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Summary and Implications

Toll-like receptors (TLR) recognize pathogen-associated molecular patterns (PAMP) of infectious microbes. Activation of TLR with PAMP can result in immune response by modulation of innate and adoptive immune system. This study aimed to investigate the acute effect of *Salmonella* challenge on TLR RNA expression in cecum and spleen of birds from different genetic lines. Chicks from broiler, Leghorn, and Fayoumi lines were challenged or mock challenged with *Salmonella*. The RNA expression levels of TLR2, TLR4 and TLR5 genes were assessed by quantitative RT-PCR in cecum and spleen tissue harvested at 2 or 18 h post-challenge. The results demonstrate a significant genetic line effect on TLR expression in the spleen of *Salmonella* infected birds, which may partly explain the genetic variability in immune response to *Salmonella enterica* serovar Enteritidis. The higher level of TLR2 and TLR4 RNA expression observed in the spleen of Fayoumi line compare to Leghorn and broiler lines in *Salmonella enterica* serovar Enteritidis challenged birds may be associated with the stronger immune response to the infection and might be useful characteristics to be considered in breeding immunocompetent chickens.

Introduction

Toll-like receptors are members of a class of cellular receptors known as pattern-recognition receptors, which recognize evolutionarily conserved molecular motifs (pathogen-associated molecular patterns, PAMP) of infectious microbes. Activation of TLR with PAMP can result in immune response by modulation of innate and adoptive immune system. At least 8 chicken homologs of mammalian TLR have been identified, so far. In vitro studies using chicken immune cells demonstrated that bacterial TLR agonists induce upregulation of RNA expression of the cytokines, nitric oxide production and oxidative burst.

There is limited information on the regulation of chicken TLR RNA expression by TLR agonist or after bacterial infection, or on genetic effect on this expression. Therefore, the current study was aimed to understand the genetic lines effect on TLR2, TLR4 and TLR5 RNA expression levels in cecal and splenic tissues after infection with *Salmonella enterica* serovar Enteritidis.

Materials and Methods

Two highly (>99%) inbred lines, Leghorn G-B2 and Fayoumi M 15.2 lines, and one outbred broiler line originated from a broiler breeder male line, were used. Twenty-four chicks from each line were equally divided into two biosafety-level-two animal rooms. At 1 day of age, the chicks in one room were intraesophageally challenged with 1 x 10^4 cfu *Salmonella* serovar Enteritidis in 0.25 ml Luria-Bertani broth, while the chicks in the second room were mock-inoculated with 0.25 Luria-Bertani broth.

At 2 or 18 h post-challenge, chicks were euthanized. The spleen and one cecum from each chick were aseptically extracted and rinsed with sterile PBS and individually quick frozen in liquid nitrogen. Total RNA was isolated from individual spleen and cecum tissue samples and TLR RNA expression were assessed by real-time quantitative RT-PCR.

Results and Discussions

A consistent pattern for line effect was observed for TLR2 and TLR4 RNA expression in the spleen of *Salmonella* challenged chicks (Figure 1). The Fayoumi chicks had significantly higher TLR2 and TLR4 RNA expression level than the Leghorns and the RNA expression level of these genes in broilers was between these two lines. The higher level of TLR2 and TLR4 RNA expression observed in spleen of Fayoumi line challenged with *Salmonella* in the current study may be associated with the stronger immune response to the infection.

In the spleen of *Salmonella* challenged birds, Fayoumi line have about the same level of TLR5 RNA expression as Leghorns, and both Fayoumis and Leghorns have significantly lower TLR5 RNA expression than broilers (Figure 1). Like in mammals and fishes, in chickens, bacterial flagellin acts as a TLR5 agonist and upregulate cytokines RNA expression. Therefore, broilers may respond more actively to the *Salmonella* flagellin stimulation in spleen compared to the Leghorn and Fayoumi lines.

In conclusion, the current study showed significant effect of genetic line on TLR RNA expression in the spleen of *Salmonella* challenged chicks that may partly

explain immune response differences between genetic lines.

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Figure 1. Line differences in TLR2, TLR4 and TLR5 RNA expression levels in cecum and spleen of *Salmonella* challenged birds. Bars represent least square means of adjusted Ct values and error bars represent standard errors (n = 22). Bars not sharing a common letter are different (P≤0.05, Tukey's test).