

COURSE OF OOCYST SHEDDING IN FAECES OF BROILERS EXPERIMENTALLY CHALLENGED WITH EIMERIA IN RELATION TO DIETARY ADMINISTRATION WITH WORMWOOD

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ABSTRACT

The aim of this work was to assess the effect of feed supplementation with *Artemisia absinthium* (wormwood) meal on the course of *Eimeria* spp. oocyst shedding in broilers following their targeted infection using area under the curve (AUC) analysis. Broilers in the control (C) group were fed basal diets without coccidiostat or wormwood herb. Broilers of the CC group were fed starter and grower basal diets supplemented with coccidiostat. Broilers in the P3 and P6 groups were fed diets containing 3 and 6% of meal from whole aerial parts of wormwood in feed, respectively. Collective faeces per pen base were gathered on days 0, 6, 13, 20 and 27 post infection to obtain the final number of oocysts per one gram of faeces (OPG). Then, the AUC analysis of OPG values was carried out. Dietary administration of the coccidiostat robenidine resulted in lower AUC values ($P < 0.05$) during the main phase of infection and confirmed its high anticoccidial efficacy. Dietary supplementation with wormwood meal did not lead to effective suppression of oocyst shedding ($P > 0.05$). However, a lower total AUC value was observed in the P6 group compared to the P3 group ($P > 0.05$); it suggests a dose-dependent effect of the tested plant-based additive on the intrinsic dynamics of the *Eimeria* infection.

Keywords: meat-type chicken; feed; *Artemisia absinthium*; oocyst; coccidiosis; area under the curve



INTRODUCTION

Traditional methods for coccidiosis control in poultry include anticoccidial chemicals, attenuated vaccines (particularly in the EU), and, alternatively, certain phyto-genic herbal supplements. Anticoccidial chemicals, coccidiostats, coccidioides and ionophores - which have been in use for decades – can be still considered the conventional method for coccidiosis control in poultry farming. Although these chemicals are an effective and economically acceptable solution, the risk of developing resistance and current consumer demands for residue-free products have recently led to the search for and development of alternative strategies for coccidiosis control in animal production (Chapman et al., 2010; Beski 2023).

Using effective phyto-genic products as an alternative to anticoccidial agents can mitigate the risks posed by certain chemical drugs. According to Srinivasu et al. (2020), certain herbs contain bioactive phytochemicals that, due to their natural immunostimulatory effects, are effective both in controlling coccidiosis in poultry and against certain other poultry diseases. The genus *Artemisia* is known for its wide spectrum of pharmacological effects, including mainly antioxidant, anti-inflammatory, immune-boosting and antiparasitic effects. The herb *Artemisia absinthium* L., known as wormwood (WM), is used primarily for its stomachic, cholagogue, antiseptic, antispasmodic, carminative, febrifuge and anthelmintic effects. Because of its rich content of interesting bioactive phytochemicals, this herb could be also a potential supplement in animal feed (Amin et al. 2022; Zapletal et al. 2024).

Despite previous studies demonstrating a number of positive effects of *Artemisia* herbs given in feed to chickens (Abdullah and Al-Barwary 2020; Wang et al. 2024), specific knowledge regarding the effect of WM as a feed additive on the shedding of *Eimeria* oocysts following coccidiosis infection remains still very limited. Therefore, the aim of this work was to assess the effect of feed supplementation with WM meal on the course of *Eimeria* spp. oocyst shedding in broilers following their targeted infection using area under the curve (AUC) analysis.

MATERIAL AND METHODS

Trial was performed on 208 one-day-old male Ross 308 chicks, which were randomly assigned to four dietary treatments, each with four replicate pens and thirteen birds per pen in the accredited stable of the Department of Animal Breeding, Animal Nutrition and Biochemistry, University of Veterinary Sciences (UVS) Brno. All broilers were housed under the same controlled housing conditions that were adjusted according to the Ross 308 guidelines. Feed and water were supplied *ad libitum* for broilers. The trial lasted for 42 days. During the trial period, starter diets were fed between days 1–14, grower diets between days 15–28, and finisher diets between days 29–42. The experiment was approved by the Animal Welfare Committee of UVS Brno.

The broilers in the control (C) group were fed basal diets without coccidiostat or WM herb. The broilers of the CC group were fed starter and grower basal diets supplemented with coccidiostat robenidine (33 mg/kg of feed); the finisher diet for the CC group didn't contain coccidiostat. The broilers in the P3 and P6 groups were fed diets containing 3 and 6% of meal from whole aerial parts of WM in feed, respectively. The P3 and P6 diets didn't contain any commercial coccidiostat. For all dietary groups, diets were designed as isoenergetic and isonitrogenous (Table 1).

Tab. 1: *Analysed nutrients composition (%) of respective diets*

Nutrient (%)	Dietary groups			
	C	CC	P3	P6
Starter diet (day 1 to 14)				
Crude protein	20.5	20.6	20.6	20.4
Crude fibre	3.6	3.5	3.7	4.0
Crude fat	3.1	3.1	3.5	3.3
Crude starch	35.6	35.4	34.5	34.1
Ash	5.9	5.9	5.9	5.8
Calcium	0.80	0.81	0.89	0.92
Inorganic phosphorus	0.59	0.57	0.56	0.55
Metabolizable energy (MJ/kg)	12.8	12.8	12.9	12.7
Grower diet (day 15 to 28)				
Crude protein	19.0	19.0	19.1	19.1
Crude fibre	3.3	3.3	3.2	3.2
Crude fat	8.0	8.0	8.0	8.1
Crude starch	35.2	35.2	35.5	35.1
Ash	5.4	5.5	5.4	5.3
Calcium	0.87	0.89	0.83	0.91
Inorganic phosphorus	0.48	0.49	0.48	0.47
Metabolizable energy (MJ/kg)	14.0	13.9	14.0	14.0
Finisher diet (day 29 to 42)				
Crude protein	18.8	18.7	18.7	18.9
Crude fibre	4.1	4.1	3.7	4.1
Crude fat	6.3	6.3	6.4	6.2
Crude starch	35.5	35.4	34.7	33.9
Ash	5.2	5.2	5.1	5.0
Calcium	0.78	0.76	0.70	0.71
Inorganic phosphorus	0.45	0.46	0.48	0.49
Metabolizable energy (MJ/kg)	13.5	13.5	13.5	13.4

At the age of 16 days, broilers in all dietary groups were infested by tenfold overdosing of live vaccine LIVACOX T (Biopharm Co., Prague, Czech Republic) to induce a mild coccidiosis infection according to procedure earlier used by Yu et al. (2021). Inoculum dose of 1 ml was orally gavaged into the crop to each broiler. Inoculum dose contained 15 000 live oocysts of *E. acervulina*, *E. tenella* and *E. maxima*. Collective faeces per pen base were gathered on days 0, 6, 13, 20 and 27 post infection (PI). The freshest faeces were collected from the pen to contain at least 15 g of faeces without bedding. The McMaster technique was further applied. An aliquot of the faecal sample was transferred in to a McMaster chamber and all the *Eimeria* oocysts were counted manually in both parts of the chamber. The sum was multiplied by a dilution factor (50) to obtain the final number of oocysts per one gram of faeces (OPG). Then, area under the curve (AUC) analysis of OPG values was performed using the trapezoidal rule (Bailer 1988).

The AUC was calculated according to the following equation:

$$\text{AUC} = \sum (y_i + y_{i+1})/2 \times (t_{i+1} - t_i)$$

where: y_i = OPG value at time point t_i ; y_{i+1} = OPG value at the subsequent time point; t_{i+1} = time interval between measurements.

Segmented AUC analysis was additionally performed for individual PI intervals (0–6, 6–13, 13–20, and 20–27 days) to evaluate temporal differences in parasite excretion dynamics among dietary groups.

The OPG values used for the current statistical re-analysis were derived from data previously published by Zapletal et al. (2025) in an IF-ranked peer-reviewed journal. Normality analysis of data distribution for AUC values was performed by a Shapiro-Wilk test; normality was not found. The non-parametric Kruskal-Wallis ANOVA was used to find differences in AUC values among dietary groups within individual PI intervals. Then, pairwise comparisons were performed using Mann-Whitney U tests without multiplicity correction due to the exploratory nature of the study and limited sample size. The significance was considered at the $P < 0.05$ level. All statistical procedures were performed by the STATISTICA CZ, vs. 10 software.

RESULTS AND DISCUSSION

The AUC represents the cumulative oocyst excretion over time and integrates both the magnitude and duration of parasite shedding during the experimental period (Velkers et al., 2012). A segmented AUC analysis of OPG levels revealed differences among the experimental groups during the early and middle phases of infection (Table 2). The results show that a significant effect ($P < 0.05$) of the broiler diet treatment on AUC values was observed from the time of infection (PI day 0) through day 20 PI; as expected, the significantly lowest AUC value (0) was observed in the CC group, which was treated with the coccidiostat robenidine,

throughout all three evaluated periods. Compared to the CC group, a significantly higher AUC value was found during the PI 0–6 day period in the P3 and P6 groups, during the PI 6–13 day period in the C, P3, and P6 groups, and during the PI 13–20 day period in the P3 group. The highest level of total oocyst shedding occurred in all infected groups during the period from PI day 6 to 20, which generally corresponds to the main phase of replication and shedding of *Eimeria* spp. In terms of total AUC, the P3 group exhibited the highest cumulative intensity of oocyst excretion, followed by the P6 and C groups.

The results show that dietary supplementation with WM meal did not lead to effective suppression of oocyst shedding; however, a lower total AUC value was observed in the P6 group compared to the P3 group ($P > 0.05$). These results suggest a dose-dependent effect of the tested plant-based additive on the intrinsic dynamics of the infection.

Published studies focusing on the use of *Artemisia* spp. in experimental and field coccidiosis in broilers have repeatedly reported a reduction in the intensity of oocyst shedding, although the effect is generally less pronounced than that of standard anticoccidial chemicals. Studies using parasitological models of *Eimeria* spp. infection found a reduction in OPG, improvement in intestinal lesions, and partial control of the infection following the administration of *Artemisia annua* or *Artemisia absinthium*. However, under the experimental conditions used, complete inhibition of oocyst shedding was not achieved compared to commercial coccidiostats (Almeida et al. 2012; Almeida et al. 2014; Coroian et al. 2022; Hezil et al. 2024), which is consistent with the results of this work. The mere inclusion of dried WM meal in this work was not associated with the expected reduction in the number of excreted oocysts.

Tab. 2: Dietary effect on oocysts shedding in chicken faeces determined by the area under the curve (AUC) values found in respective periods

PI period (days)	Dietary groups				P-value
	C	CC	P3	P6	
0–6	450 ^{ab}	0 ^b	1 275 ^a	1 313 ^a	0.013
6–13	18 681 ^a	0 ^b	47 119 ^a	29 619 ^a	0.017
13–20	23 931 ^{ab}	481 ^b	53 769 ^a	33 600 ^{ab}	0.024
20–27	6 388	3 413	8 838	6 169	0.423

PI: post infection; ^{a,b} Means within a row with different superscripts letters differ at $P < 0.05$.

The specific representation of the biologically active compounds contained in WM can be influenced by a wide range of factors, including, for example, the geographical origin of the herb, soil type, and altitude of cultivation, as well as harvest time and the duration and method of storage (Amin et al. 2022). In this context, it can be stated that the potential use of essential oil derived from WM, ideally with verified levels of β -thujone, 1,8-cineole, and p-cymene, could serve as a more reliable tool for controlling coccidiosis in poultry farming.

Segmented AUC analysis has proven to be a useful tool for evaluating the time-dependent dynamics of infection, as it integrates both the intensity and duration of oocyst shedding. Compared to the evaluation of individual time points, it allows for a more comprehensive assessment of the overall infectious load during the experiment. Although relatively high variability in OPG values was observed among individual pens (replicates), the AUC analysis confirmed a significant anticoccidial effect of the commercial coccidiostat and simultaneously suggested a partial modulatory effect of a higher dose of WM meal on the course of infection in broilers infested with *Eimeria* spp.

CONCLUSION

The results of this work showed that segmented AUC analysis represents a potential tool for the comprehensive evaluation of oocyst excretion dynamics in broilers experimentally infected with *Eimeria* spp., as it takes into account both the intensity and duration of oocyst shedding. Administration of the coccidiostat robenidine resulted in significantly lower AUC values during the main phase of infection and confirmed its high anticoccidial efficacy. In contrast, dietary supplementation with WM meal did not result in a significant reduction in oocyst shedding compared to the infected control group, although a trend toward decreased total cumulative shedding was observed at the higher dose (6%) compared to the lower dose (3%). The results suggest that the efficacy of wormwood may be influenced by the content of biologically active compounds and the form of the plant material used. Further research should therefore focus on standardized plant extracts or essential oils with a defined composition of active ingredients and on verifying their potential in controlling coccidiosis in poultry.

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REFERENCES

- Abdullah, L.N., Al-Barwary, L.T.O. (2020): Effect of *Artemisia splendens* powder and extract on broiler chicken's performance, lymphoid organ weight, gut morphology and serum biochemical during coccidiosis challenge. *Iraqi Journal of Agricultural Sciences*, 51, 611–618.
- Almeida, G.F., Horsted, K., Thamsborg, S.M., Kyvsgaard, N.C., Ferreira, J.F., Hermansen, J.E. (2012): Use of *Artemisia annua* as a natural coccidiostat in free-range broilers and its effects on infection dynamics and performance. *Veterinary Parasitology*, 186, 178–187.
- Almeida, G.F., Thamsborg, S.M., Madeira, A.M., Ferreira, J.F., Magalhães, P.M., Demattê Filho L.C., Horsted, K., Hermansen, J.E. (2014): The effects of combining *Artemisia annua* and *Curcuma longa* ethanolic extracts in broilers challenged with infective oocysts of *Eimeria acervulina* and *E. maxima*. *Parasitology*, 141, 347–355.
- Amin, I., Shubeena, S., Rashid, S.M., Gull, G., Rasool, S., Razak, R., Hussain, I., Bilal, S., Rehman, M.U., Khan, H.M., Shabir, N., Pandit, F. (2022): Biochemical estimation of *Artemisia absinthium* L. powder and qualitative phytochemical screening of its hexanic and ethanolic extracts for assessment of purity. *Annals of Phytomedicine*, 11, 517–522.
- Bailer, A.J. (1988): Testing for the equality of area under the curves when using destructive measurement techniques. *Journal of Pharmacokinetics and Biopharmaceutics*, 16, 303–309.
- Beski, S.S.M. (2023): Anti-coccidial effects of dietary chamomile against experimentally induced coccidiosis in broiler chicken. *Czech Journal of Animal Science*, 68, 30–43.
- Chapman, H.D., Jeffers, T.K., Williams R.B. (2010): Forty years of monensin for the control of coccidiosis in poultry. *Poultry Science*, 89, 1788–1801.
- Coroian, M., Pop, L.M., Popa, V., Friss, Z., Oprea, O., Kalmár, Z., Pintea, A., Borşan, S.D., Mircean, V., Lobonțiu, I., Militaru, D., Vârban, R., Györke, A. (2022): Efficacy of *Artemisia annua* against coccidiosis in broiler chickens: a field trial. *Microorganisms*, 10, 2277.
- Hezil, N., Baazize-Ammi, D., Abdelli, A., Adel, A., Kebbal, S., Gharbi, I., Djeddar, R., Guetarni, D. (2024): Effects of *Artemisia absinthium* on broiler chicken coccidiosis: a systematic review and meta-analysis. *Avian Pathology*, 53, 350–358.
- Srinivasu, B., Preetam, V.C., Gurram, S., Reddy, A.R. (2020): Comparative evaluation of herbal coccidiostat with chemotherapeutic coccidiostats on performance of broilers to control coccidiosis. *Tropical Animal Health and Production*, 52, 1985–1989.
- Velkers, F.C., Bouma, A., Stegeman, J.A., de Jong, M.C. (2012): Oocyst output and transmission rates during successive infections with *Eimeria acervulina* in experimental broiler flocks. *Veterinary Parasitology*, 187, 63–71.

- Wang, Q., Wang, L., Li, L., Sun, M., Li, P., Yu, Y., Zhang, Y., Xu, Z., Gao, P., Ma, J., Liu, X. (2024): Effects of dietary supplementation of fermented *Artemisia argyi* on growth performance, slaughter performance, and meat quality in broilers. *Poultry Science*, 103, 103545.
- Yu, M., Jeon, J.O., Cho, H.M., Hong, J.S., Kim, Y.B., Nawarathne, S.R., Wickramasuriya, S.S., Yi, Y.J., Lee, H., Wan, V., Ng, N.K.J., Tan, C.H., Heo, J.M. (2021): Broiler responses to dietary 3,4,5-trihydroxybenzoic acid and oregano extracts under *Eimeria* challenge conditions. *Journal of Animal Science and Technology*, 63, 1362–1375.
- Zapletal, D., Dobšíková, R., Kostúková, M., Šimek, V., Stříbrná, H., Koudela, B. (2025): Broilers responses to dietary wormwood administration under *Eimeria*-challenged conditions. *Czech Journal of Animal Science*, 70, 55–63.
- Zapletal, D., Dobšíková, R., Šimek, V., Kameník, J., Ježek, F. (2024): Growth performance, carcass composition, physico-chemical traits and amino acid profile of meat depending on wormwood (*Artemisia absinthium* L.) dietary supplementation in broilers. *Archives Animal Breeding*, 67, 1–12.