ejpmr, 2025, 12(5), 100-111

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION OF THE ANTICOCCIDIAL EFFICACY OF HERBAL ANTICOCCIDIAL, COCCI-00® IN BROILER BIRDS EXPERIMENTALLY INFECTED WITH *EIMERIA TENELLA*

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Article Received on 11/03/2025 **Article Revised on 01/04/2025** **Article Published on 22/04/2025**

ABSTRACT

Coccidiosis, caused by *Eimeria* spp., is a major constraint to broiler production worldwide, with *Eimeria tenella* inducing severe caecal damage, weight loss, and high mortality. Rising drug resistance and consumer demand for residue-free poultry products have spurred interest in herbal anticoccidials. This study evaluated the therapeutic efficacy of Cocci-00®, a commercial herbal formulation containing extracts of *Aegle marmelos*, *Holarrhena antidysenterica*, and *Azadirachta indica* plus vitamins A and K and prebiotics, in broiler chickens experimentally infected with *E. tenella*. Ninety broiler birds aged two weeks old were randomly assigned to six groups (n=15): Group I, uninfected control; Group II, infected-untreated control; Group III, infected and treated with Embazine Forte® (30 g/50 L water); Groups IV–VI, infected and treated with low (1 mL/2 L), medium (2 mL/L), and high (3 mL/L) dosages of Cocci-00®, respectively. Birds in Groups II–VI were orally challenged with 2×10^4 sporulated *E. tenella* oocysts. Treatments were administered via drinking water following manufacturers' guidelines. Parameters monitored included clinical signs, mortality and survival time, oocyst output, lesion scoring, growth performance (body weight gain, feed intake, feed conversion ratio), haematology, and serum protein profiles. The infected-untreated group exhibited 40% mortality, markedly elevated oocyst counts and lesion scores, and impaired weight gain and haematological indices. Embazine Forte® and Cocci-00® treatments significantly reduced mortality. Medium- and high-dose Cocci-00® (Groups V and VI) achieved oocyst counts, lesion scores, and growth performance comparable to Embazine Forte® and uninfected controls ($p > 0.05$). Haematological and serum protein parameters in these groups were restored to near-normal levels. These findings demonstrate Cocci-00® as a potent herbal alternative, effectively mitigating *E. tenella* pathogenesis while enhancing growth and metabolic recovery in a dose-dependent manner. Its multicomponent formulation offers a sustainable solution for coccidiosis control in resource-limited settings, aligning with organic farming demands and addressing antimicrobial resistance challenges.

KEYWORDS: Herbal anticoccidial; *Eimeria tenella*; Broiler chickens; Oocyst counts; Growth performance.

INTRODUCTION

Coccidiosis, a parasitic disease caused by protozoan parasites of the genus *Eimeria*, is one of the most economically significant diseases in the poultry industry, globally affecting broiler production. Among the various *Eimeria* species, *Eimeria tenella* is particularly pathogenic, leading to severe caecal coccidiosis, characterised by haemorrhagic enteritis, reduced weight gain, and high mortality in chickens (Zhang et al., 2023). The disease impairs nutrient absorption and compromises growth performance, which cumulatively results in substantial financial losses for poultry producers (Freitas et al., 2023).

The economic burden of coccidiosis is particularly profound in Nigeria, where the poultry industry is critical in ensuring food security and providing employment. With poultry contributing significantly to the agricultural sector, representing around 25% of total livestock production in the country, the impact of diseases like coccidiosis is far-reaching (Kassali et al., 2022). The cost of coccidiosis to the Nigerian economy, including costs of control, mortalities, and morbidity is estimated to be £58.67 million (Blake et al., 2020). This is exacerbated by the high cost of imported anticoccidial drugs and the growing resistance of *Eimeria* strains to conventional treatment methods, further straining the profitability of

poultry operations (Blake et al., 2020). Given these challenges, there is an urgent need for affordable, effective, and residue-free alternatives, such as herbal anticoccidials, that can be locally produced and utilized to mitigate the economic losses associated with coccidiosis in Nigeria.

The control of coccidiosis has traditionally relied on the prophylactic and therapeutic use of synthetic anticoccidials, including ionophores and chemical drugs. However, the widespread and continuous use of these agents has led to the emergence of drug-resistant *Eimeria* strains, raising concerns about the long-term efficacy of these treatments (Abbas et al., 2020). In addition, consumer preferences have shifted towards organic and residue-free poultry products, driven by increasing awareness of food safety, drug residues, and environmental sustainability (Ofuoku and Akusu, 2016; Acee-Eke and Ikegwuru, 2020). Consequently, there is an urgent need for alternative strategies, such as natural products, that can effectively manage coccidiosis while aligning with the growing demand for organic farming practices.

Herbal medicines have garnered significant attention as viable alternatives to conventional drugs due to their broad-spectrum activity, low toxicity, and minimal risk of developing resistance (Wang et al., 2023). Several studies have explored the use of plant-based compounds with anticoccidial properties, such as saponins, flavonoids, and essential oils, which have demonstrated promising results in the control of *Eimeria* infections (Quiroz-Castañeda & Dantán-González, 2015).

Cocci-00®, a commercially available herbal anticoccidial formulation, is marketed as a natural remedy for preventing and treating coccidiosis in poultry. It is composed of a blend of medicinal herbs (*Aegle marmelos*, *Holarrhena antidysenterica*, and *Azadirachta indica*) traditionally known for their antimicrobial, antiparasitic, and immune-boosting properties. Despite its increasing acceptability and use in poultry production amongst resource-poor farmers, there is limited empirical data on the efficacy of Cocci-00® against *Eimeria tenella*, and its potential to serve as an alternative to conventional anticoccidial drugs has yet to be fully elucidated.

This study was designed to investigate the therapeutic efficacy of Cocci-00® in broiler chickens experimentally infected with *Eimeria tenella*. We hypothesized that Cocci-00® would effectively mitigate the clinical manifestations of coccidiosis, reduce intestinal damage, and improve overall growth performance, thus providing a sustainable alternative to synthetic anticoccidial agents. The findings of this study are expected to contribute to the growing body of research on natural anticoccidials and offer insights into the potential use of herbal formulations in sustainable poultry farming.

MATERIALS AND METHOD

Experimental animals

One hundred and seventeen (117) day-old broiler chicks were sourced from Olam Hatcheries for this study. The chicks were brooded in a deep litter system and transferred to cages at 3 weeks of age at the pen house of the Department of Animal Production & Biochemistry, Michael Okpara University of Agriculture, Umudike. The birds were fed a commercial pelleted poultry feed (Chikun®, Chikun Feed Mills, Kaduna, Nigeria), with water provided *ad libitum*. Prior to the experimental phase, the birds were routinely vaccinated against infectious bursal disease and Newcastle disease.

Experimental Drugs

Cocci-00® (Nutricare Life Sciences Limited, India), a commercially available herbal formulation, was administered following the manufacturer's instructions. Each litre of Cocci-00® contains 5,000,000 I.U. of vitamin A, 10 g of vitamin K, 300 g of Mannan Oligosaccharides (MOS), 100 g of Fructo-Oligosaccharides (FOS), along with herbal extracts from *Aegle marmelos*, *Holarrhena antidysenterica*, and *Azadirachta indica*. A standard anticoccidial drug, (Embazine Forte® (Turner Wright Ltd, Lagos, Nigeria) was used to assess the anticoccidial efficacy of Cocci-00®.

Infective Material

During necropsy, the caeca of broiler birds diagnosed of natural clinical coccidiosis were excised, and opened, and their contents were flushed into a beaker using tap water. The contents were then centrifuged, and the resulting sediment was re-suspended in a saturated sodium chloride (NaCl) solution to isolate the oocysts, following the procedure outlined by Levine (1973). The isolated oocysts were subsequently subjected to five rounds of washing by centrifugation with water to remove any residual salt and colouring agents.

The harvested *Eimeria* oocysts were induced to sporulate by treatment with 2.5% potassium dichromate solution, following the method outlined by Levine (1973). Two coccidia-free birds were inoculated with 10^5 sporulated oocysts to propagate the infection. These birds were monitored daily for clinical signs of coccidiosis and the presence of oocysts in their faeces. Faecal samples were collected and processed using the salt flotation technique to retrieve oocysts. The collected oocysts were then sporulated and preserved in 2% potassium dichromate solution (Levine, 1973) until needed for experimental use.

A total of twenty-five 2-week-old broiler chickens were selected to determine the infective dose of sporulated *Eimeria tenella* oocysts. The birds were randomly assigned into five groups (A–E), each consisting of five birds. Groups A through D were inoculated with increasing doses of sporulated *Eimeria tenella* oocysts: 5,000, 10,000, 20,000, and 30,000 oocysts per bird,

respectively, in a 1 mL aqueous suspension administered orally using a gavage. Group E served as the uninfected control. All birds were monitored daily for clinical signs characteristic of coccidiosis, and their faecal samples were examined for the presence of oocysts. Birds in Group D, infected with 20,000 sporulated oocysts per bird, exhibited severe clinical symptoms, including weight loss, high oocyst counts, and mortality. As a result, 2×10^4 sporulated oocysts per bird was selected as the infective dose for the experimental study.

Experimental Design

Ninety broiler birds, aged two weeks old, were randomly assigned to six groups (I-VI) of fifteen birds each. Randomisation was performed using the RAND () function in Microsoft Excel to ensure unbiased group allocation. Group I served as the uninfected control, while groups II–VI were infected with 2×10^4 sporulated *Eimeria tenella* oocysts via gastric gavage. Group II was the infected-untreated control. Group III was treated with Embazine Forte® (30 g/50 litres of water) for 3 days, followed by 2 days of plain water, and another 3 days of Embazine Forte® treatment. Groups IV, V, and VI received low (1 ml/2 L), medium (2 ml/L), and high (3 ml/L) doses of Cocci-00® in their drinking water, respectively, over a five-day treatment period. Throughout the experiment, the birds were closely monitored for clinical signs of coccidiosis. Parameters such as body weight, weight gain, oocyst output, feed intake, feed conversion ratio (FCR), and lesion scores were carefully tracked. Also, haematological profiles, including leukocyte and erythrocyte counts, along with protein profiles were analysed to assess the physiological impact of the treatments.

The salt flotation technique, as outlined by Levine (1973), was used to detect the presence of oocysts in the faecal samples of each bird. Quantification of oocysts per gram of faeces was carried out daily from day 3 post-infection until the conclusion of the study using the modified McMaster method (Ministry of Agriculture, Fisheries and Food, 1977). To assess lesion severity, three birds from each group were randomly selected and humanely euthanized on day 8 post-infection. Lesion scoring followed the guidelines of Conway and McKenzie (2007), with scores ranging from 0 to 4 based on the extent of tissue damage. A score of 0 indicated the absence of visible lesions, while scores of 1, 2, 3, and 4 corresponded to mild lesions, more prominent discrete lesions, severe lesions with coalesced thickened intestinal walls, and extensive coalescence with bloody caecal contents, respectively.

The live body weight of each bird was measured daily throughout the experiment using a calibrated weighing balance. Weights were recorded every morning between 7:00 and 8:00 a.m. before feeding. Weight gain was calculated by subtracting the birds' initial weight from their final weight at the end of the study. Daily feed intake was monitored by weighing leftover feed and subtracting it from the total amount provided. Cumulative feed intake was determined at the end of the experiment, and the feed conversion ratio (FCR) for each group was calculated using the formula: FCR = total feed intake / total weight gain. Haematological profiles, including leukocyte and erythrocyte counts, as well as total protein levels, were assessed weekly following standard protocols (Doumas et al., 1971; Lubran, 1978; Coles, 1986).

Handling of Experimental Animals

Ethical clearance for this study was granted by the Institutional Animal Care and Use Committee (IACUC) of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. All procedures involving the birds adhered strictly to the ethical guidelines and regulations for the care and use of laboratory animals in biomedical research, ensuring humane treatment and minimizing discomfort throughout the experiment.

Statistical Analysis

The data were analysed using SPSS version 21 for Windows, applying one-way analysis of variance (ANOVA). Post hoc comparisons of means were performed using the Least Significant Difference (LSD) test, with p-values ≤ 0.05 considered statistically significant. The survival time of the birds was evaluated using the Kaplan-Meier survival analysis, and differences in survival times were tested with the log-rank test.

RESULTS

Clinical signs, Mortality/Survivability, Oocyst counts, and Lesion score

Following the infection of the experimental groups of birds, oocysts were observed in their faeces from day 4 post-infection (PI). The clinical signs observed include ruffled feathers, anorexia, emaciation, depression, drooping, bloody diarrhoea, and death. Following treatment of birds in groups 3 – 6, these clinical signs gradually disappeared. However, these clinical signs were marked in the infected-untreated group 2 birds.

The infected-untreated group 2 birds exhibited a 40% mortality rate (6/15 birds), whereas no deaths occurred in the uninfected-untreated control group 1 (Table 1 and Figure 1). Among treated groups, mortality rates were 6.7% (1/15) in groups 3 and 5, 13.3% (2/15) in group 4, and 0% in group 6. The mean post-infection survival time of group 2 was significantly shorter ($P < 0.05$) than all other groups (Figure 2), with no significant differences observed among treated groups.

Following infection, the infected-untreated group (Group 2) showed an exponential increase in mean oocyst counts, which remained significantly higher ($P < 0.05$) than all other groups from day 4 post-infection (PI) until the end of the study (Figure 3). Among treated groups, group 4 (infected and treated with 1 mL/L Cocci-00®)

exhibited significantly elevated ($P < 0.05$) oocyst counts on days 7, 10, and 13 PI compared to groups 3, 5, and 6. In contrast, group 3 (infected and treated with Embazine forte) had significantly lower ($P < 0.05$) oocyst counts than groups 5 and 6 on day 7 PI. Subsequently, there were no significant differences in mean oocyst counts among groups 3, 5, and 6 for the remainder of the study.

The infected-untreated group 2 birds had significantly higher mean lesion score compared to groups 1, 3, 5, and 6 (Figure 4). Among the treated groups, groups 3, 5, and 6 had comparable mean lesion score but were significantly low compared to that of group 4 birds. Also, the mean lesion scores of groups 3 and 6 were comparable to that of the uninfected control group.

Growth performance indices

The uninfected-untreated control group demonstrated the highest mean body weight throughout the study period (Figure 5). In contrast, the infected-untreated group 2 birds showed a significant reduction ($P < 0.05$) in the mean body weight compared to Group 1. Treatment with Embazine forte (Group 3) or Cocci-00 (groups 4-6) led to a marked improvement ($P < 0.05$) in body weight relative to the infected-untreated group 2 birds, nearly approximating the values of Group 1.

The mean weight gain, weight gain per day, and feed efficiency of group 2 (infected-untreated) birds was significantly low ($P < 0.05$) compared to the rest of the groups (Table 1). Group 1 (uninfected-untreated) recorded the highest mean weight gain, weight gain per day, and feed efficiency. The mean weight gain, weight gain per day, and feed efficiency of the treated groups 3 – 6 did not differ statistically. However, the Cocci-00-treated groups 4 - 6 had improved mean weight gain, weight gain per day, and feed efficiency compared to the Embazine forte-treated group 3 birds. Among the cocci-00-treated groups, group 5 had better mean weight gain, weight gain per day, and feed efficiency, followed by group 6 birds. The mean feed conversion ratio of the infected-untreated group 2 birds was significantly higher ($P < 0.05$) than the rest of the groups (Table 1). There was no statistical variation in the mean feed conversion ratios of the uninfected-untreated group, Embazine forte, and Cocci-00-treated groups.

Haematology

The mean red blood cell (RBC) count, haemoglobin concentration, and packed cell volume (PCV) significantly decreased ($P < 0.05$) in the infected-untreated group 2 birds compared to the other group of birds from day 7 PI (Table 2). Among the treated groups of birds, the mean RBC count, haemoglobin concentration, and PCV did not differ significantly except on day 14 PI, when the group 6 birds had significantly higher ($P < 0.05$) mean RBC counts and PCV. A significant increase ($P < 0.05$) in the mean white blood cell (WBC) counts was observed in the infected-untreated group compared to the uninfected control group on days 14 and 21 PI (Table 2). However, no statistical difference was found in the mean WBC counts of infected-untreated group and the treated groups of birds throughout the experiment.

The mean MCV of the treated groups of birds and the uninfected control group were comparable but were significantly lower ($P < 0.05$) than the infected-untreated group on day 21 PI (Table 3). On day 14 PI, the mean MCV of the infected-untreated group was higher ($P < 0.05$) than those of the uninfected control group and 1ml/L Cocci-00-treated group 4 but were comparable to those of groups 3, 5, and 6. The mean MCH values of the birds did not differ except on days 14 and 21 PI (Table 3). On day 14 PI, groups 1 and 6 had considerably lower ($P < 0.05$) mean MCH value compared to groups 2, 3, and 5. By day 21 PI, there were no statistical variation in the mean MHC of the uninfected control and the treated groups. The mean MCHC values of the treated groups of birds were comparable to that of the uninfected control group (Table 3). However, group 5 birds had higher ($P < 0.05$) mean MCHC value than the infected-untreated group 2 birds.

The mean lymphocyte, heterophil, monocyte, eosinophil, and basophil counts were presented in Table 4. The mean lymphocyte count was elevated ($P < 0.05$) in the infected-untreated group compared to the uninfected control group. Among the treated groups, there were no statistical difference in their mean lymphocyte counts on days 7, 14, and 21 PI. The mean heterophil counts of the birds were comparable throughout the experimental period. The mean monocyte counts of the treated groups of birds did not differ statistically. However, groups 3 and 4 had higher ($P < 0.05$) mean monocyte count than the uninfected control group on day 21 PI. The mean eosinophil and basophil count of the birds were comparable except on day 7 PI for basophil count and day 14 for eosinophil count where group 4 birds had higher ($P < 0.05$) mean counts compared to the group 5 birds.

A significant decline ($P < 0.05$) was observed in the mean serum total protein level of the infected groups of birds (Table 5). Among the treated groups, group 5 had the lowest ($P < 0.05$) mean total protein levels while no variation exists in those of groups 3, 4, and 6 at day 7 PI. By day 14 PI, the mean total protein level of groups 5 and 6 had significantly improved and were comparable to the uninfected control group but were significantly higher ($P < 0.05$) than those of groups 3 and 4. At day 21 PI, the treated groups had comparable mean total protein levels which were also comparable to that of uninfected control group except for group 3 that had lower ($P < 0.05$) mean total protein level. The mean albumin levels of the uninfected control and the treated groups were analogous except on day 14 PI where the mean albumin levels of groups 4 and 5 declined ($P < 0.05$) significantly against the uninfected group but were elevated ($P < 0.05$)

compared to the infected-untreated group 2 birds (Table 5). The mean serum globulin level of group 3 birds declined significantly ($P < 0.05$) on days 7 and 14 compared to uninfected control group (Table 5). Group 5 had comparable mean serum globulin levels with group 3 on day 7 PI which was low ($P < 0.05$) compared to those of groups 4 and 6. On day 14 PI, groups 3 and 4 had comparable mean serum globulin levels which was lower ($P < 0.05$) than those of groups 5 and 6.

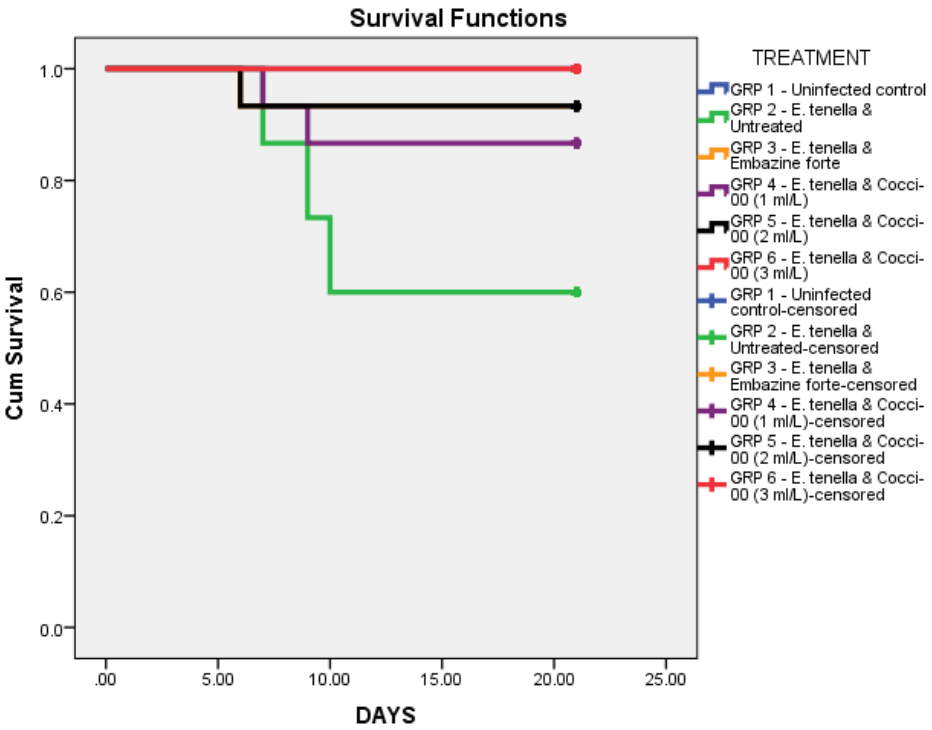

Figure 1: Kaplan-Meier survival curves of broiler birds experimentally infected with *Eimeria tenella* and treated with different doses of herbal anticoccidial - Cocci-00®

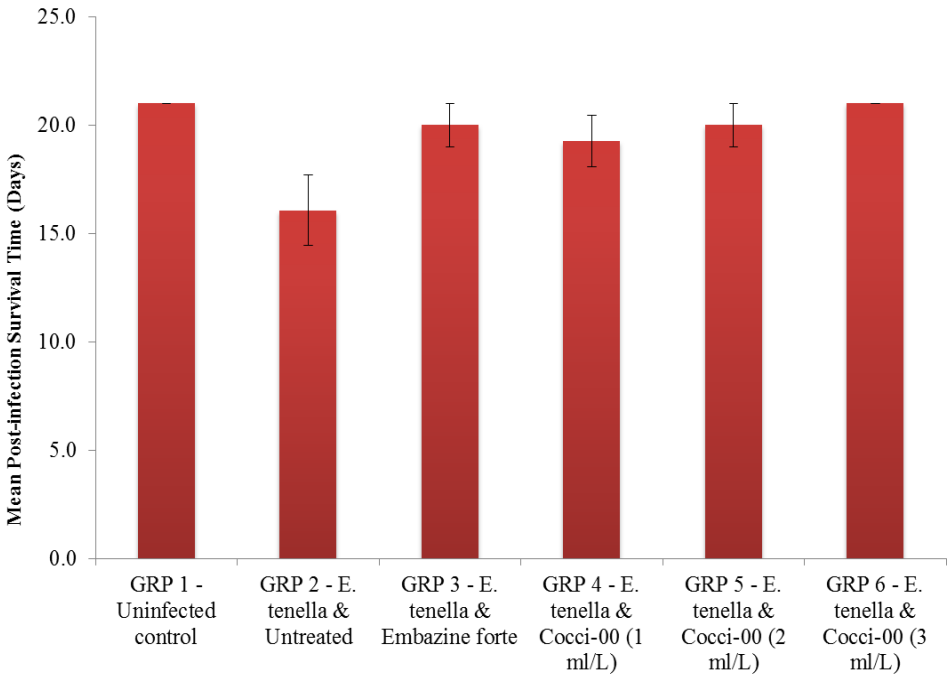

Figure 2: Mean post-infection survival times of broiler birds experimentally infected with *Eimeria tenella* and treated with different doses of herbal anticoccidial - Cocci-00®

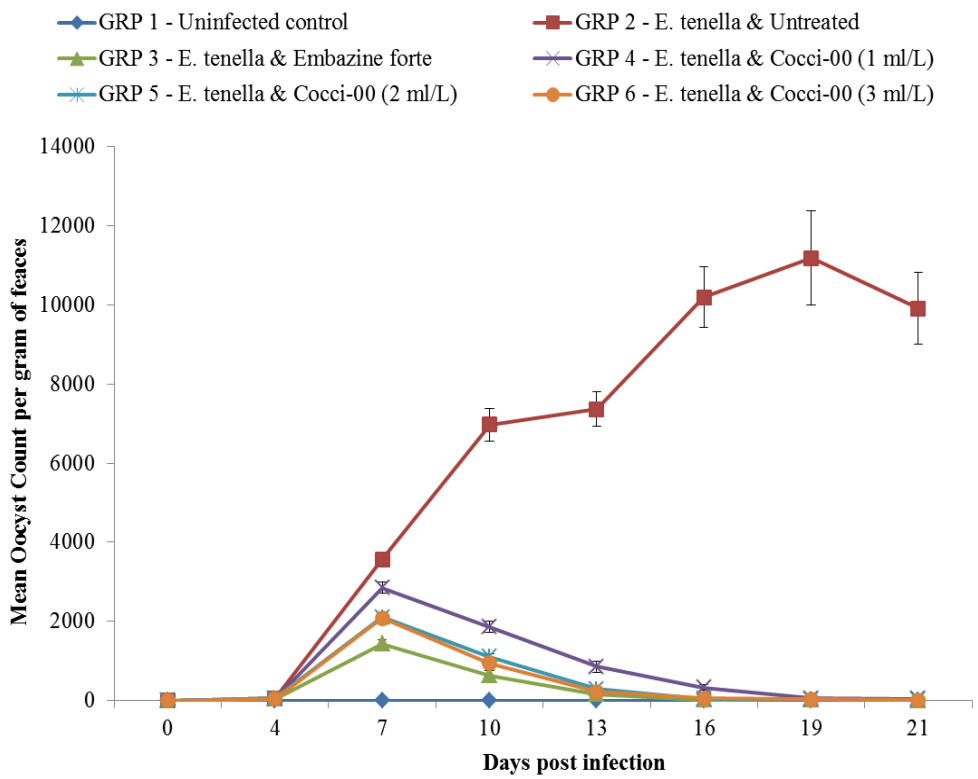

Figure 3: Mean oocyst counts of broiler birds experimentally infected with *Eimeria tenella* and treated with different doses of herbal anticoccidial - Cocci-00®

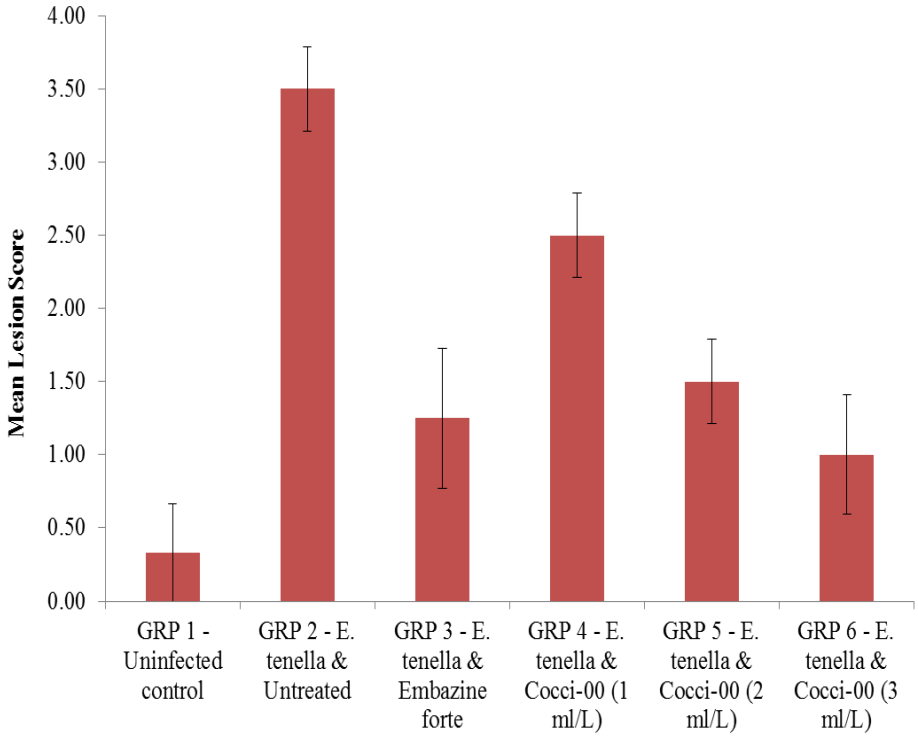

Figure 4: Mean lesion score of broiler birds experimentally infected with *Eimeria tenella* and treated with different doses of herbal anticoccidial - Cocci-00®.

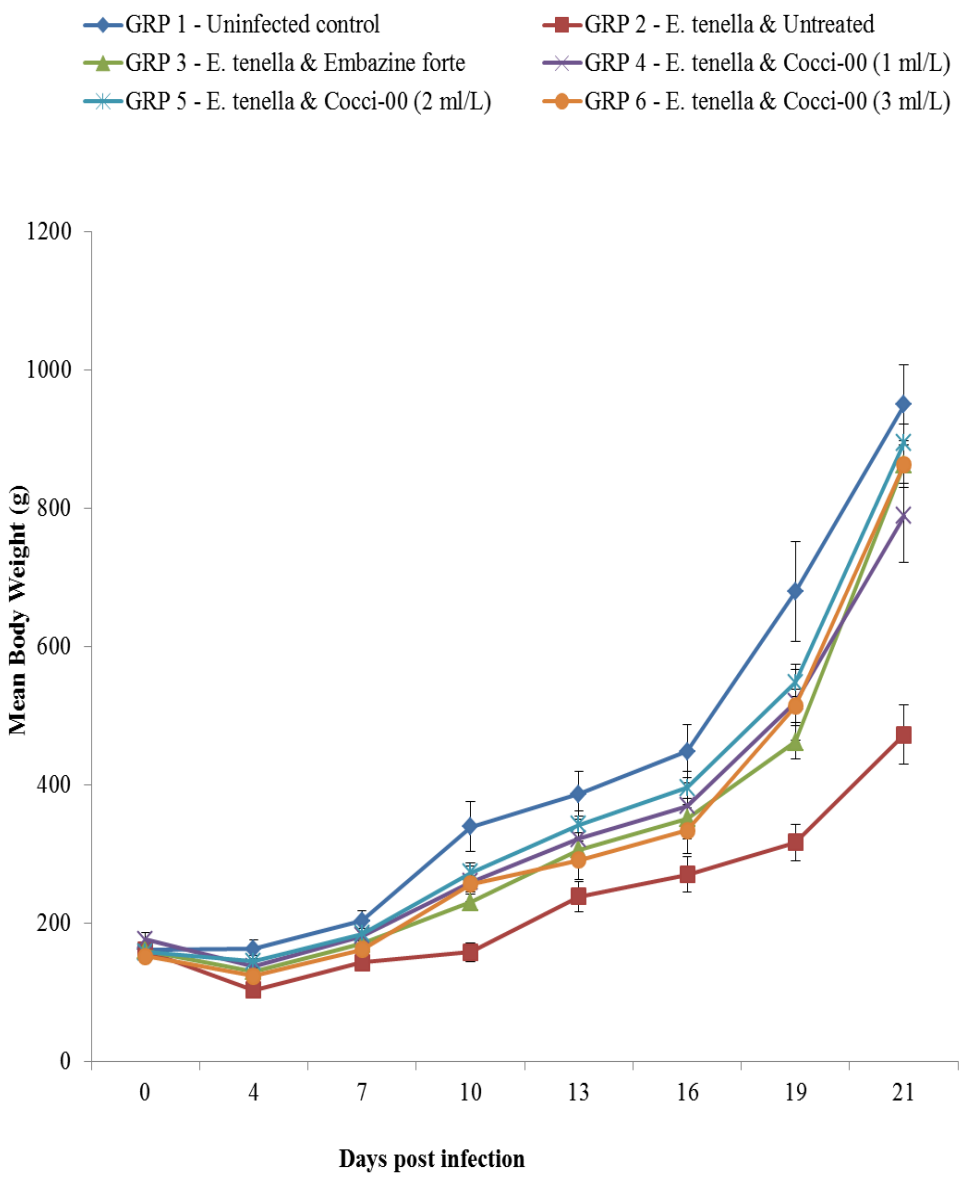

Figure 5: Mean body weight of broiler birds experimentally infected with *Eimeria tenella* and treated with different doses of herbal anticoccidial - Cocci-00®.

Table 1: Mean Weight gain, weight gain per day, feed conversion ratio (FCR), feed efficiency, and mortality rates of broiler birds experimentally infected with *Eimeria tenella* and treated with different doses of herbal anticoccidial - Cocci-00®.

	Weight gain (g)	Weight gain/day (g/day)	FCR	Feed efficiency	Mortality (%)
Group 1 - Uninfected control	517.25±70.68^{a}	27.23±3.72^{a}	4.36±0.45^{a}	25.97±3.50^{a}	0/15 (0%)
Group 2 - *E. tenella* & Untreated	156.2±29.16^{b}	8.22±1.53^{b}	13.14±2.37^{b}	10.77±2.22^{b}	6/15 (40%)
Group 3 - *E. tenella* & Embazine forte	301.35±27.87^{c}	15.86±1.47^{c}	7.01±0.68^{a}	15.26±1.20^{bc}	1/15 (6.7%)
Group 4 - *E. tenella* & Cocci-00 (1 ml/L)	343.25±59.18^{c}	18.07±3.11^{c}	8.52±2.45^{a}	18.59±3.29^{c}	2/15 (13.3%)
Group 5 - *E. tenella* & Cocci-00 (2 ml/L)	388.95±15.15^{c}	20.47±0.80^{c}	5.15±0.28^{a}	20±1.22^{ac}	1/15 (6.7%)
Group 6 - *E. tenella* & Cocci-00 (3 ml/L)	361.85±25.9^{c}	19.04±1.36^{c}	5.57±0.44^{a}	19.01±1.56^{ac}	0/15 (0%)

Different superscripts in a column represent significant differences at $P < 0.05$

Table 2: Mean red blood cell count (RBC), Haemoglobin concentration (Hb), Packed cell volume (PCV), and White blood cell count (WBC) of broiler birds experimentally infected with *Eimeria tenella* and treated with different doses of Cocci-00®.

RBC (10^6/µl)						
Days	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
0	2.85±0.13	2.9±0.17	2.99±0.14	3.15±0.09	2.89±0.16	2.8±0.16
7	3.10±0.03^{ab}	2.74±0.11^{a}	3.20±0.07^{b}	2.80±0.09^{a}	2.83±0.17^{a}	2.84±0.15^{a}
14	3.60±0.08^{ac}	2.35±0.08^{b}	3.19±0.27^{ac}	3.15±0.11^{a}	3.43±0.01^{ac}	3.65±0.15^{c}
21	3.64±0.13^{a}	2.11±0.12^{b}	3.35±0.08^{a}	3.46±0.33^{a}	3.27±0.06^{a}	3.79±0.12^{a}
Hb (g/dl)						
Days	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
0	12.51±0.10	12.13±0.36	12.40±0.12	12.20±0.27	12.15±0.31	12.20**±0.25**
7	12.87±0.41^{a}	10.93±0.24^{b}	12.93±0.18^{a}	11.60±0.13^{bc}	12.00±0.31^{ac}	12.20±0.31^{ac}
14	13±0.23^{a}	9.67±0.24^{b}	12.80±1.03^{a}	12.17±0.33^{a}	13.70±0.06^{a}	13.13±0.57^{a}
21	13.67±0.24^{a}	8.8±0.32^{b}	13.53±0.18^{a}	13.33±1.17^{a}	13.53±0.24^{a}	13.47±0.55^{a}
PCV (%)						
Days	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
0	24.7±0.4	25.1±0.2	25.3±1.3	25.3±0.8	24.5±0.2	25.1±0.3
7	28.3±0.9^{a}	24±0.6^{b}	27.7±0.7^{ab}	26±1.7^{ab}	24.7±1.3^{ab}	24.7±1.2^{ab}
14	29±1.2^{ac}	22.7±1.8^{b}	26.3±2.3^{abc}	24.7±0.7^{ab}	28.5±0.3^{ac}	30.7±1.3^{c}
21	30.7±1.5^{ad}	21±0.6^{b}	27±0.6^{ac}	28±2.1^{acd}	26.7±0.7^{c}	31±1.0^{d}
WBC (10^3/µl)						
Days	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
0	20.1±0.24	19.8±0.42	20.2±0.35	20.00±0.27	19.2±0.46	19.10±0.61
7	18.83±0.34^{a}	20.70±0.61^{ab}	20.58±0.27^{ab}	20.98±0.76^{b}	19.40±0.85^{ab}	19.32±0.11^{ab}
14	18.70±0.26^{a}	22.53±1.20^{b}	21.23±0.93^{b}	20.50±0.52^{ab}	20.95±0.32^{ab}	21.38±0.51^{b}
21	18.83±0.34^{a}	23.48±0.50^{b}	21.60±0.98^{bc}	22.15±1.43^{bc}	19.90±0.38^{ac}	21.25±0.38^{abc}

Different superscripts across a row represent significant differences at $P < 0.05$. Group 1 – Uninfected control, Group 2 – Infected-untreated, Group 3 – Infected and treated with Embazine forte, Group 4 – Infected and treated with 1 ml/L Cocci-00, Group 5 - Infected and treated with 2 ml/L Cocci-00, Group 6 - Infected and treated with 3 ml/L Cocci-00.

Table 3: Mean Corpuscular Volume (MCV), Mean Corpuscular haemoglobin (MCH), and Mean Corpuscular haemoglobin Concentration (MCHC) of broiler birds experimentally infected with *Eimeria tenella* and treated with different doses of Cocci-00®.

MCV (fL)						
Days	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
0	86.67±0.78	86.55±0.12	84.62±0.99	80.32±4.93	84.78±2.5	89.64±4.87
7	91.27±2.10	88.04±5.58	86.36±0.73	93.34±8.22	87.21±0.47	86.99±0.40
14	80.63±4.31^{a}	97.05±9.79^{b}	82.43±0.23^{ab}	78.79±4.38^{a}	83.21±0.49^{ab}	84.00±0.33^{ab}
21	84.30±2.13^{a}	99.81±4.37^{b}	80.52±0.12^{a}	82.04±8.23^{a}	81.45±.77^{a}	81.72±0.21^{a}
MCH (pg)						
Days	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
0	43.89±2.92	41.83±0.89	41.47±0.37	38.73±3.26	42.04±0.42	43.57±1.44
7	41.45±1.00	40.06±1.95	40.40±0.77	41.49±1.07	42.58±1.51	43.13±1.16
14	36.08±0.13^{a}	41.22±0.60^{b}	40.13±0.48^{b}	38.65±0.34^{ab}	40.00±0.00^{b}	36.17±2.66^{a}
21	37.67±1.41^{ad}	41.74±0.88^{b}	40.38±0.39^{abc}	38.56±0.47^{acd}	41.35±0.44^{bc}	35.53±1.48^{d}
MCHC (g/dl)						
Days	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
0	50.65±0.78	48.33±0.91	49.01±0.42	48.22±0.82	49.59±0.36	48.61±0.51
7	45.41±0.16	45.64±1.98	46.78±0.76	44.94±2.82	48.81±1.52	49.57±1.15
14	45.00±2.35	43.28±4.08	48.68±0.69	49.44±2.42	48.08±0.28	43.07±3.20
21	44.80±2.61^{ab}	41.92±1.22^{a}	50.14±0.44^{ab}	47.99±5.01^{ab}	50.77±0.44^{b}	43.49±1.92^{ab}

Different superscripts across a row represent significant differences at $P < 0.05$. Group 1 – Uninfected control, Group 2 – Infected-untreated, Group 3 – Infected and treated with Embazine forte, Group 4 – Infected and treated with 1 ml/L Cocci-00, Group 5 - Infected and treated with 2 ml/L Cocci-00, Group 6 - Infected and treated with 3 ml/L Cocci-00.

Table 4: Mean absolute lymphocytes, heterophil, monocytes, eosinophils, and basophil count of broiler birds experimentally infected with *Eimeria tenella* and treated with different doses of Cocci-00®.

Lymphocytes (10^3/µl)						
Days	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
0	11.33±0.33	11.48±0.53	11.78±0.84	11.67±0.77	10.94±0.22	10.83±0.43
7	10.65±0.34^{a}	12.14±0.35^{bc}	12.21±0.24^{bc}	12.66±0.47^{b}	11.24±0.45^{ac}	11.08±0.11^{ac}
14	11.16±0.21^{a}	13.66±0.60^{b}	12.77±0.92^{ab}	12.44±0.37^{ab}	13.10±0.38^{b}	13.33±0.38^{b}
21	11.18±0.28^{a}	14.64±0.53^{b}	13.05±0.78^{abc}	13.80±0.89^{bc}	12.02±0.48^{ac}	13.52±0.40^{bc}
Heterophils (10^3 /µl)						
Days	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
0	6.97±0.07	6.60±0.17	6.94±0.13	6.93±0.18	6.72±0.11	6.69±0.22
7	6.87±0.09	7.12±0.42	6.52±0.24	6.47±0.33	7.06±0.43	6.76±0.08
14	6.17±0.09	7.23±0.70	7.05±0.11	6.42±0.17	6.69±0.26	6.49±0.32
21	6.34±0.21	7.36±0.67	6.68±0.14	6.60±0.61	6.36±0.18	6.46±0.68
Monocytes (10^3/µl)						
Days	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
0	1.21±0.12	1.12±0.07	0.88±0.07	1.00±0.12	0.96±0.11	0.96±0.11
7	0.85±0.04	1.10±0.04	1.23±0.11	1.27±0.25	0.84±0.12	0.90±0.13
14	1.06±0.05	1.06±0.28	1.05±0.08	1.09±0.04	0.95±0.20	1.07±0.11
21	0.75±0.10^{a}	1.17±0.12^{b}	1.23±0.11^{b}	1.24±0.08^{b}	1.06±0.13^{ab}	0.92±0.14^{ab}
Eosinophils (10^3/µl)						
Days	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
0	0.53±0.13	0.59±0.11	0.54±0.18	0.33±0.13	0.58±0.11	0.63±0.06
7	0.47±0.05	0.35±0.07	0.55±0.14	0.43±0.22	0.26±0.07	0.58±0.00
14	0.31±0.06^{ab}	0.52±0.05^{ab}	0.36±0.15^{ab}	0.55±0.07^{a}	0.21±0.01^{b}	0.51±0.15^{ab}
21	0.50±0.06	0.31±0.08	0.58±0.09	0.44±0.3	0.47±0.14	0.36±0.07
Basophils (10^3/µl)						
Days	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
0	0.07±0.07	0.00±0.00	0.07±0.07	0.07±0.07	0.00±0.00	0.00±0.00
7	0.00±0.00^{a}	0.00±0.00^{a}	0.07±0.07^{ab}	0.14±0.07^{b}	0.00±0.00^{a}	0.00±0.00^{a}
14	0.00±0.00	0.07±0.07	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
21	0.06±0.06	0.00±0.00	0.07±0.07	0.07±0.07	0.00±0.00	0.00±0.00

Different superscripts across a row represent significant differences at $P < 0.05$. Group 1 – Uninfected control, Group 2 – Infected-untreated, Group 3 – Infected and treated with Embazine forte, Group 4 – Infected and treated with 1 ml/L Cocci-00, Group 5 - Infected and treated with 2 ml/L Cocci-00, Group 6 - Infected and treated with 3 ml/L Cocci-00.

Table 5: Mean total protein, albumin, and globulin levels of broiler birds experimentally infected with *Eimeria tenella* and treated with different doses of Cocci-00®.

Total protein (g/dl)						
Days	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
0	2.85±0.13	2.77±0.05	2.73±0.08	2.70±0.03	2.61±0.14	2.78±0.01
7	3.14±0.08^{a}	2.73±0.02^{b}	2.69±0.05^{b}	2.66±0.03^{ab}	2.52±0.01^{c}	2.80±0.06^{b}
14	3.30±0.09^{a}	2.57±0.13^{b}	2.77±0.03^{b}	2.83±0.13^{b}	3.12±0.05^{a}	3.40±0.01^{a}
21	3.43±0.07^{a}	2.31±0.07^{b}	2.97±0.02^{c}	3.09±0.24^{ac}	3.19±0.09^{ac}	3.31±0.01^{ac}
Albumin (g/dl)						
Days	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
0	1.55±0.01	1.43±0.11	1.65±0.08	1.42±0.13	1.49±0.16	1.51±0.04
7	1.72±0.14^{ac}	1.36±0.03^{b}	1.75±0.05^{c}	1.43±0.04^{ab}	1.56±0.15^{abc}	1.55±0.03^{abc}
14	1.76±0.03^{a}	1.38±0.04^{b}	1.65±0.03^{ac}	1.59±0.09^{c}	1.57±0.03^{c}	1.72±0.01^{ac}
21	1.75±0.04^{a}	1.30±0.03^{b}	1.68±0.06^{a}	1.73±0.02^{a}	1.75±0.04^{a}	1.75±0.13^{a}
Globulin (g/dl)						
Days	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
0	1.30±0.12	1.34±0.06	1.08±0.31	1.28±0.17	1.12±0.21	1.27±0.03
7	1.42±0.07^{a}	1.37±0.04^{b}	0.93±0.06^{b}	1.23±0.06^{a}	0.97±0.16^{b}	1.25±0.07^{a}
14	1.54±0.11^{a}	1.19±0.12^{b}	1.12±0.05^{b}	1.23±0.06^{b}	1.54±0.06^{a}	1.67±0.01^{a}
21	1.68±0.11^{a}	1.01±0.08^{b}	1.29±0.04^{ab}	1.36±0.23^{ab}	1.45±0.12^{ab}	1.56±0.12^{a}

Different superscripts across a row represent significant differences at $P < 0.05$. Group 1 – Uninfected control, Group 2 – Infected-untreated, Group 3 – Infected and treated with Embazine forte, Group 4 – Infected and treated with 1 ml/L

Cocci-00, Group 5 - Infected and treated with 2 ml/L Cocci-00, Group 6 - Infected and treated with 3 ml/L Cocci-00.

DISCUSSION

The findings of this study demonstrate that the herbal anticoccidial Cocci-00® exhibits significant efficacy in mitigating *Eimeria tenella* infection in broiler chickens, comparable to the conventional drug Embazine Forte®. The results highlight its potential as a sustainable alternative for coccidiosis control, particularly in resource-limited settings like sub-Saharan Africa, where drug resistance and high costs of synthetic anticoccidials pose major challenges to poultry production (Otu et al., 2024).

Infection with *E. tenella* in untreated birds resulted in severe clinical manifestations, including 40% mortality, bloody diarrhoea, and marked weight loss**,** consistent with previous reports on the pathogenicity of this parasite (Chapman et al., 2013). The high oocyst output observed in the infected-untreated group underscores the rapid replication cycle of *E. tenella*, which leads to extensive intestinal damage and haemorrhagic typhlitis. However, treatment with Cocci-00®, particularly at the 2 mL/L and 3 mL/L doses**,** significantly reduced oocyst excretion, with counts declining to levels comparable to those in birds treated with Embazine Forte® by day 10 post-infection. This suppression of oocyst shedding is critical in breaking the transmission cycle of coccidiosis in poultry farms, reducing environmental contamination and reinfection rates (Dalloul and Lillehoj, 2006). The reduction in lesion scores in Cocci-00®-treated birds further supports its therapeutic efficacy, as Cocci-00® appeared to facilitate mucosal healing, likely due to the anti-inflammatory and antiparasitic properties of its constituent botanicals, such as *Aegle marmelos* and *Azadirachta indica* (Kumar et al., 2012; Guatam et al., 2013; Gotep et al., 2016; Kumari et al., 2021).

One of the most striking observations was the complete prevention of mortality in birds treated with the highest dose of Cocci-00® (3 mL/L), outperforming even the standard drug Embazine Forte®, which had a 6.7% mortality rate. This suggests that Cocci-00® not only combats the parasite but also mitigates the systemic effects of infection, such as anaemia and hypoproteinaemia**,** which are major contributors to mortality in severe coccidiosis (Taylor et al., 2016). The improvement in haematological parameters indicates that Cocci-00® may possess haemostatic and erythropoietic properties**,** possibly due to its vitamin K, *Azadirachta indica*, and immunomodulatory components (Gotep et al., 2016). Also, the normalization of serum total protein and albumin levels in treated birds suggests enhanced liver function and protein synthesis, critical for recovery from the catabolic state induced by coccidiosis.

The growth performance data further reinforce the benefits of Cocci-00® in coccidiosis management. Infected birds experienced significant weight loss and poor feed conversion ratios (FCR)**,** consistent with the malabsorptive consequences of intestinal epithelial damage**.** However, birds treated with Cocci-00® exhibited near-normal weight gain and feed efficiency**,** comparable to the uninfected control group. This recovery is particularly noteworthy given that weight loss in coccidiosis is often prolonged**,** even after parasite clearance, due to persistent gut dysfunction. The rapid restoration of growth performance in Cocci-00®-treated birds may be attributed to its prebiotic components (Mannan Oligosaccharides and Fructo-Oligosaccharides)**,** which support gut microbiota balance and nutrient absorption, as well as its vitamin A content**,** known to enhance intestinal repair and immune function (Adhikari et al., 2020; Ahmad et al., 2024).

When compared to existing literature, our findings align with studies demonstrating the anticoccidial potential of phytogenic compounds**,** such as saponins and flavonoids, which disrupt parasite development (Dalloul & Lillehoj, 2006; Abbas and Alkheraije, 2023; Hussain et al., 2023; Hailat et al., 2024; Hayajneh et al., 2024). However, the dose-dependent efficacy observed in this study underscores the importance of optimizing herbal formulations for consistent results. While some studies report variability in plant-based anticoccidials due to differences in extraction methods and bioactive compound concentrations (Adjei-Mensah and Atuahene, 2022), Cocci-00®'s standardized commercial formulation appears to provide reproducible effects, making it a practical option for field use. Of particular importance is the fact that Cocci-00® offers these therapeutic benefits without the associated risks of drug residues or the development of antimicrobial resistance, challenges that are increasingly associated with long-term use of synthetic anticoccidials. The growing consumer demand for residue-free, organically produced poultry products makes Cocci-00® especially attractive as a natural and safe alternative.

Despite these promising results, certain limitations warrant consideration. The experiment focused on a controlled infection model using *E. tenella* alone, whereas in natural settings, mixed infections with multiple *Eimeria* species are more common. Future studies should evaluate the efficacy of Cocci-00® against mixed *Eimeria* infections under field conditions. Additionally, long-term safety studies and performance evaluations under varying dietary and environmental conditions would further substantiate its application in diverse poultry production systems. Investigations into the exact mechanisms of Cocci-00®'s anticoccidial activity, particularly its effects on sporozoite invasion and intracellular parasite development would also be valuable. Also, synergistic studies combining Cocci-00® with vaccines or probiotics could also enhance its efficacy and prolong protective immunity.

In conclusion, this study provides robust evidence that Cocci-00® is an effective herbal alternative for controlling *Eimeria tenella* in broilers, demonstrating comparable efficacy to synthetic anticoccidials in reducing oocyst shedding, lesion severity, and mortality while restoring growth performance and haematological parameters. Its multicomponent formulation, combining antiparasitic botanicals with immune-supportive nutrients, offers a holistic approach to coccidiosis management, particularly in regions where conventional drugs are economically or logistically inaccessible. Given the rising challenges of drug resistance and consumer demand for natural products, Cocci-00® represents a promising tool for sustainable poultry production, warranting further large-scale validation and integration into coccidiosis control programs.

Competing interest

The authors declare that they have no conflict of interest.

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