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Effects of herbal plants on ducks and quail infected with *Eimeria* species

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Abstract
The anticoccidial effects of herbal plants containing essential oils *Artemisia absinthium*, *Salvia officinalis*, *Mentha piperita*, *Matricaria chamomilla* and *Thymus serpyllum* on *E. bateri* and *E. schachdagica* were evaluated in quails (*Coturnix Coturnix*) and ducks (*Anas platyrhynchos domesticus*) after oral infection (15,000 sporulated oocysts *E. bateri* and *E. schachdagica* per birds). This study was performed using 15-day-old birds (n=120). The animals were divided into 6 groups (n =20): G1 is the untreated/infected control, G 2 is a group infected and fed *S. officinalis* (2500 mg/kg), group 3 was fed *A. absinthium* (1500 mg/kg), G4 was fed *M. piperita* (2500 mg/kg), G5 was fed *M. chamomilla* (2500 mg/kg), G6 was fed *T. serpyllum* (2500 mg/kg). The birds were fed with or without herbal plants for 1 week before and after infection. The effects of herbal plants on *Eimeria* infection of birds have been assessed by number of fecal oocysts at 4th to 14 days of infection. The *T. serpyllum* -fed ducks and *M. chamomilla* –fed quails produced a lower number of fecal oocysts than the birds who were fed the standard diet.

Keywords: *E. bateri, E. schachdagica*, ducks, quail, oocyst shedding

1. Introduction
The problem of food security has always been the center of attention of the government. For providing food security are designed and adopted the special programs in the world. Safe food supply is essential to the country's economic stability and social sustainability. Coccidiosis is recognized as the costliest parasitic disease for the poultry industry worldwide, with estimated total annual costs reaching $800 million, when the costs of prophylaxis, medications and losses of productivity due to mortality, morbidity, and lowered feed conversion are taken into account. Estimates of the global cost of its prevention in chicken alone stand at $300 million a year. Control of coccidiosis has been focused on prophylaxis with anticoccidial drugs in food (http://www.wattagnet.com/). Their extensive use has inevitably led to the development of drug resistance, and as a consequence, alternative control strategies against avian coccidiosis were studied. The new approaches include the use of natural products, probiotics, live vaccines, improved farm management practices, and modulation of the chicken immune system [9, 10]. Safe alternative anticoccidial drug to chemical feed additives are herbal extracts, because they don't results to tissue residue and drug resistance. At present the most effective way to protect farmed birds against coccidiosis is supplement anticoccidial drugs in the feed birds [1, 2, 3]. For the growth of the birds is recommended the use of feed containing plant extracts [4, 5, 6] essential oils and probiotics [7, 8] as an alternative feed additives do not harm human health.
The purpose of this study was to reveal the anticoccidial effects of the plants on ducks and quail infected with *Eimeria* species.

2. Materials and Methods
A total of 120, the 15 day old quails (*Coturnix coturnix*) and ducks (*Anas platyrhynchos domesticus*) were infected orally with 15,000 sporulated oocysts *Eimeria* (*E. bateri* - quai and *E. schachdagica* - ducks per birds). The herbal plants containing essential oils - *Artemisia absinthium*, *Salvia officinalis*, *Mentha piperita*, *Matricaria chamomilla* and *Thymus serpyllum* The animals were divided into 6 groups (n =20): G1 is the untreated/infected control, G 2 is a group infected and fed *S. officinalis* (2500 mg/kg), group 3 was fed A. *absinthium* (1500 mg/kg), G4 was fed *M. piperita* (2500 mg/kg), G5 was fed *M. chamomilla* (2500 mg/kg), G6 was fed *T. serpyllum* (2500 mg/kg).

The birds were fed with or without herbal plants for 1 week before and after infection.

The plants containing essential oils - *Artemisia absinthium*, *Salvia officinalis*, *Mentha piperita*, *Matricaria chamomilla* and *Thymus serpyllum* in the form of a dry powder was used as an additive in feed for birds.

A pure culture of *Eimeria* oocysts for infected birds was obtained from the Laboratory of biochemical aspects of host-parasite relationships of the Institute of Zoology (ANAS) (May-June 2016). The oocyst count/gm of dropping was performed at days 4th to 14 day post infection. The fresh feces (2 g) were mixed with 10 ml of saturated sodium chloride solution and were centrifuged (3000 rev/min). Then the wire loop with the surface of suspension took 0, 01 ml and observed under the x10 microscope objective (Axio Scope A1). To obtain statistically significant data was taken from each sample 5 times and counted the amount oocysts in 2 g of sample. The oocyst output was measured daily in each group. The means of OPG (oocysts per gram of faeces) output in treated groups were compared with a control group to evaluate the effects of the plant extract on avian coccidiosis induced by oocysts *E. bateri* and *E. schachdagica.* To determine the effect of plants on the reproductive parasites the oocysts isolated from birds were counted under a microscope.

3. Results and Discussions

Coccidiosis of domestic fowl is a worldwide disease caused by obligate intracellular protozoan of the genus *Eimeria*. Coccidiosis results in economic losses in poultry production [2]. This disease is characterized by enteric lesions of variable extent and severity, which reduce the absorptive function of the intestinal mucosa and lead to weight loss, diarrhea, poorer feed conversion, and a higher mortality in the affected flocks [1]. Other effective plant extracts have also been reported, although their modes of action have not been elucidated, but could consist of a combination of various pathways.

Mohammed Abdul-Aziz Kadir *et al*. [3] reported that the effect of plant extracts (*Achillea fragmentisma, Artemisea herba-alba, Cardaria draba, Mentha longifolia, Olea europea, Prosopis farcta, Punica granatum, Teucrium polium, and Ziziphus spina-christi*) on the shedding of *Crypto Oocysts* in mice.

Allen *et al*. reported a significant anticoccidial effect of *A. annua* against *E. tenella,* measured as reduced lesion scores, when fed to broiler chickens for three weeks as dried leaves at a dietary concentration of 5% [4].

From the 4th day of infection daily feces from all groups of birds were collected and calculated (Tab.1).

Table 1: Dynamics *of* shedding *E.schachdagica* oocysts (dose 15000) by ducks (Anas platyrhynchos domesticus) treated and untreated (control) (*OPG*)

Days of invasion	kontrol	A.absinthium 1500mq/kq	M.piperita 2500mq/kq	S.officinalis 2500 mq/kq	M.chamomilla 2500 mq/kq	T.serpyllum 2500mq/kq
	Group 1	Group II	Group III	Group IV	Group V	Group VI
4	4593201	4283018	4395102	4308222	4293301	4284221
5	1271801	1112580	1242610	1221521	1184415	1175632
6	1206001	1006231	1187223	1215672	1178010	1153326
7	3372003	3258065	3332105	1203459	1153875	1163498
8	1220080	889161	1285731	3202231	875220	472880
9	985011	120591	899751	889145	3171181	866950
10	58783	48880	52132	47890	475996	3178018
11	21600	19786	20836	19833	19028	18824
12	5128	3993	4466	3784	3699	3689
13	856	553	633	550	553	550
14	28	23	26	23	22	20

Oocyst outputs of the treated groups were significantly lower than that of the control group in the following days. According to our results, *T. serpyllum* as powder can be used with good results to prevent coccidiosis in quails so *T. serpyllum* also has an inhibitory effect directly on the *E. bateri* oocyst excretion**:** the number of oocysts on 12 day was 8187 from comparatively with control group - 8996 (Tab.2).

Table 2: Dynamics of shedding *E.bateri* oocysts (dose 15000) by quails (Coturnix coturnix) treated and untreated (control) (*OPG*)

Days of invasion	control	A.absinthium 1500mq/kq	M.piperita 2500mq/kq	S.officinalis 2500mq/kq	M.chamomilla 2500mq/kq	T.serpyllum2500 mq/kq
	Qrup I	Qrup II	Qrup III	Qrup IV	Qrup V	Qrup VI
4	5594320	4563917	5295282	4508312	4392311	4403129
5	1374898	1208915	1343612	1216330	1932111	1178042
6	1366899	1122640	1327110	1122581	1734251	1078102
7	3298895	1101340	3210365	1005022	1055720	1023012
8	1291780	980210	1325430	3107706	978365	1018002
9	929022	920258	928643	921223	917425	3102761
10	99445	3135041	99168	97921	544851	97432
11	48566	45247	48441	45398	3112046	44231
12	8996	8369	8631	8299	8265	8187
13	1000	821	900	814	795	812
14	32	12	25	15	11	14

Thus, the plants adverse effects on development of endogenous stages of the parasite, wherein in different groups of birds invasion peaks observed on different days of treatment. At the 14 day post infection was marginal reduction of oocyst, and at day 15 post infection oocyst shedding had ceased. In birds infected with 15000 *E.*

schachdagica and *E. bateri* oocysts and treated with the plants exhibited significantly reduced oocyst shedding. In group of birds treated with *T. serpyllum* was the best result when compared to the groups treated with *Artemisia absinthium*, *Salvia officinalis*, *Mentha piperita* and *Matricaria chamomilla*.

It was observed the dynamic of oocysts shedding of *E. schachdagica* and *E. bateri*. The study results show that by the 4th day of infection the birds from all group shedding oocysts *E. schachdagica* in the faeces. In the control group the number of secreted oocysts was - 4593201, in group were treated with *A absinthium* - 4283018, the birds of third group– *M. piperita* – 4395102, in 4th - 4308222, 5th - 4293301, 6th- 4284221 oocysts accordingly.

The largest number of oocysts inoculated by birds from the control group and the group was treated with *M. piperita*- was on the 7th day, in group were treated with *A. absinthium*- on the 7th day, with *S. officinalis* – 8th day, with *M. chamomilla*- on 9 th day, with *T. serpyllum* – on 10th day. Thus, plants such as *T. serpyllum*, *M. chamomilla* and *A. absinthium* significantly reduced *E. schachdagica* oocysts shedding. A large oocyst peak in group was treated with *T. serpyllum*, the numbers of oocysts excreted at 10th day were 3178018 oocysts and with *A. absinthium* -3258065 (in control group - 58783 oocysts) (tab. 1).

Table 2 shows oocysts excretions of infected and untreated (control) quails at 4th day invasion. On day 4 the oocyst output obtained from the control group (infected and untreated) was 5594320, in group treated with *A. absinthium*– 4563917, with *M. piperita*- 5295282, *S. officinalis* - 4508312, *M. chamomilla* - 4392311, *T. serpyllum*- 4403129. The number of oocysts from control group reached a peak on Day 7, with *A. absinthium*- on Day 10, *M. piperita*- on Day 7, *S. officinalis* - *S. officinalis* -8, *and M. chamomilla* - 11, *T.serpyllum*-9.

Herbal anticoccidials open new perspectives and proved suitable to serve as alternatives to conventional treatment, particularly in countries with limited economic potential [5]. Essential oils contain known active ingredients, including phenols, aldehydes, terpenes, and oxides, which have a direct anti-parasitic effect. One of their key modes of action is targeted against microbial membranes and cell walls, which are then disrupted. It has also been shown that essential oils could be effective in reducing mortality and fecal oocyst count following multi species *Eimeria* challenge.

The current results are found that birds fed with *T. serpyllum*, *M. chamomilla* and *A. absinthium* enhanced coccidiosis resistance as demonstrated by reduced fecal oocysts shedding compared with infected birds of control group.

4. Findings

1. It has been revealed that *T. serpyllum* significantly reduced *E. schachdagica* oocyst shedding in domestic ducks (*Anas platyrhynchos domesticus*) and *M. chamomilla* reduced *E. bateri* oocyst shedding in quails (*C. coturnix*).
2. It has been found that plants to have the anticoccidial effects agains *Eimeria* infections by reducing oocyst excretion and increasing the immune system of birds.

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