

Potential of wormwood (*Artemisia absinthium*) as a feed supplement in rabbit diet: effect on controlling rabbit coccidiosis, antioxidative systems and growth performance

Sanja J. Popović^{1*}, Ljiljana M. Kostadinović¹, Nikola M. Puvača²,
Bojana M. Kokić¹, Ivana S. Čabarkapa¹, and Olivera M. Đuragić¹

¹Institute of Food Technology, University of Novi Sad, Novi Sad, Serbia

²Patent Co. DOO, Mišičevo, Serbia

POPOVIĆ, S. J., LJ. M. KOSTADINović, N. M. PUVaČA, B. M. KOKIĆ, I. S. ČABARKAPA, O. M. ĐURAGIĆ: Potential of wormwood (*Artemisia absinthium*) as a feed supplement in rabbit diet: effect on controlling rabbit coccidiosis, antioxidative systems and growth performance. Vet. arhiv 87, 769-782, 2017.

ABSTRACT

The objective of the study was the investigation of dietary inclusion of *Artemisia absinthium* as a potential anticoccidial drug in rabbit diet, as well as a growth promoter and antioxidant in rabbit nutrition. The *in vivo* investigation was carried out on 120 male New Zealand white rabbits. The rabbits were distributed into four dietary treatments with ten replicates each. Two levels of whole wormwood plant were used: 100 g/kg (WW100) and 200 g/kg (WW200). The control treatment (C) was basal diet, and one treatment (CR) received chemical coccidiostat robenidine, supplemented in the amount of 6 g/kg. The rabbits were given ad libitum access to water and feed throughout the entire trial period. Feed intake was measured on a daily basis, while body weight was monitored at an individual level during the entire experimental period every seven days, for a total of 77 days. Blood and liver samples were collected for enzyme analyses, where oocyst counts were determined in 1 g of rabbit feces. At the end of the trial, the addition of 200 g/kg of wormwood led to the highest body weight (3047 g), as well to the lowest feed conversion ratio (3.20 kg/kg) with significant differences compared to the control treatment (3.75 kg/kg). The highest recorded glutathione reductase value was with treatment WW100, with significant differences to other experimental treatments. Consumption of coccidiostat and dried wormwood plant in different levels led to a notably lower malondialdehyde content in liver homogenate compared to the control treatment. Finally, the lowest number of oocysts was found in the treatment receiving wormwood in a concentration of 100 g/kg. On the basis of the results achieved, it may be noticed that wormwood supplementation in the diet of rabbits has a positive influence on growth performance, as well on antioxidative systems in rabbits. Subsequently, it was shown that wormwood can successfully be used in reducing the number of oocysts in rabbits.

Key words: antioxidative system, coccidiosis, growth performance, rabbit, wormwood

*Corresponding author:

MSc Sanja Popović, University of Novi Sad, Institute of Food Technology, Bulevar cara Lazara 1, Novi Sad, Serbia, Phone: +381 21 485 3775, E-mail: sanja.popovic@fins.uns.ac.rs

Introduction

Coccidiosis is a contagious sporozoal infection of the intestine, but hepatic coccidiosis is also possible (PAKANDL, 2009). Coccidiosis, caused by the *Eimeria* species, results not only in enormous economic losses in rabbit farming, when mortality rate may be between 12 and 20%, and may reach up to 50% mainly in rabbits at 4 to 8 weeks of age (RASHWAN and MARAI, 2000), but it is also responsible for a decrease in daily increment, prolonged fattening, poorer skin pigmentation, slower feed conversion and increased mortality (LI and OOI, 2008).

In the last decade the use of antibiotics, such as sulfonamides as well as salinomycin and robenidine, which are mainly used in rabbit nutrition for the treatment of coccidiosis, has been questioned. The increasing resistance of avian coccidia (protozoa) to anticoccidial drugs currently used by the animal industry has stimulated the search for new alternatives in solving such disease problems (KOSTADINOVIĆ et al., 2014). Subsequently, the ban on antibiotics in 2006 for use in animal nutrition due to the emergence of microbe resistance, has led to an intensive search for alternative solutions (STEINER, 2009) which are safer and more environmentally friendly.

Medicinal plants have recently been reported as potential alternatives to anticoccidial drugs since they have been found to be effective for a broad range of parasites, such as protozoa, arthropods and helminthes (KOSTADINOVIĆ et al., 2014; ROJHAN, 2000). *Artemisia absinthium*, known as wormwood, from the family *Compositae* (*Asteraceae*), is one of 300 species from this genus. The essential oil composition of this plant showed that its active substances include silica, two bitter substances (absinthin and anabsinthine), thujone, tannic, resinous substances, malic acid, and succinic acid (HABIBI et al., 2016). The essential oil obtained from wild plant wormwood shows antibacterial, antifeedant, antipyretic, fertility increasing, cytostatic and antimalarial activities (KOSTADINOVIĆ et al., 2014) and stimulates the digestion process (LANGHOUT, 2000). Therefore, medical herbs used in animal nutrition may have promising potential as growth and health promoters, without any adverse effects (PUVAČA et al., 2015; KOSTADINOVIĆ et al., 2014).

Increases in the level of reactive oxygen species (ROS), which are formed by living organisms as a result of normal cellular metabolism (BIRBEN et al., 2012), may lead to oxidative destruction of cells, known also as an 'oxidative stress' (MITTLER, 2002). As a consequence of oxidative stress, numerous pathological changes, such as membrane dysfunction, DNA damage, inactivation of proteins, and also cancer, arthritis and neurodegenerative disease, may occur in humans, but also in animal organisms (HAYES and McLELLAN, 1999). However, aerobic organisms possess highly effective protective systems from free radicals which consist of enzymatic and nonenzymatic antioxidants that are usually effective in blocking the harmful effects of ROS (BIRBEN et al., 2012).

The liver is an organ with a central metabolic role in the organism, known as 'the main laboratory' since it performs the major detoxification tasks (KOSTADINOVIĆ et al., 2015). Hence, various mechanisms of protection are involved during the defensive processes. Antioxidant enzymes prevent the excessive and harmful effects of reactive oxygen. For instance, superoxide dismutase (SOD) catalyzes the conversion of superoxide anion radical to H_2O_2 and reduction of H_2O_2 to water, while glutathione peroxidase (GSHPx) acts in conjunction with other enzymes to reduce H_2O_2 and to terminate lipid peroxidation (HALLIWELL and CHIRICO, 1993). Also, glutathione (GSH) plays an important role in the reduction of acute toxicity of xenobiotics and products of lipid peroxidation as a substrate for GSHPx, and can be regenerated by the enzyme glutathione reductase (GR). Lipid peroxidation is the process in which free radicals 'steal' electrons from the lipids in cell membranes, resulting in cell damage. It most often affects polyunsaturated fatty acids, since they contain multiple double bonds, between which there are methylene bridges (-CH₂-) that possess especially reactive hydrogens, leading to formation of lipid peroxides such as malondialdehyde (MDA) and hydroxyl, and hence, resulting in tissue or cell damage (OSTALOWSKA et al., 2006). For this reason, MDA content plays a major role as an indicator of the lipid peroxidation level, and as an indirect reflection of the level of cell damage.

The study was conducted in order to evaluate the *in vivo* efficacy of different doses of wormwood in lowering the number of eimerian oocysts in New Zealand White rabbits, the antioxidant efficacy, as well as growth promoter efficacy of a rabbit diet supplemented with wormwood.

Materials and methods

Pre-experimental investigation. Before the experimental trial, the symptoms of naturally infected animals were noticed in rabbits and subsequently recorded daily. All rabbits were found to be infected with *Eimeria* species in view of the increased mortality rate and bloody diarrhea which was confirmed on the basis of fecal examination.

The direct smear and the saturated sugar floatation technique were used for determining the presence and identification of eimerian oocysts (SOULSBY, 1982). The number of coccidial oocysts per gram (OPG) of feces were determined using the McMaster technique. Samples of the livers of dead rabbits were collected and examined for gross lesions, whereby the liver tissues were used for histopathological examination.

Collection and processing of plant materials. The plant used for this study was wormwood (*Artemisia absinthium*). The stem bark of the plants was collected in April and May 2014 from The Institute for Medicinal Plant Research 'Dr Josif Pančić', Belgrade. The stem bark of the plants was dried under shade for 10 days at 8 h per day, ground into powder and stored for later use in the study.

Experimental design. Experiments under *in vivo* conditions were performed on 120 seven-week-old male New Zealand white rabbits which were naturally contaminated by the *Eimeria* species, as mentioned above, with an initial body weight of 1050-1200 g. Infected rabbits were randomly divided into four dietary treatments. Each treatment consisted of 10 replicates of three rabbits housed in one cage according to the experimental design given in Table 1. Two levels of whole wormwood plant were applied: 100 g/kg (WW100) and 200 g/kg (WW200). The control treatment (C) was a basal diet (Table 2), and one treatment (CR) received chemical coccidiostat robenidine, supplemented in the amount of 6 g/kg. The rabbits were fattened from 7 to 17 weeks of age. They were housed at room temperature (22 ± 2 °C) and with a 12h light/dark cycle (lights on: 08:00-20:00 h), in wire mesh cages, which were cleaned daily. The rabbits were fed using a standard basal diet with access to water and feed ad libitum.

Table 1. Experimental design

	Treatment			
	C	CR	WW100*	WW200*
Additive	None	Coccidiostat robenidine	Wormwood	Wormwood
Concentration of additive in rabbits diet (g/kg)		6	100	200

* Medical herb is added on top on the basic diet; C-Control treatment; CR-robenidine treatment; WW100-wormwood treatment with addition of 100 g/kg of dry herb; WW200-wormwood treatment with addition of 200 g/kg of dry herb

Body weight gain, feed intake and feed conversion ratio. Each rabbit was weighed weekly from weaning to 119 days of age, while feed intake was measured on a daily basis. Daily feed intake and daily weight gain were subsequently calculated. The feed conversion ratio was calculated as the ratio between daily feed intake and daily body weight gain.

Table 2. Chemical composition of the basal diet

Ingredient	%
Proteins	15.0
Moisture	13.5
Cellulose	14.0
Ash	8.00
Ca	1.00
P	0.75
Na	0.25

In 1 kg of diet: Vitamin A 8000 IU, Vitamin D3 1200 IU, Vitamin E 25 mg, Vitamin K 1 mg, Vitamin C 100 mg, Vitamin B1 2.5 mg, Vitamin B2 6 mg, Vitamin B6 3 mg, Vitamin B12 0.02 mg, Nicotinic acid 50 mg, Pantothenic acid 15 mg, Folic acid 0.5 mg, Biotine 0.2 mg, Choline chloride 800 mg, Cu 5 mg, Fe 60 mg, Zn 60 mg, Mn 20 mg, Mg 100 mg, J 0.6 mg, Co 0.1 mg, Se 0.1 mg, Antioxidant (BHT) 100 mg.

Preparation of blood hemolysates. On day 119, two rabbits from each pen (20 per treatment) were randomly selected and blood samples were collected from a marginal ear vein of rabbits that had fasted overnight. The plasma was separated by centrifugation (3500 g, 10 min) and stored at -70 °C for analysis of biochemical parameters. The remaining red blood cells (RBCs) were washed three times with isotonic NaCl (0.15 M) prior for use in the biochemical assay. RBC hemolysates were prepared by diluting RBCs 1:10 with ice-cold double distilled H₂O, shaken vigorously to force hemolysis, and stored at -70 °C (FEBEL et al., 2008).

Preparation of liver homogenate. At the end of the trial period, the rabbits were processed after a 12 h feed withdrawal period. At sacrifice, the livers were rapidly removed and washed in cold 0.9% normal saline. One gram of the tissue was minced with scissors and homogenized in an ultratorax in 3 volumes of isotonic buffer (0.05 M Tris-HCl, 0.25 M sucrose, pH = 7.5). The homogenate was filtered through gauze into ice-cold tubes and aliquoted for further analysis (CHIU et al., 1976).

Sample preparation for glutathione determination. Proteins from freshly prepared liver homogenates were separated by adding half a volume of 10% sulfosalicylic acid and centrifugated at 5000 g, for 5 min, at 4 °C. The supernatant was stored at 4 °C, without freezing, and GSH was determined within 24 hours. The GSH content in the liver homogenate was determined from the amount of sulfhydryl residues by means of Ellmann's reagent (KAPETANOVIĆ and MIEYAL, 1979).

Determination of enzymatic activity. Superoxide-dismutase (EC1.15.1.1) 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 glutathione-peroxidase (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 the glutathione-reductase (EC 1.6.4.2.) was determined from the rate of NADPH oxidation and it was monitored by measuring the absorbance at 340 nm (LUKASZEWICZ-HUSSAIN and MONIUSZKO-JAKONIUK, 2004). Malondialdehyde concentration in the serum was measured by the 2-thiobarbituric acid assay (SIMON et al., 1974).

Oocyst count (OPG index). The fecal samples were examined for all the rabbits before administering wormwood. In weeks 7, 12 and 17, oocyst counts per gram of rabbit feces were determined.

To estimate the coccidian infection rates, fecal samples of around 30 g were collected from each cage using mosquito net mesh, and the number of oocysts in ten samples per gram of feces was determined. The same procedure was repeated for each cage in each

treatment. The samples were kept refrigerated prior to analysis. Determination of OPG and eggs per gram of feces was performed using the McMaster technique.

Statistical analyses. The mean \pm SEM values were calculated for each treatment to determine the significance of inter treatment differences. Each parameter was analyzed through analysis of variance (ANOVA) using STATISTICA 12.0 (2013). To find the difference between the dietary treatments, Fisher's LSD post-hoc test was used. Probability values lower than $P < 0.05$ were considered to be significant.

Results

Production performances. From Table 3 it may be concluded that the addition of wormwood plant to the diet of the rabbits led to significant ($P < 0.05$) differences in body weights. At the end of the trial rabbits in treatment WW200 achieved the highest body weight with significant differences ($P < 0.05$) compared to treatment CR and C (around +2% and +5%, respectively), but without any statistically significant difference ($P > 0.05$) compared to the treatment supplemented with 100 g/kg of wormwood, indicating that the addition of wormwood in treatments WW100 and WW200 exerted the stimulating effect in rabbits.

Table 3. Evaluation of the growth performance parameters of rabbits

Parameters	Treatment			
	C	CR	WW100	WW200
Live weight 35d	1103 \pm 24 ^a	1080 \pm 38 ^a	1092 \pm 78 ^a	1115 \pm 51 ^a
Live weight 119d	2890 \pm 61 ^a	2987 \pm 32 ^b	3002 \pm 41 ^c	3047 \pm 75 ^c
Daily feed intake (g/d)	75.4 \pm 3.0 ^a	75.9 \pm 5.0 ^a	77.9 \pm 2.0 ^b	85.4 \pm 7.0 ^c
Daily body weight gain (g/d)	20.1 \pm 1.5 ^a	23.2 \pm 1.1 ^b	23.9 \pm 0.9 ^b	26.7 \pm 1.8 ^c
Feed conversion rate	3.75 \pm 0.48 ^c	3.27 \pm 0.62 ^b	3.26 \pm 0.71 ^b	3.20 \pm 0.50 ^a

Results are given as mean \pm standard deviation (n = 10); ^{a-c}Means within a row with no common superscript differ significantly at $P < 0.05$; C-Control treatment; CR-robenidine treatment; WW100-wormwood treatment with addition of 100 g/kg of dry herb; WW200-wormwood treatment with addition of 200 g/kg of dry herb

Addition of coccidiostat and wormwood in treatments CR and WW100 led to a significantly ($P < 0.05$) lower feed intake compared to the WW200 treatment (around -11% and -9%, respectively). Also, the highest weight gain was recorded in the WW200 treatment and it was significantly higher compared to the other treatments. The lowest recorded daily weight gain was in the control treatment, while between treatments CR and WW100 no differences ($P > 0.05$) were noted.

The feed conversion ratio of rabbits in the experimental period was not uniform, and ranged between 3.20 and 3.75 kg of feed per kg of gain, with significant ($P < 0.05$)

differences. The addition of 200 g/kg of dry herbs led to improvement of the feed conversion rate by a half point compared to the control.

Antioxidative systems of rabbits. In the present study the antioxidant defense system (both enzymatic and nonenzymatic) in blood hemolysates (Table 4) and liver homogenates (Table 5) of the rabbits, was investigated.

As can be seen from Table 4, the content of GSH and MDA in the blood hemolysates of the rabbits treated with wormwood showed a decrease in comparison with the control (C) and robenidine (CR) treatments (around -1 point; $P < 0.05$), while glutathione peroxidase, superoxide-dismutase and glutathione reductase activities significantly increased in the WW100 treatment compared to the other diets.

Table 4. Evaluation of the antioxidative systems of rabbit's blood

Parameters	Treatment			
	C	CR	WW100	WW200
GSH (nmol/mg protein)	6.3 ± 1.0 ^b	6.1 ± 0.9 ^b	5.2 ± 0.7 ^a	4.9 ± 0.3 ^a
MDA (nmol/mL)	0.7 ± 0.1 ^b	0.6 ± 0.1 ^b	0.5 ± 0.2 ^a	0.5 ± 0.1 ^a
GSHPx (nmol/mg protein min)	7.4 ± 0.6 ^a	8.1 ± 0.9 ^a	8.4 ± 0.2 ^c	8.3 ± 0.3 ^b
SOD (nmol/mg protein min)	28.9 ± 1.7 ^a	68.4 ± 2.4 ^b	81.4 ± 1.1 ^c	70.4 ± 1.2 ^b
GR (nmol/mg protein min)	13.8 ± 1.0 ^a	15.8 ± 0.8 ^b	19.1 ± 0.6 ^c	15.9 ± 0.2 ^b

Results are given as mean ± standard deviation (n = 10); ^{a-c}Means within a row with no common superscript differ significantly at $P < 0.05$; C-Control treatment; CR-robenidine treatment; WW100-wormwood treatment with addition of 100 g/kg of dry herb; WW200-wormwood treatment with addition of 200 g/kg of dry herb

After completion of the experimental period, the lowest GSH contents achieved in liver homogenates were with treatments WW100 and WW200 (around -23%; Table 5) compared to the treatments CR and C ($P < 0.05$). Treatment with the basal diet achieved the highest MDA content compared to other treatments ($P < 0.05$).

Table 5. Evaluation of the antioxidative systems of rabbit's liver

Parameters	Treatment			
	C	CR	WW100	WW200
GSH (nmol/mg protein)	0.9 ± 0.2 ^c	0.8 ± 0.5 ^b	0.7 ± 0.3 ^a	0.6 ± 0.9 ^a
MDA (nmol/mL)	2.7 ± 0.4 ^b	1.6 ± 0.4 ^a	1.5 ± 0.9 ^a	1.7 ± 0.7 ^a
GSHPx (nmol/mg protein min)	4.3 ± 0.7 ^a	4.6 ± 0.8 ^b	5.2 ± 0.1 ^c	4.7 ± 0.2 ^b
SOD (nmol/mg protein min)	8.7 ± 0.8 ^a	9.2 ± 1.5 ^b	11.8 ± 0.2 ^c	11.1 ± 0.5 ^c
GR (nmol/mg protein min)	25.7 ± 2.0 ^a	29.6 ± 0.9 ^b	31.7 ± 1.7 ^c	29.8 ± 2.1 ^b

Results are given as mean ± standard deviation (n = 10); ^{a-c}Means within a row with no common superscript differ significantly at $P < 0.05$; C-Control treatment; CR-robenidine treatment; WW100-wormwood treatment with addition of 100 g/kg of dry herb; WW200-wormwood treatment with addition of 200 g/kg of dry herb

Like blood hemolysates, GSHPx, GR and SOD activities in the liver were systematically higher with the addition of wormwood in treatment WW100 ($P < 0.05$). There was no difference in GSHPx activity between the treatments treated with commercial coccidostat and the rabbits receiving 200 g/kg of wormwood. Furthermore, SOD activity was the same for the two levels of *Artemisia absinthium*.

Finally, it was noticed that the content of GSH in the blood and liver samples of rabbits treated with wormwood showed a significant decrease ($P < 0.05$) in comparison with the control and robenidine treatments. The same tendency was recorded for MDA content. GSHPx activity in both samples was the highest in the WW100 treatment, and was statistically significantly different ($P < 0.05$) from the other treatments. A similar tendency was observed in the activities of SOD and GR in the blood and liver samples of rabbits, where the highest values were recorded in the treatments receiving 100 g/kg of wormwood with a significant difference ($P < 0.05$) in comparison with the control and robenidine treatments.

Oocysts counts. The addition of wormwood to the diet of rabbits led to statistically significant ($P < 0.05$) differences in the number of oocysts per gram of feces (Fig. 1).

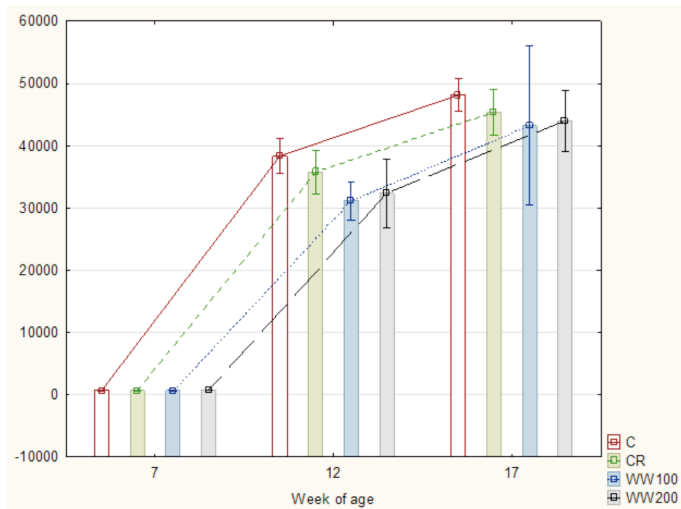


Fig. 1. Coccidiosis oocyst counts in rabbits fecal samples (number of oocyst per gram of feces). C-Control treatment; CR-robenidine treatment; WW100-wormwood treatment with addition of 100 g/kg of dry herb; WW200-wormwood treatment with addition of 200 g/kg of dry herb

At the beginning of the study, there was no statistically significant difference in the OPG between the rabbits (mean of 707 OPG; $P > 0.05$). Later, the level of contamination by oocysts in the C treatment was systematically higher compared to the other treatments

($P < 0.05$), while there was no significant difference between treatments WW100 and WW200.

Finally, at the end of the experimental period, the lowest number of oocysts achieved was with treatment WW100 (40815 oocysts per g of feces) followed by treatments WW200 (43968 oocysts per g of feces) and CR (45338 oocysts per g of feces).

Discussion

The present investigation represents a novelty in field of betel against coccidiosis in rabbits, since to the best of our knowledge there are no relevant data in the available literature on the effects of wormwood (*Artemisia absinthium*) in rabbits, but mostly in chickens. Therefore, the discussion will present our findings compared with other available published works related to coccidiosis.

The results obtained indicate that the addition of wormwood powder to rabbit diet has numerous positive effects on rabbit health. The reported results from this study showed that the inclusion of wormwood in rabbit diet produced better growth performance, including higher live weight and lower feed conversion ratio. According to PUVAČA et al. (2015) a lower feed conversion ratio shows that the addition of medicinal plants and spices has a positive influence on feed utilization and efficiency. However, the research by IQBAL et al. (2013) does not agree with the results obtained in this study, revealing that the inclusion of ethanolic extract of *Artemisia absinthium* at level of 2 g/kg BW in the diet of goat kids resulted in slightly lower body weights compared to commercial products. From this it may be inferred that the inclusion of dried wormwood whole plant in rabbit diet was satisfactorily acceptable without any adverse effects.

Antioxidant enzymes, such as glutathione peroxidase and superoxide dismutase, have the capacity to break down free-radical reactions using a chain reaction mechanism (BENZIE, 2003). Glutathione and glutathione peroxidase are among the major antioxidant defenders, wherein GSH plays a major role in reduction of the acute toxicity of xenobiotics and products of lipid peroxidation as a substrate for GSHPx (MAK et al., 1996). Subsequently, with the increased risk of lipid peroxidation in the liver, there is an increase in the enzymatic activity of GSHPx. Depletion of GSH, causes the cells to be more resistant to free radical toxicity, indicating that GSH is involved in the reduction. In this study, the GSH concentration in both types of samples, blood hemolysates and liver homogenates, was the highest in the control group, compared to the other experimental groups. Hence, the decrease in GSH levels in the experimental treatments is an indication that detoxification is active in the organism. SOD enzyme catalyzes the dismutation of superoxide radicals into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), and consequently represents an important defense mechanism against superoxide radical toxicity (ASSADY et al., 2011). Hence, SINGH et al. (2011) reported that an increase in

catalytic activity of superoxide-dismutase is expected as part of the system defense. KOSTADINOVIĆ et al. (2015) showed that addition of *Artemisia absinthium* to broiler diet led to higher SOD activity in hemolysed blood and liver homogenates, which was also in agreement with the results reported by OLANLOKUN (2008). On the other hand, KOSTADINOVIĆ et al. (2015) reported that GSHPx levels were not altered by *Artemisia absinthium* supplementation, which is not in accordance with the results obtained in this study.

Lipid oxidation plays an important role in the deterioration of physiological functions such as growth, reproduction, and immunity, inducing higher susceptibility to infectious diseases. Malondialdehyde is used as a sensitive indicator to determine the level of oxidative stress in the organism. The determination of malondialdehyde has attracted widespread interest, since it appears to offer a simple means of assessing lipid peroxidation in biological materials (DRAPER and HADLEY, 1990). CHERIAN et al. (2013) reported that addition of *Artemisia annua*, also known as a sweet wormwood, had an influence on lipid oxidation products measured as TBA reactive substances (TBARS) which were lower in the breast and thigh muscle of birds fed different doses of sweet wormwood diets, compared with the control groups. The investigation of AOUADI et al. (2014) with the use of *Artemisia herba alba* and *Rosmarinus officinalis* essential oils (EOs) to evaluate the impact of the dietary administration of EOs on the overall antioxidant capacity of the muscle and on the oxidative stability of meat over a period of refrigerated storage, showed that MDA content was significantly lower in treatments treated with medical herbs in concentrations of 400 mg/kg each, compared to control treatments. On the basis of these mentioned studies it may be concluded that medical herbs play an important role in animal nutrition in terms of the oxidative stability of organisms. This was also reported by ALUWONG et al. (2013), who showed that nutrition plays a vital role in maintaining the pro-oxidant-antioxidant balance. Moreover, CAPCAROVA et al. (2010) and RAJPUT et al., (2013) noticed that some microorganisms could help in oxidation resistance, scavenge hydroxyl radicals and increase antioxidant capacity. Thus, the increased activity of all these antioxidant enzymes in the present study may be attributed to the environmental pathogens of *Eimeria* spp. in the rabbits. The results indicate that wormwood could improve antioxidant activities and reduce the damage caused by free radicals.

Nowadays, significantly higher attention is being paid to rabbit coccidia then earlier. Moreover, medical herbs are widely being used in different studies to treat cases of parasitism in ruminants, and enviable success has been achieved in this direction against a variety of parasites. Positive results concerning oocyst reduction were recorded in the research of KOSTADINOVIĆ et al. (2014), who reported a significant decrease in the fecal oocyst count obtained from chickens which were preventively treated with wormwood in a concentration of 3 g/kg, and subsequently infected with *Eimeria* spp. In research by the same authors, the reduction of the fecal oocyst count in broiler chickens fed with

the addition of wormwood was about 20% compared to the positive control. The most active components in wormwood are trans-sabinene, α - and β -thujon, trans-sabinyl acetate, linalyl acetate and cis-chrysanthenyl acetate (BLAGOJEVIĆ et al., 2006), which are responsible for anticoccidial activity in animals. In the investigation of TARIQ et al. (2009), with the use of *Artemisia absinthium* against intestinal nematodes of sheep, *A. absinthium* had a positive effective against coccidiosis. However, the results reported by IQBAL et al. (2013) showed that *Artemisia absinthium* is not so effective in reducing the fecal oocyst count in goat kids compared to commercially available anticoccidial products. RADOSTITS et al. (2007) showed that the susceptibility to coccidiostats varied, depending on the different stages of the life cycle of *Eimeria* spp.

Conclusions

On the basis of the results obtained numerous benefits may be noticed of supplementing the diet of rabbits with dried wormwood, without any adverse effects. Modification in rabbit nutrition led to improvement of rabbit production performances, which is related to its utilization as a natural additive. The use of wormwood could be beneficial in terms of antioxidant protection in rabbits, since the ability to activate the enzymes responsible for antioxidant protection of animals was noticed. Also, wormwood supplementation in rabbit diet led to a significant decrease in the number of oocysts per gram of feces. Given that these are preliminary results, further and more detailed investigations should be carried out in order to prove the anticoccidial effects of wormwood in rabbit diet, as well as its antioxidant activity.

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.

References

- ALUWONG, T., F. B. HASSAN, M. A. RAJI, M. U. KAWU, T. J., DZENDA, O. J. AYO (2013): Effect of different levels of supplemental yeast on performance indices, serum enzymes and electrolytes of broiler chickens. *Afr. J. Biotechnol.* 12, 5480-5485.
- AOUADI, D., G. LUCIANO, V. VASTA, S. NASRI, D. M. R. BROGNA, S. ABIDI, A. PRIOLO, H. B. SALEM (2014): The antioxidant status and oxidative stability of muscle from lambs receiving oral administration of *Artemisia herba alba* and *Rosmarinus officinalis* essential oils. *Meat Sci.* 97, 237-243.
- ASSADY, M., A., FARAHNAK, A. GOLESTANI, M. R. ESHARGHIAN (2011): Superoxide dismutase (SOD) enzyme activity assay in *Fasciola* spp. parasites and liver tissue extract. *Iran J. Parasitol.* 6, 17-22.

- BENZIE, I. F. (2003): Evolution of dietary antioxidants. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 136, 113-126.
- BIRBEN, E., U. M. SAHINER, C. SACKESEN, S. ERZURUM, O. KALAYCI (2012): Oxidative stress and antioxidant defense. *World Allergy Organ J.* 5, 9-19.
- BLAGOJEVIĆ, P., N. RADULOVIĆ, R. PALIĆ, G. STOJANOVIĆ (2006): Chemical composition of the essential oils of Serbian wild-growing *Artemisia absinthium* and *Artemisia vulgaris*. *J. Agric. Food Chem.* 54, 4780-4789.
- CAPCAROVA, M., J. WEISS, C. HRNCAR, A. KOLESAROVA, G. PAL (2010): Effect of *Lactobacillus fermentum* and *Enterococcus faecium* strains on internal milieu, antioxidant status and body weight of broiler chickens. *J. Anim. Physiol. Anim. Nutr.* 94, e215-e224.
- CHERIAN, G., A. ORR, I. C. BURKE, W. PAN. (2013): Feeding *Artemisia annua* alters digesta pH and muscle lipid oxidation products in broiler chickens. *Poultry Sci.* 92, 1085-1090.
- CHIU, D. T. Y., F. H. STULTS, A. L. TAPPEL (1976): Purification and properties of rat lung soluble glutathione peroxidase. *Biochim. Biophys. Acta* 445, 558-566.
- DRAPER, H. H., M. HADLEY (1990): Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol.* 186, 421-431.
- FEBEL, H., M. MEZES, T. PALFY, A. HERMAN, J. GUNDEL, A. LUGASI, K. BALOGH, I. KOCSIS, A. BLAZOVICS (2008): Effect of dietary fatty acid pattern on growth, body fat composition and antioxidant parameters in broilers. *J. Anim. Physiol. Anim. Nutr.* 92, 369-376.
- HABIBI, H., S. FIROUZI, H. NILI, M. RAZAVI, S. L. ASADI, S. DANESHI (2016): Anticoccidial effects of herbal extracts on *Eimeria tenella* infection in broiler chickens: *in vitro* and *in vivo* study. *J. Parasit. Dis.* 40,401-407.
DOI: 10.1007/s12639-014-0517-4
- HALLIWELL B., S. CHIRICO (1993): Lipid peroxidation: its mechanism, measurement and significance. *Am. J. Clin. Nutr.* 57, 715S-725S.
- HAYES, J. D., L. I. McLELLAN (1999): Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. *Free Radic. Res.* 31, 273-300.
- IQBAL, A., K. A. TARIQ, V. S. WAZIR, R. SINGH (2013): Antiparasitic efficacy of *Artemisia absinthium*, toltrazuril and amprolium against intestinal coccidiosis in goats. *J. Parasit. Dis.* 37, 88-93.
- KAPETANOVIĆ, I. M., I. I. MIEYAL (1979): Inhibition of acetaminophen-induced hepatotoxicity by phenacetin and its alkoxy analogs. *J. Pharmacol. Exp. Ther.* 209, 25-30.
- KOSTADINOVIĆ, LJ., J. LEVIĆ, S. POPOVIĆ, I. ČABARKAPA, N. PUVAČA, O. ĐURAGIĆ, Š. KORMANJOŠ (2015): Dietary inclusion of *Artemisia absinthium* for management of growth performance, antioxidative status and quality of chicken meat. *Arch. Geflügelkd.* 79.
DOI: 10.1399/eps.2015.75
- KOSTADINOVIĆ, LJ. M., I. S. ČABARKAPA, J. D. LEVIĆ, Š. M. KORMANJOŠ, S. J. TEODOSIN, S. A. SREDANOVIĆ (2014): Effect of *Artemisia absinthium* essential oil on antioxidative systems of broiler's liver. *Food Feed Res.* 41, 11-17.

- KOSTADINOVIĆ, LJ., Z. PAVLOVSKI, S. PAVKOV (2001): Effect of amprolium on the lipid peroxidation and the activity of superoxide dismutase in broilers blood and liver. Arch. Geflügelkd. 65, 118-122.
- LANGHOUT, P. (2000): New additives for broiler chickens. World Poultr. 16, 22-27.
- LI, M. H., H. K. OOI (2008): Effect of chromium compounds on sporulation of *Eimeria piriformis* oocysts. Exp. Anim. 57, 79-83.
- LUKASZEWICZ-HUSSAIN, A., J. MONIUSZKO-JAKONIUK (2004): Liver catalase, glutathione peroxidase and reductase activity, reduced glutathione and hydrogen peroxide levels in acute intoxication with chlorfenvinphos, an organophosphate insecticide. Pol. J. Environ. Stud. 13, 303-309.
- MAK, D. H., S. P. IP, P. C. LI, M. K. POON, K. M. KO (1996): Alterations in tissue glutathione antioxidant system in streptozotocin-induced diabetic rats. Mol. Cell. Biochem. 162, 153-158.
- MITTLER, R. (2002): Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7, 405-410.
- OLANLOKUN, J. O. (2008): Protective influence of vitamin E on the antioxidant defense system in the whole blood and liver of normal and alloxan-induced diabetic rats. Indian J. Clin. Biochem. Nutr. 23, 62-66.
- OSTALOWSKA, A., E. BIRKNER, M. WIECHA, S. KASPERCZYK, A. KASPERCZYK, D. KAPOLKA, A. ZON-GIEBEL (2006): Lipid peroxidation and antioxidant enzymes in synovial fluid of patients with primary and secondary osteoarthritis of the knee joint. Osteoarthr. Cartilage 14, 139-145.
- PAKANDL, M. (2009): Coccidia of rabbit: a review. Folia Parasitol. 56, 153-166.
- PUVAČA, N., LJ. KOSTADINOVIĆ, D. LJUBOJEVIĆ, D. LUKAČ, J. LEVIĆ, S. POPOVIĆ, N. NOVAKOV, B. VIDOVIĆ, O. ĐURAGIĆ (2015): Effect of garlic, black pepper and hot red pepper on productive performances and blood lipid profile of broiler chickens. Arch. Geflügelkd. 79.
DOI 10.1399/eps.2015.73
- RADOSTITS, O. M., C. GAY, K. HINCHCLIFF, P. CONSTABLE (2007): Veterinary Medicine. A textbook of the diseases of cattle, horses, sheep, pigs, and goats. Paratuberculosis (Johne's disease). 10th ed., Saunders Elsevier, Edinburgh, London, New York, Oxford, Philadelphia, St. Louis, Sydney, Toronto, pp. 1017-1044.
- RAJPUT, I. R., L. Y. LI, X. XIN, B. B. WU, Z. L. JUAN, Z. W. CUI, D. Y. YU, W. F. LI (2013): Effect of *Saccharomyces boulardii* and *Bacillus subtilis* B10 on intestinal ultrastructure modulation and mucosal immunity development mechanism in broiler chickens. Poultr. Sci. 92, 956-965.
- RASHWAN, A. A., I. F. M. MARAI (2000): Mortality in young rabbits: A review. World Rabbit Sci. 8, 111-124.
- ROJHAN, M. (2000): Medicine and herbal treatment. 4th ed., Tehran Alavi Press, Iran, pp. 20-22.

- SIMON, L. M., Z. FATRAI, D. E. JONAS, B. MATKOVICS (1974): Study of peroxide metabolism enzymes during the development of *Phascoulus vulgaris*. *Biochem. Physiol. Pflanz.* 166, 387-392.
- SINGH, S. K., U. DIMRI, M. KATARIA, P. KUMARI (2011): Ameliorative activity of *Withania somnifera* root extract on paraquat-induced oxidative stress in mice. *J. Pharmacol. Toxicol.* 6, 433-439.
- SOULSBY, E. J. L. (1982): Helminths, arthropods and protozoa of domesticated Animals. 7th ed., ELBS and Bailliere Tindall, London, 809 pages.
- STATISTICA (Data Analysis Software System) v.12.0. 2013. Stat-Soft, Inc., USA.
- STEINER, T. (2009): Phylogenetics in animal nutrition: Natural concepts to optimise gut health and performance. Nottingham University Press, England, 192 pages.
- TARIQ, K. A., M. Z. CHISHTI, F. AHMAD, A. S. SHAWL (2009): Anthelmintic activity of extracts of *Artemisia absinthium* against ovine nematodes. *Vet. Parasitol.* 160, 83-88.

Received: 4 July 2016

Accepted: 5 July 2017

POPOVIĆ, S. J., LJ. M. KOSTADINOVIĆ, N. M. PUVAČA, B. M. KOKIĆ, I. S. ČABARKAPA, O. M. ĐURAGIĆ: Potencijal pelina (*Artemisia absinthium*) kao dodatak u hranidbi kunića: utjecaj na kontrolu kokcidioze, antioksidacijski sustav i proizvodna obilježja. *Vet. arhiv* 87, 769-782, 2017.

SAŽETAK

Cilj je ovog rada bio istražiti dodatak pelina (*Artemisia absinthium*) u hranidbi kunića kao potencijalni antikokcidostatik, ali i kao promotor rasta i antioksidans. Pokus je izveden *in vivo* na 120 bijelih novozelandskih kunića muškog spola, a bio je ponovljen četiri puta pri čemu je formirano 10 skupina. Korištene su dvije koncentracije cijele biljke pelina: 100 g/kg (WW100) i 200 g/kg (WW200). Kunići u kontrolnoj skupini (C) bili su hranjeni smjesama bez ikakvih dodataka, dok je u hranidbi pokusnih kunića (CR) dodan kemijski kokcidostatik robenidin u količini od 6 g/kg. Voda i hrana bili su im dostupni *ad libitum* tijekom cijelog pokusnog razdoblja. Utrošak hrane bio je mjeren na dnevnom razini, dok je tjelesna težina bila praćena na individualnoj razini tijekom cijelog pokusa svakih sedam dana, ukupno 77 dana. Uzorci krvi i jetre bili su prikupljeni za enzimske analize, dok je broj oocista utvrđen po 1 g fecesa. Na kraju ekperimenta utvrđeno je da je dodatak 200 g/kg pelina doveo do najveće tjelesne mase kunića (3047 g) i do najmanje konverzije hrane (3,20 kg/kg) koja se značajno razlikovala u usporedbi s kontrolom (3,75 kg/kg). Najveća zabilježena vrijednost glutation reductaze bila je u tretmanu WW100 sa značajnim razlikama u odnosu na druge eksperimentalne tretmane. Upotreba kokcidostatika i osušene biljke pelina u različitim koncentracijama dovela je do znatno manjeg sadržaja malondialdehida u homogenatu jetre u usporedbi sa kontrolnim tretmanom. Također, najmanji broj oocista bio je zabilježen u tretmanu u kome je pelin dodan u koncentraciji od 100 g/kg. Na osnovi postignutih rezultata, može se primijetiti da dodatak pelina u hranidbi kunića ima pozitivan utjecaj na njihova proizvodna obilježja, kao i na antioksidacijske sustave. Također, ustanovljeno je da se pelin može uspješno koristiti za smanjivanje broja oocista.

Ključne riječi: antioksidacijski sustav, kokcidioza, proizvodna obilježja, kunić, pelin
