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Original article

Plasma and semen antioxidant responses of West African Dwarf goats to Parquetina *nigrescens* leaf extract administration

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ABSTRACT

This study investigated the antioxidant responses in both plasma and semen of West African Dwarf (WAD) goats following the administration of *Parquetina nigrescens* leaf extract (PNLE). Twelve clinically healthy WAD bucks were randomly assigned into two groups, each receiving 0 mL (control), and 4 mL of PNLE, respectively, over a 21-day period. Blood and semen samples were collected at baseline and post-treatment to evaluate antioxidant markers including catalase, superoxide dismutase (SOD), malondialdehyde (MDA), total antioxidant capacity (TAC), lipid peroxidation (LP), and glutathione peroxidase (GPx). An antioxidant response was observed. In the control group (0 mL PNLE), significant negative correlations between baseline and post-treatment values—particularly B-Cat. vs. SOD (r= -0.973), B-Cat. vs. MDA (r= -0.990), and B-MDA vs. SOD (r= -0.999)—suggested impaired endogenous antioxidant regulation. Conversely, administration of 4 mL PNLE significantly enhanced antioxidant status, with strong positive correlations among key indices, notably catalase vs. GPx (r= 0.960**) and TAC vs. SOD (r= 0.999**), reflecting improved systemic antioxidant synergy. Semen antioxidant profiles also showed better enzymatic coordination and reduced lipid peroxidation, indicating the extract's potential to alleviate reproductive oxidative stress. Overall, *Parquetina nigrescens* (*P. nigrescens*) demonstrated marked antioxidative efficacy, supporting both systemic and reproductive redox balance in WAD goats. *P. nigrescens* leaf extract enhances seminal antioxidant status in WAD goats, thereby promoting reproductive and systemic health. This study recommends its inclusion as a phytogenic additive in goat production, especially in breeding programs prone to oxidative stress.

Keywords: antioxidants, *Parquetina nigrescens* leaf extract, plasma, semen, West African Dwarf bucks

INTRODUCTION

Livestock production, particularly small ruminants such as goats, plays an essential role in the socio-economic development of many African countries, providing meat, milk, hide, and income for rural and peri-urban communities (Khowa *et al*., 2023). Among these, the West African Dwarf (WAD) goat is a hardy and adaptable breed commonly reared for its resilience to harsh environmental conditions and its socio-cultural significance (Akintunde *et al*.,

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2024a; Oguntade *et al*., 2024). However, the reproductive efficiency and overall productivity of WAD goats are often compromised by oxidative stress, which negatively impacts spermatogenesis, semen quality, and overall male fertility (Daramola *et al*., 2016; Pintus and Ros-Santaella, 2021). Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense system, leading to lipid peroxidation, DNA damage, and impaired sperm function (Krishnamurthy *et al*., 2024; Sengupta *et al*., 2024).

Conventional antioxidant therapies, such as the use of synthetic antioxidants, have been employed to mitigate oxidative stress and improve semen quality in livestock (Bansal and Bilaspuri, 2010; Kaltsas, 2023). However, the high cost, limited accessibility, and potential side effects of synthetic agents have prompted increased interest in natural antioxidants derived from medicinal plants (Akintunde *et al*., 2023; Chaudhary *et al*., 2023). Among the plethora of African ethnomedicinal plants, *P. nigrescens* (family: Apocynaceae) has attracted attention due to its rich phytochemical profile and traditional use in managing various health conditions, including anemia, diabetes, and reproductive disorders (Adase *et al*., 2022). Notably, the aqueous leaf extracts of *P. nigrescens* are known to contain potent bioactive compounds such as flavonoids, alkaloids, tannins, and saponins, which exhibit antioxidative, anti-inflammatory, and androgenic activities (Olumide *et al*., 2022; Akintunde *et al*., 2023, 2024b, c).

Despite the traditional claims and preliminary pharmacological investigations highlighting the therapeutic potential of *P. nigrescens*, there is a paucity of scientific data on its effects on male reproductive physiology, particularly in WAD goats. Moreover, while studies have evaluated its hematological and general health benefits (Owoyele *et al*., 2008; Akintunde *et al*., 2024a, b, c), the impact of *P. nigrescens* on systemic and reproductive-specific antioxidant responses, such as plasma and semen antioxidant parameters, remains largely unexplored. This knowledge gap is critical, considering the increasing reproductive challenges faced by indigenous goat breeds in sub-Saharan Africa, which are often aggravated by environmental stressors, poor nutrition, and inadequate veterinary interventions (Daramola *et al*., 2010; Hasan *et al*., 2015; Getaneh *et al*., 2023).

Therefore, this study seeks to evaluate the plasma and semen antioxidant response of WAD goats to the administration of *P. nigrescens* leaf extract (PNLE). By investigating biomarkers of oxidative stress such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and malondialdehyde (MDA) in plasma and semen, the study aims to provide empirical data on the protective and modulatory effects of *P. nigrescens* in male goats. The justification for this study lies in its potential to validate the ethnobotanical relevance of *P. nigrescens* as a safe, affordable, and accessible natural antioxidant agent capable of enhancing reproductive performance and welfare of WAD goats, ultimately improving livestock productivity and farmer livelihoods.

The novelty of this study resides in its pioneering focus on dual compartments—plasma and semen—antioxidant responses in WAD goats, an area that has not been adequately documented in existing literature. Furthermore, the study's integration of systemic and reproductive antioxidant markers provides a holistic approach to understanding the pharmacodynamic effects of PNLE on male fertility parameters. This will offer new insights into the potential use of *P. nigrescens* as a nutraceutical intervention in animal reproductive management, filling a critical gap in veterinary phytotherapy and livestock reproductive biotechnology in Africa.

MATERIALS AND METHODS

Experimental site

This study was conducted at the Livestock Research Unit, Farm House, Babcock University, Ilishan-Remo, Ogun State, Nigeria. Babcock University is located at latitude 6.8920°

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N and longitude 3.7181° E. It is covered predominantly by rain forest and has wooden savanna in the northwest with an annual rainfall of about 1500 mm, with a mean temperature range of 27-31 °C and a very high relative humidity (above 87%). The climatic condition of the area is typically tropical with distinct wet and dry seasons.

Experimental animals and management

A total of twelve clinically healthy, sexually mature West African Dwarf (WAD) bucks, aged between 12 and 15 months, were purchased from Moniya Livestock Market, Ibadan, Oyo State, Nigeria. Upon arrival, the animals were subjected to a 3-day quarantine and acclimatization period. All animals were thoroughly washed using a mild antiseptic solution with special attention to the scrotal region to ensure external hygiene. The pens were constructed and disinfected with appropriate disinfectants before the arrival of the animals. Goats were allowed to roam and exercise freely within the farm premises during the day and were confined to well-ventilated pens at night throughout the experimental period.

Veterinary care and medication

Upon arrival, all animals were administered multivitamins (1 mL per goat per day) and broad-spectrum antibiotics (1 mL per goat per day) intramuscularly for three consecutive days to boost immunity and prevent opportunistic infections.

Dietary management

Throughout the experimental period, the goats were fed yam peels (*Dioscorea spp.*) as the basal diet. The diet was supplemented daily with floated pumpkin stems (*Telfairia occidentalis*) and freshly cut elephant grass (*Pennisetum purpureum*), offered *ad libitum*. Clean drinking water was provided throughout the study.

Collection and preparation of *P. nigrescens* leaves

Fresh leaves of *P. nigrescens* were harvested from wild populations in Ilishan-Remo Local Government Area, Ogun State, Nigeria. The leaves were authenticated by a botanist at the Department of Basic Sciences, Babcock University, Ilishan-Remo, Ogun State, Nigeria. For extract preparation, 50 g of fresh *P. nigrescens* leaves were washed, macerated in 150 mL of distilled water, and allowed to steep for 24 hours at room temperature. The mixture was filtered using Whatman No.1 filter paper, yielding 440 mL of aqueous extract. The extract was stored in sterilized amber bottles at 4°C until use. Administration of the extract commenced four days after the arrival of the animals.

Experimental design and treatment administration

The goats were randomly allocated into two treatment groups, each consisting of six goats in two replicates (three goats per replicate), and designated as follows: Group T1 (Control) did not receive the extract. Group T2 (Treatment) received 4 mL of *P. nigrescens* extract per day orally via drenching for 21 days.

Semen collection and processing

At the end of the treatment period, semen collection was carried out post-mortem. The bucks were slaughtered humanely following standard veterinary guidelines. The scrotum was dissected, and both left and right testicles were harvested. Each epididymis was carefully dissected to expose the epididymal head, body, and tail (Akintunde *et al*., 2023b). The tissues were placed separately into sterile beakers containing 0.9% normal saline. The testes were gently minced, and the released semen-rich fluid was collected into lithium heparinized tubes and placed immediately on ice. In order to determine the sperm concentration, the homogenate charged on the haemocytometer and inspected under a microscope at a magnification of x400.

Plasma collection

At slaughter, blood samples were collected directly from the jugular vein into EDTA-coated vacutainer tubes. The samples were kept in ice and transported to the laboratory where plasma was separated by centrifugation at 3000 rpm for

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15 minutes and stored at -20°C until antioxidant analysis.

Antioxidant analysis

Oxygen radical absorbance (ORAC) assay was used to measure the blood plasma TAC and assess the hydrophilic antioxidants. Total antioxidant capacity was determined using a Fenton-type reaction method and appropriate formula as described by Winterbourn (1979), Gutteridge *et al.* (1990) and Yamazaki *et al.* (1990). Peroxidative activity method was used to determine catalase with appropriate formula (Beers and Sizer, 1952) and time duration was modified to 0- and 5-minutes reading (Ewuola and Olaleye, 2015). Superoxide dismutase activity was determined using an inhibition method and appropriate formula as described (Marlund and Marklund, 1974) and modified (Soon and Tan, 2002). Glutathione peroxidase activity was estimated with appropriate formula (Rotruck *et al.*, 1973). The levels of MDA as indices of lipid peroxidation were measured in a thiobarbituric acid reactive substance (TBARS) (Yagi, 1998).

Statistical analysis

Data obtained from the study were analyzed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Mean values and standard errors were calculated for each parameter. Differences between treatment groups were analyzed using Independent Samples *t*-test. Statistical significance was declared at $p < 0.05$. Pearson's correlation statistic was used to examine association among body weights and scrotal biometry parameters and plasma and semen antioxidant parameters.

RESULTS

The effect of PNLE on body weight and scrotal biometry of WAD bucks is presented in Table 1. The mean body weight of bucks in the control group (T1) was numerically higher (8,900 ± 236.29 g) compared to the PNLE-treated group (7,716.67 ± 798.00 g), although this difference was not statistically significant (p= 0.066). Similarly, scrotal circumference, scrotal length, and scrotal width did not differ significantly between the groups (p >0.05).

Table 1 Effect of *Parquetina nigrescens* leaf extract on body weight and scrotal biometry of West African dwarf bucks

	T1 (Mean ± SEM)	T2 (Mean ± SEM)	p-value
body weight (g)	8900 ± 236.29	7716.67 ± 798.00	0.066
scrotal circumference (cm)	16.83 ± 0.73	17.00 ± 0.58	0.676
scrotal length (cm)	7.00 ± 0.29	7.93 ± 0.47	0.287
scrotal width (cm)	6.67 ± 0.33	7.23 ± 0.37	0.812

PNLE: *Parquetina nigrescens* leaf extract; T1: control with no administration of PNLE; T2: had an inclusion level of 4 mL PNLE per buck.

As presented in Table 2, the administration of PNLE did not result in any statistically significant changes (p >0.05) in plasma antioxidant parameters of WAD goats. Specifically, catalase activity was 118.00 ± 11.86 µU /L in the control group and 124.60 ± 8.72 µU /L in the PNLE-treated group (p= 0.519), while SOD was 91.93 ± 7.72 U/mL and 78.16 ± 6.43 U/mL in the control and treated groups, respectively (p= 0.624). Malondialdehyde concentration, an index of lipid peroxidation, was lower in the treated group (14.70 ± 9.60 µmol/L) compared to the control (26.00 ± 11.21 µmol/L), though the difference was not statistically significant (p= 0.703). Total antioxidant capacity was slightly higher in the treated group (439.67 ± 57.34 µmol/L) than in the control (381.33 ± 56.91 µmol/L), but also not

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significant (p= 0.963). Similarly, LP and GPx showed no significant differences between the groups, with values of 1.23 ± 0.18 µmol/L and 275.67 ± 25.83 U/L in the treated group versus 1.06 ± 0.20 µmol/L and 302.33 ± 26.17 U/L in the control group, respectively (p >0.05).

Table 2 Effect of *Parquetina nigrescens* leaf extract on blood plasma antioxidant parameters in West African dwarf bucks

	T1 (Mean ± SEM)	T2 (Mean ± SEM)	p-value
catalase (µU/L)	118.00 ± 11.86	124.60 ± 8.72	0.519
SOD (U/mL)	91.93 ± 7.72	78.16 ± 6.43	0.624
MDA (µmol/L)	26.00 ± 11.21	14.70 ± 9.60	0.703
TAC (µmol/L)	381.33 ± 56.91	439.67 ± 57.34	0.963
LP (µmol/L)	1.06 ± 0.20	1.23 ± 0.18	0.747
GPx (U/L)	302.33 ± 26.17	275.67 ± 25.83	0.960

PNLE: *Parquetina nigrescens* leaf extract; T1: control with no administration of PNLE; T2: had an inclusion level of 4 mL PNLE per buck; SOD: superoxide dismutase; MDA: malondialdehyde; TAC: total antioxidant capacity; LP: lipid peroxidase; GPx: glutathione peroxidase

Table 2 shows that PNLE had no effect on plasma antioxidant parameters; catalase, SOD, MDA, TAC, LP and GPx, as all the p-values are greater than 0.05, making them insignificant. The administration of PNLE significantly influenced (p <0.05) semen antioxidant parameters in WAD goats, as shown in Table 3. Catalase activity was significantly higher in the treated group (119.08 ± 5.44 µU/L) compared to the control group (107.48 ± 2.47 µU /L; p= 0.003). Similarly, SOD activity decreased significantly from 51.54 ± 10.81 U/mL in the control to 29.95 ± 7.71 U/mL in the treated group (p= 0.035). Malondialdehyde concentration, an indicator of LP, was significantly reduced in the PNLE-treated group (17.32 ± 0.82 µmol/L) relative to the control (25.54 ± 1.59 µmol/L; p= 0.002). Total antioxidant capacity showed a significant elevation from 374.67 ± 2.23 µmol/L in the control to 375.83 ± 36.82 µmol/L in the treated group (p <0.0001). Additionally, LP was significantly higher in the treated group (1.53 ± 0.56 µmol/L) compared to the control (1.23 ± 0.17 µmol/L; p <0.001). Glutathione peroxidase activity also decreased significantly following PNLE administration, with values of 238.17 ± 20.83 U/L in the treated group and 408.50 ± 16.84 U/L in the control group (p= 0.001). Table 4 shows that PNLE had no significant effect (p >0.05) on the sperm concentration of goats.

Table 3 Effect of *Parquetina nigrescens* leaf extract on semen antioxidant parameters in West African dwarf bucks

	T1 (Mean ± SEM)	T2 (Mean ± SEM)	p-value
Catalase (µU/L)	107.48 ± 2.47	119.08 ± 5.44	0.003
SOD (U/mL)	51.54 ± 10.81	29.95 ± 7.71	0.035
MDA (µmol/L)	25.54 ± 1.59	17.32 ± 0.82	0.002
TAC (µmol/L)	374.67 ± 2.23	375.83 ± 36.82	4.31×10^{-11}
LP (µmol/L)	1.23 ± 0.17	1.53 ± 0.56	0.000
GPx (U/L)	408.50 ± 16.84	238.17 ± 20.83	0.001

PNLE: *Parquetina nigrescens* leaf extract; T1: control with no administration of PNLE; T2: had an inclusion level of 4 mL PNLE per buck; SOD: superoxide dismutase; MDA: malondialdehyde; TAC: total antioxidant capacity; LP: lipid peroxidase; GPx: glutathione peroxidase.

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Table 4 Effect of *Parquetina nigrescens* leaf extract on sperm concentration of West African Dwarf bucks

sperm concentration	T1	T2	p-value
testes ($x10^9$/mL)	1.99 ± 0.36	1.88 ± 0.30	0.825
epididymis ($x10^9$/mL)	2.49 ± 0.72	1.19 ± 0.66	0.815

PNLE: *Parquetina nigrescens* leaf extract; T1: control with no administration of PNLE; T2: had an inclusion level of 4 mL PNLE per buck.

At 0 mL PNLE (Table 5), body weight was negatively correlated with SC, SW, and sperm concentration, while SL showed a significant negative correlation with sperm concentration in the testes (r= -0.988, p <0.05) and epididymis (r= -0.980). At 4 mL PNLE (Table 6), correlations shifted, with body weight showing positive relationships with SC, SL, and SW, but a strong negative correlation with epididymal sperm concentration (r= -0.851), while SC exhibited a strong negative correlation with testicular sperm concentration (r= -0.898).
Comparatively, the correlation patterns at 4 mL PNLE showed weakened and altered associations between body and reproductive parameters compared to the strong, significant negative correlations observed at 0 mL PNLE, indicating a possible modulatory effect of PNLE on the interrelationships among these reproductive traits.

Table 5 Correlation between body weight and scrotal biometry at 0 mL *Parquetina nigrescens* leaf extract administration

	bw	SC	SL	SW	sperm conc. (testes)	sperm conc. (epididymis)
bw	—	-0.58	0.30	-0.21	-0.45	-0.49
SC	-0.58	—	0.60	0.92	-0.47	-0.42
SL	0.30	0.60	—	0.87	-0.99*	-0.98
SW	-0.21	0.92	0.87	—	-0.78	-0.75
sperm conc. (testes)	-0.45	-0.47	-0.99*	-0.78	—	0.99*
sperm conc. (epididymis)	-0.49	-0.42	-0.98	-0.75	0.99*	—

* significant at p <0.05; bw: body weight; SC: scrotal circumference; SL: scrotal length; SW: scrotal width; sperm conc. (testes): sperm concentration in the testes; sperm conc. (epididymis): sperm concentration in the epididymis.

Table 6 Correlation between body weight and scrotal biometry at 4 mL *Parquetina nigrescens* leaf extract administration

	bw	SC	SL	SW	sperm conc. (testes)	sperm conc. (epididymis)
bw	1	0.13	0.50	0.72	0.32	-0.85
SC	0.13	1	0.92	0.78	-0.90	0.41
SL	0.50	0.92	1	0.96	-0.65	0.03
SW	0.72	0.78	0.96	1	-0.42	-0.25
sperm conc. (testes)	0.32	-0.90	-0.65	-0.42	1	-0.72
sperm conc. (epididymis)	-0.85	0.41	0.03	-0.25	-0.77	1

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* Significant at p <0.05; bw: body weight; SC: scrotal circumference; SL: scrotal length; SW: scrotal width; sperm conc. (testes): sperm concentration in the testes; sperm conc. (epididymis): sperm concentration in the epididymis.

Table 7 presents the correlation data at 0 mL PNLE, showing significant negative correlations between plasma and semen antioxidant parameters, such as plasma catalase and semen SOD (r= -0.973, p <0.01), with positive correlations observed among the semen parameters themselves (r= 0.770 to 0.991, p <0.05). Table 8 provides similar correlations at 4 mL PNLE, with several strong correlations, notably negative between plasma catalase and semen parameters (r= -0.967 to -0.999, p <0.01), while the correlation among semen antioxidant parameters is also significant but generally weaker compared to the 0 mL data. While both tables show strong correlations between plasma and semen antioxidant parameters, Table 7 at 0 mL PNLE highlights more consistent and stronger correlations, particularly in the negative direction, compared to the more variable and weaker correlations observed in Table 8 at 4 mL PNLE, suggesting that the PNLE dosage modulates antioxidant interactions.

Table 7 Correlation between blood plasma and semen antioxidant parameters at 0 mL of *Parquetina nigrescens* leaf extract administration

	B-Cat.	B-SOD	B-MDA	B-TAC	B-LP	B-GPx	catalase	SOD	MDA	TAC	LP	GPx
B-Cat.	1	-0.97	-0.99*	0.98	0.96	-0.97	-0.13	-0.08	-0.30	-0.16	-0.37	0.17
B-SOD	-0.97	1	0.99*	-0.99*	-0.99*	1.00**	0.36	0.31	0.51	-0.07	0.58	-0.39
B-MDA	-.0.99*	0.99*	1	-0.99*	-0.99*	0.99*	0.27	0.23	0.43	0.02	0.50	-0.31
B-TAC	0.98	-0.99*	-0.99*	1	0.99*	-0.99*	-0.32	-0.28	-0.48	0.03	-0.55	0.35
B-LP	0.96	-0.99*	-0.99*	0.99*	1	-1.00**	-0.39	-0.35	-0.55	0.11	-0.61	0.43
B-GPx	-0.97	1.00**	0.99*	-0.99*	-1.00**	1	0.38	0.33	0.53	-0.09	0.60	-0.41
catalase	-0.13	0.36	0.27	-0.32	-0.39	0.38	1	0.98**	0.97**	-0.89**	0.95**	-0.97**
SOD	-0.08	0.31	0.23	-0.28	-0.35	0.33	0.98**	1	0.98**	-0.83*	0.96**	-0.97**
MDA	-0.30	0.51	0.43	-0.48	-0.55	0.53	0.97**	0.98**	1	-0.79*	0.99**	-0.99**
TAC	-0.16	-0.07	0.02	0.03	0.11	-0.09	-0.89**	-0.83*	-0.79*	1	-0.74*	0.80*
LP	-0.37	0.58	0.50	-0.55	-0.61	0.60	0.95**	0.96**	0.99**	-0.74*	1	-0.99**
GPx	0.17	-0.39	-0.31	0.35	0.43	-0.41	-0.97**	-0.97**	-0.99**	0.80*	-0.99**	1

* Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level; PNLE: *Parquetina nigrescens* leaf extract; B-Cat.: catalase concentration in blood plasma; B-SOD: superoxide dismutase in blood plasma; B-MDA: malondialdehyde concentration in blood plasma; B-TAC: total antioxidant capacity in blood plasma; B-LP: lipid peroxidase in blood plasma; B-GPx: glutathione peroxidase in blood plasma; catalase: catalase concentration in semen; SOD: superoxide dismutase in semen; MDA: malondialdehyde concentration in semen; TAC: total antioxidant capacity in semen; LP: lipid peroxidase in semen; GPx: glutathione peroxidase in semen.

Table 8 Correlation between blood plasma and semen antioxidant parameters at 4 mL of *Parquetina nigrescens* leaf extract administration

	B-Cat.	B-SOD	B-MDA	B-TAC	B-LP	B-GPx	catalase	SOD	MDA	TAC	LP	GPx
B-Cat.	1	-0.88	-0.80	0.79	0.73	-0.70	-0.97	0.96	0.61	0.95	-0.72	-0.93
B-SOD	-0.88	1	0.99*	-0.99	-0.97	0.96	0.73	-0.71	-0.16	-0.69	0.30	0.64
B-MDA	-0.80	0.99*	1	-1.00**	-0.99*	0.99*	0.62	-0.60	-0.01	-0.57	0.15	0.52
B-TAC	0.79	-0.99	-1.00**	1	0.99*	-0.99*	-0.60	0.59	-0.01	0.56	-0.14	-0.51

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B-LP	0.73	-0.97	-0.99*	0.99*	1	-0.99*	-0.53	0.52	-0.09	0.49	-0.05	-0.43
B-GPx	-0.70	0.96	0.99*	-0.99*	-0.99*	1	0.50	-0.48	0.14	-0.45	0.01	0.39
catalase	-0.97	0.73	0.62	-0.60	-0.53	0.49	1	-0.97**	-0.52	-0.97**	0.90**	0.96**
SOD	0.96	-0.71	-0.60	0.59	0.52	-0.48	-0.97**	1	0.48	0.99**	-0.94**	-0.99**
MDA	0.61	-0.16	-0.01	-0.01	-0.09	0.14	-0.52	0.48	1	0.48	-0.55	-0.46
TAC	0.95	-0.69	-0.57	0.56	0.49	-0.45	-0.97**	0.99**	0.48	1	-0.95**	-0.99**
LP	-0.72	0.30	0.15	-0.14	-0.05	0.01	0.90**	-0.94**	-0.55	-0.95**	1	0.96**
GPx	-0.93	0.64	0.52	-0.51	-0.43	0.39	0.96**	-0.99**	-0.46	-0.99**	0.96**	1

* Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level; PNLE: *Parquetina nigrescens* leaf extract; B-Cat.: catalase concentration in blood plasma; B-SOD: superoxide dismutase in blood plasma; B-MDA: malondialdehyde concentration in blood plasma; B-TAC: total antioxidant capacity in blood plasma; B-LP: lipid peroxidase in blood plasma; B-GPx: glutathione peroxidase in blood plasma; catalase: catalase concentration in semen; SOD: superoxide dismutase in semen; MDA: malondialdehyde concentration in semen; TAC: total antioxidant capacity in semen; LP: lipid peroxidase in semen; GPx: glutathione peroxidase in semen.

DISCUSSION

The administration of PNLE at 4 mL per day for 21 days did not significantly influence the body weight and scrotal biometry of WAD bucks in this study. Although there were numerical reductions in body weight and slight increases in scrotal dimensions in the treated group, these differences did not reach statistical significance. This observation suggests that short-term administration of PNLE may not exert marked anabolic or catabolic effects on overall body weight but may have subtle effects on reproductive organ dimensions. These findings are consistent with the reports of Bamisaye *et al.* (2024) and Akintunde *et al.* (2025), who observed that while *P. nigrescens* has antioxidant and hematopoietic properties, its impact on growth performance and body mass may require longer administration periods or higher dosages to manifest significant effects. The slight improvement observed in scrotal dimensions could be linked to the known androgenic and fertility-enhancing properties of *P. nigrescens*, as previously reported by Ajayi *et al*. (2021), who documented increased testicular weight and improved semen characteristics in male rats administered with aqueous extracts of the plant. However, the short duration and relatively small sample size in the present study may have limited the manifestation of such reproductive benefits.

Scrotal circumference, length, and width are critical parameters associated with sperm production capacity, testicular health, and fertility potential in male livestock (Zemjanis, 1977; Oyeyemi and Okediran, 2007). The numerical increases observed in the PNLE-treated bucks, though not significant, may suggest a possible positive trend in reproductive capacity enhancement, warranting further investigation with larger cohorts and extended treatment periods. The lack of significant changes in body weight might also imply that PNLE administration at the tested dosage does not compromise the nutritional status or feed intake of the animals, corroborating the safety reports of the plant extract by Akinola *et al*. (2024, 2025) and Akintunde *et al*. (2025), who noted that PNLE is relatively safe at moderate doses. The findings in this study are consistent with similar research on the use of plant-based supplements in livestock. Akintunde *et al*. (2023b) found that herbal extracts, though beneficial in improving certain aspects of livestock health, did not always lead to significant changes in growth metrics like body weight, or reproductive traits.

Studies examining the effects of herbal treatments on antioxidant enzymes have shown mixed results. Hwang *et al*. (2016) and Uroko *et al*. (2020) found that certain plant extracts can significantly increase antioxidant enzyme activities like catalase and SOD, suggesting

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protective roles against oxidative stress. However, Aliahmat *et al*. (2012) found that the effects of plant-based antioxidants on enzyme activities can vary depending on the plant species, dosage, and duration of treatment, which aligns with the findings from this study where no significant changes were observed in the antioxidant enzymes (catalase, SOD, GPx).

In studies focusing on *P. nigrescens*, such as Akintunde *et al*. (2023), PNLE was found to have antioxidant properties *in vitro*. The contrast could be as a result of variations in the experimental animals. MDA and lipid peroxidation serve as biomarkers of oxidative damage. Oboh (2008) reported that certain plant extracts could significantly reduce MDA levels, indicating an antioxidant effect by reducing lipid peroxidation. Ayoola *et al*. (2011) and Daramola *et al*. (2025) demonstrated the effectiveness of P. *nigrescens* in mitigating oxidative stress under experimental conditions, which aligns with the slight reduction in MDA observed in this study. Given the lack of significant findings in the plasma antioxidant parameters, it appears that PNLE might not offer substantial antioxidant benefits under normal conditions in WAD goats. However, if the goats were under oxidative stress (due to environmental factors, disease, or dietary deficiencies), PNLE might demonstrate more pronounced effects. Ponnampalam *et al*. (2022) and Oke *et al*. (2024) found that the antioxidant effects of plant extracts are often more noticeable when animals are subjected to stress or are fed a suboptimal diet.

The absence of statistically significant results could be due to the relatively short duration of the treatment. Allison *et al*. (2025) suggested that the full benefits of herbal treatments may require prolonged use, as antioxidant effects are often cumulative over time. Therefore, longer studies might be required to observe more definitive antioxidant effects. Moreover, examining other biomarkers of oxidative stress, such as advanced glycation end products (AGEs), protein carbonylation, or DNA damage markers, could provide a more comprehensive view of the antioxidant effects of PNLE.

Studies on the effects of plant extracts on semen quality and antioxidant enzyme activities have shown variable results. Shakeri *et al*. (2024) found that certain herbal extracts enhanced catalase and SOD activity, which could improve sperm motility and overall semen quality. Previous research by Adase *et al*. (2022) showed that *P. nigrescens* exhibits antioxidant properties in various tissues, but the effects on semen have not been as well explored. The findings of the current study suggest that PNLE may have a mixed effect on antioxidant enzymes in semen, enhancing catalase activity while reducing GPx and SOD activities.

Malondialdehyde and LP are common biomarkers for oxidative stress and sperm dysfunction (Wang *et al*., 2025). Zhou *et al*. (2025) showed that plant extracts can reduce MDA levels in semen, leading to improved sperm quality. The significant reduction in MDA levels in treatment group of this study suggests that PNLE might have protective effects against oxidative damage to sperm cells, potentially enhancing fertility in goats. TAC is a comprehensive measure of the semen's ability to resist oxidative damage. Palani and Alahmar (2020) demonstrated that increased TAC in semen can improve sperm motility and protect against oxidative stress. The significant increase in TAC observed in treatment group suggests that PNLE might boost the overall antioxidant capacity of semen, potentially contributing to enhanced sperm quality and reproductive performance in goats.

Oxidative stress negatively impacts semen quality by damaging sperm DNA, lipids, and proteins, leading to reduced fertility. Agarwal *et al*. (2014) reported that reducing oxidative stress in semen improves sperm function. In this study, the reduction in MDA and the increase in TAC in treatment group are promising indicators that PNLE might be protecting semen from oxidative stress, despite the mixed effects on individual antioxidant enzymes. The findings suggest that PNLE may improve the antioxidant defense mechanisms in semen by increasing catalase activity and enhancing TAC. This could potentially protect sperm from oxidative damage,

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improving fertility outcomes in WAD goats. These results align with the literature, where plant-derived antioxidants have been shown to improve semen quality in various species.

The study also reveals a reduction in SOD, GPx, and an increase in LP in treatment group, suggesting that PNLE might induce a complex response in the semen's antioxidant system. The increased lipid peroxidation might indicate that PNLE could trigger a higher production of ROS than the antioxidant enzymes can handle. This imbalance could negatively affect semen quality and fertility in some cases, which warrants further exploration of the optimal dosage and administration time for PNLE in reproductive applications. The improvement in catalase and TAC levels alongside the reduction in MDA suggest that PNLE may have a beneficial effect on sperm quality by protecting against oxidative damage. However, the mixed results for other antioxidant parameters (SOD, GPx, LP) indicate that PNLE's effects may vary depending on the specific antioxidant systems in play. Thus, further research on the precise mechanisms through which PNLE influences sperm quality is necessary to better understand its role in fertility.

Sperm concentration is a direct indicator of testicular function and semen quality. According to Akporhuarho *et al*. (2018), WAD bucks typically have relatively moderate sperm concentration compared to Red Sokoto breeds, which is influenced by nutritional and environmental factors. *P. nigrescens*, in particular, has been reported to exhibit antioxidant and aphrodisiac properties in rodents and Japanese quails (Kayode *et al*., 2022; Akintunde *et al*., 2023b), with suggested benefits to testicular function and spermatogenesis. However, these positive effects are not reflected in this study's WAD goat data, potentially due to species-specific responses or suboptimal dosage or duration.

The findings from Table 4 indicate that administration of PNLE had no statistically significant effect on sperm concentration in both the testes ($1.99 \pm 0.36 \times 10^9$/mL vs. $1.88 \pm 0.30 \times 10^9$/mL; p= 0.825) and the epididymis ($2.49 \pm 0.72 \times 10^9$/mL vs. $1.19 \pm 0.66 \times 10^9$/mL; p= 0.815) of WAD goats. This suggests that although PNLE elicited notable changes in semen antioxidant parameters, as previously demonstrated in Table 3, these alterations did not translate into improvements in sperm production or concentration. The significant increase in semen antioxidant enzymes such as catalase and the reduction in oxidative stress marker MDA observed in the PNLE-treated goats aligns with the antioxidative potential of *P. nigrescens* previously reported in studies by Ayoola *et al*. (2011) and Akintunde *et al*. (2023a), who demonstrated that the plant's bioactive constituents possess potent free radical scavenging activities. However, despite these favorable changes in the antioxidant defense system of the semen, the lack of impact on sperm concentration might suggest that sperm production is influenced by other physiological and endocrine factors beyond oxidative stress balance alone. These findings have critical implications for the application of *P. nigrescens* as a reproductive booster in male goats. While PNLE appears to confer protective benefits against oxidative stress in semen, which is essential for preserving sperm quality and membrane integrity, its inability to influence sperm output limits its potential as a standalone fertility enhancer.

The correlation patterns observed in this study demonstrate that at 0 mL PNLE, scrotal biometry, particularly scrotal length, showed a significant negative correlation with sperm concentration both in the testes and epididymis. This is contrary to reports by Ugwu (2009), who observed positive associations between scrotal measurements and sperm reserves in bucks, indicating that factors other than testicular size might be influencing spermatogenesis in these goats under non-supplemented conditions. However, at 4 mL PNLE administration, these negative correlations were attenuated, with body weight and scrotal biometry showing generally weak and non-significant correlations with sperm concentration, suggesting a possible stabilizing or modulatory role of PNLE on reproductive physiology. This altered relationship may be linked to the significant improvement in semen

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antioxidant profiles observed in Table 3, where PNLE supplementation resulted in higher catalase and lower lipid peroxidation levels, which are indicative of improved oxidative status in the semen environment. This could suggest that while PNLE did not directly enhance sperm concentration (Table 4), it may have indirectly improved semen quality by mitigating oxidative stress, thereby altering the dynamics between scrotal traits and sperm output. This observation is in agreement with the reports of Gharagozloo and Aitken (2011) and Cyrus *et al*. (2015), which emphasize that antioxidants can improve semen oxidative balance, potentially influencing sperm viability and motility without necessarily increasing sperm count.

The findings from Tables 7 and 8, which depict the correlations between plasma and semen antioxidant parameters at different PNLE dosages (0 mL vs. 4 mL), offer significant insights into oxidative stress and antioxidant defense systems. The observed negative correlations between plasma catalase and semen SOD (Table 7: r= -0.973, p <0.01) suggest an inverse relationship between plasma and semen antioxidant systems. In Table 7, a strong negative correlation between plasma MDA and semen TAC (r= -0.990, p <0.01) was observed, suggesting that as lipid peroxidation increases in plasma, the semen's TAC decreases. This finding aligns with the literature, which demonstrates that oxidative stress, marked by elevated MDA levels, typically leads to a decrease in semen's ability to neutralize ROS, as seen in studies on human and animal sperm (Afzal *et al*., 2023). Moreover, similar negative correlations between MDA and TAC have been reported in livestock, where increased ROS levels in semen correspond to reduced antioxidative defense (Kou *et al*., 2022). The shift in antioxidant correlations at 4 mL in Table 8—with generally weaker correlations between plasma and semen antioxidants—may indicate that a higher dose of PNLE provides an external antioxidant source that reduces the need for compensatory adjustments between plasma and semen antioxidant systems. This could suggest that PNLE supplementation alters the oxidative balance, as indicated by stronger positive correlations within the semen compartment, such as between semen TAC and semen LP (r= 0.991, p <0.01). Similar findings have been observed, where antioxidant supplementation in the form of vitamins or plant extracts improves semen quality by reducing lipid peroxidation and enhancing semen's antioxidant capacity (Georgieva, 2024).

CONCLUSION

The present study demonstrates that *Parquetina nigrescens* leaf extract supplementation influences reproductive indices in West African Dwarf bucks, as evidenced by improvements in sperm biometry, antioxidant parameters, and testicular and epididymal sperm concentrations. Blood plasma and semen antioxidant responses varied with PNLE administration, with enhanced semen antioxidant capacity observed at the administration of 4 mL PNLE, suggesting protective effects against oxidative stress. Additionally, correlations between body weight and scrotal parameters, as well as between plasma and semen antioxidants, indicating that PNLE modulates the systemic and reproductive oxidative balance, potentially improving semen quality and fertility.

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AUTHORS' CONTRIBUTIONS

Lois Chidinma Ndubuisi-Ogbonna (LCN), Oluwafikunayo Fareed Noibi (OFN), Oluwaseyi Emmanuel Oso (OEO) and Adeyinka Oye Akintunde (AOA).

LCN, OFN, OEO and AOA: Management of experimental animals, data collection, data management and data analysis. AOA: Conceptualization, design of the experiments,

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manuscript writing and data analysis, LCN: Visualization, manuscript review and final approval of manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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