Effects of *Artemisia annua* and *Pimpinella anisum* on *Eimeria tenella* (Phylum Apicomplexa) low infection in chickens

Liviu Drăgan¹, Adriana Titilincu²*, Iosif Dan³, Ioan Dunca⁴, Maria Drăgan¹, Viorica Mircean²

1 – Veterinary and Food Safety Unit Mureș, Animal Health Service, 10 Podeni Street, 540253 Târgu Mureș, Romania.
2 – University of Agricultural Science and Veterinary Medicine, Faculty of Veterinary Medicine, Parasitology and Parasitic Diseases Department, 3-5 Calea Mănăștur, 400372 Cluj-Napoca, Romania.
4 – Agency of Reproduction in Animal Breeding Mureș, 76 Budiului Street, 540390 Târgu Mureș, Romania.

*Correspondence:* Tel. +40.264.596384 int. 165, Fax +40.264.593792, E-mail titilincua@yahoo.com

**Abstract.** The anticoccidial effect of *Artemisia annua* and *Pimpinella anisum* on *Eimeria tenella* infection (1500 oocysts/chicken) has been studied. The products were used as oil administered 7.5% in water and as powder 1.5% in food. The effects of *A. annua* and *P. anisum* on *E. tenella* infection were assessed by clinical signs, mortality, fecal oocyst shedding, lesional score, body weight gain and feed conversion. Chickens treated with *A. annua* produced significantly reduced fecal oocysts (90.76%) (*p*<0.009-0.002) and lesion score (0.8) when compared to the *E. tenella*-infected group fed standard diet. In chickens treated with *P. anisum* a reduction in fecal oocyst output of 58.83% was noticed and the lesional score was 1.2. The highest body weight gain at 7 days after challenge was registered in chickens treated with *P. anisum* and *A. annua* as oils. At the end of experiment (32 days post infection) chickens treated with *A. annua* as powder had the highest body weight gain and the best feed conversion among the experimental groups.

**Keywords:** *Eimeria tenella*; *Artemisia annua*; *Pimpinella anisum*; Anticoccidial effect.

Received 02/03/2010. Accepted 31/05/2010.

**Introduction**

Avian coccidiosis is one of the most economically important diseases of the poultry industry caused by intestinal infection with several species of *Eimeria*, with *E. tenella* being one of the most pathogenic species (McDougald and Reid, 1991). Infection with *E. tenella* is followed by cecal lesions (petechiae, thickening, ecchymoses, accumulation of blood and caseous necrotic material in the cecum) and bloody diarrhea.

The effective use of anticoccidial feed additives over the past 60 years has played a major role in the growth of the poultry industry. Their extensive use has inevitably led to the development of drug resistant *Eimeria* strains (Chapman, 1997), and they are responsible for subclinical coccidiosis and economic losses due
to poor weight gain and high food consumption. It is estimated that the annual loss worldwide is more than 3 billion US$ (Dalloul and Lillehoj, 2006). Consequently, there is increasing interest in the development of alternative strategies of disease prevention (Dalloul and Lillehoj, 2006). As part of this effort, there are studies on the effect of natural products on *Eimeria* infections and on protective immunity against these infections (Dalloul et al., 2006; Hassan et al., 2008; Lee et al., 2008).

*Artemisia annua* is a plant whose dried leaves have been used in traditional Chinese medicine for over 2 millennia (Klayman, 1985). Nowadays, extensive studies have shown that artemisinin, an extract from *A. annua*, exhibits high efficacy against several stages of *Plasmodium* (Golenser et al., 2006). Also, multiple studies have demonstrated the anticoccidial effects of *A. annua* in experiments performed with chickens infected with several species of *Eimeria* (Allen et al., 1997; 1998; Youn and Noh, 2001; Arab et al., 2006; Naidoo et al., 2008).

*Pimpinella anisum* has been long used as a flavor herb. It has active ingredients represented by volatile oil (1-4%), of which 72-90% is trans-anethole. Anethole has potent antimicrobial properties against bacteria, yeast, and fungi (De Vincenzi et al., 2002). *In vitro*, anethole has anthelmintic action on eggs and larvae of the sheep gastrointestinal nematode *Haemonchus contortus* (Camurça-Vasconcelos et al., 2007), and is also a promising insecticide (Sampson et al., 2005).

In this study, we investigated the anticoccidial effects of *A. annua* (as oil and powder) and *P. anisum* (as oil) in diets fed to chickens challenged with *E. tenella*. Oocyst shedding per gram of feces, body weight, feed conversion, clinical signs and mortality rate were measured.

**Materials and methods**

**Animals and management**

One hundred and sixty, day-old broilers (Ross 308) were obtained from S.C. OpREA Avicom S.R.L. (Venchi-SiGişoara) hatchery. They were reared as a single group from day old to 9-days and housed in a special place in S.C. Transapicola S.R.L. (Târgu Mureş) farm. Two vaccinations were performed, first in 8-days-old for Newcastle disease and second in 12-days-old chickens for Marek disease. Before being divided in experimental groups, broilers were tagged. Diet formulas contained no anticoccidial feed additives. Chickens were offered feed and water ad libitum, and were exposed to continuous light.

**Parasites**

*Eimeria tenella* (Hougton strain) was obtained from Veterinary Laboratory Agency (Parasitology Unit) New Haw, UK. Oocysts were propagated, isolated and sporulated using standard procedures (Raether et al., 1995) to the Departmnet of Parasitology and Parasitic Diseases from the Faculty of Veterinary Medicine, Cluj-Napoca. All challenge infections were initiated by administering by gavage, a suspension of the requisite number of sporulated oocysts in a volume of 1 ml to each chicken.

**Experimental design**

At the age of 9 days, chickens were randomly divided by tag-number into 8 groups (table 1) of 20 birds each. The experimental groups were placed in a house from S.C. Transapicola S.R.L. farm (Târgu Mureş), in isolated spaces measuring 130x130 cm. At the 10 days, chickens were infected with 1500 *E. tenella* oocysts. All treatments, except treatment with lasalocid, started in the same day with infection and continued till the end of the study (32 days). Lasalocid (125 g/tonne of food) was administered from one-day-old till 37-days-old. Oil extracts were given in water mixed with Tween 20 (1:1; 0.15 ml/l water; 7.5% oil), and powder mixed with feed (15 kg/tonne feed; 1.5%).

**Observations and analytical procedures**

Efficacy of *A. annua* and *P. anisum* against *E. tenella* infection was evaluated by oocysts shedded per gram of feces (McMaster counting technique), lesional score, body weight, feed conversion ratio and mortality rate. For
oocysts counting, pooled fecal samples were collected daily from 5 to 18 days post-challenge, and then on day 20, 24, 28 and 30 post-challenge from each group. Lesional score was evaluated at 7 days (10 chickens) post-challenge using a score of 0-4 (Johnson and Reid, 1970).

Table 1. Experimental groups and treatments applied

<table>
<thead>
<tr>
<th>Group</th>
<th>Infected</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neg</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Pos</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>T</td>
<td>YES</td>
<td>Tween 20</td>
</tr>
<tr>
<td>Las</td>
<td>YES</td>
<td>Lasalocid</td>
</tr>
<tr>
<td>Aa-p</td>
<td>YES</td>
<td>A. annua - powder</td>
</tr>
<tr>
<td>Aa-o</td>
<td>YES</td>
<td>A. annua - oil</td>
</tr>
<tr>
<td>Pa</td>
<td>YES</td>
<td>P. anisum - oil</td>
</tr>
<tr>
<td>Pa+Aa</td>
<td>YES</td>
<td>P. anisum + A. annua - oil</td>
</tr>
</tbody>
</table>

Statistical analysis

Data were statistically analyzed by t-test using Statistica 9.0 software (StatSoft ®).

Results

Clinical signs and mortality

At 4 days post-challenge in group T and group Pos we registered clinical signs as inappetence, polydypsia, and weakness, followed at 7 days by diarrhea with brown feces. The same clinical signs, but with lower severity, appeared later in experimental groups (Pa, Aa-o, Aa-p, Las). No clinical signs were registered in group Las and group Neg. Mortality was registered in groups Pa (1/10), T (1/10) and Pos (2/10) after 8-15 days of infection. Death was caused by infection with E. tenella in groups T and Pos, the lesional score being 4, respectively 5 (hemorrhagic typhlitis and core of fibrin in cecum). Dead chicken from group Pa showed lesions of pulmonary congestion, fibrinous airsacculitis and typhlitis with spotted hemorrhages (lesional score 2). In this case, the cause of mortality was respiratory failure. No mortality was observed in groups treated with Artemisia and lasalocid.

Oocysts shedding and lesional score

As shown in figure 1, the chickens from groups Pa, Aa-o, Aa-p, Las and Neg exhibited significantly reduced oocyst shedding when compared to the control groups (T, Pos) (p≤0.05). The highest reduction in fecal oocyst output following E. tenella infection showed group Aa-p (90.76%), followed by groups Aa-o (79.23%) and Pa (58.83%). No oocysts were detected in feces obtained from the Las group.

![Figure 1. Effect of A. annua and P. anisum on the fecal oocyst output following E. tenella low infection (1500 oocysts)](image)

The lesional scores within the experimental groups were uniformly low and were not significantly different from control groups (table 2). A lesional score less than 1 was seen in groups Neg, Las, Pa+Aa and Aa-p.

Table 2. Lesional score, body weight gain and feed conversion in experimental groups following A. annua and P. anisum diets and E. tenella infection

<table>
<thead>
<tr>
<th>Group</th>
<th>Lesional score</th>
<th>Weight gain (g/day) Day 7</th>
<th>Day 32</th>
<th>Feed conversion (kg food/kg spore)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pa</td>
<td>1.2</td>
<td>40.71</td>
<td>65.62</td>
<td>1.94</td>
</tr>
<tr>
<td>Aa-o</td>
<td>1</td>
<td>39.43</td>
<td>66.5</td>
<td>1.90</td>
</tr>
<tr>
<td>Pa+Aa</td>
<td>0.6</td>
<td>37.43</td>
<td>66</td>
<td>1.95</td>
</tr>
<tr>
<td>Aa-p</td>
<td>0.8</td>
<td>35.86</td>
<td>66.72</td>
<td>1.88</td>
</tr>
<tr>
<td>Las</td>
<td>0.4</td>
<td>45.71</td>
<td>67.5</td>
<td>1.85</td>
</tr>
<tr>
<td>T</td>
<td>1.6</td>
<td>32.71</td>
<td>61.22</td>
<td>2.06</td>
</tr>
<tr>
<td>Pos</td>
<td>1.8</td>
<td>29.71</td>
<td>59.31</td>
<td>2.21</td>
</tr>
<tr>
<td>Neg</td>
<td>0.2</td>
<td>46.57</td>
<td>67.37</td>
<td>1.96</td>
</tr>
</tbody>
</table>
Weight gain and feed conversion

At 7 and 32 days after challenge, the weight gain was the highest in Neg and Las groups and lowest in Pos group. In the case of experimental groups, there were some differences between those two periods. At 7 days post infection, the highest weight gain was registered in groups Pa and Aa-o and contrary at 32 days post infection in Aa-p and Aa-o groups (table 2).

All experimental groups, including Las and Neg, used less then 2 kg food for one kg spore, while Pos and T groups used more then 2 kg food. Anyway, among experimental groups the best feed conversion was in chickens from groups Aa-p and Aa-o (table 2).

Discussions

Coccidiosis remains one of the most economically important diseases in poultry industry. Control of coccidiosis has been focused on prophylaxis with anticoccidial drugs in food. 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 (Allen and Fetterer, 2002; Dalloul and Lillehoj, 2005).

The purpose of this experiment was to evaluate the anticoccidial activity of A. annua and P. anisum on E. tenella infection in chickens. A. annua was of great interest because it is known to contain artemisinin, a compound with antimalarial activity attributable in part to its potential to elicit oxidative stress (Krungkrai and Yuthavong, 1987; Levander et al., 1989; Meshnick et al., 1989), mechanism which is also effective in controlling infection with E. tenella (Allen et al., 1996). P. anisum was studied more for its physiological effects, antimicrobial and antifungal activity and less for the antiparasitic activity.

A. annua had good anticoccidial activity, mainly as powder. It significantly reduced fecal oocyst output (90.76%; p=0.002), lesion score (0.8), and improved weight gain (66.72 g/day) and food conversion (1.88 kg feed/kg spore) comparing with infected and untreated chicken (lesion score 1.6/1.8; weight gain 61.22/59.31 g/day; food conversion 2.06/2.21 kg feed/kg spore). It didn't have the same effects in combination with P. anisum, when only the lesional score was low (0.4).

Effect of A. annua on coccidial infection in chickens was extensively studied in the latest years. These studies have shown a great variety of results as a consequence of artemisinin concentration caused by different biological phase of plant, various methods of extraction and routes of administration, and finally as a consequence of experimental design (Eimeria species, oocysts dose, etc.). Briefly, most of them pointed out a good effect in case of E. tenella (Allen et al., 1997; Arab et al., 2006) and E. necatrix (Titilincu et al., 2008) infection but not for E. acervulina and E. maxima (Allen et al., 1997; Arab et al., 2006).

Another study showed that A. annua improved feed conversion in quails infected with Eimeria spp. (Baciu et al., 2006).

It seems that artemisinin alters the process of oocysts wall formation resulting in an incomplete oocyst wall (organized at two opposite poles), with death of developing oocysts and reduction in the sporulation rate (Del Cacho et al., 2010). This alteration is caused by reduction of SERCA (sarco/endoplasmic reticulum calcium ATPase) expression in macrogametes, that plays a role in calcium homeostasis with effects in the secretion of wall-forming bodies, which is a calcium – dependent mechanism (Del Cacho et al., 2010). Artemisia annua also has an inhibitory effect directly on the oocysts sporulation and damaging effect of sporulated oocysts. Chickens infected with Eimeria spp. oocysts sporulated in a medium that contained A. annua, presented a low lesional score and a higher weight gain comparatively with positive control groups (Ma et al., 2001; Titilincu et al., 2008).

Chickens treated with P. anisum presented an oocyst output reduction of only 58.83%, and the lesion score was over 1 (1.2). Good results were registered for weight gain (65.62 g/day) and feed conversion (1.94 feed/kg spore).
Two separate modes of action can be attributed to the efficacy of plant extracts for treating parasitic infections: their immunomodulatory properties and their antiparasitic effects (Anthony et al., 2005). In our case *P. anisum* didn’t have a good anticoccidial effect but improved the weight gain and feed conversion.

According to our results, *A. annua* as powder can be used with good results to prevent coccidiosis in chickens, at least for small groups of animals and in organic farming system, but further analysis must be done with a higher infective doses.

References


