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Inclusion of *Moringa oleifera* into Rooster Diets Improves Semen Quality and Fertility under *Escherichia coli* Infection

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ABSTRACT

Reproductive performance in poultry is highly influenced by both nutritional and environmental factors. *Escherichia coli* (EC) infection is one of the most common bacterial challenges that negatively affect male fertility and semen quality in breeder flocks. This research examined the effect of dietary *Moringa oleifera* (MO) supplementation on fertility and semen quality of roosters with EC infections. Four equal groups (n=10) of forty Lohmann roosters were assigned to the following treatments: control (C), moringa therapy (MO), *Escherichia coli* treatment (EC), and moringa plus *Escherichia coli* treatment (MO+EC). The MO and MO+EC groups were provided a baseline diet supplemented with 50g/kg MO powder for six weeks. In contrast, the C and EC rooster groups were administered a baseline diet without moringa therapy. By the end of the sixth week, 0.5mL of a suspension containing 10 million Colony-Forming Units of EC was administered intraperitoneally (IP) to each rooster in the EC and MO+EC groups, whereas 0.5mL of saline was administered IP to the roosters in the C and MO groups. Serum samples were collected one week after infection. Sperm quality and fertility characteristics were assessed using collected semen pools. The results showed that EC infection substantially ($P \leq 0.05$) reduced serum testosterone, antioxidant biomarkers, sperm quality, and reproductive features. The EC-deterioration effects were considerably ($P \leq 0.05$) reversed by MO supplementation. It is determined that MO may be used as a possible natural supplement in roosters' diet to increase fertility and semen output, particularly in cases when EC infection is a problem.

Keywords: *Moringa oleifera*, *Escherichia coli*, Semen quality, Fertility, Rooster chickens

INTRODUCTION

The poultry industry constitutes a major component of people's food supply and economic income worldwide (Kleyn and Ciacciariello 2021). Nevertheless, the endotoxin stress induced by *Escherichia coli* (EC) infection may cause a tremendous economic loss in this sector (Hashem et al. 2022). The infection with EC deteriorates the reproductive performance of chicken breeder males and females (Joseph et al. 2023). Chicken roosters, which are an essential component in the reproduction and preservation of species, could be infected by EC, affecting sperm motion and fertilizing capability (Joseph et al. 2023; Tvrdá et al. 2023). Additionally, some interleukins and cytokines were released by the immune system of EC-infected chickens to initiate inflammation and consequent immune responses (Elnagar et al. 2021). The presence of lipopolysaccharides in EC-membranes may also cause immunosuppression, oxidative stress, and inflammation in the infected birds (da Rosa et al. 2020; Wang et al. 2022).

The tropical *Moringa oleifera* (MO) is known for its possible anti-inflammatory, anti-oxidant, and disease-resistant properties (Singh et al. 2023). MO was incorporated in animal and poultry nutrition as a vital source of proteins, fats, minerals, and vitamins (Amad and Zentek 2022). It was concluded that addition of MO to the poultry diets at a level 1-5% can improve the productive performance of layer chickens (Ruelas et al. 2023) and broiler chickens (Meel et al. 2021; Wahab et al. 2020).

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Particularly, Sebola et al. (2022) reported that including the basal diet with 70% MO leaf meal remarkably increased sperm motility and velocity, and semen concentration and pH in chicken roosters. The beneficial effects of MO on testicular tissues, testosterone secretion and sperm quality have been documented in various animals (Gafer et al. 2023; Liang et al. 2023). Moreover, it was found that MO can modulate the performance of male rats under stress (Abd et al. 2020; Mohlala et al. 2023).

Due to the documented beneficial impacts of MO supplementation in animal and poultry nutrition, it is hypothesized that dietary supplementation with MO would mitigate the detrimental effects of EC infection on the physiological status and reproductive performance of the roosters. However, there is insufficient information regarding the use of MO as a reproductive promoter in poultry species, especially when affected by EC and experiencing endotoxin stress. Thus, the current study aimed to assess whether feeding MO could mitigate the decline in male reproduction among Lohman chicken roosters infected with EC.

MATERIALS AND METHODS

Moringa preparation and analysis

The MO plant was obtained from a greenhouse (The Agricultural and Veterinary Research center, King Faisal University, Saudi Arabia). The MO leaves were separated and put for 2h in an oven for drying at 35°C. The dried leaves were quashed by using a hammermill into a powdered form of MO. The basic nutritional composition of the MO powder was detected using the AOAC procedures (Latimer Jr., 2023). The total phenols, flavonoids, and antioxidant capacity of MO samples were analyzed as previously outlined by Yaméogo et al. (2011). In brief, a methanolic extraction of 100mg MO was first prepared, then complemented with aliquots of sodium carbonate and Folin-Ciocalteu reagent. After 2 hours, the total phenols absorbance in this complement was determined at 765nm based on a gallic acid equivalents (GAE) standard curve. Flavonoids were assessed using a mixture of the methanolic MO extraction with 5% sodium nitrite solution and 10% aluminum chloride. After incubation of each step at 25°C for 5min, 1.0M sodium hydroxide was supplemented to the mixture, and the absorbance was determined at 510nm. Flavonoid content was expressed using a standard quercetin equivalent (QE) curve. The antioxidant capacity of the MO-methanol extract was evaluated using the β-carotene bleaching methodology. A solution of β-carotene linoleic acid was added to the MO extract in a test tube supplemented with distilled water saturated with oxygen. After incubation at room temperature for up to 48h, the inhibition rate of β-carotene coloration was measured at 490nm, comparing the results with those obtained by ascorbic acid and blank as a positive and negative control, respectively. The MO analysis data is displayed in Table 1.

Escherichia coli preparation

The O157:H7 EC strain from the Department of Microbiology and Parasitology at King Faisal University was cultivated for 24hours at 37°C in Oxoid MacConkey broth (Thermo Fisher Scientific Inc., Hampshire, UK). According to the methods outlined by Soliman et al. (2021), the EC cells were harvested, cleaned, and homogenized using a sterile saline solution at a concentration of 2×10^7CFU/mL.

Table 1: Chemical analysis of *Moringa oleifera* powder (MO)

Item	Values per 100g DM
Moisture (g)	3.1
Ash (g)	18.7
Proteins (g)	28.4
Lipids (g)	41.5
Carbohydrates (g)	10.2
Crude fiber (g)	13.4
Gross energy (MJ)	1.2
Calcium (g)	3.1
Phosphorus (g)	0.3
Phenols (mg GAE)	138.6
Flavonoids (mg QE)	15.2
Antioxidant capacity (%)	98.7

GAE, gallic acid equivalent; QE, quercetin equivalent.

Experimental design

Forty Lohmann roosters of 45 weeks old and 2.750kg average weight were granted by Al Wataniya Poultry Co. (Riyadh, Saudi Arabia) for this study. The roosters were placed in separate cages measuring 50×50×60cm and provided with identical ambient conditions of photoperiod (16L:8D), temperature (22–25°C), and unrestricted access to feed and water. The roosters were provided with a basal diet formulation according to NRC standards to meet Lohman's nutritional needs (Table 2). The roosters were distributed into four equal groups (n = 10 roosters per group) consisting of control (C), *Moringa oleifera* treatment (MO), *Escherichia coli* treatment (EC), and MO+EC treatment. The C and EC groups' roosters were given a baseline diet without moringa treatment for six consecutive weeks, while those in the MO and MO+EC groups were given a baseline diet complemented with 50g/kg MO powder. By the end of the sixth week, 0.5mL of a suspension containing 10 million Colony-Forming Units (CFU) of EC was administered intraperitoneally (IP) to each rooster in the EC and MO+EC groups, whereas 0.5mL of saline was administered IP to the roosters in the C and MO groups. A week later, each rooster's brachial vein was used to draw blood into glass tubes and left for 30 minutes to aid in clotting at room temperature. The blood serum was harvested by centrifuging at 2000×g for 10min at 4°C and then stored at −20°C until assay. Furthermore, the roosters' semen was extracted using the dorso-abdominal massage every two days (Elomda et al. 2024). To prevent individual variations, the ejaculates from the roosters in each group were combined (six replicate pools per group) and maintained in a water bath (37°C) in the lab to assess the qualities of the semen, as explained below.

Blood parameters

Testosterone

A chicken ELISA kit (Elabscience Biotechnology Inc., Houston, TX, USA) was utilized to measure testosterone (TST) levels in serum samples under the manufacturer's instructions. To summarize, 50µL of blood samples, standard solutions, or the blank were mixed with the same volume of HRP Conjugate working solution, and then put into microplate wells. After that,

the microplates were put into an incubator at 37°C for 60 minutes. After decanting, the wells were washed five times using 350µL of the washing buffer solution. The wells were filled with 90µL of the substrate reagent and then incubated at 37°C for 20 minutes. The absorbance at 450nm was then explored with a microplate ELISA scanner after adding 50µL of stop solution to each well. There were 5.1% and 4.8% intra- and inter-assay CVs, respectively. The assay's detection range was 0.31–20ng/mL, and its sensitivity was 0.13ng/mL.

Table 2: Nutritional ingredients and composition of the baseline diet

Ingredients	g/kg as Fed
Yellow Corn	600
Wheat bran	126
Barley	90
Soybean meal 44%	74
Wheat	72
Oyster shell	15
Dicalcium phosphate	12.5
Premix *	6
Salt	4.5
Nutritional composition	
Crude protein	122.3
Metabolizable energy (MJ)	11.7
Lysin	4.9
Methionine	2.6
Calcium	9.0
Available phosphorus	3.9

* Vitamins per kg of diet: 13200IU vitamin A, 4200IU vitamin D3, 120IU vitamin E, 6mg vitamin K3, 66mg niacin, 14.4mg riboflavin, 3.0mg thiamin, 18mg pantothenic acid, 2.4mg folic acid, 4.8mg pyridoxine, 0.04mg vitamin B12, and 0.3mg biotin. Elements per kg of diet: 60mg Fe, 148mg Mn, 120mg Zn, 12mg Cu, 2.4mg KI, and 0.36mg Se.

Antioxidant biomarkers

Malondialdehyde (MDA) and total antioxidant capacity (TAOC) levels were assessed using colorimetric test kits (Elabscience Biotechnology Inc., Houston, TX, USA). One millilitre of buffer reagent solution, 2mL of chromogenic reagent, and half a millilitre of ferric salt reagent were added to 100µL of serum in the TAOC test sample tube. After 30 minutes of incubation at 37°C, 100µL of the stop solution reagent was appended to the tubes. After that, 100µL of the serum was added to the control tube. Using a spectrophotometer set at 520nm, the OD was measured after 10 minutes at room temperature. The intra-assay CV was 2.7%, while the inter-assay CV was 8.2%; the detection range was 0.62–145.2U/mL. In glass tubes containing 100µL of the clarity reagent and 3mL of the acid reagent, serum samples, standard solutions, and 100µL of the blank (absolute ethanol) were pipetted for the MDA test. One milliliter of 50% glacial acetic acid was added to the control tube. One milliliter of the chromogenic reagent was added to the tubes. After 40 minutes of incubation at 95–100°C, the tubes were cooled to room temperature using running water. The tubes underwent a 10-minute centrifugation at 3100xg. Using a 1cm optical path cuvette, the supernatant OD was measured at 532nm using a spectrophotometer (CE1010, Cecil Instruments Limited, Cambridge, UK). The intra-assay and inter-assay CVs were 4.9% and 8%, respectively, and the detection range was 0.38–133.33nm/mL.

Inflammatory cytokines

The blood concentrations of tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) were evaluated following the guidelines of ELISA kits specifically designed for poultry species and produced by MyBioSource Inc. in San Diego, California, USA. To summarize, 100µL of standard dilutions or blood samples were placed into the corresponding wells of microplates pre-coated with antibodies. The microplates were then incubated at a temperature of 37°C for one to two hours. 100µL of the first detection reagent were introduced to the mixture after the decantation of the liquid from the wells. The contents were then incubated at 37°C for one hour. After the wells were rinsed three times, 100µL of the second detection reagent were applied. Subsequently, the wells were incubated for an additional thirty minutes at 37°C. Subsequent to five washings, 90µL of the substrate solution were dispensed into each well, then incubated at 37°C for twenty minutes. After adding 50µL of stop solution to each sample well, the microplate was promptly scanned at a wavelength of 450nm. The coefficients of variation (CV) for both the intra-assay and inter-assay were determined to be below 10% and 12%, respectively, for both measurements. TNF-α and IL-1β exhibited 7.8–500pg/mL and 15.6–1000pg/mL of detection ranges, respectively.

Semen processing and evaluation

Volume and concentration

Each group's semen volume was measured using a graduated insulin syringe, and the sperm concentration was measured using a hemocytometer chamber per the procedure outlined by Mehaisen et al. (2022). A 10% eosin solution with phosphate buffer saline (PBS) was used to dilute the pooled sperm sample 1:200v/v. 10µL of sperm suspension were added to the hemacytometer and settled for three minutes. Sperm cell counts in five of the chamber's big squares were counted using a 400× magnification microscope.

Motility assay

A 10µL aliquot from the diluted samples (1:50v/v) with chicken semen extender (Mehaisen et al. 2020) was deposited on a pre-heated glass slide and topped with an 18×18mm cover slip to assess sperm motility. The slide was heated on a hot plate for 30 seconds at 37°C. A phase contrast microscope was used to quantify the proportion of motile spermatozoa. Subjective ratings were assessed on a scale from 0 to 100% for each sample (Rakha et al. 2017).

Viability assay

With a bit of modification from a previous study (Murugesan and Mahapatra 2020), Eosin-Nigrosin stain (Bio-Diagnostic, Inc., Giza, Egypt) was used to determine the proportion of viable sperm in each treatment group. A total of 190µL of staining solution was gently vortexed with 10µL of pooled semen, and the resultant combination was incubated for 30 seconds at ambient temperature. A ten-microliter portion of this blend was applied onto a sterile microscopic slide and left to air dry. Sperm that were pink-stained and unstained were identified as dead and living, respectively.

Acrosome integrity

The acrosome integrity was explored with Giemsa stain, according to Rakha et al. (2016) with a slight alteration. 10µL of pooled semen were applied to a sterile glass slide and fixed with a solution consisting of 100mL of 35% formalin, 9g of NaCl, and 12g of dibasic/anhydrous Na2HPO4, complemented with 900mL of distilled water. After that, the slide was submerged in a 25% Giemsa stain (Bio-Diagnostic) solution for 90 minutes. After being cleaned with running tap water, the slide was air dried. To ascertain the proportion of acrosome-intact sperm (ACRI) that exhibit blue-stained acrosomal caps in 200 sperm.

Plasma membrane status

The techniques outlined by Rakha et al. (2016) for the hypo-osmotic swelling test (HOST) were slightly modified to assess the plasma membrane integrity (PMI). In conclusion, 100µL of HOST solution (100mOsmol/kg), consisting of 1.375g fructose and 0.75g sodium citrate dihydrate in 100mL distilled water, was incubated with 10µL of pooled semen for 30 minutes at 37°C. A 10µL drop of the incubated solution was placed on a sterile glass slide and fixed with a formal saline solution. Spermatozoa exhibiting coiled and expanded tails were identified as normal spermatozoa with integrated plasma membranes. Each experiment saw a minimum of 200 sperm using a phase-contrast microscope with oil immersion at a magnification of 1000×.

Fertility trial

After dilution at a rate of 1:2 v/v using the poultry semen extender, 300µL of the pooled semen was used to inseminate the Lohman hens (n=10 hens per treatment group). Eggs were collected daily from each hen throughout the period beginning on the second day after the first insemination and concluding two days after the last. The collected eggs were stored at 18°C with a continuous diagonal movement before being placed in the setter. A total of one hundred eggs were chosen randomly for each treatment, disinfected, and incubated for eighteen days in a setter (at 37.5°C and 65% relative humidity (RH)), then transferred to a hatchery for three days (at 37.4°C and 70% RH). The quantity of hatching eggs was documented. Unhatched eggs were examined and categorized as infertile, early mortality embryos, late mortality embryos, or pipped eggs.

Statistical Analysis

For serum testosterone, MDA, TAOC, IL-1β, and TNF-α, n = 10 samples per treatment group were analyzed using the General Linear Model (GLM). Six pools of semen ejaculates served as experimental units and were evaluated for semen volume, concentration, motility, and other quality tests through GLM analysis. A chi-square test assessed the fertility statistics between the therapy groups. Differences were deemed statistically significant at P≤0.05. All percentage values were standardized using an arcsine transformation. All data were analyzed using SPSS 22.0 (IBM Corp., Armonk, NY, USA 2013).

RESULTS

Blood parameters

Table 3 represents the impact of dietary MO addition on the levels of inflammatory cytokines, antioxidant biomarkers, and testosterone in Lohman chicken roosters infected with EC. The findings revealed that the EC group's serum TST was markedly (P≤0.05) lower than the control groups. When MO was added, the TST level in the MO+EC group rose considerably (P≤0.05) relative to the EC group. Comparing the TAOC to the control, the EC infection considerably (P≤0.05) lowered it, while the MO supplementation significantly (P≤0.05) enhanced it. Unlike the C group, the EC infection markedly (P≤0.05) raised TNF-α, IL-1β, and MDA concentrations in the blood in the EC and MO+EC groups. On the other hand, the MO+EC group showed a substantial (P≤0.05) drop in TNF-α, IL-1β, and MDA levels relative to the EC group.

Semen quality traits

The impact of dietary MO addition on the semen quality parameters of EC-infected Lohman chicken roosters is shown in Table 4. The semen volume of the MO group was markedly (P≤0.05) greater than that of the EC and MO+EC groups. The EC group had the lowest sperm concentration (P≤0.05) relative to the other groups. The motility and viability of sperm dramatically (P≤0.05) decreased in the EC and MO+EC groups relative to the C and MO groups. Additionally, the MO+EC group's motility and viability were better (P≤0.05) than the EC groups. While the MO treatment increased the PMI in the infected roosters, the EC infection decreased (P≤0.05) the PMI in sperm compared to the C and MO groups. There was no discernible difference in the sperm ACRI among the groups.

Table 3: Impact of dietary *Moringa oleifera* (MO) addition on the testosterone, antioxidant biomarkers, and inflammatory cytokines of Lohman chicken roosters infected with *Escherichia coli* (EC)

Parameters	C	MO	EC	MO+EC	Sig.
TST (ng /mL)	6.3±0.34^{ab}	6.8±0.21^{a}	2.2±0.36^{c}	4.8±0.24^{b}	**
TAOC (U/mL)	8.3±0.29^{b}	10.1±0.27^{a}	6.8±0.56^{c}	7.7±0.37^{bc}	*
MDA (nM/mL)	2.5±0.17^{c}	2.3±0.16^{c}	5.3±0.15^{a}	3.7±0.13^{b}	***
IL-1β (pg/mL)	230.5±18.12^{c}	257.6±10.17^{c}	803.7±15.47^{a}	660.3±10.59^{b}	***
TNF-α (pg/mL)	104.2±5.07^{c}	93.3±4.45^{d}	156.5±6.11^{a}	133.1±4.77^{b}	***

Data express mean±SE of 10 observations per treatment group. Means with uncommon superscripts, within a parameter, are significantly different at *(P≤0.05), **(P≤0.01), or ***(P≤0.001). The C (control) and EC rooster groups were given a baseline diet without moringa treatment, whereas MO and MO+EC rooster groups were given a baseline diet supplemented with 50g/kg MO powder for 6 consecutive weeks. After the sixth week, the EC and MO+EC rooster groups were intraperitoneally (IP) administered a 0.5mL suspension containing 10 million CFU of EC, while the C and MO rooster groups were IP injected with 0.5mL saline. TST, testosterone; TAOC, total antioxidant capacity; MDA, malondialdehyde; IL-1β, interleukin-1 beta; TNF-α, tumor necrosis factor-alpha.

Table 4: Impact of dietary *Moringa oleifera* (MO) addition on the semen quality traits of Lohman chicken roosters infected with *Escherichia coli* (EC)

Parameters	C	MO	EC	MO +EC	Sig.
Volume (mL)	0.44±0.134^{ab}	0.56±0.021^{a}	0.28±0.036^{b}	0.34±0.024^{b}	*
Concentration (10^9/mL)	7.4±0.37^{a}	7.8±0.82^{a}	5.2±0.22^{b}	6.9±0.44^{a}	*
Motility (%)	84.0±2.22^{a}	85.4±1.77^{a}	68.2±1.02^{c}	72.5±1.14^{b}	*
Viability (%)	93.3 ±0.68^{a}	94.6±0.77^{a}	75.61 ±0.50^{c}	88.7±0.85^{b}	*
ACRI (%)	87.5±1.12	89.6±1.17	86.7±2.17	87.3±1.19	NS
PMI (%)	87.8±1.88^{a}	88.3±2.45^{a}	82.6±2.11^{b}	85.8±2.07^{ab}	*

Data express mean0±SE of 10 observations per treatment group. Means with uncommon superscripts, within a parameter, are significantly different at *(P≤0.05), **(P≤0.01), or ***(P≤0.001). The C (control) and EC rooster groups were given a baseline diet without moringa treatment, whereas MO and MO+EC rooster groups were given a baseline diet supplemented with 50g/kg MO powder for 6 consecutive weeks. After the sixth week, the EC and MO+EC rooster groups were intraperitoneally (IP) administered a 0.5mL suspension containing 10 million CFU of EC, while the C and MO rooster groups were IP injected with 0.5mL saline. ACRI, acrosome integrity; PMI, plasma membrane integrity.

Table 5: Impact of dietary *Moringa oleifera* (MO) addition on the sperm fertility traits of Lohman chicken roosters infected with *Escherichia coli* (EC)

Parameters	C	MO	EC	MO +EC	Sig.
Incubated eggs (n)	100	102	110	105	
Fertile eggs (%)[1]	81 (81.0)^{a}	84 (82.4)^{a}	73 (66.4)^{c}	82 (78.1)^{b}	**
Hatched eggs (%)[2]	79 (97.5)^{a}	83 (98.8)^{a}	44 (60.3)^{c}	78 (95.1)^{a}	***
Embryonic death (%)[2]	2.5^{b}	1.2^{b}	39.7^{a}	4.9^{b}	***

[1] Means as a percentage of the total incubated eggs. [2] Means as a percentage of the fertile eggs. Means with uncommon superscripts, within a parameter, are significantly different at *(P≤0.05), **(P≤0.01), or ***(P≤0.001). The C (control) and EC rooster groups were given a baseline diet without moringa treatment, whereas MO and MO+EC rooster groups were given a baseline diet supplemented with 50g/kg MO powder for 6 consecutive weeks. After the sixth week, the EC and MO+EC rooster groups were intraperitoneally (IP) administered a 0.5mL suspension containing 10 million CFU of EC, while the C and MO rooster groups were IP injected with 0.5mL saline.

Sperm fertility traits

The impact of dietary MO supplementation on the sperm fertility characteristics of Lohman chicken roosters infected with EC is shown in Table 5. As compared to the C and MO groups, the EC and MO+EC groups' sperm fertility was considerably (P≤0.05) lower due to EC infection. The EC group had the highest rate of embryonic mortality and the lowest hatchability of fertilized eggs related to the other groups. The MO treatment considerably enhanced the reproductive features of the sick roosters.

DISCUSSION

One of the common harmful bacteria that infects poultry is *Escherichia coli*, which is inhaled or consumed through contaminated surfaces (Gomes et al. 2022). Clinically, EC can translocate and colonize across mucosal layers of the male reproductive tissues via the hematogenous route (Okeleji et al. 2024). Since the reproductive and digestive systems share the cloaca, EC may naturally develop in poultry semen and deteriorate male fertility (Joseph et al. 2023).

In the present study, EC infection negatively affects the fertility and hatchability of the roosters and substantially increases embryonic death. According to the semen evaluation assay, the infected roosters produced lower semen volume, sperm concentration, motility, viability, and PMI than the non-infected control roosters. These findings corroborate those of other research, which showed that EC infection impairs the sperm motility of avian semen (Tvrdá et al. 2023; Joseph et al. 2023), boar semen (Contreras et al. 2022; Keeratikunakorn et al. 2024), and ram semen (Wang et al. 2023). Chromosomal aberration, cell membrane impairment, acrosome damage, and mitochondrial dysfunction are among the symptoms of bacterial infection (Henkel 2024; Neto et al. 2024). These results imply that EC and similar pathogenic bacteria probably affect the sperm's ability to swim (Marchiani et al. 2021) and seriously harm the sperm plasma membrane, acrosome, mitochondria, and DNA (Henkel 2024; Elomda et al. 2024). Such occurrences can thus explain the high rates of infertility and embryonic mortality, as well as the poor chick production in the infected roosters in the current study.

On the other hand, our results declare that the low fertility of the EC-infected roosters coincided with a low testosterone level (Table 3). Also, the infected birds had high MDA, IL-1β, and TNF-α levels, indicating high oxidative stress and inflammation. Like previous studies (Wang et al. 2021), EC inhibits the antioxidant enzyme synthesis and TAOC. It was reported that some EC-components, such as lipopolysaccharides and porins, when interacting with the genital cells, can induce oxidative stress, inflammation, and apoptosis (Palladino et al. 2018), and deteriorate sperm quality and DNA integrity (Tvrdá et al. 2022). Male infertility and epididymal damage are linked to the elevated oxidative stress caused by EC (Lynch 2018). Furthermore, several inflammatory cytokines, including TNF-α and IL-1β, cause spermatogenic cells to die, which has a detrimental effect on Leydig cells' ability to produce testosterone and carry out spermatogenesis (Wang et al. 2021).

The current study displayed a positive effect for dietary MO supplementation on the roosters' fertility and ameliorated the reduction in fertility in the EC-infected roosters (Table 5). The chemical assay of MO in the present study displayed that it contains high flavonoid contents, and the antioxidant capacity reaches 98.7% (Table 1). The flavonoid compounds extracted from natural products have a potential effect as aromatase inhibitors (Zhao et al. 2020).

Due to the aromatase inhibition that prevents androgens from being bio-transformed into estrogens, this might be the reason for the rise in serum testosterone levels in the MO groups (Yang et al. 2022). Other studies reported that herbs like MO nourished the hypothalamic-pituitary-gonadal axis and promote the male reproductive function (Chukwu et al. 2025). Consistent with other researchers (Saalu et al. 2011; Sebola et al. 2022), we found a considerable increase in the semen volume and concentration when incorporating MO in the diet of the Lohman roosters. Additionally, the compensation of ATP and element needs by MO may have contributed to the beneficial impact of food supplementation of MO on sperm motility of the infected roosters in the current investigation (Sebola et al. 2019).

Our results indicated that MO improved the antioxidant biomarkers and alleviated the elevation of the MDA and inflammatory cytokines in the EC-infected roosters. At the same time, MO treatment improved sperm viability and plasma membrane integrity. In agreement with these results, Ragab et al. (2024) manifested that the phenolic components and antioxidant potential of the MO leaves shielded the rats from the oxidative, spermatogenic, and morphologic alterations brought on by harmful substances. The positive effects of MO on roosters' sperm motility and quality traits improved the results of egg fertility and hatchability in the present study (Table 5). In addition, the antioxidant capacity of MO improved the survival indices of chicks (Seger 2025). The high zinc levels in the MO could explain the improvement of fertility and hatchability rates obtained in the EC-infected roosters, since zinc has a crucial role in the protection of sperm chromatin structure and maintenance of embryonic development (Allouche-Fitoussi and Breitbart 2020).

Conclusion

The current study's results presented a convincing case for the harmful effects of E. coli infection on oxidative stress, inflammation, semen output, sperm quality, and reproductive features in roosters raised on Lohman farms. On the other hand, adding 5g/kg of *Moringa oleifera* to the rooster diets enhanced blood testosterone and antioxidant indicators while lowering inflammatory cytokines. Furthermore, MO can effectively reverse the adverse effects of EC infection on sperm quality and fertility. As a result, this study highlights the use of MO as a natural supplement in rooster diet to minimize the need for antibiotic therapy while simultaneously maximizing fertility and semen production, particularly in context of the EC-infection threat. Further studies are recommended to determine the optimal inclusion level and duration of MO supplementation for maximizing semen quality and fertility without compromising other physiological functions. In addition, investigating the molecular pathways through which MO modulates oxidative stress, inflammatory signaling, and steroidogenesis would provide deeper mechanistic insights.

DECLARATIONS

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Conflict of Interest: The authors declare no conflict of interest.

Data Availability: All authors declare that the data supporting the findings of this study are available upon request.

Ethics Statement: If any suffering remarks were observed on the birds due to EC administration, a protocol of euthanasia was immediately applied. The Research Ethics Committee of King Faisal University in Saudi Arabia has authorized the current animal study protocol (Ref. No. KFU-REC-2024-MAR-EA000545).

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