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Seminal Morphology and Organ Morphometrics of Rabbit Bucks Fed *Piliostigma thonningii* Essential Oil Supplemented Diet

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Abstract

This study aimed to investigate whether the inclusive supplementation of *Piliostigma thonningii* essential oil in rabbit diet was beneficial to their seminal morphometrics. Forty-five clinically healthy weaned male Dutch rabbits of about five weeks of age were used for the experiment. The rabbits were divided into three treatment groups, with fifteen rabbits per group and balanced for their body weight such that rabbits in each group had similar average initial BW of 0.27 kg. The experimental rabbits were administered *P. thonningii* essential oil at 0, 2 and 4 ml/kg for treatments T1, T2 and T3, respectively for 12 weeks. The results showed that left testis weight increased with increasing levels of *P. thonningii* essential oil supplementation. The normal cells, right testis weight and right and left testis volume were higher in T2 and T3 treatments than in T1. Right and left testis lengths were higher in T3 treatment than T1, with T2 being intermediate between T1 and T3. Mid-piece droplet, coiled tail, detached head, free tail, bent tail, right and left epididymis volume, head weight and length, body weight and length, weight and tail length and weight were not affected by treatments. It is concluded that *P. thonningii* essential oil supplementation improved some organ morphometrics without necessarily affecting sperm morphology in the experimental rabbit bucks.

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Introduction

Rabbits as a pseudo ruminant are characterized by rapid growth and high fertility rates, thereby categorizing them as an excellent source of meat and protein of animal origin [1]. Rabbit meat is known to be of high quality due to adequate animal protein and polyunsaturated fatty acids and low calories [2]. Tropical climate characterized by its extreme high temperature (heat) known to be a major climatic factor invariably affecting rabbit production [1]. Lipid peroxidation is a major problem in rabbit production caused by oxidative stress leading to end product (meat) deterioration [2]. Furthermore, lipid oxidation adversely impacts meat quality as it causes the formation of some toxic compounds and malondialdehyde (MDA). The latter has been reported to negatively influence human health [3, 4]. Recently, feed additives other than antibiotics and ionophores have been used to enhance feed utilization, and the productive and reproductive performance of rabbits [5, 6]. Feed additives have been utilized in the diets of ruminants [7] and recently in the diets of monogastric such as horses [8] and rabbits [9] to improve feed efficiency and animal health. Feed additives, such as essential oils (EOs), spices and herbs, have been successfully utilized in animal feed to decrease total mesophilic aerobes, *Pseudomonas* spp. and *Entero bacteriaceae* [4, 6].

The recent ban on antibiotic growth promoters by several countries and the jeopardy of antibiotic-resistant bacteria have compelled the search for alternatives to improving animal productivity and minimizing adverse effects on human consumers. Due to this ban, extensive research has been conducted to explore the use of phytogenics as alternate feed additives in animal nutrition. Phytogenic substances such as essential oils are generally regarded as safe and are frequently used in the food and feed industries [1]. Generally, phytogenic substances are generally known to be safe and are frequently used in the food and feed industries [1]. The impact of essential oils (EOs) as a phytogenic feed substance on antioxidant status and antimicrobial activity in livestock is considered vital for biological activities [10], As well as reproductive health in terms of quality semen production [11]. Evaluation of semen morphology and morphometric characteristics of the animals may give some insight into the potential or the extent to which dietary treatment meets adequate reproductive standards of animals. Although a good number of EOs have been evaluated in livestock diets with positive results, nothing or little is known about the effect of Camel's foot EO on the productive and reproductive performance of rabbits.

Materials and Methods

Experimental site

The experiment was carried out at the Monogastric Unit of the University of Abuja Teaching and Research Farm located in Abuja, Nigeria, West Africa. The project site lies between latitudes 08051' and 09037'N and longitudes 007020' and 007051' E. Annual rainfall ranges from 1145–1631 mm. The temperature in the dry season is between 36–42°C and 25.8–30.2°C during the rainy season. Relative humidity is about 60% during the rainy season and 30% during the dry season.

Collection of *P. thonningii* seed and the extraction of essential oil

Piliostigma thonningii seeds were sourced from the Guinea Savannah agroecological zone. It was identified and authenticated by a certified taxonomist at the Department of Biological Science, Forestry Research Institute of Nigeria (FRIN). The *P. thonningii* seeds were shade-dried, finely ground and stored at room temperature until extraction. The essential oil was extracted using a steam distillation method with a Clevenger apparatus using the method described by Mohamed et al. [12]. About 100 g of ground sample was suspended in 700 ml of distilled water, where a steam distillation process was employed at 100°C for about 3 hours by placing in a steel apparatus. The setup was heated up after connecting the condenser to a water inlet and outlet. This process allowed for softening of the sample and letting (discharging) the end product essential oil in vaporized form. The vaporized essential oil droplets formed and mixed with the steam (the carrier) and subsequently converged into a cooling system. Following convergence in the cooling system, the essential oil was then collected via a collection tube. The percentage of the oil content was calculated using the formulae below: oil content (%, v/w) = volume of the oil extracted (ml) / weight of the sample taken (g) x 100% [1].

Experimental animals, management and treatment

Forty-five clinically healthy weaned male Dutch rabbits of about five weeks of age were used for the experiment. The rabbits were purchased from the

National Animal Production and Research Institute, Ahmadu Bello University, Zaria, Nigeria. Two weeks before the arrival of the rabbits, the hutches and their immediate surroundings were cleaned (swept and washed) thoroughly and disinfected with antiseptic (Morigad) and Hypo® (sodium hypochlorite, caustic soda and de-mineralized water). All the rabbits were weighed individually using a weighing scale to determine their initial body weight (BW). The animals were quarantined for two weeks and administered prophylactic treatment. The treatment included the administration of anti-stress (Vitalyte®) in drinking water, subcutaneous injection with an anti-parasitic drug (Avomec®) at 0.5 mg/kg of the animal BW for the control of endo and ecto parasites and injection with oxytetracycline HCl (a broad-spectrum antibiotic) at 1.0 mL/10 kg BW via the parenteral intramuscular route. Rabbits were also treated subcutaneously with a coccidiostat (Sulphadimidine sodium BP solution) once at the beginning of the experiment at 1 ml/rabbit according to the manufacturer's recommendation. The rabbits were housed in individual open-sided metabolic hutches, which can separate feces from urine. A basal control diet was formulated according to NRC requirements for growing rabbits [13]. Both water and feed were provided *ad libitum* for 12 weeks while feeding was done twice a day at 08:00 hrs and 16:00 hrs. Rabbits in the first treatment were fed a basal control diet. In the other treatments, the control diet was supplemented with 2 ml and 4 ml of *P. thonningii* essential oil (PEO)/kg of the diet, respectively (T1, 0 ml PEO/kg diet; T2, 2 ml PEO and T3, 4 ml PEO).

Testicular and epididymal morphometry

Semen was collected using an artificial vagina filled with a warm liquid of about 45°C. The doe was fitted with this device and presented to bucks in their respective hutches [14]. The sperm morphology was determined from already washed 95 slide smears initially stained with Giemsa at 7.5% (Doles Laboratory), diluted in distilled water and dipped (immersed) in this solution for two hours. The slides were subsequently kept in an upright position until they dried completely and finally viewed under the microscope to ascertain the normal and abnormal sperm percentages. Normal spermatozoa and spermatozoa abnormalities were classified according to principles used for rabbits by Barth and Oko [15]. At the end of the experiment in the 13th week, the animals were anesthetized and sacrificed and their reproductive tracts were dissected. The testes and epididymides were carefully removed and weighed. The left and right testes were weighed separately using a highly sensitive weighing scale (Metler balance) recording to the nearest 0.001g. Testis length, epididymal head length, body length and tail lengths were measured with the aid of a pair of vernier calipers, while the testis volume was measured by water displacement according to Archimedes principle [11, 16-18].

Statistical analyses

The obtained result for semen morphology; mid piece droplet, coiled tail, detached head, free tail, bent tail and normal cell and seminal morphometrics; right and left testis length, weight, epididymal length and weight were subjected to analysis of variance (ANOVA) in a completely randomized design using SPSS (23.0). Duncan's multiple range test was used to test the significant difference between the means at $P \leq 0.05$.

Results and Discussions

Sperm morphology of rabbits

Table 1 below shows the sperm morphology of rabbits fed PEO supplemented diet. Mid-piece droplet, coiled tail, detached head, free tail, and bent tail did not differ (P>0.05) among treatment groups. Normal cells showed no significant difference (P>0.05) which was lower in T1 but higher in T2 and T3. The middle piece defect, coiled tail and detached head are regarded as teratozoospermia, also regarded as sperm abnormalities. In the current study, these abnormalities were not significant among treatments. The middle piece connects the head to the tail, which

Table 1 Sperm morphology of rabbits fed with *Piliostigma thonningii* essential oil-supplemented diet.

Parameter (%)	T1	T2	T3
Middle piece defect	0.00	0.00	0.00
Coiled tail	11.60	9.20	7.00
Detached head	5.00	4.60	3.80
Free tail	7.00	6.00	4.80
Bent tail	6.80	4.80	4.20
Normal cells	65.00^{b}	75.00^{a}	79.80^{a}

Means with the different superscripts along the row are significantly (P<0.05) different. Also, means without the different superscripts along the row do not differ at P>0.05. T1, 0 ml *P. thonningii* essential oil; T2, 2 ml *P. thonningii* essential oil; T3, 4 ml *P. thonningii* essential oil/kg diet.

functions as the sperm body. The coiled-tail sperm are sperm cells exposed to a certain level of bacteria [19]. These sperm cells lack the ability for capacitation, which is their ability to swim via the female reproductive tract to fertilize an egg. The coil tails are damaged tails that are generally linked to poor management, stress, infection and disease [11]. The most common cause of abnormal detached head could be attributed to testicular hypoplasia, testicular degeneration and spermatozoa senescence due to sexual inactivity. Therefore, it could be adduced that toxic hydrocarbons present in PEO did not have any effect on the middle piece defect, coiled tail and detached head, implying the levels of PEO, especially 4 ml/kg PEO, used in the present study did not affect the quality of sperm produced by the rabbit [11].

The unaffected free and bent tail implies that treatments did not induce stress in the rabbit. Barth and Oko reported that tail defects, such as the free and bent tails, were mostly found in higher numbers in stressed animals [15]. The stress could be in the form of oxidative/environmental stress and that could occur by the passage of sperm via the epididymis [11]. The lack of significant variation in the free and bent tails in rabbits supplemented with PEO as compared to control proves that the activity of beta-pinene and beta mycerne which were found in higher numbers and having antioxidative properties reducing the expectation of any form of tail abnormality [1]. Though the normal sperm cells differed significantly among treatments, those were significantly higher in the group with the highest administration of PEO. The normal sperm cells among all the treatments were good but were best in T3 treatment, which goes further to indicate higher fertility potential without the occurrence of oligozoospermia or azoospermia. The result of the current study did not follow the pattern of a previous report [20], which demonstrated that TEO increased the normal sperm cells as compared to control groups. Due to the antioxidant properties in the PEO bioactive oleic acid, heptadecanoic acid and hexadecane as constituents of sesquiterpenes and monoterpene hydrocarbons such as alpha-pinene and beta-caryophyllene, semen morphometric values were numerically enhanced along treatment groups.

Seminal morphometrics of rabbits

Table 2 shows the sperm morphology of rabbits fed PEO supplemented diet. Right and left testis length was higher in T3, lower in T1 (P<0.05) but was similar (P>0.05) between T1(control) and T2 (2 ml/kg PEO), and T2 and T3 (4 ml/kg PEO). The values for the right testis weight, right and left testis volume were higher (P>0.05) in T2 and T3 than in T1, while the left testis weight increased (P<0.05)

Table 2 Seminal morphometrics of rabbits fed with *Piliostigma thonningii* essential oil supplemented diet.

Parameter	T1	T2	T3
Testis length R (cm)	10.00^{b}	11.00^{ab}	12.53^{a}
Testis length L (cm)	9.00^{b}	11.20^{ab}	11.93^{a}
Testis weight R (g)	14.00^{b}	17.00^{a}	18.47^{a}
Testis weight L (g)	12.00^{c}	14.00^{b}	16.00^{a}
Testis volume R (ml)	5.00^{b}	7.00^{a}	8.93^{a}
Testis volume L (ml)	5.00^{b}	6.83^{a}	7.00^{a}
Epididymis volume R (ml)	7.79	8.00	9.00
Epididymis volume L (ml)	6.67	7.00	8.00
Epididymis head weight R (g)	0.25	0.27	0.30
Epididymis head weight L (g)	0.42	0.47	0.51
Epididymis head length R (cm)	3.33	4.00	5.00
Epididymis head length L (cm)	3.00	4.00	4.00
Epididymis body weight R (g)	0.20	0.23	0.30
Epididymis body weight L (g)	0.01	0.01	0.03
Epididymis body length R (cm)	2.00	2.16	3.00
Epididymis body length L (cm)	2.23	2.30	2.67
Epididymis tail weight R (g)	0.25	0.29	0.40
Epididymis tail weight L (g)	0.30	0.33	0.43
Epididymis tail length R (cm)	2.00	2.00	2.33
Epididymis tail length L (cm)	2.16	2.67	3.00
Epididymis weight R (g)	0.60	0.70	0.83
Epididymis weight L (g)	0.50	0.54	0.63

Means with the different superscripts along the row are significantly (P<0.05) different. Also, means without the different superscripts along the row do not differ at P>0.05. T1, 0 ml *P. thonningii* essential oil; T2, 2 ml *P. thonningii* essential oil; T3, 4 ml *P. thonningii* essential oil/kg diet.

with increasing level of PEO supplementation. Results for the left testis length, right epididymis volume, left epididymis volume, right epididymis head weight, left epididymis head weight, right epididymis head length, left epididymis head length, right epididymis body weight, left epididymis body weight, right epididymis body length, left epididymis body length, right epididymis tail weight, left epididymis tail weight, right epididymis tail length, left epididymis tail length, right epididymis weight and left epididymis weight did not differ (P>0.05) among treatments. Ewuola reported and placed specific emphasis on the two important factors that guide breeders in selecting breeding males [17]. These factors coherently include the number or quantity of good quality spermatogenic cells alongside good sperm livability produced in seminiferous tubules by the testis, and its ability to effectively and efficiently reserve (store) the produced spermatozoa. Oyeyemi et al. also opined that adequate information on the morphometric traits of the reproductive organs of a particular breeding male is a vital and critical marker (pointer) to its breeding value and fecundity [21]. This is due to the reported and documented evidence (fact) that the spermatozoa-storing ability of the testis of a male animal determines its fertility level. Tubular measurement has often been adopted as the traditional method used in assessing spermatogenic activity in any investigation involving testicular activity and functions [22, 23]. The values for right (R) and left (L) testis weight, right and left testis volume and length were generally higher in rabbits supplemented with PEO is related to the increased semen volume, motility and concentration in earlier results in the current study. Oguike and Uwalaka who supplemented biotin in diets of rabbits explained that the larger the size (weight and length) of testicular parameters, the better the capacity of sperm cells to produce semen during spermatogenesis [24]. They further implicate semen concentration, progressive motility and percentage of live cells and normal cells to be responsible for fertility in male rabbits [24]. From the results of the current study, there exists a positive relation between the testicular and epididymal size and semen parameters. Right epididymis head weight, epididymis volume right and left, epididymis head weight (R and L), epididymis head length (R and L), epididymis body weight (R and L), the epididymis body length (R and L), epididymis tail (R and L), Epididymis tail length (R and L) and finally the epididymis weight (R and L) further proves that the supplementation of PEO in rabbits did not negatively affect reproductive function.

Conclusion

The findings presented shows that the inclusive administration of PEO in the diet of rabbits was beneficial and posed no threat as shown by the significantly better right and left testis length, right testis weight, right and left testis volume.

Conflict of interest

The authors claim no conflicts of interest.

References

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

[2] El-Gogary MR, El-Said EA, Mansour AM. Physiological and immunological effects of rosemary essential oil in growing rabbit diets. J. Agric. Sci. 2018; 10:485.

[3] Ali R, Ali S, Azeem T, Umar W, Khan MI, Ali A, Shah MA, Umar S. Effect of Chromium on meat and egg production in poultry-A review. Sci Lett 2014; 2(1):1-4.

[4] Koné AP, Cinq-Mars D, Desjardins Y, Guay F, Gosselin A, Saucier L. Effects of plant extracts and essential oils as feed supplements on quality and microbial traits of rabbit meat. World Rabbit Sci 2016; 24:107-119.

[5] Falowo AB. Potential of medicinal plants as Hypocholesterolemic agents in chicken meat production. Sci Lett 2022; 10(1):24-31.

[6] Tufarelli V, Casalino E, D'Alessandro AG Laudadio V. Dietary phenolic compounds: biochemistry, metabolism and significance in animal and human health. Curr Drug Metab 2017; 18:905-913.

[7] Morsy TA, Kholif AE, Matloup OH, Abu Elella A, Anele UY, Caton JS. Mustard and cumin seeds improve feed utilisation, milk production and milk fatty acids of Damascus goats. J. Dairy Res 2018; 85:142–151.

[8] Elghandour MMY, Kholif AE, López S, Mendoza GD, Odongo NE, Salem AZM. *In vitro* gas, methane and carbondioxide productions of high fibrous diet incubated with fecal inocula from horses in response to the supplementation with different live yeast additives. JEVS 2016; 38:64–71.

[9] Cervantes-Valencia ME, Alcala-Canto Y, Salem AZM, Kholif AE, Ducoing-Watty AM, Bernad-Bernad MJ, Gutiérrez-Olvera C. Influence of curcumin (*Curcumalonga*) as a natural anticoccidial alternative in adult rabbits. Italian J Anim Sci 2015; 14:299–303.

[10] Abdel-Wareth AAA, Metwally AE. Effects of dietary phytogenic supplementation on performance, semen quality and serum biochemical of rabbits. Res. Sq. 2020; 10(10):E1844.

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

[12] Mohamed A, Ferhet Brahim Y, Meklati JS, Farid C. An improved microwave Clevenger apparatus for distillation of essential oil from orange peel. J Chromatogr 2006; 1112:121-126.
[13] NRC. Nutrient requirements of poultry. 9th ed. Nat. Res. Coun., Nat. Acad. Press, Washington, D.C., USA;1984.
[14] Boiti C, Castellini C, Besenfelder U, Theau-Clément M, Liguori L, Renieri T, et al. Guidelines for handling of rabbit bucks and semen. World Rabbit Sci 2005; 13(2):71-91.
[15] Barth AD, Oko RJ. Abnormal morphology of Bovine spermatozoa. Iowa University Press, Ames, IO, USA; 1989.
[16] Egbunike GN, Holtz W, Endell W, Smidt D. Reproductive capacity of German Landrace boars. I. Gonadal and extra-gonadal sperm reserves. Zuththygiene 1975; 2:35-37.
[17] Ewuola EO. Daily sperm production, gonadal and extra-gonadal sperm reserves of rabbits fed prebiotic and probiotic supplemented diets. Int J App Agric Apic Res 2013; 9:48-53.
[18] Igboeli G, Rakha AM. Gonadal and extragonadal sperm reserves of indigenous central African bulls. J Reprod Fert 1971; 25:107-109.
[19] Loma Linda University-LLU. Center for fertility and IVF 2023.
[20] Oyeyemi MO, Oke A, Olusola C, Ajala O, Oluwatoyin O, Idehen CO. Differences in testicular parameters and morphological characteristics of spermatozoa as related to age of West African Dwarf bucks. Trop J Anim Sci 2002; 5(1):99-107.
[21] Oyeyemi MO, Oke A, Olusola C, Ajala O, Oluwatoyin O, Idehen CO. Differences in testicular parameters and morphological characteristics of spermatozoa as related to age of West African Dwarf bucks. Trop J Anim Sci 2002; 5:99-107.
[22] Bitto II. Seasonal changes in the physiological and reproductive responses of the West African Dwarf buck in Ibadan. Ph.D. Thesis, University of Ibadan, Ibadan Nigeria; 1989.
[23] Silva Jr VA, Vieira CFP, Paula TAR, Palma MB, Amorim AA, Manhâes RC. Neonatal treatment with naloxone increases the population of Sertoli cells and sperm production in adult rats. Reprod Nutr Dev 2006; 46:157-166.
[24] Oguike MA, Uwalaka C. Influence of biotin on semen and testicular characteristics of rabbit bucks. Nig Agric J 2008; 39:55-60.