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# Necrotic enteritis and coinfection with different coccidia species with unveiling the effect of *Moringa* extract in improving their negative impact in broiler chickens

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**ABSTRACT**

A severe co-infection in poultry is produced by *Clostridium perfringens* (*C. perfringens*) and coccidia, which results in fatalities and bleeding diarrhea. This study aimed to detect the effect of *M. oleifera* extract against *C. perfringens* and *Eimeria*. A total of 200 samples were collected from eight broiler farms to isolate *C. perfringens* and detect *Eimeria* spp. from suspected diseased poultry and litter. Thirteen percent of the samples were positive for *C. perfringens*, and 87% and 50% of the confirmed isolates had the enterotoxin genes *cpe* and *NetB*. *Eimeria* spp. accounted for 60% of the population, with *E. acervulina*, *E. necatrix*, *E. tenella*, and *E. maxima* being the most frequently identified species. Furthermore, we determined the relative efficacy of the plant extracts by examining the efficacy of *Moringa oleifera* extraction on broilers experimentally infected with *C. perfringens* and mixed *Eimeria* species. We divided the 30 birds into three groups, each containing 10 birds. Group 1 (G1) was used as a control, Group 2 (G2) had *C. perfringens* type A and *Eimeria* spp. Infections for the last 3 weeks, and Group 3 (G3) had the same infections as Group 2 and had been fed *M. oleifera* extract (150 mg/kg BW). At the experimental infection level, the animals' growth performance parameters became better than those of the infected group, with a significant variation ($P < 0.05$) in G3. Additionally, they had fewer *C. perfringens* infections in their intestines and faeces, and their feces shed fewer oocysts. In conclusion, *M. oleifera* extract is effective against *C. perfringens* and *Eimeria*-challenged birds and enhances their performance.

## Introduction

Necrotic enteritis (NE) disease in poultry is a result of infection by the anaerobic, Gram-positive, spore-forming, rod-shaped bacteria known as *C. perfringens* (Lee *et al*., 2013). The primary mechanism by which *C. perfringens* causes the disease is through the secretion of six main extracellular toxins, known as Alpha (α), Beta (β), Epsilon (ε), Iota (ι), Enterotoxin (*cpe*), and *NetB*. *C. perfringens* is categorized into 7 toxicogenic categories, A - G, based on the synthesis of the abovementioned toxins (Johnston *et al*., 2022). *C. perfringens* has more than 20 toxins and has been associated with many gastrointestinal disorders in animals, including chicken necrotic enteritis. Previous studies have shown that the levels of expression of this previously mentioned toxin are considerably higher (92%) in NE chicken isolates associated with healthy birds (29%), confirming its role in the transmission of the disease (Mehdizadeh Gohari, 2018). According to Cooper and Songer (2010) and Salem *et al.* (2021), acute NE attacks broilers between the ages of two and six weeks, leading to a daily mortality rate of 1% and a cumulative mortality rate of about 10–40%. Characteristics of necrotic enteritis in gas-filled, enlarged small intestines (Timbermont *et al*., 2011; Shakal *et al*., 2024). The main elements associated with *C. perfringens* that are linked to NE include α-toxin, *NetB*, and TpeL toxins (Olkowski *et al*., 2008). Environmental stresses and high-protein diets are other known NE risk factors; these factors can alter the microbiota, the host's immune system, and the likelihood of infection with the poultry parasite *Eimeria* (Yang *et al*., 2019).

According to Attia *et al.* (2023) and Muñoz-Gómez *et al.* (2024), coccidiosis is a major illness affecting the global chicken production industry. The apicomplexan parasite causes harm to the chicken gut's enterocytes, which in turn causes decreased feed intake, malabsorption, inadequate growth, and higher death (Nahed *et al*., 2022). From $3 billion to $14 billion each year has been the projected global loss during the last quarter of a century (Blake *et al*., 2020). Infection by coccidia, particularly *E. maxima*, makes birds more susceptible to NE, causing lesions and affecting zootechnical parameters (Emami and Dalloul, 2021).

Antibacterial growth promoters (AGPs) were found to efficiently limit the pr*oleifera*tion of clostridial infection (Zou *et al*., 2018). However, because of the growing request for antibiotic-free or organic broiler production and the development of coccidia resistance to anti-coccidial feed additives, its use has significantly reduced and the use of APGs be more, there are worries that coccidia vaccinations may not be as active as traditional anti-coccidial food additives and could potentially hinder performance and raise the risk of NE in chickens (Eckert *et al*., 2021).

There is a rising interest in the production and assessment of herbal antioxidants extracted from tropical plants for animal health. Of those tropical plants, the species *Moringa* has shown remarkable potential (Boukandoul *et al*., 2018). Many tropical and subtropical nations find *Moringa oleifera* (*M. oleifera*), a highly high-quality plant. It has a wide range of medical applications and is very nutritious (Ullah *et al*., 2024). The plant *M. oleifera* is highly valued for its medicinal properties. Indigenous medical systems, particularly in South Asia, use their leaves, roots, seeds, bark, fruit, flowers, and immature capsules among many parts to treat a variety of illnesses. These parts also have antioxidant, antibacterial, antimalarial, and antifungal properties. The plant's diverse constituents are an abundant reservoir of vitamins and proteins, β-carotene, amino acids, antioxidants, and phenol compounds, and they possess a range of vital minerals (Ullah *et al*., 2024). Furthermore, the water-purifying abilities and significant nutritional value of the product have been well documented (Anwar *et al*., 2007). So, this study aimed to detect the effect of *M. oleifera* against *C. perfringens* and *Eimeria* species.

## Materials and methods

### *Ethical approval*

The Research Ethics Committee of the Faculty of Veterinary Medicine, Cairo University, Egypt, authorized the experimental procedures follow-


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ing the applicable standards and regulations (Vet CU18042024930).

*Samples collection and preparation*

Between July 2023 and January 2024, intestines, liver, and litter samples were collected to provide evidence of an NE outbreak in eight broiler farms in El-Menoufia governorate, Egypt. We collected one hundred samples of the intestines and livers (50 of each organ) from live birds that were either sick or suspected of being sick based on subclinical and clinical symptoms for postmortem analysis as shown in (Fig. 1), additionally, fifty litter samples were collected from the same farms. Furthermore, we collected fifty intestine samples from healthy birds. The samples were promptly transmitted to the Laboratory of Microbiology at the Animal Health Research Institute on ice for identification and isolation from *C. perfringens*. The samples were collected in 10 mL of a meat preparation medium (Oxoid, England). Additionally, the presence of *Eimeria tenella*, *Eimeria necatrix*, *Eimeria acervulina*, *Eimeria brunetti*, and *Eimeria maxima* was detected in intestinal samples from naturally infected birds. Their classification was determined by the oocyst morphology, colonization site, pathology, and clinical symptoms (Chapman, 2014).

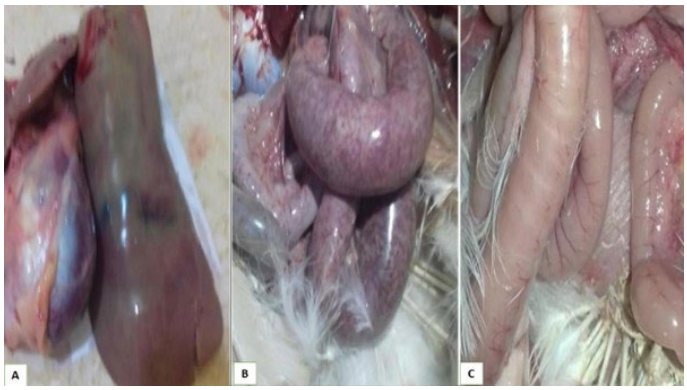

Fig. 1. Postmortem examination of freshly dead birds showing A: liver subcapsular haemorrhage and necrosis, B: pepper and salt appearance in the intestine and C: ladder shape like appearance in the intestine.

*Isolation and Identification of C. perfringens*

Samples comprising cooked meat were anaerobically incubated at 37°C for 36-48 hours in an Oxoid, anaerobic container (Thermo Fisher Scientific Inc, UK) with an anaerobic GasPak (Oxoid) to mainly enhance *C. perfringens*. After that, the samples that had already been pre-enriched were placed on 5% sheep blood agar along with 2 mg/l of neomycin sulfate and then left to incubate anaerobically for one day. The colonies were believed to have originated from *C. perfringens* due to the presence of double zones of beta hemolysis: one zone with complete hemolysis and another zone with partial hemolysis. Gram staining revealed Gram-positive, big bacilli in the colonies that were suspected of being bacteria; the catalase test came back negative; Naglar's response came back positive; and indole testing was conducted to gain further confirmation. After conducting two or three subcultures, a pure culture was obtained. Following this, the isolated colonies were grown in brain-heart infusion broth (BHI) (Oxoid). All isolates were frozen at -80°C so that they could be easily examined later.

*Molecular identification of C. perfringens*

Extraction of DNA: Following prior research, template DNA was prepared (Kumar *et al*., 2016). In a 1.5 ml microcentrifuge tube, 3-5 pure bacteriological colonies were suspended in 150 µl of ultrapure water. The mixture was heated at 100°C for 10 minutes and then cooled to -20°C for 5 minutes. Afterward, the cell lysates were spun at 12,000 rpm for 5 minutes. The DNA-containing supernatant, which amounted to 100 µl, was then transferred to a small tube and kept at -20°C for further examination.

*Confirmation of C. perfringens by using 16S rRNA species-specific gene and identification of the virulence genes*

Traditional polymerase chain reaction (PCR) with species-specific 16S rRNA primers allowed for molecular detection. For each reaction, 50 µl of the master mix was put into PCR tubes. This mix had 25 µl of Taq Green PCR Master Mix (2X) from Thermo Scientific®, USA, 5 µl of template DNA, 2 µl of forward and reverse primers, and 18 µl of sterile de-ionized water. According to Wang *et al*. (1994), the following PCR conditions were done for 16S rRNA *C. perfringens* confirmation: 35 cycles of denaturation at 94°C for 1 minute, annealing at 53°C for 1 minute, and extension at 72°C for 1 minute. The virulence genes for different toxins, including enterotoxin *Cpe* and *NetB*, were identified using a PCR as shown in Table 1. The 1.5% agarose gel electrophoresis method, applied with the Gel Doc® EZ Imager (Bio-Rad, USA), allowed for the visualization of all PCR results.

*Extraction of Moringa oleifera*

*Moringa oleifera* (*M. oleifera*) leaves were obtained from the National Research Institute in Egypt. They were subsequently identified and verified in the herbarium of the National Research Institute's Department of Botany. Leaves were dried in the open air. The dried leaves were ground into a fine powder using a milling machine, then extractions were made from these ground leaves. The extraction process involved maceration in 70% acetone with sporadic shaking for 72 hours at a solvent-to-dry-weight ratio of 10:1 (Eloff, 1998). The samples were filtered using Whatman No. 1 filter paper and a funnel. Once extracted, the acetone was transferred to a dark container and kept at 4°C until needed.

*Experimental Design*

Thirty Cobb broiler chicks, one day old, were imported from an Egyptian commercial poultry company and raised on a concrete floor using a deep litter system with around 10 cm of fresh wood shavings as bedding. Throughout the five weeks of observation, the chicks were kept in an ideal environment with a consistent light source and controlled temperatures, humidity, and ventilation. Fresh, clean water was always available, and the birds were given a balanced diet that included starter, grower, and finisher rations. No additives were added to their food. At the 14-day

Table1. Primers utilized for the amplification of genes that generate specific toxins, the anticipated amplicon size, and the annealing temperature in the PCR.

| Target gene | Primers sequences | Amplified segment (bp) | Amplification (35 cycles) | | | | Final extension | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | Primary Denaturation | Secondary denaturation | Annealing | Extension | | |
| Enterotoxin (*cpe* gene) | ACATCTGCAGATAGCTTAGGAAAT<br>CCAGTAGCTGTAATTGTTAAGTGT | 247 bp | 94˚C<br>5 min. | 94˚C<br>30 sec. | 55˚C<br>30 sec. | 72˚C<br>30 sec. | 72˚C<br>7 min. | Kaneko *et al*. (2011) |
| *NetB* | CGCTTCACATAAAGGTTGGAAGGC<br>TCCAGCACCAGCAGTTTTTCCT | 316 bp | 94˚C<br>5 min. | 94˚C<br>30 sec. | 55˚C<br>40 sec. | 72˚C<br>40 sec. | 72˚C<br>10 min. | Bailey *et al*. (2013) |

age, thirty chicks were randomly assigned to one of three groups, with ten birds in each. There were two equal replicas of each group, with five birds in each. The categories were classified as follows: G1: negative control; G2: infected with *C. perfringens* type A which was received from the Poultry Diseases Department of Cairo University's Faculty of Veterinary Medicine (Salem *et al*., 2021). G3: was infected with *C. perfringens* type A with *Eimeria* spp. and treated with *Moringa oleifera* extract. The birds in this group were given 150 mg/kg BW of extract continuously for the last three weeks. *Eimeria* were administered orally to infected groups for two days in a row at 14 days of age by 5,000 sporulated oocysts which were obtained from the Parasitology Department of Saddat University's Faculty of Veterinary Medicine.

*Evaluation of Bird Performance Morbidity and Mortality Rates*

The performance of the growth process was evaluated by weighing each treatment group of birds every day during the treatment period. Weight gain was used as a measure of growth performance. We measured the average and individual weight gains, body weight (BW), weight gain (BWG), feed consumption (FC), and feed conversation ratio (FCR). The percentage mortality was calculated by dividing the total number of dead chicks in the group by the beginning number of birds, then multiplying by 100. The birds were then analyzed for death rate and hazard after being exposed to the infectious agent, both with and without the herbal extract. After the first symptoms appeared, a score ranging from 0 (not present) to 3 (very present) was used to determine the severity of bloody or brown diarrhea. The normal status was zero, while 33%, 33-66%, and 66-0.9% blood/tan color in total feces were represented by the numbers +, ++, and +++, respectively. This was carried out using the method provided by Abbas *et al*. (2010).

*Intestinal and caecal counts of C. perfringens*

Each bird, with 3 birds per group, had its intestinal and caecal samples collected individually 7 days after infection. The samples were diluted in sterile PBS using a serial dilution method, with dilution ratios of 1:100, 1:1000, and 1:10000. A 0.1 ml sample from every dilution was subsequently streaked on plates containing egg yolk emulsion (EYE) agar (Oxoid, USA), 5% sheep blood agar (SBA), and reinforced *Clostridium* medium (RCM) (Oxoid, USA), respectively. These plates were then incubated at 37°C for 24-36 hours under anaerobic conditions, as previously mentioned. *C. perfringens* was hypothesized based on double-zoned β-hemolysis colonies on SBA and opalescent growth on EYE agar plates. Plates were counted, and CFU per gram was recorded. The colonies were chosen and confirmed based on Garrido *et al*. (2004).

*The output of Eimeria species oocysts*

The harvest of oocysts in the feces was measured by pooling daily oocyt counts per set of birds using the methodology outlined by holdsworth *et al*. (2004). The birds' group housing made it impossible to count individual oocysts. From the first day of treatment to the last day of treatment, a total of 10 g of feces was collected and processed every day. The oocysts per gram (OPG) were estimated using the modified McMaster technique, as detailed by vassilev (2002).

*Histopathological Examination*

For histological assessment, representative tissue samples were obtained from the intestines of infected and treated with plant groups (G2, G3). The samples were initially preserved in 10% Neutral buffer formalin. Then the samples were processed for paraffin embedding with alcohol as a drying agent and xylene as a clearing agent. Harris hematoxylin and eosin were used to stain the sections after they were cut at a thickness of 4 to 5 mL, following the method described by bancroft and Gamble (2008).

*Statistical analysis*

The SPSS 18 program (SPSS Inc.) was used for all statistical analysis. Means and standard error of means were summarizing the study's results. It was determined whether there was a significant difference between the treatment groups by comparing the average weights per group for each time point using ANOVA. To discover statistically significant differences between samples, chi-square is employed.

## Results

*Bacterial identification of isolates*

*C. perfringens* is identified as a Gram-positive bacteria rod-shaped, straight bacillus that has blunt extremities. On egg yolk agar medium, it shows opalescence; sheep blood agar with neomycin grows with a double hemolysis zone. We identified the *C. perfringens* isolates as negative for indole and catalase, positive for nitrate reduction, VP, and oxidase, and positive for $H_2S$ and gelatin liquefaction.

Table 2 presents the isolation of 26 *C. perfringens* isolates from 200 samples, representing a prevalence of 13%, using SBA and EYE culture media. When inoculated in blood agar, those colonies displayed a double zone of hemolysis, indicating their gram-positive status. The isolation between the various sampling organs is not significant.

From a total of 150 samples, 90 *Eimeria* spp. isolates were found to be positive, as shown in Table 3. There were 68 species of *Eimeria* in the

Table 2. Prevalence of *C. perfringens* isolated from examined samples.

| Species | Total no. of samples | No of Positive samples | % | $X^2$ | *P* value |
|---|---|---|---|---|---|
| Intestine Diseased chickens | 50 | 11 | 22 | | |
| Intestine Apparently healthy chickens | 50 | 4 | 8 | | |
| Liver | 50 | 3 | 6 | 7.25 | .064* |
| Litter | 50 | 8 | 16 | | |
| Total | 200 | 26 | 13 | | |

X2The chi-square; *: The result is not significant at p < .05

Table 3. Prevalence of *Eimeria* spp. isolated from examined samples.

| Species | Total no. of samples | No of Positive samples | % | $X^2$ | *P* value |
|---|---|---|---|---|---|
| Intestine | 100 | 68 | 68 | | |
| Litter | 50 | 22 | 44 | 8 | .004* |
| Total | 150 | 90 | 60 | | |

X2The chi-square; *: The result is not significant at p < .05

intestine, with 21 of them being *E. acervulina* (30.8%), 18 being *E. tenella* (26.4%), 17 being *E. necatrix* (25%), and 12 being *E. maxima* (17.6%). In the litter, there were 22 isolates, with 10 being *E. maxima* (45.5%), 7 being *E. acervulina* (331.8%), 3 being *E. tenella* (13.6%), and 2 being *E. necatrix* (9%). There was a significant difference in the isolation percentage between different sampling types.

*Molecular identification and genotyping of C. perfringens*

Table 4 confirms that 26 *C. perfringens* isolates (13%) were identified in 200 samples by using 16S rRNA. Multiplex PCR confirms isolates by detection of *Cpe* and *NetB* genes. All intestines from diseased chickens were positive for both *Cpe* and *NetB* genes (100%); samples from apparently healthy chickens were only positive for the *Cpe* gene (50%); liver samples were positive for both *Cpe* and *NetB* genes (100% and 66.6%, respectively); and litter samples were only positive for the *Cpe* gene (62.5%). Fig. 2 displays the agarose gel for multiplex PCR of toxins (*Cpe* and Net B genes).

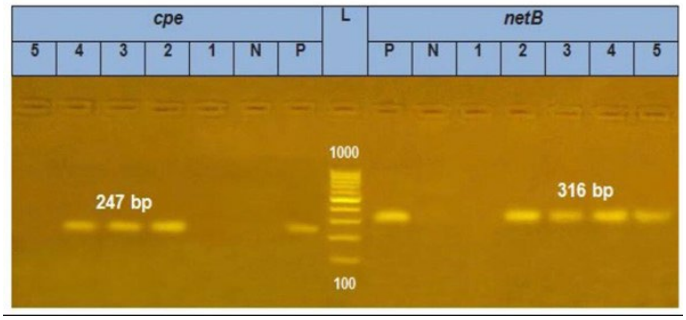


Fig. 2. Agarose gel electrophoresis result for the PCR assay to identify the presence of *cpe* gene and net B gene in the isolates, lane 2,3,4 and 5 the net B gene is present at 316 bp, and lanes 2,3 and 4 the *cpe* gene is present at 247 bp.

*Growth performance*

Compared to animals challenged with *C. perfringens* and *Eimeria* (G2), birds treated with *Moringa oleifera* had no clinical symptoms (clinical lesion score = 0). The mortality rate was the highest in the infected group (G2), reaching 70%, while the treated group with *M. oleifera* (G3) had the lowest, at 20% (Table 5).

Table 6 displays the comprehensive growth performance results. During the first two weeks of life, there were no differences in BW, BWG, and FCR among the groups at different levels. However, following that, at weeks. 3, 4, and 5, there were significant ($P < 0.05$) differences in body weights and FC between the groups treated with *M. oleifera*, the other controlled groups, and the negative control group. Remarkably, the infected controls had lower body weights than the *M. oleifera*-treated group ($P < 0.05$). We observed notable improvements ($P < 0.05$) compared to the birds that tested positive. Group 3 had the highest values across all trial weeks for BW and BWG, which increased linearly with age.

The effect of *M. oleifera* on the infected group was shown in Fig. 3, there was a significant difference between groups (infected or treated) ($P < 0.05$). In addition, the current study's findings indicated that the *M. oleifera* supplied to the group had significantly lower cecal *Eimeria* spp. counts than the infected group ($P = 0.000$). The study concluded that the intestinal *Eimeria* count was significantly lower in group 3, which had a significantly lower count (Fig. 4).

As shown in Fig. 5, Group 2 showed severe lesions and a heavy oocyte infestation in the histopathological section of cecum from broiler chickens fed 5000 sporulated oocyts by mouth. However, G3 showed mild lesions, with a few parasitic affections. The *Moringa*-treated group showed moderate lesions (H & E, X40).

Table 4. Genotyping of *C. perfringens* isolates from different poultry sources.

| Samples | *C. perfringens* positive % | 16S rRNA | Genotypes of *C. perfringens* isolates positive % | |
|---|---|---|---|---|
| | | | *Cpe* | Net B |
| Intestine (Diseased chickens) | (11/50)22 | √ | (11/11)100 | (11/11)100 |
| Intestine (Apparently healthy chickens) | (4/50)8 | √ | (2/4)50 | 0 |
| Liver | (3/50)6 | √ | (3/3)100 | (2/3)66.6 |
| Litter | (8/50)16 | √ | (5/8)62.5 | 0 |
| Total | 26 | √ | (21/26)80.7 | (13/20)50 |

Table 5. The effects of different treatments on the Macroscopic lesion score and survivability of birds.

| Bird number/ Groups | 3rd week | | | 4th week | | | Total mortality (%) |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1 | 2 | 3 | |
| G1 | 0 | 0 | 0 | 0 | 0 | 0 | 3/10(30) |
| G2 | + + + | ++ | ++ | + | + | ++ | 7/10(70) |
| G3 | 0 | 0 | 0 | 0 | 0 | 0 | 2/10(20) |

Table 6. Effect of *M. oleifera* supplementation on body weight, body weight gain, feed intake, and feed conversion ratio in broilers.

| Group | Body weight at age /g | | | | | Body weight gain/g | | | | Cumulative FC (g) | Cumulative FCR |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1st week | 2nd week | 3rd week | 4th week | 5th week | 1st week | 2nd week | 3rd week | 4th week | | |
| G1 | 153 | 462 | 863[b] | 1482[a] | 2221[a] | 310 | 400[b] | 618[b] | 740[a] | 2562 | 1.47[b] |
| G2 | 159 | 465 | 771[c] | 1124[b] | 1750[b] | 305 | 307[c] | 353[c] | 610[b] | 2502 | 1.91[a] |
| G3 | 162 | 493 | 931[a] | 1593[a] | 2184[a] | 330 | 440[a] | 665[a] | 620[b] | 2533 | 1.51[b] |
| SEM | 0.00 | 0.01 | 0.03 | 0.05 | 0.07 | 6.9 | 5.6 | 4.25 | 15.67 | 0.03 | 8 |
| *P* value | 0.25 | 0.21 | 0.00 | 0.00 | 0.02 | 0.32 | 0.03 | 0.00 | 0.00 | 0.31 | 0.00 |

Groups: G1, control group; G2, chicks infected with *C. perfringens* and coccidia spp.; G3, infected chicks treated with *M. oleifera*.
FC, feed consumption; FCR, feed conversion ratio (g of feed/g of body weight gain
a,b,c Mean values or percentages with different superscripts in the same column are significantly different ($P < 0.05$), SEM, standard error of mean.

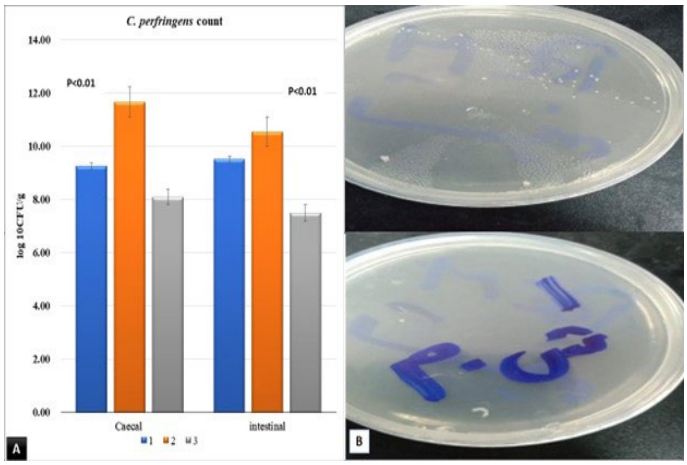


Fig. 3. A: Effect of *Moringa oleifera* on the intestinal and caecal *C. perfringens* count; 3-B: Effect of *M. oleifera* on the intestinal and caecal *C. perfringens* counts using RCM media.

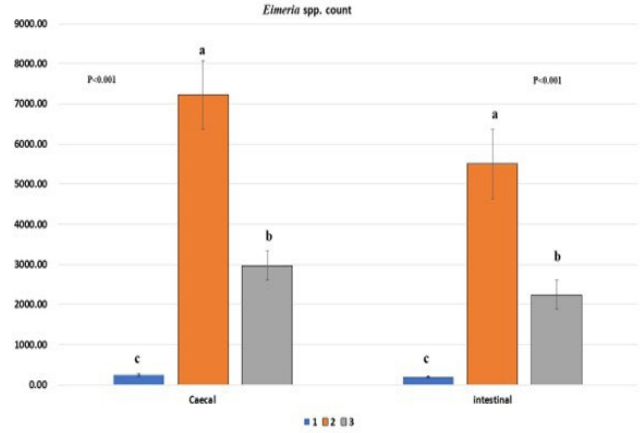


Fig. 4. Effect of *Moringa oleifera* on the intestinal and caecal *Eimeria* spp count.

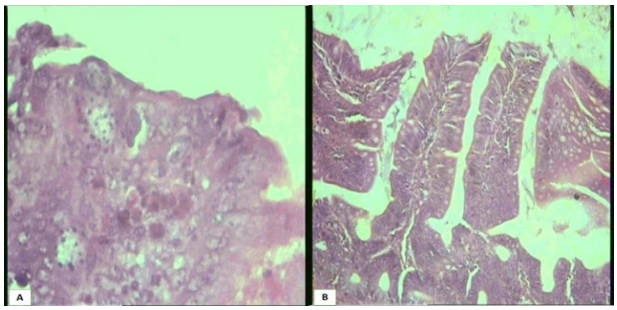


Fig. 5. Histopathological section in caecum of broiler chickens infected with 5000 sporulated oocysts/bird orally (A)showing severe lesions and heavy oocyst infestation (B) showed, mild lesions with the presence of few parasitic affection in the group treated with *Moringa* (H & E , X 40).

## Discussion

Enteric diseases in poultry can have catastrophic impacts on the economy. NE is poultry's most frequent clostridial enteric illness, mainly caused by *C. perfringens* (Shakal *et al*., 2024). Animals and birds typically harbor anaerobic bacteria called *Clostridium* species. During intestinal dysbiosis, the *Clostridium* species produced exotoxins that caused severe intestinal damage, either with or without hepatic damage (Khelfa *et al*., 2012, a, b, c; Kiu and Hall, 2018).

In the current study, we isolated 26 *C. perfringens* strains, representing an incidence of 13%. We sourced the isolates from the intestine (15%), the liver (6%), and the litter (16%). According to Fan *et al*. (2016), the isolation rate for *C. perfringens* was 9.9% (43/435), while El-Helw *et al*. (2014) and Elyazeed *et al*. (2015) reported a higher incidence of *C. perfringens* (33.33%) and 45.9%, respectively. These variations are relatively common when one considers the diverse management and sanitation practices and procedures implemented on various farms. Additionally, chickens frequently infect the environment and poultry litter with *C. perfringens*, thereby posing a risk of reinfection for them. Chickens and other animals are susceptible to cross-infection through feces, litter, refuse from poultry, feed, and water (Praveen *et al*. 2019). Moreover, according to Mohiuddin *et al*. (2016), the occurrence of *C. perfringens* could experience a substantial rise when chickens have a clinical or asymptomatic coccidia infestation in the intestine. The current study included a total of 90 *Eimeria* spp. isolates, accounting for 60% of the total. *Eimeria* spp. was prevalent in the intestine at 68/100 (68%), followed by *E. acervulina* at 21 (30.8%), *E. tenella* at 18 (26.4%), *E. necatrix* at 17 (25%), and *E. maxima* at 12 (17.6%). The litter was 22/50 (44%), with *E. maxima* 10 (45.5%), *E. acervulina* 7 (31.8%), *E. tenella* 3 (13.6%), and *E. necatrix* 2 (9%). This finding is in keeping with the data reported by Saroj Kumar *et al*. (2015), who isolated *Eimeria* spp. at a rate of 66.7%, and he reported that the prevalence of *E. acervulina*, *E. tenella*, *E. necatrix*, *E. mitis*, and *E. praecox* was 77.6, 12.1, 27.6, 70.7, and 1.7%, respectively. Also, isolation rate recognized by Lew *et al*. (2003) were *E. acervulina* (94.3%), *E. tenella* (97.2%), *E. necatrix* (94.6%), *E. maxima* (98.5%), E. praecox (11.4%), and E. brunetti (11.4%).

Diseases like avian coccidiosis are very costly for many birds because they target particular organs, tissues, and immune systems (Salem *et al*., 2022). The parasite species and dosage determine the severity of *Eimeria* infection (El-Shall *et al*., 2022). When more sporulated oocysts are consumed, the gut becomes infested with parasites, and the production of oocysts increases. Boulton *et al*. (2018) found that production decreased with increasing doses due to the crowding effect and that excessive humidity and inadequate ventilation are related to poor hygiene.

Toxigenic typing indicates that all strains of *C. perfringens*, including Type A, produce the alpha (a) toxin. Apart from alpha (α) toxins, beta (β) and epsilon (ε) toxins are produced by types B and C, respectively. Type D also produces epsilon (ε) toxin, type F enterotoxin (*Cpe*), type E iota (ι) toxin, and type G *NetB* toxin (Gautam *et al*., 2024). The current investigation identified the enterotoxin gene *Cpe* and *NetB* genes in the isolated *C. perfringens* strains. According to Table 4, intestine samples from diseased chickens were all positive for both the *Cpe* and Net B genes (100%). Intestine samples from apparently healthy chickens were only positive for the *Cpe* gene (50%). Liver samples were positive for both *Cpe* and Net B genes (100% and 66.6%, respectively). Litter samples were only positive for the *Cpe* gene (62.5%). (Fig. 2) illustrates the agarose gel used for the multiplex PCR of toxins (*Cpe* and *NetB* genes). Even now, in the developed world, it's the third most common cause of food poisoning (Johnston *et al*., 2022). The progress of intestinal illness in many species, including humans, is influenced by enterotoxin (*Cpe*). Recent research has shown that the *Cpe* gene is found on both chromosomes and plasmids in most *C. perfringens* type A food poisoning isolates and *Cpe*-associated nonfoodborne human gastrointestinal disorders (Omer *et al*., 2020; McClane, 1996). Un similar to our results, Fan *et al*. (2016), found that every single isolate belonged to *C. perfringens* type A and had just one gene, the cpa gene, which codes for toxin α.

The antioxidant properties of medicinal plants' constituents, which are occasionally linked to polyphenolic compounds, have also been attributed to their ability to prevent or control disease (Batool *et al*., 2020). There is an increasing focus on the development and assessment of natural antioxidants for animal husbandry as feed additives derived from tropical plant materials. Numerous investigations have demonstrated the antibacterial properties of *Moringa*. Dodiya *et al*. (2015) confirmed that the aqueous extracts of *M. oleifera* leaves possess antimicrobial properties. The plant is an abundant biological resource due to the secondary metabolites it contains, as discovered by Pal and Thakur (2024) and Khan *et al*. (2021). In *Moringa* trees, there is a diversity of other metabolites such as alkaloids, flavonoids, glycosides, terpenoids, and sterols. The primary compound responsible for *Moringa*'s emerging antidiabetic properties

is 4-(α-Lrhamnopyranosyloxy)-benzyl glucosinolate. The clinical lesion scores of three birds per experimental group were analyzed in Table 5 to determine the impact of treatments. In group 3, the birds exhibited no clinical indications during the entire trial period, despite being infected and treated with *M. oleifera*. In the infected group (G2), the mortality rate was the greatest, reaching 70%. Conversely, the treated group (G3) with *M. oleifera* was the lowest, at 20%.

The BW and BWG values increased with age, with the treated group (G3) exhibiting the highest values among the other two groups during all experimental weeks. Table 6 displays the performance parameters of the experimental broilers. The results indicate that birds treated with *Moringa* (G3) exhibited substantially higher body weight (P = 0.001) and weight gain (P < 0.000) than infected birds (G2). Significant enhancements in the FCR of birds that established treatment were also observed (P < 0.001). These discoveries could potentially lead to improvements in broiler growth performance (Tawanda, 2018). Research by Khan *et al*. (2021) observed the effects of *M. oleifera* on the gut's general health, nutritional digestibleness, antioxidant benefits, antibacterial capabilities, as well as immunological reply when used as a natural feed supplement.

By testing the effectiveness of *M. oleifera* extracts on both naturally infected broiler chickens by *C. perfringens* and mixed *Eimeria* species (*Eimeria maxima, Eimeria tenella, Eimeria necatrix*), we were able to ascertain the relative effectiveness of the extracts against NE in birds. Cecal *C. perfringens* counts were much lower in the group that received *M. oleifera* (G3) compared to the infected group (G2) (P = 0.000). (G1). Also, we found that the intestinal *C. perfringens* count was significantly lower in that group. According to research by Ibrahim and Altammar (2024), *M. oleifera* may be able to decrease the growth of harmful strains of *C. perfringens* in livestock. According to Dzotam *et al*. (2015) and Faustin Evaris *et al*. (2022), *M. oleifera* leaf extracts can cure a wide range of infectious diseases, either on their own or in combination with other antibiotics, it was effective against *S. aureus* and *E. coli*. El Banna *et al*. (2016) verified the anti-coccidial activity of *Moringa* extract. Elbarbary *et al*. (2023) found that thyme oil exhibited a superior anti-coccidial effect compared to *Moringa* oil. In this regard, thyme oil may serve as an alternative product for rabbit coccidiosis management. The current study's findings indicated that the *M. oleifera* supplied to the group had significantly lower cecal *Eimeria* spp. counts than the infected group (P <0.001). The study concluded that the intestinal *Eimeria* count was significantly lower in group 3, which had a significantly lower count (P < 0.05).

At the histopathological section of caecum from broiler chickens that were given 5000 sporulated oocysts by mouth, Group 2 had severe lesions and a heavy oocyte infestation. However, group 3 exhibited mild lesions, with a few parasitic affections. The *Moringa*-treated group showed moderate lesions (H & E, X 40). This outcome supported the results of Anwar *et al*. (2007), who stated that *M. oleifera* exhibited no signs of severe toxicity. *M. oleifera* is utilized due to its anti-malaria properties, and *Eimeria* is classified under the phylum Apicomplexa, which is also the phylum of the Plasmodium parasite. The group that received 5.0 g/kg BW of *M. oleifera* extract on the seventh day of the study demonstrated the best results, with a 99.8% success rate, in stopping *Eimeria* oocyst discharge in the feces. The acetone extract of *M. oleifera* leaves helped broiler chickens diseased with mixed species of coccidia gain a lot more weight. This was shown by the significant dose-response. As a result of the decreased oocyst shedding in the feces, the intestinal lining will suffer less harm, resulting in a lower incidence of hemorrhage. Abbas *et al*. (2010) found that *E. tenella*-infected broiler chickens had fewer cases of bloody diarrhea. Day 14 of our trial involved administering 5.0 g/kg BW to the birds. The capacity of *M. oleifera* extracts to cause weight gain and decrease OPG, similar to toltrazuril, suggests that they may be useful in the treatment of coccidiosis. This provides strong evidence in favor of further research into the anticoccidial effectiveness of plant extracts from *M. oleifera*. The preventative treatment of coccidiosis could potentially benefit from this plant's use as an addition to feed.

## Conclusion

Coinfection with *C. perfringens* and coccidiosis is a serious problem in Egyptian farms with a prevalence of 13% for *C. perfringens* and 60% for *Eimeria* spp. On the other hand, the dietary inclusion of extract *Moringa oleifera* (150 mg /kg BW) is a promising safe natural growth enhancer as it increases birds' growth performance with anti-clostridial and anti-coccidia impact via decreasing the intestinal and caecal clostridial counts and limited the coccidia oocyst shedding in the experimentally challenged birds.

## Conflict of interest

The authors have no conflict of interest to declare.

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