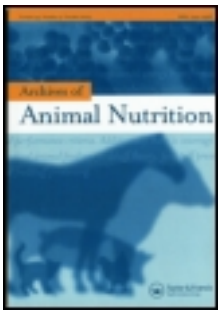

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EFFECT OF DIETARY SUPPLEMENTATION WITH OREGANO ESSENTIAL OIL ON PERFORMANCE OF BROILERS AFTER EXPERIMENTAL INFECTION WITH *EIMERIA TENELLA*

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A study was carried out to examine the effect of dietary supplementation of oregano essential oil on performance of broiler chickens experimentally infected with *Eimeria tenella* at 14 days of age. A total of 120 day-old Cobb-500 chicks separated into 4 equal groups with three replicates each, were used in this study. Two groups, one infected with $5{\cdot}10^4$ sporulated oocysts of *E. tenella* and the other not, were given a basal diet and served as controls. The other two groups also infected with *E. tenella* were administered diets supplemented with oregano essential oil at a level of 300 mg/kg, or with the anticoccidial lasalocid at 75 mg/kg. Following this infection, survival rate, bloody diarrhoea and oocysts excretion as well as lesion score were determined. Throughout the experimental period of 42 days, body weight gain and feed intake were recorded weekly, and feed conversion ratios were calculated. Two weeks after the infection with *E. tenella* supplementation with dietary oregano oil resulted in body weight gains and feed conversion ratios not differing from the non-infected group, but higher than those of the infected control group and lower than those of the lasalocid group. These parameters correspond with the extent of bloody diarrhoea, survival rate, lesion score and oocyst numbers and indicated that oregano essential oil exerted an anticoccidial effect against *E. tenella,* which was, however, lower than that exhibited by lasalocid.

Keywords: *Oreganum vulgare*; Essential oil; Coccidiosis; *Eimeria tenella*; Lasalocid; Feed supplements; Performance; Broilers

1. INTRODUCTION

It is an undisputed fact that the world's poultry industry would not achieve the high productivity levels, measured as rapid growth rate in the fattening period, high feed efficiency and low mortality, without the high genetic potential of broilers, optimal diet composition, and the use of anticoccidial substances. Coccidiosis is considered as one of the most severe health and welfare problems in poultry that costs chicken producers

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worldwide at least 1.5 billion US$ annually (Stevens, 1998). Commercially reared broilers are particularly vulnerable to the disease due to intensive production.

During the last few years, concern has been expressed regarding the future long-term prospects for control of coccidiosis. Due to the rapid and continuous development of anticoccidial drug resistance and the increasing pressure of consumers for more healthy food, several antibiotic growth promoters have been banned in the European Union and, presently, only the antibiotics avilamycin and flavomycin and certain anticoccidial drugs remain as non-prescription feed additives (European Commission Regulation, 1997). If anticoccidial feed additives were withdrawn from use, alternative feeding strategies should probably be introduced to offset any possible adverse effects on production.

Certain dietary factors may diminish the negative effects of avian coccidiosis (Kolveit, 1969; Murillo *et al*., 1976; Colnago *et al*., 1984). A number of nutrients including vitamin A (Panda *et al*., 1964), vitamin K (Davies and Joyner, 1963), selenium (Jensen *et al*., 1978) and polyunsaturated fatty acids (Allen *et al*., 1996), have been shown to change the severity of coccidial infections. Some herb extracts have been also found effective against parasites, *e.g*. *Plasmodium spp*. (Klayman *et al*., 1984; Klayman, 1985; Dutta *et al*., 1989; Lin *et al*., 1987), *Schistosoma mansoni* (Shuhua and Catto, 1989), *Toxoplasma gondii* (Qu-Yang *et al*., 1990), and helminths (Matsuda *et al*., 1989). Akhtar and Rifaat (1987) examined the anticoccidial effect of extracts of *Melia azedarach* Linn. (Bakain) in naturally infected chickens. Allen *et al*. (1997) reported that dried leaves of *Artemisia annua* provided significant protection against lesions due to *Eimeria tenella* infection. However, Youn and Noh (2001) showed that *Sophora flavescens* extracts were more effective than *Artemisia annua* against *E. tenella* infection. Recently, Waldenstedt (2001) investigated the possibility of rearing broilers without growth promoters and coccidiostats by incorporating in their diets oregano essential oil. In this experiment, however, oregano oil was not examined as an anticoccidial compound but as an antibacterial agent against intestinal colonization by *Clostridium perfringens*.

The essential oil of oregano which is obtained by steam-distillation of *Origanum vulgare ssp*. *Hirtum* plants, comprises more than 30 ingredients, most of which are phenolic compounds with varying antioxidative, antimicrobial or antifungal activity (Economou *et al*., 1991; Sivropoulou *et al*., 1996; Adam *et al*., 1998; Tsimidou *et al*., 1995; Botsoglou *et al*., 2002). Major components are carvacrol and thymol that constitute about 78–82% of the total oil (Adam *et al*., 1998) and exhibit considerable antimicrobial and antifungal activity (Sivropoulou *et al*., 1996). The activity of other main constituents such as the two monoterpene hydrocarbons, γ-terpinene and p-cymene, which often contribute to about 5% and 7% of the total oil, respectively, is uncertain, as it is an effect of all the constituents working together (Adam *et al*., 1998).

The aim of this study was to investigate the potential use of dietary oregano essential oil at the inclusion rate of 300 mg/kg in broiler feed against *Eimeria tenella*, a highly pathogenic *Eimeria* species that causes caecal coccidiosis.

2. MATERIAL AND METHODS

2.1. Animals and housing

A total of 120 day-old Cobb-500 chicks, males and females with the proportion as hatched, were randomly divided into four groups of 30 birds each. Every group was

further randomly separated into three subgroups (replicates) of 10 birds each. All twelve subgroups were housed in separate cages, each equipped with an infrared lamp. Ambient temperature was controlled, and light provided continuously 24 h per day. All birds were vaccinated against Newcastle disease and infectious bronchitis on day 10 of age and against Gumboro disease on day 17 of age.

2.2. Dietary treatments

To meet the nutrient requirements of the chickens during the experimental period, a complete basal diet was formulated. Table I presents the ingredients and the calculated composition of the basal diet, which was in a mashed form. Proximate chemical analysis showed no considerable deviations from calculated values.

Based on this basal diet, two additional diets were prepared by supplementation with either oregano essential oil (300 mg/kg) or lasalocid (75 mg/kg feed). Feed and drinking water were offered *ad libitum*.

Oregano essential oil was obtained from Ecopharm Hellas S.A. (Kilkis, Greece). Oregano oil was in the form of a powder called Orego-Stim (Meriden Animal Health Ltd. (Luton, UK) that contains 5% essential oil of *Origanum vulgare* subsp. *hirtum* plants and 95% natural feed grade inert carrier.

TABLE I Composition of the basal diet

Ingredients	Composition [g/kg]
Wheat grains	560.0
Soybean meal	310.0
Soybean oil	50.0
Brewer's yeast	25.0
Herring meal	20.0
Limestone, pulverized	14.7
Dicalcium phosphate	8.6
Biolysine	3.3
DL-methionine	2.9
Sodium chloride, iodized	2.6
Vitamin premix[1]	2.2
Trace-mineral premix[2]	0.5
Natugrain (arabinoxylanases plus glucanases)	0.2
Total	1000.0
Calculated composition	
Dry matter	892.5
Crude protein	220.0
Crude fat	74.2
Crude fibre	35.4
Ash	54.2
Calcium	9.3
Phosphorus (total)	7.0
Lysine	13.0
Methionine + cystine	10.0
Metabolizable energy [MJ/kg]	13.20

[1]Provided per kg of diet: 12 000 IU vit. A, 5000 IU vit. D_3, 80 mg vit. E, 1 mg vit. B_1, 5 mg vit. B_2, 3 mg vit. B_6, 20 μg vit. B_{12}, 1 mg vit. K_3, 30 mg nicotinic acid, 10 mg pantothenic acid, 0.8 mg folic acid, 0.05 mg biotin, vit. C, 10 mg, 480 mg choline chloride.
[2]Provided per kg of diet: 100 mg Zn, 120 mg Mn, 20 mg Fe, 15 mg Cu, 0.2 mg Co, 1 mg I, 0.3 mg Se

2.3. Experimental design

Four experimental groups, each comprising 30 chickens, were separated into three subgroups (replicates). Two groups, one infected with *E. tenella* and the other not, were given the basal diet without any anticoccidial or other antimicrobial feed additive and served as controls. From the remaining two groups, which were infected with *E. Tenella* at day 14 of age, one was fed a diet supplemented with oregano essential oil and the other a diet supplemented with the anticoccidial lasalocid. Each experimental group was given the corresponding diet from day 1 to day 42 of age.

All chicks were individually weighed at day 1, and at days 7, 14, 21, 28, 35 and 42 of age. Feed consumption within each subgroup was also determined weekly. Mortality was recorded daily in each subgroup.

Chickens were infected with *E. tenella* at 14 days of age. Bloody diarrhoea and excreted oocysts were determined daily from day 17 to 21 of age, and day 20 to day 27 of age, respectively. Seven days after the infection, the lesion score was estimated in all groups by evaluating caecal intestine lesions of nine chicks per group.

2.4. *Eimeria tenella* infection

A reference stock of *E. tenella* oocysts was provided from the Division of Parasite and Vector Biology at the Liverpool School of Tropical Medicine, UK. For the needs of the experiment, *E. tenella* oocysts were propagated in specific pathogen-free chickens. The oocysts were preserved in 2% potassium dichromate solution to induce sporulation, and kept in a refrigerator (3 – 5°C) until use. The coccidial infection was induced by administering a 2-ml dose of suspended $5{\cdot}10^4$ sporulated oocysts of *E. tenella* directly into the crop by using a plastic syringe fitted with a plastic cannula.

2.5. Performance parameters

The survival rate was calculated as the percentage of the surviving to the initial number of chickens, concerning only the first week after infection with *E. tenella*.

The lesion score in each group was determined by evaluating caecal intestine lesions of nine chicks per group one week after infection, according to Johnson and Reid (1970).

The extent of bloody diarrhoea was determined according to Youn and Noh (2001) by assigning it one of the five degrees, from 0 to 4, where 0 is the normal status, and 1, 2, 3, and 4 correspond to 25, 26 – 50, 51 – 75, or over 75% bloody in total faeces, respectively.

Counts of oocysts were determined in excreta samples taken from each subgroup daily from day 7 to day 13 after infection. Sampling was carried out by collecting randomly 50 g samples of excreta, ten times per day from each cage. Counts of oocysts were also determined in excreta samples from each subgroup 7 days before and on the day of infection with *E. tenella*. Samples collected daily from each subgroup were placed in separate airtight plastic bags, homogenized thoroughly by a domestic mixer, and kept refrigerated until assessed for total oocyst counts. Homogenized samples were ten-fold diluted with tap water to be further diluted with saturated NaCl solution at a ratio of 1 : 10. Oocyst counts were determined using McMaster chambers and presented as the number of oocysts per g of excreta (Hodgson, 1970).

2.6. Statistical analysis

All data concerning body weight gain, feed intake, feed conversion ratio, lesion scores and oocyst count numbers, were subjected to analysis of variance in the general linear model of SPSS statistical package (SPSS 10.05, SPSS Ltd., Woking, Surrey, UK). When significant treatment effects were disclosed, statistically significant differences among means were identified using Duncan's multiple range tests. Differences between means were considered significant at $P < 0.05$ (Hubbard, 1990).

3. RESULTS AND DISCUSSION

For the first two weeks of age, groups did not differ in body weight gain, feed intake and feed conversion ratio (Table II). Two weeks after the infection with *E. tenella* body weight gain and feed conversion ratio of the infected control group were significantly lower than those of all other groups. However, these performance parameters did not differ between the infected oregano group and the non-infected control group. The lasalocid group exhibited a significantly better body weight gain and feed conversion ratio among all treatments. Daily feed intake did not differ among treatments. At 42 days of age, this profile did not change with the exception of the daily feed intake of the lasalocid group, which was significantly higher than all other groups. On the basis of the significantly improved body weight and feed conversion ratio, dietary supplementation with oregano oil apparently reduces the adverse effects after a infection with *E. tenella*.

The lower body weight of the oregano group compared to the lasalocid group could be due to higher anticoccidial efficacy of lasalocid although possible toxic effects on chicken health by the phenolic constituents of oregano oil cannot be excluded. By antimicrobial effects of phenols, known for more than a century, the structure of bacterial cell walls is affected. Phenolic compounds may also exert their activity on the

TABLE II Daily body weight gain [g], daily feed intake (FI) [g], and feed conversion ratio as a function of the dietary treatment, infection with E. tenella, and age of chickens

Age of chickens	Infected control group	Non-infected control group	Infected oregano oil group	Infected lasalocid group
Day 0–14				
BWG	31.8 ± 2.7^{a}	32.3 ± 2.9^{a}	32.5 ± 2.1^{a}	32.4 ± 2.7^{a}
FI	37.2 ± 1.7^{a}	37.5 ± 0.9^{a}	38.5 ± 1.2^{a}	37.3 ± 1.4^{a}
FCR	1.16 ± 0.03^{a}	1.16 ± 0.02^{a}	1.18 ± 0.03^{a}	1.15 ± 0.03^{a}
Day 0–28				
BWG	46.2 ± 2.5^{a}	51.1 ± 2.8^{b}	51.4 ± 3.4^{b}	54.9 ± 2.9^{c}
FI	73.0 ± 3.8^{a}	75.5 ± 3.2^{a}	75.8 ± 3.3^{a}	75.8 ± 2.7^{a}
FCR	1.58 ± 0.03^{a}	1.47 ± 0.04^{b}	1.47 ± 0.03^{b}	1.38 ± 0.03^{c}
Day 0–42				
BWG	50.3 ± 3.26^{a}	54.9 ± 4.12^{b}	53.6 ± 3.16^{b}	60.5 ± 3.16^{c}
FI	98.9 ± 5.70^{a}	102.5 ± 5.30^{a}	100.9 ± 6.50^{a}	108.3 ± 6.80^{b}
FCR	1.96 ± 0.04^{a}	1.87 ± 0.06^{b}	1.91 ± 0.04^{b}	1.79 ± 0.03^{c}

Means in the same line with a common superscript do not differ significantly ($P > 0.05$)

host enterocytes (Weber and De Bont, 1996). Phenols 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*., 1999). Carvacrol and thymol, the major components of oregano essential oil, might therefore have a toxic effect on the upper layer of mature enterocytes of the intestinal mucosa. The hydrophobic character of carvacrol suggests interaction with the membranes (Sikkema *et al*., 1994). When the concentration of carvacrol increases, more of this compound is expected to accumulate and interact in the phospholipid belayer, affecting the membrane fluidity (Weber and De Bont, 1996).

Four days after infection, bloody diarrhoea occurred in all infected groups with the exception of the lasalocid group where it was delayed one day and was very weak (Table III). The extent of bloody diarrhoea in the oregano group was milder than that of the infected control group. Seven days after the infection, the survival rate of the non-infected control group was 100%, but that of the infected control group was 80%. Chicks of the oregano and the lasalocid group survived at 90% and 96.7%, respectively (Table III). The lesion score of the infected oregano group, although significantly higher than that of the lasalocid group, was significantly lower than that of the infected control group. No lesions were noted in the non-infected control group (Table III).

Table IV presents the effect of diets on oocyst excretion of broiler chickens infected with *E. tenella* on day 14 of age. The number of oocysts per g of excreta in the infected oregano group was lower than in the infected control group, but significantly higher than in the lasalocid group.

The extent of bloody diarrhoea, survival rate, lesion score and the number of oocysts in excreta suggested that oregano essential oil exerted a protective effect against *E. tenella* infection, but lower than that exhibited by lasalocid.

4. CONCLUSIONS

The results of the present study suggest that oregano essential oil exerts an anticoccidial effect against *E. tenella,* but less than that exhibited by lasalocid. Under the trade name Ecodiar, oregano oil is an appetite enhancer for poultry and pigs and approved as feed additive by the European legislation. Until now, there is no restriction or advisable

TABLE III Bloody diarrhea, survival rate and lesion score of chickens as a function of the dietary treatment, infection with *E. tenella*, and the days post-infection

	Blood in faeces Days post infection						
Experimental groups	3	4	5	6	7	Survival Rate [%]	Lesion score 7 d after challenge (Means ± SD, n = 9)
Non-infected control	–	–	–	–	–	100	0.0 ± 0.0^a
Infected control	–	+	+ + +	+ +	+	80	3.7 ± 0.6^b
Infected oregano oil	–	+	+ +	+ +	–	90	2.8 ± 0.9^c
Infected lasalocid	–	–	+	–	–	96.7	1.8 ± 0.7^d

Means in the same column with a different superscript letter differ significantly ($P < 0.05$)

TABLE IV Effect of dietary treatments on excretion of oocysts in broiler chickens infected with *E. tenella* on day 14 of age

Age of chicks [d]	Oocysts excretion [10^3/g of excreta]			
	Non-infected control group	Infected control group	Infected oregano oil group	Infected lasalocid group
7	0	0	0	0
14	0	0	0	0
20	0	25.2 ± 0.5^{a}	8.2 ± 0.5^{b}	5.2 ± 0.5^{b}
21	0	160.0 ± 1.0^{a}	23.3 ± 0.3^{b}	16.3 ± 0.5^{c}
22	0	42.1 ± 1.1^{a}	19.2 ± 0.4^{b}	12.2 ± 0.7^{c}
23	0	38.0 ± 0.8^{a}	15.6 ± 0.6^{b}	7.6 ± 0.5^{c}
24	0	30.6 ± 1.2^{a}	11.5 ± 0.8^{b}	6.5 ± 0.6^{c}
25	0	22.8 ± 0.9^{a}	10.7 ± 0.7^{b}	2.7 ± 0.7^{c}
26	0	16.5 ± 0.8^{a}	7.5 ± 1.1^{b}	2.5 ± 1.1^{c}
27	0	5.9 ± 1.1^{a}	2.3 ± 0.5^{b}	1.3 ± 0.1^{b}

Means in the same column with a different superscript letter differ significantly ($P < 0.05$)

withdrawal time for Ecodiar in food producing animals. However, the bioavailablility of the major phenolic constituents of oregano oil deposited in broiler meat cannot be directly demonstrated, since currently no analytical methods have been developed for the quantification of such residues in meat. The hypothesis of rearing broilers without coccidiostats (Ekstrand *et al.*, 1994) appears to be of promise for further investigations on a large scale. Further studies are needed to investigate the activity of different incorporation levels of oregano oil and its efficacy against different *Eimeria* species.

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