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Efficacy of dietary supplements of *Glycyrrhiza glabra* (Licorice) and maduramicin alone or in combination with *Eimeria tenella* infected chicks: A clinical study and molecular docking

Sawsan S. Elbasuni[1]*, Hanan A. A. Taie[2], Samah M. Abdel Gawad[3], Reda E. L. Kamar[4], Hala El Daous[5], Marwa Darweish[6], Mai O. Nada[7], Walaa F. SaadEldin[8] and Marwa I. Abdel Haleem[1]

[1]*Department of Avian and Rabbit Diseases, Faculty of Veterinary Medicine, Benha University, Benha, Egypt*
[2]*Plant Biochemistry Department, National Research Centre, Dokki, Giza, Egypt*
[3]*Department of Parasitology, Faculty of Veterinary Medicine, Benha University, Benha, Egypt*
[4]*Department of Histology, Faculty of Veterinary Medicine, Benha University, Benha, Egypt*
[5]*Department of Hygiene and Veterinary Management, Faculty of Veterinary Medicine, Benha University, Benha, Egypt*
[6]*Department of Pathology, Faculty of Veterinary Medicine, Benha University, Benha, Egypt*
[7]*Department of Veterinary Pharmacology, Animal Health Research Institute-Benha Branch, Agriculture Research Center (ARC), Benha, Egypt*
[8]*Educational Veterinary Hospital, Faculty of Veterinary Medicine, Zagazig University, Egypt*

ABSTRACT

Background: Coccidiosis is one of the most economically significant poultry diseases worldwide, caused by the pathogenic *Eimeria* species, and is characterized by decreased weight gain (WG) and failure to grow due to malabsorption, low feed conversion rate, bloody diarrhea, and dehydration.

Aim: This study investigated the effectiveness of licorice root extract (LRE) in controlling cecal coccidiosis to determine whether its combination with maduramicin could help alleviate the pathological, biochemical, and histopathological effects of cecal coccidiosis in Sasso broiler chicks.

Methods: A total of 125 one-day-old Sasso broiler chicks were categorized into five equal groups (n = 25), each consisting of five replicates (n = 5 per replicate). G1-LE received a basal diet supplemented with LRE (3 g/kg); G2-ME received a basal diet containing maduramycin (0.5 g/kg); and G3-LME received a basal diet containing LRE and maduramicin together with the same rates. G4-E (positive control) and G5-N (negative control) received no additives in their feed. Birds in groups (G1-4) were challenged on day 14 of the experiment by orally intercropping a 1 ml suspension of *Eimeria tenella* sporulated oocysts.

Results: Groups of birds fed on LRE and maduramicin separately or together appeared to be in good condition where no deaths or clinical abnormalities were observed, based on the analysis of clinicopathological examination. Compared with the G4-E positive control, the dropping scoring and oocyst shedding of groups G1-LE, G2-ME, and G3-LME along the 10th-day post-challenge (dpc), as well as macroscopic and microscopic lesions scoring at the 7th dpc, was considerably lower. The dual supplementation use of LRE and maduramicin in G3-LME's reduced the harmful effects of coccidian, which appeared only as a mononuclear cellular infiltration and a small number of oocysts invading the intestinal glands. Molecular docking revealed that LRE and maduramicin interacted with *E. tenella* DNA polymerase, *E. tenella* apical membrane antigen 1, and microneme protein binding sites resulting in reduced *E. tenella* replication and invasion.

Conclusion: The inclusion of LRE and maduramicin, individually or in combination, in the diet might effectively mitigate the detrimental effects of coccidiosis.

Keywords: Chicken, Coccidiosis, *Glycyrrhiza glabra*, Licorice, Maduramicin.

Introduction

The annual cost of coccidiosis to the poultry industry is estimated at £10.4 billion (Hussain, *et al.,* 2017; Blake *et al*., 2020) making it one of the most economically significant poultry illnesses worldwide. The prevalence of coccidian infection in commercial poultry production ranges from 5% to 70% in developing countries, and Egypt is one of those countries (Du and Hu, 2004; Al-Gawad *et al*., 2012; Abbas *et al*., 2017). Seven different species of the obligate intracellular parasite *Eimeria* produce

***Corresponding Author:** Sawsan S. Elbasuni. Departmessnt of Avian and Rabbit Diseases, Faculty of Veterinary Medicine, Benha University, Benha, Egypt. Email: *Sowsan.mohamed@fvtm.bu.edu.eg*

coccidiosis in chickens, leading to widespread illness and death (Berezin *et al.*, 2010; Moryani *et al*., 2021).

Clinical manifestations of coccidiosis, are caused by exposure to the pathogenic sporulated *Eimeria* species oocysts in the environment (Sundar *et al*., 2017; Kalkal *et al*., 2021). Free radical oxidants are produced by the host's cellular immunological response because of *Eimeria* entry into intestinal cells at various locations (Allen *et al*., 1998) but pathogenic oxidative stress and disruption of the ecological redox balance may result from exposure to excessive levels of infection (Georgieva *et al*., 2006).

A natural chemical substance called maduramicin helps manage coccidiosis, by blocking ion transport channels and disrupting the osmotic balance of the parasite. Drug resistance and negative effects on both beneficial organisms and human consumers from drug residues in the environment or in chicken by-products are two drawbacks of the widespread use of these chemical agents in chicken farm feed and/or water to combat coccidiosis (Williams, 1998; Peek and Landman, 2011; Kadykalo *et al.*, 2018). In recent years, medicinal plants have been used to fight coccidiosis by lowering stress levels and relieving oxidative stress, resulting in improved nutrient uptake, overall health, and increased yield (Awais *et al*., 2018; Kadykalo *et al.*, 2018; Pop *et al.*, 2019). Licorice or *Glycyrrhiza glabra* (*G. glabra*), has been used medicinally for centuries, and its numerous active components including saponin, triterpenes, flavonoids, coumarins, sugars, starch, amino acids, choline, tannins, phytosterols, ascorbic acid, and others give it a wide range of biological and pharmaceutical applications. Extracts of *G. glabra* have been tested in vitro at varying concentrations for their purported role in controlling cecal coccidiosis, where the number of sporulated and unsporulated oocysts was significantly reduced. In a dose-dependent manner, *G. glabra* extracts alone or in combination with other substances have been shown to have therapeutic and preventative benefits on single and combined infections of several *Eimeria* species (Pop *et al*., 2019; Hussain *et al*., 2022; Ghafouri *et al*., 2023).

The objective of this study is to investigate the efficacy of herbal remedies, specifically *G. glabra*, in the management of cecal coccidiosis. In addition, we aimed to determine whether combining these herbal remedies with chemotherapeutic agents such as maduramicin could help mitigate the pathological, biochemical, and histopathological effects of coccidiosis in Sasso broiler chicks. Integrating this supplementary value enables the application of traditional medicine knowledge in clinical contexts.

Material and Methods

Birds, and management

A total of 125 one-day-old Sasso broiler chicks were procured from a local hatchery in Egypt. At the Faculty of Veterinary Medicine, Benha University, the chicks were kept in clean and sanitized conditions. The birds were housed in a floor system using the standard management procedures (Abdel Haleem *et al*., 2019). There were enough feeders and waterers for each compartment. From 14 to 24 days of age, plastic sheets were placed over the bedding material to allow dropping scoring and collection for oocyst counting. All chicks were fed a broiler starter ration for three weeks, then a broiler grower ration until the 35th day. Ration formulation was in accordance with the guidelines outlined in the NRC (1994) (Table 1).

Table 1. Basal diet composition and its chemical analysis.

Item	Starter ration (0–21 d)	Grower ration (22–35 d)
Yellow corn%	56.92	60.22
Soyabean meal 46%	35	33.50
Corn gluten meal%	2.3	0.00
Vegetable oil%	0.95	2.00
Sodium chloride%	0.0.215	0.26
DL-Methionine%	0.36	0.34
L-Lysine%	0.40	0.215
Limestone%	1.67	1.55
Mono-calcium phosphate%	1.20	1.025
Sodium bicarbonate%	0.295	.20
Vitamin and mineral premix%	0.30	0.30
Crude protein%	23.03	21.02
MEn (Kcal/kg)	3009.47	3102.61
Crude fat%	3.51	4.56
Lysine%	1.40	1.20
Lysine digestible%	1.28	1.09
Methionine%	0.69	0.63
Methionine digestible%	0.66	0.60
Methionine + cysteine%	1.03	0.95
Methionine + cysteine digestible%	0.94	0.86
Threonine%	0.95	0.88
Threonine digestible%	0.83	0.77
Calcium%	0.96	0.88
Available phosphorus%	0.49	0.44
Chloride%	0.24	0.23
Sodium%	0.17	0.16
Potassium%	0.88	0.85

Experimental design

The chicks were randomly divided into five distinct groups (*n = 25* per group), each consisting of five replicates (*n = 5* per replicate). The first group (G1-LE) received a basal diet supplemented with licorice root powder extract at a dosage of 3 g/kg (Rashidi *et al*., 2020), the second group (G2-ME) was provided with the basal diet supplemented with maduramicin (Atco-pharma, Egypt) at a dosage of 0.5 g/kg. Finally, the third group (G3-LME) was given the basal diet supplemented with a combination of licorice root extract (LRE) and maduramicin throughout the experiment. The control positive group (G4-E) and control negative group (G5-N) did not receive any medications in their feed and the G5-N group maintained in isolation from the experimental area. Birds in groups (G1-4) were subjected to a challenge on the 14th day of the experiment. This challenge involved intercropping a 1 ml (5×10^4 oocysts) suspension of *Eimeria tenella* sporulated oocysts (Pop *et al.*, 2019), while G5-N received 1 ml of normal saline solution intracrop.

Eimeria tenella oocysts

The oocysts of *E. tenella* were obtained from a commercial broiler flock that experienced cecal coccidiosis in Qalyubia Governorate, Egypt. The oocysts were isolated using a combination of sieve and sedimentation methods (Longstaffe, 1984). The identification of oocysts was conducted based on their morphological properties (Al-Gawad *et al.*, 2012). The viability of the oocysts was verified through the production of a distinctive manifestation of cecal coccidiosis in experimental chicks (Swayne *et al*., 2020). The suspension containing fresh sporulated oocysts was preserved in a refrigerator (4°C) using a 2.5% potassium dichromate solution until use. The quantification and microscopic identification of sporulated oocysts in the culture were performed on the fourteenth day of the experiment directly before challenge. To minimize manufactured errors and improve the reproducibility of experiments, we have opted to designate a sample size of five avian subjects for each test as mentioned in the statistical analysis.

Licorice root extract preparation and phytochemical analysis

The powdered aqueous ethanolic extract (70%) of licorice root (LRE) was purchased from Al-Abji Factory, Cairo, Egypt, for the extraction of vegetables and essential oils. The Folin-Ciocalteu procedure was used to determine the total phenol (TP) and total tannin (TT) content and results were expressed in milligrams of gallic acid equivalent (GAE) per gram of dry extract weight (Cheel *et al*., 2007). The total flavonoid content was conducted (Ordoñez *et al*., 2006)**.** The quantification of overall flavonoid content was conducted by ascertaining the quercetin equivalent through the utilization of a calibration curve. The total saponin content was measured calorimetrically (Makkar *et al*., 1997). With minor modifications, total terpenoids were estimated and results were expressed in milligrams of linalool (LE) per gram of dry sample (Koleva *et al*., 2002).

Clinicopathological assessments

Body performance parameter

Feed intake (FI), body weight (BW), weight gain (WG), and feed conversion ratio (FCR) were estimated at day 10 post-challenge (dpc) (Abdel Haleem *et al.*, 2019), as well as mortalities**.** All birds were weighed before and after the challenge, and their clinical symptoms were monitored daily. In cases where deaths were recorded, autopsies were performed. To evaluate the efficacy of different interventions, the anticoccidial index (ACI) was calculated using the following parameters according to De Pablos *et al*. (2010**)**.

Dropping scoring

Bird droppings 10 dpc were graded on a scale of 0 to 4 according to consistency and the presence of mucus and/or blood (Morehouse and Baron, 1970; De Pablos *et al.*, 2010)

Oocyst shedding (oocysts per gram) (OPG)

Fresh fecal samples (*n* = 5 per group) were collected daily from the covered plastic lid starting at 3–10 dpc. Fecal samples were individually placed in sealed plastic bags homogenized, and subsequently stored at 4°C. OPG were counted using the McMaster counting chamber. This method has also been used to determine the oocyst index in different groups, compared to the OPG of the control positive group (Dommels *et al.*, 2007).

Pathological assessments

On the 10th dpc, five birds from each group were randomly selected and euthanized by neck dislocation. To alleviate any potential distress experienced by the birds, sodium pentobarbital (50 mg/kg) was provided via intraperitoneal injection before this particular stage to detect macroscopic changes in the cecum and scored from 0 to 4 using Johnson and Reid (1970) (Table 2). Ceca were preserved in 10% formalin and routinely processed according to the method of Bancroft *et al.* (1996), as well as, scoring according to the nature and extent of the lesion and recurrence, (Shackelford *et al*., 2002) (Table 2), which was used to calculate the histological injury score (HIS) and lesion index (Dommels *et al.*, 2007)**.**

Biochemical parameters

Blood samples (*n* = 5 per group) were collected at 14, 21, and 35 days of age from the right jugular vein of the neck using sterile plane blood collecting tubes, and the serum was centrifuged out of the blood samples and stored at −20°C for biochemical analysis.

Biomarkers of oxidative stress (Biodiagnostic kit's guidelines), such as serum malondialdehyde (MDA) were determined using a colorimetric technique (Kei, 1978), serum total superoxide dismutase (T-SOD) (Nishikimi *et al*., 1972), and catalase enzyme (Aebi, 1984). Lipid profile was evaluated as follows: total cholesterol (Richmond, 1973), high-density lipoprotein cholesterol (HDL-C) (Lopes-Virella *et al*., 1977), low-density lipoprotein cholesterol (LDL-C) (Wieland

Table 2. Grading scores of Clinicopathological changes.

Scoring Grade	0	1	2	3	4	References
Dropping scoring	Normal color and consistency	Mild mucoid to watery droppings	Moderate mucoid to watery with abnormal color	All watery, bloody-tinged color	Watery bloody droppings	Morehouse and Baron (1970)
Macroscopic Lesion scoring	No lesions	Small scattered petechiae	Numerous petechiae	Extensive hemorrhage	Extensive hemorrhage a dark color	Johnson and Reid (1970)
Microscopic Lesion scoring	No lesions	0%–10% of tissue involved)	11%–20% of tissue involved)	21%–40% of tissue involved)	41%–100% of tissue involved)	Shackelford *et al.* (2002)

and Seidel, 1983), and triacylglycerol (Fossati and Prencipe, 1982), and liver function was monitored by measuring serum aspartate transferase (AST) and alanine transferase (ALT) (Young, 1997). Meanwhile, creatinine and urea were measured (Tovar *et al.*, 2002).

Molecular docking

Three-dimensional (3D) structures of *E. tenella* DNA polymerase, *E. tenella* apical membrane antigen 1 (EtAMA1), and *E. tenella* microneme protein 3 (EtMIC3) proteins were retrieved from the AlphaFold Protein Structure Database (*https://www.uniprot.org/*). In addition, the 3D structure of maduramicin was obtained from the PubChem database (*https://pubchem.ncbi.nlm.nih.gov/*), while the 3D structures of licorice bioactive compounds were retrieved from the LOTUS database (*https://lotus.naturalproducts.net/*).

Protein preparation, protein-ligands interactions, and visualization were performed using Molecular Operating Environment software (MOE 2022.02, Chemical Computing Group, Montreal, QC, Canada).

Statistical analysis

Differences between groups were analyzed using One-Way ANOVA and Tukey's multiple comparison Post hoc tests (Duncan, 1955), and statistical analysis was performed using the statistical software package SPSS for Windows (version 20.0; SPSS Inc., Chicago, IL, USA). Statistical significance between mean values was set at $p < 0.05$.

A sample of at least five in each group and a total sample of 25 chicks (divided into five groups) are required to estimate an effect size = 1.029 (Hussain *et al*., 2022) and a normal mortality rate (dropout) of 10% with a significance level of 5%, which will provide 90% power. The sample size is calculated using G-Power Software (3.0.10).

Ethical approval

The Animal Welfare Committee of the Faculty of Veterinary Medicine, Benha University (BUFVTM 04-02-22), granted approval for all operations performed in this study, which included bird handling and maintenance.

Result

Phytochemical analysis

Several bioactive components were found in a chemical investigation of LRE. Table 3 displays the main components, TPs 47.32 ± 0.27 mg gallic/g, total flavonoids 15.44 ± 0.24 mg quercetin/g, and TTs 8.83 ± 0.10 mg gallic/g. The total terpenoids in the sample were 11.740.25 mg linalool/g, while the total saponin content was 239.641.88 mg diosgenin/g. The relatively high content of total saponin in the investigated extract was documented as 239.64 ± 1.88 mg diosgenin/g, while total terpenoids were 11.74 ± 0.25 mg linalool/g.

Table 3. Active compounds of the LRE.

Items	Quantity*
TPs	47.32 ± 0.27 mg gallic/g D.W
TT	8.83 ± 0.10 mg gallic/g D.W
Total Flavonoids	15.44 ± 0.24mg querstine/g D.W
Total saponin	239.64 ± 1.88 mg diosgenin/g D.W
Total terpenoids	11.74 ± 0.25 mg linalool/g D.W

*Values are given as mean (n = 5 standard deviation (absolute value).

Clinicopathological assessments

In the current study, the G3-LME group showed the most significant decrease in FI and FCR while registering the highest performance compared to other groups, at the same time, it had the highest average WG during the challenge period, and in contrast, G4-E had the lowest WG and highest FI and FCR.

In terms of dropping scoring, oocyst output, FCR, WG, cecal damage, and ACI values, they were more potent in G3-LME compared to groups fed LRE and maduramicin individually (Table 4).

Depression, ruffled feathers, lethargy, and bloody diarrhea were frequently seen in G4-E-challenged birds. Other gross cecal changes included enlargement and discoloration, numerous mucosal hemorrhages, and sausage-like bloody contents (Fig. 1). In addition, histological analysis showed that loss of intestinal villi, degeneration of intestinal glands laden with coccidian oocysts, mononuclear cell infiltrate, and hemorrhages all contributed to the deterioration of the cecal architecture. Therefore, as indicated in Tables 5–7 and Figures 1–3, it was evident that the mean oocyst shedding and dropping scores, macroscopic, and microscopic lesions in this group recorded the highest values.

Table 4. Effect of LRE and maduramicin alone or in combination On the performance of Sasso broilers challenged with *Eimeria tenella.*

Groups	FI	WG	FCR	Mortalities
G1-L*E*	773.3 ± 8.8[b]	278.0 ± 0.6[d]	2.8 ± 0.03[b]	0[a]
G2-M*E*	720.0 ± 5.8[c]	294.0 ± 0.6[c]	2.4 ± 0.02[c]	0[a]
G3-LM*E*	623.3 ± 3.3[e]	319.3 ± 2.9[a]	2.0 ± 0.02[e]	0[a]
G4-*E*	837.0 ± 28.5[a]	260.0 ± 3.1[e]	3.2 ± 0.13[a]	0.3 ± 0.3[a]
G5-N	673.3 ± 8.8[d]	309.0 ± 2.6[b]	2.2 ± 0.01[d]	0[a]

"[a,b,c]"Means that the different indices between groups are significantly different for $p < 0.05$. Values are given as the mean ($n = 5$) ± SE. G1-LE: Supplemented with LRE at a dosage of 3 g/kg; G2-ME: Supplemented with maduramicin at a dosage of 0.5 g/kg; G3-LME: Supplemented with a combination of LRE and maduramicin; G4-E: Control positive; G5-N: Control negative.

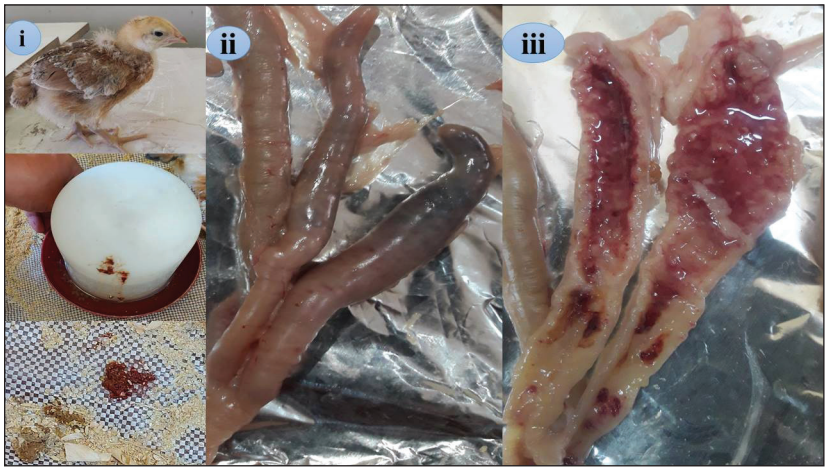

Fig. 1. Clinical and pathological findings of the cecal coccidiosis in Sasso chickens observing (i) depressed bird with ruffled feather and bloody droppings, (ii) enlarged and sausage appearance ceca, and (iii) cecal core with bloody contents.

Birds fed LRE and/ or maduramicin appear to be healthy; no deaths or clinical abnormalities were observed, according to the analysis of clinicopathological markers. Compared with G4-E, the dropping scoring and oocyst shedding of groups G1-LE, G2-ME, and G3-LME along the 10th dpc, as well as the macroscopic and microscopic lesions scored at the 7th dpc, was considerably reduced (Table 5). The cecum of birds in G1-LE showed modest desquamation of the intestinal villi epithelial cells and reduced oocyst invasion of the intestinal glands (Fig. 2d). The intestinal blood vessels of birds in group G2-ME showed substantial mononuclear cellular infiltration, either diffuse or cluster-aggregated congestion, as well as a modest oocyst invasion (Fig. 2e). A mononuclear cellular infiltration and a small number of oocysts invading the intestinal glands were recorded in group G3-LME (Fig. 2f).

Biochemical parameters

Effects of individual and combined doses of LRE (3 g/kg) and maduramicin (0.5 g/kg) on oxidative stress biomarkers of E. *tenella*-challenged birds were recorded at 14, 21, and 35 days of the experiment (Fig. 4). After *E. tenella* infection, MDA, SOD, and catalase were higher than the negative control. The trial showed increased antioxidant enzymes in G3-LME after 35 days, with MDA, SOD, and catalase levels of 3.09 ± 0.05, 3.10 ± 0.03 (nmol/ml), 158.38 ± 0.88, 164.11 ± 0.31 (U/mg), and 16.64 ± 0.25, 16.19 ± 0.22 (U/mg) for G1-LE and G2-ME The MDA, SOD, and catalase levels in G3-LME was close to the control negative group (2.38 ± 0.04, 141.56 ± 1.01, and 15.06 ± 0.67). The G4-E had significantly higher total cholesterol (202.65 ± 1.19mg/dl), triacylglycerol (110.07 ± 0.18 mg/dl), and LDL-C (58.88 ± 0.61 mg/dl), but lower HDL-C (56.44 ± 0.37 mg/dl) at 35 days. Throughout the trial period, the lipid profile of the challenged broilers was gradually improved in G1-LE, G2-ME, and G3-LME. Total cholesterol was increased in G1-LE and G2-ME (158.77 ± 0.86 and 160.70 ± 0.46 mg/dl, respectively), but the combined treatment G3-LME showed the largest increase (152.51 ± 0.75 mg/dl) compared to the negative control at 35 days of age. The greatest elevation in HDL-C was observed in G1-LE (76.84 ± 0.56 mg/dl) followed by G2-ME (66.11 ± 0.79 mg/dl) and G3-LME (65.12 ± 0.37 mg/dl). Triacylglycerol levels were slightly different between the infected and feed-treated groups (Fig. 4).

Table 5. Effect of LRE and maduramicin alone or in combination on the dropping scores of *E. tenella* challenged groups along 10 days post-challenge.

Groups	Dropping scoring							
	3rd Day	4th Day	5th Day	6th Day	7th Day	8th Day	9th Day	10th Day
G1-L*E*	1.7 ± 0.3 ^{a}	1.0 ± 0 ^{b}	1.0 ± 0 ^{b}	0.3 ± 0.3 ^{b}	0.7 ± 0.3 ^{ab}	0.3 ± 0.3 ^{bc}	0 ± 0^{a}	0 ± 0^{a}
G2-M*E*	1.3 ± 0.3 ^{a}	1.0 ± 0.0 ^{b}	1.0 ± 0 ^{b}	0.7 ± 0.3 ^{b}	0.3 ± 0.3 ^{ab}	0.3 ± 0.3 ^{bc}	0 ± 0 ^{a}	0 ± 0^{a}
G3-LM*E*	1.0 ± 0.6 ^{ab}	0.7 ± 0.3 ^{b}	0.7 ± 0.3 ^{b}	0.3 ± 0.3 ^{b}	0.7 ± 0.3 ^{ab}	1.3 ± 0.3 ^{a}	0.7 ± 0.3 ^{a}	0 ± 0^{a}
G4-*E*	2.0 ± 0.0 ^{a}	2.0 ± 0.0 ^{a}	2.0 ± 0 ^{a}	2.7 ± 0.3 ^{a}	1.0 ± 0^{a}	1.0 ± 0 ^{ab}	0.7 ± 0.3 ^{a}	0.3 ± 0. 3 ^{a}
G5-N	0 ± 0^{b}	0 ± 0^{c}	0 ± 0^{b}	0 ± 0^{b}	0 ± 0^{b}	0 ± 0^{c}	0 ± 0^{a}	0 ± 0^{a}

"$^{a, b, c}$"Means that the different indices between groups are significantly different for $p < 0.05$. Values are given as the mean ($n = 5$) ± SE. G1-LE: Supplemented with LRE at a dosage of 3 g/kg; G2-ME: Supplemented with maduramicin at a dosage of 0.5 g/kg; G3-LME: Supplemented with a combination of LRE and maduramicin; G4-E: Control positive; G5-N: Control negative.

Table 6. Effect of LRE and maduramicin alone or in combination on the oocyst shedding for ten days post challenged with *E. tenella.*

Groups	Oocyst shedding (OPG)							
	3rd Day	4th Day	5th Day	6th Day	7th Day	8th Day	9th Day	10th Day
G1-L*E*	0^{b}	20.15 ± 4^{b}	3826.5 ± 30.9^{b}	2770 ± 23.1^{b}	40 ± 7.51^{b}	0^{b}	0^{b}	0^{b}
G2-M*E*	0^{b}	4.43 ± 4.4^{cd}	233.33 ± 66.4^{c}	276.67 ± 202.7^{c}	13.3 ± 0^{b}	0^{b}	0^{b}	0^{b}
G3-LM*E*	0^{b}	17.87 ± 4.6^{bc}	40 ± 7.5^{c}	80 ± 7.51^{c}	13.3 ± 0^{b}	0^{b}	0^{b}	0^{b}
G4-*E*	30.1 ± 3^{a}	40.15 ± 7.6^{a}	8017.67 ± 369.9^{a}	6313.3 ± 347.2^{a}	1015.7 ± 31^{a}	426.5 ± 12^{a}	221 ± 30.8^{a}	70 ± 23^{a}
G5-N	0^{b}	0^{d}	0^{c}	0^{c}	0^{b}	0^{b}	0^{b}	0^{b}

"$^{a, b, c}$"Means that the different indices between groups are significantly different for $p < 0.05$. Values are given as the mean ($n = 5$) ± SE. G1-LE: Supplemented with LRE at a dosage of 3 g/kg; G2-ME: Supplemented with maduramicin at a dosage of 0.5 g/kg; G3-LME: Supplemented with a combination of LRE and maduramicin; G4-E: Control positive; G5-N: Control negative.

Table 7. Effect of LRE and maduramicin alone or in combination on the pathological change scores in the cecum of the Sasso chicken for 7 days post-challenge with *E. tenella.*

Groups	Pathological scoring of the cecum		
	Macroscopic lesion scoring	Microscopic lesion scoring	Histological injury scoring *
G1-L*E*	1.4 ± 0.51^{b}	4 ± 0^{b}	6.33 ± 0.33^{c}
G2-M*E*	0.6 ± 0.24^{bc}	7.67 ± 0.33^{a}	8.67 ± 0.33^{b}
G3-LM*E*	0.2 ± 0.2^{c}	3.33 ± 0.33^{b}	6 ± 0^{c}
G4-*E*	2.6 ± 0.51^{a}	8 ± 0.58^{a}	11 ± 0.58^{a}
G5-N	0 ± 0^{c}	0 ± 0^{c}	4 ± 0^{d}

"$^{a, b, c}$" Means that the different indices between groups are significantly different for $p < 0.05$. Values are given as the mean ($n = 5$) ± SE. * Histological injury scoring: HIS; the sum of scores for the inflammation (×2), tissue destruction, and tissue reparation in the cecal sections. G1-LE: Supplemented with LRE at a dosage of 3 g/kg; G2-ME: Supplemented with maduramicin at a dosage of 0.5 g/kg; G3-LME: Supplemented with a combination of LRE and maduramicin; G4-E: Control positive; G5-N: Control negative.

Following the *E. tenella* challenge, AST and ALT levels increased directly as recorded in the positive control G4-E group (161.890.69 and 76.330.18 (U/l), respectively). In contrast, the highest drop in both enzymes was listed in the G3-LME group, reaching 133.53 ± 0.42 and 60.48 ± 0.47 (U/l), respectively, while the G5-N group was found to have values of 139.65 ± 0.18 and 55.66 ± 0.42 (U/l). During several periods of the experiment, G1-LE and G2-ME showed a decrease in liver enzymes throughout the experiment, reaching a significant decrease on the 35th day of the experiment (Fig. 4).

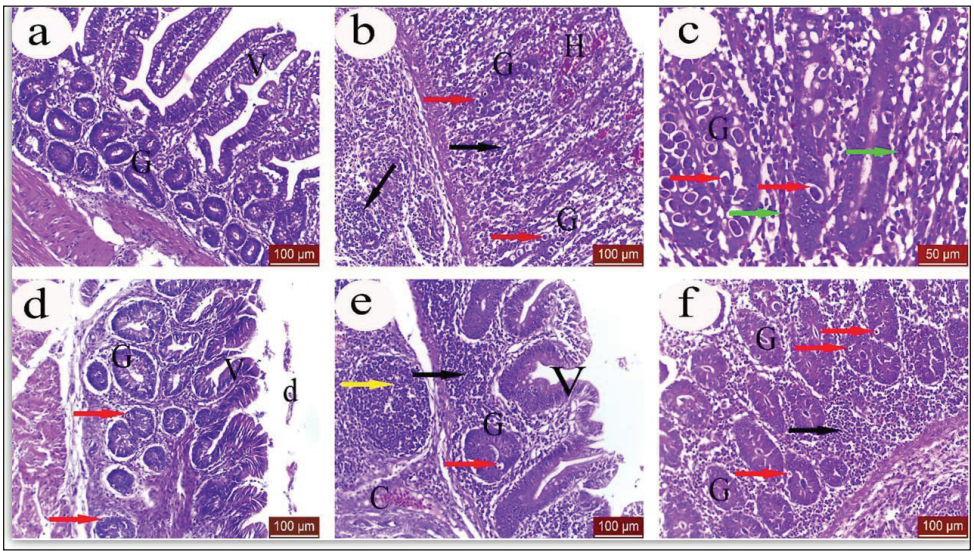

Fig. 2. Effect of LRE and maduramicin alone or in combination on the histopathological findings in the cecal tissues of Sasso chickens challenged with *E. tenella* oocysts observing (a) cecum of the control negative group showing normal intestinal villi (V) and intestinal glands (G), (H & E stain, X 20 lens). (b) Cecum of the control positive groups showed atrophied intestinal glands (G), hemorrhage (H), mononuclear cellular infiltration (black arrow) and oocyst (red arrow). (H & E stain, X 20 lens). (c) Higher magnification of showed atrophied intestinal glands (G), oocyst (red arrow) R.B. Cs in congested blood capillaries (green arrow). (H & E stain, X 40 lens). (d) cecum of birds fed on the licorice treated diet showed intestinal villi (V) with desquamated epithelial cells (d), intestinal glands (G) and some oocyst invading intestinal glands (red arrow). (H & E stain, X 20 lens). (e) Cecum of maduramicin treated groups showed normal intestinal villi (V), intestinal glands (G) with some oocyst (red arrow), congestion of blood vessels (C), mononuclear cellular infiltration either diffuse (black arrow) or nodular (yellow arrow). (H & E stain, X 20 lens). (f) Cecum of combined treated groups with licorice and maduramicin displayed little affected intestinal glands which invaded by low number of oocyst (red arrow), diffuse cellular infiltration (black arrow). (H & E stain, X 20 lens).

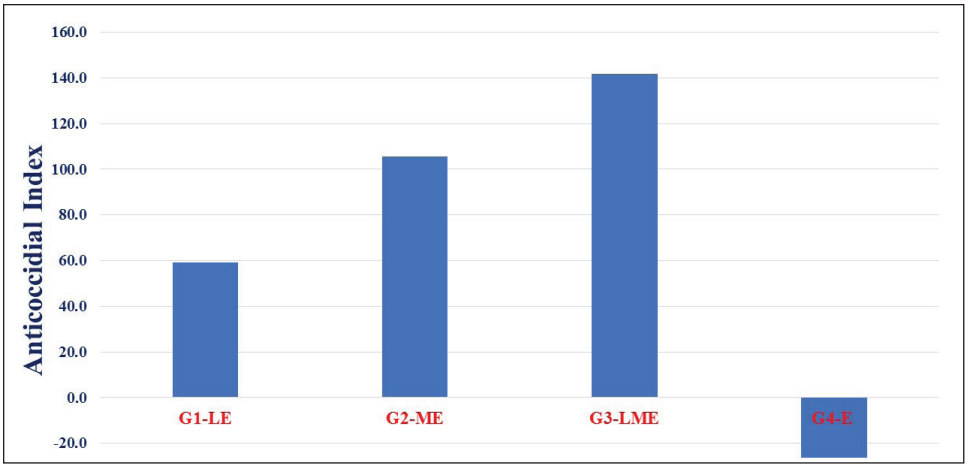

Fig. 3. The ACI value was lower than 120 which means "lack of anticoccidial activity," at 120–140 "mild effective," at 140–160 "moderate effective" and at higher than 160 "marked effective." The ACI is calculated according to the equation of subscripting for the sum of the lesion index (LI) and oocyst index (OI) from the sum for the percentages of survival and relative gain weight %S + %RGW) − (LI + OI) (De Pablos *et al.* 2010), where LI is the lesion index as the HIS multiplied by 10 and OI is the oocyst index as (OPG output of each experimental group/OPG output of the infected untreated control) × 100.

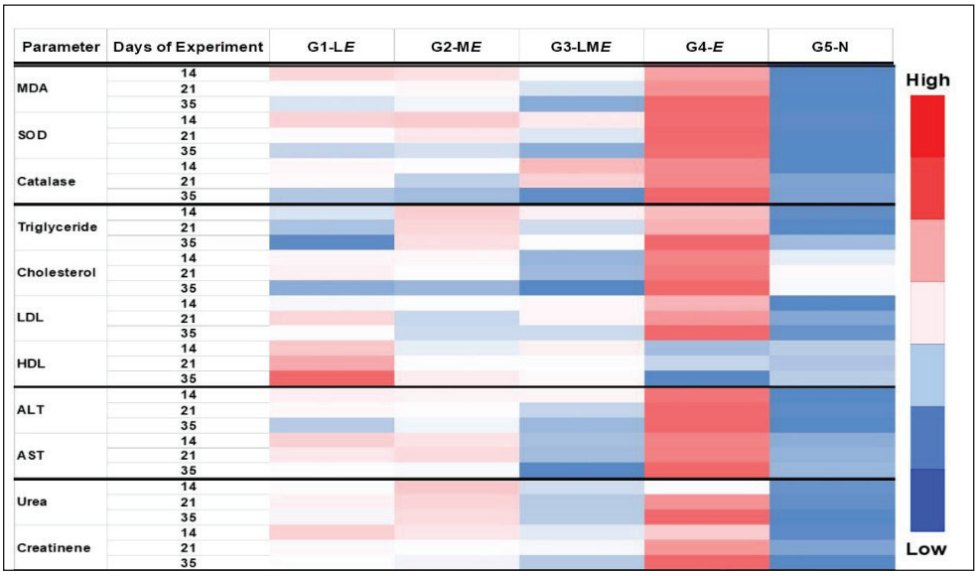

Fig. 4. Heat map showing the effect of LRE and maduramicin alone or in combination on mean values for antioxidant and lipid profiles and liver and kidney function tests for different experimental groups.

Table 8. Molecular docking scores of licorice' bioactive compounds and *E. tenella* DNA polymerase, *E. tenella* apical membrane antigen 1 (*EtAMA1*), and *EtMIC3* binding sites.

Lotus ID	Compounds	*E. tenella* DNA polymerase	EtAMA1	EtMIC3
LTS0135577	3′-methoxyglabradin	−6.30	−6.85	−6.71
LTS0158119	3-isothujone	−4.64	−4.77	−5.24
LTS0032845	Rutin	−7.86	−8.42	−7.84
LTS0200538	4′-o-methylglabridin	−6.07	−6.57	−6.48
LTS0251224	5-deoxyflavanone	−5.31	−5.76	−5.70
LTS0014786	6,8-diprenylgenistein	−6.78	−7.60	−7.83
LTS0182567	(-)-lavandulol	−5.03	−5.01	−5.54
LTS0072900	(-)-naringenin	−6.39	−6.26	−5.96
LTS0191687	(-)-vestitol	−5.73	−5.85	−6.04
LTS0225956	Abyssinone ii	−6.14	−7.05	−7.00
LTS0275427	Afformosin	−6.41	−6.19	−6.30
LTS0044471	Amylfuran	−5.31	−4.94	−5.30
LTS0068303	Asahina	−6.27	−6.05	−6.15
LTS0249588	Astragalin	−6.72	−7.62	−8.54
LTS0012882	Carvacrol	−4.75	−5.13	−4.86
LTS0181568	Cymene	−4.32	−4.78	−5.06
LTS0069837	Cynaroside	−7.59	−7.14	−8.16
LTS0138668	Echinatin	−5.60	−6.03	−6.57
LTS0222995	Enoxolone	−7.19	−6.01	−6.41
LTS0180128	Euchrenone a5	−6.77	−6.98	−7.31
LTS0048628	Euchrestaflavanone a	−7.48	−7.52	−7.58
LTS0126716	Fenchone	−4.52	−4.75	−4.38
LTS0073369	Formononetin 7-o-glucoside	−7.45	−7.65	−7.69

Continued

Lotus ID	Compounds	*E. tenella* DNA polymerase	EtAMA1	EtMIC3
LTS0082756	Formononetin	−5.45	−6.09	−6.43
LTS0210648	Galangin	−5.56	−5.86	−6.12
LTS0094683	Gancaonin f	−6.51	−6.61	−6.57
LTS0003159	Gancaonin g	−6.75	−6.85	−7.30
LTS0077774	Gancaonin h	−7.37	−7.74	−7.82
LTS0072777	Gancaonin l	−6.37	−6.66	−6.83
LTS0106538	Genistein	−5.61	−5.95	−6.08
LTS0250433	Glabranin	−6.39	−6.91	−6.27
LTS0232975	Glabrene	−6.35	−6.39	−6.94
LTS0075616	Glabridin	−6.35	−6.50	−6.40
LTS0151626	Glabrocoumarin	−6.67	−6.81	−6.58
LTS0262018	Glabrocoumarone a	−5.92	−6.54	−6.33
LTS0274460	Glabrocoumarone b	−5.91	−6.45	−6.94
LTS0267055	Trifolin	−7.72	−6.68	−7.37
LTS0164961	Glabrol	−7.72	−7.16	−7.82
LTS0075204	Glabrone	−6.75	−6.16	−7.13
LTS0186848	Glycycoumarin	−6.19	−6.82	−6.78
LTS0087818	Glycyrin	−7.37	−6.55	−8.14
LTS0198644	Glycyrrhetinic acid	−7.12	−6.80	−6.74
LTS0193131	Glycyrrhisoflavanone	−7.09	−6.53	−6.96
LTS0121878	Glycyrrhizin	−11.11	−8.14	−9.16
LTS0090907	Glyinflanin a	−7.39	−6.94	−6.53
LTS0133651	Glyinflanin b	−6.72	−6.81	−7.19
LTS0241667	Glyinflanin g	−7.17	−7.53	−7.70
LTS0179228	Guaiacol	−4.56	−4.48	−4.94
LTS0267683	Hispaglabridin a	−7.56	−7.02	−7.52
LTS0155248	Hispaglabridin b	−6.96	−7.24	−7.40
LTS0257369	Hydroxywighteone	−6.88	−5.86	−6.78
LTS0223233	Isobavachromene	−6.27	−6.49	−7.19
LTS0066952	Isoglycycoumarin	−6.98	−6.89	−7.04
LTS0264727	Isolicoflavonol	−6.72	−6.93	−6.52
LTS0051422	Isoliquiritin	−7.43	−7.63	−7.60
LTS0181160	Vicenin 2	−7.95	−8.01	−7.42
LTS0136408	Wighteone	−7.36	−6.53	−7.20
LTS0254337	Isoquercetin	−8.27	−7.27	−7.49
LTS0087575	Isorhamnetin 3-galactoside	−7.26	−7.28	−7.66
LTS0137002	Isorhamnetin 3-o-glucoside	−8.05	−8.31	−7.58
LTS0157117	Isoschaftoside	−8.98	−8.17	−7.67
LTS0035187	Isovitexin	−7.81	−7.09	−7.60
LTS0075703	Kanzonol b	−6.22	−6.81	−6.92
LTS0266469	Kanzonol c	−7.90	−7.86	−7.86
LTS0012990	Kanzonol d	−7.01	−6.88	−6.91
LTS0138968	Kanzonol y	−7.37	−7.83	−7.31

Continued

Lotus ID	Compounds	*E. tenella* DNA polymerase	EtAMA1	EtMIC3
LTS0018267	Kumatakenin	−6.40	−6.34	−6.38
LTS0106634	Licoagrochalcone a	−6.39	−6.70	−6.91
LTS0020333	Licoagrochalcone b	−6.62	−7.10	−7.56
LTS0187725	Licoagrochalcone c	−6.86	−7.50	−7.50
LTS0270336	Licoagrochalcone d	−7.19	−7.21	−7.72
LTS0018907	Licochalcone a	−7.02	−7.23	−6.63
LTS0192338	Licochalcone b	−6.69	−6.68	−6.67
LTS0183214	Licochalcone c	−7.56	−6.77	−7.34
LTS0139725	Xambioona	−7.46	−7.55	−7.35
LTS0063487	Yinyanghuo d	−6.63	−6.95	−6.89
LTS0132019	Licocoumarone	−7.49	−6.59	−6.81
LTS0244117	Licoflavanone	−6.83	−7.22	−6.80
LTS0004664	Licoflavone a	−6.90	−6.89	−6.59
LTS0122155	Licoflavone b	−7.74	−7.49	−7.76
LTS0219719	Licoflavonol	−7.02	−7.18	−6.83
LTS0263391	Licoisoflavone a	−7.19	−7.12	−6.94
LTS0055944	Licoisoflavone b	−7.00	−6.69	−6.85
LTS0048734	Licopyranocoumarin	−6.93	−7.17	−7.28
LTS0274337	Licoricidin	−8.02	−7.96	−8.18
LTS0132318	Licuroside	−8.78	−8.53	−8.00
LTS0188438	Liquiritin apioside	−8.64	−7.97	−9.33
LTS0103894	Liquiritin	−7.23	−7.78	−7.55
LTS0142270	Liquorice	−10.75	−9.59	−9.05
LTS0211446	Lupalbigenin	−7.83	−7.70	−8.23
LTS0256952	Lupeol	−6.38	−6.82	−6.48
LTS0229079	Lupiwighteone	−7.51	−6.69	−6.16
LTS0261149	Medicarpin, (-)-	−6.49	−4.91	−5.94
LTS0215385	Morachalcone a	−6.66	−6.78	−6.56
LTS0202475	Myrtenal	−5.01	−4.97	−4.46
LTS0031098	Naringenin	−5.91	−6.04	−6.10
LTS0089772	Neoliquiritin	−7.19	−7.21	−7.65
LTS0237730	Odoratin	−6.33	−6.29	−6.33
LTS0235553	Ononin	−6.87	−7.50	−7.47
LTS0014950	Paeonol	−5.19	−4.84	−5.24
LTS0124936	Parvisoflavone b	−7.42	−6.62	−6.63
LTS0151338	Phaseol	−6.54	−6.17	−6.34
LTS0010732	Pinit	−5.63	−5.33	−5.14
LTS0194724	Pinitol	−5.55	−5.02	−5.01
LTS0141508	Pinocembrine	−6.06	−5.83	−5.80
LTS0261766	Prunetin	−6.31	−6.01	−6.00
LTS0119297	Pseudoionone	−5.38	−6.08	−5.39
LTS0186298	Quercitrin	−7.75	−7.12	−6.75

Continued

Lotus ID	Compounds	*E. tenella* DNA polymerase	EtAMA1	EtMIC3
LTS0104338	Schaftoside	−7.40	−7.64	−7.54
LTS0128805	Shinflavanone	−7.33	−7.12	−7.35
LTS0058527	Sophoraflavanone b	−6.79	−6.59	−6.63
LTS0182499	Soyasaponin i	−8.79	−9.67	−8.15
LTS0184048	Soyasaponin ii	−10.58	−8.82	−8.74
LTS0152081	Talmon	−4.55	−4.24	−4.75
LTS0027534	Tephrinone	−7.61	−6.88	−7.01
LTS0168527	Thymol	−5.38	−4.95	−5.10

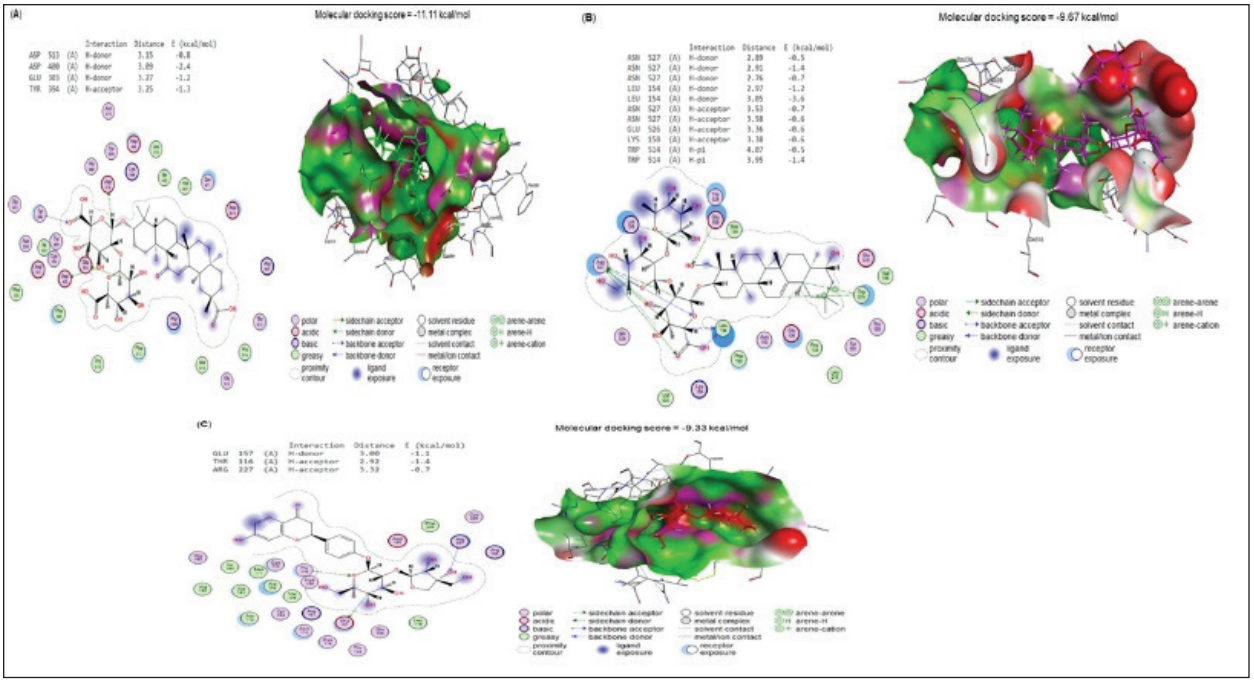

Fig. 5. (A) Molecular interaction of glycyrrhizin with *E. tenella* DNA polymerase binding site, (B) molecular interaction of soyasaponin i with *E. tenella* apical membrane antigen 1 (EtAMA1) binding site, and (C) molecular interaction of liquiritin apioside EtMIC3 binding site.

The positive control group had greater serum urea and creatinine concentrations than the negative control group at all experimental time points (14, 21, and 35 days). All examined feed regimens improved kidney functioning by lowering serum urea levels. The highest reduction in serum urea was noticed in G3-LME, followed by G1-LE and G2-ME which came in second and third place, with 7.26 ± 0.12 and 8.09 ± 0.17 mg/dl, respectively. The G1-LE, G2-ME, and G3-LME showed significantly lower serum creatinine values (0.55 ± 0.03, 0.54 ± 0.02 and 0.50 ± 0.01 mg/dl), respectively (Fig. 4).

Molecular docking

Molecular docking interactions of licorice's bioactive compounds are represented in Table 8 and Figure 5. Glycyrrhizin interacted with *E. tenella* DNA polymerase binding site by energy of −11.11 kcal/mol with ASP513 (H-donor), ASP400 (H-donor), GLU303 (H-donor), and TYR394 (H-acceptor) (Fig. 5A). −9.67 kcal/mol is the molecular docking score of soya saponin with EtAMA1 binding site interaction through H-donor (ASN527 and LEU154), H-acceptor (ASN527, GLU526, and LYS158), and H-pi (TRP514) (Fig. 5B). In addition, liquiritin apioside bound with EtMIC3 binding site with energy value of −9.33 kcal/mol through interaction with GLU157 (H-donor), THR116 (H-acceptor), and ARG227 (H-acceptor) (Fig. 5C).

Maduramicin interacted with *E. tenella* DNA polymerase binding site with an energy of −11.54 kcal/mol through interaction with ILE183 (H-donor),

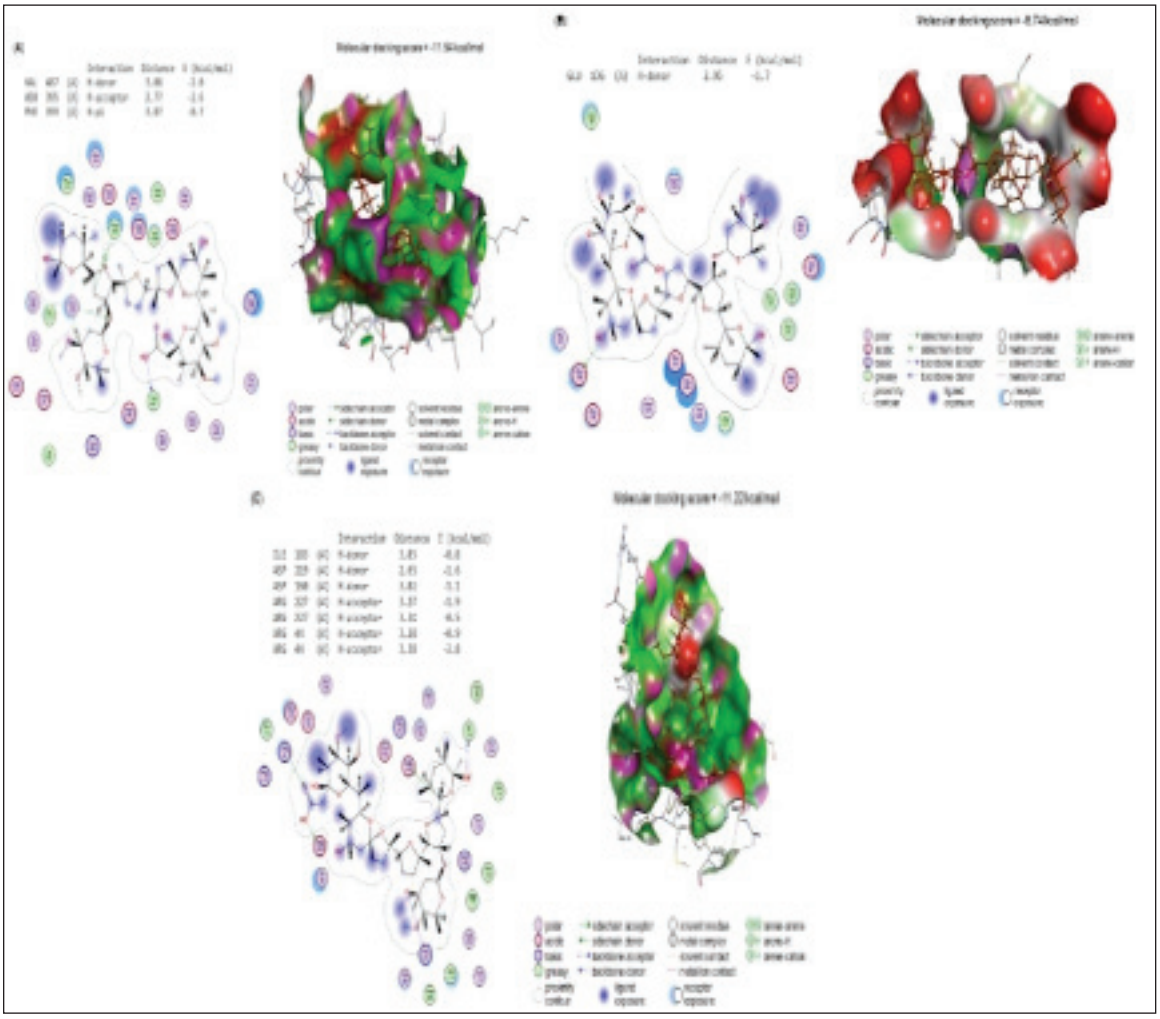

Fig. 6. (A) Molecular interaction of maduramicin with *E. tenella* DNA polymerase binding site, (B) molecular interaction of maduramicin with *E. tenella* apical membrane antigen 1 (EtAMA1) binding site, and (C) molecular interaction of maduramicin with EtMIC3 binding site.

ASP219 (H-donor), ASP160 (H-acceptor), ARG227 (H-acceptor), and ARG44 (H-acceptor), as presented in Figure 6A. In addition, maduramicin bound with GLU136 (H-donor) residue in EtAMA1 binding site with energy value of −8.74 kcal/mol (Fig. 6B). By energy value of −11.22 kcal/mol, maduramicin interacted with EtMIC3 binding site by H-donor (ILE183, ASP219, and ASP160) and H-acceptor (ARG227 and ARG44) (Fig. 6C).

Discussion

Avian coccidiosis, a cosmopolitan protozoal infection, has been linked to significant financial losses in the poultry sector (Moryani *et al.*, 2021). To address the negative effects of anticoccidial feed additives on poultry diets on bird health; a new trend has emerged in natural alternatives due to their high safety rates and positive impacts. Licorice is one of the oldest and most well-known medicinal plants that have anticoccidial activities on several *Eimeria* species (Pop *et al*., 2019; Hussain *et al*., 2022; Ghafouri *et al*., 2023) anticoccidials are generally used as feed additives. However, the frequent usage has given rise to the occurrence of resistant strains to available anticoccidial drugs. Botanicals may work as substitutes to anticoccidial drugs. The current research was designed to evaluate the efficacy of aqueous methanol extracts of Glycyrrhiza glabra (licorice. Hence, in this study, the clinical, biochemical, and histopathological effects of LRE alone or in combination with maduramicin were evaluated in Sasso broilers infected with *E. tenella*.

The chemical examination of the LRE in this study showed numerous crucial secondary metabolites. These included phenols, flavonoids, tannins, saponins, and terpenoids. Similarly, a large body of research has stated that phenolic compounds and flavonoids as well as saponins and terpenoids found in LRE are abundant (Rodino *et al*., 2015; Soliman and El-Genaidy, 2021).

Many medicinal effects have been attributed to plants rich in phenols, flavonoids, and tannins (Rajpurohit *et al*., 2017), this could explain why it has therapeutic effects.

According to the findings of the current study, the infected Sasso chicks in G4-E gained less weight with high FCR and ruffled feathers in comparison to other groups. It is backed up by reports indicating that coccidian infection reduces the bird performance (Logan *et al*., 1993; Hashmi *et al*., 1994; Tipu *et al*., 2002). These findings could be attributed to the parasite's mechanism of destroying the absorptive mucosal surface and competing for micronutrients which in turn results in a metabolic disturbance and detrimental effect on nutrition utilization (Ali *et al*., 2019)

Birds in G3-LME had superior clinicopathological scores than the other infected groups in terms of FCR and BW with decreased OPG count, severity of cecal lesions at 7th dpc, and absence of bloody secretion. Similar claims were made about the efficacy of adding LRE or maduramicin to feed on reducing symptoms of coccidiosis (Williams, 1998; Peek and Landman, 2011; Kadykalo *et al*., 2018; Pop *et al*., 2019; Hussain *et al*., 2022; Ghafouri *et al*., 2023). Despite this, the moderate ACI was recorded in the G3-LME with a value of 141.8, while the ACI in G1-LE and G2-ME was 59.1 and 105.5, respectively, being insufficient. The therapeutic and prophylactic effect of licorice extracts alone or in combination with other compounds has been demonstrated in a dose-dependent manner in broilers against single and mixed infections of different *Eimeria* species by oral administration, and the treatments have a positive effect on the intestinal lesions, oocyst outputs, and performance parameters (Pop *et al*., 2019; Hussain *et al*., 2022; Ghafouri *et al*., 2023). These results could be attributed to the effect of the phenolic contents of licorice and the chemical elements of maduramicin which increases intestinal mucosal secretion and block ion transport channels, respectively, which weakens the parasite's osmotic balance and leads to the impairment in the vital processes of coccidian, and finally, their death (Sikkema *et al*., 1995; Aly *et al*., 2005; Arczewska-Wlosek *et al*., 2012).

Regarding antioxidant biomarkers, linear trends were seen between coccidiosis and the clear elevation of MDA, SOD, and catalase enzymes. Indicators of infection-induced oxidative stress were gradually reduced by supplementing the feed with LRE alone or combined with maduramicin. Active secondary metabolites of licorice extract may be responsible for reducing the negative consequences of coccidiosis infection, as flavonoids (isoflavonoids and liquiritin), glycyrrhizic acid, liquiritigenin, triterpenes (glycyrrhizin), and saponins in licorice root have been reported to have antioxidant activity (Vlaisavljević *et al.*, 2018)**.** The present results were consistent with previous work which stated that polyphenol administration in chicken drinking water has been shown to improve antioxidant processes. Similar to previous data reported by Pabón *et al*. (2003) on the onset of oxidative stress in parasitic diseases, Georgieva *et al*. (2006) suggested that the increased blood MDA concentrations in 20-day-old broiler chickens infected with *E. tenella* may be attributable to the occurrence of oxidative stress with increased s production, leading to lipid peroxidation. Moreover, sick chicks in the study had higher blood CAT and lower SOD concentrations compared to controls. Following oxidative stress, an increase in CAT levels has been suggested to disrupt the ecological oxidative balance. Serum MDA concentrations as well as the actions of ALP, AST, ALT, and GGT are frequently utilized as markers of oxidative stress and tissue damage. According to some previous findings, Mahmoodzadeh *et al*. (2017) and Pastorino *et al*. (2018) suggested that the active components of licorice, such as flavonoids, saponins, sugars, coumarins, amino acids, starch, tannins, phytosterols, choline, and vitamins, may be correlated to the improved immunity and oxidative states in birds.

In the current study, decreased HDL-C with increased serum triacylglycerol and total cholesterol content were indicators of the adverse effects of coccidiosis on broilers. All treatments improved the lipid profile of broiler serum, including total cholesterol, HDL-C, LDL-C, and triacylglycerols, and to some extent mitigated the negative effects of coccidiosis infection. Compared to the control group, feeding birds LRE significantly reduced total cholesterol and enhanced HDL-C. The obtained results were in harmony with a previous study that reported that licorice consumption gradually decreased the levels of total cholesterol, LDL-C, and triacylglycerols in a dose-dependent way (Aghdam *et al*., 2018). Moreover, a prior investigation verified that feeding birds diets with licorice powder reduced cholesterol, triacylglycerols, and low-density lipoprotein (Fuhrman *et al.*, 2002; Sharifi *et al*., 2013). The challenged birds in G3-LME showed improvement in lipid profile, which may be attributed to the synergistic effects between LRE and maduramicin. The positive impacts of LRE alone or in combination with maduramicin could be attributed to licorice's ability to reduce LDL oxidation while inhibiting lipid peroxidation, lipoxygenase, and cyclooxygenase enzyme activity (Fuhrman *et al.*, 2002). Increased release of cholesterol, bile acids, neutral sterols, and hepatic bile acid concentration are thought to be responsible for the cholesterol-lowering effects of licorice (Sharifi *et al*., 2013). In addition, licorice's active ingredient, saponin, can block the development of lipid peroxides, increase the rate of cholesterol conversion into bile acids, and accelerate the hepatic clearance of cholesterol.

The current acquired results showed that daily use of licorice powder, either as a solo supplement or when combined with maduramicin, had a favorable impact on both the aminotransferase enzymes (AST and

ALT) and kidney functions (urea and creatinine). LRE and maduramicin together had a positive synergistic effect on AST, ALT, urea, and creatinine. All bioactive secondary metabolites, which are believed to be the main cause of antioxidant, anti-inflammatory, anticancer, and so on effects, may contribute to the effects of licorice extract (Vlaisavljević *et al.*, 2018). The hepatoprotective effect of licorice relates to an improvement in antioxidant defense and anti-inflammatory response, according to prior research on serum ALT and AST activity and triacylglycerols. The outcomes were consistent with studies by Abd-Al-Sattar (2016)**,** which established that aspartate aminotransferase activity, which was raised by CCl4 in rats to generate acute hepatotoxicity, is inhibited by supplementation with aqueous methanolic extracts of *G. glabra.* Moreover, oral LRE administration reduced the levels of kidney function parameters (urea, uric acid, and creatinine) in comparison to the control group. In addition, another report evaluated the anti-nephritis activity of glabradin, a pyramid of lavan isolated from *G. glabra*, after oral administration in glomerular disease-challenged mice, and their results showed a significant decline in the amount of urinary protein excretion, blood urea nitrogen, and serum creatinine levels (Fukai *et al*., 1998).

Molecular docking assessment revealed that licorice's bioactive compound and maduramicin effectively bind to the binding site of DNA polymerase of *E. tenella*, EtAMA1, and EtMIC leading to the reduction of *E. tenella* replication and invasion because EtAMA1 and EtMIC3 facilitate its invasion to the host cells (Jiang *et al.*, 2012; Chen *et al.*, 2021).

Conclusion

Herbal medicine is effective in treating and preventing several parasitic infections. Herbal remedies show promise as a viable option for treating coccidiosis. Despite the wealth of data available on traditional medicine, little has been done to understand and promote its usage in clinical settings, particularly in conjunction with modern medications that have anticoccidial qualities. In this research, LRE was found to be effective against coccidiosis in chickens when administered simultaneously with maduramicin. We base our recommendation on the bioactive components found in licorice, which include phenols, flavonoids, tannins, saponins, and terpenoids and are believed to be the main reason for licorice's medicinal benefits.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Author contributions

Conceptualization: Sawsan S. Elbasuni, Marwa I. Abdel Haleem, Hanan A.A. Taie, Samah M. Abdel Gawad, Reda E.L. Kamar, Hala El Daous, Marwa Darweish, Mai O. Nada, Walaa Fathy SaadEldin; Methodology: Sawsan S. Elbasuni, Marwa I. Abdel Haleem, Hanan A.A. Taie, Samah M. Abdel Gawad, Reda E.L. Kamar, Hala El Daous, Marwa Darweish, Mai O. Nada, Walaa Fathy SaadEldin; Resources: Sawsan S. Elbasuni, Marwa I. Abdel Haleem, Hanan A.A. Taie, Samah M. Abdel Gawad, Reda E.L. Kamar, Hala El Daous, Marwa Darweish, Mai O. Nada, Walaa Fathy SaadEldin. All authors participated in writing the manuscript: Sawsan S. Elbasuni, Marwa I. Abdel Haleem, Hanan A.A. Taie, Samah M. Abdel Gawad, Reda E.L. Kamar, Hala El Daous, Marwa Darweish, Mai O. Nada, Walaa Fathy SaadEldin; Review and editing: Sawsan S. Elbasuni, Marwa I. Abdel Haleem, Hanan A.A. Taie, Hala El Daous. Preparation of all tested diets: Marwa I. Abdel Haleem, Sawsan S. Elbasuni. All authors drafted and approved the final version of the manuscript. Sawsan S. Elbasuni, Marwa I. Abdel Haleem, Hanan A.A. Taie, and Hala El Daous carried out the statistical analysis.

Data availability

Data are available on request.ss

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