VETERINARSKI ARHIV 86 (2), 253-264, 2016

Influence of *Artemisia absinthium* essential oil on antioxidative system of broilers experimentally infected with *Eimeria* oocysts

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KOSTADINOVIĆ, LJ. M., S. J. POPOVIĆ, N. M. PUVAČA, I. S. ČABARKAPA, Š. M. KORMANJOŠ, J. D. LEVIĆ: Influence of *Artemisia absinthium* essential oil on antioxidative system of broilers experimentally infected with *Eimeria* oocysts. Vet. arhiv 86, 253-264, 2016.

ABSTRACT

The aim of this study was to investigate the effects of *Artemisia absinthium* essential oil (AAEO) on the enzymatic activity of superoxide-dismutase (SOD), glutathione-peroxidase (GSHPx), glutathione-reductase (GR), peroxidase (POD) and xanthine-oxidase (XOD) and the content of lipid peroxides (LPx) and glutathione (GSH) in broilers infected with an oocyst mixture of *Eimeria tenella*, *Eimeria mitis* and *Eimeria necatrix,* compared to coccidicide salinomycine. The investigation was carried out on 240 Arbour acres broilers of both sexes. Broilers were distributed completely randomly into four treatment groups: treatment group A was uninfected and untreated; treatment group B was infected and kept untreated; treatment group C preventively received coccidicide salinomycine in a dose of 60 mg/kg of feed and inoculated with an oocyst mixture on the 21st day-of-age; treatment group D received AAEO in their feed in a dose of 3 g/kg and were infected with the oocyst mixture on the 21st day of age. During the study, bloody diarrhoea was observed from the 3rd to 9th day after the challenge. After six days of infection, the most intensive bloody diarrhoea was noticed in the un-medicated treatment group. In order to evaluate the effects of essential oil on poultry coccidiosis induced by *Eimeria* spp., oocysts per gram of faeces (OPG) were also investigated in all treatment groups. During the experiment, the oocyst output and mortality rate were significantly lower ($P<0.05$) in the AAEO treatment group (D_2) in comparison to the positive control (B), while significant excretion of oocysts was noticed in the faeces of non-treated broilers infected with *Eimeria* spp. The broilers treated with salinomycin (C_2) showed complete reduction of oocysts in their faeces at 30 days of age. The results obtained in this study indicate changes in the content and the activity of the non-enzymatic and enzymatic antioxidative protective systems in blood hemolysates of infected chickens. The positive preventive effects of AAEO, applied in a concentration of 3g/kg of feed, were high on the antioxidative system of erythrocytes. On the basis of the obtained results, it was concluded that AAEO was effective in lowering the intensity of bloody diarrhoea, as well in reducing the oocyst

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ISSN 0372-5480
Printed in Croatia

output of the preventively treated and infected broilers; hence it may be used as a prophylactic feed additive. Moreover, AAEO showed an important role in the activation of antioxidative protection systems in infected broilers, which is of great interest since free radicals and lipid peroxides, formed as a result of lower food intake and exhaustion of the organism induced by diarrhoea, could cause cellular membrane damage.

Key words: *Artemisia absinthium*, coccidiosis, prophylactic feed additive, antioxidative system, salinomycin

Introduction

Coccidiosis is the acute invasion and destruction of intestinal mucosa by protozoa of the genus *Eimeria,* with oocysts often present in the environment wherever poultry are raised (CHAPMAN et al., 2010). Coccidiosis is one of the most economically damaging diseases of the poultry industry, resulting in major economic losses by reducing poultry performance and lowering productivity (CHAPMAN et al., 2010; McDONALD and SHIRLEY, 2009; PEEK and LANDMAN, 2011). Chickens are hosts to seven species of *Eimeria* that develop at specific sites along the digestive tract (McDONALD and SHIRLEY, 2009). These pathogens may cause damage to the intestinal tissue, decrease feed intake and absorption of nutrients, and also increase susceptibility to secondary bacterial infections (MORRIS et al., 2007; COOPER and SONGER, 2009; KOSTADINOVIĆ et al., 2015a).

Coccidiosis is mainly controlled using prophylactic coccidicides, administered in the feed (CONSTANTINOIU et al., 2008; SHIRLEY et al., 2005). These coccidicides are now in widespread use on chicken farms, bringing high levels of development and prosperity to the poultry industry. The prevention/treatment of chicken coccidiosis relies on the availability and effective use of coccidicides. Therefore, coccidicides play an important role in coccidiosis prevention in the commercial broiler industry. However, the extensive use of these compounds over the past 50 years has resulted in the development of drug resistance by *Eimeria* spp. (BEREZIN et al., 2008; MOLAN et al., 2009; WILLIAMS, 2006; YADAV and GUPTA, 2001). Cross-resistance and multi-drug resistance have reduced the effectiveness of coccidicides.

Subsequently, with increasing demands for high-protein meat and increased consumer concerns over the side effects of conventional anticoccidial drugs on poultry, the toxicity of some of these drugs on other animal species, and public health concerns about tissue residues of anticoccidial drugs, the search has intensified for alternative strategies against coccidiosis. One of the potential candidates is the use of medicinal plants, such as the *Artemisia* species, or their extracts (KOSTADINOVIĆ et al., 2015a; KOSTADINOVIĆ et al., 2015b). The genus *Artemisia* belongs to the *Compositae* (*Asteraceae)* family with over 300 species spread worldwide. The essential oil obtained from the wild plant *Artemisia absinthium* shows antibacterial (JUTEAU et al., 2003; LOPES-LUTZ et al., 2008; SENGUL et al., 2011), antifeedant (as a naturally occurring substance in certain plants that adversely

affects insects or other animals that eat them), antipyretic, fertility increasing, cytostatic and antimalarial activities (KHATTAK et al., 1985).

Considering the aforementioned positive aspects of *Artemisia absinthium* essential oil, the aim of this study was to compare the prophylactic efficacy of the conventional coccidicide (salinomycin) and *Artemisia absinthium* essential oil in broilers artificially infected with coccidiosis. The comparative assessment was based on the clinical symptoms and changes in the catalytic activity of the important oxidative protection enzymes in the blood hemolysates of healthy and artificially infected broilers.

Materials and methods

Chickens and housing. The experimental protocol was approved by the Ethics Committee of the University of Novi Sad, Faculty of Medicine (EC/15/05/432-6) and the principles of animal protection and welfare were strictly followed. Experiments under *in vivo* conditions were performed on 240 broilers of both sexes from the heavy Arbour acres strain. One-day-old chicks were raised in a clean and disinfected room under standard conditions. The broilers were fed a standard basal diet with access to water and food *ad libitum.* Faecal samples were taken daily to monitor the possibility of infection. The temperature and lighting regimens were in accordance with the recommendations of the breeder. The initial room temperature (32-33 °C) was reduced weekly by 1 °C to a final temperature of 28 °C.

The broilers were randomly divided into non-infected and infected treatment groups. The broilers in the infected treatment groups were exposed to a mixture of sporulated oocysts of the *E. tenella*, *E. mitis* and *E. necatrix* genus, collected from infected chicken farms. Coccidial oocysts of *E. tenella, E. mitis* and *E. necatrix* were obtained from the guts of infected chickens, and they were preserved in 2.5 % potassium dichromate solution to induce sporulation, and kept in a refrigerator at 2-5 °C until use. The oocyst mixture consisted of 20000 oocysts per ml (5000 *E. tenella* oocysts per ml; 5000 *E. mitis* oocysts per ml and 10000 *E. necatrix* oocysts per ml). The challenge infection of 21-day-old chickens was performed by oral administration of a 1 ml oocyst suspension.

Artemisia absinthium essential oil was obtained from the Dr Josif Pancic Institute for Medicinal Plant Research , Belgrade, Serbia.

Experimental protocol. One-day-old broilers, randomly selected, were divided into four treatment groups (Table 1), each containing 60 individuals, further divided into three replicates each, respectively:

Treatment Group A: uninfected and un-medicated broilers - the negative control. Blood sampling and decapitation of 10 broilers was carried out at 30 days of age.

Table 1. Experimental design with broilers

Experimental treatment	Components received by broilers		
	Coccidiostatic salynomicine (60 mg/kg)	*Artemisia absinthium* essential oil (3 g/kg)	Eimeria oocysts* (1 ml oocyst suspension)
A - Negative control treatment group	-	-	-
B - Positive control treatment group	-	-	+
C_1 -Preventive coccidicide salinomycine	+	-	-
C_2 - Broilers inoculated with laboratory derived coccidia species	+	-	+
D_1 -Preventive *Artemisia absinthium* essential oil	-	+	-
D_2 - Broilers infected with *Eimeria* oocysts	-	+	+

*Broilers were infected with *Eimeria* oocysts at 21 days of age

Treatment Group B: infected and un-medicated broilers - the positive control. Inoculation of 21-day-old broilers was performed by p.o. application of 1 ml of the oocyst mixture. Nine days later (30 days of age), when the first clinical signs of disease appeared (the broilers were bristling, showing decreased food conversion, white mucous, later bloody diarrhoea appeared, appetite decreased etc.), blood sampling and decapitation of 10 broilers was carried out.

Treatment Group C: broilers which preventively received the coccidicide salinomycine in a dose of 60 mg/kg of feed (Group C_1) and the remaining broilers were inoculated with a laboratory derived coccidian species at 21 days of age. Blood sampling and decapitation of 10 broilers was carried out at 30 days of age (Group C_2).

Treatment Group D: broilers which received AAEO in a dose of 3 g/kg (Group D_1) and the remaining broilers infected with a *Eimeria* oocyst mixture at 21 days of age. Blood was collected at 30 days of age (Group D_2). The essential oil was given to the broilers three times a day.

During the experiment the broilers were regularly controlled, autopsies were performed and all findings were carefully recorded. The oocyst output, after the infection, was measured every third day during the period from 21 to 30 days of age in each group.

The means of oocysts per gram of faeces (OPG) in the treated treatment groups were compared with OPG values for non-treated control treatment groups in order to evaluate the effects of the plant essential oil on avian coccidiosis induced by *Eimeria* spp.

Bloody diarrhoea was investigated from the 3rd to 9th day after the challenge. The bloody diarrheal score was described using numerical values from 0 to 3. Zero corresponded to normal status, whereas 1, 2 and 3 corresponded to 33; 33-66; 66-99 % of blood in total faeces, respectively.

A commercial test (”Dialab“, Vienna, Austria) was used for determination of haemoglobin level, which is an important indicator of enzyme activity in haemolysed blood. This method was performed on a spectrophotometer (Multiscan MCC 340, Finland). Protein content was determined by the method of PRAKASH et al. (2010).

Preparation of blood haemolysate. Blood was collected by heart puncture of broilers into heparinized test tubes. After centrifugation (10 min at 3500 rpm and 4 °C) and plasma removal, the erythrocytes were rinsed 3 times in saline. The resulting erythrocyte pellet was suspended in an equal volume of double distilled water and vortexed. After incubation for 1 hour at room temperature, the haemolysate was centrifuged for 15 min at 3500 rpm and the supernatant was collected for further analysis (KOSTADINOVIĆ, 1998).

Sample preparation for glutathione (GSH) determination. Proteins from freshly prepared haemolysate were separated by adding half the volume of 10 % sulphosalicylic acid and centrifuged at 5000 rpm for 5 min, at 4 °C. The supernatant was stored at 4 °C, without freezing, and GSH determined within 24 hours. The GSH content in the blood haemolysate was determined from the amount of sulfhydryl residues by means of Ellmann’s reagent (KAPETANOVIC and MIEYAL, 1979).

Determination of enzymatic activity. Superoxide-dismutase (SOD) (EC 1.15.1.1) activity was determined by the spectrophotometric method, based on the inhibition of adrenaline reduction to adrenochrome at pH 10.2 (KOSTADINOVIĆ et al., 2001). The GSHPx (EC 1.11.1.9) activity was determined by spectrophotometric measurement of absorbance at 412 nm, with cumenhydroperoxide as the substrate (CHIU et al., 1976).

Activity of glutathione-reductase (GR) (EC 1.6.4.2.) was determined from the rate of NADPH oxidation, and was monitored by measuring the absorbance at 340 nm (LUKASZEWICZ-HUSSAIN and MONIUSZKO-JAKONIUK, 2004).

Content of lipid peroxides (LPx) was determined by the thiobarbituric acid (TBA) test. The oxidation of cellular membrane lipids was measured via the reaction of lipid peroxides with thiobarbituric acid (PIRONI et al., 2003).

The determination of peroxidase (POD) (EC 1.11.1.7) activity was based on the catalytic oxidation of guayacole by hydrogen peroxide as an electron acceptor (KOSTADINOVIĆ et al., 2011). The reaction of xanthine oxidation of uric acid was used

for determination of xanthine-oxidase (XOD) (EC 1.17.3.2) activity. Spectrophotometric measurement was performed in 0.1 mmol/dm^3 phosphate buffer, pH 7.5, at 295 nm (KOSTADINOVIĆ et al., 2011).

Data analysis. The results given in the tables are reported as the mean ± standard deviations (SD) of a number (n) of independent determinations. The one way ANOVA analysis and Tukey post hock test were performed to assess data differences between various groups, using Statistica software, version 12 (STAT SOFT inc. 2013; USA). All the analyses were carried out in triplicate for each experimental treatment. The data means were considered statistically significantly different at $P<0.05$.

Results

Bloody diarrhoea was observed from the third to the ninth day after the infection with *Eimeria* spp in all experimental groups, except the uninfected experimental treatment groups.

It was observed that the bloody diarrhoea was of the same intensity in all infected treatment groups, except in the negative controlt, on the third day of infection (Table 2). Six days after the infection, the most intensive bloody diarrhoea was noticed in the un-medicated treatment group (B). The intensity of bloody diarrhoea was lower in the treatment group treated with salinomycine (C_2) compared to the other treatment groups on the 27th day of age.

Table 2. Intensity of bloody diarrhoea in chickens challenged with Eimeria spp. mixture and treated with prophylactic dose of salinomycine and AAEO

	Day of infection	After infection		
Treatment	21	24	27	30
A	-	-	-	-
B	-	1	3	1
C_1	-	-	-	-
C_2	-	1	+	-
D_1	-	-	-	-
D_2	-	1	2	-

(0) - normal status; (1) - 33 %, (2) - 33-66 %, (3) - 66 -99 % blood in total faeces; AAEO - *Artemisia absinthium* essential oil

During the experiment, the non-treated broilers infected with *Eimeria* spp. showed significant excretion of oocysts in their faeces, which is shown in Table 3. The broilers treated with salinomycin (C_2) showed complete reduction of oocysts in faeces on the 30th day. In the AAEO treatment group (D_2) oocysts output and mortality rate were

significantly lower (P<0.05) in comparison to the positive control treatment group (B). Hence, it may be concluded that AAEO was effective in reducing the oocyst output of the preventively treated and infected broilers.

Table 3. Effectiveness of salinomycine and AAEO on faecal oocyst count (means±SE) and mortality rate in different treatment groups of broilers

Treatment Group	Average oocyst count (per g)				Mortality rate (%)
	Day of infection	After infection			
	21 day	24 day	27 day	30 day	
A	0	0	0	0	3
B	21025.4 ± 838^{b}	34536.1 ± 177^{c}	37747.0 ± 420^{c}	39485.0 ± 364^{b}	12
C_2	10538.0 ± 1220^{a}	1019.2 ± 23.8^{a}	106.1 ± 18.3^{a}	0	5
D_2	17031.0 ± 1050^{b}	11200.0 ± 156^{b}	4200.8 ± 140^{b}	106.8 ± 12^{a}	7

Results are given as means ± standard deviation (n = 3); $^{a-c}$ Means within a column with no common superscript differ significantly at P < 0.05; AAEO - *Artemisia absinthium* essential oil; A - negative control; B-positive control; C_2 - salinomycin 60 mg/kg of feed and infected; D_2 - AAEO 3g/kg of feed and infected

Enzymatic activity in blood haemolysates. The GSH and LPx levels and enzymatic activity of blood haemolysates from the control treatment groups (A and B) and the experimental treatment groups (C_1, C_2, D_1, D_2) are shown in Table 4.

Table 4. GSH and LPx content and the activity of GSHPx, POD, SOD, GR and XOD in blood haemolysates

Treatment	GSH (μmol/g Hb)	LPx (μmol/g Hb)	GSHPx (μmol/g Hb min)	POD (μmol/g Hb min)	SOD (μmol/g Hb min)	GR (μmol/g Hb min)	XOD (μmol/g Hb min)
A	5.3 ± 1.2^{c}	0.4 ± 0.1^{a}	8.2 ± 2.4^{a}	64.8 ± 3.9^{b}	81.4 ± 7.3^{d}	13.0 ± 6.1^{a}	27.1 ± 2.9^{c}
B	2.4 ± 0.2^{a}	6.4 ± 0.2^{c}	13.8 ± 6.8^{e}	98.3 ± 5.8^{d}	55.4 ± 7.0^{c}	19.4 ± 3.9^{c}	11.0 ± 6.6^{a}
C_1	4.1 ± 0.8^{b}	0.4 ± 0.1^{a}	10.7 ± 4.3^{c}	56.7 ± 3.0^{a}	57.1 ± 2.0^{c}	13.8 ± 1.5^{a}	25.1 ± 7.5^{b}
C_2	5.9 ± 0.2^{c}	0.2 ± 0.1^{b}	9.2 ± 1.3^{b}	58.1 ± 9.6^{a}	21.2 ± 3.9^{a}	20.5 ± 7.5^{c}	25.4 ± 8.7^{b}
D_1	6.1 ± 1.1^{d}	0.4 ± 0.03^{a}	11.7 ± 0.6^{d}	59.8 ± 2.5^{a}	35.8 ± 9.5^{b}	17.0 ± 9.1^{b}	27.0 ± 3.2^{c}
D_2	7.9 ± 1.3^{e}	0.3 ± 0.1^{b}	11.9 ± 4.2^{d}	78.3 ± 2.8^{c}	22.0 ± 7.5^{a}	23.6 ± 5.9^{d}	28.6 ± 7.4^{c}

Results are given as means ± standard deviation (n = 3); $^{a-d}$ Means within a column with no common superscript differ significantly at P < 0.05; GSH- glutathione; LPx - lipid peroxides; GSHPx - glutathione-peroxidase; POD - peroxidase; SOD -superoxide-dismutase; GR - glutathione-reductase; XOD - xanthine-oxidase

The obtained results indicate a significant ($P<0.05$) increase in GSH content and higher catalytic activity of GR in the blood haemolysates of the infected broilers. Moreover, the increase in the GSHPx and POD activity was also significant ($P<0.05$) in group C_1 compared to group C_2. The only exception was the catalytic activity of XOD and SOD, which showed a statistically very significant reduction in the positive control treatment group compared to the negative control treatment group.

The preventive doses of coccidicide salinomycin indicated a statistically significant ($P<0.05$) decrease in GSH content, a statistically significant ($P<0.05$) increase in the activity of GSHPx and a statistically significant ($P<0.05$) reduction in catalase-activity of SOD and POD compared to treatment group A. The increase of LPx content and the activity of GR were not statistically significant ($P>0.05$) in treatment group C_1 compared to treatment group A.

Infection in the treatment group of broilers C_2, nine days later (30 days of age) resulted in a statistically very significant ($P<0.05$) increase in GSH content and higher catalase-activity of XOD compared to the treatment group B. The decrease in LPx content was also statistically significant ($P<0.05$) and amounted to 0.4 and 0.2 in treatment groups C_1 and C_2, respectively. The activity of the other enzymes investigated (GSHPx, POD, SOD) were statistically very significant ($P<0.05$) in treatment group C_1 compared to treatment group C_2. Induction and inhibition of the catalytic activity of antioxidant defences in the blood haemolysates of treatment group C_2 were carried out to achieve the basic level of activity, characteristic in broilers of a control treatment group.

The content of erythrocyte GSH and activity of GSHPx and GR in the blood haemolysates of broilers fed a diet supplemented with AAEO in a dose of 3g/kg (Group D_1) were significantly higher compared to treatment groups A and C_1. The addition of AAEO did not affect the LPx content and activity of POD and XOD in the haemolysates of the broilers. Broilers in the AAEO treatment group had greater ($P<0.05$) activity of SOD than broilers in the control and salinomycine treatment groups. Comparing the results of the effects of preventive doses of salinomycin or AAEO on the activity of antioxidative enzymes in blood haemolysate, it was concluded that good agreement was achieved.

Discussion

Some herbal extracts used as feed additives have been applied to the control of coccidiosis on some chicken farms, obtaining satisfying results (DU and HU, 2004). Medicinal herbs and their extracts are of interest for coccidiosis since several studies have shown substantial antimicrobial and antioxidative activity (ALIYU et al., 2012). The biological activity of these extracts has been mainly attributed to their phenolic components. *In vivo* and *in vitro* tests have shown (WILLIAMS and LOSA, 2001) that

phenols may be specifically used as oocysticides against *Eimeria* spp. It is known that phenols interact with the cytoplasmic membrane by changing its permeability for captions, such as H^+ and K^+. The dissipation of ion gradients leads to the impairment of essential processes in the cell and allows leakage of cellular constituents, resulting in water unbalance, collapse of the membrane potential, inhibition of ATP synthesis, and finally cell death (ULTEE et al., 1999).

The most likely explanation for the observed phenomena, presented in Table 4, is that the pathological alterations intensify free radical processes by stimulating the catalytic activities of enzymes involved in antioxidative protection, POD, GSHPx and GR. However, during the disease period, lipolysis from the lipid depots is increased due to lower food intake and exhaustion of the organism by diarrhoea, which leads to intensification of the free radical processes and formation of larger quantities of lipid peroxides in the blood. Newly formed lipid peroxides and their degradation products are transported by the blood stream to inactive organs and tissues, having a toxic effect on them and generating cellular membrane damage. In order to protect itself, the organism activates its antioxidative protection system. The reduction in catalytic activity of SOD is expected and in agreement with the literature data (SHANKER et al., 2011). Concomitantly with the increased risk of lipid peroxidation in the blood, there is an increase in the enzymatic activity of GSHPx. GSH plays an important role in reducing the acute toxicity of the xenobiotic and the products of lipid peroxidation as a substrate for GSHPx. Addition of salinomycin to the feed increases GSHPx activity and reduces the need for high levels of GSH content, which take part in the detoxification of harmful compounds in the body. A statistically significant decrease in POD activity, compared to the corresponding control group, was expected, since POD catalyses the oxidation of various proton donors with hydrogen peroxide. Salinomycine is an ionophore coccidiocide and does not act as a proton donor.

Conclusions

On the basis of the obtained results, it may be concluded with certainty that the addition of *Artemisia absinthium* essential oil to the broilers' diet has a positive effect on lowering the bloody diarrhoea intensity. Also, it may be concluded that the significant reduction in the oocyst count resulting form this medical herb supplementation to broiler diet indicates that *Artemisia absinthium* essential oil could be used as a prophylactic feed additive. Moreover, *Artemisia absinthium* essential oil showed an important role in the antioxidative protection of broilers infected with coccidiosis, which is also of great importance in treating coccidiosis.

Acknowledgements

The paper is a part of the research work on the project III 46012 financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

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Received: 21 January 2015
Accepted: 10 December 2015

KOSTADINOVIĆ, LJ. M., S. J. POPOVIĆ, N. M. PUVAČA, I. S. ČABARKAPA, Š. M. KORMANJOŠ, J. D. LEVIĆ: Utjecaj eteričnog ulja bijeloga pelina (*Artemisia absinthium*) na antioksidativni status tovnih pilića invadiranih oocistama *Eimeria* spp. Vet. arhiv 86, 253-264, 2016.

SAŽETAK

U radu je istražen utjecaj eteričnog ulja bijeloga pelina (*Artemisia absinthium*) (AAEO) na enzimsku aktivnost superoksid dismutaze (SOD), glutation peroksidaze (GSHPx), glutation reduktaze (GR), peroksidaze (POD) i ksantin-oksidaze (XOD), kao i sadržaj lipidnih peroksida (LPx) i glutationa (GSH) u tovnim pilićima inficiranim smjesom oocista *Eimeria tenella*, *Eimeria mitis* i *Eimeria necatrix* u odnosu na kokcidiostatik salinomicin. Istraživanje *in vivo* provedeno je na 240 pilića Arbor acres, oba spola. Pilići su bili podijeljeni u četiri skupine. Skupina A je bila neinficirana i netretirana (negativna kontrola). Skupina B je bila inficirana i netretirana (pozitivna kontrola). Skupina C je preventivno dobivala kokcidiostatik salinomicin u količini od 60 mg/kg hrane te je bila inokulirana kokcidijama 21. dana starosti. Skupina D je u hrani dobivala AAEO u količini 3 g/kg te je 21. dana starosti bila inficirana mješavinom oocisti *Eimeria* sp. Tijekom istraživanja krvava dijareja javljala se od 3. do 9. dana nakon infekcije. Šest dana nakon inficiranja, najintezivnija krvava dijareja zapažena je u netretiranih pilića. U cilju procjene djelovanja eteričnog ulja bijeloga pelina na kokcidiozu kod peradi izazvanu *Eimeria* oocistama, broj oocista po gramu fecesa (OPG) također je bio istražen u svim skupinama. Tijekom pokusa utvrđeno je da je broj oocista i razina smrtnosti bila znatno niža ($P<0,05$) u AAEO skupini (D2) u usporedbi s pozitivnom kontrolom (B), dok je značajno izlučivanje oocista uočeno u fecesu pilića koji nisu bili tretirani. Pilići koji su dobivali salinomicin (C2) pokazali su potpunu redukciju oocista u fecesu 30. dana starosti. Rezultati dobiveni u ovoj studiji pokazali su promjene u sadržaju i aktivnosti neenzimskih i enzimskih sustava zaštite u krvi inficiranih tovnih pilića. Zapaženi su pozitivni učinci preventivne primjene AAEO u koncentraciji 3 g/kg hrane na antioksidativni sustav eritrocita. Na osnovi dobivenih rezultata zaključeno je da je eterično ulje *Artemisia absinthium* vrlo učinkovito u smanjenju jačine krvavog proljeva kao i u smanjenju broja oocista u pilića koji su preventivno tretirani s AAEO. Stoga se može zaključiti da eterično ulje bijeloga pelina može biti davano kao profilaktički dodatak hrani za životinje. Također, AAEO ima pozitivan utjecaj na aktiviranje antioksidativnog sustava zaštite u krvi pilića, što je vrlo značajno s obzirom na to da slobodni radikali i lipidni peroksidi, koji nastaju kao rezultat manjeg unosa hrane i iscrpljenosti organizma uzrokovanog dijarejom, mogu izazvati oštećenje stanične membrane.

Ključne riječi: *Artemisia absinthium*, kokcidioza, dodatak hrani, antioksidativni sustav, salinomicin