



## RESEARCH ARTICLE

### Evaluation of Anticoccidial Activity of Cumin (*Cuminum cyminum*) Essential Oil in Broiler Chicks

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#### ABSTRACT

Avian coccidiosis, a parasitic disease caused by *Eimeria* species, poses a significant economic importance in poultry production. The emergence of drug-resistant *Eimeria* strains prompts the need to investigate effective and safe natural substitutes, including essential oils from plant sources. Thus, the study aimed to determine the anticoccidial and growth-promoting properties of (*Cuminum cyminum*) cumin essential oil (CEO) in broiler chicks that were experimentally infected with *Eimeria* spp. To this end, 270 day-old Hubbard broilers were randomly assigned to six experimental groups; three groups were given CEO at 1, 2, or 3% in feed, respectively; an infected, non-medicated control; a positive control with toltrazuril (Symcox®) on board; and a non-infected, non-medicated control. At day 14, the birds, except the neutral control, were orally inoculated with a mixed population of *Eimeria* spp. ( $1 \times 10^5$  sporulated oocysts/chick). The shedding of oocysts, lesion scores, growth performance, hematological parameters, and serum biochemistry were evaluated through standard protocols. The group supplemented with 3% CEO showed a significant ( $P < 0.05$ ) reduction in oocyst per gram (OPG) of feces ( $54.8 \pm 0.42 \times 10^3$ ), lesion scores ( $1.33 \pm 0.47$ ), and mortality (6.66%), which was as effective as the standard drug, toltrazuril. A better body weight gain, feed conversion ratio and hematological indices (e.g., PCV, Hb, RBC) were also observed in the 3% CEO group compared to the infected, untreated group ( $P < 0.05$ ). The effects were not significant ( $P > 0.05$ ) on serum biochemical parameters. Overall, CEO having 3% dietary supplement shows considerable anticoccidial and growth-promoting effects on broiler chickens, which makes it a promising natural intervention for combating coccidiosis.

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#### INTRODUCTION

Coccidiosis is one of the major health threats to commercial poultry chickens (Hayajneh *et al.*, 2024). It can cause huge economic losses as the disease can cause high mortality in broiler and layer chickens (Rahmani *et al.*, 2024). Multiple species of *Eimeria* are the etiological agents of chicken coccidiosis. Each species of *Eimeria* causes infection at different sites of the chicken intestine. The most famous species include *E. necatrix*, *E. praecox*, *E. tenella*, and *E. brunetti* (Nasiri *et al.*, 2024). These *Eimeria* species can cause infection at different sites of the intestine and cecum of the chicken.

Multiple anticoccidial drugs have exhibited promising results in controlling coccidiosis (Bharti *et al.*, 2025). However, the regular use of the same drugs causes multiple problems, including drug resistance, which is the major health threat of the era, worldwide (Gao *et al.*, 2024). Drug-resistant species of *Eimeria* demand more doses of the anticoccidial drugs, which results in huge economic losses (Abbas *et al.*, 2024; Blake, 2025). Furthermore, the more dosages of the anticoccidial drugs result in the presence of drug residues in the meat of chicken (Ke *et al.*, 2024). Nevertheless, vaccines were still a superior way to prevent the disease, yet that was an option only to the breeder and parent flock of the birds (Zaheer *et al.*, 2025). This illustrates the desperate need of innovation as this is a loophole in

practical, large-scale commercial flock preventive measures. There is a significant need to find alternative strategies to prevent coccidiosis that should be economically suitable and have potent results (Mathis *et al.*, 2024).

Scientists have now focused on alternative treatment and prevention strategies to combat these major issues. The alternative strategies include the use of organic acids, vitamins, botanical compounds, immunogens, peptides, probiotics, etc. Botanical compounds have multiple medicinal and therapeutic activities. Scientists have tested multiple botanical compounds against coccidiosis and found the most promising results (Hussain *et al.*, 2021; Rizwan *et al.*, 2022; Hou *et al.*, 2024; Ristanti *et al.*, 2024). However, various parts of the plants were experimented in different research projects (Zemedie *et al.*, 2024). Essential oils are aromatic liquids that are highly concentrated with the active ingredients, which makes them more preferable choice to be tested against coccidiosis (Amer *et al.*, 2023). Multiple experiments have proved the promising effects of the different essential oils against coccidiosis (Ewais *et al.*, 2024; Huang *et al.*, 2025; Zoroaster *et al.*, 2025).

Cumin (*Cuminum cyminum*) is a common spice that is popularly grown as a botanical herb having antimicrobial, antioxidant and anti-inflammatory pharmacological effects (Sanaei-Hoveida *et al.*, 2024). Its essential oil contains bioactive compounds like cuminaldehyde,  $\gamma$ -terpinene and p-cymene among others, which have been proven to be effective against several pathogens. Nevertheless, its implementation and the use as a dietary anticoccidial agent in chickens are underutilized. Thus, the present study hypothesized that nutritional supplementation of cumin essential oil would alleviate the pathological and productive effects of experimental coccidiosis in broiler chicks. The aim of the study was to determine dose dependent effects of CEO on shedding of oocysts, intestinal pathology, growth performance, and health biomarkers, hence establishing the potential of CEO as a novel phytopathogenic anticoccidial feed additive.

## MATERIALS AND METHODS

**Isolation and sporulation of coccoidal oocyst:** Chicken guts were collected from multiple local chicken sale shops of the study area. Microscopic examination of the intestine and ceca of collected samples was performed for presence of different species of *Eimeria*. Oocytes of *Eimeria* were isolated, identified and sporulated according to methods described by Ryley *et al.* (1976). Briefly, the isolated species of *Eimeria* proceeded with the sporulation process. The samples were incubated at 30°C for 72 hours. Doses were prepared by using the harvested sporulated oocyst and stored in a refrigerator at 4°C for experimentation.

**Extraction and characterization of essential oils:** Seeds of cumin were purchased from the local market and identified by a botanist. Seeds were then ground to make them in powder form. The powdered form was soaked in the water to perform hydro distillation, as described by Gavahian *et al.* (2012). The resulting essential oil was dried using anhydrous sodium sulfate and stored in airtight amber vials at 4°C until further use. The chemical profiling was done using gas chromatography-flame ionization detection (GC-FID) (Agilent 7890B/5977A) fitted with HP-5MS column.

**Birds Management:** A total of 270 Hubbard® chicks were purchased to conduct the research from Alpha Chicks®, Sheikhupura. All the birds were at one day old stage and their management was performed at the experimental station of the Islamia University of Bahawalpur. Preparation of the poultry house was done with the disinfectants (formalin fumigation and potassium permanganate) before the arrival of chicks. Feed and water were provided in each pen in an adequate amount, and the temperature of the house was maintained at 34°C as per guidelines. The humidity in the poultry house was maintained between 50-70%. However, the feeding protocols were followed same as described by Saeed *et al.* (2023).

**Experimental design and treatments:** A total of 270-day-old Hubbard broilers chicks were purchased, housed and randomly assigned to six groups as follows. The doses (1-3%) were selected on the basis of range-finding trials and previously available literature on the incorporation of dietary essential oils in poultry.

**Group CEO-1%:** Basal diet + 1% (v/w) CEO

**Group CEO-2%:** Basal diet + 2% CEO

**Group CEO-3%:** Basal diet + 3% CEO

**Group INF + (Positive Control):** Infected, basal diet + Toltrazuril (1mL/L drinking water)

**Group INF- (Negative Control):** No additive, basal diet, infected

**Group NC (Neutral Control):** Non-infected, non-additive basal diet

**Anticoccidial parameters:** All the experimental groups were evaluated for various anticoccidial parameters. Briefly, faecal score was determined by the daily observations of fecal material made from day 3 to 7 post infection. The faecal score was measured in each of the six groups. The fecal material with heavy blood, which is considered as the worst consistency, was given a score of 4. However, the normal consistency of the fecal sample was given 0 score according to the method of Johnson and Reid (1970). For lesion score, the examination of lesions was done at the 7<sup>th</sup> day of the infection. Guts of the chicken were opened and intestinal/cecal lesions were given a score between 0-4, as represented visually. The oocyst score was carried out 7<sup>th</sup> day of post-infection according to the procedures described by Ryley *et al.* (1976). Furthermore, oocyst per gram (OPG) was calculated of fecal samples and processed following the quantitative techniques described by Zaman *et al.* (2012).

**Performance parameters:** Calculation of the feed intake by the birds of each group was made very precisely and their average was taken. Weight gain of each bird was measured on infection day and continued for 1 week. Evaluation of the feed efficiency was done by calculating the FCR (Abbas *et al.*, 2019). FCR is calculated by the given formula:

$$\text{FCR} = \text{Feed intake (g)} / \text{weight gain (g)}.$$

Weight of the internal organs of the birds was calculated by the percentage of the body weight on the 35<sup>th</sup> day of our trial. The internal organs were collected after the slaughtering of all the remaining birds (Abbas *et al.*, 2019). The calculation of the mortality rates of birds was made between day 1 of the infection to the 7<sup>th</sup> day of infection. On postmortem examination, only the birds which showed coccidiosis lesions were considered.

**Hematological parameters:** Hemocytometer was used to calculate the Packed Cell Values (PCV), Hemoglobin (Hb) Concentration (MCHC), and Corpuscular Hemoglobin (MCH). While Natt and Herrick technique was used to calculate the White Blood Cells (WBCs), Red blood cell counts (RBC), and differential leukocytes (Natt and Herrick, 1952).

**Serum chemistry:** Spectrophotometry was performed for the calculation of Aspartate transferase (AST), lactate dehydrogenase (LDH), serum albumins (SA), Alkaline Phosphatases (ALKP), total serum proteins (TSP), and serum globulins (SG) (Abbas *et al.*, 2019).

**Statistical analysis:** R statistical software (v 4.5.0) was used to analyze all the data. One-way analysis of variance (ANOVA) was used to analyze performance and oocyst data. In case of any major difference ( $P<0.05$ ), post-hoc mean was separated with the Tukey Honestly Significant Difference (HSD) test. Kaplan-Meier survival analysis was used to analyze mortality data. The results are expressed in mean  $\pm$  standard error of the mean.

## RESULTS

**Anticoccidial parameters:** Cumin essential oil was observed and statistically compared with the control groups to determine the anticoccidial activities. Our results have determined that the CEO has shown significant  $P<0.05$  activities against coccidiosis at all the dose concentrations provided. The group of birds that were provided with the 3% concentration of the essential oil have the values of OPG, faecal, oocyst and lesion scores, nonsignificant ( $P>0.05$ ) to group D and statistically significant ( $P<0.05$ ) than the negative control birds (Table 1).

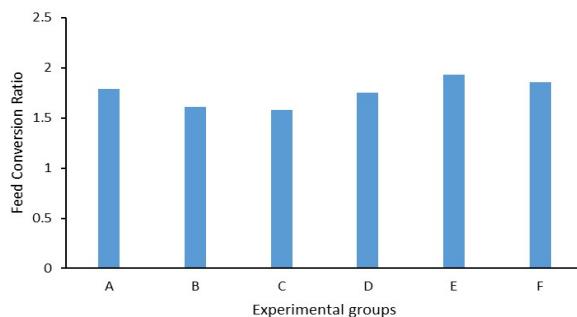
**Table 1:** Effect of cumin on oocyst per gram of faeces (OPG), oocyst score and lesion scores in experimental chicken

Treatment	Oocyst Score	OPG ( $\times 10^3$ )	Lesion Scoring
A	1.33 $\pm$ 0.47 <sup>bc</sup>	12.0 $\pm$ 0.15 <sup>d</sup>	1.33 $\pm$ 0.47 <sup>bc</sup>
B	2.33 $\pm$ 0.47 <sup>ab</sup>	32.5 $\pm$ 0.50 <sup>c</sup>	2.66 $\pm$ 0.50 <sup>ab</sup>
C	3.33 $\pm$ 0.47 <sup>a</sup>	54.8 $\pm$ 0.42 <sup>b</sup>	3.66 $\pm$ 0.47 <sup>a</sup>
D	0.66 $\pm$ 0.47 <sup>e</sup>	7.8 $\pm$ 0.16 <sup>de</sup>	0.33 $\pm$ 0.47 <sup>c</sup>
E	4 $\pm$ 0.81 <sup>a</sup>	102.0 $\pm$ 0.39 <sup>a</sup>	3.66 $\pm$ 0.47 <sup>a</sup>
F	0 $\pm$ 0 <sup>c</sup>	0 $\pm$ 0 <sup>e</sup>	0 $\pm$ 0 <sup>c</sup>

Mean values denoted by identical letters within the same column indicate no statistically significant difference ( $P>0.05$ ).

**Performance parameters:** Feed intake, FCR and weight gain were calculated and presented as FCR. The performance of birds that were given 3% of the essential oil has exhibited the best value among all groups of birds (Fig. 1). The mortality of the birds is shown in Table 3. While the group that was provided with the infection, but no treatment had shown the poorest values. After statistical analysis of organ weight ratio of the birds, the group C

showed a significant difference ( $P<0.05$ ) from the birds of group E (Table 4).



**Fig. 1:** Effects of cumin essential oil at different concentrations on feed conversion ratio of experimental birds.

**Table 2:** Effect of cumin oil on the fecal scores in experimental chicken

Group	Day 4	Day 5	Day 6	Day 7
A	3.66 $\pm$ 0.47 <sup>ab</sup>	3.66 $\pm$ 0.47 <sup>ab</sup>	3.66 $\pm$ 0.47 <sup>ab</sup>	3 $\pm$ 0 <sup>ab</sup>
B	3 $\pm$ 0.81 <sup>abc</sup>	2.66 $\pm$ 0.47 <sup>bc</sup>	2 $\pm$ 0.81 <sup>cde</sup>	2 $\pm$ 0.81 <sup>cde</sup>
C	1 $\pm$ 0 <sup>def</sup>	0.66 $\pm$ 0.47 <sup>ef</sup>	0.33 $\pm$ 0.47 <sup>def</sup>	0.33 $\pm$ 0.47 <sup>ef</sup>
D	0.66 $\pm$ 0.47 <sup>ef</sup>	0 $\pm$ 0 <sup>ef</sup>	0 $\pm$ 0 <sup>ef</sup>	0 $\pm$ 0 <sup>f</sup>
E	2.33 $\pm$ 1.69 <sup>a</sup>	2.33 $\pm$ 1.69 <sup>a</sup>	2.66 $\pm$ 1.88 <sup>ab</sup>	2.66 $\pm$ 1.88 <sup>a</sup>
F	1.33 $\pm$ 1.88 <sup>f</sup>	1.33 $\pm$ 1.88 <sup>f</sup>	1.33 $\pm$ 1.88 <sup>f</sup>	1.33 $\pm$ 1.88 <sup>f</sup>

Mean values denoted by identical letters within the same column indicate no statistically significant difference ( $P>0.05$ ).

**Table 3:** Effect of Cumin oil on the percent mortality of the experimental chicken

Groups	Mortality	No of birds died						Mortality (%)	
		Days	3	4	5	6	7		
A			3	3	7	5	0	18	40.00
B			0	2	2	1	1	6	13.33
C			0	1	0	1	1	3	6.66
D			1	1	0	1	1	4	8.88
E			2	6	9	7	2	26	57.77
F			1	0	0	0	0	1	2.22

**Table 4:** Effect of cumin on the percent organ weight ratio of experimental chicken

Group	Liver	Kidney	Heart	Intestine	Bursa	Spleen	Gizzard
A	2.52 $\pm$ 0.06 <sup>a</sup>	0.87 $\pm$ 0.1 <sup>a</sup>	0.48 $\pm$ 0.02 <sup>a</sup>	5.8 $\pm$ 0.21 <sup>b</sup>	0.22 $\pm$ 0.01 <sup>b</sup>	0.07 $\pm$ 0.01 <sup>a</sup>	1.96 $\pm$ 0.09 <sup>b</sup>
B	2.7 $\pm$ 0.16 <sup>b</sup>	1.06 $\pm$ 0.12 <sup>a</sup>	0.44 $\pm$ 0.04 <sup>b</sup>	6.23 $\pm$ 0.52 <sup>b</sup>	0.25 $\pm$ 0 <sup>b</sup>	0.08 $\pm$ 0.02 <sup>b</sup>	1.66 $\pm$ 0.12 <sup>b</sup>
C	3.13 $\pm$ 0.09 <sup>a</sup>	1.03 $\pm$ 0.04 <sup>a</sup>	0.56 $\pm$ 0.05 <sup>a</sup>	7.53 $\pm$ 0.41 <sup>a</sup>	0.29 $\pm$ 0.01 <sup>a</sup>	0.11 $\pm$ 0 <sup>a</sup>	2.06 $\pm$ 0.12 <sup>a</sup>
D	2.89 $\pm$ 0.15 <sup>a</sup>	0.98 $\pm$ 0.01 <sup>a</sup>	0.48 $\pm$ 0.01 <sup>a</sup>	7.43 $\pm$ 0.41 <sup>a</sup>	0.27 $\pm$ 0.01 <sup>a</sup>	0.1 $\pm$ 0.01 <sup>a</sup>	1.78 $\pm$ 0.06 <sup>a</sup>
E	1.93 $\pm$ 0.41 <sup>b</sup>	0.86 $\pm$ 0.05 <sup>a</sup>	0.42 $\pm$ 0.02 <sup>b</sup>	4.23 $\pm$ 0.52 <sup>b</sup>	0.07 $\pm$ 0.01 <sup>c</sup>	0.07 $\pm$ 0 <sup>a</sup>	1.78 $\pm$ 0.02 <sup>b</sup>
F	2.96 $\pm$ 0.33 <sup>a</sup>	0.96 $\pm$ 0.04 <sup>a</sup>	0.58 $\pm$ 0.01 <sup>a</sup>	6.83 $\pm$ 0.24 <sup>b</sup>	0.25 $\pm$ 0.03 <sup>b</sup>	0.1 $\pm$ 0.01 <sup>a</sup>	2.01 $\pm$ 0.06 <sup>a</sup>

Mean values denoted by identical letters within the same column indicate no statistically significant difference ( $P>0.05$ ).

**Hematological profile:** Multiple concentrations of the essential oil were provided to estimate the blood profile. MCH, MCHC, PCV, Hb, RBC, Heterophils, Lymphocytes, Eosinophils, Monocytes, Basophils, and WBC values were compared statistically. A significantly different value ( $P<0.05$ ) of group C had seen in the birds of group E (Table 5 and 6).

**Table 5:** Effects of cumin on haematological parasites of experimental chickens

Groups	MCH (pg)	MCHC (%)	PCV (%)	Hb	RBC ( $\times 10^6$ /uL)
A	44.1 $\pm$ 1.37 <sup>a</sup>	29.39 $\pm$ 1.1 <sup>a</sup>	26.75 $\pm$ 1.05 <sup>b</sup>	8.79 $\pm$ 0.97 <sup>a</sup>	2.48 $\pm$ 0.31 <sup>ab</sup>
B	43.17 $\pm$ 5.19 <sup>a</sup>	28.77 $\pm$ 2.41 <sup>a</sup>	29.51 $\pm$ 1.86 <sup>a</sup>	9.46 $\pm$ 1.36 <sup>a</sup>	2.57 $\pm$ 0.29 <sup>ab</sup>
C	41.12 $\pm$ 5.32 <sup>ab</sup>	33.19 $\pm$ 1.97 <sup>a</sup>	32.33 $\pm$ 1.81 <sup>a</sup>	11.39 $\pm$ 0.97 <sup>a</sup>	3.19 $\pm$ 0.26 <sup>a</sup>
D	43.52 $\pm$ 1.17 <sup>a</sup>	31.35 $\pm$ 4.46 <sup>a</sup>	34.14 $\pm$ 1.44 <sup>a</sup>	11.06 $\pm$ 0.63 <sup>a</sup>	3.16 $\pm$ 0.16 <sup>a</sup>
E	31.82 $\pm$ 1.2 <sup>b</sup>	25.48 $\pm$ 1.28 <sup>b</sup>	23.71 $\pm$ 1.03 <sup>c</sup>	8.3 $\pm$ 0.82 <sup>b</sup>	2.34 $\pm$ 0.04 <sup>b</sup>
F	43.94 $\pm$ 2.21 <sup>a</sup>	32.44 $\pm$ 1.84 <sup>a</sup>	32.09 $\pm$ 1.76 <sup>a</sup>	11.53 $\pm$ 1.06 <sup>a</sup>	3.06 $\pm$ 0.26 <sup>a</sup>

Mean values denoted by identical letters within the same column indicate no statistically significant difference ( $P>0.05$ ).

**Table 6:** Effects of cumin on differential leukocytes counts (WBC per  $\mu$ L)

Groups	Heterophils	Lymphocytes	Eosinophils	Monocytes	Basophils	WBC
A	777.25 $\pm$ 20.87 <sup>b</sup>	2049.36 $\pm$ 76.73 <sup>ab</sup>	130.58 $\pm$ 3.87 <sup>a</sup>	160.97 $\pm$ 3.57 <sup>ab</sup>	15.52 $\pm$ 0.96 <sup>a</sup>	3222.4 $\pm$ 164.23 <sup>ab</sup>
B	691.87 $\pm$ 33.24 <sup>b</sup>	1769.15 $\pm$ 49.11 <sup>bc</sup>	120.28 $\pm$ 6.16 <sup>ab</sup>	145.98 $\pm$ 4.24 <sup>ab</sup>	14.91 $\pm$ 1.52 <sup>a</sup>	2804.05 $\pm$ 107.1 <sup>bc</sup>
C	604.44 $\pm$ 103.36 <sup>b</sup>	1428.45 $\pm$ 127.17 <sup>c</sup>	104.3 $\pm$ 22.84 <sup>ab</sup>	133.14 $\pm$ 18.14 <sup>ab</sup>	15.99 $\pm$ 0.85 <sup>a</sup>	2273.52 $\pm$ 321.12 <sup>c</sup>
D	567.07 $\pm$ 45.7 <sup>b</sup>	1551.72 $\pm$ 71.38 <sup>bc</sup>	88.51 $\pm$ 4.43 <sup>b</sup>	127.3 $\pm$ 8.61 <sup>b</sup>	12.42 $\pm$ 1.06 <sup>a</sup>	2327.42 $\pm$ 55.08 <sup>c</sup>
E	917.77 $\pm$ 36.86 <sup>a</sup>	2567.27 $\pm$ 324.29 <sup>a</sup>	126.74 $\pm$ 3.38 <sup>a</sup>	163.33 $\pm$ 12.64 <sup>a</sup>	13.15 $\pm$ 2.6 <sup>a</sup>	3846 $\pm$ 250.93 <sup>a</sup>
F	640.46 $\pm$ 91.56 <sup>b</sup>	1793.51 $\pm$ 111.85 <sup>bc</sup>	91.39 $\pm$ 2.84 <sup>b</sup>	130.22 $\pm$ 4.38 <sup>ab</sup>	11.09 $\pm$ 2.41 <sup>a</sup>	2610.33 $\pm$ 270.16 <sup>bc</sup>

Mean values denoted by identical letters within the same column indicate no statistically significant difference ( $P>0.05$ ).

**Table 7:** Effects of cumin on serum parameters of experimental chickens

Groups	Alt (IU/L)	AST (IU/L)	ALKP (IU/L)	total proteins (g/dL)	Albumins (g/dL)	Globulins (g/dL)	Urea mmol/L	LDH U/L
A	7.87 $\pm$ 0.85 <sup>bc</sup>	330.74 $\pm$ 21.66 <sup>a</sup>	2017.51 $\pm$ 49.18 <sup>a</sup>	3.76 $\pm$ 0.07 <sup>a</sup>	1.75 $\pm$ 0.11 <sup>a</sup>	1.95 $\pm$ 0.07 <sup>a</sup>	4.36 $\pm$ 0.06 <sup>a</sup>	342.22 $\pm$ 8.36 <sup>ab</sup>
B	7.77 $\pm$ 0.43 <sup>bc</sup>	274.41 $\pm$ 7.11 <sup>b</sup>	1832.1 $\pm$ 75.74 <sup>bc</sup>	3.52 $\pm$ 0.08 <sup>a</sup>	1.69 $\pm$ 0.05 <sup>a</sup>	1.95 $\pm$ 0.11 <sup>a</sup>	4.44 $\pm$ 0.15 <sup>a</sup>	295.85 $\pm$ 15.99 <sup>bc</sup>
C	9.47 $\pm$ 0.24 <sup>a</sup>	240.24 $\pm$ 8.01 <sup>b</sup>	1749.43 $\pm$ 73.75 <sup>c</sup>	3.59 $\pm$ 0.17 <sup>a</sup>	1.83 $\pm$ 0.09 <sup>a</sup>	1.73 $\pm$ 0.22 <sup>a</sup>	4.33 $\pm$ 0.13 <sup>a</sup>	269.42 $\pm$ 9.87 <sup>c</sup>
D	9.38 $\pm$ 0.16 <sup>ab</sup>	274.15 $\pm$ 14.4 <sup>b</sup>	1806.99 $\pm$ 35.84 <sup>bc</sup>	3.37 $\pm$ 0.24 <sup>a</sup>	1.82 $\pm$ 0.07 <sup>a</sup>	1.55 $\pm$ 0.27 <sup>a</sup>	4.17 $\pm$ 0.14 <sup>a</sup>	265.11 $\pm$ 7 <sup>c</sup>
E	7.28 $\pm$ 0.36 <sup>c</sup>	352.71 $\pm$ 13.47 <sup>a</sup>	1976.27 $\pm$ 38.34 <sup>ab</sup>	3.25 $\pm$ 0.18 <sup>a</sup>	1.55 $\pm$ 0.07 <sup>a</sup>	1.68 $\pm$ 0.16 <sup>a</sup>	4.46 $\pm$ 0.11 <sup>a</sup>	370.67 $\pm$ 24.28 <sup>a</sup>
F	9.07 $\pm$ 0.49 <sup>ab</sup>	334.85 $\pm$ 22.4 <sup>a</sup>	1735.07 $\pm$ 56.22 <sup>c</sup>	3.44 $\pm$ 0.09 <sup>a</sup>	1.81 $\pm$ 0.08 <sup>a</sup>	1.62 $\pm$ 0.22 <sup>a</sup>	4.29 $\pm$ 0.16 <sup>a</sup>	305.24 $\pm$ 16.06 <sup>bc</sup>

Mean values denoted by identical letters within the same column indicate no statistically significant difference ( $P>0.05$ ).

**Serum chemistry:** Statistical comparison was performed between the birds receiving essential oil and the control groups. ALT, AST, ALKP, total proteins, Urea, Albumins, Globulins, and LDH values were compared statistically, and no specific trends were found in the treatment groups of essential oil ( $P>0.05$ ) (Table 7).

## DISCUSSION

Coccidiosis remains among the most severe enteric diseases, impacting commercial poultry production globally with a significant implication on the intestinal integrity, nutrient utilization, immune competence and overall flock performance (Mathis *et al.*, 2024; Blake, 2025). *Eimeria* species cause massive destruction of the epithelium, hemorrhage, inflammatory infiltration and oxidative stress that result in diarrhea, reduced growth, anemia, and high mortality (Razavi *et al.*, 2024). The classical pathological and productive effects of *Eimeria* spp. were effectively reproduced in the present study through experimental challenge using mixed population of *Eimeria* spp. as demonstrated by high oocyst shedding, gross lesions, impaired growth performance, distorted hematological indices and high mortality rates in the infected untreated group. Supplementation with CEO especially at the dose of 3% greatly reduced these negative effects and emphasized its considerable anticoccidial and growth-promoting nature.

During current investigation, the OPG of feces and intestinal lesion scores were significantly reduced in CEO treated birds. The shedding of oocysts is a parameter of great importance in coccidiosis, as it indicates the extent of parasite multiplication in the host, as well as the level of environmental contamination that keeps the cycles of infection in the poultry house (Gao *et al.*, 2024). The birds fed 3% CEO showed significant decrease in OPG with values that were statistically similar with those of the birds treated with toltrazuril. This observation points out to the conclusion that CEO successfully disrupts the *Eimeria* life cycle and inhibits propagation of parasites. The dose dependent effect in the current study, where higher concentrations of CEO led to higher anticoccidial activity, is not new since other studies have argued that essential oils need adequate levels of inclusion to achieve antiparasitic effects (Imran and Alsayeqh, 2022; Ewais *et al.*, 2024).

The anticoccidial efficacy of CEO has been explained by its complicated phytochemical state, which comprises of cuminaldehyde, 7-terpinene and p-cymene as the primary bioactive compounds. The compounds have well reported antimicrobial, antiparasitic and antioxidant properties (Alem, 2024). It is evident from the literature that CEO disrupts cell membrane of parasites, impairs mitochondrial respiration and disrupts intracellular development stages (i.e., schizogony and gametogony) of parasite (Zhai *et al.*, 2024). Other essential oils such as oregano, garlic, eucalyptus and citrus oils have also been suggested to have similar mechanisms and have shown efficiency against *Eimeria* spp. in broiler chickens (Sidiropoulou *et al.*, 2020; Chang *et al.*, 2021; Huang *et al.*, 2025; Zorraster *et al.*, 2025).

The protective effect on the health of the gut is further demonstrated by the decrease in severity of intestinal lesions and improvement of fecal consistency in CEO treated birds. The lesions of intestine due to the *Eimeria* infection interfere with the epithelial continuity and tight junction integrity, leading to hemorrhage, malabsorption and diarrhea (Liu *et al.*, 2023; Hayajneh *et al.*, 2024). In the current experiment, birds that were fed 3% CEO had lower lesion scores and normal fecal scores were regained within the period of days 4 to 7 post infection. These results indicate that CEO does not only reduce the extent of damage caused by parasites, but also can stimulate intestinal restoration and healing. Essential oils are proved to have a positive impact on the mucosal integrity, alter the composition of the gut microbiota and prevent the development of secondary bacterial infections, all of which help to increase intestinal functionality in an enteric challenge (Calik and Ergun, 2023; Saleh *et al.*, 2024).

Maintaining the intestinal integrity was indicated by the enhanced performance of the CEO-treated birds in terms of growth. The body weight gain, better feed intake and better FCR in broilers from 3% CEO were much greater than those of untreated infected birds. The reduction of nutrient absorption, the elevated metabolic energy in reaction to inflammation and anorexia are the main factors behind growth suppression during coccidiosis (Rahmani *et al.*, 2024). CEO may have increased the efficiency of nutrient use by minimizing intestinal damage and inflammation. Moreover, it is also recognized fact that essential oils trigger the release of the enzymes of the digestive system and the bile that further promotes the

digestion of the feed and the absorption of the nutrients (Kiczonowska *et al.*, 2024; Qaid and Abdelrahman, 2024). The performance of birds in the 3% CEO group was similar to toltrazuril-treated birds stating the high level of practical applicability of CEO as a natural growth-promoting anticoccidial.

One of the strongest outcomes of this research is mortality reduction. Mortality rate was over 50% in the untreated infected group, which indicated the pathogenicity of the mixed *Eimeria* infection challenge. Birds that were fed 3% CEO on the other hand, had a mortality rate of just 6.66% which was at par with the toltrazuril-treated group or slightly higher. Extensive intestinal hemorrhage, dehydration, electrolyte imbalance, and secondary infections are usually linked to high mortality during coccidiosis (Mathis *et al.*, 2024). The survival of the host dramatically improves, indicating that CEO does not only inhibit the reproduction of the parasites, but also increases the resilience of the host in the acute infection. This protective action can be explained by a complex combination of decreased intestinal damage, enhanced immune competence and alleviation of oxidative stress (Grenier and Applegate, 2023; Zhai *et al.*, 2024)

Supplementation with CEO has additional hematological data to its positive effects. The intestinal hemorrhage that is caused by coccidiosis usually causes anemia, with a low PCV, low Hb levels and low RBC counts (Hayajneh *et al.*, 2024). In the current experiment, the untreated infected birds had significant decreases in these indices and birds that received 3% CEO had much better hematological profiles. The process of erythrocyte parameters restoration indicates the decrease in blood loss and the enhancement of erythropoietic activity. In addition, the high levels of white blood cells and heterophil in the untreated infected birds are signs of systemic inflammation and stress. There were regularization of leukocyte counts and differentials which is an indication of reduced hyperinflammatory responses (Surai and Kochish, 2023; Bachaya *et al.*, 2024)

The reported shifts in weights of various immune related organs give additional understanding on the immunomodulatory action of CEO. Major coccidiosis is reported to damage the development of lymphoid organs, specifically the bursa of Fabricius and spleen, which results in weakening the immune system (Lillehoj *et al.*, 2023). In the current research, the relative weights of these organs were much greater in birds treated with CEO in comparison with infected untreated birds which implies the maintenance of immune organ integrity. Essential oils are also said to regulate innate and adaptive immune functions, improving disease resistance and avoiding excessive stimulation of inflammatory systems (Amer *et al.*, 2023). This effect on immunomodulation could also be one of the factors that have enhanced disease tolerance in CEO treated birds (Amer *et al.*, 2023; Saeed *et al.*, 2023).

The generation of excessive reactive oxygen species by host immune systems, which aggravate the epithelial damage and function of cells, is a key element of the pathogenesis of coccidiosis (Razavi *et al.*, 2024). The antioxidant activity of CEO is very promising, which might have been a significant factor in the recorded benefits of cumin oil on intestinal health, hematological parameters and growth performance. CEO can disrupt the pathogenic

process of oxidative injury and inflammation of severe *Eimeria* infection by scavenging free radicals and enhancing intrinsic antioxidant defense mechanisms (Huang *et al.*, 2025). This antioxidant activity supplements its direct antiparasitic effect and increases the overall host recovery (Ewais *et al.*, 2024; Bachaya *et al.*, 2024)

Notably, the supplementation of the CEOs did not trigger any negative changes in the serum biochemical parameters, such as liver enzymes, kidney functioning indicators and protein patterns. The fact that there were no notable modifications in the concentrations of ALT, AST, urea and total protein levels shows that CEO can be safely ingested at dietary levels up to 3%. This safety profile is specifically applicable due to the concerns with drug residues, toxicity and withdrawal times with conventional anticoccidials (Ke *et al.*, 2024). The good serum chemistry in this study indicates the suitability of CEO to serve a long-term diet in the rearing of broilers.

Practically, the results of this research are very applicable to sustainable systems of producing poultry. The rising limitations of using anticoccidial drugs, rising resistance concerns and consumer demands that the poultry products available to them should be residue free have increased the pursuit of natural alternatives (Bharti *et al.*, 2025; Zorer *et al.*, 2025). The fact that CEO can equal toltrazuril in controlling experimental coccidiosis highlights potentials in the integration of coccidiosis management programs using this phytogenic additive as a standalone phytogenic feed ingredient or alongside the reduced drug program.

The current research indicates that dietary supplementation with CEO, especially at 3%, is effective in reducing the pathological, productive as well as physiological effects of experimental coccidiosis in broiler chickens. Antiparasitic, antioxidant and immunomodulatory properties of CEO are also associated with the reduction of oocyst shedding, increased intestinal health, improved growth performance and increased survival without causing metabolic harm.

**Conclusions:** Finally, this study shows that dietary supplementation of CEO oil at 3% level is effective in controlling experimental coccidiosis in broiler chicken through the reduction of the oocyst shedding, low lesion score and enhanced survival. Simultaneously, it improves growth performance and major hematological indices without significantly impacting serum biochemistry, which demonstrates its safety at this inclusion level. The findings indicate the use of CEO as a potential natural anticoccidial agent. However, the understanding of its exact mechanism of action should also be explored. Furthermore, determining its effectiveness in the field conditions, its economic viability, carrying out cost-benefit studies and long-term effects to commercial poultry production.

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