



EFFECTS OF DAFLON 500 MG ON ERYTHROCYTE OSMOTIC FRAGILITY AND
MALONDIALDEHYDE CONCENTRATION IN BROILER CHICKENS INFECTED WITH *EIMERIA*
TENELLA

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ABSTRACT

The study was carried out to evaluate the effects of Daflon[®] 500 mg (DF) on erythrocyte osmotic fragility (EOF) and malondialdehyde (MDA) concentration of broiler chickens infected with *Eimeria tenella*. Twenty-five chickens randomly allotted into five groups of five each were used. Group A was uninfected untreated, Group B served as the infected-untreated, Groups C and D were first pretreated orally for three days with DF at 100 and 200 mg/kg, respectively, after which they were infected and treatment with DF continued for 11 more days. Group E birds were infected and treated with amprolium at 150 g/L of drinking water for five days. All the infected groups were experimentally inoculated with 20,000 sporulated *Eimeria tenella* oocysts. At day 7 and 14 post-infection, blood was collected into plain and ethylenediaminetetraacetic acid containing sample bottles for the evaluation of MDA and EOF, respectively. The percent haemolysis (at 0.9%, 0.7% and 0.5% NaCl concentration) and MDA concentration were significantly ($p < 0.05$) lower in the DF treated groups when compared to the infected untreated. The administration of DF ameliorated the oxidative stress induced by infection with *E. tenella* in broiler chickens evident by the significant decrease recorded in the values of EOF and MDA.

Keywords: Daflon[®]500mg, erythrocyte EOF, MDA, *Eimeria tenella*

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INTRODUCTION

Avian coccidiosis is a disease of great economic importance in the poultry industry caused by the intracellular protozoan parasite of the genus *Eimeria* [1]. The global economic loss attributed to coccidiosis is estimated at approximately 13 million US dollars, encompassing expenses related to drugs, disinfectants, vaccines, low feed conversion rate, decrease productivity and mortality [2, 3]. Erythrocyte osmotic fragility is said to be an indirect method of assessing oxidative stress as it gives information about the total status of red cell metabolism and membrane stability [4, 5]. Lipid peroxidation, the oxidation of polyunsaturated fatty acids, is a central feature of oxidative stress, a phenomenon increasingly implicated as causative in numerous pathological conditions [6], including coccidiosis. Lipid peroxidation can be assessed by measuring the products of lipid peroxidation such as malondialdehyde concentration in biological samples [7]. Infection with *Eimeria* parasite induces severe inflammatory response, leading to tissue oxidative stress characterized by increased production of reactive oxygen species (ROS) thereby causing lipid peroxidation damage and cytotoxicity and partially contributing to the pathology of infection [8].

The control of coccidiosis mostly depends on chemoprophylaxis using anticoccidial drugs [9]. However, due to frequent and irrational use of these anticoccidial drugs, varying degree of resistance has developed against almost all available anticoccidial drugs in various parts of the world [8, 10]. Moreover, drug residues in meat, eggs and poultry by-products may be potentially toxic to human beings [2, 11]. To overcome the alarming situation of resistance, integrated approaches such as the use of phytochemical substances with antioxidant activity may be adopted for the control of coccidiosis in poultry.

Daflon[®] 500 mg, a micronized purified flavonoid fraction consisting of 90% diosmin and 10% hesperidin, has gained considerable attention in human for its antioxidant, anti-inflammatory, immunomodulatory and venoprotective properties. In recent times, it has been reported to ameliorates the anaemia and lipoperoxidative changes induced by *Trypanosoma brucei brucei* infection in Wistar rats [12, 13]. Despite these promising benefits, the use of Daflon[®] 500 mg in poultry, particularly against coccidiosis, remains largely unexplored. The antioxidant and anti-inflammatory effects of Daflon[®] 500 mg may mitigate the oxidative stress and inflammation associated with *Eimeria* infection, thereby

reducing the severity of the disease and improve the overall health of infected birds. Therefore, with the increasing prevalence of drug-resistant *Eimeria* strains and the need for alternative treatment options, investigating the efficacy of Daflon® 500 mg against *Eimeria tenella* could provide valuable insights into new strategies for managing coccidiosis in poultry.

MATERIALS AND METHODS

Experimental animals

Twenty-five (25) day-old-broiler chicks were obtained from OLAM Farms Ltd, Kaduna, Nigeria. They were fed with pelleted starter mash for broilers (Chikun feed®) that does not contain any coccidiostat. Drinking water was given *ad libitum*. They were raised as a single group from one-day old till day 21. The chicks were housed at the poultry house of the Veterinary Teaching Hospital, Ahmadu Bello University, Zaria, Nigeria, under proper condition for animal rearing in terms of light and ambient temperature and strict sanitation practices were put in place within their environment before and during the course of the experiment.

Source of *Eimeria tenella*

PCR-identified and pure (100%) preserved isolate of *E. tenella* oocysts in 2.5 % potassium dichromate solution were obtained from the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. The oocysts were preserved in the refrigerator until needed. The experimental birds were challenged with 20,000 sporulated oocysts of *E. tenella* in 1 mL physiological saline per bird. The oocysts number was determined using the McMaster counting apparatus as described by Cedric *et al.* [14].

Experimental design

At 21 days old, the twenty-five broiler chickens were allotted into five groups of five each and treated as follows:

Group A: uninfected and untreated.

Group B: infected with 20,000 sporulated oocysts of *E. tenella* in 1 mL physiological saline by oral administration and left untreated.

Group C: pretreated with Daflon® 500 mg (100 mg/kg) for 3 days, infected with 20,000 sporulated oocysts of *E. tenella* in 1 mL physiological saline by oral administration. Treatment with Daflon® 500 mg continued for 11 more days.

Group D: pretreated for 3 days with Daflon® 500 mg (200 mg/kg), infected with 20,000 sporulated oocysts of *E. tenella* in 1 mL physiological saline by oral administration. Treatment with Daflon® 500 mg continued for 11 more days.

Group E: infected with 20,000 sporulated oocysts of *E. tenella* in 1 mL physiological saline by oral

administration, and by day 7 post infection (when infection was established), Amprolium (150 g/L) was administered in drinking water for 5 days.

Sample collection

Blood samples were collected at day 7 and 14 post-infection into plain and ethylenediaminetetraacetic acid (EDTA) containing sample bottles for the evaluation of erythrocyte osmotic fragility (EOF) and malondialdehyde (MDA) concentration, respectively.

In vitro evaluation of erythrocyte osmotic fragility

Erythrocyte osmotic fragility was determined as described by Faulkner and King [15] and as modified by Oyewale [16]. Sodium chloride (pH 7.4) was prepared at varying concentrations, 0.0%, 0.1%, 0.3%, 0.5%, 0.7%, and 0.9%. Five milliliters (5mL) of each NaCl concentration was placed in labeled test tubes serially in a rack. Twenty microliters of blood sample from each bird were pipetted into each of the tubes. The contents of the tubes were mixed gently by inverting the tubes and allowed to stand at room temperature for 30 minutes. The tubes were centrifuged at 200 revolutions for 5 minutes using a centrifuge machine. The supernatant obtained from each tube was transferred to a clean glass cuvette and the absorbance of the supernatant measured using UV spectrophotometer at 540 nm. The percentage hemolysis from each sample was calculated using the formula:

$$\text{Percentage (\%) hemolysis} = \frac{\text{Optical density of test solution}}{\text{Optical density of standard solution}} \times 100$$

Determination of malondialdehyde concentration

Serum malondialdehyde concentration was evaluated using the method of Draper and Hadley [17] as modified by Altuntas *et al.* [18]. The principle of the method was based on spectrophotometric measurement of the colour produced during the reaction of thiobarbituric acid (TBA) with malondialdehyde. Briefly, 2.5 mL of 100 g/L trichloroacetic acid solution was added to 0.5 mL of serum in a centrifuge tube and placed in a boiling water bath for 15 min. After cooling in tap water, the mixture was centrifuged at 1000 x g for 10 min, and 2 mL of the supernatant was added to 1 mL of 6.7 g/L TBA in a test tube and placed in a boiling water bath for 15 min. The solution was then cooled in tap water and its absorbance measured using a UV spectrophotometer (Jenway, 6405 Model, Japan) at 532 nm. 1 mL of 10% trichloroacetic acid (TCA) and 1 mL of 0.67% thiobarbituric acid (TBA) was used as the blank.

Data analysis

Values obtained were expressed as mean ± standard error of the mean (mean ± SEM). Data were subjected

to one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. All the data were analyzed using Graphpad Prism, version 12.0 for windows (Graphpad Software, San Diego, California, USA). Values of $P < 0.05$ were considered significant

RESULTS

Effects of treatment on erythrocyte osmotic fragility at day 7 post-infection

The effect of treatment on the erythrocyte osmotic fragility of broiler chickens at day 7 post-infection is shown in Figure 1. At 0.9%, 0.7% and 0.5% NaCl concentration, the EOF of birds in the infected untreated and amprolium (Groups B and E) treated groups were significantly ($p < 0.05$) higher when compared to other treatment groups.

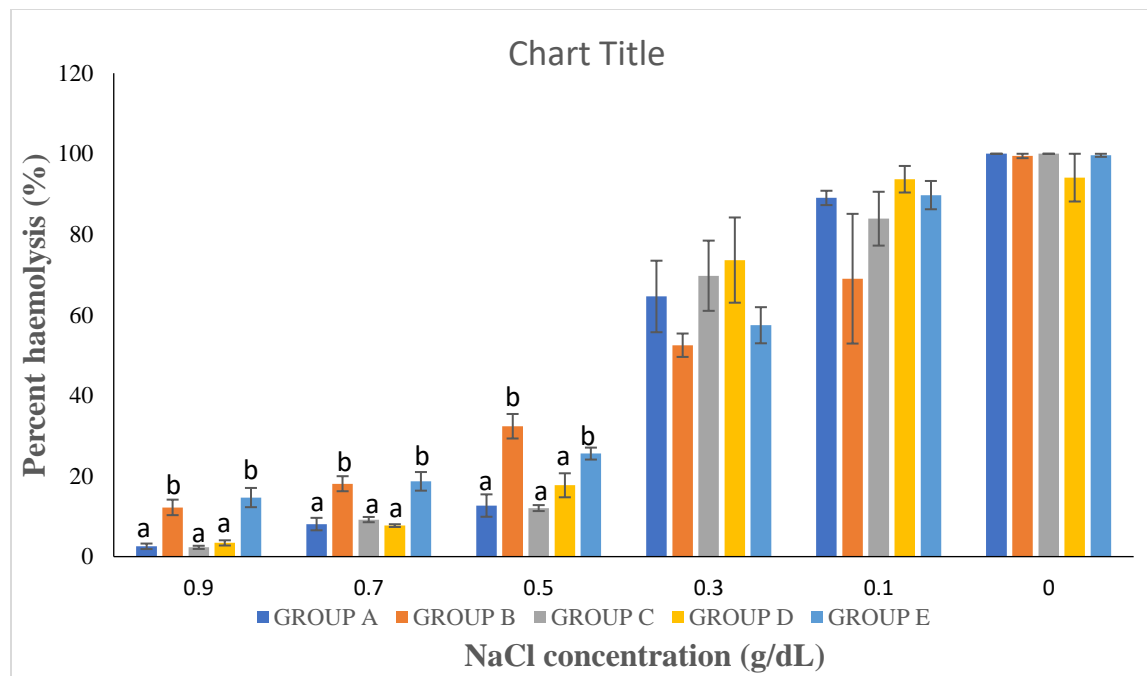


Figure 1: Effects of treatment with Daflon® 500 mg on erythrocyte osmotic fragility of broiler chickens infected with *Eimeria tenella* at day 7 post-infection.

a, b = Means with different superscript letters are significantly ($P < 0.05$) different. Values are mean \pm SEM of 3 animals per group.

KEY: Group A = uninfected untreated; Group B = infected untreated; Group C = daflon (100 mg/kg) + *E. tenella*; Group D = daflon (200 mg/kg + *E. tenella*; Group E = *E. tenella* + amprolium.

Effects of treatment on the erythrocyte osmotic fragility at day 14 post-infection

The EOF (0.9%, 0.7% and 0.5% NaCl concentration) of birds in group B was significantly ($p < 0.05$) higher at day 14 post-infection when compared to other treatment groups. Although not significant, the EOF (at 0.9%, 0.7% and 0.5% NaCl concentration) of the amprolium treated group (Group E) was relatively higher when compared to the uninfected untreated (Group A) and DF (Groups C and D) treated groups (Figure 2).

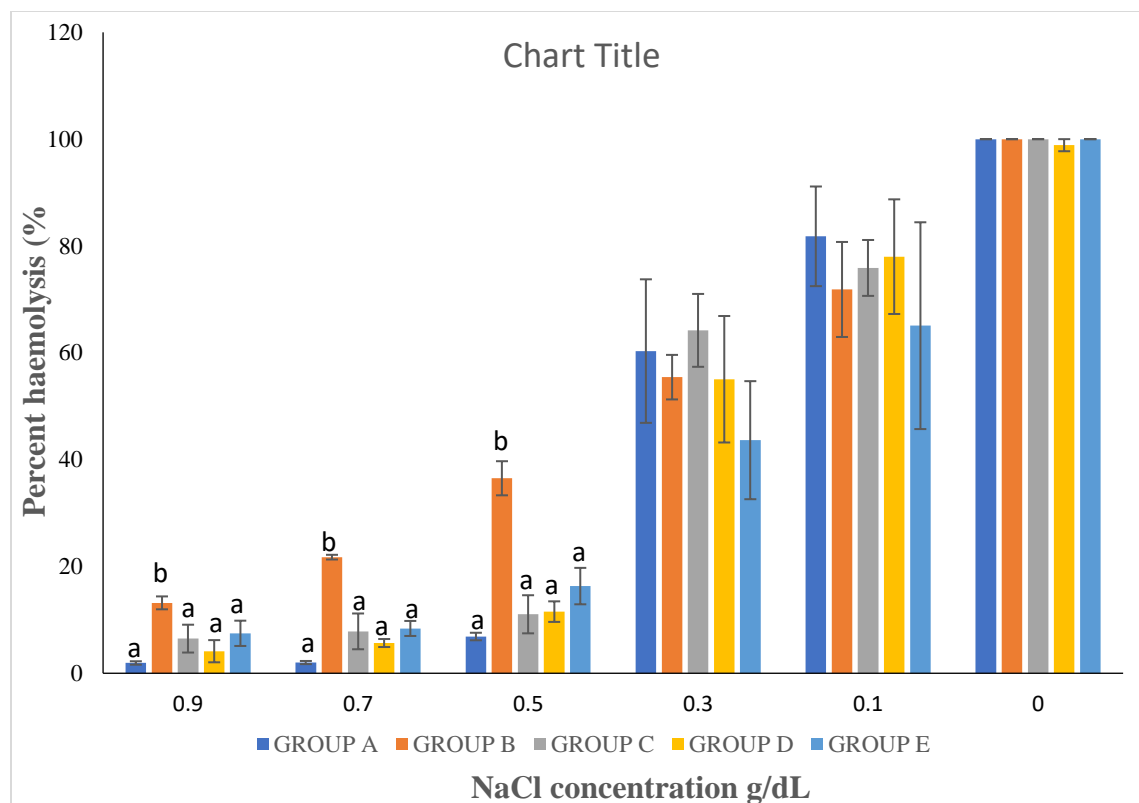


Figure 2: Effects of treatment with Daflon® 500 mg on erythrocyte osmotic fragility of broiler chickens infected with *Eimeria tenella* at day 14 post-infection

a, b, c = Means with different superscript letters are significantly ($P < 0.05$) different. Values are mean \pm SEM of 3 animals per group.

KEY: Group A = uninfected untreated; Group B = infected untreated; Group C = daflon (100 mg/kg) + *E. tenella*; Group D = daflon (200 mg/kg + *E. tenella*; Group E = *E. tenella* + amprolium

Effects of treatment on malondialdehyde concentration at days 7 and 14 post-infection

At day 7 post-infection, the MDA concentration of birds in the infected untreated and amprolium (Groups B and

E) treated groups were significantly ($p < 0.05$) higher when compared to the DF (Groups C and D) treated groups. The MDA concentration of birds in the amprolium treated group (Group E) decreased significantly ($p > 0.05$) at day 14 post-infection as compared to other treatment groups. Although not significant, the group treated with DF at 200 mg/kg (Group d) showed a relative decrease in MDA concentration in comparison to those treated with 100 mg/kg (Group C) of birds treated with 200 mg of DF (Group D) when compared to those treated with 100 mg/kg (Group C) at days 7 and 14 post-infection (Figure 3).

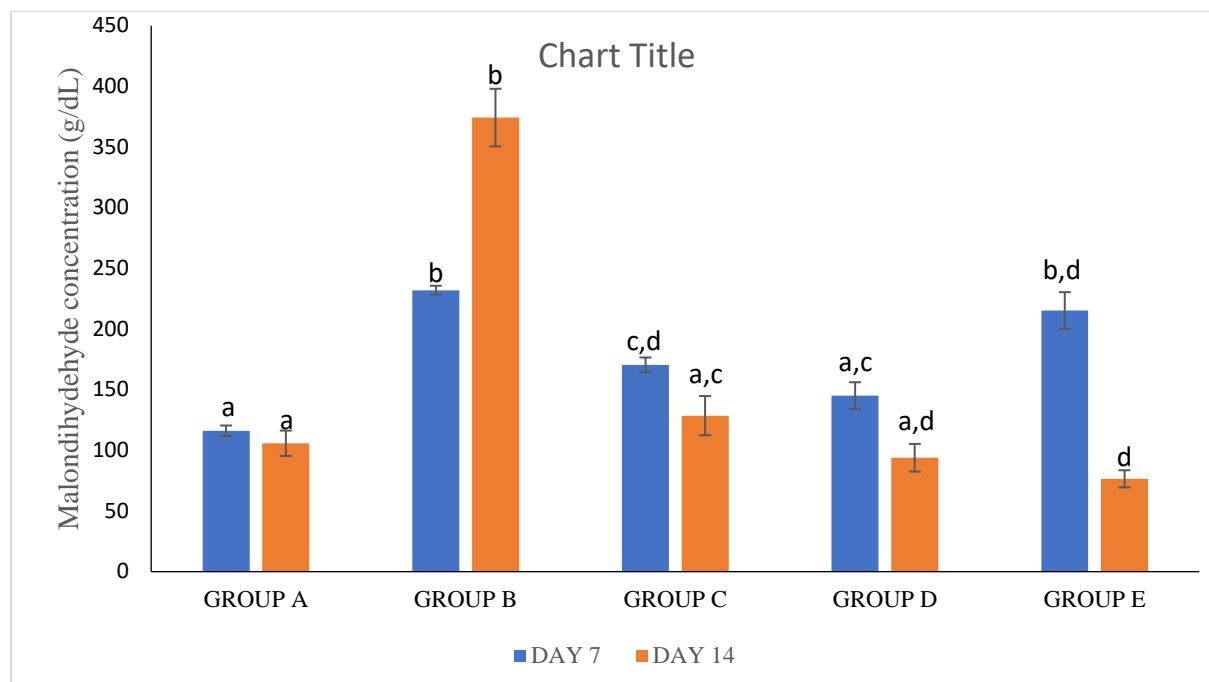


Figure 3: Effects of treatment with Daflon® 500 mg on malondialdehyde concentration of broiler chickens infected with *Eimeria tenella* at days 7 and 14 post-infection.

a, b, c, d = Means with different superscript letters are significantly ($P < 0.05$) different. Values are mean \pm SEM of 3 animals per group.

KEY: Group A = uninfected untreated; Group B = infected untreated; Group C = daflon (100 mg/kg) + *E. tenella*; Group D = daflon (200 mg/kg + *E. tenella*; Group E = *E. tenella* + amprolium.

DISCUSSION

Coccidiosis remains a significant threat in poultry production, particularly in young birds leading to severe morbidity and mortality due to the pathogenic effects of *Eimeria tenella* on the intestinal tract. The result of this study has shown that infection with *E. tenella* causes increase in erythrocyte osmotic fragility due to increased lipoperoxidation of erythrocytes membranes. The normal erythrocyte membrane is remarkable for its durability however, its tolerance is altered under certain pathologic conditions [4, 19]. Lipid peroxidation occurs when the formation of free radicals exceeds the value compensated by the cells and the cell defense systems are insufficient to destroy these compounds. Malondialdehyde a by-product of lipid peroxidation is generated by the action of reactive oxygen species (ROS) on polyunsaturated fatty acids [20]. Its concentration in blood and tissues are directly proportional to cellular damages caused by ROS and is, for this reason a useful marker in the analysis of oxidative stress [21, 22]. The significant increase in MDA concentration and erythrocyte fragility recorded

in the infected untreated group at days 7 and 14 post infection is in agreement with Pajic *et al.* [21] and Ogwiji *et al.* [22] who reported an increase in MDA concentration of birds infected with different *Eimeria* species. The increase in MDA is an indication of increased generation of reactive oxygen species (ROS) which may have led to an increase in peroxidation of erythrocytes membranes and hemolysis. Infection with various pathogens may alter the host's antioxidant defense against ROS, resulting in over-production of ROS or depletion of antioxidants [8, 23]. Treatment with DF significantly decreased erythrocyte fragility and MDA concentration as seen in groups C and D at days 7 and 14 post-infection. The decrease recorded may be attributed to the antioxidant effects of DF that scavenged the ROS produced by *E. tenella* infection while simultaneously improving the serum antioxidant capacity [24] thereby preventing lipid peroxidation and hemolysis. The administration of amprolium (Group E) decreased the concentration of malondialdehyde at day 14 post-infection. This observation is in accordance with the work of Pajic *et al.* [21] in broiler chickens who reported that anticoccidials influenced the decrease in the production of ROS and thus led to reduced lipid peroxidation.

CONCLUSION

Infection of broiler chickens with *E. tenella* increased erythrocyte fragility due to elevated lipoperoxidative changes in the erythrocyte membranes which may

increase the vulnerability of erythrocytes to haemolysis and contributes to the anaemia associated with avian coccidiosis. Treatment with DF ameliorated the lipid peroxidation and erythrocyte fragility caused by *E. tenella* infection in broiler chickens.

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REFERENCES

1. NAWARATHNE, S.R., YU, M. & HEO, J.M. (2021). Poultry coccidiosis – A concurrent overview on etiology, diagnostic practices and preventive measures. *Korean Journal of Poultry Science*, **48**(4): 297-318.
2. RISTANTI, R., HAMID, P.H., NUGROHO, H.A., NURINGTYAS, T.R., WIBOWO, S., FERDIAN, P.R., AMALIA, R.R., CARO, T.M., KUSUMARINI, S., WARDHANA, A.H. & MATSUBAYASHI, M. (2024). Anticoccidial activities of *Piper betle* L essential oil on *Eimeria tenella* oocysts. *Scientific Reports*, **14**:25568. <https://doi.org/10.1038/s41598-024-76754-7>.
3. TCHODO, F.G., DAKPOGAN, H.B., SANVEE, S., ADJEI-MENSAH, B., KPOMASSE, C.C., KAROU, S., PITALA, W., TONA, K. & BAKOMA, B. (2024). The anticoccidial in vitro effects and antioxidant properties of several plants traditionally used for coccidiosis in Togo. *Veterinary Science*, **11**: 345. <https://doi.org/10.3390/vetsci11080345>.
4. IGBOKWE, N.A. (2018). A review of the factors that influence erythrocyte osmotic fragility. *Sokoto Journal of Veterinary Sciences*, **16**(4): 1-23.
5. UCHENDU, C., AMBALI, S.F., AYO, J.O., ESIEVO, K.A.N. & UMOSEN, A.J. (2014). Erythrocyte osmotic fragility and lipid peroxidation following chronic co-exposure of rats to chlorpyrifos and deltamethrin, and the beneficial effects of alpha-lipoic acid. *Toxicological Reports*, **1**: 373-378.
6. YIN, H., MUSIEK, E.S. & MORROW, J.D. (2006). Quantification of isoprostanes as an index of oxidative stress: An update. *Journal of Biological Sciences*, **6**(3): 469-479.
7. JUAN, C.A., PÉREZ DE LA LASTRA, J.M., PLOU, F.J. & PÉREZ-LEBEÑA, E. (2021). The Chemistry of Reactive Oxygen Species (ROS) Revisited: Outlining Their Role in Biological Macromolecules (DNA, Lipids and Proteins) and Induced Pathologies. *International Journal of Molecular Sciences*, **22**: 4642. <https://doi.org/10.3390/ijms22094642>.
8. MASOOD, S., ABBAS, R.Z., IQBAL, Z., MANSOOR, M.K., SINDHU, Z., ZIA, M.A. & KHAN, J.A. (2013). Role of natural antioxidants for the control of coccidiosis in poultry. *Pakistan Veterinary Journal*, **33**(4): 401-407.
9. TEWARI, A.K. & MAHARANA, B.R. (2011). Control of poultry coccidiosis: changing trends. *Journal of Parasitic Diseases*, **35**: 10-70.
10. FELICI, M., TUGNOLI, B., GHISELLI, F., BALDO, D., RATTI, C., PIVA, A. & GRILLI, E. (2023). Investigating the effects of essential oils and pure botanical compounds against *E. tenella* in vitro. *Poultry Science*, **102**: 102898. <https://doi.org/10.1016/j.psj.2023.102898>.
11. OWUSU-DOUBREH, B., APPAW, W.O. & ABE-INGE, V. (2023). Antibiotic residues in poultry eggs and its implication on public health: A review. *Scientific African*, **19**: e01456. <https://doi.org/10.1016/j.sciaf.2022.e01456>.
12. KOBO, P.I., AYO, J.O., ALUWONG, T., ZEZI, A.U., MAIKAI, V. & AMBALI, S.F. (2014). Flavonoid mixture ameliorates increase in erythrocyte osmotic fragility and malondialdehyde concentration induced by *Trypanosoma brucei brucei*-infection in Wistar rats. *Research in Veterinary Science*, **96**: 139-142.
13. KOBO, P.I., AYO, J.O., ALUWONG, A., ZEZI, A.U. & MAIKAI, V.A. (2015). Haematological changes in *Trypanosoma brucei brucei* infected Wistar rats treated with a flavonoid mixture and/or diminazene aceturate. *Biology and Medicine*, **6**: 213. Doi: 10.4172/0974-8369.1000213.
14. CEDRIC, Y., PAYNE, V.K., NADIA, N.A.C., KODJIO, N., KOLLINS, E., MEGWI, L. & KUIATE, J.R. (2018). In vitro antioxidant and anticoccidial studies of *Pentaclethra macrophylla* extract against *Eimeria magna*, *Eimeria flavescens*, *Eimeria intestinalis*, *Eimeria stiedae* oocyst and sporozoites of rabbits. *Journal of Advance Parasitology*, **5**(3): 38-48.
15. FAULKNER, W. R. & KING, J.W. (1970). Manual of Clinical Laboratory Procedures, Chemical Rubber Company, Cleveland, Ohio, U.S.A., 346pp.
16. OYEWALE, J.O. (1991). Osmotic fragility of erythrocyte of guinea fowls at 21 and 156 weeks of age. *Veterinarski Arhiv*, **61**(1): 49-56.

17. DRAPER, H.H. & HADLEY, M. (1990). Malondialdehyde determination as index of lipid peroxidation. *Methods in Enzymology*, **186**: 421-431. Doi: 10.1021/tx0499259
18. ALTUNTAS, I., DELIBAS, N. & SUTCU, R. (2002). The effects of organophosphate insecticide methidathion on lipid peroxidation and anti-oxidant enzymes in rat erythrocytes: Role of vitamin E and C. *Human Experimental Toxicology*, **21**: 681-685.
19. SHARMA, B., RAI, D.K., RAI, P.K., RIZVI, S.I. & WATAL, G. (2010). Determination of erythrocyte fragility as a marker of pesticide-induced membrane oxidative damage. *Advanced Protocols in Oxidative Stress II: Methods. Molecular Biology*, **594**(1):123-128.
20. YILMAZ, S., ISSSI, M., KANDEMIR, F.M. & GUL, Y. (2014). Malondialdehyde and total antioxidant levels and haematological parameters of beef cattle with coccidiosis. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, **25**(2): 41-45.
21. PAJIC, M., ALEKSIC, N., VEJNOVIC, B., POLACEK, V., NOVAKOV, N., ANDRIC, D.O. & STANIMIROVIC, Z. (2019). Influence of anticoccidials on oxidative stress, production performance and faecal oocysts count in broiler chickens infected with *Eimeria* species. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, **25**(3): 379-385.
22. OGWIJI, M., JATAU, I.D., NATALA, A.J., MOHARY, M., AL-REJAIE, S.S. AND ZHU, M. (2024). Effects of prebiotic, probiotic, and symbiotic products on oxidative status, performance, and parasitological parameters of broiler chickens induced with cecal coccidiosis. *The Journal of Applied Poultry Research*, **33**: 100472. <https://doi.org/10.1016/j.jarpr.2024.100472>.
23. UMAR, I.A., OGENYI, E., OKODASO, D., KIMENG, E., STANCHEVA, G.I., OMAGE, J.J., ISAH, S. & IBRAHIM, M.A. (2007). Amelioration of anaemia and organ damage by combined intraperitoneal administration of vitamins A and C to *Typanosoma brucei*-infected rats. *African Journal of Biotechnology*, **6**(18): 2083–2086.
24. ZAHRA, Z., HEIDI, A. & BLASSAN, P.G. (2024). Flavonoids: Antioxidant powerhouses and their role in nanomedicine. *Antioxidants*, **13**: 922. <https://doi.org/10.3390/antiox13080922>.