

Impacts of *Salmonella* Enteritidis Infection on Liver Transcriptome in Broilers

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Summary and Implications

Salmonella is a bacterium that can infect chickens, contaminating meat and eggs, resulting in production losses and illness in consumers. This study was designed to analyze the metabolic effects of *Salmonella* infection in broilers. Livers were harvested from broilers at ten days post-infection with *Salmonella*. RNA was isolated and then transformed into cDNA. Genes were analyzed on a global level using microarray technology. Among the differentially expressed genes with known function, gene expression of 29 out of 30 genes was lower in *Salmonella*-challenged birds than non-challenged birds. Genes associated with the cell cycle along with DNA replication, recombination, and repair functions were the most significantly differentially expressed genes. *Salmonella* infection resulted in reduced expression of genes involved in cell death, protein recycling, blood pressure increase, cell proliferation, extracellular kinase activation and suppression of immune signaling. The revealed pathways help to determine the systemic effects of *Salmonella* infection, which may lead to genome-directed methods of disease control in poultry.

Introduction

Salmonella is a gram negative bacterium which results in production losses and illness in consumers. *Salmonella* infection can occur in the egg or after hatch. Contamination of eggs occurs through maternal exposure in infected hens or contact with fecal material from infected chickens. Transmission in chickens can occur through environmental factors such as infected fecal material, air, litter, unclean facilities, and vectors, such as rodents and humans. Once the chicken is infected, *Salmonella* colonizes the intestines and spreads throughout the body, reaching the liver and spleen within a day of infection. Bacterial infections can have generalized impacts on physiology. *Salmonella* infection results in a reduction in feeding, reduced body weights, and malnutrition. Malnutrition and metabolic disease both can lead to fatty liver pathology in chickens.

Although several studies have determined the effects of *Salmonella* infection on expression of immune genes and the associated gene networks in chickens, less is known about how *Salmonella* infection affects genes and gene

networks involved in general metabolic processes. The objective of this study is to provide insight into the systemic metabolic effects of *Salmonella* infection in broilers.

Materials and Methods

The samples

Broiler hens of approximately 5 months were used. All birds were determined to be free of *Salmonella* before the study began. Eight birds were challenged orally with *Salmonella*, and 8 were mock-challenged. Livers were harvested 10 days after infection.

Microarray Analysis

Total RNA was isolated from each liver sample. RNA samples were purified, labeled, and hybridized to a 44k microarray. After hybridization, gene expression was analyzed.

Quantitative Real Time PCR Validation

The expression levels of 8 genes differentially expressed by microarray were analyzed in triplicate by qRT-PCR. The adjusted cycle threshold (Ct) value of each sample was analyzed using ANOVA and JMP 8.0.2 software.

Results and Discussion

Forty-four genes were differentially expressed in response to *Salmonella*. At a suggestive level, 183 genes were differentially expressed in response to *Salmonella*. Among the genes with known function, there was a predominant trend of reduced gene expression in response to *Salmonella* (gene expression was higher in 29 of 30 genes in the challenged birds). Two gene networks were created using pathway analysis. One network involved cell morphology, cell cycle, organismal injury and abnormalities genes (Figure 1) and the other network involved metabolic disease, cardiovascular system development and function, and urological disease genes (Figure 2).

Genes associated with the cell cycle along with DNA replication, recombination, and repair functions were the most significantly differentially expressed genes (Figure 3). *Salmonella* infection resulted in a reduction in expression of genes involved in cell death, protein recycling, blood pressure increase, protein precursor activation, activation of extracellular-regulated kinase activation and suppression of immune signaling. The 8 most differentially expressed genes were confirmed by PCR analysis.

The reduced expression of four genes (a gene involved in cell death, a cell cycle checkpoint gene, and two genes that are responsible for the breakdown of recycling of proteins) from The Cell Morphology, Cell Cycle,

