Differential immunological response detected in mRNA expression profiles among diverse chicken lines in response to *Salmonella* challenge

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ABSTRACT *Salmonella enterica* serovar Enteritidis is a bacterial pathogen that contributes to poultry production losses and human foodborne illness. The bacterium elicits a broad immune response involving both the innate and adaptive components of the immune system. Coordination of the immune response is largely directed by cytokines. The objective of the current study was to characterize the expression of a select set of cytokines and regulatory immune genes in three genetically diverse chicken lines after infection with *S.* Enteritidis. Leghorn, Fayoumi and broiler day-old chicks were orally infected with pathogenic *S.* Enteritidis or culture medium. At 2 and 18 h postinfection, spleens and ceca were collected and mRNA expression levels for 7 genes (GM-CSF, IL2, IL15, TGF-β1, SOCS3, P20K, and MHC class IIβ) were evaluated by real-time quantitative PCR. Genetic line had a significant effect on mRNA expression levels of IL15, TGF-β1, SOCS3 and P20K in the spleen and on P20K and MHC class IIβ in the cecum. Comparing challenged vs. unchallenged birds, the expression of SOCS3 and P20K mRNA were significantly higher in the spleen and cecum, while MHC class IIβ mRNA was significantly lower in spleen. Combining the current RNA expression results with those of previously reported studies on the same samples reveals distinct RNA expression profiles among the three genetic chicken lines and the 2 tissues. This study illustrates that these diverse genetic lines have distinctively different immune response to *S.* Enteritidis challenge within the spleen and the cecum.

Key words: chicken, S*almonella enteritidis*, immune response, genetic difference

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INTRODUCTION

A zoonotic pathogen, *Salmonella enterica* serovar Enteritidis (***S.* Enteritidis**) can be responsible for production losses in poultry and can cause foodborne illness in humans (Guard-Petter, 2001). Improvements in poultry production practices have reduced the occurrence of *S.* Enteritidis contamination in the food chain, however, further reduction in bacterial burden is desirable and attainable (Doyle and Erickson, 2006). A greater understanding of the chicken immune response to *S.* Enteritidis, including better understanding of host immune response differences between genetic lines, will provide the basis to reduce the *S.* Enteritidis burden in poultry through genetic selection.

The natural route of *S.* Enteritidis infection is via oral exposure. Once ingested, the bacteria can transverse the intestinal epithelial cell layer and eventually invade cells, primarily macrophages (Desmidt et al., 1998; Eckmann and Kagnoff, 2001). Therefore, the initial site of innate and inflammatory immune activation includes the epithelial cells and gut-associated lymphoid tissue (**GALT**) of the intestinal wall including lamina propria, Peyer patches, and cecum (Schat and Myers, 1991; van Immerseel et al., 2002). The GALT of newly hatched chicks undergoes maturation with basal levels of cytokine expression detectable as early as 1-d of age (Bar-Shira et al., 2003; Bar-Shira and Friedman, 2018). After *S.* Enteritidis has invaded macrophages, the infection becomes systemic via macrophage migration throughout the circulatory system, leading to internal organ colonization and further stimulation of an adaptive immune response. The chicken's immune response to *S.* Enteritidis is largely a pro-inflammatory response (Tang et al., 2018). The proinflammatory response to *S.* Enteritidis has been demonstrated to be more pronounced than the response to *S.* Pullorum in chicken spleen and cecum (Tang et al., 2018). The evaluation of cecal and splenic cytokine

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expression can be considered representative of localized and systemic host response to *S.* Enteritidis, respectively.

In addition to their role in the initial innate response to pathogen challenge, macrophages are involved in transitioning to adaptive immune responses (Qureshi, 2003). Granulocyte-macrophage colony-stimulating factor (**GM-CSF**) is a pivotal cytokine for the initial activation of macrophages and other phagocytic cells and has been reported to drive differentiation of dendritic cells in mammals and avian bone marrow cells (Avery et al., 2004). The Major Histocompatibility Complex (**MHC**) class II is central in the initiation of an adaptive immune response. The MHC class II receptor is involved with antigen presentation between antigen presenting cells and T-cells. The MHC class II is a heterodimer comprised of alpha and beta chains expressed primarily on antigen presenting cells including macrophages, dendritic cells, and phagocytes. Variation among avian MHC haplotypes contributes to host resistance or susceptibility to many pathogens (for review see Miller and Taylor, 2016).

In avian species, as in mammals, a balanced adaptive immune response centers on properly coordinated Th1 and Th2 cell activation and regulation by Treg cells (Degen et al., 2005; Mayne et al., 2007). In chickens Interleukin 2 (**IL2**) and IL15 are cytokines with primary activities of T cell proliferation and natural killer cell activity enhancement (Lillehoj et al., 2001). A controlled and balanced inflammatory response is maintained in part by anti-inflammatory Treg cytokines such as transforming growth factor-β1 (**TGF-β1**) and IL10 (Rothwell et al., 2004; Belkaid and Rouse, 2005; Swaggerty et al., 2006; He et al., 2011). In poultry, splenic expression levels of IFN- γ and TGF-β1 mRNA were elevated during early stages of an initial immune response to *S.* Typhimurium (Beal et al., 2004). In an in vitro study, *S.* Enteritidis-exposed heterophils from a *Salmonella* resistant line had elevated proinflammatory cytokine mRNA expression but reduced TGF-β1 when compared to a susceptible line (Swaggerty et al., 2006). In mice, suppressor of cytokine signaling 3 (**SOCS3**) modulates expression of proinflammatory cytokines such as IL6 in murine macrophages and neutrophils (Fujimoto and Naka, 2003). In mouse dendritic cells, SOCS3 inhibits IL12 expression, thus directing the adaptive immune response toward a Th2 response (Li et al., 2006).

Genetic line differences have been described for the immune response in chickens to *S.* Enteritidis (Swaggerty et al., 2014), *Campylobacter jejuni* (***C. jejuni***, Swaggerty, 2017). In these studies, lines were differentially selected for high and low proinflammatory cytokines expression and the resulting high line was more resistant to both *S.* Enteritidis and *C.* jejuni. Additionally, genetic line differences have been demonstrated for resistance to *S.* Pullorum when comparing Rhode Island Red, local Chinese line and a dwarf layer synthetic line (Li et al., 2018). The objective of this study was to determine genetic line differences in the mRNA expression level of a select set of cytokines and immune regulatory genes after early age exposure of chickens to *S.* Enteritidis to gain more insight into the genetics of host immune response.

MATERIALS AND METHODS

Animals and Experimental Design, Salmonella *Challenge and Tissue Collections*

Three genetically distant chicken lines were utilized. They originated from a closed-population broiler breeder male line, a highly inbred Leghorn line and a highly inbred Fayoumi line (Lamont, 2003). The protocols for housing, Salmonella challenge and tissue collections have previously been described (Abasht et al., 2009). Briefly, 24 chicks from each line were equally divided into two BSL-2 animal rooms. The chicks were housed on wood chip-covered floors, received no vaccinations, and were given access to water and feed ad libitum that met or exceeded National Research Council requirements (1994). At 1 d of age, the chicks were either challenged with 1×10^4 cfu of *S.* Enteritidis via intraesophageally inoculation in 0.25 mL of Luria-Bertani broth, or mock-inoculated with 0.25 mL of Luria-Bertani broth. At 2 or 18 h postinoculation (**PI**), chicks were euthanized by cervical dislocation. The spleen and 1 cecum from each chick were aseptically extracted and rinsed with sterile PBS. The cecal samples were flushed with sterile PBS to remove the internal contents. Samples were snap-frozen in liquid nitrogen and stored at −80°C until RNA extraction. All animal procedures were approved by the Institutional Animal Care and Use Committee and conducted accordingly.

Total RNA Isolation and Quantitative PCR

Total RNA was isolated following the manufacturer's directions with an RNAqueous Kit (QIAGEN, Germantown, MD). Expression levels were evaluated for GM-CSF, IL2, IL15, and TGF-β1 mRNA and 28S rRNA using published primers (Kaiser et al., 2000; Kaiser and Lamont, 2002; Kogut et al., 2003; Avery et al., 2004). Using the web-based software, Primer3, primers were designed for SOCS3 (F- 5′GCCCCAGGTGATGGTGTA3', R- 5′CTTAGAGCTGAACGTCTTGAGG3' from AF424806), P20K (F- 5′ CTAGGGAGCGGAACTACACG'3, R- 5′GTTTGGGAAGCAGCATTCAT3' from NM205422.1) and MHC class IIβ (F- 5′GTGCAGAGGAGCGTGGAG3', R- 5′CGTTCAGGAACCACTTCACC3' from U02881). The genes were selected because they represented classical immune response to intercellular pathogens and/or were differentially expressed in prior microarray experiments of chickens challenged with *S.* Enteritidis (Zhou and Lamont, 2007). Quantitative reverse

transcriptase PCR (**qRT-PCR**) was performed using a QuantiTect SYBR Green RT-PCR kit (QIAGEN) as previously described (Kaiser et al., 2006). The relative mRNA expression levels were adjusted for PCR efficiency and the starting template concentration was normalized with 28S rRNA qRT-PCR values.

Adjusted Ct value is calculated by:

$$\text{Adj. Ct} = \{40 - [(\text{Mean Ct}_{\text{test gene}} + [(\text{Median}_{28S}) - (\text{Mean Ct}_{28S})]) * (\text{Slope}_{\text{test gene}}/\text{Slope}_{28S})]\}$$

Statistical Analysis

Quantitative PCR statistical analyses were performed with JMP (SAS Institute, 2000). For each gene, the General Linear Model was used where y = adjusted Ct values of gene and fixed main effects were line (broiler, Leghorn and Fayoumi), time post-infection (2 or 18 h) and challenge (*S.* Enteritidis or mock-infected) and random effect of PCR plate. All 2-way interactions were tested; the final model for each gene removed all 2-way interactions of fixed main effects for which $P \geq 0.15$. Comparisons within significant variables were ranked by Tukey Honestly Significant differences test (**HSD**; SAS, 2000; Cary, NC).

Cluster analysis was conducted on the combined data of the current study with that of 2 previous studies (Cheeseman et al., 2007; Abasht et al., 2009) that evaluated expression of different genes on the same RNA samples. Genes were assigned into five immune function categories (Th1, Th2, Treg, inflammation, and innate responses; Table 1). Recognizing the pleotropic nature of some cytokines, each gene was assigned to only one primary, functional category. The adjusted Ct values were imported into R (version 4.1.0) and log2 fold changes were calculated as the difference of the challenged vs. nonchallenged average Ct values within the 6 groups (3 lines × 2 tissues). Times were pooled because of little effect on gene expression. Hierarchical clustering of the genes was performed by tissue, plotted with heatmap.2 function from the gplots package in R and the optimal number of clusters was determined with the average silhouette method from the NbClust package in R.

Table 1. Functional grouping by immune response and source of RNA expression data for the purpose of conducting combined analysis.

Immune functional group	Gene	Source of data
Innate	TLR4	Abasht et al., 2009
	TLR2	Abasht et al., 2009
	TLR5	Abasht et al., 2009
Inflammation	GM-CSF	Current study
	MIP-1β	Cheeseman et al., 2007
	IL8	Cheeseman et al., 2007
	IL1β	Cheeseman et al., 2007
Th_1	IL15	Current study
	IL2	Current study
	IL12α	Cheeseman et al., 2007
	IL12β	Cheeseman et al., 2007
	IL18	Cheeseman et al., 2007
	IFN-γ	Cheeseman et al., 2007
	MHC IIβ	Current study
T_{reg}	TGF-β1	Current study
	SOCS3	Current study
	P20K	Current study
Th_2	IL10	Cheeseman et al., 2007
	IL6	Cheeseman et al., 2007

RESULTS

Effect of S. Enteritidis Challenge on Immune Gene mRNA Expression Levels

The *S.* Enteritidis challenged chicks had significantly higher SOCS3 and P20K mRNA expression in both spleen and cecum than did unchallenged chicks (Table 2, Figures 1A and 1B). For MHC class IIβ, however, challenged chicks had significantly lower splenic mRNA expression than unchallenged chicks (Figure 1A).

Effect of Genetic Line on Immune Gene mRNA Expression Levels

Genetic line had a significant effect on the mRNA expression of IL15, TGF-β1, SOCS3, and P20K in the spleen (Table 2; Figure 1C). Leghorn spleens had significantly lower IL15 and significantly higher SOCS3 mRNA expression levels than the other 2 lines. Broiler spleens had significantly lower TGF-β1 mRNA expression than the other lines. Leghorn spleens had significantly greater P20K mRNA expression than Fayoumi while the broilers were intermediate. Line had a significant effect on mRNA expression levels of MHC class IIβ and P20K in the cecum (Table 2; Figure 1D). Both Fayoumi and Leghorns had higher MHC class IIβ mRNA expression in the cecum than the broiler lines, while the Leghorn only had higher P20K mRNA expression than the other lines.

Effect of Tissue-Harvest Time on Immune Gene mRNA Expression Levels

Tissue-harvest time had little effect on mRNA expression, with significant effects on only 2 genes in the spleen (Table 2) and none in the cecum (Table 2). Interleukin IL15 and P20K mRNA expression levels in spleens were significantly greater at 18 than 2 h.

Table 2. Effect of genetic line, *S.* Enteritidis challenge, and tissue-harvest time on mRNA expression levels from spleen and cecum of three chicken lines (significance levels [$P(F)$] of values derived from GLM).

		Gene assay						
Tissue	Variable	GM-CSF	IL2	IL15	TGF-β1	SOCS3	P20K	MHC IIβ
Spleen	Line	0.62	0.71	**0.00**[1]	**0.01**	**0.01**	**0.02**	0.26
	Challenge	0.93	0.89	0.97	0.90	**0.00**	**0.00**	**0.03**
	Time	0.43	0.66	**0.00**	0.98	0.27	**0.02**	0.66
Cecum	Line	0.49	0.12	0.36	0.57	0.32	**0.00**	**0.00**
	Challenge	0.56	0.87	0.13	0.49	**0.01**	**0.01**	0.57
	Time	0.09	0.67	0.15	0.39	0.84	0.50	0.46

[1]Bolded values are $P < 0.05$.

Interactions of Fixed Effects on Immune Gene mRNA Expression Levels

Of the 42 two-way interactions, only 6 (14%) were significant at $P \leq 0.05$ and therefore included in the final statistical model. There were two interactions of line by time postinfection. The Leghorn spleens at 2 h postinfection expressed significantly lower IL15 mRNA levels than all other harvest time by line groups ($P = 0.03$, data not shown) and for MHC class IIβ ($P = 0.02$) expression in the cecum, Leghorns at 18 h were higher than broilers at either time point (Figure 1E). The three significant interactions for line by challenge were on expression of SOCS3 ($P < 0.01$), P20K ($P < 0.01$), and MHC class IIβ ($P < 0.04$) in the spleen (Figure 1F). In the main model, there was a single significant interaction of time postinfection and challenge; for IL2 expression in the spleen ($P = 0.02$, data not shown) which did not differ when ranked by Tukey HSD.

Joint Cluster Analysis of Three Studies on Immune Gene Expression Response to Infection in Spleen and Cecum Among the Three Genetic Lines

Figure 2 displays the results of cluster analysis for each of the tissues with sample times pooled within tissue since sample times did not statistically differ. Hierarchical clustering analysis established the gene placement and distance across the heat map independent of the 5 predefined immune functional group. For both the spleen and cecum, gene expression profiles in response to *S.* Enteritidis challenge were line and tissue dependent (Figure 2). Overall, the cecum showed stronger RNA expression changes relative to the spleen for the immune genes as shown in the bolder coloring of the fold changes in the heat maps. Cluster analysis with 19 genes from the 3 studies combined showed 5 clusters for spleen (Figure 2A) and 4 clusters for cecum (Figure 2B). The gene clusters are mostly composed of multiple functional groups rather than being based on a priori information about each gene's functional grouping, except for Cluster 4 of spleen, where 3 of the 4 inflammation genes (MIP-1β, IL1β, and IL8) were grouped (Figure 2A).

In the spleen (Figure 2A), Cluster 4 (MIP-1β, IL1β, and IL8) and 5 (SOCS3, IL12α, P20K, IL10, IL6, and IFN-γ) showed a strong increase in expression after *S.* Enteritidis infection for the Leghorn line that was generally absent in the broiler and Fayoumi lines. Cluster 1 (IL18, GM-CSF, IL2, TGF-β1, and MHC IIβ) and 3 (IL15, TLR4, and TLR2) were genes that had increased expression that was generally unique for the Fayoumi line with Cluster 3 genes being depressed in the Leghorn line. Cluster 2 contained IL12β and TLR5 that did not show a strong response in the spleen for all 3 lines of birds.

In the cecum (Figure 2B), Cluster 3 (SOCS3 and IL2), and 4 (TGF-β1, IL15, MHC IIβ, IL18, TLR4, and P20K) showed an increase in expression for the Leghorn line; broiler, and Fayoumi lines also showed increased expression for Cluster 4 genes. Cluster 4 consisted of genes from Th1 (IL15, MHC IIβ and IL 18), Treg (TGF-β1 and P20K), and Innate (TLR4) categories. Cluster 1 (IL12α, IL1β, IFN-γ, IL12β, and MIP-1β) contained genes that had decreased expression for all 3 lines. Cluster 2 (IL6, TLR2, TLR5, IL10, IL8, and GM-CSF) contained genes that did not show a strong response in the cecum for all 3 lines of birds.

DISCUSSION

Role of S. Enteritidis Challenge on GM-CSF, IL2, IL15, SOCS3, P20K, MHC Class IIβ, and TGF-β1 mRNA Expression

After oral infection, the initial colonization occurs in the digestive tract, including the cecum. Colonization of the cecal lumen by *S.* Enteritidis can occur as early as 3 h postinfection whereas colonization of internal organs occurs sometime after 24 h postinfection (van Immerseel et al., 2002). A more rapid inflammatory cytokine response was observed in the intestinal tract (jejunum, ileum, and cecal tonsils) than spleen for 1-day-old chicks after exposure to *S.* Typhimurium (Withanage et al., 2004). Of the RNA expression levels measured in the current study, an overall strong Th1 (MHC IIβ) and Treg (P20K and SOCS3) response to *S.* Enteritidis challenge was observed in the spleen while a primarily Treg (SOCS3 and P20K) response was detected in the cecum. A distinct line effect of Th1 (MHC IIβ) and Treg (P20K) associated immune genes also had increased in response to *S.* Enteritidis challenge in the cecum of Leghorn and Fayoumi, suggesting the activation of a Th1 and Treg adaptive immune response in these lines.

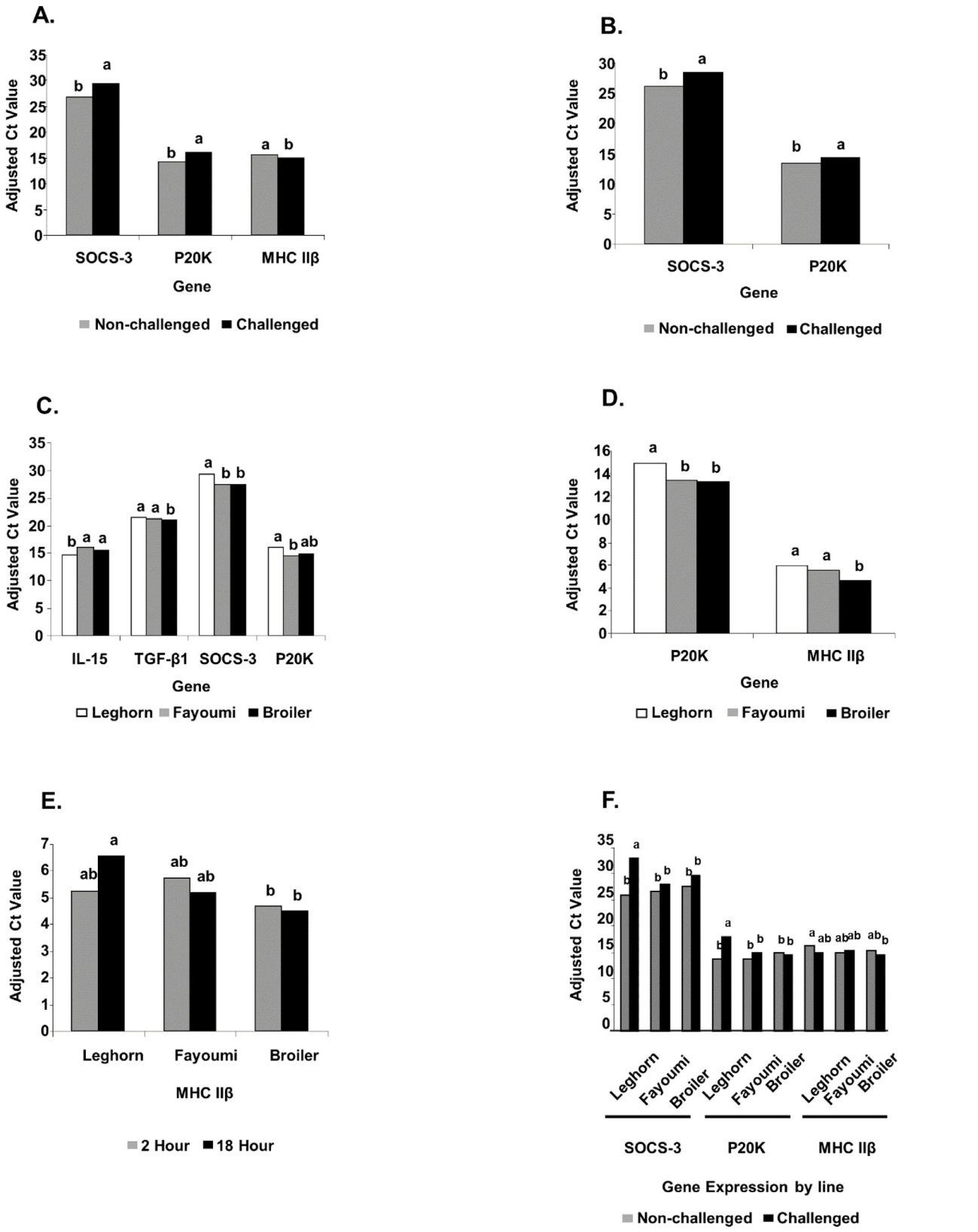

Figure 1. Mean mRNA expression levels for experimental variables with significant ($P \leq 0.05$) effect: *S.* Enteritidis challenge effect on (A) spleen and (B) cecum; line effect on (C) spleen and (D) cecum; (E) interaction of tissue-harvest time and line on cecum MHC class IIβ mRNA expression levels and (F) interaction of line and *S.* Enteritidis challenge on spleen SOCS-3, P20K, and MHCIIβ mRNA expression levels. For comparisons within a gene, different letters indicate a significant difference at $P \leq 0.05$ as determined by Tukey HSD test.

Given the previously reported minimum interval of 3 h between *S.* Enteritidis exposure and tissue colonization of the cecal lumen (van Immerseel et al., 2002), it would be expected that at 18 h postinfection, the cecum would be colonized with *Salmonella* while the spleen may not yet be colonized. The mRNA expression data from the current study suggest that Th1 (IL15) and Treg (P20K) immune activation in the spleen is greater

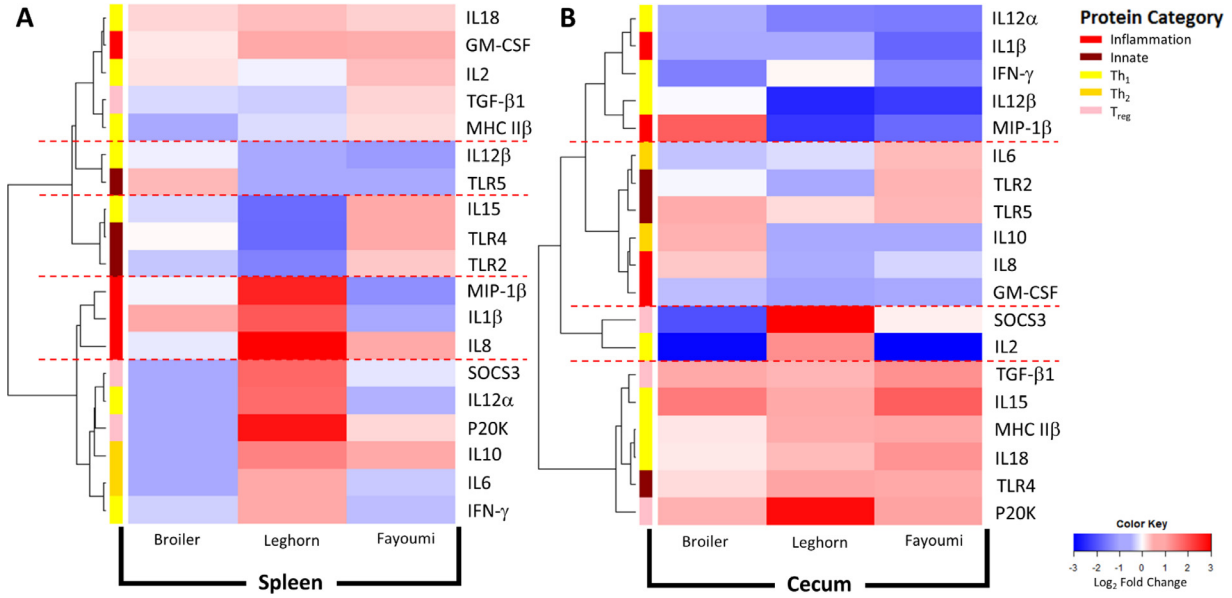

Figure 2. The log2 fold change heat maps for combination of genes from Cheeseman et al., Abasht et al. and the current study for (A) spleen and (B) cecum and the three chicken lines. Genes are clustered based on hierarchical clustering and clusters are demarcated by dotted red lines. Red color represents increase log2 fold change and blue color represents decrease log2 fold change. Functional protein categories are shown to the left of the heat maps for inflammatory response (red), innate immune response (dark red), Th1 associated cytokines (yellow), Th2 associated cytokines (orange), and Treg associated cytokines (pink).

at 18 h than 2 h postinfection. To the best of the authors' knowledge, this is the first report of quantified RNA response to *S.* Enteritidis challenge 2 h postinfection in either the spleen or cecum of chickens. Furthermore, the mRNA expression profiles demonstrate the tissue-specificity of immune-related gene expression at a systemic (spleen) vs. a localized (cecum) site. Immune response differences of the systemic and localized systems have been demonstrated to be independent of each other in mice (Alpan et al., 2001), as well as suggested in chickens (Kaiser and Lamont, 2001). The early differential expression of immune genes, particularly in the spleen, following pathogen exposure underscores the importance of coordinated immune response in early systemic pathogenic challenge.

In mice, dendritic cells transduced with SOCS3 decreased surface expression of MHC class II (Li et al., 2006). This suggests that the elevated expression of SOCS3 observed in challenged chicks may be suppressing MHC class IIβ expression in chicken spleen, thus shifting the Th1/Th2 paradigm toward that of a Th2 immune response to *S.* Enteritidis challenge via negative regulation of the JAK/STAT pathway. In mice, MHC class IIβ mRNA expression is upregulated by IFN-γ (Trinchieri and Perussia, 1985). One method by which a pathogen attempts to escape the immune system may be to induce SOCS3, thereby inhibiting IFN-γ activation, in turn downregulating MHC class IIβ expression (Bertholet et al., 2003; He et al., 2011). We hypothesize that *S.* Enteritidis, an intracellular pathogen, induces SOCS3 as a means to interrupt the IFN-γ pathway, which would include IL12, IL18, and MHC class IIβ, thereby circumventing activation of the host Th1 immune system.

Genetic lines significantly differed for mRNA expression levels of immune-related genes in both spleen and cecum. Of the 3 lines evaluated in this study, the Leghorn mRNA expression profile was the most unique, in that the expression profile of the Leghorn line tended to differ from the other two lines within the 5 functional gene groups. Leghorn spleens had higher mRNA expression levels for both regulatory genes (TGF-β1 and SOCS3) and the quiescent-related gene (P20K), but lower expression of IL15. Previous studies using the same spleen tissue samples demonstrated line differences in which Leghorn had lower TLR2, TLR4, and TLR5 mRNA expression levels in *S.* Enteritidis challenged chicks (Abasht et al., 2009) and overall higher mRNA expression levels of combined challenged and non-challenged chicks for proinflammatory chemokines (CXCLi2, CCLi2), the Treg cytokine IL10 and the Th1 cytokine IL12α (Cheeseman et al., 2007). Leghorn ceca were previously reported to express greater mRNA levels of IL18 and lower levels of CCLi2, IL12α, and IL12β than the other lines (Cheeseman et al., 2007).

Outcome of Joint Cluster Analysis on the mRNA Expression Profiles Following S. Enteritidis Challenge

RNA expression data from the current study was combined with data from two previous studies that evaluated RNA expression of different genes on the same samples (PSJ101605) to perform hierarchical cluster analysis. Immune gene RNA expression level in response to *S.* Enteritidis differed across the 3 genetic lines and was also tissue-dependent.

In the spleen, the Leghorn line responded to *S* Enteritidis challenge primarily through increased expression of inflammatory genes (MIP-1β, IL1β, and IL8) and genes from Th1 (IL12α and IFN-γ), Th2 (IL6 and IL10) and Treg (SOCS3 and P20K) suggesting an inflammatory response with some T-helper cells involvement. In contrast, the Fayoumi line responded in the spleen primarily through mild increase expression of innate genes (TLR4 and TLR2), Th1 (IL2, IL15, IL18, and MHC IIβ), and Treg (TGF-β1) suggesting a greater innate immune response with a different set of T-helper cells involvement from the Leghorn line. The broiler line response was generally milder, which was more similar to the Fayoumi line.

In the cecum, the genetic effect among the 3 lines was much more consistent including T-cell mediated response with increased expression of genes from Th1 (IL15, IL18, MHC IIβ) and Treg (TGF-β1 and P20K) groups and decreased expression of genes from Th1 (IL12α, IL12β, and IFN-γ) and inflammation (IL1β and MIP-1β) groups. There were a couple of notable exceptions; the Leghorn line had a strong increased expression for SOCS3 (Treg) and IL2 (Th1) that had decreased expression in both broiler and Fayoumi lines.

Combined analysis of the data from the current study and previous companion studies suggest that there are genetic differences in immune response to *S.* Enteritidis in which the Leghorn line utilizes more of an adaptive immune response than the Fayoumi and broiler lines. These genetic differences in immune response mRNA profiles could also be associated with disease resistance (Swaggerty et al., 2017). It has been suggested as a general phenomenon that indigenous breeds have greater disease resistance through natural selection over multiple generations (Schou et al., 2010). The mRNA expression profile identified in this study from the non-commercially selected Fayoumi line, which is generally more resistant to pathogen challenge (Deist et al., 2017; Wang et al., 2014), may thus constitute a desirable profile of gene expression for disease resistance. The nonselected Fayoumi line has also been reported to be more immunologically responsive to *S.* Enteriditis when compared to the same Leghorn and broiler lines (Redmond et al., 2009). In that study, heterophils of the 3 lines were challenged in vitro with *S.* Enteritidis and the Fayoumi line had greater expression level of IL10, IL6, TGR-β1, and GM-CSF with an intermediate expression of TLR4 RNA.

Previously it has been demonstrated that macrophages isolated from the Leghorn line utilized in the current study are hyporesponsive to LPS stimulation and have low expression levels of inducible nitric oxide synthase (**iNOS**) mRNA, compared to another Leghorn line and a macrophage cell line, MQ-NCSU (Hussain and Qureshi, 1997). Additionally, production of nitric oxide in HD11 cells is suppressed by *S.* Enteritidis challenge (He et al., 2011). The lower expression profile of Leghorn for the innate functional genes, in particularly TLR4 at a systemic infection site (spleen) supports the Leghorn's relative hyporesponse to LPS, compared to the Fayoumi line. As a pivotal antigen of Gram negative bacteria such as *S.* Enteritidis, immune response to LPS has been used to model a Gram negative challenge (Monson et al., 2019). Additionally, macrophages from a *Salmonella*-resistant chicken line had a more active proinflammatory response to *S.* Gallinarum and *S.* Typhimurium than a *Salmonella*-susceptible line, suggesting that the resistant chicken line may be more capable of inducing a cellular response to *Salmonella* challenge (Wigley et al., 2006). This hypothesis suggests that, for the current study, the Fayoumi and Leghorn lines, which have an overall more immune activated response compared to the broiler line in the cecum, are more resistant to *S.* Enteritidis challenge than the broiler.

Chickens that are susceptible or resistant to *S.* Enteritidis challenge have differences in RNAseq data that demonstrate immune responsiveness differences (P. Li et al., 2018). The combined joint analysis illustrates that the 3 lines have differing mRNA expression profiles in response to *S.* Enteritidis challenge, thus demonstrating genetic variation altering the overall immune response. The genetic variation in immune response among these lines is further supported by the previous report that these three genetic lines differed in their immune gene expression profiles in response to having β-glucan or ascorbic acid added to the diet as an immune modulator (Redmond et al., 2010).

Collectively, these data demonstrate that the genetic lines differ in immune response to *S.* Enteritidis at both local and systemic immune sites. Furthermore, these genetic line-based immune response profile differences to *S.* Enteritidis challenge vary in magnitude across the various facets of the immune response. Therefore, immune response differences to *S.* Enteritidis infection are likely driven by specific immune functions which are preferentially activated in different tissues in a line-dependent manner. These differences in immune response profiles help to further define disease resistance/susceptibility within specific chicken lines.

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DISCLOSURES

The authors have no conflicts of interest to disclose.

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