Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

# *Rosmarinus officinalis* Essential Oils as an Alternative to Anticoccidial Drugs in Broiler Chicken against *E. maxima* and *E. tenella* Infection

**Saba B. Badar*[1], Gulbeena S. Saleem*[1], Asim A. Chaudhry[1] and Kamran A. Ashraff[2]**

[1]*Department of Pathology, Faculty of Veterinary Sciences, University of Veterinary & Animal Sciences, Lahore, Pakistan.*

[2]*Department of Parasitology, Faculty of Veterinary Sciences, University of Veterinary & Animal Sciences, Lahore, Pakistan.*

**Abstract**

IN the current study, *Rosmarinus officinalis* essential oil (REO) was used as an alternative to anticoccidial drugs in broilers infected with *E. maxima* and *E. tenella*. A total of 700-day-old straight run broiler chicks were randomly assigned to seven groups having 5 treatments of 20 birds each according to completely randomized design. Treatments were consisted of negative control (CN); positive *E. maxima* (PCM); positive *E. tenella* (PCT); *E. maxima* treated with anticoccidial drug (SM); *E. tenella* treated with anticoccidial drug (ST); Rosemary-fed *E. maxima* challenged group (RM); Rosemary-fed *E. tenella* challenged group (RT). At 14 days of their age, growth performance, oocyst number, haematopathology, corticosterone levels, gut microbiota modulation were measured. GC/MS analysis showed that extracted REO had various antioxidant as well as anticoccidial compounds such as 1,8-Cineole (53.94%), α-Pinene (18.45%), β-Pinene (4.57%) and Camphor (6.08%). Birds fed with REO, presented higher weight gain, reduced fecal oocyst count, best haematological parameters, least lesion score, corticosterone level and number of *Salmonella typhimurium* colonies, as compared to all other groups ($P<0.05$). Both *E. maxima* and *E. tenella* were observed as equally pathogenic to broilers ($P>0.05$). We concluded that *E. maxima* and *E. tenella* are equally pathogenic, and REO can be a good alternative to anticoccidial drugs as it improves bird performance by inducing intestinal healing and restoration.

**Keywords:** Anticoccidial alternate, Corticosterone, Green herb, Haematopathology, Phytogenics.

## Introduction

Coccidiosis is a parasitic disease that has a significant impact on the economy of poultry farming [1, 2, 3]. Protozoan parasites, specifically those belonging to the genus *Eimeria* are responsible for causing significant health and productivity problems in chickens [4]. Coccidiosis attacks susceptible chickens when they consume sporulated oocysts found in the surroundings. After ingestion, acidic conditions in the gizzard of the host trigger the process of excystation of the oocyst [5]. This leads to the liberation of sporozoites, which then invade intestinal cells and cause damage to the intestinal mucosa [6, 7]. Consequently, clinical symptoms such as decreased feed intake, bloody diarrhea, and weight loss are displayed by the infected birds [8, 9]. Coccidiosis is most common in the avian population of three and eight weeks of age but it can affect chickens of any age [10]. Of the nine avian *Eimeria* species, only two of them i.e., *E. tenella* and *E. maxima* are considered as most prevalent and pathogenic coccidian species affecting domestic poultry [10, 11].

Intensive farming practices and higher stocking densities at broiler farms are the triggering factors in the spread of coccidiosis [2, 3]. To prevent the disease from spreading, the poultry industry is spending a lot of money on anticoccidial medications given in feed or water, which is a financial challenge for the producers. The effectiveness of anticoccidial drugs has been diminished due to the developing drug resistance in various regions across the globe [12, 13]. Vaccines have been employed to control the spread of coccidiosis but one potential limitation of live vaccination is that it can induce a temporary early decline in the growth of chickens under certain commercial conditions [14]. This decline in growth due to live vaccine is frequently accompanied by an elevated risk of secondary enteritis, and in certain cases, it can also cause necrotic enteritis [3]. Secondly, the usage of anti-coccidial drugs in poultry is discouraged due to the growing demand of

*Corresponding authors: Gulbeena S. Saleem, E-mail:gulbeena.saleem@uvas.edu.pk, Tel.: 92-300-940-0919
(Received 19 March 2025, accepted 01 August 2025)
DOI: 10.21608/ejvs.2025.369584.2714

consumers for drug-free poultry products and the emergence of drug-resistant strains of *Eimeria* species [13, 15]. Nowadays, probiotics and natural dietary supplements are being used as alternative strategies for the control of coccidiosis [13, 15]. The utilization of phytoextracts and various active ingredients from plants has gained significant attention of scientists. These alternative strategies are primarily driven by the increasing efforts to mitigate the environmental impact of drug usage and to combat antimicrobial resistance. The plant extracts have been suggested for use as feed supplements due to their nutraceutical, probiotic, and immunoregulatory attributes, as well as their potential positive-impact on the quality of the final product [16].

Rosemary (*Rosmarinus officinalis*) is classified as a member of the *Lamiaceae* family and a perennial evergreen herb that possesses needlelike leaves with a distinct fragrance. Recently, the application of Rosemary essential oil in the livestock industry has gained a lot of attention [17, 18]. *Rosmarinus officinalis* essential oil (REO) has various compounds which work against inflammation, germs, and viruses, and also regulates the immune system [17]. The study conducted by Norouzi *et al.*, (2015) [18] examined the impact of varying dietary amounts of Rosemary herb powders on the growth performance, carcass characteristics, and ileal microbiota of chickens. They concluded that the efficacy of using the Rosemary herb at a concentration of 0.5% was better than the other concentrations to trigger the immune response in chickens [17]. However, there is a need to explore the potential effects of supplementing the REO in chicken diets [19, 20]. The present study was designed to investigate the effects of feed supplementation with REO as an alternative to anti-coccidial drugs to improve the performance, haematopathology and anti-stress responses in broilers against *E. maxima* and *E. tenella* infection.

## Material and Methods

### *Materials*

Fresh leaves were randomly picked from a mature Rosemary shrub, grown in Lahore, rinsed with water, and dried in the dark at 25°C for four weeks before being pulverized using an electronic processor (MoulinexOvatio 2, Serris, France). Oil was extracted through microwave-aided hydro-distillation (MAH) in a domestic microwave oven [21, 22, 23]. An HP 5890 (II) gas chromatograph connected to an HP 5972 mass spectrometer at 70 eV of electron impact ionization energy was used to conduct the GC/MS study of volatile chemicals. An HP-5 MS capillary column with dimensions 30 m. 0.25 mm, coated with 5% phenyl methyl silicone, 95% dimethylpolysiloxane, 0.25 mm film thickness (Hewlett-Packard, Palo Alto, CA, USA), was programmed to rise from 50°C to 240°C at 5°C/minute while the ion source, quadrupole, and front inlet temperatures were 230°C, 150°C, and 250°C respectively. Carrier gas Helium flowed at 1.2 ml/min and had a 60:1 split ratio. Scan length was 1s and mass spectra range was 40–300 m/z at 3.62 amu/scan. The peaks of the volatile compounds were identified by cross-referencing the data with published literature the 8th edition of the Wiley Registry of Mass Spectra [24].

### *Study design*

A total of 700 day-old (Ross-308; 40±0.2g Male) broiler chicks were purchased from a hatchery in Lahore, Pakistan, and raised under standard managerial protocols at the experimental facility, Department of Pathology, University of Veterinary & Animal Sciences Lahore, Pakistan. Floor dividers were employed to provide individual housing for each treatment group (n=20, 5 replicates). The chicks were vaccinated against Newcastle disease (ND Lasota IB Mass, Zoetis Poultry Vaccines. United States of America) using a nasal spray when they were two days old. They were fed starter diets from birth until 11 days of age, and then switched to finisher diets from 12 to 23 days of age. Initially set at 33°C for the first week, the room temperature was reduced to 22°C by day 14 and maintained at that level. The lighting program implemented was a continuous 23-hour light cycle. On the 14$^{th}$ day of age (0 day post infection (dpi), the chickens were distributed at random into seven groups, negative control (NC), Positive *Eimeria maxima* (PCM), Positive *E. tenella* (PCT), *E. maxima* treated with anticoccidial drug (SM), *E. tenella* treated with anticoccidial drug (ST), Rosemary fed *E. maxima* challenged group (RM), Rosemary fed *E. tenella* challenged group (RT). It is of considerable significance to acknowledge that the manifestation of *Eimeriosis* in avian livestock is primarily ascribed to the existence of *E. tenella* and *E. maxima* [11]. Since day 0, 40 birds in the RT and RM groups were fed a diet enriched with 100 parts per million (ppm) of REO, the weighed amount of feed was mixed with REO quantity before given to the birds in RT and RM groups. Previous studies in broilers helped to establish the optimal dietary supplementation level of REO [25]. On day 14 of their lives, birds fed non-REO feed were separated in groups PCM, PCT, ST, SM (n=20) and REO fed birds in 2 groups RT and RM, were given experimental gametocytes through oral gavage: 1 ml of PBS solution containing 35,000 sporulated oocytes. PBS 1ml, was given to the NC group, which served as the negative control. The absence of coccidia in chickens was confirmed by coprological examination. On day 16 (2dpi), anticoccidial drug was administered to SM and ST birds (Symans. Amprovil: Amprolium HCl: 900 gm. 1g/16 liters water, administered orally for 5 days from 2dpi to 6dpi). The entire duration of the

experiment was 23 days. Experimental treatment studies are dependent on the evaluation of different post-challenge factors include the examination of specific lesions that indicate the presence of a disease, analysis of blood parameters, measurement of parasite replication through oocyst output, and assessment of productivity indicators like body weight gain and feed conversion rates.

*Methods*

*The assessment of live body weight in different groups*

Live body weights of birds were recorded on day of arrival, $0^{th}$, $5^{th}$, $7^{th}$ and $9^{th}$ dpi. Weighted amounts of feed were given to the animals in the morning and evening during the study period to gauge their daily feed consumption; the leftovers were gathered the following day, weighed, and deducted from the feed supplied. Death tolls were tallied at the time of occurrence (no mortality reported after 14 day of age).

*The fecal examination*

Fecal samples were collected from all groups on $0^{th}$, $5^{th}$, $7^{th}$, and $9^{th}$ dpi. A total of 500 grams of fresh fecal droppings were collected randomly by hand from both the experimental and control groups. Flotation solution of saturated sodium chloride (specific gravity 1.18–1.2) was used in the flotation analysis of the fecal samples in order to identify the oocysts of the *Eimeria* species. The positive samples were then additionally analyzed using the modified McMaster technique in order to determine the oocyst count per gram (OPG). For every fecal sample, duplicate counts of duplicate fecal slurries were conducted. The OPG was computed following the McMaster technique with duplicate counts of fecal samples. FCR of birds in all groups was calculated using formula according to Zhenhua (2018) [26].

FCR= Feed intake in grams/ body weight gain in grams

Postmortem examination for coccidiosis was performed on Intestinal tissue samples and ceacal pouches for gross and histopathological analysis. Birds from each group were humanely slaughtered on $0^{th}$, $5^{th}$, $7^{th}$ and $9^{th}$ dpi to analyze the lesions in the intestine and ceaca for *E. maxima* and *E. tenella* respectively. From 0 to 4, the Johnson and Reid (1970) [27] lesion scoring system was followed. As 0 for nearly no lesions, 1 for rare scattered lesions, 2 for twofold lesions, 3 when lesions consolidate, intestinal contents present a bloody tinge and intestinal wall thickening, 4 for spotting unsporulated oocysts of *E. maxima* and *E. tenella* during magnification of the contents, presence of blood-tinged ceacal and intestinal contents with enteric wall thickening. The histopathology was carried out in accordance with the recommendations made by Luna (1968) [28] and H&E staining after Urara *et al.,* (2005) [29]. Histopatholgical examination was conducted for both *E. maxima* and *E. tenella* oocysts groups using a compound microscope at 40X magnification (BZ-X810; Keyence, Osaka, Japan).

Intestinal contents (ceacal and ileal) were collected on $0^{th}$, $5^{th}$, $7^{th}$ and $9^{th}$ dpi in the sterile tubes. Following the method of Norouzi *et al.,* (2015) [18], 10 folds serial dilutions were prepared from 1 gram of the ileal and ceacal contents in a phosphate buffer solution ($10^{-1}$–$10^{-6}$). 100 µl from $10^{-4}$, $10^{-5}$ and $10^{-6}$ were poured onto the labeled petri-dishes containing culture medium i.e., *Salmonella-Shegiella* agar and incubated at 37°C for 72 hrs. Effects of REO on gut microbiota were measured in terms of increase or decrease of the population of *Salmonella typhimurium* and confirmed with Gram staining [30].

Blood samples (3ml) were collected in EDTA vacutainers from the wing vein of birds, on $0^{th}$, $5^{th}$, $7^{th}$ and $9^{th}$ dpi. Haematological analysis was carried out to examine Haematological parameters Total erythrocyte count (TEC) ($10^6$/micro liter), leukocytes, differential leukocyte count (DLC), packed cell volume (PCV) and haemoglobin concentration (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular haemoglobin concentration (MCHC) and Mean Corpuscular haemoglobin (MCH). Haematological parameters: Total red blood cells (RBCs), packed cell volume (PCV), hemoglobin (Hb), total white blood cells (WBCs), and differential leukocytic counts were performed in accordance with standard avian haematological protocols using hematology analyzer (HEMA-A5-7010). Following formulae were used after Odunitan-Wayas *et al.,* (2018) [31].

MCV (fL) =Ht (%)×10RBC (millions / µL)

MCH (pg)=Hb (g / dL)×10RBC (millions / µL)

MCHC (g/dL)=Hb (g / dL)×100Ht (%)

Blood samples (3ml) were collected on the $0^{th}$, $5^{th}$, $7^{th}$ and $9^{th}$ dpi for determination of corticosterone level. The samples were centrifuged for 20 minutes at 1,500×g and 4°C to obtain the serum, which was then preserved at -20°C for further investigation. ELISA kits were used to assess serum corticosterone concentrations (Cortisol AccuBind ELISA Kit, sensitivity: 0.25µg/dL, Product#3625-300. Monobind Inc., Lake Forest, CA) following instructions from the manufacturer.

The data from all groups were subjected to analysis, and recorded parameter values were treated as variables for each sampling day. The data collected from all groups were tested with Multivariate Analysis of Variance (MANOVA) using SPSS 23.0 software (International Business Machines Corporation, Armonk, NY, USA). The results were expressed as mean values±standard deviation. The significance of the difference in results was tested using Duncan's test, with a *P*-

*value<0.05*. Following mathematical model was applied:

$Yij = \mu + \tau i + \epsilon ij$

Where,

Yij = observation of dependent variable recorded on $i^{th}$ treatment group

μ = population mean

τi = effect of $i^{th}$ treatment group (i= 1, 2, 3, 4, 6, 7)

ϵij = residual effect of $j^{th}$ observation recorded on $i^{th}$ treatment group, NID ~ 0, σ2

## Results

Birds in RT and RM groups showed increase in body weight gain compared with ST and SM, negative control and positive control groups post-coccidiosis challenge with *E. maxima* and *E. tenella* (*P*< 0.05) (Table 1). Birds from ST, SM, PCT and PCM showed reduced weight gain post challenge till the end of the trial period. Birds from RT and RM groups showed better FCR as compared to birds from PCT, PCM, NC, ST and SM groups (P<0.05) (Table 2). The FCR exhibited by NC birds was not superior to that of RT and RM birds, whereas RT and RM birds gained weight on the day of challenge and until the conclusion of the trial, on $9^{th}$ dpi.

There was no trace of oocysts in the feces of negative control broilers when the samples collected on days 0, $5^{th}$, $7^{th}$ and $9^{th}$ dpi. The birds showed highest OPG count from positive control groups PCM and PCT on $5^{th}$, $7^{th}$ and $9^{th}$ dpi (Table 3). RT and RM groups recorded the lowest value of OPG during the entire recording period (P<0.05).

Gross pathological evaluation of infected groups revealed that lesions in various intestinal segments varied with the sporulated *Eimeria* species used for inoculation. Petechial and inflated haemorrhages were visible on the enteric serosal surface by the $5^{th}$ dpi. Intestinal and caecal contents of all non-infected control chickens showed no pathological alterations.

At $5^{th}$, $7^{th}$, and $9^{th}$ dpi, the mean score of gross intestinal lesions in birds ST and SM groups, was significantly lower than in the positive controls (P<0.05) (Table 4). Birds from RT and RM groups, had a significantly reduced intestinal lesion score $7^{th}$ dpi in the anterior intestine and cecum (P<0.05) than PCT, PCM, ST and SM birds for all the sampling days. Also that demonstrated earlier recovery from the infection than anticoccidial drug administrated birds. The rate of infection clearance varied significantly across treatment groups. When compared to the positive control group, the lesion score of ST, SM, RT and RM dropped significantly. At $9^{th}$ dpi, there were no statistically significant differences in the mean gross intestinal lesion score of RT and RM and that of the negative controls, indicating that the intestines of the surviving birds had healed earlier as compared to ST and SM birds (P>0.05). For PCT and PCM mean lesion score was 4 on $9^{th}$ dpi. Lesion score of CN remained zero as well.

The PCT and PCM birds had the highest mean histopathologic score (P<0.05). On $5^{th}$ dpi, the celiac and intestinal lesion scores of birds in the RT and RM groups were 3 that is significantly lower than those of the PCT, PCM, ST, and SM groups. On the $7^{th}$ day post-challenge, the lesion score in RT and RM dropped to 2, and by the $9^{th}$ day post-challenge, full healing had been recorded, making this group much less vulnerable than the other challenged and treated groups. On the $5^{th}$ dpi, histopathology of birds in the ST and SM groups revealed a mean lesion score of 3.5, and this value rose to 4 on the $7^{th}$ dpi. With a lesion score of 4, unsporulated *Eimeria* oocysts could still be found in the tissues on $9^{th}$ dpi in ST and SM histopathological sections. The differences in histopathologic intestinal lesions of challenged birds were narrowed for both *Eimeria* species in all groups and reached to insignificant differences among all challenged and treated birds (ST and SM, PCT and PCM).

RT and RM groups exhibited a significant (P<0.05) increase in TEC, PCV (%), MCV (fL), Hb (g/L), MCH (pg), MCHC (g/L), PCV percent (Tables 5, 6, 7, 8, 9, 10), heterophils and leucocytes, DLC (Tables 11, 12, 13, 14, 15). Both the RT and RM groups demonstrated a statistically significant (P<0.05) increase in TLC from $0^{th}$ to $9^{th}$ dpi. Compared to haematological measures in RT and RM birds, haematological parameters in ST and SM birds showed a substantial (P<0.05) reduction.

RT and RM groups showed minimal corticosterone level in blood (Table 16), before infection and after infection in both *Eimeria* species (P>0.05). The hormone remained consistent with only slight increase at $5^{th}$ dpi in RT and RM birds, although significantly lower (P<0.05) than ST and SM groups. The corticosterone levels in the negative control group increased barely with age. On $0^{th}$ dpi, all groups except RT and RM exhibited the same number of colonies. Essential oil was found to reduce the number of live *Salmonella typhimurium* bacteria in the intestines of broilers by a statistically significant number (Table 17). The number of *Salmonella* colonies was lowest in the RT and RM groups (P <0.05), followed by the NC group, which had the fewest colonies (P<0.05).

## Discussion

Several pathogenic species of *Eimeria* cause coccidiosis infection in broiler chickens. *Eimeria* species are categorized after their pathogenicity and *E. maxima* and *E. tenella* are the most pathogenic species [11, 32, 33, 34]. The evolutionary process and mutations in *Eimeria* could potentially lead to the development of resistance against anticoccidial

drugs [2, 12, 13]. Therefore, reliable alternatives to anticoccidial drugs were examined in the current experiment. The success of experimental treatments relies on the assessment of various factors like analyzing blood parameters and measuring the rate of parasite replication through oocyst output, histopathology, chicken growth performance and immune potential post-infection [15, 35, 36].

GC/MS analysis of Rosemary essential oil showed 1, 8-Cineole (53.94%), α- Pinene (18.45%), β-Pinene (4.57%), Borneol (1.50%), Camphor (6.08%), Camphene (4.38%) and β-Caryophyllene (1.65%) as major components of the REO. The extraction procedures, region of the Rosemary herb, the part of the aromatic plant from where the oil is extracted, are reported to affect the yield of oil and its composition [23]. Different regions of the Asia show same composition of Rosemary oil as compared to other continents [23, 37, 38, 39, 40]. The above-mentioned compounds exhibit strong antioxidant as well as anticoccidial properties and are found in various essential oils [41, 42].

Chickens from RT and RM groups showed the lowest OPG number than all other groups, although there was no trace of oocysts in NC birds throughout the study. The results agree with previous findings that essential oils have a positive impact on performance and coccidial parameters, leading to reported improvement [1, 3, 43, 44, 45]. RT and RM birds showed not only a good FCR but also a significant difference from NC, PCT, PCM, ST, and SM groups; this agrees with the previous findings [13]. There was a profound anticoccidial properties difference in groups fed REO and non-REO fed birds when challenged with *Eimeria*, agreeing with the findings of Gumus and Gelan (2023) [25], yet some studies support that it is known to produce no effect on weight gain and FCR [46]. The birds fed with REO showed less lesion development, better growth, and less inflammation in the intestine. This is by findings of other researchers [13, 47, 48, 49] that rosemary herb-fed broilers showed good weight gain at later stages of life at a low level in the feed.

RT and RM groups showed significantly lower lesion scores than the other challenged groups. This result coincides with the findings of Pop *et al.,* (2019) [1], who used Rosemary to control coccidiosis from *E. maxima* and *E. tenella* species. Also, Bozkurt *et al.,* (2014), Saeed *et al.,* (2023), and Ding *et al.,* (2022) [13, 50, 51] recommended that an in-feed mixture of essential oils can be beneficial in reducing lesions in coccidial challenges and found significantly low lesion score in essential oil fed birds as compared to other supplements.

Our results showed a significant difference among the REO-fed groups, non.REO fed anticoccidial treated (ST and SM) and PCT, PCM groups for haematological analysis (Hb, MCH, MCHC, MCV, TEC) ($P<0.05$). Both species of *Eimeria* were not significantly different from each other ($P>0.05$) for all treatments. The results indicate that Rosemary oil protects against disease losses caused by pathogenic *Eimeria* species. This finding is consistent with a study by Li *et al.,* (2020) [52].

The white blood cell count (WBC) decreased after administering the anticoccidial drug until the 9th dpi, indicating a reduction in natural immunity. This reduction was significantly different ($P<0.05$) compared to the group fed with REO on the same day of sampling, which had a normal WBC count. This suggests that the immune system is naturally restored or by the birds themselves after the challenge, without the use of any medication. This finding aligns with the research conducted by Aouadi *et al.,* , Abd El-Hack *et al.,* and Saeed *et al.,* [13, 23, 41]. Due to inflammation and damage to the epithelium earlier in infection, the anticoccidial group could not recover the H/L ratio as early as REO-fed groups, as shown on the $9^{th}$ dpi. This is in agreement with the findings of Amera *et al.,* [46]. Amera *et al.,* [46] claimed that Rosemary essential oils have no positive impact on the hematology of broiler birds.

REO group showed minimal corticosterone levels in the blood, on $0^{th}$ dpi and after infection in both RT and RM birds. The hormone remained consistent, slightly increasing at $5^{th}$ dpi in RT and RM birds, significantly lower than in ST and SM groups. PCT and PCM birds showed the highest corticosterone levels. The results of the present study agree with Scanes, (2016) [53]. The negative control group showed no change in cortisol levels. Our results agree with other studies that claim that *Rosmarinus officinalis* is an antistressent and reduces stress as shown by the corticosterones levels in different groups, which is a stress hormone [13, 45, 51]. The use of essential oils is among the top priority as far as herbal or plant extracts are concerned as medicine replacements [48]. RT and RM birds showed the highest number of colonies on the $5^{th}$ dpi, reduced on the $7^{th}$ dpi, and no colonies on the $9^{th}$ dpi, compared to the NC, ST, and SM birds. This could be attributed to the antibacterial aspects of the essential oils as supported by other findings that the use of essential oils could manipulate the gut microbiota, thus influence the immune responses of the birds [13, 23, 41, 46, 54].

## Conclusion

Rosemary essential oil has been found to exhibit diverse effects on performance enhancement, modulation of the digestive systems, and modulation of microbial populations when incorporated into

broiler rations, which could help to control and prevent coccidiosis. The study found that using REO enhanced performance, improved carcass parameters, positively affected haematological parameters, improved gut health, and reduced bacterial count. The treatment groups that received REO supplementation demonstrated the most favorable outcomes compared to other groups. Thus, the potential impact of REO on the physiological processes of the gastrointestinal system, hematopathology, and immunity may play a crucial role in enhancing the efficiency of energy and nutrient digestion, growth performance, and stress relief pre- and post-coccidiosis challenge in broiler birds. Thus, REO has the potential to outperform anticoccidials.

The inherent quality of Rosemary essential oils to be readily absorbed necessitates exploring various methodologies to facilitate their controlled release at peculiar anatomical locations within the gastrointestinal tract. Further investigations are imperative to unravel the intricate workings underlying the modus operandi of *Rosmarinus officinalis* essential oil and its bioactive constituents against coccidian parasites. This elucidation is crucial for their prospective integration into forthcoming coccidiosis control initiatives.

*Acknowledgments*

Not applicable.

*Author's contribution*

S. Badar conducted research, written the manuscript. G. Saleem and S. Badar conceptualize the research. G. Saleem supervised the research. S. Badar , A. Aslam and K. Ashraf reviewed and helped in conducting the research work.

*Conflict of interest*

Authors declare there is no conflict of interest in their research work.

*Ethical of approval*

Animal ethical and welfare regulations and institutional requirements of the Advanced Studies and Research Board (ASRB), University of Veterinary and Animal Sciences were strictly adhered to throughout all processes under letter issued DR/609-A Dated 16-12-21.

**TABLE 1. Effects of REO and anticoccidial drug on body weight (grams) of broiler chickens infected with *E. maxima* and *E. tenella.***

| Days (dpi) | CN | PCT | PCM | ST | SM | RT | RM |
|---|---|---|---|---|---|---|---|
| 0 | 327.2±23.1$^{b}$ | 326.6±34.2$^{b}$ | 328.1±25.6$^{b}$ | 328.5±35.6$^{b}$ | 326.4±18.8$^{b}$ | 335.4±25.2$^{a}$ | 338.8±28.0$^{a}$ |
| 5 | 465.3±32.4$^{b}$ | 325.1±33.0$^{c}$ | 324.6±27.0$^{c}$ | 461.5±48.6$^{b}$ | 431.4±25.6$^{b}$ | 557.4±33.0$^{a}$ | 544.8±46.5$^{a}$ |
| 7 | 502.0±35.0$^{ab}$ | 328.7±33.4$^{c}$ | 324.6±27.0$^{c}$ | 479.9±50.5$^{b}$ | 448.5±26.6$^{b}$ | 579.6±34.4$^{a}$ | 566.5±48.3$^{a}$ |
| 9 | 541.6±37.7$^{b}$ | 332.3±33.7$^{d}$ | 331.7±27.6$^{d}$ | 499.1±52.5$^{bc}$ | 466.4±27.7$^{c}$ | 602.7±35.7$^{a}$ | 595.1±50.3$^{a}$ |

a-d: different alphabets within a row indicate significant difference (P<0.05) between treatments within the same day (dpi). Values were expressed as mean±standard deviation (SD). CN: Negative control; PCM: Positive *E. maxima*; PCT: Positive *E. tenella*; SM: *E. maxima* treated with anticoccidial drug; ST: *E. tenella* treated with anticoccidial drug; RM: Rosemary fed *E. maxima* challenged group; RT: Rosemary fed *E. tenella* challenged group.

**TABLE 2. Effects of dietary supplemented Rosemary essential oils and anticoccidial drug on FCR of broiler chickens infected with *E. maxima* and *E. tenella***

| Days (dpi) | CN | PCT | PCM | ST | SM | RT | RM |
|---|---|---|---|---|---|---|---|
| 0 | 1.57 ± 0.11$^{a}$ | 1.69 ± 0.13$^{a}$ | 1.63± 0.15$^{a}$ | 1.61 ± 0.02$^{a}$ | 1.56± 0.15$^{a}$ | 1.26 ± 0.30$^{b}$ | 1.24 ± 0.08$^{b}$ |
| 5 | 1.58 ± 0.17$^{b}$ | 2.54 ± 0.19$^{a}$ | 2.03± 0.09$^{a}$ | 1.74 ± 0.31$^{b}$ | 1.78± 0.52$^{b}$ | 1.18 ± 0.03$^{c}$ | 1.15 ± 0.02$^{c}$ |
| 7 | 1.41± 0.05$^{bc}$ | 2.01± 0.01$^{a}$ | 1.99± 0.45$^{a}$ | 1.51± 0.31$^{b}$ | 1.65± 0.52$^{b}$ | 1.36± 0.12$^{c}$ | 1.41± 0.15$^{bc}$ |
| 9 | 1.31± 0.12$^{b}$ | 2.10± 0.15$^{a}$ | 2.11± 1.21$^{a}$ | 2.15± 0.35$^{a}$ | 2.05± 0.23$^{a}$ | 1.45± 0.21$^{b}$ | 1.31± 0.08$^{b}$ |

a-c: different alphabets within a column indicate significant difference (*P<0.05*) between treatments within the same day (dpi). Values were expressed as mean±standard deviation (SD). CN: Negative control; PCM: Positive *E. maxima*; PCT: Positive *E. tenella*; SM: *E. maxima* treated with anticoccidial drug; ST: *E. tenella* treated with anticoccidial drug; RM: Rosemary fed *E. maxima* challenged group; RT: Rosemary fed *E. tenella* challenged group

**TABLE 3. Effects of REO and anticoccidial drug on OPG (x10$^4$) of broiler chickens infected with *E. maxima* and *E. tenella***

| Days (dpi) | CN | PCT | PCM | ST | SM | RT | RM |
|---|---|---|---|---|---|---|---|
| 0 | 0.00±0.00a | 0.00±0.00$^a$ | 0.00±0.00$^a$ | 0.00±0.00$^a$ | 0.00±0.00$^a$ | 0.00±0.00$^a$ | 0.00±0.00$^a$ |
| 5 | 0.00±0.00d | 11.20±0.39$^a$ | 10.89±0.47$^a$ | 9.52±0.30$^{ab}$ | 8.94±0.52$^b$ | 2.10±0.15$^c$ | 1.57±0.02$^c$ |
| 7 | 0.00±0.00d | 15.54±0.66$^a$ | 16.54±0.54$^a$ | 9.99±0.57$^b$ | 9.88±0.47$^b$ | 0.15±0.47$^c$ | 0.18±0.31$^c$ |
| 9 | 0.00±0.00c | 16.21±1.11$^a$ | 15.85±0.47$^a$ | 6.82±2.10$^b$ | 6.78±1.02$^b$ | 0.00±0.01$^c$ | 0.00±0.01$^c$ |

a-d: different alphabets within a row indicate significant difference (*P<0.05*) between treatments within the same day (dpi). Values were expressed as mean±standard deviation (SD). CN: Negative control; PCM: Positive *E. maxima*; PCT: Positive *E. tenella*; SM: *E. maxima* treated with anticoccidial drug; ST: *E. tenella* treated with anticoccidial drug; RM: Rosemary fed *E. maxima* challenged group; RT: Rosemary fed *E. tenella* challenged group

**TABLE 4. Effects of REO and anticoccidial drug on lesion scores of broiler chickens infected with *E. maxima* and *E. tenella***

| Days (dpi) | CN | PCT | PCM | ST | SM | RT | RM |
|---|---|---|---|---|---|---|---|
| 0 | 0.00±0.00$^a$ | 0.00±0.00$^a$ | 0.00±0.00$^a$ | 0.00±0.00$^a$ | 0.00±0.00$^a$ | 0.00±0.00$^a$ | 0.00±0.00$^a$ |
| 5 | 0.00±0.00$^f$ | 3.37±0.52$^c$ | 3.87±0.35$^a$ | 3.50±0.53$^{ab}$ | 3.75±0.46$^b$ | 1.50±0.53$^e$ | 1.62±0.52$^d$ |
| 7 | 0.00±0.00$^d$ | 4.00±0.00$^a$ | 4.00±0.35$^a$ | 3.50±0.53$^b$ | 3.65±0.46$^b$ | 0.25±0.46$^c$ | 0.27±0.47$^c$ |
| 9 | 0.00±0.00$^c$ | 4.00±0.03$^a$ | 4.00±0.02$^a$ | 3.52±0.53$^b$ | 3.50±0.53$^b$ | 0.00±0.00$^c$ | 0.00±0.00$^c$ |

a-f: different alphabets within a row indicate significant difference (*P<0.05*) between treatments within the same day (dpi). Values were expressed as mean±standard deviation (SD). CN: Negative control; PCM: Positive *E. maxima*; PCT: Positive *E. tenella*; SM: *E. maxima* treated with anticoccidial drug; ST: *E. tenella* treated with anticoccidial drug; RM: Rosemary fed *E. maxima* challenged group; RT: Rosemary fed *E. tenella* challenged group.

**TABLE 5. Effects of REO and anticoccidial drug on TEC (10$^6$ /microliter) of broiler chickens infected with *E. maxima* and *E. tenella***

| Days (dpi) | CN | PCT | PCM | ST | SM | RT | RM |
|---|---|---|---|---|---|---|---|
| 0 | 2.85±0.28$^b$ | 2.85±0.21$^b$ | 2.85±0.21$^b$ | 2.75±0.275$^{bc}$ | 2.70±0.18$^c$ | 3.98±0.22$^a$ | 3.99±0.22$^a$ |
| 5 | 2.74±0.17$^b$ | 1.78±0.23$^c$ | 1.78±0.23$^c$ | 1.56±0.16$^d$ | 1.57±0.13$^d$ | 3.77±0.17$^a$ | 3.78±0.16$^a$ |
| 7 | 2.75±0.23$^b$ | 1.84±0.18$^c$ | 1.75±0.18$^{cd}$ | 1.62±0.33$^d$ | 1.59±0.19$^e$ | 4.02±0.28$^a$ | 4.03±0.27$^a$ |
| 9 | 2.66±0.24$^c$ | 1.74±0.19$^d$ | 1.74±0.19$^d$ | 1.12±0.11$^e$ | 1.13±0.012$^e$ | 3.69±0.019$^b$ | 3.71±0.28$^a$ |

a-e: different alphabets within a row indicate significant difference (*P<0.05*) between treatments within the same day (dpi). Values were expressed as mean±standard deviation (SD). CN: Negative control; PCM: Positive *E. maxima*; PCT: Positive *E. tenella*; SM: *E. maxima* treated with anticoccidial drug; ST: *E. tenella* treated with anticoccidial drug; RM: Rosemary fed *E. maxima* challenged group; RT: Rosemary fed *E. tenella* challenged group.

**TABLE 6. Effects of REO and anticoccidial drug on Hb (g/L) of broiler chickens infected with *E. maxima* and *E. tenella*.**

| Days (dpi) | CN | PCT | PCM | ST | SM | RT | RM |
|---|---|---|---|---|---|---|---|
| 0 | 10.45±0.78$^a$ | 10.52±0.05$^a$ | 10.54±0.35$^a$ | 10.35±0.15$^a$ | 10.55±0.14$^a$ | 10.75±0.25$^a$ | 10.78±0.15$^a$ |
| 5 | 10.55±0.24$^a$ | 6.25±0.21$^b$ | 6.85±0.18$^b$ | 6.65±0.65$^b$ | 6.57±0.21$^b$ | 9.54±0.25$^a$ | 9.76±0.21$^a$ |
| 7 | 10.67±0.15$^a$ | 5.54±0.05$^c$ | 5.98±0.25$^c$ | 6.57±0.96$^b$ | 6.78±0.55$^b$ | 10.01±0.10$^a$ | 10.99±0.05$^a$ |
| 9 | 10.43±0.21$^a$ | 5.65±0.21$^b$ | 5.56±0.51$^b$ | 5.71±0.11$^b$ | 5.91±0.25$^b$ | 11.00±0.10$^a$ | 10.57±0.21$^a$ |

a-c: different alphabets within a row indicate significant difference (*P<0.05*) between treatments within the same day (dpi). Values were expressed as mean±standard deviation (SD). CN: Negative control; PCM: Positive *E. maxima*; PCT: Positive *E. tenella*; SM: *E. maxima* treated with anticoccidial drug; ST: *E. tenella* treated with anticoccidial drug; RM: Rosemary fed *E. maxima* challenged group; RT: Rosemary fed *E. tenella* challenged group.

**TABLE 7. Effects of dietary supplemented Rosemary essential oils and anticoccidial drug on MCV (fL) of broiler chickens infected with *E. maxima* and *E. tenella*.**

| Days (dpi) | CN | PCT | PCM | ST | SM | RT | RM |
|---|---|---|---|---|---|---|---|
| 0 | 121.40±5.64$^{a}$ | 124.21±8.91$^{a}$ | 119.24±7.54$^{a}$ | 122.37±5.89$^{a}$ | 124.65±5.87$^{a}$ | 132.65±5.65$^{a}$ | 131.98±4.35$^{a}$ |
| 5 | 125.61±6.05$^{a}$ | 95.91±8.05$^{b}$ | 90.65±8.78$^{b}$ | 95.61±9.94$^{b}$ | 94.89±8.79$^{b}$ | 119.94±6.10$^{a}$ | 117.87±7.65$^{a}$ |
| 7 | 129.21±7.89$^{a}$ | 88.98±7.53$^{b}$ | 89.91±9.41$^{b}$ | 96.71±8.12$^{b}$ | 94.69±8.98$^{b}$ | 129.65±7.89$^{a}$ | 125.34±8.18$^{a}$ |
| 9 | 131.24±8.55$^{a}$ | 80.35±9.19$^{bc}$ | 78.91±7.45$^{c}$ | 99.94±7.41$^{b}$ | 98.99±7.53$^{b}$ | 132.34±5.49$^{a}$ | 133.31±5.81$^{a}$ |

a-c: different alphabets within a column indicate significant difference ($P<0.05$) between treatments within the same day (dpi). Values were expressed as mean±standard deviation (SD). CN: Negative control; PCM: Positive *E. maxima*; PCT: Positive *E. tenella*; SM: *E. maxima* treated with anticoccidial drug; ST: *E. tenella* treated with anticoccidial drug; RM: Rosemary fed *E. maxima* challenged group; RT: Rosemary fed *E. tenella* challenged group.

**TABLE 8. Effects of dietary supplemented Rosemary essential oils and anticoccidial drug on MCH (pg/L) of broiler chickens infected with *E. maxima* and *E. tenella***

| Days (dpi) | CN | PCT | PCM | ST | SM | RT | RM |
|---|---|---|---|---|---|---|---|
| 0 | 43.25±1.32$^{a}$ | 43.42±1.12$^{a}$ | 43.45±1.11$^{a}$ | 43.65±1.12$^{a}$ | 43.61±1.29$^{a}$ | 45.24±0.65$^{a}$ | 45.59±0.39$^{a}$ |
| 5 | 42.15±1.45$^{a}$ | 34.42±0.05$^{b}$ | 34.45±2.10$^{b}$ | 35.65±1.10$^{b}$ | 34.61±1.27$^{b}$ | 42.54±0.10$^{a}$ | 42.36±0.95$^{a}$ |
| 7 | 42.35±0.41$^{a}$ | 33.75±1.00$^{b}$ | 33.56±1.19$^{b}$ | 36.61±1.20$^{b}$ | 37.85±0.57$^{b}$ | 43.34±0.25$^{a}$ | 44.41±1.19$^{a}$ |
| 9 | 43.31±1.12$^{a}$ | 31.11±0.12$^{c}$ | 30.31±0.75$^{c}$ | 37.34±1.01$^{b}$ | 36.69±0.24$^{b}$ | 45.66±0.24$^{a}$ | 45.95±0.57$^{a}$ |

a-c: different alphabets within a column indicate significant difference ($P<0.05$) between treatments within the same day (dpi). Values were expressed as mean±standard deviation (SD). CN: Negative control; PCM: Positive *E. maxima*; PCT: Positive *E. tenella*; SM: *E. maxima* treated with anticoccidial drug; ST: *E. tenella* treated with anticoccidial drug; RM: Rosemary fed *E. maxima* challenged group; RT: Rosemary fed *E. tenella* challenged group.

**TABLE 9. Effects of dietary supplemented Rosemary essential oils and anticoccidial drug on MCHC (g/dL) of broiler chickens infected with *E. maxima* and *E. tenella***

| Days (dpi) | CN | PCT | PCM | ST | SM | RT | RM |
|---|---|---|---|---|---|---|---|
| 0 | 31.49±0.67$^{a}$ | 31.68±1.30$^{a}$ | 31.79±0.38$^{a}$ | 31.45±0.05$^{a}$ | 30.99±1.02$^{a}$ | 33.55±0.35$^{a}$ | 32.98±0.65$^{a}$ |
| 5 | 31.55±0.04$^{a}$ | 22.66±1.11$^{c}$ | 22.65±1.24$^{c}$ | 24.69±0.57$^{d}$ | 24.54±1.21$^{d}$ | 28.99±1.36$^{b}$ | 29.31±1.51$^{b}$ |
| 7 | 31.51±1.05$^{a}$ | 21.56±1.23$^{c}$ | 21.01±1.09$^{c}$ | 25.71±0.63$^{b}$ | 25.51±1.01$^{b}$ | 30.53±1.24$^{a}$ | 31.65±1.31$^{a}$ |
| 9 | 31.25±0.68$^{a}$ | 20.65±0.54$^{c}$ | 20.71±1.14$^{c}$ | 26.04±0.54$^{b}$ | 26.40±1.08$^{b}$ | 33.65±1.54$^{a}$ | 32.59±1.25$^{a}$ |

a-d: different alphabets within a column indicate significant difference ($P<0.05$) between treatments within the same day (dpi). Values were expressed as mean±standard deviation (SD). CN: Negative control; PCM: Positive *E. maxima*; PCT: Positive *E. tenella*; SM: *E. maxima* treated with anticoccidial drug; ST: *E. tenella* treated with anticoccidial drug; RM: Rosemary fed *E. maxima* challenged group; RT: Rosemary fed *E. tenella* challenged group

**TABLE 10. Effects of REO and anticoccidial drug on PCV (%) of broiler chickens infected with *E. maxima* and *E. tenella***

| Days (Dpi) | CN | PCT | PCM | ST | SM | RT | RM |
|---|---|---|---|---|---|---|---|
| 0 | 31.54±0.99$^{b}$ | 31.69±0.2$^{b}$ | 30.99±0.41$^{b}$ | 31.66±0.22$^{b}$ | 30.79±0.54$^{b}$ | 33.54±0.51$^{a}$ | 33.63±0.78$^{a}$ |
| 5 | 30.64±0.61$^{b}$ | 23.60±2.1$^{d}$ | 22.79±0.31$^{d}$ | 26.61±0.54$^{c}$ | 25.64±1.51$^{c}$ | 31.78±0.45$^{a}$ | 31.94±0.91$^{a}$ |
| 7 | 31.69±0.89$^{b}$ | 22.45±1.0$^{d}$ | 21.77±0.12$^{d}$ | 27.55±1.91$^{c}$ | 26.50±2.64$^{c}$ | 32.51±0.32$^{a}$ | 32.04±0.41$^{a}$ |
| 9 | 31.55±0.51$^{b}$ | 19.61±1.11$^{d}$ | 19.89±0.50$^{d}$ | 28.01±2.11$^{c}$ | 27.61±1.05$^{c}$ | 33.45±0.15$^{a}$ | 33.51±0.21$^{a}$ |

a-d: different alphabets within a row indicate significant difference ($P<0.05$) between treatments within the same day (dpi). Values were expressed as mean±standard deviation (SD). CN: Negative control; PCM: Positive *E. maxima*; PCT: Positive *E. tenella*; SM: *E. maxima* treated with anticoccidial drug; ST: *E. tenella* treated with anticoccidial drug; RM: Rosemary fed *E. maxima* challenged group; RT: Rosemary fed *E. tenella* challenged group

**TABLE 11. Effects of dietary supplemented Rosemary essential oils and anticoccidial drug on Leucocytes (x10$^{10}$/microL) of broiler chickens infected with *E. maxima* and *E. tenella***

| Days (dpi) | CN | PCT | PCM | ST | SM | RT | RM |
|---|---|---|---|---|---|---|---|
| 0 | 2619.6±45.7$^{a}$ | 2617.6±64.3$^{a}$ | 2618.6±59.4$^{a}$ | 2579.2±24.1$^{a}$ | 2651.2±13.2$^{a}$ | 2671.5±25.5$^{a}$ | 2654.5±35.4 |
| 5 | 2671.7±35.48$^{c}$ | 3843.5±21.1$^{a}$ | 3938.2±34.0$^{a}$ | 3756.5±68.1$^{a}$ | 3657.2±21.2$^{a}$ | 2930.1±12.1$^{b}$ | 3044.5±13.4$^{b}$ |
| 7 | 2548.6±25.50$^{d}$ | 3851.1±35.1$^{a}$ | 3798.2±34.2$^{a}$ | 3261.1±81.9$^{b}$ | 3250.4±23.0$^{b}$ | 2804.2±15.2$^{c}$ | 2788.5±14.1$^{c}$ |
| 9 | 2606.8±30.1$^{b}$ | 3962.6±54.3$^{a}$ | 3954.5±23.3$^{a}$ | 2587.2±12.2$^{b}$ | 2665.5±10.5$^{b}$ | 2354.3±20.1$^{c}$ | 2321.5±2.2$^{c}$ |

a-c: different alphabets within a column indicate significant difference (*P<0.05*) between treatments within the same day (dpi). Values were expressed as mean±standard deviation (SD). CN: Negative control; PCM: Positive *E. maxima*; PCT: Positive *E. tenella*; SM: *E. maxima* treated with anticoccidial drug; ST: *E. tenella* treated with anticoccidial drug; RM: Rosemary fed *E. maxima* challenged group; RT: Rosemary fed *E. tenella* challenged group

**TABLE 12. Effects of dietary supplemented Rosemary essential oils and anticoccidial drug on Heterophils (x10$^{10}$/microL) of broiler chickens infected with *E. maxima* and *E. tenella***

| Days (dpi) | CN | PCT | PCM | ST | SM | RT | RM |
|---|---|---|---|---|---|---|---|
| 0 | 637.8±40.3$^{a}$ | 641.5±13.5$^{a}$ | 631.3±13.5$^{a}$ | 627.3±31.2$^{a}$ | 638.3±12.2$^{a}$ | 640.5±34.1$^{a}$ | 639.2±14.1$^{a}$ |
| 5 | 639.4±21.4$^{d}$ | 1025.2±40.5$^{a}$ | 998.3±40.2$^{a}$ | 952.5±32.2$^{a}$ | 879.4±40.3$^{b}$ | 780.5±35.8$^{c}$ | 774.5±32.4$^{c}$ |
| 7 | 638.3±34.2$^{c}$ | 1090.4±25.1$^{a}$ | 1019.5±35.1$^{a}$ | 850.21±45.3$^{b}$ | 920.3±41.2$^{b}$ | 680.5±21.3 $^{c}$ | 690.2±31.2$^{c}$ |
| 9 | 634.4±21.4$^{c}$ | 990.8±24.1$^{a}$ | 1004.5±35.4$^{a}$ | 720.54±35.3$^{b}$ | 750.4±35.1$^{b}$ | 655.2±21.3$^{c}$ | 659.3±15.5$^{c}$ |

a-d: different alphabets within a column indicate significant difference (*P<0.05*) between treatments within the same day (dpi). Values were expressed as mean±standard deviation (SD). CN: Negative control; PCM: Positive *E. maxima*; PCT: Positive *E. tenella*; SM: *E. maxima* treated with anticoccidial drug; ST: *E. tenella* treated with anticoccidial drug; RM: Rosemary fed *E. maxima* challenged group; RT: Rosemary fed *E. tenella* challenged group

**TABLE 13. Effects of dietary supplemented Rosemary essential oils and anticoccidial drug on eosinophils (x10$^{10}$/microL) of broiler chickens infected with *E. maxima* and *E. tenella***

| Days (dpi) | CN | PCT | PCM | ST | SM | RT | RM |
|---|---|---|---|---|---|---|---|
| 0 | 88.91±1.01$^{a}$ | 89.11±1.45$^{a}$ | 89.12±3.10$^{a}$ | 87.12±1.50$^{a}$ | 89.15±1.10$^{a}$ | 90.11±0.45$^{a}$ | 90.13±0.51$^{a}$ |
| 5 | 89.11±1.12$^{c}$ | 132.25±1.12$^{a}$ | 131.21±0.21$^{a}$ | 126.02±022$^{ab}$ | 125.04±0.41$^{ab}$ | 107.31±0.11$^{bc}$ | 105.45±0.10$^{bc}$ |
| 7 | 90.01±0.45$^{c}$ | 130.65±0.14$^{a}$ | 129.58±0.51$^{a}$ | 116.65±.3.22$^{ab}$ | 117.89±3.22$^{ab}$ | 100.41±1.01$^{b}$ | 101.11±0.11$^{bc}$ |
| 9 | 90.45±3.11$^{b}$ | 129.08±1.15$^{a}$ | 129.05±1.14$^{a}$ | 106.50±2.51$^{ab}$ | 100.10±3.51$^{ab}$ | 91.70±0.11 $^{b}$ | 90.31±0.21$^{b}$ |

a-c: different alphabets within a column indicate significant difference (*P<0.05*) between treatments within the same day (dpi). Values were expressed as mean±standard deviation (SD). CN: Negative control; PCM: Positive *E. maxima*; PCT: Positive *E. tenella*; SM: *E. maxima* treated with anticoccidial drug; ST: *E. tenella* treated with anticoccidial drug; RM: Rosemary fed *E. maxima* challenged group; RT: Rosemary fed *E. tenella* challenged group

**TABLE 14. Effects of dietary supplemented Rosemary essential oils and anticoccidial drug on lymphocytes (x10$^{10}$/microL) of broiler chickens infected with *E. maxima* and *E. tenella***

| Days (dpi) | CN | PCT | PCM | ST | SM | RT | RM |
|---|---|---|---|---|---|---|---|
| 0 | 1714.3±35.2$^{a}$ | 1718.3±50.5$^{a}$ | 1765.9±35.6$^{a}$ | 1786.6±55.5$^{a}$ | 1696.6±65.3$^{a}$ | 1787.3±15.3$^{a}$ | 1781.4±31.2$^{a}$ |
| 5 | 1717.8±35.4$^{c}$ | 2673.4±54.6$^{a}$ | 2556.2±35.1$^{a}$ | 2345.1±45.5$^{a}$ | 2373.4±41.2$^{a}$ | 1815.6±29.0$^{b}$ | 1827.7±18.6$^{b}$ |
| 7 | 1797.3±45.2s$^{c}$ | 2782.3±34.5$^{a}$ | 2841.2±35.5$^{a}$ | 2425.2±34.5$^{b}$ | 2573.2±35.2$^{ab}$ | 1797.5±31.6$^{c}$ | 1775.2±34.6$^{c}$ |
| 9 | 1756.3±45.2$^{c}$ | 2871.4±33.5$^{a}$ | 2954.3±35.5$^{a}$ | 2537.5±31.2$^{bc}$ | 2561.3±23.3$^{bc}$ | 1757.7±35.5$^{c}$ | 1778.5±45.5$^{c}$ |

a-c: different alphabets within a column indicate significant difference (*P<0.05*) between treatments within the same day (dpi). Values were expressed as mean±standard deviation (SD). CN: Negative control; PCM: Positive *E. maxima*; PCT: Positive *E. tenella*; SM: *E. maxima* treated with anticoccidial drug; ST: *E. tenella* treated with anticoccidial drug; RM: Rosemary fed *E. maxima* challenged group; RT: Rosemary fed *E. tenella* challenged group

**TABLE 15. Effects of dietary supplemented Rosemary essential oils and anticoccidial drug on monocytes (x10$^{10}$/microL) of broiler chickens infected with *E. maxima* and *E. tenella***

| Days (dpi) | CN | PCT | PCM | ST | SM | RT | RM |
|---|---|---|---|---|---|---|---|
| 0 | 125.31±5.39$^{b}$ | 125.69±3.10$^{b}$ | 125.99±1.12$^{b}$ | 125.01±1.01$^{b}$ | 124.99±2.10$^{b}$ | 126.65±1.12$^{a}$ | 127.59±1.12$^{a}$ |
| 5 | 126.21±1.10$^{c}$ | 171.65±9.10$^{a}$ | 174.51±10.74$^{a}$ | 142.64±5.21$^{b}$ | 143.25±1.50$^{b}$ | 128.91±1.21$^{c}$ | 128.20±3.01$^{c}$ |
| 7 | 125.99±1.01$^{c}$ | 165.21±10.54$^{a}$ | 164.82±7.58$^{a}$ | 135.21±0.21$^{b}$ | 137.54±3.51$^{b}$ | 128.23±2.10$^{c}$ | 127.99±0.21$^{c}$ |
| 9 | 126.24±2.11$^{b}$ | 150.31±14.51$^{a}$ | 155.21±8.188$^{a}$ | 131.31±2.10$^{b}$ | 132.58±4.50$^{b}$ | 127.98±2.10$^{b}$ | 127.39±1.51$^{b}$ |

a-c: different alphabets within a column indicate significant difference (*P<0.05*) between treatments within the same day (dpi). Values were expressed as mean±standard deviation (SD). CN: Negative control; PCM: Positive *E. maxima*; PCT: Positive *E. tenella*; SM: *E. maxima* treated with anticoccidial drug; ST: *E. tenella* treated with anticoccidial drug; RM: Rosemary fed *E. maxima* challenged group; RT: Rosemary fed *E. tenella* challenged group

**TABLE 16. Effects of REO and anticoccidial drug on serum corticosterone levels (ng/ml) of broiler chickens infected with *E. maxima* and *E. tenella***

| Days (dpi) | CN | PCT | PCM | ST | SM | RT | RM |
|---|---|---|---|---|---|---|---|
| 0 | 1.09±0.19$^{a}$ | 1.08±0.02$^{a}$ | 1.08±0.15$^{a}$ | 1.08±0.015$^{a}$ | 1.08±0.015$^{a}$ | 1.08±0.015$^{a}$ | 1.08±0.015$^{a}$ |
| 5 | 1.09±0.14$^{d}$ | 1.82±0.022$^{a}$ | 1.82±0.22$^{a}$ | 1.82±0.012$^{a}$ | 1.70±0.005$^{b}$ | 1.17±0.020$^{c}$ | 1.16±0.005$^{c}$ |
| 7 | 1.11±0.16$^{c}$ | 1.82±0.024$^{a}$ | 1.83±0.024$^{a}$ | 1.83±0.011$^{a}$ | 1.82±0.023$^{a}$ | 1.18±0.011$^{b}$ | 1.17±0.009$^{b}$ |
| 9 | 1.14±0.22$^{c}$ | 1.85±0.12$^{a}$ | 1.84±0.023$^{a}$ | 1.82±0.005$^{a}$ | 1.61±0.020$^{b}$ | 1.16±0.01$^{c}$ | 1.16±0.01$^{c}$ |

a-c: different alphabets within a row indicate significant difference (*P<0.05*) between treatments within the same day (dpi). Values were expressed as mean±standard deviation (SD). CN: Negative control; PCM: Positive *E. maxima*; PCT: Positive *E. tenella*; SM: *E. maxima* treated with anticoccidial drug; ST: *E. tenella* treated with anticoccidial drug; RM: Rosemary fed *E. maxima* challenged group; RT: Rosemary fed *E. tenella* challenged group

**TABLE 17. Effects of REO and anticoccidial drug on number of *Salmonella typhimurium* colonies (log of 10$^{6}$CFU/g) of broiler chickens infected with *E. maxima* and *E. tenella*.**

| Days (dpi) | CN | PCT | PCM | ST | SM | RT | RM |
|---|---|---|---|---|---|---|---|
| 0 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 5 | 0.00±0.00$^{c}$ | 5.77 ± 0.19$^{a}$ | 5.69±2.61$^{a}$ | 5.64 ± 0.31$^{a}$ | 5.45±3.54$^{a}$ | 3.78 ± 0.03$^{b}$ | 2.78 ± 0.02$^{b}$ |
| 7 | 0.00±0.00$^{d}$ | 7.88±3.81$^{a}$ | 8.99±1.34$^{a}$ | 6.76±3.21$^{b}$ | 6.54±3.58$^{b}$ | 1.58±0.22$^{c}$ | 1.41±1.04$^{c}$ |
| 9 | 0.00±0.00$^{d}$ | 9.83±4.12$^{a}$ | 9.87±4.48$^{a}$ | 7.57±1.64$^{b}$ | 7.87±1.21$^{b}$ | 0.01±0.02$^{cd}$ | 0.04±0.01$^{cd}$ |

a-d: different alphabets within a row indicate significant difference (*P<0.05*) between treatments within the same day (dpi). Values were expressed as mean±standard deviation (SD). CN: Negative control; PCM: Positive *E. maxima*; PCT: Positive *E. tenella*; SM: *E. maxima* treated with anticoccidial drug; ST: *E. tenella* treated with anticoccidial drug; RM: Rosemary fed *E. maxima* challenged group; RT: Rosemary fed *E. tenella* challenged

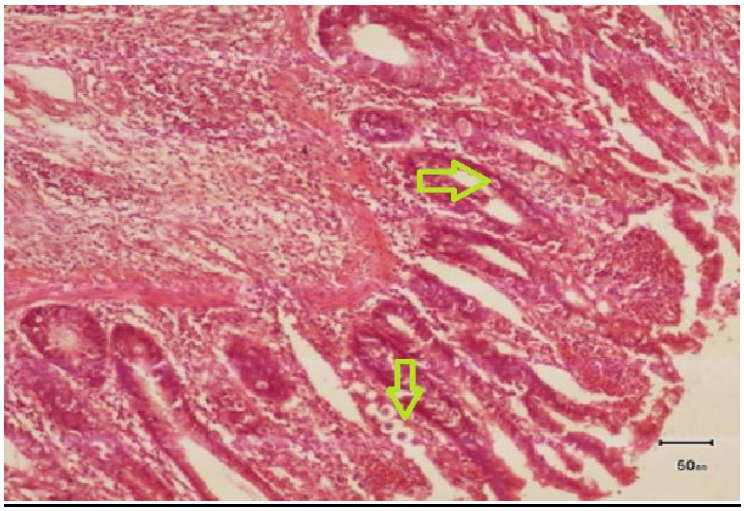


**Fig. 1. Histopathology of Ceacal pouches' section on 9th dpi from SM bird. Arrows showing presence of *E. tenella* oocytes. 100X magnification.**

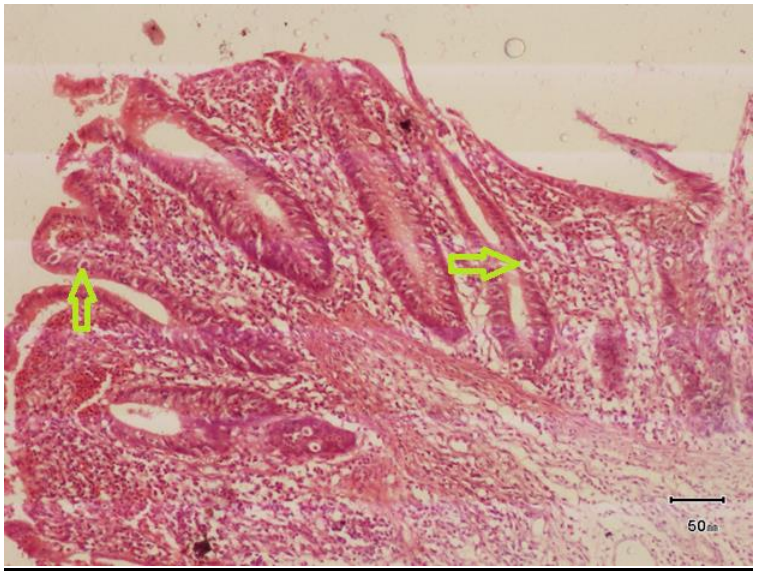


**Fig. 2. Histopathology of Intestinal section on 9th dpi from ST bird. Arrows showing presence of *E. maxima* oocytes. 100X magnification.**

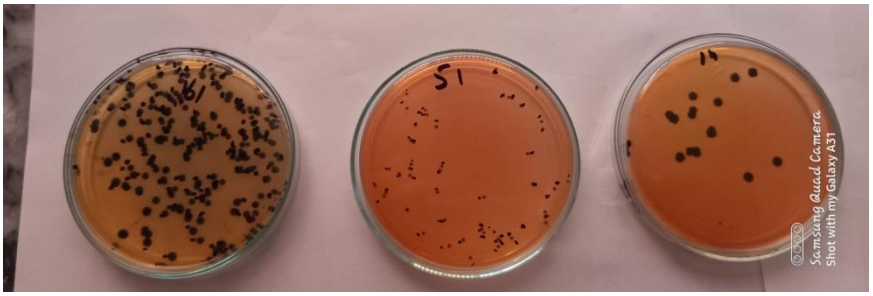


**Fig. 3. Colonies of *Salmonella typhimurium* on SSA 10-6 dilution on 9$^{th}$ dpi (a) Positive control maxima group (b) ST group (c) SM group**

**References**


1. Pop, L.M., Varga, E., Coroian, M., Nedișan, M.E., Mircean, V., Dumitrache, M.O., Farczádi, F.I., Croitoru, M.D., Fazakas, M. and Györke, A. Efficacy of a commercial herbal formula in chicken experimental coccidiosis. *Parasites and Vectors*, **12**, 343 (2019).
2. Blake, D.P., Knox, J., Dehaeck, B., Huntigton, B., Rathinam V., Ayoade S., Gilber W., Adebambo, A.O., Jatau, I.D., Raman, M., Parker, D., Rushton, J. and Tomley, F.M. Re-calculating the cost of coccidiosis in chickens. *Veterinary Research*, **51**, 115 (2020).
3. Lee, J.W., Kim, D.H., Kim, Y.B., Jeong, S.B., Oh, S.T., Cho, S.Y. and Lee, K.W. Dietary encapsulated essential oils improve production performance of coccidiosis-vaccine-challenged broiler chickens. *Animals*, **10**, 481 (2020).
4. Imran, A. and Alsayeqh, A. Anticoccidial efficacy of *Citrus sinensis* essential oil in broiler chicken. *Pakistan Veterinary Journal*, **42**, 461-466 (2022).
5. Chapman, H.D. Milestones in avian coccidiosis research: a review. *Poultry Science*, **93**(3), 501-11 (2014).
6. Deplazes, P., Eckert, J., Mathis, A., Samson-Himmelstjerna, G.V. and Zahner, H. Parasitology in veterinary medicine. Wageningen Academic Publishers, Netherland. *Parasitolology in Veterinary Medicine*, **1**, 650 (2016).
7. López-Osorio, S., Chaparro-Gutiérrez, J.J. and Gómez-Osorio, L.M. Overview of poultry *Eimeria* life cycle and host-parasite interactions. *Frontiers in Veterinary Science*, **7**, 384 (2020).
8. Burrell, A., Tomley, F.M., Vaughan, S. and Marugan-Hernandez, V. Life cycle stages, specific organelles and invasion mechanisms of *Eimeria* species. *Parasitology*, 147(3), 263-278 (2020).
9. Rotolo, J.L., Snyder, R.P., Imai, R.K., Répérant, J.M. and Barta, J.R. Description of a new *Eimeria* species *(Apicomplexa: Eimeriidae*) responsible for clinical coccidiosis in commercial Chukar partridge *(Alectoris chukar)*. *Journal of Parasitology*, **107**(4), 648-657 (2021).
10. Melkamu, S., Chanie, M. and Asrat, M. Haematological changes caused by coccidiosis in experimentally infected broiler chickens. *International Journal of Advanced Research in Biological Sciences*, **5**(5), 196-202 (2018).
11. Amin, Y., Aslam, A., Anwar., Pervez, K. and Ali, Z. Seasonal prevalence of *Eimeriosis* in broiler chicken. *Advances in Life sciences*, **1**(3), 160-164 (2014).
12. Nahed, A., Abd El-Hack, M.E., Albaqami, N.M., Khafaga, A.F., Taha, A.E., Swelum, A.A., El-Saadony, M.T., Salem, H.M., El-Tahan, A.M. and AbuQamar, S.F. Phytochemical control of poultry coccidiosis: a review. *Poultry Science*, **101**, 10154, (2022).
13. Saeed, Z., Abbas, R.Z., Khan, M.K. and Saleemi, M.K. Anticoccidial activities of essential oil of *Amomum subulatum* in broiler chicks. *Pakistan Journal of Agricultural Sciences*, **60**, 2 (2023).
14. Flores, R.A., Nguyen, B.T., Cammayo, P.L.T. et *et al.*, Epidemiological investigation and drug resistance of *Eimeria* species in Korean chicken farms. *BMC Veterinary Research*, **18**, 277 (2022).
15. de Oliveira, J.R., SEA, C. and De Oliveira, L.D. *Rosmarinus officinalis L* (Rosemary) as therapeutic and prophylactic agent. *Journal of Biomedical Science*, **26**(1), 5 (2019).
16. Barbarestani, S.Y., Jazi, V., Mohebodini, H., Ashayerizadeh, A., Shabani, A. and Toghyani, M. Effects of dietary lavender essential oil on growth performance, intestinal function, and antioxidant status of broiler chickens. *Livestock Science*, **233**, 103958 (2020).
17. Shang, R., Chen, L., Xin, Y., Wang, G., Li, R., Li, S. and Li, L. Evaluation of rosmarinic acid on broiler growth performance, serum biochemistry, liver antioxidant activity, and muscle tissue composition. *Animals (Basel)*, **12**(23), 3313 (2022).
18. Norouzi, B., Ali, A.A.Q., Seidavi, A., Schiavone, A. and Martínez, M.A.L. Effect of different dietary levels of Rosemary (*Rosmarinus officinalis*) and Yarrow (AchilleaMillefolium) on the growth performance, carcass traits and ileal micro-biota of broilers. *Italian Journal of Animal Science*, **14**, 3 (2015).
19. de Sousa, D.P., Damasceno, R.O.S., Amorati, R., Elshabrawy, H.A., de Castro, R.D., Bezerra, D.P., Nunes, V.R.V., Gomes, R.C. and Lima, T.C. Essential oils: chemistry and pharmacological activities. *Biomolecules*, **13**, 1144 (2023).
20. Patra, J.K., Das, G., Bose, S., Banerjee, S., Vishnuprasad, C.N., Rodriguez-Torres, M.D.P. and Shin, H.S. Star anise *(Illicium verum)*: Chemical compounds, antiviral properties, and clinical relevance. *Phytotherapy Research*, **34** (6), 1248-1267 (2020).
21. Akhbari, M., Masoum, S., Aghababaei, F. and Hamed, S. Optimization of microwave assisted extraction of essential oils from Iranian *Rosmarinus officinalis* L. using RSM. *Journal of Food Science and Technology*, **55**(6), 2197–2207 (2018).
22. Elyemni, M., Bouchra, L., Nechad, I., Taha, E., Abdelhak, B., Mustapha, T., Mahdi, C. and Noureddine, E. Extraction of essential oils of *Rosmarinus officinalis* L. by two different methods: Hydrodistillation and Microwave Assisted Hydrodistillation. *Science World Journal*, **6**, (2019).
23. Aouadi, M., Sebai, E., Saratsis, A., Kantzoura, V., Saratsi, K., Msaada, K., Sotiraki, S. and Akkari, H. Essential oil of *Rosmarinus officinalis* induces in vitro anthelmintic and anticoccidial effeacts against *Haemonchus contortus* and *Eimeria* spp. in small ruminants. *Veterinary Medicine*, **66**(04), 146–155 (2021).
24. Ghozlan, S.A., El-Far, A.H., Sadek, K.M., Abourawash, A.A. and Abdel-Latif, M.A. Effect of Rosemary (*Rosmarinus officinalis*) dietary supplementation in broiler chickens concerning immunity, Antioxidant status, and performance. *Alexandrian Journal of Veterinary Sciences*, **55**(1), 152-161 (2017).

25. Gumus, R. and Gelen, S.U. Effects of dietary Thyme and Rosemary essential oils on performance parameters with lipid oxidation, water activity, pH, colour and microbial quality of breast and drumstick meats in broiler chickens. *Archives Animal Breeding*, **9**(66), 17-29 (2023).

26. Zhenhua, Y., Xing, L., Luo, W., Xu, Z., Ji, C., Zhang, Y., Nie, Q., Zhang, D. and Zhang, X. Feed conversion ratio, residual feed intake and cholecystokinin type A receptor gene polymorphisms are associated with feed intake and average daily gain in a Chinese local chicken population. *Journal of Animal Science and Biotechnology*, **9**, 50 (2018).

27. Johnson, J. and Reid, W.M. Anticoccidial drugs, lesion scoring techniques in battery and floor-pen experiments with chickens. *Experimental Parasitolology*, **28**(1), 30 (1970).

28. Luna, L.G. Manual of histologic staining methods of the Armed Forces Institute of Pathology. 3rd Edition, McGraw-Hill, New York, (1968).

29. Urara, K., Bordin, E.L., de Lima, C.A. and Dias, L.A.V. Characterisation and histopathological observations of a selected Brazilian precocious line of *Eimeria acervulina*. *Veterinary Parasitology*, **131**(1-2), 4-14 (2005).

30. Yang, C., Li, H., Tao, Z., Chu, Y., Zou, J. and Chen, D. Study on antibiotic susceptibility of *Salmonella typhimurium* L forms to the third and fourth generation Cephalosporins. *Scientific Reports*, **10**, 3042 (2020).

31. Odunitan-Wayas, F., Kolanisi, U. and Chimonyo, M. Haematological and serum biochemical responses of ovambo chickens fed provitamin A Biofortified Maize. *Rev. Bras. Cienc. Avic. (Brazilian Journal of Poultry Science)*, **20**, 03 (2018).

32. Venkatas, J. and Adeleke, M.A. Emerging threat of *Eimeria* operational taxonomic units (OTUs) on poultry production. *Parasitology*, **146**(13), 1615-1619 (2019).

33. Attree, E., Arsuaga, G.S., Jones, M., Xia, D., Hernandez, V.M., Blake, D. and Tomley, F. Controlling the causative agents of coccidiosis in domestic chickens; an eye on the past and considerations for the future. *CABI Agriculture and Bioscience*, **2**, 37 (2021).

34. Macdonald, S.E., Van Diemen, P.M., Martineau, H., Stevens, M.P., Tomley, F.M., Stabler, R.A. and Blake, D.P. Impact of *Eimeria tenella* coinfection on *Campylobacter jejuni* colonization of the chicken. *Infection and Immunity*, **87**, 18–24 (2019).

35. Adhikari, P., Kiess, A., Adhikari, R. and Jha, R. An approach to alternative strategies to control avian coccidiosis and necrotic enteritis. *Journal of Applied Poultry Research*, **29**(2), 515-534 (2020).

36. Soutter, F., Werling, D., Tomley, F.M. and Blake, D.P. Poultry Coccidiosis: Design and interpretation of vaccine studies. *Frontiers in Veterinary Science*, **7**, 101 (2020).

37. Akrout, A., Hajlaoui, H., Mighri, H., Najja, H., Jani, H.E., Slah, Z. and Neffati, M. Chemical and Biological Characteristics of Essential oil of *Rosmarinus officinalis* cultivated in Djerba. *Journal of Essential Oil-bearing Plants*, **13**(4), 398-411 (2010).

38. Szumny, A., Figiel, A., Gutierrez-Ortiz, A. and Carbonell-Barrachina, A.A. Composition of Rosemary essential oil *(Rosmarinus officinalis)* as affected by drying method. *Journal of Food Engineering*, **97**(2)**,** 253-260 (2010).

39. Elyemni, M., El Ouadrhiri, F., Lahkimi, A., Elkamli, T., Bouia, A. and Eloutassi, N. Chemical composition and antimicrobial activity of essential oil of wild and cultivated *Rosmarinus officinalis* from two Moroccan localities. *Journal of Ecological Engineering*, **23**(3), 214-222 (2022).

40. Boukhari, E.R. and Fatimi, A.A. review of the patentability of rosemary-derived drugs and bioactive compounds. *Drugs Drug Candidates*, **2**, 172-188 (2023).

41. Abd El-Hack, M.E., El-Saadony, M.T., Saad, A.M., Salem, H.M., Ashry, N.M., Ghanima, M.M.A., Shukry, M., Swelum, A.A., Taha, A.E. and El-Tahan, A.M. Essential oils and their nanoemulsions as green alternatives to antibiotics in poultry nutrition: a comprehensive review. *Poultry Science*, **101**, 101584 (2022).

42. Raza, Q.S., Saleemi, M.K., Gul, S., Irshad, H., Fayyaz, A., Zaheer, I., Tahir, M,W., Fatima, Z. and Chohan, T.Z., Imran, M. Role of essential oils/volatile oils in poultry production—A review on present, past and future contemplations. *Journal of Agrobiological Records*, **7**, 40-56 (2022).

43. Puvaca, N., Horvatek, T.D., Popović, S., Đorđević, S., Brkić, I., Lalić, N., Kika, T.S. and Lika, E. Influence of tea tree (*Melaleuca alternifolia*) essential oil as feed supplement on production traits, blood oxidative status and treatment of coccidiosis in laying hens. *Veterinarski Arhive (Veterinary Archive)*, **90**, 331-340 (2020).

44. Sidiropoulou, E., Skoufos, I., Marugan-Hernandez, V., Giannenas, I., Bonos, E., Aguiar-Martins, K., Lazari, D., Blake, D.P. and Tzora, A**.** In vitro anticoccidial study of oregano and garlic essential oils and effects on growth performance, fecal oocyst output, and intestinal microbiota in vivo. *Frontiers in Veterinary Science*, **7**, 420 (2020).

45. Chang, L.Y., Di, K.Q., Xu, J., Chen, Y.F., Xi, J.Z., Wang, D.H., Hao E.Y., Xu, L.J., Chen, H. and Zho, R.Y. Effect of natural garlic essential oil on chickens with artificially infected *Eimeria tenella*. *Veterinary Parasitology*, **300**, 109614 (2021).

46. Amera, S.A., Saleh, N.S., Allam, T.S. and Ghazy, E.W. The effects of Rosemary *(Rosmarinus officinalis)* and Garlic (*Allium sativum*) essential oils on performance, haematological, Biochemical and Immunological parameters of broiler chickens. *British Poultry Science*, **2**(2), 16-24 (2013).

47. Ghazalah, A.A. and Ali, A.M. Rosemary leaves as a dietary supplement for growth in broiler chickens. *International Journal of Poultry Science*. **7**, 234-239 (2008).

48. Murakami, A.E. and Eyng, C., Torrent, J. Effects of functional oils on coccidiosis and apparent metabolizable energy in broiler chickens. *Asian Australas Journal of Animal Sciences*, **27**(7), 981-989 (2014).

49. ELnaggar, A.S. and E-Tahawy, W.S. Productive performance, physiological and immunological response of broiler chicks as affected by dietary aromatic plants and their essential oils. *Egyptian Poultry Science Journal*, **38**(3), 773-795 (2018).

50. Bozkurt, M., Aysul, N., Küçükyilmaz, K., Aypak, S., Ege, G., Çatli, A.U., Akşit, H., Çöven, F., Seyrek, K. and Çına, M. Efficacy of in-feed preparations of an anticoccidial, multienzyme, prebiotic, probiotic, and herbal essential oil mixture in healthy and *Eimeria* spp.-infected broilers. *Poultry Science*, **93**(2), 389-399 (2014).

51. Ding, Y., Hu, Y., Yao, X., He, Y., Chen, J., Wu, J., Wu, S., Zhang, H., He, X. and Song, Z. Dietary essential oils improves the growth performance, antioxidant properties and intestinal permeability by inhibiting bacterial proliferation, and altering the gut microbiota of yellow- feather broilers. *Poultry Science*, **101**, 102087 (2022).

52. Li, A.L., Ni, W.W., Zhang, Q.M., Li, Y., Zhang, X., Wu, H.Y., Du, P., Hou, J.C. and Zhang, Y. Effect of Cinnamon essential oil on gut microbiota in the mouse model of dextran sodium sulfate-induced colitis. *Microbiology and Immunology*, **64**, 23-32 (2020).

53. Scanes C.G. Biology of stress in poultry with emphasis on glucocorticoids and the heterophil to lymphocyte ratio. *Poultry Science*, **95**, 2208–2215 (2016).

54. Oakley, B.B., Vasconcelos, E.J.R., Diniz, P.P.V.P., Calloway, K.N., Richardson, E., Meinersmann, R.J., Cox, N.A. and Berrang, M.E. The cecal microbiome of commercial broiler chickens varies significantly by season. *Poultry Science*, **97**(10), 3635-3644 (2018).