Effects of artemisinin in broiler chickens challenged with Eimeria acervulina, E. maxima and E. tenella in battery trials

ARTICLE in VETERINARY PARASITOLOGY · OCTOBER 2015
Impact Factor: 2.46 · DOI: 10.1016/j.vetpar.2015.10.011

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Effects of artemisinin in broiler chickens challenged with *Eimeria acervulina*, *E. maxima* and *E. tenella* in battery trials

Loredana Pop a,1, Adriana Györke a,⁎,1, Alexandru Flaviu Tăbăran b, Mirabela Oana Dumitrache a, Zsuzsa Kalmár a, Cristian Magdaș a, Viorica Mircean a, Diana Zagon a, Anamaria Balea a, Vasile Cozma a

a Department of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Manastur Street, Cluj-Napoca 400372, Romania

b Department of Anatomic Pathology, Necropsy and Forensic Medicine, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Manastur Street, Cluj-Napoca 400372, Romania

ARTICLE INFO

Article history:
Received 13 May 2015
Received in revised form
24 September 2015
Accepted 9 October 2015

Keywords:
Artemisinin
Eimeria
Chicken
Coccidiosis

ABSTRACT

Four experiments were conceived in order to test the efficacy of artemisinin, a sesquiterpene lactone derived from *Artemisia annua*, in single experimental infection of broiler chickens with *Eimeria acervulina* (1 × 10⁵ oocysts), *Eimeria maxima* (5 × 10⁶ oocysts) or *Eimeria tenella* (1 × 10⁶ oocysts), and mixed infection with all 3 species (3.2 × 10⁶ *Eimeria* spp. oocysts). For each experiment, three different dosages of artemisinin (5, 50 and 500 ppm) were compared with a negative control (uninfected, unmedicated), a positive control (infected, unmedicated) and a classical anticoccidial (monensin). The weight gain (WG), feed conversion ratio (FCR), oocysts shedded per gram of feces (OPG), lesion score, oocysts sporulation rates and mortality rate were recorded in all groups. The dosage of 5 ppm of artemisinin improved the WG and FCR for the chickens infected with *E. acervulina*. The OPG was significantly decreased in all the groups medicated with artemisinin and challenged with a mixed infection (*p* ≤ 0.01). The lesion score of the chickens challenged with *Eimeria* was reduced by different concentrations of artemisinin, depending on the species involved, but this compound did not have a positive effect on the lesions caused by *E. acervulina*. Histopathological analysis revealed superficial erosions of the intestinal mucosa, mixt. mononuclear and heterophilic inflammatory infiltrate in the lamina propria and intralesional presence of various developmental stages of parasite in groups infected with *Eimeria* spp. The sporulation rate of *E. acervulina* and *E. maxima* oocysts was significantly affected by 500 ppm of artemisinin, whilst the dosage of 5 ppm affected the sporulation of *E. tenella* oocysts. These data suggest that artemisinin is not effective against single eimerian infections but could be used as an alternative in mixed coccidiosis, especially if its effect on the oocysts sporulation would be fully investigated.

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1. Introduction

Chicken coccidiosis is one of the most economically devastating diseases of poultry industry. Worldwide, the costs with low productivity, mortality, prophylaxis and treatment exceed 3 billion US dollars, annually (Dalloul and Lillehoj, 2006). This disease caused by seven different species of *Eimeria* (*Eimeria acervulina*, *Eimeria tenella*, *Eimeria maxima*, *Eimeria necatrix*, *Eimeria brunetti*, *Eimeria mitis*, *Eimeria praecox*) affects the intestinal tract of chickens, producing diarrhea, low weight gain, poor feed conversion efficiency, and in severe cases, mortality (Williams, 2002; Williams et al., 2009). In broiler chickens the most prevalent species are *E. acervulina*, *E. tenella* and *E. maxima* (Györke et al., 2013), of which *E. tenella* is highly pathogenic, causing hemorrhagic diarrhea and being responsible for greatly reduction of weight gain and considerable mortality. *E. maxima* has moderate pathogenicity producing economical losses and mortality whilst *E. acervulina* is mildly pathogenic, but it is the most common species in chickens and causes poor feed conversion and mortality only in heavy infections (McDougald and Fitz-Coy, 2008).

The control of coccidiosis is based mainly on in-feed anticoccidials, but the emergence of drug-resistance to all know substances and the concerns regarding residues in poultry products have led to
the search of new effective and safer alternatives (Chapman, 1997). Vaccination was proven to be an effective solution, but live vaccines may produce severe reactions, affecting the performance of chickens, and attenuated vaccines are expensive to produce. Another downside of vaccination is that one vaccine strain may not be efficient in all geographical areas (Chapman, 2000; Abbas et al., 2012a).

Therefore, several studies have directed toward the antischistosomal activity of natural products such as essential oils and plant extracts (Tewari and Maharana, 2011; Abbas et al., 2012b; Zaman et al., 2012). Among them, artemisinin, a sesquiterpene lactone produced by aerial parts of Artemisia annua, has been proven to be effective against several species of Eimeria in chickens (Oh et al., 1995; Allen et al., 1997; Arab et al., 2006; Del Cacho et al., 2010). The mode of action of artemisinin most likely implies the production of free radicals due to cleavage of its endoperoxide bridge resulting in the inhibition of the coccidial sarcoc/endoplasmic reticulum calcium ATP-ase (Del Cacho et al., 2010). Artemisinin is being used for over 1000 years in malaria treatment, being efficient even against multi-drug-resistant strains of this parasite (Dhingra et al., 1999). This compound has efficacy also on other protozoan parasites like Toxoplasma gondii, Neospora caninum, Theileria equi or Leishmania donovani (Ke et al., 1990; Yang and Liew, 1993; Kim et al., 2002; Kumar et al., 2003).

In the present study we aimed to test the antischistosomal effect of different concentrations of artemisinin in chickens infected with various species of Eimeria.

2. Materials and methods

2.1. Chickens

One day-old ROSS 308 hybrid chickens were purchased from S.C. VIS AVIS S.A., Vadu Crișului and housed in batteries in dedicated facilities of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Until the beginning of the experiments chickens were fed with standard starter feed free of antischistosomal. Water and feed were provided ad libitum and light was continuous.

2.2. Medication

Pure artemisinin (purity min. 98%), powder, extracted from A. annua, was purchased from INTTRADE Chemicals GmbH, Germany and was introduced in chicken’s standard grower feed in concentrations of 5, 50 and 500 ppm from 12 days until 28 days of age. We used a 10 fold drug dose escalation in order to detect the dose–response relationship.

Monensin (Coxidin® 200, Huvepharma) was administered in chicken’s diet from 12 days age until 28 days of age at a concentration of 125 mg/kg feed.

2.3. Parasites

We used for experimental infection, Houghton and Weybridge strains of E. acervulina, E. tenella and E. maxima, kindly provided by Ralph Marshal from Animal Health and Veterinary Laboratories Agency (UK). Eimeria oocysts were propagated through experimental infections in 14 days-old chickens at the Parasitology and Parasitic Diseases Department, Faculty of Veterinary Medicine Cluj-Napoca, then isolated and sporulated in 2.5% potassium dichromate using standard procedures (Raether et al., 1995). The number of oocysts per ml was determined using a Fuchs-Rosenthal chamber and adjusted according to sporulation rate.

Table 1

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Group</th>
<th>BWG (g)</th>
<th>FCR</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>First period</td>
<td>Second period</td>
</tr>
<tr>
<td>I</td>
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<tr>
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<td>22.3 ± 1.24ab</td>
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<td>35.7 ± 4.35ab</td>
</tr>
<tr>
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<td>27.9 ± 2.59ab</td>
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<tr>
<td>MON+1000</td>
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<td>33.0 ± 1.28ab</td>
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<td>MON+3000</td>
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<td>39.1 ± 2.35ab</td>
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<tr>
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<tr>
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<td>22.4 ± 1.56ab</td>
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<td>27.77 ± 1.14ab</td>
<td>34.46 ± 2.52ab</td>
<td>30.9 ± 1.90ab</td>
</tr>
</tbody>
</table>

Table 1 The effect of artemisinin on performance parameters in experimental groups of chickens challenged with E. acervulina (experiment 1), E. maxima (experiment 2), E. tenella (experiment 3), Eimeria spp. (experiment 4) compared with control groups.

Values with no common superscript in a column within an experiment were significantly different (p < 0.05); results are expressed as means ± SEM;
I: 1st period = 0–5 days; 2nd period = 5–12 days; total period = 0–12 days;
II: 1st period = 0–6 days; 2nd period = 6–13 days; total period = 0–13 days;
III/IV: 1st period = 0–7 days; 2nd period = 7–14 days; total period = 0–14 days;
BWG: Body weight gain (g/day/chicken);
FCR: Feed conversion ratio – kg feed consumed/kg weight gain in the specified periods.

2.4. Experimental design

Four independent experiments were designed in order to verify the effectiveness of artemisinin against *E. acervulina*, *E. maxima*, *E. tenella*, and mixed infection with all three species of *Eimeria*.

For each experiment, 126 chickens were randomly divided in six groups each with three replicates of seven chickens/cage (*n* = 21). The groups were: negative control (NC)—uninfected and untreated; positive control (PC)—infected and untreated; monensin control (MC)—infected and treated with 125 ppm monensin; ART5—infected and treated with 5 ppm artemisinin; ART50—infected and treated with 50 ppm artemisinin; and ART500—infected and treated with 500 ppm artemisinin. Experimental infection was done on day 14 by oral gavage with a known number of sporulated oocysts/chicken in a volume of 1 mL as follows: experiment 1: 1 × 10⁵ oocysts of *E. acervulina*; experiment 2: 2–5 × 10⁴ *E. maxima* oocysts; experiment 3: 3–5 × 10⁴ *E. tenella* oocysts; experiment 4: 3.2 × 10⁴ *Eimeria* spp. oocysts (*E. acervulina* 20,000, *E. maxima* 10,000 and *E. tenella* 2000 sporulated oocysts). Monensin and artemisinin, in specified doses, were introduced in the diet two days prior experimental infection until the end of the experiments.

The efficacy of artemisinin was evaluated by recording the weight gain, feed conversion ratio, oocysts shedded per gram of feces, lesion score, histological examination of the intestine, mortality rate and oocysts sporulation rates compared with control groups (Holdsworth et al., 2004).

All experiments were approved by the Animal Ethics Committee of our institution (protocol no. 4/19.09.2013).

2.4.1. Mortality rate

Mortality was recorded throughout the entire experimental period as it occurred, and the exact cause of death was investigated by necropsy examination.

2.4.2. Weight gain (WG) and feed conversion ratio (FCR)

Chickens were weighted individually at the beginning, middle and end of the experiments, in order to calculate the weight gain achieved by each chicken. For assessing the feed consumption the amount of feed given to the chickens was weighted daily per cage. The feed conversion was calculated per cage as the ratio between the amount of feed consumed per weight gain of the chickens.

2.4.3. OPG

After 3 days of experimental infection we started coproparasitological examination by flotation technique using saturated sodium chloride solution (specific gravity 1.28). When we found oocysts, fecal samples were collected daily until the end of the experiments and the number of oocysts per gram of feces was determined by duplicate counts of duplicate fecal slurries from each cage by using the McMaster method (12 chambers counted for every group).

2.4.4. Lesion score

Ten chickens from each group were euthanized on different days postinfection according to the experiment. The lesions were macroscopically evaluated by using a scoring system (Johnson and Reid, 1970). Grades from 0 to 4 were given, depending on the severity of lesions in specific portions of the intestine which corresponded to the species involved in the experimental infection. The entire intestine was pull out from the chickens, and both the mucosal surface...
and the unopened serosal surface were analyzed for lesions (the duodenum and jejunum were examined for the infection with *E. acervulina*, the duodenum, entire jejunum and ileum for infection with *E. maxima*, both the caeca for *E. tenella*, and for the mixed infection all the above described intestinal portions were examined, and scored separately).

2.4.5. Histological analysis

The samples of duodenum, ileum and caecum (duodenum for *E. acervulina*, ileum for *E. maxima* and caecum for *E. tenella*), were collected immediately after euthanasia injected in the lumen with 10% neutral buffered formalin solution (NBF) and after washing out the intestinal content immersed in fresh NBF fixative for 48 h. Longitudinally and transversely trimmed samples were dehydrated in ethanol and embedded in paraffin wax following the laboratory routine protocol. Four micron sections were cut from the paraffin blocks with a rotary microtome (Leica RM2125) and routinely stained with hematoxylin–eosin (H&E). Optical images were captured with an Olympus UC30Camera mounted on an Olympus BX41 optical microscope and finally processed by Stream Basic analysis software.

2.4.6. Statistical analysis

Arithmetic mean and standard deviation were calculated for body weight gain, feed conversion ratio, oocysts per gram feces and lesion score in Excel 2007. Then all data were analyzed by one-way ANOVA and Tukey HSD test using online software (Assaad et al., 2014). The level of significance was set at 0.05.

3. Results

3.1. Mortality rate

None of the chickens died following infection with *Eimeria* spp. One death occurred in the uninfected, unmedicated group from experiment 4, but this was due to colibacillosis.

3.2. Experiment 1

Chickens infected with *E. acervulina* and medicated with 5 ppm of artemisinin had the highest WG recorded in the periods 5–12 days post infection and 0–12 days post infection, which was even greater than the uninfected group. In the first period investigated (0–5 days post infection) the groups treated with 5 and 50 ppm of artemisinin had weight gains comparable with the positive control group, but higher than the group treated with monensin. The group supplemented with 500 ppm of artemisinin had the lowest WG in all the periods recorded (Table 1).

The FCR was consistent with the WG. The group supplemented with 5 ppm of artemisinin had the best FCR, even greater than the negative control group, during the entire period investigated. The group treated with 500 ppm of artemisinin had the worst use of feed in all the periods recorded (Table 1).

In the second day of shedding, the OPG was surprisingly higher in all the groups supplemented with artemisinin compared with the control group. Except the group medicated with 5 ppm of artemisinin, which eliminated fewer oocysts of *E. acervulina* than the control group in days 7, 8 and 10 post infection (*p* < 0.02), the two other groups who received artemisinin in their diet had higher OPG compared with the untreated group. Monensin proved his effectiveness by significantly diminishing the number of oocysts shedded starting with day 7 post infection (Fig. 1a).

Another surprising fact was that the lesion score and sporulation rate were in contradiction with the data produced for the recording performance and oocysts shedding. The lesion score was lower than the control group only in the group supplemented with 500 ppm artemisinin of the artemisinin treated groups, but without statistical significance (*p* = 0.1). The group medicated with monensin had significantly less intestinal lesions (a reduction of 89.25% compared with the untreated group) (Table 2).

According to the data recorded in the sporulation rate, artemisinin in concentration of 500 ppm, significantly alters the sporulation of *E. acervulina* oocysts (*p* < 0.001), the percentage of sporulated oocysts being lower not only as against the control group but even lower than the monensin treated group (Table 3).

3.3. Experiment 2

The chickens infected with *E. maxima* and medicated with artemisinin recorded lower WG compared with the control groups in all the periods investigated. The group medicated with monensin had good weight gains, comparable with the negative control group (Table 2).

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>The effect of artemisinin on lesion score in experimental groups of chickens challenged with <em>E. acervulina</em> (experiment I), <em>E. maxima</em> (experiment II), <em>E. tenella</em> (experiment III), <em>Eimeria</em> spp. (experiment IV) compared with control groups.</strong></td>
</tr>
<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td><strong>Lesion</strong></td>
</tr>
<tr>
<td>NC</td>
</tr>
<tr>
<td>PC</td>
</tr>
<tr>
<td>ART5</td>
</tr>
<tr>
<td>ART50</td>
</tr>
<tr>
<td>ART500</td>
</tr>
<tr>
<td>MON</td>
</tr>
</tbody>
</table>

Values with no common superscript in a column were significantly different (*p* < 0.05); results are expressed as means ± SEM;

I: lesion score at 5 days post infection;
II: lesion score at 6 days post infection;
III: lesion score at 7 days post infection.

**Table 3**

<p>| <strong>The effect of artemisinin on oocysts sporulation rate in experimental groups of chickens challenged with <em>E. acervulina</em> (experiment I), <em>E. maxima</em> (experiment II), <em>E. tenella</em> (experiment III), compared with control groups.</strong> |</p>
<table>
<thead>
<tr>
<th><strong>Group</strong></th>
<th><strong>I</strong></th>
<th><strong>II</strong></th>
<th><strong>III</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PC</td>
<td>87.3 ± 0.76a</td>
<td>82.7 ± 2.02a</td>
<td>95.42 ± 0.74a</td>
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<td>ART5</td>
<td>87.8 ± 1.28a</td>
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<td>81.4 ± 1.24a</td>
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</table>

Values with no common superscript in a column were significantly different (*p* < 0.05); results are expressed in percentages as means ± SEM;

I: sporulation rate at 6 days p.i.;
II: sporulation rate at 7 days p.i.;
III: sporulation rate at 8 days p.i.

The FCR was higher for all the groups medicated with artemisinin than the negative control group in all the periods investigated. Only the chickens who received monensin in their diet recorded feed conversions comparable with the uninfected group (Table 1).

The number of oocysts/g of feces shedded in the first day was lower in the experimental groups than in the control group. However, the following days the groups medicated with artemisinin shedded higher number of oocysts compared with the untreated group, except the days 9 and 11th post infection when the chickens who received 5 ppm of artemisinin in their diet shedded significantly lower oocysts than the control group. As expected, monensin reduced significantly the OPG (Fig. 1b).

The groups medicated with 50 and 500 ppm of artemisinin had significantly lower lesion score than the control group, the highest reduction being recorded in the 50 ppm treated group (p = 0.001). The chickens treated with monensin had very few intestinal lesions (Table 2).

Just as in the case of *E. acervulina*, the sporulation rate of *E. maxima* oocysts was significantly affected by artemisinin in the dosage of 500 ppm (p = 0.002). In the other 2 groups medicated with artemisinin, the percentage of sporulated oocyst was also lower than in the control and monensin groups, but without statistical significance (Table 3).

3.4. Experiment 3

The chickens infected with *E. tenella* and medicated with 5 and 50 ppm of artemisinin had weight gains comparable with the positive control group in the second period investigated, but this recordings did not exceed the values registered for the uninfected group. For the rest of the periods the chickens who received in their diet artemisinin had lower weight gains than the control groups. The highest WG was recorded for the group medicated with monensin in all periods taken into consideration (Table 1).

The FCR was consistent with the WG. The chickens from the groups ART5 and ART50 had a more efficient use of feed in the period 8–14 days post infection in comparison with the other two periods and the group ART500, but FCR was worse than the negative control group. The best feed conversion was recorded for the uninfected group in the second period investigated (Table 1).

The chickens from the groups medicated with 50 and 500 ppm of artemisinin shedded more oocysts, except for the first and last day of shedding, the values being considerable higher for the group ART500 than all the other groups. In day 7 post infection, the second day of shedding, the OPG in the chickens who received 5 ppm of artemisinin was lower than in the positive control group, but without statistical significance, and this event did not occur the following days. Although an increase was seen in the number of oocysts shedded in the 7th day post infection in the chickens medicated with monensin, the OPG in this group was considerably lower than all the other groups for the entire period investigated (Fig. 1c).

Cecal lesions were reduced by 5 and 50 ppm of artemisinin (p = 0.02; p = 0.01), but the highest dosage of this compound, respectively 500 ppm, had an adverse effect on the intestinal lesions, the lesion score for the chickens in this group being almost twice as the one for the positive control group. This data are in accordance with the aspects recorded for the OPG. As expected, the chickens from the group medicated with monensin had a much lower lesion score than all the other groups (Table 2).

The percentage of sporulated oocysts of *E. tenella* is also affected by artemisinin in all given concentrations, but the lowest number of sporulated oocysts was recorded for the group medicated with 5 ppm of artemisinin, contrary to the data recorded for *E. acervulina*.

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and *E. maxima*. Monensin also affected the sporulation of *E. tenella* oocysts (Table 3).

### 3.5. Experiment 4

Artemisinin did not have a positive effect on WG neither in the chickens infected with the suspension containing all three species of *Eimeria*. In all the periods recorded the groups who received artemisinin in their diet showed lower body weight gains than the control groups (Table 1). Although, in the period 7–14 days post infection, the chickens medicated with 5 ppm of artemisinin showed a FCR comparable with the control groups, this was not the case for the total period, in this situation the feed conversion being worse than the control groups for all artemisinin treated groups. The best FCR was recorded, as expected, in the uninfected group, but only in the second and total periods investigated, while in the first period, the chickens medicated with monensin showed a better feed conversion than the control group (Table 1).

Contrary to the data recorded for WG and FCR, the results registered for the OPG and lesion score were encouraging. In day 5 post infection, the day with the highest oocyst output, in all the groups medicated with artemisinin the number of oocysts per gram of feces was significantly lower than the control group (*p* ≤ 0.01). The dosage of 500 ppm of artemisinin seemed to have the most remarkable effect on reducing the oocysts shedding (*p* = 0.0002) (Fig. 1d).

The lesion score assessed for the duodenum was significantly lower for the chickens medicated with 5 ppm of artemisinin (*p* = 0.05), this aspect being recorded also in the caecum. The dosage of 500 ppm of artemisinin had also reduced the intestinal lesions in the caecum (*p* = 0.05) and as well in the jejunum. Surprisingly, the dosage of 50 ppm of artemisinin exacerbated the lesions, the chicks that received this diet showed a higher lesion score even than the positive control group (Table 2).

### 3.6. Histological analysis

The main histopathological findings observed in the experimental groups consist in superficial erosions of the intestinal mucosa with variable fusion of the intestinal villi and mixt. mononuclear and heterophilic inflammatory infiltrate in the lamina propria which in severe cases expands to the submucosa. Rarely the inflammatory process had a transmural pattern. In all cases the intestinal changes were associated with intraluminal presence of various developmental stages of *Eimeria* spp. All the described elements fluctuate largely between experimental groups (Figs. 2–4), with significant reduction of the number of parasites in certain artemisinin groups. A particular aspect was observed in the case of *E. tenella* infection in which multiple polygonal or ovoid cells harboring schizonts were observed deep in the lamina propria, sometimes associated with small foci of intestinal wall mineralization.

### 4. Discussions

In spite of the advances in pharmacological industry, chicken coccidiosis remains one of the greatest threats for poultry productivity. The discovery of novel effective anticoccidials is essential in order to keep in control this devastating disease.

Artemisinin, the main compound from the plant *A. annua*, has been studied for its effects on cancer and viral infections, and primarily for its antimalarial properties but also for its activity against other protozoan parasites. It seems to have an effect on chicken coccidiosis by reducing the infection risks (Allen et al., 1997; Goodarzi et al., 2004; Arab et al., 2012).

Thus, we intended to test artemisinin effect on most prevalent species of *Eimeria* in chickens, with single and mixed experimental infections, in order to find a natural product that can be used with success as a feed additive in broiler industry.

Unlike the results of Allen et al. (1997) who obtained a significantly drop of the oocyst coproelimation rates in the chickens medicated with artemisinin and single infected with *E. acervulina* and *E. tenella*, in the current study artemisinin did not diminish the oocyst output in single infections. This negative result may be due to the fact that a higher range between dosages (5–50–500 ppm) was used in the present study. In the case of the study made by Allen et al. (1997) the most effective dosage in reducing the OPG was 17 ppm of artemisinin, but Goodarzi et al. (2004) obtained good results also with the dosage of 80 ppm of artemisinin. In a study on gastrointestinal nematodes in sheep, artemisinin had also nonsignificant EPG reductions (Calá et al., 2014). However, in the present study, a significantly drop in the oocysts production was noticed for all artemisinin medicated groups in the chickens challenged with the mixed suspension, but the number of oocysts inoculated of each species was five times lower than in the single infections. The study of Arab et al. (2006) showed that artemisinin in concentrations of 1 and 2.5 mg/kg body weight significantly reduces the number of oocysts of *E. acervulina* and *E. tenella*, which is not consistent with the data obtained in the present study. However, the authors did not register a drop in the oocyst output in the case of *E. maxima* infection, aspect that was encountered in the present study also.

Artemisinin seems to alter the process of *E. tenella* oocyst wall formation, leading to reduced sporulation rates, thus the reproduction of this parasite is severely affected (Del Cacho et al., 2010). Fatemi et al. (2015) showed that *A. annua* extracts inhibit sporulation of mixed oocysts of *E. acervulina*, *E. necatrix* and *E. tenella* in vitro. In the present study it was shown that artemisinin decreases the percentage of sporulated oocysts, not only for *E. tenella*, but also for *E. acervulina* and *E. maxima*. It is known that the ingestion of sporulated oocysts by chickens is essential for the occurrence of the disease and it is also a very important factor in the severity, and spread of coccidiosis in broilers (Kaboutari et al., 2014).

The dosages used by Allen et al. (1997), respectively 2, 8.5 and 17 ppm of artemisinin, did not have any negative effect on the weight gains of the infected chickens, which is in contradiction with what was obtained in the present study for all experiments except for the chickens infected with *E. acervulina* and medicated with 5 ppm artemisinin. This issue encountered may be related to the bitter taste of *A. annua* (de Almeida et al., 2012), and artemisinin, its active substance, may have the same problem in higher concentrations, leading to a reduced feed consumption and as a consequence lower weight gains. It is not the case in our study, because the feed intake did not differ significantly from control groups (data unshown). Otherwise, Engberg et al. (2012) noticed a reduction in the feed intake and weight gains of chickens, when they used 500 mg/kg feed of *n*-hexane extract of *A. annua*, aspects that are consistent with the recordings from the present study (unshown data).

The data recorded for the lesion scores for *E. acervulina* and *E. tenella* are in accordance with the study of Allen et al. (1997), artemisinin did not have a positive effect on the intestinal lesions produced by *E. acervulina* in any concentration, but 5 and 50 ppm dosages of artemisinin decreased the lesion scores in the case of *E. tenella*. The histopathological findings in this study are equivalent with the lesions produced by infection with *E. acervulina*, *E. maxima* and *E. tenella* (McDougald and Fitz-Coy, 2008). Also, the lesions and the number of parasites observed at histological examination are in accordance with the recordings for the macroscopic lesion score and the oocysts counts for all the experiments designed. The identified cells harboring schizonts of *E. tenella* in the lamina propria.
Fig. 3. Representative histological images of ileum at 6 days following *E. maxima* infection and chronically oral intake of 5, 50 and 500 ppm of artemisin: Image A (positive control)—massive number of intracellular parasites in various developmental stages (arrows) located in the middle and upper third of the villi associated with erosions of the mucosa. Lamina propria contain a moderate number of inflammatory cells represented by lymphocytes and rare heterophils; Images B–D—endogenous stages of *E. maxima* (arrows) associated with superficial mucosal erosions (B and D) and minimal to mild inflammatory infiltrate. The inset images present schizonts, gamonts and oocysts; H&E stain objective ×20 and respectively ×100 for the insets.

Fig. 4. Representative histological images of caecum at 7 days following *E. tenella* infection and chronically oral intake of 5, 50 and 500 ppm of artemisin: Image A (positive control)—massive number of intracellular parasites in various developmental stages (arrows) associated with lymphocytic and heterophilic infiltration of the lamina propria and villous atrophy. Image B–D—intracellular *E. tenella* (arrows) in various developmental stages associated with moderate inflammatory infiltrate (mainly lymphocytes and plasma cells). The insets present details of the intestinal *Eimeria* infection. H&E stain objective ×20 and respectively ×100 for the insets.
have been proved to be epithelial cells infected by merozoites of first generation, that detach themselves from the crypts and enter the lamina propria, where the development into schizonts takes place (López-Bernad et al., 1998).

5. Conclusions

Although artemisinin affects the sporulation process of *Eimeria* spp., reduces the lesion score and decreases the oocysts output in mixed infections, this compound seems to have an adverse effect on the weight gain and also on the feed conversion in challenged chickens, which are the most important parameters for broiler industry. If the cause of this downside will be investigated and straighten, artemisinin could be used as an alternative for broiler coccidiosis produced by *E. acervulina, E. tenella* and *E. maxima*.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

This work was supported by a grant of the Romanian National Authority for Scientific Research, CNCDI–UEFISCDI, project number 110/2012 and was published under the frame of European Social Found, Human Resources Development Operational Programme 2007–2013, project POSDRU/159/1.5/S/136893. We thank to Ralph Marshall for supplying *Eimeria* strains.

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