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Dietary oregano essential oil alleviates experimentally induced coccidiosis in broilers

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ABSTRACT

An experiment was conducted to determine the effects of oregano essential oil on growth performance and coccidiosis prevention in mild challenged broilers. A total of 250 1-d-old chicks were used in a completely randomized design with 5 treatments and 5 replicates with 10 birds in each replication. Experimental treatments included: (1) negative control (NC; unchallenged), (2) positive control (PC; challenged with sporulated oocysts of *Eimeria*), (3) PC fed 200 ppm Diclazuril in diet, (4) PC fed 300 ppm oregano oil in diet, and (5) PC fed 500 ppm oregano oil in diet. At 22 d of age, all the experimental groups except for NC were challenged with 50-fold dose of Livacox T as a trivalent live attenuated coccidiosis vaccine. On d 28, two birds were slaughtered and intestinal coccidiosis lesions were scored 0–4. Moreover, dropping was scored in the scale of 0–3, and oocysts per gram feces (OPG) were measured. Oregano oil at either supplementation rate increased body weight gain ($P=0.039$) and improved feed conversion ratio ($P=0.010$) from d 22 to 28, when compared with PC group. Using 500 ppm oregano oil in challenged broilers diet increased European efficiency factor than PC group ($P=0.020$). Moreover, challenged broilers fed 500 ppm oregano oil or Diclazuril in diets displayed lower coccidiosis lesions scores in upper ($P=0.003$) and middle ($P=0.018$) regions of intestine than PC group, with the effect being similar to unchallenged birds. In general, challenged birds fed 500 ppm oregano oil or Diclazuril in diets had lower OPG ($P=0.001$), dropping scores ($P=0.001$), litter scores ($P=0.001$), and pH of litter ($P=0.001$) than PC group. It could be concluded that supplementation of oregano oil at the dose of 500 ppm in diet may have beneficial effect on prevention of coccidiosis in broilers.

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1. Introduction

Coccidiosis is an important parasitic disease of the intestinal tract of chickens caused by coccidian protozoa of the genus *Eimeria*. The disease especially affecting young chicks and spreads from one chick to another by contact with infected dropping. Parasite proliferation in the epithelium cells causes tissue destruction that increased

incidence of diarrhea, intestinal hemorrhage and may over-set digestive processes, leading to decreased body weight gain and feed consumption, and impaired feed conversion ratio (Williams, 2005; Hafez, 2008). Furthermore, it is responsible for significant economic loss in the poultry industry in many parts of the worlds (Chapman et al., 2010). Therefore, coccidiosis is known as the most costly transmissible disease in commercial poultry flocks.

Applying coccidiostat drugs and attenuated vaccines, are the common approaches to prevent and control this disease. The frequent use of anticoccidial medications leading to the egress of drug-resistant *Eimeria* strains, and the

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vaccines are expensive to produce (Peek and Landman, 2003; Abbas et al., 2011). Therefore, developing safe and inexpensive methods to control coccidiosis in poultry are needed. The use of herbals and their derived compounds could be a potential alternative to control poultry coccidiosis (Abbas et al., 2012).

The essential oil of *Origanum vulgare* is famous for its antimicrobial, antifungal, and antiprotozoal activity (Milhau et al., 1997; Adam et al., 1998; Botsoglou et al., 2002). It contains majorly carvacrol and thymol that constitute almost 78–82% of the total oil (Lee et al., 2004), which has shown antiprotozoal effect *in vitro* (Milhau et al., 1997), and *in vivo* (Giannenas et al., 2003, 2013; Tsinas et al., 2011), though there is some available information about the effect of oregano essential oil on avian coccidiosis, more investigations are needed to detect efficacious dose of administration for alleviating harmful effects of *Eimeria* spp. (Tsinas et al., 2011). The objective of this study was to test the efficacy of oregano essential oil in comparison with an anticoccidial drug (Diclazuril) on growth performance and coccidiosis prevention in challenged broiler chickens.

2. Materials and methods

2.1. Birds, diets and management

The trial was conducted at the agricultural experiment station of University of Guilan from January to February 2014. Two hundred and fifty straight-run 1-d-old Ross 308 broiler chicks, with average body weight of 46.8 ± 0.8 g were used in this study. The chicks were feather sexed and distributed into 25 homogenous groups of floor pens (1 m × 1 m) according to their sex and initial weight. Pens were randomly assigned to five treatments with each treatment having five replicates of 10 birds. Chicks were kept on wood shavings at 32 °C during first 7 d of age, and then temperature was stepped down to 23 °C by 21 d of age. There was continuous light regimen during the first 2 d, then 23 h lighting was applied up to 42 d of age. Light intensity was 20 lux at the first 2 d then was decreased to 5 lux for the remaining period (3–42 d). All the chicks were vaccinated based on a routine program. Diets were provided in mash form and formulated according to Ross 308 recommendations without including any antimicrobial product. Feed and fresh water offered *ad libitum* throughout the experiment. Diets were prepared at pilot feed mill at University of Guilan. Table 1 describes the diet ingredients and nutrient contents of the basal diets. The two control treatments (NC; unchallenged, and PC; challenged group) included no measure for coccidiosis prevention, however, other groups challenged with coccidiosis received a dietary treatment. As a reference treatment, Diclazuril (Clinacox® 0.5%; Huverpharma, Inc.) at 200 ppm was administered. Oregano essential oil was tested at 2 rates of 300 and 500 ppm. Oregano oil was in the form of a powder called Orego-Stim® (Meriden Animal Health Ltd., Luton, UK) that contains 5% essential oil of *O. vulgare* subsp. *Hirtum* plants and 95% natural feed grade inert carrier (Giannenas et al., 2003). All the dietary treatments were fed continuously for 42 d from 1 d old.

2.2. Inoculation

At d 22, all the experimental groups except NC, were challenged with 50 doses of the Livacox T (Biopharm Co., Prague, Czech Republic) orally, to produce a mild coccidiosis infection as described by Mansoori and Modirsanei (2012). Each dose of vaccine contained 300–500 sporulated oocysts of each of *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella* in 0.01 ml distilled water. Birds of NC were sham-inoculated with 0.5 ml of distilled water.

2.3. Collection of samples and measurements

Body weight of the birds and feed consumption were recorded weekly by replicate, and mortality was recorded and weighed as produced. From these data, body weight gain (BWG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated by week and for the entire experimental period. At the end of experiment (d 42), the European efficiency factor (EEF) was calculated using the following formula: $BW(kg) \times \%liveability \times 100 / FCR \times trial\ duration(d)$ (Huff et al., 2013).

Coccidial lesion scoring was carried out 6 d after challenge using the method described by Johnson and Reid (1970). At d 28, two birds per pen were randomly selected, weighed, and slaughtered humanely by knife. Three regions (upper duodenum, middle jejunum, and cecum) of intestinal tract were examined for coccidial lesions. Generally, these regions correspond grossly to natural predilection sites for *E. acervulina*, *E. maxima*, and *E. tenella*, respectively. Based upon severity of the lesions, a score of 0 (no lesions), 1 (mild lesions), 2 (moderate lesions), 3 (severe lesions) or 4 (extremely severe lesions) was recorded for each chicken. A cumulative lesion score was used to judge overall multiple species anticoccidial efficacy. This cumulative lesion score was calculated by adding the lesion scores of each of the three coccidial lesion scored regions.

Excreta samples of each pen were collected on d 28 (6 d after challenge infection), and the oocysts per gram feces (OPG) counting performed by means of a modification of the McMaster counting chamber technique of Hodgson (1970) and Peek and Landman (2003). Droppings and litter scores was scored visually on d 28 by 3 observers blind to the treatments, as described by Morehouse and Baron (1970) and Benabdeljelil and Ayachi (1996), respectively. The means of the 3 values were used for statistical analysis. Droppings scored 0–3, in which 0 was assigned for normal dropping, 1 for few droppings were purplish or brownish in color, 2 for more reddish droppings, some dropping mixed with flakes of blood, and 3 for bloody droppings, absence of normal fecal content. The scale used for litter varied from 1 to 5, with a score of 1 indicating no caking and extremely dry litter and a score of 5 indicating heavy caking and extremely wet litter.

Litter samples for measuring pH were collected from five locations within each pen (4 peripheral samples and 1 central one) and thoroughly mixed to obtain material representative of the entire pen. The pH of each litter type was measured using a pH meter after litter samples of nearly

Table 1
Composition and calculated nutrient composition of the basal diets (% as fed basis).

Item	Starter (d 0–10)	Grower (d 11–24)	Finisher (d 25–42)
<i>Ingredient</i>			
Corn	53.31	56.15	59.61
Soybean meal, 44% CP	39.26	35.72	31.90
Soybean oil	2.83	4.16	4.73
Dicalcium phosphohate	2.00	1.78	1.68
Calcium carbonate	1.18	0.95	0.93
Common salt	0.36	0.29	0.30
L-Lysine HCl, 78%	0.18	0.08	0.04
DL-Methionine, 98%	0.30	0.24	0.20
L-Threonine, 99%	0.08	0.03	0.01
Vitamin premix ^a	0.25	0.25	0.25
Mineral premix ^b	0.25	0.25	0.25
Sodium bicarbonate	0.00	0.10	0.10
Total	100	100	100
<i>Calculated composition</i>			
Metabolizable energy, kcal kg ⁻¹	2870	3000	3080
Crude protein, %	21.82	20.48	19.06
Digestible lysine, %	1.20	1.05	0.93
Digestible methionine, %	0.60	0.52	0.47
Digestible methionine + cystine, %	0.89	0.80	0.73
Digestible threonine, %	0.79	0.70	0.63
Calcium, %	1.00	0.86	0.82
Available phosphorus, %	0.47	0.43	0.40
Total phosphorus, %	0.75	0.70	0.67
Sodium, %	0.16	0.16	0.16
Potassium, %	0.91	0.85	0.79
Chloride, %	0.29	0.23	0.23
DEB ^c , meq kg ⁻¹	221	222	207

^a Vitamin premix provided the following per kilogram of diet: vitamin A (*trans*-retinyl acetate), 10000 IU; vitamin D₃ (cholecalciferol), 2000 IU; vitamin E (DL- α -tocopherol acetate), 45 IU; vitamin K₃ (bisulfate menadione complex), 3 mg; thiamine (thiamine mononitrate), 3 mg; riboflavin, 9 mg; nicotinic acid, 30 mg; pantothenic acid (D-calcium pantothenate), 10 mg; vitamin B₆, 4 mg; D-biotin, 0.1 mg; folic acid, 2 mg; vitamin B₁₂ (cyanocobalamin), 0.02 mg and choline (choline chloride), 1000 mg.

^b Mineral premix provided the following per kilogram of diet: iron (FeSO₄·7H₂O), 55 mg; iodine (Ca(IO₃)₂), 1.3 mg; manganese (MnSO₄·H₂O), 100 mg; zinc (ZnO), 85 mg; copper (CuSO₄·5H₂O), 13 mg; selenium (Na₂SeO₃), 0.2 mg.

^c DEB = dietary electrolyte balance; Na + K – Cl.

5 g were suspended for 30 min in 45 ml of distilled water (1 part material: 9 parts water) and stirred for 5 min (Abd El-Wahab et al., 2012).

2.4. Statistical analyses

All data were analyzed as a completely randomized design with five treatments using the General Linear Model procedure of SAS (SAS Institute, 2002). The following model was fitted:

$$Y_{ij} = \mu + T_i + e_{ij},$$

where Y_{ij} was the trait of interest for chicken, μ was the overall mean, T_i was the treatment effect, and e_{ij} was the residual error. Normal distribution of residuals and variance homogeneity of the data was tested by UNIVARIATE procedure and the Levene's test, respectively. The experimental unit was the slaughter chicks for coccidiosis lesion scores, constituting two observations in each replicate. For all the remaining studied traits including BWG, ADFI, FCR, EEf, OPG, dropping score and litter quality, the pen used as the experimental unit. Differences were considered significant at $P < 0.05$. Significant differences between means were separated by Tukey test.

3. Result

3.1. Performance

Mortality was <1% and was not related to the treatments. For the first three weeks of age and the entire period, all groups displayed similar BWG (Tables 2–4). On 28 d, a week after *Eimeria* inoculation, BWG of the unchallenged group or challenged broilers fed diets supplemented with oregano oil was significantly higher ($P = 0.039$) than the PC group, but was similar among the broilers fed supplemented diet and unchallenged birds. From d 29 to 35, the average BWG of unchallenged birds was higher than the birds in the other groups ($P = 0.004$) and there was no significant difference between challenged treatments. The ADFI was not affected by the treatments in any periods (Tables 2–4).

Feed conversion ratio from d 22 to 28 was significantly different between treatments ($P = 0.010$; Table 3), but was similar among all the treatments in the other periods (Tables 2 and 3). The unchallenged group and challenged birds fed diets supplemented with Diclazuril or 500 ppm oregano oil showed better FCR than PC group (1.73 vs. 1.99; $P = 0.010$), but was not different between challenged birds fed diets supplemented with 300 ppm oregano oil and PC group.

Table 2

Effect of oregano oil and Diclazuril on average body weight gain (BWG), average daily feed intake (ADFI), and feed conversion ratio (FCR) from 1 to 21 d of age.

Item	1–7 d			8–14 d			15–21 d		
	BWG, g	ADFI, g	FCR	BWG, g	ADFI, g	FCR	BWG, g	ADFI, g	FCR
NC ^a	18.8	27.0	1.44	32.1	53.3	1.66	49.8	82.5	1.67
PC ^b	17.4	26.2	1.51	33.0	53.2	1.62	52.8	85.9	1.63
PC + Diclazuril	18.5	26.6	1.43	32.0	50.6	1.58	53.3	85.4	1.60
PC + Oregano ^c 300	18.1	25.5	1.47	34.7	52.8	1.53	49.7	80.2	1.62
PC + Oregano 500	17.9	26.1	1.46	33.3	50.7	1.55	52.1	83.7	1.61
SEM ^d (n = 5)	0.43	0.54	0.038	0.70	1.33	0.042	2.25	2.87	0.026
P-value	0.204	0.142	0.254	0.082	0.413	0.172	0.694	0.634	0.519

^a Negative control; unchallenged.

^b Positive control; challenged with sporulated oocysts of *Eimeria*.

^c Oregano oil.

^d Standard error of mean.

Table 3

Effect of oregano oil and Diclazuril on average body weight gain (BWG), average daily feed intake (ADFI), and feed conversion ratio (FCR) from 22 to 42 d of age.

Item	22–28 d			29–35 d			36–42 d		
	BWG, g	ADFI, g	FCR	BWG, g	ADFI, g	FCR	BWG, g	ADFI, g	FCR
NC ^c	74.1 ^a	128.6	1.74 ^b	94.2 ^a	171.8	1.82	99.6	204.4	2.06
PC ^d	63.0 ^b	125.2	1.99 ^a	89.3 ^b	170.5	1.90	103.8	217.5	2.12
PC + Diclazuril	70.2 ^{ab}	120.7	1.72 ^b	84.8 ^b	161.9	1.91	98.6	208.3	2.12
PC + Oregano ^e 300	72.5 ^a	135.2	1.87 ^{ab}	88.7 ^b	170.7	1.93	102.3	224.5	2.19
PC + Oregano 500	77.0 ^a	133.1	1.73 ^b	88.2 ^b	168.2	1.91	96.9	212.0	2.18
SEM ^f (n = 5)	2.99	3.53	0.056	1.44	3.50	0.037	4.00	5.55	0.068
P-value	0.039	0.057	0.010	0.004	0.301	0.343	0.743	0.131	0.634

^{a,b} Means within a column with different superscripts are significantly different ($P < 0.05$).

^c Negative control; unchallenged.

^d Positive control; challenged with sporulated oocysts of *Eimeria*.

^e Oregano oil.

^f Standard error of mean.

Table 4

Effect of oregano oil and Diclazuril on average body weight gain (BWG), average daily feed intake (ADFI), feed conversion ratio (FCR), and European efficiency factor (EEF) on 1–42 d of age.

Item	1–21 d			22–42 d			1–42 d			EEF ^h
	BWG	ADFI	FCR	BWG	ADFI	FCR	BWG	ADFI	FCR	
NC ^d	33.6	54.3	1.62	88.7	166.5	1.88 ^b	60.5	109.0	1.80 ^c	364.9 ^a
PC ^e	34.4	55.1	1.60	84.5	168.7	2.00 ^a	58.8	110.6	1.88 ^{ab}	340.5 ^b
PC + Diclazuril	34.6	54.2	1.57	83.8	161.4	1.93 ^{ab}	58.6	106.5	1.82 ^{bc}	349.9 ^{ab}
PC + Oregano ^f 300	34.2	52.9	1.55	87.1	174.4	2.01 ^a	60.0	112.2	1.87 ^{ab}	353.3 ^{ab}
PC + Oregano 500	34.4	54.2	1.58	86.9	169.1	1.94 ^{ab}	60.1	110.2	1.84 ^{abc}	362.1 ^a
SEM ^g (n = 5)	0.87	1.09	0.017	1.65	3.22	0.032	0.95	1.72	0.020	5.07
P-value	0.921	0.697	0.052	0.237	0.113	0.048	0.577	0.239	0.047	0.020

^{a–c} Means within a column with different superscripts are significantly different ($P < 0.05$).

^d Negative control; unchallenged.

^e Positive control; challenged with sporulated oocysts of *Eimeria*.

^f Oregano oil.

^g Standard error of mean.

^h European efficiency factor.

The experimental diet had no effect on BWG and ADFI from 1 to 21 d, 22 to 42 d, and 1 to 42 d of age and FCR from 1 to 21 d (Table 4). Unchallenged broilers had better FCR than PC group from 22 to 42 d and 1 to 42 d of age (1.88 and 1.80 vs. 2.00 and 1.88, respectively) but was not different between challenged birds fed diets supplemented with Diclazuril or oregano oil and unchallenged group.

Challenged broilers fed diets supplemented with 500 ppm oregano oil was equal to unchallenged group in EEF and were higher compared with PC group (365 and

362 vs. 341, respectively; $P = 0.020$), but was not different between PC group and challenged broilers fed diets supplemented with Diclazuril or 300 ppm oregano oil (Table 4).

3.2. Coccidiosis lesion scores

Lesion scores in the upper ($P = 0.003$) or middle ($P = 0.018$) region of intestine differed significantly between treatments, but there was no significant effect between the experimental groups in cecal region (Table 5).

Table 5

Effect of oregano oil and Diclazuril on coccidiosis lesion score on d 28.

Item	Coccidiosis lesion score on d 28			
	Upper region (<i>Eimeria acervulina</i>)	Middle region (<i>Eimeria maxima</i>)	Cecal region (<i>Eimeria tenella</i>)	Overall mean
NC ^d	0.78 ^c	0.84 ^b	0.42	0.68 ^d
PC ^e	2.16 ^a	1.93 ^a	0.60	1.55 ^a
PC + Diclazuril	1.12 ^{bc}	1.34 ^{ab}	0.58	1.01 ^{bc}
PC + Orego ^f 300	1.68 ^{ab}	1.42 ^{ab}	0.66	1.25 ^b
PC + Orego500	1.24 ^{bc}	0.90 ^b	0.62	0.91 ^{cd}
SEM ^g (n = 10)	0.224	0.218	0.094	0.101
P-value	0.003	0.018	0.475	0.001

^{a–c} Means within a column with different superscripts are significantly different ($P < 0.05$).^d Negative control; challenged.^e Positive control; challenged with sporulated oocysts of *Eimeria*.^f Oregano oil.^g Standard error of mean.

The unchallenged birds had lower lesion scores in upper region of small intestine than PC group (0.78 vs. 2.16; $P = 0.003$). Challenged birds fed diets supplemented with Diclazuril or 500 ppm oregano oil had similar coccidiosis lesion scores in upper region of small intestine when compared to unchallenged group, and differences between PC group and challenged birds received 300 ppm oregano oil was not significant.

Lesion scores values of the unchallenged group in middle region of intestine were lower than these of PC (0.84 vs. 1.93; $P = 0.018$), and the other supplemental groups had same lesions as the unchallenged group.

The overall mean lesion scores of PC group were the highest and significantly different from all the other treatments ($P = 0.001$), and challenged broilers fed diets containing 500 ppm oregano oil were similar to unchallenged group.

3.3. Oocytes per gram and droppings scores

The oocytes present per gram feces (OPG) of challenged birds fed diets supplemented with Diclazuril was similar to unchallenged birds and were lower in comparison with all other treatments (Table 6). However, supplementation of 500 ppm oregano oil reduced OPG in challenged birds, they had higher OPG than unchallenged group or challenged birds fed diets supplemented with Diclazuril (1360 vs. 300

and 340, respectively; $P = 0.001$). There was no significant difference for OPG between PC group and challenged broilers fed 300 ppm oregano oil in diet.

The birds of PC group had a significantly ($P = 0.001$) higher dropping score than the other groups (Table 6). Oregano oil reduced dropping score of challenged birds when supplemented at 300 or 500 ppm, but these values were higher than those of unchallenged birds or those fed Diclazuril. However, using 500 ppm of oregano oil appears to be more efficacious than 300 ppm and equal to Diclazuril in reducing dropping scores.

3.4. Litter scores and pH

The birds of the PC group had a significantly ($P = 0.001$) higher litter score than those of the other groups (Table 6). Either 300 or 500 ppm of oregano oil reduced litter scores in challenged birds, but birds fed Diclazuril were more similar to unchallenged birds in litter score value.

The litter pH of challenged group was the highest and significantly different from all other treatments ($P = 0.001$), and was similar among all experimental groups except for the challenged birds fed diets supplemented with 300 ppm oregano oil (Table 6). The challenged birds fed diets containing 300 ppm oregano oil had a significantly higher litter pH than unchallenged group or challenged birds fed diets supplemented with Diclazuril (2.8 vs. 1.4 and 2.0,

Table 6

Effect of oregano oil and Diclazuril on oocysts per gram feces (OPG), litter quality, and litter pH on d 28.

Item	OPG	Dropping score ⁱ	Litter score ⁱ	Litter pH
NC ^e	300 ^c	0.2 ^d	1.4 ^c	7.68 ^c
PC ^f	2720 ^a	2.8 ^a	3.8 ^a	8.47 ^a
PC + Diclazuril	340 ^c	0.4 ^{cd}	2.0 ^{bc}	7.69 ^c
PC + Orego ^g 300	1760 ^{ab}	1.6 ^b	2.8 ^b	8.11 ^b
PC + Orego500	1360 ^b	1.0 ^{bc}	2.6 ^b	7.91 ^{bc}
SEM ^h (n = 5)	337.6	0.24	0.32	0.120
P-value	0.001	0.001	0.001	0.001

^{a–d} Means within a column with different superscripts are significantly different ($P < 0.05$).^e Negative control; challenged.^f Positive control; challenged with sporulated oocysts of *Eimeria*.^g Oregano oil.^h Standard error of mean.ⁱ Subjective scores: (0): normal dropping, (1): few droppings were purplish or brownish in color, (2): more reddish droppings, some dropping mixed with flakes of blood, (3): bloody droppings, absence of normal fecal content.^j Subjective scores: 1 = dry litter to 5 = heavy caking.

respectively; $P=0.001$), but was not different between two levels of oregano oil.

4. Discussion

4.1. Performance

Coccidiosis causes serious problems, such as malabsorption, dysentery and affected performance in broilers (Peek and Landman, 2003). In the current study, oregano oil specially when supplemented at 500 ppm in the diet of challenged broilers with *Eimeria* parasites displayed the ability to reduce coccidiosis detrimental effects on performance. Results of this study are in good agreement with Tsinas et al. (2011) who found that oregano oil represents anticoccidial effects against *E. acervulina* and *E. maxima*. Various authors indicated that broilers could be reared without coccidiostat drugs when their diets supplement by oregano essential oil (Giannenas et al., 2003, 2004; Silva et al., 2009). In a recently published study, Tsinas et al. (2011) reported that the dietary oregano oil supplementation either at 300 or 600 ppm, attained BWG and FCR similar to those of the broilers fed ionophorous antibiotic, salinomycin. In contrast, Scheurer et al. (2013) reported that the use of oregano oil at 200 ppm had not significant effect on performance compared to a coccidiostat, Narasin. In the current experiment, due to the mild challenge, after a week, the birds were able to overcome the parasites. Scheurer et al. (2013) indicated that performance parameters did not differ between treatments from d 22 to 39 when birds challenged on d 15. They concluded that challenged broilers recovered from the *Eimeria* challenge during this period. Gadzirayi et al. (2005) reported that the anticoccidial effects of *Aloe excelsa* were comparable with sulphachlopyrazine sodium monohydrate in term of improved BWG. Upper layer of enterocytes constantly shed and replenished every 4–7 d. Giannenas et al. (2003) reported that oregano oil as a source of thymol and carvacrol, may have toxic effect on the upper layer of mature enterocytes of the intestinal mucosa due to the hydrophobic character of carvacrol, and accelerates this natural renewal process (2–3 d). Therefore, sporozoite-infected cells are shed before development of merozoite phase, which causes the main clinical signs of coccidiosis. It is also suggested that phenolic compounds may exert their activity on the host enterocytes (Weber and de Bont, 1996). Phenols such as thymol and carvacrol interact with the cytoplasmic membrane by changing its permeability for cations, like H^+ and K^+ . The dissipation of ion gradients leads to impairment of essential processes in the cell, allows leakage of cellular constituents, resulting in water unbalance, collapse of the membrane potential and inhibition of ATP synthesis, and finally cell death (Ultee et al., 2002).

The rate of supplementation of essential oils was also variable in previous studies. For example, the carvacrol content of a blend of essential oils studied by Kucukyilmaz et al. (2012) was much lower (19 ppm) than that declared by Giannenas et al. (2003) (300 ppm) and Ibrir et al. (2009) (250 ppm). Therefore, it could be a reason for controversy of results in previous researches. This can be attributed to

the fact that essential oils vary considerably with respect to their phenolic compound contents (Marino et al., 1999; Faleiro et al., 2003), and this variation can adversely affect the metabolism, performance and immunity of broilers (Lee et al., 2004; Brenes and Roura, 2010). Mortality rate also depends on the infection level. High rate of mortality has been reported when broilers had severe coccidial infection (Giannenas et al., 2003; Christaki et al., 2004), and it was considerably lower when the infection dose was more limited (Kucukyilmaz et al., 2012). From this explanations it could be concluded that oregano oil by cutting the parasites life cycle, may control and prevent coccidiosis. Moreover, oregano oil leads to lower contamination of the emerging enterocytes, by this way may improve absorption capacity for nutrients.

4.2. Coccidiosis lesion scores and oocytes per gram, and droppings scores

Lesion scores, oocytes per gram, and droppings scores are some of measurements studied by various investigators to judge efficacy of coccidiosis (Conway and McKenzie, 2007). In the current study, lesion scores for unchallenged group were higher than 0, indicated that these birds also infected by *Eimeria* during the period of study. The infection probably may be due to native oocysts or by cross contamination from infected birds, since the study was conducted using open pens and not isolated units (Scheurer et al., 2013).

We observed that supplemental oregano oil generally had beneficial effects on coccidiosis lesion scores and improved dropping scores in challenged broilers with coccidiosis *Eimeria*. Essential oil of *O. vulgare* spp. Hirtum plants is composed by 30 or more ingredients, mainly phenolic compounds, with the main substances being carvacrol and thymol which composed almost 78–82% of total oil (Adam et al., 1998). Antibacterial mechanisms proposed for thymol and carvacrol are disruption of the cellular membrane, inhibition of ATPase activity and release of intracellular ATP (Lambert et al., 2001; Oussalah et al., 2006; Giannenas et al., 2013). These substances enter into the phospholipid bilayer and get aligned between the fatty acid chains, which appear to make the cell membrane permeable (Lambert et al., 2001). This modification results in expansion and destabilization of the membrane and increase membrane fluidity, which in turn increase passive permeability (Ultee et al., 2002; Negi, 2012). Moreover, hydrophobic substance such as thymol and carvacrol because of low molecular mass can diffuse inner side of cell wall, by making hydrogen bonds with cell wall and show permeability change affect cell membrane. In fact, Ultee et al. (2002) showed different mode of actions for carvacrol. They showed that hydroxyl substances of phenolic compounds carry ions and protons in and out from cell membrane similar to ionophore antibiotics. This mode of action related to hydroxyl substances only for aromatic compounds. However, carvacrol and thymol disrupt the lipid structure of the cell membrane, making it permeable to ions and leading to, the inhibition of enzyme and metabolic activity, followed by death of the cell. Due to

this unique mode of action, the micro-organisms cannot develop resistance to phenols (Weber and de Bont, 1996).

It has been proposed that Diclazuril has a coccidiacidal effect toward all *Eimeria* spp. of the chicken; however the exact mode of action has not been unraveled yet. It is a nucleoside analog thought to be involved in acid nucleic synthesis, possibly affecting later phases of coccidian differentiation (Verheyen et al., 1988). It has been shown that this effect on parasite wall synthesis resulting in the formation of an abnormally thickened, incomplete oocyst wall and zygote necrosis in both, *E. brunette* and *E. maxima* (Verheyen et al., 1989). In this respect, Abbas et al. (2011) proposed that Diclazuril affected *Eimeria* life cycle at both sexual and asexual stages.

Tsinas et al. (2011) reported that the use of dietary 300 or 600 ppm oregano oil decreased overall mean lesion scores than broilers challenged by *E. acervulina* and *E. maxima*. In contrast, Scheurer et al. (2013) did not observe significant difference for overall mean lesion scores either between treatments containing oregano oil, or unchallenged and challenged birds. In the current study, we did not observe significant differences in cecal region between any experimental groups. This perception may partially be explained by the pathogenicity of the applied *Eimeria* strain (Scheurer et al., 2013). Judging by the results of oocyst counts in feces, the experimental infection was successful. Possibly supplementation of 500 ppm of oregano oil to the diet through altering the life cycle of the *Eimeria* strains decreased the oocyst counts in feces. The use of higher levels of oregano oil may be able to reduce OPG. Yim et al. (2011) reported that dietary supplementation of *Aloe vera* resulted in significantly lower gut lesion scores and reduced OPG of *E. maxima* in broiler chickens. These authors suggested that reduced OPG, a protective role against *Eimeria* infection, in *Aloe*-based chicken diets could be associated more with cell-mediated responses than antibody responses. Allen (2003) indicated that broilers fed diet containing *Echinacea purpurea* had fewer coccidial lesions after a mixed challenge infection with *E. acervulina*, *E. maxima*, *E. tenella* and *E. necatrix*. Carvacrol and thymol, the main ingredients of oregano oil, have anticoccidial action against *E. tenella* (Giannenas et al., 2003), *E. acervulina* (Ibrir et al., 2009) and mixed *Eimeria* spp. infection (Oviedo-Rondón et al., 2006). However, it should be noticed that the protective effect and recovery effect was generally lower, compared to chemical anticoccidials.

4.3. Litter scores and pH

Litter conditions influence broilers performance. Dry litter helps to control ammonia levels, and provides a healthy flock environment. When litter begins to retain moisture it will clump together, which is referred to as caking. One of the most important signs characterizing a coccidial infection is watery excreta due to diarrhea which causes wet litter problems (Hafez, 2008). Therefore, the use of additives for prevention of poultry coccidiosis might improve litter quality. We observed that dietary supplementation of oregano oil decreased gut lesion and dropping scores and OPG which has resulted beneficial effects on broilers coccidiosis incidence. Moreover, Abd El-Wahab et al. (2012)

reported that OPG had closely correlate with the changes in dry matter content of excreta which is in accordance with our results. It is stated that wet litter was associated with a higher pH compared with dry litter (Lerner, 1996). Litter pH affects ammonia release and should be below 7 to reduce volatilization. Moreover, litter not treated with an acidifying agent will often have a pH near 8 or slightly higher. Results suggest that the supplementation of challenged broilers diet with 500 ppm oregano oil decreased litter scores and pH due to reduce detrimental effects of coccidiosis in broilers. One of the major factors causing wet litter is diarrhea. This can be a result of different infection in the intestinal tract; for example, protozoal *Eimeria* spp. infection (Mayne, 2005). Abd El-Wahab et al. (2012) reported that coccidial infection resulted in markedly lowered dry matter contents of excreta and litter. They showed that induced diarrhea caused by coccidial infection led to poor litter quality. Similarly, in this current study, increases of moisture litter due to coccidiosis resulted in a higher litter pH value. On the contrary, using dietary oregano oil produced the lowest litter moisture and pH value. In the current study, coccidiosis infection increases OPG and produced watery litter. Similar to our study, Abd El-Wahab et al. (2012) indicated that OPG were closely correlated with the changes in dry matter content of excreta.

5. Conclusion

Challenge of broilers on d 22 with *Eimeria* (*acervulina*, *maxima*, and *tenella*) impaired growth performance, mainly from 22 to 28 d of age. Inclusion of 500 ppm oregano oil in diet of challenged broilers alleviated the detrimental effects of coccidiosis same as Diclazuril. Therefore, according to the results of this study, and harm effects known for using anticoccidiosis drugs, supplementation of broilers diet with 500 ppm oregano oil could be a trustworthy alternative for coccidiostat medications.

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