



## RESEARCH ARTICLE

### Anticoccidial Activity of Chinese Cardamom (*Lanxangia tsao-ko*) Essential Oil in Broiler Chicken

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#### ABSTRACT

Coccidiosis, a parasitic disease caused by *Eimeria*, has a catastrophic effect on the world's poultry industry. Synthetic anticoccidial drugs are being used commercially for the prevention and treatment of disease and are being stopped in their normal use because of drug resistance and problems affecting the population's health. Several alternatives are being proposed, with essential oils being some of the novel substances to be used against this disease experimentally. The application of the essential oil of Chinese cardamom (*Lanxangia tsao-ko*) in the prevention of protozoal infection in broiler chickens was tested in this experiment. A total of 270 broiler chickens were reared and grouped into 6 groups, with each group to obtain randomization. Essential oil of Chinese cardamom was administered to three groups at 1, 2, and 3% concentrations, and the remainder of the three was medicated control, infected 006Eon-medicated control, and non-infected non-medicated control group, respectively. The parameters on the effect on the coccidial parameters (lesion score, oocyst score, oocyst per gram of feces, and fecal score), performance parameters (feed intake, weight gain, feed ratio, organ weight ratios and percent mortality), and hematological and serum parameters were estimated. The results showed that the 3% essential oil significantly ( $P < 0.05$ ) decreased lesion scores (1.33 vs. 4.0), oocyst scores, oocyst per gram ( $1.33 \times 10^4$  vs.  $3.66 \times 10^4$ ), and mortality (4.44 vs. 35.55%). It also improved weight gain and feed conversion when compared to the infected non-medicated control group. Following the anticoccidial potential of the essential oil of Chinese cardamom, its application can be considered in the control programs of coccidiosis in broiler chickens.

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#### INTRODUCTION

Coccidiosis has remained one of the most economically devastating parasitic diseases that afflict the poultry industry in the world (Zaheer *et al.*, 2022; Rahmani *et al.*, 2024). It is an apicomplexan protozoon of the genus *Eimeria*, which invades and reproduces in the intestinal epithelium of birds and causes severe enteritis, malabsorption, hemorrhagic diarrhea, and high mortality rates (Gao *et al.*, 2024; Bharti *et al.*, 2025). The disease poses a severe threat to broiler chickens because of intense rearing methods that contribute to the rapid transmission and large oocyst loads. There are seven *Eimeria* species known to infect chickens, among which *E. tenella*, *E. necatrix*, *E. maxima*, and *E. brunetti* are the most harmful,

resulting in cecal and small intestine lesions (Xu *et al.*, 2022; Kassaw *et al.*, 2025). Coccidiosis represents a persistent and significant threat to poultry producers globally, with estimated annual worldwide losses reaching into the billions of dollars because of factors such as mortality, reduced weight gain, and less efficient feed conversion ratios.

Synthetic anticoccidial drugs and ionophores have been the primary source for the control of coccidiosis in poultry feed over the decades (Chapman and Rathinam, 2022; Martins *et al.*, 2022). These include toltrazuril, amprolium and monensin, which have proved to be useful in the reduction of clinical outbreaks and an increase in productivity (Ogwiji *et al.*, 2022). Nevertheless, their extensive and usually unselective application has resulted

in the development of drug-resistant strains of *Eimeria*, and the issue has been growing more acute over the years (Abbas *et al.*, 2008; Abbas *et al.*, 2023). Drug uptake, target site mutations, and increased efflux pumps are some mechanisms of resistance that have made many traditional anticoccidials ineffective (Abbas *et al.*, 2011a). Moreover, drug residues in poultry meat and eggs are a health risk to people, and they may cause cancer and alter the intestinal microbes of a person (Abbas *et al.*, 2014). As a result, regulatory bodies in several countries have limited the application of some anticoccidial agents, leading to the search for more sustainable and safer alternatives (Aguiar-Martins *et al.*, 2023; Rodriguez *et al.*, 2025). Vaccination has also turned out to be a potential option, with live attenuated and recombinant vaccines being produced against different *Eimeria* species. Nevertheless, vaccines tend to be costly, need special care, and may not offer full protection against all strains of the field, particularly at times of high challenge pressure. Moreover, the immunity produced by vaccines is prone to immunosuppressive conditions that are usually found on commercial poultry farms. Therefore, there is still a strong demand for effective, affordable, and residue-free substitutes, which can be incorporated into coccidiosis control programs (Saeed and Alkheraije, 2023).

Plant-derived botanicals and their essential oils have received significant interest among natural alternatives because of their wide range of bioactivity, such as antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory (Ali *et al.*, 2025; El-Saadony *et al.*, 2025). Essential oils are volatile, lipophilic secondary metabolites which can penetrate biological membranes easily and have synergistic effects in a variety of ways. Some of these essential oils, including oregano, garlic, thyme, and citrus, have been found to lower coccidial lesion scores, oocyst shedding and enhance growth performance in experimentally infected chickens (Felici *et al.*, 2023; Khukhodziinai *et al.*, 2024). It is also believed that the anticoccidial effect of these oils is because of their capacity to scavenge reactive oxygen species (ROS) and indirectly mediate oxidative damage caused by the immune response of the host and parasite replication, and directly disrupt the parasite life cycle (Ahmed *et al.*, 2025).

Chinese cardamom (synonym *Amomum tsao-ko*) is a perennial herb of the Zingiberaceae family, native to the highlands of Southwest China, Vietnam, and Laos (Sim *et al.*, 2019; Hazitai *et al.*, 2024). Its dried fruits are commonly consumed as a spice in Asian cuisine and as traditional medicine to treat digestive disorders, malaria, and inflammation (Abdullah *et al.*, 2022). *L. tsao-ko* oil is a source of bioactive monoterpenes and sesquiterpenes, with 1, 8-cineole,  $\alpha$ -terpinyl acetate, geraniol, and limonene as the most common ones (Noumi *et al.*, 2018). These polymers have proven to be strong antioxidants, antimicrobials, and anti-inflammatory agents in various *in vivo* and *in vitro* models. As an example, 1,8-cineole was found to decrease the production of pro-inflammatory cytokines and increase the mucosal protective barrier, whereas  $\alpha$ -terpinyl acetate presents excellent radical-scavenging activity (Alam *et al.*, 2021). With such properties, it is reasonable to expect that the *Lanxangia tsao-ko* essential oil may have positive effects against enteric pathogens like *Eimeria*. Although *L. tsao-ko*

essential oil has been known to be bioactive, the anticoccidial effects of this product in poultry have never been explored.

Hence, the aim of the research was to assess the effectiveness of dietary supplementation of *L. tsao-ko* essential oil at three concentrations (1, 2, and 3%) on coccidial parameters, growth performance, hematological profile, and serum biochemistry of broiler chickens experimentally infected with mixed *Eimeria* species. The results of the current study are anticipated to help in the creation of natural and sustainable options for coccidiosis prevention in the poultry sector.

## MATERIALS AND METHODS

**Parasite material:** Coccidiosis-infected intestinal contents of chickens were obtained by Avian Research Center, King Faisal University. Morphological features were used to identify oocysts under the microscope (Ryley *et al.*, 1976). Mixed *Eimeria* species (*E. tenella*, *E. necatrix*, *E. maxima*, and *E. mivati*) were used. Oocysts were sporulated in 2.5 percent potassium dichromate at 30°C for 72 hours, washed, and kept at 4°C until used. At the age of 7 days, 100,000 sporulated oocysts were administered to each of the infected groups orally. Each chicken was inoculated with ND, IBD, and IB vaccines, adhering strictly to the vaccination schedule detailed in the Hubbard manual.

**Essential Oils:** Seeds of Chinese Cardamom were purchased and identified. The seeds were crushed into a powder and hydro distilled in a Clevenger-type distillation apparatus for 4 hours, as reported in research (Gavahian *et al.*, 2012). The essential oil yield was approximately 2.0% (w/w), which is in line with the previous reports for *L. tsao-ko* (Sim *et al.*, 2019). The resulting essential oil was stored in airtight amber glass bottles at 25°C until use.

**Ethical statement:** Birds were monitored daily to ensure humane handling and minimal discomfort. All procedures complied with King Faisal University research ethical guidelines (Approval No. KFU-REC-2026-Jan-ETHICS4781).

**Management of birds:** Two hundred and seventy one-day-old broiler chickens (Hubbard) were kept in a bio-secure facility at the Experimental Research Center, King Faisal University, Al-Ahsa. Potassium permanganate and formalin were used to fumigate the house before chicken arrived (Mitchell *et al.*, 2000). Birds were confined to floor pens where they had *ad libitum* access to food and water. The temperature was set at 34°C in the first week and was slowly lowered, relative humidity maintained at 50-70 percent. The basal diet was prepared based on the standard broiler needs and was devoid of anticoccidials.

**Experimental Design:** On the 7th day of age, the chickens were randomly separated into 6 experimental groups (A-F) consisting of three replicates of 15 chickens each and replicates were distributed across different floor locations to minimize spatial bias. The 100,000 sporulated oocysts/birds were inoculated into groups A-E, and group F was left uninoculated. The following were the treatment groups:

Group A (P1): Infected chickens given treatment of 1% Chinese Cardamom essential oil in feed

Group B (P2): Infected chickens given treatment of 2% Chinese Cardamom essential oil in feed

Group C (P3): Infected chickens given treatment of 3% Chinese Cardamom essential oil in feed

Group D (C1): Infected chickens given toltrazuril (Symocox®, containing 25 mg toltrazuril per mL, at 1 mL/L in drinking water)

Group E (C2): Infected chickens given no treatment (negative control)

Group F (C3): uninfected chickens given no treatment (neutral control)

The essential oil was added to the feed manually and was mixed daily to maintain even distribution using the technique of incremental dilution, in which a small portion of oil is mixed with feed before combining complete cluster to ensure homogeneity. Toltrazuril was administered in drinking water on two consecutive days since day 7 after infection.

## Parameters

### Coccidial Parameters

1. **Fecal scoring:** Fecal scoring was done between days 4-7 after infection based on the scoring system of Johnson and Reid's study, in which the normal consistency of feces was given 0 while the worst consistency was given 4 (Johnson and Reid, 1970).

2. **Lesion scoring:** On the 7<sup>th</sup> day of infection, three birds per replicate were euthanized, and the intestinal and cecal lesions scored from 0 to 4 as prescribed by the method of Johnson and Reid (1970). Specifically, the ceca segment was examined for *E.tenella*, the midgut segment (duodenum to jejunum) for *E.maxima*, and the entire small intestine segment for *E.necatrix* and *E.mivati*.

3. **Oocyst scoring:** Microscopic scoring of unsporulated oocysts in fecal smears from 0 to 4 was done on 7<sup>th</sup> day after infecting birds (Ryley *et al.*, 1976).

4. **Oocyst per gram (OPG):** Fecal samples were taken after 7 days of infection, and OPG was established through McMaster counting method.

### Growth parameters

1. **Weight gain:** The weight gain of the chickens during the experiment was recorded from the first day of infection to the 7<sup>th</sup> day post-infection (Abbas *et al.*, 2019).

2. **Feed intake:** The feed intake of the chickens was determined, and the average was taken during the experimental period (Abbas *et al.*, 2019).

3. **Feed Conversion Ratio:** For evaluating the feed efficiency of the birds, FCR was calculated by the given formula

FCR= feed intake of the birds (g)/ weight gain of the birds (g) (Abbas *et al.*, 2019).

4. **Mortality rates:** During the observational period, the mortality rates were calculated from the day of infection to 7 days post infection, and birds developing coccidiosis lesions on post-mortem examinations were taken into consideration. The formula is

% Mortality: Number of dead birds in the group/ total no of birds × 100.

5. **Organ weight ratio:** At the 35<sup>th</sup> day of trial, all the surviving birds were slaughtered, and the weights of the

internal organs were determined using the percent of body weight.

**Hematological parameters:** On day 14, blood was taken from the brachial vein of 3 chickens in each group. An auto hematology analyzer (XN-1000V, Sysmex, Japan) was used to measure packed cell volume (PCV), hemoglobin (Hb), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). The Natt and Herrick technique was used to establish the red blood cell (RBC) and white blood cell (WBC) counts (Natt and Herrick, 1952). Differential leukocyte counts were determined on Giemsa-stained blood smears.

**Serum Biochemistry:** Clotted blood samples were separated and stored at -20°C awaiting analysis. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALKP), total serum protein (TSP), albumin, globulins, urea, and lactate dehydrogenase (LDH) were measured using commercial kits and a spectrophotometer (Abbas *et al.*, 2019).

**Statistical analysis:** Microsoft Excel was used to calculate weight gain, FCR, and feed intake. The remaining parameters were arranged in Microsoft Excel and processed for Analysis of Variance (ANOVA). Means were computed through Tukey's test, and ANOVA was applied using Minitab 20.3. Results are presented as mean±standard error of the mean (SEM).

## RESULTS

The effects of Chinese cardamom essential oil on lesion score, oocyst score, and OPG are presented in Table 1. The research revealed that all groups subjected to treatment demonstrated substantial progress when juxtaposed with the control group (C2), which did not administer any medication and had been infected. The 3% essential oil group (P3) had the lowest lesion scores (1.33±0.47), oocyst scores (1.56±0.49), and OPG (1.33±0.47)×10<sup>4</sup>, which were comparable to the medicated control (C1) and significantly (P<0.05) better than the infected control.

Fecal scores also follow the same trend (Table 2). The fecal score for the 3% essential oil group, measured at 0.33±0.47, showed no significant difference compared to the medicated control (0±0) after 7 days post-infection.

**Table 1:** Effects of Chinese Cardamom essential oil on lesion score, oocyst per gram of feces and oocyst score in broiler chickens

Treatment	Lesion Score	Oocyst scoring	Oocyst per gram (×10 <sup>4</sup> )
P1	3.33±0.13 <sup>a</sup>	3.66±0.33 <sup>b</sup>	3.33±0.13 <sup>a</sup>
P2	2.33±0.67 <sup>ab</sup>	2.66±0.67 <sup>c</sup>	2.66±0.33 <sup>ab</sup>
P3	1.33±0.47 <sup>bc</sup>	1.33±0.33 <sup>d</sup>	1.33±0.67 <sup>bc</sup>
C1	0.66±0.33 <sup>bc</sup>	0.66±0.33 <sup>d</sup>	0.33±0.13 <sup>c</sup>
C2	4±0.33 <sup>a</sup>	3.67±0.68 <sup>a</sup>	3.66±0.47
C3	0±0 <sup>c</sup>	0±0 <sup>e</sup>	0±0 <sup>c</sup>

Values are mean±SEM (n=3 replicates per group). Non-identical superscripts mean statistically significant differences (P<0.05). P1: chickens receiving @1% essential oil; P2: chickens receiving @ 2% essential oil; P3: chickens receiving @ 3% essential oil; C1: chickens receiving medicated control (toltrazuril); C2: chickens infected with non-treated control; C3: chickens receiving no-treated control and not infected.

Fig. 2 shows weight gain, feeding intake, and FCR. In the 3% essential oil group (P3), the highest weight gain and feed intake were found, along with the highest FCR, equal to the medicated control group. The non-medicated control (C2) that was infected performed the worst weight gain, feed intake, and FCR, respectively.

The infected non-medicated control group had the highest mortality (35.55%), and the 3% essential oil group had 4.44% mortality, as given in Fig. 1, which was the same as the medicated control (4.44%).

**Table 2:** Effects of Chinese Cardamom essential oil on a fecal score in broiler chickens

Treatment	Day 4	Day 5	Day 6
P1	3.33±0.47 <sup>a</sup>	3.00±0.81 <sup>a</sup>	2.33±0.47 <sup>ab</sup>
P2	2.33±0.47 <sup>ab</sup>	2.00±0.81 <sup>b</sup>	1.66±0.47 <sup>b</sup>
P3	1.00±0.81 <sup>b</sup>	0.66±0.47 <sup>c</sup>	0.33±0.47 <sup>c</sup>
C1	0.33±0.47 <sup>c</sup>	0.00±0 <sup>c</sup>	0.00±0 <sup>c</sup>
C2	3.66±0.47 <sup>a</sup>	4.00±0.81 <sup>a</sup>	3.33±0.47 <sup>a</sup>
C3	0.00±0 <sup>c</sup>	0.00±0 <sup>c</sup>	0.00±0 <sup>c</sup>

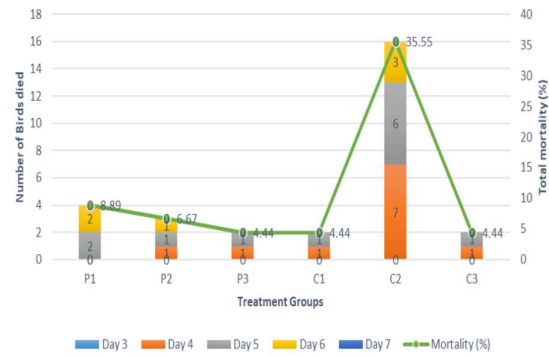
Values are mean±SEM (n=3 replicates per group). Non-identical superscripts mean statistically significant differences (P < 0.05). P1: chickens receiving @1% essential oil; P2: chickens receiving @ 2% essential oil; P3: chickens receiving @ 3% essential oil; C1: chickens receiving medicated control (toltrazuril); C2: chickens infected with non-treated control; C3: chickens receiving no-treated control and not-infected.

P1: chickens receiving @1% essential oil; P2: chickens receiving @ 2% essential oil; P3: chickens receiving @ 3% essential oil; C1: chickens receiving medicated control (toltrazuril); C2: chickens infected with non-treated control; C3: chickens receiving no-treated control and not-infected.

The weight ratios of all organs revealed that the 3 percent essential oil group (P3) had significantly (P<0.05) greater relative weights of heart, liver, intestine, gizzard, bursa, kidney and spleen compared to those of the infected control, indicating superior overall wellness as given in Table 3 given below.

Table 4 demonstrates packed cell volume, hemoglobin, MCH, MCHC, and RBC counts. The 3% essential oil group (P3) had values (PCV 33.02±1.11%, Hb 11.07±0.62g/L, RBC 3.32±0.11 × 10<sup>6</sup>/μL) that were significantly higher (P<0.05) than the infected control and like the medicated control. The same trend was followed by MCH and MCHC.

**Effects of Chinese Cardamom essential oil on mortality**



**Fig. 1:** shows the effects of Chinese Cardamom essential oil on mortality (%) in broiler chickens

**Table 3:** Effects of Chinese Cardamom essential oil on percent organ weight ratio (% of body weight) in broiler chickens

Treatment	Heart	Liver	Intestine	Gizzard	Bursa	Kidney	Spleen
P1	2.43±0.08 <sup>c</sup>	0.89±0.03 <sup>b</sup>	0.41±0.02 <sup>c</sup>	4.56±0.16 <sup>de</sup>	0.25±0.01 <sup>c</sup>	0.08±0 <sup>b</sup>	1.72±0.07 <sup>b</sup>
P2	2.7±0.09 <sup>bc</sup>	1.02±0.01 <sup>a</sup>	0.5±0.01 <sup>b</sup>	5.73±0.71 <sup>cd</sup>	0.29±0 <sup>ab</sup>	0.1±0 <sup>a</sup>	1.82±0.03 <sup>ab</sup>
P3	3.04±0.12 <sup>a</sup>	1.08±0.04 <sup>a</sup>	0.56±0.02 <sup>a</sup>	7.98±0.23 <sup>a</sup>	0.31±0 <sup>a</sup>	0.12±0 <sup>a</sup>	1.90±0.1 <sup>ab</sup>
C1	2.97±0.08 <sup>ab</sup>	1.09±0.01 <sup>a</sup>	0.55±0.01 <sup>ab</sup>	7.56±0.45 <sup>ab</sup>	0.29±0 <sup>ab</sup>	0.12±0 <sup>a</sup>	1.95±0.03 <sup>a</sup>
C2	2.06±0.07 <sup>d</sup>	0.9±0 <sup>b</sup>	0.42±0 <sup>c</sup>	3.98±0.23 <sup>e</sup>	0.1±0 <sup>d</sup>	0.07±0 <sup>b</sup>	1.77±0.07 <sup>ab</sup>
C3	2.91±0.12 <sup>ab</sup>	1.06±0.04 <sup>a</sup>	0.52±0.01 <sup>ab</sup>	6.44±0.34 <sup>bc</sup>	0.26±0.01 <sup>bc</sup>	0.11±0 <sup>a</sup>	1.94±0.02 <sup>ab</sup>

P1: chickens receiving @1% essential oil; P2: chickens receiving @ 2% essential oil; P3: chickens receiving @ 3% essential oil; C1: chickens receiving medicated control (toltrazuril); C2: chickens infected with non-treated control; C3: chickens receiving no-treated control and not-infected. Non-identical superscripts mean statistically significant differences (P<0.05).

**Table 4:** Influence of the Chinese cardamom essential oil on the packed cell volume, the erythrocyte count, and the hemoglobin parameters in broilers chickens

Treatment	MCH (pg/L)	MCHC (g/dL)	PCV (%)	Hb (g/L)	RBC (×10 <sup>6</sup> /μL)
P1	43.77±1.36 <sup>ab</sup>	29.85±0.67 <sup>ab</sup>	26.87±0.42 <sup>b</sup>	8.07±0.26 <sup>c</sup>	2.61±0.04 <sup>cd</sup>
P2	42.78±1.28 <sup>b</sup>	29.26±1.12 <sup>ab</sup>	29.19±0.7 <sup>b</sup>	9.32±0.08 <sup>bc</sup>	2.84±0.08 <sup>bcd</sup>
P3	46.59±0.96 <sup>a</sup>	33.86±0.64 <sup>a</sup>	33.02±1.11 <sup>a</sup>	11.07±0.62 <sup>a</sup>	3.32±0.11 <sup>ab</sup>
C1	45.58±0.24 <sup>ab</sup>	31.66±3.42 <sup>a</sup>	34.61±0.68 <sup>a</sup>	11.8±0.5 <sup>a</sup>	3.4±0.12 <sup>a</sup>
C2	34.52±0.57 <sup>c</sup>	25.97±0.58 <sup>b</sup>	23.64±0.94 <sup>c</sup>	8.19±0.19 <sup>c</sup>	2.41±0.08 <sup>d</sup>
C3	43.47±0.68 <sup>ab</sup>	32.04±1.19 <sup>a</sup>	32.79±0.99 <sup>a</sup>	10.5±0.77 <sup>ab</sup>	3.12±0.32 <sup>abc</sup>

P1: chickens receiving @1% essential oil; P2: chickens receiving @ 2% essential oil; P3: chickens receiving @ 3% essential oil; C1: chickens receiving medicated control (toltrazuril); C2: chickens infected with non-treated control; C3: chickens receiving no-treated control and not-infected. Non-identical superscripts mean statistically significant differences (P < 0.05).

**Table 5:** The influence of Chinese cardamom essential oil on total and differentiated leukocytes in broilers chickens

Treatment	Heterophils (×10 <sup>6</sup> /μL)	Lymphocytes (×10 <sup>6</sup> /μL)	Eosinophils (×10 <sup>6</sup> /μL)	Monocytes (×10 <sup>6</sup> /μL)	Basophils (×10 <sup>6</sup> /μL)	Total WBC (×10 <sup>3</sup> /μL)
P1	777.36±17.52 <sup>ab</sup>	1973.39±89.24 <sup>b</sup>	132.64±2.72 <sup>a</sup>	161.3±3.81 <sup>ab</sup>	15.03±0.92 <sup>a</sup>	3059.73±81.45 <sup>b</sup>
P2	708.07±14.95 <sup>bc</sup>	1811.42±53.43 <sup>b</sup>	121.88±2.32 <sup>ab</sup>	146.21±3.25 <sup>abc</sup>	14.98±1.55 <sup>a</sup>	2802.57±68.4 <sup>bc</sup>
P3	590.85±94.05 <sup>bc</sup>	1502.27±65.71 <sup>b</sup>	106.57±21.43 <sup>abc</sup>	131.24±17.28 <sup>bc</sup>	16.49±0.74 <sup>a</sup>	2347.43±144.61 <sup>c</sup>
C1	576.65±20.17 <sup>c</sup>	1569.02±54.88 <sup>b</sup>	89.59±3.13 <sup>c</sup>	125.62±4.39 <sup>c</sup>	12.06±0.31 <sup>a</sup>	2372.95±82.33 <sup>c</sup>
C2	929.74±41.02 <sup>a</sup>	2586.07±309.51 <sup>a</sup>	126.69±6.14 <sup>a</sup>	165.22±10.55 <sup>a</sup>	12.97±2.51 <sup>a</sup>	3820.69±336.17 <sup>a</sup>
C3	631.06±86.77 <sup>bc</sup>	1823.43±204.95 <sup>b</sup>	91.23±3.86 <sup>bc</sup>	127.92±5.41 <sup>c</sup>	11.48±2.7 <sup>a</sup>	2685.14±235.78 <sup>bc</sup>

P1: chickens receiving @1% essential oil; P2: chickens receiving @ 2% essential oil; P3: chickens receiving @ 3% essential oil; C1: chickens receiving medicated control (toltrazuril); C2: chickens infected with non-treated control; C3: chickens receiving no-treated control and not-infected. Values are mean±SEM (n=3 replicates per group). When the superscript letters above the means in a column are not identical, it indicates a statistically significant difference between those means, with a P-value less than 0.05.

Table 5 expresses the total and differential counts of leukocytes. The total WBC, heterophils, and lymphocytes were significantly increased in the infected non-medicated control (C2) ( $P < 0.05$ ), suggesting the presence of an inflammatory response. The 3% essential oil group (P3) demonstrated much less numerating values, which were similar to the medicated and neutral controls and thus indicated less inflammation.

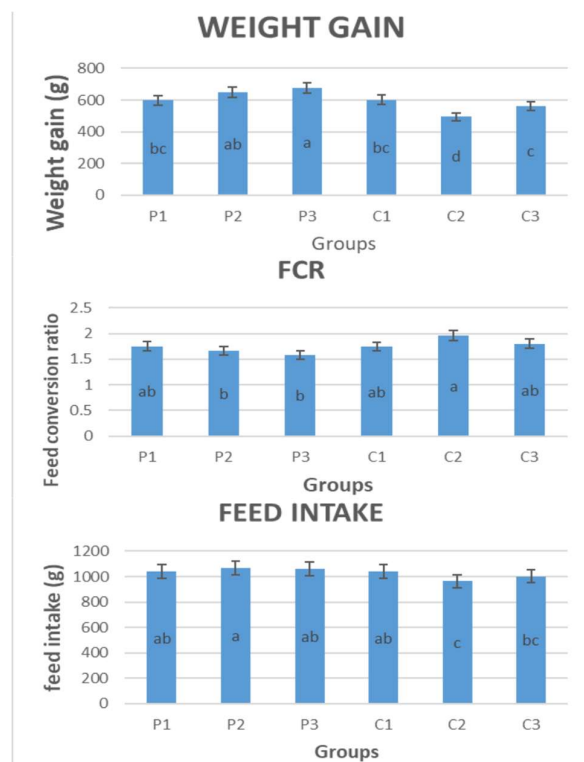
All groups had normal serum physiological parameters, as shown in Table 6. There was no substantial difference ( $P > 0.05$ ) in ALT, AST, ALKP, total protein, albumin, globulins, urea, or LDH between the 3 percent essential oil group and medicated or neutral controls, indicating that the essential oil did not cause liver or kidney damage. C2, the infected, non-medicated control group, showed considerably elevated AST ( $347.48 \pm 7.59$  IU/L) and LDH ( $367.62 \pm 17.93$  IU/L) levels ( $P < 0.05$ ) when contrasted with the 3% essential oil group (P3) (AST:  $248.12 \pm 10.44$  IU/L). No significant differences ( $P > 0.05$ ) were observed between the P3 group and the medicated control (C1) or uninfected control (C3) for ALT, ALKP, total protein, albumin, globulins, and urea. Importantly, ALT levels in P3 ( $9.58 \pm 0.16$  IU/L) were like those in C1 ( $9.44 \pm 0.26$  IU/L) and C3 ( $9.20 \pm 0.13$  IU/L), but significantly greater than in C2 ( $7.11 \pm 0.10$  IU/L), indicating improved overall health rather than liver toxicity. ALKP values were slightly elevated in P1 ( $1970.31 \pm 66.34$  IU/L) but remained within acceptable limits. The P3 group showed restored globulin and albumin levels (albumin:  $1.82 \pm 0.06$  g/L). Overall, these outcomes confirm that Chinese cardamom essential oil does not induce liver or kidney damage even at the highest concentration (3%) and may help in reversing the mild enzyme elevation lined with coccidial infection.

## DISCUSSION

The current work experimentally tested the anticoccidial dietary effect of *Lanxangia tsao-ko* (Chinese cardamom) essential oil dietary supplementation on broiler chickens infected with mixed *Eimeria* species. The outcomes indicate clear evidence that the essential oil containing a 3 percent concentration led to a substantial decrease in the severity of coccidial infection (e.g., 66% reduction in the lesion scores which drops from 4.0 to 1.33, and oocyst per gram reduced by 64% from  $3.66 \times 10^4$  to  $1.33 \times 10^4$ ), improvement in growth performance (e.g., nearly 88% reduction in the mortality from 35.55% to 4.44%, with comparable improvements in feed conversion and weight gain), and restoration of hematological parameters, and a safer serum biochemistry demonstration. These were similar effects to those of the regular anticoccidial drug, toltrazuril, which underscored the

prospects of *L. tsao-ko* essential oil as a natural substitute for coccidiosis control.

The 3% essential oil's anticoccidial activity was demonstrated by the high percentage reduction in the lesion scores, oocyst scores, and OPG when compared to the infected non-medicated control (Table 1, 2). Reduced lesion scores demonstrate less intestinal damage, whereas a decrease in oocyst output demonstrates the inhibition of parasite multiplication and shedding (Hafeez *et al.*, 2023; Santos *et al.*, 2024). The results are consistent with the literature on essential oils of oregano (*Origanum vulgare*), garlic (*Allium sativum*), and citrus (*Citrus sinensis*) that also led to fewer coccidial lesions and oocysts in chickens (Abbas *et al.*, 2011b; Idris *et al.*, 2017; Saeed *et al.*, 2023). It is possible that the mechanism entails several pathways. Essential oils may have a direct inhibitory effect on the process of *Eimeria* sporulation or invasion, as in vitro research indicated. More significantly, their powerful



**Fig. 2:** explored how varying quantities of Chinese cardamom essential oil influenced key broiler chicken metrics, including their weight gain, daily feed intake, and overall feed conversion efficiency. Values are mean  $\pm$  SEM. Bars with different letters differ significantly ( $P < 0.05$ ). Group codes: P1: 1% essential oil; P2: 2% essential oil; P3: 3% essential oil; C1: medicated control (toltrazuril); C2: infected, non-medicated control; C3: uninfected, non-medicated control.

**Table 6:** Effect of Chinese cardamom essential oil on serum parameters in broiler chickens

Treatment	ALT (IU/L)	AST (IU/L)	ALKP (IU/L)	Total Protein (g/L)	Albumins (g/L)	Globulins (g/L)	Urea (mmol/L)	LDH (IU/L)
P1	$7.65 \pm 0.63^b$	$321.45 \pm 15.49^a$	$1970.31 \pm 66.34^a$	$3.7 \pm 0.08^a$	$1.75 \pm 0.08^{ab}$	$1.94 \pm 0.01^a$	$4.25 \pm 0.07^a$	$339.98 \pm 5.6^{ab}$
P2	$7.9 \pm 0.33^b$	$266.66 \pm 6.62^b$	$1792.19 \pm 44.48^{bc}$	$3.5 \pm 0.14^a$	$1.55 \pm 0.07^b$	$1.94 \pm 0.14^a$	$4.31 \pm 0.15^a$	$295.97 \pm 8.11^{bc}$
P3	$9.58 \pm 0.16^a$	$248.12 \pm 10.44^b$	$1792.83 \pm 62.24^{bc}$	$3.57 \pm 0.17^a$	$1.82 \pm 0.06^a$	$1.74 \pm 0.22^a$	$4.28 \pm 0.06^a$	$275.82 \pm 4.82^c$
C1	$9.44 \pm 0.26^a$	$266.38 \pm 9.61^b$	$1758.03 \pm 35.41^c$	$3.3 \pm 0.26^a$	$1.87 \pm 0.06^a$	$1.42 \pm 0.26^a$	$4.2 \pm 0.07^a$	$262.35 \pm 8.08^c$
C2	$7.11 \pm 0.1^b$	$347.48 \pm 7.59^a$	$1943.72 \pm 18.66^{ab}$	$3.22 \pm 0.22^a$	$1.53 \pm 0.08^b$	$1.68 \pm 0.19^a$	$4.44 \pm 0.18^a$	$367.62 \pm 17.93^a$
C3	$9.2 \pm 0.13^a$	$331.39 \pm 23.09^a$	$1780.65 \pm 50.9^{bc}$	$3.3 \pm 0.09^a$	$1.83 \pm 0.05^a$	$1.47 \pm 0.15^a$	$4.36 \pm 0.14^a$	$299.31 \pm 24.25^{bc}$

P1: chickens receiving @ 1% essential oil; P2: chickens receiving @ 2% essential oil; P3: chickens receiving @ 3% essential oil; C1: chickens receiving medicated control (toltrazuril); C2: chickens infected with non-treated control; C3: chickens receiving no-treated control and not-infected. Values are mean  $\pm$  SEM (n=3 replicates per group). When the superscript letters above the means in a column are not identical, it indicates a statistically significant difference between those means, with a P-value less than 0.05.

antioxidant effects neutralize the host immune system's oxidative stress production processes during parasite replication, safeguarding intestinal epithelial integrity (Medhat *et al.*, 2026). Key active ingredients of *L. tsao-ko* essential oil, including 1,8-cineole and alpha-terpinyl acetate, are reported to be free-radical scavengers and anti-inflammatory disease agents. The essential oil may prevent hemorrhage, diarrhea, and false nutrient malabsorption by oxidative damage, resulting in better clinical results.

This interpretation is supported by data on growth performance as given in Fig. 2. Chicken fed on 3 percent essential oil gained more weight, fed better, and had lower mortality than the infected control, and approximated the values of both the medicated and uninfected controls (Table 3). Reduced intestinal pathology directly leads to improved performance, as more nutrients can be absorbed more efficiently. The large percentage change in the relative weights of the liver, intestine, gizzard, bursa, kidney, and spleen in the 3% essential oil group also points to improved overall health and development of the immune organs (Table 3). These results align with growth-promoting properties of other essential oils on coccidiosis models (Almahallawi *et al.*, 2024; Abbas *et al.*, 2025).

Essential hematological parameters were used as an extra indication of the positive action of the oil. The chickens that were infected showed the manifestation of anemia, which was evidenced by low PCV, Hb, and RBC numbers, a typical symptom of cecal hemorrhage because of *Eimeria*. Essential oil group with the 3% essential oil demonstrated the same restored value as the medicated control, indicating that the essential oil was effectively used in reducing blood loss. In contrast, the 1 and 2% essential oil groups demonstrated only partial improvement with hematological parameters to remain intermediate between the positive control (C1) and the infected non-medicated control (C2), reflecting a dose-dependent response. In addition, total and differential leukocyte counts were normalized in the 3% group (Table 5). The increased heterophils and lymphocytes in the infected control indicated that there has been an incessant inflammatory reaction to the parasite (El-Saied *et al.*, 2024). The decreased number of cells in the 3% essential oil group suggests that the oil could potentially control the immune response and avoid excessive inflammation that can result in collateral tissue damage (Table 4) (Abbas *et al.*, 2017; Ismail *et al.*, 2023). The same immunomodulatory effect has been outlined regarding a few essential oils and their constituents.

One of the most important factors about any feed additive is its safety. The serum biochemistry findings (ALT, AST, ALKP, urea, LDH) were in the normal range in all the treatment groups, and no significant difference was seen between the 3% essential oil group and the controls (Table 6). These enzymes are liver and kidney function markers; their stability shows that the essential oil, regardless of the highest concentration, did not cause hepatotoxicity or nephrotoxicity (Alkhalifah *et al.*, 2022; Sarawi *et al.*, 2025). The safety profile given is critical for commercial use and compliant with literature on other important oils implemented in poultry.

The 1 percent and the 2 percent concentration treatment groups also had certain positive effects, but the largest effects were always brought about by the 3 percent concentration, implying a relation of dose effect. The trend is a characteristic of essential oils, with the higher the concentration, the greater the effectiveness because pure substances are more available. Toltrazuril has been regarded as one of the most effective anticoccidials; however, the development of pharmacological resistance to the medication and the issues of residues highlight the importance of natural alternatives. A promising alternative would be *L. tsao-ko* essential oil, as it can be added to feed without complex delivery.

To sum up, the study is quite effective in supporting the hypothesis that Chinese cardamom essential oil at a dietary level of 3% is successful in preventing coccidiosis in broilers, which is both effective and safe in improving performance and health. Its potential as a preventative should be studied in the future, the inclusion will be most appropriate, and its cost-effectiveness when utilized in large-scale production systems. Also, research that explains the exact antiparasitic action mechanisms, and host regulation would help to further justify the use of the compound as a sustainable anticoccidial agent.

**Conclusions:** The study has proven that the essential oil of Chinese cardamom (*Lanxangia tsao-ko*) has a potent anticoccidial and growth-promoting activity in broiler chickens when used at a percentage concentration of 3, and its activity is as effective as that of the widely used anticoccidial toltrazuril. The risk oil may be used safely with this dose and does not cause any adverse effects on serum biochemistry. More research should be conducted to assess the economics, long-term use, preventive willingness, and the working principles of action of Chinese cardamom essential oil prior to its application.

**Conflict of interest:** The Authors declare that there is no conflict of interest.

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**Authors contribution:** AOA, AAA, AA, MS, HMS, and ESM conceived and designed the study and executed the experiment. AOA, AAA, AA, MS, HMS, and ESM validated and analyzed the data. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

**Generative AI statement:** The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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