	0.54	28-40
MCH (pg)	8.03^b	11.77^a	11.91^a	0.61	8-12
MCHC (g/dL)	31.27^b	33.73^{ab}	34.00^a	1.20	31-34
White blood cells (x10^9/L)	8.23^c	10.40^b	11.58^a	0.31	4-12
Eosinophils (%)	4.80^c	7.43^b	8.56^a	0.27	0-10
Basophils (%)	0.94	1.30	1.44	0.03	0-3
Monocytes (%)	2.56	3.06	3.28	0.20	0-6
Neutrophils (%)	24.96^c	32.19^b	35.88^a	0.41	10-50
Lymphocytes (x10^9/L)	49.95^c	72.22^b	74.82^a	0.60	40-75
Neutrophil/lymphocyte ratio	0.49^a	0.45^b	0.47^b	0.00	0.30-0.50

Means with the different superscripts along the row are significantly ($P < 0.05$) different.
T1, 0% MREO; T2, 6ml/kg MREO; T3, 12ml/kg MREO. SEM: Standard error of the mean, RV: Reference values, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration.

likely through improved iron metabolism and antioxidant defense mechanisms [4]. The similar trends in PCV and Hb values are expected, as both parameters are interdependent and indicative of erythrocyte function and oxygen-carrying capacity. Erythrocyte indices, MCH, MCV, and MCHC, remained within normal physiological limits [43] and are consistent with findings by Bassiony et al. [42]. The elevated MCH and MCHC values in the MREO-supplemented groups suggest improved oxygen delivery efficiency, enhanced red blood cell integrity, and better nutritional status [44]. These indices serve as diagnostic tools for identifying the type and severity of anemia and for monitoring the hematopoietic response to dietary interventions.

The WBC count and differential leukocyte profiles (lymphocytes, neutrophils, and eosinophils) were significantly higher in the MREO-fed groups, signifying a stronger immune response. Increased lymphocyte and neutrophil counts suggest enhanced immunological vigilance and pathogen resistance. These findings are consistent with earlier studies by [9,10,13,45], which linked phytogenic compounds to improved gut health, microbial balance, and immune cell activity in small ruminants. Elevated WBC values in this study also fall within the normal range of 4–12 × 10^9/L for healthy goats [43]. Lymphocytes, critical to both humoral and cellular immunity, were significantly higher in T2 and T3, indicating a potential enhancement in antibody production and immunoregulation [22, 46]. Eosinophil counts—also within the accepted physiological range—were highest in T3, which may indicate heightened cytotoxic and allergic response capability, as these cells are key effectors in parasite resistance and inflammatory regulation [47]. Monocyte and basophil levels, though not significantly affected, remained within their respective reference ranges (0–6%) and underscore a maintained baseline readiness of the innate immune system for phagocytosis and allergen response. The stability of these indices across all groups further supports the safety and immune compatibility of MREO at the inclusion levels used. The results of this study demonstrate that dietary inclusion of MREO at 6 and 12 mL/kg diet improves haematological indices, enhances immune functionality, and poses no toxicity risk to WAD does. All measured parameters were within acceptable physiological ranges, reinforcing MREO's potential as a natural, health-promoting feed additive for sustainable small ruminant production.

Conclusion

The findings from this study affirm that dietary supplementation with Milne-Rech Essential Oil (MREO) exerts a markedly positive impact on the overall health status, physiological function, and productivity of West African Dwarf (WAD) does. Inclusion of MREO in the diets significantly improved dry matter and nutrient intake, enhanced feed efficiency, and elevated key hematological and immunological parameters, indicating superior nutrient utilization and improved systemic health. Importantly, no clinical signs of toxicity, stress, or mortality were observed throughout the feeding period, underscoring the safety and physiological compatibility of MREO at the administered doses. The supplementation level of 12 mL/kg of diet emerged as the optimal inclusion rate, promoting enhanced hematological profiles, robust immune response, and overall improved performance in WAD without compromising animal welfare. These outcomes underscore the potential of MREO as a viable, eco-friendly, and sustainable alternative to conventional synthetic growth promoters and antimicrobials in small ruminant production systems. Moreover, the strategic use of MREO supports global initiatives aimed at reducing antimicrobial resistance and promoting the use of safe phytogenic feed additives. Therefore, MREO represents a promising non-conventional additive for enhancing livestock health, productivity, and sustainability in tropical smallholder farming systems.

Conflict of interest

The authors declare no conflict of interest.

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