VETERINARSKI ARHIV 90 (4), 331-340, 2020 DOI: 10.24099/vet.arhiv.0638

Influence of tea tree (*Melaleuca alternifolia*) essential oil as feed supplement on production traits, blood oxidative status and treatment of coccidiosis in laying hens

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PUVAČA, N., D. HORVATEK TOMIĆ, S. POPOVIĆ, S. ĐORĐEVIĆ, I. BRKIĆ, N. LALIĆ, T. SHTYLLA KIKA, E. LIKA: Influence of tea tree (*Melaleuca alternifolia*) essential oil as feed supplement on production traits, blood oxidative status and treatment of coccidiosis in laying hens. Vet. arhiv 90, 331-340, 2020.

ABSTRACT

Aim of this experiment was to investigate the effect of tea tree (*Melaleuca alternifolia*) essential oil in the nutrition of laying hens on table egg production, antioxidant activity and potential as a natural anticoccidial drug. A total of 360 Lohmann Brown laying hens aged 54 weeks were divided into 3 different treatment diets, supplemented with 0 (T1), 40 (T2) and 80 mg/kg (T3) tea tree essential oil, respectively. Each treatment was replicated 4 times with 30 birds each. The experiment lasted 56 days in total (55 to 62 weeks of hen age). The 56 day experimental period was divided into 2 periods of 28 days duration each: period 1 (55 to 58 weeks of hens age) and period 2 (59 to 62 weeks of hens age). The hens' blood oxidative status, including glutathione peroxidase (GSHPx), superoxide dismutase (SOD) activity and concentration of malondialdehyde (MDA), and the anticoccidial effects of tea tree on *Eimeria spp.* compared to the unsupplemented control treatment, were evaluated. With the diet supplemented with tea tree essential oil, hen-day egg production improved significantly ($P<0.05$). Dietary addition of tea tree as a natural coccidiostatic in treatments T2 and T3 indicated a statistically significant ($P<0.05$) increase in SOD and GSHPx activity compared with control treatment T1, while significant changes in MDA concentration were not recorded ($P>0.05$). The anticoccidial activity of tea tree essential oil caused a significant ($P<0.05$) decrease in the output of *Eimeria spp.* oocysts per hen. Based on the results, it may be concluded that dietary tea tree essential oil was effective in increasing hen-day egg production and reducing coccidia oocysts output, hence it can be used as a prophylactic feed additive. Also tea tree oil showed an important role in the activation of antioxidative protection systems in hens.

Key words: hens; tea tree; *Melaleuca alternifolia;* coccidiosis; eggs; atioxidative protection; nutrition

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ISSN 0372-5480
Printed in Croatia

Introduction

The last decade has without doubt been the most revolutionary ever regarding animal welfare in the poultry industry, especially in some parts of the world. This has been the case in all the poultry production sectors, both meat and eggs. However, the egg sector has probably undergone the greatest changes (PUVAČA et al., 2013; NIKOLOVA and KOCEVSKI, 2018).

Essential oils have been used in poultry nutrition for their antimicrobial, antibacterial, antioxidant and digestive stimulant properties. Over the past decade, essential oils have been regarded as a possible antibiotic substitute in poultry (PUVAČA et al., 2013) and nowadays they are in the spotlight of the scientific community (POPOVIĆ et al., 2016, PUVAČA, 2018). Tea tree oil is commonly used as a topical antiseptic agent because of its antimicrobial properties, and it is also known to reduce inflammation and may be effective in the treatment of fungal infections (HAMMER et al., 2006). Tea tree oil should not be ingested in large amounts due to its toxicity. OLGUN (2016) showed that egg weight, egg mass and eggshell thickness were positively affected by essential oil supplementation to laying hens' diet, while NIKOLOVA and KOCEVSKI (2018) in their research showed the technological aspects of eggshell quality. ÇABUK et al. (2006) reported that dietary supplementation of essential oil significantly increased egg production and improved feed conversion ratio compared with the control.

Coccidiosis is an acute invasion with destruction of the intestinal mucosa caused by protozoa of the genus *Eimeria*, with the oocysts often present in the environment wherever poultry are raised. 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 (PEEK and LANDMAN, 2011; POPOVIĆ et al., 2017). Poultry 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 the susceptibility to secondary bacterial infections (KOSTADINOVIĆ et al., 2015).

Coccidiosis is mainly controlled using prophylactic anticoccidial drugs administered in the feed (CONSTANTINOIU et al., 2008), which are now in widespread use on poultry farms, bringing a high level of development and prosperity to this industry. The prevention and treatment of poultry coccidiosis relies on the availability and effective use of anticoccidial drugs, so they play an important role in commercial poultry industry (KOSTADINOVIĆ et al., 2016). However, the extensive use of these compounds over the past 50 years has resulted in the development of drug resistance in *Eimeria* spp. (BEREZIN et al., 2008; MOLAN et al., 2009). Cross-resistance and multi-drug resistance have reduced the effectiveness of the anticoccidial drugs.

In Europe, consumer trends are increasingly demanding, clearly chosing cage-free production. For example, in The Netherlands, Germany, Austria and Sweden everything or almost everything is produced in cage-free systems, in response to clear consumer demand. In fact, the poultry system has become one of the fundamental criteria when it comes to choosing what to buy at the supermarket.

Definitively, this global welfare demand comes more strongly from the food companies than the legislators themselves, and this is clearly a consequence of consumer pressure for cage-free eggs. The evolution of these production systems with an ever-increasing percentage of laying hens that are floor-reared or reared in systems like aviaries also has an influence on diseases and how we have to prevent them. Specifically, preventing coccidiosis in these systems has already become one of the many challenges that egg producers will have to face in the near future. In laying hens it is fundamental to maintain the best intestinal health and, as a consequence, preventing coccidiosis will be one of the main focuses.

Having in mind the aforementioned positive aspects of *Melaleuca alternifolia* essential oil, the aim of this experiment was to investigate the effect of tea tree essential oil in laying hens' nutrition on table egg production, antioxidant activity, and its potential as a natural anticoccidial drug.

Materials and methods

Animals, housing and feeding. This biological experiment with laying hens was performed in accordance with EU legislation and the principle of the Three Rs within Directive 2010/63/EU. A total of 360 Lohmann Brown laying hens, aged 54 weeks, were divided into 3 different groups. The non-treated group served as control (T1), while the other two groups were treated with 40 (T2) and 80 mg/kg (T3) of dietary tea tree essential oil (Planet Fresh d.o.o., Montenegro), respectively. This tea tree oil includes Terpinen-4-ol 40.0%, γ-Terpinene 23.0% and α-Terpinene 10.4% as the active components. Each treatment was replicated four times with 30 birds each. All hens were housed in an environmentally controlled experimental facility, with the temperature maintained at approximately 22˚C. The house had controlled ventilation and lighting, according to the hybrid specifications. All hens were supplied with a diet of 110 g of feed/hen/day, while water, at an average temperature of 20˚C, was provided *ad libitum*. The hens were fed diets in mash form during the experiment. The basal diet was formulated according to the hybrid specifications, with and without on top addition of tea tree essential oils in the mixtures. The chemical composition of the diets used is shown in Table 1.

Table 1. Nutritive value of basal diet for nutrition of laying hens

Nutrients*	Content, %
Crude protein	15.5
Crude fat	4.6
Calcium	3.8
Phosphorus (available)	0.37
Lysine	0.79
Methionine	0.35
Cystine	0.19
Threonine	0.60
Tryptophan	0.18
Metabolizable Energy MJ/kg	11.0

*The value of crude protein, crude fat and calcium was analysed and the value of metabolizable energy was calculated, others were calculated values

Preparation of blood samples. Blood was collected from the wing vein of laying hens into heparinized test tubes, and at the end of the experiment from six laying hens of each group in a total of eighteen blood samples. 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 blood haemolysate was centrifuged for 15 min at 3500 rpm, and the supernatant was collected for further analysis (KOSTADINOVIĆ et al., 2016).

Determination of enzymatic activity. The glutathione peroxidase (GSHPx) (EC 1.11.1.9) activity was determined by spectrophotometric measurement of absorbance at 412 nm with cumenhydroperoxide as the substrate (POPOVIĆ et al., 2017). 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., 2016). The content of lipid peroxides was determined by thiobarbituric acid reactive substances (TBARS) test through the concentration of MDA (PUVAČA et al., 2016).

Preparation of faeces samples. Faecal samples of hens from treatments T1, T2 and T3 in the amount of 3 g were collected at the beginning of the 55th week of age and at the end of the 58th and 62nd weeks of age of the laying hens. Collected samples were weighed into 50 mL centrifuge tubes, and each of the 36 samples per each treatment, 108 samples in total, were prepared and stored at 4 °C until further analyses.

Coccida oocysts counting. Coccidia oocytes were counted according the Weybridge method, using gauze to screen the faeces. Each test sample was prepared whereby 3g of faeces were transferred from the centrifuge tube into a glass beaker, and spiked with 1 mL of the nominal stock concentration in 2% (w/v) potassium dichromate, before adding 41 mL of water, which was used simultaneously to rinse any remaining faeces from the tube into the beaker. First the faeces were allowed to soak thoroughly, after which the suspension was homogenized using an electric mixer.

Table 2. The effects of tea tree essential oil on production performance of laying hens

Parameter	Dietary treatments with tea tree essential oil, mg/kg			SEM	P-value
	0 (T1)	40 (T2)	80 (T3)		
Feed intake, g/hen/day					
55 to 58 week	123.0^{a}	123.2^{a}	124.1^{a}	1.354	0.489
59 to 62 week	120.5^{a}	120.5^{a}	121.2^{a}	1.198	0.483
55 to 62 week	122.0^{a}	121.9^{a}	122.7^{a}	1.076	0.382
Feed conversion ratio					
55 to 58 week	2.51^{a}	2.45^{a}	2.43^{a}	0.042	0.672
59 to 62 week	2.49^{a}	2.36^{b}	2.40^{b}	0.025	0.004
55 to 62 week	2.50a	2.41a	2.41^{a}	0.027	0.081
Hen-day egg production, %					
55 to 58 week	78.5^{a}	80.2^{a}	81.6^{a}	1.353	0.622
59 to 62 week	77.0^{b}	79.5^{a}	80.5^{a}	1.076	0.034
55 to 62 week	77.8^{b}	79.9^{b}	81.1^{a}	1.098	0.016
Egg weight, g					
55 to 58 week	62.6^{a}	63.0^{a}	63.0^{a}	0.319	0.558
59 to 62 week	62.8^{a}	62.9^{a}	62.7^{a}	0.322	0.631
55 to 62 week	62.7^{a}	63.0^{a}	62.9^{a}	0.297	0.529

Different small letters indicate a significant (P<0.05) difference within a row

Table 3. The effects of tea tree essential oil on activity of GSHPx, SOD and content of MDA in blood haemolysates of laying hens

Parameter	Dietary treatments with tea tree essential oil, mg/kg			SEM	P-value
	0 (T1)	40 (T2)	80 (T3)		
GSHPx, µmol/g Hb min	8.62^{b}	10.12^{a}	10.93^{a}	0.861	0.029
SOD, µmol/g Hb min	78.58^{b}	82.41^{a}	83.11^{a}	1.039	0.041
MDA, µmol/g Hb	0.47^{a}	0.42^{a}	0.49^{a}	0.021	0.526

Different small letters indicate significant (P<0.05) difference within a row

Table 4. The effects of tea tree essential oil on oocyst count in the faeces of laying hens

Average oocyst count/g of faeces	Dietary treatments with tea tree essential oil, mg/kg			SEM	P-value
	0 (T1)	40 (T2)	80 (T3)		
Start of 55th week	99.0^{a}	118.0^{a}	94.0^{a}	0.076	0.921
End of 58th week	152.0^{a}	31.0^{b}	0.0^{c}	0.911	0.018
End of 62nd week	139.0^{a}	16.0^{b}	0.0^{b}	0.343	0.038

Different small letters indicate significant (P<0.05) difference within a row

The suspension was then poured through a coarse gauze, via a funnel, into a glass beaker. The filtrate was mixed thoroughly and transferred to a centrifuge tube using a plastic pipette. The volume of fluid was marked and then centrifuged for 5 min at 1000 g. After removing the supernatant, the pellet was re-suspended in the same volume of saturated salt solution as the supernatant. The tube was inverted several times to obtain a homogeneous suspension before a subsample was transferred using a disposable plastic pipette from the central level of the centrifuge tube into a McMaster chamber, with grid dimensions of 1 cm × 1 cm = 1 cm^2, and with the volume of liquid below the grid of 0.15 mL. The tube contents were mixed again before the second chamber of the McMaster slide was filled with a second subsample. The prepared sample was not moved for 5 min before counting, allowing the oocysts to float up. All oocysts under the grid of each chamber were counted using 10 objectives, and the average mean of the two counts was calculated (HAUG et al., 2006).

Statistical analyses. The data obtained in the experiment firstly were tested for the normality of data spread, and then analysed by one-way ANOVA using the statistical software Statistica 12 (TIBCO Software Inc, USA), to determine whether variables differed between treatments. When the ANOVA showed statistical significance, Duncan's multiple range test was conducted, and P<0.05 indicated a significant difference.

Results

The effect of tea tree essential oil on laying hen production performance is shown in Table 2. Over the total 56 days of the experimental trial only egg production (EP) recorded statistically significantly different (P<0.05) results, while egg weight (EW), feed intake (FI) and feed conversion ratio (FCR) were not affected by the addition of essential oils. Hen-day egg production and the FCR were significantly improved (P<0.05) in the 59^{th} to the 62^{nd} week of the experimental period.

The GSHPx and SOD enzymatic activity and MDA concentration in blood haemolysates from the laying hens in control treatment T1 and experimental treatments, T2 and T3, supplemented with 40 and 80 mg/kg tea tree essential oil, respectively, are shown in Table 3.

Enzymatic activity of GSHPx and SOD increased significantly in treatments T2 and T3 compared to treatment T1 (P<0.05). The addition of dietary tea tree oil did not lead to significant (P>0.05) changes in MDA concentration between the control and the experimental groups.

The results in Table 4 show the statistically significant (P<0.05), dose dependent, influence of dietary tea tree essential oil on the reduction of oocysts in the faeces of laying hens.

The numbers of oocysts in the faeces of laying hens at the beginning of the experiment in the 55^{th} week of age of the laying hens were uniform and ranged between 90.0 (T3) to 118.0 (T2) per g, without statistically significant differences (P>0.05). The laying hens supplemented with dietary tea tree essential oil in group T2 at the end of the 58^{th} week of age showed a statistically significant (P<0.05) reduction in the total number of oocysts compared to the control treatment T1, and the same tendency was maintained until the end of the experimental period, in the 62^{nd} week. Dietary supplementation of tea tree essential oil in group T3 led to statistically significant (P<0.05) and total elimination of total oocysts at the end of the 58^{th}, as well at the end of the 62^{nd} week of the hens' life. Therefore, it can be concluded that dietary tea tree essential oil was effective in both concentrations in reducing (T2) and eliminating (T3) the total oocyst output in laying hens.

Discussion

Generally it is assumed that essential oils, herbs, spices and medicinal plants (PUVAČA et al., 2018) might improve the palatability of feed, due to their flavourful characteristics, and hence could promote feed intake when added to poultry diets (PUVAČA et al., 2016). RADWAN et al. (2008) compared four herbs (thyme, oregano, rosemary, turmeric) at an inclusion level of 5 or 10 g/kg diet in a study with 28-week old laying hens, for 12 weeks. They found no differences in feed intake between the treated groups and furthermore did not observe any differences when comparing the treatments to the untreated control group, what is in agreement with our research. Similar results were obtained when garlic was included in diets fed to laying hens, in concentrations of 20, 40, 60, 80 or 100 g/kg diet at nearly the same age (27-28 weeks), but for only 5-6 weeks (CHOWDHURY et al., 2002). However, ABDO et al. (2010) found that the average feed intake was significantly reduced in hens fed either 10 to 50 g/kg of green tea essential oil when compared to a non-supplemented control treatment. However, in the present study, hen-day egg production and the FCR improved significantly in weeks 59 to 62, however, during the total of 56 days of the feeding trial, egg production recorded significant ($P<0.05$) results, while egg weight, feed intake and FCR were not affected with or without dietary supplementation of tea tree essential oils, which is in agreement with other research (BÖLÜKBAŞI et al., 2009; BÖLÜKBAŞI et al., 2010). Essential oils as performance enhancers for laying hens, have primarily been supplemented to increase the utilization of the limit-fed diet and, in turn, improve egg production. Some studies showed positive effects on performance traits, such as egg production rate, which was also the case in our research, then egg weight and egg mass output, whereas some other studies demonstrated no effect of essential oil addition (NASIR et al., 2005).

TORKI et al. (2018) conducted an experiment to determine the effects of adding essential oils of rosemary, dill and chicory extract to diets, on the performance, egg quality and enzyme activity of laying hens subjected to heat stress. Dietary dill had a significant effect on egg index under heat stress conditions. The birds given rosemary, dill and chicory alone exhibited lower serum cholesterol and triglyceride concentration under thermo neutral and heat stress conditions, compared to those fed the basal diet ($P<0.05$). The synergistic effects between dietary rosemary, dill and chicory significantly ($P<0.05$) increased GSHPx activity compared to those that received only rosemary, dill or chicory, where it was not recorded. ABD EL-HACK and ALAGAWANY (2015) in their research evaluated the effect of thyme (*Thymus vulgaris* L.) on productive performance, egg quality, blood profile, immune function and antioxidant enzyme activity in laying hens. Their research has showed that dietary supplementation with thyme produces a significantly positive effect on parameters related to immunity and lipid profile. Serum superoxide dismutase (SOD) activity and reduced glutathione concentration were significantly increased in groups fed diets with thyme, which was in accordance with our research with dietary tea tree oil. The malondialdehyde (MDA) concentration decreased in the experimental groups in comparison with the control, while in our research with tea tree oil, no changes in MDA concentrations were reordered.

BASMACIOGLU et al. (2004) conducted an experiment to compare the effects of two essential oils, fed individually or in combination with alpha-tocopheryl acetate (alpha-TA) on performance parameters and lipid oxidation in broiler meat enriched with n-3 PUFAs. They came to the conclusion that essential oil shows a positive influence on lipid oxidation as measured in TBARS, and that the essential oils could be usefully used as a natural antioxidant to inhibit lipid oxidation. The beneficial effects of turmeric (*Curcuma longa*) on liver health, and its immunomodulatory and antioxidative effects in poultry nutrition were elaborated in research by KHAN et al. (2012).

Biochemical blood parameters are usually related to health status. These parameters are vital indicators of the nutritional and physiological status of laying hens. The effects of thyme supplementation on the blood metabolites of laying hens in the research by ABD EL-HACK and ALAGAWANY (2015) showed that blood protein, albumin, urea, total cholesterol, IgG and IgA were significantly

($P<0.05$) influenced by thyme supplementation, while HDL-cholesterol and IgM were not affected. Dietary supplementation of thyme exhibited a significantly positive impact on parameters related to immunity and lipid profile, which is in accordance with the findings of HASHEMIPOUR et al. (2013), as well as with the findings in our research. In the poultry industry, it is important to stimulate the immune system to reduce or prevent infectious diseases. The usage of immune enhancers is a key solution in improving immunity and reducing susceptibility to infectious diseases on poultry farms (PUVAČA, 2018). Tea tree essential oils, which are rich in flavonoids, extend the biological activity of ascorbic acid, act as antioxidants and may enhance immune function in poultry (PUVAČA et al., 2018).

Adding natural herbal additives to layer diets may improve the immune system due to the increase in immunoglobulin concentrations (IgG, IgA and IgM) in layers given diets with medicinal and aromatic plants, versus hens fed a control diet. It is likely that a lower dose of natural phytogenic feed additives may be needed to stimulate the humoral immune response. Previous studies showed that medicinal plants, spices, aromatic plants and their ingredients could activate immune functions, such as lymphocyte proliferation, phagocytosis, and red and white blood cell counts and haemoglobin levels (HASHEMIPOUR et al., 2013; KOSTADINOVIĆ et al., 2016).

The effect of Mentha piperita essential oil was investigated on performance in poultry after experimental infection with coccidia *Eimeria tenella* (5×10^4 oocytes/bird) compared with salinomycin. It was concluded that the essential oil of Mentha piperita significantly reduced *E. tenella,* but these effects were still lower in the coccidiostatic salinomycin (KOSTADINOVIĆ et al., 2010). GIANNENAS et al. (2003) examined the anti-coccidial effect of the essential oils of oregano, and found that the essential oil of oregano, added to broiler nutrition at an inclusion rate of 300 mg/kg, caused the suppression of coccidiosis caused by *E. tenella*. YOUN and NOH (2001) reported the most pronounced anti-coccidial effect by *Sophora flavescens* extract against *E. tenella,* of fifteen plants studied, which was even stronger than *Artemisia annua*.

KHAN et al. (2008) compared the effect of selected botanical supplements (*Polygonum bistorta* and *Agele marmelos*) with homeopathic preparations (*Mercurius solubilis* and *Darvisul liquid*) on the suppression of coccidiosis in poultry. They concluded that the herb extracts examined expressed anti-coccidial activity and increased feed digestibility, daily weight gain and reduced poultry mortality rate, which is in accordance with our investigation with tea tree essential oil. The influence of medicinal plant mixtures (*Artemisia absinthium*, *Thymus vulgaris*, *Menthae piperitae* and *Thymus serpyllum*) and their beneficial effects in broiler nutrition on biochemical blood status, was described in research by POPOVIĆ et al. (2018).

An *in vivo* study, testing the anti-coccidial activities of artemisinin isolated from the plant *Artemisia sieberi* on broiler chickens, showed that the extract reduced the number of *E. tenella* and *E. acervulina* oocytes, but not *E. maxima* (ARAB et al., 2006). The anti-coccidial activity of the plants *Artemisia annua* and *Pimpinella anisum* on *E. tenella* oocytes were examined by DRAGAN et al. (2010). *Artemisia annua* caused a significant reduction (90.7%) in the number of oocytes in the faeces of broilers infected with *E. tenella,* compared with the infected control group that was fed a standard diet.

According to SOARES et al. (2004) coccidiosis continues to affect caged layers worldwide, despite the fact that they are kept on wire throughout their productive life. Coccidiosis in caged layers, diagnosed either in clinical or subclinical forms, has one feature in common: unpredictability. Despite the use of a vaccine, coccidiosis remains problematic, particularly at the beginning of laying, probably in relation to the low protective immunity because of the lack of vehicles for recycling.

Ten or fifteen years ago, when most laying hens were reared in conventional cages, coccidiosis hardly occurred at all. It was very rare that the birds had the chance to come into contact with their faeces, and a few cases appeared on those occasions when hens could peck the manure belt of the floor above. The greatest evidence of this new situation is that coccidiosis vaccination in laying hens has increased considerably in the EU, but also outside the EU. Many egg producers have taken the decision to vaccinate all their flocks against coccidiosis, as the disease has started to appear more frequently in these semi-floor type rearing systems.

Having in mind drug residues in eggs for human consumption, further perspectives should be addressed to find natural and safe alternatives (PUVAČA et al., 2018), such as for example tae tree essential oil, which showed very promising results in this experiment.

Conclusions

On the basis of the results obtained, it can be concluded that the dietary addition of tea tree (*Melaleuca alternifolia*) essential oil in the nutrition of laying hens had a positive effect on the productive results of the hens, and led to a significant reduction in the number of oocysts, indicating that tea tree essential oil could be used as a prophylactic feed additive. Nevertheless, *Melaleuca alternifolia* essential oil showed important role in antioxidative protection of laying hens, which is of great importance in the treatment of coccidiosis.

Acknowledgements

The paper is part of the research work in 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 February 2019
Accepted: 1 April 2019

PUVAČA, N., D. HORVATEK TOMIĆ, S. POPOVIĆ, S. ĐORĐEVIĆ, I. BRKIĆ, N. LALIĆ, T. SHTYLLA KIKA, E. LIKA: Utjecaj esencijalnog ulja čajevca (*Melaleuca alternifolia*) kao dodatka prehrani na proizvodna svojstva, oksidacijski status krvi i liječenje kokcidioze u kokoši nesilica. Vet. arhiv 90, 331-340, 2020.

SAŽETAK

Cilj ovoga rada bio je istražiti utjecaj esencijalnog ulja čajevca (*Melaleuca alternifolia*) u prehrani kokoši nesilica na proizvodnju jaja, antioksidacijsku aktivnost te kao mogući lijek za kokcidiozu. Ukupno 360 kokoši nesilica Lohmann Brown u dobi od 54 tjedna podijeljeno je u tri različite skupine s obzirom na prehranu. Skupina T1 nije dobivala ulje čajevca, skupina T2 dobivala je 40 mg/kg, a skupina T3 80 mg/kg esencijalnog ulja čajevca. Svaka je skupina bila podijeljena u četiri podskupine po 30 kokoši. Pokus je trajao 56 dana (od 55. do 62. tjedna starosti kokoši). Tih je 56 dana podijeljeno u dva razdoblja od 28 dana: prvo razdoblje (55. do 58. tjedan starosti kokoši) i drugo razdoblje (59. do 62. tjedan starosti kokoši). Ustanovljen je krvni oksidacijski status kokoši, uključujući glutation-peroksidazu (GSHPx), aktivnost superoksid-dismutaze (SOD) i koncentraciju malondialdehida (MDA) te antikokcidijalni učinak čajevca na parazit *Eimeria* spp. u usporedbi s kontrolnom skupinom koja nije dobivala ulje čajevca. U skupinama koje su dobivale esencijalno ulje čajevca dnevna proizvodnja jaja znakovito je povećana ($P < 0,05$). Dodatak ulja čajevca kao prirodnog kokcidiostatika u skupinama T2 i T3 pokazao je statistički znakovit porast ($P < 0,05$) aktivnosti SOD-a i GSHPx u usporedbi s kontrolnom skupinom T1, dok u koncentraciji MDA-a nisu uočene znakovite promjene ($P > 0,05$). Antikokcidijalna aktivnost esencijalnog ulja čajevca utjecala je na znakovito smanjenje ($P < 0,05$) broja oocista *Eimeria* spp. po kokoši. Na temelju rezultata ovoga istraživanja može se zaključiti da je dodatak esencijalnog ulja čajevca povećao dnevnu proizvodnju jaja i smanjio formiranje oocista, stoga se ono može upotrijebiti kao profilaktički dodatak prehrani kokoši nesilica. Također se pokazalo da je ulje čajevca važno u aktivaciji antioksidacijskog zaštitnog sustava u kokoši.

Ključne riječi: kokoši; ulje čajevca; *Melaleuca alternifolia*; kokcidioza; jaja; antioksidacijska zaštita; prehrana