Broiler breeders with an efficient innate immune response are more resistant to *Eimeria tenella*

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ABSTRACT In previous studies we characterized the innate immune response of 2 parental broiler lines (A and B) and compared their resistance against *Salmonella*, *Enterococcus*, and *Campylobacter* challenges. In all cases, line A was more responsive and more resistant than line B. In the present study, we sought to determine whether this trend was also observed following challenge with the protozoan parasite *Eimeria tenella*. In 3 separate experiments, 14-d-old chickens from lines A and B were challenged orally with 15 to 50 $\times 10^3$ *E. tenella* oocysts. Birds were killed 6 d postchallenge and the ceca was removed and scored for lesions and weight gain compared with noninfected controls. Line A birds were more resistant to intestinal pathology as demonstrated by lower lesion scores compared with line B birds. As might be expected, the lower lesion scores in line A chickens were often accompanied by higher weight gain compared with line B chickens, thus reducing potential revenue loss associated with low carcass weights often observed with coccidia-infected birds. The results from this study showed that in addition to having enhanced resistance against bacterial infections, line A chickens were also more resistant to coccidial infections compared with line B birds. Taken together with all of our earlier studies using these lines of birds, an efficient innate immune response protects against a broad range of foodborne and poultry pathogens, including costly coccidial infections.

Key words: chicken, coccidiosis, *Eimeria tenella*, innate immunity

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INTRODUCTION

Coccidiosis is caused by protozoan parasites that are members of the genus *Eimeria*. Coccidiosis is one of the most widely reported and economically important diseases affecting commercial poultry production, with an estimated cost of more than $800 million annually in the United States alone and upward of $3 billion worldwide (De Gussem, 2007; Sharman et al., 2010). At least 70% of that cost is a result of subclinical coccidiosis characterized by substandard flock performance, including decreased weight gain and increased feed conversion. Seven different species of *Eimeria* are known to infect chickens: *Eimeria tenella*, *Eimeria acervulina*, *Eimeria maxima*, *Eimeria brunetti*, *Eimeria necatrix*, *Eimeria praecox*, and *Eimeria mitis*. Each species is pathogenic to chickens in varying degrees; however, *E. acervulina*, *E. maxima*, and *E. tenella* are the most pathogenic and prevalent within the broiler industry (McDougald, 1998). Each of these species targets a distinct region of the gut: *E. acervulina* pathology is characterized by white lesions in the upper small intestine, *E. maxima* results in petechiae in the midgut, and *E. tenella* targets the cecal pouches and results in bloody feces (Hammond and Long, 1973).

Current prevention and control measures are limited to the addition of anticoccidials, often referred to as coccidiostats, to the feed and vaccinations (De Gussem, 2007; Sharman et al., 2010). Over the past 60 to 70 yr, virtually every poultry producer has extensively used these drugs to control coccidiosis (Akira et al., 2006; Chapman, 2009). However, under such heavy use pressures the drugs are losing efficacy. Additionally, public outcry exists for removal of all drugs from poultry feed, further pressuring the poultry industry to find suitable alternative control and preventative measures (McDougald, 1998; Akira et al., 2006). One of the contributing factors that make coccidiosis so costly and difficult to control is that little to no cross-protection exists between the different *Eimeria* spp. and, further, protection may not even exist following infection with a different strain of the same species (Martin et al., 1997; Chapman, 2008); therefore, employing an effective and efficient vaccination protocol is proving to be difficult. Consequently, identifying a line of chickens

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with increased natural resistance to coccidiosis would be extremely valuable to the poultry industry in combating this very costly disease.

For the past several years, we have been profiling 2 parental broiler lines (A and B) with regard to their heterophil-mediated innate immune function and cytokine and chemokine responses and comparing their resistance or susceptibility against *Salmonella enteritidis* (Ferro et al., 2004; Swaggerty et al., 2005a), vancomycin-resistant *Enterococcus gallinarum* (Swaggerty et al., 2005b), and *Campylobacter jejuni* (Li et al., 2008) challenges. In all cases, line A chickens and their heterophils were more responsive at the cellular level and the birds were more resistant to bacterial challenges than line B chickens. The objective of the current study was to determine whether the same trend of enhanced resistance in line A chickens was observed following challenge with 2 field isolates of *E. tenella*.

MATERIALS AND METHODS

Experimental Chickens

Parental broiler chickens used in this study were obtained from a commercial primary breeder. To maintain confidentiality, lines were designated A and B. Fertilized eggs were incubated and hatched under standard conditions (Stromberg, 1975). On day of hatch, straight-run chicks from each line were placed in separate floor pens (4 m × 4 m) within the same room. Each pen contained wood shavings, supplemental heat, water, and a balanced, unmedicated corn and soybean meal based chick starter diet ad libitum. The feed was calculated to contain 23% protein and 3,200 kcal of ME/kg of diet, and all other nutrient levels met or exceeded the requirements established by NRC (1994). Experiments were conducted according to guidelines established by the USDA animal care committee.

Parasite Preparation and Experimental Design

Eimeria tenella was propagated and prepared as described previously (Hammond and Long, 1973). Briefly, *E. tenella* oocysts were collected from the ceca of 21-d-old broiler chickens 7 d postinfection, sporulated, sterilized by 5% sodium hypochlorite, washed 3 times with PBS, and enumerated using a hemocytometer before experimental infections. Oocysts were kindly provided by Mark Jenkins, USDA-ARS, Beltsville, Maryland (Beltsville strain) and Lorraine Fuller, Department of Poultry Science, University of Georgia, Athens (Penn State strain). At 13 d of age, birds were leg banded, weighed, and randomly assigned to control or challenge groups. The following day, chicks from lines A and B were challenged orally with 15 to 50 $\times 10^3$ oocysts and killed by cervical dislocation 6 d postchallenge. The Beltsville strain of *E. tenella* was used in experiments 1 and 2 and birds (n = 20 and 25/dose, respectively) were administered 0, 15, 30, or 45 $\times 10^3$ oocysts. The Penn State strain was used in experiment 3 and birds were given 0, 25, or 50 $\times 10^3$ oocysts (n = 25/dose). On the last day of the experiment, the birds were weighed and the ceca were removed and scored for lesions by a standard method (Johnson and Reid, 1970). The experimental challenges were conducted a total of 3 times with chicks from separate hatches (2 biological replicates with the Beltsville strain and 1 replicate with the Penn State strain). All birds administered the same dose were housed in the same room, but the lines were maintained in different pens. The birds were not vaccinated at any time during the experiment nor did they receive any medications.

Lesion Scoring

Lesions were blindly scored by a single person to eliminate bias. Lesions were scored on a scale of 0 to 4. A score of 0 indicated normal healthy tissue; a score of 1 was characterized by a few scattered petechiae, no thickening of the cecal wall, and normal brownish colored content; a score of 2 was characterized by increased numbers of petechiae, some bleeding at the mucosal surface, relatively normal cecal content, and slight thickening of the cecal wall; a score of 3 was defined by increased bleeding with clotting at the distal end of the pouch that became hardened as the sloughed mucosal surface united with bloody cecal content, and marked thickening of the cecal wall; and a score of 4 was accompanied by severe bleeding, severe thickening of the wall, and eroding of the mucosal surface featuring a hard core of material inside the cecal pouch (Johnson and Reid, 1970).

Statistical Analyses

Three separate challenges were conducted using chickens from a different flock for each trial, and 20 to 25 birds/dose were used for each experiment. Each experiment was analyzed separately to support the consistency of the results. All analyses were performed with SAS 9.2 software (SAS Institute, Cary, NC).

Contingency table analyses were performed for each line of birds within each experiment. The numbers of birds are shown in Tables 1 through 3. Because both the response variable (lesion score) and the treatments (dose) were ordinal in nature we used the Mantel-Haenszel ψ^2 to test for patterns of association. When a level of response was all 0 (i.e., experiment 1 line A had no lesion scores of 0 in the treatment groups), this level was removed to attain statistical results. Average treatment lesion scores were compared by simple ANOVA as a supplemental interpretation to support the contingency table analysis. Weight gain in each experiment was analyzed as a 2-way ANOVA. Means were compared using a Tukey-Kramer adjustment for type-I error when making multiple comparisons. Regression and correlation analyses were used to explore association

patterns between individual birds' weight gain and the severity of the lesions in the ceca.

RESULTS

Experiment 1

Chickens from line A were more resistant to *E. tenella* infection compared with line B chickens as indicated by reduced cecal lesion scores. The average lesion scores following administration of 30 and 45 $\times 10^3$ oocysts of the Beltsville strain were lower ($P \leq 0.05$) for line A than line B ($30 \times 10^3 = 1.43$ and 3.05, respectively; $45 \times 10^3 = 1.86$ and 2.9, respectively; Table 1). The lesion scores for the 15×10^3 dose were not significant but were numerically lower for line A (1.74) than line B (2.15). All line A birds showed some degree of infection response, but no significant association existed between the dose administered and the severity of the lesions (Mantel-Haenszel ψ^2 $P = 0.54$); a strong dose response association was found for line B (Mantel-Haenszel ψ^2 $P = 0.01$). The average lesion score for line A birds were not different at any dose (15, 30, and 45 $\times 10^3$ oocysts) whereas line B birds showed an increasing trend in the severity of lesions with the corresponding increase in oocytes administered. No significant differences were found in overall weight gain between the 2 lines at any dose (Figure 1); however, weight gain was significantly lower with increasing dose ($P < 0.0001$). A significant correlation (r = −0.63) was found between the severity of lesion score and weight gain for line B birds ($P < 0.0001$), whereas no significant correlation was found for line A birds (r = 10.18, $P = 0.11$). The slope of the regression analysis for line A birds was −12.95 g of weight gained for each unit increase in lesion score and line B had a slope of −31.97 g of weight gained for each unit increase in lesion score. It should be noted that before challenge line B birds were heavier than line A birds, but following challenge, line A birds maintained more of their potential weight compared with line B birds.

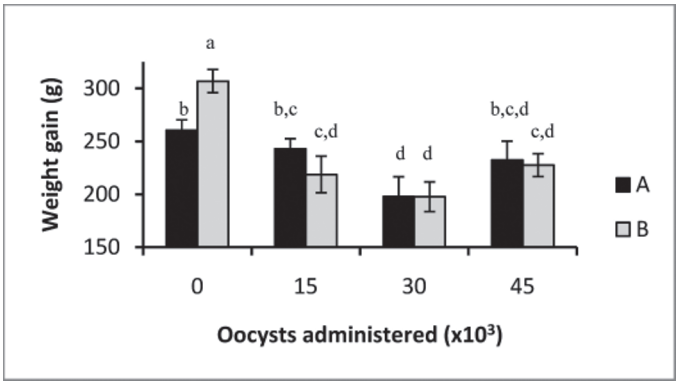

Figure 1. Experiment 1 weight gain in line A and B broilers administered the Beltsville strain of *Eimeria tenella* at 14 d of age and killed 6 d postchallenge (n = 20/dose). Each bar represents the mean ± SEM for each group. Means lacking a common letter differ ($P \leq 0.05$).

Experiment 2

The lesion scores following administration of all doses of the Beltsville strain were lower ($P \leq 0.05$) for line A than line B ($15 \times 10^3 = 0.15$ and 0.72, respectively; $30 \times 10^3 = 0.6$ and 1.28, respectively; $45 \times 10^3 = 0.68$ and 1.65, respectively; Table 2). Although a statistical dose response was found in line A birds ($P = 0.0006$), the severity of the infection was low (i.e., lesion scores of ≤2). Line B birds also showed a significant dose response and experienced a greater severity of lesions ($P < 0.0001$; Table 2). It is possible that the lower overall lesion scores for experiment 2 compared with what was

Table 1. Experiment 1 distribution (no. of chickens) and average lesion scores

Line-dose[1] (×10³)	Lesion score[2] 0	1	2	3	4	Average lesion score (±SEM)
A-0	20	0	0	0	0	0 ± 0^{a}
B-0	19	0	0	0	0	0 ± 0^{a}
A-15	0	8	8	3	0	1.74 ± 0.16^{b}
B-15	0	6	7	5	2	2.15 ± 0.22^{b}
A-30	0	12	9	0	0	1.43 ± 0.11^{bc}
B-30	2	0	2	6	9	3.05 ± 0.28^{d}
A-45	0	7	11	2	1	1.86 ± 0.18^{bc}
B-45	0	0	5	12	3	2.9 ± 0.14^{d}

[a–d]Means lacking a common superscript differ ($P \leq 0.05$).

[1]Parental broiler chickens used in this study were obtained from a commercial primary breeder. To maintain confidentiality, lines were designated A and B. Birds were administered 0, 15, 30, or 45 $\times 10^3$ oocysts of *Eimeria tenella*.

[2]Lesions were scored on a scale of 0 to 4. 0 = normal healthy tissue. 1 = a few scattered petechiae, no thickening of the cecal wall, and normal brownish colored content. 2 = increased numbers of petechiae, some bleeding at the mucosal surface, relatively normal cecal content, and slight thickening of the cecal wall. 3 = increased bleeding with clotting at the distal end of the pouch that becomes hardened as the sloughed mucosal surface unites with bloody cecal content, and marked thickening of the cecal wall. 4 = severe bleeding, severe thickening of the wall, and eroding of the mucosal surface featuring a hard core of material inside the cecal pouch (Johnson and Reid, 1970).

Table 2. Experiment 2 distribution (no. of chickens) and average lesion scores

Line-dose ($\times 10^3$)	Lesion score					Average lesion score (±SEM)
	0	1	2	3	4	
A-0	22	0	0	0	0	0 ± 0^{a}
B-0	25	0	0	0	0	0 ± 0^{a}
A-15	22	4	0	0	0	0.15 ± 0.07^{b}
B-15	9	14	2	0	0	0.72 ± 0.12^{c}
A-30	10	15	0	0	0	0.60 ± 0.10^{c}
B-30	4	11	9	1	0	1.28 ± 0.16^{d}
A-45	10	11	3	0	0	0.68 ± 0.14^{c}
B-45	0	14	6	5	0	1.65 ± 0.16^{d}

[a–d]Means lacking a common superscript differ ($P \leq 0.05$).

[1]Parental broiler chickens used in this study were obtained from a commercial primary breeder. To maintain confidentiality, lines were designated A and B. Birds were administered 0, 15, 30, or 45 $\times 10^3$ oocysts of *Eimeria tenella*.

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observed in experiment 1 was attributable to natural variation in chicken flocks or to changes in the *E. tenella* stock following passage or storage or both. In addition to reduced cecal lesion scores, we also observed significant differences in the final weight gained between the lines at different doses ($P = 0.02$); this interaction was likely driven by the increased weight gain by line B birds at the 30×10^3 dose. Line A birds administered 15 and 45 $\times 10^3$ oocysts had higher ($P \leq 0.05$) weight gain compared with dose-matched line B birds (Figure 2). The correlation between weight gained and lesion score was less profound in this experiment; line A revealed no correlation ($r = 0.07$, $P = 0.51$) and line B suggested a trend toward an association ($r = -0.18$, $P = 0.07$). The regressions between weight gained and lesion scores for line A had a nonsignificant slope of −5.24 ($P = 0.51$) and for line B a slope of −12.08 ($P = 0.07$).

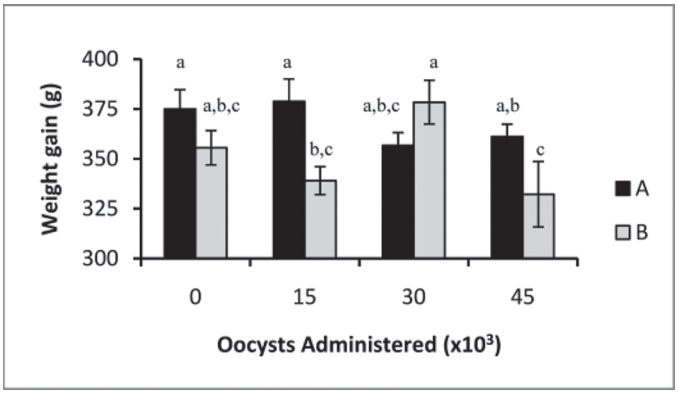

Figure 2. Experiment 2 weight gain in line A and B broilers administered the Beltsville strain of *Eimeria tenella* at 14 d of age and killed 6 d postchallenge (n = 25/dose). Each bar represents the mean ± SEM for each group. Means lacking a common letter differ ($P \leq 0.05$).

Experiment 3

To show that line A chickens were generally more resistant than line B chickens, we used a different field isolate of *E. tenella* for experiment 3 (Penn State strain). As observed with the Beltsville strain, line A birds showed enhanced resistance against cecal pathology. The severity of the lesion scores was lower (mostly 1 and 2) for line A and no association existed between the 2 doses ($P = 0.12$); however, a dose response was found for line B birds ($P = 0.03$) that was accompanied by more severe lesions (22 birds had lesions that scored 3 or 4). The average lesion scores reflect this following administration of 50×10^3 oocysts, which were significantly ($P \leq 0.05$) lower (1.4 and 2.6 for lines A and B, respectively) whereas the 25×10^3 dose was numerically less (1.72 and 2.21 for A and B, respectively; Table 3). No significant differences were found in final weight gain between the 2 lines at any dose ($P = 0.47$; Figure 3), although a significant overall dose response ($P < 0.0001$) correlation existed between weight gained and lesion scores, which were significant for both line A and line B ($r = -0.52$, $P < 0.0001$ for line A; $r = -0.76$, $P < 0.0001$ for line B). The regression slopes for lines A and B were −34.1 and −34.9, respectively.

DISCUSSION

Line A and B chickens are 2 commercial parental broiler lines that, to our knowledge, have not been selected for enhanced disease resistance against any poultry or foodborne pathogen. In this study, lower cecal lesion scores were often accompanied by improved weight gain in line A birds infected with 2 separate field isolates of *E. tenella* when compared with line B. The increased resistance observed in line A chickens has been consistent regardless of the challenge: *Salmonella enteritidis*

Table 3. Experiment 3 distribution (no. of chickens) and average lesion scores

Line-dose ($\times 10^3$)	Lesion score					Average lesion score (±SEM)
	0	1	2	3	4	
A-0	30	0	0	0	0	0 ± 0^{a}
B-0	20	0	0	0	0	0 ± 0^{a}
A-25	0	8	7	3	0	1.72 ± 0.15^{bc}
B-25	0	5	7	5	2	2.21 ± 0.21^{b}
A-50	0	12	8	0	0	1.4 ± 0.10^{bc}
B-50	2	1	1	6	9	2.6 ± 0.27^{bd}

[a–d]Means lacking a common superscript differ ($P \leq 0.05$).

[1]Parental broiler chickens used in this study were obtained from a commercial primary breeder. To maintain confidentiality, lines were designated A and B. Birds were administered 0, 25, or 50 $\times 10^3$ oocysts of *Eimeria tenella*.

[2]Lesions were scored on a scale of 0 to 4. 0 = normal healthy tissue. 1 = a few scattered petechiae, no thickening of the cecal wall, and normal brownish colored content. 2 = increased numbers of petechiae, some bleeding at the mucosal surface, relatively normal cecal content, and slight thickening of the cecal wall. 3 = increased bleeding with clotting at the distal end of the pouch that becomes hardened as the sloughed mucosal surface unites with bloody cecal content, and marked thickening of the cecal wall. 4 = severe bleeding, severe thickening of the wall, and eroding of the mucosal surface featuring a hard core of material inside the cecal pouch (Johnson and Reid, 1970).

(Ferro et al., 2004; Swaggerty et al., 2005a), *Enterococcus gallinarum* (Swaggerty et al., 2005b), *Campylobacter jejuni* (Li et al., 2008), and now against the parasite *E. tenella*. Additionally, while fertile eggs were available, we conducted 1 experiment each with *E. acervulina* and *E. maxima*; line A was more resistant against intestinal pathology and maintained higher weights in those 2 trials (Swaggerty and Kogut, unpublished data).

A comparison study found that reduced weight loss and lower fecal parasite numbers are coupled with increased expression of IL-1β, IL-6, and IL-8 following infection with *E. maxima* (Kim et al., 2008). Other studies also report that IL-1β and IL-6 increase after exposure to *E. tenella* (Lynagh et al., 2000; Laurent et al., 2001), further indicating that a strong proinflammatory response limits this parasite. Although we did not address gene expression in the current study, we know that the more resistant line (A) has higher basal levels of IL-1β, IL-6, and IL-8 (now referred to as CXCLi2) compared with line B (Swaggerty et al., 2004) and the differences are maintained following oral challenge with *S. enteritidis* (Ferro et al., 2004). One could speculate that line A responded with a more robust proinflammatory cytokine and chemokine response that limited colonization and therefore contributed, in part, to lower cecal lesion scores and reduced weight loss observed herein. This would be in agreement with another study that shows the Fayoumi line of chickens also has a strong cytokine response against *S. enteritidis*, indicating that a vigorous cytokine response is critical in controlling both *Salmonella* and *Eimeria* in chickens (Redmond et al., 2009).

We observed differences in bird weights between lines as well as between the 3 experiments. Line A bird weights varied more than line B weights, where the control weights at the conclusion of the experiments ranged from 260 to 375 g for line A and 306 to 355 g for line B. The differences are likely attributable to normal variation that would be expected between flocks and farms. The source of fertile eggs varied for each experiment (i.e., the lines were provided by different farms across a wide geographical location). In addition to bird weight variation, we also observed variability in the *E. tenella* stocks and their ability to induce lesions. A marked reduction occurred in lesion scores between experiments 1 and 2 (both using the Beltsville strain). It is likely that the variation is attributable to a reduction in pathogenicity of the *E. tenella* stock as a result of storage or passage both, or, less likely, the birds may have been generally more resistant than those used in experiment 1. However, the distribution of lesion scores (Tables 1 and 2) indicates the *E. tenella* stock had lost some of its pathogenicity given that no lesion scores of 4 were observed for either line in experiment 2, whereas 15 individuals in experiment 1 were scored as a 4. Regardless of the cause of the variation, the overall trend observed for the lines remained consistent where line A was more resistant than line B.

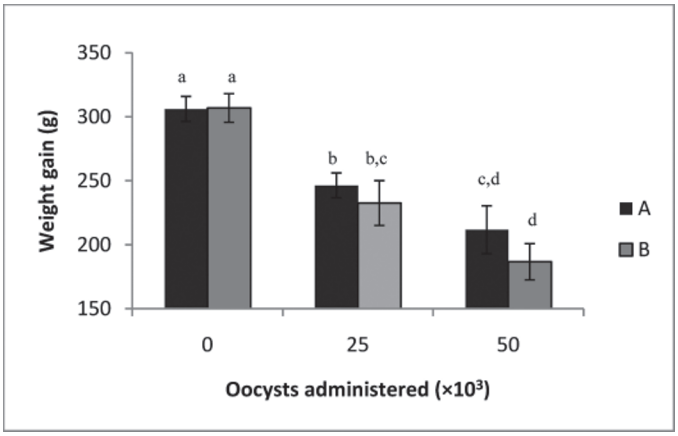

Figure 3. Experiment 3 weight gain in line A and B broilers administered the Penn State strain of *Eimeria tenella* at 14 d of age and killed 6 d postchallenge (n = 25/dose). Each bar represents the mean ± SEM for each group. Means lacking a common letter differ ($P \leq 0.05$).

In addition to the value of lines A and B as parental broilers, these data in conjunction with our earlier studies indicate the experimental value of these 2 lines of chickens. Future functional genomic and phenotypic comparisons that dissect the molecular and immunological mechanisms that contribute to the differential response between lines A and B would provide valuable information to the poultry industry with respect to identifying key components that contribute to increased resistance against both poultry and foodborne pathogens.

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