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Comparative analysis of evaluation parameters in *E. acervulina*, *E. maxima* and *E. tenella*-infected broilers

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ABSTRACT

Three parameters, body weight gain (BWG), intestinal lesion score (LS) and fecal oocyst shedding, were compared in broilers infected with major parasitic species; *Eimeria acervulina*, *E. maxima*, and *E. tenella*. First, two- and three-week-old chickens with Eimeria infection showed LS of approximately 3, but two-week-old chickens were more correlated with BWG. Second, significant differences in BWG were observed between male and female broilers challenged with *Eimeria*. Finally, *E. maxima*-infected broilers among three *Eimeria* species showed a higher relationship between BWG and LS, suggesting three considerations such as genders, age and *Eimeria* species for *Eimeria* experiments.

Keywords: Broilers; chickens; *Eimeria* spp; evaluation of parameters

INTRODUCTION

Avian coccidiosis, one of the most economically important diseases in chickens, is an intracellular parasitic disease caused by seven species of the apicomplexan protozoa *Eimeria* [1]. 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 [2].

To reduce avian coccidiosis caused by *Eimeria* infection, vaccines, anticoccidial drugs and natural products have been used worldwide [1]. 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 [3].

Anticoccidial effect of these strategies was commonly assessed by three parameters; body weight gain (BWG), lesion score (LS) or fecal oocyst shedding [4,5], However, there is few

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Conflict of Interest

The authors declare no conflicts of interest.

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studies on comparative analysis of these evaluation parameters in *Eimeria*-infected birds [6,7]. Therefore, this study aimed to compare these parameters in broilers infected with *E. acervulina*, *E. maxima* and *E. tenella*.

MATERIALS AND METHODS

Ethics statement

All animal maintenance and experimental procedures were approved by the Institutional Animal Care and Use Committee (GNU-191111-C0058). Humane endpoint criteria were set for all animals. All remaining animals were euthanized at specific time points post inoculation.

Animals and infections

ROSS 308 broilers (Samhwa, Korea) were raised in wire cages in a temperature-controlled environment, with unlimited access to anticoccidial/antibiotic-free feed and water. The wild-type strains of *Eimeria* species were developed and maintained in 2.5% potassium dichromate (Daejung Chemicals and Metals Co. Ltd, Korea) until use at Gyeongsang National University (Korea). Prior to infection, sporulated oocyst were cleaned by flotation using 5.25% sodium hypochlorite, washed 3 times with PBS, and pellet containing the oocyst were resuspend to PBS. Chickens were orally infected using a gavage 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.* Similarly, control birds were orally given with the same amount of PBS. Low infection dose was adapted from previous reports [7] while the high doses that can cause a LS of approximately 3 have been performed in the laboratory.

Evaluation of BWG, LS and fecal oocyst shedding

BWG was measured between 6 and 9 days after infection. LS was determined from duodenum for *E. acervulina*, jejunum for *E. maxima*, and cecum for *E. tenella* on day 7 after *Eimeria* infection. Each chicken received a numerical LS from 0 (none) to 4 (severe) based on scoring techniques [8]. Feces was collected from 6 to 9 days post infection. Oocyst numbers were calculated from the average of three counts per sample using a McMaster counting chamber.

Histopathological analysis

Chickens were orally infected using a gavage at 3 weeks of age with 1.5×10^5 sporulated oocysts of *E. acervulina*, 7×10^4 sporulated oocysts of *E. maxima,* or 5×10^4 sporulated oocysts of *E. tenella.* Similarly, control birds were orally given with PBS. Duodenum for *E. acervulina*, jejunum for *E. maxima*, and ceca for *E. tenella* on day 7 after *Eimeria* infection were rapidly removed, fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin wax for sectioning. Sections of intestinal tissues were cut to 3-μm thickness. The sections were stained with hematoxylin and eosin. Five microscopic fields (400×) were randomly selected to measure villi length and crypt depth.

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 ± SE.

RESULTS

BWG was compared between normal, uninfected female and male broilers. BWG differed significantly between normal female and male chickens ($p < 0.01$) at all evaluated time points (**Supplementary Table 1**). In female and male broilers infected with 1×10^4 oocysts of *E. tenella*, the infected females (596.3 ± 47.2) showed significantly lower BWG compared with males (654.4 ± 33.2) on day 9 post infection. No significant difference between sexes was observed in LS or oocyst outputs. The mean LS of infected females (2.2 ± 0.9) was similar to that of infected males (2.2 ± 1.7). Oocyst output by the infected females ($656.4 \times 10^3 \pm 111.9 \times 10^3$ oocysts/bird) was similar to that of infected males ($572.2 \times 10^3 \pm 21.1 \times 10^3$ oocysts/bird) (**Supplementary Fig. 1**). No lesion or fecal oocysts were observed in uninfected chickens used as controls (data not shown).

In evaluating parameters in male broilers infected with low dose (1×10^4 oocysts) of *Eimeria* species, the initial BWG before infection showed no significant differences among groups ($p > 0.05$) (data not shown). BWG measured on days 6 and 9 post infection were significantly lower in *E. maxima*-infected group, but not *E. acervulina* or *E. tenella*-infected groups, compared to uninfected controls (**Fig. 1A**). LS was significantly higher for *E. tenella*-infected group (2.2 ± 1.8) compared with *E. maxima*-infected group (0.8 ± 0.4), but was similar to that of *E. acervulina*-infected group (1.8 ± 0.5) (**Fig. 1B**). Oocyst shedding was significantly lower in *E. tenella*-infected group than *E. acervulina*-infected group, but was higher than in *E. maxima*-infected group (**Fig. 1C**). No lesion or fecal oocysts were observed in uninfected control chickens (data not shown). To determine whether sex-based differences existed in infected broilers, female chickens were also infected with 1×10^4 oocysts of *E. acervulina*, *E. maxima,* or *E. tenella.* The patterns of BWG, LS, and oocyst shedding in *Eimeria*-infected female chickens were similar to those observed in male chickens (**Supplementary Fig. 2**). These observations showed that *E. maxima* induced more relationship between LS and BWG compared to *E. acervulina* and *E. tenella*.

To monitor whether increased LS correlated with BWG, 2-week-old male chickens were orally infected with high doses of *Eimeria* species such as 1.5×10^5 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. BWG measured on day 9 post infection were significantly lower in all infected groups compared to uninfected group (**Fig. 2A**). Initial BWG showed no significant differences among the groups, including uninfected chickens ($p > 0.05$) (data not shown). The mean LS of *E. tenella*-infected group (3.6 ± 0.5) was significantly higher than that of *E. maxima*-infected group (2.8 ± 0.4), but was similar to that of *E. acervulina*-infected group (3.1 ± 0.4) (**Fig. 2B**). Oocyst sheddings in *E. maxima*- and *E. tenella*-infected groups were significantly lower compared with *E. acervulina*-infected group (**Fig. 2C**).

Furthermore, to determine whether age can influence three parameters, 3-week-old male chickens were infected as described above. BWG measured on day 9 post infection was significantly lower in both *E. acervulina*- and *E. maxima*-infected groups compared to uninfected group. However, BWG of *E. tenella*-infected group was similar to BWG of uninfected group (**Fig. 2D**). The mean LS was significantly higher in *E. tenella*-infected group (3.2 ± 1.1) compared with *E. maxima*-infected group (2.2 ± 0.5), but was similar to that of *E. acervulina*-infected group (3.6 ± 0.5) (**Fig. 2E**). Moreover, oocyst shedding was significantly lower in *E. tenella*-infected group compared with *E. acervulina*-infected group, but was similar to that of *E. maxima*-infected group (**Fig. 2F**). No lesion or fecal oocysts were observed in uninfected chickens used as controls (data not shown).

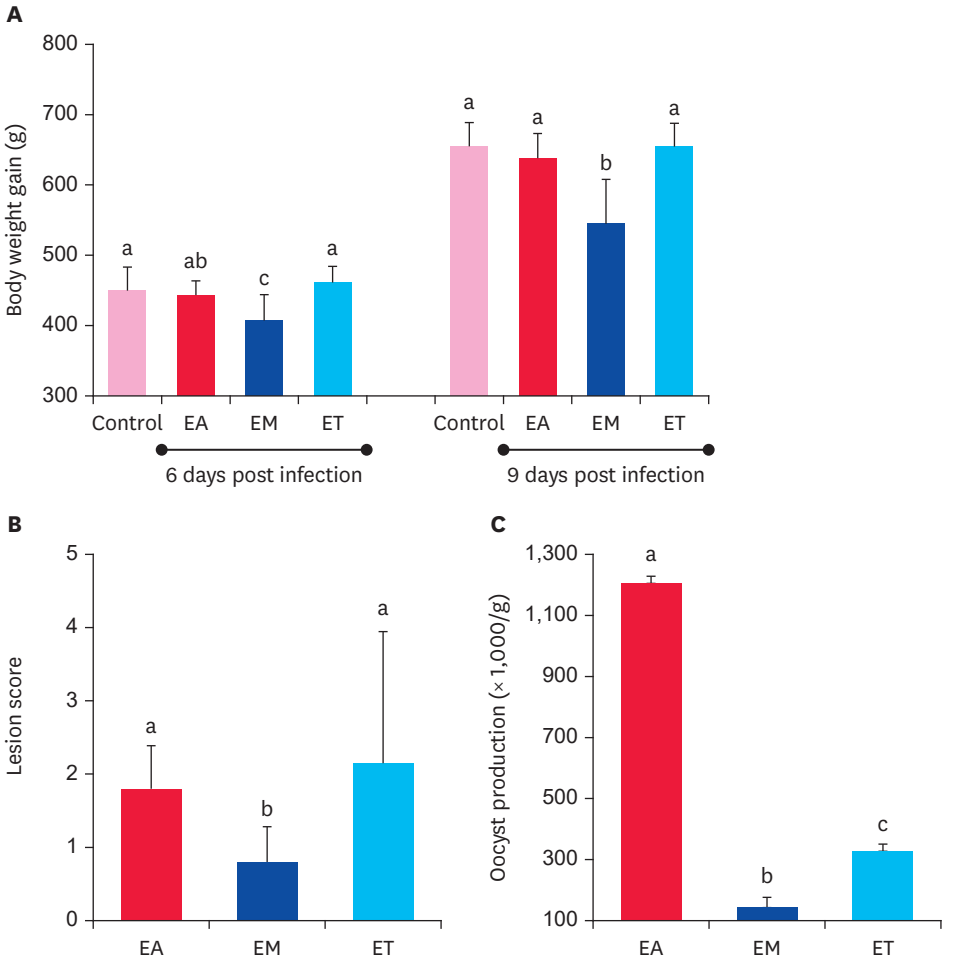

Fig. 1. Comparison of clinical symptoms in male broilers infected with low dose of *Eimeria* species. One-week-old ROSS 308 male chickens were orally infected with 1×10^4 sporulated oocysts of EA, EM, or ET. (A) Body weights (n = 20) were measured at days 6 and 9 post infection. (B) Five chickens were randomly selected for gut lesion scoring 7 days post infection. (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 ± SE for one of two independent experiments producing similar results.
EA, *Eimeria acervulina*; EM, *E. maxima*; ET, *E. tenella*.

Compared with uninfected and normal controls of 3-week-old male broilers, histopathological observation revealed that villi length was reduced approximately 11.5% (2,178 ± 661 vs. 1,950 ± 194) in the duodenum infected with *E. acervulina* and 36.3% (1,392 ± 284 vs. 888 ± 277) in the jejunum infected with *E. maxima*, respectively. Ceca, the site of *E. tenella* infection, has no villi. Therefore, the length of the villi for *E. tenella* was not measured (**Fig. 3** and **Table 1**). Crypt depth was increased approximately 188% (211 ± 96 vs. 397 ± 86) in the duodenum infected with *E. acervulina*, 182% (210 ± 55 vs. 382 ± 32) in the jejunum infected with *E. maxima*, and 141% (353 ± 51 vs. 499 ± 151) in the ceca infected with *E. tenella*, respectively (**Fig. 3** and **Table 1**).

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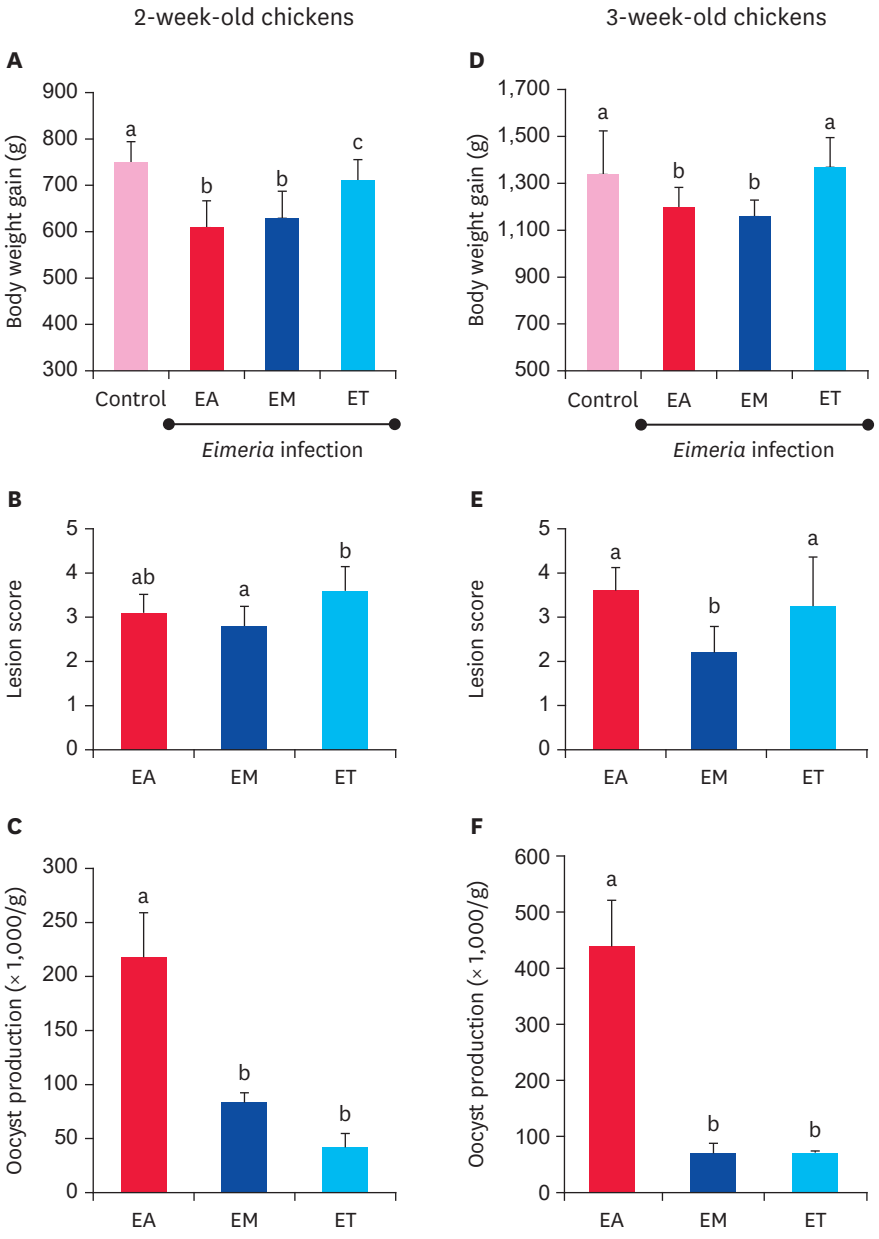

Fig. 2. Comparison of clinical symptoms in 2- and 3-week-old broilers infected with a high dose of *Eimeria* species. Two-week-old and three-week-old ROSS 308 male chickens were orally infected with 1.5×10^5 sporulated oocysts of EA, 7×10^4 sporulated oocysts of EM, or 5×10^4 sporulated oocysts of ET. (A) Body weights (n = 20) were measured at day 9 post infection. (B) Ten chickens were randomly selected for gut lesion scoring 7 days post infection. (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 ± SE for one of two independent experiments producing similar results.
EA, *Eimeria acervulina*; EM, *E. maxima*; ET, *E. tenella*.

Table 1. Villi length and crypt depth in *Eimeria* infected broilers

Eimeria species	Villi length (um)		Crypt depth (um)	
	Control	Infected	Control	Infected
E. acervulina	2,178 ± 661	1,950 ± 194	211 ± 96	397 ± 86
E. maxima	1,392 ± 284	888 ± 277	210 ± 55	382 ± 32
E. tenella	ND	ND	353 ± 51	499 ± 151

Data represent the mean ± standard deviation (n = 5).
ND, not determined.

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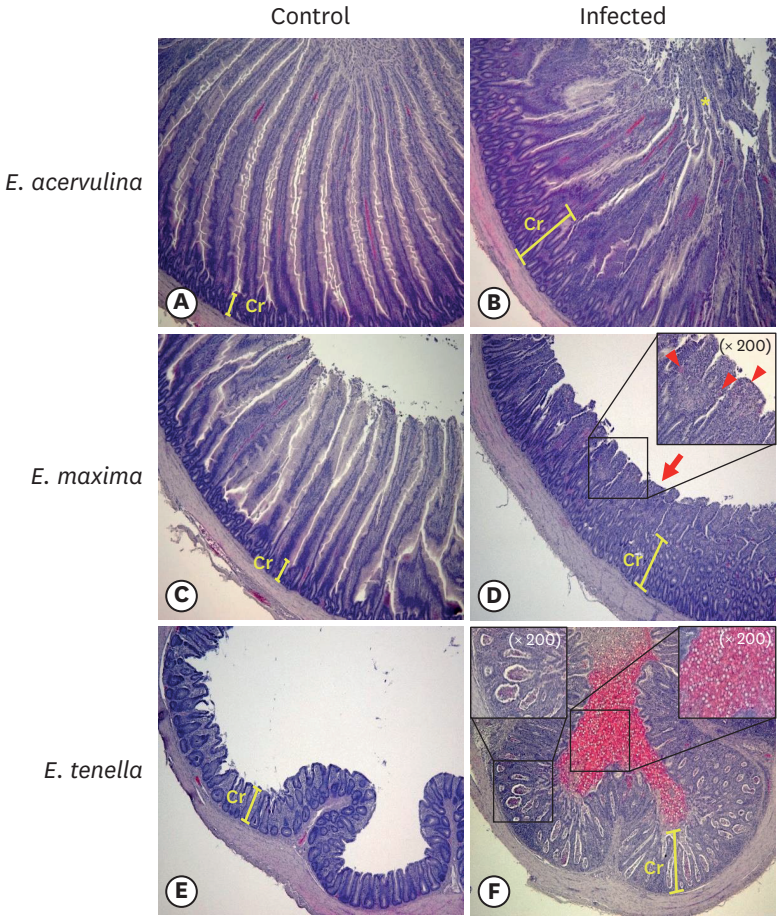

Fig. 3. Villi length and crypt depth in *Eimeria* infected broilers. Chickens were orally infected at 3 weeks of age with 1.5×10^5 sporulated oocysts of EA (B), 7×10^4 sporulated oocysts of EM (D), or 5×10^4 sporulated oocysts of ET (F). Similarly, control birds were orally given with the same amount of PBS (A, C, E). Duodenum for EA (A, B), jejunum for EM (C, D), and ceca for ET (E, F) on day 7 after *Eimeria* infection were rapidly removed and fixed in 10% neutral buffered formalin. The sections were stained with hematoxylin and eosin. Five microscopic fields (400×) were randomly selected to measure villi length and crypt depth. Yellow star (B) and arrow (D) indicated that the tip of the villi is falling off. Arrowheads in the inset (D) and the inset (F) indicated oocysts of EM and ET, respectively.
EA, *Eimeria acervulina*; EM, *E. maxima*; ET, *E. tenella*; Cr, crypt.

DISCUSSION

Eimeria spp. infection causes one of the most expensive diseases in the poultry industry worldwide. In practice, anticoccidial drugs have been used in 60% to 99% of affected flocks to reduce the economic losses related to the infection [1]. However, the emergence of drug-resistant strains and the increasing demand of antibiotic free poultry products direct new approaches such as vaccines, probiotics, and naturally derived products to control the disease. Traditionally, efficiency evaluation of the potential anticoccidial activity of these alternatives is assessed using BWG, LS and oocyst shedding. In this study, we compared these parameters in broiler chickens infected with three major *Eimeria* species- *E. acervulina*, *E. maxima*, and *E. tenella*.

Due to the lack of information regarding the dependence of genders on avian coccidiosis, a sex-based evaluation of parameters was performed using normal and *E. tenella*-infected broilers. These data demonstrated a fundamental difference between the sexes with respect

to BWG in both normal and *E. tenella*-infected broilers. However, no significant difference between the sexes was observed in LS or oocyst production in *E. tenella*-infected broilers. In sex-based evaluation of BWG, LS, and oocyst shedding in broilers infected with 1×10^4 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. Similarly, after *E. acervulina* and *E. tenella* infections, the chickens showed significant sex differences only in initial and final BWG, but not in LS, mortality, or packed red cell volume [9]. Furthermore, significant differences in BWG were observed between *E. maxima*-infected male and female broilers. However, there were no significant sex effects on oocyst shedding measured on days 6 and 9 post infection [10]. Taken together, these findings indicate that the sex of *Eimeria*-infected broilers should be considered in experiments including BWG as a parameter.

In all experiments, BWG of broilers infected with 1×10^4 and 7×10^4 oocysts of *E. maxima* were significantly reduced compared with uninfected control birds. Similarly, when two genetic lines of broilers were infected with *E. acervulina*, *E. maxima* and *E. tenella,* only *E. maxima*-infected broilers showed decreased BWG as compared with control broilers [11]. Generally, higher inoculation doses of *E. maxima* resulted in a lower BWG compared to lower inoculation doses [10]. In broilers infected with *E. maxima*, broilers with a higher LS (2.39) lost more BWG than broilers with a lower LS (1.72) [12].

On the other hand, broilers infected with low dose (1×10^4 oocysts) of *E. acervulina* and *E. tenella* showed a similar BWG compared to uninfected birds but a significantly reduced BWG with high dose infection. Consequently, mean LSs were 1.8 (low dose) and 3.1 (high dose) in *E. acervulina*-infected broilers and 2.2 (low dose) and 3.6 (high dose) in *E. tenella*-infected broilers. Similar to the present results, *E. tenella*-infected broilers with LS less than 2 had similar BWG compared to the uninfected control group, whereas infected broilers with LS of 3.5 showed significantly reduced BWG [13]. It is interesting to note that while the susceptibility of commercial broiler parent lines to *E. tenella* infection varies, an increased LS on infected birds was accompanied with reduced BWG on these birds, and vice versa [14]. Additionally, a correlation between BWG and LS of approximately 3 was observed in 12 major histocompatibility complex congenic lines of chickens infected with *E. tenella* [15].

Depending on the condition of the small intestine and large intestine, each *Eimeria* species exhibits different characteristic lesions [8]. Although it is difficult to directly compare LS and number of shedding oocysts, histopathological observation of intestinal tissues infected with *E. acervulina, E. maxima* or *E. tenella* was made. Villi length was decreased more in *E. maxima*-infected chickens than in *E. acervulina*-infected chickens, and crypt depth increased more in both *E. acervulina*-infected and *E. maxima*-infected chickens than in *E. tenella*-infected chickens. In conclusion, these results indicated that considerations such as genders, *Eimeria* species and birds' age are necessary in *Eimeria* experiments for evaluating anticoccidial effects of products.

SUPPLEMENTARY MATERIALS

Supplementary Table 1

Body weight of normal ROSS 308 broiler chickens

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Supplementary Fig. 1

Comparison of clinical symptoms in male and female broilers following *Eimeria 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 from 0–4. (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. Within each graph, bars not sharing the indicated letters are significantly different ($p < 0.05$) between male and female chickens. Data represent the mean ± SE for one of two independent experiments producing similar results.

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Supplementary Fig. 2

Comparison of clinical symptoms in female broilers infected with EA, EM and ET. One-week-old ROSS 308 female chickens were orally infected with 1×10^4 sporulated oocysts of EA, EM and ET. (A) Body weights (n = 20) were measured at days 6 and 9 post infection. Within each graph, bars not sharing the indicated letters are significantly different ($p < 0.01$). (B) Five chickens were randomly selected for gut lesion scoring 7 days post infection. Lesion scores were from 0–4. Within each graph, 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. Within each graph, bars not sharing the indicated letters are significantly different ($p < 0.05$). Data represent the mean ± SE for one of two independent experiments producing similar results.

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REFERENCES

1. Min W, Kim WH, Lillehoj EP, Lillehoj HS. Recent progress in host immunity to avian coccidiosis: IL-17 family cytokines as sentinels of the intestinal mucosa. Dev Comp Immunol. 2013;41(3):418-428.
PUBMED | CROSSREF
2. Venkatas J, Adeleke MA. A review of *Eimeria* antigen identification for the development of novel anticoccidial vaccines. Parasitol Res. 2019;118(6):1701-1710.
PUBMED | CROSSREF
3. Zhu JJ, Lillehoj HS, Allen PC, Yun CH, Pollock D, Sadjadi M, et al. Analysis of disease resistance-associated parameters in broiler chickens challenged with *Eimeria maxima*. Poult Sci. 2000;79(5):619-625.
PUBMED | CROSSREF
4. Pop LM, Varga E, Coroian M, Nedișan ME, Mircean V, Dumitrache MO, et al. Efficacy of a commercial herbal formula in chicken experimental coccidiosis. Parasit Vectors. 2019;12(1):343.
PUBMED | CROSSREF
5. Ritzi MM, Abdelrahman W, Mohnl M, Dalloul RA. Effects of probiotics and application methods on performance and response of broiler chickens to an *Eimeria* challenge. Poult Sci. 2014;93(11):2772-2778.
PUBMED | CROSSREF
6. Chasser KM, Duff AF, Wilson KM, Briggs WN, Latorre JD, Barta JR, et al. Research Note: Evaluating fecal shedding of oocysts in relation to body weight gain and lesion scores during Eimeria infection. Poult Sci. 2020;99(2):886-892.
PUBMED | CROSSREF
7. You MJ. The comparative analysis of infection pattern and oocyst output in *Eimeria tenella, E. maxima* and *E. acervulina* in young broiler chicken. Vet World. 2014;7(7):542-547.
CROSSREF

8. Johnson J, Reid WM. Anticoccidial drugs: lesion scoring techniques in battery and floor-pen experiments with chickens. Exp Parasitol. 1970;28(1):30-36.
PUBMED | CROSSREF
9. Mathis GF, Washburn KW, McDougald LR. Genetic variability of resistance to *Eimeria acervulina* and *E. tenella* in chickens. Theor Appl Genet. 1984;68(5):385-389.
PUBMED | CROSSREF
10. Zhu JJ, Lillehoj HS, Allen PC, Yun CH, Pollock D, Sadjadi M, et al. Analysis of disease resistance-associated parameters in broiler chickens challenged with *Eimeria maxima*. Poult Sci. 2000;79(5):619-625.
PUBMED | CROSSREF
11. Gilbert ER, Cox CM, Williams PM, McElroy AP, Dalloul RA, Ray WK, et al. *Eimeria* species and genetic background influence the serum protein profile of broilers with coccidiosis. PLoS One. 2011;6(1):e14636.
PUBMED | CROSSREF
12. Hamzic E, Bed'Hom B, Juin H, Hawken R, Abrahamsen MS, Elsen JM, et al. Large-scale investigation of the parameters in response to *Eimeria maxima* challenge in broilers. J Anim Sci. 2015;93(4):1830-1840.
PUBMED | CROSSREF
13. Zhao P, Li Y, Zhou Y, Zhao J, Fang R. *In vivo* immunoprotective comparison between recombinant protein and DNA vaccine of *Eimeria tenella* surface antigen 4. Vet Parasitol. 2020;278:109032.
PUBMED | CROSSREF
14. Swaggerty CL, Genovese KJ, He H, Duke SE, Pevzner IY, Kogut MH. Broiler breeders with an efficient innate immune response are more resistant to *Eimeria tenella*. Poult Sci. 2011;90(5):1014-1019.
PUBMED | CROSSREF
15. Caron LA, Abplanalp H, Taylor RL Jr. Resistance, susceptibility, and immunity to *Eimeria tenella* in major histocompatibility (B) complex congenic lines. Poult Sci. 1997;76(5):677-682.
PUBMED | CROSSREF