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Body Thermoregulatory Adaptation and Blood Metabolic Profile of Rabbits Supplemented with Rolfe (*Daniellia oliveri*) Leaf Extract-Based Diet

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Abstract

This study evaluated the impact of *Daniellia oliveri* leaf extract (DOLE) on hematological parameters and thermoregulation in growing bucks. Forty-five rabbits were randomly assigned to three treatment groups (15 per group) with balanced initial body weights (average 0.27 kg). The treatments included a control diet (T1), the control diet plus 5 ml DOLE/kg feed (T2), and the control diet plus 10 ml DOLE/kg feed (T3), in a completely randomized design. Blood samples were analyzed using an Abacus Ross hematology analyzer at the end of the trial. Rectal temperature was significantly higher in T1, while T2 and T3 showed better thermoregulation. Hematological results showed improved values of hemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), and mean corpuscular hemoglobin concentration (MCHC) in the order T3 > T2 > T1. Similar trends were observed for packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), lymphocytes, monocytes, and platelets, with T1 showing the lowest values. Eosinophils and neutrophils were highest in T3, while the neutrophil/lymphocyte ratio was unaffected by treatment. Heart rate (243.50–249.60 bpm) and respiratory rate (54.25–55.26 cycles/min) did not differ significantly among groups. Overall, DOLE supplementation enhanced blood profiles and supported body temperature regulation, with 10 ml DOLE/kg diet identified as the most effective dosage for improving physiological health in rabbits.

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Introduction

Rabbits are little animals that are relatively easy and inexpensive to keep at low cost. They can be produced on a huge scale or in a backyard farm with proper management [1]. Similar to chicken, they produce white, appetizing meat that is moderately higher in protein (approximately 21%), lower in fat and cholesterol, and well-flavored [2]. One extremely temporary environmental element that affects livestock, particularly rabbit production in the tropics, is high temperatures [3]. The rabbit's ability to adapt to hot climates is limited because it relies heavily on respiratory evaporation for physiological regulation of its body temperature [2, 4]. Convection and radiation both release heat; however, the rabbit's hairy covering significantly limits these processes. According to Adeyemi [5], New Zealand White rabbits' larger ears and shorter fur serve a physiological and physical purpose in aiding their cooling process. These workers claimed that ambient (environmental) temperatures of 28.3°C and above hindered growth and development. In general, the agitative disruption of the rabbit's physiological activity and function increases with the ambient temperature [6, 7].

There is sufficient evidence to support the claim that high ambient temperatures can negatively impact rabbits' overall performance. Anaso and Alhassan [8] claim that the current antimicrobial resistance epidemic in Africa, coupled with the danger of synthetic antibiotic abuse and noncompliance with withdrawal periods, particularly in Nigeria, and the subsequent prohibition of antibiotic growth promoters by several nations, has forced the search for alternatives (plant-based antioxidants and antimicrobials) to increase animal productivity and reduce negative effects on human consumers [9]. The use of phytogenics as alternative feed additives in animal nutrition and physiology has been extensively studied as a result of this restriction [10, 11]. Using medicinal plants, which are natural, less harmful, residue-free, and have been shown to contain several bioactive compounds that give them the capacity to carry out a variety of biological functions, is one such option [12, 13].

Daniellia oliveri (Rolfe) is one of the possible plants; it is abundant in vitamins, minerals, amino acids, and other secondary metabolites. *D. oliveri*, a member of the Fabaceae family, is widely available as an uncultivated copal tree in West Africa, especially in Nigeria's savannah region [14]. The plant can also be found in the Amazon region, South America, Africa, and temperate and tropical regions of the world (Gentry, an uncultivated, evergreen tree that is widely available, especially in Nigeria's savannah zone) [14]. Additionally, the plant can be found in the temperate and tropical parts of the planet [15]. Osuntokun et al. [16] claim that *D. oliveri* leaves produce a liquid oleoresin that is made up of a variety of phytochemicals, particularly oleoresins, in substantial but variable concentrations. Oleresins have long been utilized to treat genito-urinary tract and skin conditions as well as an anti-inflammatory [15]. Additionally, the leaves are abundant in bioactive substances including flavonoids, alkaloids, tannins, and saponins, which provide them anti-inflammatory, antibacterial, antifungal, and antioxidant properties [17, 18]. Even so, a lot of research has been done on the advantages of certain plant extracts for poultry nutrition [19, 20]. Although there isn't substantial research on feeding *D. oliveri* to rabbits, doing so will also help close the gap between food safety and production. There is no information on the body thermoregulatory and hematological parameters of rabbits supplemented with plant extract. The objectives of the present research were, therefore, to: 1) evaluate the thermoregulation parameters and 2) determine the hematological profile of rabbits fed with *D. oliveri* leaf extract (DELO) supplemented diet.

Materials and Methods

Collection of *Daniellia oliveri* leaves and preparation of the extract

Several strands of *D. oliveri* trees in the grounds of the Federal University of Agriculture in Mubi, Nigeria, yielded fresh and robust leaves. The mature leaves had lighter mid-veins and undersides and were dark green and slightly glossy. A certified taxonomist from the Forestry Research Institute of Nigeria's Department of Biological Science verified the plant. In order to eliminate soil and other binding particles, the *D. oliveri* leaf was thoroughly rinsed with running tap water and then distilled water. It was then allowed to air dry until it reached a consistent weight and blended into a meal. To make the leaf extract, 250 grams of *D. oliveri* powder was combined with 1000 ml of ethanol (80% BDH) and left to soak for 48 hours in an airtight container. The resulting extract was stored in a refrigerator at 4°C for additional analysis after being filtered through regular filter paper using Whatman No. 1 filter paper.

Chemical analysis

Using conventional procedures outlined by Harborne [21] and Odebiyi and Sofowora [22], the extract was subjected to qualitative phytochemical screening to detect the presence of secondary metabolites such as saponins, flavonoids, phenolics, alkaloids, steroids, and glycosides. The tannin content was determined using the protein precipitation method involving the formation of tannin-protein complexes as described by Van-Burden and Robinson [23]. Flavonoid concentration was estimated using the acid hydrolysis and spectrophotometric method reported by Boham and Kocipai [24], which quantifies flavonoid content based on absorbance at specific wavelengths. Proximate analysis of the leaf sample was conducted following the standard procedures of the Association of Official Analytical Chemists [25], which include moisture, crude protein, crude fat, ash, fiber, and nitrogen-free extract determinations.

Experimental site

In Mubi town, in the Northern Senatorial District of Adamawa State, Nigeria, at the Federal University of Agriculture Mubi Teaching and Research Farm's Monogastric Unit, the Morugo Agricultural/Research site was the study's location. Situated at the base of the Mandara Mountains, which divide Nigeria from the Republic of Cameroon, Mubi is 1906 feet above sea level and falls between latitude 10.27 and longitude 13.28 (10.2801° N, 13.2774° E).

Experimental animals, management, and treatment

Forty-five clinically certified healthy weaned male Dutch rabbits, weighing an average of 0.27 kg and approximately five weeks of age, were utilized in the experiment. The rabbits were purchased from a trustworthy source, the National Animal Production Research Institute at Ahmadu Bello University in Zaria, Nigeria. The hutches and the surrounding area were sterilized and disinfected two weeks before the rabbits arrived using Hypo® (sodium hypochlorite, caustic soda, and demineralized water) and Morigad antiseptic. The animals were quarantined for precisely two weeks and given prophylactic medication. The preventive measures included administering an anti-stress drink (Vitalyte®), injecting a parenterally administered intramuscular injection of the broad-spectrum antibiotic oxytetracycline HCl at 1.0 mL/10 kg body weight (BW), and injecting a subcutaneous dose of an anti-parasitic drug (Avomec®) at 0.5 mg/kg of the animal BW to control endo and ecto parasites. Additionally, as advised by the manufacturer, rabbits were given a single subcutaneous treatment with coccidiostat (Sulphadimidine Sodium BP solution) at the start of the study at a dose of 1 ml/rabbit. The sporadic hutches were cleaned every day using a strong disinfectant. The rabbits were methodically separated into three groups, each consisting of exactly fifteen rabbits. Once BW was balanced, each group's rabbits were randomly assigned to one of three treatments, with their starting BWs being numerically identical. Table 1 below shows the base control diet that was developed for rabbits based on the recommendations given by the NRC [26]. For 12 weeks, water and feed were given freely, with feeding occurring twice a day at 8:00 and 16:00. In the initial treatment, a baseline control food was given to the rabbits. In the other treatments, five and ten millilitres of DOLE per kilogram of the control food were added.

Table 1 Ingredient composition (%) of the experimental diet.

Ingredient	Quantity
Maize	30.00
Cowpea husk	20.00
Soybean meal	7.00
Corn bran	20.00
Groundnut cake	19.40
Bone meal	2.00
Salt	0.30
Limestone	1.00
Premix	0.30
Total	100

Body thermoregulation

Each rabbit's heart rate, respiration rate, and rectal temperature were recorded twice a week at 11:00 a.m. A digital thermometer was used to measure the rectal temperature. After being cleaned with an antibiotic and lubricated with Vaseline (a petroleum jelly), the sensory tip was placed into each animal's rectum at a depth of 2 cm, and when the thermometer's small digital screen showed "C L0," which denotes a consistent temperature. Following the digital thermometer's beeping alarm signal, the device was taken out of the rectum, and the recorded body temperature was noted [1]. Using the second hand on an analog wristwatch, the number of abdominal movements per minute was counted for one minute, and the counts were recorded to calculate the respiratory rate. A stethoscope was positioned at the left side of the ribcage, which is where the heart is anatomically located, to measure heart rate. Using the second hand on an analog wristwatch for one minute and the rates recorded suitably as specified by

[27, 28], the "lob dob" sound, which denoted a full heartbeat, was the typical heart sound.

Blood collection and analysis

On the final day of the study, blood was drawn from each rabbit in each treatment. Blood samples were taken from the marginal veins in the ears of the rabbits in each treatment group before their morning access to food and drink. The blood samples were quickly taken to the lab for analysis after being collected into many 5 ml vacuum tubes and refrigerated with ice packs. An Abacus Ross haematology analyzer (Model 212, Indian) was used to perform a haematological examination four hours following collection. The complete count was used to calculate the hemoglobin concentration, red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), and their differentials. Mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) were calculated using the following formulas:

MCV = PCV × 10/RBC,

MCH = Hb × 10/RBC

MCHC = Hb × 100/PCV)

as described by [29].

Statistical analyses

Using SPSS (23.0), data on semen characteristics and haematological markers were analyzed for variance in a completely randomized fashion. The significance of the mean difference at the $P<0.05$ level was evaluated using the Duncan multiple range test in the same program/software.

Results and Discussion

Body thermoregulation of rabbits supplemented with Daniellia oliveri leaf extract

Table 2 shows the body thermoregulation of rabbits administered a DOLE-supplemented diet. Rectal temperature varied from 38.29 to 40.08°C and higher ($P < 0.05$) in T1 compared to T2 and T3, which were unaffected ($P > 0.05$). Heart rate varied from 243.50 to 249.60 beats per minute and respiratory rate from 54.25 to 55.26 cycles per minute. The parameters were unaffected ($P > 0.05$) by the treatments. Utilizing environmentally friendly dietary supplements to mitigate the detrimental effects of heat stress is still a critical concern, as heat stress is a significant stressor that impairs productive performance [1, 3, 30]. The physiological adaptation mechanism is described by the following parameters: rectal temperature (RT), heart rate (HR), and respiratory rate (RR). In domestic animals, RT and RR are associated with haematological parameters [27]. The experimental animals' RT, HR, and RR fell within the typical limits for rabbits in good health [28]. The fact that the experimental rabbits appeared healthy and showed no significant symptoms of disease during the trial may have been the deciding factor in this. According to Hassan and Hassan [28] and Veterinary Science from UC Davis, healthy rabbits should have RT, HR, and RR between 37.5 and 39.5 °C, 130 and 325 beats per minute (bpm), and 32 and 60 cycles per minute (cpm), respectively. Additionally, the results fell within the Merck's Veterinary Manual [31] specified ranges of 38.6 to 40.1 °C, 210 to 230 bpm, and 45 cpm for the RT, HR, and RR, respectively.

The extract's antioxidative capacity to lower reactive oxygen species and their subsequent reaction with body cells is demonstrated by the reduced rectal temperature in rabbits supplemented with 5 ml and 10 ml of DOLE. Even though there is significant variance in the core of the body at different times, rectal temperature is thought to be a good indicator of body temperature [1, 9]. El-Ratel et al. [32] reported that rabbits typically range in temperature from 38.0 °C to 40.5 °C. In general, both internal and external factors can have an impact on the body's heat balance. The physiological functions of the organism, including feed metabolism, are examples of internal circumstances. The rabbits' RT in this investigation was within the allowed range and was not adversely affected by the addition of DOLE. According to Okoikhian et al. [33], an animal's increased RT also reflects the degree of heat stress it is experiencing. Consequently, the rabbits' RT suggests that they were not under any heat stress. Overall, the RT results fell within the range and were consistent with earlier research by Anaso and Alagbe [1], who examined the effects of the essential oil extracted from Schum (*Piliostigma thonningii*) seeds on the bucks' body thermoregulation and serum mineral markers. Additionally, the outcome was in line with the findings of another researcher [34], who found that adding extra virgin olive oil (EVOO), betaine (BET), lemongrass essential oil (LGEO), gallic acid (GA), vitamin C (VC), and vitamin E (VE) to the diet decreased rabbit temperatures in several ways, EVOO in particular.

The experimental animals' respiratory rates were within normal norms for healthy rabbits and did not significantly differ between treatments. According to

Table 2 Body thermoregulation of the rabbits fed with *Daniellia oliveri* leaf extract and *Piliostigma thonningii* essential oil-supplemented diets.

Parameters	Treatments				
	T1	T2	T3	SEM	RV
Rectal temperature (^{0}C)	40.08^a	38.39^b	38.29^b	0.36	37.5-40.0
Heart rate (bpm)	243.50	249.44	249.60	12.40	130-325
Respiratory rate (cpm)	55.26	54.25	55.07	1.05	30-60

^{abc} along the row in superscripts show significant ($P < 0.05$) difference; T1: 0 ml DOLE; T2: 5 ml DOLE; T3: 10 ml DOLE; SEM: Standard error of mean; RV: Reference values as stated by Hassan and Hassan (2003)

Al-Haidary et al. [35], RR is a measure of heat stress and can be used to calculate the negative impacts of ambient temperature. According to Anaso and Alagbe [1] and Okoruwa et al. [36], RR is a useful and accurate indication of heat load, and a value or range over the normal range indicates heat stress. Consequently, the rabbits' normal respiratory rates, regardless of their diets, suggest that they were not under heat stress and appeared to be in good health. The experimental animals' heart rates, which fell within the range recommended for healthy rabbits, indicate that blood was circulated to peripheral tissues effectively throughout the trial.

Hematological parameters of the rabbits administered with *Daniellia oliveri* leaf extract supplemented diet

Table 3 shows the hematological parameters of the rabbits of rabbits administered a *D. oliveri* leaf extract supplemented diet. Hb, RBC, WBC, and MCHC varied among treatment groups in the order: T3 > T2 > T1 (P<0.05). PCV, MCV, MCH, lymphocytes, monocytes, and platelets for T1, T2, and T3, respectively, varied with T1 having lower values (P<0.05) than T2 and T3, which had similar values (P>0.05). Eosinophils were higher (P<0.05) in T3 than in T1 and T2, which were similar (P>0.05). Neutrophil was higher (P<0.05) in T3 than in T1, but was similar (P>0.05) between T1 and T2, and T2 and T3, while Neutrophil/lymphocyte ratio showed no significant (P>0.05) difference among treatment groups. In terms of the quality of the food an animal consumes and the nutrients provided to meet its physiological needs, hematological parameters serve as an indicator and a reflection of the impacts of diet on the animal [37, 38]. It is significant to note that the experimental animals in the study did not exhibit any overt clinical symptoms of illness, especially those who were fed a diet supplemented with *D. oliveri* leaf extract. The fact that the animals showed no symptoms of illness, morbidity, or death indicates that the dosage of *D. oliveri* leaf extract utilized in this investigation was safe. Additionally, Mahmoud et al. [40] demonstrated and concluded that dietary essential oils, including thyme extract, were not harmful to test animals. According to Ogbiko et al. [40], leaf extract from *Piliostigma thonningii* is safe to take orally up to a dosage of 2 g/kg body weight. In general, treatment groups supplemented with *D. oliveri* leaf extract had greater levels of PCV, HB, RBC, MCHC, WBC, neutrophils, and eosinophils. Increases in WBC, RBC, Hb, and lymphocytes were comparable to the findings of other researchers [12, 41], who found that thymol and cinnamon aldehyde greatly enhanced these metrics. PCV is one important measure of anaemia. For clinically healthy rabbits, the PCV values were within the specified range of 33%-50% [31]. Given that PCV helps determine the protein status of a fed diet, the higher PCV values for the T2 and T3 show that DELO supplementation at 5 and 10 ml/kg food allowed for the provision of high-quality protein to the rabbits [42]. Perhaps as a result of increased protein intake and digestion, DOLE improved the PCV, indicating better protein use. The absence of harmful substances such as haemagglutinin, which negatively impacts blood formation, is suggested by the normal PCV values for all treatments that fell within the authorized range [12]. The higher value of T3 and T2 than T1 implies that DELO enhanced the rabbit's PCV compared to T1. This further justifies the non-toxicity of the high DELO.

PCV and hemoglobin concentration are also markers of adaptability to unfavorable circumstances, such as oxidative stress. Low hemoglobin levels indicate that the body's peripheral tissues are not receiving enough oxygen from red blood cells. Despite being within the recommended range of 13–18 g/dL for healthy rabbits, the noticeably higher levels of Hb in T2 and T3 are indicative of DELO's antioxidative properties, which ensure less oxidative stress. The outcome also shows that the DELO-supplemented meals improved iron utilization for hemoglobin production. Nevertheless, T3 improved iron utilization more than T2, suggesting that DELO at 10 ml/kg diet was preferable. Both test results

Table 3 Hematological parameters of rabbits with *Daniellia oliveri* leaf extract-supplemented diet.

Parameter	T1	T2	T3	SEM	RV
Packed cell volume (%)	38.27^{b}	47.41^{a}	49.57^{a}	1.24	33-50
Hemoglobin concentration (g/dl)	13.29^{c}	15.48^{b}	17.84^{a}	0.35	13-18
Red blood cell count (10^6/l)	11.81^{c}	14.95^{b}	18.00^{a}	0.30	11-18
White blood cell count (10^9/l)	7.40^{c}	9.37^{b}	10.48^{a}	0.20	5-12.5
Mean corpuscular volume (fl)	58.33^{b}	64.45^{a}	65.15^{a}	0.76	58-67
Mean corpuscular hemoglobin (pg)	17.44^{b}	22.41^{a}	23.48^{a}	0.46	17-24
MCHC (%)	28.71^{c}	33.30^{b}	35.97^{a}	0.66	29-37
Lymphocyte (%)	50.83^{b}	73.39^{a}	76.35^{a}	2.00	30-85
Monocyte (%)	1.44^{b}	2.62^{a}	3.32^{a}	0.30	1-4
Neutrophil (%)	22.45^{b}	29.16^{ab}	32.78^{a}	7.04	20-75
Eosinophil (%)	1.37^{b}	1.68^{b}	2.52^{a}	0.14	1-4
Platelet (10^3/ul)	373.39^{b}	553.91^{a}	582.37^{a}	20.3	250-650
Neutrophil: lymphocyte ratio	0.55	0.50	0.61	0.009	0.34-2.64

^{abc} along the row in superscripts show significant ($P < 0.05$) difference; T1: 0 ml DOLE; T2: 5 ml DOLE; T3: 10 ml DOLE; SEM: Standard error of mean; RV: Reference values as stated by Hassan and Hassan (2003)

showed similar trends, indicating a clear link between PCV and Hb [43]. The bioactive compounds in DOLE may be responsible for the improved performance of rabbits given DOLE, which is comparable to that of other essential oils and phytogenics. For example, tannins have a variety of biological activities, such as antiviral and antibacterial qualities that are primarily attributed to their antioxidant and antiradical activity. When combined with other secondary metabolites, such as flavonoids, alkaloids, saponins, and phenols, they work in concert to lessen the burden of disease on the flock, which increases the likelihood that the flock will perform as intended [14]. The MCHC, MCH, and MCV values were within the physiological ranges listed [31] for healthy growing rabbits and are similar to those reported by Bassiony et al. [41]. The absence of anemia, especially a hypochromic microcytic type, is further confirmed by the normal readings in the three therapies [12, 44]. The MCV readings falling within the designated reference range suggested that the animals were not at risk for anemia and hemoconcentration [45]. The higher MCH values in the animals fed DELO-supplemented diet demonstrate enhanced oxygen carrying capacity of the RBC because MCH is an indication of the oxygen carrying capacity of the RBC [12, 45]. When assessing the physiological impact of DELO on the health and welfare of the experimental animals, changes in the total RBC count and its indicators (MCV and MCHC) are useful.

Better activation of the body's defense and immunity against infections or hazardous substances is indicated by the increased WBC, lymphocytes, and neutrophil levels in treatments (5- and 10-ml PEO) compared to the control. The rabbits' improved gut-associated lymphoid tissue development during the feeding period and lack of severe disease may have resulted from a shift in the microbial ecology that favors beneficial microbial species in the gut and a decrease in pathogenic bacteria brought on by bioactive components [2, 11, 37, 46]. The higher number of advantageous Lactobacilli in the caecum may be the cause of this. The values reported by [41] are consistent with WBC counts. According to Merck's Veterinary Manual [31], the WBC counts for healthy rabbits similarly fell within the range of 5–12.5 × 10^3/µl. The elevated platelet and lymphocyte count in T2 and T3 suggests that the rabbits were better equipped to repel invasive pathogens. Merck's Veterinary Manual [31] states that lymphocytes are a type of white blood cell that directly combats pathogens, viruses, and poisons. They also control other immune system components and generate antibodies that either neutralize invaders or mark them for elimination by other immune system agents. Mahgoub et al. [47] state that both humoral and cell-mediated immune responses depend on lymphocytes. According to Jelalu [49] and Anaso and Olafadehan [48], differential leukocytes are in charge of an organism's defense, and low lymphocyte counts suggest a weak immune system, whilst high lymphocyte counts signify an ongoing illness.

For clinically healthy rabbits, the eosinophil concentration fell within the range specified in [31]. Rabbits treated with 10 ml of DELO had a greater eosinophil count, which may indicate more activated immunological and cytotoxic processes [12]. Monocytes are essential to the animal immune system because they are the precursors of macrophages [12]. The results were consistent with the 1–4% range for rabbits given in [31]. A normal monocyte result shows that the biological system maintains homeostasis and plays a crucial role in tissue

development. The greater monocyte counts in T2 and T3, however, suggest that they were equally capable of recognizing dangerous germs, viruses, and poisons, combating alien materials, and supplying everything required for the rabbit's well-being, survival, and improved performance.

Conclusions

DELO supplementation improved the rabbits' hematological blood profile while lowering rectal temperature. Since all the investigated hematological parameters fell within the normal ranges for healthy rabbits and they showed no clinical signs or symptoms of illness, it can be said that DELO can be added to grower rabbits' diets as a supplement to improve their hematological indices without posing a health risk or resulting in intoxication.

Ethical Approval

The Guinea Savannah agro-ecological zone is where the leaves of *Daniellia oliveri* were obtained. The study for experimentation complies with the ethical standards for plant materials. The study for analysis complies with the ethical criteria for animals.

Conflicts of Interest

The author declares no conflicts of interest.

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