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Anticoccidial effects of artemisinin in broiler chickens

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Abstract

Coccidiosis is an economically devastating disease in broiler industry. Due to development of drug resistance against *Eimeria spp.*, there is an increasing demand for alternative solutions in controlling this illness and artemisinin, the main bioactive compound from *Artemisia annua*, a plant used for centuries in malaria treatment, seems to be an effective choice. Therefore, in the present study, four experiments were conceived in order to test the efficacy of artemisinin in single experimental infection of broiler chickens with *E. acervulina* ($1x10^5$oocysts), *E. maxima* ($5x10^4$ oocysts) or *E. tenella* ($1x10^4$ oocysts), and mixed infection with all 3 species ($3.2x10^4$ *Eimeria* spp. oocysts). For each experiment, three different dosages of artemisinin 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, mortality rate and oocysts sporulation rates were recorded in all groups in order to investigate the anticoccidial effect of artemisinin. The dosage of 5 ppm of artemisinin improved the weight gain and feed conversion ratio for the chickens infected with *E. acervulina.* The oocyst output was significantly decreased in all the groups medicated with artemisinin and challenged with a mixed

infection ($p \leq 0.01$). The lesion score of the chickens challenged with *Eimeria* was reduced by different concentrations of artemisinin, depending on the species incriminated, but this compound did not have a positive effect on the lesions caused by *E. acervulina*. 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.

Keywords: Artemisinin, *Eimeria*, chicken, coccidiosis.

1. Introduction

Chicken coccidiosis is one of the most economically important diseases of poultry industry. Worldwide, the costs with low productivity, mortality, prophylaxis and treatment exceed 3 billion dollars, annually (Dalloul and Lillehoj, 2006).

This disease caused by seven different species of *Eimeria* (*E. acervulina*, *E. tenella*, *E. maxima*, *E. necatrix*, *E. brunetti*, *E. mitis*, *E. 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, 2012).

Therefore, several studies have directed towards the anticoccidial activity of natural products such as essential oils and plant extracts (Tewari and Maharana, 2011). 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 coccidian sarco/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 & Liew, 1993; Kim et al, 2002; Kumar et al, 2003).

In the present study we aimed to test the anticoccidial 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 Crişului and housed in standard conditions in dedicated facilities of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Until the beginning of the experiments chickens were fed with starter feed free of anticoccidials. Water and feed were provided *ad libitum* and light was continuous.

2.2. Medication

Pure artemisinin (purity min. 98%), powder, extracted from *Artemisia annua*, was purchased from INTATRADE Chemicals GmbH, Germany and was introduced in chicken's feed in concentrations of 5, 50 and 500 mg/ kg feed from 12 days until 28 days of age.

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.

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 (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 artemisnin; Art50 - infected and treated with 50 ppm artemisnin; and Art500 - infected and treated with 500 ppm artemisinin (Table 1). 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 - $1x10^5$ oocysts of *E. acervulina;* experiment 2 - $5x10^4$ *E. maxima* oocysts; experiment 3 - $1x10^4$ *E. tenella* oocysts; experiment 4 - $3.2x10^4$ *Eimeria* spp. oocysts (*E. acervulina* 20.000, *E. maxima* 10.000 and *E. tenella* 2.000 sporulated oocysts) (Table 2). 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, mortality rate and oocysts sporulation rates comparing with control groups (Holdsworth et al., 2004).

All experiments were approved by the Animal Ethics Committee of our institution.

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 begining, middle and end of the experiments (Table 2), in order to calculate the weight gain achieved by each chicken. For assesing 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 tehnique using saturated sodium chloride solution (specific gravity 1.28). When we found oocysts, faecal samples were collected daily until the end of the experiments (Table 2) and the number of oocysts per gram of faeces was determined by duplicate counts of duplicate homogenates 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 euthanasied on different days postinfection according to the experiment (Table 2). The lesion score was evaluated according to Johnson and Reid (1970).

Table 1. Experimental groups and treatments applied during the experiments

Group	Challenge	Prophylaxis
Negative control (NC)	-	-
Positive control (PC)	+	-
ART5	+	Artemisinin 5 ppm
ART50	+	Artemisinin 50 ppm
ART500	+	Artemisinin 500 ppm
MON	+	Monensin 125 ppm

Table 2. Experimental design for testing the anticoccidial efficacy of artemisinin in chickens

	Infection	WG, FCR	OPG	Lesion score	Sporulation rate
Experiment 1	$1x10^5$ *E. acervulina*	0-5, 5-12	5-12	5	6
Experiment 2	$5x10^4$ *E. maxima*	0-6, 6-13	6-13	6	7
Experiment 3	$1x10^4$ *E. tenella*	0-7, 7-14	6-14	7	8
Experiment 4	$3.2X10^4$ *Eimeria* spp.	0-7, 7-14	4-14	7	-

151 **3. Results**

152 ***3.1. Mortality rate***

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

155 ***3.2. Experiment 1***

156 Chickens infected with *E. acervulina* and medicated with 5 ppm of artemisinin had the
157 highest weight gain recorded in the period 5-12 days post infection, even greater than the
158 uninfected group. In the first period investigated (0-5 days post infection) the groups treated with
159 5 and 50 ppm of artemisinin had weight gains comparable with the positive control group, but
160 higher than the group treated with monensin. The group supplemented with 500 ppm of
161 artemisinin had the lowest weight gain in this period and also in the other 2 periods investigated.
162 During the full period investigated (0-12 days post infection) the highest weight gain was
163 achieved by the chickens from the group medicated with 5 ppm of artemisinin. The other 2
164 groups medicated with artemisinin had lower weight gains compared with the control groups
165 (Table 3).

166 The feed conversion ratio was consistent with the weight gain. The group supplemented
167 with 5 ppm of artemisinin had the best feed conversion ratio, even greater than the negative
168 control group, during the entire period investigated. In the period 0-5 days post infection the
169 group medicated with 50 ppm of artemisinin had also a better feed conversion ratio than the
170 control and monensin groups. The group treated with 500 ppm of artemisinin had the worst use
171 of feed in all the periods investigated (Table 3).

172 In the second day of shedding, the number of oocysts per gram of faeces was surprisingly
173 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 ($p \leq 0.02$), the other two groups who received artemisinin in their diet had a 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. 1).

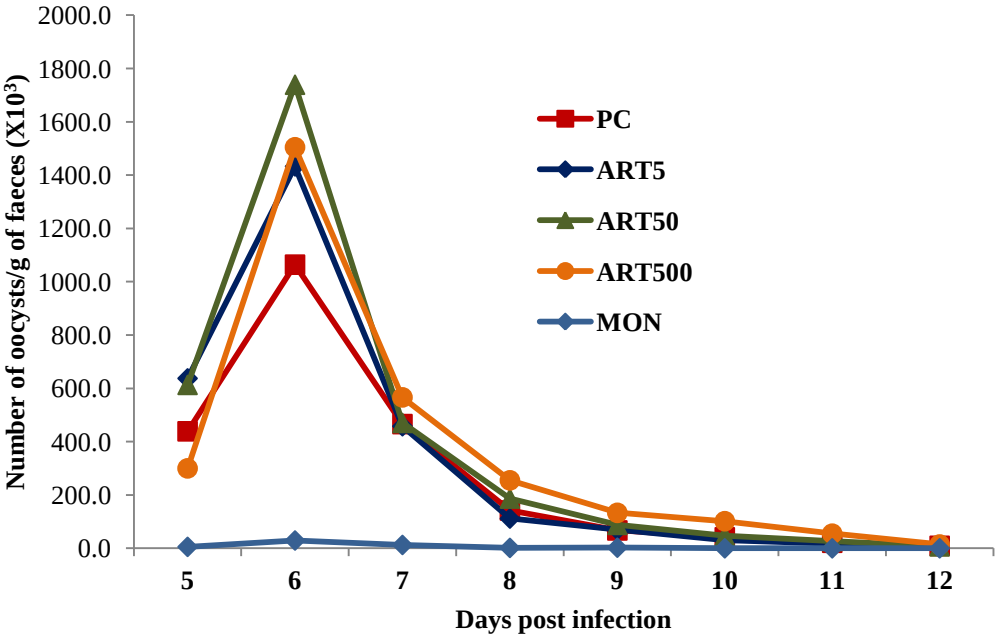


Fig.1. Dynamics of mean oocysts number/g of faeces in experimental groups of chickens infected with *E. acervulina* and treated with artemisinin compared with group treated with monensin

Another surprising fact was that the lesion score and sporulation rate were in contradiction with the data recorded for the production 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 89.29% compared with the untreated group) (Table 3).

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 weight gains compared with the control groups in all the periods investigated. In the first period (0-6 days post infection) the groups treated with 5 and 50 ppm of artemisinin had weight gains comparable with the infected unmedicated group, but significantly lowers than the uninfected group. The group medicated with monensin had good weight gains, comparable with the negative control group (Table 3).

The feed conversion ratio 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 3).

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 11^{th} 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. 2).

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).

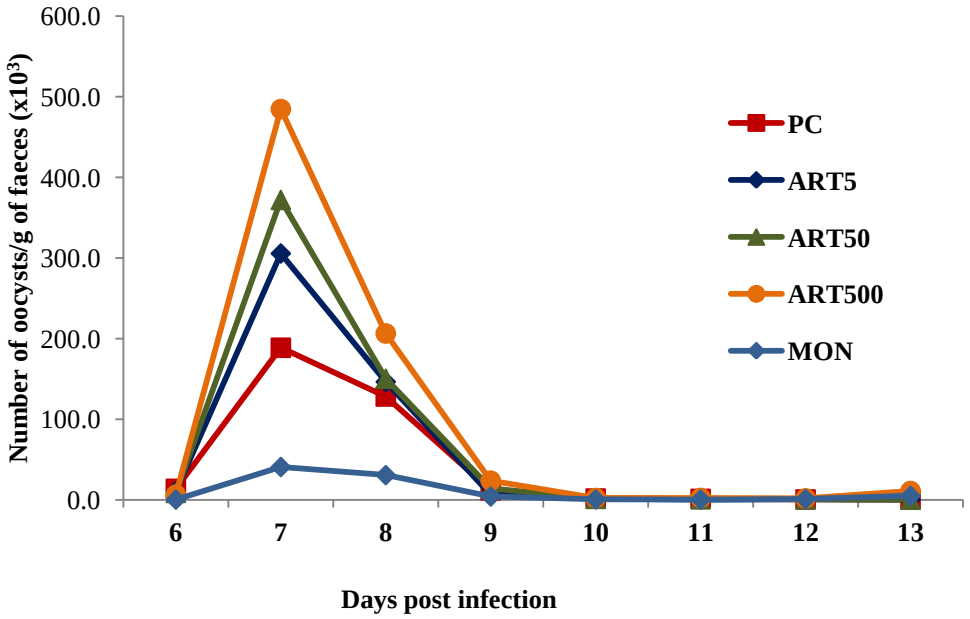


Fig.2. Dynamics of mean oocysts number/g of faeces in experimental groups of chickens infected with *E. maxima* and treated with artemisinin compared with group treated with monensin

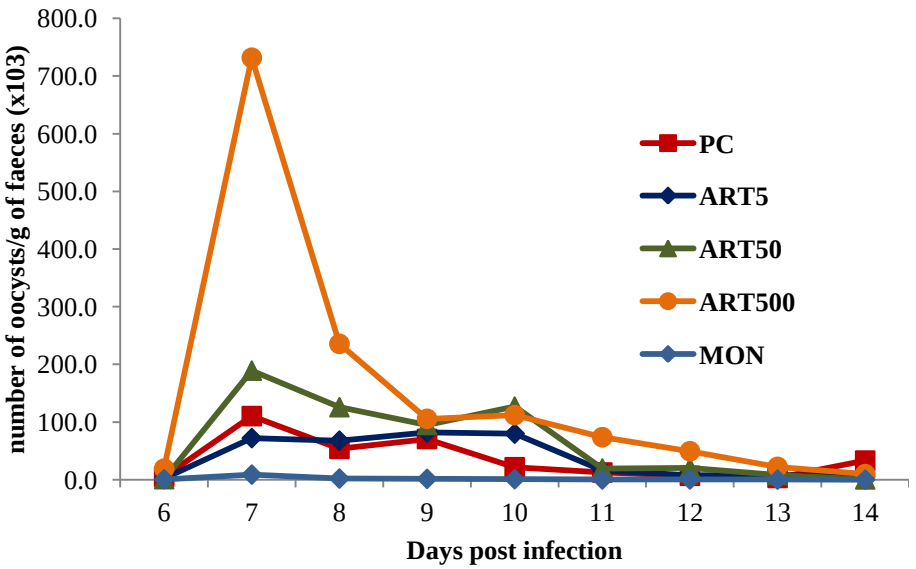
3.4. Experiment 3

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 unifected group. For the rest of the periods the chickens who received in their diet artemisinin had lower weight gains than the

224 control groups. The highest weight gain was recorded for the group medicated with monensin in
225 all three periods taken into consideration (Table 3).

226 The feed conversion was consistent with the weight gain. The chickens from the groups
227 ART5 and ART50 had a more efficient use of feed in the period 8-14 days post infection in
228 comparison with the other two periods and the group ART500, but the feed conversion ratio was
229 worse than the negative control group. The best feed conversion was recorded for the uninfected
230 group in the second period investigated (Table 3).

231 The chickens from the groups medicated with 50 and 500 ppm of artemisinin shedded
232 more oocysts, except for the first and last day of shedding, the values being considerable higher
233 for the group ART500 than all the other groups. In day 7 post infection, the second day of
234 shedding, the OPG in the chickens who received 5 ppm of artemisinin was lower than in the
235 positive control group, but without statistical significance, and this event didn't occur the
236 following days. Although an increase was seen in the number of oocysts shedded in the 7^{th} day
237 post infection in the chickens medicated with monensin, the OPG in this group was considerably
238 lower than all the other groups for the entire period investigated (Fig. 3).



239

240 Fig.3. Dynamics of mean oocysts number/g of faeces in experimental groups of chickens infected with *E.*
241 *tenella* and treated with artemisinin compared with group treated with monensin

242 Coecal lesions were reduced by 5 and 50 ppm of artemisinin ($p = 0.02$; $p = 0.01$), but the
243 highest dosage of this compound, respectively 500 ppm, had an adverse effect on the intestinal
244 lesions, the lesion score for the chickens in this group being almost twice as the one for the
245 positive control group. This data are in accordance with the aspects recorded for the OPG. As
246 expected, the chickens from the group medicated with monensin had a much lower lesion score
247 than all the other groups (Table 3).

248 The percentage of sporulated oocysts of *E. tenella* is also affected by artemisinin in all
249 given concentrations, but the lowest number of sporulated oocysts was recorded for the group
250 medicated with 5 ppm of artemisinin, contrary to the data recorded for *E. acervulina* and *E.*
251 *maxima*. Monensin also affected the sporulation of *E. tenella* oocysts (Table 3).

252 ***3.5. Experiment 4***

253 Artemisinin didn't have a positive effect on the weight gain neither in the chickens
254 infected with the suspension containing all three species of *Eimeria*. In all the periods recorded

255 the groups who received artemisinin in their diet showed lower body weight gains than the
256 control groups. In the period 0-7 days post infection, the group treated with monensin exhibited
257 the highest weight gains even than the uninfected group (Table 3).

258 In the first period recorded, the feed conversion ratio for the artemisinin treated groups
259 was very high in comparison with the control groups. Although, in the period 7-14 days post
260 infection, the chickens medicated with 5 ppm of artemisinin showed a feed conversion
261 comparable with the control groups, this was not the case for the total period, in this situation the
262 feed conversion being worse than the control groups for all artemisinin treated groups. The best
263 feed conversion was recorded, as expected, in the uninfected group, but only in the second and
264 total periods investigated, while in the first period, the chickens medicated with monensin
265 showed a better feed conversion than the control group (Table 3).

266 Contrary to the data recorded for the weight gain and feed conversion, the situation
267 registered for the OPG and lesion score was encouraging.

268 In day 5 post infection, the day with the highest oocysts output, in all the groups
269 medicated with artemisinin the number of oocysts per gram of faeces was significantly lower
270 than the control group ($p \leq 0.01$). The dosage of 500 ppm of artemisinin seemed to have the most
271 remarkable effect on reducing the shedding ($p = 0.0002$) (Fig. 4).

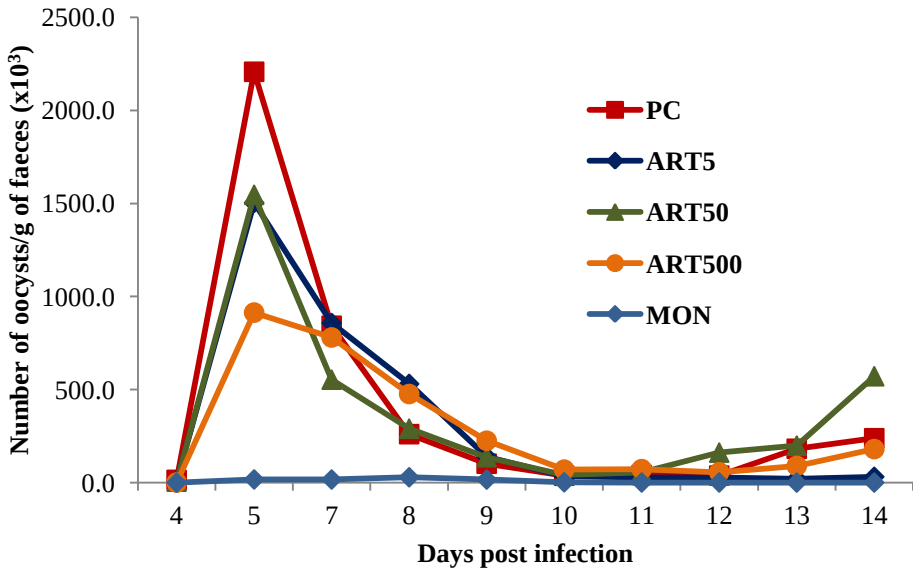


Fig.4. Dynamics of mean oocysts number/g of faeces in experimental groups of chickens infected with *Eimeria* spp. and treated with artemisinin compared with group treated with monensin

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 showing a higher lesion score even than the positive control group (Table 3).

Table 3. Effect of artemisinin comparing with control groups on performance parameters, lesion score and oocysts sporulation rate in experimental groups of chickens challenged with *E. acervulina* (experiment 1), *E. maxima* (experiment 2), *E. tenella* (experiment 3), *Eimeria spp.* (experiment 4) – means ± standard error

Experiment	Group	BWG[e]			FCR[f]			Lesion score	Sporulation rate (%)
		First period	Second period	Total period	First period	Second period	Total period		
I[a]	NC	28.7±0.96	32.9±1.35	31.3±1.09	2.88±0.06	2.65±0.12	2.73±0.08	0	-
	PC	26.8±1.54	34.2±1.91	31.1±1.67	2.68±0.33	2.76±0.006	2.59±0.18	2.8±0.25	87.3±0.76
	ART5	26.1±1.04	40.0±2.56	34.7±2.11	2.58±0.09	2.38±0.24	2.47±0.20	3.5±0.22	87.8±1.28
	ART50	26.1±1.41	33.8±2.58	29.5±2.16	2.57±0.27	2.72±0.04	2.74±0.11	3.1±0.31	87.8±1.16
	ART500	20.6±1.00	23.9±1.25	22.3±1.24	3.12±0.27	3.50±0.04	3.45±0.03	1.9±0.41	79.7±0.68
	MON	24.6±0.95	29.6±1.58	27.0±1.32	2.77±0.18	2.90±0.04	2.85±0.11	0.3±0.15	84.0±1.01
II[b]	NC	37.7±2.24	52.4±2.30	45.2±1.86	2.62±0.52	2.24±0.04	2.36±0.16	0	-
	PC	27.9±2.64	47.4±3.18	39.1±3.25	3.63±0.20	2.81±0.46	3.12±0.38	2.3±0.21	82.7±2.02
	ART5	27.8±1.61	41.1±3.30	33.9±2.57	3.34±0.28	4.27±2.06	3.64±1.14	2.0±0.26	78.1±1.17
	ART50	27.4±2.21	42.8±6.59	35.7±4.35	3.46±0.35	2.85±0.05	3.08±0.08	0.9±0.18	78.5±1.11
	ART500	25.3±2.20	30.4±2.72	27.9±2.59	3.67±0.31	4.22±1.10	3.91±0.69	1.6±0.16	72.7±0.97
	MON	36.6±2.73	50.1±2.54	46.5±2.04	2.62±0.52	2.71±0.59	2.67±0.55	0.3±0.15	81.4±1.24
III[c]	NC	23.99±1.25	41.36±1.99	33.09±1.28	3.18±0.08	2.11±0.07	2.54±0.05	0	-
	PC	29.6±1.16	34.77±2.41	31.63±1.62	2.48±0.02	2.71±0.33	2.59±0.17	1.05±0.05	95.42±0.74

	ART5	24.40±1.29	36.51±2.44	29.97±1.71	3.39±0.29	2.19±0.04	2.82±0.14	0.6±0.18	87.83±1.19
	ART50	22.36±0.99	37.16±3.10	29.06±1.44	3.72±0.29	2.59±0.05	3.14±0.16	0.75±0.11	92.17±0.67
	ART500	17.26±1.49	23.06±2.58	20.24±1.51	4.63±0.09	3.68±0.28	4.16±0.21	1.95±0.31	93.8±0.57
	MON	28.27±1.13	41.80±3.04	36.11±2.12	2.82±0.20	2.27±0.07	2.44±0.03	0.35±0.18	87.3±0.86
IV[d]	**NC**	23.99±1.25	41.36±1.99	33.09±1.28	3.18±0.08	2.11±0.07	2.54±0.05	0	-
	PC	19.22±0.99	38.78±2.35	29.65±1.78	3.85±0.11	2.52±0.18	2.85±0.12	1.6±0.37	-
	ART5	15.12±1.25	32.18±2.60	22.44±1.56	5.10±0.56	2.74±0.98	3.59±0.67	0.5±0.22	-
	ART50	18.95±1.05	25.59±2.93	23.19±1.60	4.20±0.28	3.56±0.05	3.75±0.10	2.1±0.28	-
	ART500	15.35±0.78	22.61±1.74	19.15±1.09	5.22±0.05	4.01±1.50	4.43±0.91	0.7±0.33	-
	MON	27.77±1.14	34.46±2.52	30.39±1.90	2.87±0.23	3.17±0.88	3.03±0.56	0.1±0.1	-

[a] 1st period = 0-5 days; 2nd period = 5-12 days; total period = 0-12 days; lesion score at 5 days p.i. and sporulation rate at 6 days p.i.

[b] 1st period = 0-6 days; 2nd period = 6-13 days; total period = 0-13 days; lesion score at 6 days p.i. and sporulation rate at 7 days p.i.

[c/d] 1st period = 0-7 days; 2nd period = 7-14 days; total period = 0-14 days; lesion score at 7 days p.i. and sporulation rate at 8 days p.i.

[e] Body weight gain (g/day/chicken);

[f] Feed conversion ratio - kg feed consumed/ kg weight gain in the specified periods

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 *Artemisia 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.

Artemisinin didn't diminish the oocyst output in single infections, unlike the results of Allen et al. (1997) who obtained a significantly drop of the oocyst coproelimination rates in the chickens medicated with artemisinin and single infected with *E. acervulina* and *E. tenella*. This negative result may be due to the fact that a higher range between dosages (5-50-500 ppm) was used, in the case of the study made by Allen et al., 17 ppm of artemisinin was the most effective in reducing the OPG, but Goodarzi et al. (2004) obtained good results also with the dosage of 80 ppm of artemisinin. 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 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* (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. Engberg et al. (2012) noticed the same 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 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). 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).

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 of the chickens and also on the feed conversion, 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.

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