Research Article

Three Coccidiocidal Drugs, Three Different Responses Observed in Broiler Chickens Challenged with *Eimeria tenella* Oocyst

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ABSTRACT

Background: The successes in the poultry industry are being diminished by ravaging effect of coccidiosis that result in great mortality of birds and economic losses to farmers. Here, we describe the responses of three coccidiocidal drugs commonly used by poultry farmers in tropical regions. **Materials and Methods:** The weight performance, faecal oocysts count, haematological parameters and histopathological architecture were investigated. Twenty-five days old broiler chickens of both sexes were randomly assigned to five groups. Of these 5 groups, groups 1-4 were orally inoculated with $4x10^3$ (0.2ml) sporulated oocysts of *Eimeria tenella* as a single gavage, while group 5 (negative control) were administered water. Whereas groups 1-3 were treated with same regime (100%) of drugs, group 4 served as the positive control. **Results and Conclusion:** The results indicated that inoculated birds had significantly reduced growth rate and varied haematological parameters, but increased faecal oocysts shedding with sloughing, and also had necrosis in the intestine. These parameters were however reversed after nine days of treatment with significant improvement observed in all the treated groups with the highest effects in groups 1 and 2. Statistical analysis showed a significant difference at $P< 0.05$. In conclusion, we report that the three drugs worked effectively in the boosting of the listed parameters. But importantly, Embazin had best effects on eliminating oocyst in the faeces, while Diclazuril had the most improved effect on the haematological and histological architectures. We therefore propose that a combination of the three synthetic drugs could offer the best result since they could complement the shortfall of each other.

Keywords: *Eimeria tenella*, Drug regimes, Weight performance, Oocysts count, Haematogical parameters, Histological architecture.

INTRODUCTION

Coccidiosis is one of the major diseases affecting the poultry industry resulting in a worldwide annual economic loss of over 800 million US dollars.[1-3] The causative organism of coccidiosis in domestic fowl is Eimeria species, which affects the gastrointestinal channels and is characterized by diarrhoea, weight loss, reduce egg production and in many cases death.[2-3] The seven genera of Eimeria species usually found in chickens are differentiated based on morphology of oocyst, virulent competence and sites of infection in the digestive system.[4] The ability of Eimeria oocysts to adapt easily to the environment,

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makes control processes challenging. In addition, the commonly used prophylactic anticoccidial feed additives has resulted in a wide spread anticoccidial resistance.[5]

Faecal examinations are usually advocated for the diagnosis, detection and identification of oocyst.[6-7] Mundt *et al*.[8] and Bangoura *et al*.[9] both suggested in their reports that a threshold of 500 oocysts per gram of faeces (OPG) of pathogenic Eimeria species is significant to causing clinical disease at the herd level.

Sporozoites which are released from sporocysts in the upper intestine usually initiate infection in the intestine as they migrate to their favorite locations of development and also invade villus enterocytes. The mechanisms deployed by sporozoites to reach their destination is precisely not known, but it is suggested they must have been transported from the villi within the host lymphocytes particularly the intra-epithelial,[10] cytotoxic T-cells and macrophages.[11-12]

The availability of several anticoccidial compounds and their effectiveness at controlling or decreasing the severity of coccidiosis in chickens and cattle has been reported. But the number of approved medicines available in many countries is inadequate and variable.[7,13-17]

Triazones, diclazuril and toltrazuril are some of the compounds advocated for treatment and control of chicken as well as bovine coccidiosis in many countries.[18] In bovine coccidiosis, Diclazuril is used for metaphylactic control of *Eimeria* infections due to its low absorption and its rapid excretion, whereas Toltrazuril although similar and chemically related, is slowly absorbed, has a long half-life and a much longer period of excretion, and reported persistent activity.[19]

These chemical coccidiostats/coccidiocidals are also effective for the control of chicken coccidiosis when added to their feeds.[20] But this routine practice and abuse of these drugs lead toward Eimeria strains' emersion with drug-resistant properties[1,21] with many other negative implications of these drugs.[22] Along with the drug resistance, anticoccidial drug residues are also present in poultry products, which have potentially harmful effects on public health and food safety concerns.[23]

The quest to comparatively evaluate the individual potency/efficacy of some of these coccidiostatic and/or anticoccidiocidal synthetic drugs (Diclazuril, Embarzine forte and Toltrazuril- [DET]) in a tropical climate could unravel the limits of their individual potency and serve as a precursor to mitigating coccidiosis *in vivo* in infected chickens.

MATERIALS AND METHODS

Study Area: This research was carried out at the National Veterinary Research Institute Vom, Plateau State, Nigeria. The large Animal House within the Institute was used to house the birds. Data analysis was conducted at the Applied Entomology and Parasitology Unit of the Department of Zoology, University of Jos, Nigeria.

Vaccination of Experimental animals: 25 day old broiler birds were allowed to acclimatize in the Animal House for 26 days before the commencement of the study. During this period, using the NVRI Vom vaccination schedule, the birds were vaccinated in the first, second and third weeks against common viral diseases like infectious bursal disease (IBD) also known as Gumboro 1, Newcastle Disease (La Sota) 1 and Gumboro 2 on days 7, 14 and 21 respectively. This was to nullify the possible occurrence of viral infections. The vaccines were administered in their drinking water after about 12hrs of water starvation.

Ethical clearance: All experiments were conducted in accordance with the principles and guidelines for the care and use of laboratory animals[24] and approved by the Animal Ethics Committee of NVRI, Vom.

Innoculation of Experimental Animals with Coccidia: On the day 26, birds were randomly assigned to five (5) groups each of five (5) birds, with groups 1-3 being experimental groups and groups 4 and 5 being the positive and negative controls respectively. Sporulated oocysts of *Eimeria tenella* was orally given at a dose of $4x10^3$= 0.2ml as a single gavage to each bird except the negative control which was not infected, but was given feed and water *ab initio* throughout the experimental period.

Drug Treatments: Three conventional drugs: Embazin forte, Diclazuril and Toltrazuril were used individually (in single form) to comparatively determine their respective potencies in thetreatment of *E. tenella* infection in the broiler chickens. The administration of the various drugs to the treated groups (i.e. groups 1-3) was done based on the manufacturer's guide. The groups and the respective treatments regimen are thus:

Group '1' -- infected and treated with 100% Embazin(E) in 4litres of H2O
Group '2' --infected and treated with 100% Diclazuril(D) in 4litres of H2O.
Group '3' --infected and treated with 100% ToltrazurilT) in 4litres of H2O.
Group '4' --infected and not-treated (Positive Control).
Group '5' --not-infected and not-treated (Negative Control).

Experimental Design

Parasitological examination (Oocyst Count): Faecal samples were collected into clean labelled containers on day 3 post infection subsequently after every 3 days and then taken to parasitology laboratory for *Eimeria* oocyst identification and count per gram (opg) of faeces. The mean number of oocyst per gram faeces for each bird was determined using the McMaster counting technique according to the method described by Long and Truiscot;[25] Long and Rowell.[26]

Body weights: Weight of each bird was taken on the day prior to treatment (day-1), recorded and repeated after every three days and day 55 post-treatment, which was the last day of the trial.

Parasitaemia and Haematology: 2ml of veinous blood from three birds from each group was randomly collected for haematology after the establishment of parasitaemia. The haematological evaluation to determine Red Blood Cell counts (RBC), Packed Cell Volume (PCV), White Blood Cell counts (WBC), Lymphocytes (LYM) and Haemoglobin Concentration (HBC) was then carried out.

Clinical observations: Treatment commenced on day 9 post infection following the observations of clinical signs such as bloody diarrhoea, depression, pale combs, ruffled feathers, loss of appetite and establishment of parasitaemia level (90%). General behaviour, desire for food and poor health of all birds were examined daily throughout the entire study period (day -1 to day 25 post-treatment) by the same farm staff on the farm. Individual rectal faecal samples (minimum 5 g) were collected after every three days for the entire trial duration, and stored at +4°C until shipped to the laboratory for analysis. Visual faecal score was also recorded throughout on the farm, on a scale 0–4, where 0=normal faeces; 1=pasty to semi liquid; 2=liquid; 3=liquid with blood; and 4=liquid with blood and tissue.

Treatments and Histology: The Embazin forte was measured using sensitive weighing scale (PB 153 Mettler Toledo weighing scale) and applied in the birds' drinking water. While, Diclazuril (D) and Toltrazuril (T) were measured using 5ml syringe. Both powdered and liquid drugs were applied orally via drinking water. Overall, treatment lasted for nine days. For histological examination, experimental birds were randomly selected five days post treatment for sacrifice by cervical dislocation and for necropsy at the Central Diagnostic Laboratory NVRI, Vom. Their intestines were harvested immediately after the sacrifice.

Statistical Analysis

The data obtained were statistically analysed using SPSS version 20.0. Analysis of variance (ANOVA) and comparing groups was performed using the Least Significance Difference (LSD) at $P< 0.05$ as recommended by Petrie and Watson.[27] The criteria used to measure the degree of coccidial infection and the efficacy of Embazin, Diclazuril and Toltrazuril were*:* Potency of the three individual coccidiostatic and/or coccidiocidal drugs [Diclazuril, Embazine forte and Toltrazuril (D, E, T)] in infected chickens; the mean weight gain in the various groups; Haematological parameters of various groups; Histological analysis of the intestines. The experiment lasted for 55days.

RESULTS

The general observation post infection indicated that majority of the infected birds experienced reduction in weight, decreased appetite, tangled feathers and bloody diarrhoea. These clinical observations were compared to those of the control group. Prior to treatment, no mortality was recorded as a result of parasitaemia.

Parasitological (oocyst counts): Table 1, shows the comparative efficacy of the individual standard drug (E, D & T) on *E. tenella oocysts* (oocyst counts) in broiler chickens. On day 3 post infection, there was shedding of oocysts by all the infected groups except the negative control which were not infected with the parasite. On days 3 to 6 post infection, there was an increase in oocysts output in all the infected groups. After exposure to the three (3) individual standard drugs (E, D, T), there was a significant difference ($P< 0.05$) in the effect of the treatments on the final oocysts counts on day 9 post treatment. Post hoc test revealed that group 1 differed insignificantly from 2 and 3.

However, these groups (2 & 3) showed a significant decrease in the total oocysts counts in comparison with the positive control (group 4). Worthy of note, was the strong efficacy of Embazin on *E. tenella* oocysts in the broiler chickens which was evident from the non-presence of oocysts in group 1 on day 9 post treatment. However, no oocyst was shed in the negative control (grp 5) through-out the experimental period.

Haematological evaluation: Table 2, shows the comparative efficacies of the individual treatments (E, D, T) on the groups (1, 2, and 3) of broilers and their corresponding control groups (4 & 5) with respect to blood parameters.

Table 1: Comparative efficacy of individual standard drugs (E, D & T) on *E. tenella* oocysts (Parasitaemia level) in Broiler chickens.

		Post infection		Post treatment		
Group	**Treatment**	**egg count on day 3**	**egg count on day 6**	**egg count on day 3**	**egg count on day 6**	**egg count on day 9**
1	100% Embazin	$3.33^{ab}\pm0.67$	30.33± 13.86	38.33± 18.56	$11.00^{a}\pm 2.2$	$0.00^{a}\pm 0.00$
2	100% Declazuril	$4.66^{b}\pm 1.76$	26.67± 9.28	16.67± 6.01	$9.00^{a}\pm 3.21$	$17.33^{c}\pm 1.20$
3	100% Toltrazuril	$4.00^{ab}\pm1.15$	89.00± 80.81	24.33± 8.29	$3.33^{a}\pm 1.20$	$10.00^{b}\pm 2.67$
4	Positive control	$4.00^{ab}\pm 1.53$	30.00± 7.64	68.33± 9.28	$166.67^{b}\pm 44.10$	$167.68.00^{e}\pm 43.20$
5	Negative control	$0.00^{a}\pm0.00$	0.00± 0.00	0.00± 0.00	0.00± 0.00	$0.00^{a}\pm 0.00$
		0.00	NS	NS	0.00	0.00

NS- No significant difference, E- Embazin, D- Declazuril, T- Toltrazuril, entries with same superscripts on the same columns share no significant difference, Critical P value-0.05

Table 2: Comparative Effect of individual standard drug (E, D, T) on haematological parameters of Broiler chickens

Group/ Treatment		**Pre-inoculation**					**Post-inoculation**					**Post-treatment**				
Grp	**Treament**	**PCV(%)**	**RBCcells/L**	**WCB×10⁹/L**	**HB(g/L)**	**LYM**	**PCV (%)**	**RBCcells/L**	**WCB×10⁹**	**HB(g/L)**	**LYM**	**PCV (%)**	**RBCCells/L**	**WCB×10⁹**	**HB(g/L)**	**LYM**
1	100% Embazin	26.17+ 0.73	8.00+ 0.50	4.13+ 0.09	8.00+ 0.50	55.00+ 2.87	40.00^{e}+ 0.58	2.73^{e}+ 0.12	5.50^{c}+ 0.06	13.3^{a}+ 0.06	31.00^{d}+ 0.58	21.00^{b}+ 0.58	2.30^{b}+ 0.06	5.50^{c}+ 0.06	13.30^{c}+ 0.06	14.00^{a}+ 6.02
2	100% Diclazuril	40.00+ 0.58	13.23+ 0.43	5.43+ 0.09	13.23+ 0.43	60.+ 2.89	28.33^{c}+ 0.89	1.33^{b}+ 0.02	3.80^{a}+ 0.06	9.13^{d}+ 0.19	18.00^{a}+ 0.58	32.00^{c}+ 0.58	4.50^{e}+ 0.17	3.80^{b}+ 0.06	9.13^{e}+ 0.19	30.00^{c}+ 2.89
3	100% Toltr-azuril	37.00+ 0.58	12.5+ 0.29	4.00+ 0.08	12.50+ 0.29	56.66+ 3.33	24.67^{a}+ 0.88	2.13^{cd}+ 0.09	4.30^{b}+ 0.11	7.70^{e}+ 0.06	21.0^{ab}+ 0.58	18.00^{a}+ 0.58	3.20^{c}+ 0.11	4.30^{d}+ 0.11	7.70^{b}+ 0.06	19.33^{a}+ 0.88
4	Positive control	36.67^{c}+ 0.88	2.67+ 0.19	2.67^{e}+ 0.19	2.50+ 0.06	25.00 + 0.58	28.00^{bc}+ 0.58	2.00^{a}+ 0.00	6.23^{d}+ 0.03	8.23^{d}+ 0.09	20.33^{a}+ 0.33	28.00^{c}+ 0.58	2.00^{a}+ 0.00	6.23^{d}+ 0.03	9.20^{e}+ 0.06	24.33^{a}+ 0.58
5	Negative control	31.33^{e}+ 0.33	4.00+ 0.06	4.00+ 0.06	10.26+ 0.09	26.23 + 2.11	31.00^{d}+ 0.58	4.20^{f}+ 0.06	10.10^{e}+ 0.03	5.47^{bc}+ 0.81	25.00^{c}+ 0.58	31.00^{c}+ 0.58	4.20^{d}+ 0.06	10.13^{e}+ 0.03	8.23^{bc}+ 0.09	25.00^{a}+ 0.58
	P(O.O5)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Key: NS- No significant difference, E- Embazin, D- Declazuril, T- Toltrazuril, While entries with same superscripts on the same columns share no significant difference

A significant difference was observed across all heamatological parameters post-infection. For packed cell volume (PCV), the highest percentage was recorded in group 1; 100% (Embazin) post infection, while the least was observed in group 3; 100% (totrazuril). 1 differed significantly from 3 post infections. The red blood cell count (RBC) was lowest in group 2 and significantly different from the rest of the treatments post infection. On the haemoglobin concentration, there was a significant difference observed in all the treatments with group 1; 100% (Embazin) having the highest, while the negative control had the least haemoglobin concentration post infection.

The lymphocyte counts post infection was observed to have the highest value recorded in group 1; 100% (Embazin)=31.00±0.58 while the least was recorded in group 2; 100% (Diclazuril)=18.00 ± 0.58, where 1 differed significanlty with all other groups. Post treatment showed a siginificant difference across all the haematological parameters. For PCV, the highest percentage among the treatments (groups 1, 2 & 3) was observed in group 2; 100% (Diclazuril)=32.00 ± 0.58, while the least was observed in group 3; 100% (Toltrazuril)=18.00± 0.52. In the red blood cell count (RBC) post treatment, a significant difference was observed among the treatments with group 2; 100% (Diclazuril) having the highest RBC count = 4.50± 0.17. The lymphocyte counts post treatment as observed had the highest level recorded in group 2; 100% (Diclazuril)=30.00± 2.8. The haemoglobin concentration post treatment was also significant, with group 1; 100% (Embazin)=13.30± 0.06 having the

Table 3: Comparative Efficacy of individual standard drugs (E, D, T) on the Weight of Broiler chickens.

		Pre-inoculation			Post-inoculation			Post-treatment		
Group	Treatment	Day 3	Day 6	Day 9	Day 3	Day 6	Day 9	Day 3	Day 6	Day 9
1	100% Embazin	1.07+0.09	1.53+0.09	1.90+0.6	1.70^{d}+0.12	1.60^{a}+0.58	1.47+0.09	1.77^{b}+0.18	1.80^{ab}+0.06	2.23+0.07
2	100% Declazuril	1.50+0.31	1.63+0.22	2.00+0.17	1.80^{d}+0.12	1.93^{a}+0.15	1.90+0.12	2.03^{b}+0.12	2.10^{bc}+0.06	2.27+0.07
3	100% Toltrazuril	1.30+0.21	1.37+0.35	0.97+0.95	0.77^{a}+0.03	1.43^{a}+0.35	1.43+0.24	1.03^{a}+0.24	1.60^{ab}+0.40	1.93+ 0.30
4	Positive control	1.53+0.15	1.53^{ab}+0.09	1.77+0.15	1.50^{bc}+0.06	1.67^{a}+0.07	1.57+0.12	1.63^{b}+0.07	1.53^{a}+ 0.19	1.53^{bc}+0.60
5	Negative control	0.90+0.06	0.87+ 0.12	1.07+0.12	1.33^{bc}+0.28	1.37^{a}+0.18	1.70+0.12	1.80^{c}+0.08	1.84^{cd}+0.06	2.87^{c}+0.48
	P(0.05)				0.00	NS	NS	0.037	0.00	NS

Key: NS- No significant difference, E- Embazin, D- Declazuril, T- Toltrazuril , entries with same superscripts on the same columns share no significant difference , Critical P value-0.05

highest concentration, thus being significantly different from the rest of the treatments and statistically same as the negative control post treatment.

Effects of Treatments on Weight of Broiler Chickens: Table 3 shows the comparative effects of the individual treatments (E, D, T) on the weights of broiler chickens. On days 3 to 9 pre-inoculation, there was a significant weight gain across all the groups (treatments and control groups), except in group 3 which had decreased mean weights on day 9 pre-inoculation. On day 3 post-infection, there was a loss of weight in birds in all groups. From days 6 to 9 post infection, there was an insignificant loss of body weight across all infected groups compared to their negative control which had a weight gain.

Histopathology: At the time of collection, birds in all the groups had coccidiosis and were recovering except the negative control (Plate 11 & 12) which was neither infected nor treated". The intestinal epithelial cells were sloughed as a result of invasion by the oocysts of *Eimeria* spp. Numerous goblet cells which form part of the digestive juices in the intestine as well as secrete mucus that forms a protective barrier were seen (Plates 1-10). Their presence shows part of the efforts of the intestine to provide defense for the intestinal architecture against the invading oocysts. The breakdown of our findings is as demarcated in the chart below.

The caecum had a sloughing of the epithelia and clusters of oocysts invading the villi which consequently affect the blood capillaries beneath the villi, resulting to leakage of blood into the intestine, hence, the presence of blood in the faeces (Plates 4, 6, 9 & 10). Most of the caecal portions especially in the negative control (Plate 13) were intact with short and broad villi and no pathology observed. Crypts of lieberkuhn with several goblet cells were revealed in the ilieum with no pathology observed (Plate 3). The duodenum had clusters of oocyst mingled with epithelial debri (Plates 1, 2, 5, 7 & 8). The absence of oocysts in the intestine despite the sloughing of the intestine is attributed to healing process (occurrence of recovery).

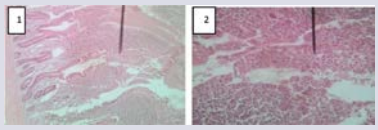
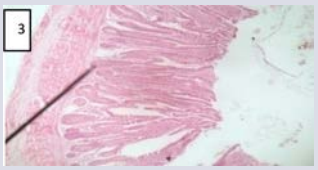
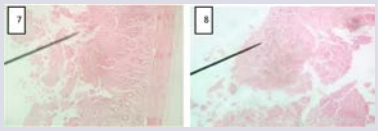
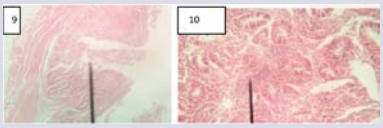
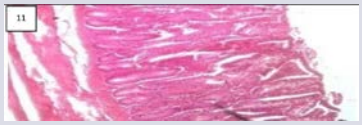
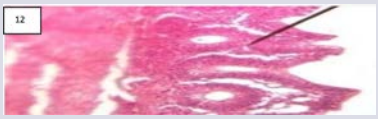
It was carefully and clearly observed that the treated groups (Plates 1, 2, 3, 4, 5 & 6) showed some level of interventions in terms of oocyst reduction as compared to the positive control (Plates 7, 8, 9 & 10) "infected but not treated" which had clusters of oocysts along with epithelial debri, lymph node in the outer longitudinal muscle and infiltration of the caecum being an indicator of infection.

Overall, Embazin had best effect on eliminating oocyst in the faeces (caecum and ileum), while Diclazuril had the most improved effect on the haematological and histological architectures (Plates 3 & 4).

DISCUSSION

Oocyst Count: Many studies have documented the prevalence of *Eimeria* species in poultry with 80% of vicinity examined discovered to have poultry excreting oocyst [28-31]. The levels of oocyst excretion in poultry were usually high and recordings of the different sample of *Eimeria* species reported with the significant correlation between their presence and the incidence of blood dysentery and diarrhoea noted.[32-33]

In this study conducted on poultry challenged with Eimeria oocyst, oocyst shedding were found to be as high level as compared to those naturally infected.[34] The degrees of challenge experienced by all treated groups of poultry were reflected in the untreated control group of poultry. In order to lessen the burden of an estimated variation in oocyst output between individual poultry, collection of oocyst was carried out three times weekly so that both lowest and highest output from each poultry may take place on a non-sampling day. Thus, the group mean oocyst counts result presented was considered as trend rather than absolute values.

Treatment	Small Intestine		Large Intestine
Various Drugs	Duodenum	Ileum	Caecum
Embazin	 **Plate 1:** Duodenum revealing sloughing of intestinal epithelia by invading oocysts. H&E **Stain** x100. **Plate 2:** Duodenum revealing invasion of intestinal villi by oocysts. H&E Stain x400.	No oocyst infection recorded	No oocyst infection recorded
Diclazuril	No oocyst infection recorded	 **Plate 3:** Ileum with arrow pointing at the crypt of lieberkuhn and several goblet cells revealed. No pathology observed. H&E Stain x100.	 **Plate 4:** Caecum with short and broad villi. No pathology observed. H&E Stain x100
Toltrazuril	 **Plate 5:** Duodenum with mild sloughing of the epithelial lining. H&E Stain x100	No oocyst infection recorded	 **Plate 6:** Caecum revealing short and broad villi with no pathology observed H&E Stain. x100
Positive control	 **Plate 7:** Duodenum with sloughing of epithelia. H&E Stain x100. **Plate 8:** Duodenum with clusters of oocysts along with epithelial debri. H&E Stain x400	No oocyst infection recorded	 **Plate 9:** Caecum with sloughing of epithelia. H&E Stain x100. **Plate 10:** Caecum with Leukocyte infiltration, indicative of infection. H&E Stain x100
Negative control	 **Plate 11:** Duodenum with intact villi. No pathology observed. H&E Stain x400	No oocyst infection recorded	 **Plate 12:** Caecum with intact villi and no pathology observed. H&E Stain x400

Overcrowding and poor hygiene with attendant stressful situation are generally linked to the cause of coccidiosis in birds which usually occur between six days after birds get infected.[35]

On day three (3) Post infection, the mean oocyst counts were similar for all the treated three groups with no significant difference observed in oocyst counts within 72 hr when compared to the Positive and Negative controls. However, this changed 6 days after Post Infection. On treatment, the Post treatment indicate that while no significant difference was observed three days Post Treatment (PT), 9 days PT however showed fewer number of egg counts were recovered from the Embazine treated groups as compared to the other groups of treatment.

Peaks of oocyst counts were observed in Toltrazuril treated groups until the 3rd day Post Treatment but reduced thereafter to lower levels throughout the rest

of the study period with a mean total oocyst counts of 10.00 OPG between days 3 to 9 PT. In contrast however, the Diclazuril treated birds had significantly lower oocyst counts 3 days Post Infection but thereafter oocyst counts remained consistently and significantly higher than in the control, Toltrazuril and Embazin groups until 9 days Post Treatment with several peaks of oocyst counts coinciding with increased frequency of diarrhoea during this period.

Mean oocyst counts in the untreated control group were high on several occasions from 6 days PT to 9 days PT (mean oocyst counts of 167.88 OPG). When compared to reports in calves, a different scenario was observed by Philippe *et al*.[36] who noted that the mean oocyst counts for Toltrazuril treated cows were higher than both Diclazuril-treated and untreated groups.

With the high mean oocyst counts in untreated birds, the possibility of the newly birthed chicks getting infected with coccidiosis few days after birth coinciding with periods of maximum oocyst shedding could be very high. Generally, depending on the timing of exposure and levels of challenge, incidence of disease are usually extremely variable, but could reduce except where vulnerability is increased through impaired immune response or devastating levels of oocyst challenge.[37] It has been reported by Beach and Corl[38] that chickens exposed to sufficient oocyst challenge will usually develop an effective immunity to homologous re-infection, mediated by increased levels of interferon-gamma following primary challenge.[39-40]

This study reveals that with the commencement of various treatments, the potency of each of the standard drugs (E, D, T) induced anticoccidial/coccidiostatic effect against *Eimeria tenella*, with Embazin having more coccidiocidal effect than Diclazuril and Toltrazuril as seen from the drastic reduction and elimination of oocysts in the faeces of the treated chickens. These results are however at variance with those previously findings by El-Banna *et al*.[41] and El-Shazly *et al*.[42] who reported that diclazuril in the drinking water was appropriate for use in the prevention and treatment of Eimeria infected chickens indicated by decrease in the oocyst number.

Haematology

In the blood parameters, a significant reduction in the packed cell volume, haemoglobin concentration and red blood cell counts was observed post infection on the haematological parameters table (Table 2). This result agrees with Witlock;[43] Adulugba *et al*.[44] who both observed a significant decrease in red blood cells, haemoglobin concentration and packed cell volume of *E. tenella* infected chickens and suggested that the decline may be due to the severe bleeding and tissue damage in the mucosa of duodenum originated from invasion of *E. tenella*.

Ogbe *et al*.[45] observed a slight drop in packed cell volume in the broilers infected with the Hougton strain of *E. tenella* on the 7th day post infection and attributed it to the virulent nature of *E. tenella* in chickens. An increase in the lymphocyte and white blood cell counts was also recorded among the infected groups of birds. These parameters changed significantly post treatment where an increase in the red blood cells count and haemoglobin concentration was recorded, but with a reduction in lymphocyte counts.

The RBC counts and percentage of PCV were highest in group 2 (100% Diclazuril) post treatment as compared to the results in the other two treatments (i.e. 100% Embazin and 100% Toltrazuril). Anaemia caused by *E. tenella* was characterized by the decrease in number of red blood cells and packed cell volume. This effect was ameliorated by 100% Diclazuril which recorded the highest RBC after treatment. The increase in lymphocyte count may be attributed to the effect of the sloughing of the intestine.

Histological Architecture

The photomicrograph of sections of the intestines showed varying degrees of epithelial sloughing and depletion which was as a result of the parasitic infection by *E. tenella* and evidently seen in the varying stages of their development. This is in sync with studies by Maskerem *et al*.[46] who observed excessive tissue damage, haemorrhage, presence of clusters of large schizonts and merozoites in the caeca of birds infected with *E. tenella* and *E. brunette*.

However, due to the treatment administered to the birds, there was a moderate sloughing and degeneration of intestinal epithelial lining in group 3. Obviously, group 2 (100% Diclazuril) had the best architecture of intestinal epithelial lining compared to the rest of the groups. Group 4 had a severe destruction of its intestinal crypts due to lack of intervention.

Weight

In this study, the planned treatments with the three drugs had no significant consequences on chicken performance, based on mean bodyweights and mean weight gains, in chickens **o**n day 3 post treatment, where it was observed that some treated groups (1 & 2) had a similar weight gain except group 3 which had a continued weight loss. From days 6 to 9 post treatment, all the groups (treated and controls) had a mean

weight gain with group 2 (100% Diclazuril) having the highest weight gain. Worthy of note was the fluctuating (inconsistent) weight gain and loss in positive control (group 4) with eventual possession of least weight gain and diarrhoea on day 9 post treatment as compared to negative control (group 5) which had weight gain all through the experimental period. Fascinatingly, whereas clinical symptoms and the number of diarrhoea days were lower in all treatments, the number of diarrhoea days in the untreated group kept increasing.

Overall, the results the attendant body weights were affected by coccidial oocyst load but decreased with treatments. This corroborates the finding of Akhter *et al.*[47] who reported a negative co-relationship between coccidial oocyst load and body weight, invariably, that body weight of birds decreased significantly when protozoal load increases.

CONCLUSION

With the evaluation of the effects of Embazin, Diclazuril and Toltrazuril, we have been able to further confirm the anticoccidial activities of these drugs against *E. tenella* as evident from reduction with even elimination of oocysts in the faeces, improvement in weight gain, haematological parameters and recovering of intestinal architecture after distortion via sloughing due to the infection (coccidiosis). The severity of coccidiosis observed based on the oocysts counts, haematology, histopathology and weight gain was high, therefore, the disease caused by *E. tenella* if untreated has a destructive effect on broiler chickens. The untreated group (group 4) of the birds had the lowest parameters with increased oocyst number, distorted intestinal architecture which is an indication of the efficacy of the various treatments.

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Authors Contribution

MJA: literature search, clinical studies, experimental studies, data acquisition and manuscript preparation. **GON:** concept, design, definition of intellectual content, literature search, clinical studies, experimental studies, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing and manuscript review. **DA:** clinical studies, experimental studies, data acquisition. **JBN:** clinical studies, experimental studies, data acquisition. **HBM:** clinical studies, experimental studies, data acquisition. **AOR:** clinical studies, experimental studies, data acquisition. **USS:** clinical studies, experimental studies, data acquisition. **EOC:** clinical studies, experimental studies, data acquisition. **IOS:** clinical studies, experimental studies, data acquisition **AYM:** clinical studies, experimental studies, data acquisition. **AGY:** clinical studies, experimental studies, data acquisition **NN:** clinical studies, experimental studies, data acquisition. **GGY:** clinical studies, experimental studies, data acquisition. **AHO:** manuscript preparation, manuscript editing and manuscript review. **IGN:** data analysis, statistical analysis, manuscript preparation, manuscript editing and manuscript review. **MBM:** data analysis, statistical analysis manuscript preparation, manuscript editing and manuscript review.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Shirley MW, Smith AL, Blake DP. Challenges in the successful control of the avian coccidia. Vaccine. 2007;25(30):5540-7. doi: 10.1016/j.vaccine.2006.12.030, PMID 17224208.
2. Conway DP, McKenzie ME. Poultry coccidiosis and effect of coccidiosis diagnostic and testing procedures'. 3rd ed. Oxford: Blackwell Publishing; 2007.
3. Guo FC, Kwakkel RP, Williams CB, Suo X, Li WK, Verstegen MW. Coccidiosis immunization: Effects of mushroom and herb polysaccharides on immune responses of chickens infected with *Eimeria tenella*. Avian Dis. 2005;49(1):70-3. doi: 10.1637/7227-062504R1. PMID 15839415.
4. Collier CT, Hofacre CL, Payne AM, Anderson DB, Kaiser P, Mackie RI, *et al.* Coccidia-induced mucogenesis promotes the onset of necrotic enteritis by supporting Clostridium perfringens growth. Vet Immunol Immunopathol. 2008;122(1-2):104-15. doi: 10.1016/j.vetimm.2007.10.014, PMID 18068809.
5. Leemans I, Hooshmand-Rad P, Uggla A. The indirect fluorescent antibody test based on schizont antigen for study of the sheep parasite Theileria lestoquardi. Vet Parasitol. 1997;69(1-2):9-18. doi: 10.1016/s0304-4017(96)01098-9. PMID 9187025.
6. Jolley WR, Bardsley KD. Ruminant coccidiosis. Vet Clin North Am Food Anim Pract. 2006;22(3):613-21. doi: 10.1016/j.cvfa.2006.07.004, PMID 17071356.
7. Daugschies A, Agneessens J, Goossens L, Mengel H, Veys P. The effect of a metaphylactic treatment with diclazuril (Vecoxan) on the oocyst excretion and growth performance of calves exposed to a natural *Eimeria* infection. Vet Parasitol. 2007;149(3-4):199-206. doi: 10.1016/j.vetpar.2007.08.003, PMID 17850970.
8. Mundt HC, Bangoura B, Mengel H, Keidel J, Daugschies A. Control of clinical coccidiosis of calves due to *Eimeria bovis* and *Eimeria zuernii* with toltrazuril under field conditions. Parasitol Res. 2005a;97;Suppl 1:S134-42. doi: 10.1007/s00436-005-1457-9, PMID 16228270.
9. Bangoura B, Mundt HC, Schmäschke R, Westphal B, Daugschies A. Prevalence of Eimeria bovis and *Eimeria zuernii* in German cattle herds and

factors influencing oocyst excretion. Parasitol Res. 2012;110(2):875-81. doi: 10.1007/s00436-011-2569-z, PMID 21808979.
10. Fernando MA, Rose ME, Millard BJ. Eimeria spp. of domestic fowl: The migration of sporozoites intra- and extra-enterically. J Parasitol. 1987;73(3):561-7. doi: 10.2307/3282137, PMID 3598806.
11. Trout JM, Lillehoi HS. Evidence of a role for intestinal CD8+ lymphocytes and macrophages in transport of Eimeria Acervulina sporozoites. J Parasitol. 1993;79(5):790-2. doi: 10.2307/3283625, PMID 8105047.
12. Trout JM, Lillehoj HS. Eimeria Acervulina infection: Evidence for the involvement of CD8+ T lymphocytes in sporozoite transport and host protection. Poult Sci. 1995;74(7):1117-25. doi: 10.3382/ps.0741117, PMID 7479488.
13. Conlogue G, Foreyt WJ, Wescott RB. Bovine coccidiosis: Protective effects of low-level infection and coccidiostat treatments in calves. Am J Vet Res. 1984;45(5):863-6. PMID 6732015.
14. Fitzgerald PR, Mansfield ME. Effect of decoquinate on the control of coccidiosis in young ruminating calves. Am J Vet Res. 1986;47(1):130-3. PMID 3946892.
15. Hoblet KH, Charles TP, Howard RR. Evaluation of lasalocid and decoquinate against coccidiosis resulting from natural exposure in weaned dairy calves. Am J Vet Res. 1989;50(7):1060-3. PMID 2774324.
16. Epe C, Von Samson-Himmelstjerna G, Wirtherle N, Von Der Heyden V, Welz C, Beening J, *et al*. Efficacy of toltrazuril as a metaphylactic and therapeutic treatment of coccidiosis in first-year grazing calves. Parasitol Res. 2005;97;Suppl 1:S127-33. doi: 10.1007/s00436-005-1456-x, PMID 16228269.
17. Mundt HC, Bangoura B, Rinke M, Rosenbruch M, Daugschies A. Pathology and treatment of *Eimeria zuernii* coccidiosis in calves: Investigations in an infection model. Parasitol Int. 2005b;54(4):223-30. doi: 10.1016/j.parint.2005.06.003, PMID 16023406.
18. Taylor MA, Bartram DJ. The history of decoquinate in thecontrol of coccidial infections in ruminants. J Vet Pharmacol Ther. 2012;35(5):417-27. doi: 10.1111/j.1365-2885.2012.01421.x.
19. Veronesi F, Diaferia M, Viola O, Fioretti DP. Long-term effect of toltrazuril on growth performances of dairy heifers and beef calves exposed to natural *Eimeria zuernii* and *Eimeria bovis* infections. Vet J. 2011;190(2):296-9. doi: 10.1016/j.tvjl.2010.10.009, PMID 21144780.
20. Allen PC, Fetterer RH. Recent advances in biology and immunobiology of *Eimeria* species and in diagnosis and control of infection with these coccidian parasites of poultry. Clin Microbiol Rev. 2002;15(1):58-65. doi: 10.1128/CMR.15.1.58-65.2002, PMID 11781266.
21. McDougald LR, Fitz-Coy SH. Coccidiosis. In: Saif YM, editor, Disease of poultry. 12th ed. Oxford: Blackwell Publishing; 2008. p. 1068-80.
22. Hafeez A, Ullah Z, Khan RU, Ullah Q, Naz S. Effect of diet supplemented with coconut essential oil on performance and villus histomorphology in broiler exposed to avian coccidiosis. Trop Anim Health Prod. 2020;52(5):2499-504. doi: 10.1007/s11250-020-02279-6, PMID 32377969.
23. Mortier L, Huet AC, Charlier C, Daeseleire E, Delahaut P, Van Peteghem C. Incidence of residues of nine anticoccidials in eggs. Food Addit Contam. 2005;22(11):1120-5. doi: 10.1080/02652030500199355, PMID 16332635.
24. National Research Council Guide for the Care and Use of Laboratory animals; 1996.
25. Long JR, Truscott RB. Necrotic enteritis in broiler chickens. III. Reproduction of the disease. Can J Comp Med. 1976;40(1):53-9. PMID 187291.
26. Long PL, Rowell JG. Counting oocysts of chicken coccidia. Lab Pract. 1958;7(15):5-18.
27. Petrie A, Watson P. Statistics for veterinary and animal science. 3rd ed The Black Well Science Ltd. United Kingdom; 2013. p. 110-5.
28. Michels MG, Bertolini LC, Esteves AF, Moreira P, Franca SC. Anticoccidial effects of coumestans from *Eclipta alba* for sustainable control of *Eimeria tenella* parasitosis in poultry production. Vet Parasitol. 2011;177(1-2):55-60. doi: 10.1016/j.vetpar.2010.11.022, PMID 21177038. vetpar.2010.11.022.
29. Jadhav BN, Nikam SV, Bhamre SN, Jaid EL. Study of Eimeria necatrix in broiler chicken from Aurangabad district of Maharashtra State India. Inter Multidiscip Res J. 2011;1(11):11-2.
30. Blake DP, Knox J, Dehaeck B, Huntington B, Rathinam T, Ravipati V, *et al* Re-calculating the cost of coccidiosis in chickens. Vet Res. 2020;51(1):115. doi: 10.1186/s13567-020-00837-2, PMID 32928271.
31. Goselle ON, Adulugba IA, Ajayi OO, Lawan SB, Jwanse RI, Ike ME. The efficacy of phyto-synthetic drug of coccidian as evidenced from oocysts counts and histopathology. Asian J Sci Technol. 2017;8(8):5139-43.
32. Vegad JL. Poultry coccidiosis. In: Poultry Diseases, a guide for farmers and poultry professionals. India: International Book Distributing Company; 2004. p. 186-97.
33. Wakenell PS. Hematology of chickens and turkeys. In: Weiss DJ, Wardrop KJ, editors. Veterinary hematology. 6th ed. Chichester: John Wiley and Sons; 2010. p. 957-67.
34. Kinunghi SM, Tilahun G, Hafez HM, Woldemeskel M, Kyule M, Grainer M, *et al*. Assessment of economic impact caused by poultry coccidiosis in small and large poultry farms in DebreZeit, Ethiopia. Internal. J Poult Sci. 2004;3(11):715-8.
35. SMK, . GT, . HMH, . MW, . MK, . MG *et al*. Assessment of Economic Impact Caused by Poultry Coccidiosis in Small and Large Scale Poultry Farms in Debre Zeit, Ethiopia. Int J Poult Sci. 2004;3(11):715-8. doi: 10.3923/ijps.2004.715.718.
36. Philippe P, Alzieu JP, Taylor MA, Dorchies Ph. Comparative efficacy of diclazuril (Vecoxan®) and toltrazuril (Baycox bovis®) against natural infections of *Eimeria bovis* and *Eimeria zuernii* in French calves. Vet Parasitol. 2014;206(3-4):129-37. doi: 10.1016/j.vetpar.2014.10.003.
37. Greif G. Immunity to coccidiosis after treatment with toltrazuril. Parasitol Res. 2000;86(10):787-90. doi: 10.1007/s004360000218, PMID 11068809.
38. Beach JR, Corl JC. Studies in the control of avian coccidiosis. Poult Sci. 1925;4(3):83-93. doi: 10.3382/ps.0040083.
39. Edgar SA, King DE. Breeding and immunizing chickens for resistance to coccidiosis. 62nd and 63rd. Annu rep Alabama Agr Exp stat. 1952; p. 36-7.
40. Wallach M. Role of antibody in immunity and control of chicken coccidiosis. Trends Parasitol. 2010;26(8):382-7. doi: 10.1016/j.pt.2010.04.004, PMID 20452286.
41. Elbanna H, El Latif AA, Soliman M. Anticoccidial activity of *Allium sativum* and *Aloe vera* in broiler chickens. Intern'l J Agro Vet. Med Sci. 2013;7(4):177-25.
42. El-Shazly KA, El-Latif AA, Abdo W, El-Morsey A, El-Aziz MIA, El-Mogazy H. The anticoccidial activity of the fluoroquinolone lomefloxacin against experimental *Eimeria tenella* infection in broiler chickens. Parasitol Res. 2020;119(6):1955-68. doi: 10.1007/s00436-020-06692-6, PMID 32399722.
43. Witlock DR. Physiologic basis of blood loss during *Eimeria tenella* infection. Avian Dis. 1983;27(4):1043-50. doi: 10.2307/1590205, PMID 6606420.
44. Adulugba IA, Goselle ON, Ajayi OO, Pam KC, Friday SE, Tanko JT. Phyto-synthetic combination as great enhancers of haematological parameters: A case study in poultry. Am J Phytomed Clinl Ther Imedpub J. 2017;5(1: 9):1-6.
45. Ogbe AO, Atawodi SE, Abdu PA, Sannusi A, Itodo AE. Changes in weight gain, faecal oocyst count and packed cell volume of *Eimeria tenella*-infected broilers treated with a wild mushroom (*Ganoderma lucidum*) aqueous extract: Article. J S Afr Vet Assoc. 2009;80(2):97-102. doi: 10.4102/jsava.v80i2.179.
46. Maskerem A, Chaiwat B, Nirat G. Montaken. V. Haematological, biochemical and histopathological changes caused by coccidiosisin chickens. Nat Sci. 2013;47:238-46.
47. Akhter MJ, Aziz FB, Hasan MM, Islam R, Parvez MMM, Sarkar S, *et al*. Comparative effect of papaya (*Carica papaya*) leaves' extract and toltrazuril on growth performance, hematological parameter, and protozoal load in Sonali chickens infected by mixed *Eimeria* spp. J Adv Vet Anim Res. 2021;8(1):91-100. doi: 10.5455/javar.2021.h490, PMID 33860018.

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