

# The effects of dietary oregano essential oil on live performance, carcass yield, serum immunoglobulin G level, and oocyst count in broilers

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**Primary Audience:** Nutritionists, Feed Additive Companies, Veterinarians, Researchers, Production Managers

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## SUMMARY

The present study was designed to evaluate the effect of dietary oregano essential oil on the live performance, carcass yield, and serum IgG levels of broilers and to examine its anticoccidial effect. In a completely randomized design, 1,200 straight-run Ross 308 broilers were allocated into 3 experimental groups, each consisting of 5 replicates. The first group received a basal diet with an anticoccidial (Cygro) at a level of 100 mg/kg of the feed. The second group received diets supplemented with oregano essential oil (Orego-Stim) at a level of 300 mg/kg of the feed, whereas the third group received neither the coccidiostat nor oregano oil (negative control). Throughout the experimental period of 42 d, individual BW and pen feed intake were recorded weekly, and FCR were calculated. Blood samples were collected on d 1 and 42 to determine serum IgG levels. Oocyst counts were determined in excreta samples taken at 10-d intervals from each replication. Results indicated that dietary oregano essential oil and anticoccidial supplementation improved the FCR significantly ( $P < 0.05$ ) from 21 to 42 d and 1 to 42 d of age, respectively, compared with the negative control diet. Birds fed the oregano oil diet consumed significantly less feed compared with those fed the negative control diet from 21 to 42 d and from 1 to 42 d of age. No dietary effect was observed on the preslaughter weight, carcass yield, or serum IgG level ( $P > 0.05$ ) at 42 d. Although not significant, there was a slight increase in the serum IgG levels of broilers fed the oregano oil diet. Although the dietary oregano oil significantly ( $P < 0.05$ ) lowered the excreta oocyst counts compared with those of birds fed the negative control diet, its anticoccidial effect was significantly less than the effects in birds fed the diet with the anticoccidial. In conclusion, oregano essential oil may provide an alternative to conventional anticoccidial additives in broiler feeds.

**Key words:** broiler, coccidiosis, growth performance, oregano essential oil, serum immunoglobulin G

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## DESCRIPTION OF PROBLEM

Herbal compounds, mainly essential oils, have been evaluated as possible feed additives for animal and poultry production, especially considering their *in vitro* antimicrobial activity [1–3]. The proposed mode of action of plant products is attributed to their antimicrobial properties [3, 4], oxidative-resistant activity [5], enhancement of the immune system [6], control of coccidial infections [7], and, consequently, improvement in poultry performance.

Lee et al. [4] reviewed the classification and synthesis of essential oils and their biological effects on chicken growth. They indicated that results are conflicting regarding the effects of essential oils on growth performance in chickens. This might be partly because plant essential oil concentrations and compositions are variable.

Oregano, or scientifically named, *Origanum vulgare* L., is a common species of *Origanum*, an aromatic herbal product, which, because of its highly potent chemical nature, has been used as an alternative growth promoter in the poultry industry. It contains mainly carvacrol, thymol, and their precursors [4, 8, 9].

Lee et al. [10] reported that a dietary concentration of 200 ppm of carvacrol vs. thymol fed to broilers lowered BW gain and feed intake but improved the feed:gain ratio at 4 wk of age. Botsoglou et al. [5] indicated that dietary oregano oil showed no growth-promoting effect on broilers when administered at 50 or 100 mg/kg of feed. Oviedo-Rondon et al. [11] evaluated the efficacy of 2 specific essential oil blends (Crina Poultry and Crina Alternate) on coccidia-challenged birds. They reported that the Crina Poultry blend helped to lower coccidial lesion scores in the duodenum, whereas the Crina Alternate reduced the lesions in the cecum.

The objective of the current study was to determine the effects of dietary oregano essential oil [12] on growth performance, carcass yield, and the serum IgG levels in broilers. The anti-coccidial effect of dietary oregano essential oil was also investigated.

## MATERIALS AND METHODS

A total of 1,200 straight-run Ross 308 (Ross × Ross) day-old broiler chicks obtained from a commercial hatchery were randomly divided

into 3 treatment groups consisting of 400 birds each. Every group was further divided into 5 replicates of 80 birds each. They were then housed in floor pens (2.40 × 3.25 m) equipped with deep wood shavings as the litter material. The birds used in this study were not challenged with coccidiosis, and the experiment was conducted in the months of May and June. The lighting regimen provided 23 h of light per day. Commercially mixed diets were formulated to meet or exceed the minimum NRC [13] requirements for broilers and were in mash form (Table 1). The negative control diet contained no coccidiostat or oregano oil, whereas the other 2 diets contained either oregano essential oil at a level of 300 mg/kg or a coccidiostat [14] at the level of 100 mg/kg feed. The commercial oregano oil (Orego-Stim), which was in powder form [12], contained 5% essential oil of *O. vulgare* ssp. *hirtum* plants and 95% natural feed grade inert carrier. The starter and finisher diets were provided from 1 to 21 d and 21 to 42 d of age, respectively. Feed and water were provided at all times. Body weights of the birds were measured individually, and feed intake per pen was recorded weekly. Mortality was recorded as it occurred. Total feed consumption per bird was adjusted according to daily mortalities. At 42 d, all broilers were weighed. Two male birds randomly selected from each pen were weighed and then slaughtered by cutting the carotid artery and jugular vein on the side of the neck, with subsequent exsanguination. Preslaughter weights and cold carcass weights of the selected birds were recorded, and percentage carcass weights were calculated. All bird-handling procedures were approved by the Ethics Committee of the Faculty of Veterinary Medicine, University of Istanbul.

### Determination of Serum IgG Level

On d 1, an additional 40 chicks were killed by cervical dislocation to determine the baseline levels of IgG in the blood serum. Again, before processing of the experimental birds for carcass performance, 2 birds from each replicate were randomly selected for collection of blood samples for IgG determination on d 42. The ELISA method used previously by Erhard et al. [15] and Li et al. [16] was modified in the present study by

**Table 1.** Composition of the experimental broiler diets (% , as-is basis)

Item	Starter			Finisher		
	Negative control	Coccidiostat	Oregano oil	Negative control	Coccidiostat	Oregano oil
<b>Ingredient</b>						
Ground corn	51.00	51.09	51.27	52.65	52.74	52.92
Wheat	3.95	3.85	3.65	4.30	4.30	4.30
Wheat bran fine middlings	—	—	—	4.75	4.65	4.45
Soybean meal (46% CP)	24.60	24.60	24.60	19.00	19.00	19.00
Full-fat soybeans	7.50	7.50	7.50	7.00	7.00	7.00
Fish meal (67% CP)	6.60	6.60	6.60	5.50	5.50	5.50
Sunflower oil, 8,950 kcal/kg	3.00	3.00	3.00	4.00	4.00	4.00
Limestone	1.15	1.15	1.15	1.00	1.00	1.00
Dicalcium phosphate <sup>1</sup>	1.35	1.35	1.35	0.95	0.95	0.95
Vitamin-mineral premix <sup>2</sup>	0.35	0.35	0.35	0.35	0.35	0.35
Salt	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.15	0.15	0.15	0.15	0.15	0.15
Antioxidant	0.10	0.10	0.10	0.10	0.10	0.10
Coccidiostat <sup>3</sup>	—	0.01	—	—	0.01	—
Oregano essential oil powder <sup>4</sup>	—	—	0.03	—	—	0.03
Total	100.00	100.00	100.00	100.00	100.00	100.00
<b>Calculated and analyzed values</b>						
ME, kcal/kg	3,165	3,167	3,160	3,230	3,231	3,230
CP, % (analyzed)	23.59	23.60	23.57	21.02	21.03	21.02
Lysine, %	1.31	1.31	1.31	1.11	1.11	1.11
Methionine, %	0.54	0.54	0.54	0.50	0.50	0.50
Methionine + cysteine, %	0.86	0.86	0.86	0.77	0.77	0.77
Calcium, % (analyzed)	1.11	1.11	1.11	0.90	0.90	0.90
Available phosphorus, %	0.50	0.50	0.50	0.40	0.40	0.40

<sup>1</sup>Contains 24% calcium and 17.5% available phosphorus.

<sup>2</sup>Supplied the following per kilogram of diet: vitamin A, 15,000 IU; cholecalciferol, 1,500 ICU; vitamin E, 30.0 IU (DL- $\alpha$ -tocopheryl acetate); menadione, 5.0 mg; thiamine, 3.0 mg; riboflavin, 6.0 mg; niacin, 20.0 mg; pantothenic acid, 8.0 mg; pyridoxine, 5.0 mg; folic acid, 1.0 mg; vitamin B<sub>12</sub>, 15  $\mu$ g; manganese, 80.0 mg; zinc, 60.0 mg; iron, 30.0 mg; copper, 5.0 mg; and iodine, 2.0 mg; and selenium, 0.15 mg.

<sup>3</sup>Cygro (Alpharma Animal Health, Inc., NJ).

<sup>4</sup>Orego-Stim (Meriden Animal Health Ltd., Luton, UK).

using monoclonal anti-chicken IgG, which was diluted (2, 5, and 10  $\mu$ g/mL) in a coating buffer of 0.05 M carbonate-bicarbonate at 9.6 pH. The polystyrene ELISA plates were coated at 4°C with 100  $\mu$ L of the various dilutions of monoclonal anti-chicken IgG. The plates were then blocked with 100  $\mu$ L of PBS containing 2.0% BSA and were incubated at 37°C for 1 h. Serum samples were diluted from 1:50 to 1:6,400, and 100  $\mu$ L of each dilution was added to the wells. One hundred microliters of each 2-fold dilution of standard chicken IgG [17], from 40 to 0.306  $\mu$ g in PBS-Tween 20, was added to the control wells. The plates were then incubated at 37°C for 80 min. Peroxidase-conjugated rabbit anti-chicken IgG was diluted in PBS-Tween 20 to 1:20,000, and 100  $\mu$ L of this dilution was added

as the secondary antibody. The plates were then incubated at 37°C for 70 min. They were washed 4 times with PBS-Tween 20 after each incubation. One hundred microliters of peroxidase substrate (1:10 wt/vol; peroxidase tablet [17]) was added to all the wells, and the plates were read at an optical density of 492 nm after 20 min. The test serum samples were analyzed at a dilution of 1:200 using the 5  $\mu$ g/mL monoclonal antibody-coated plates.

### *Oocyst Counts of Excreta Samples*

Approximately 50 g of excreta sample was randomly collected from each pen on d 10, 20, 30, and 40 of the experiment. Samples from each pen were placed in separate airtight plastic bags,

homogenized thoroughly, and refrigerated until assayed for total oocyst counts. Homogenized samples were diluted 10-fold with tap water and further diluted with a saturated sodium chloride solution at a ratio of 1:10. Oocysts counts were determined using McMaster chambers [18] and expressed as the number of oocysts per gram of excreta [19].

### **Statistical Analysis**

All data were subjected to one-way ANOVA using the GLM procedure of SAS [20]. The statistical differences between treatments were determined by a Tukey test. Comparison of the oocyst counts at different times among groups was done using the nonparametric Kruskal-Wallis test. Mortality percentage data were analyzed after arcsine transformation. All statements of significance are based on a probability of less than 0.05 [20].

## **RESULTS AND DISCUSSION**

### **Broiler Performance and Carcass Yield**

The composition of the experimental diets is shown in Table 1. Analyzed CP and calculated lysine values of the starter and finisher diets were above the NRC [13] requirements, whereas other nutrients were within the recommended range. The effects of dietary treatments on BW, feed intake, and FCR at d 21 and 42 of the experiment are presented in Table 2. The experimental diets had no effect on BW or BW gain from 1 to 42 d of age. These results were consistent with the findings of Botsoglou et al. [5]. A significant difference ( $P < 0.05$ ) in feed consumption at 21 and 42 d of the experiment was noted between birds fed the negative control diet and the oregano oil diet, whereas the feed intake of birds fed the diet with coccidiostat was intermediate. Birds consuming the negative control diet had a significantly higher ( $P < 0.01$ ) feed:gain (FCR) ratio than did birds fed the other diets from 21 to 42 d and 1 to 42 d of age, respectively. The significantly improved FCR of birds fed the diet containing oregano oil agreed with the results reported by Basset [8] and Alçiçek et al. [21]. Basset [8] also reported that supplementing oregano essential oil in the drinking water increased BW and improved

the FCR of birds. Similarly, improved broiler performance was reported by Ather [22] and Hertrampf [23]. The improved feed utilization with oregano oil in the present study could be due to a stimulating effect of essential oils on the digestion process, as reported by Langhout [1] and Williams and Losa [24]. There is evidence to suggest that herbs, spices, and various plant extracts have appetite- and digestion-stimulating properties [25]. Plant extracts containing various molecules have intrinsic bioactivities on broiler physiology and metabolism [26]. However, the intestinal microflora of birds, and internal and external stress factors during grow-out trials also contribute to conflicting results when using essential oil extracts in experimental diets for poultry [4].

Dietary treatment with oregano oil or the coccidiostat had no effect on preslaughter weight, carcass weight, or carcass yield (Table 3) in the present study. In contrast with our results, Alçiçek et al. [21] reported that supplementing essential oils at a concentration of 48 or 72 mg of an essential oil combination/kg of feed significantly improved carcass yield.

The total mortalities were 4.0, 11.25, and 8.25% for the coccidiostat, negative control, and oregano oil treatments, respectively, at 42 d (data not shown). The birds receiving no dietary additives (negative control) had the highest mortality rate, and birds receiving the coccidiostat had the lowest, whereas the mortality rate was intermediate for birds receiving the oregano oil. Because no necropsy was performed on the dead birds, it is difficult to establish a correlation between mortality and the presence of oocysts in the excreta in all treatments.

### **Serum IgG Levels**

Blood serum IgG at d 1 was determined to be 14.60  $\mu\text{g/mL}$ , and it decreased over time (Table 4). The supplementation of oregano oil or coccidiostat did not have a significant effect ( $P > 0.05$ ) on IgG levels in the blood serum of broilers. However, the level of IgG was increased at d 42 in groups that received oregano oil. This point warrants further investigation. The antimicrobial activities of Spanish oregano (*Coridothymus capitatus*), Chinese cinnamon (*Cinnamomum cassia*), and savory (*Satureja montana*) essential

**Table 2.** Growth performance of broilers (n = 5)

Item	Period, d	Negative control	Coccidiostat	Oregano oil	SEM	P-value
BW, g	1	37.7	37.5	37.8	0.1	0.899
	21	809	816	814	2.1	0.676
	42	2,260	2,295	2,276	7.9	0.345
BW gain, g/d	1 to 21	771	778	777	3.7	0.660
	21 to 42	1,451	1,479	1,461	8.9	0.390
	1 to 42	2,222	2,257	2,237	10.7	0.341
Feed intake, g	1 to 21	1,247	1,217	1,209	10.2	0.189
	21 to 42	3,162 <sup>a</sup>	3,015 <sup>ab</sup>	2,984 <sup>b</sup>	35.6	0.028
	1 to 42	4,410 <sup>a</sup>	4,233 <sup>ab</sup>	4,193 <sup>b</sup>	42.0	0.023
Feed:gain, g/g	1 to 21	1.62	1.56	1.56	0.01	0.106
	21 to 42	2.18 <sup>a</sup>	2.04 <sup>b</sup>	2.04 <sup>b</sup>	0.02	0.001
	1 to 42	1.98 <sup>a</sup>	1.87 <sup>b</sup>	1.87 <sup>b</sup>	0.02	0.001

<sup>a,b</sup>Means within the same row with no common superscripts differ significantly.

oils on *Escherichia coli* O157:H7 and *Listeria monocytogenes* were investigated by Oussalah et al. [27] and found to be effective in decreasing the number of bacteria in vitro. In addition, a Chinese herbal polysaccharide (Achyranthan) has been reported as a feed additive to improve the immunity of broilers [28]. However, data are currently not available on the effect of dietary oregano oil on the blood serum IgG of broilers.

### Anticoccidial Effect

Figure 1 illustrates the effect of dietary treatments on *Eimeria* oocyst counts on d 10, 20, 30, and 40 of the experiment. Significant oocyst counts occurred among treatment groups on d 20 and 40 ( $P < 0.05$ ), whereas no significant differences were observed on d 10 and 30. On d 20 and 40, the highest oocyst counts were found in the negative control group, whereas the lowest count was measured in birds receiving the coccidiostat-supplemented diet. Oocyst counts were significantly lower from the birds receiving the oregano oil-supplemented diet compared with those receiving the negative control diet. These results indicate that oregano oil may not be as

effective as the anticoccidial agent; however, it is still effective in reducing oocysts beyond those of birds in the negative control treatment. The active ingredient in Cygro [14] is maduramicin, a monoglycoside polyether derived from the fungus *Actinomadura rubra*. It is classified as an ionophore and is predominantly used as an anticoccidial agent [29]. Ionophore compounds are thought to work by affecting the movement of cations across membranes, causing an influx of sodium and calcium and an efflux of potassium. This in turn results in pH changes within the cell, affecting metabolic processes and damaging organelles. Coccidia are reported to be more sensitive to these changes [30]. Numerous findings can be found on the mechanisms of chemical anticoccidial agents, whereas data are limited on the mechanism of action of herb extracts on coccidia. Williams [31] showed that phenols used as disinfectants exhibit oocysticidal activity against *Eimeria tenella* in tests in vivo and in vitro. Terpenes and phenylpropenes are the 2 major compounds that are mostly responsible for the antimicrobial activity of *O. vulgare* L. [4]. Giannenas et al. [9] experimentally infected broilers with *E. tenella* at 14 d of age and

**Table 3.** Carcass characteristics of broilers (n = 5)

Item	Negative control	Coccidiostat	Oregano oil	SEM	P-value
Preslaughter weight, g	2,343	2,325	2,344	22.36	0.940
Cold carcass weight, g	1,691	1,686	1,695	17.31	0.985
Carcass yield, %	72.2	72.5	72.3	0.16	0.430

**Table 4.** Serum IgG levels of broilers (µg/mL; n = 5)

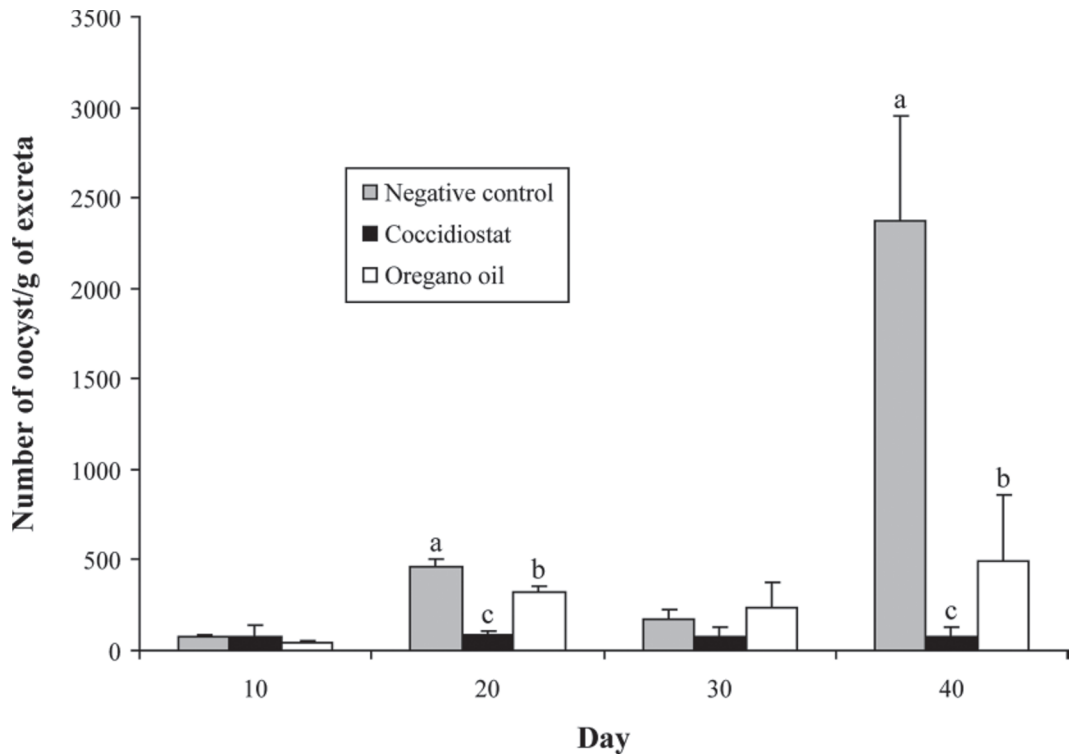
Period	Negative control	Cocciostat	Oregano oil	SEM	P-value
1 d <sup>1</sup>	14.60	14.60	14.60	—	—
42 d	7.68	7.41	8.09	0.15	0.316

<sup>1</sup>Determined using a total of 40 birds for all treatments.

investigated the survival rate, lesion scores, and number of oocysts in the excreta 7 d after the challenge when birds were fed diets containing oregano oil, lasalocid, or no agent. They found that birds receiving oregano and lasalocid survived at rates of 90 and 96.7%, respectively, whereas the infected birds receiving no treatment had an 80% survival rate. Infected birds receiving no treatment had significantly higher oocyst counts than infected birds receiving the lasalocid, whereas infected birds receiving the oregano oil diet were intermediate. Although the birds were not challenged with *Eimeria* in the present study, the effects of oregano oil on the oocyst counts were similar.

**CONCLUSIONS AND APPLICATIONS**

1. No significant effects of dietary oregano essential oil were observed on BW or BW gain at the end of the experiment. However, birds receiving the dietary oregano essential oil had less feed intake and a better FCR than did birds in the negative control group.
2. Further research is needed to investigate the optimal dietary inclusion level of oregano essential oil to achieve optimal growth performance and anticoccidial benefits.
3. On the basis of the serological data from the study herein, dietary additions of



**Figure 1.** Fecal oocyst numbers of broilers at the different measurement periods. Bars within each measurement period with different letters (a–c) are significantly different ( $P < 0.05$ ).

oregano essential oil did not significantly change the serum IgG levels in broilers. However, the increase in IgG levels found at d 42 in groups given oregano oil is interesting and requires further investigation.

- Oregano essential oil exerted an anticoccidial effect, albeit at a lower level than that exhibited by the commercial anticoccidial agent. Oregano essential oil may offer an alternative to using the conventional anticoccidial agents currently used in broiler diets.

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