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Haematological Indices and Fertility Potential of Rabbits Receiving Camels Foot (*Piliostigma thonningii*) Essential Oil Supplemented Diet

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ABSTRACT

The current study aimed to evaluate *Piliostigma thonningii* seeds-derived essential oil (PTO) effect on hematological and reproductive parameters in rabbits. Three groups consisting of 15 animals each were randomly assigned and with an average initial body weight (BW) of 262.89 ± 22.36 g in a fully randomised experimental design. Group 1 received the control diet, while for groups 2 and 3 the basal control diet was supplemented with 2 mL PTO/kg diet and 4 mL PTO/kg diet, respectively. At the end of the experiment, blood samples were collected and the blood was analysed using the ABACUS ROSS haematology analyser. The results indicated significant differences in rabbits receiving PTO supplemented feed, namely; Packed cell volume, red blood cells, white blood cells, mean corpuscular haemoglobin concentration, neutrophil increased ($P<0.05$) with increasing level of PEO supplementation. Mean corpuscular volume and haemoglobin, lymphocyte, monocyte and platelet were higher ($P<0.05$) in T2 and T3 than in T1. Neutrophil/lymphocyte ratio was higher ($P<0.05$) in T3 than T1 with T2 being intermediate ($P>0.05$) between T1 and T3. Semen volume, concentration and motility were higher ($P<0.05$) in T2 and T3 than in T1. While semen abnormalities and bucks' reaction time to does (libido) were greater ($P<0.05$) in T1 than in T2 and T3, Live dead ratio was higher ($P<0.05$) in T3 relative to T1 while T2 was intermediate between T1 and T3 ($P>0.05$). semen color and pH were not affected ($P>0.05$) by treatments. It was therefore concluded that *P. thonningii* essential oil supplementation enhanced both haematological and fertility potential of the experimental rabbits.

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Introduction

Rabbit farming in Africa, mainly Nigeria have been tremendously challenging due to several multitudinous problems, which have resulted to a gross insufficiency of animal products to meet up the increasing population challenge in the country (Anaso, 2023a).

In West Africa, precisely in Nigeria, the acute scarcity of meat due to farmer-herder crises, the past global pandemic effects and poor economic policies caused by corruption have adherently compelled livestock farmers to improve feed resources utilisation, health status and meat production from their cattle, goat and sheep (Anaso et al., 2021), however there still exist a shortage, which can be bridged by the farming of highly prolific monogastrates with short production cycles like rabbits.

Rabbits generally possess high fertility and rapid growth rates, thereby characterizing them an excellent source of meat and protein of animal origin (Anaso et al., 2024). Rabbit meat is characterized as high quality due to adequate animal protein and polyunsaturated fatty acids, and low calories (El-gogary et al., 2018).

Anaso (2023a and 2023b), described have essential oils to have potentially important aromatic characteristic of plant when harvested, extracted and isolated. *Piliostigma thonningii* Schum., or camel's foot Milne-Rech (Caesalpiniaceae) is a fairly little tree that grows crookedly and has a characteristic dark brown to black fissured bark.

It is commonly found in savannah areas. *Piliostigma thonningii* is widely accessible and has been used for many years by African and Nigerian traditional healers as a treatment for dermatosis and later malaria. Recent research has revealed that it also possesses normal flavouring, antioxidant, insecticidal, and antibacterial qualities Anaso (2023a). Ogbiko et al. (2021) reported no mortality at 2 g/kg p.o after 24 hours and showed no sign of delayed toxicity or mortality after 14 days of observation. Ajiboye et al. (2017) concluded from their findings that the seed oils of *P. thonningii* may be of significant importance medicinally and industrially, and could, therefore, also be used as a substitute for other oils or blended with other vegetable oils. Jimoh and Oladiji (2005) reported Seeds of *P. thonningii* were also found to be rich in protein (crude protein), carbohydrate, and several essential mineral elements. Mineral analysis of *P. thonningii* showed the seed to be an exceptionally excellent source of antioxidant micronutrients such as iron, calcium, selenium, zinc and manganese. The phytochemical investigation (screening) of the seed showed the presence of saponins, flavonoids, phenolics, glycosides, anthraquinones as well as cardiac glycosides while tannins, steroids, phylobatannins and triterpenes were absent (Anaso, 2023a). Elghalid et al. (2020) reported that administration of lower (LA) and higher doses (HA) of a newly developed mixture of herbal plants and spices enriched with special extracts and essential oils decreased blood cholesterol, triglycerides and low-density lipoproteins; however, the LA treatment increased high-density lipoproteins and total antioxidant capacity but decreased malondialdehyde relative to the control treatment. The rabbits were fed a basal diet without additives (Control rabbits) or supplemented with 0.5 mL (LA) or 1mL (HA) of the additives mixture per litre of drinking water.

Anaso (2023b) presented in Table 1 showing the bioactive ingredients.

Anaso (2023b) explains that Beta pinene was the most abundant and are well-known representatives of the monoterpenes group, and are found in many plants' essential oils. A wide range of pharmacological activities of β-pinene have been reported to include antibiotic resistance modulation, anticoagulant, antitumor, antimicrobial, antimalarial, antioxidant, anti-inflammatory, anti-*Leishmania* and analgesic effects (Anaso 2023b; Bahare et al., 2019). Pandi et al. (2021) reported limonene as monoterpenes which possesses antioxidant, antidiabetic, anticancer, anti-inflammatory, cardioprotective, gastroprotective, hepatoprotective, immune modulatory, anti-fibrotic and anti-genotoxic properties. Gamma terpineol, hexenyl acetate, fenchol and camhene have functions similar to the flavouring and aroma characteristic of the β-pinene (Anaso, 2023b). Similarly, n-nonanol has relative flavouring characteristic but can be harmful to internal body organs when large doses are administered and ingested.

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

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

Trans-pinocarveol and Cis- pinocarveol are harmful when ingested in large quantity (Anaso, 2023b). Similarly, Alpha cubebene, alpha yiangene, alpha selinene, chrysanthenine, alpha allytoluene and alpha muurolene are generally susquiterpene hydrocarbons exhibiting antifungal, antioxidant and antiinsecticidal properties and may be harmful in large quantity (SCLabs, 2016).

The current epidermic of antimicrobial resistance challenge and subsequent ban on antibiotic growth promoters by several countries have compelled the search for alternatives of improving animal productivity and minimizing adverse effects on human consumers. Due to this ban, a great deal of study has been done to explore the use of phytogenics as alternate feed additives in animal nutrition Anaso (2023a).

There is no information on the hematological and fertility potential of rabbits supplemented PEO. The objectives of the present research were, therefore, to: 1) Evaluate the haematological parameters and 2) Determine the fertility potentials of rabbits fed PEO supplemented diet

Materials And Methods

Ethical Approval

Ethical approval was granted by the Animal Ethics and Conduct Board of the Department of Animal Science University of Abuja, Nigeria following the presentation of the research proposal on the 17th Day of November 2022, with approval registration number 19/501/ANSJ/002. The approval granted before the thesis proceeded and was strictly adhered to and scrutinized to conform with international standards for conducting research on rabbits, acknowledging the current international recommendations on the rational use of antibiotics.

Assemblage of P. thonningii Seeds and Subsequent Essential Oil Extraction

Piliostigma thonningii seeds were carefully obtained from the University of Abuja's surroundings and they were later verified by a licensed taxonomist at the Department of Biological Science at the Forestry Research Institute of Nigeria.

The Clevenger apparatus was used to extract the essential oil in accordance with Anaso's (2023b) and Mohamed et al. (2006) technique. The *P. thonningii* seeds were carefully ground, dried in enough shade, and kept at room temperature until they were needed again (extraction). Laboratory procedure involved placing the ground seed sample in a steel apparatus and allowing it to soften and produce the essential oil forms by intermittently heating up after connecting the condenser to a water inlet and outlet, precisely 100 g of dried ground sample was suspended in precisely 700 ml of distilled water using distillation process at about 100°C for three hours. The resultant essential oil droplets were congregated in a cooling system after prior mixing with steam and passage through the carrier.

Experimental Site

The study was conducted at the University of Abuja Teaching and Research Farm's Monogastric Unit, which is situated in Giri inside the Gwagwalada Area Council in the Federal Capital Territory of Abuja, Nigeria. Rabbits were housed in individual open sided metabolic hutches which can separate faeces from urine.

Experimental Animals, Management and Treatment

For the experiment, 45 clinically certified healthy weaned male Dutch rabbits weighing an average of 262.89 ± 22.36 g were used, they were about five weeks old. A reliable source (The National Animal Production Research Institute at Ahmadu Bello University in Zaria, Nigeria), is where the rabbits were bought. Two weeks prior to the arrival of the rabbits, the hutches and Hypo® (sodium hypochlorite, caustic soda, and de-mineralized water) and antiseptic (Morigad) were used to sterilize and disinfect the area around them. The animals received preventative medication and were placed in quarantine for exactly two weeks. The prophylactic measures comprised injecting a subcutaneous dose of an anti-parasitic medication (Avomec®) at 0.5 mg/kg of the animal body weight (BW) to control endo and ecto parasites, administering an anti-stress drink (Vitalyte®), and administering a parenterally administered intramuscular injection of the broad-spectrum antibiotic oxytetracycline HCl at 1.0 mL/10 kg BW. Additionally, at the beginning of the trial, rabbits received a single subcutaneous treatment with coccidiostat (Sulphadimidine Sodium BP solution) at a dose of 1 mL/rabbit per manufacturer's advice.

Daily sanitation of the intermittent hutches was carried out with a strong disinfectant. With precisely fifteen rabbits in each group, the rabbits were systematically divided into three groups. After balancing for body weight (BW), the rabbits in each group were assigned to one of three treatments in a completely randomised manner, with their beginning BWs being numerically equal.

Based on the guidelines provided by the NRC (1984), a base control diet was formulated for growing rabbits. For a duration of 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, two and four milliliters of PEO per kilogram of the control food were added.

Blood Collection and Analyses

Blood samples were taken from each treatment's individual rabbits on the last day of the trial. Before the rabbits in each treatment group had access to food and water in the morning, blood samples were drawn from the marginal veins in their ears. After being collected into multiple 5 ml vacuum tubes and chilled using ice packs, the blood samples were promptly transported to the laboratory for examination. Four hours after collection, a haematological analysis was conducted utilizing an ABACUS ROSS haematology analyzer (Model 212, Indian). The packed cell volume (PCV), hemoglobin concentration, red blood cell (RBC), white blood cell (WBC), and their differentials were all determined using the entire count. The following values were obtained: mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) using the following formulas;

$$MCV = PCV \times 10/RBC,$$

$$MCH = Hb \times 10/RBC, \text{ and}$$

$$MCHC = Hb \times 100/PCV) \text{ as described by Jain (1986).}$$

Semen Collection and Characteristics, and Libido Test

An improvised artificial vagina (AV) filled with a warm water at about 45°C was used in collecting semen from bucks, as the doe was fitted with the AV and presented to bucks. Samples that were collected were examined immediately for semen volume via of millimetre direct reading, expressed in millilitre (mL). The samples were then kept in water bath at 37°C, and evaluations were made in sequence according to Colegio Brasileiro de Reproduçâo (CBRA, 1998) animal manual. The appearance of the semen (color) was determined by visualization of consistence of ejaculates and classified as: creamy marble, creamy, thick milky, milky and watered. Smear of each sample of semen was prepared, allowed to air dry, labeled, and stored for additional analysis.

As soon as the sample was taken, live and dead sperm were identified by making a smear of the corresponding sample from each replicate using the eosin-nigrosin stain. A drop of individual semen sample was placed on a clean glass slide using automatic pipette. A drop of the eosin-nigrosin solution was then placed alongside the drop of semen on the slide. A gentle circular mixing (turning) of the slide was done to allow a uniform mixture of the two samples. One-quarter of the part of another clean slide was held in place, on top of the first sample on the slide at a 45-degree angle, to make contact with the semen sample slide and carefully drawn apart to prepare and produce a thin smear. This was then left and allowed to dry and thereafter respectively labelled. This process was carried out on separate samples. After that, the slides were finally mounted unto the microscope to count the live and dead sperm cells. The principle of the stain and procedure was that the dead sperm cells accepted the stain and appeared stained while the live sperm cells rejected the stain and remain unstained. The procedure above was developed by Hancock (1951) and modified by Anaso et al. (2024).

Libido/male reaction time to female was determined by exposing bucks to estrogenized doe in enclosed area. A stop watch was adopted to take the time for mounting without intromission and ejaculation, and duly recorded as described by Angel-Gracia et al. (2015).

Statistical Analyses

Data on haematological parameters and semen characteristics were subjected to analysis of variance in a completely randomized design using the SPSS (23.0). The same software's Duncan multiple range test was performed to assess the significance of the mean difference at the $P \leq 0.05$ level.

The statistical model is shown below

$Yij = \mu + ti + eij$

Where:

Yij = the overall response to the specific parameter under investigation,

μ = the general mean peculiar to each observation,

tij = the fixed effect of the dietary treatments (i = 3) on the observed parameters and

eij = the random error term for each estimate

Results

Haematological Parameters of Rabbits Fed Piliostigma Thonningii Essential Oil Supplemented Diet

Table 2 shows the haematological parameters of the rabbits fed PEO supplemented diet. PCV varied from 38.00 to 49.95%, Hb from 13.13 to 18.50 g/dL, RBC from 11.87 to 17.52 $\times 10^6$/L, WBC from 5.60 to 8.77 $\times 10^9$/L, MCHC from 29.80 to 35.91% and neutrophil from 22.45 to 32.78% among treatment groups in the order: T3 > T2 > T1 ($P<0.05$). MCV, MCH, lymphocytes, monocytes and platelets were 58.02, 64.02 and 65.12 fl; 17.46, 22.45 and 23.67 pg, 49.80, 70.40 and 73.07%; 1.28, 2.40 and 2.72% and 340.16, 530.74 and 580.11 $\times 10^3$/uL for T1, T2 and T3 respectively with T1 having lower values ($P<0.05$) than T2 and T3, which had similar values ($P>0.05$). Eosinophil varied from 1.36 to 2.22% and was higher ($P<0.05$) in T3 than in T1 and T2, which were similar ($P>0.05$). Neutrophil/lymphocyte ratio (0.41 - 0.45) was higher ($P<0.05$) in T3 than and T1 but was similar ($P>0.05$) between T1 and T2, and T2 and T3.

Semen Characteristics of Rabbits Fed Piliostigma thonningii Essential Oil Supplemented Diet

Table 3 shows the semen parameters of rabbits fed PEO supplemented diet. The semen colour was similar (creamy) across treatments (T1, T2 and T3). Semen pH was 6.00 for each of the treatments and was not affected by treatments. Ejaculatory volume varied from 0.53 to 1.08 ml, sperm motility from 68.33 to 86.00 % and semen concentration varied from 222.20 to 321.80 ($\times 10^6$), presenting lower ($P<0.05$) values in T1 compared to T2 and T3 which were similar ($P>0.05$).

Table 2. Haematological parameters of rabbits fed Piliostigma thonningii essential oil supplemented diet

Parameter	T1	T2	T3	SEM	RV
Packed cell volume (%)	38.00^c	45.25^b	49.95^a	0.96	33-50
Haemoglobin concentration (g/dL)	13.13^c	16.57^b	18.50^a	0.48	13-18
Red blood cell count (10^6/L)	11.87^c	14.72^b	17.52^a	0.53	11-18
White blood cell count (10^9/L)	5.60^c	7.55^b	8.77^a	0.23	5-12.5
Mean corpuscular volume (fl)	58.02^b	64.02^a	65.12^a	2.03	58-67
Mean corpuscular haemoglobin (pg)	17.47^b	22.45^a	23.67^a	0.58	17-24
MCHC (%)	29.80^c	33.57^b	35.91^a	0.63	29-37
Lymphocyte (%)	49.80^b	70.40^a	73.07^a	1.34	30-85
Monocyte (%)	1.28^b	2.40^a	2.72^a	0.27	1-4
Neutrophil (%)	22.45^c	29.16^b	32.78^a	0.90	20-75
Eosinophil (%)	1.36^b	1.60^b	2.22^a	0.17	1-4
Platelet (10^3/uL)	340.16^b	530.74^a	580.11^a	26.1	250-650
Neutrophil:lymphocyte ratio	0.44^b	0.41^{ab}	0.45^a	0.01	0.34-2.64

^{abc} means along the row with the various superscripts are significantly ($P<0.05$) different; T1, 0 ml *P. thonningii* essential oil; T2, 2 ml *P. thonningii* essential oil; T3, 4 ml *P. thonningii* essential oil/kg diet. RV: reference values as stated Beers (2006).

Table 3. Semen characteristics of rabbits fed *Piliostigma thonningii* essential oil supplemented diet

Parameter	T1	T2	T3	SEM
Semen colour	Cream	Cream	Cream	
pH	6.00	6.00	6.00	0.01
Semen volume (ml)	0.53^{b}	0.83^{a}	1.08^{a}	0.12
Motility (%)	58.33^{b}	70.00^{a}	76.00^{a}	6.11
Semen concentration ($x10^6$)	222.20^{b}	290.60^{a}	321.80^{a}	27.26
Abnormalities (%)	19.52^{a}	11.83^{b}	9.95^{b}	1.62
Live dead ratio	1.50^{b}	2.53^{ab}	3.29^{a}	0.58
Libido/reaction time (secs)	20.33^{a}	12.33^{b}	11.67^{b}	2.31

^{abc} Means with the different superscripts along the row are significantly (P<0.05) different; T1, 0 ml *P. thonningii* essential oil; T2, 2 ml *P. thonningii* essential oil; T3, 4 ml *P. thonningii* essential oil/kg diet.

Sperm live cell (live spermatozoa) varied between 60.00 to 76.67% and live dead ratio (1.50 to 3.29) with T3 higher and T1 lower (P<0.05) but was similar (P>0.05) between T1 and T2 and T2 and T3. Contrarily, dead sperm cell (23.33 to 40.00%) varied with T3 lower and T1 higher (P<0.05) but was similar (P>0.05) between T1 and T2 and T2 and T3. Reaction time from 11.67 to 20.33 secs and sperm abnormalities 9.95 to 19.52% presented higher (P<0.05) values in T1 compared to T2 and T3 which were similar (P>0.05).

Discussions

Haematological Parameters of Rabbits Fed Piliostigma thonningii Essential Oil Supplemented Diet

Haematological parameters are an index and are a reflection of the dietary effects on an animal with respect to the quality of the diet consumed and the nutrient supplied to satisfy the physiological requirements of the animal. It is important to note that the study's experimental animals, particularly those that were fed *P. thonningii* EO based diet, did not show apparent clinical signs of ill health. The absence of signs and symptoms of ill health, morbidity and mortality in the animals suggests the used doses of *P. thonningii* EO in the current study were not toxic. Mahmoud et al. (2016) also illustrated and concluded that thyme extract and other dietary essential oils were not toxic to experimental animals. Ogbiko et al. (2021) reports *Piliostigma thonningii* leaf extract to be orally safe up to a dose of 2 g/kg body weight.

In general results obtained for PCV, HB, RBC, MCHC, WBC, neutrophils and eosinophils were highest in treatment groups supplemented with 4 ml PEO. Highest WBC, RBC, Hb, and lymphocytes were similar to work of Bassiony et al. (2015) who reported cinnamaldehyde and thymol to significantly improve these parameters.

One significant indicator of anemia is PCV. The values obtained for PCV were within the stipulated range 33-50% for clinically healthy rabbits (Beers, 2006). The higher PCV values for the T2 and T3 indicate that PEO supplementation at 2 and 4 ml/kg diet enabled provision of quality protein to the rabbits, as PCV is useful in assessing protein status of a fed diet (Ayoade et al., 2015). PEO enhanced the PCV suggesting improved protein utilization, possibly due to enhanced protein intake and digestibility. The normal PCV values for all the treatments which were stipulated range, is suggestive of the absence of toxic factors such haemagglutinin which adversely affects blood formation (Ayoade et al., 2015). The higher value of T3 than T2 implies that PEO at 4ml/kg diet enhanced the rabbit's PCV compared to T2. This further justifies the non-toxicity of the high PEO.

Haemoglobin concentration and PCV are also indicative of adaptation to adverse situations in form of oxidative stress conditions. Low hemoglobin levels are a sign that not enough red blood cells are supplying oxygen to the body's peripheral tissues. The significantly higher levels of Hb in T2 and T3, although within the stipulated range of 13-18 g/dL for healthy rabbits is, therefore, indicative of antioxidative property of PEO ensuring reduced oxidative stress. The result also indicates better iron utilisation for the formation of haemoglobin by the PEO supplemented diets. However, T3 enhanced iron utilisation than T2, indicating superiority of PEO at 4ml/kg diet. There is a direct correlation between PCV and Hb, as seen by the comparable trends that both test results showed (Olafadehan, 2011).

The MCHC, MCH, and MCV values are comparable to those disclosed by Bassiony et al. (2015) and were within the physiological ranges stated in Beers (2006) for healthy growing rabbits. The normal values in the three treatments further confirm the absence of anaemia, particularly a hypochromic microcytic type (Olafadehan et al., 2014). The MCV values within stipulated reference range implied that the animals did not stand the risk of haemoconcentration and anaemia (Brian, 2009). The higher MCH values in the animals fed PEO supplemented diet show enhanced oxygen carrying capacity of the RBC because MCH is an indicator of the oxygen carrying capacity of the RBC (Brain, 2009). Changes in total RBC count and its indices (MCV and MCHC) are of value in determining the physiological effect of PEO to the experimental animals' health and well-being.

The higher WBC, lymphocytes and neutrophils values in treatments (2 and 4ml PEO) are indicative of improved activation of the body defense and immunity to infections or toxic substances than in the control. The absence of overwhelming disease in the rabbits throughout the feeding period showed improved gut associated lymphoid tissue development, this tissue development may have been possibly due to the decrease in the pathogenic bacteria as a result of bioactive components leading to a change in the microbial ecology, which favours, beneficial microbial species in the gut (Anaso et al., 2023: Ismail and Bealish, 2014). This could be linked to the increased count of beneficial Lactobacilli in the caecum. WBC counts are in agreement with the values reported by Bassiony et al. (2015). The WBC counts also fell within the stipulated range of 5-12.5 $10^3/\mu l$ for healthy rabbits as stipulated by Beers (2006) Merck's Veterinary Manual.

The high lymphocytes and platelets values in T2 and T3 imply that the rabbits had a better potential to ward off invading disease-causing organisms. According to the Beers (2006) Merck's Veterinary Manual, lymphocytes are a variety of WBC that directly attack disease-causing bacteria, viruses and toxins and regulate the other part of the immune system as well as produce antibodies, which neutralize invaders or mark them for destruction by other agents of the immune system. According to Mahgoub et al. (2009), lymphocytes are necessary for humoral and cell-mediated immune responses. Jelalu (2014) explained that differential leukocytes are responsible for the defence of an organism and depressed levels of lymphocytes indicate a depleted immune system while an elevated level indicates an active infection.

The eosinophil content was within reference values in the Beers Merck's Veterinary Manual (2006) for clinically healthy rabbits. The higher eosinophil count in rabbits supplemented 4 ml of the EO suggests a more induced immunological and cytotoxic processes (Chattopadhyay et al., 2007).

Since monocytes are the progenitors of macrophages, they are vital to the animal immune system. The values obtained were in line with 1 to 4% reported by Merck's Veterinary Manual (2006) for rabbits. The normal monocyte value indicates the critical role played in tissue development and homeostasis is performed at the cellular system. However, the higher monocytes values in T2 and T3 imply that they were equally equipped to identify harmful bacteria, viruses and toxins, attack foreign substances and provided all that were needed for rabbit's health, survival and enhanced performance.

It can be concluded that *P. thoninngii* EO can be added to grower rabbits' diets as a supplement to improve their haematological indices without endangering their health or causing intoxication, as all the studied haematological parameters were within the normal ranges for healthy rabbits and they did not exhibit any clinical signs or symptoms of illness.

Semen Characteristics of Rabbits Fed Piliostigma thonningii Essential Oil Supplemented Diet

According to studies by Anaso et al. (2024), Anaso et al. (2023) and Ososanyo et al. (2013), who noted a creamy colour characteristic for domestic animals, the experimental rabbits' semen had a similar colour. High-quality semen has a creamy white appearance. Low concentration is indicated by translucent semen, while contamination or poor quality is indicated by blood stains and strange colour. Thus, the similar semen colour of all the rabbits irrespective of treatment indicates good quality semen. This further indicates that PEO could be supplemented up to 4ml/kg diet without compromising the semen quality.

Semen pH is crucial factor affecting sperm motility and viability in term of its ability to fertilize an egg (Zhou et al., 2015). The pH difference in the semen was not statistically significant, which was in line with the findings of Abdel-Wareth and Metwally (2021) who found no significant variation in the pH of the semen from animals fed thyme essential oil (TEO) in comparison to the control group. The pH value in rabbit is between 6.00-6.33 which is slightly acidic (Abdel-Wareth and Metwally, 2020). According to Zhou et al. (2015), abnormal pH values above the range may indicate an underlying infection It might therefore have an impact on its viability.

El-Gindy et al. (2020) observed improved semen characteristics, including higher volume, motility, concentration, and fewer abnormalities, after feeding potato peel extract, a strong antioxidant, to growing rabbits. The study conducted by Abdel-Wareth and Metwally (2020) revealed a noteworthy enhancement in the semen characteristics of the treatment groups of growing rabbits that were administered dietary thyme essential oil and phytogenic supplement. Reduced ejaculate volume cases may result from season, collectors' factor, pooled volume, collecting frequency, or management (Anaso et al., 2023). They may also result from poor nutrition. Therefore, the oxidative and antibacterial activities of the bioactive chemicals in PEO may be the cause of a slight change in semen volume.

Sperm motility is connected to sperm viability, which is a measure of sperm count that may be high or low, claims Osinowo (2016). In general, motile cells are always viable, and determining whether non-motile cells are alive or dead depends on their viability. According to Osinowo (2016), sperm motility above 65% is deemed good. Strong, progressive motility, which is frequently observed as swirling, wave-like motions in highly concentrated ejaculates, is an important indicator of the survivability of the sperm.

Elevated feed intake is directly correlated with high semen concentration, which can also be caused by variations in temperature and frequency of ejaculation (Anaso et al., 2024). PEO supplementation's strong antioxidant activities of beta-pinene and caryophyllene may therefore be responsible for the enhanced delivery of nutrients for the spermatogenic process and higher sperm concentration in T2 and T3. Comparable to the present results, El-Ratel et al. (2021) observed an improvement in the progressive motility, vitality, sperm cell concentration, sperm outputs, and fertility in developing rabbits fed extra virgin olive oil (EVOO), betaine (BET), and ginger (GIN) as natural antioxidants.

Higher libido score in animals supplemented PEO proves the resultant effect of the bioactive constituent (components) of the essential oil, mainly in terms of it antioxidative property. The libido score is a function of the reaction time, higher libido is inversely proportional to lower reaction time. Libido test is becoming a common practice in breeding soundness examination for domestic animals, compared to other livestock species, increasing rabbits' sexual performance is currently the subject of more research and interest. Generally, libido (sex drive) is an important component of male fertility. It is independent on gonadal and extragonadal sperm reserves, semen quality, BW, growth rate or masculinity.

Conclusion

P. thonningii essential oil supplementation improved the haematological parameters and reproductive potentials under the circumstances of the current findings, suggesting a beneficial influence and thus encouraging its usage in other domestic animals. The study established 4 ml *P. thonningii* essential oil supplementation per kg of basal diet as the optimum dose for the rabbits due to their improved physiological response and reproductive potentials.

Declarations

The author declares no conflict of interest in the design, collection, writing of manuscript and decision to publish this work

References

Abdel-Wareth, A.A.A. and Metwally, A.E. (2020). Effects of dietary phytogenic supplementation on performance, semen quality and serum biochemical of rabbits. https://doi.org/10.21203/rs.3.rs-28978/v1.

Ajiboye, A.T., Lawal, S.O., Otun, K.O., Bale, A.T. and Arowon, M.T. (2017). Evaluation of physico-chemical and antimicrobial studies of *Delonix regia* and *Piliostigma thonningii* seed oils. *Jordan Journal of Chemistry* 12(1): 21-32.

Anaso EU, Olafadehan OA, Chibuogwu IC, Alagbe JO. Seminal mophology and organ morphometrics of rabbit bucks fed Piliostigma thinningii essential oil supplementd diet. Science Letters 2024; 12(2):70-75.

Anaso EU, Olafadehan. OA and Chibuogwu, IC. Semen characteristics of rabbits fed Camel's foot (Piliostigma thonningii) essential oil supplemented diet. Discovery 2023; 59: e36d1037.

Anaso EU. Bioactive compounds of *piliostigma thonningii* essential oil detected by the gas chromatography-mass spectrometry. EJABS, 2023b: 3(2): 68-72.

Anaso EU. Phytogenics and essential oil supplementation in rabbit and monogastrate production, a panacea to improved animal production and antibiotic resistance challenge in Nigeria. EJABS, 2023a: 3(2):45-51.

Anaso, E.U., Olafadehan, O.A. and Oluwafemi, R.A. (2021). Biodegradation of agro industrial by products as a panacea to feed scarcity in Nigeria amidst farmer insecurity and global pandemic. *Proceedings of the 46th Annual Nigerian Society for Animal Production Conference, Dutsin-Ma,* Nigeria, pp. 616 - 620.

Angel-Gracia, O., Meza- Herrera, C.A., Guillen-Munoz, J.M., Carillo-Castellanos, E., Luna- Orozko, J.R., Mellado, M. and Veliz- Deraz, F.G. (2015). Seminal characteristics, libido and serum testosterone concentrations in mixed –breed goat bucks receiving testosterone during the non-breeding period. *Journal of Applied Animal Research* 43(4): 457-461.

Ayoade, J.A., Wuanor, A.A. and Ochepo, G.O. (2015). Performance, haematology and serum biochemistry of grazing Bunaji bulls supplemented varying levels an agro industrial by- product based diet. IOSR *Journal of Agriculture and Veterinary Science* 8: 95-100.

Bahare, S., Upadhyay, I., Erdogan, O., Arun Kumar, J, Sumali, L.D.J., Daniel A.D., Farukh, S., Yasaman, T., Natália, M., Navid, B., William, C. and Cho, J.S. (2019). Antidiabetic potential of medicinal plants and their active components**.** *Biomolecules* 30;9(10):551. doi: 10.3390/biom9100551.

Bassiony, S.S.M., Elhindawy, M.M., Attia, A.E. and Ismail, I.E. (2015). Effect of some bioactive components of essential oils on growing rabbits performance. *Zagazig Journal of Agricultural Research* 42(5).

Beers, M.H. (2006). The Merck manual of diagnosis and therapy. 18th ed. Whitehouse Station, N.J. Merck Research Laboratories.

Brian, T. (2009). Red blood cells. Microsoft Encartha (DVD) Redmond, W.A. Microsoft Corporation.

CBRA-Colegio Brasileiro de Reprodução (1998). Animal. Manual de exame e avaliação de sêmen animal. 2. ed. Belo Horizonte: CBRA.

Chattopadhyay, B.P., Mahata, A., Kundu, S., Alan, J., Dars, S. and Roychawdhury, A. (2007). A study on blood eosinophil level and ventilatory pulmonary function of the workers exposed to storage grain dust. *Indian Journal of Occupational and Environmental Medicine* 11(1): 21–25.

Elghalid, O.A., Kholif, A.E., El-Ashry, G.M., Matloup, O.H., Olafadehan, O.A., El-Raffaa, A.M and AbdEl-Hadya, A.M. (2020). Oral supplementation of the diet of growing rabbits with a newly developed mixture of herbal plants and spices enriched with special extracts and essential oils affects their productive performance and immune status. *Livestock Science* 238: 104082.

El-Gindy, Y.M., Abu Hafsa, S.H. and Dosoky, W.M. (2020) Effects of potato peel extract on semen quality, sex hormones and immunity of rabbit bucks under intensive breeding system. *Journal of the American Oil.* https://doi.org/10.1111/and.13869

El-Gogary, M.R., El-Said, E.A. and Mansour, A.M. (2018). Physiological and immunological effects of rosemary essential oil in growing rabbit diets. *Journal Agricultural Science* 10: 485.

El-Ratel, I.T., Attia, K.A.H., El-Raghi, A.A. and Fouda, S.F. (2021). Relief of the negative effects of heat stress on semen quality, reproductive efficiency and oxidative capacity of rabbit bucks using different natural antioxidants. *Animal bioscience* 34(5): 844-854.

Hancock, J.I. (1951). A staining technique for the study of temperature shock in semen. Nature, London 197: 323-343.

Ismail, I.E. and Bealish, A.M.A. (2014). Effect of acetic acid addition on growth and some physiological and immunological characteristics of growing rabbits. *Egyptian Journal of Rabbit Science* 24 (1): 19 – 34.

Jain N. C. (1986). Scanning electron micrograph of blood cell. In Schalm's Veterinary Haematology, D.J. Weiss and K.J. Wardrop Eds. John Willey and Sons Inc. New York Pp. 63-70.

Jelalu, K. (2014). Laboratory manual and review of clinical pathology. OMICS group ebooks, USA. pp. 5-21.

Jimoh, F.O. and Oladiji, A.T. (2005). Preliminary Studies on Piliostigma thonningii Seeds: Proximate Analysis, Mineral Composition and Phytochemical Screening. *African Journal of Biotechnology*, 4, 1439-1442.

Mahgoub, A.M., Aufy, M.G., Saadi, M.G. and Adel Elmallawany, M. (2009). Risk factors predisposing to Toxoplasmosis in chronic renal patients and renal transplant recipients. *Journal of Egyptian Society for Parasitology* 36: 945-958.

Mahmoud, M.A., Shehata, S.S.A and Khalil, B.A. (2016). Effect of water thyme extract on growing rabbit's performance. *Zagazig Journal of Agricultural Research* 43 (6A): 2023-2035.

Mohamed, A., Ferhet Brahim, Y., Meklati, J.S. and Farid, C. (2006). An improved microwave Clevenger apparatus for distillation of essential oil from orange peel. *Journal of chromatography* 1112: 121-126.

NRC (1984). Nutrient requirements of poultry. 9th Ed. Nat. Res. Coun., Nat. Acad. Press, Washington, D.C., USA.

Ogbiko C1., Chukwunwejim, C. R. and Okezie, U. (2021). Phytochemical Composition, Acute Toxicity and Lipid Profile of the Methanol Leaf Extract of *Piliostigma Thonningii* (Fabaceae). *Journal of Pharmaceutical and Allied Sciences* 18 (1) 3430 – 3436.

Olafadehan O. A. (2011). Haematological Parameters, Serum Constituents and Organ Development of Growing Rabbits as Affected by Feeding of Processed Cassava Peels. *Animal Nutrition and Feed Technology* Vol. 11(1) Pp 41- 51.

Olafadehan, O.A., Adewumi, M.K. and Okunade, S.A. (2014). Effects of feeding tanning- containing forage in varying proportion with concentrate on the voluntary intake, haematological and biochemical indices of goats. *Trakia Journal of Sciences* 12: 73- 81.

Osinowo, O.A. (2016). Introduction to Animal Reproduction, Sophie Academics Services Limited. 2nd edition.

Ososanya, T. O., Adewumi, M. K., Oyeyemi, M. O. and Adeosun, A. O. (2013). Semen characteristics of pubertal West Africa dwarf rams fed pineapple waste silage as replacement for dried cassava peel. *African Journal of Agricultural Research* 9(4): 501- 506.

Oyeyemi. M.O., Leigh, O.O. and Fakayode, M.O. (2011). Spermiogram and changes in body parameters of West African Dwarf bucks fed dussa- poultry waste combination. *Global Veterinaria* 6(2): 136-139.

Pandi N, Sonawane SH, Anand Kishore K. Synthesis of cellulose nanocrystals (CNCs) from cotton using ultrasound-assisted acid hydrolysis. Ultrason Sonochem. 2021 Jan;70:105353. doi: 10.1016/j.ultsonch.2020.105353. Epub 2020 Sep 19. PMID: 33007536; PMCID: PMC7786604.

Romero, C., P.G. Rebollar, A. Dal Bosco, C. Castellini and R. Cardinali (2011). Dietary effect of short-chain organic acids on growth performance and development of intestinal lymphoid tissues in fattening restricted rabbits. *World Rabbit Science* 19: 133 – 142.

SCLabs. (2016). Beyond Aroma: Terpenes in cannabis Archived 2016-06-15 at the Wayback Machine.

Zhou, L., Zhao, Y., Wang, S., Han, S and Liu, J., (2015). Lead in the soil–mulberry (Morus alba L.)–silkworm (Bombyx mori) food chain: Translocation and detoxification. Chemosphere, 128: 171–177.