

Comparative Analysis of Evaluation Parameters in Broiler Chickens Infected With Major Parasitic Species of Eimeria

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Abstract

Background: Avian coccidiosis is a major disease within the poultry industry caused by species of *Eimeria*, an intestinal protozoan parasite. Body weight gain, intestinal lesion score, and fecal oocyst shedding are parameters that have been used to assess the protective effects of various treatments in *Eimeria*-infected chickens. The objective of this study was to compare the aforementioned parameters in broiler chickens infected with major parasitic species of *Eimeria*, such as *E. acervulina*, *E. maxima* and *E. tenella*.

Results: The results of the body weight gain, intestinal lesion score, and fecal oocyst shedding showed similar patterns between female and male broilers infected with *Eimeria* species. However, there was a difference in body weight gain between normal females and males, and also between *Eimeria*-infected females and males. When broilers were infected with 1×10^4 sporulated oocysts of *Eimeria* species, each *Eimeria* species induced distinct changes in body weight gain, lesion score, and fecal oocyst shedding. In addition, a lesion score of approximately 3 was more closely related to body weight gain than a lesion score of approximately 2.

Conclusion: These results suggest that certain levels of lesion severity are more closely related to body weight gain.

Background

Avian coccidiosis, one of the most economically important diseases in chickens, is an intracellular parasitic disease caused by several species of the apicomplexan protozoa *Eimeria* [1-3]. Various studies have indicated that the worldwide prevalence of *Eimeria* infections varies from 10% to 90% in the poultry industry [4-5]. There are seven species of *Eimeria* known to infect chickens, including *E. acervulina*, *E. maxima*, *E. necatrix*, *E. brunetti*, *E. praecox*, *E. mitis* and *E. tenella*. Each species invades the intestinal epithelial tissues of the host, eliciting a variety of clinical effects in infected chickens, including necrotic gut lesions, reduced feed conversion rate and weight gain, increased mortality, and greater susceptibility to secondary pathogens [5-6].

Current strategies to alleviate avian coccidiosis include vaccines, anticoccidial drugs and natural products. Anticoccidial drugs have long been a mainstream strategy to control avian coccidiosis in modern poultry farms. However, as the emergence of drug-resistant parasites is widespread, government regulations have increasingly demanded for a reduced use of anticoccidial drugs [7-8]. Vaccines composed of one or more strains of attenuated or non-attenuated *Eimeria* species have been successful in controlling avian coccidiosis in commercial production facilities. Live vaccines may be produced by the chickens themselves, but this approach results in increased vaccine production costs and limited production capacity [5, 9]. Natural products have emerged as a complementary or alternative methods to restrict avian coccidiosis outbreaks [10-12].

While developing these strategies, it is important to consider many factors that can influence efficacy assessments. Disease susceptibility and the induction of protective immunity to *Eimeria* infection depends on many factors, including host genetics, polymorphism in *Eimeria* resistance genes, host age, host immune status, parasite virulence factors, and parasite inoculation dose [9, 13-14]. Parameters, such as body weight gain, intestinal lesion score, or fecal oocyst shedding, are widely utilized in efficacy assessments and in experimental studies of *Eimeria* infections [15-16]. The experiments presented here evaluate values of body weight gain, intestinal lesion score, and oocysts per gram of feces (OPG) in broiler chickens infected with three major species of *Eimeria*: *E. acervulina*, *E. maxima* and *E. tenella*.

Results

Comparison of parameters in male and female broiler chickens

Body weight gain is one of three parameters widely used to evaluate *Eimeria* infection. First, we compared body weight between normal, uninfected female and male broiler chickens. Body weight differed significantly between normal female and male chickens ($P<0.01$) at all evaluated time points [see additional file 1). Next, female and male broilers were infected with 1×10^4 sporulated oocysts of *E. tenella*, and their parameters compared. On day 9 post infection, the infected females (596.3 ± 47.2) showed significantly lower body weight gain compared with males (654.4 ± 33.2). However, no significant difference between sexes was observed in lesion scores or fecal oocyst outputs. The mean lesion score of the infected females (2.2 ± 0.9) was similar to that of the infected males (2.2 ± 1.7). Fecal oocyst shedding by the infected females ($656.4\times 10^3\pm 111.9\times 10^3$ oocysts/bird) was similar to that of the infected males ($572.2\times 10^3\pm 21.1\times 10^3$ oocysts/bird) (Fig. 1). No lesion or fecal oocysts were observed in the uninfected chickens used as controls (data not shown). These results suggest that evaluation of parameters, such as body weight gain, should consider gender differences in their analysis.

Evaluation of parameters in broiler chickens infected with low dose of *Eimeria* species

Male chickens were infected with 1×10^4 sporulated oocysts of *Eimeria*, and the three parameters were monitored. The initial body weight of chickens measured before infection showed no significant differences among groups ($P>0.05$) (data not shown). Body weight gain measured on days 6 and 9 post infection were significantly lower in the *E. maxima*-infected group, but not the *E. acervulina* or the *E. tenella*-infected groups, compared to the uninfected controls (Fig. 2A). Intestinal lesion scores were significantly higher for the *E. tenella*-infected group (2.2 ± 1.8) compared with the *E. maxima*-infected group (0.8 ± 0.4), but was similar to that of the *E. acervulina*-infected group (1.8 ± 0.5) (Fig. 2B). Fecal oocyst shedding was significantly lower in the *E. tenella*-infected group than the *E. acervulina*-infected group, but was higher than in the *E. maxima*-infected group (Fig. 2C). No lesion or fecal oocysts were observed in the uninfected control chickens (data not shown). To determine whether sex-based differences existed in the infected broiler chickens, female chickens were infected with 1×10^4 sporulated oocysts of *E. acervulina*, *E. maxima*, or *E. tenella*, and the same three parameters were monitored. The patterns of body weight gain, lesion score, and oocyst shedding were similar in *Eimeria*-infected female

chickens compared with those observed in male chickens (See additional file 2). These observations showed that each *Eimeria* species induced distinct changes in the evaluated parameters, such as body weight gain, lesion score or fecal oocyst shedding, when broilers were infected with the same number of oocysts from different species of *Eimeria*.

Evaluation of parameters in broiler chickens infected with high dose of *Eimeria* species

We investigated whether increased lesion score correlated with body weight gain. Based on preliminary experiments (data not shown), 2-week-old male chickens were orally infected with 1.5×10^5 sporulated oocysts of *E. acervulina*, 7×10^4 of *E. maxima*, or 5×10^4 of *E. tenella*, to induce lesions with a score of approximately 3. Body weight gains measured on day 9 post infection were significantly lower in all the infected groups compared to the uninfected group (Fig. 3A). Initial body weights showed no significant differences among the groups, including the uninfected chickens ($P > 0.05$) (data not shown). The mean intestinal lesion score of the *E. tenella*-infected group (3.6 ± 0.5) was significantly higher than that of the *E. maxima*-infected group (2.8 ± 0.4), but was similar to that of the *E. acervulina*-infected group (3.1 ± 0.4) (Fig. 3B). Fecal oocyst shedding in the *E. maxima*- and *E. tenella*-infected groups was significantly lower compared with the *E. acervulina*-infected group (Fig. 3C).

Next, to determine whether age can influence the three parameters, 3-week-old male chickens were infected as described above. Body weight gain measured on day 9 post infection was significantly lower in both the *E. acervulina*- and *E. maxima*-infected groups compared to the uninfected group. However, the body weight gain in the *E. tenella*-infected group was similar to weight gain in the uninfected group (Fig. 4A). The mean intestinal lesion score was significantly higher in the *E. tenella*-infected group (3.2 ± 1.1) compared with the *E. maxima*-infected group (2.2 ± 0.5), but was similar to that of the *E. acervulina*-infected group (3.6 ± 0.5) (Fig. 4B). Moreover, fecal oocyst shedding was significantly lower in the *E. tenella*-infected group compared with the *E. acervulina*-infected group, but was similar to that of the *E. maxima*-infected group (Fig. 4C). No lesion or fecal oocysts were observed in the uninfected chickens used as controls (data not shown). Taken together, these results suggest that lesion severity and/or age is important for body weight gain during an infection with *Eimeria* species.

Discussion

Invasion of chicken intestinal epithelial cells by *Eimeria* species causes one of the costliest diseases that affects the poultry industry worldwide. To reduce economic losses caused by the seven species of *Eimeria*, anticoccidial drugs have been used in 60% to 99% of chicken herds [1, 3]. Due to the emergence of drug-resistant parasites and increasing public concern regarding the presence of drug residue in chicken products, vaccines, probiotics and natural products have been considered as potential alternatives for coccidiosis control [9, 11, 17-18]. Although the effectiveness of these alternatives has been evaluated in commonly assessed parameters, such as body weight gain, intestinal lesion score, or fecal oocyst shedding [16, 18-20], few studies have included these parameters when evaluating *Eimeria*-infected birds [15, 21-22]. Therefore, the present study aimed to compare the aforementioned parameters

in broiler chickens infected with three major *Eimeria* species, including *E. acervulina*, *E. maxima* and *E. tenella*.

Due to the lack of information regarding the dependence of sex on avian coccidiosis, in our first experiment we carried out a sex-based evaluation of the parameters using normal and *E. tenella*-infected broiler chickens. These data demonstrated a fundamental difference between the sexes with respect to body weight gain in both normal and *E. tenella*-infected broilers. However, no significant difference between the sexes was observed in lesion scores or fecal oocyst production in *E. tenella*-infected broilers (Fig. 1). Similarly, after *E. acervulina* and *E. tenella* infections, the chickens showed significant sex differences only in initial and final weight gain, but not in lesion scores, mortality, or packed red cell volume [23]. Furthermore, significant differences in body weight gain were observed between male and female broiler chickens challenged with *E. maxima*. However, there were no significant sex effects on fecal oocyst shedding or plasma carotenoid concentration measured 6 and 9 days post infection [24]. Generally, plasma carotenoid values were inversely related to the *Eimeria* oocyst inoculation dosage. Decreases in total plasma carotenoid coincided with significant reductions in the lesion score of *E. tenella*-infected broilers [24-25]. In our sex-based evaluation of body weight gain, intestinal lesion score, and fecal oocyst shedding in broilers infected with 1×10^4 sporulated oocysts of *E. acervulina*, *E. maxima* or *E. tenella*, the patterns of these parameters in infected females were similar to those of the infected males. Taken together, these findings indicate that the sex of *Eimeria*-infected broilers should be taken into account for experiments that include body weight gain as a parameter.

Broilers infected with 1×10^4 and 7×10^4 oocysts of *E. maxima* exhibited mean lesion scores of 0.8 and 2.8, respectively, and their body weight gains were significantly decreased compared with the uninfected birds (Fig. 2, 3, and 4). Similarly, when two genetic lines of broiler chickens were infected with *E. acervulina*, *E. maxima* and *E. tenella*, only *E. maxima*-infected broilers showed decreased body weight gains as compared with control broilers [26]. Generally, higher inoculation doses of *E. maxima* resulted in a lower body weight gain compared to lower inoculation doses [24, 27]. In broiler chickens infected with *E. maxima*, broilers with a higher lesion score (2.39) lost more body weight than broilers with a lower lesion score (1.72) [28]. However, Chasser et al. reported that weight gain in Ross broilers infected with *E. maxima* on day 14 after hatching, with a macroscopic lesion score of 0.9 (assessed on day 19), was similar to that of uninfected birds [15]. Additionally, for broilers inoculated with three isolates of *E. maxima*, each having different pathogenicity, there was no relationship between microscores and body weight gain on day 6 post infection [21].

In our study, broilers infected with 1×10^4 sporulated oocysts of *E. acervulina* and *E. tenella* had mean lesion scores of 1.8 and 2.2, respectively, and showed a similar body weight gain compared to uninfected birds. Broilers infected with a higher dose of *E. acervulina* and *E. tenella* had mean lesion scores of 3.1 and 3.6, respectively, and showed significantly decreased body weight gain compared to uninfected birds. Similar to the present results, *E. tenella*-infected broilers with lesion scores less than 2 had similar body weight gain compared to the uninfected control group, whereas infected broilers with lesion score of 3.5 showed significantly reduced body weight gain [29]. It is interesting to note that, for most outbred lines of

chickens infected with *E. tenella*, decreases in body weight gain were greater in birds with lesion scores of 4 than those with lesion scores of 3, which indicated some correlation between lesion score and body weight gain [30]. Additionally, a correlation between body weight gain and lesion scores of approximately 3 was observed in 12 major histocompatibility complex congenic lines of chickens infected with *E. tenella* [31].

Conclusions

Although several experimental factors have been shown to influence weight gain, lesion severity, and oocyst shedding in *Eimeria*-infected chickens, knowing how these parameters are interrelated is important to better understand the effects of vaccines, probiotics, and natural products on coccidiosis. Our data indicate that greater lesion severity is more closely related to body weight gain. Further investigations regarding the relationship among parameters, including other *Eimeria* species, will greatly improve our understanding of the effects of coccidiosis-inhibiting substances.

Methods

Animal ethics statement

All animal maintenance and experimental procedures were performed according to Gyeongsang National University Guidelines for the Care and Use of Experimental Animals, and approved by the Institutional Animal Care and Use Committee (IACUC) of Gyeongsang National University (GNU-191111-C0058). Humane endpoint criteria were set for all animals, such that moribund animals exhibiting severe weight loss and tremors, or became unresponsive and unaware of stimuli, were euthanized immediately by atlanto-occipital dislocation. All remaining animals were euthanized at specific time points post inoculation.

Animals, parasites and infections

ROSS 308 broiler chicks (Samhwa, Korea) were raised in wire cages in a temperature-controlled environment, with unlimited access to anticoccidial/antibiotic-free feed and water. Constant light was provided for the duration of the experiments. Infected birds were housed in separate cages from non-infected birds. The wild-type strains of *E. acervulina*, *E. maxima*, and *E. tenella* were developed and maintained at the Gyeongsang National University (Jinju, Korea), and were cleaned by flotation on 5.25% sodium hypochlorite, and washed 3 times with phosphate buffered saline. Sporulated oocysts for experimental infections were enumerated using a McMaster counting chamber. Chickens were orally infected at 1-3 weeks of age with 1×10^4 or 1.5×10^5 sporulated oocysts of *E. acervulina*, 1×10^4 or 7×10^4 sporulated oocysts of *E. maxima*, or 1×10^4 or 5×10^4 sporulated oocysts of *E. tenella*.

Evaluation of body weight gain, lesion score and fecal oocyst production

Chicken body weights were measured between 6 and 9 days after infection. Chickens were randomly chosen for gut lesion scoring 7 days after *Eimeria* infection. Lesion scores were determined from intestinal tissues (duodenum for *E. acervulina*, jejunum for *E. maxima*, and cecum for *E. tenella*). Each chicken received a numerical lesion score from 0 (none) to 4 (severe) from a total of three independent, blinded observations, based on scoring techniques previously described elsewhere (Johnson and Reid, 1970). Fecal materials were collected from 6 to 9 days post infection, weighed, and the number of oocysts were counted using a McMaster counting chamber. Fecal oocyst number, expressed as oocysts per gram of feces, was calculated from the average of three counts per sample.

Statistical analysis

Data were analyzed with Student's *t*-test, or with one-way ANOVA and Dunnett's multiple comparison test, using InStat statistical software (GraphPad, USA). Differences were considered statistically significant at $P < 0.05$. Data were expressed as the mean \pm SE.

List Of Abbreviations

Not applicable.

Declarations

Ethics approval and consent to participate

All animal maintenance and experimental procedures were performed according to Gyeongsang National University Guidelines for the Care and Use of Experimental Animals, and approved by the Institutional Animal Care and Use Committee (IACUC) of Gyeongsang National University (GNU-191111-C0058).

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available with the corresponding author, and can be accessed on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

RAF, WHK, and WM designed the experiments. RAF, BTN, PLTC and AR performed the experiments. RAF, CPF-C, SK, and WM analyzed the data. RAF, WHK and WM contributed to the writing, review and editing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Figures

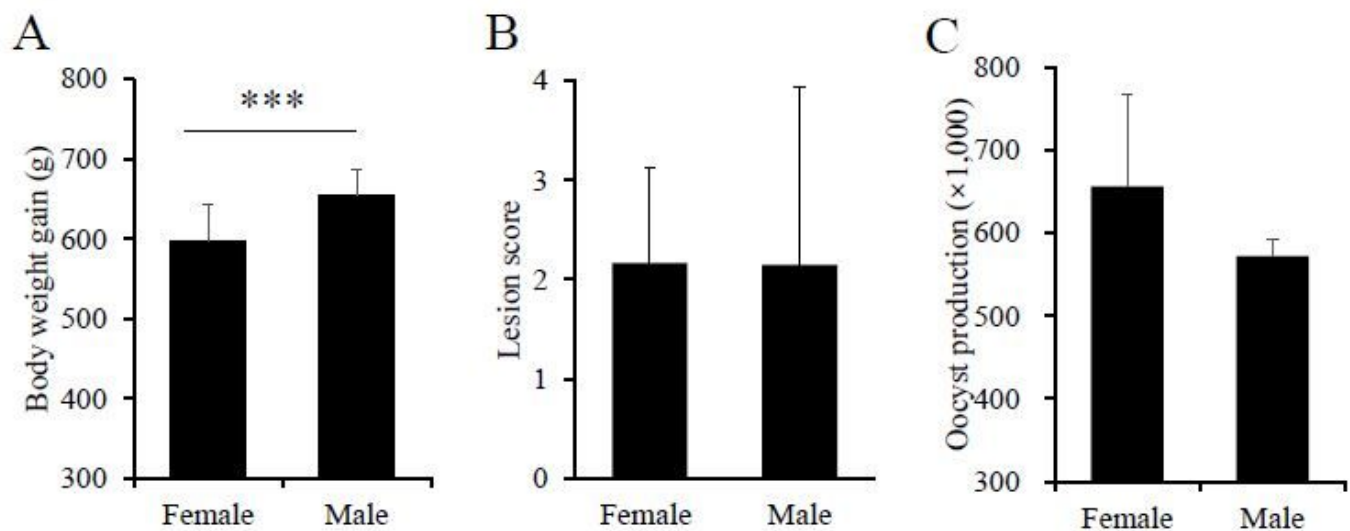


Figure 1

Comparison of clinical symptoms in male and female broilers following *E. tenella* infection. One-week-old ROSS 308 male and female chickens were orally infected with 1×10^4 sporulated *E. tenella* oocysts. (A) Body weights (n=20) were measured at day 9 post infection. (B) Five chickens were randomly selected for gut lesion scoring 7 days post infection. Lesions were scored (0-4) as previously described (Johnson and Reid, 1970). (C) Fecal oocyst production in chickens (n=30). The oocysts per gram feces were determined for fecal samples collected from day 6 to day 9 post infection. *** $P < 0.001$ indicates a significant difference between male and female chickens. Data represent the mean \pm SE for one of two independent experiments producing similar results.

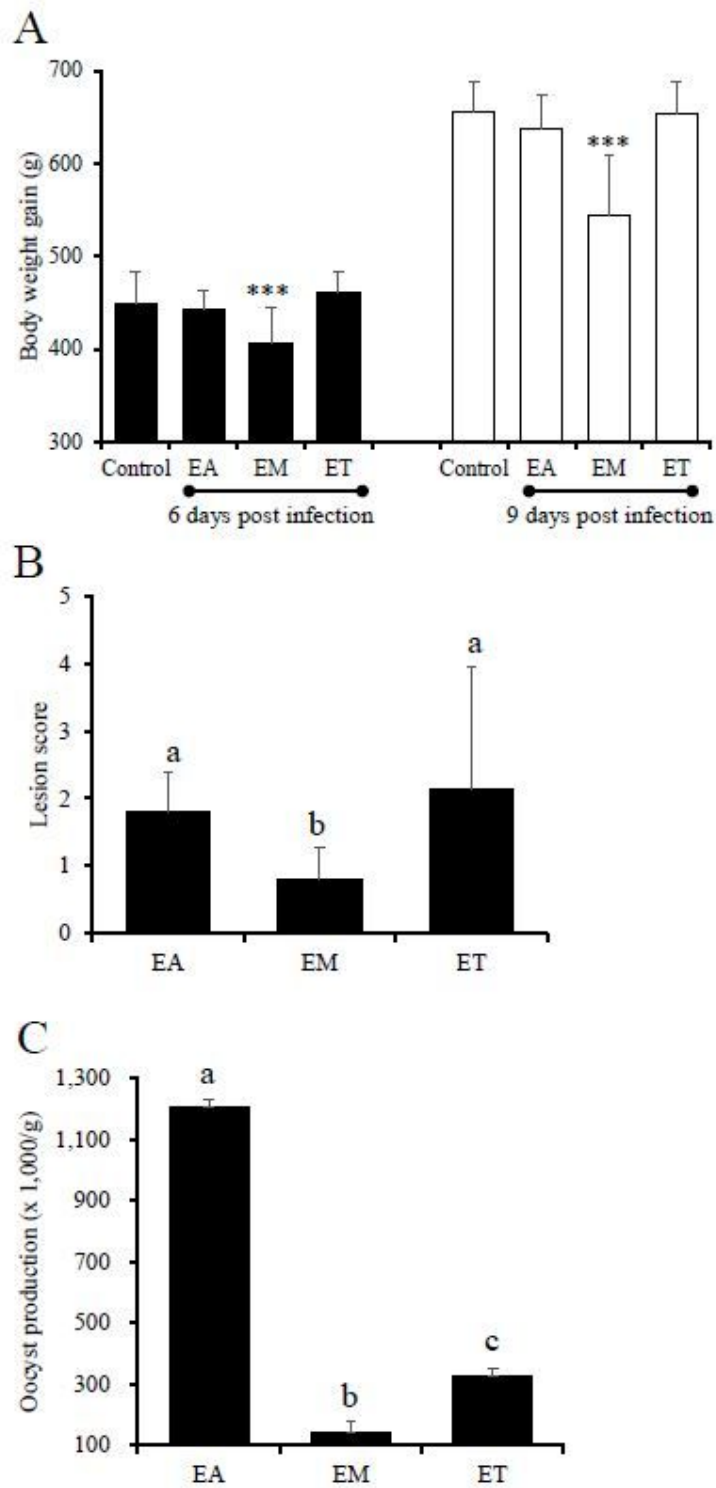


Figure 2

Comparison of clinical symptoms in male broilers infected with *E. acervulina*, *E. maxima* and *E. tenella*. One-week-old ROSS 308 male chickens were orally infected with 1×10^4 sporulated oocysts of *E. acervulina* (EA), *E. maxima* (EM), or *E. tenella* (ET). (A) Body weights ($n=20$) were measured at days 6 and 9 post infection. *** $P < 0.001$ indicates significant difference between the infected groups and the uninfected group (control). (B) Five chickens were randomly selected for gut lesion scoring 7 days post

infection. Bars not sharing the indicated letters are significantly different ($P<0.05$). (C) Fecal oocyst production in chickens ($n=20$). The oocysts per gram feces were obtained from fecal samples collected from day 6 to day 9 post infection. Bars not sharing the indicated letters are significantly different ($P<0.05$). Data represent the mean \pm SE for one of two independent experiments producing similar results.

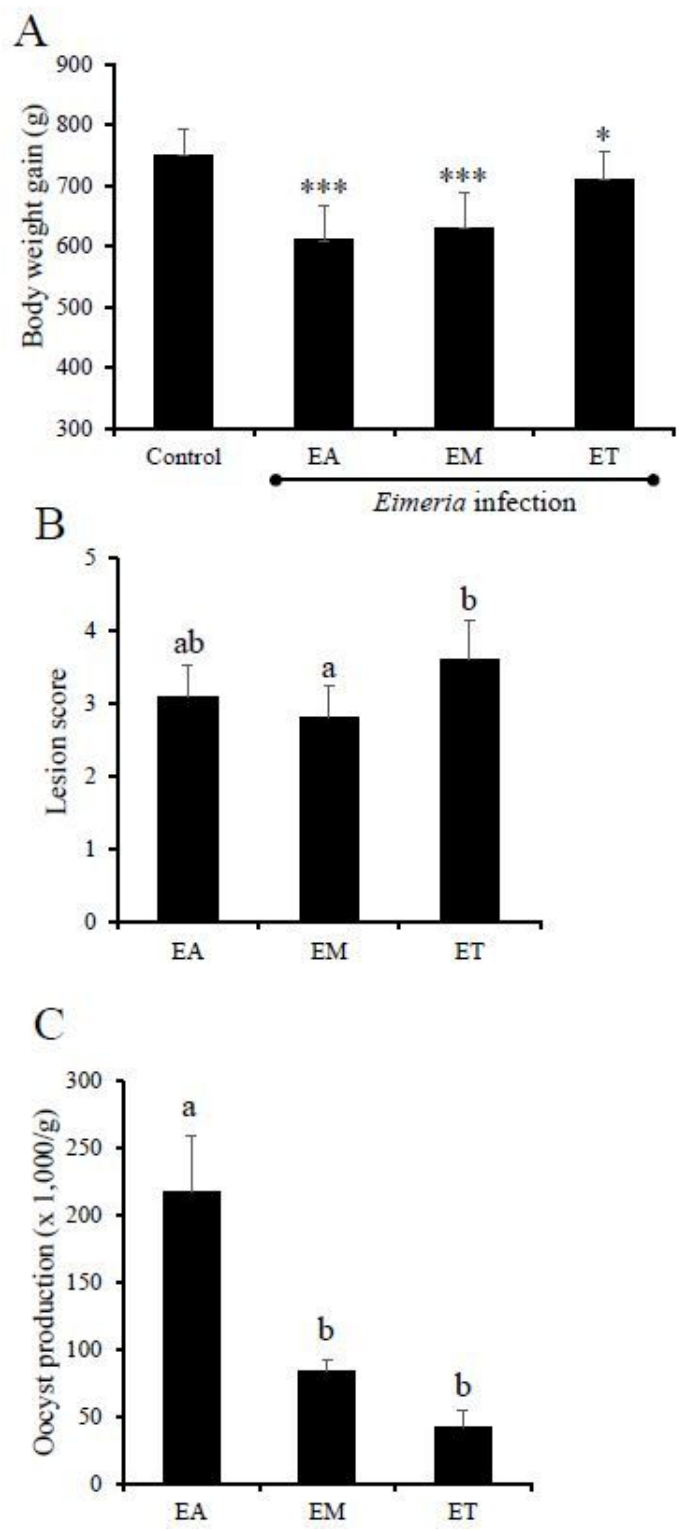


Figure 3

Comparison of clinical symptoms in male broilers infected with a high dose of *E. acervulina*, *E. maxima* and *E. tenella*. Two-week-old ROSS 308 male chickens were orally infected with 1.5×10^5 sporulated oocysts of *E. acervulina* (EA), 7×10^4 sporulated oocysts of *E. maxima* (EM), or 5×10^4 sporulated oocysts of *E. tenella* (ET). (A) Body weights ($n=20$) were measured at day 9 post infection. * $P<0.05$ and *** $P<0.001$ between the infected groups and the uninfected group (control). (B) Ten chickens were randomly selected for gut lesion scoring 7 days post infection. Bars not sharing the indicated letters are significantly different ($P<0.05$). (C) Fecal oocyst production in chickens ($n=15$). The oocysts per gram feces were determined for fecal samples collected from day 6 to day 9 post infection. Bars not sharing the indicated letters are significantly different ($P<0.05$). Data represent the mean \pm SE for one of two independent experiments producing similar results.

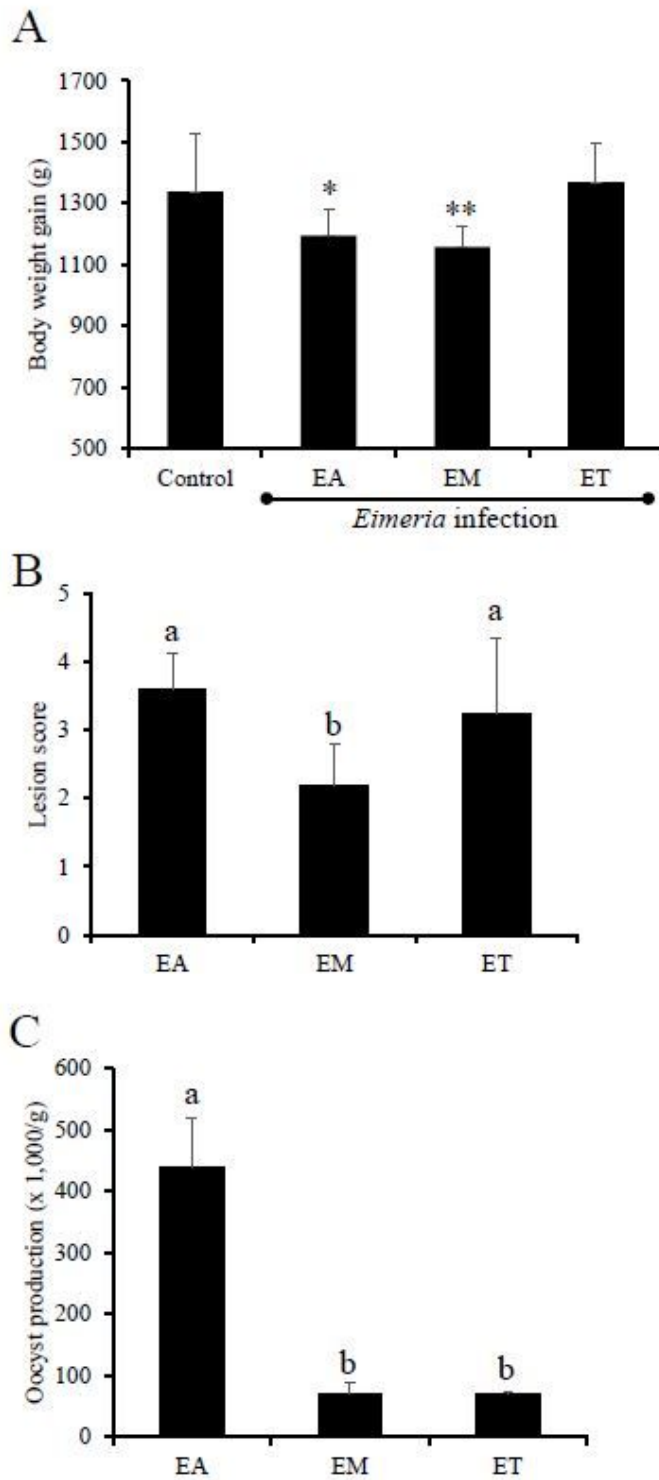


Figure 4

Comparison of clinical symptoms in male broilers infected with a high dose of *E. acervulina*, *E. maxima* and *E. tenella*. Three-week-old ROSS 308 male chickens were orally infected with 1.5×10^5 sporulated oocysts of *E. acervulina* (EA), 7×10^4 sporulated oocysts of *E. maxima* (EM), or 5×10^4 sporulated oocysts of *E. tenella* (ET). (A) Body weights ($n=20$) were measured at day 9 post infection. * $P < 0.05$ and ** $P < 0.01$ between the infected groups and the uninfected group (control). (B) Ten chickens were randomly selected

for gut lesion scoring 7 days post infection. Bars not sharing the indicated letters are significantly different ($P < 0.05$). (C) Fecal oocyst production in chickens ($n = 15$). The oocysts per gram feces were determined for fecal samples collected from day 6 to day 9 post infection. Bars not sharing the indicated letters are significantly different ($P < 0.05$). Data represent the mean \pm SE for one of two independent experiments producing similar results.

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