Heat Stress of Commercial Producing Laying Hens Affects Gene Expression in Cecal Tonsil

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Summary and Implications

In this experiment, producing layers were exposed to 4 weeks of cyclic heat to better understand the effects of heat stress on the gene expression in the cecal tonsil (CT). CT is the immune tissue located at the junction between the ileum and the ceca within the digestive tract. CT has been suggested to have a role in the regulation of the cecal content, which may have an impact on nutrient absorption. Knowledge of specific changes in gene expression at the CT highlights potential mechanisms that the tissue utilizes to alleviate the effect from heat stress.

Introduction

One of the hallmark signs of heat stress in poultry is decreased intake of feed. For producing layers, reduced feed intake directly results in decreased egg production. When layers first enter into egg production, there are physiological changes that occur, especially in the balance between production and maintenance. The CT serves the role of regulating the cecal content, but there is currently very little known about the gene expression profile for CT, let alone for CT in layers under stress from coming into production immediately followed by prolonged heat stress. In this study, we utilized RNA sequencing (RNA-seq) to get a snapshot of the gene expression changes in the CT during a 4 week heat stress experiment on newly producing layers.

Materials and Methods

A total of 24 CT samples were collect from 24 to 28 week old Hy-Line W-36 producing laying hens. Half of the samples (n=12, "heat") were from birds subjected to daily heat treatment of 7 hours at 35°C and 17 hours of relaxed temperature of 30°C, and the other half of the samples (n=12, "control") were from birds kept at a constant 21°C. Four birds were sampled from both heat and control groups at 3 times post heat treatment: 3 hours (hrs), 2 weeks (wks), and 4 wks. The birds had *ad libitum* access to feed and water.

RNA was isolated from the CT samples, converted to cDNA libraries, and sequenced on the Illumina HiSeq 3000 sequencer to generate millions of short sequence reads. The reads were processed computationally, aligned to the chicken genome, and gene expression was inferred based on the number of reads that aligned to each of the genes. Two types of comparison between the groups were made to determine a list of differentially expressed (DE) genes: 1) within a time period where the heat group was contrasted against the control group, and 2) within a treatment group where 3 different time points were contrasted.

Results and Discussion

For the 3 contrasts across time within the control group, there was only 1 DE gene detected: *CLEC2B*, a cell adhesion protein involved in cell signaling. Another 18 DE genes were detected in the remaining 6 contrasts involving the heat group, as listed by functional categories in Table 1.

As expected for layers that recently started egg production, we detected changes in genes related to cholesterol biosynthesis (*DHCR24*) and cholesterol metabolism (*INSIG1*). A surprising result is the large number of genes involved with epigenetic regulators that were found to be DE. Although epigenetic regulators are currently not well studied in chickens, epigenetic regulatory mechanisms such as histone modification (*ANKRD11*, *BAZ1A*, and *FAM208B*) and alternative splicing (*SON* and *USP49*) have been shown in mammals to have dramatic impact on gene expression changes.

In all, the 19 DE genes represent pathways and mechanisms that layer CT tissue utilizes under heat stress that were previously unknown. This result will add to the breadth of knowledge for potential gains in layer production through future investigation of specific pathways associated with heat stress in different tissues.

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Table 1. Differentially expressed genes of the cecal tonsil from the heat contrasts of the layer heat stress experiment

	Gene ID	Full Name	Main Function
Cellular Metabolism			
	DHCR24	24-Dehydrocholesterol Reductase	Cholesterol biosynthesis, protects cells from oxidative stress by reducing caspase 3 activity during apoptosis
	INSIG1	Insulin Induced Gene 1	Regulation of cholesterol metabolism, lipogenesis, and glucose homeostasis
	SLC7A1	Solute Carrier Family 7 Member 1	Transport of cationic amino acids in non-hepatic tissues
Epigenetic Regulation of Gene Expression			
	ANKRD11	Ankyrin Repeat Domain 11	Chromatin regulator which modulates histone acetylation and gene expression in neural precursor cells
	BAZ1A	Bromodomain Adjacent To Zinc Finger Domain 1A	Component of the ACF complex that regulates spacing of nucleosomes during DNA replication
	FAM208B	Family With Sequence Similarity 208 Member B	Component of the HUSH complex that mediates epigenetic repression through H3K9me3 transcriptional silencing
	SON	SON DNA Binding Protein	mRNA splicing cofactor for many cell-cycle and DNA-repair transcripts
	USP49	Ubiquitin Specific Peptidase 49	Regulation of mRNA splicing through deubiquitination of histone H2BK120Ub
Cellular Signaling			
	HSPB11	Heat Shock Protein Family B Member 11	Component of the IFT complex B required for SHH signaling for cell differentiation
	MAP3K9	Mitogen-Activated Protein Kinase Kinase Kinase 9	Serine/threonine kinase that is part of the MAP kinase signal transduction pathway
	PLEKHH1	Pleckstrin Homology Domain Containing Family H Member 1	Component of the cytoskeleton
	PTPN13	Protein Tyrosine Phosphatase, Non-Receptor Type 13	Tyrosine phosphatase which negatively regulates FAS-induced apoptosis and NGFR-mediated pro-apoptotic signaling
Immune-Related			
	ENSGALG00000031118	CD82 Antigen-Like	Associates with CD4 or CD8 and delivers costimulatory signals for the TCR/CD3 pathway
	USP18	Ubiquitin Specific Peptidase 18	Regulation of inflammatory response to interferon type 1
Small RNA with Unknown Function			
	ENSGALG00000032037	Novel lincRNA	lincRNA
	ENSGALG00000036605	Novel lincRNA	lincRNA
	gga-mir-1600	gga-mir-1600	microRNA
	SNORA31	Small nucleolar RNA, H/ACA Box 31	snoRNA