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Article

Influence of Wormwood Seeds on Enzymatic and Non–enzymatic Activity in Blood of Broilers with Coccidiosis

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Abstract: Wormwood plants have been of great botanical and pharmaceutical interest and are employed in folk medicine for a long time. Wormwood has been used successfully for many years as a remedy against malaria and has been reported to exhibit antiprotozoal potential. Having in mind that, this study aimed to investigate the effects of wormwood seeds on enzymatic activity and non–enzymatic activity in broilers infected with coccidiosis. A biological experiment with broiler chickens was performed on a total of 360 Ross 308 broiler chickens. Chickens were divided into three experimental treatments with six replication each, respectively. Treatment I (WW_1) was uninfected with coccidia oocyst and untreated, treatment II (WW_2) was infected with coccidia oocyst and was kept untreated, while treatment III (WW_3) was infected with coccidia oocyst and received wormwood seeds in feed in the quantity of 1.5%. Blood was collected for the evaluation of antioxidant status. Results of bloody diarrhea intensity of chickens have ranged from 0% (WW_1; day 26^{th}) to 99% (WW_2; day 28^{th}), respectively. Regarding the influence of dietary wormwood seeds on fecal oocyst counts obtained results showed the highest oocyst in treatment WW_2 on the 30^{th} day of the experiment (45115.7 oocyst number/g), which was significantly higher ($p<0.05$) when compared to treatments WW_3 (623.3 oocyst number/g), and WW_1 (0 oocyst number/g) on the same experimental day. Our results indicate a significant ($p<0.05$) increase in non-enzymatic activity and higher catalytic enzymatic activity in blood hemolysates of infected broilers. Based on our research it can be concluded that wormwood seeds can be used in feed for broilers as a prophylactic feed additive to prevent coccidia oocyst development.

Keywords: Wormwood; *Artemisia absinthium*; antioxidants; poultry; parasites; enzymes.

1. Introduction

The search for alternative strategies against coccidiosis has intensified due to increasing consumer concerns regarding conventional anticoccidial drugs' side effects on birds, the toxic effects of some of these drugs on animal species, and public health concerns about tissue residues [1–4]. Many common infectious diseases have been treated with plants since ancient times [5,6]. In herbal medicine, extracts and biologically active compounds isolated from plants have gained considerable attention because of the side effects and resistance pathogenic microbes can build up against antibiotics and many drugs available today [7–10].

There is a possibility that a medicinal plant or extract from an *Artemisia* species might be one of these potential candidates [11,12]. More than 300 species of *Artemisia* can be found worldwide in the family *Asteraceae*. One of these numerous plants is wormwood (*Artemisia absinthium*), more commonly known in the UK and France as absinthe, which has antibacterial, antifeedant, antipyretic, fertility-increasing, cytostatic, and antimalarial properties [13]. In folk medicine, wormwood leaves have been used for various ailments and have been of great botanical and pharmaceutical interest

[14]. There has been evidence that wormwood contains antiprotozoal properties and has been used successfully by the French army in Algeria for many years to treat malaria [15].

As a result of protozoal infection, such as coccidiosis, the intestinal mucosa is invaded and destroyed by oocysts of the genus *Eimeria*, which is found and spread in poultry farming environments [16,17]. A major economic loss occurs when coccidial disease reduces animal performance and lowers productivity in the poultry industry [18,19]. There are seven species of *Eimeria* in chickens that develop in specific areas of their digestive tracts [20]. Pathogens such as these can damage intestinal tissue, decrease feed intake and nutrient absorption, and make individuals more susceptible to secondary bacterial infections.

In feed, coccidiostats are used primarily to prevent coccidiosis [21]. The poultry industry has experienced high levels of development and prosperity because of these coccidiostats. The availability and effective use of coccidiostats are vital to the prevention of chicken coccidiosis [22]. In the commercial broiler industry, therefore, coccidiostats play a crucial role in preventing coccidiosis. Due to their extensive use over the last 50 years, *Eimeria* species have become resistant to these compounds [23,24]. The effectiveness of coccidiostats has been reduced by multi-drug resistance and cross-resistance [25,26].

As a natural dietary remedy against coccidiosis, wormwood has not been reported for its antiparasitic activity despite many pharmacological investigations. Using artificially infected broilers with coccidiosis, this study investigated the nutritional effects of wormwood seeds.

2. Materials and Methods

Experimental design

Animal welfare and animal protection principles were followed throughout the experimental protocol, which was approved by the University Ethics Committee. Three hundred and sixty one-day-old broilers of the hybrid line Ross 308 of both sexes were used in the biological experiments. Breeding was carried out under standard conditions recommended by the producer of hybrid chicks on a floor system with deep bedding. Broilers were fed by standard commercial complete diet with access to water and feed *ad libitum.* The possibility of infection was monitored daily by taking fecal samples. As recommended by the breeder of the broiler hybrid, temperature and lighting were also followed. The initial temperature of 32 °C was reduced weekly by 1 °C to a final temperature of 28 °C.

Infected and non-infected chickens were randomly assigned to treatment groups. Sporulated oocysts were collected from infected farms and administered to broilers in infected treatments. A suspension of 1 cm^3 oocyst was administered orally to chickens for infection.

Seeds of wormwood were obtained from the Institute for Medicinal Plant Research "Dr. Josif Pancic", Belgrade, Serbia.

Biological experiment

Randomly selected one-day-old broilers were divided into three dietary treatments consisting of 120 individual birds per treatment in six replicates:

WW_1: uninfected and unmedicated broilers – negative control group.

WW_2: infected and unmedicated broilers – positive control group.

WW_3: infected broilers which received wormwood seeds in the quantity of 1.5%.

Chickens were regularly monitored during the experiment, autopsies were conducted, and all findings are meticulously documented. The oocyst output was measured daily in each treatment on the 26^{th}, 27^{th}, 28^{th}, 29^{th}, and 30^{th} day of age, respectively.

To evaluate the effects of wormwood seed on avian coccidiosis induced by *Eimeria* spp., the mean number of oocysts per gram of feces in treated treatments was compared to the non-treated control treatment. The bloody diarrheal score was described as bloody diarrheal intensity and expressed in percentages.

Blood was collected at the 30th day of age, and hemoglobin level in haemolysed blood was determined using a commercial test (Dialab, Vienna, Austria) using the spectrophotometer (Multiscan MCC 340, Finland). Protein content was determined by the method of Surapneni and Chandrasada Gopan [27]. In haemolysed blood, glutathione content, products of lipid peroxidation, and the activities of antioxidant enzymes were determined.

Preparation of blood hemolysate

Heparinized test tubes were used to collect blood from broiler hearts. As soon as the erythrocytes were centrifuged (10 minutes at 3500 rpm and 4 °C) and the plasma was removed, erythrocytes were rinsed 3 times in saline to remove any remaining serum. In the second step, the erythrocyte pellets were suspended in double distilled water and vortexed. For further analysis, the hemolysate was centrifuged at 3500 rpm for 15 minutes after incubation for one hour at room temperature [28].

Determination of enzymatic activity

Spectrophotometric measurements were performed at pH=10.2 to determine the superoxide dismutase activity. As a substrate, cumene hydroperoxide was used for spectrophotometric measurements of glutathione-peroxidase activity at 412 nm. An absorbance measurement at 340 nm was used to determine the glutathione–reductase activity. As an electron acceptor, hydrogen peroxide was used as a catalyst to oxidize guaiacol catalytically. To determine the activity of xanthine-oxidase, uric acid was oxidized with xanthine and at 295 nm, spectrophotometer measurements were made in pH=7.5 phosphate buffer containing 0.1 mmol/dm^3.

Determination of non-enzymatic activity

A thiobarbituric acid (TBA) test was used to determine the content of lipid peroxides. By reacting lipid peroxides with thiobarbituric acid, the oxidation of cellular membrane lipids was measured [29].

In addition to 10% sulfosalicylic acid, half the volume of freshly prepared hemolysates was centrifuged at 5000 rpm for 5 min, at 4°C, to separate proteins. Glutathione was determined within 24 hours after the supernatant was stored at 4 °C without freezing. Based on Ellmann's reagent's determination of sulfhydryl residues in blood hemolysate, glutathione content has been determined.

Statistical analysis

The one-way ANOVA analysis was performed to assess data differences between various groups using Statistica software version 13 (StatSoft inc. 2013; USA). The data means were considered different at $p<0.05$.

3. Results and Discussion

Anticoccidial activity of wormwood seeds

Bloody diarrhea was observed on the 26th, 27th, 28th, 29th, and 30th day of the experiment, while chickens were infected with *Eimeria* spp., on the 21st day of the experiment.

Results in Figure 1. show that the intensity of bloody diarrhea was lower in treatment WW_3 in chickens with dietary wormwood seeds in concentrations of 1.5%, in comparison to other experimental treatments WW_1, and WW_2. Treatment WW_1 showed 0% of bloody diarrhea intensity between the 26th and 30th day of the experiment, while in the same period chickens in treatment WW_2 recorded the highest bloody diarrhea intensity of 99% on the 28th day of the experiment. An average of 66% of bloody diarrhea intensity was recorded in treatment WW_3 with wormwood seeds on the 28th day of the experiment.

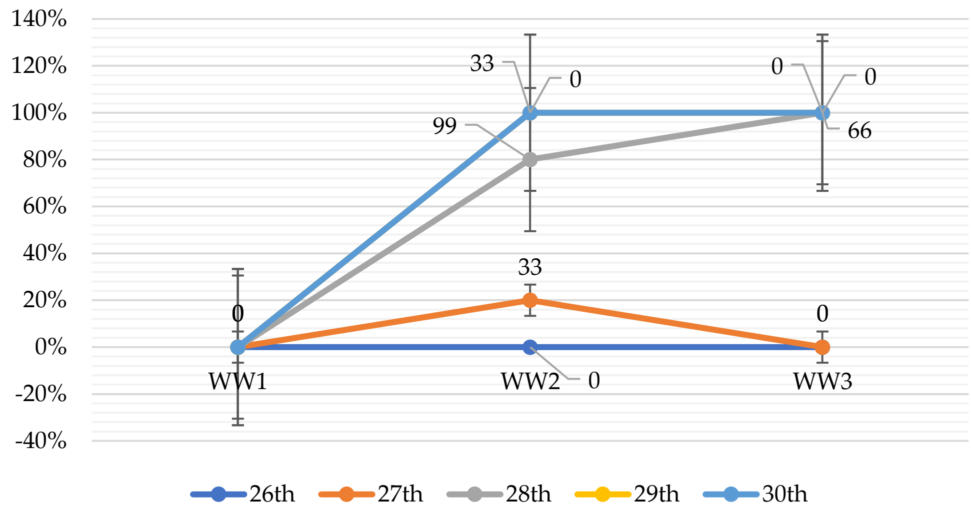

Figure 1. The intensity of bloody diarrhea of chickens in the experiment.

Results given in Table 1 show that non-treated chickens infected with *Eimeria* spp. excreted oocysts in their feces, which was significantly ($p<0.05$) higher than chickens with the addition of wormwood seeds. In WW_3 treatment the oocysts output and mortality rate were significantly lower ($p<0.05$) in comparison to positive control treatment WW_2. This suggests that adding dietary wormwood seeds to daily broilers' diet infected with protozoa decreased their oocyst output.

Table 1. Influence of dietary wormwood seeds on fecal oocyst counts and mortality rate of chickens.

Treatment	**Average oocyst count (per g)**					**Mortality rate (%)**
	26th day	**27th day**	**28th day**	**29th day**	**30th day**	
WW_1	0	0	0	0	0	0.2
WW_2	22167.2±354[a]	36778.2±489[a]	39123.5±982[a]	43800.3±542[a]	45115.7±784[a]	13.8
WW_3	16764.8±566[b]	9659.2±670[b]	5681.2±544[b]	1126.9±67[b]	623.3±401[b]	4.3

[a-b] - Means within a column with no common superscript differ significantly at $p<0.05$.

A feed additive containing herbal extracts has been successfully used to control coccidiosis on some chicken farms [30,31]. Many studies have shown that herbal extracts and medicinal herbs have antimicrobial and antioxidant properties [32] that may be useful for treating coccidiosis [33,34]. There has been evidence that phenolic components are responsible for this biological activity. The use of phenols as oocysticides against *Eimeria* spp. has been demonstrated *in vivo* and *in vitro* tests [35,36]. It is known that H+ and K+ cations interact with phenols by altering their permeability to the cytoplasmic membrane. As ion gradients dissipate, indispensable cellular processes are impaired, constituents leak out, water balance is compromised, membrane potential collapses and adenosine triphosphate synthesis is inhibited, ultimately leading to cell death.

Enzymatic activity in blood hemolysates

Enzymatic activity of blood hemolysates from the controls (WW_1 and WW_2) and experimental (WW_3) treatments are shown in Figure 2.

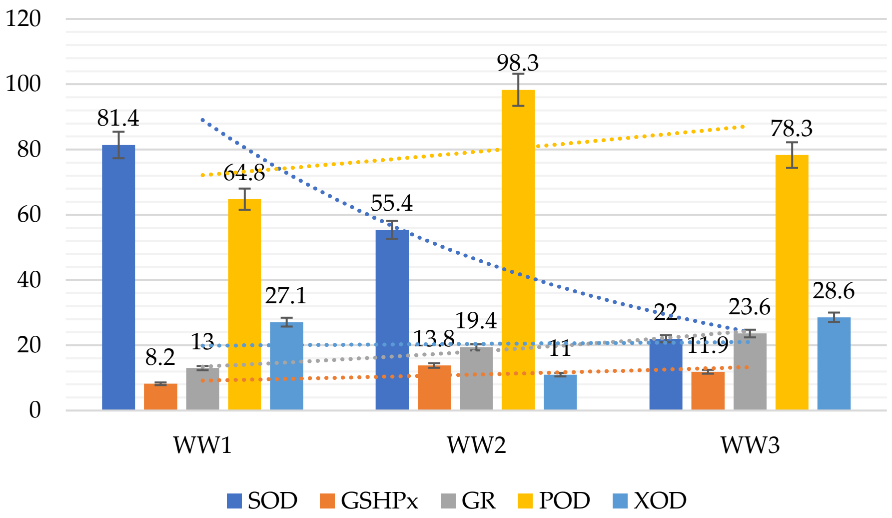

Figure 2. Enzymatic activity of blood hemolysates, µmol/g Hb min. SOD - superoxide–dismutase; GSHPx - glutathione–peroxidase; GR - glutathione–reductase; POD – peroxidase; XOD - xanthine–oxidase.

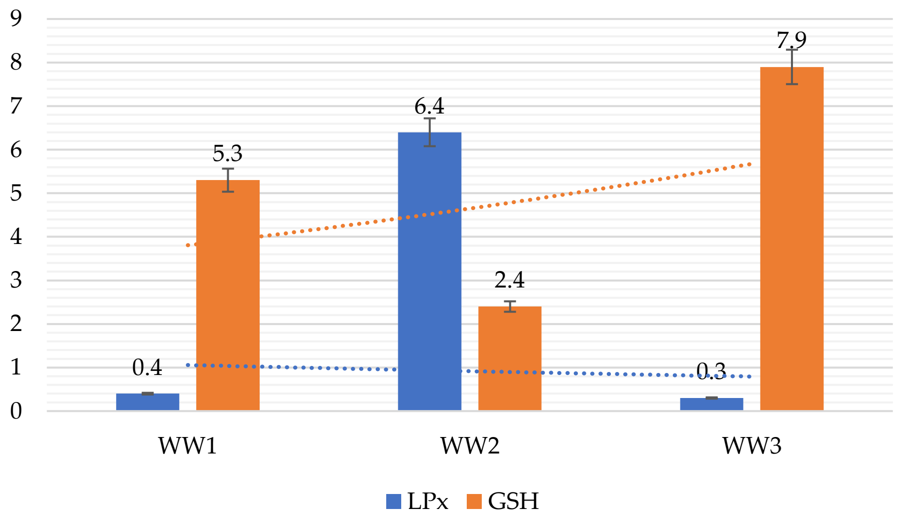

Figure 3. The non-enzymatic activity of blood hemolysates, µmol/g Hb min. LPx - the content of lipid peroxides; GSH - glutathione.

The obtained results indicate a significant (*p<0.05*) increase in GSH (7.9 µmol/g Hb min) content (Figure 3) and higher catalytic activity of GR (23.6 µmol/g Hb min) in blood hemolysates of infected broilers (Figure 2). There was one exception, the catalytic activity of SOD (55.4 µmol/g Hb min) and XOD (11.0 µmol/g Hb min) in the positive control treatment (WW_2) was significantly lower (*p<0.05*) than in the negative control treatment (WW_1). Probably, this is a result of pathological alterations, which stimulate enzymes involved in antioxidant protection - POD, GSHPx, and GR, to increase their catalytic activities. As a result of decreased feed intake and exhaustion from diarrhea during the disease period, lipolysis from the lipid depots increases, causing the formation of lipid peroxides to increase. Furthermore, inactive organs and tissues are damaged by newly formed lipid peroxides and their degradation products transported by the bloodstream. Antioxidative protection is activated by the organism to protect itself [37,38]. Following the literature data, the catalytic activity of SOD is expected to be reduced [39,40]. It has been established that the enzymatic activity of GSHPx also increases in conjunction with the increased risk of lipid peroxidation in blood [41,42].

Through its role as a substrate for GSHPx, GSH reduces xenobiotic toxicity and lipid peroxidation products. Due to POD's ability to oxidize various proton donors with hydrogen peroxide, a statistically significant ($p<0.05$) decrease in POD activity (from 98.3 and 78.3 μmol/g Hb min in WW_2 and WW_3 to 64.8 μmol/g Hb min in WW_1) was expected.

In blood hemolysates from broilers treated with dietary wormwood seeds supplemented at 1.5%, the concentration of erythrocyte GSH (7.9 μmol/g Hb min) and the activity of GSHPx (11.9 μmol/g Hb min) and GR (23.6 μmol/g Hb min) was significantly higher than in WW_1 ($p<0.05$).

4. Conclusions

These results suggest that wormwood seeds could function as a potential source of anti-coccidiosis protection agents, as they reduce oocyst output in infected and preventive treated broilers.

Obtain results showed an increase in free radical processes in blood hemolysates from artificially infected broilers. It appears that blood hemolysates display a greater level of non-enzymatic and enzymatic antioxidative protective activity. Results also revealed that the antioxidative system of erythrocytes was positively affected by dietary wormwood seed addition in the concentration of 1.5% to chicken daily nutrition. In addition, dietary wormwood seed retains a protective effect against *Eimeria* infection in broilers after infection with *Eimeria* oocysts, indicating that wormwood seeds can be used in feed for broilers as a prophylactic feed additive to prevent coccidia oocyst development.

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References

1. Dalloul, R.A.; Lillehoj, H.S. Poultry Coccidiosis: Recent Advancements in Control Measures and Vaccine Development. *Expert Review of Vaccines* **2006**, *5*, 143–163, doi:10.1586/14760584.5.1.143.
2. Lillehoj, H.S.; Lee, K.W. Immune Modulation of Innate Immunity as Alternatives-to-Antibiotics Strategies to Mitigate the Use of Drugs in Poultry Production. *Poultry Science* **2012**, *91*, 1286–1291, doi:10.3382/ps.2012-02374.
3. Tsiouris, V.; Giannenas, I.; Bonos, E.; Papadopoulos, E.; Stylianaki, I.; Sidiropoulou, E.; Lazari, D.; Tzora, A.; Ganguly, B.; Georgopoulou, I. Efficacy of a Dietary Polyherbal Formula on the Performance and Gut Health in Broiler Chicks after Experimental Infection with Eimeria Spp. *Pathogens* **2021**, *10*, 524, doi:10.3390/pathogens10050524.
4. Vapa Tankosić, J.; Puvača, N.; Giannenas, I.; Tufarelli, V.; Ignjatijević, S. Food Safety Policy in the European Union. *J Agron Technol Eng Manag* **2022**, *5*, 712–717, doi:10.55817/EMRK6646.
5. Lika, E.; Kostić, M.; Vještica, S.; Milojević, I.; Puvača, N. Honeybee and Plant Products as Natural Antimicrobials in Enhancement of Poultry Health and Production. *Sustainability* **2021**, *13*, 8467, doi:10.3390/su13158467.
6. Hall-Stoodley, L.; Costerton, J.W.; Stoodley, P. Bacterial Biofilms: From the Natural Environment to Infectious Diseases. *Nat Rev Microbiol* **2004**, *2*, 95–108, doi:10.1038/nrmicro821.
7. Vinay Kumar Antibacterial Activity of Crude Extracts of Spirulina Platensis and Its Structural Elucidation of Bioactive Compound. *J. Med. Plants Res.* **2011**, *5*, doi:10.5897/JMPR11.1175.
8. Plaza, M.; Herrero, M.; Cifuentes, A.; Ibáñez, E. Innovative Natural Functional Ingredients from Microalgae. *J. Agric. Food Chem.* **2009**, *57*, 7159–7170, doi:10.1021/jf901070g.
9. Giordano, R.; Saii, Z.; Fredsgaard, M.; Hulkko, L.S.S.; Poulsen, T.B.G.; Thomsen, M.E.; Henneberg, N.; Zucolotto, S.M.; Arendt-Nielsen, L.; Papenbrock, J.; et al. Pharmacological Insights into Halophyte Bioactive Extract Action on Anti-Inflammatory, Pain Relief and Antibiotics-Type Mechanisms. *Molecules* **2021**, *26*, 3140, doi:10.3390/molecules26113140.

10. Nastić, N.; Gavarić, A.; Vladić, J.; Vidović, S.; Aćimović, M.; Puvača, N.; Brkić, I. Spruce (Picea Abies (L.). H. Karst): Different Approaches for Extraction of Valuable Chemical Compounds. *J Agron Technol Eng Manag* **2020**, *3*, 437–447.
11. Arab, H.A.; Rahbari, S.; Rassouli, A.; Moslemi, M.H.; Khosravirad, F. Determination of Artemisinin in Artemisia Sieberi and Anticoccidial Effects of the Plant Extract in Broiler Chickens. *Trop Anim Health Prod* **2006**, *38*, 497–503, doi:10.1007/s11250-006-4390-8.
12. Sharafati-Chaleshtori, R.; Nickdasti, A.; Mortezapour, E.; Pourhanifeh, M.H.; Ghazanfari, M.; Movahedpour, A.; Khatami, A.; Ashrafizadeh, M.; Zarrabi, A.; Mahabady, M.K.; et al. Artemisia Species as a New Candidate for Diabetes Therapy: A Comprehensive Review. *Current Molecular Medicine* **2021**, *21*, 832–849, doi:10.2174/1566524020999210101234317.
13. Kshirsagar, S.G.; Rao, R.V. Antiviral and Immunomodulation Effects of Artemisia. *Medicina* **2021**, *57*, 217, doi:10.3390/medicina57030217.
14. Batiha, G.E.-S.; Olatunde, A.; El-Mleeh, A.; Hetta, H.F.; Al-Rejaie, S.; Alghamdi, S.; Zahoor, M.; Magdy Beshbishy, A.; Murata, T.; Zaragoza-Bastida, A.; et al. Bioactive Compounds, Pharmacological Actions, and Pharmacokinetics of Wormwood (Artemisia Absinthium). *Antibiotics* **2020**, *9*, 353, doi:10.3390/antibiotics9060353.
15. Abiri, R.; Silva, A.L.M.; de Mesquita, L.S.S.; de Mesquita, J.W.C.; Atabaki, N.; de Almeida, E.B.; Shaharuddin, N.A.; Malik, S. Towards a Better Understanding of Artemisia Vulgaris: Botany, Phytochemistry, Pharmacological and Biotechnological Potential. *Food Research International* **2018**, *109*, 403–415, doi:10.1016/j.foodres.2018.03.072.
16. Attree, E.; Sanchez-Arsuaga, G.; Jones, M.; Xia, D.; Marugan-Hernandez, V.; Blake, D.; Tomley, F. Controlling the Causative Agents of Coccidiosis in Domestic Chickens; an Eye on the Past and Considerations for the Future. *CABI Agric Biosci* **2021**, *2*, 37, doi:10.1186/s43170-021-00056-5.
17. Toledo, G.A.; Almeida, J.D. de M.; Almeida, K. de S.; Freitas, F.L. da C. Coccidiosis in Broiler Chickens Raised in the Araguaína Region, State of Tocantins, Brazil. *Rev. Bras. Parasitol. Vet.* **2011**, *20*, 249–252, doi:10.1590/S1984-29612011000300014.
18. Popović, S.J.; Kostadinović, L.M.; Puvača, N.M.; Kokić, B.M.; Čabarkapa, I.S.; Đuragić, O.M. Potential of Wormwood (Artemisia Absinthium) as a Feed Supplement in Rabbit Diet: Effect on Controlling Rabbit Coccidiosis, Antioxidative Systems and Growth Performance. *Vet. arhiv* **2017**, *87*, 769–782, doi:10.24099/vet.arhiv.160704a.
19. Puvača, N.; Horvatek Tomić, D.; Popović, S.; Đorđević, S.; Brkić, I.; Lalić, N.; Shtylla Kika, T.; Lika, E. Influence of Tea Tree (Melaleuca Alternifolia) Essential Oil as Feed Supplement on Production Traits, Blood Oxidative Status and Treatment of Coccidiosis in Laying Hens. *Veterinary Archives* **2020**, *90*, 331–340.
20. Allen, P.C.; Fetterer, R.H. Recent Advances in Biology and Immunobiology of Eimeria Species and in Diagnosis and Control of Infection with These Coccidian Parasites of Poultry. *Clinical Microbiology Reviews* **2002**, *15*, 58–65, doi:10.1128/CMR.15.1.58-65.2002.
21. Agunos, A.; Deckert, A.; Léger, D.; Gow, S.; Carson, C. Antimicrobials Used for the Therapy of Necrotic Enteritis and Coccidiosis in Broiler Chickens and Turkeys in Canada, Farm Surveillance Results (2013–2017). *Avian Diseases* **2019**, *63*, 433–445, doi:10.1637/11971-091718-Reg.1.
22. Martins, R.R.; Silva, L.J.G.; Pereira, A.M.P.T.; Esteves, A.; Duarte, S.C.; Pena, A. Coccidiostats and Poultry: A Comprehensive Review and Current Legislation. *Foods* **2022**, *11*, 2738, doi:10.3390/foods11182738.
23. Zhang, J.J.; Wang, L.X.; Ruan, W.K.; An, J. Investigation into the Prevalence of Coccidiosis and Maduramycin Drug Resistance in Chickens in China. *Veterinary Parasitology* **2013**, *191*, 29–34, doi:10.1016/j.vetpar.2012.07.027.
24. Kamci Tekin, G.; Bicer, B.T. Evaluating Seed Dressing and Soil Application of Vermicompost on Pea (Pisum Sativum) Growth and Development. *J Agron Technol Eng Manag* **2022**, *5*, 835–840, doi:10.55817/KNLY5326.

25. Ljubojević, D.; Puvača, N.; Pelić, M.; Todorović, D.; Pajić, M.; Milanov, D.; Velhner, M. Epidemiological Significance of Poultry Litter for Spreading the Antibiotic-Resistant Strains of *Escherichia Coli*. *Worlds Poult Sci J* **2016**, *72*, 485–494, doi:10.1017/S004393391600043X.
26. Puvača, N.; Vapa Tankosić, J.; Ignjatijević, S.; Carić, M.; Prodanović, R. Antimicrobial Resistance in the Environment: Review of the Selected Resistance Drivers and Public Health Concerns. *J Agron Technol Eng Manag* **2022**, *5*, 793–802, doi:10.55817/CSCQ3326.
27. Surapneni, K.M.; Chandrasada Gopan, V.S. Lipid Peroxidation and Antioxidant Status in Patients with Rheumatoid Arthritis. *Indian J Clin Biochem* **2008**, *23*, 41–44, doi:10.1007/s12291-008-0010-x.
28. Kostadinović, L.M.; Pavkov, S.T.; Lević, J.D.; Galonja-Coghill, T.A.; Dozet, G.K.; Bojat, N.Č. Effect of Sulphachloropyrazine on Antioxidative Systems in Blood and Liver of Broilers. *Acta Vet. Brno* **2011**, *80*, 165–170, doi:10.2754/avb201180020165.
29. Puvača, N.; Kostadinović, Lj.; Popović, S.; Lević, J.; Ljubojević, D.; Tufarelli, V.; Jovanović, R.; Tasić, T.; Ikonić, P.; Lukač, D. Proximate Composition, Cholesterol Concentration and Lipid Oxidation of Meat from Chickens Fed Dietary Spice Addition (Allium Sativum, Piper Nigrum, Capsicum Annuum). *Anim. Prod. Sci.* **2016**, *56*, 1920–1927, doi:10.1071/AN15115.
30. Peek, H.W.; Landman, W.J.M. Coccidiosis in Poultry: Anticoccidial Products, Vaccines and Other Prevention Strategies. *Veterinary Quarterly* **2011**, *31*, 143–161, doi:10.1080/01652176.2011.605247.
31. Kadykalo, S.; Roberts, T.; Thompson, M.; Wilson, J.; Lang, M.; Espeisse, O. The Value of Anticoccidials for Sustainable Global Poultry Production. *International Journal of Antimicrobial Agents* **2018**, *51*, 304–310, doi:10.1016/j.ijantimicag.2017.09.004.
32. Aćimović, M.; Puvača, N. Tanacetum Vulgare L. – A Systematic Review. *J Agron Technol Eng Manag* **2020**, *3*, 416–422.
33. Naidoo, V.; McGaw, L.J.; Bisschop, S.P.R.; Duncan, N.; Eloff, J.N. The Value of Plant Extracts with Antioxidant Activity in Attenuating Coccidiosis in Broiler Chickens. *Vet Parasitol* **2008**, *153*, 214–219, doi:10.1016/j.vetpar.2008.02.013.
34. Rizwan, H.M.; Khan, M.K.; Mughal, M.A.S.; Abbas, Z.; Abbas, R.Z.; Sindhu, Z. ud D.; Sajid, M.S.; Ain, Q. ul; Abbas, A.; Zafar, A.; et al. A New Insight in Immunomodulatory Impact of Botanicals in Treating Avian Coccidiosis. *J Parasit Dis* **2022**, *46*, 1164–1175, doi:10.1007/s12639-022-01519-w.
35. Jitviriyanon, S.; Phanthong, P.; Lomarat, P.; Bunyapraphatsara, N.; Porntrakulpipat, S.; Paraksa, N. In Vitro Study of Anti-Coccidial Activity of Essential Oils from Indigenous Plants against Eimeria Tenella. *Veterinary Parasitology* **2016**, *228*, 96–102, doi:10.1016/j.vetpar.2016.08.020.
36. Murshed, M.; Al-Quraishy, S.; Qasem, M.A. Evaluation of the Anticoccidial Activity of Sheep Bile against Eimeria Stiedae Oocysts and Sporozoites of Rabbits: An In Vitro Study. *Veterinary Sciences* **2022**, *9*, 658, doi:10.3390/vetsci9120658.
37. Lee, J.; Koo, N.; Min, D.B. Reactive Oxygen Species, Aging, and Antioxidative Nutraceuticals. *Comprehensive Reviews in Food Science and Food Safety* **2004**, *3*, 21–33, doi:10.1111/j.1541-4337.2004.tb00058.x.
38. Ristow, M. Unraveling the Truth About Antioxidants: Mitohormesis Explains ROS-Induced Health Benefits. *Nat Med* **2014**, *20*, 709–711, doi:10.1038/nm.3624.
39. Ujowundu, C.O.; Anaba, P.-O.I.; Ulinasombu, N.B.; Ujowundu, F.N.; Igwe, K.O.; Ogbuagu, H.D. Attenuation of Paraquat-Induced Nephrotoxicity and Dysfunction in Male Wistar Albino Rats. *Innov Biosyst Bioeng* **2020**, *4*, 26–35, doi:10.20535/ibb.2020.4.1.191259.
40. Sharifi-Rigi, A.; Heidarian, E. Therapeutic Potential of Origanum Vulgare Leaf Hydroethanolic Extract against Renal Oxidative Stress and Nephrotoxicity Induced by Paraquat in Rats. *Avicenna J Phytomed* **2019**, *9*, 563–573, doi:10.22038/AJP.2019.13466.

41. Okutan, H.; Ozcelik, N.; Ramazan Yilmaz, H.; Uz, E. Effects of Caffeic Acid Phenethyl Ester on Lipid Peroxidation and Antioxidant Enzymes in Diabetic Rat Heart. *Clinical Biochemistry* **2005**, *38*, 191–196, doi:10.1016/j.clinbiochem.2004.10.003.
42. Casado, Á.; Encarnación López-Fernández, M.; Concepción Casado, M.; de La Torre, R. Lipid Peroxidation and Antioxidant Enzyme Activities in Vascular and Alzheimer Dementias. *Neurochem Res* **2008**, *33*, 450–458, doi:10.1007/s11064-007-9453-3.

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