Efficacy of in-feed preparations of an anticoccidial, multienzyme, prebiotic, probiotic, and herbal essential oil mixture in healthy and *Eimeria* spp.-infected broilers

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ABSTRACT The efficacies of 5 widely used dietary supplements were investigated on performance indices, fecal oocyst excretion, lesion score, and intestinal tract measurements in healthy and *Eimeria* spp.-infected birds by using a comparative model. This study included 2,400 sexed Ross 308 broiler chicks that were equally divided in 2 groups: the infected group, experimentally infected with oocysts of mixed *Eimeria* spp. at 14 d of age, and the healthy controls. The birds in both groups were further divided equally into 6 groups, of which one was fed a basal diet and served as control without treatment and the other 5 served as experimental treatments. These 5 groups were fed 5 diets containing preparations of 60 mg/kg of anticoccidial salinomycin (SAL), 1 g/kg of multienzyme (ENZ), 1 g/kg of probiotic (PRO), 1 g/kg of prebiotic (PRE), and 40 mg/kg of an herbal essential oil mixture (EOM). Body weight gain and feed conversion ratio (FCR) showed significant improvement in the infected animals, which indicates that dietary supplemental regimens with SAL, ENZ, PRO, and PRE initiated in 1-d-old chicks reduced adverse effects after challenge with coccidiosis; however, chicks that were administered EOM failed to show such improvement. Uninfected chickens showed significant improvement in FCR with supplements SAL, PRE, and EOM, which signifies significant ($P < 0.01$) infection by supplement interactions for BW gain and FCR. In the infected group, all of the supplements reduced the severity of coccidiosis lesions ($P < 0.01$) induced by mixed *Eimeria* spp. through the middle and lower regions of the small intestines, whereas supplementation with SAL or EOM alone was effective ($P < 0.01$) in reducing oocyst excretion compared with the control treatment. The data indicated that use of these subtherapeutically efficacious supplements (except EOM) in broiler production can lessen the depression in growth due to coccidial challenge.

Key words: anticoccidial, multienzyme, prebiotic, probiotic, essential oil

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INTRODUCTION

Avian coccidiosis is caused by several species of *Eimeria*, which are infectious protozoa that penetrate and damage the epithelial cells of intestinal tissue, resulting in intestinal inflammation and hemorrhage (Lillehoj and Trout, 1996; Cook, 1998). The intestinal damage results in decreased feed intake (**FI**) and retarded growth as well as suppression of cell-mediated immune response and low survival, all of which have significant adverse implications for the commercial poultry industry (Lillehoj and Lillehoj, 2000; McDougald, 2003). Coccidiosis results in the greatest financial liability to the poultry industry, with the majority of money being spent toward prevention of this condition by using synthetic (chemical) and ionophoric anticoccidial feed additives (Williams, 1999; Shirley et al., 2007).

Anticoccidial drugs added to the feed constitute a good preventative measure and are convenient for large-scale use, but prolonged use of these drugs inevitably leads to the emergence of *Eimeria* strains that are resistant to all anticoccidial drugs, including ionophores (Chapman, 1998; Peek and Landman, 2003). Despite the emerging resistance, feed compounders continue to add polyether antibiotics and chemicals as anticoccidial agents to poultry feeds over the past 5 decades (Chapman, 2008; Chapman et al., 2010).

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Concomitantly with the ban of antibiotic growth promoters in animal production, the European Union has put into question the use of anticoccidials from the year 2012 onward (Wallace et al., 2010). This public debate has led to an urgent need for searching new methods of coccidiosis control that would replace anticoccidial drugs (Dalloul and Lillehoj, 2006; Abbas et al., 2011, 2012). Therefore, recent studies have given prime consideration to feed additives of natural origins (i.e., herbal remedies, probiotic microorganisms, and prebiotic preparations) as an alternative means of disease control (Lee et al., 2007; Abbas et al., 2012; Taherpour et al., 2012). There is still an increasing need for highly effective, nonantibiotic products that are cost-effective, stable, and widely usable.

Common in-feed performance enhancers, such as enzymes, prebiotics, probiotics (**PRO**), and essential oils (**EO**) of medicinal herbs, have been used in broiler nutrition with considerable success (Bedford, 2000; Hooge, 2004, Yang et al., 2009; Brenes and Roura, 2010). Scientific evidence has shown that these supplements may potentially be used to optimize the health of animals by positive manipulation of the gastrointestinal tract [i.e., balancing the intestinal microbial community, improving intestinal histomorphology, and stimulating specific and nonspecific immunity (Tellez et al., 2006; Mountzouris et al., 2010, 2011)]. The intentional choice of these in-feed supplements as anticoccidial agents is based on their antimicrobial mode of action (Yang et al., 2009). The antimicrobial property of these microbial cultures or yeast fermentation product (i.e., probiotics and enzymes or prebiotics) may be assumed to contribute to the protection of the intestinal epithelium from coccidial damage and is found to be responsible for the antiparasitic activity (Abbas et al., 2011). The ability of plant bioactives with antimicrobial and antioxidant properties to limit *Eimeria*-induced damage to the intestinal wall during proinflammatory reaction can be responsible for a less damaged gut (Christaki et al., 2004; Bozkurt et al., 2012c). Few studies have reported the anticoccidial effects of these preparations, which could be because the interest in the use of microbial cultures and yeast cell wall derivatives (oligosaccharides) as anticoccidial additives in feed has increased in the recent years. Some recent trials have shown that probiotic and prebiotic preparations have an inhibitory effect on *Eimeria* infection (Elmusharaf et al., 2007; Giannenas et al., 2012; Taherpour et al., 2012), which indicates an indirect effect of these dietary supplements on the reduction of *Eimeria* lesions. However, thus far, few reports have described the potential effects of enzymes with anticoccidial activity.

There is an increasing amount of scientific evidence to prove the antiparasitic effects of medicinal plants and their associated extracts and EO. The majority of studies available thus far demonstrate the efficacy of phytogenic compounds on shedding of *Eimeria* spp. and reducing related intestinal lesions and their effect on growth. The improvement of immunity against the *Eimeria* parasite in birds treated with extracts from plants is of increasing interest as an alternative to anticoccidial agents (Lee et al., 2010; Küçükyılmaz et al., 2012).

All of these novel approaches may provide opportunities for the control of coccidiosis (Wallace et al., 2010; Abbas et al., 2011, 2012) and also of concurrent enteritis problems in the field of poultry (Williams, 2005). However, until now, there has been no comparative experimental evidence for overall anticoccidial efficacy of the above-mentioned supplements, with promising anticoccidial activity.

Hence, further investigations are required to understand the exact mechanism underlying the effects of these feed additives, which are still in use in broiler nutrition as performance enhancers and as agents for controlling coccidiosis. A comparative model was designed in this study to assess the effectiveness of the in-feed commercial preparations, an ionophore anticoccidial agent, enzymes, prebiotics, probiotics, and a defined herbal essential oil mixture (**EOM**), in chickens experimentally infected with mixed *Eimeria* spp. and in uninfected chickens. Performance indices, fecal oocyst excretion, lesion score, and intestinal tract measurements were determined.

MATERIALS AND METHODS

All procedures involving animals were approved by the Intuitional Animal Care and Use Committee of Adnan Menderes University.

Birds and Housing

Two thousand four hundred 1-d-old broiler chicks (Ross 308) of mixed sexed were used in this experiment. In a 2 × 6 factorial arrangement, chicks were fed 6 dietary supplements either in coccidial infection procedure or left uninfected. Chicks were vaccinated against infectious bursal disease virus and Newcastle disease virus with Gumbopest (Merial, St Priest, France) at arrival of the house. Chicks were housed in a broiler house (22 m length and 7 m width) divided equally into 2 similar areas with separate drinking, feeding, and management facilities to avoid cross-infection. Half of the broilers (600 male and 600 female) were randomly allocated to 6 dietary treatments for 6 wk. Each treatment had 5 replicates of 40 broilers (20 males and 20 females). The other half were allocated to the same dietary treatments in the other section of the house. Each replicate was assigned to a clean floor pen (2.2 × 1.5 m) equipped with one hanging bell drinker that was cleaned daily, 2 tube-type feeders, and electric heaters. Birds were reared in pens (12 birds per m^2 floor space) provided with litter (pine wood shavings) to a depth of 5 to 6 cm. The room temperature was gradually decreased from 33°C on d 1 to 22°C on d 21 and then kept constant to trial termination on d 42. Light

was provided at 23L:1D. The house was naturally ventilated with adjustable windows, and efforts were made to copy commercial conditions as much as possible. The management procedure was the same in both sections of the house. A service room was provided in the middle with 2 separate entrances and used as an isolation barrier between the sections. Each section was serviced by separate labor and equipment without permitting entrance to the other side. Strict biosecurity procedures were maintained between treatment groups. In the infection room, caretaking was conducted on a treatment basis to minimize cross-contamination between pens of different treatment groups. For this aim, separate boots and gloves were used for each treatment group. The experiment was terminated when the birds were 42 d old. Throughout, experimental diets and drinking water were available ad libitum.

Experimental Design

Six of the 12 groups were infected with *Eimeria* spp., whereas the other 6 were uninfected. In both the 6 infected and 6 uninfected treatment groups, one was given the control diet, and the other 5 diets were supplemented with preparations of an anticoccidial, salinomycin, multienzyme, probiotic, prebiotics, and an herbal essential oil mixture (**EOM**).

Experimental Diets

The basal diet was a typical corn-wheat-soybean diet that was formulated to meet or exceed all nutrient recommendations published in the Ross rearing guideline (Aviagen, 2007). The experimental period was divided into 3 phases: a starter phase (1 to 14 d), a grower phase (15 to 28 d), and a finisher phase (29 to 42 d). The ingredient composition and nutrient content of the basal diets for 3 experimental phases are presented in Table 1. These diets contained no antibiotics, anticoccidials, or growth enhancers and were isoenergetic and isonitrogenous. The basal mash diet was prepared every 2 wk and was stored in sacks in a cool place. Chemical composition was determined according to the protocols stated by AOAC (1990). All of the feed samples were analyzed for DM (934.01), ash (942.05), nitrogen (Kjeldahl procedure: 988.05), ether extract (920.39), crude fiber (962.09), calcium (927.02), and total phosphorus (965.17). Treatment diets relative to experimental periods were also analyzed to guarantee that they were identical regarding chemical composition with the exception of the supplements.

Pens were randomly assigned 1 of 6 experimental diets. Dietary treatments were 1) a basal diet with no anticoccidials or growth enhancers (**CNT**), 2) CNT + 60 mg/kg of ionophore anticoccidial (**SAL**; Sacox 120, Huveparma, Sofia, Bulgaria), 3) CNT + 1 g/kg enzyme complex (**ENZ**; Karyzyme 8601, Kartal Chemistry Inc. Company, Gebze-İzmit, Turkey), 4) CNT + 1g/kg probiotic (**PRO**; Primalac, Star Labs Inc., Clarksdale, MO), 5) CNT + prebiotic, mannan oligosaccharide (**PRE**; Bio-Mos, Alltech Inc., Nicholasville, KY), and 6) CNT + 24 mg/kg EOM. When preparing experimental diets, saw dust was included in the CNT diet to match the addition of the supplements. All of the supplements (i.e., SAL, ENZ, PRE, PRO, EOM), in the form of a premix powder, were preparations of 1 kg weight. Each preparation was added to an equal amount of fine-ground soybean meal and homogenized by mixer, and then the premixture was added to the main mixture.

The in-feed ionophore anticoccidial preparation (Sacox 120) contains salinomycin at a concentration of 12% (120 g/kg). The active substance belongs to the group of polyether ionophore antibiotics and is produced by *Streptomyces albus*. The approved dose range is 50 to 70 mg/kg of complete feed in the European Union. In this study the treatment level of salinomycin was 60 mg/kg of feed, representing the highest level of its usual commercial usage in European field practice for the control of coccidiosis. The enzyme complex

Table 1. Composition of the basal starter, grower, and finisher diets and their nutrient profile

	Diet		
Item	Starter	Grower	Finisher
Ingredient, g/kg			
Corn	369.78	398.02	431.50
Wheat	200.00	200.00	200.00
Soybean meal	355.69	319.42	284.62
Soy oil	34.50	45.76	49.27
Dicalcium phosphate	17.50	16.70	15.84
Limestone	11.34	9.10	8.72
Sodium chloride	2.40	2.75	2.64
L-Lysine HCl	1.00	0.00	0.00
DL-Methionine	2.05	2.75	2.21
L-Threonine	0.64	0.50	0.20
Vitamin premix[1]	2.50	2.50	2.50
Mineral premix[2]	1.00	1.00	1.00
Sodium bicarbonate	0.60	0.50	0.50
Sawdust	1.00	1.00	1.00
Analyzed value,[3] %			
DM	88.98	89.23	89.52
CP	22.61	20.94	19.63
Ether extract	5.73	7.11	7.39
Crude fiber	3.28	3.13	3.04
Crude ash	6.30	5.90	5.69
Ca	1.09	0.98	0.89
P (total)	0.71	0.69	0.62
Calculated value			
ME, kcal/kg	3,013	3,161	3,203
Lysine, %	1.26	1.07	0.97
Methionine, %	0.55	0.52	0.49
Methionine + cysteine, %	0.96	0.92	0.84
Threonine, %	0.89	0.82	0.73
Linoleic acid, %	2.81	3.45	3.69

[1]Provided per kilogram of diet: *trans*-retinol, 12,000 IU; cholecalciferol, 1,500 IU; α-tocopherol acetate, 75 mg; vitamin K_3, 5 mg; vitamin B_1, 3 mg; vitamin B_2, 6 mg; vitamin B_6, 5 mg; vitamin B_{12}, 0.03 mg; nicotineamide, 40 mg; pantothenic acid, 10 mg; folic acid, 0.75 mg; D-biotin, 0.075 mg; and choline, 375 mg.

[2]Provided per kilogram of diet: Mn, 80 mg; Fe, 40 mg; Zn, 60 mg; Cu, 5 mg; I, 0.5 mg; Co, 0.2 mg; Se, 0.15 mg.

[3]Analyzed values refer to basal (control) diets.

(ENZ) contained 46,800 IU of xylanase/g, 36,000 IU of amylase/g, 120 IU of protease/g, 120 IU of cellulose/g, 600 IU of β-glucanase/g, and 48 IU of mannanase/g as determined by the manufacturer. The probiotic preparation (PRO) contained *Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterococcus faecium*, and *Bifidobacterium bifidum*; the microbial blends and concentrations are proprietary. Bio-Mos is derived from a select strain of the yeast *Saccharomyces cerevisiae* by a proprietary process developed by Alltech Inc. Bio-Mos comes from the outer membrane, rich in mannan, that is extracted from the whole cell yeast. The specific essential oil blend was provided by Herba Ltd. Co. (Seferihisar, İzmir-Turkey). It contained carvacrol (33.0%), 1,8-cineole (20.0%), camphor (15.1%), and thymol (5.9%) as the main active components. Thus, the EOM preparation provides 7.93 mg of carvacrol, 4.80 mg of 1,8-cineole, 3.62 mg of camphor, and 1.42 mg of thymol per each kilogram of diet. These 3 essential oils were derived from selected herbs growing in Turkey: oregano oil (*Origanum* spp.), laurel leaf oil (*Laurus nobilis*), and lavender oil (*Lavandula stoechas*). Active compounds in essential oil mixture are shown in Table 2. The composition of the EOM was determined using the GC/MS (HP 6890GC/5973 MSD) system. The EOM was distilled from the ground feed samples using the Clevenger distillation apparatus in accordance with United States Pharmacopoeia (**USP**) methods and USP 23 NF18 (1995). First, the EOM was obtained by using the Clevenger apparatus to distill 100 g of the ground feed samples in water for 2 h. The oil was then diluted with n-hexane (1:100) and injected into the Gas Chromatograph/Mass Selective Detector (Hewlett Packard 6890 GC System Gas Chromatograph with 5973 Mass Selective Detector) system [injection temperature: 250°C; injection split: 1/100; column: DB-17 30m, 0.25 μm, 0.32 mm (Agilent Technologies, Santa Clara, CA); initial oven temperature: 70°C, at a rate of 8°C/min; final oven temp: 200°C; injection volume: 1 μL]. The proportion of each in the mixture was 1:1, and the essential oil preparation used was 960 g of zeolite as a feed-grade inert carrier for each 40 g of EOM.

Broiler Performance Responses

Chicks were weighed pen basis on d 1, 14, 28, and 42 to determine BW gain (**BWG**) through relevant experimental periods. Feed intake within each subgroup was calculated at d 14, 28, and 42 by subtracting residual feed from the offered feed. The feed conversion ratio (**FCR**) was calculated as the ratio of FI to BWG (g of feed/g of gain). Mortality was recorded daily and expressed as a percentage of the initial number of chicks. The FCR was adjusted for mortality and calculated on a per pen basis. Any bird that died was weighed and the FCR values were calculated by dividing total FI by BWG of live plus dead birds. Necropsies were performed on birds that died during the current study.

Eimeria Infection and Fecal Oocyst Measurements

Chicks were infected at 14 d age with a standard oral inoculum containing 5×10^5 sporulated oocysts from field isolates of *Eimeria acervulina*, *maxima*, *tenella*, *mitis*, *brunetti*, and *praecox*. The reference stocks in the current experiment were provided by the Department of Parasitology at the Veterinary Medicine Faculty of Ankara University, Turkey. These reference stocks were maintained by periodic passage through coccidia-free chicks and sporulated in 2% potassium dichromate by standard operation in Ankara University.

The inoculum was washed several times with tap water to remove potassium dichromate then a 2-mL suspension of 5×10^5 sporulated oocysts administered directly into the crop by oral gavage by using a plastic syringe fitted with a plastic cannula.

Sampling was carried out by collecting approximately 400- to 500-g samples of excreta from each replicate pen. Oocyst counts were determined in samples of excreta obtained from each subgroup at 10 and 14 d of age [i.e., before infection, and determined daily from d 19 (5 dpi) to d 36 (22 dpi) only for infected groups (**dpi** = days postinfection)]. Test samples excreta were collected from the uninfected pens on d 24 and 36. Random collection of the samples obtained from each pen was necessary to obtain an accurate estimate of oocyst excretion. All areas of the pen floor were sampled, and a representative amount of excreta was collected. Samples were collected daily and placed in separate airtight plastic bags, homogenized thoroughly with a domestic mixer, and kept refrigerated until total oocyst counts were determined (Christaki et al., 2004). Homogenized samples were diluted 10-fold with tap water and further diluted with saturated saline solution at a ratio of 1:10. Oocyst counts were determined using McMaster cham-

Table 2. Bioactive components of the essential oil mixture (analyzed values)

Compound	Value, %
Carvacrol	33.07
1,8-Cineole	20.00
Camphor	15.12
Thymol	5.94
Myrteny acetate	1.81
(+) Borneol	1.45
Linalool	1.42
α-Pinene	1.29
p-Cymene	1.13
Bornylester	1.12
β-Bisabolene	1.07
α-Terpineol	0.83
Camphene	0.70
Trans caryophylene	0.64
Limonen	0.62
Others[1]	13.79

[1]Includes 17 other active components ranging in a regularly decreasing order.

bers and expressed as the number of oocysts per bird (Hodgson, 1970).

Measurements of Intestinal Regions

Ten days after the inoculation (d 24), 3 birds whose BW were similar to the group mean were selected from each replicate pen (15 birds per treatment group) after feed deprivation for 10 h. The 180 sampled birds were electrically stunned and slaughtered. After 3 min suspension for exsanguination, birds were eviscerated, and their complete intestines, liver, and pancreas were removed. The total length of the small intestine (duodenum, ileum, and jejunum) and large intestines (colon) provided the intestinal length. The weight of intestines, liver, pancreas, and ceca was expressed as a percentage of live BW.

Lesion Score

Complete intestines of the same birds used for determination of intestinal measurements were also examined for degree of presence of coccidial lesions. Five different sections of the chick intestine (i.e., duodenum, jejunum, ileum, cecum, and colon) were examined for lesions. Lesion scores were observed and recorded according to the system of Johnson and Reid (1970). A lesion score was assigned from 0 to 4, where 0 corresponds to normal status with no gross lesions, 1 to small scattered petechiae, 2 to numerous petechiae, 3 to extensive hemorrhage, and 4 to extensive hemorrhage that gives a dark color to the cecal intestine.

Statistical Analysis

Data on growth performance parameters (BWG, FI, FCR, and mortality) and number of oocysts excreted were analyzed on a pen basis, whereas data on organ measurements and intestinal lesion scores were based on individual broilers. Data regarding growth performance parameters were analyzed on a 2-factorial ANOVA using the GLM procedure (SAS Institute Inc., 2001). The main effects of coccidial infection, diet, and the infection × diet interaction were tested. Because related symptoms observed only in the infected chicks, data regarding number of oocyst excreted and intestinal lesion scores were subjected to ANOVA using the GLM procedure of SAS system (SAS Institute Inc., 2001). Duncan's multiple-range test was carried out to detect differences among treatments. All differences were considered significant at $P < 0.05$. Because the oocyst yields and lesion scores were not distributed normally, the Kruskal-Wallis nonparametric analysis (SAS Institute Inc., 2001) was employed. Arcsin transformation was applied to the percentage values (i.e., mortality and relative weights of digestive organs) before testing for differences.

RESULTS AND DISCUSSION

Broiler Growth Performance

Data regarding performance traits in 2-wk intervals are shown in Table 3. The average BW of newly hatched broiler chicks was 46.9 ± 0.96 g and did not differ among the different treatment groups ($P = 0.4663$). For the first 2 wk, all of the supplements led to increased BWG ($P < 0.01$), in association with increased FI ($P < 0.01$), and thus decreased FCR ($P < 0.01$), compared with the results obtained from CNT birds (Table 3). The ENZ-treated chickens showed the highest BWG (614 g) and lowest FCR (1.41) during the challenge-free period. Mortality was not significantly different in any of the groups ($P > 0.05$) before the challenge was implemented.

Broiler chicks differed in response to dietary supplements in terms of performance traits, including BWG, FI, and FCR under the coccidial challenge or sanitary conditions during 15 to 28, 29 to 42, and 1 to 42 d (Table 3). The differences in responses resulted in significant ($P < 0.01$) infection × diet interactions for the above-mentioned traits in both the postinfection and complete growth period excluding the period of 29 to 42 d. These results clearly indicate that these supplements have different efficacies under unchallenged and parasitic disease challenge conditions.

In the untreated CNT group, the chicks infected with mixed *Eimeria* spp. on d 14 presented subclinical signs of coccidiosis with a strong reduction in BWG (185 g) and FI (156 g) as well as worsened FCR (0.11) at 15 to 28 dpi. On the basis of these results, coccidial infection encountered at even a subclinical level is sufficient to preclude birds from achieving their genetic potential. However, soon after the experimental infection, all of the supplements were successful in alleviating growth retardation due to coccidial challenge compared with CNT chicks. Under unchallenged conditions, ENZ, PRO, and PRE showed significant improvement in growth throughout the entire experimental period compared with the untreated group. Related data indicate that the extent of positive responses to supplements during the 1 to 42 d, with respect to performance indices, were more pronounced under coccidial challenge conditions rather than under unchallenged conditions (Table 3). The observed significant improvements in BWG and, to a lesser extent, in FCR with respect to dietary administration of SAL, ENZ, PRO, PRE, and EOM preparations are in agreement with reports that indicate a significant increase in productivity with the use of these additives under unchallenged conditions (Bedford, 2000; Mountzouris et al., 2010; Bozkurt et al., 2012a; Yang et al., 2012).

The supplementation diet with ENZ, PRO, and PRE promoted whole FI of uninfected birds ($P < 0.01$) compared with CNT birds, but SAL and EOM showed no effect (Table 3). However, under the *Eimeria* challenge,

Table 3. Body weight gain (BWG; g), feed intake (FI; g), feed conversion ratio (FCR; g of feed/g of gain), and overall mortality (%) after broilers were infected with an inoculum containing 5×10^5 oocysts of *Eimeria* at 14 d of age and provided with diets supplemented with anticoccidial, enzyme mixture, probiotic, prebiotic, and an essential oil mixture

		d 1 to14			d 15 to 28			d 29 to 42			d 1 to 42			
Item	Diet[1]	BWG	FI	FCR	BWG	FI	FCR	BWG	FI	FCR	BWG	FI	FCR	Mortality
Infection														
−	CNT	383	566	1.47	998^{c}	1,629^{b}	1.63^{d}	1,148	2,342	2.04	2,520^{d}	4,547^{bc}	1.80^{cd}	2.56
	SAL	420	600	1.42	1,014^{bc}	1,619^{b}	1.59^{e}	1,139	2,284	2.00	2,573^{c}	4,516^{bc}	1.75^{f}	2.04
	ENZ	423	600	1.41	1,036^{ab}	1,694^{a}	1.63^{d}	1,176	2,403	2.04	2,638^{a}	4,712^{a}	1.78^{de}	2.66
	PRO	423	610	1.44	1,031^{ab}	1,677^{a}	1.62^{de}	1,179	2,398	2.03	2,625^{ab}	4,708^{a}	1.79^{de}	2.05
	PRE	419	604	1.43	1,054^{a}	1,697^{a}	1.61^{de}	1,151	2,281	1.98	2,648^{a}	4,694^{a}	1.77^{ef}	2.05
	EOM	411	594	1.44	1,028^{abc}	1,676^{a}	1.63^{d}	1,139	2,316	2.03	2,582^{bc}	4,579^{b}	1.77^{ef}	2.50
+	CNT	385	576	1.49	813^{g}	1,473^{ef}	1.81^{a}	1,139	2,351	2.06	2,347^{f}	4,392^{de}	1.87^{a}	2.50
	SAL	423	602	1.42	936^{d}	1,535^{cd}	1.64^{de}	1,138	2,295	2.01	2,500^{d}	4,422^{d}	1.76^{ef}	1.50
	ENZ	426	601	1.41	894^{e}	1,563^{c}	1.74^{c}	1,177	2,416	2.05	2,498^{d}	4,569^{bc}	1.82^{bc}	2.00
	PRO	426	618	1.45	837^{fg}	1,492^{de}	1.78^{b}	1,171	2,420	2.06	2,442^{e}	4,509^{c}	1.84^{b}	2.00
	PRE	415	602	1.45	852^{f}	1,448^{f}	1.79^{ab}	1,173	2,392	2.04	2,419^{e}	4,332^{e}	1.79^{de}	2.00
	EOM	414	598	1.44	802^{g}	1,443^{f}	1.79^{ab}	1,145	2,309	2.01	2,355^{f}	4,358^{de}	1.85^{ab}	2.00
SEM[2]		4.07	5.26	0.01	12.61	15.19	0.01	17.88	19.91	0.02	17.80	23.21	0.01	0.57
Infection														
−		415	599	1.44	856	1,665	1.74	1,157	2,364^{a}	2.02	2,427	4,430	1.82	2.00
+		413	596	1.44	1,027	1,492	1.62	1,152	2,337^{b}	2.04	2,598	4,626	1.78	2.31
Diet														
CNT		384^{c}	571^{c}	1.48^{a}	906	1,551	1.72	1,143	2,347^{b}	2.05	2,434	4,469	1.84	2.53
SAL		422^{a}	601^{b}	1.42^{cd}	975	1,577	1.61	1,139	2,289^{c}	2.01	2,537	4,469	1.76	1.77
ENZ		425^{a}	601^{b}	1.41^{d}	965	1,629	1.69	1,177	2,410^{a}	2.04	2,568	4,640	1.81	2.33
PRO		424^{a}	614^{a}	1.44^{b}	934	1,584	1.70	1,175	2,409^{a}	2.05	2,534	4,608	1.82	2.02
PRE		417^{ab}	603^{b}	1.44^{b}	953	1,572	1.65	1,162	2,337^{b}	2.01	2,533	4,513	1.78	2.02
EOM		412^{b}	596^{b}	1.44^{b}	915	1,560	1.71	1,142	2,312^{bc}	2.02	2,468	4,468	1.81	2.25
P-value[3]														
Infection		0.488	0.208	0.478	0.001	0.001	0.001	0.841	0.027	0.173	0.001	0.001	0.001	0.353
Diet		0.001	0.001	0.001	0.001	0.001	0.001	0.129	0.001	0.285	0.001	0.001	0.001	0.822
Infection × diet		0.945	0.866	0.768	0.001	0.001	0.001	0.956	0.067	0.756	0.001	0.001	0.003	0.987

[a–g]Values within a column not sharing the same superscript are different at $P < 0.05$.

[1]The broilers were fed a control diet (CNT) containing no anticoccidial and performance enhancer and supplemented with preparations of anticoccidial salinomycin (SAL; 60 mg/kg of diet), enzyme mixture (ENZ; 60 mg/kg of diet), probiotic (PRO; 1 g/kg of diet), prebiotic (PRE; 1 g/kg of diet), and an essential oil mixture (EOM; 40 mg/kg of diet).

[2]Data are means of 5 replicate pens with 200 chicks each per treatment.

[3]Data were analyzed as a 2 × 6 arrangement.

ENZ and PRO were effective in alleviating the depression in FI during the 15 to 28 and 29 to 42 dpi ($P < 0.01$). However, birds fed PRE and EOM tended to consume less feed than their untreated controls during the same postinfection periods.

Feed was more efficiently utilized by the treated chicks compared with the CNT chicks (Table 3). Overall, FCR significantly decreased ($P < 0.01$) in birds fed PRE and EOM under unchallenged conditions; however, this was the case for ENZ and PRE treatments in infected birds. The SAL was shown to be the best supplement for improving overall FCR of treated broiler chickens compared with CNT treatment, both under unchallenged (1.75 versus 1.80) and challenge (1.76 versus 1.87) conditions. Indeed, the ability of the performance-enhancing feed additives to improve nutrient utilization by positive manipulation of the gastrointestinal tract is well understood (Tellez et al., 2006; Yang et al., 2009; Chapman et al., 2010), but the manner by which they benefit chickens exposed to coccidial infection remains almost unknown. However, with regard to salinomycin, the mode of action and the subsequent benefits on production performance are far better understood (Chapman, 1998).

A general impairment of nutritional elements (Turk, 1972) and digestive enzyme activities (Major and Ruff, 1978) occurs in birds infected with coccidia. In this study, lessened intestinal lesion scores in response to administration of a diet with supplements might have contributed to the improvement in feed conversion efficiency under the coccidial challenge.

Scientific evidence shows that the most common effects of coccidiosis in poultry are reduction of BWG, in association with diminished FI, and a concomitant adverse effect on FCR (Cook, 1998; McDougald, 2003). In the present study, chicks subjected to mixed *Eimeria* infection benefited from the dietary provision with those supplements in terms of performance enhancers. Considering the significantly increased BWG regarding d 1 to 42, we showed that dietary supplementation with SAL, ENZ, PRO, and PRE, but not EOM, appeared to reduce the adverse effects after the challenge with *Eimeria* spp. This agrees with the report of Taherpour et al. (2012) that indicates that optimal response in growth and feed efficiency in chickens occur with PRO and PRE during a coccidial infection.

Despite the improvements in the fecal oocyst excretion and intestinal lesions from the application of EOM (Figure 1), there was no significant improvement in the broiler overall growth performance. Contrary to our observations, dietary supplementation with oregano EO generally has positive effects on both performance and anticoccidial action in broilers infected with *Eimeria* spp. (Giannenas et al., 2003; Waldenstedt, 2003; Reisinger et al., 2011; Bozkurt et al., 2012c). It is understood that, beyond the anticoccidial mode of action, the magnitude of improvement in growth performance will depend on other factors related to gut ecology (e.g., gut microflora and histomorphology, gut maintenance, mucus production, and host immune response) that consume part of the energy and nutrients the host otherwise uses for production purposes (Koutsos and Arias, 2006; Mountzouris et al., 2011). It is important to consider that some phytochemicals might elicit undesirable adverse interactions with other dietary nutrients (Greathead, 2003) and even cause harmful effects on the performance of uninfected (Lee et al., 2004; Bozkurt et al., 2012b) and infected chickens (Küçükyılmaz et al., 2012).

Unfortunately, there has been no specific attempt to replace anticoccidial drugs with an enzyme preparation, to ascertain the efficacy of exogenous enzymes under a coccidial challenge. From our results, it can be concluded that a multi-ENZ preparation was effective

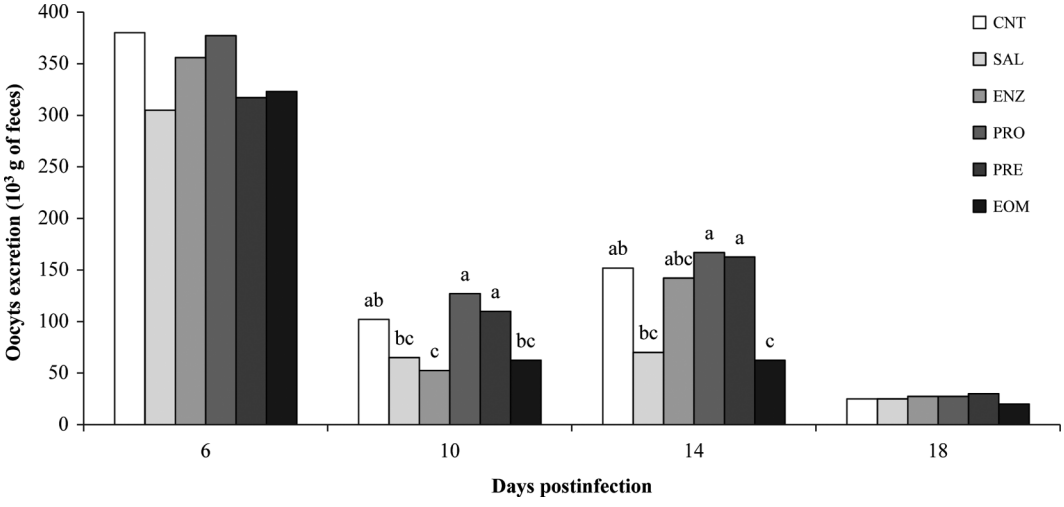

Figure 1. Daily (as measured at 6, 10, 14, and 18 dpi) fecal oocyst output (means of 5 measurements each per treatment) in chicks given diet supplemented with feed additives after broilers were infected with an inoculum containing 5×10^5 oocysts of *Eimeria* at 14 d of age. The broilers were fed a control diet (CNT) containing no anticoccidial and performance enhancer and supplemented with preparations of anticoccidial salinomycin (SAL; 60 mg/kg of diet), enzyme mixture (ENZ; 60 mg/kg of diet), probiotic (PRO; 1 g/kg of diet), prebiotic (PRE; 1 g/kg of diet), and an essential oil mixture (EOM; 40 mg/kg of diet). Within the same day, means with different letters (a–c) differ significantly ($P < 0.05$).

Table 4. The effects of feed additives on length of intestines and cecum, and relative weight (%) of intestines, cecum, liver, and pancreas of birds as measured at 24 d of age (10 d postinfection)

	Relative weight (%)				Length[1] (cm)	
Main effect	Intestines	Cecum	Liver	Pancreas	Intestines	Cecum
Infection						
−	5.31[b]	0.44[b]	2.32[b]	0.35[b]	163[b]	15.20[b]
+	6.60[a]	0.49[a]	2.89[a]	0.39[a]	187[a]	15.86[a]
Diet[2]						
CNT	6.02[ab]	0.49	2.63	0.37	168[bc]	14.93[bc]
SAL	5.69[bc]	0.47	2.61	0.36	166[c]	14.81[c]
ENZ	6.13[ab]	0.45	2.59	0.36	177[ab]	15.37[abc]
PRO	5.36[c]	0.45	2.42	0.38	176[ab]	16.18[a]
PRE	6.21[ab]	0.46	2.73	0.38	180[a]	16.06[a]
EOM	6.32[a]	0.45	2.64	0.37	183[a]	15.82[ab]
Pooled SEM[3]	0.294	0.026	0.105	0.022	5.00	0.461
P-value[4]						
Infection	0.001	0.003	0.001	0.006	0.001	0.017
Diet	0.014	0.313	0.097	0.923	0.005	0.011
Infection × diet	0.099	0.064	0.672	0.212	0.677	0.836

[a–c]Values within a column not sharing the same superscript are different at $P < 0.05$.

[1]The total length of the duodenum, ileum, jejunum, and colon provided the intestinal length.

[2]The broilers were fed a control diet (CNT) containing no anticoccidial and performance enhancer and supplemented with preparations of anticoccidial salinomycin (SAL; 60 mg/kg of diet), enzyme mixture (ENZ; 60 mg/kg of diet), probiotic (PRO; 1 g/kg of diet), prebiotic (PRE; 1 g/kg of diet), and an essential oil mixture (EOM; 40 mg/kg of diet).

[3]Data are means of 15 measurements each per treatment.

[4]Data were analyzed as a 2 × 6 arrangement.

in ameliorating the decrease in BWG and impairment in FCR associated with coccidiosis.

Neither the supplements nor the coccidial infection affected mortality rate ($P > 0.01$) during the entire course of the study (Table 3). The overall mortality rate ranged between 1.5 and 2.5%, even in infected broilers, indicating that coccidial infection encountered in this study was indeed mild and subclinical. In confirmation, postmortem examination of the birds revealed no abnormalities in the gross pathology of major organs. However, higher mortality rates were observed in similar preliminary studies (Giannenas et al., 2003; Christaki et al., 2004), even though the inoculum had a markedly lower number of oocysts than that used in this study. Presumably, the lower mortality was because of the lower pathogenicity of field inoculum used in this study as compared with the severe pathogenicity of laboratory-type inoculum. Therefore, the inoculum dose (5×10^5) applied in the present study did affect performance but did not cause mortality, as in the case of the more severely challenged models.

Intestinal Measurements

Coccidial infection induced substantial ($P < 0.01$) increases in relative weights of the small intestine (25%), cecum (11%), liver (24%), and pancreas (11%) compared with the uninfected birds (Table 4). A similar pattern was observed in the length of the small intestine ($P < 0.01$; 187 vs. 163 cm) and cecum ($P < 0.05$; 15.8 vs. 15.2 cm). No significant infection × diet interaction was found for any of the measurements of digestive organs ($P > 0.05$). Main effects reveal that supplements had significant effects on relative weight and length of the small intestines and cecum length ($P < 0.05$), but they had no significant effects on the weights of the cecum, liver, or pancreas ($P > 0.05$).

Earlier studies mainly focused on the pathological lesions in the intestinal wall due to coccidiosis, but information is lacking on histomorphological effects that might alter the size of the digestive organs. The marked increase in the weight of the small intestine and cecum of the infected chicks appears to be caused by the thickening of the mucosal wall, which might in turn be triggered by the parasitic infection and concurrent bacterial infections (Williams, 2002, 2005). The reduction in the weight of the small intestine and cecum, after administration of a fortified diet with the polyether antibiotic, SAL, correlates well with the mechanism purported that in-feed antibiotics minimize the adverse effect of pathogenic bacteria, lessen the mucosal infection, and thus improve the gut function (Gaskins et al., 2002; Dibner and Richards, 2005). Studies have correlated improvements in balanced cecal microbial community and intestinal integrity with administration of a PRO-supplemented diet (Iji et al., 2001; Yang et al., 2012); this may explain the reduction in the weight of the small intestine and cecum as well as the lesion scores observed in birds treated with PRO.

Previous reports have reported a decrease in the activities of digestive enzymes (Major and Ruff, 1978) and an increase in the digesta passage time (Aylott et al., 1968) in birds challenged with coccidiosis; their findings may help explain our findings of increased relative weights of the liver and pancreas of infected animals compared with their uninfected counterparts. We

presume that our findings were a result of the increased functional activities of these organs, in an attempt to overcome the deficiencies in the production of digestive enzymes and bile salts and to maintain hepatic turnover under the conditions of parasitic infection. Indeed, the deficiency in the production of enzymes and bile salts despite increased relative weights of the liver and pancreas is contradictory. It is possible that production of bile salts (or enzymes, or both) is not deficient per se, but the activity could be impaired due to deconjugation as a result of bacterial activity.

Lesion Score

No lesion score was observed at 24 d of age in the upper and cecal sections of the intestinal tract of birds challenged at 14 d (Table 5). Compared with the CNT group, administration of supplemented diets significantly decreased the intestinal lesion score ($P < 0.01$), with the jejunum and ileum showing a similar pattern. The reduction in lesion score ($P < 0.01$) was more pronounced in birds fed diets containing PRE and EOM than in those fed diets containing other supplements. The total score showed a tendency similar to that of lesion scores observed in the jejunum and ileum, which suggests that these in-feed preparations provided adequate protection from the *Eimeria* infection. Indeed, in the present study, the worst total lesion score (1.37) determined in the control group was markedly lower than 4, which indicates the most severe lesions associated with coccidiosis, according to the scoring system of Johnson and Reid (1970).

Contrary to our observations, most preliminary studies have reported that concurrent heavy infections of coccidiosis produced more severe pathogenic effects (Giannenas et al., 2003; Oviedo-Rondón et al., 2006). The small and insignificant sectional lesions observed in this study indicate that the coccidial challenge procedure we used did not cause apparent severe damage to the intestinal surface. Significant protection against lesions caused by *Eimeria* challenge was provided by preparations of PRO (Giannenas et al., 2012) and PRE (Elmusharaf et al., 2007), or a combination of these supplements (Taherpour et al., 2012). The beneficial effects of these supplements might be related to antimicrobial and competitive exclusion properties of PRO microorganisms and mannan oligosaccharides in the intestinal lumen of birds (Fernandez et al., 2002; Mountzouris et al., 2011; Yang et al., 2012). The favorable effects of multi-ENZ preparation on lessening the total lesions might be driven by a weakening effect on pathogenic bacteria (Bedford, 2000), which might allow the intestinal barrier to inhibit penetration of oocysts through the epithelium. Alleviation of lesion severity due to coccidiosis infection has been ascribed to oregano EO when fed at 15 and 30 mg/kg in diet (Tsinas et al., 2011). Oregano EO and some EO blends were shown to be as effective as the ionophores lasalocid (Giannenas et al., 2003) and bacitracin + monensin (Oviedo-Rondón et al., 2006) in reducing the expression of single or mixed coccidial infection. However, a total eradication of *Eimeria* was not obtained in any of the above-mentioned studies, but rather a lessening of lesion severity was obtained.

Fecal Oocyst Output

The daily fecal oocyst yield from 6 to 18 dpi (20–32 d of age) is shown in Figure 1. Oocysts were not detected in the excreta obtained from noninfected groups. Infected CNT birds excreted the highest number of oocysts. Feeding diets containing SAL decreased ($P < 0.05$) the number of oocyst per gram of feces at 10 dpi; however, this was the case for the EOM treatment at 14 dpi compared with the infected CNT treatment. With respect to fecal oocyst output, no significant difference was found among the treatments at 6 and 18 dpi ($P > 0.05$).

The daily oocyst excretion showed a fluctuating course, rather than a consistent reduction in number of oocysts with time (Figure 1). The pattern of oocyst shedding peaked at 6 and 14 dpi, and then declined steadily through the end of the entire measurement period (6–22 dpi).

In contrast, earlier studies have shown that fecal oocyst yields exhibited a peak with a linear increase after *Eimeria* spp. infection, and then exhibited a gradual decrease that lasted for a maximum of 10 d until the oocyst excretion stopped. An explanation given by McDougald (2003), which suggests that each species cycles at different rates in a mixed rather than single species

Table 5. Intestinal lesion scores of chicks as measured at 24 d of age (10 dpi) subjected to infection with an inoculum containing 5×10^5 oocysts of Eimeria at 14 d of age and provided with diets supplemented with anticoccidial, enzyme mixture, probiotic, prebiotic, and an essential oil mixture

	Intestinal lesion score[1]		
Item	Jejunum	Ileum	Total[2]
Diet[3]			
CNT	1.00[a]	0.37[a]	1.37[a]
SAL	0.31[b]	0.19[b]	0.50[b]
ENZ	0.50[b]	0.12[b]	0.62[b]
PRO	0.50[b]	0.12[b]	0.62[b]
PRE	0.12[c]	0.06[c]	0.18[c]
EOM	0.12[c]	0.06[c]	0.18[c]
Pooled SEM[4]	0.08	0.08	0.09
P-value	0.001	0.001	0.001

[a–c]Values within a column not sharing the same superscript are different at $P < 0.05$.

[1]A lesion score was assigned from 0 (no gross lesions) to 4 (extensive hemorrhage) according to the system of Johnson and Reid (1970).

[2]Data are means of 15 measurements each per treatment.

[3]The broilers were fed a control diet (CNT) containing no anticoccidial and performance enhancer and supplemented with preparations of anticoccidial salinomycin (SAL; 60 mg/kg of diet), enzyme mixture (ENZ; 60 mg/kg of diet), probiotic (PRO; 1 g/kg of diet), prebiotic (PRE; 1 g/kg of diet), and an essential oil mixture (EOM; 40 mg/kg of diet).

[4]Total values of coccidial intestinal lesion scores (no lesion was observed through the intestinal section of duodenum, colon, and cecum).

infection, might account for the discrepancy between ours and earlier results. Girgis (2007) also reported that several outbreaks of coccidiosis can occur at different times in the same flock due to the lack of cross-immunity between *Eimeria* species.

Several earlier experiments showed that birds receiving the diet with PRO (Giannenas et al., 2012) and PRE (Elmusharaf et al., 2007) excreted fewer oocysts, compared with the control birds, which was comparable with the results exhibited after anticoccidial lasalocid treatment. However, these findings differed from our studies in that a similar oocyst number was observed in PRO and PRE treatments and the control groups at all time intervals.

Databased on the evaluation of oocyst output and lesion scores suggests that treatment with a proprietary product (Orego-Stim) at 2 supplemental levels, 300 and 600 mg/kg of diet (which delivers 15 and 30 mg of oregano oil per kg of diet, respectively), could alleviate the impact of coccidial infection from *E. acervulina* and *E. maxima* (Tsinas et al., 2011). However, researchers observed that the exerted coccidiostatic effect against *Eimeria* infection was considerably lower than that exhibited by SAL (60 mg/kg of diet). In contrast, our results showed a similar level of coccidiostatic effect of EOM compared with SAL, verified by excretion of oocysts after mixed *Eimeria*. spp. challenge and treatment with 40 mg of EOM/kg (which provides 13.3 mg of oregano oil per kg of diet). The above-mentioned studies with oregano EO point out that coccidiostatic action can be obtained using oregano oil in the chicken diet within a range from 13 to 30 mg/kg of diet. It is not known why feeding PRO and PRE failed to reduce fecal oocyst output, yet both of them were effective in alleviating the impact of parasite infection as well as lowering lesion score. Considering their potential performance-enhancing effect under coccidial challenge, it can be postulated that PRO and PRE preparations have the potential to lessen the severity of the infection and, at the same time, maintain oocyst production, which is crucial for the reinfection and maintenance of immunity stimulated by the intestinal infection (Elmusharaf et al., 2007). Such a phenomenon, described as a trickle infection, was shown to be very effective in stimulating protective immunity (Joyner and Norton, 1976). The fact is that fecal oocyst output is generally considered to correlate poorly with production performance, but the reduction in lesion score is measurement of success in evaluating coccidiostatic action against *Eimeria* spp.

In conclusion, supplemental intake of SAL, ENZ, PRO, and PRE by chickens on exposure to experimental coccidiosis alleviated the influence of disease and positively influenced growth and feed conversion efficiency. The anticoccidial efficacy of SAL, in terms of lowering oocyst output and overall feed conversion ratio, was more pronounced than any other supplement after an experimental infection at 14 d of age with oocysts of mixed *Eimeria* spp. These observations further support current scientific evidence that these supplements may act as performance enhancers with remarkable benefits in coccidiosis-free broiler chickens reared up to 42 d of age. Further studies are needed to understand whether the ameliorative effect provided by supplements is driven by the specific anticoccidial mode of action or modulator effect. Moreover, the data related to performance indices in this study should not discourage the scientific community from continuing investigations of medicinal plant extracts as alternatives to anticoccidial agents.

REFERENCES

Abbas, R. Z., D. D. Colwell, and J. Gilleard. 2012. Botanicals: An alternative approach for the control of avian coccidiosis. World's Poult. Sci. J. 68:203–215.

Abbas, R. Z., Z. Iqbal, D. Blake, M. N. Khan, and M. K. Saleemi. 2011. Anticoccidial drug resistance in fowl coccidia: The state of play revisited. World's Poult. Sci. J. 67:337–350.

AOAC. 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists Inc., Arlington, VA.

Aviagen. 2007. Broiler Nutrition Specifications. Aviagen Inc., Newbridge, Scotland, UK.

Aylott, M. V., O. H. Vestal, J. F. Stephens, and D. E. Turk. 1968. Effect of coccidial infection upon passage rates of digestive tract contents of chicks. Poult. Sci. 47:900–904.

Bedford, M. R. 2000. Exogenous enzymes in monogastric nutrition—Their current value and future benefits. Anim. Feed Sci. Technol. 86:1–13.

Bozkurt, M., K. Küçükyılmaz, A. U. Çatlı, M. Çınar, M. Çabuk, and A. Alçiçek. 2012a. Effects of administering an essential oil mixture and an organic acid blend separately and combined to diets on broiler performance. Arch. Geflügelk. 2:81–87.

Bozkurt, M., K. Küçükyılmaz, A. U. Çatlı, Z. Özyıldız, M. Çınar, M. Çabuk, and F. Çöven. 2012b. Influences of an essential oil mixture supplementation to corn versus wheat-based practical diets on growth, organ size, intestinal morphology and immune response of male and female broilers. Ital. J. Anim. Sci. 11:290–297.

Bozkurt, M., N. Selek, K. Küçükyılmaz, H. Eren, E. Güven, A. U. Çatlı, and M. Çınar. 2012c. Effects of dietary supplementation with a herbal extract on performance of broilers infected with a mixture of *Eimeria* species. Br. Poult. Sci. 53:325–332.

Brenes, A., and E. Roura. 2010. Essential oils in poultry nutrition: Main effects and modes of action. Anim. Feed Sci. Technol. 158:1–14.

Chapman, H. D. 1998. Evaluation of the efficacy of anticoccidial drugs against *Eimeria* species in the fowl. Int. J. Parasitol. 28:1141–1144.

Chapman, H. D. 2008. Coccidiosis in the turkey. Avian Pathol. 37:205–223.

Chapman, H. D., T. K. Jeffers, and R. B. Williams. 2010. Forty years of monensin for the control of coccidiosis in poultry. Poult. Sci. 89:1788–1801.

Christaki, E., P. Florou-Paneri, I. Giannenas, M. Papazahariadou, N. A. Botsoglou, and A. B. Spais. 2004. Effect of mixture of herbal extracts on broiler chickens infected with *Eimeria tenella*. Anim. Res. 53:137–144.

Cook, G. C. 1998. Small intestinal coccidiosis and emergent clinical problems. J. Infect. 16:213–219.

Dalloul, R. A., and H. S. Lillehoj. 2006. Poultry coccidiosis: Recent advancements in control measures and vaccine development. Expert Rev. Vaccines 5:143–163.

Dibner, J. J., and J. D. Richards. 2005. Antibiotic growth promoters in agriculture: History and mode of action. Poult. Sci. 84:634–643.

Elmusharaf, M. A., H. W. Peek, L. Nollet, and A. C. Beynen. 2007. The effect of an in-feed mannanoligosaccharide preparation (MOS) on a coccidiosis infection in broilers. Anim. Feed Sci. Technol. 134:347–354.

Fernandez, F., M. Hinton, and B. Van Gils. 2002. Dietary mannan oligosaccharides and their effect on chicken caecal microflora in relation to *Salmonella enteritidis* colonization. Avian Pathol. 31:49–58.

Gaskins, H. R., C. T. Collier, and D. B. Anderson. 2002. Antibiotics as growth promotants: Mode of action. Anim. Biotechnol. 13:29–42.

Giannenas, I., P. Florou-Paneri, M. Papazahariadou, E. Christaki, N. A. Botsoglou, and A. B. Spais. 2003. Effect dietary supplementation with oregano essential oil on performance of broilers after experimental infection with *Eimeria tenella*. Arch. Anim. Nutr. 57:99–106.

GiannenasI.PapadopoulosE.TsalieE.TriantafillouE.HeniklS.TeichmannK.TontisD. 2012. Assessment of dietary supplementation with probiotics, on performance, and intestinal morphology and microflora of chickens infected with *Eimeria tenella*. Vet. Parasitol. 188:31–40.

Girgis, G. 2007. Coccidiosis: A field problem with many aspects to consider. World Poult. 8:44–45.

Greathead, H. 2003. Plants and plant extracts for improving animal productivity. Proc. Nutr. Soc. 62:279–290.

Hodgson, J. N. 1970. Coccidiosis: Oocyst-counting technique for cocccidiosis evaluation. Exp. Parasitol. 28:99–102.

Hooge, D. M. 2004. Meta-analysis of broiler chicken pen trials evaluating dietary mannan oligosaccharide, 1993–2003. Int. J. Poult. Sci. 3:163–174.

Iji, P. A., A. A. Saki, and D. R. Tivey. 2001. Intestinal structure and function of broiler chickens on diets supplemented with a mannan oligosaccharide. J. Sci. Food Agric. 81:1186–1192.

Johnson, J., and W. M. Reid. 1970. Anticoccidial drugs: Lesion scoring techniques in battery and floor-pen experiments with chickens. Exp. Parasitol. 28:30–36.

Joyner, L. P., and C. C. Norton. 1976. The immunity arising from continuous low-level infection with Eimeria maxima and *Eimeria acervulina*. Parasitology 72:115–125.

Koutsos, E. A., and V. J. Arias. 2006. Intestinal ecology: Interactions among the gastrointestinal, nutrition and the microflora. J. Appl. Poult. Res. 15:161–173.

Küçükyılmaz, K., M. Bozkurt, N. Selek, E. Güven, H. Eren, A. Atasever, E. Bintaş, A. U. Çatlı, and M. Çınar. 2012. Effects of vaccination against coccidiosis, with and without a specific herbal essential oil blend, on performance, oocyst excretion and serum IBD titers of broilers reared on litter. Ital. J. Anim. Sci. 11:1–8.

Lee, K. W., H. Everts, H. J. Kappert, H. Wouterse, M. Frehner, and A. C. Beynen. 2004. Cinnamaldehyde, but not thymol, counteracts the carboxymethyl cellulose-induced growth depression in female broiler chickens. Int. J. Poult. Sci. 9:608–612.

Lee, S. H., H. S. Lillehoj, R. A. Dalloul, D. W. Park, Y. H. Hong, and J. J. Lin. 2007. Influence of *Pediococcus*-based probiotic on coccidiosis of broiler chickens. Poult. Sci. 86:63–66.

Lee, S. H., H. S. Lillehoj, S. I. Jang, D. K. Kim, C. Ionescu, and D. Bravo. 2010. Effect of dietary *curcuma*, *capsicum*, and *lentinus* on enhancing local immunity against *Eimeria acervulina* infection. Jpn. Poult. Sci. 47:89–95.

Lillehoj, H. S., and E. P. Lillehoj. 2000. Avian coccidiosis. A review of acquired intestinal immunity and vaccination strategies. Avian Dis. 44:408–425.

Lillehoj, H. S., and J. M. Trout. 1996. Avian gut-associated lymphoid tissues and intestinal immune responses to *Eimeria* parasite. Clin. Microbiol. Rev. 9:349–360.

Major, J. R., and M. D. Ruff. 1978. Disaccharidase activity in the intestinal tissue of broilers infected with coccidia. J. Parasitol. 64:706–711.

McDougald, L. R. 2003. Coccidiosis. Pages 974–991 in Poultry Diseases. Y. M. Saif, H. J. Barnes, A. M. Fadly, J. R. Glisson, L. R. McDougald, and D. E Swayne, ed. Iowa State University Press, Ames.

Mountzouris, K. C., V. Paraskevas, P. Tsirtsikos, I. Palamidi, and T. Steiner. 2011. Assessment of a phytogenic feed addtive effect on broiler growth performance, nutrient digestibility and caecal microflora composition. Anim. Feed Sci. Technol. 168:223–231.

Mountzouris, K. C., P. Tsirtsikos, I. Palamidi, A. Arnaviti, M. Mohnl, G. Schatzmayr, and K. Fegeros. 2010. Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition. Poult. Sci. 89:58–67.

Oviedo-Rondón, E. O., M. E. Hume, C. Hernández, and S. Clemente-Hernández. 2006. Intestinal microbial ecology of broilers vaccinated and challenged with mixed *Eimeria* species, and supplemented with essential oil blends. Poult. Sci. 85:854–860.

Peek, H. W., and W. J. Landman. 2003. Resistance to anticoccidial drugs of Dutch avian *Eimeria* spp. field isolates originating from 1996, 1999 and 2001. Avian Pathol. 32:391–401.

Reisinger, N., T. Steiner, S. Nitsch, G. Schatzmayr, and T. J. Applegate. 2011. Effects of a blend of essential oils on broiler performance and intestinal morphology during coccidial vaccine exposure. J. Appl. Poult. Res. 20:272–283.

SAS Institute Inc. 2001. SAS User's Guide. Version 8 ed. SAS Institute Inc., Cary, NC.

Shirley, M. W., A. L. Smith, and D. P. Baker. 2007. Challenges in the successful control of the avian coccidia. Vaccine 25:5540–5547.

Taherpour, K., H. Moravej, H. R. Taheri, and M. Shivazad. 2012. Effect of dietary inclusion of probiotic, prebiotic and butyric acid glycerides on resistance against coccidiosis in broiler chickens. Jpn. Poult. Sci. 49:57–61.

Tellez, G., E. Higgins, A. M. Donoghue, and B. M. Hargis. 2006. Digestive physiology and the role of microorganisms. J. Appl. Poult. Res. 15:136–144.

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

Turk, D. E. 1972. Protozoan parasitic infections of the chick intestine and protein digestion and absorption. J. Nutr. 102:1217–1221.

Waldenstedt, L. 2003. Effect of vaccination against coccidiosis in combination with an antibacterial oregano (*Origanum vulgare*) compound in organic broiler production. Acta Agric. Scan.Sect. A Anim. Sci. 53:101–109.

Wallace, R. J., W. Oleszek, C. Franz, I. Hahn, K. H. C. Başer, A. Mathe, and K. Teichmann. 2010. Dietary plant bioactives for poultry health and productivity. Br. Poult. Sci. 51:461–487.

Williams, R. B. 1999. A compartmentalized model for the estimation of the cost of *Cocccidiosis* to the world's chicken production industry. Int. J. Parasitol. 29:1209–1229.

Williams, R. B. 2002. Anticocccidial vaccines for broiler chickens: Pathways to success. Avian Pathol. 31:317–353.

Williams, R. B. 2005. Intercurrent coccidiosis and necrotic enteritis of chickens. Rational, integrated disease management by maintenance of gut integrity. Avian Pathol. 34:159–180.

Yang, C. M., G. T. Cao, P. R. Ferket, T. T. Liu, L. Zhou, L. Zhang, Y. P. Xiao, and A. G. Chen. 2012. Effects of probiotic, *Clostridium butyricum*, on growth performance, immune function cecal microflora in broiler chickens. Poult. Sci. 91:2121–2129.

Yang, Y., P. A. Iji, and M. Choct. 2009. Dietary modulation of gut microflora in broiler chickens: A review of the role of six kinds of alternatives to in-feed antibiotics. World's Poult. Sci. J. 65:97–114.