Research Article

Leverage of *Moringa oleifera* on Blood Profile and Sperm Quality in Duroc Boars

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Abstract | Semen quality and quantity are essential for successful boar herd management. Oxidative stress, nutritional imbalances, and inflammation can impair boar fertility and semen quality. We investigated the impact of *Moringa oleifera* leaf extract (MOLE) supplementation on blood parameters, serum antioxidant status, and sperm quality in 24 Duroc boars at 1.0-1.5 years. The boars were randomly divided into four groups of three replicates each and their feed was supplemented with MOLE at concentrations of 0, 10, 50, and 250 mg/kg body weight (BW). Supplementing Duroc boras with MOLE increased leukocyte, erythrocyte, and hemoglobin levels, and significantly influenced serum antioxidant concentrations. The 50 mg/kg showed the highest serum antioxidant capacity, followed by 10 mg/kg. The boars in the MOLE-supplemented group exhibited a significant (P=0.004) reduction in the percentage of total sperm abnormalities. MOLE in the diet can notably enhance blood parameters in breeding boars and improve antioxidant status and sperm quality.

Keywords | Antioxidant status, Blood indices, Boars, *Moringa oleifera leaf* extract, Sperm quality

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INTRODUCTION

Moringa oleifera (MO), known as the miracle tree, has gained significant attention due to its impressive nutritional and medicinal properties (Islam et al., 2021). Rich in vitamins, minerals, antioxidants, and bioactive compounds, MO has a long history of use in traditional medicine for a wide range of health benefits (Kashyap et al., 2022). Recently, research has expanded to investigate its potential effects on animal health and performance (Abd El-Hack et al., 2018), including its impact on blood parameters, antioxidant capacity, and reproductive factors in pigs (Serem et al., 2017; Sun et al., 2022). Animal studies have shown that MOLE can act as antioxidants. MOLE increased glutathione peroxidase (GPx) levels in the testicles (Akunna et al., 2012; Mansour et al., 2020) and decreased the activity of glutathione S-transferase (GST) (Elblehi et al., 2019). MO significantly raised catalase (CAT) and superoxide dismutase (SOD) while reducing malondialdehyde (MDA) levels (Mansour et al., 2020). MO contains abundant antioxidants, including phenolic compounds, flavonoids, and vitamins C and E, which have been shown to combat oxidative stress and improve semen quality in both humans and various animal models (Halaby et al., 2013; Joung et al., 2017; Peñalver et al., 2022; Mohlala et al., 2023).

MOLE demonstrated beneficial impacts on the semen

qualities and surviving spermatozoa of male rats (Ododo et al., 2019), count, motility, and a reduction in sperm abnormalities (Elblehi et al., 2019; Mansour et al., 2020), as well as a decrease in sperms with morphological damage (Akunna et al., 2012). *Moringa oleifera* leaf (MOL) has demonstrated protective properties in spermatogonial cells and can mitigate cell damage in mice exposed to cyclophosphamide injections (Nayak et al., 2016). Additionally, the hexane extract derived from MOL has been observed to enhance the performance of seminiferous tubules, epididymis, testes, and seminal vesicles in male mice (Cajuday and Pocsidio, 2010). The testosterone levels of male rats supplemented with MO also increase (Akunna et al., 2012; Elblehi et al., 2019). MOLE boosted the concentration of serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone (Ododo et al., 2019; Mansour et al., 2020). MO supplements may positively affect boar health and reproductive parameters by enhancing hematological aspects, bolstering antioxidant capabilities, and positively influencing sperm characteristics.

The boar population plays a central role in the swine industry, exerting a significant influence over genetic advancements and the overall productivity of pig populations. Maintaining boars' well-being and reproductive capability cannot be overstated, given their direct impact on seminal quality and quantity– a critical aspect in the success of breeding programs (Lopez Rodriguez et al., 2017). However, the intricacies of boar reproductive health are subject to a range of factors that can hinder their capability. Oxidative stress, inflammation, and nutritional deficiencies can compromise boar vitality and semen quality (Pintus and Ros-Santaella, 2021; Rungruangsak et al., 2021).

Delving into the potential of MOL as a viable supplement to enhance boar health and reproductive prowess emerges as a promising avenue in the swine industry. Discovering the inherent benefits encapsulated within this "miracle tree" can unlock a wealth of knowledge poised to elevate boar well-being. Thus, we aimed from the current study to investigate the effects of MOLE-supplementation on blood parameters, serum antioxidant status, and sperm quality.

MATERIALS AND METHODS

Moringa oleifera leaf extract

Fresh MOLs were collected from Thailand's Ayutthaya, Phitsanulok, and Phichit provinces. The fresh leaves were immediately cleaned, dried at 40°C, ground into coarse powder and extracted with 95% ethanol (PC drug, Bangkok, Thailand), filtered, and lyophilized to obtain the crude extract. The percentage yield of the extract was 26.05%. The extract was stored at -20°C in a dark container for further experiments.

MOL wet granulation

MOL wet granulation was performed by blending the MOL extract with maltodextrin (PC drug, Bangkok, Thailand), and microcrystalline cellulose PH101 (PC drug, Bangkok, Thailand), using the Moisture-Activated Dry Granulation technique (Shanmugam, 2015).

Determination of total phenolic content

The total phenolic content (TPC) was determined using the Folin-Ciocalteu's method as previously described (Zhu et al., 2010; Agbor et al., 2014). Briefly, A standard calibration curve of gallic acid was prepared. Twenty µL of the MOLE (1 mg/mL) was mixed with 100 µL of Folin-Ciocalteu's reagent (Merck, Darmstadt, Germany) in the dark for 5 min. Subsequently, 80 µL of Na_2CO_3 (Sigma Aldrich, MO, USA) was added to the samples, followed by incubation for 30 min at room temperature in dark place. The absorbance was measured at a wavelength of 765 nm using Varioskan™ LUX multimode microplate reader (Thermo Scientific, USA). The TPC was determined using the calibration curve established with gallic acid (Sigma Aldrich, MO, USA) as the standard. The results were expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract).

Determination of total flavonoid content

The MOLE's total flavonoid content (TFC) was assessed using the aluminum chloride colorimetric method (Umbert et al., 2020). Briefly, catechin (Sigma Aldrich, MO, USA) was first prepared as a standard flavonoid solution in absolute ethanol. Five hundred microliters of the tested samples were mixed with 75 µL of 5% $NaNO_2$ (Sigma Aldrich, MO, USA), 150 µL of 10% $AlCl_3$ (Sigma Aldrich, MO, USA), 500 µL of 1 M NaOH (Sigma Aldrich, MO, USA) and 275 µL of distilled water, respectively, in a centrifuge tube. One hundred microliters of the mixture were then transferred into 96 well-plate and incubated at room temperature for 30 minutes. The absorbance was measured at a wavelength of 510 nm using Varioskan™ LUX multimode microplate reader (Thermo Scientific, USA). The TFC was determined using the calibration curve established with catechin as the standard. The results were expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g extract).

Determination of DPPH radical scavenging activity

The antioxidant properties of MOLE were assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay according to the modified method described by Brand-Williams et al. (1995). Briefly, 180 µL

of a 0.2 mM DPPH solution (Sigma Aldrich, Saint Louis, USA) was combined and incubated with 12 µL of the samples (4 mg/mL), Trolox (Sigma Aldrich, MO, USA) (in six different serial dilutions ranging from 0 to 125 µM), and 50% methanol (blank) individually in a 96-microwell plate for 30 min at 37°C in the dark. Subsequently, the absorbance value for each mixture were measured at 517 nm using Varioskan™LUX multimode microplate reader (Thermo Scientific, USA). Trolox was used as positive control. The results were expressed as µM Trolox equivalent/mg of sample (µM TE/mg sample).

Analysis of volatile compounds using gas chromatography-mass spectrometry (GC-MS)

The ethanol extract from MOL was analyzed using a GC7890B and an MSD 5977A mass detector (Agilent Technologies, Inc., California, USA). Helium was used as the carrier gas at a flow rate of 1 mL/min;1 µL of the sample supernatant was injected into the GC. The GC oven temperature was programmed to increase from 80 to 200°C at a rate of 15°C/min, and then increase from 200 to 280°C at 5 °C/min, with a final hold of 5 min at 280°C. The ionization voltage and the ion source were established at 70 eV and 230°C, respectively (Ibrahim et al., 2021).

Analysis of phenolic compounds profile using high-performance liquid chromatography (HPLC)

The study was conducted using a High-Performance Liquid Chromatography (HPLC) model 20AT (Shimadzu Corporation, Kyoto, Japan) system operating in isocratic mode with a 20 µL injection loop. Signal detection was achieved using a UV-Vis detector model SPD-20A (Shimadzu Corporation, Kyoto, Japan). The analytical conditions involved a mobile phase methanol: water (70: 30), a flow rate of 0.7 mL/min, a 20 µL sample injection, and a 15-minute analysis time. The column used was an HPLC Column Hypersil GOLD (Thermo Scientific™, Massachusetts, USA) with dimensions of 4.6 x 250 mm and a particle size of 5 µm. The standards used for preparation were cryptochlorogenic acid (4-O-caffeoylquinic acid), isoquercetin (quercetin 3-β-D-glucoside), and kaempferol (kaempferol 3-β-D-glucopyranoside) (Sigma Aldrich, MO, USA). Sample preparation involved weighing 0.0020 g of the sample, dissolving it in 1 mL of ethanol, and then filtering it through a 0.22 µm syringe filter.

Animals, general management, and experimental design

We used 24 Duroc boars at 1-1.5 years with an average weight ranging from 135 to 160 kg. The study was conducted on a commercial swine herd in the central region of Thailand from March to May 2021, which is the summer season. The boars were fed twice daily, receiving 2.5 kg of commercial feed per day. The feed was a standard boar diet (Hygro 568F, Charoen Pokphand Foods Public Company Limited, Bangsaothong, Samutprakarn, Thailand) that was specifically formulated for boars and contained 3,000 kcal of energy per kg, 14.0% crude protein, 5.8% fat, 5.8% crude fiber, 0.65% lysine, 0.85% calcium, and 0.35% phosphorus. To evaluate the effectiveness of MOLE-supplementation, the boars were randomly allocated to four experimental groups of three replicates each. The treatment groups were as follows: G1: commercial diet (control); G2: commercial diet + 10 mg MOLE/kg BW; G3: commercial diet + 50 mg MOLE/kg BW; and G4: commercial diet + 250 mg MOLE/kg BW. The MOLE quantities were based on the research findings of Prabsattroo et al. (2015). The MOLE was supplemented into each feed by topping up the commercial feed daily (Liao et al., 1985; Kaewma et al., 2021). The testing period was eight weeks. Water was available freely through drinking nipples. The boars were housed individually in pens measuring 9m^2 per boar, within a conventional open housing system that followed the Thai good agricultural practices for pig farms (Ministry of Agriculture and Cooperatives, 2009). The average temperature and humidity were approximately 26.21 to 36.18°C and 88.87%, respectively. The boars were vaccinated and dewormed before the start of the experiment, following the recommendations of the Department of Livestock Development, Thailand. All the boars included in this study were established sires frequently used for artificial insemination. Their participation was conducted with the official approval of the Institutional Animal Care and Use Committee (IACUC), following the Rajamangala University of Technology Suvarnabhumi regulations, as outlined in animal use protocol No. IAU-RUS63-006.

Blood sample collection and analyses

Blood samples were collected from the ear vein of all boars on days 28 and 56 of the experiment for serum oxidation analysis and day 56 for hematological analysis. Two types of tubes were used: The first tube was treated with ethylenediamine tetra-acetic acid (EDTA) and used for hematological parameters such as red blood cells (RBC), white blood cells (WBC), hemoglobin, hematocrit, platelets, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), and they were assessed using a hematological analyzer (Cell Dyn 3700, Wiesbaden, Hesse, Germany). The second tube was collected without EDTA, centrifuged at 3,000 rpm/ 10 min, and the serum was aliquoted into tubes and stored at -20°C until serum antioxidant levels measurement.

Serum antioxidant assays

Superoxide dismutase (SOD) levels were assessed following Nishikimi et al. (1972). This method relies

on SOD's ability to hinder the reduction of nitroblue tetrazolium dye mediated using phenazine methosulphate. The Malondialdehyde (MDA) assay is grounded in the reaction of thiobarbituric acid with MDA under acidic pH conditions at 95°C for 30 min, resulting in a thiobarbituric acid-reactive product (Satoh, 1978). The optical density of the resultant pink hue was measured at 534 nm using Varioskan™LUX multimode microplate reader (Thermo Scientific, USA). Serum glutathione (GSH) was measured using the method of Beutler et al. (1963). The values of GSH were expressed as µmol/ml.

Boar semen collection and analyses

Ejaculates were collected manually using the gloved-hand technique at one-week intervals for four weeks before the start of feeding and from the eighth week onwards after the start of feeding. Sampling was performed in the morning before feeding at approximately 6:00 to 7:00 AM. Eight ejaculates were collected from each of the twenty-four boars for a total of 192 samples. The semen collection was performed by a trained technician and performed by the modified method described in Kondracki et al. (2021). Briefly, the ejaculates from the boars were collected and were filed to discard the gel fraction. The freshly collected ejaculates were immediately transferred to the laboratory, where they underwent standard laboratory assessment for ejaculate volume (mL), percentage of sperm motility, and sperm concentration (×10^6 sperm per mL) photometrically using a SpermaCue Porcine photometer (Minitube, GmbH, Tiefenbach, Germany) at a light wavelength of 0.7 µm. In the laboratory, semen was smeared and stained with Giemsa (Blom, 1973), then sperm morphologies were examined for sperm abnormality rate (%) (Watson, 1975).

Statistical analysis

A completely randomized design (CRD) was employed. We used Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test, following the guidelines outlined by SAS Institute Inc. (SAS Institute, Cary, NC, USA), at a significance level of 95%. The results present the least square mean values for various treatment groups, their corresponding SEM values and the associated probabilities of error (*P*-values).

RESULTS AND DISCUSSION

Total phenolic, total flavonoid content, and antioxidant activity of MOL extract

The MOLE exhibited a total phenolic content of 38.26±2.23 mg GAE/g extract and a total flavonoid content of 12.54±0.98 mg QE/g extract. Notably, the flavonoid content was lower than that of phenolic in the ethanolic extraction. Additionally, the antioxidant capacity of the MOLE was measured at 17.69±1.07 µM TE/mg extract.

Gas chromatography-mass spectrometry and high-performance liquid chromatography analysis

GC-MS analysis of the ethanol extract of MOL resulted in detecting of 87 compounds, of which hydroxyprogesterone showed the highest peak area. Hydroxyprogesterone was the major compound identified, followed by vitamin E, octadecatrienoic acid, and di-anhydromannitol (Table 1, Figure 1). Isoquercetin, astragalin, and cryptochlorogenic acid, the major active antioxidant compounds in MOLE, were separated and identified by chromatographic and spectroscopic techniques and yielded 1.085, 0.907, and 0.843% (w/w) of MOLE, respectively.

Table 1: Gas chromatography-mass spectrometry analysis of MOLE.

No	Name of the compound	Molecular formula	Retention time	Peak area (%)
1	Hydroxyprogesterone	$C_{27}H_{40}O_4$	72.84	75.34
2	Vitamin E (Tocopherol)	$C_{29}H_{50}O_2$	73.71	59.68
3	Octadecatrienoic acid	$C_{20}H_{34}O_2$	50.33	38.03
4	Dianhydromannitol	$C_6H_{10}O_4$	19.04	32.30

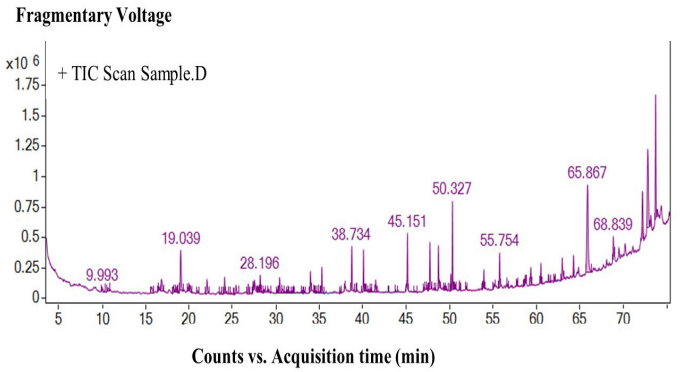

Figure 1: Gas chromathography-mass spectrometry chromatogram of MOLE used in the diet of boar.

Hematological characteristics

The supplementing the diet with 50 mg of MOLE/kg BW (Table 2) significantly (P<0.05) increased WBCs, RBCs, neutrophils, hemoglobin concentration, MCV, MCH, and mean MCHC.

Serum antioxidant activity

The results in Table 3 showed that a dose of 50 mg/kg BW reduced MDA when supplemented in the diet for four weeks, and it affected the overall serum antioxidant activity in the eighth week. Boars receiving 50 mg/kg of BW of MOLE exhibited the highest SOD and GSH activity. Furthermore, this group had lower levels of MDA.

Sperm qualities

Supplementing the diet with MOLE (Table 4) did not affect sperm quality parameters, except for the total sperm

Table 2: Effect of MOLE supplementation for 8 weeks on blood hematological parameters of boars.

Items	MOLE (mg/kg BW)				p value
	0	10	50	250	
RBC (10^{12}/L)	4.66±0.41^{b}	4.64±0.17^{b}	5.59±0.18^{a}	4.57±0.36^{b}	0.037
Hemoglobin (g/L)	9.47±0.25^{b}	9.63±0.25^{a}	9.84±0.06^{a}	8.89±0.57^{c}	<0.001
Hematocrit (L/L)	30.45±0.89^{a}	30.24±0.26^{a}	31.25±0.19^{a}	28.29±0.26^{b}	<0.001
MCV (fL)	54.31±0.89^{b}	54.94±0.27^{b}	55.92±0.36^{a}	51.42±0.57^{c}	<0.001
MCH (pg)	16.66±0.32^{b}	16.46±0.44^{b}	18.25±0.20^{a}	15.83±0.21^{b}	<0.001
MCHC (g/dL)	30.48±0.59^{c}	31.05±0.28^{ab}	32.88±0.13^{a}	30.12±0.24^{c}	0.001
WBC (10^{9}/L)	15.72±0.33^{b}	15.37±0.51^{b}	16.32±0.27^{a}	13.88±0.35^{c}	<0.001
Neutrophils (%)	25.67±1.15^{a}	26.67±2.08^{a}	27.67±4.51^{a}	15.33±1.53^{b}	0.017
Lymphocytes (%)	69.67±1.53	66.33±4.04	72.00±3.61	77.33±3.06	0.878
Monocytes (%)	3.00±1.73	2.67±0.58	2.33±0.58	2.67±0.58	0.452
Eosinophils (%)	1.33±0.58	1.67±1.15	1.00±1.00	2.33±1.15	0.512
Basophils (%)	0.43±0.12	0.50±0.20	0.30±0.20	0.37±0.12	0.478

0: commercial diet (control); 10: commercial diet + 10 mg MOLE/kg BW; 50: commercial diet + 50 mg MOLE/kg BW; 250: commercial diet + 250 mg MOLE/kg BW. RBCs, Red blood cells; MCV, Mean corpuscular volume; MCH, Mean corpuscular haemoglobin; MCHC, Mean corpuscular; WBCs, White blood cells. $^{a, b, c}$ Means in the same row followed by different letters differ significantly (P≤0.05).

Table 3: Effect of MOLE- supplementation on plasma antioxidant status of boars.

Items	MOLE (mg/kg BW)				p value
	0	10	50	250	
Week 4					
SOD (U/ml)	2.47 ± 0.18	2.75 ± 0.25	2.85 ± 0.35	2.64 ± 0.38	0.184
MDA (nmol/ml)	10.08 ± 0.52^{a}	9.52 ± 0.24^{b}	9.39 ± 0.30^{b}	9.64 ±0.22^{ab}	0.014
GSH (µmol/ml)	2.04 ± 0.17	2.16 ± 0.15	2.34 ± 0.21	2.18 ± 0.18	0.068
Week 8					
SOD (U/ml)	2.54 ± 0.33^{c}	3.30 ± 0.28^{b}	3.76 ± 0.46^{a}	3.06 ± 0.34^{b}	0.001
MDA (nmol/ml)	10.87 ± 0.31^{a}	9.58 ± 0.31^{b}	9.42 ± 0.28^{b}	10.18 ± 0.67^{a}	<0.001
GSH (µmol/ml)	2.09 ± 0.26^{c}	2.76 ± 0.27^{b}	3.51± 0.29^{a}	3.08 ± 0.48^{a}	<0.001

0: commercial diet (control); 10: commercial diet + 10 mg MOLE/kg BW; 50: commercial diet + 50 mg MOLE/kg BW; 250: commercial diet + 250 mg MOLE/kg BW. SOD, Superoxide dismutase; MDA, Malondialdehyde; GSH, Glutathione. $^{a, b, c}$ Means in the same row followed by different letters differ significantly (P≤0.05).

Table 4: Effect of MOLE-supplementation on boar semen qualities.

Items	MOLE (mg/kg BW)				p-value
	0	10	50	250	
Week 4					
Volume (ml)	204.39 ± 6.97	217.59 ± 8.03	218.80 ± 3.49	213.86 ± 8.62	0.086
Concentration (×10^{6} sperm per ml)	297.66 ± 26.23	313.31 ± 17.19	314.01 ± 14.89	309.57 ± 8.17	0.379
Individual motility (%)	81.06 ± 0.67	81.95 ± 0.83	82.20 ± 0.71	81.42 ± 0.86	0.075
Total sperm (abnormality (%)	4.61 ± 0.85	3.82 ± 0.19	3.75 ± 0.83	4.12 ± 0.59	0.140
Week 8					
Volume (ml)	210.04 ± 10.20^{c}	225.88 ± 10.84^{ab}	234.75 ± 14.11^{a}	216.14 ± 10.85^{bc}	0.007
Concentration (×10^{6} sperm per ml)	307.82 ± 10.80	317.19 ± 9.17	321.63 ± 13.77	309.62 ± 28.10	0.481
Individual motility (%)	81.58 ± 0.90	81.92 ± 0.42	82.44 ± 0.59	81.95 ± 0.58	0.185
Total sperm abnormality (%)	4.54 ± 0.73^{a}	3.77 ± 0.58^{bc}	3.24 ± 0.18^{c}	4.13 ± 0.58^{ab}	0.004

0: commercial diet (control); 10: commercial diet + 10 mg MOLE/kg BW; 50: commercial diet + 50 mg MOLE/kg BW; 250: commercial diet + 250 mg MOLE/kg BW. SOD, Superoxide dismutase; MDA, Malondialdehyde; GSH, Glutathione. $^{a, b, c}$ Means in the same row followed by different letters differ significantly (P≤0.05).

abnormality rate. The group receiving 50 mg of MOLE/kg BW exhibited a lower (P< 0.01) total sperm abnormality rate compared to the 10, 250 mg, and control groups.

Moringa oleifera is a renowned plant widely recognized for its extensive use in traditional medicine due to its diverse pharmacological properties. Recent investigations have emphasized its impressive role as a modulator with antioxidative, and anti-inflammatory potential (Zeng et al., 2019), and its ability to improve fertility markers (Greish et al., 2021). Within MOL, a variety of chemical compounds possessing antioxidant, anti-carcinogenic, and antimicrobial attributes have been identified, including tannins, saponins, glycosides, terpenoids, and flavonoids (Ayoola et al., 2008; Mahmood et al., 2010). MO extract is particularly rich in phenolic compounds such as quercetin, kaempferol, and rutin, which are classified as flavonoid compounds. The results reveal that flavonoid content was lower than phenolic content in all fractions, substantiating the theoretical understanding that flavonoids are a subgroup of phenolics contributing to the overall phenolic composition (Dai and Mumper, 2010).

Research on organic compounds derived from plants and their associated functions is on the rise, primarily due to their potential as sources of innovative pharmaceuticals. GC-MS has emerged as a valuable tool for reliably identifying bioactive components in plant-based investigations (Balamurugan et al., 2015). Among the key compounds detected in the MOLE are hydroxyprogesterone and vitamin E. Hydroxyprogesterone is a crucial protein in synthesizing steroid hormones through the Δ4 or Δ5 pathway. In the Δ4 pathway, the C27 cytochrome P450 side-chain cleavage enzyme (CYP11A1) governs the conversion of cholesterol into pregnenolone. Subsequently, pregnenolone enters the smooth endoplasmic reticulum, where it is further transformed into progesterone, under the regulation of 3β-HSD. The 17-alpha (α)-hydroxylase enzyme (CYP17A1) catalyzes the conversion of progesterone into 17-alpha (α)-hydroxyprogesterone, which is then further metabolized into androst-4-ene-3,17-dione. Finally, the conversion of androst-4-ene-3,17-dione into the testosterone hormone is regulated by 17-beta hydroxysteroid dehydrogenase (Clark and Stocco, 2014; Beattie et al., 2015; Chung et al., 2020).

Vitamin E is also a vital nutrient for male fertility. It is a precursor to specific substances such as prostaglandins, thromboxanes, leukotrienes, and immunoglobulins. Consequently, these substances promote spermatogenesis and maintain acrosomal integrity (Umesiobi, 2009). In sheep, vitamin E enhanced spermatogenesis by controlling the expression of proteins like FLNA, SPCS3, YBX3, and RARS, which are linked to the plasma membranes and the production of protamines in sperm cells (Gao et al., 2021). The presence of hydroxyprogesterone and vitamin E in MOLE may benefit male fertility.

Based on the HPLC chromatogram, the main compounds identified in the MOLE are isoquercetin, astragalin, and cryptochlorogenic acid. This follows the findings of Vongsak et al. (2014), an HPLC quantitative analysis using MOL collected from various regions of Thailand. However, it differs from the studies of Verma et al. (2009) and Amaglo et al. (2010). They reported the major compounds of MOL in Ghana and India were rutin and chlorogenic acid. This variation may be due to differences in plantation regions or the genetic diversity of MOL. Isoquercetin is a monoglycosylated derivative of quercetin, found in various plants, including MO. Upon ingestion, isoquercetin accumulates in the intestinal mucosa more than quercetin, where it is converted to quercetin. Isoquercetin and quercetin have anticoagulant, immunomodulatory, anti-inflammatory, and antioxidant properties, as evidenced by physiological studies (Mbikay and Charitien, 2022).

Our findings indicated that dietary MOLE-supplementation improved blood indices such as RBCs, neutrophils, hemoglobin concentration, MCV, MCH, and MCHC concentration, and this influence might stem from a combination of antioxidative properties and regulation of metabolism-involved pathways (Al-Masruri et al., 2022; Al-Mufarji and Mohammed, 2022). Adding MOLE to boars' diets at 50 mg/kg BW led to a significant rise in RBC count on day 56. Conversely, supplementing MOLE at quantities of 10 and 250 mg/kg BW in the boars' diet did not impact RBC count compared to the non-supplemented group. This observation aligns with the study by Serem et al. (2017), which experimented with supplementing dried MOL at varying levels in pig diets. They found that a 6% supplementation positively influenced RBC count (8.70 ± 0.25 x 10^6/mm^3), while a 12% supplementation trended toward reducing RBC count (7.10 ± 0.09 x 10^6/mm^3), although not statistically different from the non-supplemented boars. The variations in the effects of increasing RBC count with MOLE supplementation could be attributed to the increase in iron content or the level of folates in the bloodstream, potentially promoting the release of immature RBCs into circulation, leading to a decrease in MCV (Al-Mufarji and Mohammed, 2022). Furthermore, the quercetin content in MOL can enhance iron absorption in the apical enterocyte region while decreasing iron efflux in the basolateral area and reducing the expression of ferroportin (FPN) in the intestine. Over the long term, excessive amounts of quercetin and polyphenols can also function as iron chelators (Lesjak et al., 2014).

When boars were provided with a supplementary diet containing MOLE at a dose of 50 mg/kg BW over 56

days, it was observed that the extract supplementation positively impacted the WBC count and the percentage of neutrophils. This aligns with Gupta et al. (2012), who revealed that MOL is rich in vitamins and proteins that contribute to developing the animal's immune system, including cells responsible for safeguarding the body against foreign agents. The heightened levels of immune system cells resulting from the supplementation of MOLE or leaves in the diet could be attributed to increased in WBC precursors. This prepares the animals' bodies to fend off foreign agents, as evidenced by the research conducted by Gaikwad et al. (2011) and Stohs and Hartman (2015). MOL has been found to stimulate both cellular and humoral immune systems, which can be attributed to its content of flavonoids, polyphenols, and terpenoids, all of which promote immune system activity (Mousa et al., 2019). MOL's enhancement of granulocytes, which play a protective role against bacterial infections, results in MOL exhibiting inhibitory properties against various bacterial strains. This results in the suppression of bacterial growth and ultimately leads to reduced levels of these immune cells (Serem et al., 2017). MO has been reported to diminish the production of TNF-α, IL-6, and IL-8 in various disease conditions, confirming MO as an effective therapy for inflammatory reactions (Kou et al., 2018; Abubakar et al., 2023).

Antioxidants and reactive oxygen species (ROS) are involved in male infertility. Spermatozoa are exceptionally vulnerable to ROS due to immanent limitations in the activity of intracellular antioxidant enzymes, making the body's antioxidant capacity crucial for sperm protection (Ayad et al., 2022). The supplementation of MOLE at 50 mg/kg BW significantly impacted the overall serum antioxidant activity compared to boars without extract supplementation. This finding is follows those of Verma et al. (2009), who administered MOLE at 50 and 100 mg/kg BW to rats exposed to CCl_4. Their study demonstrated that supplementing with 50 mg/kg BW of MOLE led to increased SOD levels in the blood of rats subjected to CCl_4, while supplementation with 100 mg resulted in even higher SOD levels in the bloodstream, similar to the group supplemented with 50 mg of vitamin E/kg BW. These results are due to the presence of antioxidative compounds including catechins and flavonoids in MOLE (Benavente-Garcia et al., 1997).

In sequence, the addition of MOLE-supplementation resulted in an increased in serum MDA. Correspondingly, a study by Verma et al. (2009) reported reduced MDA levels in animals following the administration of MO, which aligns with our findings. The beneficial effects of MO may arise from its ability to mitigate oxidative stress and enhance antioxidant capacity. This effect could be attributed to the phytochemical composition of MO, initiating a process of recovery from associated tissue damage. The supplementation of MOLE led to a substantial increase in glutathione levels in the blood circulation of boars over 56 days, demonstrating a significant alignment with the report by Cui et al. (2018). Their research uncovered that supplementing MOL at levels of 1, 2, 5, 10, and 15% over 21 and 42 days resulted in heightened glutathione levels in the blood of broiler chickens. Glutathione and protein thiols protect sperm plasma membrane lipids from oxidation and inhibit the formation of free radicals during the reconstruction of thiol groups (–SH). Glutathione deficiency may cause decreased sperm motility by deteriorating the midpiece of spermatozoa (Walczak-Jedrzejowska et al., 2013).

According to Zeng et al. (2019), supplementing 4 and 8% MOL in the diet of one-month-old mice for 30 days reduced the abnormality rate of sperm bodies compared to those not receiving MOL supplementation. In this study, supplementing MOLE for boars at 10, 50, and 250 mg/kg BW for eight weeks did not reveal significant differences in semen concentration or individual motility compared to the control. However, supplementing 50 and 10 mg MOLE/kg BW for eight weeks led to a reduction in total sperm abnormality in agreement with the findings of Prabsattroo et al. (2015). Moreover, during the 8th week, boars receiving MOLE supplements at 10 and 50 mg/kg BW exhibited a significantly higher semen volume than the other groups. These findings are in accordance with those of Ajuogu et al. (2018), who observed that MO extracts also increased sperm count, motility, and semen volume in post-pubertal rabbits. Furthermore, MO significantly increased serum testosterone levels, the primary androgen in the testis that regulates spermatogenesis and upregulates expressions of follicle-stimulating hormone and luteinizing hormone (Khalifa et al., 2016). Zheng et al. (2019) found that MOLE reduced the occurrence of sperm abnormalities and the expression of Bcl2-associated X protein (Bax) in the testes. Moichela et al. (2021) revealed that MO inhibited the development of intracellular sperm ROS, reduced the proportion of sperm with fragmented DNA, and elevated the proportion of immotile and acrosome-intact spermatozoa.

Our study found that dietary MOLE-supplementation significantly improved blood parameters, antioxidant status, and sperm quality in breeding boars for eight weeks of supplementation. In boars, the cyclic rhythm of spermatogenesis consists of individual cycles lasting 8.6–9.0 days, culminating in an overall spermatogenic duration of about six weeks (França et al., 2005). This study is the first to investigate the effects of MOLE supplementation in the form of granules in the diet of Duroc boars. Previous studies on MOLE supplementation have typically investigated the effects of MOLE on the semen

quality of boars extended with Beltsville thawing solution (BTS) (Omodewu et al., 2021). Recently, Shai et al. (2023) reviewed evidence that MOLE supplementation for two weeks at 100–300 mg/kg BW levels improved sperm characteristics in male ruminants.

CONCLUSIONS AND RECOMMENDATIONS

Our investigation explored the beneficial effects of MOLE, including blood indices, antioxidant capacity, and sperm quality in boars, particularly in the group supplemented with 50 mg MOLE /kg BW. We highlighted the positive influence of dietary MOLE on the overall health of boars. Incorporating MOLE into the diet resulted in elevated levels of leukocytes, erythrocytes, hemoglobin, and serum antioxidants, demonstrating its potential to enhance various physiological markers. MOLE-supplementation exhibited a positive correlation with improved semen quality in boars. The dietary inclusion of MOLE increased boar serum SOD and GSH levels, reinforcing antioxidant defenses. MOLE-supplementation was associated with a marked reduction in sperm abnormalities, suggesting a potential enhancement in sperm quality.

It is imperative to acknowledge that while our study sheds light on the promising effects of MOLE, there is still room for further exploration. The impact of MOLE on swine production remains multifaceted and calls for continued investigation to uncover its comprehensive potential.

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NOVELTY STATEMENT

To our knowledge, this is the first study of the supplementation of MOLE to improve sperm qualities in Duroc boars.

AUTHOR'S CONTRIBUTION

WK: Formal analysis, methodology, validation, visualization, writing review, and editing.
KM: Conceptualization, data curation, formal analysis, funding acquisition resources, investigation, methodology, resources, software, supervision, validation, writing - original draft, project administration.
The final manuscript was read and approved by all authors.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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