Anim. Res. 53 (2004) 137–144

DOI: 10.1051/animres:2004006

Original article

Effect of a mixture of herbal extracts on broiler chickens infected with *Eimeria tenella*

Efterpi CHRISTAKI[a], Panagiota FLOROU-PANERI[a], Ilias GIANNENAS[a], Margarita PAPAZAHARIADOU[b], Nikolaos A. BOTSOGLOU[a]*, Alexandros B. SPAIS[a]

[a] Laboratory of Animal Nutrition, Faculty of Veterinary Medicine, Aristotle University, 54006 Thessaloniki, Greece
[b] Laboratory of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, Aristotle University, 54006 Thessaloniki, Greece

(Received 22 May 2003; accepted 9 February 2004)

Abstract – The effect of dietary supplementation with Apacox (Apa-CT, s.r.l. Italy), a commercial preparation of herbal extracts, on the performance of broiler chickens experimentally infected with 6×10^4 sporulated oocysts of *Eimeria tenella* at 14 days of age, was evaluated. A total of 150 day-old Cobb-500 chicks separated into 5 equal groups with three replicates each, were used. Two of the groups, one challenged with *E. tenella* oocysts and the other not, were given a basal diet and served as controls. The remaining groups that were also challenged with *E. tenella* were administered a basal diet supplemented with Apacox at levels of 0.5 or 1.0 g·kg^{-1} or the anticoccidial lasalocid at 75 mg·kg^{-1}. Throughout the experimental period from day 1 to day 35, performance parameters including body weight gain, feed intake, feed conversion ratio, mortality, caecal lesion score, bloody diarrhoea and oocyst output were recorded. Dietary supplementation with Apacox attained higher body weight gain and lower feed conversion ratio values than the non-supplemented challenged control group. The lasalocid and the non-challenged control groups exhibited body weight gain and feed conversion ratio values that did not significantly differ from each other, and were better than in the Apacox groups. Bloody diarrhoea was observed in all challenged groups except for the Apacox group at the dose of 1.0 g·kg^{-1} diet, where it was milder and the lasalocid group where it was very weak. The mortality in the challenged control group was 23.4% whereas in the Apacox group at the dose of 1.0 g·kg^{-1}, 13.4%. The caecal lesion scores of the Apacox groups were not significantly different from that of the challenged control group. The numbers of oocysts per bird in the Apacox groups were lower than that in the challenged control group but higher than that in the lasalocid group. These results indicate that Apacox exerted a coccidiostatic effect against *E. tenella*. This effect was, however, significantly lower than that exhibited by lasalocid.

broiler chickens / herbal extracts / coccidiosis / performance / *Eimeria tenella*

Résumé – Effet d'un mélange d'extraits d'herbes incorporé dans l'aliment de poulets de chair, sur le développement d'une coccidiose due à *Eimeria tenella.* Dans ce travail, nous avons étudié

* Corresponding author: bots@vet.auth.gr

l'effet de l'incorporation dans l'aliment d'une préparation commerciale, contenant un mélange d'extraits d'herbes (Apacox), sur le développement d'une coccidiose expérimentale due à *Eimeria tenella* (6×10^4 oocystes) réalisée à 14 jours d'âge chez des poulets de chair. Dans ce but, 150 poussins Cobb 500 d'un jour ont été séparés en 5 groupes de 30 poussins chacun avec 3 répliques de 10 oiseaux. Deux groupes témoins, dont l'un a été infecté avec des oocystes d'*E. tenella* et l'autre pas, ont consommé l'aliment de base. Les trois autres groupes ont été infectés avec *E. tenella* ; l'un a consommé l'aliment de base supplémenté avec l'Apacox à la dose de 0,5 $g \cdot kg^{-1}$, l'autre à la dose de 1,0 $g \cdot kg^{-1}$ et le troisième avec un anticoccidien (75 $mg \cdot kg^{-1}$ de lasalocide de sodium). Au cours de la période expérimentale, de 1 à 35 jours d'âge, le gain de poids des poulets, la consommation d'aliment, et l'efficacité alimentaire étaient estimés chaque semaine. Après infection, la mortalité, les diarrhées sanglantes et l'excrétion d'oocystes, ainsi que le score lésionnel au niveau du caecum ont été déterminées. L'incorporation d'Apacox a conduit à un poids vif et un indice de consommation supérieurs à ceux du groupe témoin infecté, mais inférieurs à ceux du groupe recevant le lasalocide et à ceux du groupe témoin non-infecté. Ces deux derniers groupes n'ont pas différé. Des diarrhées sanglantes ont été observées dans tous les groupes infectés, excepté dans le groupe recevant l'Apacox à 1 $g \cdot kg^{-1}$ qui a conduit à des diarrhées plus faibles et le groupe lasolacide qui a eu de très faibles diarrhées. La mortalité dans le groupe témoin infecté a été de 23,4 % alors que pour le groupe Apacox à la dose de 1 $g \cdot kg^{-1}$, elle s'est élevée à 13,4 %. Bien que numériquement plus faibles, les lésions caecales des groupes Apacox n'ont pas été différentes de celles observées dans le groupe témoin infecté. Le nombre d'oocystes excrété dans les groupes Apacox a été plus faible que dans le groupe témoin infecté, mais plus élevé que dans le groupe lasalocide. Ces résultats indiquent que l'Apacox a un effet coccidiostatique modéré contre *E. tenella,* mais significativement inférieur à celui du lasalocide.

poulets de chair / extraits végétaux / coccidiose / performance / *Eimeria tenella*

1. INTRODUCTION

Coccidiosis is one of the most detrimental diseases in poultry due primarily to impaired feed conversion, depressed growth rate and, sometimes, increased mortality [13]. In order to avoid the potential for coccidiosis outbreak and the resulting financial loss, broiler chickens are continuously medicated with coccidiostatic drugs, predominately ionophore antibiotics, added in feeds. However, concern has been expressed regarding the routine use of these antibiotics in feeds due mainly to the emergence of resistant coccidial strains [5]. In addition, the use of antibiotic feed additives in general is being phased out in Europe and only a few coccidiostatic drugs remain as non-prescription feed additives [8]. If all coccidiostatic feed additives are withdrawn from use, alternative feeding strategies should probably be introduced to restrict the adverse effects of coccidia on production, although vaccines are already available. There is, therefore, a need for intensive research into the identification and evaluation of alternatives to traditional coccidiostatics that would satisfy consumer demands and would be closer to environmentally friendly farming practices.

Some natural products have already been tested for their potential to provide protection against or modulate the effects of coccidial infections. Allen et al. [1] reported that dried leaves of *Artemisia annua* could provide significant protection against intestinal lesions caused by *E. tenella*. Youn and Noh [20] found that *Sophora flavescens* extracts were more effective than *Artemisia annua* against *E. tenella* infection in chickens. Giannenas et al. [9] reported that the essential oil of oregano, an aromatic plant of the *Labiatae* family, exhibited coccidiostatic action against *E. tenella* when incorporated into chicken diets at the level of 300 $mg \cdot kg^{-1}$.

The aim of the present study was to investigate the effect of a dietary mixture of herbal extracts on the performance of broiler chickens experimentally infected with *Eimeria tenella*, a highly pathogenic *Eimeria* species that causes caecal coccidiosis. The

herbal extracts used in this study constitute the active components of Apacox (APA-CT, s.r.l., Italy), a naturally derived nutrition enhancer that contains extracts from the plants *Agrimonia eupatoria, Echinacea angustifolia, Ribes nigrum* and *Cinchona succirubra*.

2. MATERIALS AND METHODS

2.1. Animals and housing

A total of 150 day-old Cobb-500 chicks were randomly allocated into 5 equal groups with three subgroups of 5 males and 5 females each. Each subgroup was housed in separate wire suspended cage equipped with an infrared lamp. Temperature was gradually decreased from 32 °C on day 1 to 22 °C on day 21 and then kept constant. The lighting regimen provided 24 h of continuous light per day. The 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 broiler chickens during the experimental period from day 1 to day 35, a complete basal diet was formulated. Table I presents the ingredients and the composition of the basal diet. It contains neither antibiotics (or growth enhancers) nor coccidiostats. The chemical composition was determined according to AOAC [2]. The diet was presented in a mashed form. Based on this basal diet, additional diets were prepared by incorporating Apacox at levels of 0.5 and 1.0 $g \cdot kg^{-1}$ feed or the anticoccidial lasalocid sodium at 75 $mg \cdot kg^{-1}$ feed, at the expense of corn gluten feed.

Two of the five groups, one challenged with *E. tenella* and the other not, were given the basal diet, and served as controls. From the remaining three groups that were challenged with *E. tenella,* two were administered the Apacox supplemented diets, while the third the lasalocid diet. Each diet was given from day 1 to day 35 of age. Feed and drinking water were offered to birds ad libitum.

Table I. Composition of basal diet.

Ingredients	$g \cdot kg^{-1}$
Wheat grains	580.0
Soybean meal	284.0
Soybean oil	44.0
Herring meal	15.0
Corn Gluten feed	40.0
Limestone, pulverised	14.9
Dicalcium phosphate	7.8
Biolysine -BASF	3.3
DL-Methionine	2.9
Sodium chloride, iodised	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	892.5
Crude protein (N × 6.25)	223.5
Crude fat	74.0
Crude fibre	38.2
Ash	56.8
Calculated analysis	
Calcium	9.3
Phosphorus (total)	6.7
Lysine	13.0
Methionine + Cystine	10.2
Metabolisable energy in $kcal \cdot kg^{-1}$	3160.0

[1] Supplying per kg feed: 12 000 IU vitamin A, 5 000 IU vitamin D_3, 80 mg vitamin E, 7 mg vitamin K, 5 mg thiamine, 6 mg riboflavin, 6 mg pyridoxine, 0.02 mg vitamin B_{12}, 60 mg niacin, 15 mg pantothenic acid, 1.5 mg folic acid, 0.25 biotin, 10 mg vitamin C, 500 mg choline chloride.
[2] Supplying per kg feed: 100 mg Zn, 120 mg Mn, 20 mg Fe, 15 mg Cu, 0.2 mg Co, 1 mg I, and 0.3 mg Se.
[3] According to AOAC [2].

2.3. Experimental infection with *E. tenella*

Challenge of chickens with *E. tenella* was carried out at 14 days of age. A reference stock of *E. tenella* oocysts propagated in chickens was used. To induce sporulation, the 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* in saline water directly into the crop via an oral gavage. All birds of the non-challenged control group were also administered a 2-mL suspension of saline water.

2.4. Performance parameters

All chicks were individually 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, the 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 evaluation of the caecal lesions was carried out in nine chicks of each group. A lesion score was assigned from 0 to 4, where 0 corresponds to the normal status with no gross lesions, 1 to small scattered petechiae, 2 to numerous petechiae, 3 to extensive haemorrhage, and 4 to extensive haemorrhage that gives a dark colour to the caecal intestine [11]. 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 [20] by assigning it one of five levels, where the zero level 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 excreta samples taken from each subgroup at days 7 and 14 of age, and daily from day 20 to day 26 of age. A clean polyethylene sheet placed daily under each cage was used for the collection of excreta for oocyst analysis. Total faecal samplings over each 24 h from each subgroup, were placed in separate airtight plastic bags, homogenised thoroughly with a domestic mixer, and kept refrigerated until assessed for total oocyst counts. Homogenised 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 bird [10].

2.5. Statistical analysis

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 a probability level of $P < 0.05$, the Tukey test was applied in order to determine the statistical differences between means [16]. The homogeneity of the variances was tested by the Bartlett test. For values not normally distributed, the non-parametric analysis of Kruskal-Wallis was employed.

3. RESULTS

Body weight gain, cumulative feed intake and feed conversion ratio values at the age of 14 days did not differ among treatments (Tab. II). At the age of 21 days that is 7 days after the challenge with *E. tenella*, mean body weight gain in the challenged control group turned out to be lower than in all other groups. At this age, mean body weight gain values in the Apacox groups, although higher than in the challenged control group, were lower compared to the non-challenged control group and the lasalocid group that in turn did not significantly differ from each other. Feed conversion ratio values in the non-challenged

Table II. 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* at day 14 of age.

Age of chickens	Non-challenged control group[1]	Challenged control group[1]	Challenged Apacox group[1] at 0.5 g·kg^{-1}	Challenged Apacox group[1] at 1.0 g·kg^{-1}	Challenged Lasalocid group[1] at 75 mg·kg^{-1}	Pooled SEM	*P* value
7 days							
BWG (g)	124	127	124	122	122	0.9	0.979
FI (g)	129	127	130	131	133	1.7	0.872
FCR	1.04	1.00	1.05	1.07	1.09	0.01	0.07
14 days							
BWG (g)	399	386	394	405	396	3.1	0.43
FI (g)	530	522	529	534	534	7.3	0.982
FCR	1.33	1.35	1.34	1.32	1.35	0.01	0.91
21 days							
BWG (g)	737[a]	596[c]	686[b]	678[b]	719[a]	13.6	0.000
FI (g)	1005	932	983	976	992	11.6	0.368
FCR	1.36[c]	1.56[a]	1.43[b]	1.44[b]	1.38[c]	0.02	0.000
28 days							
BWG (g)	1166[a]	952[c]	1046[b]	1056[b]	1145[a]	21.3	0.000
FI (g)	1772[a]	1694[b]	1726[a b]	1721[a b]	1729[ab]	15.2	0.044
FCR	1.52[c]	1.78[a]	1.65[b]	1.63[b]	1.51[c]	0.03	0.000
35 days							
BWG (g)	1806[a]	1442[c]	1634[b]	1640[b]	1809[a]	36.8	0.000
FI (g)	2998[a]	2670[c]	2876[b]	2880[b]	2967[ab]	36.3	0.009
FCR	1.66[c]	1.85[a]	1.76[b]	1.75[b]	1.64[c]	0.02	0.000

[1] n = 3.
[a, b, c] Values in the same row with a superscript in common do not differ significantly ($P > 0.05$) by the Tukey test.

control group and the lasalocid group were the lower among all groups. The Apacox groups exhibited better feed conversion ratio values compared to the challenged control group. At the ages of 28 and 35 days, these profiles did not change.

Four days after the challenge, bloody diarrhoea was observed in all challenged groups except for the lasalocid group where it started one day later and was very weak (Tab. III). The intensity of bloody diarrhoea was lower in the Apacox groups than in the challenged control group, and was similar between the two doses of Apacox.

Seven days after the challenge, mortality in the non-challenged control and the lasalocid groups was zero, and in the challenged control group was 23.4% (Tab. III). The Apacox group at 0.5 g·kg^{-1} diet presented a mortality of 16.7%, whereas that at 1.0 g Apacox·kg^{-1} a mortality of 13.4%. The challenged control group and both the Apacox groups did not significantly differ in mortality.

The caecal lesion score in the Apacox groups did not significantly differ from that of the challenged control group. The lasalocid group presented a lower caecal lesion score compared to all other challenged groups except the Apacox group at 1.0 g·kg^{-1} (Tab. III). The numbers of oocysts per bird in the Apacox groups (Tab. IV) were lower than in the challenged

Table III. Bloody diarrhea, mortality, and lesion score of broiler chickens in response to diet and infection with *E. tenella* at the age of 14 days.

Experimental groups	Level of bloody diarrhea[1] (n = 3)					Mortality at day 21 (%)	Lesion score at day 21 (n = 3)
	Days post infection						
	3	4	5	6	7		
Non-challenged control	0	0	0	0	0	0	0
Challenged control	0	1.3	3.3 [a]	2.3 [a]	0.7	23.4	3.6[a]
Challenged Apacox at 0.5 g·kg^{-1} diet	0	0.7	2.0 [b]	1.7 [a]	0	16.7	2.6[a]
Challenged Apacox at 1.0 g·kg^{-1} diet	0	1.0	1.7 [bc]	0.3 [b]	0	13.4	2.1[ab]
Challenged Lasalocid at 75mg·kg^{-1}diet	0	0	0.7 [c]	0	0	0	1.2[b]
Pooled SEM		0.2	0.3	0.3	0.1		0.3
P value		0.059	0.000	0.000		0.177*	0.011

[a, b, c] Values in the same column with a superscript in common do not differ significantly ($P > 0.05$) by the Tukey test.
[1] The extent of bloody diarrhoea was determined according to Youn and Noh [20] by assigning it one of five levels, where the zero level 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.
*Values in the column do not differ significantly ($P > 0.05$) by non parametric analysis of Kruskal-Wallis.

Table IV. Effect of diet on litter oocyst excretion in broiler chickens experimentally infected with *E. tenella* at the age of 14 days.

Oocysts excretion (× 10^6)·bird^{-1}							
Age of chickens (days)	Non-challenged control group	Challenged control group	Challenged Apacox group at 0.5 g·kg^{-1}	Challenged Apacox group at 1.0 g·kg^{-1}	Challenged Lasalocid group	Pooled SEM	*P* value
7	0	0	0	0	0		
14	0	0	0	0	0		
20	0	2.4[a]	2.0[a]	1.7[a]	0.6[b]	0.2	0.000
21	0	25.4[a]	11.7[b]	7.3[c]	2.2[d]	2.6	0.000
22	0	16.4[a]	4.3[b]	2.6[c]	1.5[d]	1.8	0.000
23	0	7.6[a]	2.8[b]	1.8[c]	1.1[d]	0.8	0.000
24	0	2.9 [a]	1.3[b]	0.9 [b]	0.6[c]	0.3	0.000
25	0	2.5[a]	1.0[b]	0.8 [b]	0.5[c]	0.8	0.000
26	0	1.5[a]	0.6[b]	0.5[b]	0.3[c]	0.1	0.000

[a, b, c, d] Values in the same row with a superscript in common do not differ significantly ($P > 0.05$) by the Tukey test.

control group from day 21 to day 26 of age but similar at day 20. Moreover, Apacox at 1.0 g·kg^{-1} had lower numbers of oocyst output compared to Apacox at 0.5 g·kg^{-1} at days 21 to 23 of age. The numbers of oocysts per bird in the lasalocid group were always lower compared to all other challenged groups (Tab. IV).

4. DISCUSSION

Infection with *E. tenella* significantly reduced body weight gain and feed intake, and increased feed conversion ratio values highlighting the detrimental effect of the infection with this parasite on broiler performance. The Apacox treatment exerted a beneficial effect by significantly improving body weight gain and feed conversion ratio values compared to the challenged control group. However, there were no significant effects on mortality and caecal lesion score. The numbers of excreted oocysts in the Apacox groups were significantly lower than in the challenged control group from day 21 to day 26 of age.

Treatment with Apacox at 1.0 g·kg^{-1} significantly reduced the amount of blood observed in the faeces compared to both the Apacox at 0.5 g·kg^{-1} and the challenged control groups, whereas it presented a comparable caecal lesion score with the lasalocid group. It also had a significantly lower number of oocyst output compared to Apacox at 0.5 g·kg^{-1} at days 21 to 23 of age. Based on these results, one could speculate that there might be a dose response trend for Apacox to reduce the impact of infection to chickens by exerting a coccidiostatic effect against *E. tenella.* However, there were no significant differences between the Apacox levels for body weight gain, feed intake, feed conversion ratio values, lesion score and mortality. Additional experimentation with the administration of Apacox to unchallenged chickens might give an answer to whether the increase in body weight and the corresponding effect on feed conversion ratio was due to an appetite enhancing effect or an anticoccidial effect, since the appetite enhancing effect of herbal extracts is well known and documented. However, such a treatment was not included in the experimental protocol.

Some herbal extracts have already been shown to possess a coccidiostatic activity [1, 20]. Extracts and essential oils from aromatic plants are of interest for coccidiosis because several studies have shown substantial antimicrobial and antioxidative activity [3, 4, 7, 12, 15, 19]. This biological activity has been mainly attributed to phenolic components. In vivo and in vitro tests have shown [18] that phenols can be specifically used as oocysticides against *E. tenella*. The antimicrobial effects of phenols, known for more than a century, are targeted against the bacterial cell wall affecting the cell wall structure. Phenols interact with the cytoplasmic membrane by changing its permeability for cations, like H+ and K+ [14]. The dissipation of ion gradients leads to the 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 [17].

Herbal extracts may easily become popular, because they are not synthetic products. However, they have to be extensively investigated in terms of their mechanisms of action, efficacious level of administration and clinical effects. Halofuginone, for example, is derived from an extract of the *Dichroa febrifuga*. The original extract is known for antimalarial and coccidiostatic activity but for long was not marketed because of a very narrow safety margin at the dose of 3 ppm [20]. Issues of safety, toxicity and side effects for medicinal herbs should be standardised and their extraction should also be properly controlled and manufactured before wide use in animal diets [6].

5. CONCLUSION

The results of the present study suggest that treatment with Apacox could alleviate the impact of parasite infection on broiler chickens by exerting a coccidiostatic effect against *E. tenella,* which, however, was significantly lower than that exhibited by lasalocid. Further research is needed to examine the composition of the examined mixture of herbal extracts, to identify the active components and to elucidate their mechanism of action.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to the Animal Feed Company, Triantafillou Bross, SA, Thessaloniki, Greece, for supporting the present research by kindly donating the broiler feeds.

REFERENCES

[1] Allen P.C., Lydon J., Danforth H., Effects of components of *Artemisia annua* on coccidia infections in chickens, Poult. Sci. 76 (1997) 1156–1163.

[2] AOAC, Official Methods of Analysis of the Association of Official Analytical Chemists, in: Herlich K. (Ed.), A.O.A.C. International, Arlington, USA, 1990.

[3] Botsoglou N., Florou-Paneri P., Christaki E., Fletouris D.J., Spais A.B., Effect of dietary Oregano essential oil on performance of chickens and iron-induced lipid oxidation of breast, thigh and abdominal fat tissues, Brit. Poult. Sci. 42 (2002) 223–230.

[4] Botsoglou N.A., Christaki E., Fletouris D.J., Florou-Paneri P., Spais A.B., The effect of dietary oregano essential oil on lipid oxidation in raw and cooked chicken during refrigerated storage, Meat Sci. 62 (2002) 259–265.

[5] Chapman H.D., Isolates of *Eimeria tenella*: studies on resistance to ionophorous anticoccidial drugs, Res. Vet. Sci. 41 (1986) 281–282.

[6] Chang J., Medicinal herbs: drugs or dietary supplements? Biochem. Pharmacol. 59 (2000) 211–219.

[7] Economou K.D., Oreopoulou V., Thomopoulos C.D., Antioxidant properties of some plant extracts of the *Labiatae* family, J. Am. Oil Chem. Soc. 68 (1991) 109–113.

[8] European Commission Regulations (EC) No 97/6 of 30 January 1997 amending Council Directive 70/524/EC concerning additives in feedingstuffs, OJ L 96, 28/3/1998, p. 39.

[9] Giannenas A.I., Florou-Paneri P., Papazahariadou M., Christaki E., Botsoglou N.A., Spais A.B., Dietary oregano essential oil supplementation on performance of broilers challenged with *Eimeria tenella*, Arch. Anim. Nutr. 57 (2003) 99–106.

[10] Hodgson J.N., Coccidiosis: oocyst-counting technique for coccidiostat evaluation, Exp. Parasitol. 28 (1970) 99–102.

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

[12] Lambert R.J.W., Skandamis P.N., Coote P.J., Nychas G.-J.E., A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol, J. Appl. Microbiol. 91 (2001) 453–462.

[13] Mc Dougald L.R., Intestinal protozoa important to poultry, Poult. Sci. 77 (1998) 1156–1158.

[14] Sikkema J., de Bont J.A.M., Poolman B., Mechanisms of membrane toxicity of hydrocarbons, Microbiol. Rev. 59 (1995) 201–222.

[15] Sivropoulou A., Papanikolaou E., Nikolaou C., Kokkini S., Lanaras T., Arsenakis M., Antimicrobial and cytotoxic activities of Origanum Essential oils, J. Agric. Food Chem. 44 (1996) 1202–1205.

[16] Steel R.G.D., Torrie J.H., Principles and procedures of Statistics. A Biometrical Approach, 2nd ed., McGraw-Hill Book Co, New York, 1980.

[17] Ultee A., Kets E.P.W., Smid E.J., Mechanisms of action of carvacrol on the food borne pathogen Bacillus cereus, Appl. Environ. Microbiol. 65 (1999) 4606–4610.

[18] Williams R.B., Laboratory tests of phenolic disinfectants as oocysticides against the chicken coccidium *Eimeria tenella*, Vet. Rec. 141 (1997) 447–448.

[19] Yanishlieva N.V., Marinova E.M., Gordon M.H., Raneva V.G., Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems, Food Chem. 64 (1999) 59–66.

[20] Youn H.J., Noh J.W., Screening of the anticoccidial effects of herb extracts against *Eimeria tenella*, Vet. Parasitol. 96 (2001) 257–263.