Exposure to Heat Stress and an Immune Stimulus Affects Gene Expression in Chicken Immune Tissues

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Summary and Implications

Gene expression changes after exposure to acute heat stress and/or to bacterial lipopolysaccharide (LPS) were investigated by measuring global gene expression in two immune organs, bursa and thymus, from two chicken lines, Fayoumi and broiler. Over 1,600 genes had significant expression changes in response to treatment; greater numbers were identified in bursa for Fayoumi and in thymus for broilers. Heat stress suppressed gene expression responses to LPS in both tissues. Both Heat and LPS impacted expression of immune cell trafficking genes; these pathways need to be investigated for potential to improve immune responses in heat-stressed chickens.

Introduction

Heat stress in poultry reduces growth and egg production and increases mortality, which causes losses for the industry. Exposure to high temperatures can reduce immune functions, further affecting poultry health and performance. Characterizing the mechanisms by which heat stress impacts the immune system will provide a first step towards development of methods to improve disease resistance in commercial chicken populations. This study was designed to identify effects of heat stress and/or LPS in a heat-resistant inbred Fayoumi line and a heat-susceptible outbred broiler line using global gene expression levels. Bacterial LPS was included as a pro-inflammatory immune stimulant. Analyses were performed in the bursa and thymus, which are critical to lymphocyte development and can provide insight into the effects on immune functions.

Materials and Methods

Chickens from Fayoumi (n = 23) and broiler (n = 26) lines were kept at 25°C in temperature controlled rooms. On day 22, two rooms increased to 35°C (heat stress); two rooms remained at 25°C (thermoneutral). After 3.5 hours, the Thermoneutral + LPS and Heat + LPS groups received LPS (100 µg/kg) by subcutaneous injection, while the Thermoneutral + Saline and Heat + Saline groups received phosphate buffered saline. After 7 hours (post heat or thermoneutral), birds were euthanized and bursa and thymus samples were collected for RNA isolation. cDNA libraries (n = 31 total; 3-4 samples/group/line) were made from the RNA and sequenced on the Illumina HiSeq 2500, generating short sequence reads. These reads were mapped onto the chicken genome and gene expression levels were determined from the number of reads that matched each gene. Gene expression was compared between treatment groups to identify genes with significant differential expression (DE). Potential functional effects of DE were investigated using Ingenuity Pathway Analysis (IPA).

Results and Discussion

Analyses identified 1,607 genes with significant DE in response to at least one treatment. Only 123 significant genes were affected in both tissues, illustrating that there are distinct responses in bursa and thymus. Most significant genes were identified in Fayoumi for bursa (888) and in broiler for thymus (766). This pattern could indicate that humoral and cellular immune responses differ in Fayoumi and broiler chickens. Acute heat stress had less impact on expression than LPS or Heat + LPS, with 94% of changes in broiler thymus. Many interactions between heat and the immune system were still observed (Figure 1). In Fayoumi bursa, Heat + LPS suppressed DE of 364 genes that were up-regulated by exposure to LPS, suggesting high temperatures can reduce inflammatory responses. For example, exposure to LPS up-regulated genes linked to immune cell migration, except in Fayoumi after Heat + LPS (Table 1). In broiler thymus, Heat + LPS suppressed DE of 205 genes, although 316 genes were expressed similarly in both treatments (Figure 1). These expression patterns suggest that both LPS and Heat + LPS could reduce responses in broiler thymus. Consistent with this, immune cell migration genes were down-regulated in all three treatments (Table 1). Overall, global gene expression changes demonstrated unique effects of heat stress in each chicken line and highlighted that immune cell recruitment pathways should be investigated for a potential contribution to chicken disease resistance under heat stress.

Acknowledgments

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Figure 1. Impacts of heat stress on gene expression responses to LPS. Significant changes in expression (FDR-adjusted p-value < 0.05, $\log_2$ fold change ≥ 1) were compared in Heat + LPS and Thermoneutral + LPS. Three outcomes were observed: Suppressed DE (Significant in Thermoneutral + LPS, not Heat + LPS), Same DE (Significant in both), and Additional DE (Significant in Heat + LPS, not Thermoneutral + LPS). The number of genes with each outcome are shown in Fayoumi (orange) and broiler (blue) for both bursa (darker) and thymus (lighter). Differential expression (DE).

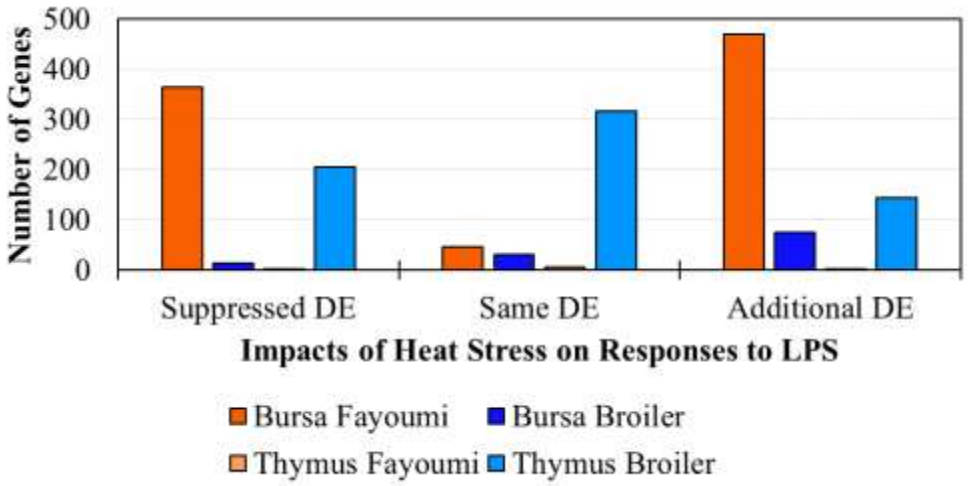

Table 1. Immune-related functions activated or inhibited in response to heat stress and/or LPS[1].

	Bursa		Thymus	
Treatment Group	**Broiler**	**Fayoumi**	**Broiler**	**Fayoumi**
Heat + Saline	---	---	⬇ cell movement ⬇ leukocyte migration	---
Thermoneutral + LPS	⬆ leukocyte activation ⬆ leukocyte migration	⬆ cell movement ⬆ cell proliferation ⬆ leukocyte migration	⬇ cell movement ⬇ endocytosis	---
Heat + LPS	⬆ leukocyte activation ⬆ leukocyte migration ⬆ quantity of Ig	⬆ lymphoma ⬆ phagocytosis ⬇ cell movement	⬇ cell movement	---

[1] Gene functions were predicted using IPA, z-score ≥ |2|, ⬆ = activated, ⬇ = inhibited, --- = none identified