

# Chicken Immune-related Gene Expression after Stimulation with Bacterial Component

## A.S. Leaflet R2999

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### Summary and Implications

The RNA expression levels of Caspase 1 and Interleukin 18 were quantified after a lipopolysaccharide (LPS) challenge. The proteins of these two genes are specifically involved with the inflammatory immunological response pathway of the chicken. Collectively, a greater understanding of RNA expression levels of immunologically related genes in the chicken genome under challenging situations could lead to increased effectiveness of future genetic selection and targeted therapeutics against prevalent diseases in the poultry industry. Results demonstrated the effectiveness of the LPS stimulation, and variation in expression among genetic lines.

### Introduction

Salmonella enteritidis (SE) is a species of gram-negative bacteria of special concern amid modern poultry production systems. Lipopolysaccharide (LPS) is a component of this and other gram-negative bacterial cell walls, and is frequently used to stimulate a mock immunological response without the biosecurity that comes with using a live pathogen. Using LPS as a model of a gram-negative bacterial infection amongst genetically distinct lines of chickens could aid in the potential identification of genetic variances these lines hold. Identification of these variances could allow advantageous immunological responses to be incorporated into modern breeding programs for commercial production through genetic selection.

The two genes studied were Caspase 1 and Interleukin 18. Caspase 1 codes for an enzyme that cleaves inactive Interleukin 18 gene product into its active form. Interleukin 18 codes for a pro-inflammatory cytokine found within macrophages. Once activated, this cytokine aids in the further activation of Natural Killer and T cells of the immune system.

### Materials and Methods

#### *Experimental design*

Thirty adult male birds were used in the experiment. Fayoumi, Leghorn and broiler genetic lines maintained at the Iowa State poultry research facilities each contributed 10

individuals in the study population. Six birds from each line were intravenously injected in the brachial wing vein with LPS, and four birds served as controls intravenously injected with phosphate buffered saline (PBS). Blood samples were collected at the 1 and 3 hour time points for each bird post-injection of LPS or PBS. White blood cells were then isolated from these blood samples and preserved in RNAlater until RNA was isolated. RNA isolations for individual samples were performed using an RNAqueous Kit from Ambion under standard protocol.

#### *Quantitative Real-Time PCR Analysis*

Quantification of the isolated RNA from the samples was performed using real-time quantitative PCR (RT-qPCR) methods via QuantiTect SYBR Green RT-PCR kit. Under this technique, we reverse transcribed RNA to cDNA for amplification and quantification. Samples were run in triplicate, and in accompaniment with the 28s gene, which is used to normalize the variation in total RNA among samples. Statistical analysis was performed via JMP software with the following model:

$$y = \text{line} + \text{LPS/PBS} + \text{time the interactions} + e$$

All interactions with a P value of  $>.05$  were excluded from the analysis. A Student's T test was used in the interaction between line and treatment for Caspase 1 RNA expression.

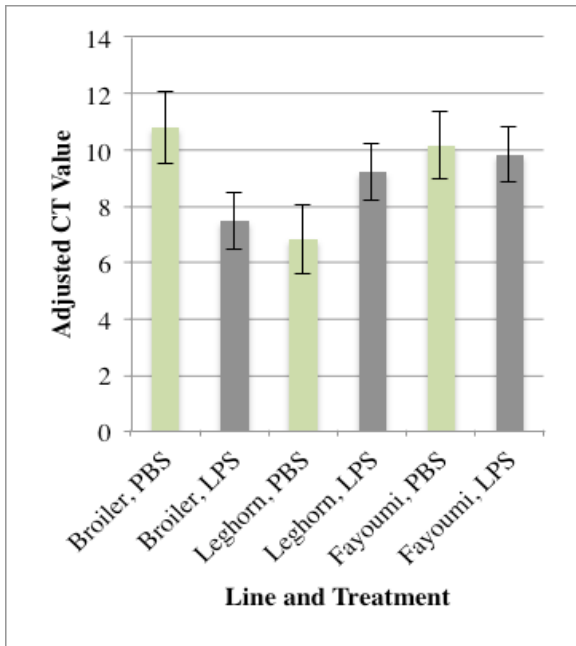
### Results and Discussion

Interaction between line and treatment for Caspase 1 RNA expression level was found to be significant. Broiler birds injected with PBS showed the highest expression of Caspase 1 amongst all lines utilized (Figure 1). This expression level was significantly different from broiler LPS stimulation. Leghorns injected with PBS had the lowest expression levels of Caspase 1 between all three lines, but showed no statistical significance in difference between PBS and LPS stimulation for this gene. Fayoumi differential RNA expression levels of Caspase 1 were not statistically significant between treatment types. Results suggest a difference in expression of Caspase 1 between different breeding populations of chickens. The variation shown could lead to increased validity when selecting for or modulating change in avian immune response through this gene in the future.

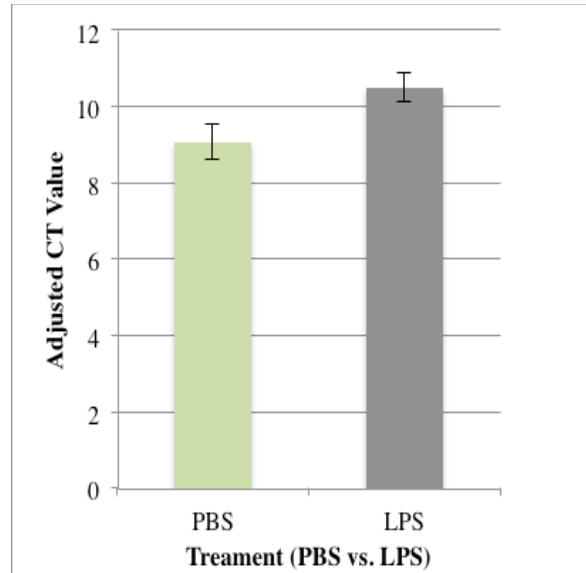
In regard to Interleukin 18 RNA expression levels, response to treatment was found to be significant. Birds injected with LPS were found to have had a greater least square mean than birds injected with PBS (Figure 2). The results suggest Interleukin 18 is up regulated under immunological challenge induced by gram-negative bacterium.

**Acknowledgements**

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**Figure 1: Interaction of line and LPS treatments on Caspase 1 RNA expression levels in chicken white blood cells (means with standard error bars).**



**Figure 2: Treatment effect of LPS on Interleukin 18 RNA expression levels in chicken white blood cells (means with standard error bars).**