

# Genetic Differences in Chicken Heterophil mRNA Expression in Response to *In-Vitro* Stimulation with *Salmonella enteritidis*

## A.S. Leaflet R2445

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### Summary and Implications

Heterophils were collected from chickens from genetically diverse lines and gene expression in response to *in vitro* stimulation with *Salmonella enteritidis* (SE) was measured for cells from each line. Heterophils are cells important in the initial detection of and mounting of an immune response to pathogens. Understanding the response of heterophils to stimulation with SE and how this response is different for diverse genetic lines may allow selection for increased resistance to this bacterial pathogen.

### Introduction

Food safety is a growing concern for both producers and consumers, with new regulations restricting use of antibiotics in animals and regular reports of human disease outbreaks due to contaminated animal and produce products. Improving the immune response of production animals is one way to combat this important issue. SE is a common pathogen transmitted from poultry to humans through the food supply. Investigating the heterophil response to SE will help better understand what immune mechanisms are important in successfully combating this bacterium.

### Materials and Methods

Inbred (Leghorn and Fayoumi) and outbred (Broiler) lines were evaluated for heterophil gene expression, with a focus on cytokines as an indicator of immune response. Heterophil samples were collected from 54 eight-week-old birds from each line and pooled within line in groups of three. Each sample was stimulated for 2 hours with or without SE and mRNA expression levels were evaluated for IL-6, IL-10, TGF- $\beta$ 4, and GM-CSF. Gene expression assays were performed by quantitative PCR with SYBR Green and the 28s housekeeping gene. A standard curve was generated using a serial dilution of template for each primer set. C(t) values were adjusted to account for the amount of template and reaction efficiency and evaluated with respect to this value as well as fold change.

### Results and Discussion

The analysis of C(t) values showed that genetic line was responsible for some of the differences in expression seen in IL-6 and GM-CSF, with higher expression levels of these genes observed in the Fayoumi line and in the broiler or Leghorn lines. The effect of exposure to SE alone was

only significant in altering the expression level of IL-10, due to lower levels of expression in the broiler and Leghorn lines and little change in the expression of the Fayoumi line. The interaction of line and exposure to SE was significant for IL-6, TGF- $\beta$ 4, TLR-4, and GM-CSF. These differences were due to the divergent responses of the broiler and Leghorn lines when compared to the Fayoumi line. In these cases the Fayoumi line showed a greater increase in expression of cytokines, which is an indicator of the initiation of an immune response.

### Acknowledgements

This research was supported the Iowa State University Center for Integrated Animal Genomics and National Research Initiative Grant no. 2004-35205-14234 from the USDA Cooperative State Research, Education, and Extension Service. SBR supported by a USDA-CSREES National Needs fellowship. Bird studies were done at the Iowa State University Poultry Research Center. Staffs at this facility and members of the Lamont lab are thanked for their help in animal care and data collection.

**Table 1. ANOVA model effects on heterophil gene expression levels (p-values).**

	IL-10	IL-6	TGF- $\beta$ 4	TLR-4	GM-CSF
Line	0.096	0.048	NS	0.056	0.0002
SE	0.004	NS	NS	NS	NS
Line*SE	NS	0.008	0.0009	0.029	0.0037
Hatch	0.013	NS	NS	NS	NS

NS = not significant ( $P > 0.1$ )

**Figure 1. Fold change in mRNA expression of stimulated heterophils over unstimulated heterophils.**



