JOURNAL OF VETERINARY AND APPLIED SCIENCES
VOLUME 15, ISSUE 1: 980 – 988 (2025)
Published by: Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria
ISSN: 2315-6856; e-ISSN: 2636-5553; Website: www.jvasonline.com

Comparison of the efficacy of three commonly used anti-coccidial drugs on broiler chickens naturally infected with *Eimeria* species

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Abstract

The comparative efficacy of Sulphadimidine, Totrazuril and Amprolium on broiler chickens naturally infected with *Eimeria* species was investigated. A total of forty day old broiler chicks were used for the study. They were randomly assigned to four groups (Groups A – D) of ten birds each. The broilers were reared on deep litter, and they came down with natural *Eimeria* infection/coccidiosis on the fourth week. Clinical signs such as bloody diarrhoea, weakness and ruffled feathers were observed following establishment of the *Eimeria* infection at the fourth week in the broilers. Birds in groups A, B and C were treated with Totralzuril, Amprolium and Sulphadimidine, respectively, while those in group D were the untreated control. Following establishment of the infection, a drop in the packed cell volume of the birds was observed. A return to normal values after treatment with the three anti-coccidial drugs was recorded. There was no significant difference ($p > 0.05$) in the mean body weights of the broilers in all the groups. The faecal oocysts output [oocysts per gramme (OPG) of faeces] of all the treated groups decreased significantly post-treatment. However, Group B birds (treated with Amprolium) showed the greatest decrease in oocyst output from 149,500 OPG to 100 OPG by day 5 of treatment. This study showed that Amprolium was more effective than Sulphadimidine and Totrazuril in reducing *Eimeria* faecal oocysts output, and thus in the treatment of coccidiosis in broilers naturally infected with *Eimeria* species.

Keywords: Coccidiosis; *Eimeria* species; Broiler chickens; Amprolium; Totralzuril; Sulphonamides.

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Article History: Initial manuscript submission received – Sept. 23, 2024; Final revised form received – April 27, 2025; Accepted for publication – May 02, 2025; Published – May 12, 2025.

Introduction

Domestic birds are reared all over the world as source of protein and income, and poultry meat is generally accepted by all tribes and religions globally (Obianuju *et al*., 2021). Meat from poultry is one of the most available and affordable source of protein in Nigeria, and this has led to its high demand and a massive expansion of the poultry industry across the country (Ngongeh *et al*., 2017). However, poultry production is constrained by the occurrence of some poultry diseases (Nzeakor *et al*., 2021), of which coccidiosis is one of the most important (Ola-Fadunsin, 2017).

Avian coccidiosis is caused by an ubiquitous and hardy Apicomplexan parasite, *Eimeria* species, which survives in infected birds and the environment for a long time (Bachaya *et al*., 2012). A prevalence of 39.5% has been reported for *Eimeria* species in domestic birds in Nsukka, Enugu State, Nigeria (Okwuonu *et al*., 2021). Coccidiosis in poultry is associated with high morbidity and mortality in young chicks between the ages of 3 to 18 weeks (Shojaei, 2014). Huge losses, estimated at over $2.4 billion annually, emanating from the cost of prevention, treatment, morbidity and mortality of poultry due to coccidiosis have been recorded globally (Sharma *et al*., 2015).

Infection of birds by the *Eimeria* parasite occurs following ingestion of viable sporulated oocysts in contaminated feed. The *Eimeria* parasitizes the intestine of birds where they multiply, resulting in damage of the intestinal walls with sequel nutrient malabsorption and blood loss (Lu *et al*., 2021). The disease manifests clinical signs such as bloody or brownish pasty diarrhoea, depression, ruffled feathers, reduced feed consumption etc. (Awais *et al*., 2012).

Poultry coccidiosis is majorly controlled by the use of chemotherapeutic anti-coccidial drugs amidst other control measures like vaccines and good husbandry/biosecurity (Soutter *et al.,* 2020). Chemotherapy of coccidiosis in Nigeria is mainly based on three classes of drugs: Amprolium, Totralzuril and Sulphonamides. These drugs are widely used by poultry farmers that rear broilers in Nigeria. The use of inappropriate dosages of these drugs, inconsistent and frequent treatments with them have been reported to lead to the emergence of drug resistant strains of *Eimeria* (Price and Barta, 2010). There is paucity of information in available literature on the comparative efficacy of the drugs commonly used for coccidiosis treatment. The present study compared the efficacy of the three commonly used anti-coccidial drugs (Amprolium, Totralzuril and Sulphonamides) for the treatment of poultry coccidiosis in naturally infected broiler chickens.

Materials and Methods

Experimental Birds: A total of forty day old broiler chicks were used for the experiment. The chicks were housed on a deep litter system. Prior to arrival of the chicks, the pen together with the equipment to be used in raising the birds were washed thoroughly and disinfected. Brooding temperature was maintained close to 35°C using an electric light bulb in their pen for the first four weeks. The broilers were fed commercial feed (Chikun Feeds, Oasis Farms & Agro Services Ltd., Port Harcourt, Nigeria) *ad libitum* starting with broiler starter feed for the first four weeks and then broiler finisher from the fourth week till the end of the experiment. The birds were vaccinated against Infectious bursal disease at day 10 and 21, and Newcastle disease at day 3 and 21 respectively.

Experimental Design: The broilers were randomly assigned to four groups (A – D) of 10 birds each. They were allowed to come down with the natural infection of coccidiosis. At the fourth week, onset of coccidiosis was suspected by the observation of bloody diarrhea of the birds and confirmed via faecal floatation using saturated salt solution. Following establishment of coccidiosis in the experimental birds, the birds in Groups A were treated with Totralzuril (Keprocox®, Kepro, Holland) at 8 mg/kg for two days; Group B broilers were treated with Amprolium (Amprolin-300 WS®, Interchemie, Netherlands) at 0.4 mg/litre for five days; while Group C broilers were treated with Sulphonamide (Triple sulphur®, Agar, Holland) at 1g/litre for five days, based on the manufacturer's instructions. Broilers in Group D were the untreated control. Faecal oocyst count, body weight, feed intake and packed cell volume (PCV) of the broilers were determined and recorded at intervals during the study.

Faecal examination for *Eimeria* oocyst: Birds in all the experimental groups were screened for coccidian oocysts from day old till the establishment of infection using the floatation technique as described by MAFF (1977). The faecal sample was mixed thoroughly for even distribution of the oocysts. One gram of the faeces was then mixed with 14 ml of saturated sodium chloride solution in a plastic test tube using a spatula. The mixture was sieved with a tea/coffee strainer to remove large particulate faecal materials. The mixture was transferred to a test tube that was then filled to form a positive meniscus with more salt solution using a Pasteur pipette. A cover slip was then placed on the test tube and allowed to stand for 30 minutes. After 30 minutes the cover slip was then carefully lifted and placed on a clean microscope slide. The slide was examined for *Eimeria* oocysts using a light microscope, at ×10 magnification.

Faecal oocysts count: The faecal oocysts count was done following the McMaster technique. Three grammes of faeces was weighed and placed in a plastic container. Forty-two millilitres of floatation fluid (saturated solution of sodium chloride) was measured out using a measuring cylinder. The faecal sample was mixed with some of the salt solution using a spatula. The mixture was filtered through a tea/coffee strainer into a beaker. The filtrate was stirred very well and a sample was collected with a Pasteur pipette to fill one chamber of the McMaster slide. The same process was repeated to fill the second chamber of the slide. The slide was then placed on the microscope stage and allowed to stand for three minutes for the contents to settle. The sample was examined under the microscope using the ×10 objective lens. The total number of oocysts counted within the two chambers was added and the number multiplied by 50 to determine the number of oocysts per gramme (OPG) of faeces. This procedure was done as described by MAFF (1977).

Body weight measurement: The individual body weights of the broilers in each group were determined weekly using a weighing balance.

Feed intake measurement: The feed intake of the experimental birds in each group was determined weekly using a weighing balance. The total weight of the feed given to each of the groups was recorded at a specific time in the morning before administering the feed. After 24 hours, the remaining of the feed was weighed at the same time in the morning the next day. The daily feed consumption was calculated by subtraction of the weight of feed given initially minus the weight remaining the next morning.

Packed cell volume determination: The packed cell volume (PCV) was determined by the microhaematocrit method (Coles, 1986). Blood was collected from the wing vein of the birds. The micro-haematocrit capillary tubes were nearly filled through capillary action and one end of the tube was sealed with plasticine. The capillary tubes were placed in a haematocrit centrifuge with the sealed ends of the tubes placed towards the rim of the centrifuge while the open ends were placed pointing towards the centre of the centrifuge. The blood samples in the capillary tubes were centrifuged for 5 minutes at 1,400 revolutions per minute. The PCV values were read using a microhaematocrit reader.

Lesions observed in the intestines at post-mortem were also noted/recorded.

Data Analysis: Quantitative data obtained from the study were subjected to analysis of variance (ANOVA). Probability values less than 0.05 were considered significant. Variant means were compared using Duncan's new multiple range test. The software used for the statistical analysis was the Statistical Package for Social Sciences (SPSS), version 15.0.

Results

Presence of intestinal linings in the faeces was seen at the first two weeks. Patency of the infection as evidenced by bloody diarrhoea was observed by the fourth week. Following treatment, the consistency and colour of the faeces passed out by the experimental birds (Group A, B and C) were observed to be chocolate brown in colour, which returned to its formed state. However, the faeces of birds in the untreated group showed a more intense reddish-brown colour.

Packed cell volume (PCV): A drop in the mean PCV was observed following the establishment of the *Eimeria* infection by week 4 (Figure 1). The PCV began rising in all the groups following treatment up to far above the pre-infection levels in Groups A and C treated with Totralzuril and Sulphonamides, respectively (Figure 1).

Faecal oocyst count (FOC): The faecal oocyst counts (FOC) continued to rise sequel to establishment of the infection. Following treatment, there was a remarkable drop in the faecal oocyst output of the broiler chickens in Group B, treated with Amprolium (from 149500 opg to 100 OPG) [Table 1]. Broiler chickens in Group A, C and D showed fluctuating oocyst output, while those in group D (infected and untreated) maintained the highest FOC till the end of the experiment [Table 1].

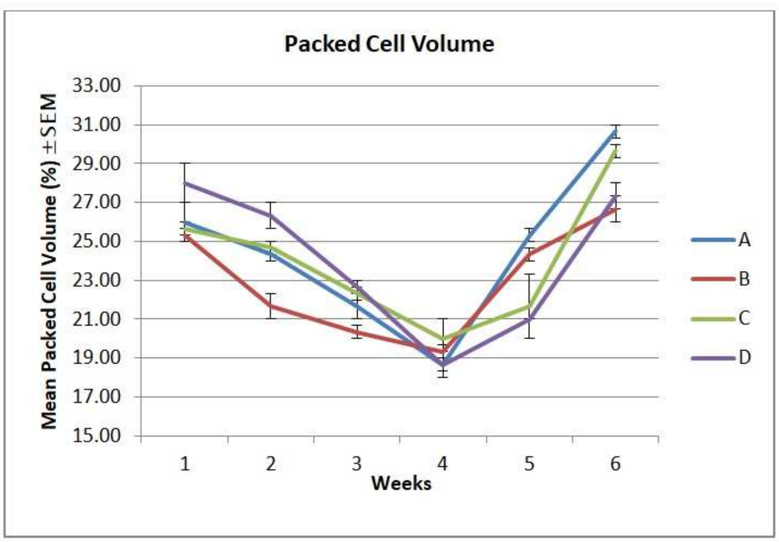

Figure 1. Packed cell volume of groups of broiler chickens naturally infected with *Eimeria* species and treated with Totralzuril, Amprolium and Sulphadimidine, compared with an untreated control. [A – Group treated with Totralzuril; B – Group treated with Amprolium; C – Group treated with Sulphadimidine; D – Untreated Control Group].

Table 1. Faecal oocysts output of broiler chickens naturally infected with *Eimeria* species and treated with Totralzuril, Amprolium and Sulphadimidine, compared with an untreated control.

Groups and their treatments	Faecal oocysts output (opg), days post commencement of treatment with anti-coccidial agents					
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Group A (Totrazuril)	2050	3250	22100	105500	650	2850
Group B (Amprolium)	149500	4600	4800	1450	200	100
Group C (Sulphadimidine)	83400	44000	16400	5650	21650	15800
Group D (Untreated Control)	4150	22900	13500	24000	14000	25000

Body weight: The mean body weights of the four groups of broiler chickens consistently increased till week 5 – 6 when the body weights of the Group D broiler chickens significantly dropped (Figure 2). Group C broilers treated with Sulphadimidine attained the highest body weight by week 6, followed by Group B (treated with Amprolium) and then Group A (treated with Totralzuril) [Figure 2].

Feed intake: A continuous increase in the feed intake was recorded for all the broiler chickens across the groups throughout the experimental period (Table 2).

Post mortem lesions: The gross lesions observed at necropsy for the *Eimeria* infected broilers included haemorrhages in the intestinal mucosa and distended caeca (Figures 4).

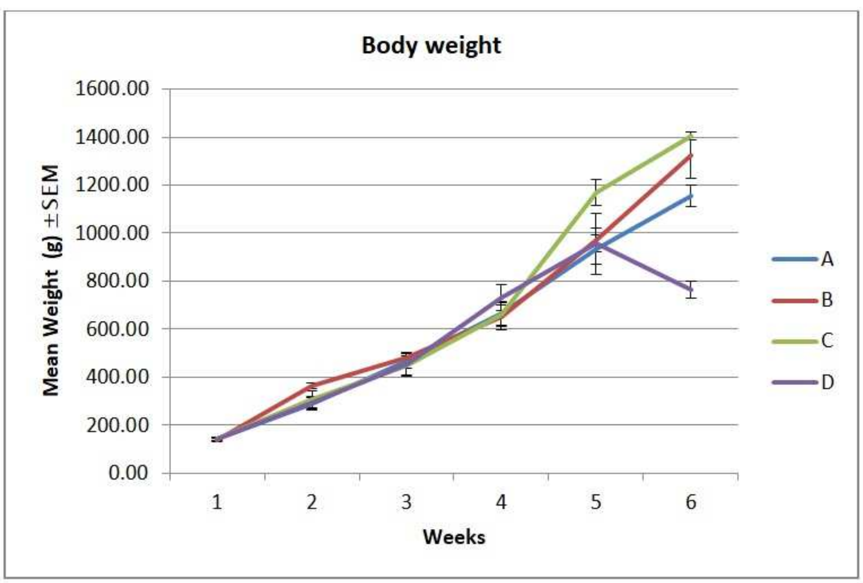

Figure 2. Mean body weights of broiler chicken groups naturally infected with *Eimeria* species and treated with Totralzuril, Amprolium and Sulphadimidine, compared with an untreated control. [A – Group treated with Totralzuril; B – Group treated with Amprolium; C – Group treated with Sulphadimidine; D – Untreated Control Group].

Table 2. Weekly feed intake of broiler chickens naturally infected with *Eimeria* species and treated with Totralzuril, Amprolium and Sulphadimidine, compared with an untreated control.

Groups and their treatments	Weekly feed intake (g) during the experimental period				
	Week 1	Week 2	Week 3	Week 4	Week 5
Group A (Totrazuril)	2261	5670	6444	7714	8516
Group B (Amprolium)	2114	6605	8223	8107	8355
Group C (Sulphadimidine)	2220	6620	8370	8356	8311
Group D (Untreated Control)	2252	5119	7880	8143	8222

Figure 4. Picture showing haemorrhages in the intestine of broiler chickens infected with *Eimeria* species.

Discussion

The clinical signs of bloody diarrhoea which is typically reported in avian caecal coccidiosis (Gharekhani *et al.,* 2014) was recorded in this present study by the fourth week. This is consistent with earlier reports of the onset of the infection by the fourth week (Abdisa *et al*., 2019). This bloody diarrhoea was followed by a drop in packed cell volume; this drop was likely as a result of blood loss due to the destruction of the intestinal tissues by the multiplying enteric protozoa and subsequent bloody diarrhoea. The PCV began to rise back to pre-infection levels in all the groups; the increase back to pre-infection rates in the treatment groups can be related to the response to treatment and a termination of the coccidial infection. The increase in the non-treated group can be attributed to rise in post-infection immunity to the coccidial infection by the birds. Birds have been reported to show some degree of natural immunity to the coccidial parasite (Wallach, 2010), thus making the infection a self-limiting one (Quiroz-Castaneda and Dantan-Gonzalez, 2017).

Contrary to previous assertions of weight loss and decreased feed intake being part of the clinical signs that is usually observed in coccidial infection (Ogbe *et al*., 2009), reduction in the feed intake and weight loss was not recorded in this experiment. Feed intake continued to rise in all the groups till the end of the experiment. The continued

Increasing feed intake was evidenced in the increasing body weight of the birds till the end of the experiment. Only birds in group D showed a significantly lower body weight than other birds at week 6. Nwosu *et al*., (2011) also reported a continuous weight gain in *Eimeria*-infected broiler chickens in an earlier research.

The fecal oocyst count (FOC) showed a huge difference in values following onset of the infection and this was attributed to the fact that the infection was allowed to occur naturally (it was not experimentally induced). The FOC increased with the establishment of the infection with some fluctuations in various groups; reduction in the FOC values was observed across all the groups. The reduction in the treated groups was attributed to the treatment given to the birds. Group B (Amprolium treated group) showed the greatest reduction with the lowest FOC by day 5 post commencement of treatment (PCT), while group A and C (treated with Totralzuril and Sulphadimidine, respectively) showed poor reduction with fluctuations in FOC; this could be a pointer to some level of resistance to these anticoccidials. Reduction in the FOC of the untreated control group can be opined to be as a result of the natural immunity of the birds and probably declining infection cycles of the *Eimeria* parasites (Quiroz-Castaneda and Dantan-Gonzalez, 2017). Some researchers however have reported more efficacy with Totralzuril (Amer *et al*., 2010; Hamed and Elad, 2011) and insinuated ongoing resistance of the *Eimeria* strains used in their experiments to Amprolium and Sulphonamides.

Amprolium [1-(4-amino-2-n-propyl-5-pyrimidinylmethyl)-2-picolinium chloride hydrochloride], a quarternized derivative of pyrimidine, is a thiamine (Vitamin B1) antagonist. Thiamine pyrophosphate is a cofactor of several decarboxylase enzymes which play a major role in cofactor synthesis (Kant *et al*., 2013). Amprolium works by blocking the thiamine receptors in the rapidly dividing coccidia thereby making it unavailable and thus preventing carbohydrate synthesis. Sulphonamides on the other hand are structural antagonists of folic acid or of para-aminobenzoic acid (PABA), which is a precursor of folic acid. They act by competing for incorporation of PABA and metabolism of folic acid by the coccidia (Kant *et al*., 2013). Toltrazuril, belonging to the class of symmetric triazines, interferes with the division of the parasite's nucleus and with the activity of mitochondria, which is responsible for the respiratory metabolism of coccidia. In the macrogamonts, toltrazuril damages the wall-forming bodies. In all intracellular developmental stages, severe vacuolization occurs due to inflation of the endoplasmic reticulum (Duszynski *et al*., 2018).

During post mortem, the caeca were distended and contained bloody fluids, and there were haemorrhages in the intestinal mucosa. The lesions observed in the intestines were attributed to the coccidial infection/life cycle. The life cycle of this protozoan parasite involves its invasion of intestinal cells with subsequent destruction of tissues (Sharma *et al.,* 2015; Kaboudi *et al.,* 2016).

Conclusion: It was concluded from the results of this research that Amprolium showed the greatest reduction in fecal oocyst count. Amprolium is thus recommended as the better choice in the treatment of poultry coccidiosis, when compared to the other two anti-coccidial agents.

Acknowledgement

The authors wish to thank the laboratory staff of the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

Conflict of interest

The authors declare no conflict of interests.

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