

## Response of 2 breeds of broiler chicks to experimental infection with low dose of *Eimeria tenella* sporulated oocysts

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Received: 10.06.2013 • Accepted: 22.04.2014 • Published Online: 17.06.2014 • Printed: 16.07.2014

**Abstract:** This study evaluated the effect of low-grade *Eimeria tenella* infections in broiler chicks of Cobb (C) and Marshal (M) breeds. Birds from the experimental group of each breed were orally infected with 3000 sporulated oocysts of *E. tenella* and examined for weight gain, feed conversion ratio (FCR), packed cell volume (PCV), total leukocyte count (WBC), total plasma protein (TP), gross lesion scores, and histopathological changes 10 days after infection. There were 31.7% and 15.7% reductions in the mean weight gain of experimental groups of C and M, respectively, compared to their controls; this was statistically significant ( $P < 0.05$ ) in C but not ( $P > 0.05$ ) in M. Differences in FCR values between infected and control groups were 0.4 and 0.04 in C and M, respectively. There were no significant differences ( $P > 0.05$ ) between the mean PCV and WBC values of the infected and control groups in either breed, but the mean TP values were significantly lower ( $P < 0.05$ ) in the experimental groups of both breeds. Mean gross lesion scores were significantly higher in C than in M ( $P < 0.05$ ) but the histopathological changes observed were similar in both breeds.

**Key words:** Response, experimental infection, *Eimeria tenella*, broilers, breeds

### 1. Introduction

Commercial poultry production has greatly expanded in many developing countries due to increases in demand for animal proteins (1). Unfortunately, the poultry industry has been adversely affected by a variety of constraints. Of these constraints, diseases play a leading role in hampering the development of poultry production (1,2). Coccidiosis is one of the most devastating poultry diseases. It causes considerable economic losses in the poultry industry and appears to be the most frequently reported disease of chickens worldwide (3).

In chickens, coccidiosis is caused by an enteric protozoan parasite of the genus *Eimeria*, of which *Eimeria tenella* is the most pathogenic and the most significant cause of outbreaks in poultry farms worldwide (4–6). The severity of coccidiosis is dependent on the number of oocysts ingested by each bird; morbidity and mortality increase with the size of the dose ingested (6,7). According to Holdsworth et al. (8) and Conway and McKenzie (5), a dose of  $10^4$  to  $10^5$  sporulated oocysts of *Eimeria tenella* is required to induce a severe clinical response in broilers.

Ingestion of low doses of sporulated environmental oocysts by birds results in low-grade infections, or

subclinical coccidiosis; because of its lack of obvious symptoms, farmers often remain unaware of the infection and as a result it is the main cause today of production losses in the poultry industry (4,9,10). Losses due to subclinical coccidiosis manifest as poor bird performance as a result of poor nutrient utilization (11). Despite the importance of subclinical coccidiosis in the poultry industry, to date there appear to be very few studies available about the pathological effects associated with this form of the infection. The present study evaluated the responses of 2 broiler breeds in terms of weight gain, feed conversion ratio (FCR), hematology, and gross and histopathological changes after being experimentally challenged with low doses (3000 oocysts) of sporulated *E. tenella* oocysts to induce subclinical infection.

### 2. Materials and methods

#### 2.1. Study area

The experiment was conducted in the poultry house of the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria ( $11^{\circ}10'N$ ,  $7^{\circ}38'E$ ).

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## 2.2. Experimental birds

Ten chicks each of Cobb (C) and Marshal (M) breeds, all 1 day old, were used for the study. The birds were brooded for 2 weeks in separate cages under strict biosecurity measures and, as is routine, were adequately vaccinated against Newcastle disease and infectious bursal disease. They were fed ad libitum on a commercial broiler starter diet throughout the period of the experiment.

## 2.3. *Eimeria tenella* isolate

The *Eimeria tenella* isolate used in this study was isolated from the cecum of a pullet that died of clinical cecal coccidiosis at the Avian Unit of the Ahmadu Bello University, Veterinary Teaching Hospital. The isolated parasite was propagated by orally infecting 10 two-week-old pullets with  $10^4$  sporulated oocysts. The infected birds showed classical signs of coccidiosis 4 days after infection, such as bloody diarrhea, anorexia, and somnolence, with a 20% mortality rate. At necropsy, the gross lesions were consistent with cecal coccidiosis, which included hemorrhages, clotted blood in the ceca, and thickening of the cecal mucosa. After 10 days of infection, sufficient oocysts were recovered from the feces of the birds using a centrifugal floatation technique (7). The isolated oocysts were allowed to sporulate in solution of 2.5% potassium dichromate at room temperature (25 °C) for 6 days and used for the experimental study.

## 2.4. Experimental grouping and infection

At 2 weeks of age, chicks from each breed were randomly selected and distributed into 2 groups of 5 birds each: Cobb experimental ( $C_A$ ), Cobb Control ( $C_B$ ), Marshal experimental ( $M_A$ ), and Marshal Control ( $M_B$ ). Birds from groups  $C_A$  and  $M_A$  were orally infected with approximately 3000 sporulated oocysts of the *E. tenella* isolate.

## 2.5. Determination of feed intake, weight gain, and feed conversion ratio

The birds were tagged for identification and weighed individually prior to experimental infection with the *E. tenella* oocysts. The daily feed given to each group of birds was weighed and the amount left in the feeders was also weighed the following day to determine the daily feed intake. At the end of the experiment, the birds were reweighed individually. Mean weight gain and FCR for each group were determined as described by Holdsworth et al. (8).

## 2.6. Observation on the clinical signs and prepatent period

The birds were monitored closely for possible observable clinical signs throughout the course of the experiment. Starting on the third day of infection, daily fecal samples were collected from each group of the experimental birds and analyzed for the presence of oocysts using a simple floatation test to establish the prepatent period of the infection in each experimental group.

## 2.7. Blood sample collection and hematological examination

On day 10 of the infection, 2 mL of blood was collected from each bird by cardiac puncture, placed into a sample bottle containing EDTA, and used for the determination of packed cell volume (PCV), total white blood cell count (WBC), and total plasma protein (TP). PCV was determined using the standard capillary method as described by Jain (12). WBC was determined using the counting method described by Campbell (13), while TP was determined using the refractometer method as described by Kerr (14).

## 2.8. Observation on the gross lesions, lesion scoring, and histopathology.

At the end of the experiment (day 10 after the infection), the birds were humanely sacrificed by injecting 3 mL of air intracardially. Postmortem examination was conducted on the dead birds for the presence of gross lesions consistent with coccidiosis. Lesions observed were assigned scores from 1 to 4 using the criteria described by Conway and McKenzie (5). Cecal segments were collected, fixed in 10% formalin, dehydrated in absolute alcohol, cleared in xylene, and embedded in paraffin for preparation of fine blocks in paraffin wax; sections of 5- $\mu$ m thicknesses were cut and subjected to routine hematoxylin and eosin staining (15). The sections were examined under 400 $\times$  magnification for histopathological lesions and for the presence of developmental stages of the parasite (schizonts, gametocytes, and oocysts).

## 2.9. Statistical analysis of the data

PCV, WBC, and TP values, as well as the lesion scores of the experimental and control groups of each breed of broilers, were summarized as mean  $\pm$  SD and subjected to statistical analysis using Student's t-test. Values of  $P < 0.05$  were considered statistically significant.

## 3. Results

### 3.1. Clinical signs and prepatent period of low-grade *E. tenella* infection in broiler chicks

The clinical signs observed in all the infected birds were very mild and included reduced activity, reduced feed intake, and mild diarrhea, which were observed between day 3 and 5 after infection; after that point, the birds appeared apparently normal. Oocysts were first detected in the feces of the infected birds on the fifth day of infection and thereafter throughout the course of the experiment in both breeds of the broilers.

### 3.2. Effect of low-grade *E. tenella* infection on weight gain and FCR

#### 3.2.1. Effect on weight gain

The low-grade infection with *Eimeria tenella* isolate affected the weight gain of the infected broilers. There were 31.7% and 15.7% reductions in mean weight gain of the experimental

groups of C and M, respectively, compared to their controls. The differences in weight gain between the infected and control groups were not statistically significant ( $P > 0.05$ ) in M but were statistically significant ( $P < 0.05$ ) in C (Table 1).

**3.2.2. Effect on FCR**

Higher values of FCR were observed in the infected groups when compared with their respective control groups. However, the difference between the values of the FCR of control and infected groups was 10 times higher in C than in M, with values of 0.4 and 0.04, respectively (Table 2).

**3.3. Effect of low-grade *E. tenella* infection on some hematological parameters in broilers**

Table 3 describes the mean values of the PCV, WBC, and total plasma protein of M and C broilers on day 10 of the low-grade *E. tenella* infection. The differences in mean PCV and WBC counts between the infected and control groups of both breeds were not statistically significant ( $P > 0.05$ ). However, there were statistically significant differences ( $P < 0.05$ ) between the mean total plasma protein values of the infected and control groups of both breeds of broiler.

**Table 1.** Effect of low-grade *E. tenella* infection on weight gain in 2 breeds of broilers 10 days after infection.

Breeds	Initial weight (g)		Final weight (g)		Weight gain		% weight gain	
	A	B	A	B	A	B	A	B
Marshal	328 ± 27.0 (302–369)	336 ± 23.0 (300–365)	547 ± 43.0 (480–581)	595.8 ± 35.0 (554–652)	219 ± 39.0 <sup>a</sup> (168–279)	259.8 ± 15.0 <sup>a</sup> (249–287)	84.3	100
	330.4 ± 20.0 (295–346)	334.6 ± 44.0 (299–409)	561.6 ± 105.0 (393–684)	673.2 ± 82.0 (571–796)	231.2 ± 89.0 <sup>a</sup> (98–345)	338.6 ± 44.0 <sup>b</sup> (272–387)		
Cobb							68.3	100

A = infected group, B = control group, ( ) = range.

<sup>a,a</sup> No significant difference between weight gain of A and B in same row ( $P > 0.05$ ).

<sup>a,b</sup> Significant difference between weight gain of A and B in same row ( $P < 0.05$ ).

**Table 2.** Effect of low-grade *E. tenella* infection on feed conversion ratio (FCR) in 2 broiler birds 10 days after infection.

Breeds	Total feed intake (g)		Total weight gain (g)		Feed conversion ratio	
	A	B	A	B	A	B
MARSHAL	3532	4141	1095	1299	3.23	3.19
COBB	3653	4861	1156	1693	3.16	2.76

A = Experimental group.

B = Control group.

**Table 3.** Mean PCV, WBC counts, and total plasma protein ± SD of Marshal and Cobb breeds of broilers at day 10 after low-grade *E. tenella* infection.

Breeds	PCV (%)		WBC ( $\times 10^3 \mu\text{L}^{-1}$ )		Total protein (g dL <sup>-1</sup> )	
	A	B	A	B	A	B
Marshal	26.80 ± 3.42 <sup>a</sup> (23–32)	26.40 ± 4.51 <sup>a</sup> (21–32)	26.74 ± 12.81 <sup>a</sup> (11.1–44.5)	26.06 ± 11.98 <sup>a</sup> (15.1–45.0)	4.24 ± 0.54 <sup>a</sup> (3.8–5.0)	8.16 ± 1.66 <sup>b</sup> (5.8–10.0)
	26.40 ± 3.5 <sup>a</sup> (23–32)	25.00 ± 2.00 <sup>a</sup> (22–27)	21.72 ± 6.56 <sup>a</sup> (13.0–30.0)	23.62 ± 9.34 <sup>a</sup> (11.1–35.0)	5.16 ± 0.80 <sup>a</sup> (4.0–6.0)	7.28 ± 0.77 <sup>b</sup> (6.2–8.2)

A = infected group, B = control group, ( ) = range.

<sup>a,a</sup> No significant difference between means of A and B in same column ( $P > 0.05$ ).

<sup>a,b</sup> Significant difference between means of A and B in same column ( $P < 0.05$ ).

**3.4. Effect of low-grade *E. tenella* infection in broilers on gross lesion score**

The gross lesions observed in the infected birds at necropsy ranged from slight ballooning of the ceca, thickening of the mucosa, sloughing of the mucosal surface, and petechial hemorrhages to bloody cecal contents in some cases. The mean scores of the observed gross lesions were higher in C than in M and there was a statistically significant difference between the mean lesion scores observed in the 2 breeds ( $P < 0.05$ ). Gross lesions were not observed in all the birds necropsied from the control groups of both breeds (Table 4).

**3.5. Histopathological lesions of low-grade *E. tenella* infection in broilers**

The histopathological lesions observed in C were: a reduced number of submucosal glands, thickened muscular layers, thinner glandular layers, hemorrhagic areas, desquamation of villi, necrosis of submucosal glands, presence of developmental stages of the parasite, and diffused inflammatory cellular infiltration (Figures 1a and 1b). For M they were desquamation of the villi, presence of numerous inflammatory cells, developmental stages of the parasites, and hemorrhagic areas (Figures 2a and 2b).

**4. Discussion**

Subclinical coccidiosis occurs as a result of ingesting a low dose of sporulated oocysts from the environment. It is common in poultry houses that use anticoccidial prophylactic programs, where it is usually associated with drug-tolerant strains of the *Eimeria* parasite. Under experimental conditions, and also as observed in the

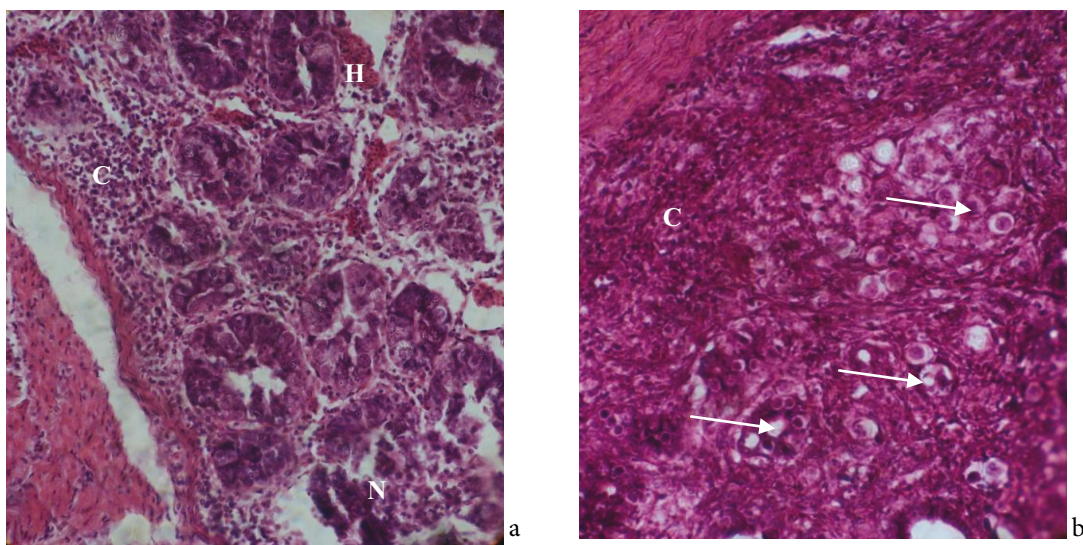
**Table 4.** Mean lesion scores of 2 breeds of broiler 10 days after low-grade *E. tenella* infection.

Breed	Mean lesion scores
Marshal	0.8 ± 0.45 <sup>a</sup>
Cobb	1.6 ± 0.55 <sup>b</sup>

<sup>a,b</sup> Significant difference between means ( $P < 0.05$ ).

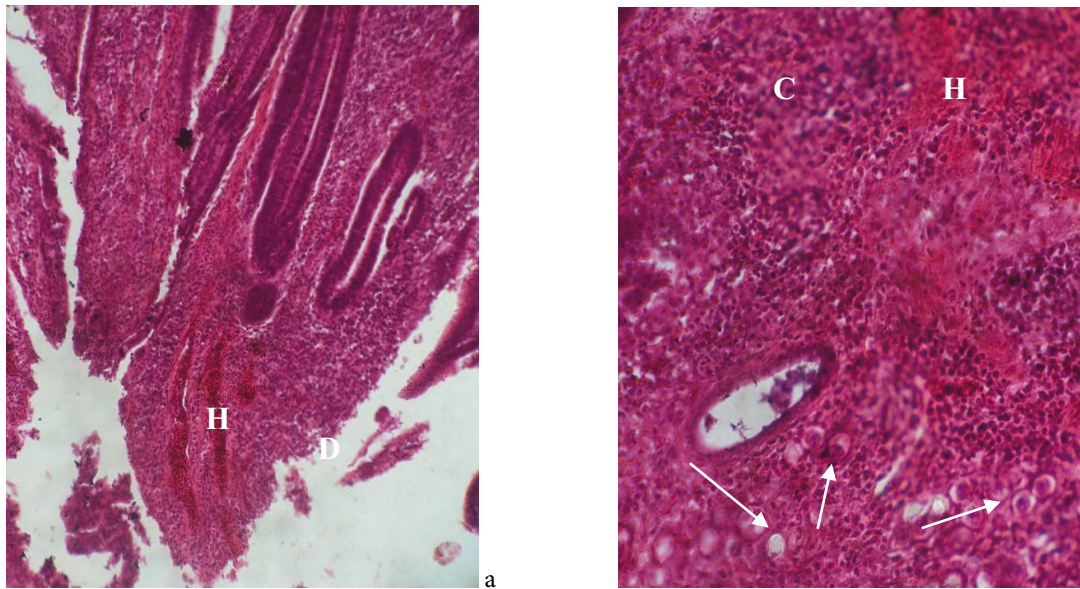
present study, subclinical coccidiosis can be induced by infecting chicks with low doses of sporulated oocysts (16). Broussard et al. (17) and Shaban (16) reported a decrease in the performance of broilers in the form of decreased weight gain and increased FCR in birds with subclinical *E. tenella* infections. These reports conform to the findings of the present study. The decreased weight gain (weight loss) observed in the infected birds may be attributed to the changes in the gut morphology and truncation of the intestinal villi as a result of injury caused by the invasion and replication of the parasite (18), thereby affecting the normal absorption of nutrients. This can further be supported by the gross and histopathological lesions observed in the infected groups. The observed decrease in weight gain could also be due to the combined effect of anorexia and muscle breakdown that occur during the acute stage of *Eimeria* infection (19).

Hypoproteinemia observed in the subclinically infected birds is consistent with the findings of Fukata et al. (20) in clinical *E. tenella* and *E. acervulina* infections; it may also be due to the nutrient malabsorption in the gut as a result of the damage caused by the parasite.

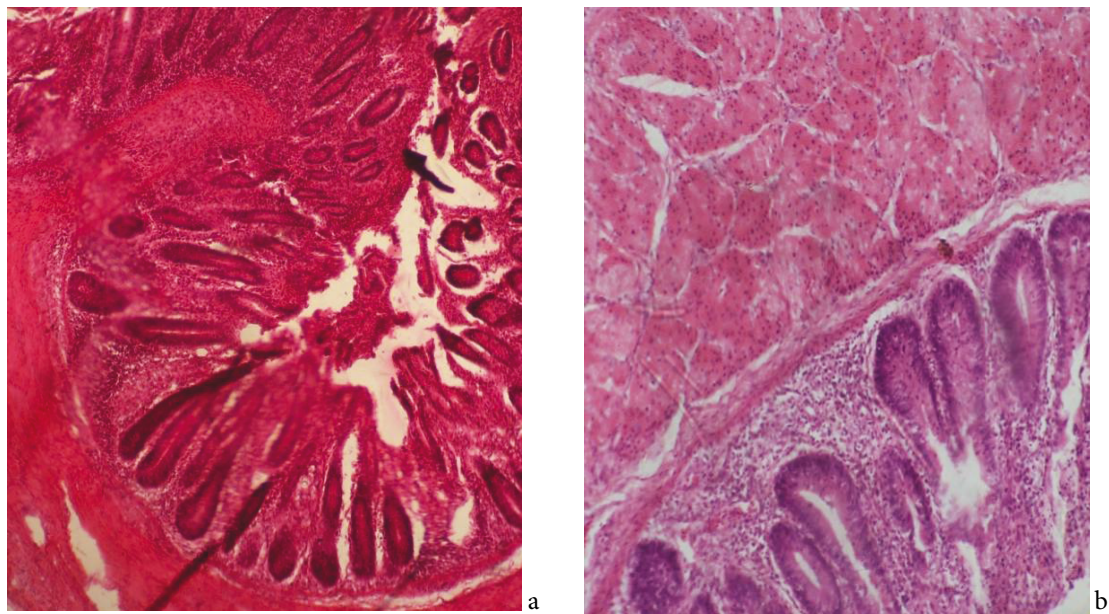


**Figure 1.** Photomicrograph of the cecum of Cobb breed of broiler with subclinical *E. tenella* infection. (a) Note hemorrhagic areas (H) cellular infiltration (C) and necrosis of glands (N). (b) Note the developmental stages of the parasite (arrows) and cellular infiltration (C).





**Figure 2.** Photomicrograph of the cecum of Marshal breed of broiler with subclinical *E. tenella* infection. (a) Note the hemorrhagic areas (H) and desquamation (D). (b) Note the areas with cellular infiltration (C), hemorrhagic areas (H), and developmental stages of the parasite (arrows).



**Figure 3.** Photomicrograph of the cecum of uninfected control Cobb (a) and Marshal (b) breeds of broilers showing normal cecal tissues.

Anemia in coccidiosis is due to blood loss in the form of hemorrhagic diarrhea as a result of severe intestinal destruction commonly seen during the acute phase (3–5 days) of clinical infection (21). Similarly, changes in leukocyte counts were reported in clinical *Eimeria* infections (22,23). However, in the present study, anemia and significant changes in leukocyte counts were not

observed in the infected groups of birds, which might be due to the mild nature of the infection.

The principal histopathological changes observed in the infected birds were consistent with those associated with infection induced by *Eimeria* species (22,24,25) and may be associated with the injuries caused by the invasive stages of the parasite. The damage caused by the invasion of

the second generation merozoites into the mucosal linings of the cecum affects the functional integrity of the cecum, which may lead to secondary bacterial infection and cellular infiltration (22,26); this may perhaps explain the diffuse cellular infiltration observed histopathologically in the infected groups of birds.

According to the World Association for the Advancement of Veterinary Parasitology, the severity of coccidia infection in birds can be measured by the effect of the infection on the weight gain and FCR (8). Based on the findings of the present study, the experimental C broilers appeared to be more susceptible to the subclinical *E. tenella* infection than the M broilers, as evidenced by the statistically significant decrease in mean weight gain, higher FCR, and more severe lesion scores. Breed susceptibility to *Eimeria* infection has long been established (11,26,27). Selection of poultry for natural resistance to coccidiosis is a promising alternative method of controlling the disease (28).

The *E. tenella* isolate caused decreased weight gain, increased FCR, and gross pathological changes in the

2 breeds of broilers at the subclinical dose of sporulated oocysts, but C appears to be more susceptible to the infection than M, as evidenced from the significant poor commercial performance and lesion score of the former in relation to the latter.

The fact that eradication of coccidiosis is almost practically impossible (especially in birds raised under deep litter system) due to the ubiquitous nature of the parasites, the underperformance of the affected flocks as observed in the present study and elsewhere as a result of infection, and the increased prevalence of drug-resistant strains of the parasites in the field mean that subclinical coccidiosis will continue to be a problem in broiler industries in the foreseeable future. It is therefore recommended that prevention and control methods must be optimized in order to minimize the negative impact of subclinical coccidiosis on broiler flock performance. In addition, poultry farmers should source their birds from hatcheries that produce breeds of birds with established histories of resistance to coccidiosis.

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