The Effect of Salmonella Enteritidis on Immune Genes in Three Different Lines of Chickens

A.S. Leaflet R2482

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Summary and Implications

Spleens from twelve Broiler, twelve Leghorn, and twelve Fayoumi chickens were randomly chosen from a group of 212 birds that were either unchallenged birds or had been given *Salmonella Enteritidis*. The birds were 5-7 month old and were given 1×10^8 *Salmonella* dose 3 times by way of mouth. The spleens from these birds were removed 10 days after the infection. The expression levels of the mRNA of IL-6, IL-8, IL-10, IL-18, MIP1β, INF-y, TGFβ1, and Rantes were analyzed by qRT-PCR analysis.

Line, challenge, and line by challenge interaction were all considered fixed effects and were tested. All non-significant effects left out. The Student's T test was used to determine rankings of each effect. Two line effects along with a line by challenge interaction were observed. There was a line effect associated with INF-γ expression. There was also a line effect observed in RANTES. Fayoumis expressed RANTES significantly lower than Broilers or Leghorns. Lastly, there was a line by challenge interaction observed for IL-6. The difference in the expression levels displayed by the three different lines may indicate the use of very distinct immune mechanisms. This could be used in future breeding strategies to produce a bird that is resistant to Salmonella Enteritidis and other pathogens.

Introduction

Salmonella is a pathogen that can be found in chickens has serious financial and health implications. *Salmonella* can infect the consumer of meat or eggs produced by infected chickens. *Salmonella Enteritidis* and *Salmonella Typhimurium* are the most common forms of *Salmonella* isolated from humans (Patrick et al., 2004). In order to reduce the occurrence of Salmonella contamination in the layer and broiler industry there has been considerable research conducted. *Salmonella Enteritidis* has been shown to affect the expression of many genes associated with innate and acquired immune responses. The objective of this study is to examine the gene expression of IL-6, IL-8, IL-10, IL-18, MIP1β, INF-γ, TGFβ1, and RANTES in three genetically distinct lines (Broiler, Leghorn, and Fayoumi) in order to see if one line is more susceptible than the others, in relation to *Salmonella Enteritidis* infection.
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Materials and Methods

The samples

Spleens from twelve Broiler, twelve Leghorn, and twelve Fayoumi chickens were randomly chosen from a group of 212 birds that had been either challenged with *Salmonella* or were unchallenged birds. The birds were mature (5-7 month old) and were orally inoculated with 1×10^8 *Salmonella* dose 3 times. The spleens from these birds were removed 10 days after infection.

Quantitative Real Time PCR Analysis

The expression levels of the mRNA of IL-6, IL-8, IL-10, IL-18, MIP1β, INF-y, TGFβ1, and Rantes were analyzed by qRT-PCR analysis. The qRT-PCR was performed on the twelve spleen samples from each line that represented six challenged and six unchallenged birds. Each of the reactions were run in duplicate and consisted of approximately 50ng of total RNA, 12.5ml of QuantiTect SYBR Green master mix, 0.25ml QuantiTect RT mix, forward and reverse primers, and RNAse-free water for a final volume of 25ml. An Opticon 2 machine was used to run each reaction.

Statistical Analysis

The expression level of each sample ran in duplicate was analyzed using JMP software (SAS Institute, 2004). Line, challenge, and line by challenge interaction were all considered fixed effects and were tested. All non-significant effects ($P > 0.01$) were excluded. To determine rankings amongst classes within the fixed effects (line, challenge, and line by challenge) Student's t test was used.

Results and Discussion

Line effects along with a line by challenge interaction were observed. There was a line effect associated with INF-γ expression. Leghorns expressed INF-γ significantly lower than Broilers and Fayoumis. The Least Square Means values were -0.728, -0.743, and -2.72 for Broilers, Fayoumis, and Leghorns, respectively ($P = 0.0218$) (refer to Figure 1). There was also a line effect observed in RANTES. Fayoumis expressed RANTES significantly lower than Broilers or Leghorns. The Least Square Means values were 17.72, 15.78, and 17.22 for Broilers, Fayoumis, and Leghorns, respectively ($P = 0.0086$) (refer to Figure 2). There was a line by challenge interaction observed for IL-6. Challenged Fayoumis expressed IL-6 significantly lower than challenged as well as, unchallenged Broilers and Leghorns. The Least Squares Means are as follows 12.34, 11.18, 12.14, 12.47, 10.19, and 12.8 for challenged broilers, unchallenged broilers, challenged leghorns, unchallenged

leghorns, challenged Fayoumis, and unchallenged Fayoumis, respectively ($P = 0.0122$) (refer to Figure 3).

There are several important factors to consider when thinking about the varying gene responses of the three different lines. The first factor to consider is the time point in which the spleens were extracted. There were no Challenge effects observed and this could be in part due to the reduction of chemokine action as the time after infection is increased. Secondly, the different gene expression could be a result of these birds using a different mechanism to combat *Salmonella Enteritidis* infection. Lastly, the higher expression of certain genes during certain times may render certain lines to be more susceptible at specific time points of infection. The analysis of the expression levels of all of these genes can be considered and used in order to create future lines that can be resistant or have increased resistance to *Salmonella Enteritidis* and possibly other pathogens. This would result in increased production of layers, increased feed efficiency of Broilers, and an overall reduction in mortality of all lines and breeds.

Gene	Line Effect	Challenge Effect	Line-by-Challenge Interaction
IL-6	0.3870	0.2284	**0.0122**
IL-8	0.7601	0.6970	0.4620
IL-10	0.8526	0.7834	0.1430
IL-18	0.6729	0.7045	0.5043
INF-γ	**0.0218**	0.6883	0.2998
MIP1β	0.9524	0.4087	0.1214
RANTES	**0.0244**	0.6396	0.4536
TGFβ1	0.3378	0.6550	0.2494

Table 1. *P* values for IL-6, IL-8, IL-10, IL-18, INF-γ, MIP1β, RANTES, and TGFβ1, in the spleen of mature birds that have been infected with *Salmonella Enteritidis*. Significant P values in bold.
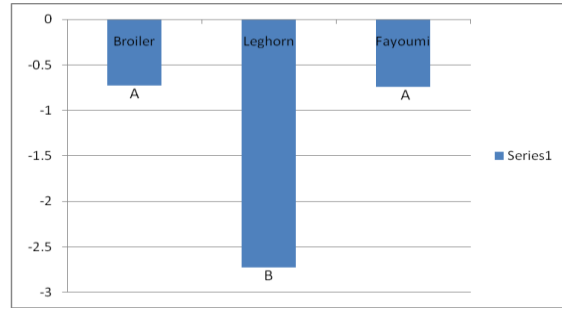

Figure 1. Mean INF-γ mRNA expression levels by breed, in the spleen of mature birds. Bars not sharing a letter are shown to be significantly different by Student's t test.
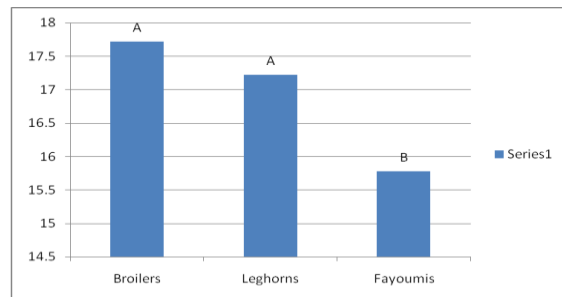

Figure 2. Mean RANTES mRNA expression levels by breed, in the spleen of mature birds. Bars not sharing a letter are shown to be significantly different by Student's t test.
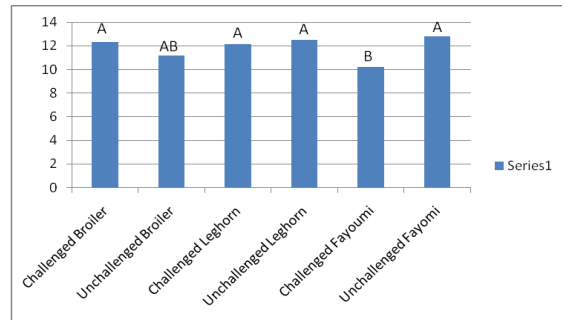

Figure 3. Mean IL-6 mRNA expression levels by breed, in the spleen of mature birds. Bars not sharing a letter are shown to be significantly different by Student's t test.