Efficacy Of *Artemisia Annua* Against *Eimeria* Spp. Infection In Broiler Chickens In A Battery Trial

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Abstract.

The goal of this study was to evaluate the efficacy of A. annua as essential oil in preventing Eimeria infection in broilers. Sixty broilers of fourteen days were randomly divided in 3 groups of 20 chickens as follows: negative control (NC) (uninfected, unmedicated), positive control (PC) (infected, unmedicated) and experimental group (EG) (infected, medicated). Experimental infection was done with $1x10^4$ oocysts of Eimeria spp. and treatment with 0.15 ml essential oil of A. annua/l water for 27 days. The chickens were kept in batteries, fed and watered ad libitum, and were exposed to continuous light. Essential oil of A. annua was obtained from a Romanian variety through a hydro-distillation process of plants vegetative parts, blooms and flowers. Efficacy of A. annua against Eimeria spp. infection was evaluated by: (i) oocysts shedded per gram of feces; (ii) lesional score; (iii) body weight gain; (iv) feed conversion ratio; and (v) mortality rate.

The inoculum used for experimental infection consisted in E. tenella 35.0%, E. acervulina 32.5%, E. mitis 25.0%, and E. maxima 7.5%. All chicks survived in NC and EG, in PC 2 chickens died , at 7 and 10 days after challenge. Chickens treated with essential oil of A.annua produced with 97.1% less oocysts than chickens from PC, while the mean lesion score was lower only with 35.3% (EG = 0.86; PC = 1.33). Following the intestinal segment the highest reduction of lesion score in EG was registered in duodenum (1 and 2 in PC) and caeca (0.4, and 1 in PC), while in the jejunum was higher than in PC (1.2, and 1 in PC). Body weight gain and feed conversion ratio were higher in chickens medicated with A. annua and in chickens from NC at 7 days post-infection, but at 27 days post-infection chickens from PC presented a higher body weight gain and a lower feed conversion ratio.

According to our results, A. annua Romanian variety as essential oil can be used with good results to treat coccidiosis mainly caused by E. tenella and E. acervulina in chickens, and not for prevention. Further analysis must be done.

Keywords: coccidiosis, *Eimeria*, poultry, *Artemisia*.

Introduction

Coccidiosis is one of the most important diseases of the poultry industry, caused by intracellular protozoan parasites of the genus *Eimeria.* It produces severe economic losses worldwide due to expensive and ineficient control measures leading to increased mortality, growth depression and poor feed conversion in broiler chickens. Combined, this losses exceed 3 billion US$ annually in the entire world (Dalloul and Lillehoj, 2006).

There are seven recognized species of *Eimeria* that affect poultry: *E. acervulina, E. maxima, E. tenella, E. necatrix, E. praecox, E. mitis* and *E. brunetti* (Williams et al., 2009). In broiler chickens, *E. acervulina, E. maxima,* and *E. tenella* are found frequently (Ogedengbe et al., 2011).

Control of coccidiosis is based on the use of in-feed anticoccidial drugs and rarely of live vaccines (Peek and Landman, 2011). The extensive use of prophylactic anticoccidial drugs has led to the development of drug resistant strains of *Eimeria* against all products introduced (Chapman, 1997). Live vaccines can restore drug sensitivity and proved to be

efficient in controlling the disease, but they are expensive and have adverse effects on early chick growth (Williams, 2002).

Therefore, there is an increasing need in discovering new alternatives for coccidiosis control. In the last decade, many different natural products such as mushrooms, and plant extracts have been tested for anticoccidial activity (Tewari and Maharana, 2011). Amongst them, *Artemisia annua*, a traditional Chinese medicine used for malaria treatment, was found to be effective against *E. tenella* and *E. acervulina* (Allen et. al, 1997). It is believed that acts by inducing oxidative stress (Allen et al., 1998). There are studies showing that artemisinin, a sesquiterpene lactone from *A. annua*, can significant decrease the number of oocysts shedded by chickens infected with *Eimeria* spp. (Arab et al., 2006; Naidoo et al., 2008).

In this study, we investigated the anticoccidial effects of *A. annua* as essential oil in broilers kept in batteries.

Materials and methods

The research has been conducted during 14th May – 12th June 2007 in the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca – Faculty of Veterinary Medicine, Department of Parasitology and Parasitic Diseases.

We aimed to assses the efficacy of *A. annua* as essential oil (0.15 ml/l of water) in experimental infection with 1x10^4 *Eimeria* spp. sporulated oocysts, in broiler chickens kept in batteries.

Animals. One day old chickens (hybrid Ross 308) were purchased from S.C. Oprea Avicom S.R.L. hatchery (Venchi-Sighişoara, Mureş county), and housed in animal facilities until the experimental infection to avoid spontaneous infection with *Eimeria* spp. Chickens were fed with starter feed without anticoccidial feed aditives until the date of the experiment. Feed and water were provided *ad libitum*, and the lighting was continuous.

***Artemisia* product.** The essential oil of *A. annua* was obtained from a Romanian variety, from the green herb right after harvest, through hydro-distillation process using an artisanal machinery. The herb was cultivated in 2006, started from seeds in middle March, planted in early June, and harvested in early October, when plants were in late blooming - beginning of flowering stage.

Parasites. We used for experimental infection a suspension of mixed *Eimeria* species. The strains were obtained from died chicks in intensive broiler farms by scraping the intestinal content. Oocysts were propagated, isolated and sporulated in 2.5% potassium dichromate using standard procedures (Raether et al., 1995). The species were identified based on morphology of sporulated oocysts using microphotographs. We took microphotographs at Olympus BX61 microscope using 400x magnification with Olympus ODP72 camera, and then we sized, and analyzed 50 oocysts with CELL-F software.

Experimental design. At 13-days-old, chicks were subsequently randomly divided into 3 groups of 20 birds each as follows: negative control group (NC) - uninfected, unmedicated; positive control group (PC) – infected, unmedicated; experimental group (EG) – infected and medicated with essential oil of *A. annua* in water.

At 14-days-old chicks from PC and EG groups were infected with 1x10^4 *Eimeria* spp. sporulated oocysts in a volume of 1 mL, through gavage into the crop. The treatment in EG group started in the same day with the experimental infection.

The essential oil of *A. annua* was administered 0.15 ml/l water during the entire period of experiment, until 27 days after challenge. The essential oil was solubilised in water, by adding Tween 70, 0.75 ml/10 l of water.

Analytical procedures. Efficacy of *A. annua* essential oil was evaluated by recording the following: (i) clinical signs; (ii) mortality percentage; (iii) number of oocysts

shedded/g of faeces (OPG) and the reduction percentage of OPG compared to the positive control; (iv) lesion score and the reduction percentage of the lesion score compared to the positive control; (vi) body weight gain, and (vii) feed conversion ratio (Holdsworth et al., 2004).

We used for oocysts counting McMaster method, and sodium chloride (sp.gr. 1.20) solution as flotation solution. Fecal samples were collected daily from 5 to 18 days post-challenge from each group. Lesion score was evaluated at 7 (5 chicks/group) and 27 days post-challenge using a score of 0–4 (Johnson and Reid, 1970). Body weight gain was assessed at 7 and 27 days pi, and the feed conversion ratio at the end of the experiment.

Statistical analysis. In order to identify the statistically significant differences, the data obtained were processed by student test (T) for comparison of the averages of two equal populations (Drugan et al., 2003), in Excel 2003. This statistical processing was applied for OPG, and lesion score. The normal distribution of data was verified before statistical processing (Drugan et al., 2003).

Results

Eimeria species

The oocysts suspension used for experimental infection contained 4 species based on oocysts morphology (Fig. 1): *E. tenella* (35%), *E. acervulina* (32,5%), *E. mitis* (25%), and *E. maxima* (7,5%) (Fig. 2). As we used 10^4 oocysts/chicken, one dose had 3500 oocysts of *E. tenella* , 3250 *E. acervulina*, 2500 *E. mitis*, and 750 *E. maxima* respectively.

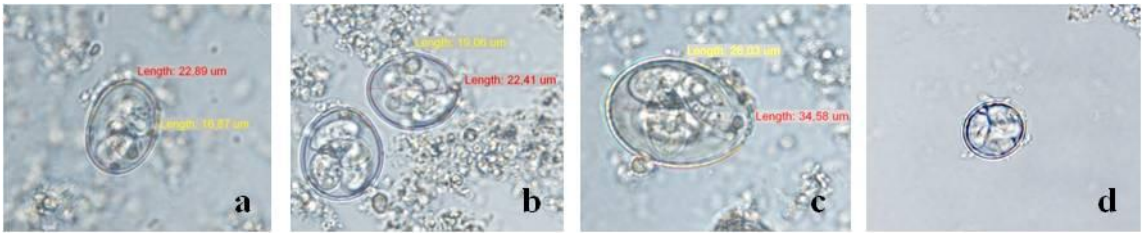


Fig. 1: Species of *Eimeria* in the inoculum based on morphology of the sporulated oocysts. *E. acervulina* (a), *E. tenella* (b), *E. maxima* (c) and *E. mitis* (d).

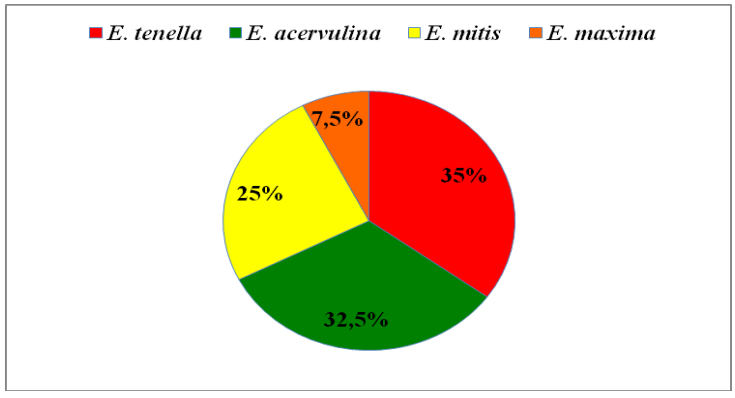


Fig. 2: The structure of eimerian population used for experimental infection

Clinical signs and mortality percentage

After experimental infection with 10.000 oocysts of *Eimeria* spp., clinical changes were visible in positive control group, at 3 days after challenge. These were represented by

decreased appetite, polydipsia, and sleepiness. These clinical changes diminished and then dissapeared at 10 days after challenge. In the group treated with *Artemisia*, especially in the first part of the experiment, an increase in the daily feed consumption and a more pronounced state of liveliness of the chickens was noted.

The mortality rate was 0 in negative control, and experimental groups. In positive control group 2 deaths were recorded, the mortality rate being 10%. The first mortality case was recorded 7 days after challenge, and the second 10 days respectively. At necropsy were recorded typical lesions of infection with *E. acervulina* in the duodenum (lesion score 1, and 2), (Fig. 3) and hemorrhagic typhlitis (lesion score 3, and 4) (Fig. 3).

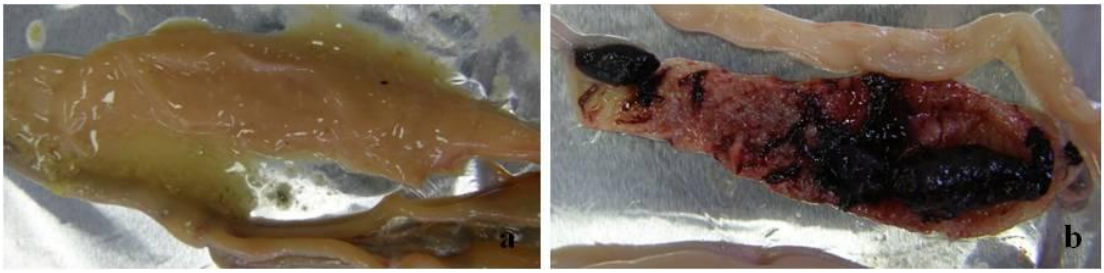


Fig. 3: Characteristic lesions in the duodenum and caecum in chickens from positive control group died 7-10 days postinfection. (a) White streaks oriented transversely across the intestine , lesion score 2. (b) Hemorrhagic typhlitis, lesion score 4.

OPG and lesion score

The dynamics of oocysts shedded per groups and days, beginning with day 5 after challenge is presented in fig. 4. The highest oocysts number was recorded in positive control group, with an average of 71980 oocysts/g of faeces. The chickens from the group treated with essential oil of *A. annua* have shedded an approximately equal number of oocysts (an average of 2079 ooccysts/g of faeces) to the one of the chickens from negative control group (an average of 2757 oocysts/g of faeces). In dynamics, the differences were observed in 5-9 days afer challenge, after that, in all 3 experimental groups, OPG was situated at the same level.

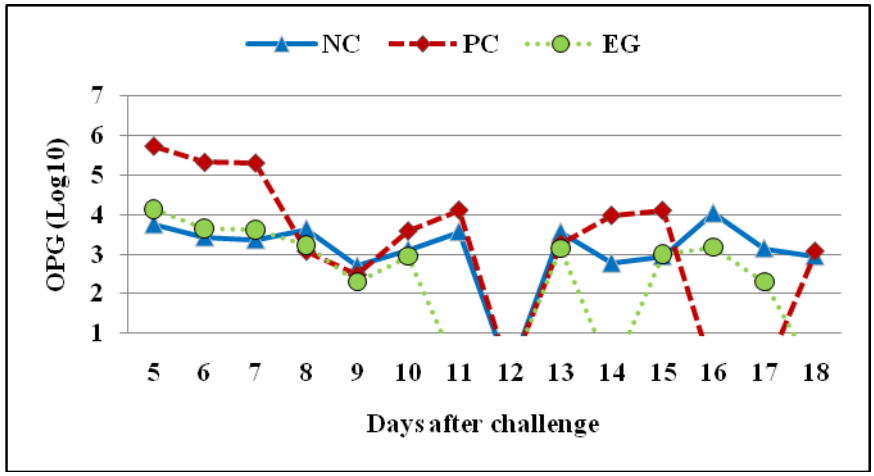


Fig. 4: Dynamics of OPG in experimental groups. NC = negative control; PC = positive control; EG = group treated with A. annua essential oil 0,15 ml/l of water.

The lesion score at 7 days after challenge was higher in positive control group (1.33), while no significant differences have been recorded between the 3 experimental groups at 27 days after challenge (Tab. 1). The lesion score in the duodenum and caecum was generally higher in positive control group, whilst in the jejunum it was higher for EG group (Fig. 5).

Tab. 1

Average of lesion score, body weight gain and feed conversion in experimental groups following treatment with *A. annua* and *Eimeria* infection

Experimental group	Lesion score		Weight gain (g/day)		Feed conversion (kg feed/kg spore)
	7 days a.c.	27 days a.c.	7 days a.c.	27 days a.c.	
NC	0,13	1,06	41,97	41,75	2,16
PC	1,33	0,9	31,42	50,87	2,19
EG	0,86	0,8	41,45	41,57	2,31

Legend: NC (negative control) – uninfected and unmedicated; PC (positive control) – infected and unmedicated; EG – infected and medicated with *A. annua* essential oil 0,15 ml/l of water

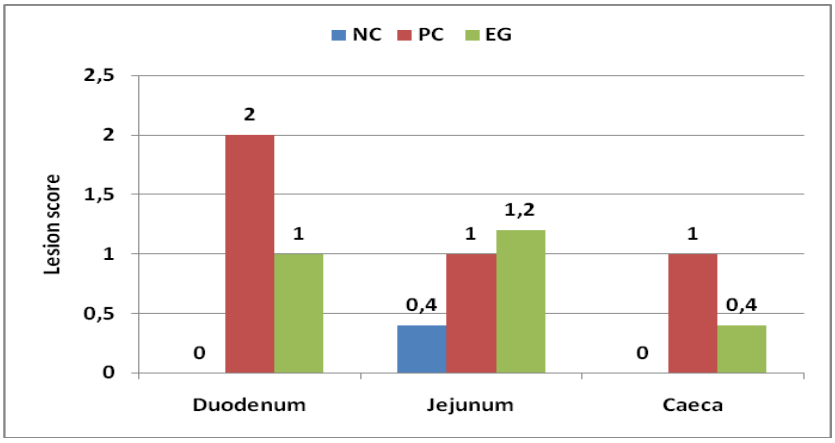


Fig. 5: Lesion score in experimental groups at 7 days after challenge in different parts of gut. NC - negative control; PC - positive control; EG - group treated with *A. annua* essential oil 0,15 ml/l of water.

Body weight gain and feed conversion ratio

The body weight gain was different in the 2 evaluation periods (Tab. 1), being higher in NC group (41,97 g/day) and EG (41,45 g/day) at 7 days after challenge (PC = 31,42 g/day), whilst at 27 days after challenge a recovery in PC group (50.87 g/day) from the NC group (41,75 g/day) and EG (41,57 g/day) was observed. Also, the feed consumption (Tab. 1) was lower in positive control group (2,19 kg feed/kg spore) as against group treated with *A. annua* under the form of essential oil 3.75% in water (2,31 kg feed/kg spore).

Discussions

Coccidiosis remains one of the most costly diseases in poultry. Due to emergence of chimioresistant strains of *Eimeria*, eforts were directed towards finding inexpensive and efficient alternative treatments.

In this study we aimed to evaluate the prophylactic and therapeutic efficacy of *A. Annua* as essential oil in broiler coccidiosis. The results showed decreased OPG output, lesion score and mortality rate and higher body weight gain in experimental group, revealing

the fact that chicken coccidiosis caused by *E. tenella* and *E. acervulina* can be treated with essential oil of *A. annua.*

A. annua is a traditional Chinese medicine used for malaria treatment. It is believed that the anticoccidial activity of this plant is based on artemisinin, it's active substance, which could be lethal to parasites by inducing oxidative stress (Allen and Fetterer, 2002). *A. annua* also contains flavonoids, which have high antioxidant activity, and may enhance the effect of artemisinin, or have anticoccidial effects on their own (Ferreira et. al., 2010)

Oh et al. (1995) are the first to report that *A. annua* has an anticoccidial effect by improving body weight gain, feed conversion rate and lesion score in broilers challenged with *E. tenella.* Two years later, Allen et al. (1997) demonstrated that dried leaf supplements of *A. annua* have a positive effect on lesion score in chickens infected with *E. tenella.* However, they found no protective effect against *E. acervulina* and *E. maxima*. According to lesion score in the jejunum, neither in our study, *A. annua* had no effect against *E. maxima.*

In our previous study (Drăgan et al., 2010), chickens treated with *A. annua* as oil and powder produced significantly reduced oocysts output and lesion score when compared to the *E. tenella*-infected group fed standard diet. The group treated with *A. annua* as powder had the highest body weight gain and the best feed conversion among the experimental groups.

Treatment with artemisinin alters the process of oocysts wall formation, as demonstrated by del Cacho et al. (2010). The consequence of this was death of developing oocysts and reduced sporulation rate. Through immunofluorescent studies the authors showed that artemisinin reduced sarcoplasmic–endoplasmic reticulum calcium ATPase (SERCA) expression in macrogametes. SERCA plays a role in calcium homeostasis so treatment with artemisinin may affect the secretion of wall-forming bodies, which is a calcium-dependent mechanism. This leads to abnormal oocyst wall formation and death of the developing oocysts (del Cacho et al., 2010).

Almeida et al. (2012a) found that during infection with *Eimeria spp.* in free-range broilers supplemented with *A. annua* dried leaves, the number of excreted oocysts significantly reduced, but no significant difference was noted in feed consumption between groups. In the present study, the feed consumption was lower in positive control group than the group treated with *A. annua*.

In a study made by Almeida et al. (2012b), the supplementation of *A. annua* in the diet (3% inclusion based on feed weight) influenced negatively the growth rates of the pullets and did not affect OPG. Ethanolic extract of *A. vulgaris* supplemented before appearance of clinical symptoms of disease showed a trend to reduce oocysts excretion in the late infection. The authors believed that the failure of suppressing oocysts excretion was due to the fact that pullets were challenged before a reasonable period of plant consumption.

Kaboutari et al. (2013) showed that a granulated formulation of *Artemisia* extract significantly reduced mortality, diarrhea, OPG output and lesion score in the treated groups, but no significant difference between the prophylactic and therapeutic groups was noticed.

As proven by several studies, *A. annua* is efficient against *Eimeria* in chickens, but further studies need to be done to establish the mechanism of action and the plant compound that has the best effect.

Conclusions

According to our results, *A. annua* Romanian variety as essential oil can be used with good results to treat coccidiosis mainly caused by *E. tenella* and *E. acervulina* in chickens, and not for prevention. Essential oil of *A. annua* Romanian variety had no effect against *E. maxima,* according to lesion score in the jejunum. Further analysis must be done.

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