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Combinatorial Effect of Dietary Oregano Extracts and 3,4,5-Trihydroxy Benzoic Acid on Growth Performance and Elimination of Coccidiosis in Broiler Chickens

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We aimed to compare the combinatorial effect of 3,4,5-trihydroxybenzoic acid (THB) and oregano extracts (OE) with THB alone on the growth performance and elimination of deleterious effects in coccidiosis-infected broilers. A total of 210 one-day-old broilers were randomly assigned to one of five dietary treatments, with six replicates each, for 35 days. Dietary treatments were: 1) non-challenged, non-treated (NC); 2) challenged, non-treated (PC); 3) PC+Salinomycin (0.05 g/kg; AB); 4) PC+THB (0.1 g/kg; THB); and 5) PC+THB+OE (0.1 g/kg; COM). On day 14, all groups except for NC were challenged with a 10-fold dose of Livacox® T anticoccidial vaccine to induce mild coccidiosis. All treatments significantly improved ($P<0.05$) body weight, average daily gain, and average daily feed intake, compared to PC, on days 21, 28, and 35. However, all treatments significantly reduced ($P<0.05$) the feed conversion ratio of PC by more than 14.60% on day 35, 11.76% during growing period, and 10.36% through the entire period. Broilers receiving anticoccidial treatments had 54.23% and 51.86% lower lesion scores ($P<0.05$) at 4 and 7 days post-infection, respectively, compared to PC. Additionally, the villus height of COM was significantly longer ($P<0.05$) than that of THB. Although the molecular action of COM remains unclear, OE addition to THB reduced the shedding of oocysts better than THB alone ($P<0.05$, 9–11 days post-infection). Most importantly, COM effectively minimized the mortality of challenged birds from as high as 11.90% (PC) to 0%, a level similar to NC and AB, while THB maintained a mortality of 2.38%. In conclusion, the anticoccidial effect of THB can be enhanced by the addition of OE for better animal performance and the elimination of deleterious effects from coccidiosis-infected broilers for 35 days.

Key words: broiler, coccidiosis, elimination, growth performance, oregano extracts, 3,4,5-trihydroxy benzoic acid

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Introduction

Poultry coccidiosis is considered a major parasitic disease caused by coccidia, which comprise a wide variety of single-celled protozoa, particularly those of the genus *Eimeria* (Conway and McKenzie, 2007; Ali *et al*., 2019). Coccidiosis leads to high mortality rates and reduced performance, including impaired feed intake, feed efficiency, and the need for excessive medication (Tanweer *et al*., 2014; Chand *et al*., 2016; Abudabos *et al*., 2017), resulting in considerable economic losses (Amerah and Ravindran, 2014; Tanweer *et al*., 2014). *Eimeria* parasites colonize and reproduce in the intestinal regions and infect the digestive tract, causing tissue destruction of the epithelial cells and, eventually, lesion formation (Gilbert *et al*., 2011; Soutter *et al*., 2020). This phenomenon may lead to bloody diarrhea and osmotic stress in birds. Consequently, infected birds exhibit reduced digestibility and malabsorption of nutrients, eventually result-

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ing in higher morbidity and mortality rates (Persia *et al*., 2006; Perez-Carbajal *et al*., 2010; Arczewska-Wlosek and Swiatkiewicz, 2012).

Immunity boosting through vaccination and anticoccidial medication is commonly practiced to prevent coccidiosis in poultry (Chand *et al*., 2016). Broad-spectrum anticoccidials are supplemented in feed on a prophylactic basis to strengthen the immunity of birds against multiple *Eimeria* species (Chapman, 1998; Allen and Fetterer, 2002; Conway and McKenzie, 2007). Anticoccidial products can be classified into three major groups based on their origin: chemically synthesized compounds, ionophores/polyether antibiotics (produced by fermentation of *Streptomyces* spp. or *Actinomadura* spp.), and a mixture of synthetic compounds and ionophores (Allen and Fetterer, 2002; Peek and Landman, 2011). Polyether antibiotics are effective against *Eimeria*, as they destroy the sodium/potassium concentration gradient across the parasitic cell membrane. This alters the intracellular pH, increases osmotic pressure (followed by osmotic swelling), induces cell vacuolization, and impedes mitochondrial function (Lavine and Arrizabalaga, 2011; Kala *et al*., 2014; Antoszczak *et al*., 2019). Moreover, a popular global strategy has been the addition of antimicrobial compounds to broiler feed at sub-therapeutic levels to improve the growth performance of birds in terms of body weight (BW) and feed efficiency (Lin *et al*., 2013). Nonetheless, haphazard use of antimicrobial compounds has led to the development and spread of antimicrobial resistance against bacterial diseases, which adversely affects consumer perception and the quality of the final product (Stanley *et al*., 2004; Samuel *et al*., 2017). To prevent the transmission of antimicrobial resistance genes and antibiotic residue accumulation in the food chain, the European Union, South Korea, and the United States banned the use of antimicrobial growth promoters in animal feed in 2006, 2010, and 2017, respectively (Maron *et al*., 2013; Alloui *et al*., 2014; Giannenas *et al*., 2014; Salim *et al*., 2018; Roth *et al*., 2019). Consequently, there is a tremendous demand for alternatives to antimicrobial growth promoters, such as phytogenics, to reduce the incidence of poultry coccidiosis (Tanweer *et al*., 2014; Mohiti and Ghanaatparast, 2015; Ali *et al*., 2019).

Phytogenic compounds are a broad range of plant-derived natural bioactive composites that can be supplemented in broiler diets, either as essential oils or herbal extracts, to enhance animal growth performance (Hashemi and Davoodi, 2010; Puvača *et al*., 2013). Due to their unique biological and chemical properties, including antimicrobial, antioxidative, and antiparasitic properties, phytogenic compounds are emerging as naturally sourced ingredients used to combat poultry coccidiosis. The plant-derived phenolic compound 3,4,5-trihydroxybenzoic acid (THB), or gallic acid, can mitigate the negative impact of coccidiosis in broilers and improve gut health, BW, and feed efficiency, while reducing the number of oocysts in excreta (Samuel *et al*., 2017; Tinh *et al*., 2019). The antioxidative nature of THB and its ability to scavenge reactive oxygen species (ROS) helps mitigate coccidiosis conditions and serves as a natural antimicrobial agent (Kroes *et al*., 1992; Naidoo *et al*., 2008; Özçelik *et al*., 2011).

Furthermore, feeding a diet supplemented with essential oils derived from *Origanum vulgare* improves growth performance and intestinal integrity of broiler chickens when coccidiosis occurs (Giannenas *et al*., 2003; Tsinas *et al*., 2011; Alp *et al*., 2012). Oregano extracts (OEs) comprising terpenoids (isoprenoids), such as carvacrol and thymol, have demonstrated antiprotozoal and antimicrobial properties against *Eimeria*. These extracts increased cell membrane permeability, resulting in cytoplasmic coagulation, proton motive force reduction, and protein molecule denaturation in parasitic cells (Nazzaro *et al*., 2013; Gavaric *et al*., 2015; Felici *et al*., 2020).

To the best of our knowledge, few studies have examined the efficacy of OE and THB combinations in the treatment of coccidiosis in broilers. Therefore, this study was designed to investigate and compare the effects of the combination of THB+OE and THB alone on the growth performance and elimination of coccidia in infected broilers. The hypothesis is that synergistic anticoccidial effects and improved growth performance can be achieved by supplementing a specific combination of natural phytochemicals (i.e., THB and OE) with divergent anticoccidial mechanisms in infected broilers.

Materials and Methods

The rearing of all birds complied with the guidelines of the Ross® broiler management handbook (Aviagen, 2014a). All animal handling procedures adhered to the guidelines of the Animal Care and Use Committee (Protocol No. 202006A-CNU-092), Chungnam National University, Republic of Korea.

Birds and Management

The experiment was conducted using 210 one-day-old male "Ross® 308" (a trademark of Aviagen Group, Huntsville, AL, USA) broiler chickens for 35 days. Birds were randomly allocated to five dietary treatments as: non-challenged, non-treated(NC), challenged, non-treated (PC), PC+Salinomycin (AB), PC+THB (THB), and PC+THB+OE (COM) (Table 1). Each dietary treatment consisted of six replicate pens. Seven birds with similar initial BWs (44.61±0.07 g) were housed in each raised battery cage (76×61×46 cm). The initial temperature was maintained at 30±1℃ for the first 3 days of the experiment and then gradually decreased to 25±1℃ until day 14. Thereafter, a temperature of 25±1℃ was maintained throughout the experimental period. Each pen was equipped with two nipple drinkers and a metal feeding trough. Birds were offered the experimental diets on an *ad libitum* basis via a metal trough of the pen and had free access to fresh clean drinking water throughout the experimental period.

Induction of Coccidial Infection

All the birds were challenged (except those in the NC group) by an overdose (10× recommended dose) of the Livacox® T anticoccidial vaccine (Biopharm, Research Institute of Biopharmacy and Veterinary Drugs, Jílové u Prahy, Czech Republic) on day 14. One milliliter of inoculum (i.e., 0.1 mL of vaccine with 0.9 mL of sterile distilled water) was orally gavaged directly into the crop of each bird in the challenged groups, while those in the NC group were ad-

Table 1. **Dietary treatments**

Treatment	Identity	Details
1	NC	Control diet with no coccidiosis challenge or anti-coccidiosis treatment / non-challenged, non-treated
2	PC	Control diet with coccidiosis challenge and no anti-coccidiosis treatment / challenged, non-treated
3	AB	PC+Salinomycin (0.05 g/kg)
4	THB	PC+THB (0.1 g/kg)
5	COM	PC+THB+OE (0.1 g/kg)

Table 2. **Composition (g/kg, as-fed basis) of the experimental diets**

Item	Starting period (Days 1–21)	Growing period (Days 22–35)
Corn	48.51	60.96
Wheat, Hard Red Winter	8.40	4.12
Wheat bran	4.10	—
Soybean meal 48%	31.15	27.62
Vegetable Oil	3.30	3.30
Limestone	1.20	0.95
Mono-calcium Phosphate	1.65	1.39
Salt	0.30	0.35
Vitamin-Mineral Premix[1]	0.30	0.30
Lysine-HCl	0.34	0.30
DL-Methionine	0.20	0.19
L-Threonine	0.13	0.11
L-Cystine	0.12	0.11
Cr_2O_3	0.30	0.30
Calculated values[2]		
Crude protein, %	22.0	20.0
Metabolizable energy, kcal/kg	3050	3200
Lysine, %	1.40	1.20
Methionine, %	0.62	0.60

[1] Provided per kilogram of diet: vitamin A, 12,000 IU; vitamin D3, 2,500 IU; vitamin E, 30 IU; vitamin K3, 3 mg; D-pantothenic acid, 15 mg; nicotinic acid, 40 mg; choline, 400 mg; and vitamin B12, 12 µg; Fe, 90 mg from iron sulfate; Cu, 8.8 mg from copper sulfate; Zn, 100 mg from zinc oxide; Mn, 54 mg from manganese oxide; I, 0.35 mg from potassium iodide; Se, 0.30 mg from sodium selenite.
[2] The values were calculated according to the values of feedstuffs in NRC (1994) and "Ross 308" Broiler Nutrition Specification (2013).

ministered the same amount of sterile distilled water. The Livacox® T vaccine contains *Eimeria acervulina, E. maxima*, and *E. tenella* strains, which were responsible for coccidiosis in the respective broilers.

Experimental Design, Diets, and Treatments

The experiment was conducted according to a completely randomized design. Five dietary treatments were used (Table 1). Both THB and THB+OE additives were supplied with the product name, "VANTIPEARLTM 201" and "ORSENTIALTM Extend" from Kemin Industries Asia Pte Ltd., Singapore, respectively. All the diets were formulated based on corn and soybean meal to meet the Ross 308 nutrition specification (Aviagen, 2014b; Table 2). All additives were top-dressed to the basal diet. Chromium oxide powder (Cr_2O_3, >99.9% purity; Sigma-Aldrich, St. Louis, MO, USA) was added at a concentration of 0.3% for all experimental diets as an internal indigestible marker for digestibility analysis.

Growth Performance Evaluation

The BWs of the birds were recorded individually on day 1 and weekly thereafter. Feed disappearance on a pen basis was measured weekly. The mortality-corrected average daily feed intake was calculated. The feed conversion ratio was calculated based on the feed intake and weight gain every week.

Slaughtering of Birds, Sample Collection, and Sample Preparation for Laboratory Analysis

Six birds per treatment (one bird per cage) that were closer to the mean BW were selected and euthanized by cervical dislocation for sample collection on days 18 and 21 (4- and 7-days post-infection). The birds were necropsied and ileum samples for morphological analysis were excised and prepared as described by Wickramasuriya *et al*. (2019) and Pelicano *et al*. (2005). A 3 cm fragment of the ileum (i.e., identified as the intestinal segment from the Meckel's diverticulum to the ileocecal junction) was collected from each sacrificed bird, rinsed with phosphate buffered saline, stored in 10% formaldehyde, and dehydrated with increasing concentrations of ethanol. Thereafter, the intestinal segments were embedded into paraffin wax blocks to obtain ring-shaped dehydrated ileal tissues. Finally, six diagonal histological sections (4–6 μm) were sliced, stained with hematoxylin and eosin, and placed on glass slides. The height of ten well-aligned villi and their associated crypts were observed using an Eclipse TE2000 inverted microscope (Eclipse TE2000, Nikon Instruments Inc., Melville, NY, USA). The height of the villi and the depth of the crypts were measured by analyzing images of histological sections made from the computerized image-capture software (NIS-Elements Viewer software, Version: 4.20; NIS-Elements, Nikon Instruments Inc.). The height of the villi was defined as the distance from the tip to the base. The depth of the crypt was defined as the distance from the top of the crypt to the muscularis mucosa (Seyyedin and Nazem, 2017).

Ileal digesta samples from each sacrificed broiler were collected and stored at −20℃ until further analysis for ileal nutrient digestibility. Before analysis, the samples were oven-dried at 55℃ for 24 h, followed by fine grinding and strained through a sieve of $<$0.75 mm (Wickramasuriya *et al*., 2019). AOAC (2005) standard analytical methodologies were used to determine the dry matter, crude protein, and energy content in digesta samples, and the concentration of Cr_2O_3 in the feed and digesta was analyzed as described by Huang *et al*. (2005) and Wickramasuriya *et al*. (2019). The coefficient of digestibility was calculated as 1-[(ID×AF)/(IF×AD)], where ID is the concentration of the indigestible marker in the diet, IF is the concentration of the indigestible marker in the digesta, AD is the particular nutrient concentration in the diet, and AF is the particular nutrient concentration in the digesta.

The dressing percentage was determined from the properly eviscerated hot carcass of broilers (Jahejo *et al*., 2016). Subsequently, breast meat and drumstick samples were obtained to evaluate the relative weights corresponding to the carcass weights of broilers.

Lesion Scoring

Lesion scores in the experimental broilers were determined by separately observing the lesions in the digestive tract, including the jejunum (from the insertion of duodenal mesentery to the Meckel's diverticulum), ileum, and ceca. Generally, lesions in these regions are caused by *E. acervulina*, *E. maxima*, and *E. tenella*, respectively (Conway and McKenzie, 2007). The scoring was performed on a scale of 0 to 4, according to the assessment method of Johnson and Reid (1970), where 0=no lesion, 1=mild lesion, 2=moderate lesion, 3=severe lesion, and 4=extremely severe lesion. The total lesion score for the intestine was calculated using the separate lesion scores from the three different intestinal segments.

Fecal Analysis of the Number of Oocysts Per Gram (OPG Count)

Clean excreta (free from feathers and feed) were collected separately on days 7, 8, 9, 10, and 11 post-infection to enumerate oocysts from each cage. The collected samples were refrigerated until analysis. The OPG count was measured using the McMaster method of Soulsby (1982) and according to the procedures of Mwale and Masika (2011) with slight modifications. Briefly, 4 g of fecal sample was suspended in a 56 mL saturated salt solution (flotation solution) and the mixture was filtered carefully using a sterilized cheesecloth. The filtrate was loaded into both chambers of the McMaster counting slide using a micropipette and kept aside for 5 min before counting. The number of total oocysts in the chambers was counted separately by observation at 10× magnification using an Eclipse TE 2000 compound microscope. The OPG count in each replicate was calculated by multiplying the number of oocysts counted in both chambers by a factor of 50. The final results were expressed as $\log_{10}$ oocysts/g feces for each treatment.

Statistical Analyses

Data were analyzed according to a completely randomized design using a general linear model procedure of one-way ANOVA using SPSS software (Version 26; IBM, Armonk, NY, USA). A single pen was used as the experimental unit for all growth performance measurements and OPG counting. Selected individual birds were considered the experimental unit for the mean lesion score, gut morphology, digestibility, and carcass trait analyses. When treatment effects were significant ($P<0.05$), means were separated using Tukey's multiple range test in SPSS software.

Results

Growth Performance

No mortality was observed in the NC, AB, and COM groups during the 35-day trial. All deaths in the other groups were reported following the coccidial challenge, with a record of 2.38% and 11.90% for THB and PC, respectively.

There was no difference ($P>0.05$) in the BW of broilers among the dietary treatments within 14 days after hatching (Table 3). The BWs of NC and PC birds were the highest ($P<0.05$) and lowest ($P<0.05$), respectively, compared to the birds in the other groups, from days 21 to 35. Supplementation with antibiotics significantly improved ($P<0.05$) the BW of broilers, compared to those fed THB and COM, from days 21 to 35. The COM group displayed numerically improved ($P<0.1$) BW of coccidia-challenged broiler chickens compared to the THB group by 2.03% on day 28 and 4.24% on day 35.

NC broilers had the highest ADG and ADFI (both $P<0.05$), followed by the AB-treated birds during the starting, growing, and whole phases of the experimental periods. In contrast, PC broilers had the lowest ADG and ADFI (both $P<0.05$) at the same time points. The COM group displayed improved ADG during the growing period and the overall experimental period (both $P<0.01$), compared to the THB group. In addition, the COM group displayed numerically enhanced ($P>$

Table 3. **Effect of dietary treatments on growth performance of coccidiosis challenged broiler chickens.**[1]

Period	Experimental Diets					SEM[2]	P-value
	NC	PC	AB	THB	COM		
Body weight, g							
Day 1	44.67	44.67	44.57	44.50	44.62	0.106	0.989
Day 7	158.19	145.02	148.17	146.88	143.40	2.241	0.258
Day 14	333.31	310.98	326.43	328.17	310.29	5.580	0.611
Day 21	788.11[c]	537.53[a]	725.83[bc]	695.21[b]	689.32[b]	17.217	<0.001
Day 28	1376.40[d]	881.50[a]	1253.23[c]	1142.23[b]	1165.37[bc]	32.855	<0.001
Day 35	2003.39[d]	1253.37[a]	1840.81[c]	1692.12[b]	1763.94[bc]	48.721	<0.001
Average daily gain, g/d							
Day 7	16.22	14.34	14.80	14.63	14.11	0.318	0.254
Day 14	25.02	23.71	25.47	25.90	23.84	0.704	0.841
Day 21	64.97[c]	32.36[a]	57.06[bc]	52.43[b]	54.15[b]	2.237	<0.001
Day 28	84.04[d]	49.14[a]	77.72[cd]	63.86[b]	68.01[bc]	2.567	<0.001
Day 35	89.57[c]	53.12[a]	83.94[bc]	78.55[b]	85.51[bc]	2.677	<0.001
Day 1–21	35.40[c]	23.47[a]	32.44[bc]	30.99[b]	30.70[b]	0.820	<0.001
Day 22–35	86.81[d]	51.13[a]	80.83[cd]	71.21[b]	76.76[bc]	2.382	<0.001
Day 1–35	55.96[d]	34.53[a]	51.80[c]	47.08[b]	49.12[bc]	1.397	<0.001
Average daily feed intake, g/d							
Day 7	23.40	22.67	23.00	22.57	22.89	0.305	0.936
Day 14	39.16	38.32	38.00	38.46	35.87	0.601	0.513
Day 21	92.59[c]	58.90[a]	86.18[bc]	81.86[b]	81.39[b]	2.365	<0.001
Day 28	121.91[d]	80.27[a]	114.12[cd]	96.60[ab]	101.08[bc]	3.180	<0.001
Day 35	131.23[c]	91.37[a]	123.77[bc]	117.13[b]	125.33[bc]	2.932	<0.001
Day 1–21	51.71[c]	39.96[a]	49.06[bc]	47.63[b]	46.72[b]	0.821	<0.001
Day 22–35	126.57[d]	85.82[a]	118.94[cd]	106.86[b]	113.21[bc]	2.797	<0.001
Day 1–35	81.66[c]	58.31[a]	77.01[bc]	71.32[b]	73.31[b]	1.567	<0.001
Feed conversion ratio, g/g							
Day 7	1.45	1.60	1.58	1.55	1.65	0.039	0.582
Day 14	1.57	1.64	1.50	1.54	1.56	0.043	0.903
Day 21	1.43[a]	1.83[b]	1.51[a]	1.58[ab]	1.53[a]	0.039	0.006
Day 28	1.46	1.64	1.48	1.51	1.49	0.024	0.142
Day 35	1.47[a]	1.73[b]	1.48[a]	1.49[a]	1.47[a]	0.024	<0.001
Day 1–21	1.48[a]	1.69[b]	1.53[a]	1.56[ab]	1.58[ab]	0.024	0.047
Day 22–35	1.47[a]	1.68[b]	1.48[a]	1.50[a]	1.48[a]	0.019	<0.001
Day 1–35	1.48[a]	1.69[b]	1.51[a]	1.53[a]	1.54[a]	0.018	<0.001

[1] Values are the mean of six replicates per treatment; [a–d] Values in a row with different superscripts differ significantly ($P<0.05$). NC, non-challenged, non-treated; PC, challenged, non-treated; AB, PC+Salinomycin; THB, PC+3,4,5-trihydroxybenzoic acid; COM, PC+3,4,5-trihydroxybenzoic acid+oregano extracts
[2] Pooled standard error of the mean.

0.05) ADFI during the growing period of infected broilers in favor of the THB diet.

Feed efficiency was significantly different among treatments during the starting, growing, and overall phases of the experimental periods (all $P<0.05$). NC broilers had better FCR ($P<0.05$) than PC broilers during these phases. However, no significant difference was found ($P>0.05$) between challenged birds fed a diet supplemented with antibiotics, THB, COM, and NC birds. Feed efficiency was markedly improved ($P<0.05$) by supplementing infected birds with COM for the growing period compared to those fed THB. Interestingly, broilers fed COM exhibited a similar feed efficiency to AB broilers during the growing period ($P>0.05$).

Compared to COM, THB numerically improved the feed efficiency of infected birds by 1.28% and 0.65% for the starting period and overall experimental period, accordingly (1.58 vs. 1.56 and 1.54 vs. 1.53, respectively).

Lesion Score

The effect of anticoccidial dietary supplementation on the overall mean lesion score in broiler chickens for 35 days was determined (Table 4). No lesions were found in the digestive tract of non-infected birds (NC), whereas PC broilers had the most severe lesions ($P<0.05$) at either 4 or 7 days post-infection (dpi). AB broilers had the lowest ($P<0.05$) scores among the infected groups. The differences between THB- and COM-fed birds were not significant. COM numerically

Table 4. **Effect of dietary treatments on overall mean lesion score of coccidiosis challenged broiler chickens.[1]**

Period	Experimental diets					SEM	P-value
	NC	PC	AB	THB	COM		
Lesion Score							
4 dpi[3]	0.00[a]	3.24[d]	0.46[b]	2.06[c]	1.93[c]	0.314	<0.001
7 dpi[3]	0.00[a]	3.58[d]	0.67[b]	2.36[c]	2.14[c]	0.425	<0.001

[1] Values are the mean of six replicates per treatment; [a–d] Values in a row with different superscripts differ significantly ($P<0.05$).
NC, non-challenged, non-treated; PC, challenged, non-treated; AB, PC+Salinomycin; THB, PC+3,4,5-trihydroxybenzoic acid; COM, PC+3,4,5-trihydroxybenzoic acid+oregano extracts
[2] Pooled standard error of the mean
[3] Days post-infection

Table 5. **Effect of dietary treatments on lesion score in caeca, jejunum, and ilium of coccidiosis challenged broiler chickens.[1]**

Period	Experimental diets					SEM	P-value
	NC	PC	AB	THB	COM		
Caeca							
4 dpi[3]	0.00[a]	3.33[d]	0.78[b]	2.00[c]	1.83[c]	0.309	<0.001
7 dpi[3]	0.00[a]	3.58[d]	0.83[b]	2.33[c]	2.25[c]	0.419	<0.001
Jejunum							
4 dpi[3]	0.00[a]	2.83[e]	0.44[b]	2.28[d]	1.89[c]	0.294	<0.001
7 dpi[3]	0.00[a]	3.33[c]	0.50[a]	2.42[bc]	2.08[b]	0.416	<0.001
Ileum							
4 dpi[3]	0.00[a]	3.56[c]	0.17[a]	1.89[b]	2.06[b]	0.359	<0.001
7 dpi[3]	0.00[a]	3.83[d]	0.67[b]	2.33[c]	2.08[c]	0.449	<0.001

[1] Values are the mean of six replicates per treatment; [a–e] Values in a row with different superscripts differ significantly ($P<0.05$).
NC, non-challenged, non-treated; PC, challenged, non-treated; AB, PC+Salinomycin; THB, PC+3,4,5-trihydroxybenzoic acid; COM, PC+3,4,5-trihydroxybenzoic acid+oregano extracts
[2] Pooled standard error of the mean
[3] Days post-infection

Table 6. **Effect of dietary treatments on oocysts per gram in feces of coccidiosis challenged broiler chickens.[1]**

Period	Experimental diets					SEM	P-Value
	NC	PC	AB	THB	COM		
OPG count (Log_{10} Oocysts)							
7 dpi[3]	0.00[a]	4.06[d]	2.75[b]	3.64[c]	3.55[c]	0.391	<0.001
8 dpi[3]	0.00[a]	4.11[d]	3.01[b]	3.78[c]	3.68[c]	0.401	<0.001
9 dpi[3]	0.00[a]	4.16[e]	3.16[b]	3.85[d]	3.76[c]	0.408	<0.001
10 dpi[3]	0.00[a]	4.09[e]	2.96[b]	3.75[d]	3.61[c]	0.398	<0.001
11 dpi[3]	0.00[a]	4.02[e]	2.62[b]	3.63[d]	3.46[c]	0.387	<0.001

[1] Values are the mean of six replicates per treatment; [a–e] Values in a row with different superscripts differ significantly ($P<0.05$).
NC, non-challenged, non-treated; PC, challenged, non-treated; AB, PC+Salinomycin; THB, PC+3,4,5-trihydroxybenzoic acid; COM, PC+3,4,5-trihydroxybenzoic acid+oregano extracts
[2] Pooled standard error of the mean
[3] Days post-infection

improved gut integrity by suppressing lesions at both 4 and 7 dpi in infected birds (1.93 and 2.14, respectively) when compared to THB (2.06 and 2.36, respectively). The data in Table 5 are the separate lesion scores for ceca, jejunum, and ilium in the digestive tract of broilers. COM-fed broilers had lower lesion scores in all intestinal segments compared to those fed THB, with a significant difference detected in the jejunum at 4 dpi ($P<0.05$).

OPG Counts

As expected, oocysts were undetected in the excreta ob-

Table 7. **Effect of dietary treatments on nutrient digestibility coefficient of coccidiosis challenged broiler chickens.**[1]

Period	Experimental Diets					SEM[2]	P-value
	NC	PC	AB	THB	COM		
Dry Matter							
4 dpi[3]	0.70	0.57	0.69	0.64	0.65	0.020	0.228
7 dpi[3]	0.77	0.57	0.71	0.65	0.67	0.026	0.137
Crude Protein							
4 dpi[3]	0.83^{b}	0.60^{a}	0.80^{b}	0.73^{ab}	0.76^{ab}	0.024	0.012
7 dpi[3]	0.87^{b}	0.66^{a}	0.81^{ab}	0.75^{ab}	0.78^{ab}	0.024	0.049
Energy							
4 dpi[3]	0.75	0.57	0.76	0.62	0.67	0.027	0.099
7 dpi[3]	0.80	0.60	0.77	0.66	0.73	0.028	0.121

[1] Values are the mean of six replicates per treatment; $^{a-b}$ Values in a row with different superscripts differ significantly ($P<0.05$).
NC, non-challenged, non-treated; PC, challenged, non-treated; AB, PC+Salinomycin; THB, PC+3,4,5-trihydroxybenzoic acid; COM, PC+3,4,5-trihydroxybenzoic acid+oregano extracts
[2] Pooled standard error of the mean
[3] Days post-infection

Table 8. **Effect of dietary treatments on villus height, crypt depth and V: C ratio of coccidiosis challenged broiler chickens.**[1]

Period	Experimental Diets					SEM[2]	P-value
	NC	PC	AB	THB	COM		
Villus height (µm)							
4 dpi[3]	974.24^{e}	605.84^{a}	880.04^{d}	753.73^{b}	821.50^{c}	19.247	<0.001
7 dpi[3]	1133.91^{d}	693.91^{a}	955.09^{c}	817.11^{b}	898.59^{c}	23.004	<0.001
Crypt depth (µm)							
4 dpi[3]	76.96^{a}	109.54^{c}	90.20^{b}	94.14^{b}	93.44^{b}	1.783	<0.001
7 dpi[3]	85.34^{a}	118.38^{c}	94.83^{ab}	99.08^{b}	95.70^{ab}	2.003	<0.001
Villus height: Crypt depth ratio (V: C)							
4 dpi[3]	12.71^{d}	5.55^{a}	9.38^{c}	8.39^{b}	8.81^{bc}	0.358	<0.001
7 dpi[3]	13.28^{d}	5.87^{a}	10.22^{c}	8.64^{b}	9.13^{bc}	0.390	<0.001

[1] Values are the mean of six replicates per treatment; $^{a-e}$ Values in a row with different superscripts differ significantly ($P<0.05$).
NC, non-challenged, non-treated; PC, challenged, non-treated; AB, PC+Salinomycin; THB, PC+3,4,5-trihydroxybenzoic acid; COM, PC+3,4,5-trihydroxybenzoic acid+oregano extracts
[2] Pooled standard error of the mean
[3] Days post-infection

tained from the non-infected group, while PC birds had the highest OPG count from 7 to 11 dpi ($P<0.05$; Table 6). The oocyst shedding pattern was increased ($P<0.05$) at 7, 8, and 9 dpi, but was decreased ($P<0.05$) at 10 and 11 dpi in all treatments. The COM diet reduced ($P<0.05$) the OPG count in the coccidia-infected birds at 9, 10, and 11 dpi. These values were higher ($P<0.05$) than those fed AB. The collective findings indicate that supplementation with COM appears to be more efficacious than THB alone in reducing the OPG count in infected broilers.

Ileal Digestibility of Nutrients

There were no differences ($P>0.05$) in the digestibility coefficients of dry matter and energy of infected broilers at both 4 and 7 dpi. The birds in the NC group had a higher ($P<0.05$) crude protein digestibility coefficient than those of the other groups at both 4 and 7 dpi (Table 7). Both THB and COM improved protein digestibility in challenged birds, when compared to PC birds on both test dates ($P<0.05$). However, birds fed AB had similar protein digestibility profiles to those of NC birds at 4 dpi.

Ileal Morphology

The effects on the gut morphology of coccidia-challenged broiler chickens fed diets containing different dietary treatments are summarized in Table 8. There was an effect of diet ($P<0.05$) on villus height, crypt depth, and the villus height: crypt depth (V:C) ratio of coccidia-infected broilers. NC

Table 9. **Effect of dietary treatments on dressing percentage, relative breast meat weight, and relative drumstick weight of coccidiosis challenged broiler chickens.**[1]

Period	Experimental diets					SEM[2]	P-value
	NC	PC	AB	THB	COM		
Dressing percentage[3], %							
4 dpi[6]	88.96	84.64	87.86	87.11	87.38	0.512	0.078
7 dpi[6]	89.24	85.84	89.05	87.70	87.85	1.015	0.836
Relative breast meat weight[4], %							
4 dpi[6]	25.86	23.65	25.11	24.14	24.91	0.444	0.584
7 dpi[6]	26.16	25.65	26.05	25.33	25.76	0.361	0.963
Relative drumstick weight[5], %							
4 dpi[6]	9.91^{b}	8.93^{a}	9.86^{b}	9.76^{b}	9.81^{b}	0.123	0.048
7 dpi[6]	10.64^{b}	9.67^{a}	10.39^{ab}	9.97^{ab}	10.14^{ab}	0.131	0.036

[1] Values are the mean of six replicates per treatment; $^{a-b}$ Values in a row with different superscripts differ significantly ($P<0.05$).
NC, non-challenged, non-treated; PC, challenged, non-treated; AB, PC+Salinomycin; THB, PC+3,4,5-trihydroxybenzoic acid; COM, PC+3,4,5-trihydroxybenzoic acid+oregano extracts
[2] Pooled standard error of the mean
[3] Dressing percentage=[Carcass weight/Live body weight]×100%
[4] Relative breast meat weight=[Breast meat weight/Carcass weight]×100%
[5] Relative drumstick weight=[Drumstick weight/Carcass weight]×100%
[6] Days post-infection

broilers had the healthiest ($P<0.05$) gut morphology, followed by AB-, COM-, and THB-fed birds ($P<0.05$). The PC broilers exhibited the worst ($P<0.05$) ileal morphology. Broiler-fed COM displayed longer villi ($P<0.05$) but shorter crypts, and hence a higher V:C ratio, than those fed THB at 4 and 7 dpi.

Carcass Traits

There were no differences ($P>0.05$) in dressing percentage and relative breast meat weight of broilers among dietary treatments at 4 and 7 dpi (Table 9). Nonetheless, the NC broilers had the highest relative drumstick weight, and the PC broilers had the lowest relative weight on both test dates compared to those of the other treatments (all $P<0.05$). No significant difference was observed between the NC, AB, THB, or COM treatments in terms of relative drumstick weight.

Discussion

Oregano-derived phytogenic compounds are effective against coccidiosis by suppressing adverse growth performance and ameliorating the deleterious effects of coccidiosis in broilers (Reisinger *et al*., 2011; Remmal *et al*., 2013; Alarcon-Rojo *et al*., 2017). The main active components of OE, carvacrol and thymol, are potent molecules against *Eimeria tenella*, *E. acervulina*, and a combination of *Eimeria* spp. (Giannenas *et al*., 2003; Christaki *et al*., 2004). In contrast, THB, an equally important hydrolyzable tannin component, has anticoccidial activity by stimulating immunity against *Eimeria* spp. in broilers (Choi and Kim, 2020). Nonetheless, until the present study, the effect of diet supplementation using a combination of OE and THB on coccidiosis in broilers remains unknown. It is important to consider OE and THB in broiler diets to optimize growth performance and prevent adverse reactions during treatment of poultry coccidiosis.

Several studies have reported improved growth performance (i.e., BW, feed efficiency, and feed intake) of coccidiosis-infected broiler chickens receiving diets containing dietary oregano, compared to the infected control group (Tsinas *et al*., 2011; Alp *et al*., 2012; Yitbarek, 2015; Franciosini *et al*., 2016). Despite the anti-coccidiocidal effect on animals, the improvement of growth performance in coccidia-infected broilers depends on other factors, such as gut microflora, gut histomorphology, and mucus production (Bozkurt *et al*., 2014). Oregano and its key components of carvacrol and thymol protect the integrity and microbial ecosystems in the gut, while stimulating the secretion of endogenous digestive enzymes, mucus, and saliva, which improve digestion, growth performance, and feed efficiency in challenged broilers (Silva *et al*., 2009; Alloui *et al*., 2014; Yitbarek, 2015). These effects could be achieved by enhancing the inner surface area of the intestine and prolonging the feed retention time in the gut. OEs increase nutrient absorption and transport through the villi and amplify feed efficiency in broilers (Garcia *et al*., 2007; Bozkurt *et al*., 2016; Simitzis, 2017). Hernandez *et al*. (2004) and Cross *et al*. (2007) demonstrated that dietary OE containing carvacrol and thymol improved the apparent ileal digestibility of dry matter and crude protein in broilers. Consistent with these findings, in the present study a significantly higher digestibility coefficient of crude protein with COM than with PC and a numerical improvement when compared to THB were observed. Bozkut *et al*. (2016) reported that pancreatic and intestinal chymotrypsin activities in coccidiosis challenged birds could be improved by feeding OE to increase protein digestibility. Both Jamroz *et al*. (2005)

and Yitbarek (2015) reported enhanced intestinal trypsin activity by incorporating phytogenic compounds, such as essential oils, into the broiler diet. The higher feed intake observed in the OE-fed birds (COM) in the present study might be due to the improvement in flavor and odor of the feed contributed by the essential oil components, thus enhancing dietary palatability (Frankič *et al*., 2009). Interestingly, THB-fed broilers showed numerically higher feed efficiency than COM-fed broilers, and a non-significant difference was observed with the AB-fed broilers during the entire experimental period. Tinh *et al*. (2019) reported similar results, in which birds fed a diet with THB (100 g/ton) showed an equally better feed efficiency than those fed lasalocid (75 g/ton). This finding could be attributed to the significantly lower feed intake of THB-supplemented feed, and the higher concentration of the pure form of polyphenols that improved the gut architecture in the infected birds, thus influencing effective nutrient absorption and, ultimately, obtaining better feed efficiency. Mašek *et al*. (2014) reported an improved growth performance of THB-fed broilers. This could be attributed to the increased short-chain fatty acid concentration, which aids nutrient digestion and proper absorption (Liao *et al*., 2020; Iqbal *et al*., 2020), in turn leading to improved gut morphology in broilers. These findings suggest that OE and THB provided individually can improve the growth performance of either infected or non-infected chickens, with synergistic effects evident with their combined use in broiler diets.

Supplementation of OE can reduce the severity of gut lesions caused by *Eimeria* spp. (Giannenas *et al*., 2003; Tsinas *et al*., 2011; Mohiti and Ghanaatparast, 2015; Pop *et al*., 2019). This could be due to the antioxidative compounds in oregano, which reduces the cytotoxic effect caused by ROS and helps to mitigate the intestinal damage caused by *Eimeria* invasion (Idris *et al*., 2017). Carvacrol and thymol can scavenge hydroxyl radicals and convert them to stable compounds, thus diminishing oxidative stress and in turn reducing the severity of intestinal lesions in infected broilers (Giannenas *et al*., 2003; Alagawany *et al*., 2015; Bozkurt *et al*., 2016; Upadhaya *et al*., 2019). In addition, the antiparasitic property of OE might reduce coccidia oocysts, thereby reducing protozoa-induced lesions (Scheurer *et al*., 2013; Mohiti-Asli and Ghanaatparast-Rashti, 2015). Similarly, Tosi *et al*. (2016) and Qaid *et al*. (2021) reported reductions in gut lesions of *Eimeria*-infected broilers receiving diets supplemented with dietary plant-based meals and extracts, such as THB, when compared to the infected control group. THB has wound healing abilities in mammals by activating specific healing factors, such as focal adhesion kinases, cytoplasmic protein tyrosine kinase, c-Jun N-terminal kinases, and extracellular signal-regulated kinases (Yang *et al*., 2016; Pal, 2018). Moreover, the ferric-reducing antioxidative activity and the ability to scavenge hydroxyl radicals of dietary THB could attenuate intestinal lesions and cytotoxicity caused by *Eimeria* spp. (Archer *et al*., 2019; Qaid *et al*., 2021). Hence, enhanced antioxidative activities and altered gut structure due to *Eimeria* invasion could be better ameliorated by combining OE with THB, compared to THB alone.

Phytogenic compounds inhibit the growth of *Eimeria* spp. in different phases of the parasitic life cycle (i.e., sporogony and merogony stages). This in turn reduces the production and release of oocysts in feces (Muthamilselvan *et al*., 2016). The hydrophobic nature and high lipid solubility of carvacrol and thymol could reduce the fluidity of the phospholipid bilayer in the cell membrane of microorganisms, including *Eimeria* spp., thereby decreasing the permeability of vital cations, such as K+ and H+ (Ultee *et al*., 1999; Ochoa-Velasco *et al*., 2021). Consequently, parasitic cell destruction occurs through energy loss, ion leakage (particularly calcium), and cellular constituents commensurate with a decrease in adenosine triphosphate generation (Ultee *et al*., 2002; Jitviriyanon *et al*., 2016; Sidiropoulou *et al*., 2020). Numerous studies have reported that carvacrol and thymol are responsible for the inhibition of glycoprotein synthesis and the cysteine protease cruzain enzyme in parasites (Dos Santos *et al*., 2018). This restricts propagation and diminishes survival in the parasitic life cycle (Stokes *et al*., 2007; Monzote *et al*., 2012). Several studies (Chand *et al*., 2016; and Ali *et al*., 2019) reported similar oocyst shedding patterns as those of the present study (see Table 6). The higher biological potential and the short prepatent period of the parasites may decrease the rate of oocysts during the initial infection stage, and the acquisition of immunity during the latter period reduces oocyst excretion through chicken feces (Pattison *et al*., 2007). A reduction in OPG counts emphasized the protective role of an additive against *Eimeria* infection (Yim *et al*., 2011). In addition, several studies have reported that OE containing thymol and carvacrol contributes to a reduction in the number of oocysts shed through the feces of infected broilers (Giannenas *et al*., 2003; Küçükyilmaz *et al*., 2012; Mohiti and Ghanaatparast, 2015). The finding agrees with our results and suggests that OE constituents have a defensive capability against *E. tenella*, *E. acervulina*, and a combination of *Eimeria* spp. (Giannenas *et al*., 2003; Christaki *et al*., 2004; Küçükyilmaz *et al*., 2012). In the present study, PC birds had significantly higher oocyst counts than AB-treated birds, whereas COM birds had intermediate-level counts that were significantly lower than the THB-treated birds. Alp *et al*. (2012) obtained a similar observation by providing a diet containing 300 mg/kg of oregano to infected birds. We concur with the authors' recommended use of OE as a phytogenic anticoccidial agent. Although this approach was not as effective as antibiotics, it was still effective in reducing oocyst excretion beyond that of infected non-treated birds. Similarly, a series of recent studies have reported that either plant meals or phytogenic plant extracts that contain THB could reduce the OPG count of coccidiosis-infected birds, compared to infected non-treated groups (Naidoo *et al*., 2008; Lee *et al*., 2012; Lahlou *et al*., 2021). Tannin derivatives (including THB) can improve the resistance and resilience of animals against gastrointestinal parasitic infections by generating complexes with essential parasitic enzymes, inhibiting oxidative phosphorylation or electron transport (thus decreasing parasite metabolism), and chelating metal iron to make them unavailable for vital biological activities (i.e., heme formation, reduction of the ribonucleotide precursor of DNA) for the parasite (Chung *et al*., 1998; Min and Hart, 2003). Because THB has a low molecular weight and poor affinity to proteins (and thus is easily hydrolyzed), it can be easily absorbed into the intestinal bloodstream, consequently providing antioxidative and anti-inflammatory benefits to chickens (Manach *et al*., 2005; Choi and Kim, 2020).

Ileal morphology is an indicator of both broiler gut health and nutrient absorption capacity (Apperson and Cherian, 2017). Villus height, crypt depth, and V:C ratio are histomorphometric parameters that can be used to assess the development and functional status of the small intestine (Laudadio *et al*., 2012; Nabizadeh, 2012; Seyyedin and Nazem, 2017). Coccidiosis in broilers distresses intestinal integrity, causing tissue damage in the mucosa and submucosa, shortening the villi, and impairing digestive enzyme activities (Kettunen *et al*., 2001; Williams, 2005; Perez-Carbajal *et al*., 2010). Consequently, detrimental effects can occur during digestion and nutrient absorption in the broiler gut (Amerah and Ravindran, 2015; Ali *et al*., 2019). Earlier studies reported that broilers fed diets with OE at concentrations of 12 and 24 mg/kg, and with a mixture of carvacrol and thymol at concentrations of 100 and 200 mg/kg, increased villus height and V:C ratio, and reduced crypt depth in an experimental infection of *Eimeria* spp. in broiler chickens for 42 days (Hashemipour *et al*., 2013; Bozkurt *et al*., 2016). Moreover, the commensal relationship between intestinal bacteria and THB in mammals improves the diversity of the gut microbiome and enhances short-chain fatty acid synthesis (i.e., butyric acid), which maintains proper gut integrity (Leeson *et al*., 2005; Bortoluzzi *et al*., 2020). Additionally, carvacrol and thymol reportedly increased the production and secretion of mucin from the villi surface, which improved the morphology and pathogenic activities of the broiler gut (Jamroz *et al*., 2006). Samuel *et al*. (2017) reported a significant improvement in the V:C ratio with a decrease in crypt depth by incorporating THB into the broiler diet at a level of 75–100 mg/kg, compared to that of the equivalent control group. A reduction in crypt depth indicates slow cellular turnover in the intestine, which is an indication of good intestinal health and overall growth of the birds (Oso *et al*., 2019).

The results of the present study regarding carcass traits agree with those of Eler *et al*. (2019), who described that the relative breast meat weight was unaffected by dietary oregano supplementation, although carcass yield and relative leg meat weight differed. Hong *et al*. (2012) did not detect a difference in carcass dressing percentage and hot carcass yield of OE-fed chickens, which was consistent with our results, indicating that birds fed COM did not show any differences in dressing percentage. However, increased carvacrol and thymol levels may reduce the visceral organ weight in broiler chickens (Cázares-Gallegos *et al*., 2019), which aids in amplifying the dressing percentage. The present findings do not support this concern. Furthermore, improved breast muscle yields were reported from broilers fed a diet supplemented with ≥100 mg/kg THB (Samuel *et al*., 2017) and *Rumex nervosus* leaves meal (the active compound in the leaves is THB) (Azzam *et al*., 2020).

In conclusion, COM outperformed THB alone in achieving better coccidia elimination in broiler chickens in the 35-day study. These results could provide useful data in related fields and support our hypothesis that a synergistic anticoccidial effect can be achieved through a specific combination of natural phytochemicals in challenged broilers.

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Author Contributions

Writing - Original Draft, Shan Randima Nawarathne and Dong-Myung Kim; Methodology, Hyun-Min Cho, Junseon Hong and Yubin Kim; Software and Investigation, Myunghwan Yu; Formal analysis,Young-Joo Yi; Investigation and Conceptualization, Hans Lee; Data curation and Formal analysis, Vannie Wan and Noele Kai Jing Ng; Validation and Conceptualization, Chuan Hao Tan; Conceptualization and Writing - review & editing, Jung-Min Heo

Conflict of Interest

The authors Hans Lee, Vannie Wan, Noele Kai Jing Ng, and Chuan Hao Tan are employees of Kemin Industries Asia Pte Ltd. and declare no conflicts of interest.

References

Abudabos AM, Alyemni AH, Swilam EO and Al-Ghadi M. Comparative anticoccidial effect of some natural products against *Eimeria* spp. infection on performance traits, intestinal lesion and oocyte number in broiler. Pakistan Journal of Zoology, 49: 1989–1995. 2017.

Alagawany M, El-Hack M, Farag MR, Tiwari R and Dhama K. Biological effects and modes of action of carvacrol in animal and poultry production and health-a review. Advances in Animal and Veterinary Sciences, 3: 73–84. 2015.

Ali M, Chand N, Khan RU, Naz S and Gul S. Anticoccidial effect of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) against experimentally induced coccidiosis in broiler chickens. Journal of Applied Animal Research, 47: 79–84. 2019.

Allen PC and Fetterer RH. Recent advances in biology and immunobiology of *Eimeria* species and in diagnosis and control of infection with these coccidian parasites of poultry. Clinical microbiology reviews, 15: 58–65. 2002.

Alloui MN, Agabou A and Alloui N. Application of herbs and phytogenic feed additives in poultry production-A Review. Global Journal of Animal Scientific Research, 2: 234–43. 2014.

Alp M, Midilli M, Kocabağlı N, Yılmaz H, Turan N, Gargılı A and Acar NÜ. The effects of dietary oregano essential oil on live performance, carcass yield, serum immunoglobulin G level, and oocyst count in broilers. Journal of Applied Poultry Research, 21: 630–6. 2012.

Alarcon-Rojo AD, Janacua-Vidales H and Renteria-Monterrubio A. In: Active Ingredients from Aromatic and Medicinal Plants (Hany A and Ei-Shemy eds.). vol. 1. pp. 225–229. InTech.

Rijeka. 2017.

Amerah AM and Ravindran V. Effect of coccidia challenge and natural betaine supplementation on performance, nutrient utilization, and intestinal lesion scores of broiler chickens fed suboptimal level of dietary methionine. Poultry Science, 94: 673–680. 2015.

Antoszczak M, Steverding D and Huczyński A. Anti-parasitic activity of polyether ionophores. European Journal of Medicinal Chemistry, 166: 32–47. 2019.

Apperson KD and Cherian G. Effect of whole flax seed and carbohydrase enzymes on gastrointestinal morphology, muscle fatty acids, and production performance in broiler chickens. Poultry Science, 96: 1228–1234. 2017.

AOAC. Official Methods of Analysis, 18th. ed. Association of Official Analytical Chemists International, Maryland. 2005.

Archer MA, Agyei A, Mintah S, Adjei P, Kumadoh D and Asiedu-Larbi J. Medicinal uses of Cassia sieberiana; a review. International Journal of Sciences: Basic and Applied Research, 48: 161–80. 2019.

Arczewska-Wlosek A and Swiatkiewicz S. The effect of a dietary herbal extract blend on the performance of broilers challenged with *Eimeria* oocysts. Journal of Animal and Feed Sciences, 21: 133–142. 2012.

Aviagen. Ross 308: Broiler Management Handbook. Aviagen Inc., Huntsville, AL. 2014a.

Aviagen. Ross 308: Nutrition Specification. Aviagen Inc., Huntsville, AL. 2014b.

Azzam MM, Qaid MM, Al-Mufarrej SI, Al-Garadi MA, Albaadani HH and Alhidary IA. Rumex nervosus leaves meal improves body weight gain, duodenal morphology, serum thyroid hormones, and cecal microflora of broiler chickens during the starter period. Poultry Science, 99: 5572–5581. 2020.

Bortoluzzi C, Vieira BS and Applegate TJ. Influence of dietary zinc, copper, and manganese on the intestinal health of broilers under Eimeria challenge. Frontiers in Veterinary Science, 7: 13. 2020.

Bozkurt M, Aysul N, Küçükyilmaz K, Aypak S, Ege G, Catli AU, Akşit H, Çöven F, Seyrek K and Çınar 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: 389–399. 2014.

Bozkurt M, Ege G, Aysul N, Akşit H, Tüzün AE, Küçükyılmaz K, Borum AE, Uygun M, Akşit D, Aypak S and Şimşek EM. Effect of anticoccidial monensin with oregano essential oil on broilers experimentally challenged with mixed *Eimeria* spp. Poultry Science, 95: 1858–1868. 2016.

Cázares-Gallegos R, Silva-Vázquez R, Hernández-Martínez CA, Gutiérrez-Soto JG, Kawas-Garza JR, Hume ME and Méndez-Zamora GM. Performance, Carcass Variables, and Meat Quality of Broilers Supplemented with Dietary Mexican Oregano Oil. Brazilian Journal of Poultry Science, 21: 1–10. 2019.

Chand N, Faheem H, Khan RU, Qureshi MS, Alhidary IA and Abudabos AM. Anticoccidial effect of mananoligosacharide against experimentally induced coccidiosis in broiler. Environmental Science and Pollution Research, 23: 14414–1421. 2016.

Chapman HD. Evaluation of the efficacy of anticoccidial drugs against *Eimeria* species in the fowl. International journal for parasitology, 28: 1141–1144. 1998.

Christaki E, Florou-Paneri P, Giannenas I, Papazahariadou M, Botsoglou NA and Spais AB. Effect of a mixture of herbal extracts on broiler chickens infected with *Eimeria tenella*. Animal Research, 53: 137–144. 2004.

Choi J and Kim WK. Dietary Application of Tannins as a Potential Mitigation Strategy for Current Challenges in Poultry Production: A Review. Animals. 2020 Dec; 10(12): 2389.

Chung KT, Lu Z and Chou MW. Mechanism of inhibition of tannic acid and related compounds on the growth of intestinal bacteria. Food and Chemical Toxicology. 1998 Dec 1; 36(12): 1053–60.

Conway DP and McKenzie ME. Poultry coccidiosis: diagnostic and testing procedures. 3rd ed. Blackwell Publishing Ltd. Oxford. 2007.

Cross DE, McDevitt RM, Hillman K and Acamovic T. The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. British Poultry Science, 48: 496–506. 2007.

Dos Santos AM, Cianni L, De Vita D, Rosini F, Leitão A, Laughton CA, Lameira J and Montanari CA. Experimental study and computational modelling of cruzain cysteine protease inhibition by dipeptidyl nitriles. Physical Chemistry Chemical Physics, 20: 24317–24328. 2018.

Eler G, Gomes AV, Trindade BS, Almeida LS, Dilelis F, Cardoso VS and Lima CA. Oregano essential oil in the diet of broilers: performance, carcass characteristics, and blood parameters. South African Journal of Animal Science, 49: 753–762. 2019.

Felici M, Tugnoli B, Ghiselli F, Massi P, Tosi G, Fiorentini L, Piva A and Grilli E. In vitro anticoccidial activity of thymol, carvacrol, and saponins. Poultry Science, 99: 5350–5355. 2020.

Franciosini MP, Casagrande-Proietti P, Forte C, Beghelli D, Acuti G, Zanichelli D, dal Bosco A, Castellini C and Trabalza-Marinucci M. Effects of oregano (*Origanum vulgare L.*) and rosemary (*Rosmarinus officinalis L.*) aqueous extracts on broiler performance, immune function and intestinal microbial population. Journal of Applied Animal Research, 44: 474–479. 2016.

Garcia V, Catala-Gregori P, Hernandez F, Megias MD and Madrid J. Effect of formic acid and plant extracts on growth, nutrient digestibility, intestine mucosa morphology, and meat yield of broilers. Journal of Applied Poultry Research, 16: 555–562. 2007.

Frankič T, Voljč M, Salobir J and Rezar V. Use of herbs and spices and their extracts in animal nutrition. Acta argiculturae Slovenica, 94: 95–102. 2009.

Gavaric N, Mozina SS, Kladar N and Bozin B. Chemical profile, antioxidant and antibacterial activity of thyme and oregano essential oils, thymol and carvacrol and their possible synergism. Journal of Essential Oil-Bearing Plants, 2015; 18: 1013–1021.

Giannenas I, Florou-Paneri P, Papazahariadou M, Christaki E, Botsoglou NA and Spais AB. Effect of dietary supplementation with oregano essential oil on performance of broilers after experimental infection with *Eimeria tenella*. Archives of Animal Nutrition, 57: 99–106. 2003.

Giannenas I, Papaneophytou CP, Tsalie E, Pappas I, Triantafillou E, Tontis D and Kontopidis GA. Dietary supplementation of benzoic acid and essential oil compounds affects buffering capacity of the feeds, performance of turkey poults and their antioxidant status, pH in the digestive tract, intestinal microbiota and morphology. Asian-Australasian Journal of Animal Sciences, 27: 225–236. 2014.

Gilbert ER, Cox CM, Williams PM, McElroy AP, Dalloul RA, Ray WK, Barri A, Emmerson DA, Wong EA and Webb Jr KE. Eimeria species and genetic background influence the serum protein profile of broilers with coccidiosis. PloS ONE, Jan 31; 6: e14636. 2011.

Hashemi SR and Davoodi H. Phytogenics as new class of feed additive in poultry industry. Journal of Animal and Veterinary

Advances, 17: 2295–2304. 2010.

Hashemipour H, Kermanshahi H, Golian A, Raji A and Van Krimpen MM. Effect of thymol+ carvacrol by next enhance 150® on intestinal development of broiler chickens fed CMC containing diet. Iranian Journal of Applied Animal Science, 3: 567–576. 2013.

Hernandez F, Madrid J, Garcia V, Orengo J and Megias MD. Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. Poultry Science, 83: 169–174. 2004.

Huang RL, Yin YL, Wu GY, Zhang YG, Li TJ, Li LL, Li MX, Tang ZR, Zhang J, Wang B and He JH. Effect of dietary oligochitosan supplementation on ileal digestibility of nutrients and performance in broilers. Poultry Science, 84: 1383–1388. 2005.

Idris M, Abbas RZ, Masood S, Rehman T, Farooq U, Babar W, Hussain R, Raza A and Riaz U. The potential of antioxidant rich essential oils against avian coccidiosis. World's Poultry Science Journal, 73: 89–104. 2017.

Iqbal Y, Cottrell JJ, Suleria HA and Dunshea FR. Gut Microbiota-Polyphenol Interactions in Chicken: A Review. Animals. Aug; 10(8): 1391. 2020.

Jahejo AR, Rajput N, Rajput NM, Leghari IH, Kaleri RR, Mangi RA, Sheikh MK and Pirzado MZ. Effects of heat stress on the performance of Hubbard broiler chicken. Cells, Animal and Therapeutics, 2: 1–5. 2016.

Jamroz D, Wiliczkiewicz A, Wertelecki T, Orda J and Skorupińska J. Use of active substances of plant origin in chicken diets based on maize and locally grown cereals. British Poultry Science, 46: 485–493. 2005.

Jamroz D, Wertelecki T, Houszka M and Kamel C. Influence of diet type on the inclusion of plant origin active substances on morphological and histochemical characteristics of the stomach and jejunum walls in chicken. Journal of Animal Physiology and Animal Nutrition, 90: 255–268. 2006.

Jitviriyanon S, Phanthong P, Lomarat P, Bunyapraphatsara N, Porntrakulpipat S and Paraksa N. In vitro study of anti-coccidial activity of essential oils from indigenous plants against *Eimeria tenella*. Veterinary Parasitology, 228: 96–102. 2016.

Kala S, Gattani A, Kumar A and Samantaray S. Evaluation of polyether ionophores against coccidiosis in broiler chicken. Haryana Veterinarian, 52: 63–67. 2014.

Kettunen H, Tiihonen K, Peuranen S, Saarinen MT and Remus JC. Dietary betaine accumulates in the liver and intestinal tissue and stabilizes the intestinal epithelial structure in healthy and coccidia-infected broiler chicks. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 130: 759–769. 2001.

Kırkpınar F, Ünlü HB and Özdemir G. Effects of oregano and garlic essential oils on performance, carcase, organ and blood characteristics and intestinal microflora of broilers. Livestock Science, 137: 219–225. 2011.

Kroes BV, Van den Berg AJ, Van Ufford HQ, Van Dijk H and Labadie RP. Anti-inflammatory activity of gallic acid. Planta Medica, 58: 499–504. 1992.

Lahlou RA, Bounechada M, Mohammedi A, Silva LR and Alves G. Dietary use of Rosmarinus officinalis and Thymus vulgaris as anticoccidial alternatives in poultry. Animal Feed Science and Technology, Jan 12: 114826. 2021.

Laudadio V, Passantino L, Perillo A, Lopresti G, Passantino A, Khan RU and Tufarelli V. Productive performance and histological features of intestinal mucosa of broiler chickens fed different dietary protein levels. Poultry Science, 91: 265–270. 2012.

Lavine MD and Arrizabalaga G. The antibiotic monensin causes cell cycle disruption of Toxoplasma gondii mediated through the DNA repair enzyme TgMSH-1. Antimicrobial Agents and Chemotherapy, 55: 745–755. 2011.

Lee JJ, Kim DH, Lim JJ, Kim DG, Min W, Kim GS, Lee HJ, Rhee MH, Park H, Kim SC and Chang HH. Anticoccidial effect of supplemental dietary Galla Rhois against infection with Eimeria tenella in chickens. Avian Pathology, 41: 403–407. 2012.

Leeson S, Namkung H, Antongiovanni M and Lee EH. Effect of butyric acid on the performance and carcass yield of broiler chickens. Poultry science, 84: 1418–1422. 2005.

Liao X, Shao Y, Sun G, Yang Y, Zhang L, Guo Y, Luo X and Lu L. The relationship among gut microbiota, short-chain fatty acids, and intestinal morphology of growing and healthy broilers. Poultry Science, 99: 5883–5895. 2020.

Lin J, Hunkapiller AA, Layton AC, Chang YJ and Robbins KR. Response of intestinal microbiota to antibiotic growth promoters in chickens. Foodborne Pathogens and Disease, 10: 331–337. 2013.

Manach C, Williamson G, Morand C, Scalbert A and Rémésy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. American Journal of Clinical Nutrition, 81: 230S–42S. 2005.

Maron DF, Smith TJ and Nachman KE. Restrictions on antimicrobial use in food animal production: an international regulatory and economic survey. Globalization and Health, 9: 8. 2013.

Mašek T, Starčević K and Mikulec Ž. The influence of the addition of thymol, tannic acid or gallic acid to broiler diet on growth performance, serum malondialdehyde value and cecal fermentation. Europian Poultry Science, 78: 64. 2014.

Min BR and Hart SP. Tannins for suppression of internal parasites. Journal of Animal Science, 81(E. Suppl. 2): E102–109. 2003.

Mohiti-Asli M and Ghanaatparast-Rashti M. Dietary oregano essential oil alleviates experimentally induced coccidiosis in broilers. Preventive Veterinary Medicine, 120: 195–202. 2015.

Mohiti-Asli M and Ghanaatparast-Rashti M. Comparing the effects of a combined phytogenic feed additive with an individual essential oil of oregano on intestinal morphology and microflora in broilers. Journal of Applied Animal Research, 46: 184–189. 2018.

Monzote L, Alarcón O and Setzer WN. Antiprotozoal activity of essential oils. Agriculturae Conspectus Scientificus, 77: 167–175. 2012.

Muthamilselvan T, Kuo TF, Wu YC and Yang WC. Herbal remedies for coccidiosis control: A review of plants, compounds, and anticoccidial actions. Evidence-based Complementary and Alternative Medicine: eCAM. 2016; 2016.

Mwale M and Masika PJ. Point prevalence study of gastro-intestinal parasites in village chickens of Centane district, South Africa. African Journal of Agricultural Research, 6: 2033–2038. 2011.

Nabizadeh A. The effect of inulin on broiler chicken intestinal microflora, gut morphology, and performance. Journal of Animal Feed Science, 21: 725–734. 2012.

Naidoo V, McGaw LJ, Bisschop SP, Duncan N and Eloff JN. The value of plant extracts with antioxidant activity in attenuating coccidiosis in broiler chickens. Veterinary Parasitology, 153: 214–219. 2008.

Nazzaro F, Fratianni F, De Martino L, Coppola R and De Feo V. Effect of essential oils on pathogenic bacteria. Pharmaceuticals, 6: 1451–1474. 2013.

Ochoa-Velasco CE, Pérez-Pérez JC, Varillas-Torres JM, Navarro-Cruz AR, Hernández-Carranza P, Munguía-Pérez R, Cid-Pérez

TS and Avila-Sosa R. Starch Edible Films/Coatings Added with Carvacrol and Thymol: In Vitro and In Vivo Evaluation against Colletotrichum gloeosporioides. Foods, 10: 175. 2021.

Oso AO, Suganthi RU, Reddy GM, Malik PK, Thirumalaisamy G, Awachat VB, Selvaraju S, Arangasamy A and Bhatta R. Effect of dietary supplementation with phytogenic blend on growth performance, apparent ileal digestibility of nutrients, intestinal morphology, and cecal microflora of broiler chickens. Poultry Science, 98: 4755–66. 2019.

Özçelik B, Kartal M and Orhan I. Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids. Pharmaceutical Biology, 49: 396–402. 2011.

Trees AJ. Parasitic disease. In: Poultry Diseases (Pattison M, McMullin P, Bradbury JM and Alexander D eds.). 6th ed. pp. 444–456. Elsevier Health Sciences. London. 2008.

Pal SM, Avneet G and Siddhraj SS. Gallic acid: Pharmacogical promising lead molecule: A review. International Journal of Pharmacognosy and Phytochemical Research, 10: 132–138. 2018.

Peek HW and Landman WJ. Coccidiosis in poultry: anticoccidial products, vaccines and other prevention strategies. Veterinary Quarterly, 31: 143–161. 2011.

Pelicano ER, Souza PA, Souza HB, Figueiredo DF, Boiago MM, Carvalho SR and Bordon VF. Intestinal mucosa development in broiler chickens fed natural growth promoters. Brazilian Journal of Poultry Science, 7: 221–229. 2005.

Perez-Carbajal C, Caldwell D, Farnell M, Stringfellow K, Pohl S, Casco G, Pro-Martinez A and Ruiz-Feria CA. Immune response of broiler chickens fed different levels of arginine and vitamin E to a coccidiosis vaccine and *Eimeria* challenge. Poultry Science, 89: 1870–1877. 2010.

Persia ME, Young EL, Utterback PL and Parsons CM. Effects of dietary ingredients and *Eimeria acervulina* infection on chick performance, apparent metabolizable energy, and amino acid digestibility. Poultry Science, 85: 48–55. 2006.

Pop LM, Varga E, Coroian M, Nedişan ME, Mircean V, Dumitrache MO, Farczádi L, Fülöp I, Croitoru MD, Fazakas M and Györke A. Efficacy of a commercial herbal formula in chicken experimental coccidiosis. Parasites & Vectors, 12: 343–351. 2019.

Puvača N, Stanaćev V, Glamočić D, Lević J, Perić L, Stanaćev V and Milić D. Beneficial effects of phytoadditives in broiler nutrition. World’s Poultry Science Journal, 69: 27–34. 2013.

Qaid MM, Al-Mufarrej SI, Azzam MM, Al-Garadi MA, Albaadani HH, Alhidary IA, Aljumaah RS. Anti-Coccidial Effect of Rumex Nervosus Leaf Powder on Broiler Chickens Infected with Eimeria Tenella Oocyst. Animals. 2021 Jan; 11(1): 167.

Reisinger N, Steiner T, Nitsch S, Schatzmayr G and Applegate TJ. Effects of a blend of essential oils on broiler performance and intestinal morphology during coccidial vaccine exposure. Journal of Applied Poultry Research, 20: 272–283. 2011.

Remmal A, Achahbar S, Bouddine L, Chami F and Chami N. Oocysticidal effect of essential oil components against chicken *Eimeria* oocysts. International Journal of Veterinary Medicine, 2013: 1–8. 2013.

Roth N, Käsbohrer A, Mayrhofer S, Zitz U, Hofacre C and Domig KJ. The application of antibiotics in broiler production and the resulting antibiotic resistance in Escherichia coli: A global overview. Poultry Science, 98: 1791–804. 2019.

Salim HM, Huque KS, Kamaruddin KM and Haque Beg A. Global restriction of using antibiotic growth promoters and alternative strategies in poultry production. Science Progress, 101: 52–75. 2018.

Samuel KG, Wang J, Yue HY, Wu SG, Zhang HJ, Duan ZY and Qi GH. Effects of dietary gallic acid supplementation on performance, antioxidant status, and jejunum intestinal morphology in broiler chicks. Poultry Science, 96: 2768–2775. 2017.

Scheurer W, Spring P and Maertens L. Effect of 3 dietary phytogenic products on production performance and coccidiosis in challenged broiler chickens. Journal of Applied Poultry Research, 22: 591–599. 2013.

Seyyedin S and Nazem MN. Histomorphometric study of the effect of methionine on small intestine parameters in rat: an applied histologic study. Folia Morphologica, 76: 620–629. 2017.

Sidiropoulou E, Skoufos I, Marugan-Hernandez V, Giannenas I, Bonos E, Aguiar-Martins K, Lazari D, Blake D and Tzora A. In vitro anticoccidial study of oregano and garlic essential oils and effects on growth performance, faecal oocyst output and intestinal microbiota in vivo. Frontiers in Veterinary Science, 7: 420–451. 2020.

Silva MA, Pessotti BM, Zanini SF, Colnago GL, Rodrigues MR, Nunes LD, Zanini MS and Martins IV. Intestinal mucosa structure of broiler chickens infected experimentally with *Eimeria tenella* and treated with essential oil of oregano. Ciência Rural, 39: 1471–1477. 2009.

Simitzis PE. Enrichment of animal diets with essential oils—a great perspective on improving animal performance and quality characteristics of the derived products. Medicines, 4: 1–21. 2017.

Soutter F, Werling D, Tomley FM, Blake DP. Poultry coccidiosis: design and interpretation of vaccine studies. Frontiers in Veterinary Science, Feb 26; 7: 101. 2020.

Stanley VG, Gray C, Daley M, Krueger WF and Sefton AE. An alternative to antibiotic-based drugs in feed for enhancing performance of broilers grown on Eimeria spp.-infected litter. Poultry Science, 83: 39–44. 2004.

Stokes SL, Cole RA, Rangelova MP, Haber WA and Setzer WN. Cruzain inhibitory activity of the leaf essential oil from an undescribed species of Eugenia from Monteverde, Costa Rica. Natural Product Communications, 2: 1211–1213. 2007.

Tanweer AJ, Saddique U, Bailey CA and Khan RU. Antiparasitic effect of wild rue (*Peganum harmala L.*) against experimentally induced coccidiosis in broiler chicks. Parasitology Research, 113: 2951–2960. 2014.

Tinh N, Tan BF and Wan V. Comparison of diet supplementation of THB and Lasalocid on growth performance of native broilers in Vietnam. Journal of Poultry Science Advance Publication. February 25, doi: 10.2141/jpsa.0210116. 2022.

Tosi G, Massi P, Antongiovanni M, Buccioni A, Minieri S, Marenchino L and Mele M. Efficacy test of a hydrolysable tannin extract against necrotic enteritis in challenged broiler chickens. Italian Journal of Animal Science. 2013 Jan 1; 12(3): e62.

Tsinas A, Giannenas I, Voidarou C, Tzora A and Skoufos J. Effects of an oregano based dietary supplement on performance of broiler chickens experimentally infected with *Eimeria acervulina* and *Eimeria maxima*. Journal of Poultry Science, 48: 194–200. 2011.

Ultee A, Bennik MH and Moezelaar RJ. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. Applied and Environmental Microbiology, 68: 1561–1568. 2002.

Ultee A, Kets EP and Smid EJ. Mechanisms of action of carvacrol on the food-borne pathogen Bacillus cereus. Applied and Environmental Microbiology, 65: 4606–4610. 1999.

Upadhaya SD, Cho SH, Chung TK and Kim IH. Anti-coccidial effect of essential oil blends and vitamin D on broiler chickens vaccinated with purified mixture of coccidian oocyst from *Eimeria tenella* and *Eimeria maxima*. Poultry Science, 98: 2919–2926. 2019.

Wickramasuriya S, Kim E, Shin TK, Cho HM, Kim B, Patterson R, Yi YJ, Park S, Balasubramanian B and Heo JM. Multi-carbohydrase addition into a corn-soybean meal diet containing wheat and wheat by products to improve growth performance and nutrient digestibility of broiler chickens. Journal of Applied Poultry Research, 28: 399–409. 2019.

Williams RB. Intercurrent coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. Avian Pathology, 34: 159–180. 2005.

Yang DJ, Moh SH, Son DH, You S, Kinyua AW, Ko CM, Song M, Yeo J, Choi YH and Kim KW. Gallic acid promotes wound healing in normal and hyperglucidic conditions. Molecules, 21: 899. 2016.

Yitbarek MB. Phytogenics as feed additives in poultry production: a review. International Journal of Extensive Research, 3: 49–60. 2015.