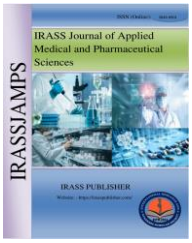
IRASS Journal of Applied Medical and Pharmaceutical Sciences
Abbriviate Title- IRASS J App Med Pharm Sci
ISSN *(Online) 3049-0901*
https://irasspublisher.com/journal-details/IRASSJAMPS
Vol-2, Iss-3(March-2025)



Effects of methanolic extract of Vernonia amygdalina leaf and its fractions on endogenous stages and recovered secondary oocysts of Eimeria tenella in broiler chickens

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Article History

Received: 26 / 02 / 2025

Accepted: 11 / 03 / 2025

Published: 14 / 03 / 2025

Abstract: The aim of the study was to evaluate the effects of the methnolic leaf extract of Vernonia amygdalina and its major fractions on the endogenous life cycle stages of Eimeria tenella in exprimentally-infected broiler chickens. Experimental birds were divided into seven groups of two replicates and dosed with 105sporulated oocysts of E.tenella and treated with extracts. Two birds from each replicate were sacrificed on days 5 and 6 postinfection for histopathological examination of caecal sections. In addition, the secondary oocysts recovered from treated birds at 7 days postinfection were evaluated for viability by their ability to sporulate after incubation in 2.5% potassium dichromate. Histopathological investigation of sections of the ceaca for schizonts and gametocytes revealed reduced number of schizonts and gametocytes in the caeca of birds of the treated groups compared to those of the positive control. Of interest is the efficacy of the mehanolic extract and the butanol fraction, greatly diminishing the number of schizonts and gametocyte to remarkable levels. Most of the secondary oocyts recovered from chickens treated with methanol extract, hexane fraction, butanol fraction, and aqueous residue fraction had only 33.78, 1.94, 0.36 and 0.96% non-viable oocysts, respectively, compared with those recovered from birds in the positive control group, with observed sporulation rate of 95.46%.

Keywords: Vernonia amygdalina, methanolic extract, fractions, endogenous stages, secondary oocysts, chickens, Eimeria tenella.

How to Cite: Biallah, M. B., Okubanjo, O. O., Abdu, P. A., Kaze, P. D., Dogo, G. A., (2025). Anticoccidial efficacy of Vernonia amygdalina methanolic leaf extract and its major fractions against experimental Eimeria tenella challenge in broiler chickens. *IRASS Journal of Applied Medical and Pharmaceutical Sciences, 2(3),9-14.*

Introduction

According to Chapman (2014), coccidios is the parasite illness that has the biggest financial impact on the chicken business globally. According to recent estimates of production losses brought on by the disease and the financial impact of prophylactic measures, coccidiosis costs the worldwide poultry sector more than $14 billion USD a year (Blake et al., 2020).

Of the sevens pecie s of *Eimeria*, including *E.acervulina*, *E.brunetti*, *E.maxima*, *E.mitis*, *E.necatrix* and *E.tenella* which infect chickens, *E.tenella* is the most frequently studied and mostly used as a model in the study of coccidiosis in chickens (Chapman and Shirley, 2003). The species inhabits the caecum causing caecal coccidiosis that is recognized by the accumulation of blood in the caecal lumen and haemorraghic diarrhea (McDougald and Fitz-Coy, 2013).The entire development of *E.tenella*occurs in the caeca of infected chickens with life cycle stages similar to other species (Chapman and Shirley, 2003). The life cycle has basically two stages: the exogenous phase (sporogony) and the endogenous phase (schizogony and gametogony). It begins with the ingestion of sporulated oocysts in contaminated feed or water which release the sporozoites when digested. The sporozoites infect the epithelial cells of the caecum and transform to the first merogonic generation. One or more merogonic divisions lead to the formation of gametocytes. The microgametocyte fertilizes the surrounding macrogametocyte, forming the zygote and subsequently the oocysts which are released into the environment via the faeces (Conway and McKenzie, 2007).

The traditional methods of controlling coccidiosis in chickens is the administration of chemoprophylactic drugs, and to a limited extent, by vaccination, but both have their limitations including the development of resistance reduction in productivity (Kadykalo *et al*, 2018). Recently, interest has shifted toward the development of alternative strategies, such as natural products to control coccidiosis (Muthamilselvan *et. al*., 2016).

Numerous plants with anticoccidial qualities have been reported in the literature, and some of these claims have been confirmed by contemporary scientific techniques (Ahad et al., 2017). The evaluation of anticocidial drugs' effects on endogenous

phases has been used to test their effectiveness (Alzahrani et al., 2016).

*Vernonia amygdalina*is a plant widely used in ethnomedicine and ethnoveterinary practice as extensively reviewed (Yeap *et. al*., 2010). Whilst the anticoccidial of *V. amygdalina* has been documented (Al-Fifi *et. al*., 2007), an indepth study, using parasitological parameters remain to be desired.

Materials and methods

Plant Collection, Identification and Processing

At the flowering stage, a single batch of fresh, disease-free V. amygdalina leaves was gathered from a private garden in Jos town. Jos is located between latitude $8^{o}24$'N, and longitude $8^{o}32$ and 10^{o} 38E. Its average elevation is 1,250 m above sea level and consists of plains, hills and depressions of various sizes. October through April is the dry season, and May through September is the wet season. Temperatures in December and January are below 15°C, and there is an average of 1,100 mm of rainfall per year (Plateau Agricultural Development Prograamme, 2002). The voucher sample, number 7183, was placed in the department's herbarium for future use after being identified and verified by Ahmadu Bello University's Department of Biological Sciences in Zaria. After being cleaned with tap water, the leaves were allowed to air dry on galvanized wire screens in the shade, occasionally moving, until their weight remained consistent. After being ground into a powder using a mechanical grinder, a sample of ten kilograms (10 kg) of leaves was kept in an airtight plastic container until it was needed.

Extraction and Fractionation of V. amygdalina leaves

To create a methanolic extract, a 2 kg quantity of the powdered V. amygdalina leaves was extracted using 10 L of absolute methanol in a Soxlet apparatus at 70 oC. A rotary evaporator was used to dry the extract into a powder at 40 oC. It was then refrigerated until it was needed and kept in an airtight amber-colored glass bottle (Momoh et al., 2010).

Phytochemical Analysis

Using established protocols as outlined by Hashemi et al. (2008), chemical tests were conducted to screen and identify the bioactive chemical elements of the plant's ethanolic leaf extract.

Parasite propagation and purification

The study made use of a local strain of E. tenella that had previously undergone molecular characterisation and was kept at Ahmadu Bello University's Department of Veterinary Parasitology and Entomology in Zaria (Jatau et al., 2016). As advised by Holdsworth et al. (2004), five broiler chickens were given an oral dose of a seed stock of 1x104 oocysts suspended in 1 milliliter of distilled water at two weeks of age in order to multiply the oocysts.

Sporulated oocysts were cleaned and counted by the McMaster technique (Shirley *et. al*., 1995) The required concentration of sporulated oocysts (100,000/mL) was maintained with phosphate buffered saline.

Experimental birds, housing and management

To avoid contamination from extraneous coccidial infections, three hundred (300) heads of one-day-old broiler chicks were acquired, raised, and cared for in a separate fly-proof cage. Wood shaving litter was present in the brooder pen down to a depth of 5 cm. To meet the nutrient requirement of the broilers during the experimental period, a coccidiostat-free commercial diet formulated for the two stages of growth and water were provided *ad libitum* and birds were routinely vaccinated against Newcastle disease and Gumboro disease

Experimental design

The experiment was carried out in randomized completely block design. Two hundred and ten (210) broilers with similar body weight were selected for the study as recommended by Holdsworth *et al*. (2004)). Except group A which served as negative control.The treatments were as follows: A) Non medicated non infected, B) infected non medicated, C) infected and medicated with amprolium at 125mg/kg, D) infected and medicated with methanol extract at 1000mg/kg, E) infected and medicated with hexane fraction at 500mg/kg, F) infected and medicated with butanol fraction at 500mg/kg and G) infected and medicated with aqueous residue fraction at 500mg/kg respectively.

Effects of extracts on endogenous stages of the parasite

The anticoccidial effects of the methanolic extract of *V.amygdalina* leaf and its fractions on two of the endogenous stages of *E.tenella* was assessed using the schizonts and the gametocytes as well as the viability of secondary oocysts recovered from treated chickens.

Effects of extracts on schizonts

One bird from each replicate was sacrificed by cervical dislocation at 5days postinfection and the caeca were harvested for histopathological processing (Suprihati and Yunus, 2018). Briefly, tissue from the mid-caecum each bird was fixed informalin, processed and sectioned at 5µm thick.The sections were stained with haematoxylin and eosin (H and E) and examined for schizonts under 40X objective of a light microscope. Pictures were taken using a digital microscope camera.The schizonts,which appear as round bodies containing the merozoites that are closely packed like segments of an orange (Soulsby,1986) were counted under the microscope.

Effects of extracts on gametocytes

To asses the effect of the extracts on gametocytes, one chick from each replicate was sacrificed at 6days postinfection per group (Chen *et. al*., 2020). A section of the infected caeca was processed for histopathological study after staining with haematoxylin and eosin to visualize gametocytes in mucosal tissues.The gametocytes which have large central nucleus and wall forming bodies at the periphery as described by Soulsby (1986) were counted and recorded.

Assessment of viability of secondary oocysts recovered fromtreated broiler chickens

The viability of the oocysts that was recovered from treated broiler chickens was assessed by their ability to sporulate in 2.5% potassium dichromate after 7days of incubation was carried out (El-Ashram and Suo, 2017). Briefly, 20g of faecal material was macerated and centrifuged at 1200g for 15min and there covered oocysts were washed three times in distilled water. Oocysts were dispensed into petridishes containing 2.5% potassium dichromate and incubated for 7days at room temperature with constant

aeration. Aliquot soft the incubated oocysts were dispensed on glass slides and the sporulated oocysts were counted under the microscope.The examined oocysts were considered sporulated when four sporocysts were apparently distinguishable in each oocyst (DelCacho *et. al*., 2010).

Ethical Approval

Ethical approval for use of live animals were obtained from the Ethical Committee of the National Veterinary Research Institute,Vom.

Data Analyses

The statistical analysis was performed using SAS statistical software 8.2, SASInstitute Inc. Cary, NC, USA. All data were expressed as mean values with their standard errors, and comparison of mean values was performed by one-way analysis of variance (ANOVA). Tukey test was used to separate means which differ significantly.The differences between groups was considered significant if $p<0.05$.

Results

The histopathological sections of caeca obtained from birds that have been sacrificed 5dpi showed no schizonts in the negative control (NC) group. Numerous schizonts were observed in sections of caeca obtained from untreated chickens (Figure1A). The sections obtained from the groups that were treated with the extract and its fractions showed fewer schizonts compared with the untreated group (Figure1, B-E).

The effects of the methanolic extract of *V.amygdalina* leaf and its fractions on the population of gametocytes in birds sacrificed 6dpi are presented in Figure 2A-E. Numerous gametocytes were observed in the caecal sections obtained from birds in the positive control group (Figure 2, A). The number of gametocytes obtained per field in caeca of chickens treated with methanolic extract, hexane fraction, butanol fraction and aqueous residue fraction were 11,19,7,and 22, respectively (Figure 2, B-E). The highest number (35) of gametocytes was observed per field of caecal sections of chickens in the positive control group.

The effect of the methanolic extract of *V.amygdalina* leaf and its fractions on the viability of secondary oocysts recovered from treated birds is presented in Table 2. The secondary oocysts recovered from treated birds showed minimal viability in comparison to the oocysts recovered from untreated birds. The viability of oocysts excreted by birds treated with butanol fraction was lowest (0.36%) while that of the methanol group was highest (33.78%).

Table1: Effect of treatment on endogenous stages of E. tenella in experimentally infected broiler chickens.

Group	Treatment	Endegenous stages	
		Schizonts	Gametocytes
A	Negative control	0.00	0.00
B	Positive control	13.00	35.00
C	Standard control	0.00	0.00
D	Methanol extract	6.00	11.00
E	Hexane fraction	4.00	19.00
F	Butanol fraction	5.00	7.00
G	Aqueous residue fraction	9.00	22.00

Table2: Viability of secondary oocysts of E.tenella recovered from experimental birds treated with extracts.

Group	Treatment	Unsporulated oocysts	Sporulated oocysts	Percent viability
A	Negative control	-	-	0.00
B	Positive control	2,320,000	2,214,600	95.46
C	Standard control	0.00	0.00	0.00
D	Methanol extract	355,200	12,000	33.78
E	Hexane fraction	1,417,500	27,500	1.94
F	Butanol fraction	240,000	880	0.36
G	Aqueous residue fraction	378,400	2,640	0.69

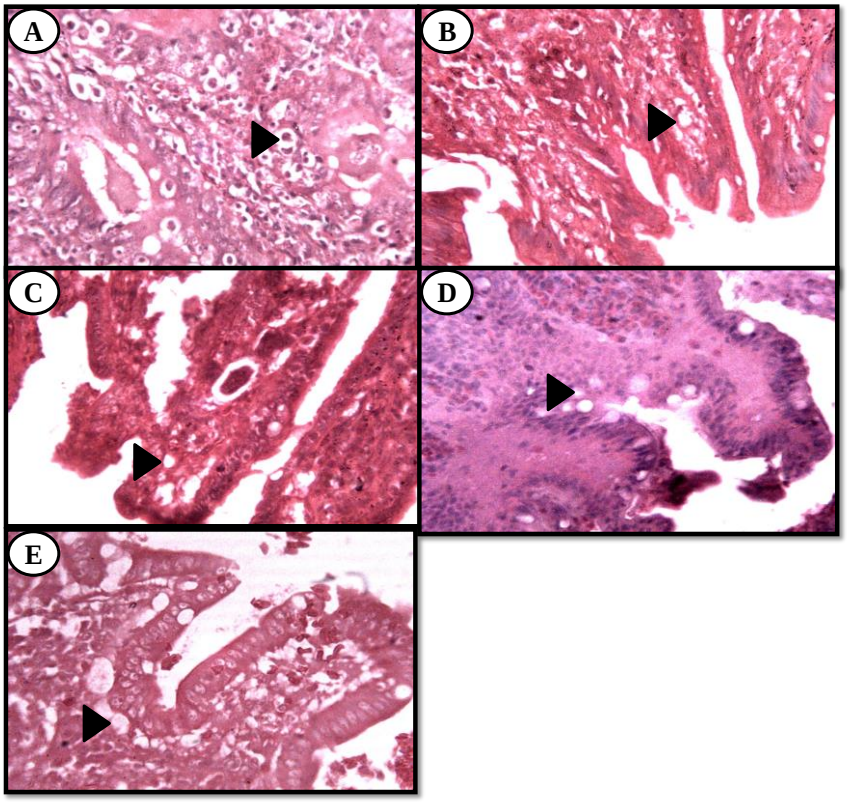

Figure1: Micrograph of caecal tissue 5days p.i A) massive number of schizonts (arrow head) in non treated bird. B) scanty schizonts (arrow head) inm ethanol extract treated chicken. C) few schizonts (arrow head) in hexane fraction treated birds. D) minimal number of schizonts (arrow head) butanol fraction treated chickens. E) some schizonts (arrow head) in aqueous fraction treated birds (H and E, X40).

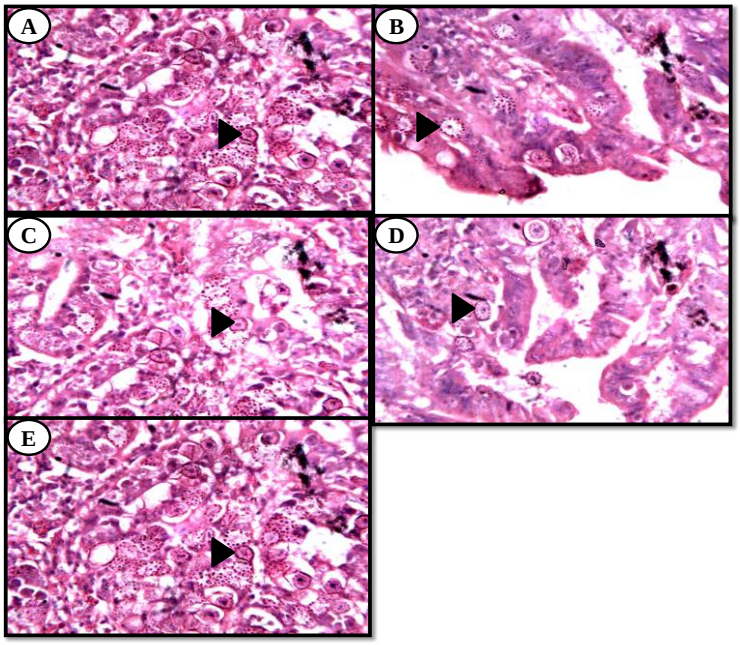

Figure2: Micrograph of caecal tissue obtained 6days p.I. A) Heavy presence of gametocytes (arrow head) in non treated birds. B) few gametocytes (arrow head) in methanol extract treated chickens. C) moderate number of gametocytes (arrow head) in hexane fraction treated birds. D) scanty number of gametocytes in butanol fraction treated chickens. E) noticeable number of gametocytes (arrow head) in aqueous fraction treated birds (H and E, X40).

Discussion

Alternative means of parasite control using plant extracts has become a hot spot in anticoccidial research in recent times

(Bozkurt *et al*. 2013, Lan *et. al*., 2016). Published reports on the number of endogenous stages of *E.tenella* in experimental caecal coccidiosis in broiler chickens are being employed as a measure of anticoccidial efficacy (Zhang *et. al*., 2012, El-Shazly *et. al*., 2020). The leaf extract of *Vernonia amygdalina* contains phytochemicals including alkaloids, tannins, saponins, and triterpenoids (Alara *et. al*., 2020). These phytochemicals have been demonstrated to reduce the number endogenous stages of *E.tenella* in treated chickens as observed in other studies (Zhang *et. al*., 2 012, Srinivasu *et. al*., 2019. Consistent with previous reports, schizonts in caecal sections from infected chickens were evident at 5days post infection with *E.tenella* (DelCacho *et. al*.,2014, Lan *et. al*., 2016, Cha *et. al*., 2018). The substantially low population of schizonts observed in histological sections of caecum in treated birds observed in this study concurs with previous findings (Kurkure *et. al*., 2006, She *et. al*., 2017). The phytochemicals contained in plants might exert their anticoccidial effect by inhibiting the multiplication of the schizonts as asserted in previous reports (Amer *et. al*., 2015, Lan *et. al*., 2016). The presence of gametocytes in the caecal sections of infected birds observed at 6days post-infection concurs with previous studies (Suprihati and Yunus, 2018).

Diminished number of gametocytes in treated chickens sacrificed at 6days postinfection has been reported in previous studies (Maes *et. al*., 1988, Liu *et. al*., 2016). Long and Jeffers (1982) suggested that continuous medication of chickens with monensin produced reduction in gametocytes mediated through the uptake of the drug by merozoites during transition from schizogony to gametogony. The number of viable oocysts in the litter is an important factor in the spread of coccidiosis during intensive rearing of poultry (Shirley *et. al*., 2005, Chapman *et. al*., 2013). In the present study, the methanolic extract of *V.amygdalina* leaf and its fractions drastically reduced the number of viable secondary oocysts recovered from treated birds as evaluated by their ability to inhibit oocysts porulation. Secondary oocysts have been reported to display low sporulation rate when recoverd from treated birds in other studies (Arakawa e*t al*., 1991, Sharma *et. al*., 2021). Fatemi *et. al*. (2015) suggested that the inhibition of the sporulation of secondary oocysts recovered from extract-treated birds could be attributable to the continued activity of unmetabolized molecules excreted in the faeces of treated birds. This claim was supported by Abba and Udo (2018) and Yang *et. al*. (2019), who observed that treatment of birds with plant extracts led to formation and shedding of non-viable oocysts.

From the result of this study, it is concluded that the administration of the methanolic extract of *Vernonia amygdalina* leaf and its fractions markedly reduce the numbers of endogenous schizonts and gametocytes, and further diminished the viability of secondary oocysts of *Eimeria tenella* recovered from infected chickens.

References

1. Abba, A.M. and Udo, E.J. (2018). *In-vivo* evaluation of anti-coccidial efficacy of aqueous extract of *Allium sativum* and *Carica papaya* in experimentally infected broiler chicks. *Open Access Journal of Biomedical Engineering and Biosciences*, 2(5)-2018, 9pages.
2. Ahad, S., Tanveer, S., Nanchoo, I.A. and Malik, T.A. (2017). Anticoccidial activity of *Artemisia vestita* (Anthemideae, Asteraceae), a traditional herb growing in Western Himalayas, Kashmir India. *Microbial Pathogenesis*, 104:289-295.
3. Alara, O.R., Abdurahman, N.H. and Olalere, A.O. (2020). Ethanolic extraction of flavonoids, phenolics and antioxidants from *Vernonia amygdalina* leaf using two-level factorial design. *Journal of King Saud University-Science*, 32:7-16.
4. Al-Fifi, Z.I.A. (2007) . Effects of leaves extract of *Carica papaya*, *Vernonia amygdalina* and *Azadirachta indica* on the coccidiosis of free-range chickens. *Asian Journal of Animal Sciences*,1(1):26-32.
5. Alzahrani,F., Al-Shaebi,E.M., Dkhil,M.A. and Al-Quraishy,S. (2016). *In vivo* anti-*Eimeria* and *in vitro* anthelmintic activity of *Ziziphusspina-christi* leaf extracts. *Pakistan Journal of Zoology*, 48(2):409-413.
6. Amer,S.O., khil,M.A., Hikal,W.M. and Al-Quraishy,S. (2015). Antioxidant and anti-inflammatory activities of pomegranate (*Punica granatum*) on *Eimeria papilatta*-induced infection in mice. *Bio. Med. Research International*, Volume 2025, Article ID 219670, 7 pages.
7. Arakawa,A., Tanaka,Y., Baba,E. and Fukata, T. (1991). Effects of clopidol on sporulation and infectivity of *Eimeria tenella* oocysts.*Veterinary Parasitology*, 38(1):55-60.
8. Blake,D., Knox,J., Dehaeck,B., Huntington,B., Rathinam,T., Ravipati,V., Ayoade,S., Gilbert,W., Adedambo,A., Jatau,I., Raman,M., Parkar,D., Rushton,J. and Tomley, F. (2020). Re-calculating the cost of coccidiosis in chickens. *Veterinary Research*, 51:115.
9. Bozkurt,M., Giannenas,I., Kucukyilmaz,K., Christaki,E. and Flouru-Paneri,P. (2013). An update on approaches to controlling coccidian in poultry using botanical extracts. *British Poultry Science*, 54(6):713-727.
10. Cha,J.O., Zhao,J., Yang,M.S., Kim,W.I., Cho,H.S., Lim,C.W., and Kim,B. (2018). Oocysts-shedding patterns of three E*imeria* species in chickens and shedding pattern variation depending on the storage period of *Eimeria tenella* oocysts. *Journal of Parasitology*, 104(1):18-22.
11. Chapman,H.D. (2014). Milestones in avian coccidiosis research: a review. *Poultry Science*, 93:501-511.
12. Chapman,H.D. and Shirley, M.W. (2003). The Houghton strain of *Eimeria tenella*: a review of the type strain selected for genome sequencing. *Avian Pathlogy*, 32:115-127.
13. Chapman,H.D., Barta,J.R., Blake,D., Gruber,A., Jenkins,M., Smith,N.C., Suo,X. and Tomley,F.M. (2013). A selective review of advances in coccidiosis research. *Advances in Parasitology*, 83:93-171.
14. Chen,H.L., Zhao,X.Y., Zhao,G.X., Huang,H.B., Li,H.R., Shi,C.W., Yang,W.T., Jiang,Y.L., Wang,J.Z., Ye,L.P., Zhao,Q., Wang,C.F., and Yang,G.L. (2020). Dissection of the cecal microbial community of chickens after *Eimeria tenlla* infection. *Parasites and Vectors*, 13:56,15 pages.
15. Conway,D.P. and McKenzie,M.E. (2007). *Poultry Coccidiosis-Diagnostic and Testing Procedures*. 3rd edn. Blackwell Publishing.
16. DelCacho,E., Gallego,M., Lillehoj,H.S., Quilez,J., Lillehoj,E.P., Ramo,A. and Sanchez-Acedo,C. (2014).

IL-17A regulates *Eimeria tenella* schizont maturation and migration in avian coccidiosis. *Veterinary Research*, 45:25, 9 pages.

17. DelCacho,E., Gallego,M., Francesch,M., Quilez,J. and Sanchez-Acedo,C. (2010). Effect of artemisinin on oocyst wall formation and sporulation during *Eimeria tenella* infection. *Parasitology International*, 59:506-511.
18. El-Ashram,E. and Suo,X. (2017). Elctrical cream separator coupled with vacuum filtration for the purification of *Eimeria* oocysts and trichostrongylid eggs. *Scientific Reports*, 7:43346.
19. El-Shazly,K.A., AbdEl-Latif,A., Abdo,W., El-Morsey,A., AbdEl-Aziz,M.I. and El-Mogazy,H. (2020). The anticoccidial activity of fluoroquinolone lomefloxacin against experimental *Eimeria tenella* infection in broiler chickens. *Parasitology Research*, 119:1953-1968.
20. Fatemi,A., Razavi,S.M., Asasi,K. and Goudarzi,M.T. (2015). Effects of *Artemisia annua* extracts on sporulation of *Eimeria* oocysts. *Parasitology Research*, 114(3):1207-1211.
21. Holdsworth,P.A., Conway,D.P., McKenzie,M.E., Dayton,A.D., Chapman,H.D., Mathis,G.R., Skinner,J., Mudt,HC., and Williams,R.B. (2004). World Association for the Advancement of Veterinary Parasitology (WAAVP) guide lines for evaluating the efficacy of anticoccidial drugs in chickens and turkeys. *Veterinary Parasitology*, 121:189-212.
22. Kadykalo,S., Roberts,T., Thompson,M., Wilson,J., Lang,M. and Espeisse,O. (2018). The value of anticoccidials for sustainable global poultry production. *International Journal of Antimicrobial Agents*, 51:304-310.
23. Kurkure, N.V., Kolte,S.W., Bhandarkar,A.G., and Kalorey, D.R. (2006). Evaluation of herbal coccidiostat 'Coxynil' in broilers. *Indian Journal of Experimental Biology*, 44:740-744.
24. Lan,L., Zuo,B., Ding,H., Huang,Y., Chen,X. and Du,A. (2016). Anticoccidial evaluation of a traditional Chinese medicine-*Bruceajavanica*-in broilers. *Poultry Science*, 95:811-818.
25. Liu,L., Chen,H., Wang,X., Zhang,W., Wang,M., Zhang,K., Zhang,L., Li,T. and Xue,F. (2016). Ultrastructural effects of acetamizuril on endogenous phases of *Eimeria tenella*. *Parasitology Research*, 115:1245-1252.
26. Long,P.L. and Jeffers,T.K. (1982). Studies on the stage of action of ionophorous antibiotics against *Eimeria*. *Journal of Parasitology*, 68:363-371.
27. McDougald,L. and Fitz-Coy,S. (2013). Coccidiosis :protozoan infections. In:Swayne,D.E (ed.). *Diseases of Poultry*, 13th edition. Blackwell, New York, pp.1148-1166.
28. Maes,L., Coussement,W., Vanprijs,O. and Marsboom,R. (1988). *In vivo* action of the anticoccidial diclzuril (Clinacox®) on the developmental stages of *Eimeria tenella*:a histology studies. *Journal of Parasitology*, 74(6):931-938.
29. Muthamilselvan,T., Kuo,T.F., Wu,Y.C. and Yang,W.C. (2016). Herbal remedies for coccidiosis control:a review of plants, compounds and anticoccidial actions. *Evidence-Based Complementary and Alternative Medicine*.Volume 2016, Article ID:2657981, 19pages.
30. Sharma,U.N.S., Fernando,D.D., Wujesundra,K.K., Manawadu,A.M., Pathirana,I. and Rajapakse,R.P.V. (2021). Anticoccidial effects of *Phyllanthus emblica* (Indian goose berry) extracts:potential for controlling avian coccidiosis. *Veterinary Parasitology:Regional Studies and Reports*, 100592.
31. She,R., Fei,C., Chen,H., Wang,X., Wang,M., Zhang,K., Zhang,L., Wang,C., Liu,Y., Zheng,W., and Xue,F. (2017). Action of nitromezuril against *Eimeria tenella* with clinically anticoccidial indices and histopathology. *Parasitology Research*, 116(8):2167-2174.
32. Shirley,M.W. (1995). *Eimeria* species and strains of chickens. In:Eckert.J, Braun,R., Shirley,M.W. Coudert,P. (Eds.). *Biotechnology–Guide lines on Techniques in Coccidiosis Research*. Luxemburg:European Commission, Pp.1-24.
33. Soulsby,E.J.L. (1986). *Helminths, Arthropods and Protozoa of Domesticated Animals*. 7th edition, Balliere Tindall, London.
34. Srivinasu,B., ChinniPreetam,V., Srinivas,G. and SrinivasRao,D. (2019). Effect of cocci ban herbal coccidiostats on haematological, fecal parameters and cecal histopathology of broiler chicken. *Tropical Animal Health and Production*, 51:1375-1381.
35. Suprihati,E. and Yunus,M. (2018). Evaluation of the antigenicity and immunogenicity of *Eimeria tenella* by reproductive index and histopathological changes of cecal coccidiosis virulent live vaccine in broiler chickens. *African Journal of Infectious Diseases*, 12(S):104-110.
36. Yang,W.C., Yang,C.Y., Liang,Y.C., Yang,M.T., Kuo,T.F., Lin,C.F., Liang,C.L. and Yang,C.L.T. (2019). Anti-coccidial properties and mechanisms of an edible herb, *Bidens pilosa*, and its active compounds for coccidiosis. *Scientific Reports*,9:2896.
37. Yeap,S.K., Ho,W.Y., Beh,B.K., Liang,W.S., Ky,H., Yousr,A.H.N. and Alitheen,N.B. (2010). *Vernonia amygdalina*, an ethnoveterinary and ethnomedical used green vegetable with multiple bioactivities. *Journal of Medicinal Plants Research*, 4(25):2787-2812.
38. Zhang, D.F., Sun, B.B., Yue, Y.Y., Yu, H.J., Zhang, H.L., Zhou, Q.J. and Du, A.F. (2012). Anticoccidial effect of halofuginone hydrobromide against *Eimeria tenella* with associated histology. *Parasitology Research*, 111:695-701.