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Anticoccidial activity of essential oils containing eugenol against *Eimeria tenella* in broiler chickens

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Abstract

The development of alternative therapies to treat chicken coccidiosis has become a hot topic because of the widespread use of conventional medicines. This study aimed to investigate the effectiveness of eugenol in treating *Eimeria tenella* infection in broilers. Broiers, at the age of 14 d, were orally infected with sporulated *Eimeria tenella* oocysts, and then, eugenol essential oil was added to chicken feed at three different dosages (0.1, 0.2 or 0.4 g/kg). The anticoccidial effects of eugenol essential oil were assessed using the anticoccidial index (ACI). As a result, eugenol exhibited a moderate anticoccidial effect, with an ACI of 167.37 at 0.2 g/kg. After eugenol treatment, the expression of occludin in the epithelial cells of the chicken cecum was significantly greater ($P < 0.05$) than that in the epithelial cells of the nontreated control (IC) group. The proportion of intestinal *Lactobacillus_agilli* increased. Eugenol therapy dramatically increased the activity of superoxide dismutase. After high-dose treatment, the expression of the proinflammatory factors IL-1β and IL-6 significantly decreased, while the expression of the cytokines IL-4 and IFN-γ significantly increased. The safety of eugenol essential oil was evaluated at the 1, 3 or 6 recommended doses. Overall, no significant differences were detected in the blood tests or serum biochemistry of the chickens between the treatment groups and the control group. As a result, eugenol essential oil can cure chicken coccidiosis by improving the intestinal microbial structure in the chicken cecum and decreasing the cecum's inflammatory reactions, thus strengthening immune function and eventually demonstrating anticoccidial properties.

Keywords Coccidiosis, *Eimeria tenella*, Eugenol essential oils, Safety test, Broiler chickens

Introduction

Chicken coccidiosis is a common parasitic disease caused by numerous *Eimeria* pathogens, the most virulent of which is *Eimeria tenella* (*E. tenella*) (Gaboriaud et al. 2021); as a result, *E. tenella* is frequently employed as a model for chicken coccidiosis research. More than £10 billion is spent on the prevention and control of chicken coccidiosis annually (Zhang et al. 2022). Currently, anticoccidials are commonly added to poultry feed (Xu et al. 2022); however, this leads to medication resistance in chickens. To address drug resistance, some researchers have proposed a scheme for rotating drugs. Subsequently, the rotating drug scheme is combined with the coccidiosis vaccine at three-month intervals (Chapman and Jeffers 2014). Additionally, veterinary drug residues present a difficulty. As a result, other treatments must be explored to address the developing issues of drug resistance and residues.

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Natural solutions are viable alternatives to chemical medications for treating *Eimeria tenella* infections. Compared with chemical drugs, natural products exhibit multiple advantages, such as minimal environmental impact, ease of access, and enhanced safety (Huang et al. 2021). Because of their long-term interactions and coevolution with other plants and species in their environment, some plants have developed the ability to produce a diverse range of secondary metabolites with distinct ecological roles. This capacity is essential for their survival (Mahizan et al. 2019). Terpenes, aldehydes, and esters constitute the majority of the secondary metabolites that make up essential oils (EOs) (Swamy et al.2016). As small hydrophobic molecules, these secondary metabolites can traverse biological barriers and biofilms (Costa et al. 2018); prevent infections, inflammation, and spasms; combat bacteria and viruses (Gucwa et al. 2018); and promote cell metabolism and regeneration. Notably, certain components of essential oils possess antiparasitic effects.

Eugenol is a natural essential oil obtained from plants that has antiparasitic effects. Many studies have shown the efficacy of eugenol against parasites such as *Trypanosoma cruzi*, *Giardia lamblia*, and *Leishmania donovani*, with a significant impact on their morphology and growth. Eugenol has the potential to be a more effective antiparasitic agent than other chemotherapeutic medicines for the treatment of various parasitic disorders. Moreover, eugenol has been reported to have antileishmanial, antimalarial and anthelmintic effects. In an in vivo investigation with mouse models, eugenol showed a synergistic impact with conventional medications, resulting in a considerable reduction (19.2%) in the percentage of drug-resistant *Schistosoma mansoni* parasites (Nisar et al. 2021).

Furthermore, a 60-min application of eugenol essential oil resulted in complete eradication (100%) of *L. amazonensis* parasites, which might be mainly attributed to the elevated levels of eugenol-induced nitric oxide (NO), which acts as an antileishmanial oxidant, thus contributing to the annihilation of *Leishmania* species (Ueda-Nakamura et al. 2006). Eugenol has demonstrated analgesic, anti-inflammatory, antibacterial and local anesthetic effects (Hosny et al., 2020). The characteristics of eugenol are beneficial for promoting intestinal health, increasing resistance to infections, and improving mucosal immunity, all of which improve host health. It has also been reported that eugenol essential oil is nonmutagenic and noncarcinogenic, according to the US Food and Drug Administration (FDA) (S Bendre and D Rajput 2016). These reports indicate the high safety profile of eugenol essential oil. In conclusion, eugenol essential oil is characterized by its antiprotozoal effects, such as antimalarial effects, and high host safety; however, there are no reports on the anticoccidial activity of eugenol essential oil against *Eimeria tenella* related to *Eimeria tenella*; thus, the anticoccidial activity of eugenol essential oil against *Eimeria tenella* has been investigated.

In this study, the anticoccidial index (ACI) was used to calculate the recommended anticoccidial dose of eugenol essential oil, and the safe dose was evaluated using a gradient safety dose evaluation test of eugenol essential oil based on 1, 3 and 6 times the recommended amount. This study also investigated the effects of oral administration of eugenol essential oil (at dosages of 100, 200 and 400 mg/kg) on intestinal barrier function in broilers. We examined physical barrier functions in broilers by determining the expression of tight junction proteins; chemical barrier functions by serum malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) indicators; microbiological barrier functions by the effect of eugenol essential oil on the cecal microbiota; and immunological barrier functions by the gene expression of cytokines in cecal tissues. This study indicates for the first time the anti-E. tenella action of eugenol essential oil, providing a potential chemical therapy for treating chicken coccidiosis.

Results

Anti-coccidia test

Anti-*Eimeria tenella* test in broilers showed that only one broiler died in each of the infected control (IC) and low-dose (LD) groups, while no broilers died in other treatment groups during the whole animal experiment. The ACI of the DC group was 189.77, which indicated that its anticoccidial effect reached an efficient level, and the greater ACI than 180 was mainly due to the oocyst output of 0 in the DC group. The ACI for the LD group was 136.34, indicating that LD had the lowest anticoccidial impact on the therapy groups.

The results of the study showed that the body weight gain (BWG) of the medium-dose (MD) group reached 99.87, and all chickens in the MD group survived, with a survival rate of 100%. The cecal lesion score in this group was 22.50, indicating that eugenol essential oil effectively improved cecal lesions. The oocyst index (OI) of this group was measured and was 10.00 after oocyst ratio conversion. Based on these four indicators, the ACI was 167.37, indicating that the medium-dose group achieved a moderate anti-*Eimeria tenella* effect. The results mentioned above demonstrated that the intermediate dose (200 mg/kg) enhanced weight gain in broilers and had a better anti-*Eimeria tenella* effect. Thus, it should be utilized as the suggested dose.

Histopathological observation of the cecum

Hematoxylin and eosin (H&E) staining were used to assess pathological damage to the cecum in each of the treatment groups. The results of cecal histopathological sections at 7 d postinfection (dpi) showed slight pathological changes in the cecum of the MD groups, but the swelling and thickening of the cecum were more obvious in the IC and LD groups (Fig. 1A, B). The IC group exhibited severe histopathological changes (Fig. 1F), including extensive necrosis of the lamina propria of intestinal villi and degeneration of intestinal epithelial cells. These findings indicate that MD therapy effectively reduced cecal lesion damage following *Eimeria tenella* infection.

Immunohistochemistry

To assess the expression level of the tight junction protein Occludin in the cecum, MOD (mean optical density) were calculated for each treatment group 7 dpi with *Eimeria tenella* by immunohistochemistry. The relevant statistical results of the immunohistochemical analysis are shown in Fig. 2A. The MOD of occludin in the MD and HD groups was significantly greater than that in the IC group ($P < 0.01$). The MOD of HD group were also slightly greater than those in the IC group. These results indicate that the addition of eugenol essential oil for 7 d was effective at increasing the expression level of occludin.

Relative changes in cytokine expression in cecal tissue

To assess the expression levels of cytokines in the cecum of each treatment group 7 dpi with *Eimeia tenella*, real-time fluorescence quantification PCR were used to detect mRNA expression of cytokines in each treatment group. Figure 2B-E shows the quantitative expression of *IL-1β*, *IL-4*, *IL-6* and *IFN-γ* genes in cecal tissue. Compared with those in the IC group, expression levels of *IL-1β* and *IL-6* in the LD, MD and HD groups were significantly lower ($P < 0.0001$). This finding suggested that the cecal cells in the eugenol essential oil-treated groups (LD, MD and HD) exhibited reduced expression of proinflammatory factors (*IL-1β* and *IL-6*), thereby attenuating the inflammatory response. IFN-γ expression in the HD groups was significantly upregulated ($P < 0.05$); *IL-4* expression in the HD and MD groups was significantly upregulated ($P < 0.05$). These findings show that 400 mg/kg of eugenol essential oil (HD group) can promote Th1 and Th2 cellular immunological responses in broilers.

Serum MDA, SOD, GSH-Px and CAT concentrations

To detect changes of oxidative stress factors in each treatment group 7 dpi with *Eimeria tenella*, concentrations of MDA, SOD, GSH-Px and CAT in the serum were detected. (Fig. 2F-I). Compared with that in the IC group, MDA concentration in the LD, MD and HD groups was significantly lower ($P < 0.05$), suggesting that the addition of eugenol essential oil ameliorates lipid peroxidation in vivo and indirectly mitigates the degree of cellular damage. SOD concentration significantly increased ($P < 0.01$), suggesting that eugenol essential oil is effective at reducing the degree of oxidative stress damage. However, contents of GSH-Px and CAT were not significantly different.

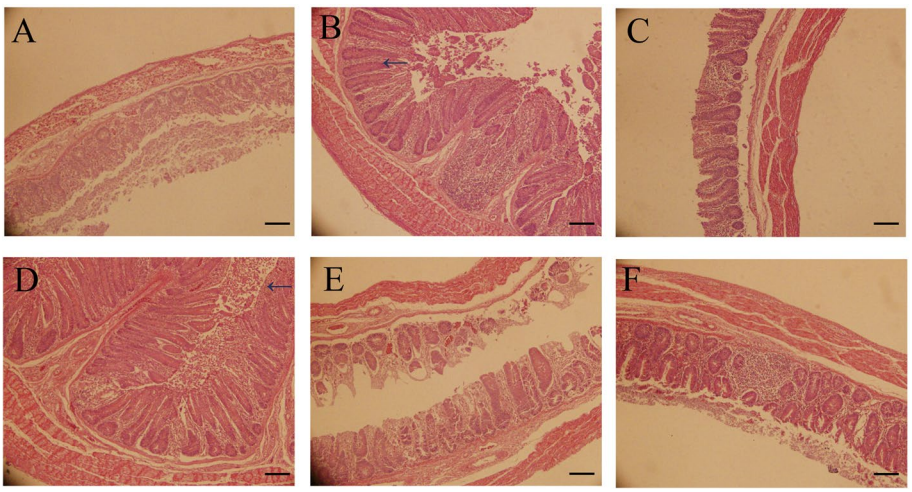

Fig. 1 Histopathological changes in cecal tissues from chickens. Typical histopathological changes in the ceca, including severe damage to intestinal villi and intestinal glands in the lamina propria, were observed in the IC control (**F**) and LD groups (**C**). The HC group (**D**) and the DC group (**E**) showed no obvious histopathological changes. The histopathological changes in the HD group (**A**) and the MD group (**B**) were not as severe as those in the IC group (**F**). Mucosal integrity can be found in the B and D sections. Arrow shows intestinal villi and intestinal glands. Scale bar = 100 μm

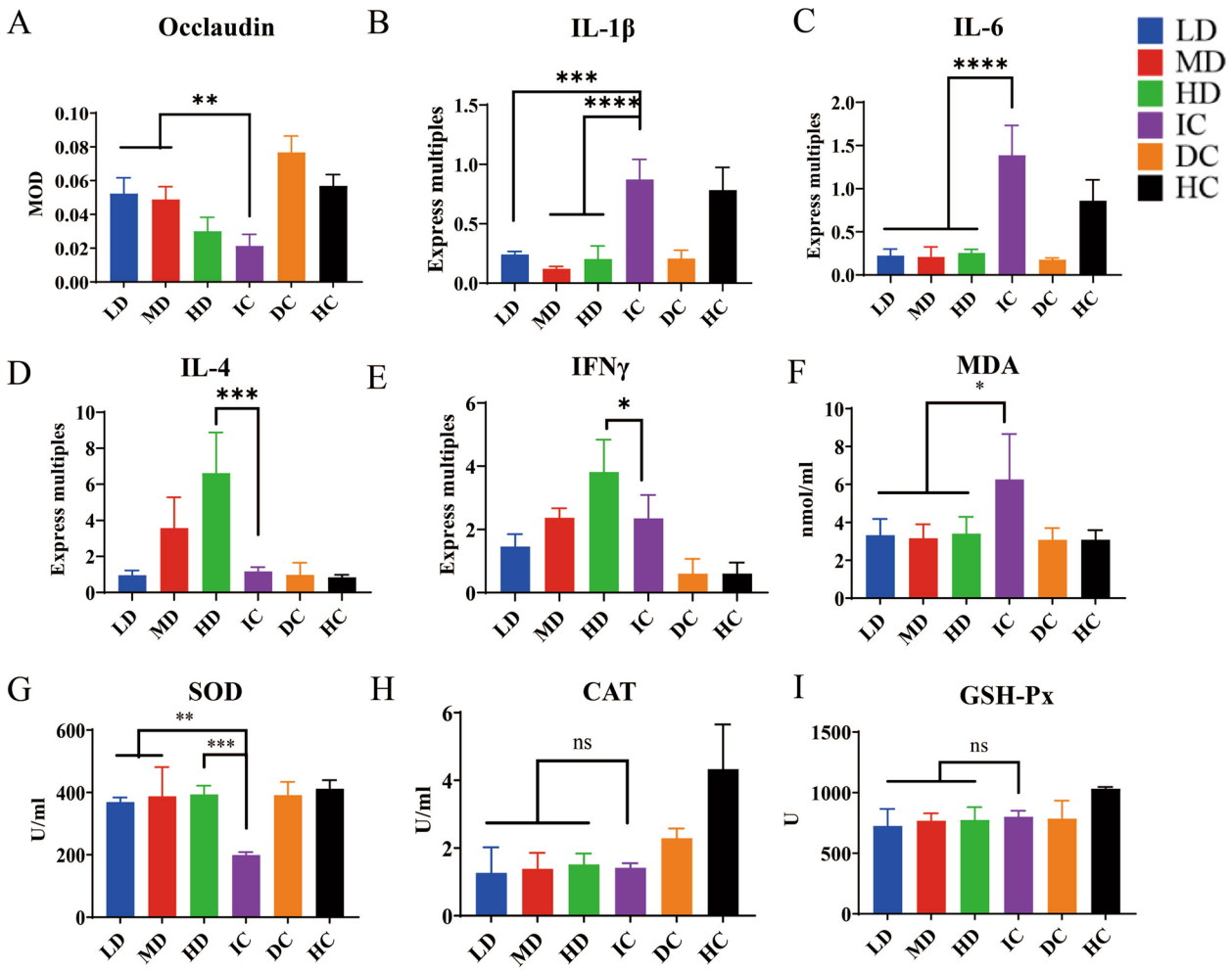

Fig. 2 Mean optical density of the occludin protein in cecum determined by immunohistochemistry. **A** MOD = IOD/Area. MOD, mean optical density; IOD, integrated optical density, which is the cumulative optical density in positively stained areas. The area in the formula MOD = IOD/Area refers to the mucosal layer pixel area. mRNA levels of cytokines in cecal tissue. At 7 dpi, the mRNA levels of IL-1β (**B**), IL-6 (**C**), IL-4 (**D**), and IFN-γ (**E**) in the cecal tissues of chickens ($n = 3$) in each group were quantified by qRT–PCR. GAPDH was used as a reference gene. The data are expressed as the means ± SDs ($n = 3$). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, no significant difference. Effects of eugenol essential oil on serum MDA, SOD, CAT, and GSH-Px concentrations in broilers. Note: (**F**): Serum MDA activity in each group; (**G**): Serum SOD level in each group; (**H**): Serum CAT activity in each group; (**I**): Serum GSH-Px level in each group. *, $P < 0.05$; **, $P < 0.01$; *** $P < 0.001$; ns, no significant difference. HD, high dose of eugenol essential oil; MD, medium dose of eugenol essential oil; LD, low dose of eugenol essential oil; HC, healthy control group (nontreated and noninfected); DC, decoxyquinate treatment control; IC, infected control group (untreated and infected with *E. tenella*)

Effect of eugenol essential oil on the cecal microbiota

To assess the structure in the intestinal flora of the cecum in each treatment group at 7 dpi, the cecal contents were collected for sequencing of the intestinal flora 16S rDNA amplicons. In this study, relative abundance histograms of the highest top 10 species at phylum level in each group were plotted (Fig. 3A). The relative abundances of the LD, MD, HD, IC, DC and HC groups at 7 dpi were 14.72%, 14.69%, 17.10%, 2.03%, 9.93% and 2.59%, respectively. In addition, common and unique feature sequences in different groups were analyzed, and the numbers of feature sequences in the LD, MD, HD, IC, DC and HC groups at 7 dpi were 2,048, 3,013, 2,555, 232, 1,913 and 252, respectively (Fig. 3B). The Chao1 index values of the LD, MD and HD groups were greater than those of the HC and IC groups, indicating that eugenol essential oil increased the richness of the microbiota in the cecum (Fig. 3C). Principal coordinate analysis (PCoA) showed that there was a large difference between the IC group and the eugenol essential oil treatment groups (LD, MD and HD groups), indicating a great difference in intestinal microbial composition between them (Fig. 3D). UPGMA cluster analysis revealed high similarity in microbial relative abundance at the phylum level among samples

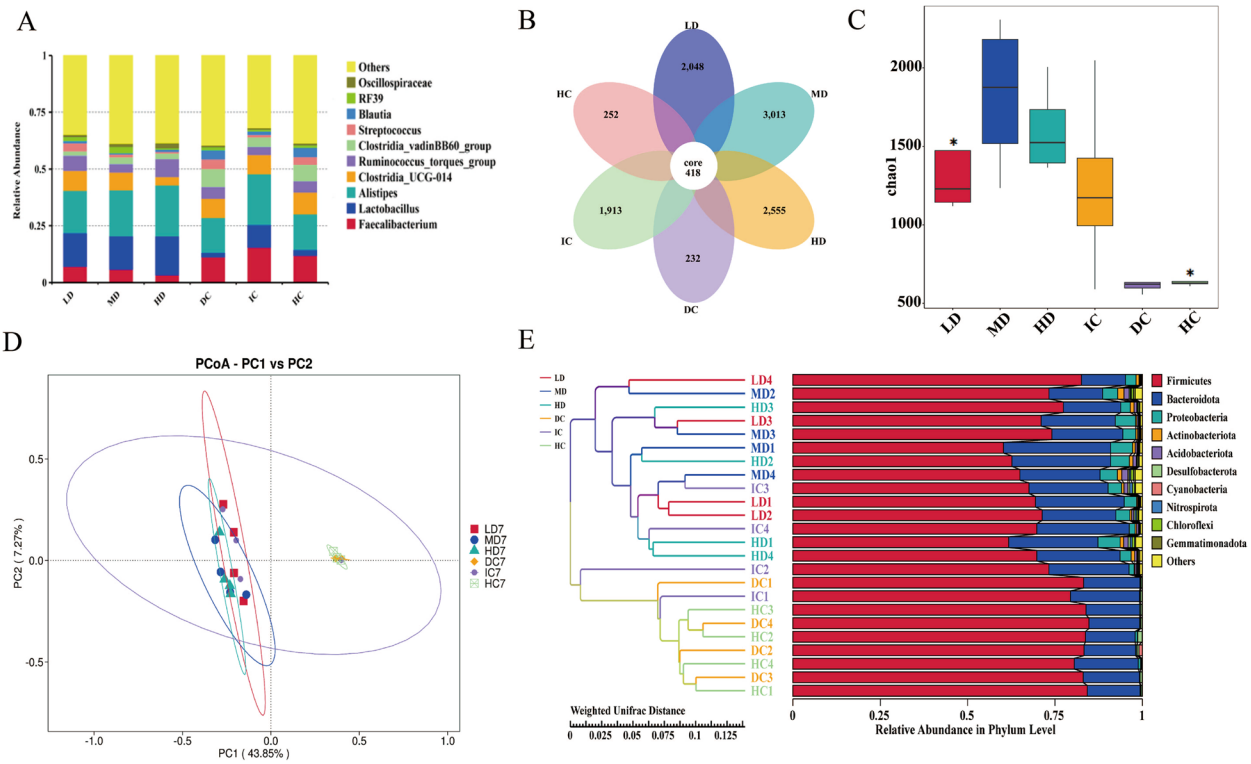

Fig. 3 Differences in intestinal microbial composition among the different groups. **A** Differences in the relative abundance of the microbial community at the phylum level. **B** Venn diagram of the unique and shared amplicon sequence variants (ASVs) in the intestinal microbiota among different groups. In the Venn diagram, one petal represents one group, the numbers in the core of the petals represent feature sequences shared by all groups, and the numbers on the petals represent feature sequences unique to each group. **C** Cecal microbial α diversity of broilers reflected by the Chao1 index. **D** Principal coordinate analysis (PCoA) of unweighted UniFrac calculated based on the relative abundance of OTUs. The data are expressed as the means ± SDs of three replicates. **E** UPGMA clustering tree based on weighted UniFrac distances

within a group (Fig. 3E). As shown in Fig. 4A and B, *Lactobacillus* at the order level and *Lactobacillus agillis* at the species level had LDA scores > 4.0 in the LD group, and *Lactobacillus* at the family level and *Lactobacillus* at the genus level had LDA scores > 4.0 in the HD group. The use of eugenol essential oil increased the percentage of probiotics in the broiler cecum, improving the structure of the cecum intestinal flora.

Dose safety test

The dose safety test revealed no aberrant behavior or death in the chickens. No obvious drug toxicity to the heart, liver, spleen, lungs or kidneys was detected on 7 and 14 d after eugenol essential oil treatment (Fig. 5, Fig. S1). Histopathological tests revealed no abnormal pathological changes in the liver or kidneys of any of the groups on 7 or 14 d after eugenol essential oil treatment (Fig. 6).

On d 7 post-eugenol essential oil treatment, G3a (with eugenol essential oil at a concentration of 1.2 g/kg) exhibited the lowest feed conversion rate (FCR = 1.790) (Table 1). On d 7 and 14 after eugenol essential oil treatment, there was no significant difference in the relative organ weight (ROW) between treated groups and the G4a or G4b group (Fig. 5, Fig. S1). On d 7 and 14 after eugenol essential oil treatment, except for $P < 0.05$, no significant differences in routine blood or serum biochemical indicators were detected between G4a and G1a, G2a or G3a (Figs. 7 and 8) or between G4b and G1b, G2bor G3b (Fig. S2), except that the TBIL of G3a was significantly different from that of G4a (Figs. 7 and 8).

Discussion

Good anticoccidial effect and high safety

Eugenol has powerful antioxidative, anti-inflammatory, antibacterial, antiparasitic and anticancer activities (Das et al. 2016) with low toxicity (Nisar et al. 2021). To date, no studies have investigated the impact of eugenol on *Eimeria tenella* (*E. tenella*). Therefore, this research systematically assessed the anticoccidial efficacy and safety profile of eugenol essential oil through anticoccidial tests and dose safety evaluations. The results showed that the ACI in the medium-dose group (MD) exceeded 160, indicating moderate anticoccidial

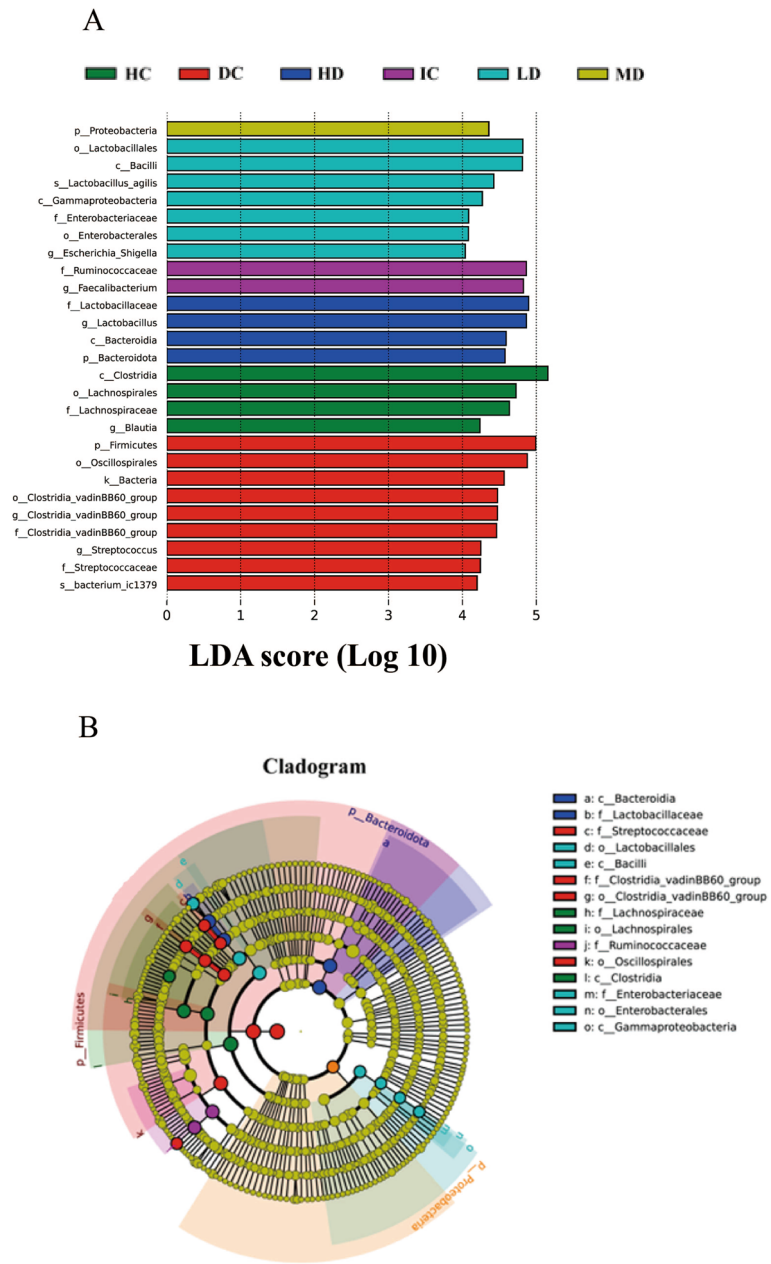

Fig. 4 Linear discriminant analysis effect size (LEfSe) among different groups. **A** LDA value distribution histogram. Only the phylotypes with LDA values > 4 are shown. **B** Evolution cladogram of intestinal microorganisms with LDA > 4

action (Table 2). The ACI is a comprehensive standard for evaluating the anticoccidial effect in chickens. In this study, the ACI was calculated by combining four indices, namely, the rBWG, survival rate, cecum lesion score, and oocyst ratio, and it was found that, as a control of chemical drugs, the ACI of the DC group reached 189.77, which was close to the excellent anticoccidial effect, while the ACI of the essential oil group was 167.37, which was smaller than that of the DC group but still achieved a good anticoccidial effect; the anticoccidial effect is still considerable.

Furthermore, when comparing the results of the present study to those of Wang's evaluation of the anticoccidial effect of artemisinin, it was discovered that eugenol essential oil had greater relative weight gain than artemisinin, which was more advantageous in terms of the economic benefits of broiler meat production (Wang et al. 2021). Our dose safety test indicated that no obvious

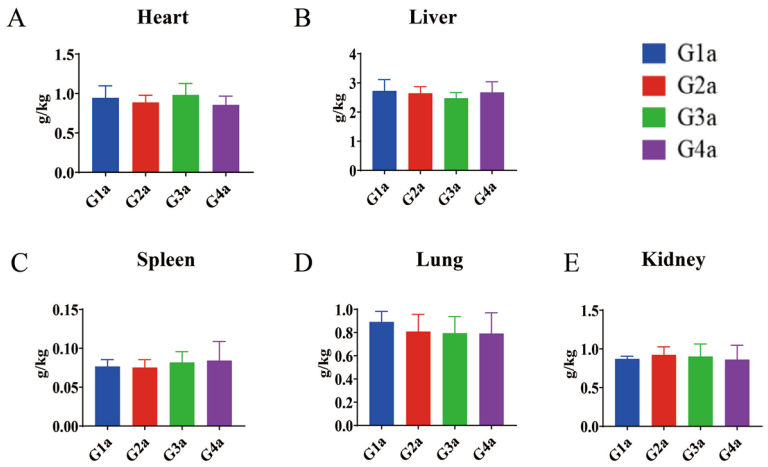

Fig. 5 Effect of eugenol essential oil on the relative organ weight (ROW) of chickens after 7 d of eugenol essential oil treatment ($n=6$). **A** Heart. **B** Liver. **C** Spleen. **D** Lung. **E** Kidney

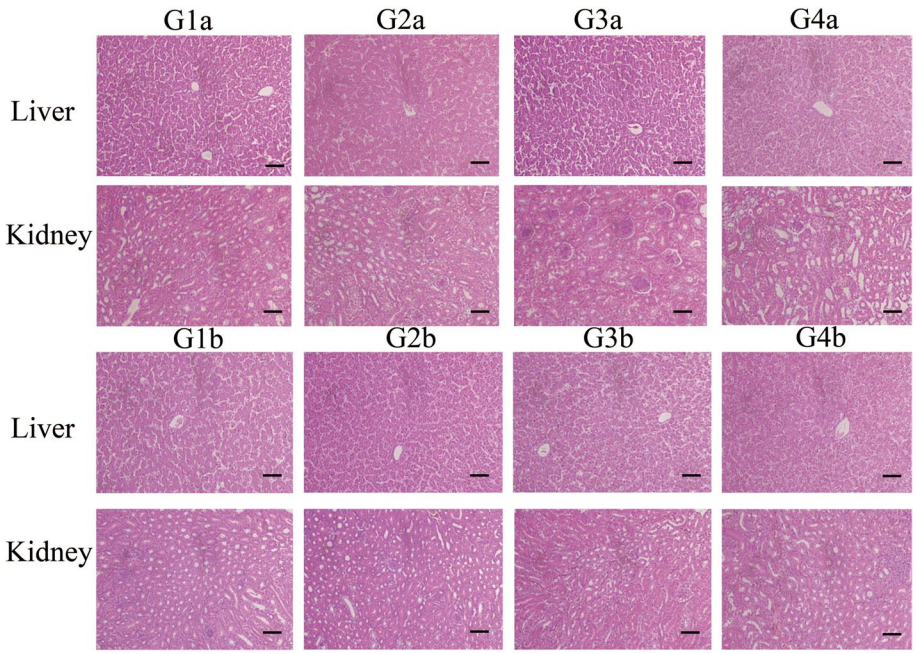

Fig. 6 Histopathological analysis of organs (livers and kidney) in the control group (G4a) and three eugenol essential oil-treated groups (G1a, G2a and G3a) after 7 d of administration by H&E staining. Histopathological analysis of organs (livers and kidneys) in the control group (G4b) and three eugenol essential oil-treated groups (G1b, G2b and G3b) after 14 d of administration. Scale bar = 50 μm

abnormalities in the heart, liver, spleen, kidneys or lungs were observed in the eugenol essential oil-treated groups. Pathology of liver and kidney sections showed that the tissue structure of the liver and kidney in each eugenol essential oil-treated group was intact, with no obvious histopathological changes. Routine blood and serum biochemical tests revealed that eugenol essential oil administration had no harmful effects on the chickens. These findings suggest that eugenol essential oil is safe for broilers.

Physical, chemical, immune and microbial barriers

The possible anticoccidial effect of eugenol may be explained by its role in gut health. It is widely recognized that intestinal health is crucial for the well-being of broilers, as the intestine not only facilitates nutrient absorption but also serves as a natural barrier to maintaining internal equilibrium. The intricate intestinal barrier comprises four key components: physical, chemical, immune and microbial barriers. In light of this, the current study

Table 1 Effect of eugenol essential oil on the growth performance of chickens ($n = 10$)

Groups	Initial group weight (g)	Final group weight (g)	Initial average weight (g)	Average final weight (g)	Weight gain (g)	Intake (g)	FCR	BWG (%)
G1a	1,501	3,081	150.10 ± 11.15[a]	308.10 ± 44.46[a]	1,580	2,883	1.825	103.26
G2a	1,496	3,036	149.60 ± 10.44[a]	303.60 ± 39.30[a]	1,540	2,780	1.805	100.65
G3a	1,496	3,103	149.60 ± 10.37[a]	310.30 ± 38.66[a]	1,607	2,877	1.790	105.03
G4a	1,503	3,033	150.30 ± 11.72[a]	303.30 ± 58.21[a]	1,530	2,820	1.843	100.00
G1b	1,498	5,354	149.80 ± 10.92[a]	535.40 ± 73.65[b]	3,856	7,534	1.954	99.51
G2b	1,497	5,319	149.70 ± 10.46[a]	531.90 ± 84.32[b]	3,822	7,710	2.017	98.63
G3b	1,494	5,004	149.40 ± 10.72[a]	500.40 ± 72.59[b]	3,510	7,031	2.003	90.58
G4b	1,502	5,377	150.20 ± 11.29[a]	537.70 ± 72.77[b]	3,875	7,440	1.920	100.00

[a, b]Values with different superscripts in the same column differ significantly ($P < 0.0001$). *FCR* feed conversion rate. *BWG* Body weight gain

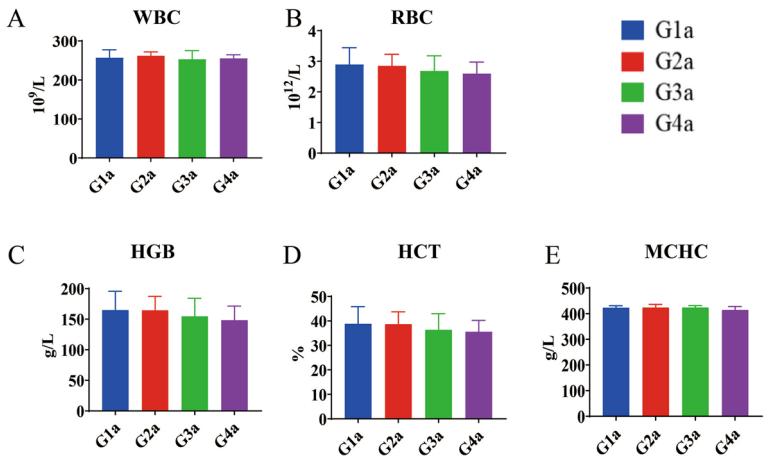

Fig. 7 Effects of eugenol essential oil on hematological indicators in chickens ($n = 6$). The WBC (**A**), RBC (**B**), HGB (**C**), HCT (**D**), and MCHC (**E**) were assessed after 7 d of eugenol essential oil treatment. *WBC* White blood cells, *RBC* Red blood cells, *HGB* Hemoglobin concentration

examined the mechanisms behind the anticoccidial properties of eugenol essential oil from four different perspectives.

Physical barrier functions of broiler chicken intestines were assessed by immunohistochemistry to detect the protein expression of occludin in the cecum. Occludin is a cellular tight junction protein that helps establish the intestinal mechanical barrier (Deng et al. 2022). This research showed that after infection with *Eimeria tenella*, the expression of occludin decreased, and occludin expression increased in the LD, MD and HD groups. Our findings indicate that eugenol essential oil can increase the expression of occludin, reducing mechanical damage to the intestinal wall caused by coccidial infection.

MDA is a major byproduct of lipid peroxidation. The concentration of serum MDA indirectly reflects the degree of cellular lipid peroxidation and the accumulation of reactive oxygen species (ROS) (Li et al. 2020). Our findings indicated that eugenol essential oil therapy might greatly boost SOD activity (Fig. 2G).

Cytokines are a group of proteins synthesized and secreted by immune cells and nonimmune cells after stimulation, and they have important biological functions. $CD4^+$ T lymphocytes are divided into Th1-type T lymphocytes and Th2-type T lymphocytes (Khramtsov et al. 2021). Th1 T lymphocytes secrete IFN-γ, which is related to the cellular immune response, and Th2 lymphocytes secrete IL-4 to regulate antibody production (Zhao et al. 2020; Zhang et al. 2016; Fatoba et al. 2022). Gene expression levels of IFN-γ and IL-4 were significantly greater in the HD group than in the IC group ($P < 0.05$) (Fig. 2D and E), suggesting that eugenol essential oil treatment could effectively activate Th1- and Th2-type cellular immune responses in chickens. Eugenol essential oil therapy reduced the gene expression of the proinflammatory factors IL-1β and IL-6, indicating its effectiveness in reducing inflammation.

Intestinal health is directly related to interactions between the microbiota and the host (He et al. 2021). The intestinal microbiota directly affects intestinal

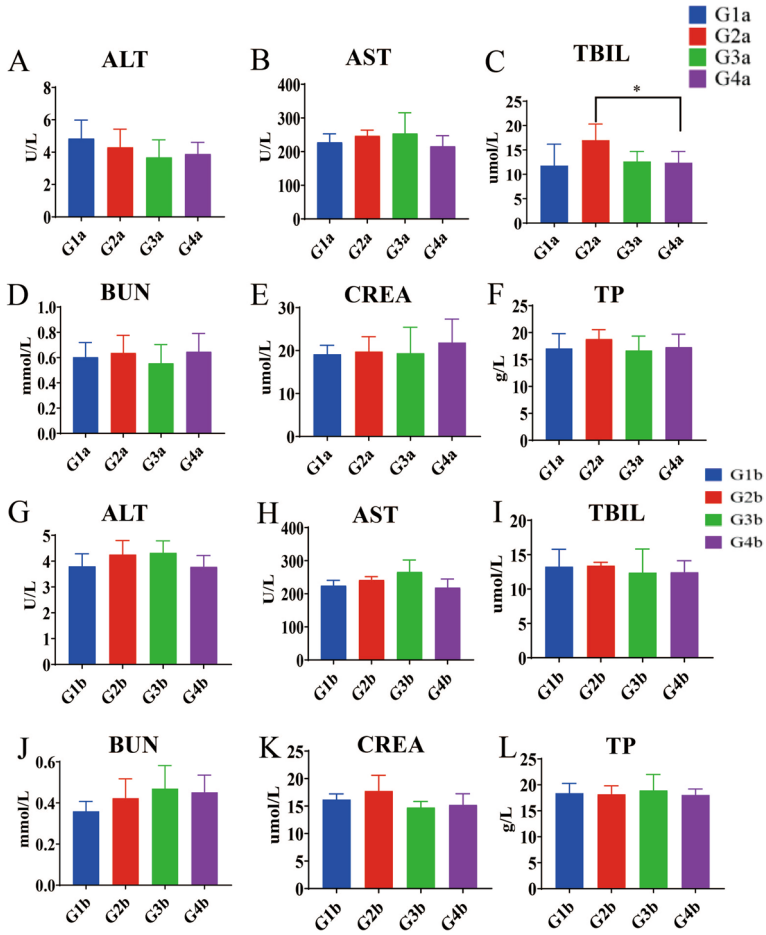

Fig. 8 Effects of eugenol essential oil on the serum biochemical indicators of chickens ($n=6$). Detection of serum biochemical indices, including ALT (**A**), AST (**B**), TBIL (**C**), BUN (**D**), CREA (**E**), and TP (**F**), 7 d after eugenol essential oil treatment. Detection of serum biochemical indices, including ALT (**G**), AST (**H**), TBIL (**I**), BUN (**J**), CREA (**K**), and TP (**L**), 14 d after eugenol essential oil treatment. *, $P<0.05$

absorption efficiency, antagonizes pathogenic bacteria, and enhances intestinal immunity (Zhen et al. 2022). Therefore, a normal intestinal microbiota is very important to the intestine. To evaluate the possible effects of eugenol essential oil on intestinal bacteria, the cecal microbial community in each group was investigated. At the genus level, compared with that in the IC group, the relative abundance of *Faecalibacterium* in the eugenol essential oil-treated groups was reduced, but that of *Lactobacillus* was increased (Fig. 3A), and the relative abundance of *Lactobacillus* in the HD group increased by 72.37%. *Lactobacillus* is known to be a beneficial bacterial genus that helps repair the intestinal immune barrier, thereby improving intestinal immunity in chickens (Wen et al. 2022). These findings suggest that eugenol essential oil enhances host health, possibly by increasing the number of beneficial bacteria while decreasing the proportion of opportunistic pathogenic bacteria.

Since eugenol essential oil can regulate the intestinal microbiota, we further studied the effect of eugenol essential oil on cecal dysbacteriosis caused by *Eimeria tenella* infection. The abundance and diversity of the cecal microbiota were assessed using alpha diversity (Chao1 index) and beta diversity analysis (PCoA). The higher the α-diversity index is, the greater the diversity of the microbiota and the more even the distribution of species (Liu et al. 2022). Beta diversity is a comparative analysis of the microbial community composition of different samples to discover differences between groups (Wang et al. 2022). Figure 3C shows that the IC group had a considerably greater Chao1 index than did the HC group ($p<0.05$), indicating increased microbial richness. The PCoA results showed that the microbial composition of IC was greatly different from that of HC (Fig. 3D), indicating that the coccidiosis model was successfully established. Furthermore, the LEfSe (LDA effect size) analysis tool was used to analyze the changing characteristics of

Table 2 Effects of eugenol essential oil on the relative BWG, lesion score, and ACI of chickens ($n = 12$)

Groups	rBWG [A]	S% [B]	LI [C]	OI [D]	ACI [E]
HD	94.32	100.00	20.83	20.00	153.49
MD	99.87	100.00	22.50	10.00	167.37
LD	93.00	91.67	38.33	10.00	136.34
HC	100.00	100.00	0.00	0.00	200.00
DC	99.77	100.00	10.00	0.00	189.77
IC	79.84	91.67	39.17	40.00	92.34

HD High dose of eugenol essential oil, *MD* Medium dose of eugenol essential oil, *LD* Low dose of eugenol essential oil, *HC* Healthy control group (nontreated and noninfected), *DC* Decoxyquinate treatment control, *IC* Infected control group (untreated and infected with *E. tenella*), *rBWG* relative body weight gain

[A] rBWG = (BWG of the infected/unmedicated control or drug-treated group ÷ BWG of healthy control) × 100

[B] Survival rate (%) = (number of surviving chickens in each group ÷ number of initial chickens in each group) × 100

[C] The lesion index (LI) of the cecum was examined on PI d 8

[D] Oocyst index (OI) of each group at 8 d postinfection (dpi). Oocyst index value = 0 (an oocyst ratio of 0–1%); oocyst index value = 5 (an oocyst ratio of 1–25%); oocyst index value = 10 (an oocyst ratio of 26–50%); oocyst index value = 20 (an oocyst ratio of 51–75%); oocyst index value = 40 (an oocyst ratio of 76–100%); oocyst ratio = (OPG in healthy control or drug-treated group) ÷ (OPG in infected/unmedicated control group) × 100%; OPG, oocyst per gram

[E] Anticoccidial index (ACI) of each group. ACI = (rBWG + survival rate) × 100 − (lesion score + oocyst index value)

the cecal microbiota in different treatment groups (Wu et al. 2022). The results of LEfSe analysis showed that at the species level, the microbiota of the LD group was rich in *Lactobacillus_agilli* (LDA > 4), and at the genus level, the microbiota of the HD group was rich in Lactobacillus (LDA > 4) (Fig. 4A and B). These findings suggest that eugenol essential oil may influence the intestinal microbiota by increasing the proportion of the beneficial bacteria *Lactobacillus*, thereby mitigating the effects of cecal dysbiosis induced by *Eimeria tenella* infection.

These results showed that eugenol essential oil has good anticoccidioidal effects and improves the functioning of physical, chemical, immunological, and microbial barriers in the chicken cecum, resulting in anticoccidial effects. Among these four barriers, the intestinal microbial barrier may play a key role in the occurrence and development of coccidiosis. As a result, modulating the intestinal microbiota may be a new approach for preventing and treating coccidiosis, opening up new avenues for coccidiosis management in chickens.

Conclusions

In this study, a medium dose (0.2 g/kg) of eugenol essential oil had a desirable anticoccidial effect (ACI = 167.37), alleviated cecal lesions, and reduced the output of oocysts, indicating that this dosage could be used for coccidiosis prevention in chickens. All of the chicks survived and exhibited no evidence of toxicity or deformities, indicating that eugenol essential oil was a safe feed supplement. The addition of eugenol essential oil to feed upregulated the expression of occludin in the cecal mucosa of chickens, suggesting a positive effect of eugenol essential oil on the intestinal physical barrier. Following treatment with eugenol essential oil, the serum MDA concentration decreased while the SOD enzyme activity increased, indicating that eugenol essential oil may have a favorable effect on the chemical barrier. Eugenol essential oil increased the proportion of beneficial bacteria (such as *Lactobacillus_agilli*) in the cecum, thereby positively affecting the intestinal microbial barrier. Eugenol essential oil treatment downregulated the gene expression of the proinflammatory factors IL-1β and IL-6 but upregulated the gene expression of the cytokines IL-4 and IFN-γ in the high-dose eugenol essential oil group, indicating that eugenol essential oil could reduce inflammatory responses and enhance host immunity. In summary, eugenol essential oil possesses strong anticoccidial properties, is exceedingly safe, and improves broiler growth performance and intestinal barrier function. This study provides feed manufacturers with an option for replacing anticoccidials with essential oils.

Methods

Drugs and essential oils

Eugenol essential oil was purchased from Shanchuan Biotechnology (Wuhan, China) Co., Ltd. Decoxyquinate solution was used as the positive control drug (batch number: 20210826, Shandong Luxi Veterinary Drug Co., Ltd., China).

Study design

The oocyst was isolated from chicken feces from Xiantao city, Hubei Province, by single oocyst isolation technology. The isolated oocysts were microscopically examined and identified as *Eimeria tenella* by sequencing rRNA in the internal transcription spacer. The isolated strain was preserved at the State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University. Oocyst passage was performed by oral administration to 9-d-old chicks every three months. The CM was collected at 6, 7 and 8 d postinfection, and oocysts were isolated from the obtained manure and subsequently stored in 2.5% potassium dichromate solution at 4°C.

Broilers

One-day-old Arbor Acre broilers (number = 200) were purchased from Zhengkang Livestock and Poultry Co., Ltd. (Jingzhou City, China). All the chickens were raised in cages ($0.7 \times 0.7 \times 0.4$ m^3) in a chicken coccidia-free

environment with free access to feed and drink at 25 ± 2°C and 55 ± 15% humidity.

Anticoccidial test

A total of 72 14-d-old broiler chickens were randomly assigned to six groups, with 12 chickens per group. Group 1 was the healthy control group (HC group), which was not infected with eugenol essential oil or drug application. Group 2 was the infected control group (IC group), which was infected but not administered drugs or eugenol essential oil. Group 3 was the decoxyquinate drug treatment control group (DC, 1.00 mL of decoxyquinate/L water). Group 4 was the low-dose eugenol essential oil treatment group (LD, 0.10 g of eugenol essential oil/kg feed). Group 5 was the middle-dose eugenol essential oil treatment group (MD, 0.20 g of eugenol essential oil/kg feed). Group 6 was the high-dose eugenol essential oil treatment group (HD, 0.40 g/kg feed). To test the dose–response relationship, we used a twofold dose increment of the eugenol essential oil. Each chicken in each group (except the healthy control group) was then orally administered 5×10^4 oocysts. The 22-d-old chickens were weighed one by one and then euthanized. Their cecal lesions were scored by a previously reported method (Johnson and Reid 1970). During the duration of the experiment, we diligently documented the occurrence of bloody diarrhea and the mortality rate of all the chicks on a daily basis.

The number of oocysts per gram of feces (OPG), survival rate (%), relative weight gain rate (%), cecum lesion score, oocyst count, and oocyst ratio were calculated and the oocyst ratio was calculated according to the methods reported by Wang et al. (Wang et al. 2021). The ACI is an index used to determine the anticoccidial effect, and this index combines several parameters, such as survival rate, weight gain, lesions, and fecal oocyst output. In this study, ACI = (relative weight gain rate + survival rate) − (cecal lesion score + oocyst ratio value) was used. An ACI > 180 indicated good protection; 160 < ACI < 179 represented moderate protection; 120 < ACI < 159 denoted limited protection; and an ACI < 120 indicated no protection (Chang et al. 2021).

Determination of serum MDA, SOD, GSH-Px, and CAT

Serum malondialdehyde (MDA) and SOD levels were measured using an MDA assay kit (A001-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and a SOD assay kit (A003-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China), following manual guidelines. Additionally, the serum levels of glutathione peroxidase (GSH-Px) and catalase (CAT) were determined by utilizing a GSH-Px assay kit (A001-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and a CAT assay kit (A007-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China), respectively, following the manufacturer's instructions (Gu et al. 2022).

Quantification of cytokines by qRT–PCR

One week after the addition of eugenol essential oil, three chickens were randomly chosen from each group and euthanized. After being sampled, the cecal tissues were snap-frozen in liquid nitrogen and kept at -80°C for additional examination. A three-dimensional cryomill (Item No.: KZ-5F-3D, Wuhan Servicebio Technology Co., Ltd., China) was used to homogenize the cecal tissues. Total RNA was extracted from the tissue homogenates using a procedure supplied by Wuhan Servicebio Technology Co., Ltd., China. The levels of chicken interleukin 1β (IL-1β) (accession NO. NM_015297469.1), IL-4 (accession NO. NM_001007079.2), IL-6 (accession NO. NM_204628.2), and chicken interferon (IFN-γ) (accession NO. NM_205149.2) were among the cytokines whose levels were ascertained via real-time PCR (qRT–PCR). The endogenous reference gene GAPDH (accession no. NM_001289745.3) was used. Table 3 contains a list of primers used for qPCR quantification. The data were normalized using the $2^{-\Delta\Delta CT}$ method (Livak et al. 2001).

Cecal pathological changes and immunohistochemistry

Hematoxylin/eosin (HE) staining of cecum samples was performed following standard procedures (Xiang et al. 2022). The obtained samples were adequately fixed using 4% paraformaldehyde to ensure that the samples were in a proper fixation state. Subsequently, the samples were trimmed, dehydrated, embedded, sectioned, stained, sealed, and finally subjected to microscopic observation following the standard protocols set by the pathology laboratory. The prepared tissue sections were heated at 60°C for 20 min, dewaxed with xylene and gradient alcohol, soaked in 98°C sodium citrate buffer for 15 min, cooled to room temperature naturally, incubated with 3% deionized water for 10 min, and rinsed with PBS three times. Normal goat serum was added dropwise to the tissue sections and incubated at 37°C for 30 min. After the serum was removed, the tissue sections were incubated with appropriately diluted primary antibodies (Occludin 1:150), incubated overnight at 4°C, rinsed with PBS three times, incubated with secondary antibody dropwise, incubated again at 37°C for 30 min, and rinsed with PBS three times. The tissue sections were subjected to color development with DAB solution for 5 min for subsequent immunohistochemistry analysis. The sections were dehydrated, sealed with neutral gum, and photographed with a microscope. Instead of a primary antibody, 0.01 mol/L PBS solution was used as a negative control. The sections

Table 3 Primer sequences for qRT–PCR quantification

Gene name	Accession No	Primer pair	Primer sequences (5′–3′)	Length of PCR products
GAPDH	NM_001289745.3	GAPDH/F	GTGAAAGTCGGAGTCAACGG	184
		GAPDH/R	CGTTCTCAGCCTTGACAGTG	
IL-1β	NM_015297469.1	IL-1β/F	GCATCAAGGGCTACAAGCTC	134
		IL-1β/R	GTCCAGGCGGTAGAAGATGA	
IL-4	NM_001007079.2	IL-4/F	CCAGCACTGCCACAAGAACC	163
		IL-4/R	AGCTAGTTGGTGGAAGAAGGTACG	
IL-6	NM_204628.2	IL-6/F	AGATGCTCGTCCGGAACAAC	130
		IL-6/R	AGGTAGGTCTGAAAGGCGAACA	
IFN-γ	NM_205149.2	IFNγ/F	AAGCTCCCGATGAACGACTTG	125
		IFNγ/R	TTGCATCTCCTCTGAGACTGGC	

in the negative control group were subjected to the abovementioned treatment steps. The intensity of DAB staining was analyzed by Image-Pro Plus (6.0) software, and the final results are expressed as the mean optical density (MOD) (MOD = IOD/area, IOD = integral optical density, area = positive optical density) (Zhang et al. 2015).

DNA extraction, PCR amplification, and 16S rRNA sequencing

DNA was extracted from the fecal and intestinal content samples utilizing the Magnetic Soil and Stool DNA Kit (TianGen, China, Catalog #: DP712). The 16SV3-V4 regions of the 16S rRNA genes were amplified using specific primers containing unique barcodes (Mei et al. 2021). The PCR system included 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM forward 515F (5′GTGCCAGCMGCCGGTAA-3′), 0.2 μM reverse primer 806R (5′GGACTACHVGGG TWTCTAAT-3′), and approximately 10 ng of template DNA. The PCR thermal cycling process involved an initial denaturation step at 98°C for 1 min, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s, and elongation at 72°C for 30 s. Finally, a final elongation step was performed at 72°C for 5 min. The PCR products and 1 × loading buffer containing SYB green were thoroughly mixed. The mixture was subjected to electrophoresis on a 2% agarose gel. and then purified using the Universal DNA Purification Kit from TianGen (China, Catalog #: DP214). Sequencing libraries were constructed using the NEBNext® Ultra™ II FS DNA PCR-free Library Prep Kit (New England Biolabs, USA, Catalog #: E7430L) following the manufacturer's instructions. The constructed libraries were quantified using a Qubit instrument, and real-time PCR and size distribution detection were performed using a bioanalyzer. After quantification, the libraries were pooled and then sequenced on Illumina platforms based on the desired effective library concentration and data volume (Martins et al. 2022).

Bioinformatics analysis

DADA2 (the Divisive Amplicon Denoising Algorithm) in the QIME2 software (Quantitative Insights into Microbial Ecology 2, Version QIIME2-202006) was used for denoising, and the sequences with an abundance < 5 were filtered to obtain the final amplicon sequence variants (ASVs) with sequence identity ≥ 97% (de Bruijn et al. 2020). Additionally, species annotation and rapid multiple sequence alignment were performed using QIIME2 software for subsequent construction of the phylogenetic tree. The number of OTUs and the Shannon, Simpson, Chao1, Good's coverage, dominance, and Pielou's evenness indices were calculated using QIIME2 software. In addition, principal coordinate analysis (PCoA) was conducted based on the weighted UniFrac distance and unweighted UniFrac distance, and the principal coordinate combination with the largest contribution rate was selected for diagram plotting. In addition, we performed an intergroup significant differential species analysis using linear effect size (LEfSe) software, with the default linear discriminant analysis (LDA) threshold set to 4 (Lu et al. 2020).

Dose safety test

The dose safety test was performed according to the *Target Animal Safety Tests of Veterinary Traditional Chinese Medicines and Natural Medicines* published by the Center for Veterinary Drug Evaluation (CVDE, China) in 2012. A total of 80 0-d-old Arbor Acre broiler chickens were used for the dose safety test. The chickens were then randomly divided into four groups. Each group had

almost the same average weight. The grouping was as follows. Group 1 (G1) was the eugenol essential oil group with 1 dose added (1×RD), in which chickens were fed a basal diet containing 0.2 g/kg of eugenol essential oil; group 2 (G2) was the eugenol essential oil group with 3 doses (3×RD), in which chickens were fed a basal diet containing 0.6 g/kg of eugenol essential oil; group 3 (G3) was the eugenol essential oil group with 6 doses (6×RD), in which chickens were fed a basal diet containing 1.2 g/kg of eugenol essential oil; and group 4 (G4) was the control group, in which chickens were fed a basal diet. Each group was further divided into two subgroups, a and b (namely, G1a and G1b, G2a and G2b, G3a and G3b, and G4a and G4b), with 10 chickens per subgroup. Chickens in G1a, G2a, G3a, and G4a were euthanized at the age of 21 d, and chickens in G1b, G2b, G3b, and G4b were euthanized at the age of 28 d. The chickens' clinical signs and mortality were observed and recorded every day. The body weight gain (BWG) of the chickens was calculated as the difference between the mean initial body weight (on d 14) and the mean final body weight (on d 21 and d 28). The feed conversion ratio (FCR) was calculated as the ratio of feed consumption to BWG.

Relative organ weight (ROW), white blood cell (WBC) counts, red blood cell (RBC) counts, hemoglobin concentration (HGB), hematocrit (HCT, %), mean corpuscular hemoglobin concentration (MCHC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), total bilirubin (TB), and total blood cell (WBC) counts were obtained. Bilirubin (TBIL), blood urea nitrogen (BUN), and creatinine (CREA) levels were measured and calculated according to the methods of Wang et al. (Wang et al. 2021).

Statistical analysis and data availability statement

Statistical differences among groups were analyzed using one-way ANOVA and Duncan's multiple range test with GraphPad Prism 8.0 (Software Inc., La Jolla, CA, USA). The raw whole-genome resequencing data were submitted to the Sequence Read Archive database under the data certification number PRJNA1035248.

Abbreviations

ACI	Anticoccidial index
IC	Infected control group
RD	Recommended dose
TBIL	Total bilirubin
E.tenella	*Eimeria tenella*
EO	Essential oil
NO	Nitric oxide
MDA	Malondialdehyde
SOD	Superoxide dismutase
GSH-Px	Glutathione peroxidase
CAT	Catalase
LD	Low-dose eugenol essential oil treatment group
DC	Decoxyquinate drug treatment control group
BWG	Body weight gain
MD	Middle-dose eugenol essential oil treatment group
OI	Oocyst index
HE	Hematoxylin/eosin
MOD	Mean optical density
IL-1β	Interleukin 1β
IL-4	Interleukin 4
IL-6	Interleukin 6
IFN-γ	Interferon γ
Th1	T helper cells 1
Th2	T helper cells 2
HC	Healthy control group
PCoA	Principal coordinate analysis
LDA	Linear discriminant analysis
ROW	Relative organ weight
ROS	Reactive oxygen species
$CD4^+$	CD4 T-lymphocytes
LEfSe	Linear effect size
OTU	Operational taxonomic units
OPG	Oocysts per gram
DAB	Diaminobenzidine
DADA	Divisive Amplicon Denoising Algorithm
QIME2	Quantitative Insights into Microbial Ecology 2
ASVs	Amplicon sequence variants
CVDE	Center for Veterinary Drug Evaluation
FCR	Feed conversion rate
HD	High-dose eugenol essential oil treatment group
WBC	White blood cells
RBC	Red blood cells
HGB	Hemoglobin concentration
EDTA	Ethylene Diamine Tetraacetic Acid
TP	Total protein
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
MCHC	Mean corpuscular hemoglobin concentration
HCT	Hematocrit
CREA	Creatinine
BUN	Blood urea nitrogen

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s44149-024-00116-z.

Additional file 1: Fig. S1. Effect of eugenol essential oil on the relative organ weight (ROW) of chickens after 14 d of eugenol essential oil treatment in the safety test (*n*=6). A. Heart. B Liver. C Spleen. D Lung. E Kidney.

Additional file 2: Fig. S2. Dose-safety test showing the effects of eugenol essential oil on hematological indicators in chickens (*n*=6). The WBC (A), RBC (B), HGB (C), HCT (D), and MCHC (E) were assessed after 14 d of eugenol essential oil treatment.

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Authors' contributions

Y.Z. and J.Z. contributed to the conception and design of this study. T.G. executed the experiments and analyzed the data. T.G. wrote the first draft of the manuscript. S.B. and R.F. edited the manuscript. X.P. and L.W. performed the animal experiments.

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Availability of data and materials

The relevant data and material in this article are available and can be requested from the corresponding authors.

Declarations

Ethics approval and consent to participate

The experimental procedures adhered to the guidelines set by the Ministry of Agriculture, China, specifically the Center for Veterinary Drug Evaluation (CVDE). The Ethics Committee of Huazhong Agricultural University reviewed and approved the study (Approval number: HZAUCH-2020–0017).

Consent for publication

Not applicable.

Competing interests

All contributing authors declare no competing interests. Author Bang Shen was not involved in the journal's review or decisions related to this manuscript.

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References

Chang, L.Y., K.Q. Di, J. Xu, Y.F. Chen, J.Z. Xi, D.H. Wang, E.Y. Hao, L.J. Xu, H. Chen, and R.Y. Zhou. 2021. Effect of natural garlic essential oil on chickens with artificially infected *Eimeria tenella*. *Veterinary Parasitology* 300: 109614. https://doi.org/10.1016/j.vetpar.2021.109614.

Chapman, H.D., and T.K. Jeffers. 2014. Vaccination of chickens against coccidiosis ameliorates drug resistance in commercial poultry production. International journal for parasitology. *Drugs and Drug Resistance* 4 (3): 214–217. https://doi.org/10.1016/j.ijpddr.2014.10.002.

Costa, S., C. Cavadas, C. Cavaleiro, L. Salgueiro, and M. do Céu Sousa. 2018. In vitro susceptibility of *Trypanosoma brucei brucei* to selected essential oils and their major components. *Experimental Parasitology* 190: 34–40. https://doi.org/10.1016/j.exppara.2018.05.002.

Das, B., D. Mandal, S.K. Dash, S. Chattopadhyay, S. Tripathy, D.P. Dolai, S.K. Dey, and S. Roy. 2016. Eugenol provokes ROS-mediated membrane damage-associated antibacterial activity against clinically isolated multidrug-resistant *Staphylococcus aureus* strains. *Infectious Diseases* 9: 11–19. https://doi.org/10.4137/IDRT.S31741.

de Bruijn, V., C. Behr, S. Sperber, T. Walk, P. Ternes, M. Slopianka, V. Haake, K. Beekmann, and B. van Ravenzwaay. 2020. Antibiotic-Induced changes in microbiome-related metabolites and bile acids in rat plasma. *Metabolites* 10 (6): 242. https://doi.org/10.3390/metabo10060242.

Deng, L., H. Guo, S. Wang, X. Liu, Y. Lin, R. Zhang, and W. Tan. 2022. The attenuation of chronic ulcerative colitis by (R)-salbutamol in repeated DSS-induced mice. *Oxidative Medicine and Cellular Longevity* 2022: 9318721. https://doi.org/10.1155/2022/9318721.

Fatoba, A.J., V.T. Adeleke, L. Maharaj, M. Okpeku, A.A. Adeniyi, and M.A. Adeleke. 2022. Design of a multiepitope vaccine against chicken anemia virus disease. *Viruses* 14 (7): 1456. https://doi.org/10.3390/v14071456.

Gaboriaud, P., G. Sadrin, E. Guitton, G. Fort, A. Niepceron, N. Lallier, C. Rossignol, T. Larcher, A. Sausset, R. Guabiraba, et al. 2021. The absence of gut microbiota alters the development of the apicomplexan parasite *Eimeria tenella*. *Frontiers in Cellular and Infection Microbiology* 10: 632556. https://doi.org/10.3389/fcimb.2020.632556.

Gu, X., Q. Long, W. Wei, J. Tong, Z. Li, Z. Zhang, and Y. Jiao. 2022. Number 2 feibi recipe inhibits H_2O_2-mediated oxidative stress damage of alveolar epithelial cells by regulating the balance of mitophagy/apoptosis. *Frontiers in Pharmacology* 13: 830554. https://doi.org/10.3389/fphar.2022.830554.

Gucwa, K., S. Milewski, T. Dymerski, and P. Szweda. 2018. Investigation of the antifungal activity and mode of action of *Thymus vulgaris*, *Citrus limonum*, *Pelargonium graveolens*, *Cinnamomum cassia*, *Ocimum basilicum*, and *Eugenia caryophyllus* essential oils. *Molecules (basel, Switzerland)* 23 (5): 1116. https://doi.org/10.3390/molecules23051116.

He, Q., T. Zou, J. Chen, J. He, L. Jian, F. Xie, J. You, and Z. Wang. 2021. Methyl-donor micronutrient for gestating sows: Effects on gut microbiota and metabolome in offspring piglets. *Frontiers in Nutrition* 8: 675640. https://doi.org/10.3389/fnut.2021.675640.

Hosny, K.M., and N.A. Alhakamy. 2020. Nasal gel loaded with amphotericin nanotransferosomes as antifungal treatment for fFungal sinusitis. *Pharmaceutics* 13 (1): 35. https://doi.org/10.3390/pharmaceutics13010035.

Huang, S.Y., N. Yao, J.K. He, M. Pan, Z.F. Hou, Y.M. Fan, A. Du, and J.P. Tao. 2021. In vitro antiparasitic activity of *Pelargonium X. asperum* essential oil against toxoplasma gondii. *Frontiers in Cell and Developmental Biology* 9: 616340. https://doi.org/10.3389/fcell.2021.616340.

Johnson, J., and W.M. Reid. 1970. Anticoccidial drugs: Lesion scoring techniques in battery and floor-pen experiments with chickens. *Experimental Parasitology* 28 (1): 30–36. https://doi.org/10.1016/0014-4894(70)90063-9.

Khramtsov, P., M. Bochkova, V. Timganova, A. Nechaev, S. Uzhviyuk, K. Shardina, I. Maslennikova, M. Rayev, and S. Zamorina. 2021. Interaction of graphene oxide modified with linear and branched PEG with monocytes isolated from human blood. *Nanomaterials (basel, Switzerland)* 12 (1): 126. https://doi.org/10.3390/nano12010126.

Li, P., K. Li, C. Zou, C. Tong, L. Sun, Z. Cao, S. Yang, and Q. Lyu. 2020. Selenium yeast alleviates ochratoxin A-induced hepatotoxicity via modulation of the PI3K/AKT and Nrf2/Keap1 signaling pathways in chickens. *Toxins* 12 (3): 143. https://doi.org/10.3390/toxins12030143.

Liu, M.M., N. Zhou, N. Jiang, K.M. Lu, C.F. Wu, and J.K. Bao. 2022. Neuroprotective effects of oligosaccharides from periplaneta americana on parkinson's disease models In vitro and In vivo. *Frontiers in Pharmacology* 13: 936818. https://doi.org/10.3389/fphar.2022.936818.

Livak, K.J., and T.D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods (san Diego, Calif.)* 25 (4): 402–408. https://doi.org/10.1006/meth.2001.1262.

Lu, Q., Y. Lin, T. Chen, H. Lv, F. Diao, C. Liu, M. Peng, X. Ling, H. Li, Y. Wang, Y. Wei, J. Du, G. Jin, Y. Xia, H. Ma, X. Liu, H. Shen, and Z. Hu. 2020. Alternations of gut microbiota composition in neonates conceived by assisted reproductive technology and its relation to infant growth. *Gut Microbes* 12 (1): 1794466. https://doi.org/10.1080/19490976.2020.1794466.

Mahizan, N.A., S.K. Yang, C.L. Moo, A.A. Song, C.M. Chong, C.W. Chong, A. Abushelaibi, S.E. Lim, and K.S. Lai. 2019. Terpene derivatives as a potential agent against antimicrobial resistance (AMR) pathogens. *Molecules (basel, Switzerland)* 24 (14): 2631. https://doi.org/10.3390/molecules24142631.

Martins, F.H., A. Rajan, H.E. Carter, H.R. Baniasadi, A.W. Maresso, and V. Sperandio. 2022. Interactions between *Enterohemorrhagic escherichia coli* (EHEC) and gut commensals at the interface of human colonoids. *Mbio* 13 (3): e0132122. https://doi.org/10.1128/mbio.01321-22.

Mei, Q.X., J.H. Hu, Z.H. Huang, J.J. Fan, C.L. Huang, Y.Y. Lu, X.P. Wang, and Y. Zeng. 2021. Pretreatment with chitosan oligosaccharides attenuate experimental severe acute pancreatitis by inhibiting oxidative stress and modulating intestinal homeostasis. *Acta Pharmacologica Sinica* 42 (6): 942–953. https://doi.org/10.1038/s41401-020-00581-5.

Nisar, M.F., M. Khadim, M. Rafiq, J. Chen, Y. Yang, and C.C. Wan. 2021. Pharmacological properties and health benefits of eugenol: A comprehensive review. *Oxidative Medicine and Cellular Longevity* 2021: 2497354. https://doi.org/10.1155/2021/2497354.

S Bendre, R., & D Rajput, J. 2016. Outlooks on medicinal properties of eugenol and its synthetic derivatives. *Natural Products Chemistry & Research*, 04(03). https://doi.org/10.4172/2329-6836.1000212.

Swamy, M.K., M.S. Akhtar, and U.R. Sinniah. 2016. Antimicrobial properties of plant essential oils against human pathogens and their mode of action: An updated review. *Evidence-Based Complementary and Alternative Medicine: Ecam* 2016: 3012462. https://doi.org/10.1155/2016/3012462.

Ueda-Nakamura, T., Mendonça-Filho, R. R., Morgado-Díaz, J. A., Korehisa Maza, P., Prado Dias Filho, B., Aparício Garcia Cortez, D., Alviano, D. S., Rosa, M.doS., Lopes, A. H., Alviano, C. S., et al. 2006. Antileishmanial activity of Eugenol-rich essential oil from Ocimum gratissimum. *Parasitology International*, 55(2), 99–105. https://doi.org/10.1016/j.parint.2005.10.006.

Wang, L., W. Guo, S.U. Haq, Z. Guo, D. Cui, F. Yang, F. Cheng, X. Wei, and J. Lv. 2021. Anticoccidial Activity of Qinghao Powder Against *Eimeria tenella* in Broiler Chickens. *Frontiers in Veterinary Science* 8: 709046. https://doi.org/10.3389/fvets.2021.709046.

Wang, F., N. Sun, H. Zeng, Y. Gao, N. Zhang, and W. Zhang. 2022. Selenium deficiency leads to inflammation, autophagy, endoplasmic reticulum sStress, apoptosis and contraction abnormalities via affecting intestinal flora in intestinal smooth muscle of mice. *Frontiers in Immunology* 13: 947655. https://doi.org/10.3389/fimmu.2022.947655.

Wen, K., L. Liu, M. Zhao, T. Geng, and D. Gong. 2022. The changes in microbiotic composition of different intestinal tracts and the effects of supplemented *Lactobacillus* during the formation of goose fatty liver. *Frontiers in Microbiology* 13: 906895. https://doi.org/10.3389/fmicb.2022.906895.

Wu, R., Q. Shen, P. Li, and N. Shang. 2022. Sturgeon chondroitin sulfate restores the balance of gut microbiota in colorectal cancer bearing mice. *International Journal of Molecular Sciences* 23 (7): 3723. https://doi.org/10.3390/ijms23073723.

Xiang, B., R. Geng, Z. Zhang, X. Ji, J. Zou, L. Chen, and J. Liu. 2022. Identification of the effect and mechanism of Yiyi Fuzi Baijiang powder against colorectal cancer using network pharmacology and experimental validation. *Frontiers in Pharmacology* 13: 929836. https://doi.org/10.3389/fphar.2022.929836.

Xu, L., Z. Yu, K. He, Z. Wen, M.T. Aleem, R. Yan, X. Song, M. Lu, and X. Li. 2022. PLGA nanospheres as delivery platforms for *Eimeria mitis* 1a protein: A novel strategy to improve specific immunity. *Frontiers in Immunology* 13: 901758. https://doi.org/10.3389/fimmu.2022.901758.

Zhang, D.D., J.G. Zhang, X. Wu, Y. Liu, S.Y. Gu, G.H. Zhu, Y.Z. Wang, G.L. Liu, and X.Y. Li. 2015. Nuciferine downregulates Per-Arnt-Sim kinase expression during its alleviation of lipogenesis and inflammation on oleic acid-induced hepatic steatosis in HepG2 cells. *Frontiers in Pharmacology* 6: 238. https://doi.org/10.3389/fphar.2015.00238.

Zhang, J.M., F.E. Feng, Q.M. Wang, X.L. Zhu, H.X. Fu, L.P. Xu, K.Y. Liu, X.J. Huang, and X.H. Zhang. 2016. Platelet-derived growth factor-BB protects mesenchymal stem cells (MSCs) derived from immune thrombocytopenia patients against apoptosis and Ssenescence and maintains MSC-mediated immunosuppression. *Stem Cells Translational Medicine* 5 (12): 1631–1643. https://doi.org/10.5966/sctm.2015-0360.

Zhang, H., L. Zhang, H. Si, X. Liu, X. Suo, and D. Hu. 2022. Early transcriptional response to monensin in sensitive and resistant strains of *Eimeria tenella*. *Frontiers in Microbiology* 13: 934153. https://doi.org/10.3389/fmicb.2022.934153.

Zhao, P., Y. Li, Y. Zhou, J. Zhao, and R. Fang. 2020. In vivo immunoprotective comparison between recombinant protein and DNA vaccine of *Eimeria tenella* surface antigen 4. *Veterinary Parasitology* 278: 109032. https://doi.org/10.1016/j.vetpar.2020.109032.

Zhen, R., J. Feng, D. He, Y. Chen, T. Chen, W. Cai, Y. Xiong, Y. Qiu, Z. Jiang, L. Wang, and H. Yi. 2022. Effects of niacin on resistance to enterotoxigenic *Escherichia coli* infection in weaned piglets. *Frontiers in Nutrition* 9: 865311. https://doi.org/10.3389/fnut.2022.865311.

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