IOWA STATE UNIVERSITY
Digital Repository

Animal Science Publications | Animal Science

4-25-2016

# Comparing the immune responses of two genetically B-complex disparate Fayoumi chicken lines to Eimeria tenella

S.-H. Lee
*United States Department of Agriculture and National Academy of Agricultural Science*

X. Dong
*United States Department of Agriculture and China Agricultural University*

H. S. Lillehoj
*United States Department of Agriculture*

Susan J. Lamont
*Iowa State University*, sjlamont@iastate.edu

X. Suo
*Iowa State University*

*See next page for additional authors*

Follow this and additional works at: https://lib.dr.iastate.edu/ans_pubs

 Part of the Agriculture Commons, Genetics Commons, Poultry or Avian Science Commons, and the Veterinary Infectious Diseases Commons

The complete bibliographic information for this item can be found at https://lib.dr.iastate.edu/ans_pubs/555. For information on how to cite this item, please visit http://lib.dr.iastate.edu/howtocite.html.

This Article is brought to you for free and open access by the Animal Science at Iowa State University Digital Repository. It has been accepted for inclusion in Animal Science Publications by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.

# Comparing the immune responses of two genetically B-complex disparate Fayoumi chicken lines to Eimeria tenella


## Abstract

1. The present study was conducted to compare the susceptibility of congenic Fayoumi lines to *Eimeria tenella* infection and to assess genetic differences in *Eimeria* egression.
2. Chickens were orally inoculated with 5 × 104 sporulated *E. tenella* oocysts and challenged with 5 × 106 oocysts on the 10th day after the primary infection.
3. The Fayoumi M5.1 line exhibited higher levels of body weight gain, less oocyst shedding and higher percentages of B and CD4+/CD8+ T cells than the M15.2 chickens.
4. These results demonstrate that M5.1 line is more resistant to *E. tenella* infection than M15.2 line. Furthermore, the percentage of sporozoite egress from peripheral blood mononuclear cells (PBMCs) was higher in the M5.1 line.
5. The results of this study suggest that enhanced resistance of Fayoumi M5.1 to *E. tenella* infection may involve heightened cell-mediated and adaptive immunity, resulting in reduced intracellular development of *Eimeria* parasites.



## Disciplines

Agriculture | Genetics | Poultry or Avian Science | Veterinary Infectious Diseases

## Comments

This is the Version of Record of an article published by Taylor & Francis in *British Poultry Science* on April 25, 2016, available online DOI: 10.1080/00071668.2016.1141172.

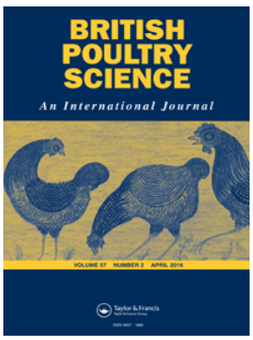


## Authors

S.-H. Lee, X. Dong, H. S. Lillehoj, Susan J. Lamont, X. Suo, D. K. Kim, K.-W. Lee, and Y. H. Hong



This article is available at Iowa State University Digital Repository: https://lib.dr.iastate.edu/ans_pubs/555



British Poultry Science

ISSN: 0007-1668 (Print) 1466-1799 (Online) Journal homepage: https://www.tandfonline.com/loi/cbps20

# Comparing the immune responses of two genetically *B*-complex disparate Fayoumi chicken lines to *Eimeria tenella*

S.-H. Lee, X. Dong, H. S. Lillehoj, S. J. Lamont, X. Suo, D. K. Kim, K.-W. Lee & Y. H. Hong

**To cite this article:** S.-H. Lee, X. Dong, H. S. Lillehoj, S. J. Lamont, X. Suo, D. K. Kim, K.-W. Lee & Y. H. Hong (2016) Comparing the immune responses of two genetically *B*-complex disparate Fayoumi chicken lines to *Eimeria tenella*, British Poultry Science, 57:2, 165-171, DOI: 10.1080/00071668.2016.1141172

**To link to this article:** https://doi.org/10.1080/00071668.2016.1141172

Accepted author version posted online: 04 Mar 2016.
Published online: 25 Apr 2016.

Submit your article to this journal

Article views: 291

View related articles

View Crossmark data

Citing articles: 7 View citing articles

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=cbps20

*British Poultry Science*, 2016
Vol. 57, No. 2, 165–171, http://dx.doi.org/10.1080/00071668.2016.1141172

Taylor & Francis
Taylor & Francis Group

# Comparing the immune responses of two genetically *B*-complex disparate Fayoumi chicken lines to *Eimeria tenella*

S.-H. LEE[1,2]*, X. DONG[1,3]*, H. S. LILLEHOJ[1], S. J. LAMONT[4], X. SUO[4], D. K. KIM[5], K.-W. LEE[6], AND Y. H. HONG[7]

[1]*United States Department of Agriculture, Animal Bioscience and Biotechnology Laboratory, Beltsville Agricultural Research Center, Beltsville, MD, USA,* [2]*National Academy of Agricultural Science, Rural Development Administration, Jeollabuk-do, Republic of Korea,* [3]*National Animal Protozoa Laboratory & College of Veterinary Medicine, China Agricultural University, Beijing, China,* [4]*Department of Animal Science, Iowa State University, Ames, IA, USA,* [5]*C&K Genomics Inc., Seoul, Republic of Korea,* [6]*Department of Animal Science and Technology, Konkuk University, Seoul, Republic of Korea, and* [7]*Department of Animal Science and Technology, Chung-Ang University, Anseong, Republic of Korea*

**Abstract** 1. The present study was conducted to compare the susceptibility of congenic Fayoumi lines to *Eimeria tenella* infection and to assess genetic differences in *Eimeria* egression.
2. Chickens were orally inoculated with $5 \times 10^4$ sporulated *E. tenella* oocysts and challenged with $5 \times 10^6$ oocysts on the 10th day after the primary infection.
3. The Fayoumi M5.1 line exhibited higher levels of body weight gain, less oocyst shedding and higher percentages of B and $CD4^+/CD8^+$ T cells than the M15.2 chickens.
4. These results demonstrate that M5.1 line is more resistant to *E. tenella* infection than M15.2 line. Furthermore, the percentage of sporozoite egress from peripheral blood mononuclear cells (PBMCs) was higher in the M5.1 line.
5. The results of this study suggest that enhanced resistance of Fayoumi M5.1 to *E. tenella* infection may involve heightened cell-mediated and adaptive immunity, resulting in reduced intracellular development of *Eimeria* parasites.

## INTRODUCTION

Fayoumi chickens, which originated in Egypt (Ekarius, 2007), were reported to be resistant to avian leukosis and to exhibit robust protective responses against *Eimeria maxima* infection (Pinard-Van Der Laan *et al.*, 1998; Kim *et al.*, 2008, 2009). Two congenic lines, Fayoumi M5.1 and M15.2, have a 99% inbreeding coefficient and are genetically distant from broiler and Leghorn chickens (Zhou and Lamont, 1999). These two chicken lines share an identical genetic background except at the 16th chromosome, which encodes the major histocompatibility complex (MHC) (Zhou and Lamont, 1999, 2003). The chicken MHC (*B*-complex) influences resistance or susceptibility to a variety of viral, bacterial and parasitic diseases (Bacon, 1987; Lamont, 1998) through distinct immune mechanisms that involve communication between the different cellular components of the immune system, including T cells, B cells and antigen-presenting cells (Lamont, 1998). Indeed, the cause and effect relationships between MHC haplotypes and protective immune responses to coccidiosis in these chicken lines were reported previously (Clare *et al.*, 1985; Lillehoj *et al.*, 1989; Brake *et al.*, 1997; Caron *et al.*, 1997). In previous studies from our laboratory, the M5.1 chickens were more resistant to *E. maxima* than the M15.2 animals. Furthermore, the M5.1 and M15.2 lines exhibited differential expression of immune-related cytokines after infection with *E. maxima* (Kim *et al.*, 2008).

Correspondence to: Yeong Ho Hong, Department. of Animal Science & Technology, Chung-Ang University, Anseong 17546, Republic of Korea. E-mail: yhong@cau.ac.kr
Accepted for publication 10 December 2015.
*Both authors equally contributed.

© 2016 British Poultry Science Ltd

Moreover, in a recent study, it was demonstrated that lymphocytes, particularly B cells, $CD4^+$ and $CD8^+$ T lymphocytes, as well as antibodies from *Eimeria*-infected chickens effectively induce egress of intracellular *E. tenella* sporozoites from host cells, which is an essential stage in the life cycle and pathogenesis of *Eimeria* spp. (Dong *et al.*, 2011).

Apicomplexan parasites, such as *Plasmodium, Toxoplasma* and *Eimeria*, comprise a large group of human and/or animal pathogens, most of which possess an apical complex containing characteristic structures such as the conoid, rhoptries and micronemes. Organisms of the phylum *Apicomplexa* feature a complex life cycle consisting of cell invasion, intracellular development and propagation, morphogenesis and egression. In general, infectious sporozoites rapidly enter host cells aided by the glideosome, a molecular machine that powers parasite motility, migration, cell invasion and egression (Soldati-Favre, 2008). The process of postinfection egress of several apicomplexan parasites was previously studied, with the goal of identifying potential therapeutic approaches to interrupt cell exit and thereby disrupt the parasite's life cycle (Roiko and Carruthers, 2009).

Based on these results, it is hypothesised that Fayoumi M5.1 chickens are more resistant to coccidiosis than M15.2 chickens due to a distinct immune mechanism. However, there is no information regarding host–parasite interactions during *E. tenella* infection of these animals. Therefore, this study was conducted to compare the immune responses of the *B*-complex disparate Fayoumi M5.1 and M15.2 chicken lines to primary and secondary *E. tenella* infection by measuring various disease parameters, assessing lymphocyte populations and sporozoite egress from host cells.

## MATERIALS AND METHODS

### Animals, parasites and animal infection model

Two highly inbred MHC-congenic Fayoumi chicken lines (M5.1 and M15.2, 50 birds/line) were obtained from the poultry genetics program at Iowa State University. The birds were housed in Petersime starter brooder units (91 cm × 76 cm × 25 cm) (Zulte, Belgium) and provided with food and water *ad libitum* under coccidian-free conditions. Birds were fed an antibiotic free, certified organic starter diet containing 170 g crude protein (CP)/kg between d 1 and 14, followed by a standard grower diet containing 240 g CP/kg. All experiments were approved and followed by the United States Department of Agriculture (USDA)-Agricultural Research Service Beltsville Animal Care and Use Committee.

Four-week-old M5.1 and M15.2 birds were orally inoculated with $5 \times 10^4$ *E. tenella* oocysts and then challenged with $5 \times 10^6$ *E. tenella* oocysts at 10 d post-primary infection (PPI) (10 birds/group). Twenty naïve birds per line were kept in a separate room and used as a negative control. Sporulated oocysts of *E. tenella* (Beltsville strain WLR-1) were used for oral infection and for the preparation of sporozoites for the egression study (Lee *et al.*, 2013). Fresh preparation of sporozoites was carried out by excystation of sporulated oocysts, as described previously (Lee *et al.*, 2013*a*). Briefly, freshly sporulated oocysts were disrupted with 0.5 mm glass beads for 5–7 s using a Mini-beadbeater at homogeniser level (BioSpec Products, Bartlesville, OK, USA). To obtain sporozoites, the released sporocysts were purified by isopycnic centrifugation on a percoll gradient, washed in ice-cold phosphate-buffered saline (PBS), and treated at 41°C for 30 min with 0.25% trypsin and 0.014 M taurocholic acid (Sigma, St. Louis, MO, USA). The sporozoites were then collected by filtration, washed three times with Hank's Balanced Salt Solution (HBSS, Sigma), centrifuged at 3000 × *g* for 10 min at 4°C and resuspended at a concentration of $1.0 \times 10^6$ sporozoites/ml in complete RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS, Sigma), 100 units/ml penicillin and 100 µg/ml streptomycin (Sigma).

### Measurement of body weight and faecal oocysts

The body weight of each chicken was measured using a scale (Sartorius CP34001P, Goettingen, Germany) at d 0, 9 d PPI, and again at d 9 post-secondary infection (PSI). Faeces were collected between 5 and 9 d PPI or PSI, and *E. tenella* oocyst output was calculated, as previously described, using a McMaster counting chamber (Marienfeld-Superior, Lauda-Königshofen Germany) (Lee *et al.*, 2013*b*). The total number of oocysts was calculated according to the following formula:

$$\text{Total oocysts} = \text{Oocysts counted} \times \text{Dilution factor} \times (\text{Faecal sample volume}/\text{Counting chamber volume}).$$

### Antigen-specific serum antibody response

A total of 5 ml of blood samples were obtained from chickens by cardiac puncture with 0.1 M EDTA-coated 10 ml syringe (23G) following euthanasia. Blood samples from naïve birds were used as negative controls. Serum was collected by centrifugation and the levels of IgY antibodies against *E. tenella* were measured against recombinant profilin protein, which is a coccidial antigen, by enzyme-linked immunosorbent assays (ELISA),

as described by Lee *et al.* (2012*a*). Briefly, 96-well flat-bottom microtiter plates were coated with 1 µg profilin in 100 µL of 0.1 M carbonate buffer and incubated overnight. Plates were washed with 0.05% Tween in PBS (PBS-T), and blocked with 1% bovine serum albumin (BSA) in PBS-T for 1 h at room temperature on a plate shaker. Serum samples were then added to the plates, incubated for 2 h with continuous gentle shaking and washed with PBS-T. PBS containing 0.1% BSA was included as a negative control. A total of 100 µl of horseradish peroxidase (HRP)-conjugated rabbit anti-chicken IgY (Sigma) was added to each well and incubated for 30 min at room temperature with gentle shaking. The plates were washed with PBS-T and incubated with 100 µl of the 3,3´,5,5´-Tetramethylbenzidine (TMB) substrate (Sigma), and the optical density (OD) was read at 450 nm using an automated microtiter plate reader (Bio-Rad, Hercules, CA, USA).

### Flow cytometric analysis of lymphocyte subpopulations

Spleens were harvested from three chickens per group at d 7 PSI. Splenic single-cell suspensions were prepared and resuspended in FACS buffer ($Ca^{2+}$- and $Mg^{2+}$-free (CMF)-HBSS without phenol red, containing 3% FBS and 0.01% sodium azide), according to the method described by Lee *et al.* (2012*b*). Samples of the single-cell suspensions ($1.0 \times 10^6$ cells/sample) were incubated with 100 µl of CD4-, CD8α-, or Bu-1a-specific mouse anti-chicken monoclonal antibodies (mAbs) for 30 min on ice. Additionally, cells incubated with mouse mAbs specific for human HB2 (human T cell marker) and K55 (pan chicken lymphocyte marker) were utilised as negative and positive controls, respectively. The cells were then washed twice with 2.0 ml of PBS buffer, incubated on ice for 30 min with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse IgG secondary Ab (1:64) (Sigma), washed twice, resuspended in 1.0 ml FACS buffer, and analysed with a FACSCalibur flow cytometer (Becton Dickinson and Co., Franklin Lakes, NJ, USA). Data was obtained from a total of $1.0 \times 10^4$ viable cells.

### Egress assays

Egress assays were carried out as described in a previous publication (Dong *et al.*, 2011). Peripheral blood mononuclear cells (PBMCs) and splenic lymphocytes were prepared separately. PBMCs obtained from healthy M5.1 or M15.2 chickens were cultured in 24-well tissue culture plates including glass slides using 10% complete IMDM (Iscove's Modified Dulbecco's Medium) supplemented with 10% FBS, 10 mM HEPES buffer, 100 units/ml penicillin and 100 µg/ml streptomycin at 41°C. After reaching up to approximately 80% confluence, PBMCs were incubated with $1.0 \times 10^5$ freshly prepared *E. tenella* sporozoites overnight. Free parasites were removed by washing with CMF-HBSS at 41°C. Intracellular parasites were then exposed to *E. tenella*-primed or naive splenic lymphocytes for 3 h. After washing twice with CMF-HBSS, PBMCs were fixed by incubating with cold absolute methanol for 5 min and permeabilised by treatment with 0.05% Triton X-100 in PBS for 5 min. In order to measure egressed parasites, glass slides containing fixed PBMCs were incubated with mouse anti-*Eimeria* antibodies (Krucken *et al.*, 2008; Dong *et al.*, 2011) for 30 min at 4°C, washed and incubated with Alexa Fluor 488-conjugated goat anti-mouse IgG secondary antibody (Invitrogen, Carlsbad, CA, USA) for 30 min at 4°C. Intracellular parasites in PBMCs were counted by fluorescence microscope (Carl Zeiss, Thornwood, NY, USA). The percentage of egress was calculated using the following formula, as previously described (Dong *et al.*, 2011):

Egress (%) = 100 × (Number of intracellular parasites in the non-treatment group−Number of intracellular parasites in the treatment group)/ Number of intracellular parasites in the non-treatment group.

### Statistical analysis

Data were expressed as means ± SEM, and the mean values from each treatment group were compared using Student's *t*-tests. Differences between means were considered to be statistically significant at $P < 0.05$. All data were evaluated using IBM SPSS software (SPSS 20.0 for Windows, Chicago, IL).

## RESULTS

### Body weight and faecal oocyst output

We compared the body weight gains between 0 and 9 d PPI and PSI of uninfected (control) and *E. tenella*-infected Fayoumi M5.1 and M15.2 chickens. As depicted in Figure 1, there was no difference in weight gain after 9 d PPI between the M5.1 uninfected and *E. tenella*-infected groups. Conversely, the M15.2 chickens infected with *E. tenella* exhibited significantly lower weight gains than the uninfected M15.2 control group ($P < 0.05$). Meanwhile, after secondary infection with *E. tenella*, both the M5.1 and M15.2 chickens showed significantly reduced body weights between 0 and 9 d PSI compared to those of the uninfected control groups, respectively. However, the average weight loss of the M5.1 chickens was significantly less than that of the M15.2 chickens (32.7% in M5.1 [$P < 0.05$] vs. 39% in M15.2 [$P < 0.01$]) compared with their respective uninfected control animals.

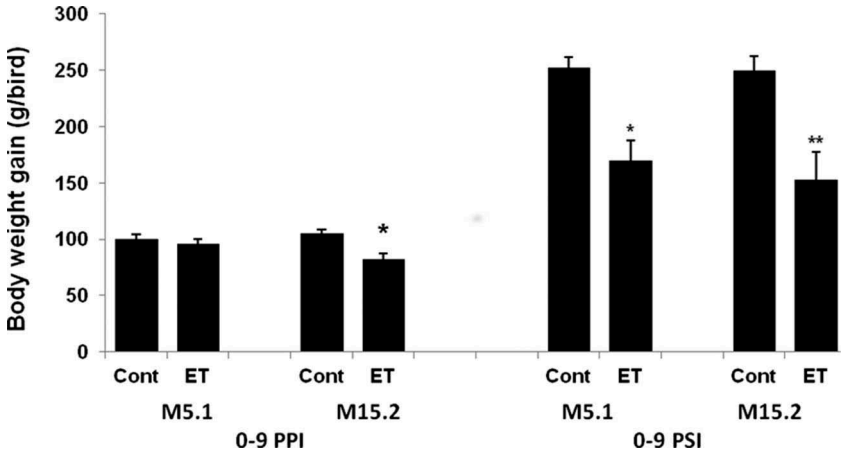



**Figure 1.** *Body weight gains of Fayoumi M5.1 and M15.2 chickens after infection with E. tenella oocysts. Body weight gain was measured by the difference of the body weights between d 0 and 9 d PPI or PSI. Each bar represents the mean ± SEM values for each group (n = 10). *P < 0.05, **P < 0.01, compared to the uninfected control according to the t-test.*

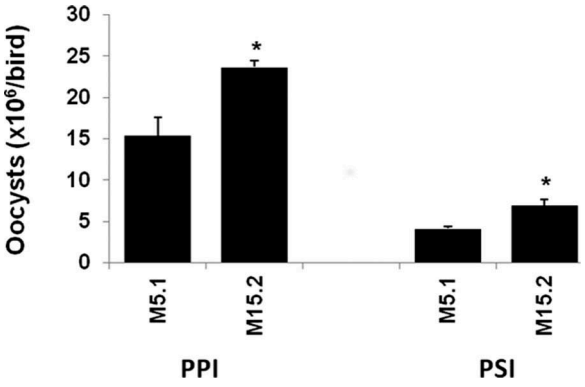



**Figure 2.** *Faecal E. tenella oocyst shedding of Fayoumi M5.1 and M15.2 chickens after infection with E. tenella oocysts. Oocyst numbers were determined using a McMaster chamber and expressed per bird. Each bar represents the mean ± SEM values for each group (n = 10). Significant difference between M5.1 and M15.2 chickens was expressed as *P < 0.05 according to the t-test.*

The faecal oocyst output of M15.2 chickens was significantly higher than that of the M5.1 animals between 5 and 9 d PPI and 5 and 9 d PSI (Figure 2). Specifically, the M15.2 group exhibited 54% higher oocyst production than the M5.1 group between 5 and 9 d PPI, and 67% higher production than the M5.1 group between 5 and 9 d PSI.

### Serum Ab response to profilin protein

The Ab response to profilin was measured using serum from chickens infected with *E. tenella* at both d 6 PPI and d 6 PSI. The M5.1 chickens exhibited significantly higher serum Ab levels than the M15.2 chickens at both time points ($P < 0.05$) (Figure 3). In other words, the M5.1 chickens produced higher serum Ab responses to profiling results from *E. tenella* than the M15.2 animals at 6 d PPI and PSI.

### Comparison of splenic lymphocyte subpopulations

Figure 4 depicts the major lymphocyte subpopulations in the spleens of uninfected and *E. tenella*-infected M5.1 and M15.2 chickens. Interestingly, only the M5.1 chickens exhibited increases in $CD4^+/CD8^+$ and Bu-1a-positive cells (approximately a 62% increase in both populations) following *E. tenella* infection compared to the number of these cells in the uninfected control chickens, but not in the M15.2 chicken.

### Sporozoite egress mediated by primed lymphocytes

PBMCs treated with M5.1-primed splenocytes for 3 h exhibited a 4.3-fold increase in sporozoite egression compared to those treated with naïve splenocytes. Meanwhile, PBMCs treated with M15.2-primed splenocytes also exhibited a 3.5-fold increase in sporozoite egression compared to those treated with naïve splenocytes at 7 d PSI (Figure 5).

## DISCUSSION

Previous studies reported that two *B*-congenic Fayoumi chicken lines, M5.1 and M15.2, which share an identical genetic background except at the MHC locus (Zhou and Lamont, 1999, 2003), displayed differences both in resistance to *E. maxima* infection and in the expression of various immune system-related genes (Kim *et al.*, 2008, 2009). Because the MHC plays an integral role in determining the T cell composition and the host immune response to pathogens, we hypothesised that many aspects of the immune response of these chickens to *E. tenella* infection, including

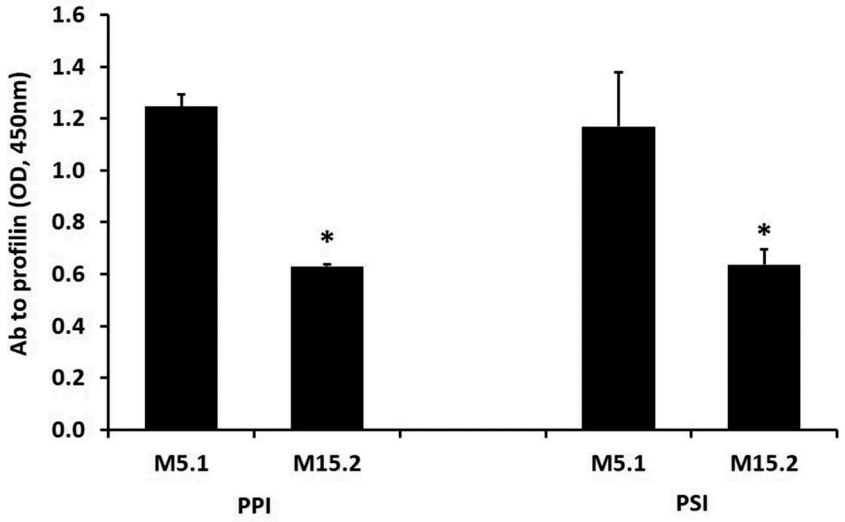



**Figure 3.** *Serum antibody response to profilin in M5.1 or M15.2 chickens infected with E. tenella. Blood serum was collected by cardiac puncture at the time of killing at 6 d PPI and d 6 PSI. Each bar represents the mean ± SEM (n = 3). Significant difference between uninfected control and infected groups in M5.1 and M15.2 chickens was expressed as *P < 0.05.*

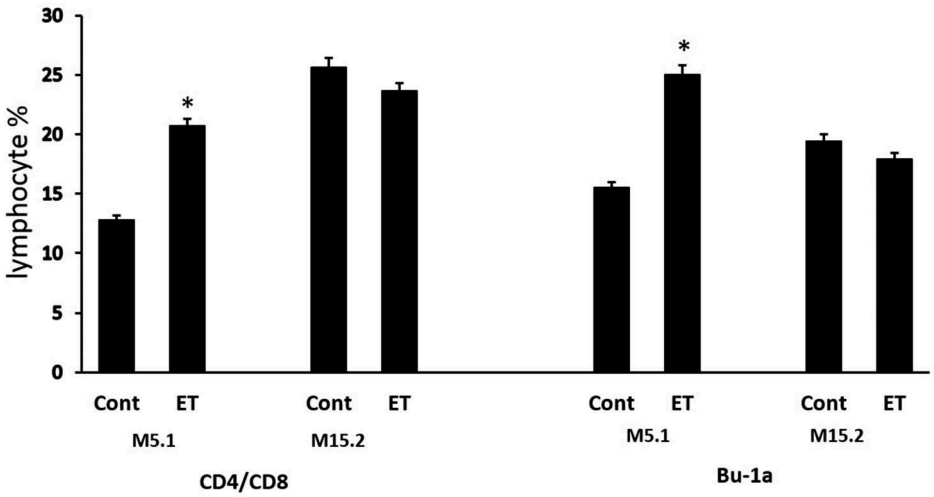



**Figure 4.** *Spleen lymphocyte subpopulations expressing CD4/CD8 and Bu-1a in M5.1 and M15.2 chickens at 7 d PSI. The spleens were collected from three birds per group at 7 d PSI (n = 3) and splenocytes were stained with mAbs to each cell surface marker. Data was obtained from a total of 1.0 × $10^4$ viable cells. CD4/CD8 was expressed as % of CD4 cell number divided by CD8 cell number. Significant difference between uninfected control and infected was expressed as * P < 0.05.*

humoral immunity, lymphocyte subsets and parasite egression, may differ.

As expected, M5.1 and M15.2 chickens infected with *E. tenella* differed from each other in both body weight change and faecal oocyst shedding, which are two of the most important disease parameters for evaluating genetic susceptibility to *Eimeria* infection. While there was no significant body weight loss in the M5.1 line following primary infection with 5 × $10^4$ oocysts of *E. tenella*, there was a significant decrease in the body weights of the infected M15.2 chickens compared with those of the uninfected chickens ($P < 0.05$). In contrast, secondary infection with high doses of *E. tenella* oocysts (5 × $10^6$) significantly decreased the body weights of both the M5.1 and M15.2 chickens between d 0 and 9. Thus, in regard to body weight gain, the two *B*-congenic Fayoumi chicken lines showed significant differences in resistance to *E. tenella*. In addition, the M15.2 line chickens produced more oocysts than the M5.1 line after both primary and secondary infections. It is likely that the differences in oocyst output after primary infection were due to differences in the efficacy of the innate immune responses of these animals.

Cell-mediated and humoral immunity were associated with resistance/susceptibility to *E. tenella* in congenic chicken lines. Indeed, chickens develop strong humoral responses following initial and secondary exposure to *E. tenella*, indicating the important role of the adaptive humoral

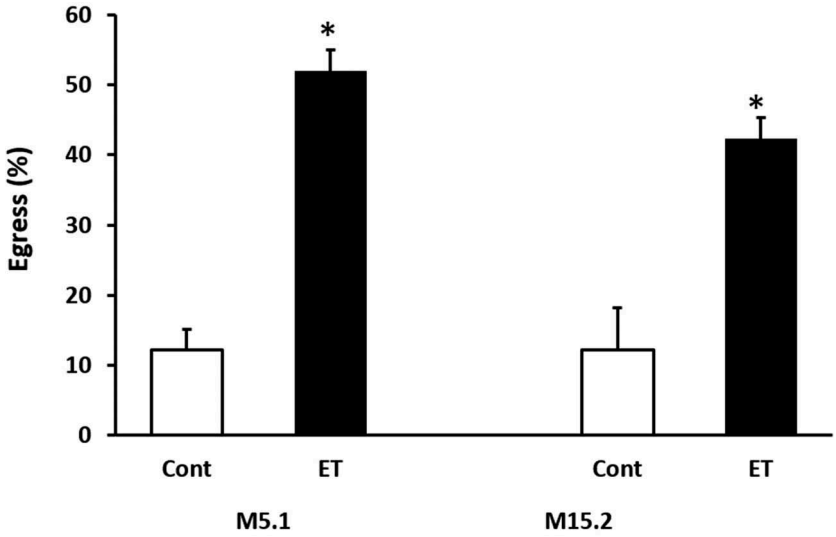



**Figure 5.** *Primed splenic lymphocyte-mediated sporozoite egress in two Fayoumi chicken lines. Splenocytes were collected from control and E. tenella-infected chickens (n = 3) at 7 d PSI. Data point represents the mean ± SEM. Significant difference between uninfected control and infected groups in M5.1 and M15.2 chickens was expressed as *P < 0.05.*

immune system in controlling coccidiosis (Lillehoj *et al.*, 1989). Notably, serum Ab responses to profilin were higher in the *E. tenella*-infected M5.1 line than in infected M15.2 chickens at both 6 d PPI and 6 d PSI.

Many studies have demonstrated an association between distinct chicken MHC loci and the levels of antibody production to various antigens (Lamont, 1998; Weigend *et al.*, 2001). Additionally, the mechanisms of protective immunity to coccidiosis are thought to depend on T cells (Elaine Rose and Long, 1971; Giambrone *et al.*, 1980; Lee *et al.*, 2011). In a previous study, we detected differences between these two Fayoumi chicken lines in the expression of immune system-related genes within the spleen after *E. maxima* infection (Kim *et al.*, 2008). Thus, the quantitative differences in the number of T cells influenced the qualitative aspects of cellular immunity. In a conflicting report, however, Cheeseman *et al.* (2004) found no significant difference in the percentages of cells expressing CD3, CD4, CD8, CD14 and Bu-1a positive leukocytes in peripheral blood leukocytes between healthy, adult Fayoumi M5.1 and M15.2 chickens. Therefore, further studies on the cellular mechanisms governing resistance to coccidiosis are necessary.

The present study was also conducted to investigate the interplay between the host's adaptive immune system and *Eimeria* egression. Egress, which is the process that certain intracellular parasites exit from parasitophorous vacuoles and host cells, is an essential component of the parasite life cycle and is central to *Eimeria* propagation and pathogenesis. Despite the importance of egress in the life cycle of intracellular parasites, the mechanism by which this process occurs is poorly understood, particularly in comparison to other steps in the life cycle, for example, invasion. In this study, premature egression of sporozoites from *E. tenella*-infected chicken PBMCs occurred when the cells were co-cultured *in vitro* with splenic lymphocytes from *E. tenella*-infected chickens, but not when they were co-cultured with splenocytes from uninfected chickens. In a previous study, *Eimeria*-specific antibodies and cytokines (IFN-γ, IL-2 and IL-15) derived from *E. tenella*-primed B and T lymphocytes, respectively, were capable of promoting premature egress of sporozoites from infected host cells. Both the host cells and parasites were viable, although the latter showed reduced reinvasion ability (Dong *et al.*, 2011). These results suggest a novel or immune-mediated mechanism that the host exploits to interrupt the normal *Eimeria* life cycle *in vivo* and thereby block the release of mature parasites into the environment. The parasite egress activity was enhanced in *E. tenella*-infected M5.1 line compared to M15.2 line. These results confirm that *E. tenella*-infected M5.1 and M15.2 chickens exhibit different levels of anti-parasite effector cell activity due to differences in the *B*-complex genes (Caron *et al.*, 1997).

In conclusion, two Fayoumi chicken lines with different MHC loci responded differently to primary and secondary *E. tenella* infections. Specifically, the M5.1 line exhibited lower oocyst shedding, higher $CD4^+/CD8^+$ T cell population, and heightened parasite egress with reduced intracellular parasite development than the M15.2 line. Although further studies are needed to investigate the association between sporozoite egress and host resistance to *E. tenella* infection in detail, these studies may provide crucial information to discern differences in the immune responses to parasitic pathogens.

## DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

## FUNDING

This project was supported, in part, by the "Investigation of the functional activity and the development of functional foods of *Allium hookeri*" (grant number PJ010490) project and Golden Seed Project (grant number PJ00992502 2016), Ministry of Agriculture, Food and Rural Affairs (MAFRA), Ministry of Oceans and Fisheries (MOF), Rural Development Administration (RDA) and Korea Forest Service (KFS).

## ORCID

*K.-W. Lee* http://orcid.org/0000-0002-3533-7979

## REFERENCES

Bacon, L.D. (1987) Influence of the major histocompatability complex on disease resistance and productivity. *Poultry Science*, **66** (5): 802–811. doi:10.3382/ps.0660802

Brake, D.A., Fedor, C.H., Werner, B.W., Miller, T.J., Taylor Jr, R.L. & Clare, R.A. (1997) Characterization of immune response to Eimeria tenella antigens in a natural immunity model with hosts which differ serologically at the B locus of the major histocompatibility complex. *Infection and Immunity*, **65** (4): 1204–1210.

Caron, L.A., Abplanalp, H. & Taylor Jr., R.L. (1997) Resistance, susceptibility, and immunity to Eimeria tenella in major histocompatibility (B) complex congenic lines. *Poultry Science*, **76** (5): 677–682. doi:10.1093/ps/76.5.677

Cheeseman, J.H., Kaiser, M.G. & Lamont, S.J. (2004) Genetic line effect on peripheral blood leukocyte cell surface marker expression in chickens. *Poultry Science*, **83** (6): 911–916. doi:10.1093/ps/83.6.911

Clare, R.A., Strout, R.G., Taylor Jr., R.L., Collins, W.M. & Briles, W.E. (1985) Major histocompatibility (B) complex effects on acquired immunity to cecal coccidiosis. *Immunogenetics*, **22** (6): 593–599. doi:10.1007/BF00430307

Dong, X., Abdelnabi, G.H., Lee, S.H., Li, G., Jin, H., Lillehoj, H.S. & Suo, X. (2011) Enhanced egress of intracellular Eimeria tenella sporozoites by splenic lymphocytes from coccidian-infected chickens. *Infection and Immunity*, **79** (8): 3465–3470. doi:10.1128/IAI.01334-10

Ekarius, C. (2007) *Storey's Illustrated Guide to Poultry Breeds*. (North Adams, MA, Storey Publishing).

Giambrone, J.J., Klesius, P.H. & Edgar, S.A. (1980) Aviancoccidiosis: evidence for a cell-mediated immunere-sponse. *Poultry Science*, **59**: 38–43. doi:10.3382/ps.0590038

Kim, D.K., Kim, C.H., Lamont, S.J., Keeler Jr., C.L. & Lillehoj, H.S. (2009) Gene expression profiles of two B-complex disparate, genetically inbred Fayoumi chicken lines that differ in susceptibility to Eimeria maxima. *Poultry Science*, **88** (8): 1565–1579. doi:10.3382/ps.2009-00012

Kim, D.K., Lillehoj, H.S., Hong, Y.H., Park, D.W., Lamont, S.J., Han, J.Y. & Lillehoj, E.P. (2008) Immune-related gene expression in two B-complex disparate genetically inbred Fayoumi chicken lines following Eimeria maxima infection. *Poultry Science*, **87** (3): 433–443. doi:10.3382/ps.2007-00383

Krucken, J., Hosse, R.J., Mouafo, A.N., Entzeroth, R., Bierbaum, S., Marinovski, P., Hain, K., Greif, G. & Wunderlich, F. (2008) Excystation of Eimeria tenella sporozoites impaired by antibody recognizing gametocyte/oocyst antigens GAM22 and GAM56. *Eukaryotic Cell*, **7** (2): 202–211. doi:10.1128/EC.00292-07

Lamont, S.J. (1998) The chicken major histocompatibility complexand disease. *Genetic Resistance to Animal Diseases*, **17**: 128–142.

Lee, S.H., Lillehoj, H.S., Jang, S.I., Lee, K.W., Baldwin, C., Tompkins, D., Wagner, B., Del Cacho, E., Lillehoj, E.P. & Hong, Y.H. (2012*b*) Development and characterization of mouse monoclonal antibodies reactive with chicken CD83. *Veterinary Immunology and Immunopathology*, **145** (1–2): 527–533. doi:10.1016/j.vetimm.2011.11.020

Lee, S.H., Lillehoj, H.S., Jang, S.I., Lee, K.W., Bravo, D. & Lillehoj, E.P. (2011) Effects of dietary supplementation with phytonutrients on vaccine-stimulated immunity against infection with Eimeria tenella. *Veterinary Parasitology*, **181** (2–4): 97–105. doi:10.1016/j.vetpar.2011.05.003

Lee, S.H., Lillehoj, H.S., Jang, S.I., Lee, K.W., Kim, D.K., Lillehoj, E.P., Yancey, R.J. & Dominowski, P.J. (2012*a*) Evaluation of novel adjuvant Eimeria profilin complex on intestinal host immune responses against live E. acervulina challenge infection. *Avian Diseases*, **56** (2): 402–405. doi:10.1637/9906-082411-ResNote.1

Lee, S.H., Lillehoj, H.S., Jang, S.I., Lillehoj, E.P., Min, W. & Bravo, D.M. (2013*b*) Dietary supplementation of young broiler chickens with Capsicum and turmeric oleoresins increases resistance to necrotic enteritis. *The British Journal of Nutrition*, **110** (5): 840–847. doi:10.1017/S0007114512006083

Lee, S.H., Lillehoj, H.S., Tuo, W., Murphy, C.A., Hong, Y.H. & Lillehoj, E.P. (2013*a*) Parasiticidal activity of a novel synthetic peptide from the core α-helical region of NK-lysin. *Veterinary Parasitology*, **197** (1–2): 113–121. doi:10.1016/j.vetpar.2013.04.020

Lillehoj, H.S., Ruff, M.D., Bacon, L.D., Lamont, S.J. & Jeffers, T.K. (1989) Genetic control of immunity to Eimeria tenella. Interaction of MHC genes and non-MHC linked genes influences levels of disease susceptibility in chickens. *Veterinary Immunology and Immunopathology*, **20** (2): 135–148. doi:10.1016/0165-2427(89)90094-9

Pinard-Van Der Laan, M.H., Monvoisin, J.L., Pery, P., Hamet, N. & Thomas, M. (1998) Comparison of outbred lines of chickens for resistance to experimental infection with coccidiosis (Eimeria tenella). *Poultry Science*, **77** (2): 185–191. doi:10.1093/ps/77.2.185

Roiko, M.S. & Carruthers, V.B. (2009) New roles for perforins and proteases in apicomplexan egress. *Cellular Microbiology*, **11** (10): 1444–1452. doi:10.1111/cmi.2009.11.issue-10

Rose, M.E. & Long, P.L. (1971) Immunity to coccidiosis: protective effects of transferred serum and cells investigated in chick embryos infected with Eimeria tenella. *Parasitology*, **63** (2): 299–313. doi:10.1017/S0031182000079610

Soldati-Favre, D. (2008) Molecular dissection of host cell invasion by the apicomplexans: the glideosome. *Parasite (Paris, France)*, **15** (3): 197–205. doi:10.1051/parasite/2008153197

Weigend, S., Matthes, S., Solkner, J. & Lamont, S.J. (2001) Resistance to Marek's disease virus in White Leghorn chickens: effects of avian leukosis virus infection genotype, reciprocal mating, and major histocompatibility complex. *Poultry Science*, **80** (8): 1064–1072. doi:10.1093/ps/80.8.1064

Zhou, H. & Lamont, S.J. (1999) Genetic characterization of biodiversity in highly inbred chicken lines by microsatellite markers. *Animal Genetics*, **30** (4): 256–264. doi:10.1046/j.1365-2052.1999.00505.x

Zhou, H. & Lamont, S.J. (2003) Chicken MHC class I and II gene effects on antibody response kinetics in adult chickens. *Immunogenetics*, **55** (3): 133–140. doi:10.1007/s00251-003-0566-9