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Effect of dietary Olympus tea (*Sideritis scardica*) supplementation on performance of chickens challenged with *Eimeria tenella*

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ABSTRACT

An experiment was carried out to examine the effect of dietary supplementation with Olympus tea (*Sideritis scardica*) on the performance of broiler chickens challenged with 6×10^4 sporulated oocysts of *Eimeria tenella* at 14 days of age. A total of 150 day-old Cobb-500 chickens were separated into 5 equal groups with three subgroups each. Two of the groups, one challenged with *E. tenella* and the other not, were given the basal diet and served as controls, two challenged groups were administered diets supplemented with ground dried plants of Olympus tea at levels of 5 and 10 g/kg of diet, and the remaining challenged group a diet supplemented with the coccidiostat lasalocid. During the experimental period that lasted 35 days, body weight and feed intake were weekly recorded, and feed conversion ratio were calculated. Following the *E. tenella* challenge on day 14 of age, mortality, caecal lesion score, bloody diarrhoea and oocyst excretion were examined in all groups from day 17 to day 26 of age. Results showed that mean body weight gain of the Olympus tea groups, although higher ($P<0.05$) than the challenged control group, were lower ($P<0.05$) compared to the non-challenged control and lasalocid groups. Feed conversion ratio of the challenged control group was the highest ($P<0.05$) among all groups. Data on the extent of bloody diarrhoea, mortality, caecal lesion score and oocyst output suggested that treatment with Olympus tea at the supplementation level of 10 g/kg diet in particular, could alleviate the impact of parasite infection on the bird by exerting a coccidiostatic effect against *E. tenella* which, however, was considerably lower than that exhibited by lasalocid.

KEY WORDS: Olympus tea, *Sideritis scardica, Eimeria tenella*, chickens, performance, lasalocid

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INTRODUCTION

The strategy of dietary administration of coccidiostatic drugs, predominantly polyether ionophore antibiotics, over the last four decades, has, in part, provided the basis for the rapid growth of the poultry industry and the increased availability of high quality, affordable poultry products to the consumer. Nevertheless, the routine use or misuse of these antibiotics has now come under increasing scrutiny because of potential development of resistant strains (Chapman, 1986) or to appearance of residues in edible tissues that are unacceptable by consumers (Botsoglou and Fletouris, 2001). Presently, in Europe, the use of antibiotic feed additives in animal production is being phased out (European Commission Regulations, 1998). A complete ban on the use of coccidiostats in animal feeds has not yet been enforced in many countries, however this day may eventually come (Government Official Reports, 1997).

Without alternatives, this ban will have a strong economic impact on the poultry industry. There has been, therefore, a growing interest in the identification and evaluation of alternative feed additives that would satisfy consumer demands and would be closer to environmentally friendly farming practices. As part of this effort, several natural products have been tested for their potential to provide protection against or modulate the effects of coccidial infections. Allen et al. (1997) reported that dried leaves of *Artemisia annua* could protect chickens against caecal lesions due to *E. tenella* infection. Youn and Noh (2001) showed that *Sophora flavescens* extracts were more effective than *Artemisia annua* against *E. tenella* infection in chickens.

In a study conducted in our laboratory on the modulation of coccidia infections by various aromatic plants of the Greek flora, we have found that oregano, an aromatic plant of the *Labiatae* family, exhibits coccidiostatic action against *E. tenella* when the essential oil or ground flowers, leaves and stems of the plant are incorporated into chicken diets (Giannenas et al., 2003, 2004). In continuation of this investigation, we are examining other plants of the *Labiatae* family, which might also have the potential to reduce the adverse effects of coccidiosis.

Plants of the genus *Sideritis* also belong to the *Labiatae* family and are commonly known as mountain tea. These plants comprise 8 species and 7 subspecies (Strid and Tan, 1991) and are often used in Mediterranean countries as tea infusions, flavouring agents, and for medicinal purposes due to their anti-inflammatory, antirheumatic, and antimicrobial activity (Villar et al., 1986; Diaz et al., 1988; Aligiannis et al., 2001). *Sideritis scardica* Griseb. subsp. *scardica*, a subspecies endemic in the mountains of North Greece, is specifically known as Olympus tea. The composition of Olympus tea has been little studied. However, the presence of carvacrol and thymol, compounds that also occur in oregano,

has been established in the essential oil of Olympus tea (Kokkalou, 1987; Komaitis et al., 1992; Baser et al., 1997).

The objective of the present study was to investigate the efficacy of ground dried flowers, leaves, stems and stalks of Olympus tea as a dietary supplement in broiler chickens against the highly pathogenic *E. tenella*, and to compare this use to the approved coccidiostat lasalocid.

MATERIAL AND METHODS

Animals and housing

A total of 150 one-day-old Cobb-500 chickens, randomly allocated into 5 equal groups with three subgroups of 5 males and 5 females each, were used in this study. All subgroups were housed in separate wire suspended cages, each equipped with an infrared lamp. Temperature was gradually decreased from 32°C on day 1 to 22°C on day 21 and kept constant by day 35 that the trial ended. The lighting regimen provided 24 h of continuous light per day for the first three days, and 23 h of light per day, thereafter. Birds were vaccinated against Newcastle disease and infectious bronchitis on day 10 of age using Hitchner B1 and Massachusetts H-120 strains, respectively, and against Gumboro disease on day 17 of age using D-78 strain. All birds were cared for in accordance with the Guide for the Care and Use of Laboratory Animals (Committee on Care and Use of Laboratory Animals, 1985).

Dietary treatments

To meet the nutrient requirements of the broilers during the experimental period, a complete basal diet was formulated. Table 1 presents the ingredients and the composition of the basal diet that was in mash form. Based on this basal diet, additional diets were prepared by incorporating either ground dried plants of Olympus tea at levels of 5.0 and 10 g/kg feed or lasalocid at 75 mg/kg feed.

Ground dried plants of Olympus tea consisted of flowered tops, leaves, stems and stalks of *Sideritis scardica* Griseb. subsp. *scardica* that had been dried and ground to pass 2 mm screen.

TABLE 1

Composition of basal diet, g/kg	
Ingredients	
wheat grains	580.0
soyabean meal	284.0
soyabean oil	44.0
herring meal	15.0
maize gluten feed	40.0
limestone, pulverized	14.9
dicalcium phosphate	7.8
biolysine - BASF	3.3
dl-methionine	2.9
sodium chloride, iodized	2.8
Natuphos - BASF (phytase)	0.1
Natugrain - BASF (arabinoxylanases plus glucanases)	0.2
vitamin premix[1]	3.0
trace-mineral premix[2]	2.0
Chemical analysis[3]	
dry matter	889.1
crude protein (N × 6.25)	231.0
crude fat	74.7
crude fibre	38.0
ash	56.0
Calculated analysis	
Ca	9.3
P (total)	6.7
lysine	13.0
methionine + cystine	10.2
metabolizable energy, kcal/kg	3160.0

[1] supplying per kg feed: vit. A 12,000 IU, vit. D_3 5,000 IU; mg: vit. E 80, vit. K 7, thiamin 5, riboflavin 6, pyridoxine 6, vit. B_{12} 0.02, niacin 60, pantothenic acid 15, folic acid 1.5, biotin 0.25, vit. C 10, choline chloride 500

[2] supplying per kg feed, mg: Zn 100, Mn 120, Fe 20, Cu 15, Co 0.2, I 1, Se 0.3

[3] according to AOAC, 1990

Experimental design

Two of the five groups, one challenged with *E. tenella* and the other not, were given a basal diet that did not contain any anticoccidial or other antimicrobial feed additive and served as controls. From the remaining groups that were all challenged with *E. tenella*; two were administered the Olympus tea supplemented diets, while the last group the lasalocid diet. Each experimental group was given the corresponding diet from day 1 to day 35 of age. Feed and drinking water were offered to birds *ad libitum*.

Eimeria tenella challenge

Challenge of chickens with *E. tenella* was carried out at 14 days of age. 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 propagated in chickens. To induce sporulation, oocysts were preserved in 2% potassium dichromate solution, and kept refrigerated at 3-5°C until use. Challenge of each bird was carried out by administering a 2-ml suspension of 6×10^4 sporulated oocysts of *E. tenella* directly into the crop using an oral gavage. The birds of the non-challenged group were sham-infected with a 2-ml suspension of saline water.

Performance parameters

All 15 subgroups of chickens were weighed at the time of their placing into the cages and on days 7, 14, 21, 28 and 35 of age. Four hours prior to bird weighing, diets were removed and feed consumption within each subgroup was determined. Feed conversion ratio values were calculated weekly as the ratio of feed intake to weight gain. Mortality was recorded daily in each subgroup.

Seven days after the challenge, the lesion score was estimated in all groups by evaluating caecal intestine lesions of 3 chickens per subgroup. Lesion score was assigned from 0 to 4, where zero score is the normal status with no gross lesions, one corresponds to small scattered petechiae, two to numerous petechiae, three to extensive haemorrhage, and four to extensive haemorrhage giving the caecal intestine a dark colour (Johnson and Reid, 1970). Dead birds were given the score of 4.

Bloody diarrhoea was determined daily from day 17 to day 21 of age. The extent of bloody diarrhoea was determined according to Youn and Noh (2001) by assigning it one of five levels, where zero is the normal status, 1 corresponds to less than 25%, 2 to 26-50%, 3 to 51-75%, and 4 to over 75% bloody faeces in total faeces over each 24 h.

Oocyst counts were determined in the excreta samples taken from each subgroup, at 7 and 14 day of age and daily from day 20 to day 26 of age. A clean polyethylene sheet placed daily under each cage was used for collection of excreta for oocyst analysis. Total faecal sampling over each 24 h 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).

Statistical analysis

Each subgroup served as the experimental unit in the statistical analysis of all data. Data were subjected to analysis of variance (ANOVA) in the general linear model using the SPSS 10.05 statistical package (SPSS Ltd., Woking, Surrey, UK). When significant treatment effects were disclosed at probability level of $P<0.05$, the Tukey's test was applied in order to determine statistical differences between means (Steel and Torrie, 1980). The homogeneity of the variances was tested by Bartlett's test. When variances were not normally distributed, non-parametric analysis was employed.

RESULTS

At the ages of 7 and 14 days, body weight gain, cumulative feed intake and feed conversion ratio values did not differ ($P<0.05$) among treatments (Table 2).

TABLE 2

Cumulative body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) values of broiler chickens in response to diet, age, and infection with *E. tenella*

Age of chickens	Control[1]	Challenged control[1]	Olympus tea		Lasalocid 75 mg/kg[1]	Pooled SEM	P value
			5 g/kg[1]	10 g/kg[1]			
7 days							
BWG, g	124[a]	127[a]	121[a]	124[a]	122[a]	0.9	0.267
FI, g	129[a]	127[a]	131[a]	133[a]	133[a]	0.9	0.177
FCR	1.04[a]	1.00[a]	1.08[a]	1.07[a]	1.09[a]	0.02	0.332
14 days							
BWG, g	399[a]	386[a]	395[a]	389[a]	396[a]	2.9	0.669
FI, g	530[a]	522[a]	537[a]	525[a]	534[a]	6.7	0.981
FCR	1.33[a]	1.35[a]	1.36[a]	1.35[a]	1.35[a]	0.02	0.564
21 days							
BWG, g	737[a]	596[b]	671[c]	680[c]	719[a]	13.8	0.000
FI, g	1005[a]	930[b]	980[a]	1006[a]	992[a]	11.3	0.048
FCR	1.36[a]	1.56[b]	1.46[c]	1.48[c]	1.38[a]	0.02	0.000
28 days							
BWG, g	1166[a]	952[b]	1056[c]	1092[c]	1145[a]	21.1	0.000
FI, g	1772[a]	1694[b]	1710[a]	1725[a]	1729[a]	12.3	0.004
FCR	1.52[a]	1.78[b]	1.62[a]	1.58[a]	1.51[a]	0.03	0.001
35 days							
BWG, g	1806[a]	1442[b]	1667[c]	1664[c]	1809[a]	36.4	0.000
FI, g	2998[a]	2670[b]	2852[a]	2830[a]	2967[a]	32.3	0.016
FCR	1.66[a]	1.85[b]	1.71[a]	1.70[a]	1.64[a]	0.03	0.006

[1] n=3

[a,b,c] values in the same row with a superscript in common do not differ significantly at $P>0.05$

At the age of 21 days, that is 7 days after the challenge with *E. tenella*, mean body weight gain of the challenged control group turned out to be lower ($P<0.05$) than in all other groups. At this age, mean body weight gain of the Olympus tea

groups were lower (P<0.05) compared to the non-challenged control and lasalocid groups that in turn did not differ (P>0.05) from each other. Also, feed conversion ratio values of the non-challenged control group and the lasalocid group were the lowest (P<0.05) among all groups. Both the Olympus tea groups exhibited better feed conversion ratio values than the challenged control group, however these values did not differ (P>0.05) from each other.

At the ages of 28 and 35 days, the rate of body weight gain did not change substantially, whereas feed conversion ratio values of both the Olympus tea groups did not differ (P>0.05) from those of the lasalocid and the non-challenged control groups. The challenged control group had the highest feed conversion ratio value.

Bloody diarrhoea the extent of which did not differ among all challenged groups was observed on day 18 of age, except for the lasalocid group where it appeared one day later. On days 19 and 20 of age, the extent of bloody diarrhoea in the Olympus tea groups was lower (P<0.05) compared to the challenged control group but similar to the lasalocid group (Table 3). On day 21, the extent of bloody diarrhoea did not differ among the challenged control and both the Olympus tea groups although it was at zero for the lasalocid group.

TABLE 3

Bloody diarrhoea, mortality and lesion score of broiler chickens in response to diet and infection with *E. tenella*

Dietary treatments	Bloody faeces, % age in days					Mortality %	Lesion score at day 21 of age
	17	18	19	20	21		
Control[1]	0	0	0	0	0	0	0
Challenged control[1]	0	1.0[a]	3.3[a]	2.3[a]	1.3[a]	23.4[a]	3.6[a]
Olympus tea 5 g/kg diet[1]	0	0.7[a]	1.7[b]	1.0[b]	0.3[a]	13.4[a]	2.8[a]
Olympus tea 10 g/kg diet[1]	0	1.0[a]	1.7[b]	0.7[b]	0.3[a]	10.0[a]	2.9[a]
Lasalocid 75 mg/kg diet[1]	0	0	0.7[b]	0.3[b]	0	0	1.2[b]
Pooled SEM		0.2	0.3	0.2	0.2		0.3
P value		0.512	0.005	0.005	0.144	0.368	0.026

[a,b,c] values in the same column with a superscript in common do not differ significantly at P>0.05

[1] n=3

Seven days after the challenge, mortality in the non-challenged control and lasalocid groups was zero, whereas in the challenged control group was at 23.4% (Table 3). The group at 5.0 g tea/kg diet presented mortality at 13.4%, whereas that at 10.0 g tea/kg diets at 10%.

The caecal lesion scores of the Olympus tea groups did not differ (P>0.05) from the score of the challenged control group. The lasalocid group presented significantly (P<0.05) lower caecal lesion score compared to all other challenged groups (Table 3). The numbers of oocysts per g of excreta in the Olympus tea groups were lower (P<0.05) than in the challenged control group from day 20 to day 23

of age, whereas they were similar on days 25 and 26. On day 24, oocyst numbers between the Olympus tea groups differed each other, the higher supplementation group presenting oocyst numbers lower ($P<0.05$) than the challenged control group while the lower supplementation Olympus tea group presenting numbers similar to the challenged control group. The numbers of oocysts per g of excreta in the lasalocid group were always lower ($P<0.05$) compared to both the Olympus tea groups and the challenged control group (Table 4).

TABLE 4

Effect of diet on litter oocyst excretion of broiler chickens infected with *E. tenella*

Age of chickens days	Oocysts excretion ($\times 10^3$)/ g excreta						
	Control[1]	Challenged control[1]	Olympus tea		Lasalocid 75 mg/kg[1]	Pooled SEM	P value
			5 g/kg[1]	10 g/kg[1]			
7	0	0	0	0	0		
14	0	0	0	0	0		
20	0	26[a]	12[b]	11[b]	7[c]	2.2	0.000
21	0	267[a]	126[b]	117[b]	25[c]	26.2	0.000
22	0	164[a]	79[b]	72[b]	17[c]	15.9	0.000
23	0	74[a]	29[b]	25[b]	11[c]	7.2	0.000
24	0	27[a]	21[a]	17[b]	6[c]	2.4	0.000
25	0	22[a]	19[a]	19[a]	5[b]	2.1	0.000
26	0	13[a]	12[a]	11[a]	2[b]	1.4	0.001

[a,b,c] values in the same row with a superscript in common do not differ significantly at $P>0.05$

[1] n=3

DISCUSSION

In the last few years, several aromatic plants have been tested for their potential to provide protection against coccidial infections. Oregano, a plant of the *Labiatae* family, has been found to exhibit coccidiostatic action against *E. tenella* when the essential oil or ground flowers, leaves and stems of the plant are incorporated into chicken diets (Giannenas et al., 2003, 2004). The mechanism of action of oregano against coccidia has not yet been extensively investigated. However, it might be postulated that the coccidiostatic activity might be attributed to the contained phenolic compounds since several *in vivo* and *in vitro* tests have shown (Williams, 1997) that phenolic compounds are effective oocysticides against *E. tenella*. In oregano, major components are the phenols carvacrol and thymol that constitute about 78-82% of the oregano essential oil (Vekiari et al., 1993), whereas γ-terpinene and p-cymene often constitute about 5 and 7% of the essential oil, respectively (Adam et al., 1998). Carvacrol and thymol have been recently shown to be capable of reducing the impact of *Eimeria acervulina* infection in chickens when they are incorporated in their diets (Ibrir et al., 2001).

Olympus tea, another plant of the *Labiatae* family, also contains phenolic compounds and monoterpene hydrocarbons like oregano. Kokkalou (1987) reported that the essential oil of Olympus tea contains α-pinene, β-pinene, myrcene and β-phellandrene as major constituents at levels of 52, 13, 13 and 4%, respectively. Komaitis et al. (1992) showed that β-caryophyllene, thymol, carvacrol, α-pinene and β-pinene constitute the major components of the essential oil of Olympus tea at levels of 8, 6, 6, 5 and 6%, respectively. Baser et al. (1997) established the presence of β-pinene, thymol, carvacrol, β-caryophyllene and α-pinene in the essential oil of Olympus tea at levels of 18, 4, 15, 6 and 7%, respectively. Therefore, Olympus tea could be a promising dietary supplement to reduce the adverse effects of coccidiosis in chickens.

Adverse effects of coccidiosis due to *E. tenella*, include bloody diarrhoea, intestinal lesions, depressed growth rate and, sometimes, high mortality. Therefore, a number of performance parameters including body weight gain, feed intake, feed conversion ratio, mortality, caecal lesion score, bloody diarrhoea and oocysts output of the experimental groups were investigated after challenging chickens at 14 days of age with sporulated oocysts of *E. tenella*.

Infection with *E. tenella* significantly reduced body weight gain, feed intake and feed conversion ratio values, highlighting the detrimental effect of infection with this parasite on bird performance. Treatment with Olympus tea was found to have beneficial effect on post infection performance improving body weight gain and feed conversion ratio values compared to the challenged control group. It also significantly reduced the amount of blood observed in faeces on days 19 and 20 of age compared to the challenged control group. The numbers of oocysts per g of excreta in the Olympus tea groups were also lower ($P<0.05$) than in the challenged control group from day 20 to 23 of age, although they were always higher ($P<0.05$) than in the lasalocid group. However, there were no significant effects on mortality and caecal lesion score.

CONCLUSIONS

In conclusion, the results of the present study suggest that treatment with Olympus tea could alleviate the impact of parasite infection on the bird by exerting a coccidiostatic effect against *E. tenella* which, however, was lower than that exhibited by lasalocid. Therefore, the hypothesis of rearing broilers without coccidiostats is thought to be of promise for more extensive studies.

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STRESZCZENIE

Wpływ dodatku herbaty Olympus (*Sideritis scardica)* do diety kurcząt zakażonych *Eimeria tenella* na ich wyniki produkcyjne

Celem doświadczenia było zbadanie wpływu dodatku do diety herbaty Olympus na wyniki produkcyjne kurcząt brojlerów zakażonych w 14 dniu życia oocystami (6×10^4) *Eimeria tenella*. Sto pięćdziesiąt jednodniowych kurcząt Cobb 500 podzielono na 5 grup o takie samej liczebności, po 3 podgrupy w każdej. Dwie grupy, z których jedną zakażono, a drugiej nie zakażono *E. tenella*, otrzymywały dietę podstawową i były traktowane jako grupy kontrolne. Pozostałe 3 grupy, zakażone *E. tenella*, żywiono dietami z dodatkiem suszonych mielonych roślin herbaty Olympus w ilości 5 i 10 g/kg, oraz dietą uzupełnioną kokcydiostatykiem, lasolocidem. W doświadczeniu, trwającym 35 dni, oznaczano co tydzień przyrost masy ciała i pobranie paszy oraz obliczono wykorzystanie paszy. Pomiędzy 17 a 26 dniem życia kurcząt, po 14 dniach od zakażenia *E. tenella*, określono śmiertelność, oznaczono w skali punktowej zmiany chorobowe w jelitach ślepych, występowanie krwawych biegunek oraz liczbę wydalanych oocyst.

Średni przyrost masy ciała kurcząt z grup otrzymujących dodatek herbaty był wprawdzie większy ($P<0,05$) niż w grupie kontrolnej zakażonej *Eimeria*, był jednak mniejszy ($P<0,05$) niż w grupie kontrolnej niezakażonej oraz w grupie z dodatkiem lasolocidu. Kurczęta z grupy kontrolnej zakażonej pasożytem wykorzystywały paszę gorzej ($P<0,05$) niż z pozostałych grup, które nie różniły się między sobą. Wyniki dotyczące występowania krwawej biegunki, upadków kurcząt, zmian chorobowych w jelitach ślepych oraz liczby wydalanych oocyst sugerują, że dodatek herbaty Olympus, szczególnie w ilość 10 g/kg diety, może złagodzić skutki zakażenia pasożytami u ptaków, jednak kokcydiostatyczny wpływ herbaty Olympus w stosunku do *E. tenella* jest znacznie mniejszy niż lasolocidu.